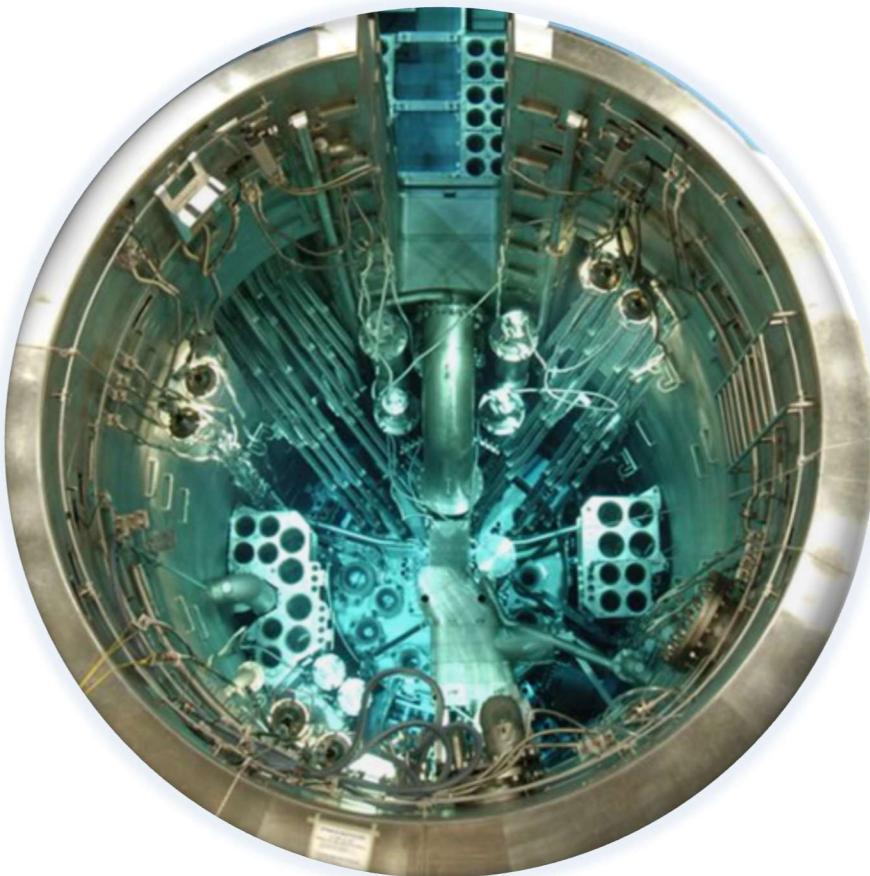




兰州大学核科学与技术学院

School of Nuclear Science and Technology, Lanzhou University



# Radiopharmaceutical Chemistry

## TEXTBOOK

Editor: Prof. Pavle Mocilac

Based on: "Radiopharmaceutical Chemistry" by J. S. Lewis, A. L. Windhorst, B. M. Zeglis (Springer, 2019)



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## Preface

You have decided to pick radiopharmaceutical chemistry as your optional course, thank you very much for your wise choice! It will certainly pay off!

The major purpose of this course is to equip students of radiochemistry with the key knowledge of radiopharmaceutical chemistry so that you can take positions and jobs in the growing radiopharmaceutical sector of China, to ignite your interest in working in a radiopharmaceutical facility, to learn about very modern and cutting-edge radiopharmaceutical concept and ideas emerging in the world as well as the newest frontiers in this area. Finally, I hope this course will inspire your creativity and innovativeness and to think about future developmental and experimental work in radiopharmaceutical chemistry so that radiopharmaceutics in China can thrive and close the gap with other developed countries.

This textbook is just loosely based on the previous course in Chinese language; however, it is heavily updated and modified: largely based on newly published modern book “Radiopharmaceutical Chemistry” written by many authors and edited by Jason S. Lewis, Albert L. Windhorst, Brian M. Zeglis (published by Springer, 2019). In fact, the textbook I your hands is a shortened version of that book. Therefore, the ideas and knowledge in this textbook are much modernised, updated for novel inventions and concepts. The focus is not so much on  $^{99m}\text{Tc}$  (as it was the case in the past), but more on other modern radionuclides for PET imaging such as  $^{18}\text{F}$ ,  $^{11}\text{C}$  and others. As this is a textbook for future radiochemists the focus is on the production of medical radionuclides and radiochemical syntheses of radiopharmaceuticals with many various examples.

It contains general introduction, a short history of radiopharmaceuticals and nuclear medicine, explanation of the key concepts in pharmacology, topics on production of radionuclides for medicinal applications, processing, and quality control. There is also an overview of equipment and instrumentation used.

However, the majority of this textbook covers detailed radiochemistry of medical radionuclides and their radiopharmaceuticals: technetium-99m, fluorine-18, carbon-11, radioiodine, gallium, indium-111, nitrogen-13 and oxygen-15, copper, zirconium, lutetium-177, yttrium, and finally heavy alpha emitters. The accents are on their radiochemistry, but also some examples of radiopharmaceuticals are given. The textbook finishes with radiation protection.

At the very end a dictionary is given with translations of the many English professional and less known words into their equivalent in Chinese language.

Prof. Pavle Mocilac, 19<sup>th</sup> January 2023



## Forewords

When asked to write a foreword for a textbook entitled by Radiopharmaceutical Chemistry in English language for Chinese students, I was quite surprised since textbooks in English language written for Chinese universities in such a discipline are a quite rare novelty.

I do not personally know the author of this book, Professor Pavle Mocilac in Lanzhou University, who is one of a very few foreign teachers in China working in the area of radiochemistry. Here in this textbook Professor Mocilac did an excellent work of summarising the key issues of modern radiopharmaceutical chemistry needed for undergraduate students to understand basic principle, methods, and clinical applications. It is written in simple wording of English language and most of students will understand it without any great difficulty. The textbook is full of dedicated figures and images that will help students to better understand the topics. It is modern and full of very recent ideas and examples tackling the student's imagination and can ignite their interest for this area.

This textbook will be of great use, not only for students majored in radiochemistry, but also for professional workers in this field. I hope this textbook will build a useful bridge between our students and their future achievements and innovations. We are looking forward to seeing these innovations and breakthroughs soon.

A handwritten signature in black ink, appearing to read "Chai".

**Professor Zhifang Chai**

Member of the Chinese Academy of Sciences  
Institute of High Energy Physics, CAS



It was a great surprise to me when I was given a copy of this textbook prepared by Professor Pavle Mocilac for the student of radiochemistry at Lanzhou University. Radiopharmaceutical chemistry is generally topic of postgraduate study, therefore a book covering area of radiopharmaceutical chemistry for undergraduate students is a brave attempt.

This book roughly follows the original large international book of the same topic but is fully tailored and much more suitable for the needs of radiochemists: it covers radionuclides, its production, synthesis, radiochemistry of the most important radionuclides as well as gives short history of radiopharmaceutical chemistry. Also, it is full of practical topics, exactly what young professional people will need once they start dealing with radiopharmaceuticals in their professional life.

China needs to ramp up application of radiological and nuclear tools in science and technology and this especially is the case in medicine. We cannot afford any more to depend on foreign imports. Therefore, new young professional experts with top-notch knowledge in modern radiopharmaceutical chemistry are urgently needed to close the gap and bring the Chinese radiopharmaceutical sector and nuclear medicine to the world level. Part of it is to develop proficiency in English language terminology and therefore develop capability to follow international literature and publish articles in international journals. This textbook is written with the emerging future in mind and therefore, I fully recommend this book to all undergraduate student, not only in the area of radiochemistry but also in general chemistry area.

A handwritten signature in black ink, appearing to read "Wangsuo Wu".

**Professor Wangsuo Wu**

School of Nuclear Science and Technology  
Lanzhou University



## Acknowledgements

Here, I wish to express my greatest gratitude to all who helped me in this project. Firstly, I would like to thank all the teachers and professors at the School of Nuclear Science and Technology for their great support and help especially to Professor Chen Zong Yuan who gave me lots of help at the beginning of my course.

Especially, I wish to say many thanks to Professor Liu Zhibo from Beijing University for giving previous advice and suggestions. I also wish to say great thanks to Professor Wu Wangsuo who helped me and motivated me for this task. A special thank is going to Tien Yi Jia for helping me making all the translations for the dictionary.

In addition, I wish to say thanks to all the students who so far attended my course, their interest in this area is my greatest motivation that this book will be useful in their careers.

The last and not the least, I wish to say thanks to my wife who supported me and motivated in my quest.

Pavle Mocilac



## Chapter I – Introduction to Radiopharmaceuticals and Nuclear Medicine

### What is radiopharmaceutical chemistry?

In short, it is the radiochemistry of nuclear medicine and covers all radiochemical topics there:

- Production and purification of medicinal radioisotopes (such as  $^{99m}\text{Tc}$ ,  $^{18}\text{F}$ ,  $^{11}\text{C}$ ...)
- Radiochemical synthesis and purification of radiopharmaceutical
- Formulation of radiopharmaceuticals
- Development of new radiopharmaceuticals as well as new synthetic methods.

This is very special type of radiochemistry because it looks both into nuclear reactions and organic synthesis. Opposite to nuclear fuel and power areas it deals with very, very tiny, minute quantities of active materials: not mg, not  $\mu\text{g}$  but ng. Preparations here are very fast and automated. It is very interdisciplinary area: a huge bridge between physical, chemical, and medical sciences.

### What are the radiopharmaceuticals?

Radiopharmaceuticals are purified and specially prepared **radioactive substances** (radiotracers compounds or radiolabelled molecules) intended to be taken internally ("*in vivo*") by human or animal patients:

- Radiopharmaceuticals may or may not resemble typical drugs or natural compound but contain one radioactive isotope instead of a stable atom.
- Radiopharmaceuticals are given to patients for diagnostic or therapeutic purposes whereby their main effect is due to their emission of ionising radiation.
- Radiopharmaceuticals can specifically target or interact with biological structures in a human or animal body and perform a diagnostic test or achieve a therapeutic effect.

When used for therapy they are often called "radiotherapeutics": radiopharmaceutical substances (agents) that can specifically target sick body structure, cells or tissue and are design to heal/treat disease, have some therapeutic effect.

Chemically speaking radiotherapeutics can be:

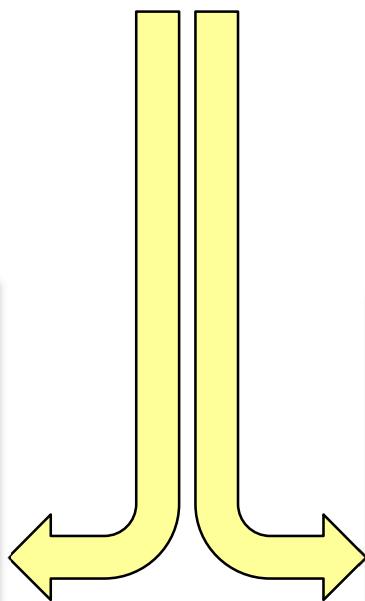
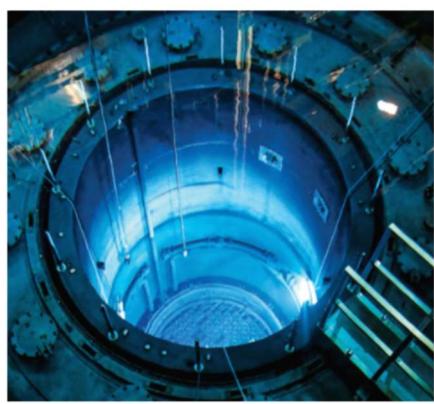
- A simple radioactive material, salt like  $\text{Na}^{99m}\text{TcO}_4$ ,  $\text{Na}^{131}\text{I}$  or  $^{223}\text{RaCl}_2$ ,
- A natural compound or a drug-like small molecule labelled with a radionuclide. Here a radionuclide atom is covalently bound onto the rest of the molecule.

- Radioactive metal ion in a complex with a chelating ligand to carry it through the body and protect it from interactions with biological structures,
- Radioactive isotope covalently bound onto a peptide, protein or even a nanoparticle.
- Radioactive metal ion in a complex with a chelating agent while the whole complex is then covalently linked with a biomolecule (protein, peptide, monoclonal antibody) that is then called bioconjugate

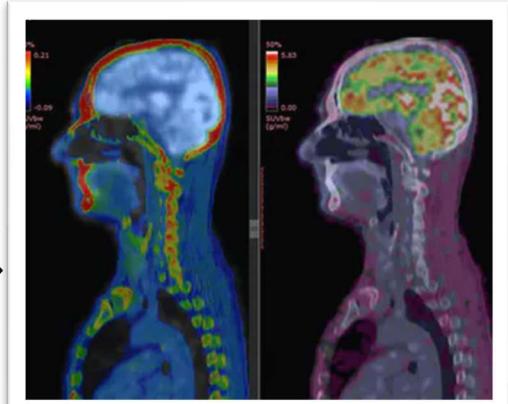
It is very important to remember that when it comes to usage of radioactive materials in the world per quantity of radioactivity (in Bq), then nuclear power section is, for sure, number one: most of radioactive materials are used by nuclear power technologies. However, nuclear medicine is the second area with the largest usage of radioactive materials. Therefore, nuclear medicine is the second biggest consumer of radioactive materials after power generation. Other areas where radioactive materials are used are industry, research, science, environment, mining, agriculture, space travel, but these human activities consume less radioactive materials than nuclear medicine:

## Radioactive materials

### 1# Nuclear Power



### 2# Nuclear Medicine





## What is the nuclear medicine?

It is classically defined as the application of radionuclides in the area of medicine. Nuclear medicine takes advantage of the unique properties of radioactive isotopes, which have significantly different physical properties compared to stable elements but identical chemical behaviour. More specifically, radioactive isotopes decay and emit ionising radiation: particles and/or photons (e.g., positrons, gamma rays, etc.). This ionising radiation can be then harnessed to facilitate some diagnostic procedure or even to cure a disease, where small quantities of ionizing radiation are used for:

- **Diagnostics (imaging or tracing)** where a patient is given a very small quantity of radiochemical substance containing photon-emitting radionuclide and this substance then concentrates in some specific organ, tissue or cells, and photons emitted from that specific location serve as signals for locating, imaging – instruments then calculate exact location of the signal source and create special maps: images of internal organs of a body.
- **Targeted radiotherapy (TRT)** where larger quantities of radioactive materials are guided onto a specific unwanted tissue or cells inside a body (like for example cancer), and then the deadly effect of ionizing radiation removes those unwanted cells and tissue thereby curing a disease.

Therefore, radiolabelled molecules, termed “radiopharmaceuticals,” are the essential elements in nuclear medicine.

## What is the nuclear imaging?

It is the use of radiopharmaceutical agents to target specific tissues or cells, and then detecting those cells or tissues in a body with gamma-detectors, creating images: locating or determining function of targeted cells/tissues. In practice, a live person receives a radiopharmaceutical imaging substance (agent) that goes specifically to the tissue it has specific affinity for: it binds to it or simply concentrates inside or around the desired cells/tissues. Then location of the emission in the body gets precisely detected and visualized by large detecting and imaging devices. There are two types of such imaging devices:

- Single-Photon Emission Computed Tomography (SPECT, also known as “gamma camera”), detects simple gamma radiation
- Positron Emission Tomography (PET) detects annihilation gamma rays emitted by annihilation of positrons and electrons.

These devices can detect the radiation, locate the source, and using computer software map the point sources of radiation thereby creating images. Nuclear imaging is based on the approximation that practically none of the biomolecules within the body are very radioactive. As a result, radiopharmaceuticals can be distinguished easily from the native molecules, providing nearly infinite contrast for

imaging. In principle, every molecule of a diagnostic radiopharmaceutical can be detected, providing extraordinary sensitivity for imaging.

In practice, however, there are limits how much radiopharmaceutical tracer can be given to a human. It is possible to generate high-quality images using radioactivity doses as low as 30–600 MBq, values which correspond to as little as nanomoles of the radioactive compound or less. These compounds usually contain highly active radioisotopes:  $^{99m}\text{Tc}$ ,  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{68}\text{Ga}$ ,  $^{111}\text{In}$ ,  $^{123}\text{I}$  and their radioactivity gives the patient usually 3–30 mSv of radiation dose. This unique property allows radiopharmaceuticals to behave as true molecular tracers without perturbing the native biochemistry of the system, following the tracer principle of De Hevesy.

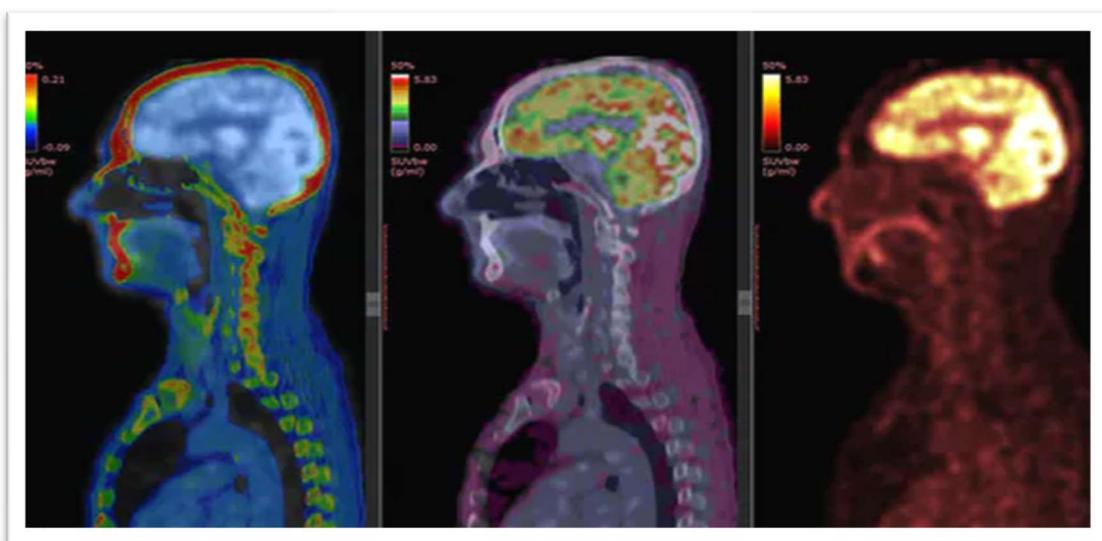


Figure 1: Nuclear imaging, PET scan overlaid with MRI scan

Here I will illustrate how tiny, minute quantities of radiopharmaceuticals are enough for nuclear imaging: we need use radioisotopes of very high activity and very short half-life. For example, a molecule named  $^{18}\text{F}$ -fluoroestradiol ( $^{18}\text{F}$ ES, Figure 2) is a female hormone estradiol labelled with highly radioactive fluorine-18 ( $^{18}\text{F}$ ).  $^{18}\text{F}$  has half-life of just 1 hour and 50 minutes. In this molecule  $^{18}\text{F}$  is covalently attached onto estrogen molecule and estrogen specifically binds in a body onto estrogen receptors. And these receptors are quite numerous on breast cancer cells. It means once in the body radioactive emission of  $^{18}\text{F}$  will be carried by pharmacological behaviour of this

whole molecule and then will be especially concentrate around breast cancer cell. Molecular weight of  $^{18}\text{F}$ ES is 289.37 g/mol, therefore specific activity is very high, 3519 PBq/g. For a good image quality, we need 185 MBq of  $^{18}\text{F}$  activity and when we calculate how much is 185 MBq in moles: it is just 5 nanomoles or just 0.845 ng. Very, very tiny quantity indeed!

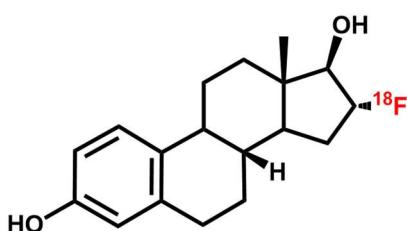


Figure 2:  $^{18}\text{F}$ -fluoroestradiol (FES)



Maximal concentration of this  $^{18}\text{FES}$  radiopharmaceutical imaging agent in the body could be around 1 nM and the lowest concentration of circulating estrogen in body is also 1 nM, therefore this additional  $^{18}\text{FES}$  will not produce any additional physiological hormonal effect in the body. In other words, body will not even see it, not even feel it. But our sensitive machines will be able to detect it and identify its location very clearly and precisely. And calculated dose of radiation is just 4 mSv and that is acceptable in the fight against cancer. So, De Hevesy principle is fulfilled: 185 MBq of  $^{18}\text{FES}$  makes a good image but does not interfere and gives acceptable level of radiation dose to patients. Nuclear imaging is a very important tool for clinical diagnostics. It is used thousands of times each day around the world either as PET or SPECT scan procedures. It is most used to detect and quantify organ function and/or abnormal physiology and molecular biochemistry in a variety of disorders.

Area	Problem/Disease	Radiopharmaceutical agent
Cancer	Aberrant glucose metabolism	$^{18}\text{F}$ -Fluorodeoxyglucose (FDG)
	Abnormal amino acid transport	$^{18}\text{F}$ -Fluciclovine, $^{11}\text{C}$ -Methionine
	Expression of cancer-specific biomarkers	$^{225}\text{Ac}$ -DOTA-PSMA 617
	New bone formation associated with cancer metastases	$^{99\text{m}}\text{Tc}$ -Methylene diphosphonate (MDP)
Endocrine disorders (glands)	Hyperthyroidism	$^{123}\text{I}$ -NaI
	Abnormal catecholamine-producing tumours such as pheochromocytomas and neuroblastoma	$^{123}\text{I}$ -meta-Iodobenzylguanidine (mIBG)
	Neuroendocrine tumours	$^{111}\text{In}$ -Pentetretide or $^{68}\text{Ga}$ -DOTATATE
Cardiovascular (heart) disease	Coronary artery disease	$^{99\text{m}}\text{Tc}$ -Sestamibi or $^{82}\text{RbCl}$
	Aberrant presynaptic cardiac innervation in heart failure and arrhythmias	$^{123}\text{I}$ -mIBG
Neurologic and psychiatric diseases (brain)	Stroke	$^{99\text{m}}\text{Tc}$ -ECD
	Alzheimer's dementia	$^{11}\text{C}$ -Pittsburgh compound B (PIB)
	Depression	$^{11}\text{C}$ -Raclopride
	Schizophrenia	$^{18}\text{F}$ -DOPA, $^{18}\text{F}$ -N-methylspiperone
	Epilepsy	$^{18}\text{F}$ -Flumazenil,
	Parkinson's disease	$^{18}\text{F}$ -DOPA, $^{18}\text{F}$ Fluoropropyl-CIT
Kidneys or liver	Renal dysfunction	$^{99\text{m}}\text{Tc}$ -MAG <sub>3</sub>
	Cholecystitis and biliary dyskinesia	$^{99\text{m}}\text{Tc}$ -Mebrofenin
Infection, trauma, etc.	Bone trauma and infection on the basis reactive new bone formation	$^{99\text{m}}\text{Tc}$ -MDP
	Infection	White blood cells labelled with $^{111}\text{In}$ -oxime

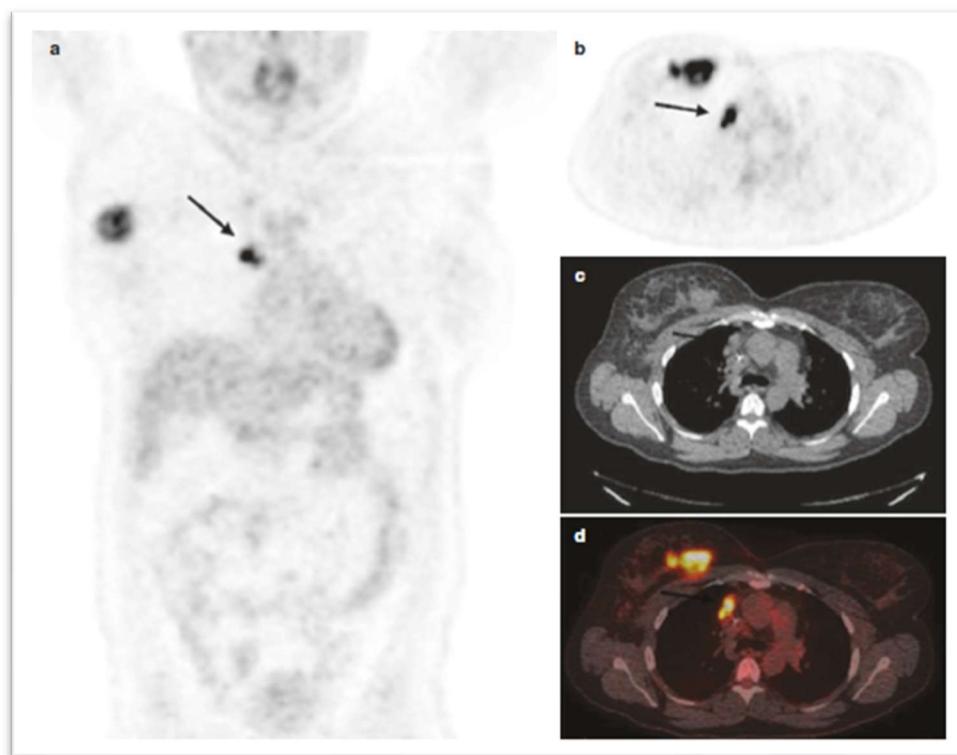
Table 1: Various radiopharmaceuticals and their medical area

Nuclear imaging is used to localize and measure specific physiologic and molecular processes associated with either normal organ function or tissue dysfunction, a disease.

Please make important difference: **it is visualizing mainly functions of organs, not so much shape of organs.** In recent years, fundamental research in molecular biology, cell biology and human physiology has led to the identification of new targets (proteins), and radiopharmaceutical chemists have used this information for the development of novel radiopharmaceuticals and the result is more accurate and precise imaging agents based on newly discovered mechanisms in molecular and cellular biology.

Table 1 presents a list of various radiopharmaceuticals, molecules labelled with certain radioisotope, and for which kind of disease it is used in clinic and area of medicine.

Figure 3 shows a patient with breast cancer that was given radiopharmaceutical agent called  $^{18}\text{F}$ luorodeoxyglucose ( $^{18}\text{FDG}$ ) and this radiolabelled molecule specifically goes to the cells with very high consumption of glucose. Since cancer cells feed themselves mostly on glucose they are known as “traps for glucose” and concentration of glucose in cancer cells is unusually high. As it takes normal glucose cancer cell will “soak-in” also  $^{18}\text{FDG}$  and finally concentration of  $^{18}\text{FDG}$  will be very high in cancer cells, much more than in other tissues (except brain and bladder).

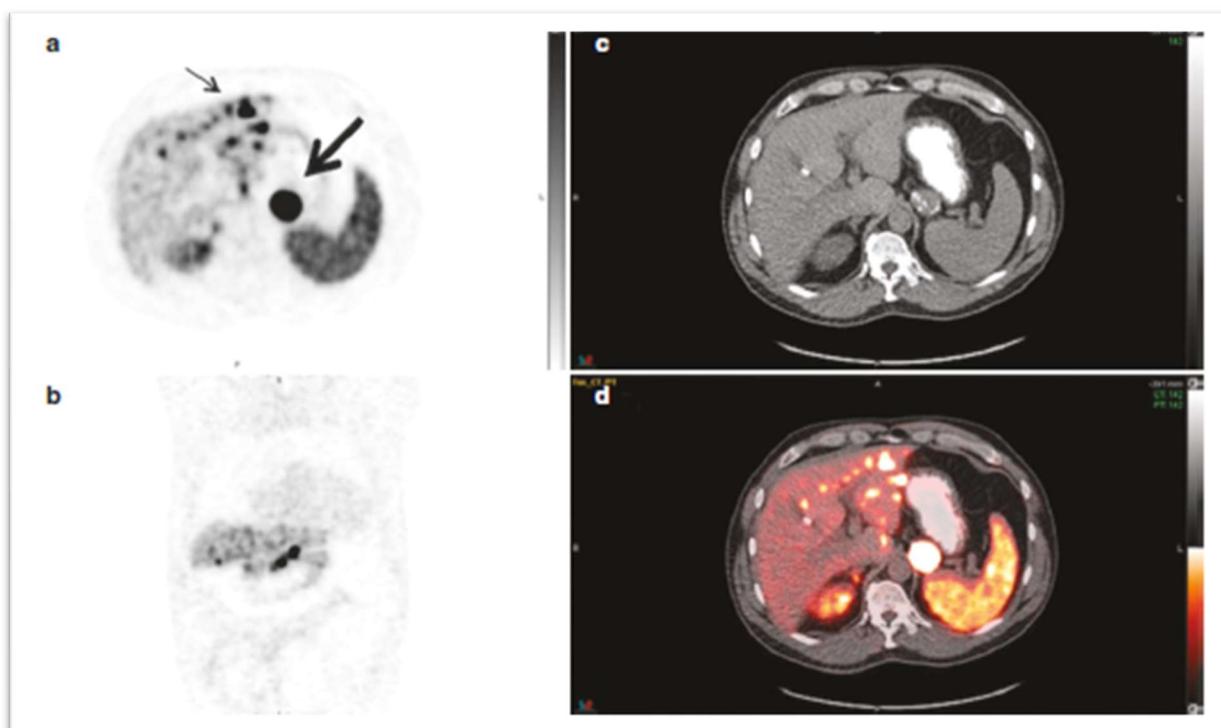


**Figure 3:**  $^{18}\text{F}$ luorodeoxyglucose (FDG) PET/CT of breast cancer demonstrates the spread of the disease to small mediastinal nodes that are not detected by CT (arrows). Image a is a coronal PET image of the regional retention of FDG; on the right, axial PET images (b) have been combined with CT in the images (c) to yield fused images overlaying PET and CT images (d)

Therefore physician will be able (using a radiation detecting device called PET) to literally “see” where in the body cancer cells are growing, and can conclude if there

is a spread of the disease or a metastasis. Sometimes it is not very clear where exactly this metastasis is on this PET scan so if we then combine it with a CT scan of the same person, same conditions, and then we can be very sure where it actually is. Next, knowing exact location of a cancer a surgeon can start planning a very precise surgery.

Another example is in the Figure 4: validation of tumour called “carcinoid”, and this is a form of tumour that slowly grows in the belly organs, but if it goes into the patient’s liver then becomes very deadly. Here  $^{68}\text{Ga}$ -DOTATE, a radiopharmaceutical agent that binds specifically to the receptor typical for this kind of tumour can be used and the cells of the tumour have lots of these receptors on their surface. The molecules of this radiopharmaceutical agent are able to seek and find cancer cells, bind directly onto the cancer cells and there from the actual location of the cancer cells are emitting gamma rays. Then, we also do CT scan and combine CT and PET images and we get precise locations of the cancer cells that look like fire spots! In this case we can see that cancer has spread to liver. You can see how in CT scan we see no cancer inside the liver. But with PET scan we can see that clearly.



**Figure 4:** The staging of tumour using  $^{68}\text{Ga}$ -DOTATE PET/CT. Imaging of somatostatin receptor-expressing carcinoid tumour deposits using a PET scan (a, b) and relate the localization of sites of radiopharmaceutical uptake to anatomic sites indicated by the accompanying CT (c) and depicted on fused PET and CT images (d).

Final example of use of nuclear imaging in diagnosis is in the Figure 5: visualisation of Alzheimer’s dementia. Two persons are given a radiopharmaceutical agent that contains  $^{18}\text{F}$ : on the left is a person with Alzheimer’s dementia and on the right is a person without Alzheimer’s dementia, both persons are given the same amount of

radiopharmaceutical agent, but this image clearly gives proof that this patient really has the Alzheimer's dementia, not some other type of dementia.

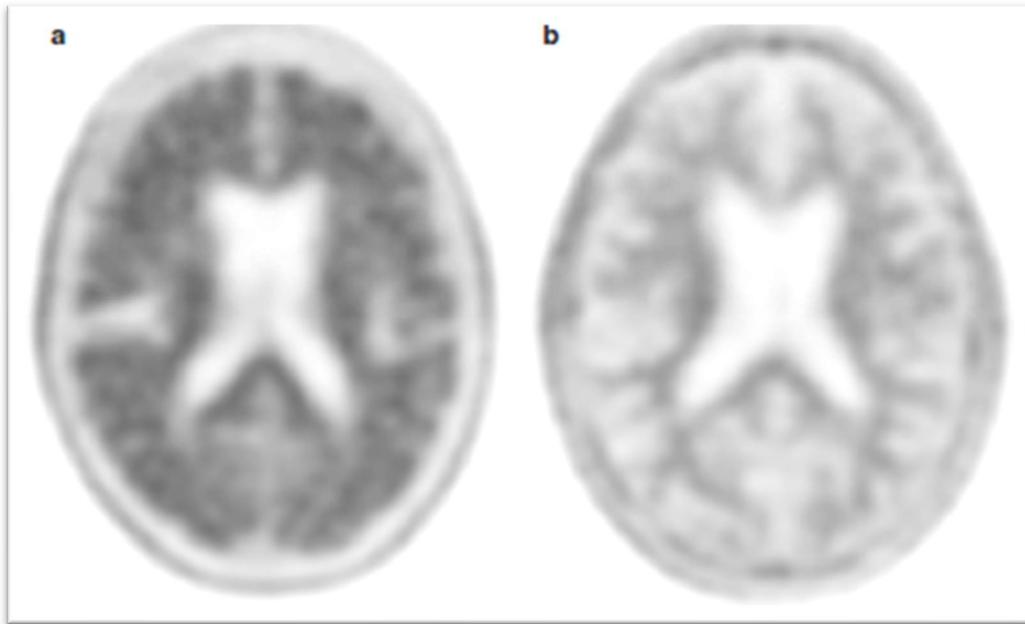


Figure 5: Imaging amyloid deposition in Alzheimer's dementia neural plaques using  $^{18}\text{F}$ -Florbetapir.  $^{18}\text{F}$ -Florbetapir PET images from an Alzheimer's disease patient (a) and a normal control subject (b) are shown.

Are the nuclear imaging methods, PET and SPECT better than the classic methods of medical imaging like classic X-rays, computed tomography X-ray scan (CT scan), Nuclear Magnetic Resonance (MRI) or ultrasound?

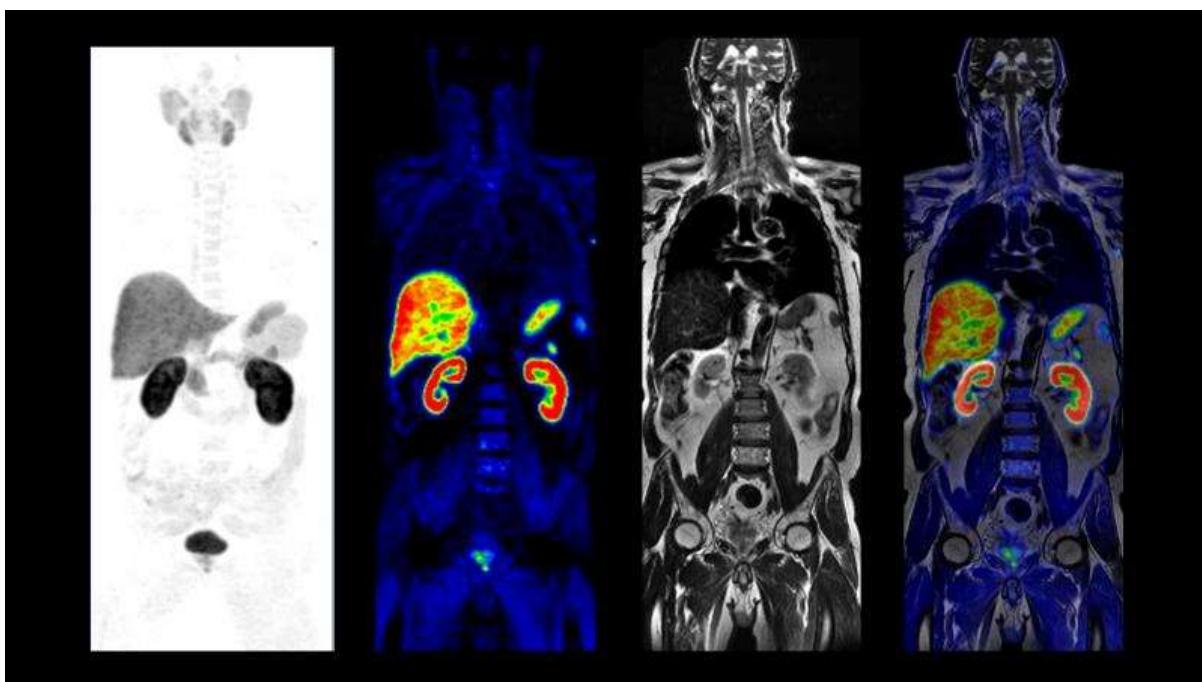


Figure 6: combination of PET and MRI scans gives physician excellent physiological-anatomic view of the disease

They are different and have advantages: in nuclear imaging the tracer principle is used which means that imaging is done without perturbing native biochemistry whatsoever. There are not many side effects, relatively low radiation dose to patients is given (3-30 mSv), but nuclear imaging is very unique because it gives high-contrast images of a certain function of an organ: nuclear imaging “sees” what classic methods cannot!



Figure 7: Single-photon emission computed tomography device (SPECT, a.k.a. gamma camera).

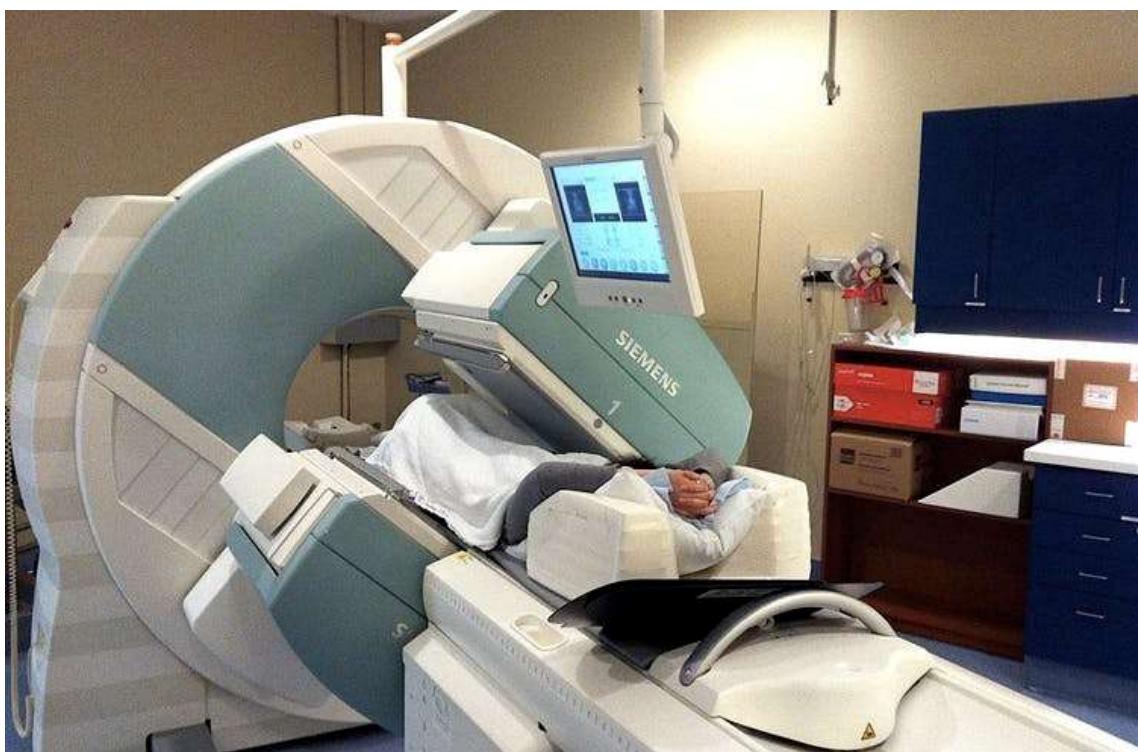


Figure 8: Single-photon emission computed tomography (SPECT) machine in action.



**Figure 9: Positron-Emission Tomography scanner (PET scanner)**

On the other hand, PET (Figure 9) and SPECT (Figures 7 and 8) cannot visualize organs well; classic MRI or CT “sees” organs and tissue shapes and locations much better than PET and SPECT. Also, it involves exposure to radiation, unlike MRI or ultrasound. In practice however, these classic technics are combined with techniques of nuclear medicine: in almost all clinical practice we have combinations: PET/CT or PET/MRI or SPECT/CT (Figure 10).



**Figure 10: Positron Emission Tomography (PET) and Nuclear Magnetic Resonance Imaging (MRI) in the same machine!**

## What is the nuclear therapy?

As previously shown, the imaging is for diagnostic procedures, to find out what kind of disease patient is suffering from, and to gather more information about the disease and the progress of disease. Therapy is a procedure to cure, alleviate disease. Therefore, nuclear therapy is the use of ionizing radiation for therapy, to cure disease. There are three types of nuclear therapy:

**a) External beam of ionizing radiation**

**b) Brachytherapy**

**c) Targeted radiotherapy (TRT)**

A) The external beam of ionising radiation is the classic treatment with gamma rays that originate from some large source of ionizing radiation, for example a large quantity of radioactive material that gives strong, penetrating gamma-rays, such as  $^{60}\text{Co}$  or  $^{137}\text{Cs}$ . A gamma ray is directed onto a cancer tissue, and the tissue is irradiated until it receives a high dose of radiation, several Sieverts of radiation, but only the specific area where the tumour is located. Another option is radiotherapy with focused beams of radiation where dose is very large only at the intersection of all these rays, while other tissues will not get much dose (so called “gamma-knife”). There are other powerful types of external therapies such as proton beam or neutron therapy.



Figure 11: External beam radiation therapy

B) Brachytherapy is when small metallic capsules filled with some radioactive materials are physically inserted into the cancer tissues and then are emitting radiation there. The trick is that many these little capsules are inserted into and around cancer tissue and then left for a while to kill all the cells around, while other tissues will stay healthy.

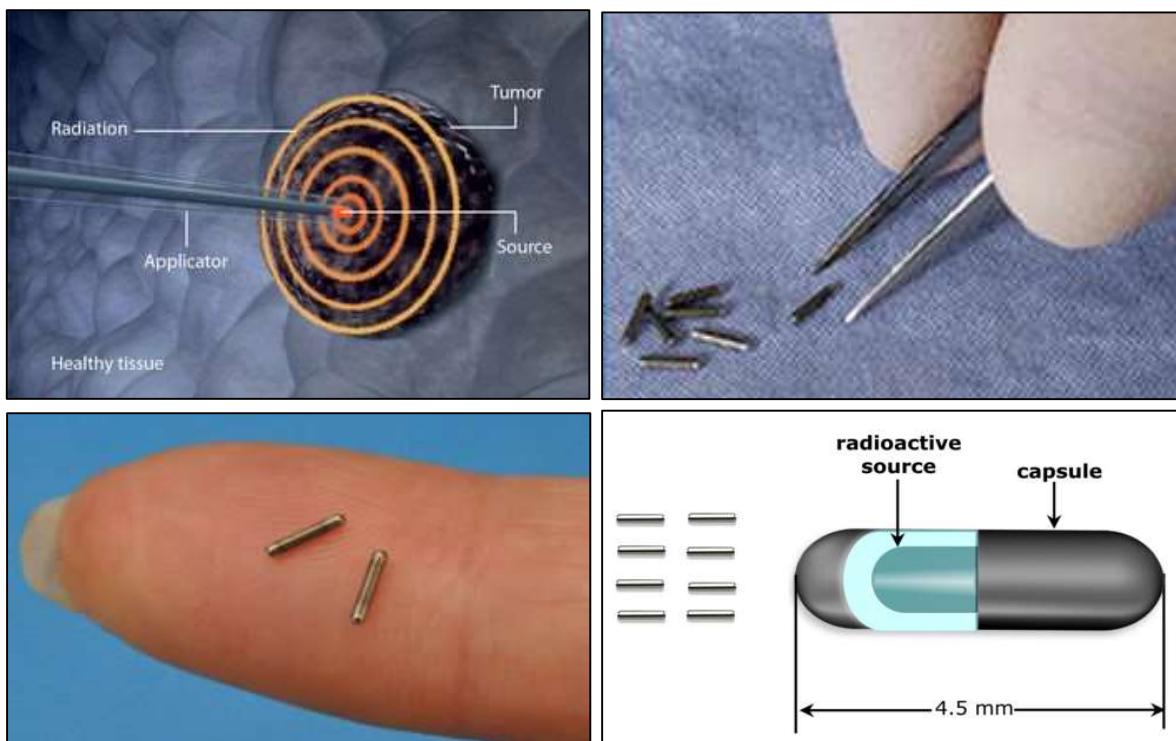


Figure 12: Brachytherapy

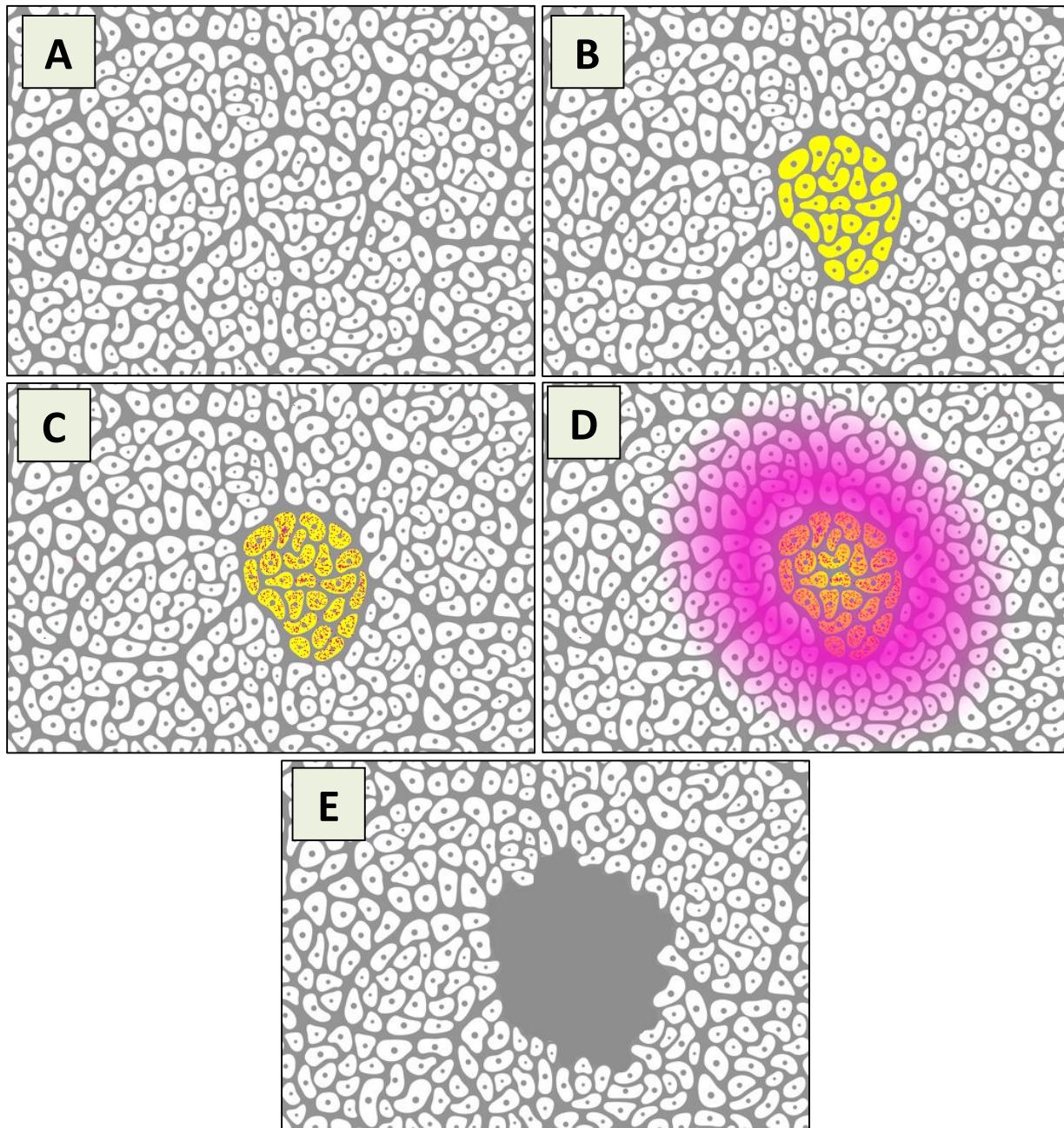
### Targeted radiotherapy (TRT)

Targeted radiotherapy or TRT is the third type of nuclear therapy and involves radiopharmaceuticals that are taken internally into the body and are freely circulating bloodstream. Therefore, here we have internal irradiation of biological structures. Literally, a patient receives a radioactive drug that then goes into blood circulation and seeks, searches the cells it specifically binds and destroys with ionising radiation. Radionuclide is carried by a radiolabelled molecule that can specifically bind to particular tissue or cells that need to be irradiated and killed. Radiopharmaceutical agent in that case is called radiotherapeutic agent and is given to a patient in the form of injection or infusion, circulates the body and seeks the targeting tissue, concentrates only there and delivers ionising radiation only there: where it has to!

Nuclear radiotherapy is based on the use of radiopharmaceuticals to deliver therapeutic radiation to a target within the body we want to eliminate, like in the case of cancer cells or tissues as shown in the Figure 13!

Idea of targeted radiotherapy was conceived many years ago and gave us a promise of powerful therapy to fully heal deadly cancer but failed to materialize so far as a

massive therapeutic option in clinical practice. In fact, it's still mainly limited and experimental option for just some forms of cancers.



**Figure 13: Basic concept of targeted radiotherapy:** a) Morphologically both healthy and cancerous tissue cells all look the same; b) but cancerous cells (yellow) even they look the same as healthy ones, have some specific antigens and different behaviour (physiology) b) radiotherapeutic agent has ability to recognize specific antigens on cancer cells and bind specifically cancer cells not healthy cells; d) ionising radiation emitted from radiotherapeutic is concentrated only around cancers cells although some healthy surrounding tissue can be affected e) finally, irradiated cells die and cancer is removed

The principle of targeted radiation therapy is to expose cancer cells to very high doses of ionising radiation in form of particles ( $\alpha$  or  $\beta$ ). This radiation is delivered just onto those cells that need to be removed from the body – up to 30 Sv! One must realise that 30 Sv is a huge dose of ionising radiation: if this 30 Sv is delivered onto whole body then death from acute radiation sickness is inevitable within days (2-3



days)! But in targeted radiotherapy up to 30 Sv of ionising radiation is delivered to cancer cells only, while the rest of the body received very little ionising radiation and suffers little or no side effects.

Targeted radiation therapy is especially attractive for non-operable and dispersed types of cancer such as for various blood cancers (for example for leukaemia, lymphoma, myeloma) but also for all sorts of cancer in metastatic stage where cancer cells spread everywhere in the body and surgery, or other kind of radiotherapy cannot be used while chemotherapeutic options are limited.

Since the basis for TRT is the precise and strong targeting of “bad guys” then specific binding of radiotherapeutic agent onto malignant cancer cell must be accomplished by using molecular recognitions: here simple radiopharmaceutical agents in the form of small molecules are usually not good enough, and the strategy needs superb recognition ability, hence peptides and proteins, especially artificial monoclonal antibodies (mABs) are used as vectors, carriers of radionuclides.

However, there is one inherent problem of targeted radiotherapy and that is that healing is achieved by only killing, removal of dysfunctional cells. The most of normal common therapeutics are not outright killing cells but are in fact just modulating their function usually interacting with some proteins in cells. TRT is on the other hand killing complete cells. This characteristic limits it to treating cancer which is still acceptable since cancer is a dreadful disease. Now, there is another group of diseases where removal of cells could cause full remission, and these are autoimmune diseases. In autoimmune diseases immune cells such as for example lymphocytes become aberrant and are attacking different healthy cells. These diseases are numerous and are affecting millions; these are for example diabetes mellitus I, multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythematosus, inflammatory bowel disease, etc. All we need is to identify proteins that are responsible for aberrant behaviours of lymphocytes and make mABs to target just aberrant cells, but not similar but healthy cells. This, in fact, is not an easy task and currently there is no radiotherapeutics able to tackle autoimmune diseases, yet one can imagine that one day this would be possible.

Which medical radionuclides are used for radiotherapy? There are limited number of them, and these are: either

- beta emitters such as  $^{177}\text{Lu}$ ,  $^{90}\text{Y}$ ,  $^{131}\text{I}$ ,  $^{67}\text{Cu}$ ,  $^{89}\text{Sr}$ ,  $^{153}\text{Sm}$ ,  $^{188}\text{Re}$  and  $^{32}\text{P}$
- alpha emitters such as  $^{225}\text{Ac}$ ,  $^{223}\text{Ra}$ ,  $^{221}\text{At}$ ,  $^{227}\text{Th}$  or  $^{212}\text{Pb}$ .

For a radionuclide to be acceptable for radiotherapy some criteria need to be met:

1. Half-life should be in days, max 15 days, not longer, but also could be as short as 7 hours.
2. The particles,  $\alpha$  or  $\beta$  should have high “linear energy transfer (LET)” value although sometimes lower LET is acceptable.



3. It should immediately give a stable daughter (but this is not often the case).
4. It should have only one decay route, and this is also sometimes hard to have.
5. There should be no strong gammas, weak ones can be good (theranostic).
6. It should be held by vector tightly and never “wander off”
7. It should be easy to be made, not overly expensive, hence should be readily available and affordable.

Therefore, the criteria are limiting our choice. The table below shows some medical radionuclides suitable for targeted radiotherapy and their characteristics. The energy of particles is quite high for betas while alphas have typical energies. Range is from 11 mm to just 47 micrometers, yet the most important characteristic is in fact the linear energy transfer (LET). This is a measure of the energy transferred to material as an ionizing particle travel through it so therefore it is a measure of force of ionizing radiation particles and ability to kill the cells. We can see that alpha particles have way higher LET than beta particles and are much more suitable for radiotherapy.

Particle type		Half-life	Max. energy (keV)	Max. range in tissue ( $\mu\text{m}$ )	LET (keV/ $\mu\text{m}$ )
$^{177}\text{Lu}$	$\beta^-$	6.65 days	500	2000	0.34
$^{90}\text{Y}$	$\beta^-$	2.67 days	2280	11 000	0.21
$^{131}\text{I}$	$\beta^-$	8.05 days	606	2000	0.25
$^{67}\text{Cu}$	$\beta^-$	2.58 days	580	2100	0.27
$^{225}\text{Ac}$	$\alpha$	9.92 days	5750	47	80
$^{223}\text{Ra}$	$\alpha$	11.4 days	5979	100	80
$^{221}\text{At}$	$\alpha$	7.21 hours	7450 ( $^{211}\text{Po}$ )	80 ( $^{211}\text{Po}$ )	98

Targeted radiotherapy offers a significant advantage over traditional systemic therapy with non-radioactive drugs and external beam radiotherapy:

- Radiopharmaceuticals can deliver high doses of ionising radiation exactly where it is needed, no other effect!
- Radiopharmaceuticals are given to patients at low molecular doses and therefore do not generate the serious side-effects typically seen in chemotherapy.
- Compared to external beam radiotherapy, molecularly targeted radiopharmaceuticals are typically able to deliver radiation to tissues more selectively than external beam radiotherapy.

Yet, targeted radiotherapy is not perfect:

- it is limited by the specificity of the vector for the targeted disease, typically cancer or endocrine disorders, and by the toxicity to while radiotherapeutic is traveling through the body to the targeted tissue



In practice targeted radiotherapy is much less used than other types of nuclear therapies and significantly much less than nuclear imaging. In the table below currently, approved radiotherapeutics are listed:

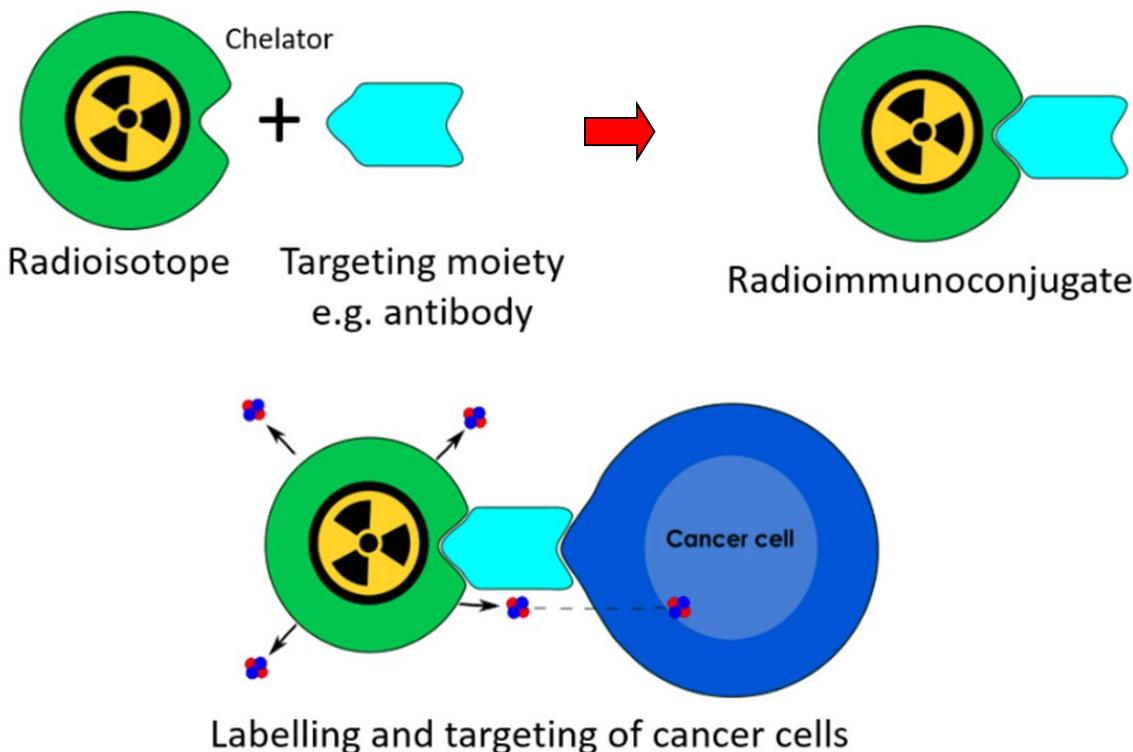
Disease	Radiotherapeutic
Hyperthyroidism	$\text{Na}^{131}\text{I}$
Thyroid cancer	$\text{Na}^{131}\text{I}$
Painful bone metastases	$^{89}\text{SrCl}_2$ , $^{223}\text{RaCl}_2$ , or $^{153}\text{Sm-Lexidroname}$
Neuroblastoma	$^{131}\text{I-mIBG}$
Malignant pheochromocytoma	$^{131}\text{I-mIBG}$
Neuroendocrine tumours	$^{177}\text{Lu}$ or $^{90}\text{Y-DOTA-octreotate}$
Non-Hodgins lymphoma	$^{90}\text{Y-Ibritumomab-tiuxetan}$ or $^{131}\text{I-Tositumumab}$
Metastatic prostate cancer	$^{225}\text{Ac-DOTA-PSMA 617}$

The first and still most common use of nuclear radiotherapy is the treatment of hyperthyroidism caused by Graves' disease and toxic nodular goitre. In this approach, modest doses of radioactive sodium iodide (contains  $^{131}\text{I}$ ) provide a safe and highly effective therapy. In Graves' disease and toxic nodular goitre large fraction of ingested iodine (30%) goes to the thyroid and tissue gets reduced by radiation with minimal radiation exposure to the rest of the body. The remaining applications of nuclear therapy largely focus on treating cancer: the small risk of modest radiation exposure to some normal tissues is acceptable taking into account considerable therapeutic efficacy in otherwise terrible disease that usually kills patient. Some examples of nuclear radiotherapy in the treatment of cancer include treatment of thyroid cancer, using  $\text{Na}^{131}\text{I}$  (typically higher doses than those needed in hyperthyroid treatments), painful bone metastases using bone-targeting agents such as  $^{89}\text{SrCl}_2$ ,  $^{223}\text{RaCl}_2$ , and  $^{153}\text{Sm-lexidroname}$ , cancers like neuroblastoma and malignant pheochromocytoma using the  $^{131}\text{I-mIBG}$ , and neuroendocrine tumours, using  $^{177}\text{Lu}$  or  $^{90}\text{Y-DOTA-octreotate}$ .

An additional type of nuclear radiotherapy is called “radioimmunotherapy” (Figure 14) and takes advantage of the specificity and affinity of monoclonal antibodies (mAB) as molecular markers of disease. Radioimmunotherapy is based on the use of therapeutic radioimmunoconjugates, most commonly labelled with beta particle-emitting radionuclides such as  $^{131}\text{I}$  or  $^{90}\text{Y}$ . The application of radioimmunotherapy to B-cell lymphoma generated considerable excitement and resulted in two approved agents (Bexxar and Zevalin) based on anti-CD20 antibodies labelled with  $^{131}\text{I}$  and  $^{90}\text{Y}$ , respectively.

There has been considerable recent excitement over the future of nuclear radiotherapy. This optimism has been driven by the increased success in generating highly targeted small molecules and peptides that have high uptake and retention in cancerous tissues e.g., PSMA-targeted radiotherapeutics for prostate cancer that are

carrying  $^{225}\text{Ac}$  was recently approved in the USA for treatment of metastatic prostate cancer.



**Figure 14:** Concept of radioimmunotherapy

### Role of radiochemistry and radiochemists in nuclear medicine

Radiopharmaceutical chemistry and nuclear medicine are very interdisciplinary area, demand knowledge in many areas and collaboration of many professions. So, what is the exact role of radiochemistry and radiochemist in nuclear medicine?

Design, building and operating reactors and accelerators is usually sole task of **nuclear physicists**, but the preparation of the targets and actual production of medical isotopes is the area where radiochemists are collaborating with nuclear physicists. **Radiochemists** exclusive areas are purification of radioisotopes and making precursors, synthesis of radiolabelled compounds or radiopharmaceuticals. When it comes to formulating radiopharmaceutical preparations and quality control of radiopharmaceuticals radiochemists are usually working with **pharmacists**, while pharmacists are working with **medical doctors** on application of radiopharmaceutical to patients. Finally, imaging, interpretation of images and general evaluation of radiopharmaceutical effects in patients, this is a sole job of medical doctors, like radiologists, oncologists, pharmacologists. We, radiochemists are not dealing with these areas.



### Nuclear physicists

- Designing, building and operating reactors and accelerators

Radiochemists

- Preparation of targets and production of medical radioisotopes

- Purification of radioisotopes (precursors)
- Synthesis of radiolabelled compounds (radiopharmaceuticals)

Pharmacists

- Preparation of radiopharmaceutical preparations
- Quality control of radiopharmaceuticals

Medical doctors

- Application of radiopharmaceuticals
- Imaging, Interpretation of images
- Evaluation of radiopharmaceutical effects

Figure 15: Nuclear medicine as an interdisciplinary area, involving nuclear physicists, radiochemists, pharmacists and medical doctors (physicians)

## Chapter II - History, current state and the future of radiopharmaceuticals

### Discovery of ionizing radiation and radioactivity

All story about radioactivity and ionising radiation started in 1895 with the discovery of X-rays by Wilhelm Roentgen, a German professor (Figure 16a). On November 8, 1895, he was studying cathode rays using an evacuated glass cathode tube (Figure 16b). The tube was covered in black paper and the room was dark, but he noticed that a fluorescent screen across the room was glowing. Remarkably, when he blocked the beam with his hand, he could see the bones in his hand projected on the screen. This discovery was an instant sensation, and immediately by the end of the next year X-rays were becoming an established tool in medicine, and in 1901, Roentgen won the Nobel Prize in Physics for his discovery.

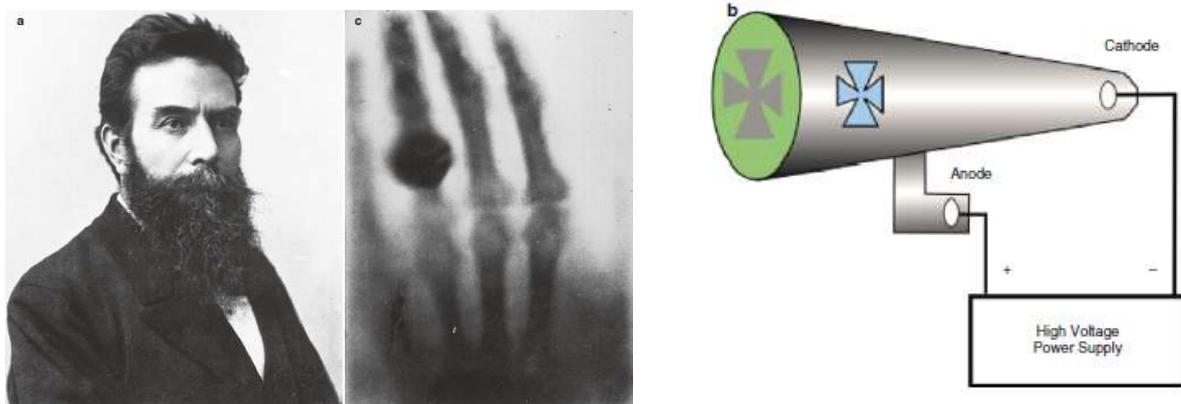


Figure 16: a) Wilhelm Roentgen, b) the first X-ray photograph of a human body (the hand belongs to Wilhelm Roentgen's wife) b) the concept of X-ray tube

Natural phenomenon of radioactivity was discovered by a French scientist Antoine Becquerel (Figure 17a) in 1896. Upon learning of Roentgen's discovery of x-rays, Becquerel chose to study these "mysterious rays". He exposed a salt of uranium to sunlight and placed it on photographic plates wrapped in black paper. When developed, the plates showed an image of the uranium crystals, and he initially believed that the sunlight was absorbed by uranium, which then, he thought, emitted X-rays. But then the photographic plates with uranium on a top of them were returned to a dark drawer and left for a while. Although Becquerel expected only faint images, they were strong and clear (Figure 17b). It was clear that uranium itself is emitting some kind of rays. He later demonstrated that the radiation emitted by uranium shared certain characteristics with X-rays, but (unlike x-rays) could be deflected by a magnetic field and, therefore, must consist of some charged particles.

In 1897, a young PhD student of Becquerel from Poland, Marie Curie (Figure 18a) decided to systematically investigate those strange uranium "rays". She discovered that thorium also emitted the same rays as uranium and that the strength of the rays

did not depend on the chemical composition, only on the amount of uranium or thorium in the sample.

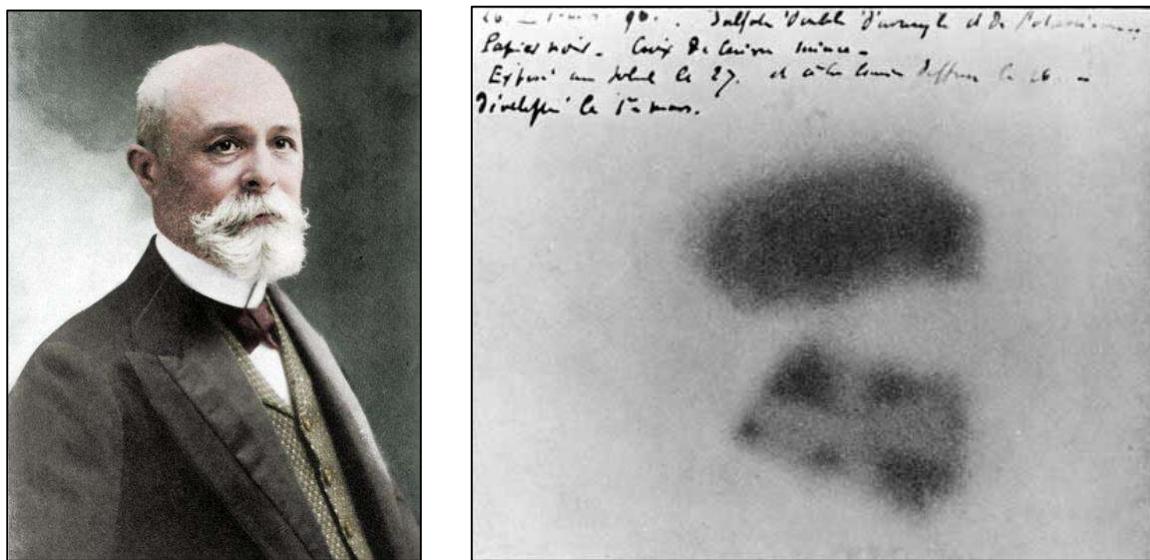


Figure 17: a) Antoine Becquerel; b) photo-plate with dark imprints of irradiation from uranium salts

She concluded that the radiation did not depend on the arrangement of the atoms in the molecule but was linked to the interior of the atoms themselves. Marie Curie then found that uranium ore was much more radioactive than it was expected based on the amount of uranium in the ore. She extracted uranium out of the uranium ore, but the waste left after extraction was more “active” than uranium itself. So, she discovered radioactive elements polonium (named for Marie’s native country, Poland) and radium (name based on the fact that it radiated very strongly). The term “radioactivity” was coined by Marie Curie herself, while the old unit of radioactivity “Curie (Ci)” is equivalent to 1 g of radium and was named in honour of Marie Curie. Marie and Pierre Curie (Figure 18b) and her supervisor Becquerel were awarded the Nobel Prize in Physics in 1903 for their work on radioactivity. Marie was later awarded a second Nobel Prize in Chemistry in 1911 for the discovery of elements radium and polonium.



Figure 18: a) Marie Curie b) Marie and Pierre Curie

## Radium-226: A wonder drug and/or just quackery?

Very soon after discovery of X-rays scientists realised that X-rays could cause a skin inflammation and therefore can interact with human live tissue. In 1896 a first physician (an American) thought that X-ray may be actually therapeutic and could treat even cancer. In 1900 it was found out that rays of radium also can cause inflammation and have a similar effect on tissues like X-rays. Year later Becquerel suffered a skin burn with a small sample of radium and then a French physician successfully treated a few cases of skin disease with radium. Soon radium was showed to be able to provide some help in certain cases of skin cancers and diseases and its usage in medicine started immediately (Figure 19). At that time, it was astonishing news: radium is a drug! Radium was soon believed to have wide curative powers and was applied to many diseases!

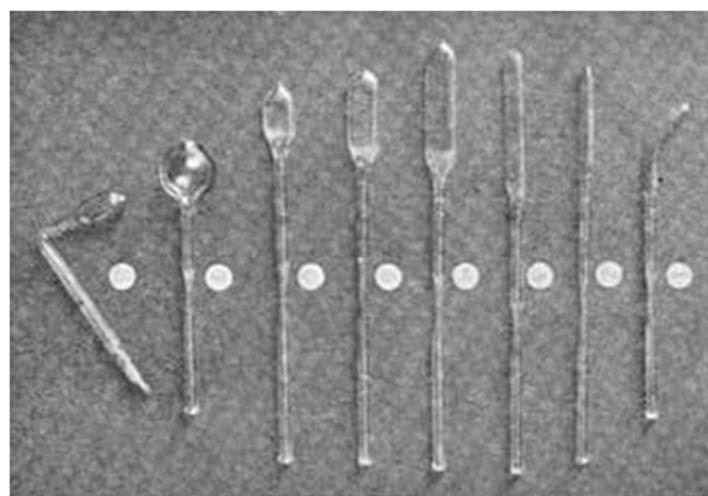


Figure 19: a) Old lady being treated with  $^{226}\text{Ra}$ ; b) tablets containing  $^{226}\text{Ra}$  and tool for application

Unfortunately, as it usually goes when people do not understand some newly discovered natural phenomenon very much, soon people started believing that radium is a miracle drug that can cure anything. The commercial exploitation of radium started in 1913 and soon went out of control. Radium was used to make quackery (a fake medicine based on pseudoscience and unsupported claims) and were sold with fantastic claims that it can cure basically anything. Typical was so called "Radithor" (Figure 20), a fake medicine that was useless in healing anything, but contained dangerously significant quantities of radiotoxic radium. It was made by a failed Harvard student William Bailey who wanted just to grab money from some unfortunate and naive patients: it was advertised as a "cure for the living dead", was very expensive and claimed to

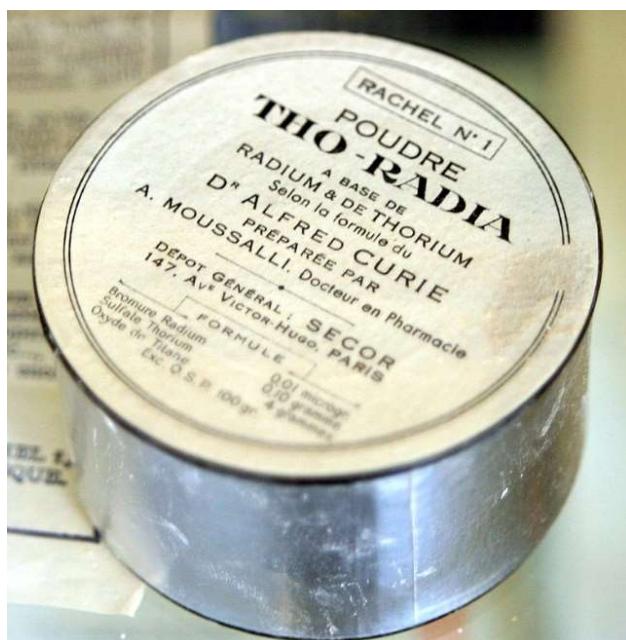


Figure 20: Radithor



be able to stimulate body functions and cure anything, including impotence. Most of people at that time believed those claims and did not even think that radium is actually very toxic.

Also, some companies started adding radioactive materials into cosmetics, such was “Doramad” toothpaste that was made in Germany and contained thorium, or “Tho-Radia” powder for face made by some person named Alfred Curie: he had the same surname as Marie Curie, but this person was not related to Curie couple at all, therefore this is an example of a pure manipulation of people by deliberately causing deception and confusion giving it a false credibility of a famous surname “Curie”. There were many other preparations that contained radium and at that time it was all fully legal.

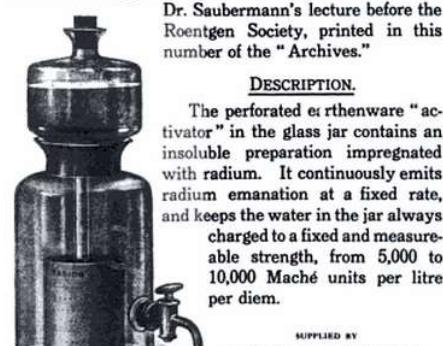


## RADIUM THERAPY

The only scientific apparatus for the preparation of radio-active water in the hospital or in the patient's own home.

This apparatus gives a high and measured dosage of radio-active drinking water for the treatment of gout, rheumatism, arthritis, neuralgia, sciatica, tabes dorsalis, catarrh of the antrum and frontal sinus, arterio-sclerosis, diabetes and glycosuria, and nephritis, as described in

Dr. Saubermann's lecture before the Roentgen Society, printed in this number of the "Archives."



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Figure 21: Three examples of radioactive quackery: a) “Tho-Radia” powder for face; b) “radium therapy” c) Doramad, radioactive toothpaste containing thorium.

Uncontrolled and dangerous use of radium and other radioactive materials in medicine went on until the case of Eben Byers (Figure 22). He was an athlete and a famous golfer who had an accident in a train and had a painful arm. He needed something to cure his pain and a doctor suggested him at that time very popular “Radithor”. Mr Byers started consuming it every day, for 3 years. He claimed that he feels good out of it, but that was mostly a so called “placebo” effect. During three



years, he ingested total of 37 MBq of  $^{226}\text{Ra}$ . As the consequence he lost weight and had headaches, and his teeth began to fall out. Soon he developed horrible symptoms: his "whole upper jaw, excepting two front teeth and most of his lower jaw had been removed" and "all the remaining bone tissue of his body was disintegrating, and holes were actually forming in his skull."

On 31<sup>st</sup> March 1932 Eben Byers died of radiation poisoning; in fact, he died from multiple cancers and bone necrosis. Since he was very famous and a celebrity many newspapers publicized his case and therefore dangers of radium became well known. There were some other cases of poisoning with radium such as the case of so called "radium girls", but their case was not so famous at that time. When Marie Curie herself died of aplastic anaemia it was a serious wakeup call that radium is not useful in therapy but instead is very toxic and deadly. Very soon use of radium-266 was fully abandoned in medicine.



Figure 22: Eben Byers

## The discovery of nucleus, protons and neutrons

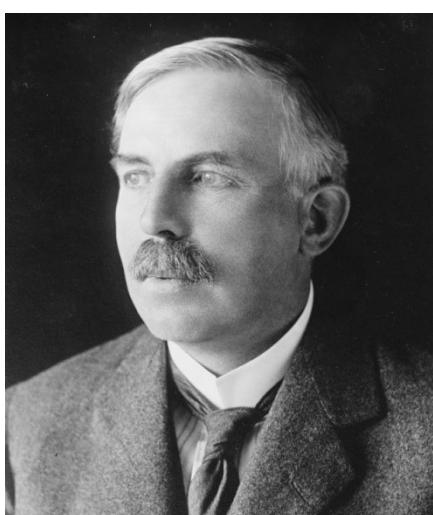


Figure 23: Ernest Rutherford

In 1911 experimenting with alpha particles from radium at the University of Manchester Ernest Rutherford (Figure 23) discovered that radiation coming from radium can be separated with electrical or magnetic field into three types based its charge: he named the positive rays as alpha ( $\alpha$ ) rays, negative as beta ( $\beta$ ) rays and the one with no charge as gamma ( $\gamma$ ) rays. This naming Rutherford gave is still in use. Soon his experiments lead him to the theory that all atoms have positively charged nucleus.

Later on, he discovered that alpha rays are nothing but fast helium nuclei. In the meantime,

Patrick Blackett, a research fellow working under Ernest Rutherford was the first to achieve transformation of one element into a different one by artificial means. After bombarding nitrogen gas with alpha particles, he noticed that sometimes the alpha particle was stopped and a proton with high kinetic energy was released. This was the first production of  $^{17}\text{O}$  via the  $^{14}\text{N} (\alpha, p)^{17}\text{O}$  nuclear reaction.

In 1930, two German scientists from the University of Giessen, Walther Bothe and Herbert Becker bombarded beryllium sheet with alpha particles emitted from



polonium and found a strange emission of highly penetrating radiation. French scientists Irène and Frédéric Joliot-Curie investigated these reactions and thought that it was just some high energy gamma rays. Two years later British scientist James Chadwick from University of Cambridge found that those were not gamma ray, but a beam of new subatomic particles. This was discovery of the neutron, and it was central to understanding atomic structure and to the advancement of nuclear science.

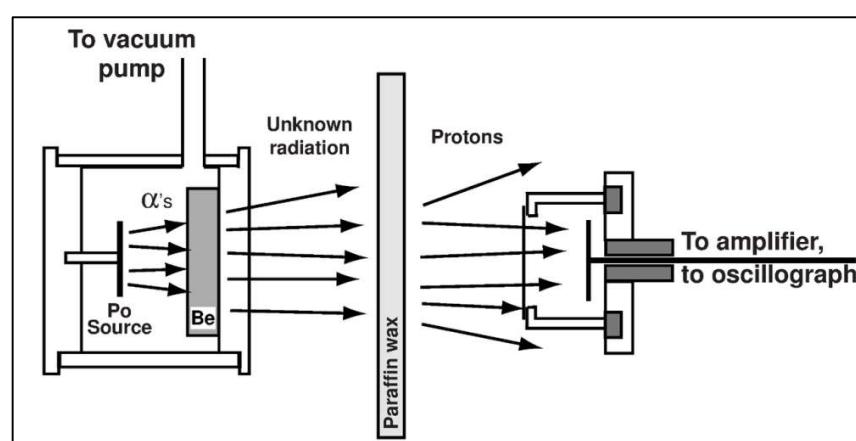


Figure 24: a) James Chadwick and b) his experiment by which he discovered neutrons

### The discovery of artificial radioactivity and radionuclides

Generally, from the year 1930 bombardment of some materials with radiation became very fashionable form of new scientific research. In 1934, Irène and Frédéric Joliot-Curie created new radionuclides by irradiating stable nuclides with alpha particles. The bombardment of aluminium by alpha particles produced radioactive phosphorus plus a neutron:



They then observed that this phosphorus decayed to silicon, releasing a new particle, a positron. Soon, then managed to isolate the positron-emitting  $^{13}N$  into a separate vessel to confirm that they had in fact artificially created a different element.

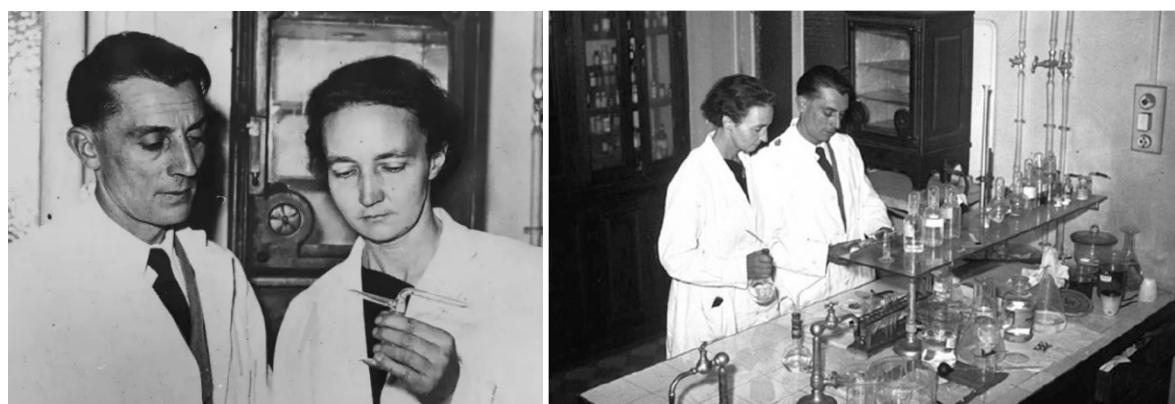


Figure 25: Irène and Frédéric Joliot-Curie



As a result, Irène and Frédéric Joliot-Curie won the Nobel Prize in Chemistry in 1935 “in recognition of their synthesis of new radioactive elements”, work that laid the foundation for modern day nuclear medicine and radiopharmaceutical chemistry. Interestingly to note: Irène Joliot-Curie was daughter of Marie and Pierre Curie.

At about the same time, American scientist Ernest Lawrence (Figure 26a) from University of California at Berkeley developed the first cyclotron (Figure 26b) and started bombarding different materials with very fast and energetic particles to see what he will get. Lawrence’s team at Berkeley discovered new radionuclides:

- $^{131}\text{I}$  - discovered in 1938 by Glenn Seaborg and John Livingood.
- $^{99}\text{Tc}$  and  $^{99\text{m}}\text{Tc}$  - discovered in 1938 by Emilio Segrè and Glenn Seaborg.

These discoveries set the stage for the use of cyclotrons for the production of radionuclides. As recognition of his work, Ernest Lawrence received the Nobel Prize in Physics in 1939 “for the invention and development of the cyclotron and for results obtained with it, especially with regard to artificial radioactive elements”



Figure 26: a) Ernest Lawrence, Ernest Lawrence with his cyclotron

## Radionuclides and life science research

The late 1930s and early 1940s were the time when many new radionuclides of light elements were artificially made: team of Ernest Lawrence made  $^{11}\text{C}$  by bombarding boron oxide with deuterons, while  $^{18}\text{F}$  was made by bombarding neon with accelerated deuterons.

Italian scientist Enrico Fermi was the first to produce isotopes using neutrons in 1934 and demonstrated that the radionuclides derived from neutron irradiation decayed by beta emission and not positron emission.

A Hungarian scientist, George de Hevesy irradiated stable sulphur with neutrons producing  $^{32}\text{P}$ . In 1938 Robley Evans used neutrons to produce  $^{128}\text{I}$ , while Glenn

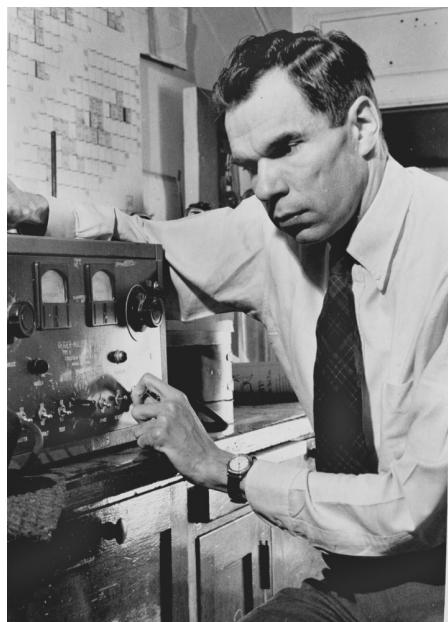


Figure 27: Glenn Seaborg

Seaborg and John Livingood used deuterons from cyclotron to bombard  $^{128}\text{Te}$  and made  $^{130}\text{I}$  and  $^{131}\text{I}$ . In 1940 Martin Kamen and Samuel Ruben made  $^{14}\text{C}$  by bombarding graphite with accelerated deuterons.

Fermi also bombarded uranium with neutrons and hoped he will produce a heavier element, but he was not able to find any heavier element: a German chemist, Ida Noddack, analysed Fermi's neutron-irradiated uranium samples and demonstrated the presence of lighter elements, not heavier. This was confirmed by Otto Hahn and Lise Meitner in 1938, and the year later in 1939 the theory of fission was developed. This theory, that atom of uranium can be split by neutron radiation had profound effect on growing

community of nuclear physicist and chemists. However, World War II was on-going, and much research was disrupted since many scientists had to shift their attention onto other areas.

### George de Hevesy and the tracer principle

Despite the failure of radiotherapy with  $^{226}\text{Ra}$  idea that radioactive materials could be used in medicine and life sciences persisted. Therefore, parallel with development of nuclear science of atom splitting development of light radionuclides and their possible use in biomedicine was developing. The most interesting was idea that radioactive isotopes should be used as tracers – to attach them onto biomolecules or drugs and then to monitored and detect them in biological systems (cells, organs, plants, microorganism) by using newly developed instrumentation for detection counting of radioactivity. A molecule chemically identical to some biological molecules but containing traceable radioactive atom that can be remotely detected by some instrument or method was called a radiotracer.

Hungarian scientist George de Hevesy is called the “father of nuclear medicine” and was the first to describe the radiotracer principle. The tracer principle states that:

- ***radiopharmaceuticals can participate in biological processes but should not alter or perturb them***



Figure 28: George de Hevesy

In this way, radiopharmaceuticals facilitate the imaging of normal and disease processes without interfering with them (if tiny amounts of radiopharmaceuticals can be detected with relative ease). The first radiotracer experiment in animals was to use  $^{210}\text{Bi}$  to follow the circulation of bismuth-containing antisyphilitic drugs in rabbits. De Hevesy received the 1943 Nobel Prize for this discovery.

### The “Manhattan project” and its impact on nuclear medicine

Approximately at that time World War II started in Europe and changed everything. Many European scientists fled to the USA where they started project of making an atomic bomb, so called “Manhattan” project. Idea of nuclear chain reaction and that a huge energy will be released by it came from Hungarian scientist Leo Szilard who conceive that idea in 1933, but at that time it was considered as impossible. Yet it was unclear which element could be used for such chain reaction of atom splitting but after Otto Hahn and Lise Meitner proved that atoms of uranium can be split by neutron radiation idea of nuclear chain reaction became much more plausible. To prove the concept of chain reaction a first experimental nuclear reactor was built out of graphite blocks and wood in Chicago (USA) and the first sustainable chain reaction of uranium atom splitting was achieved on 2<sup>nd</sup> December 1942 (Figure 29a).

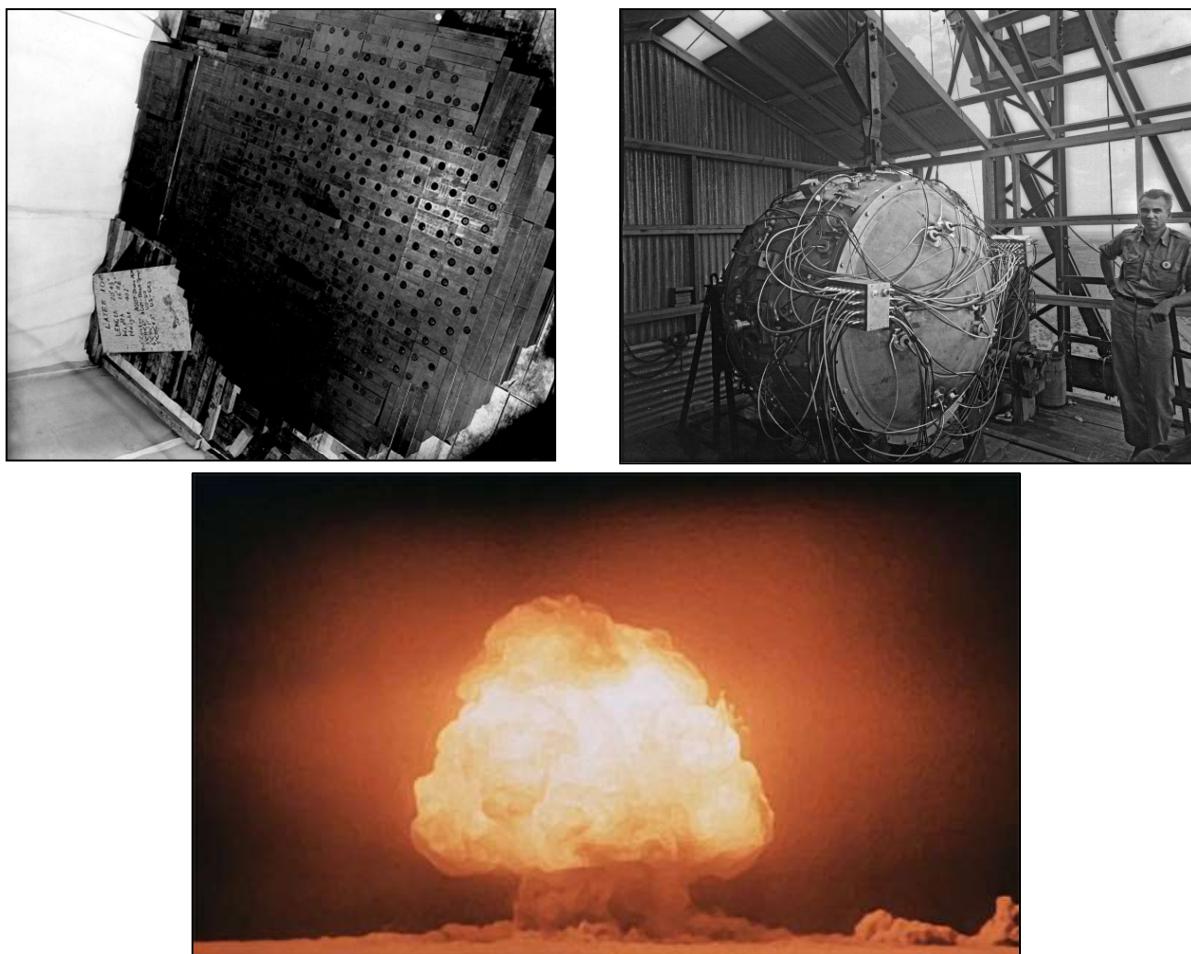


Figure 29: a) The first nuclear reactor named Chicago pile I; b) The first nuclear bomb (plutonium implosion fission) c) The first nuclear bomb test was named “Trinity”



To turn nuclear chain reaction into a bomb was considerably harder: scientists working on a secret site of Los Alamos were unsure if they will succeed, theory and calculations were showing it is possible, but it was a huge bet.

The main purpose of the nuclear bomb was not to bomb Japan but Germany especially before Germany gets its own atomic bomb. It was known by the British and Americans that Germany had at that time a nuclear project lead by Werner Heisenberg, but it was not known how successful and in which stage it is. Fortunately for Germany they surrendered before the American atomic bomb was ready. However, then Americans decided to drop the bombs on Japan, not only to force Japan to surrender, but also to scare USSR.

Despite all the politics, the project enormously expanded nuclear science: the first nuclear reactor was built in 1942; plutonium and americium were produced and discovered as well as many other discoveries and inventions. In general, this was the largest single scientific project ever, and changed the course of human history. There were many inventions and discoveries that came from this project and were pivotal for the development of nuclear science and technologies: radiochemistry was significantly improved, methods for extractions, analysis, new isotopes became available. Also new instruments such as scintillators were invented, while fission products became a spring of new yet unknown isotopes.

### **The $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator and $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals**

$^{99}\text{Tc}$  was discovered by Italian scientists Emilio Segrè (Figure 30) and Carlo Perrier in 1937 from radioactive samples of molybdenum accidentally irradiated by deuterons in Berkeley. However, its meta-stable radioisomer,  $^{99\text{m}}\text{Tc}$ , was discovered a year later (1938) also by Emilio Segrè at Berkeley in the USA. Later it was noticed that  $^{99\text{m}}\text{Tc}$  is almost an ideal isotope for the medical applications: its half-life was short (6 hours), it had gamma emission only, almost ideal energy of gamma emission detection, no beta emission at all, and had very good chemical properties. In later 1950s and 1960s Powell Richards promoted and advocated use of  $^{99\text{m}}\text{Tc}$  in the medical applications.



Figure 30: Emilio Segrè

The first  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator was created in 1958 by Walter Tucker and Margaret Greene. It was a laboratory apparatus for “milking of  $^{99\text{m}}\text{Tc}$ ”, also known as “the cow”. In fact, the first  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator was created accidentally, since the first generator for the “milking of radioisotopes” or the “cow” was in fact  $^{132}\text{Te}/^{132}\text{I}$  generator.  $^{132}\text{Te}$  was extracted from fission products out of irradiated nuclear fuel (from nuclear

reactor) and  $^{99}\text{Mo}$  was inside of it as an impurity. It was just later observed that there is something else in the eluted “milk” apart from  $^{132}\text{I}$  -  $^{99\text{m}}\text{Tc}$  was also eluted! In fact, this  $^{99}\text{Mo}$  was also fission product from nuclear reactors. In 1964 Paul Harper from the University of Chicago demonstrated the effectiveness of  $^{99\text{m}}\text{Tc}$  for the imaging of liver, brain, and thyroid and the era of  $^{99\text{m}}\text{Tc}$  began. Today 70-80% all nuclear imaging procedures are performed by using  $^{99\text{m}}\text{Tc}$ !

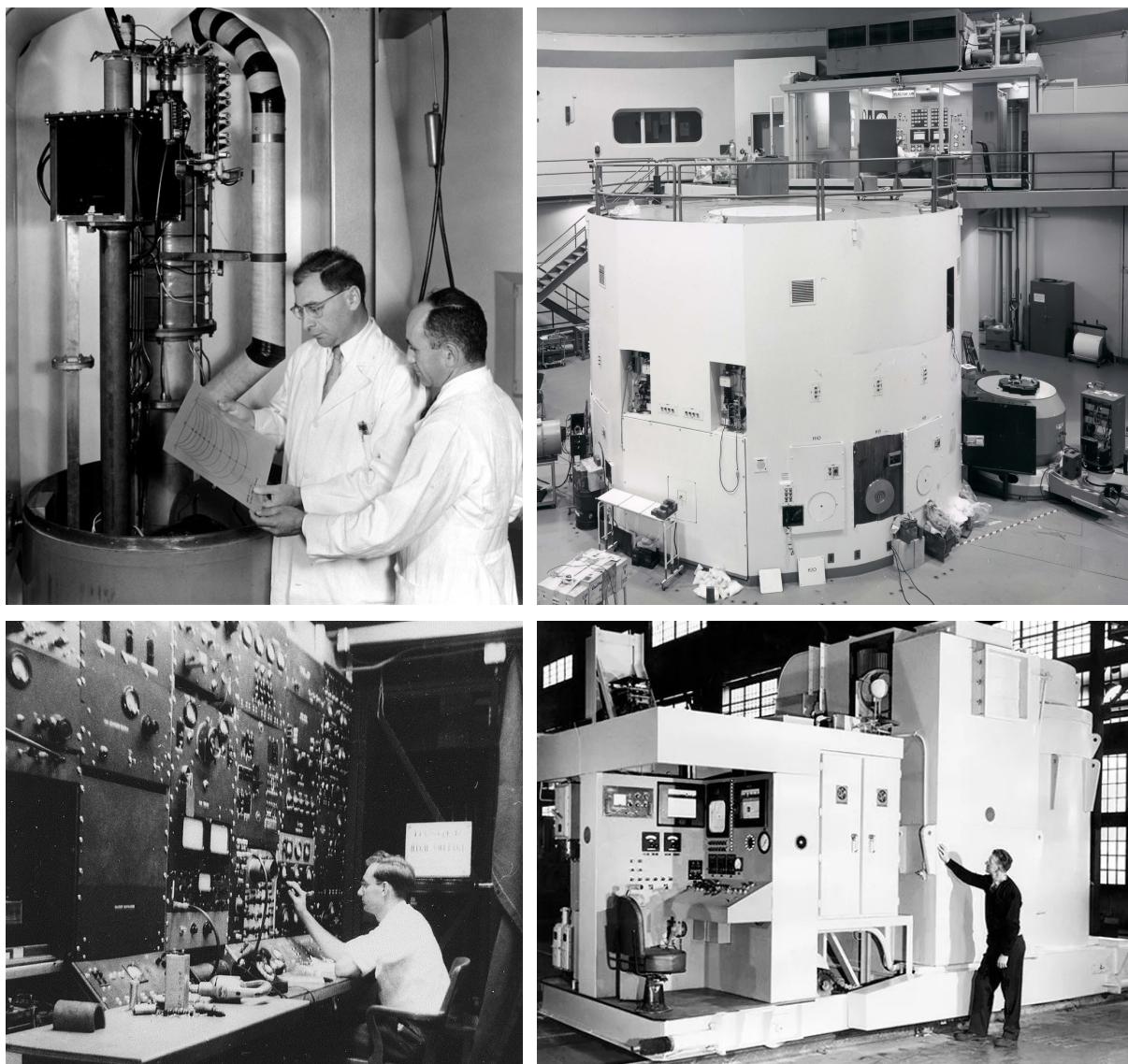


Figure 31 a) Walter Tucker (left) and Powell Richards (right); b) The first Mo-99/Tc-99m generator created in 1958 by Walter Tucker and Margaret Greene.

### The production of radionuclides – cyclotrons and reactors

The two decades, 1950s and 1960s were the “golden age of nuclear expansion” also known as “Atomic age”. Every country that had any self-confidence started developing nuclear science and did not want to be left behind: nuclear physics departments were being established in many countries and new labs are built along with reactors, cyclotrons and other equipment. Therefore, nuclear research was flourishing in USA, Britain, France, Germany but development started in USSR, India, China, Israel, Japan, South Africa, Yugoslavia, and many other countries around the world.

However, the main driver for the expansion of nuclear research was the quest for nuclear weapons due to the Cold war between the USSR and USA and the consequent feeling of insecurity. Development of nuclear power generation and nuclear medicine was only secondary. At that time there was no much restriction on nuclear research as it was widely believed that nuclear technology will soon significantly improve society. In this period nuclear and radiochemical research was very rich: power and production reactors were built, many new accelerators including cyclotrons, Also, new radiochemical and analytical methods and separations, as well as powerful detection equipment was developed.



**Figure 32: Development of nuclear science in 1950s and 1960s.**

## Development of imaging instrumentation

In the same time imaging instrumentation for detection of radiotracers was developed. In the beginning scientists and medical doctors were trying to use Geiger-Mueller counters for the detection of gammas in human organs, but it was inappropriate for gamma radiation. In the year 1944 scintillation detector was invented by Samuel Curran. In the early 1950's rectilinear scanner was invented, and also in 1953 a multidetector instrument for the localization of brain tumours with positron-emitting radionuclides was developed by Brownell and Sweet.

In 1957 Hal Anger invented the scintillation camera, known also as the gamma camera; it contained the collimator plate and an array of photomultiplier tubes. It became a basis for scintigraphy.

In 1966 the first circular array of detectors for imaging the brain was developed and was nicknamed the “head shrinker” or “hair dryer” due to its funny futuristic appearance. In the 1960s, David Kuhl and Roy Edwards developed a tomographic imaging - the predecessor of modern SPECT systems.

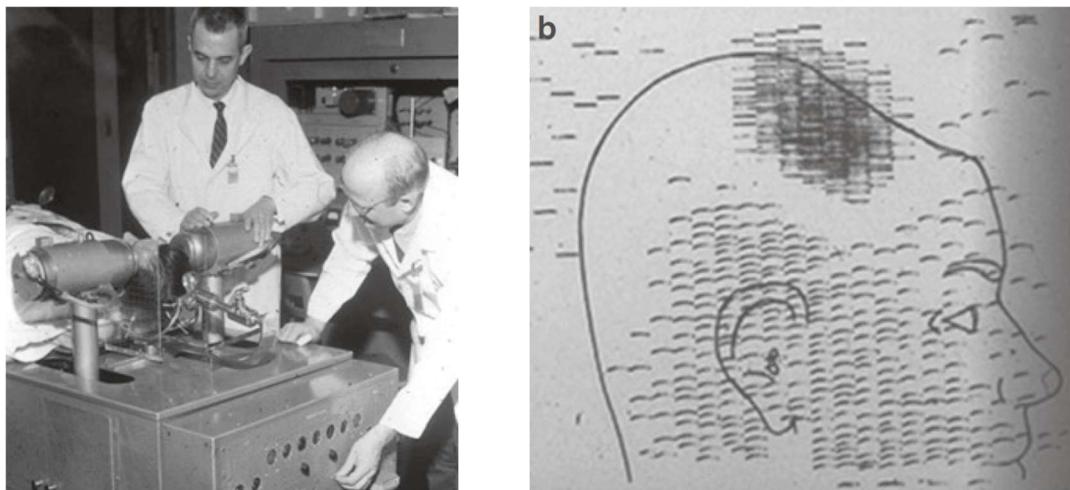


Figure 33: a) An early multidetector instrument for the localization of brain tumours with positron-emitting radionuclides developed by Brownell and Sweet, b): an image from this scanner showing the presence of a brain tumour

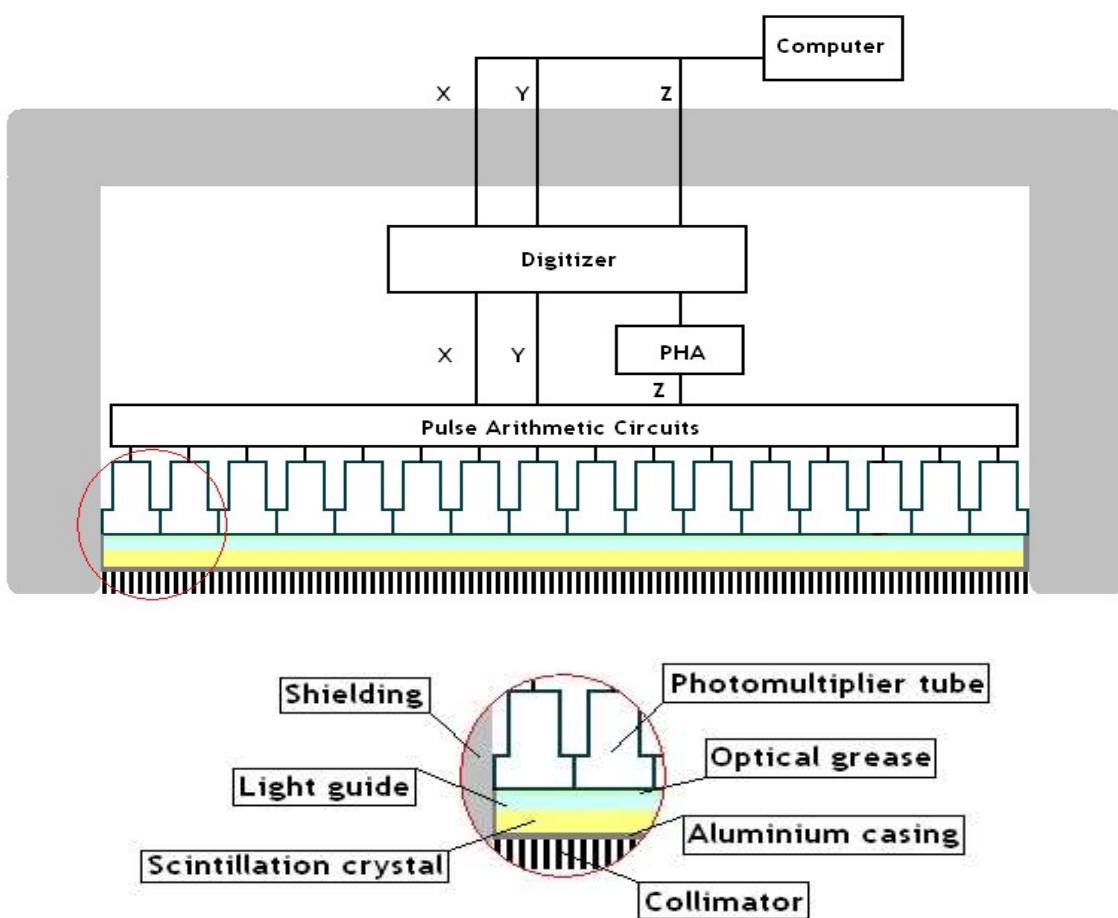
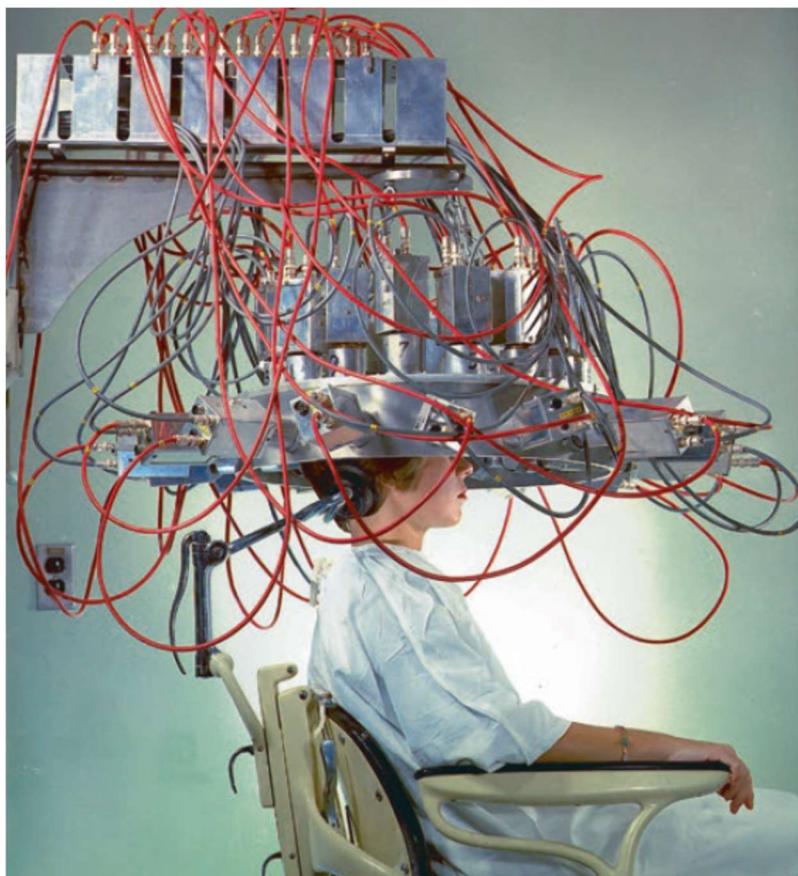
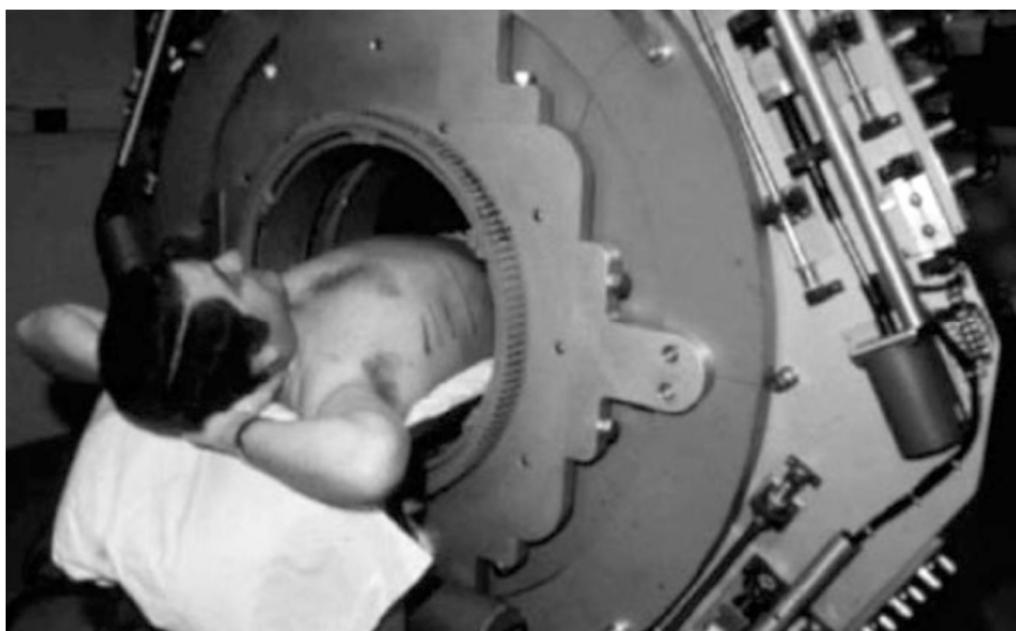


Figure 34: Concept of multidetector gamma-camera containing collimator and an array of photomultiplier tubes



**Figure 35:** Circular array of detectors used for brain imaging nicknamed “hairdryer” or “head-shrinker”

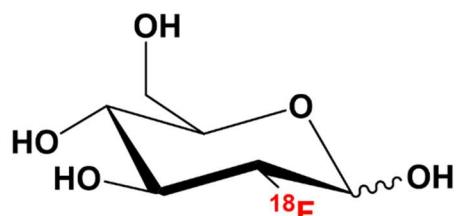
In 1975 the first PET instrument was developed by Michel Ter-Pogossian, Michael E. Phelps, and Edward J. Hoffman. In 1977 the first camera that rotates around the patient was developed.



**Figure 36:** Researcher inside one of the early PET scanners at Washington University, USA

## The discovery and applications of $^{18}\text{F}$ ludeoxyglucose ( $^{18}\text{FDG}$ )

Also, in 1970s FDG was invented and applied. What is FDG? It is 2-deoxy-2- $^{18}\text{F}$ luoro-D-glucose, a radiolabelled form of glucose in which one  $^{18}\text{F}$  atom takes the place of one hydroxyl group. It is widely used radiopharmaceutical agent for visualizing/measuring glucose metabolism in the live tissues. Because it has fluorine instead of oxygen it cannot be easily metabolized, but it concentrates in all tissues that have high metabolism of glucose and therefore always have a high concentration of glucose: it is mostly brain, but also, cancer cells and cancer tissues!



Hence it can be used to visualise and localize cancer in human body. It contains  $^{18}\text{F}$ , a positron emitter, and is therefore used in PET scans for many applications: it is the most produced and used radioactive molecule in the world; millions of applications with FDG are performed every year.

Figure 37: 2-deoxy-2- $^{18}\text{F}$ luoro-D-glucose (FDG)

FDG was developed also in the USA. In the year 1975 2-deoxy-glucose labelled with  $^{14}\text{C}$  was developed in order to investigate glucose metabolism in animals. But for studies in humans  $^{14}\text{C}$  was completely inappropriate. Then, one group of scientists had another idea, instead of  $^{14}\text{C}$  to use  $^{18}\text{F}$ : on 14<sup>th</sup> July 1975 team of Alfred Wolf (a Chinese scientist named Chung Nan Wan was also part of the team) made  $^{18}\text{F}$ -FDG by using  $^{20}\text{Ne}$  ( $p, \alpha$ )  $^{18}\text{F}$  nuclear reaction and reacted it with glucose derivative to obtain FDG. The yield was just 8%. It was tried in a PET scanner and has shown excellent results. Later on, in the year 1986 new method for the synthesis of FDG was developed that starts from  $^{18}\text{F}$ -fluoride ion. The yield was much better (50%), and all procedure was finished in just in 50 minutes. Very soon the synthesis was automatized. Today high yields of FDG can be achieved in only 30 minutes.

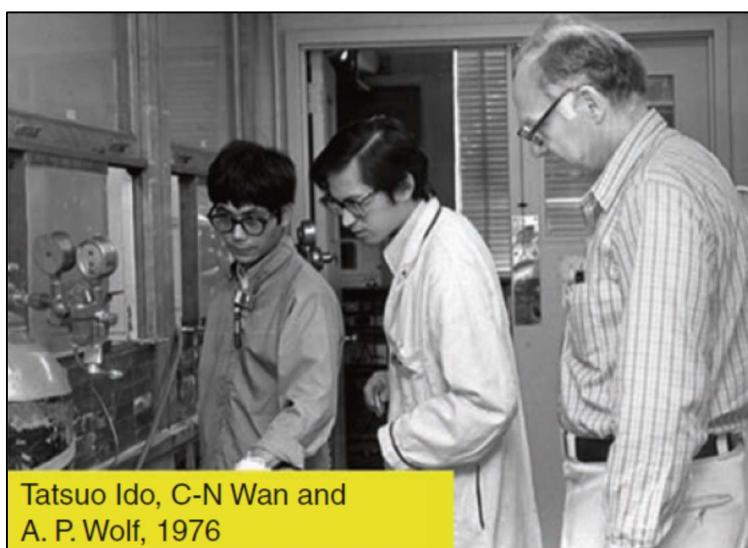


Figure 38: Inventors of FDG, Tatsuo Ido, Chung Nan Wan, and Alfred Wolf

## Modern radiopharmaceutical facilities

Today, there are plenty of radiopharmaceutical facilities around the world, mostly in USA, EU and Japan. They are usually based in hospitals, research centres, universities, but also can be independent commercial companies. Their major job is production of medical radionuclides or precursors ( $^{99m}\text{Tc}$ , radioiodine), but also there are PET centres with a cyclotron and radiochemical lab for the fast automated production of  $^{18}\text{F}$ ,  $^{11}\text{C}$  and other radiopharmaceuticals. Usually produced radiopharmaceutical preparations are immediately applied in PET/SPECT scans or they are quickly transported to hospitals.



Figure 39: Modern radiopharmaceutical facilities featuring hot cell and particle accelerators

## Nuclear medicine, medical radionuclides and radiopharmaceuticals in the world

Measurement of one country's strength and power in the area of nuclear medicine and radiopharmaceutical chemistry is often measured by number of research reactors for the production of medical isotopes (such as is  $^{99}\text{Mo}$  used to make  $^{99m}\text{Tc}$ ) as well as number of modern radiopharmaceutical facilities and suites that usually house a medical cyclotron for the instant production of light positron-emitting radionuclides (such as  $^{18}\text{F}$  and  $^{11}\text{C}$  or can be used to make some other medical radionuclides such as radiocopper, etc).

These facilities are critical for the development of nuclear medicine since allow better availability of medicinal radioisotopes and make these procedures cheaper and more affordable for the local medical system.

The map below (Figure 40) shows number of research reactors for the production of medicinal radionuclides around the world: total of thirty-nine, mostly in the USA, Russia, but then in China, India, Canada and other countries.

Countries labelled yellow have one research reactor for the production of medical radionuclides, orange have two (Ukraine, Indonesia, Argentina and Algeria), green three (Canada, Brazil, India) purple five (China), blue ten (Russia) and red is USA with total of seventeen reactors.

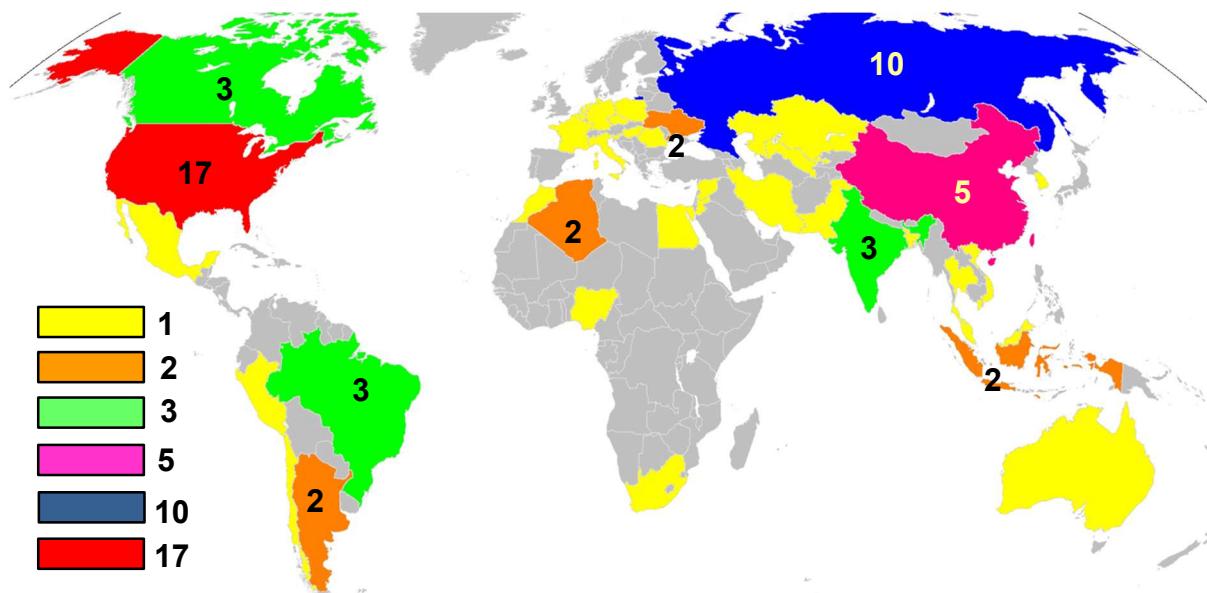


Figure 40: Nuclear reactors for the production of radioisotopes around the world (2020)

Although China has five reactors, when it is then divided by its total population number (Figure 41) it can be seen that China gets not so much per 100 million inhabitants. This is telling us that China might need to build and operate more research reactors so that medicinal radioisotopes such as  $^{99m}\text{Tc}$  can be more available for the use in nuclear medicine.

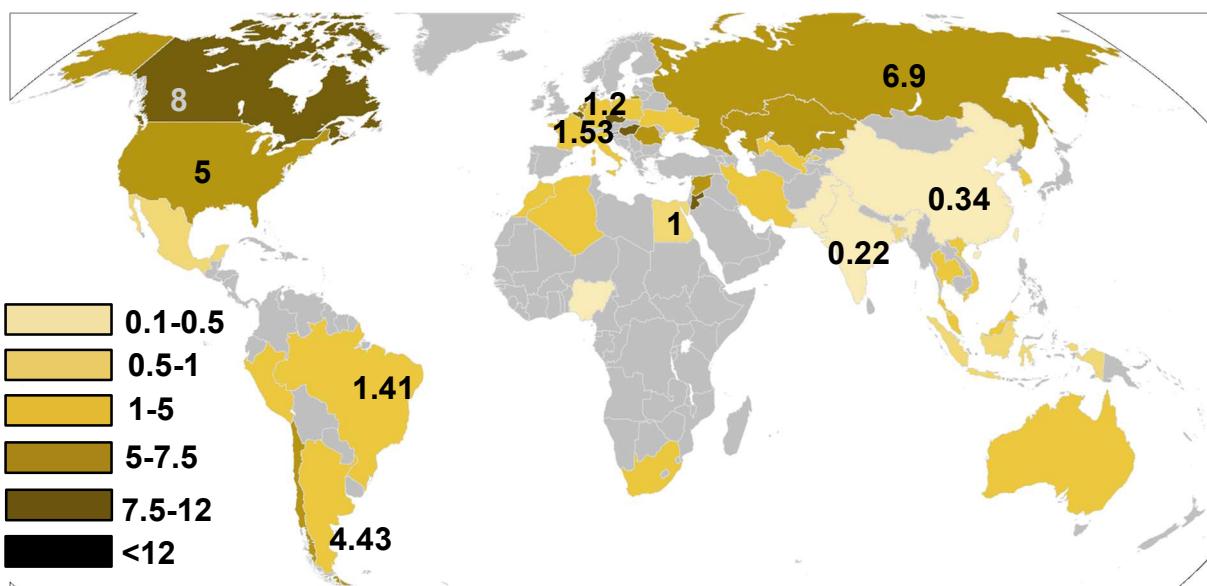


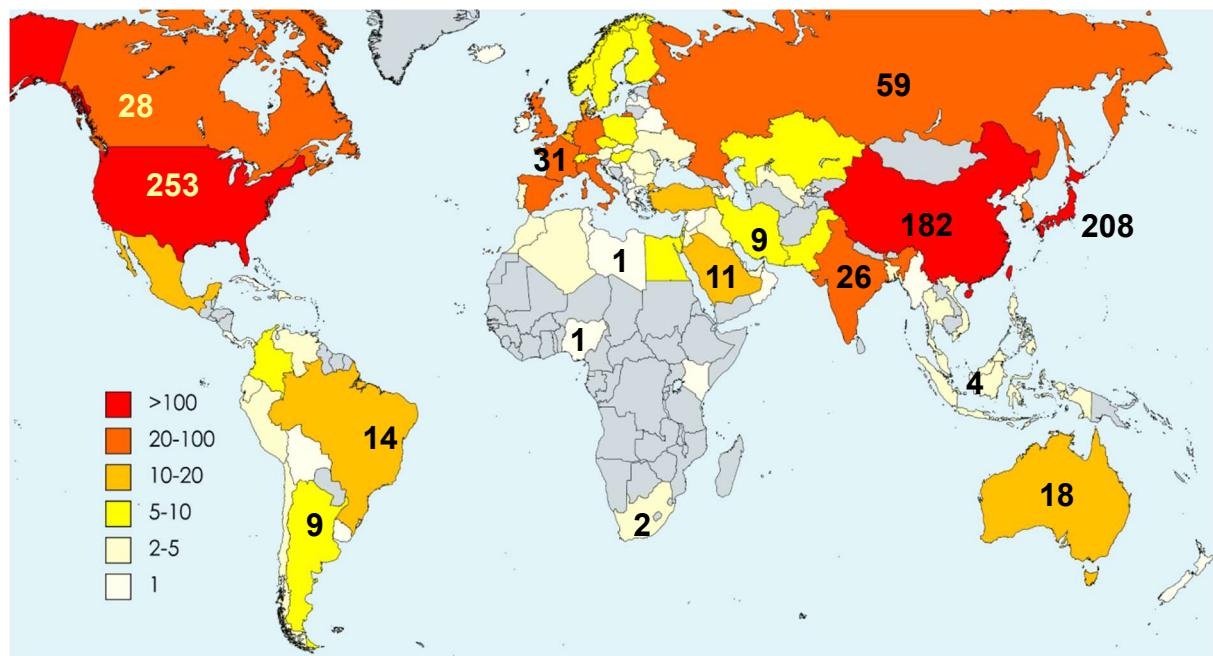
Figure 41: Reactors for the production of radioisotopes per 100 million inhabitants (2020)

In this sense some European countries and Canada have more advantage. Research reactors are usually used for the production of molybdenum-99 which is then extracted and used to get  $^{99m}\text{Tc}$ . Yet, one reactor can supply several countries as is often the case with the states in the European Union. Research reactors are large and expensive to operate and nowadays many countries prefer to abandon widespread use of  $^{99m}\text{Tc}$  and instead prefer to give priority to light positron-emitting radionuclides for PET diagnostics. This is because each individual

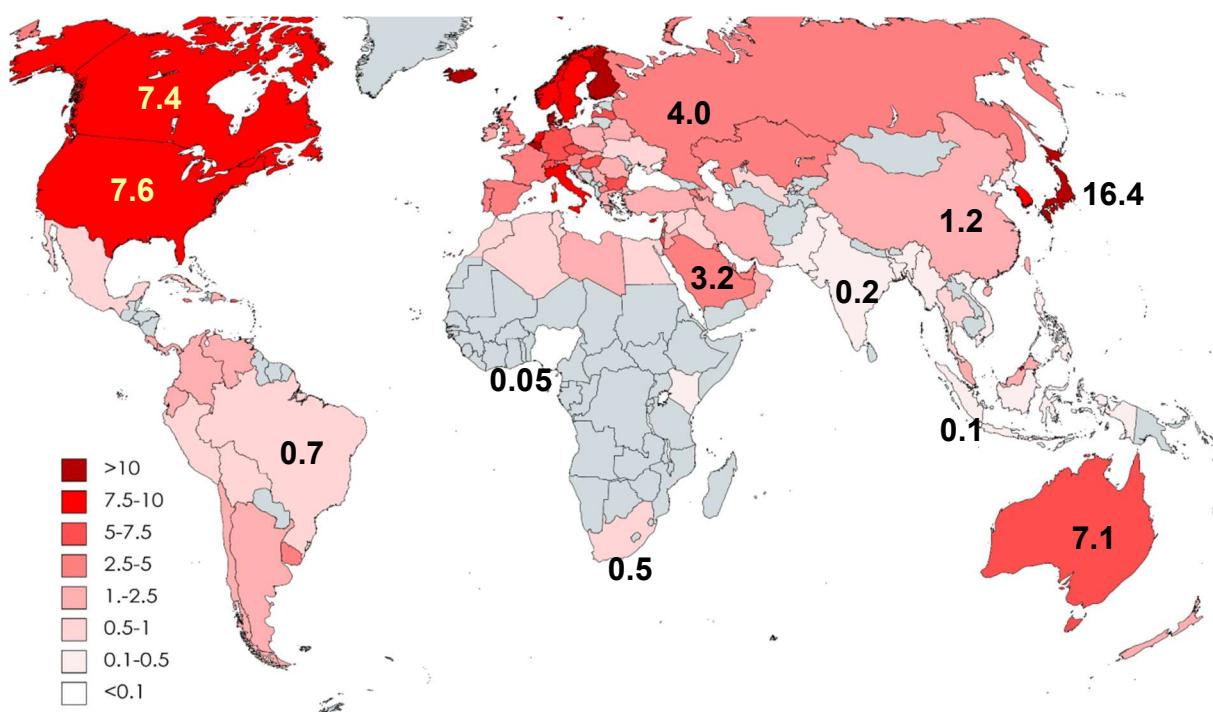


radiopharmaceutical facility with small medical cyclotron is cheaper and smaller, and if properly spread around the country can serve numerous patients by making PET diagnostic procedures as affordable as is SPECT.

If you now look into the number of cyclotrons for the production of medical radioisotopes around the world per country (Figure 42) the USA is again number one with 253, Japan is number two with 208, but immediately next China is number three with 182 cyclotrons.



**Figure 42: Cyclotrons for the production of medical radioisotopes around the world per country, data 2021**



**Figure 43: Medical cyclotrons around the world (per 10 million inhabitants), data 2021**



However, again, if you divide number of cyclotrons with number of inhabitants then you can see that number of cyclotrons per capita in China is not as high as in USA and is much less than in Japan, but better than in India or Indonesia. In Africa there are very few cyclotrons. To catch up with developed countries and make nuclear medicine procedure more available and affordable China needs to ramp up its number of cyclotrons and radiopharmaceutical facilities at least four times to reach the European level. One of the most important tasks in this effort is to develop affordable, reliable and high quality medicinal cyclotron.

### Nuclear medicine, medical radionuclides and radiopharmaceuticals in China

Important centres for the production of medical radioisotopes in China are in Beijing, the China Institute of Atomic Energy in Beijing, with two key reactors, SPR (Swimming Pool Reactor) IAE and CARR (China Advanced Research Reactor, Figure 44).

Another important centre is Nuclear Power Institute of China in Sichuan having two reactors: MJTR (Min Jiang Test Reactor) and HFETR (High Flux Experimental and Test Reactor).

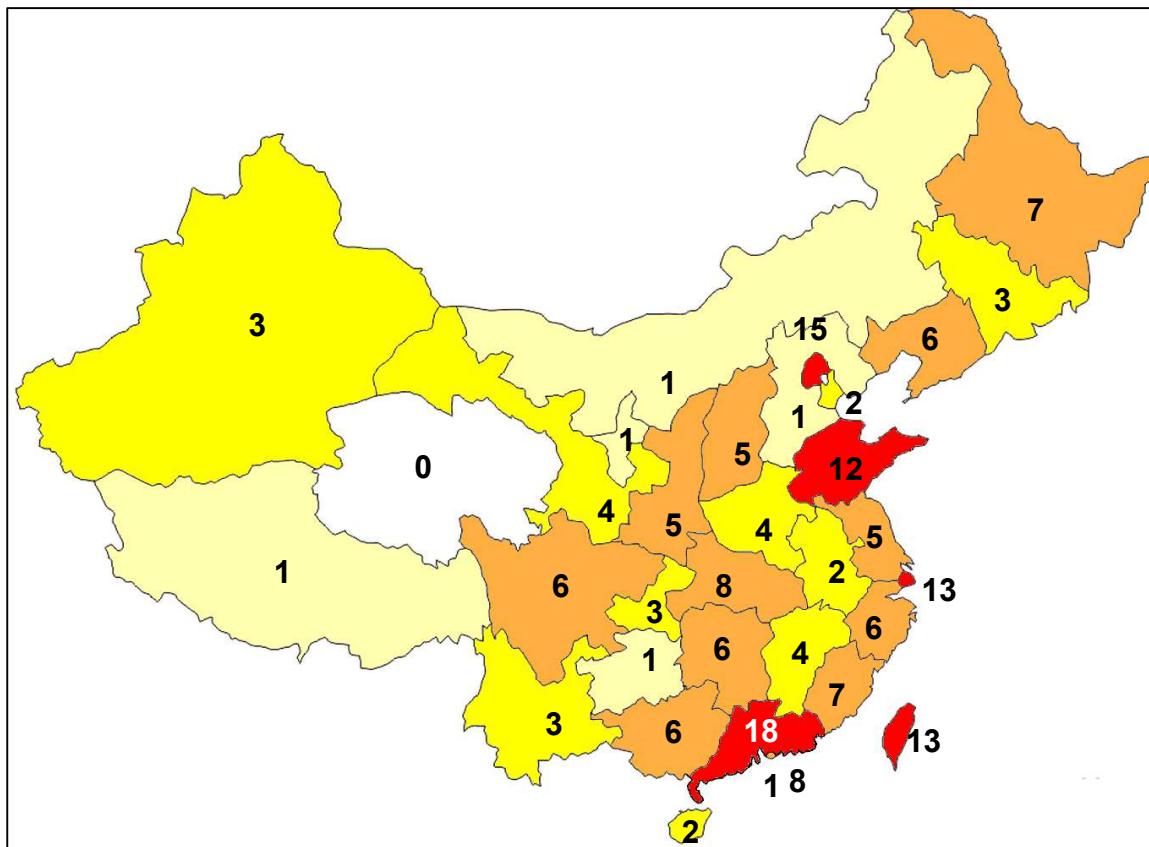


Figure 44: a) The core layout of CARR reactor, b) The view of CARR

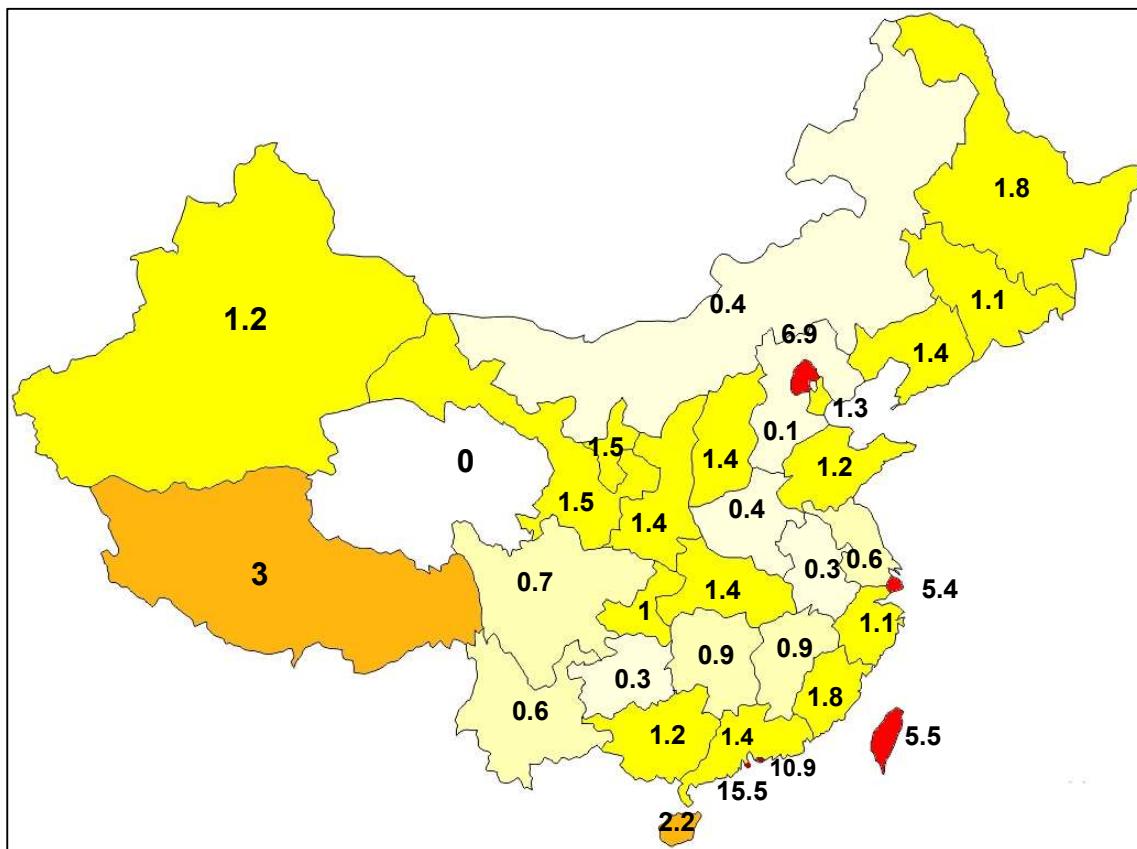
These centres are able to produce molybdenum-99 that is then used to make radionuclide generators for  $^{99m}\text{Tc}$ . Yet, need for  $^{99m}\text{Tc}$  is increasing and opening additional reactors for the production of medical radionuclides could be advantageous for the development of nuclear medicine in China.

If one looks into the distribution of radiopharmaceutical facilities with medicinal cyclotrons in China per province the map looks as in Figure 44.

As you can see the richest are Guangdong, Beijing, Shandong, Shanghai and Taiwan, while other have less machines and facilities. In Gansu there are four cyclotrons (in Lanzhou), while, for example, there is not even a single cyclotron in Qinghai.



**Figure 45: Number of cyclotrons for the production of medical radioisotopes by province**



**Figure 45: Number of cyclotrons for the production of medical radioisotopes by province**



Yet, when we divide number of cyclotrons by population of each province then we see that the richest are Beijing, Hong Kong, Macau, Shanghai and Taiwan. They are at the European level and even higher. Most of other provinces are around one cyclotron per 10 million people, some are lower, but Xizang, with only one cyclotron comes much higher because of its quite small population. Obviously, China needs much more cyclotrons to close the gap with developed countries at least 4 times more than now.

### **Future of radiopharmaceutical chemistry**

As nuclear medicine is making important advancements it is also becoming more available and affordable. Medicinal cyclotrons were rarity just few decades ago, but now number of producers are increasing, while important large medical centres can afford at least one radiochemical suite. Further automatization and artificial intelligence will make radiopharmaceutical suites fully automatic especially for several main radiopharmaceuticals increasing availability of nuclear medicine procedures

In future some radioisotopes commonly made by reactors, like  $^{99m}\text{Tc}$ , will be made by cyclotrons only, although importance of  $^{99m}\text{Tc}$  and SPECT technology will continue to fade in favour of PET radionuclides. The main reason is that PET technology gives much more clear images with higher resolution.

We will see new, quicker, and more practical ways to synthesize radiopharmaceuticals: new reactions will be optimized by artificial intelligence. Novel methods of organic chemistry will come with faster and more yielding reactions suitable for automatization. Also, we can predict entrance of nanoscience and supramolecular chemistry into the area of radiopharmaceuticals.

Automatic synthesis, purification and formulation of radiopharmaceuticals will be improved, incorporating robotic technologies (a compact production machines will be developed for the continuous production of radiotracer doses) and artificial intelligence.

New radiotracers for PET imaging (in oncology, cardiology, neurology, infection, inflammation, and others) will be developed based on new very specific monoclonal antibodies, while targeted radioimmunotherapy with alpha emitters will be improved and expanded, possibly into the area of autoimmune disease. Area of radioactive theranostics will also expand and become routine. Some exotic radionuclides such as  $^{211}\text{At}$  will become common. Radiotherapy will slowly but surely make its way into common and routine use.



## Chapter III - Key concepts in (radio)pharmacology and (radio)pharmaceuticals

### What would be an ideal medical radionuclide?

The key elements in radiopharmaceuticals are radionuclides. However, not all radionuclides can be used for medicinal purposes. In fact, most of the nuclides from the nuclear power area cannot be used in the medical area. What would be an ideal radionuclide?

There are some requirements a radionuclide has to fulfil to be considered as good for medical applications.

- A good medical nuclide has to have sufficiently short half-life. This is to avoid any unwanted accumulation in the organism. Half-life should be such to be in minutes, hours, days, definitely not in months, or years. But, neither in seconds – a too short half-life is not practical. Therefore, we cannot use long lived radionuclides such as  $^{238}\text{U}$ ,  $^{235}\text{U}$ ,  $^{232}\text{Th}$ ,  $^{226}\text{Ra}$ ,  $^{60}\text{Co}$  nor can we use radionuclides with too short half-life such as  $^{15}\text{C}$ , or  $^{212}\text{Ra}$ .
- Medical radionuclide has to have sufficiently high specific activity so that it can be used as tracer, its dose in grams should be negligible: also, specific activity has to be high to enable targeted radiotherapy to be achievable with very tiny amount of material.
- It needs to have high radionuclidic and radiochemical purity: it should be only one radioisotope of only one element. Therefore, radionuclides that are hard to be purified and usually contain radiotoxic radionuclides as impurity cannot be used.
- Emission should be proper, should penetrate to some desirable distance: we cannot use  $^{14}\text{C}$ , for example or  $^3\text{H}$  since these radionuclides emit very weak beta particles that have no energy to penetrate more than several micrometres of tissue. Therefore, it would be impossible to detect them by their external irradiation on a live person.
- It should not be a radiotoxic radionuclide: otherwise, we will end up with more harm than benefit to patient. Therefore, radiotoxic radionuclides of plutonium or  $^{241}\text{Am}$ ,  $^{210}\text{Po}$ ,  $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ , or  $^{226}\text{Ra}$  cannot be used.
- And finally, it should be cheap, convenient and readily available, which means its production should not be overly complicated, hard and expensive.



## How do we classify radiopharmaceutical agents?

There are numerous ways to do that, but here we will show examples of some common ways. Radiopharmaceutical agents can be classified based on the following categories:

- Radioisotope they contain
- Emission they emit
- Chemical structure and/or pharmacological group they belong
- Therapeutic group or clinical use

Classification of radiopharmaceuticals **based on their radionuclide** is the most common type of classification. Based on this classification we can say there are  $^{18}\text{F}$ -containing radiopharmaceuticals,  $^{11}\text{C}$ -containing radiopharmaceuticals,  $^{13}\text{N}$ ,  $^{15}\text{O}$ ,  $^{99\text{m}}\text{Tc}$ , radiocopper ( $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ ),  $^{90}\text{Y}$ , radioiodine ( $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ), radio-gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ),  $^{111}\text{In}$ ,  $^{201}\text{Tl}$ -containing radiopharmaceuticals, then those having lanthanides such as  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ , then those with heavy alpha emitters such as  $^{225}\text{Ac}$ ,  $^{223}\text{Ra}$ , and finally there are also radioactive noble gases  $^{133}\text{Xe}$  and  $^{81\text{m}}\text{Kr}$  that are used sometimes. However, there are more radioisotopes used for radiopharmaceuticals than are on this list.

Radioisotope	Radiopharmaceutical
$^{18}\text{F}$	$^{18}\text{F}$ Fludeoxyglucose, $^{18}\text{F}$ Florbetapir, $^{18}\text{F}$ Flumazenil, $^{18}\text{F}$ Fluoroestradiol, etc.
$^{11}\text{C}$	$^{11}\text{C}$ -PIB, $^{11}\text{C}$ -methionine, $^{11}\text{C}$ -DASB
$^{13}\text{N}$ , $^{15}\text{O}$	$^{13}\text{NH}_3$ , $\text{H}_2\text{O}$
$^{99\text{m}}\text{Tc}$	$^{99\text{m}}\text{Tc}$ -sestamibi, $^{99\text{m}}\text{Tc}$ -MDP, $^{99\text{m}}\text{Tc}$ -mebrofenin, $^{99\text{m}}\text{Tc}$ -MAG3
$^{64}\text{Cu}$ , $^{67}\text{Cu}$	$^{64}\text{Cu}$ -Dotatate, $^{64}\text{Cu}$ -ETS
$^{90}\text{Y}$	$^{90}\text{Y}$ -Ibritumomab tiuxetan
$^{123}\text{I}$ , $^{125}\text{I}$ , $^{131}\text{I}$	$^{123}\text{I}$ -NaI, $^{131}\text{I}$ -MIBG, $^{131}\text{I}$ -Tositumomab
$^{68}\text{Ga}$ , $^{67}\text{Ga}$	$^{68}\text{Ga}$ -DOTA-octreotate
$^{111}\text{In}$	$^{111}\text{In}$ -Imciromab, $^{111}\text{In}$ -pentetreotide, $^{111}\text{In}$ -oxime
$^{201}\text{Tl}$	$^{201}\text{TlCl}$
$^{153}\text{Sm}$ , $^{177}\text{Lu}$	$^{153}\text{Sm}$ -lexidronam, $^{177}\text{Lu}$ -oxodotreotide
$^{225}\text{Ac}$ , $^{223}\text{Ra}$	alpha emitters
$^{133}\text{Xe}$ , $^{81\text{m}}\text{Kr}$	noble gases

Table 2: Classification of radiopharmaceuticals based on their radioisotope

Another way of classifying medical radionuclides and also radiopharmaceuticals is **based on the emission they emit**: there are pure gamma emitters (these are decaying usually by electron capture, internal conversion or just stabilisation of isomers like in the case of  $^{99\text{m}}\text{Tc}$ ), then positron emitters, beta emitters and alpha



emitters. This classification actually coincides with clinical use classification: pure gammas are used in SPECT imaging, positrons in PET imaging, while betas and alphas are usually, but not only therapeutic radionuclides.

- Pure gammas ( $\gamma$ ):  $^{99m}\text{Tc}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{201}\text{Tl}$ ,  $^{111}\text{In}$ ,  $^{67}\text{Ga}$ ,  $^{81m}\text{Kr}$
- Positrons ( $\beta^+$ ):  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$ ,  $^{64}\text{Cu}$ ,  $^{68}\text{Ga}$ ,  $^{82}\text{Rb}$ ,  $^{89}\text{Zr}$ ,  $^{124}\text{I}$
- Betas ( $\beta^-$ ):  $^{177}\text{Lu}$ ,  $^{90}\text{Y}$ ,  $^{131}\text{I}$ ,  $^{67}\text{Cu}$ ,  $^{133}\text{Xe}$ ,  $^{32}\text{P}$ ,  $^{153}\text{Sm}$ ,  $^{188}\text{Re}$
- Alphas ( $\alpha$ ):  $^{225}\text{Ac}$ ,  $^{223}\text{Ra}$ ,  $^{221}\text{At}$ ,  $^{227}\text{Th}$ ,  $^{212}\text{Pb}$

Third, we can classify all radiopharmaceuticals by using **chemo-pharmacological** classification, which means, based on what kind of molecule they are, based on their chemical nature and hence the way they interact with body structures such as receptors.

Here we can define labelled analogues of natural compounds that are anyway always present in our organism and are not foreign, for example nutrients such as sugars (like glucose), amino acids or common hormones, neurotransmitters, vitamins labelled with a radioactive atom. For example, those are  $^{18}\text{FDG}$ ,  $^{18}\text{F}$ -estradiol,  $^{11}\text{C}$ -methionine, radiocobalt-labelled vitamin B<sub>12</sub>, and others.

Then we have labelled analogues of various drugs such as  $^{18}\text{Florbetapir}$ ,  $^{18}\text{Flumazenil}$  but also in this group there are labelled small molecules that are usually not registered drugs but are behaving as drugs by specifically binding onto certain cellular structures in human body (such as receptors). Some of them are even failed drugs that failed to find their way into clinical practice due to the certain reason, but their good binding onto specific cellular structures allows them to be used as tracers.

Then, there are labelled natural or artificial macromolecules such as proteins or peptides, for example  $^{18}\text{F}$ -neurotensin.

Bioconjugates are more complicated and sophisticated forms of radiopharmaceutical agents where usually a radio-metal is chelated within a chelating ligand and then the whole complex is conjugated with a large biomolecule, a protein or peptide: those are more sophisticated radiopharmaceuticals such as  $^{90}\text{Y}$ -ibritumomab tiuxetan or  $^{68}\text{Ga}$ -DOTA-octreotate.

Then, there are various complexes and chelates or more simple complexes like  $^{99m}\text{Tc}$ -sestamibi,  $^{99m}\text{Tc}$ -MDP,  $^{99m}\text{Tc}$ -DTPA.

Finally, old style simple salts like NaI, NaTcO<sub>4</sub>,  $^{223}\text{RaCl}_2$ ,  $^{89}\text{SrCl}_2$ ,  $^{82}\text{RbCl}_2$  are still in use.

Therefore, we can conclude chemo-pharmacological classification:

- Labelled analogues of natural biomolecules (sugars, amino acids, hormones, neurotransmitters):  $^{18}\text{FDG}$ ,  $^{18}\text{F}$ -estradiol,  $^{11}\text{C}$ -methionine,  $^{11}\text{C}$ -dopamine...



- Drug analogues (labelled drugs):  $^{18}\text{F}$ Florbetapir,  $^{18}\text{F}$ Flumazenil,
  - $^{11}\text{C}$ -DASB is not a registered drug although it acts as a drug
- Labelled proteins or peptides:  $^{18}\text{F}$ -Neurotensin
- Bioconjugates:  $^{90}\text{Y}$ -Ibritumomab tiuxetan,  $^{68}\text{Ga}$ -DOTA-octreotate
- Chelates or simple complexes:  $^{99\text{m}}\text{Tc}$ -sestamibi,  $^{99\text{m}}\text{Tc}$ -MDP,  $^{99\text{m}}\text{Tc}$ -DTPA...
- Simple salts: NaI, NaTcO<sub>4</sub>,  $^{223}\text{RaCl}_2$ ,  $^{89}\text{SrCl}_2$ ,  $^{82}\text{RbCl}$ ,  $^{201}\text{TlCl}$

Also, radiopharmaceuticals can be classified based on a test or area we want to investigate or cure such as cancer, endocrine disorders (hormones), cardiovascular disorders (hearth), neurological or psychological (brain), or excretory organs such as kidneys, or we look into traumas or infections. This is purely medical classification.

And finally, we can classify radiopharmaceuticals and medical radionuclides by methods of application: there are radionuclides for imaging, like those for PET (all are positron emitters), and for SPECT (pure gamma emitters). For radiotherapy, beta or alpha emitters are used, and finally, some light radionuclides like  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{45}\text{Ca}$  and  $^{51}\text{Cr}$  that are used only for laboratory tests, not in live persons.

- **For imaging:**
  - **PET:**  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$ ,  $^{64}\text{Cu}$ ,  $^{68}\text{Ga}$ ,  $^{82}\text{Rb}$ ,  $^{89}\text{Zr}$ ,  $^{124}\text{I}$
  - **SPECT:**  $^{99\text{m}}\text{Tc}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{201}\text{Tl}$ ,  $^{111}\text{In}$ ,  $^{67}\text{Ga}$ ,  $^{81\text{m}}\text{Kr}$
- **For therapy:**  $^{225}\text{Ac}$ ,  $^{223}\text{Ra}$ ,  $^{221}\text{At}$ ,  $^{227}\text{Th}$ ,  $^{212}\text{Pb}$ ,  $^{177}\text{Lu}$ ,  $^{90}\text{Y}$ ,  $^{131}\text{I}$ ,  $^{67}\text{Cu}$ ,  $^{133}\text{Xe}$ ,  $^{32}\text{P}$ ,  $^{153}\text{Sm}$ ,  $^{188}\text{Re}$
- **For laboratory tests use only:**  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{45}\text{Ca}$ ,  $^{51}\text{Cr}$

## What is pharmacology?

In short pharmacology is a science about drugs, how drugs work and how drugs behave in a live organism. This science is part of medical sciences and there are lots of chemistry, biochemistry, cell biology and physiology in this science. Pharmacology deals with molecular, cellular and organ/systems mechanisms of drug action: about systems, receptors and ligands in the body, signal transduction/cellular communication, molecular interactions, therapy, and medical applications. It also covers fate of drug in the body: absorption, distribution, metabolism, and excretion. Also, there is a section of pharmacology called clinical pharmacology: practical medical application of drugs: doses, indications, contraindications, side-effects, etc.

Pharmacology generally consists of two main branches:

- Pharmacodynamics
- Pharmacokinetics.

### What is pharmacodynamics?

Pharmacodynamics deals with how drug works at cellular and molecular level. It covers science of molecular, cellular and organ/systems mechanisms of action: for example, molecular interactions of drugs with cellular structures, proteins, receptors enzymes, and other ligands in the body. It answers questions of where and how a drug binds in a body or cell and how it produces its effect. It deals with the (bio)chemistry of drug binding (ligand-drug interaction) and with doses (concentrations) of drugs required to trigger effect.

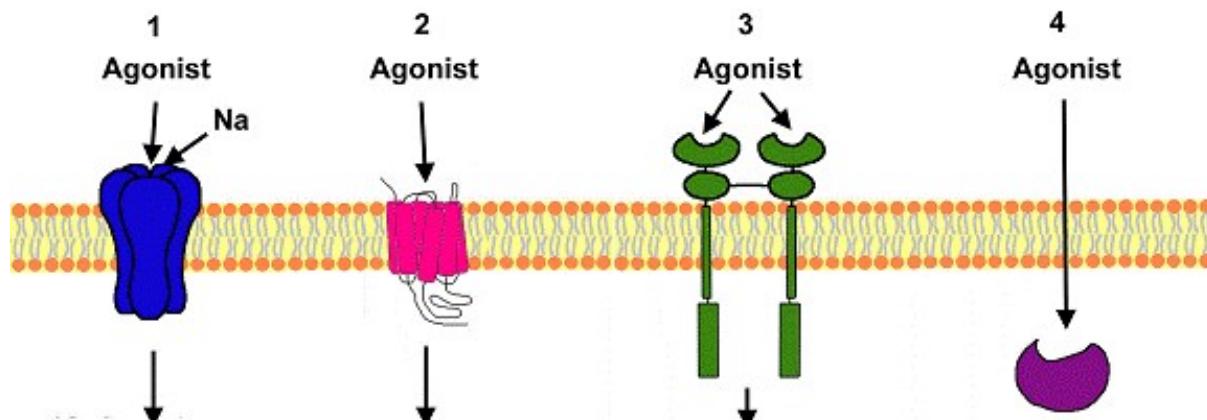
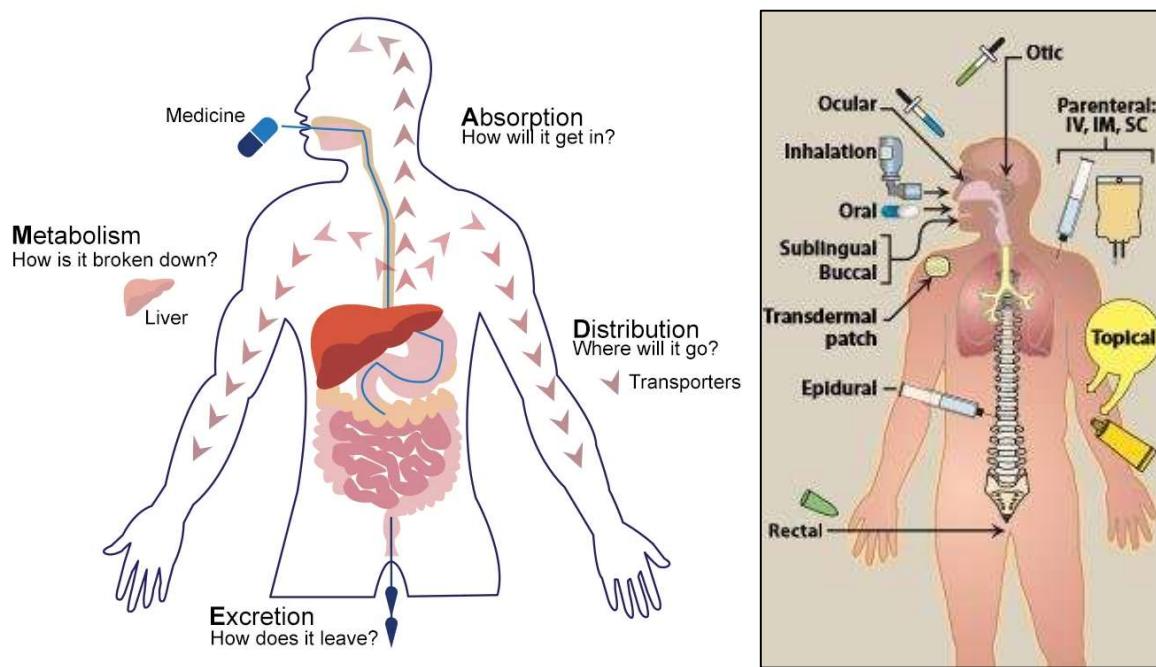


Figure 46: Pharmacodynamics deals with how drug interacts with tiny structures of cells at molecular level.

### What is pharmacokinetics?

It is the second branch of pharmacology that deals with the question of how a drug travels through the body. What is happening with a drug as it travels the body before it arrives on the site of action and after it leaves? It deals with absorption, distribution, metabolism and finally excretion or elimination of drugs. In other words: which way it enters, how quickly, where it then goes, how long it stays, what it turns into and how and how quickly leaves the body? Also, what happens to a drug in liver and other

tissues where it gets metabolised, which means chemically and biochemically modified, or broken down.



**Figure 47: a) Pharmacokinetics is dealing with the traffic of drug in human organism; b) routes for drug application in humans**

### How a drug travels though the body?

We can distinguish five stages of drug pathway through a body: absorption, metabolism, distribution, elimination, and accumulation (Figure 47a). In absorption we are interested to know how a drug enters body and how quickly it is absorbed: what is the kinetics (how fast) of absorption? For example, if a drug is taken through mouth it will be absorbed either in mouth or in stomach or in intestines. After absorption it firstly arrives into liver where it is metabolised and only then it enters the bloodstream. Absorption *via* mouth is usually slow to medium slow. Once a drug is in liver it gets metabolized, changed and covalently modified. Distribution is a stage where we want to know where a drug goes, to which organ? Some drugs prefer certain organs, while it is hard to find them in some other organs. Some drugs cannot enter brain (there is special so called “blood-brain barrier” only some drugs can enter brain if these drugs are lipophilic enough). For example, iodine goes almost exclusively to the thyroid gland, hardly can be found anywhere else. Distribution depends on pKa, lipophilicity, solubility and other factors. Elimination (also known as excretion) is the removal of a drug from organism: we wish to know parameters of the drug clearance, how and how quickly drug is removed from organism. Drugs are usually filtrated out of body in kidneys, into urine and *via* bladder they go out of our body. Another option is through colon and faeces after modification in liver where drug gets metabolised into a form that cannot be re-absorbed in intestines. It is important to know that human body has ability to recognize what is a xenobiotic – a foreign substance that is possibly toxic and should



not be in the organism and then to get rid of it. Drugs are xenobiotics, they are foreign to our body. At the end there is accumulations: some drugs tend to accumulate in the organism, usually in fatty tissue or quite often in bones.

Drugs can be taken or applied in various ways, but the final goal is to get a drug into bloodstream.

So, the most common way is to swallow it, through mouth, like tablets or capsules. We call this an oral route. A drug gets dissolved in stomach and gets absorbed in stomach or intestines, then passes liver, and then enters bloodstream.

There is an option to avoid stomach and get a drug directly into bloodstream, using a needle! This is called parenteral application, usually through injection:

- it can be directly into a vein (called intravenous),
- into an artery (intra-arterial),
- into a muscle (intramuscular),
- below skin (subcutaneous)
- a slow injection of large volume of liquid into a vein (called infusion).

Another quick way of getting a drug into bloodstream is by inhalation (through lungs) and for that a drug should be in the form of a spray or powder, or even a gas. Sometimes, drug is given into rectum and is then absorbed there (rectal application). There are other types of applications such as sublingual where drug is kept only in mouth, then transdermal where some very fatty drug gets absorbed into bloodstream directly through skin: this is usually very slow, rarely, via a patch, drug has to be very lipophilic and very potent (like fentanyl or some hormones).

## A drug and its target

Here we are going to focus on idea of a drug and its target. But what is a target in pharmacology?

One cannot fully understand it if do not have some idea of how drug works and how it targets its target. A drug works by binding or interfering with its target or ligand in a live body. A target or a ligand is usually some particular cellular structure, a protein in most of the cases or sometimes (rarely) it can be a DNA. Only some anticancer drugs bind onto and disrupt DNA. Also, a target can be a biological structure not of a human, but of bacteria, virus or other microbes like in the case of antibiotics and antiviriotics.

To list some examples based on its function a targeted protein can be:

- An enzyme: a drug can act as an inhibitor of an enzyme and many drugs work in that way.

- A receptor (usually for some natural neurotransmitters or hormones) that usually can be found on the surface of a cell but sometimes also inside the cell. A drug specifically binds it and can activate or block its function.
- An ion channel for biological ions such as sodium, potassium, calcium: a drug can block it or keep it open longer.
- A transporter protein molecule: a drug can block the transport of neurotransmitters, hormones or some other small molecules. .
- Signal transducer: a drug can block or mimic cellular signalling mechanisms.

In general, by binding onto its target protein a drug usually changes (or modulates) normal function of that target protein and of the whole cell. When function of many cells is modulated then the whole tissue or organ is affected by the drug.

This may sound like a binding of a metal and ligand in coordination chemistry, but there is a big difference! A metal usually binds many ligands, but one drug usually binds only one or very few targets: drug-target binding tends to be quite specific, just like in the case of hormones (hormones and neurotransmitters are in fact some kind of internal drugs).

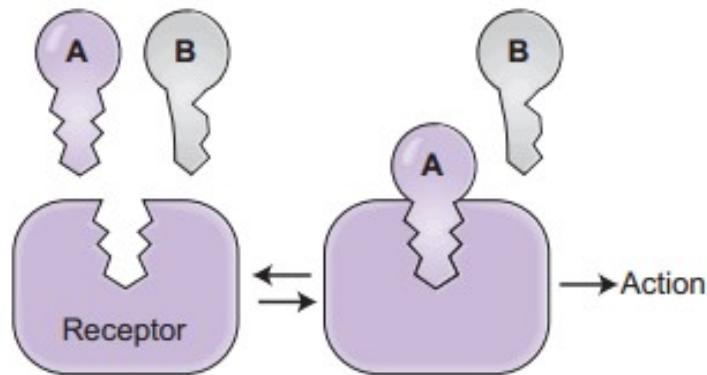


Figure 48: Concept of drug and its target is similar like the concept of a key and the lock: one drug (key) can activate a target (lock) another drug (key) cannot: Drug A binds to receptor, while drug B cannot bind to receptor!

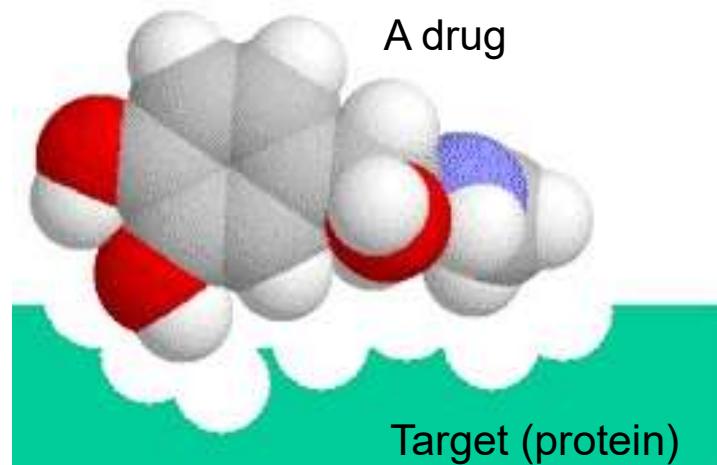


Figure 49: A drug binds onto its receptor (protein) through molecular recognition: topology of the drug fits into topology of the target!

A drug and its target look more like a key and its lock: a drug is like a key, while the target is the lock. And one key opens only one specific lock. How is this possible? Because a specific molecular structure (or shape, pattern) of a drug can bind only exactly complementary molecular structure (pattern) of just certain proteins. Therefore, one drug can bind onto very few proteins (targets): very specific drug will bind only one protein (target) out of thousands available! When one molecule perfectly complements into another and likes to bind it we call this a molecular recognition.

Here are some examples! Acetylsalicylic acid (also known as Aspirin) or ibuprofen or paracetamol: all these molecules look different (Figure 50), but they all are binding and are blocking the same family of similar enzymes: they inhibit enzymes cyclooxygenases (COX-1 and COX-2). By doing so they are acting as painkillers, alleviate pain, and decrease body temperature. Therefore, enzymes cyclooxygenases are targets of aspirin, ibuprofen and paracetamol.

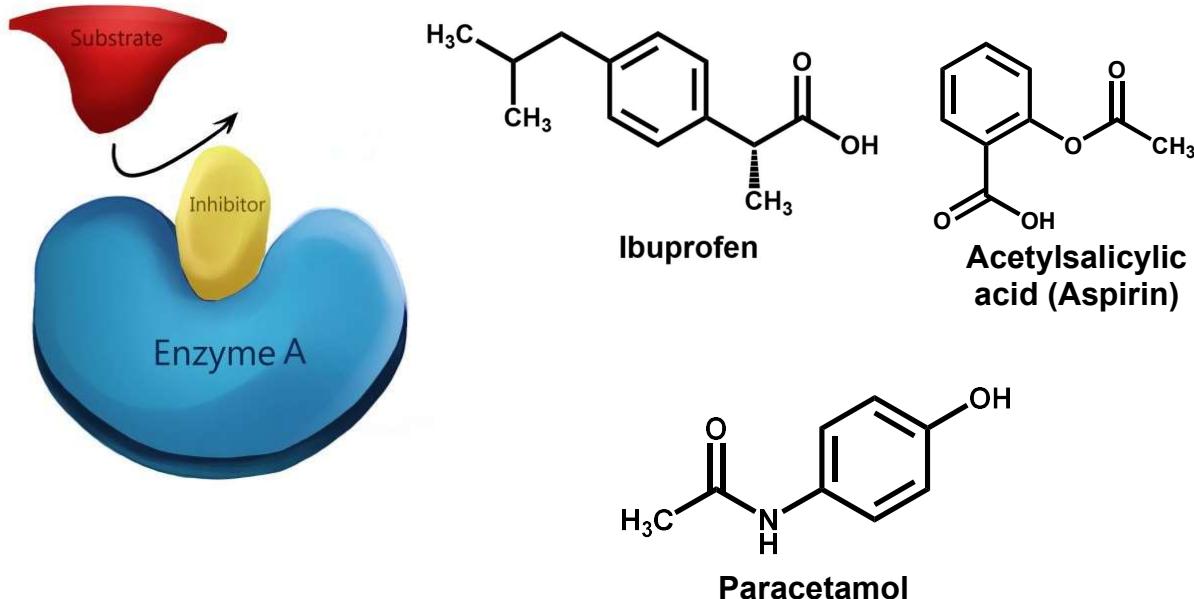


Figure 50: when a drug binds onto enzyme and prevents its substrate to bind then it is called inhibitor of an enzyme – blocks its function. Such are famous and heavily used drugs like Aspirin, Paracetamol and Ibuprofen.

Here are some other, different examples (Figure 51): morphine, tramadol, methadone and fentanyl. All these drugs have different structures but have something in common: these are all drugs that bind and activate so called  $\mu$ ,  $\delta$  and  $\kappa$  opioid receptors in brain. Since they are all binding and activating these receptors, we say they are all agonists (or activators) of  $\mu$ ,  $\delta$  and  $\kappa$  opioid receptors in brain.

Agonist is an activator: it binds a receptor and activates it. By doing so these drugs are very strong painkillers, alleviate pain, but also cause euphoria, sedation and addiction. Therefore,  $\mu$ ,  $\delta$  and  $\kappa$  opioid receptors are targets of morphine, tramadol, methadone and fentanyl.

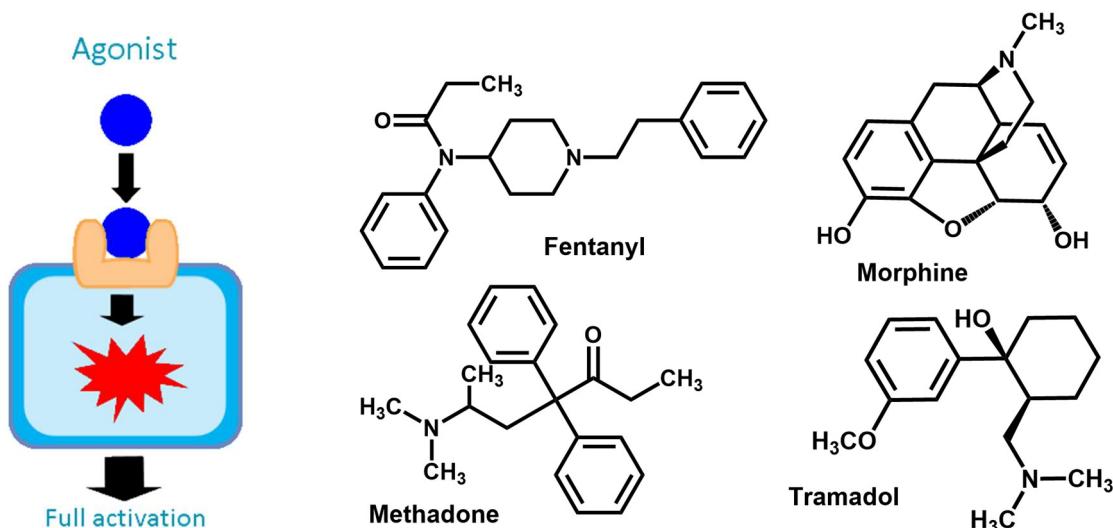


Figure 51: Agonist binds onto its receptor and activates – receptor triggers a cascade of reactor inside a cell or cellular compartment. Typical agonists are drugs like painkillers Fentanyl, Morphine, Methadone, and Tramadol.

Some other drugs bind onto receptors but then are not activating them but are blocking them: atenolol, for example, binds and blocks so called  $\beta$ -receptors in blood vessels (we call these drugs  $\beta$ -antagonist or  $\beta$ -blocker, Figure 52) as the consequence natural neurotransmitter and a hormone adrenalin cannot bind these  $\beta$ -receptor where  $\beta$ -antagonist such as atenolol “sits” on adrenalin’s place and therefore atenolol lowers blood pressure and slows heart rate.

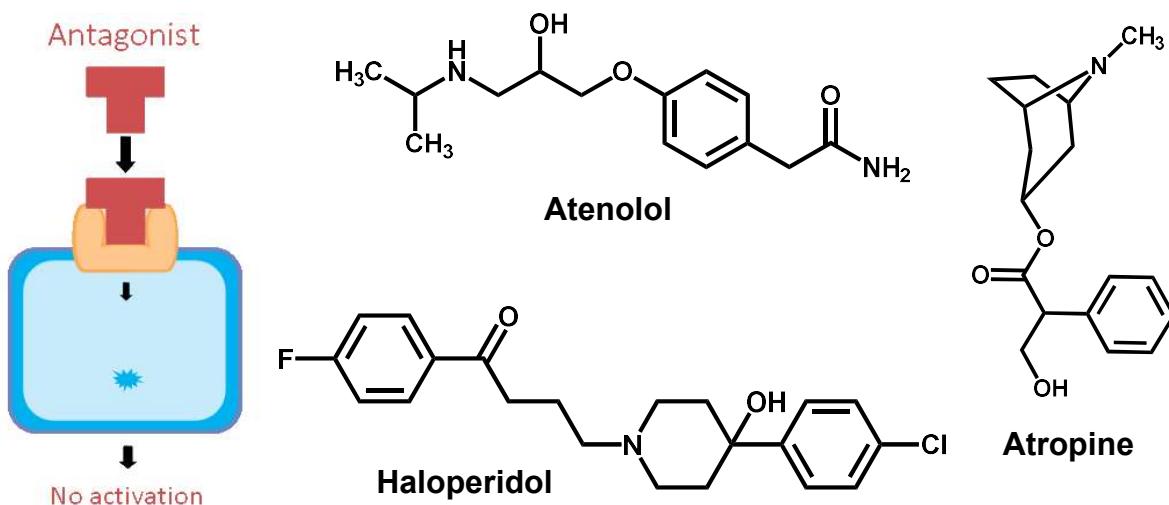


Figure 52A: Agonist binds onto its receptor and activates – receptor triggers a cascade of reactor inside a cell or cellular compartment. Typical agonists are drugs like painkillers Fentanyl, Morphine, Methadone, and Tramadol.

Haloperidol is another example of an antagonist. It binds and blocks so called D<sub>2</sub>-receptors in brain normally reserved for neurotransmitter called dopamine: dopamine then cannot bind D<sub>2</sub>-receptors, and this helps to alleviate schizophrenia (therefore we call haloperidol an antipsychotic). A natural product from a poisonous plant,

atropine binds and blocks M-receptors where usually neurotransmitter acetylcholine binds: acetylcholine therefore cannot bind M-receptors as long as atropine is bound on receptors takes acetylcholine's natural position. Hence, it is used as a drug for bradycardia, and it also increases heart rate. In total, antagonist or a blocker is a drug that binds receptor and blocks its function.

Only certain drugs bind DNA. For example, some old-style anticancer drugs are covalently binding DNA and damaging it permanently, causing death of cells that multiply a lot (like cancer cells). We call these drugs "alkylating agents". However, these drugs are not so specific, causing lots of side effects and some of these side effects are very nasty and hard to withstand. Effect of these drugs is somewhat similar as the effect of ionizing radiation.

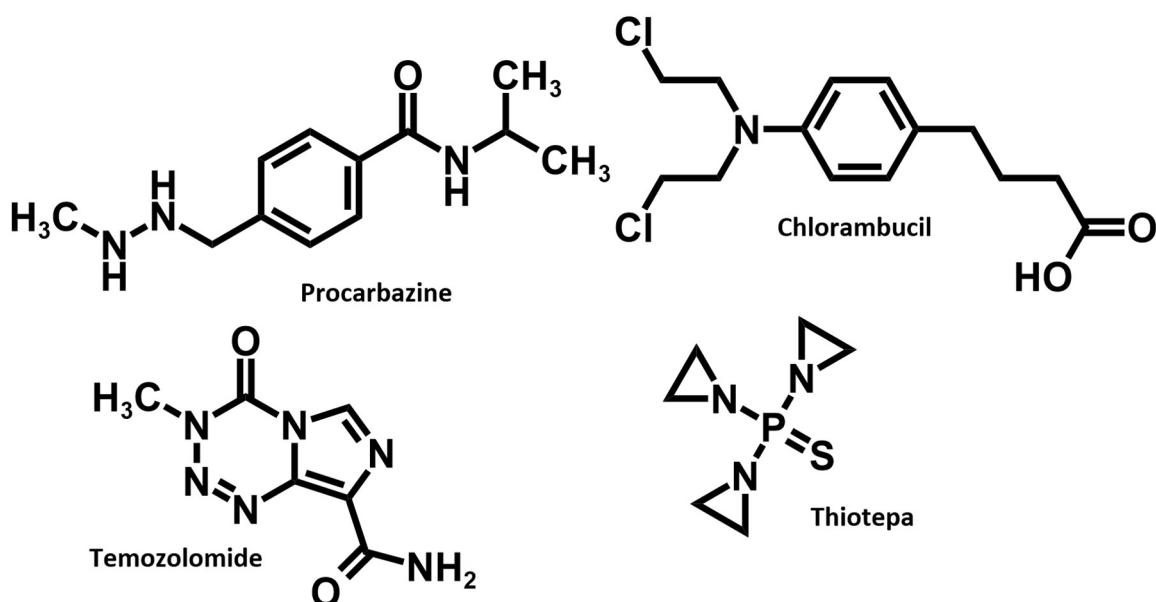
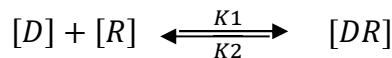


Figure 52B: Some old drugs for treating cancer: they are reactive molecules that bind and destroy DNA

What are the factors that influence drug behaviour? First and foremost, it is chemical, molecular structure of a drug (shape, topology, charge density) that is directing pharmacological effect of a drug. Pharmacodynamics of a drug depends on its molecular structure. Other factors are how soluble in water drug is (drugs should be soluble in water at least a bit), its pKa (ionisation has a huge influence on behaviour of drugs), how lipophilic it is: for example, more lipophilic molecule is able to cross cell membranes and arrive to the site of action. In general, behaviour of a drug is interplay between molecular structure, solubility, pKa, lipophilicity and some other factors.

## Doses in pharmacology and radio(pharmacology)

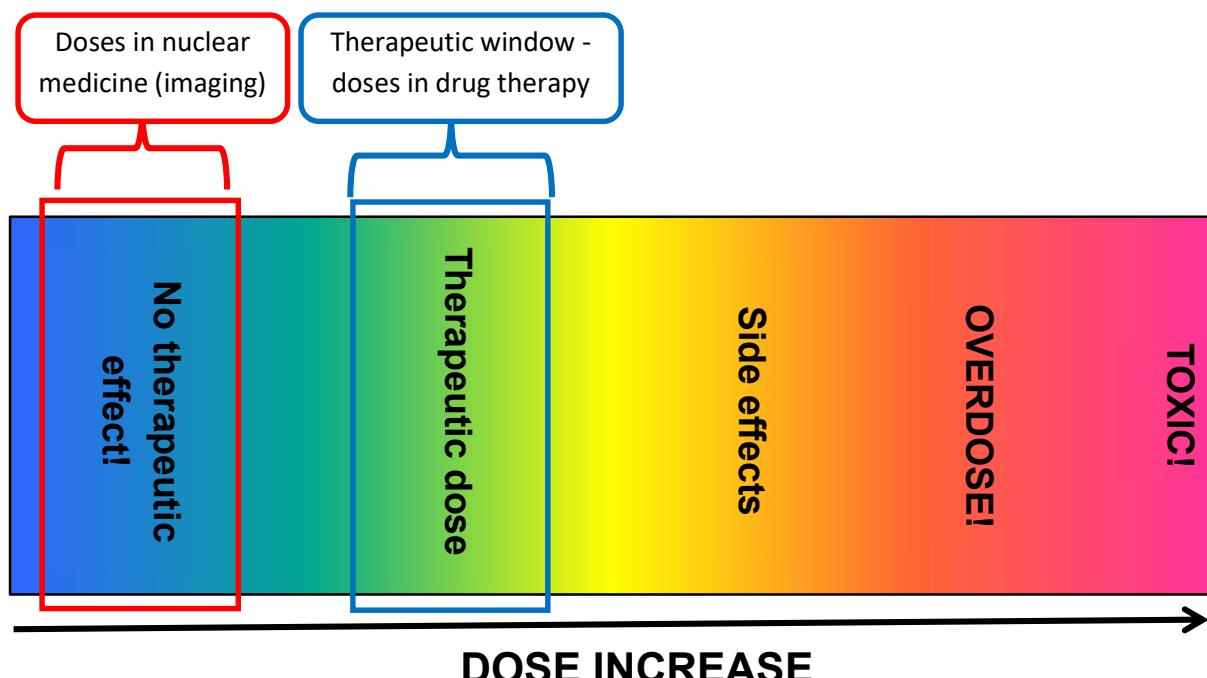
As we have seen one target can be targeted by several drugs. On the other hand, drugs that have only one target are called “specific” and have fewer side effects. However, very specific drugs are quite rare, most of drugs have not only one target, but several: more targets a drug has less specific it is and has more side effects. Each drug binds to its target with a specific affinity ( $K_1$ ):



This is an equivalent of binding affinity in coordination chemistry. The specific affinity depends of molecular structure of a drug.

However, the total pharmacological effect of a drug (sometimes expressed as  $ED_{50}$ ) depends much on concentration of a drug in the proximity of the target: the smallest amount of drug you imagine does binds to its target but, however, to achieve measurable pharmacological effect a drug has to reach certain concentration: this is called a therapeutic concentration! In order drug to have its effect molecules of drug need to fill certain number of receptors. There is also something called potency: a drug that is causing the same pharmaceutical effect with lower therapeutic concentration is more potent, stronger.

Doses in pharmacology are usually at the range from 1000 mg to 1 mg. If evenly distributed throughout a live average human body (5 L) it can reach concentration of about 200 mg/L to 0.2 mg/L, and in most of the cases it is from 1 mM down to tens of nM. This is a usual pharmacological therapeutic range (Figure 53).



**Figure 53:** With the increase of its dose the effect of a drug goes from therapeutic into toxic, it can be a drug or a poison depending on its dose. Yet, in nuclear medicine dose of radioactive drug (tracer) are far less than those required to make any physiological or pharmacological effect.



However, if the dose is too low then there will be no pharmacological effect at all, a patient does not feel any effect, nor any effect can be measured or observed. Yet, the small number of molecules of drugs that reach its targets do bind onto receptors but not in sufficient numbers.

If dose is too high then it is not good either: too much side effects, or it can be overdose, and if dose is very, very high a drug becomes a poison! Drug works the best in an optimal dose, so called therapeutic dose: just about to have a proper effect, but not side effects and not to be toxic. This is called a therapeutic window and sometimes tends to be quite narrow.

In nuclear medicine and especially in the area of nuclear imaging we want radiotracer molecule to bind onto its target and sends us its gamma-signal, but we do not want any pharmacological effect.

And this is why in nuclear medicine doses of radiopharmaceuticals are actually much lower than in classic pharmacological therapy; doses in nuclear imaging are in the area where drug has no pharmacological effect whatsoever (Figure 53): it binds receptors but exerts no effect, dose is too small.

Radiopharmaceutical doses are in MBq and mSv but if one recalculates them into molarity then it is at pM and nM levels, significantly lower than the doses in pharmacology.

In fact, radiopharmaceuticals are often called tracers not drugs, especially when applied for imaging: De Hevesy tracer principle says that radiopharmaceuticals can participate in biological processes but should not alter or perturb them! This is the reason concentration of radiopharmaceutical in organism has to be much more below those that are typical for therapeutic dose: just to bind the target, not to activate, not to inhibit, not to block it!

On the other hand, if applied for targeted radiation therapy (TRT) radiotherapeutic concentrations of radiopharmaceuticals are often smaller than pharmaceutical: radiation dose has to be high enough to kill all targeted cells, but low enough not to cause any unwanted radiation damage to healthy tissues.

### **Doses in imaging vs. doses in targeted therapy:**

It is important to remember that doses of radiopharmaceuticals are expressed in MBq, while dose of ionising radiation received by a patient or targeted cells are in mSv or Sv (Sieverts).

However, there is a big difference between imaging and therapy: imaging asks for lower, tracer doses, about 1-400 MBq (giving a patient total of just few mSv to per dose). For example, typical dose of FDG is from 200 to 400 MBq and a patient receives from 4 to 8 mSv - that is acceptable. But its dose in grams is just 1 ng, that is very, very tiny amount. Here are another such examples:  $^{99m}\text{Tc}$ -sestamibi, a typical

radiopharmaceutical for kidney function test is given at the dose from 150 to 740 MBq and it gives patient about 1.2-6 mSv. But the weight of this agent that is given to a patient is just 30 ng! Similarly,  $^{18}\text{F}$ -estradiol dose given for diagnostics of breast cancer is 185 MBq, gives just 4 mSv to a patient and it is a minute quantity of 0.845 ng.

On the other hand, targeted radiation therapy asks for large doses (from 1 to several GBq), while the targeted cells get lethal doses up to 30 Sv! For example, therapeutic dose of  $^{90}\text{Y}$ -Ibritumomab tiuxetan is up to 1.2 GBq – targeted lymphoma cells get a lethal dose of 17 Sv! What would happen if a person got whole body dose of 17 Sv? That person immediately dies. But in fact, the whole-body dose in targeted radiotherapy regimen is much lower, while 17 Sv is delivered only to the targeted malign cells that need to be destroyed. Likewise, as therapeutic dose of  $^{131}\text{I}$ -Tositumumab is 3 GBq, the lymphoma cells are hit with devastating 27 Sv and killed while whole body gets just few mSv and stays unharmed.

### Effect of ionising radiation

Is there some damaging effect of radiopharmaceuticals due to ionising radiation? As we know, radiopharmaceuticals are radioactive materials, and emit ionising radiation that can damage tissues. This negative side-effect is not incredibly significant in the radiopharmaceutical agents for imaging but tends to be more significant in targeted radiotherapy. As we know, beta and alpha particles can directly ionise biological structure and damage them, while gamma radiation is primarily ionising water, and then this ionisation of water is causing radiolysis and generation of free radicals. These free radicals are then reacting with biomolecules and are causing damage (Figure 54).

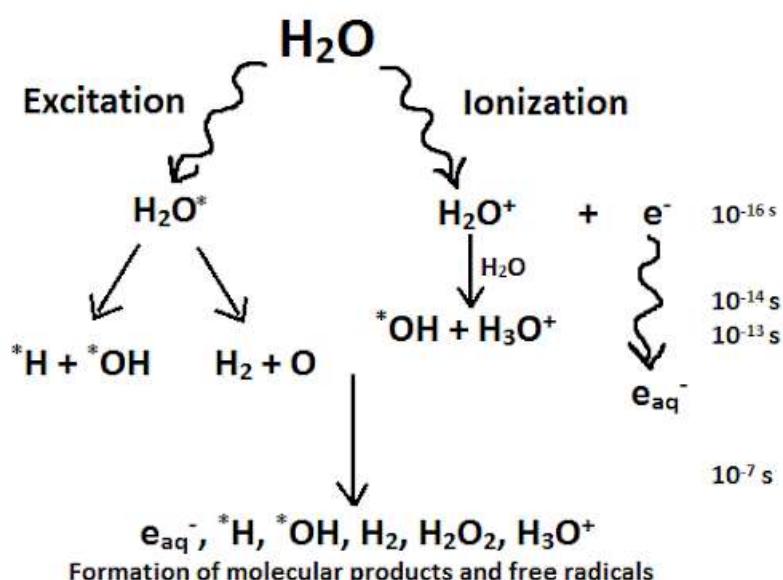


Figure 54: Gamma radiation ionises water and then reactive species (free radicals) are created that are ionising DNA, hence destruction of DNA with gamma radiation is indirect.

Toxicology is a science about poisons, while radiotoxicology is science about toxicity of a radioactive substance where one looks primarily into the toxic effect of radioactive materials and its ionising radiation inside human body, and damage it causes to the body. It is different from classic toxicology that is dealing only with chemical toxicity: radiotoxicity deals mainly with toxic effect of ionising radiation! As mentioned earlier, radiotoxicology is less important in imaging due to very low doses, but more important in targeted radiation therapy (TRT): if radionuclide is fully delivered just onto the target, then overall radiotoxicity to the whole body could be low since only targeted tissue is affected. Some radiotoxicity at the place of entrance (vein, infusion) is of concern. However, the general rule is that a radiopharmaceutical agent should not be radiotoxic, overall should not cause harm!

## What are theranostics?

It is also especially important to introduce concept of theranostics. What are theranostics? They are agents that are therapeutics and diagnostics in the same time! Name, as you can see, comes from combining “thera” (the first part of the word “therapeutic”) and “nostic” (the last part of the word “diagnostic”). How is this possible?

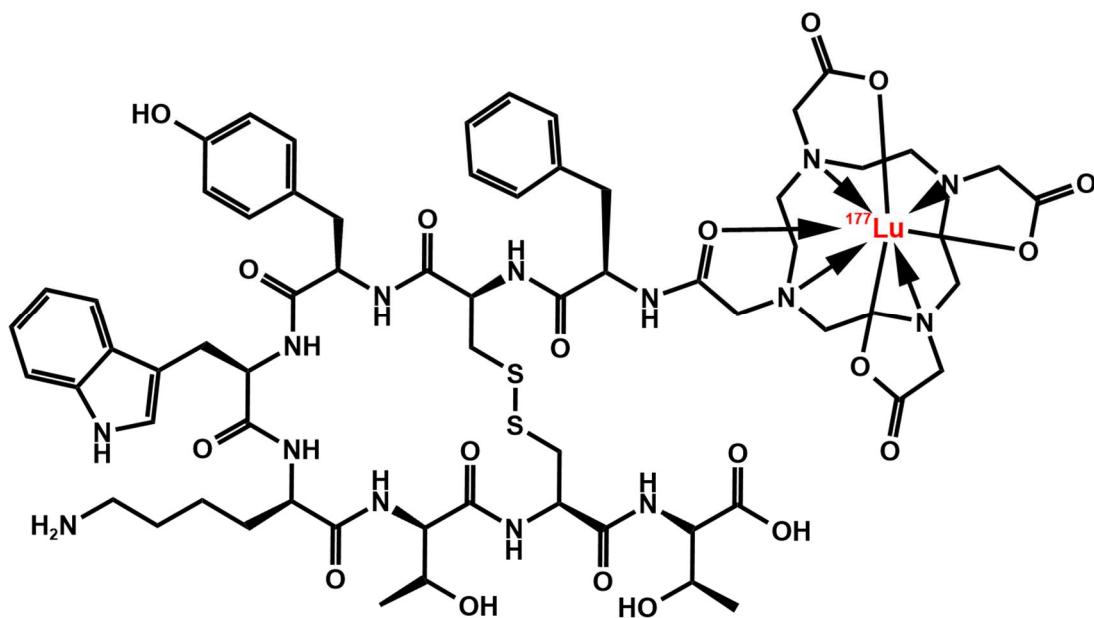


Figure 55: Theranostics Lutetium-177–DOTA-Octreotate

We can imagine a radiopharmaceutical agent that is both an imaging agent and a targeted radiotherapeutic agent at the same time. For example, a radiopharmaceutical agent can contain a radionuclide with both  $\beta^-$  and  $\gamma$  emission:  $\beta^-$  particle kills a cell, while accompanying  $\gamma$  ray serve as a location signal. These can be radionuclides such as  $^{177}\text{Lu}$  or  $^{131}\text{I}$ . (Figure 55) Another concept is to have a radiopharmaceutical with two radionuclides, one alpha, another gamma where alpha emitting radionuclide is delivering therapeutic effect while gamma emitting one serves to send imaging signal from the site of therapy.

## Chapter IV - Methods for the production of medical radionuclides

### How radionuclides for nuclear medicine are produced?

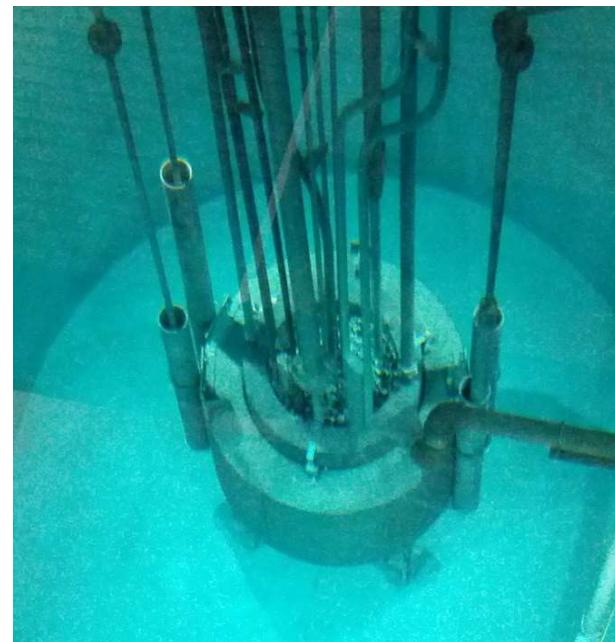
In short, all medical radionuclides are produced by nuclear reactions: particles of proper energy (usually  $p^+$ ,  $n$ ,  $d^+$ , or  $\alpha$ ) collide with a target material for transmutation. Sometimes we call those very accelerated particles “projectiles”. In general, there are three main types of methods to produce medical radionuclides and those are:

- Nuclear reactors where a target material is inserted into nuclear reactor and subjected to bombardment of thermal or fast neutrons. In this way it is possible to make  $^{99}\text{Mo}$ ,  $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$  and some other radionuclides.
- Accelerators, precisely cyclotrons where target material is bombarded by a beam or a ray if accelerated particles, usually protons. This is the most important methods for the production of radionuclides
- Radionuclide generators where medical radionuclides are made by the decay of their parent radionuclides. The most important such radionuclide generators is the one for  $^{99\text{m}}\text{Tc}$ .

### Nuclear reactors

Nuclear reactors for the production of medical radionuclides are not like the power reactors. They are usually an old research reactor so-called “pool type,” (Figure 56) but there are also some new as well. In general, they have high flux of neutrons inside a small, compact core, immersed in a pool of purified water. There are two methods for the production of medical radionuclides in reactors:

1. **Fission** where highly enriched uranium is irradiated and fissions into many products of much smaller molecular weight.
2. **Irradiation** where neutron capture nuclear reactions are making new radionuclides.



### Fission by thermal neutrons

In fission by thermal neutrons target is usually highly enriched uranium, up to 93%. This makes many various fission products (Figure 57), but  $^{99}\text{Mo}$  is quite abundant among them, makes 6% of all fission products. And  $^{99}\text{Mo}$  is the key precursor of

$^{99m}\text{Tc}$  which is one of the most important medical radionuclides. Except  $^{99}\text{Mo}$ , fission also makes  $^{131}\text{I}$ ,  $^{89}\text{Sr}$ ,  $^{133}\text{Xe}$  and  $^{90}\text{Sr}$  that can be used as a precursor for  $^{90}\text{Y}$ .

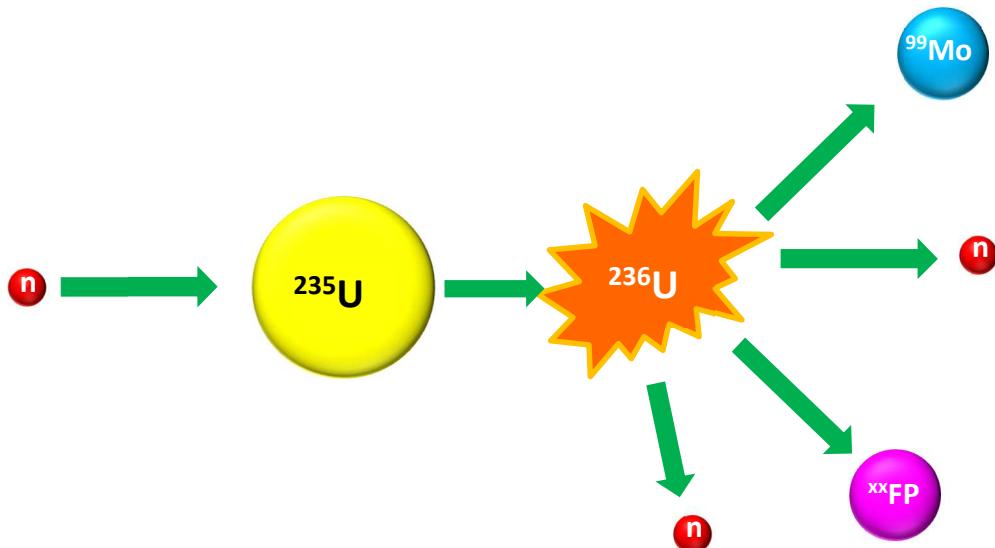
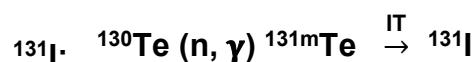
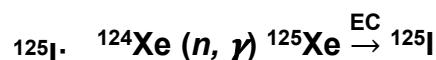


Figure 57: Schematic presentation of the fission process where a neutron hits  $^{235}\text{U}$  nucleus and it becomes unstable  $^{236}\text{U}$  and then fissions into smaller nuclei, such as  $^{99}\text{Mo}$  releasing more neutrons.

## Irradiation

Irradiation by neutron capture is the method where a target material is irradiated with a high flux of neutrons provided by the reactor reactions and then neutron capture is causing transmutation. In this way high variety of products can be made at low cost and high yield. There are two types of nuclear reactions: one is more common, capture of thermal neutrons followed by the release of gamma rays. For example,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{177}\text{Lu}$  and  $^{153}\text{Sm}$  can be made in this way:



Another, much less common nuclear reaction is the capture of a neutron followed by release of proton or alpha particle, but for this type of nuclear reaction fast neutrons are needed. In this way tritium and  $^{32}\text{P}$  can be made:



How much of radionuclide activity neutron capture irradiation can make? This is limited and depends on cross section of target, neutron flux, number of target atoms, decay constant of the product and time of irradiation. However, it is mainly limited by

the decay constant of the product: as we are irradiating the target the number of radionuclide atoms are increasing, but then their decay is becoming more important, until more radionuclides cannot be made; at one moment produced radionuclides are decaying at the same rate as irradiation is making them. This is called saturation and is described by a following equation:

$$A = \sigma\varphi N_{tgt}(1 - e^{-\lambda t})$$

In this equation A is the activity of product,  $\sigma$  is the cross section of target,  $\varphi$  is the neutron flux,  $N_{tgt}$  is the number of target atoms,  $\lambda$  is the decay constant of product and t is the time of irradiation.

When saturation forms, irradiation must be stopped, and the target taken out: longer irradiation will not make more desired radionuclide atoms.

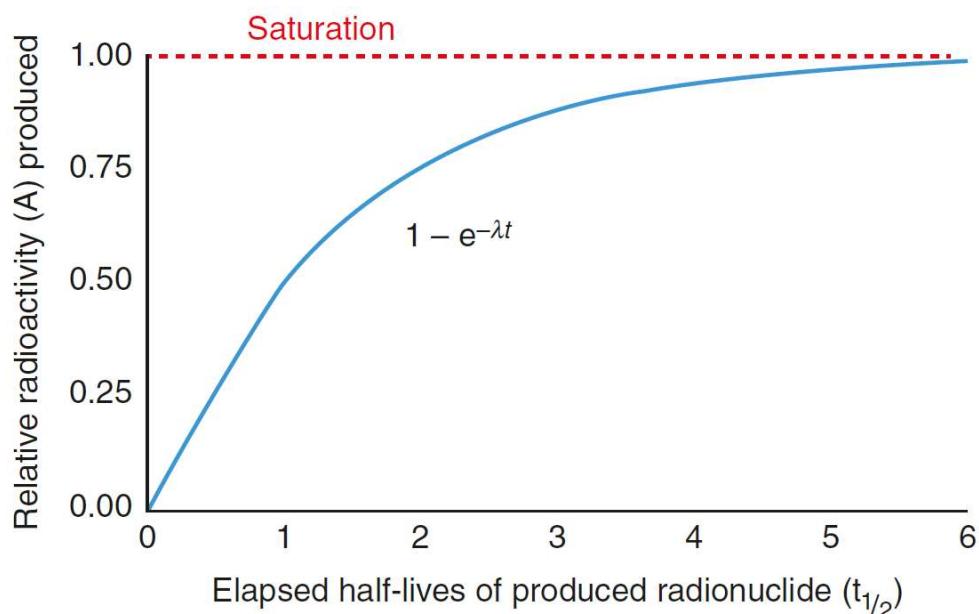


Figure 58: Diagram describing saturation as function of time

Targets for neutron irradiation are usually solid metals or oxide powders, encapsulated in an ampoule (typically aluminium or quartz, must be able to withstand very high temperatures (as much as 1000 °C). Today there are special computer programs for simulation and optimization of irradiation that seems to be very accurate: we can in advance plan how much radionuclide we wish to make.

Which radioisotopes could be produced by reactors? In general, all those that can be made by fission or neutron capture, but namely these are  $^{99}\text{Mo}$ ,  $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{177}\text{Lu}$ ,  $^{153}\text{Sm}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{131}\text{I}$ ,  $^{64}\text{Cu}$ ,  $^{32}\text{P}$  and others.

What are the drawbacks and challenges of reactors as producing instruments? Firstly, they are large and complicated devices, they are expensive to build and operate. We cannot have them in each town. Once radionuclides are produced, they must be quickly processed “on-spot”, and the transportation of materials can also be

challenging. These reactors are aging and are closing around the world, and now have become a bottleneck for the production of  $^{99}\text{Mo}$ . If  $^{99}\text{Mo}$  cannot be made, then it will be a big obstacle for the availability of  $^{99\text{m}}\text{Tc}$ . However, there are methods now for the production of  $^{99\text{m}}\text{Tc}$  using cyclotrons that can fully replace reactor-made  $^{99}\text{Mo}$ .

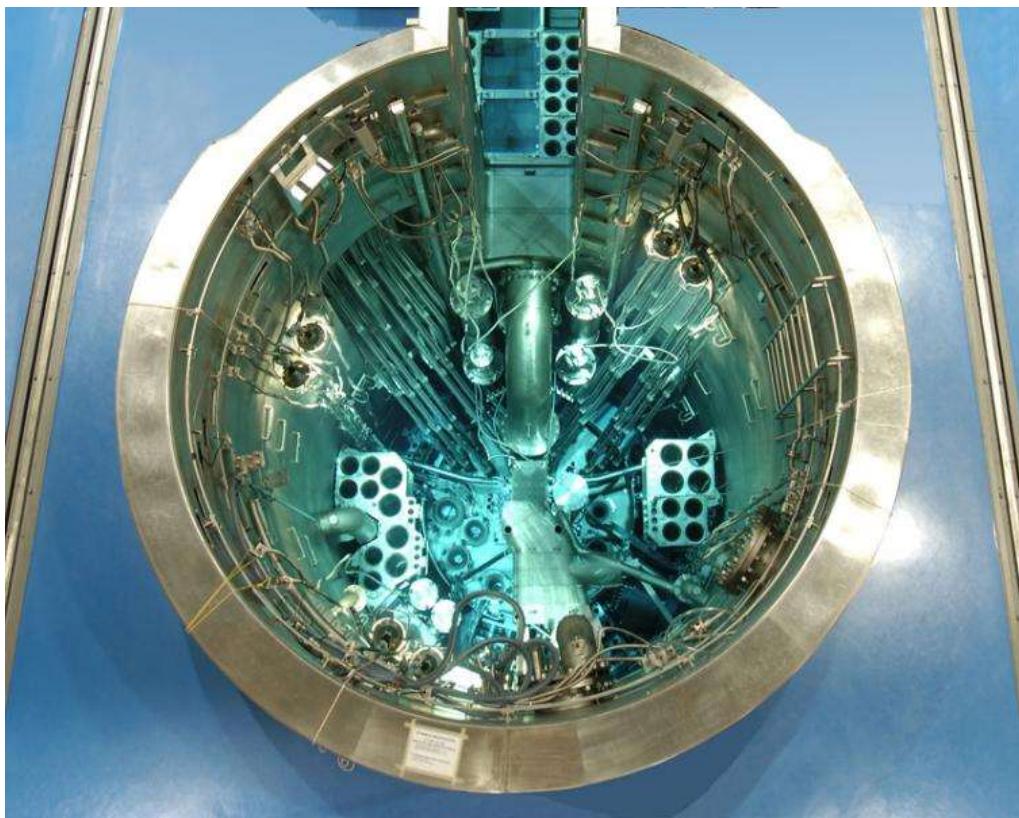


Figure 59: A view into the core of an experimental reactor used to make medical radionuclides

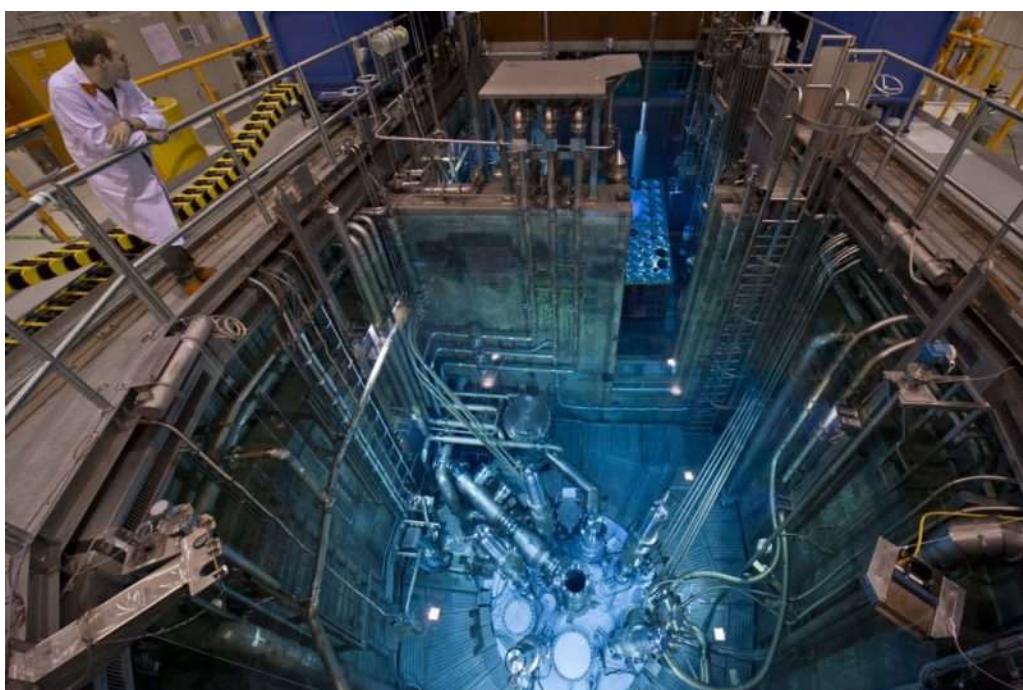


Figure 60: Another view of a small pool-type reactor

## Accelerators

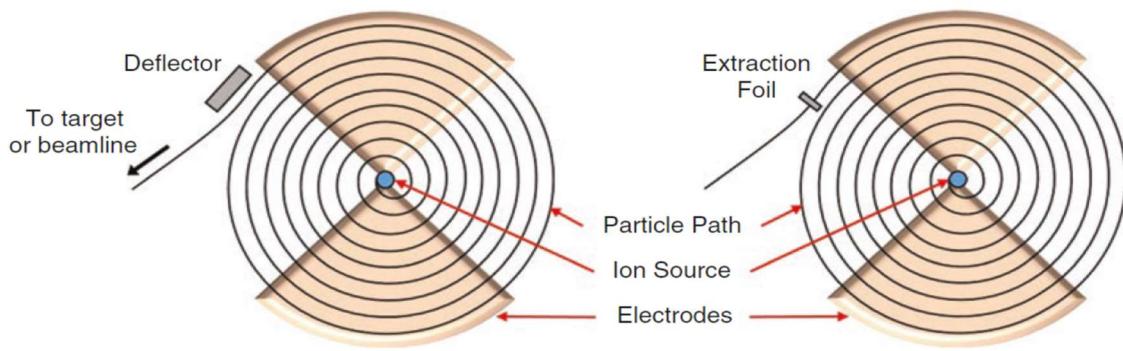
Another important method for the production of medical nuclides is accelerators. What is accelerator? It is a device that accelerates moving charged particles (such as proton,  $p^+$ ) increasing its energy. There are two main types of accelerators for the production of medical radionuclides:

- Linear accelerators (LINAC) where a particle is accelerated by moving along a line. This type is less important for medical radionuclides.
- Cyclotrons where particle is accelerated by cycling in a spiral trajectory. This is the most important for medical radionuclides and will be further discussed.

In fact, cyclotrons have become the main devices for the production of medical radionuclides: nowadays most of medical radionuclides needed in nuclear medicine can be produced using cyclotrons although reactors are still important.

Cyclotrons work by accelerating a charge particle along a spiral trajectory – by accelerating its speed a charged particle is gaining huge energy and becomes able to penetrate nucleus and cause nuclear reactions. When accelerated particles collide with a target material (gas, liquid, solid) they are causing nuclear transformation. These nuclear transformations depend on type of accelerated particle, energy (speed) of accelerated particles and the nature of the target material.

Accelerated particles can be protons ( $p^+$ ), deuterons ( $d^+$ ), alpha particles ( $\alpha$ ), but are in fact mostly hydride ions that are turned into protons when they exit accelerator chamber. To make enough of radionuclides cyclotrons need to accelerate huge number of ions into a shower of “projectiles” and use of energy is extremely high. Energies of accelerated particles are usually denoted in megaelectronvolts (MeV).

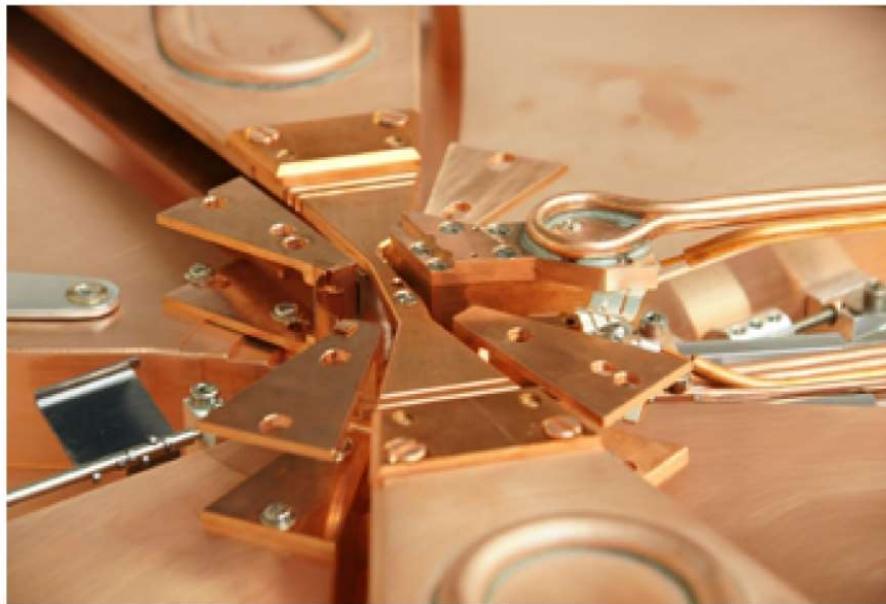


**Figure 61: Schematic explanation of accelerator principle: ion is injected into the accelerator and is speeding up faster and faster until it is ejected into the beamline. With each turn in the spiral trajectory ion is gaining speed and energy.**

Cyclotrons can be small, commercially available machines that are accelerating protons up to 8 MeV and are used only for the production of  $^{18}\text{F}$  and  $^{11}\text{C}$  but can be much more powerful: medium make protons of 11-30 MeV, while the large ones, installed in large institutions are accelerating protons to energies more than 30 MeV.

Medical cyclotrons are usually isochronous cyclotrons (Figure 61): using a combination of very strong orthogonal magnetic field and alternating electric field it is forcing injected ions to start moving in a spiral orbit and at each half-round they are moving with larger radius, hence faster and faster – accelerating and gaining enormous kinetic energy!

Beam of ions e.g. particles are coming from the plasma of ions that serves as a source of ions.



**Figure 62: Ion source in the middle of a cyclotron.**

This plasma of ions is made of feed gas, and usually it is hydrogen if we want to accelerate protons or hydride ions, or it is helium if we need to accelerate alpha particles, or even heavy hydrogen (deuterium) if we need deuteron particles. This plasma is generated by the flux of electrons colliding with the feed gas and the formed cloud of ions are injected into the centre of cyclotron chamber.

The main chamber of the cyclotron is at very high vacuum, and is placed between two very strong magnet poles, and inside are half-circular electrodes alternating polarity at high frequencies (10s of MHz). In the early stages of development of cyclotrons these electrodes were in the form of Latin letter “D” so they are called “dees”, but nowadays electrodes have slightly different shape.

The ions injected into the centre of accelerator chamber accelerate to high energies: 3 MeV to 70 MeV and even more (500)! This shower, or ray of very fast and energetic particles is called a beam. When cyclotrons work, they consume lots of energy: to accelerate 160  $\mu$ A of protons to 16.5 MeV a cyclotron consumes 75 kWh of electric energy!

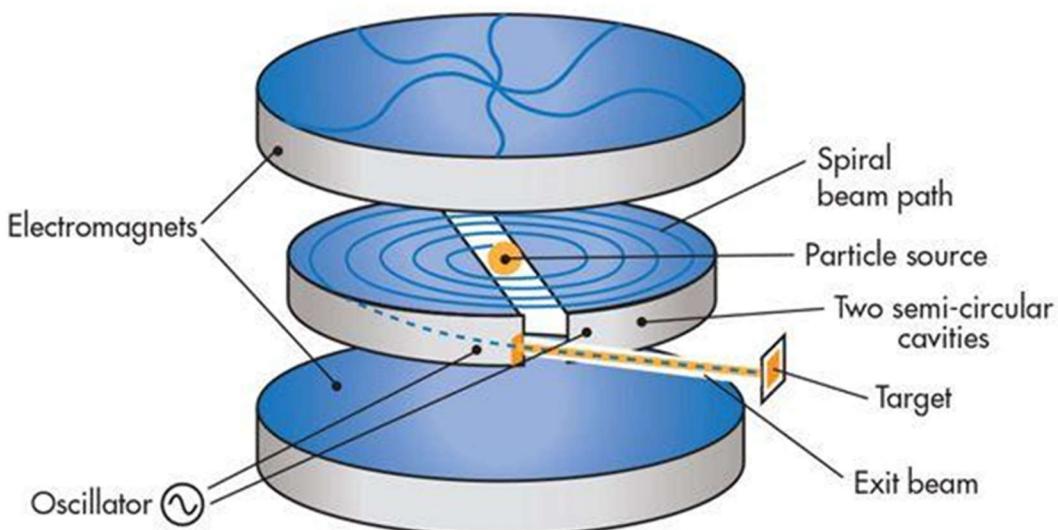
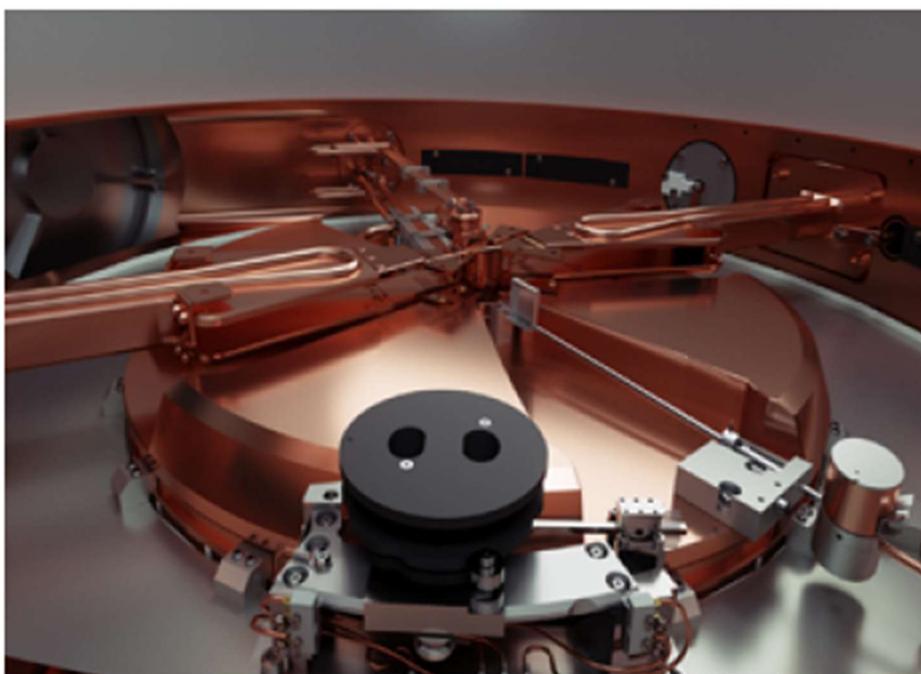


Figure 63: Schematic diagram showing elements of cyclotron: magnetic poles, electrodes (“dees”), and the spiral trajectory of accelerated particles

Once the fully accelerated particles reach the outmost orbit they are extracted from the circular path and are either directed onto a target or down a beamline. The extraction of positive ions such as protons can be achieved by an electromagnetic deflector, while the extraction of negative ions such as hydride ions is achieved by an extraction foil, usually made of graphite:  $H^-$  passes extraction foil, gets stripped off electrons, becomes  $p^+$ , and then exits the cyclotron towards the target! In both cases the result are very fast, energetic protons heading towards the target!



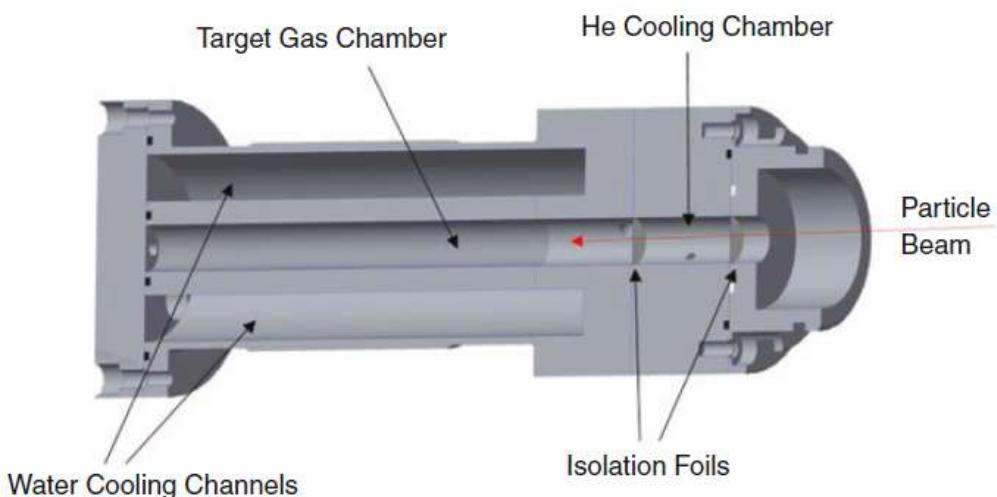
Figure 64: A view into an opened cyclotron



**Figure 65: Extraction foil carousel for the extraction of accelerated hydride ions from cyclotron**

Accelerator targets can be in all states: gas, liquid or solid. When the target is a gas then it is kept in a sealed metal casing (Figures 66 and 67) and is very intensively cooled since ion beam hitting the target release lots of heat! A typically gas target can be nitrogen gas enriched with  $^{14}\text{N}$  isotope: by using such target we can make  $^{11}\text{C}$  or  $^{15}\text{O}$  via following nuclear reactions using accelerated protons or deuterons:

- $^{14}\text{N}(p,\alpha)^{11}\text{C}$
- $^{14}\text{N}(d,n)^{15}\text{O}$



**Figure 66: Schematic cross-section of a metal casing for gas target.**



Figure 67: Actual casing for gas target.

Targets also can be liquids: for example, water. If water enriched with  $^{18}\text{O}$  is bombarded with protons we are going to get fluoride-18 ( $^{18}\text{F}^-$ ) ions. If we bombard plain, normal water containing mainly  $^{16}\text{O}$  we will get  $^{13}\text{N}$  however this reaction is not typically used to make  $^{13}\text{N}$ . These important nuclear reactions can be summarised as  $^{18}\text{O}$  ( $p, n$ )  $^{18}\text{F}$  and  $^{16}\text{O}$  ( $p, n$ )  $^{13}\text{N}$ . As in the case of the gas targets, this one also needs to be intensively cooled.

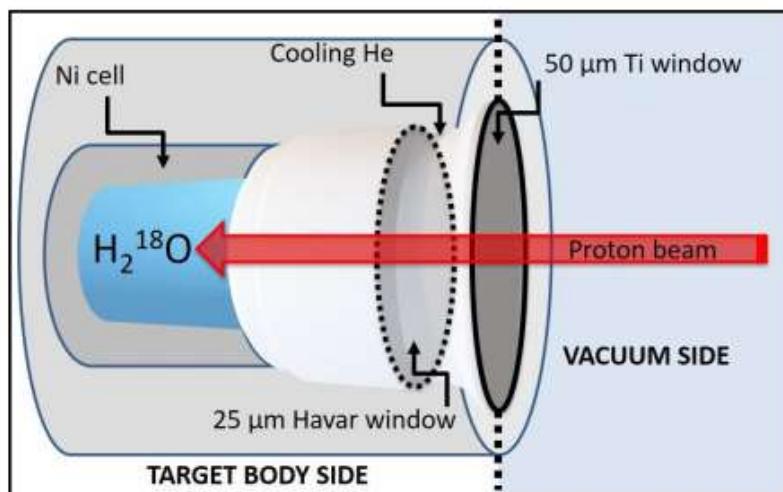


Figure 68: Schematic presentation of a target chamber for liquid target (up); actual appearance of a chamber for liquid targets down)

Accelerator targets also can be solid. In fact, most of radionuclides are made by bombarding solid targets. Target materials are usually very thin: electroplated onto small “coins” made of inert metal such as tantalum or niobium. Alternatively, solid targets can be foils or pressed powders. Many important medical radionuclides are made using solid targets, including  $^{99m}\text{Tc}$  that can be made by using cyclotrons by bombarding  $^{100}\text{Mo}$  with protons. However, majority of  $^{99m}\text{Tc}$  used is still from  $^{99}\text{Mo}$  obtained in nuclear reactors. Here is the list of nuclear reactions and medical radionuclides routinely produced by bombarding solid targets in cyclotrones:

- $^{64}\text{Ni}(p,n)^{64}\text{Cu}$
- $^{89}\text{Y}(p,n)^{89}\text{Zr}$
- $^{111}\text{Cd}(p,n)^{111}\text{In}$
- $^{100}\text{Mo}(p,2n)^{99m}\text{Tc}$
- $^{226}\text{Ra}(p,2n)^{225}\text{Ac}$

As the case with other targets solid targets also must be intensively cooled.

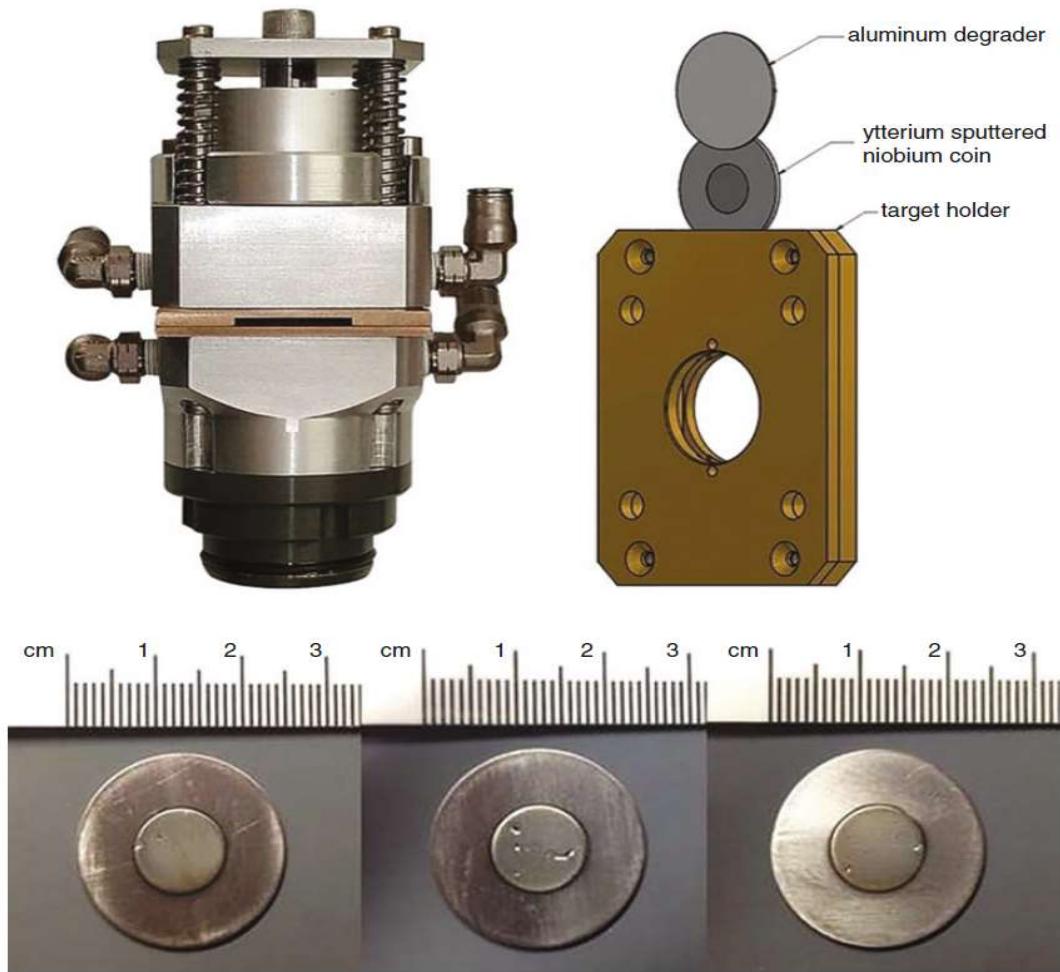


Figure 69: Target holder for solid targets: solid targets (down) are in form of a coin and are placed into the holder.



Which medical radionuclides can be made by cyclotron? Almost all important! Each radioisotope is made by using a defined target (sometimes isotopically enriched) and defined conditions such as accelerated particle type, energy of particles, particle current (in  $\mu\text{A}$ ), etc. Activity of produced radionuclide depends on particle current but has a saturation effect as seen in reactors: longer irradiation will not make significantly more radionuclides. In general, activities produced ranges from 1 GBq to 10 TBq. Also, produced radionuclides probably may contain some radionuclide impurities. However, this can be predicted based on conditions: today there are computer programmes for the quite accurate simulations and optimisations of radionuclide production in cyclotrons.

Medical cyclotrons are today produced serially and can be small sized, middle sized, large and very large. There are at least eleven producers of commercially available medical cyclotrons such as General Electric, Siemens, IBA and Sumimoto. Most of them are from the West or Japan or Korea, however a Chinese brand of medical cyclotrons will be presented soon!

Brand	Country of origin
<b>General Electric</b>	USA
<b>Siemens</b>	Germany
<b>IBA</b>	Belgium
<b>Sumimoto</b>	Japan
<b>TCC</b>	USA
<b>ABT</b>	USA
<b>ACSI</b>	Canada
<b>NIEFA</b>	Russia
<b>Scanditronix</b>	Sweden
<b>Philips</b>	Netherlands
<b>Kotron</b>	Korea

Table 3. List of medical cyclotron brands and their countries of origin.



Figure 70: GENtrace, General Electric, small cyclotron

Cyclotrons differ by type, power and energy of their particles. Small, weak cyclotrons such as the one in the Figure 70 make protons up to 8 MeV for the production of only  $^{18}\text{F}$  and  $^{11}\text{C}$ .

The middle-sized cyclotrons (Figures 71, 72) can accelerate protons from 5 to 30 MeV; however these can make whole range of various radionuclides depending on target, particle and its energy for example  $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$ ,  $^{18}\text{F}$ ,  $^{61}\text{Cu}$ ,  $^{64}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ge}$ ,  $^{89}\text{Zr}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{123}\text{I}$ ,  $^{201}\text{Tl}$ ,  $^{211}\text{At}$ , and  $^{225}\text{Ac}$ . These cyclotrons can have gas, liquid or solid targets.

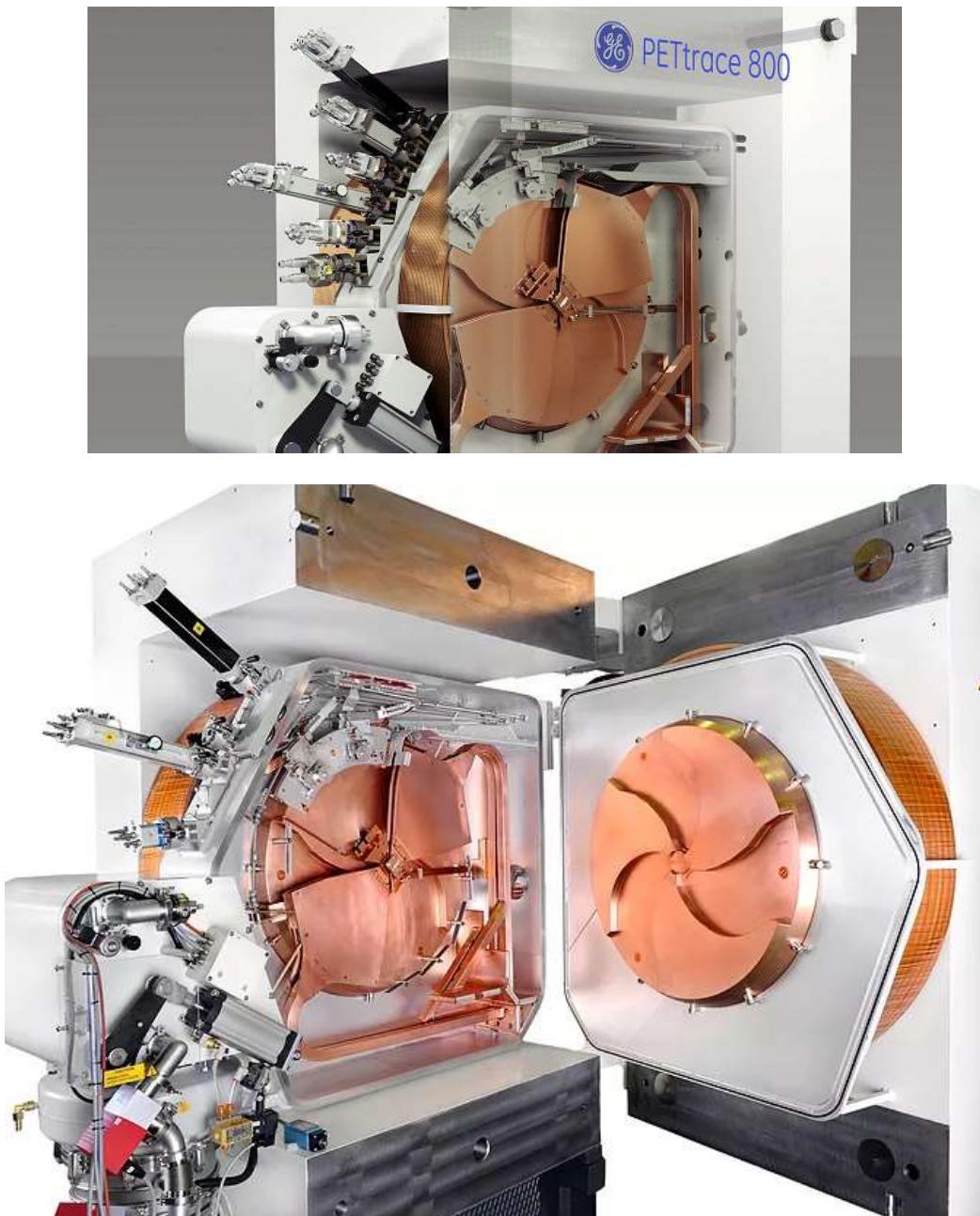


Figure 72: PETtrace 800, General Electric, USA



Figure 73: Middle-sized cyclotron Cyclone KIUBE, IBA, Belgium

Also, there are large ones (Figure 74) with energies up to 70 MeV that can make very wide range of radionuclides.

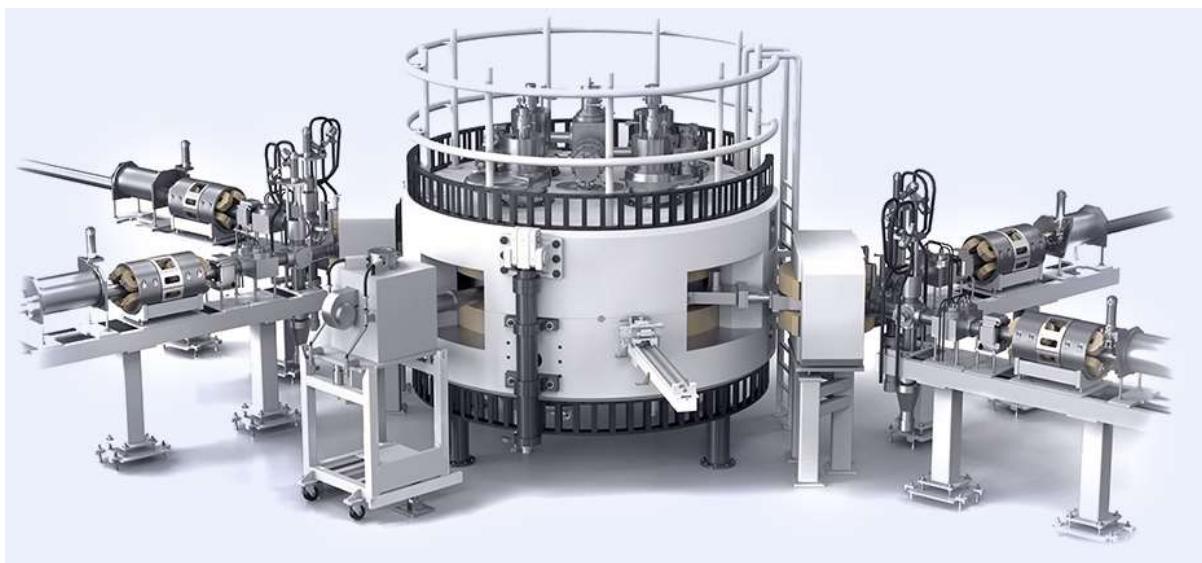


Figure 74: Large cyclotron Cyclone 70, IBA, Belgium

And there are very large ones (Figure 75) that can accelerate protons to 500 MeV, and these are used for very special radionuclides.



Figure 75: Very large cyclotrons are usually custom made and specially installed in large institutes.

## Radionuclide generators

The third type of devices for the production of medical radionuclides are radionuclide generators. They are small, simple apparatuses where a longer-lived parent radionuclide is kept immobilized on a solid support, and decays into a chemically different, shorter-lived daughter nuclide, which can subsequently be separated easily from the parent by elution.

The removal of daughter radionuclide by washing (or elution) is also called “milking” in slang, while radionuclide generators are called “cows”. The most common radionuclide generator is  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator nicknamed “Moly cow” because it contains **molybdenum** and  $^{99\text{m}}\text{Tc}$  is “milked” out of it every morning. There are other radionuclide generators such as  $^{68}\text{Ge}/^{68}\text{Ga}$ , and some other not very common for example  $^{82}\text{Sr}/^{82}\text{Rb}$ ,  $^{188}\text{W}/^{188}\text{Re}$ ,  $^{90}\text{Sr}/^{90}\text{Y}$ , and  $^{81}\text{Rb}/^{81\text{m}}\text{Kr}$ . But make no mistake: radionuclide generation is just an extension of a nuclear reactor or accelerator where it is not available. To make the parent radionuclide you still need a nuclear reactor or an accelerator.

Yet, the most famous and the most important radionuclide generator is the one that gives  $^{99m}\text{Tc}$ . Therefore, the principle of radionuclide generators will be explained by using the example of  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator.

Originally extracted from fission products,  $^{99}\text{Mo}$  is in the form of molybdate ion ( $\text{MoO}_4^{2-}$ ) and is adsorbed onto alumina ( $\text{Al}_2\text{O}_3$ ) or as zirconium molybdate gel ( $\text{ZrMo}_2\text{O}_7$ ). It cannot be eluted with water, it is immobilized.

However, its daughter,  $^{99m}\text{Tc}$  builds up as pertechnetate ion ( $\text{TcO}_4^-$ ); it is very soluble in water and can be easily washed out from alumina or gel, hence, it is mobile. The pertechnetate ion is eluted with saline solution ( $\text{NaCl}$ , 0.91%) with certain efficiency and filtered through a special membrane. Once obtained it is pure, carrier free, sterile, and ready for further reactions or direct application.

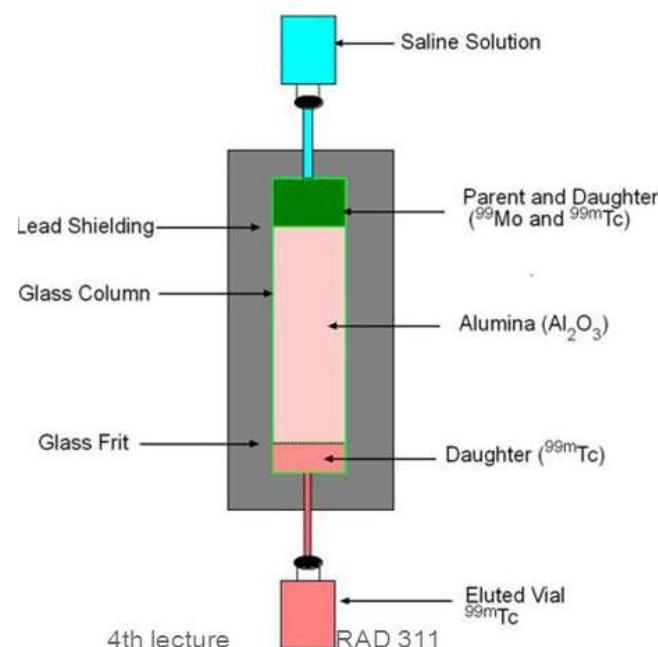
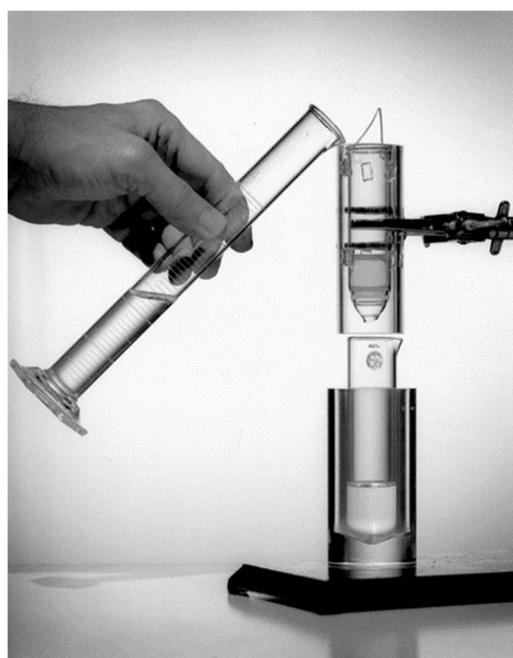
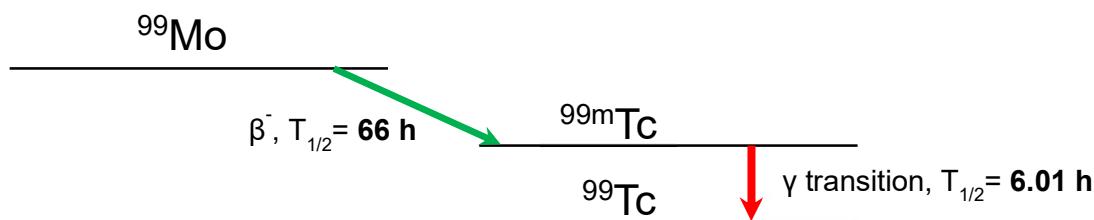
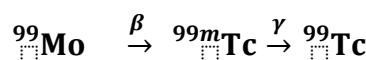


Figure 76: left) The first  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator, right) schematic explanation of  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator.

Molybdenum-99 ( $^{99}\text{Mo}$ ) decays into  $^{99m}\text{Tc}$  and then into  $^{99}\text{Tc}$  via following decay equation and scheme:



The  $^{99m}\text{Tc}$  is a meta-stable radioisomer of  $^{99}\text{Tc}$ . Half-life of  $^{99}\text{Mo}$  is 66 hours and the half-life of  $^{99m}\text{Tc}$  is eleven times less, just 6 hours. This ratio of half-lives brings these

two radionuclides into so-called transient equilibrium. This type of equilibrium can be mathematically described using the following lengthy equation, but the diagram (Figure 77) is visualizing the equation for better understanding. This is how  $^{99}\text{Mo}$  activity and  $^{99\text{m}}\text{Tc}$  activity are related in a transient equilibrium.

$$A_T = \text{BR} \left( \frac{\lambda_T}{\lambda_T - \lambda_M} \right) A_M^0 (e^{-\lambda_M t} - e^{-\lambda_T t}) + A_T^0 \times e^{-\lambda_T t}$$

“ $A_T$ ” is the activity of  $^{99\text{m}}\text{Tc}$ , “BR” is the branching ration, “ $\lambda_T$ ” is the  $^{99\text{m}}\text{Tc}$  decay constant ( $0.01 \text{ h}^{-1}$ ), “ $\lambda_M$ ” is  $^{99}\text{Mo}$  decay constant ( $0.115 \text{ h}^{-1}$ ), “ $A_M^0$ ” is activity of  $^{99}\text{Mo}$  at time 0, “ $A_T^0$ ” is the activity of  $^{99\text{m}}\text{Tc}$  at time 0 and “ $t$ ” is time.

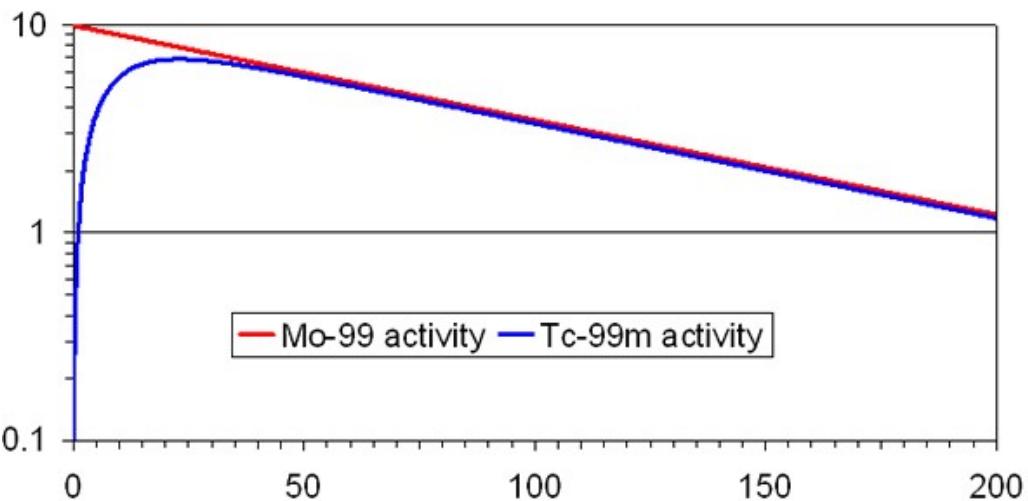


Figure 77: Diagram of the transient equilibrium of  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  radionuclide pair

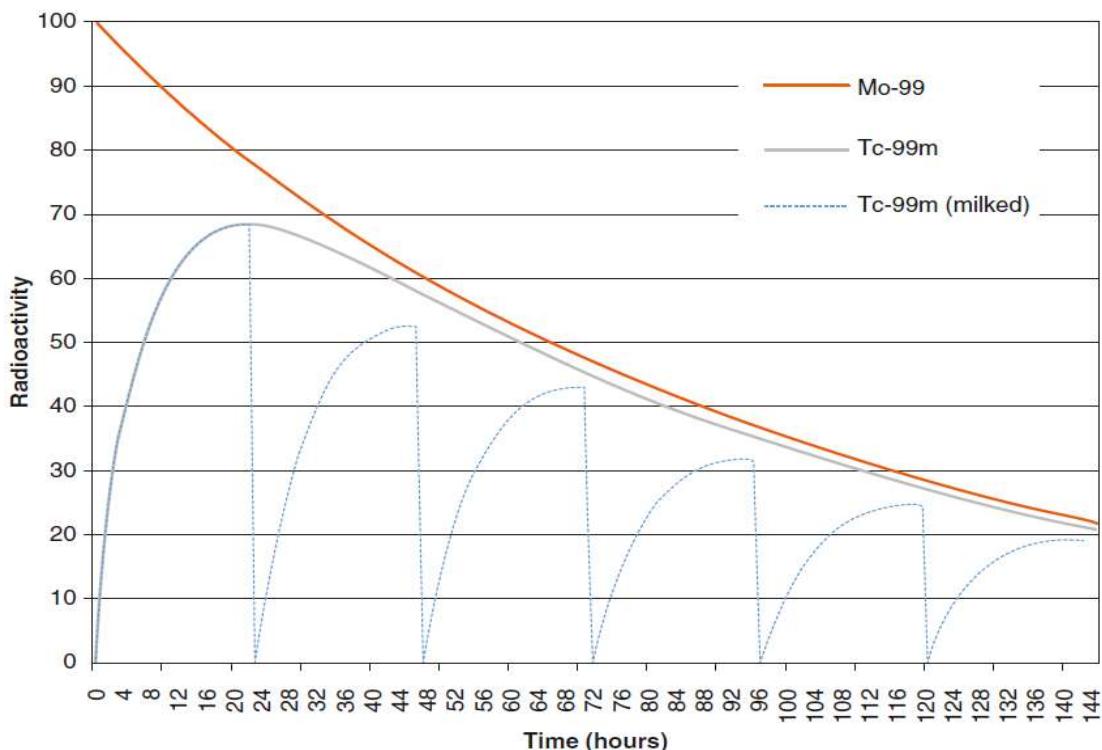


Figure 78: Equilibrium forms again every 24 hours but total activity is fading away.

The equation is telling us that if all the suddenly  $^{99m}\text{Tc}$  is removed from its mixture with its parent,  $^{99}\text{Mo}$ , (as it happens in the moment of “milking”) the new equilibrium will form within approximately 24 hours, a day. Therefore, you can remove newly formed  $^{99m}\text{Tc}$  every morning. Yet, activity of  $^{99}\text{Mo}$  is decreasing day by day and in 6 days you are at 1/5<sup>th</sup> of the original activity (Figure 78). Eventually all  $^{99}\text{Mo}$  will decay and  $^{99m}\text{Tc}$  with it.

How these generators and milking works in practice? It is illustrated in the Figures 79 and 80. The vial with  $^{99}\text{Mo}/^{99m}\text{Tc}$  absorbed onto alumina is encased into a big mass of lead that serves as a shielding, to make work with these generators safe. One tube brings eluent from the first vial, and another tube takes eluted  $^{99m}\text{Tc}$  through a filter into a shielded vial. Two vials are inserted: firstly, the one with a pure saline solution (eluent) and only then the second empty under vacuum: vacuum serves as force that runs eluent. Vacuum sucks eluent that flows through the column inside, eluting (washing) out  $^{99m}\text{Tc}$ . Eluent with  $^{99m}\text{Tc}$  flows through a filter into a shielded product container until it is fully filled. This procedure (milking) can be repeated every day!

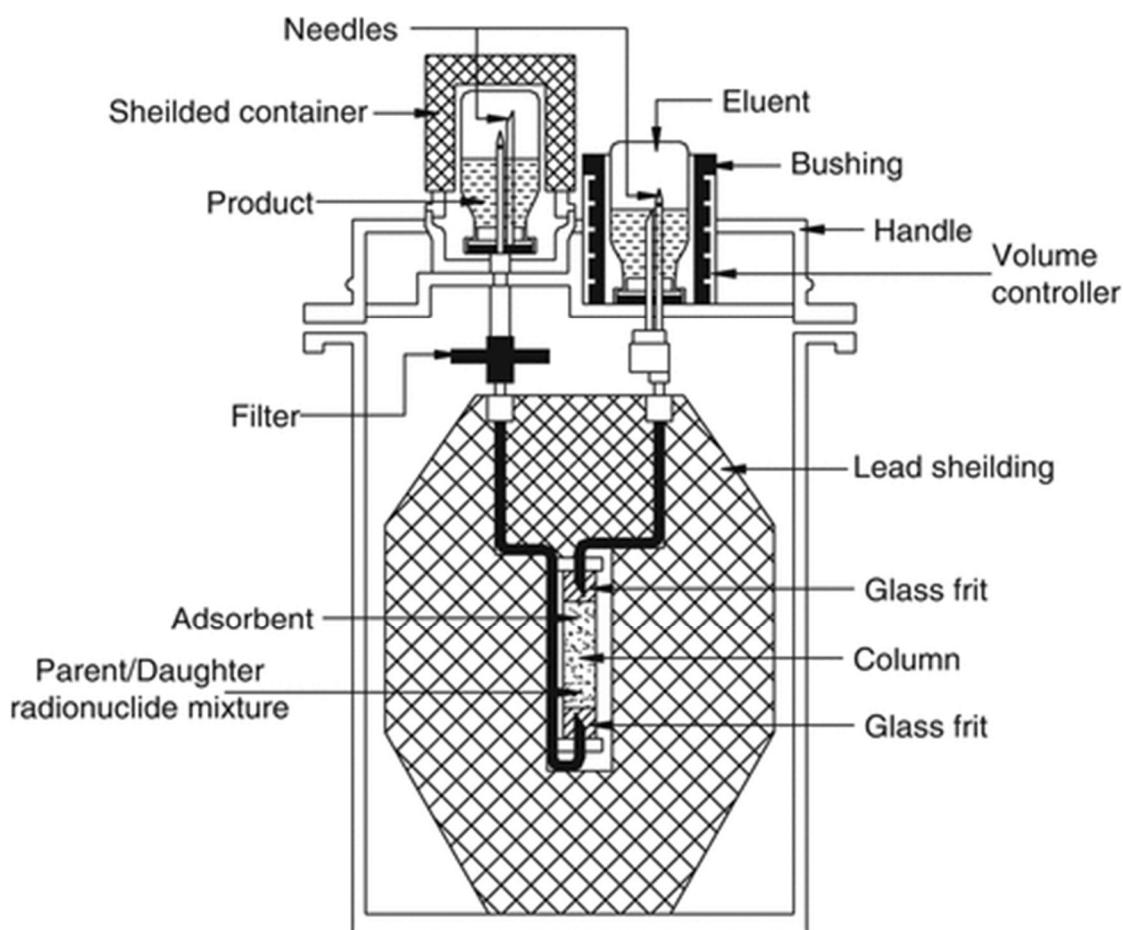


Figure 79: Scheme of practical use of  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator

How the generator looks like in the real life? It looks like a small, cylindrical box (Figures 80 and 81). Most of the box is shielding lead.

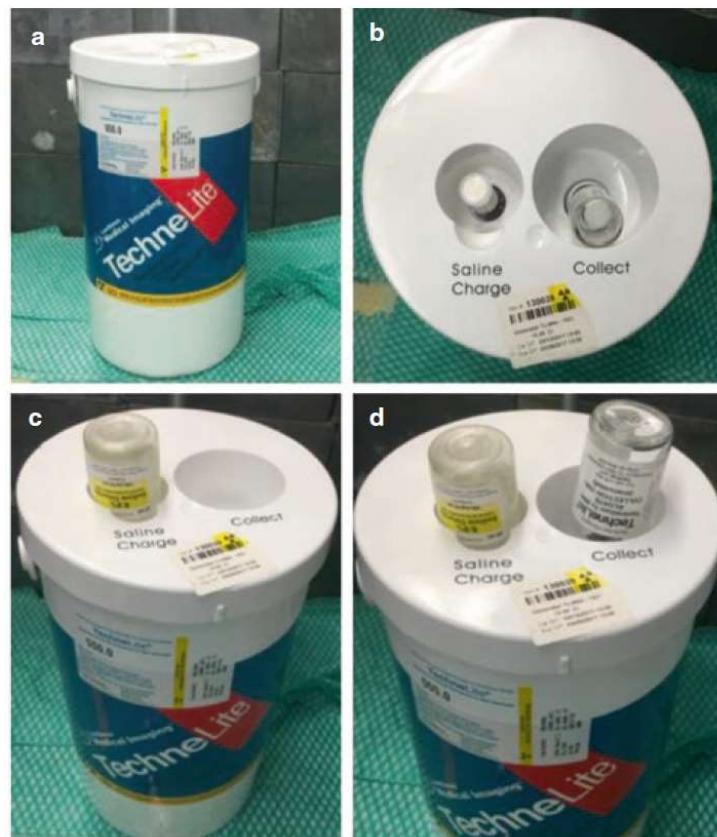


Figure 80: Practical use of  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  radionuclide generator



Figure 81: Some examples of  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  radionuclide generator

There are also some other radionuclide generators, such as  $^{68}\text{Ge}/^{68}\text{Ga}$  generator (Figure 82) for obtaining  $^{68}\text{Ga}$ , an important radionuclide for PET scans. Its parent radionuclide,  $^{68}\text{Ge}$  can be made using an accelerator. The half-life of  $^{68}\text{Ge}$  is 271 days or more than 6500 hours, while the one of  $^{68}\text{Ga}$  is just 1.13 hour. With such huge difference the two radionuclides are in so called secular equilibrium. This means that  $^{68}\text{Ge}/^{68}\text{Ga}$  generator lasts much longer than  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator and is made much more robust. In practice  $^{68}\text{Ge}$  is absorbed into titanium-dioxide or tin-dioxide powder while  $^{68}\text{Ga}^{3+}$  ions are eluted using HCl solution.



Figure 82: An example of  $^{68}\text{Ge}/^{68}\text{Ga}$  radionuclide generator

Other radionuclide generators are much less common but  $^{90}\text{Sr}/^{90}\text{Y}$  radionuclide generator that is still under development would be a great breakthrough in the area of radiotherapy: if potential  $^{90}\text{Sr}/^{90}\text{Y}$  generator could give  $^{90}\text{Y}$  of acceptable radionuclide purity it would revolutionize availability of targeted radiotherapy based on  $^{90}\text{Y}$ .

## Chapter V - Processing of medical radionuclides, labelling, and quality control

This chapter will be generally about the processing of medical radionuclides into radiopharmaceuticals. The radiopharmaceutical process (Figure 83) starts with production of radionuclides, and this is covered it in the previous chapter. After radionuclide is produced it is then extracted out and sent into a radiochemical lab where, firstly, precursors are made.

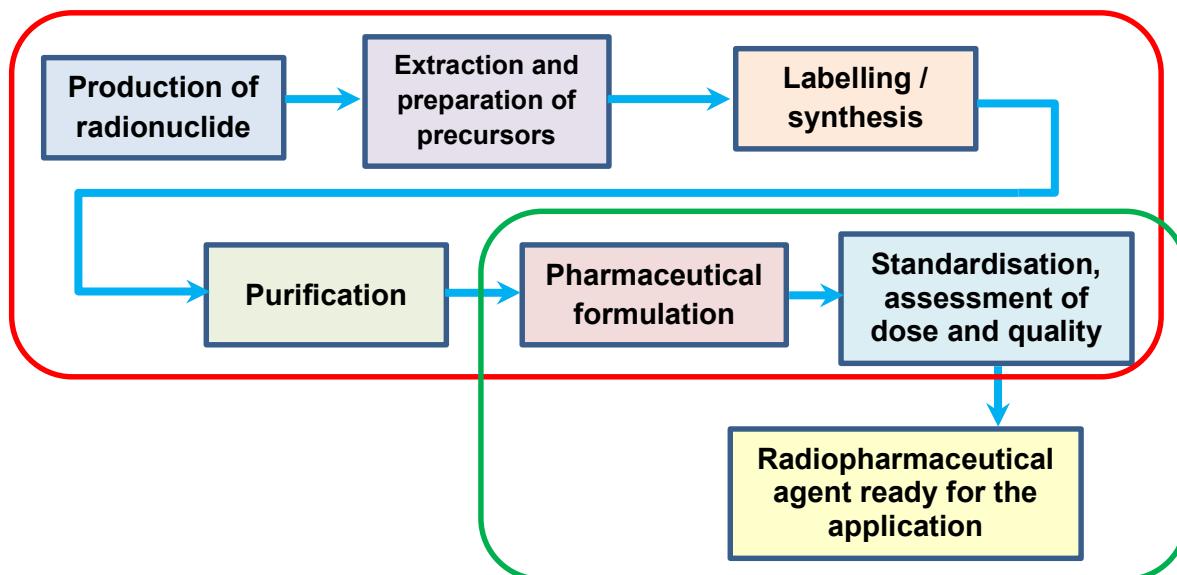


Figure 83: Radiopharmaceutical production process, from making radionuclide to radiopharmaceutical agent ready for application

These precursors then take part in the key step called labelling by which a radiopharmaceutical compound is chemically prepared. Next, the radiopharmaceutical compound is purified and then formulated into a radiopharmaceutical preparation (or agent). The final step is the standardization and assessment of quality after which it is ready for the application. The whole process takes approximately 30 minutes and sometimes even shorter. Part encircled by red line (Figure 83) is usually the responsibility and competence of radiochemists, while last two steps can be also done by pharmacists.

### Radionuclides with and without carrier

Medical radionuclides can be produced and extracted with or without a carrier. What is a carrier?

It is chemically same or similar compound or isotope present in the raw product, usually much more abundant than the trace amount of produced medical radionuclide. The carrier can be so-called isotopic carrier, for example trace quantities of  $\text{Na}^{131}\text{I}$  in 10 mg of stable, non-radioactive  $\text{NaI}$ , therefore stable  $\text{NaI}$  is an isotopic carrier or the same element.



On the other hand, the carrier can also be a non-isotopic carrier: a different stable element that is chemically similar to the radionuclide. For example, few nanograms of  $^{89}\text{SrCO}_3$  in 20 mg of  $\text{CaCO}_3$ . In this case  $\text{CaCO}_3$  is a carrier of a different element. A newly made radionuclide is “carrier-free” if no other stable nuclides of the same element is present in the product than the radionuclide itself. Carrier-free radionuclides are, in practice typical for accelerator-produced radionuclides; if no carrier is added during the separation process, the nuclide is considered to be carrier-free. Carrier-free radionuclides have a high specific activity (Bq/g), while a radionuclide with carrier has much lower specific activity.

### Purity of radiopharmaceuticals – radionuclidic purity

Radiopharmaceutical agents generally require high standards of purity. There are several levels of purity in the area of radiopharmaceuticals, and the first one is the radionuclidic purity. It is defined as a “percentage of the radioactivity of the desired radionuclide in a sample out of the total radioactivity of the sample”. For example, presence of few percentages of  $^{99}\text{Mo}$  activity in  $^{99\text{m}}\text{Tc}$  is considered to be radionuclidic impurity.

Any radionuclide impurities may increase the radiation dose received by the patient and may also degrade the quality of any medical procedure performed. Radionuclide should be as pure as possible, containing only one desired radionuclide, but this often is not the case, since it can contain small activity of other radionuclides as impurity. For example, there can be some small activity of  $^{99}\text{Mo}$  in an eluted  $^{99\text{m}}\text{Tc}$  sample, but requirement is that 99% of the radioactivity must be from  $^{99\text{m}}\text{Tc}$ . The Equation for radionuclide purity is as follows (taken  $^{99\text{m}}\text{Tc}$  as an example):

$$\boxed{^{99\text{m}}\text{Tc}_{(R.P.)} = \frac{A_{99\text{mTc}}}{A_{99\text{mTc}} + A_{99\text{Mo}}} \times 100 > 99.9\%}$$

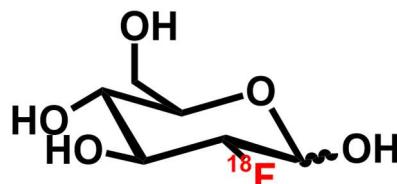
Sometimes radionuclidic impurities can be tolerated to the certain extent but sometimes it cannot at all since impurity can be radiotoxic even in trace quantities. The example of such situation is presence of  $^{90}\text{Sr}$  impurity in its daughter  $^{90}\text{Y}$ .

### Labelling

The next stage in the process of producing radiopharmaceuticals is the one that is the central and most important and that is **labelling**. What is the labelling? In our case it is the substitution of one or more atoms or chemical groups of a compound by a radioisotope(s). In other words, labelling is the addition of the radionuclide onto its vector.

And what is a vector? In radiopharmaceutical chemistry it is the stable part of the molecule of a radiopharmaceutical agent, onto which radionuclide is bound and then carried: vector gets “labelled” with a radionuclide and carries the radionuclide through

the body. Vector is responsible for the physiological/pharmacological behaviour of the whole radiopharmaceutical agent, while the radionuclide attached to the vector is responsible for imaging or radiotherapeutic effect. An example is given in the Figure 84: in FDG most of the glucose molecule is the vector onto which  $^{18}\text{F}$ , the radionuclide, is attached.



**Figure 84: Glucose is a vector for  $^{18}\text{F}$ : glucose equips the molecule with the chemical and biological behaviour of a sugar, while  $^{18}\text{F}$  give it gamma-emitting property.**

The vector is a stable molecule usually prepared in advance and waits for the radionuclide to be produced and turned into a proper reactive form and then the final labelling reaction is usually when the vector and a radionuclide are reacted. Vector is often made somewhere else and could be commercially available.

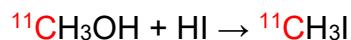
### Precursors

Precursor is a chemical compound that is to be used as a starting material for the synthesis of some desired new compound. So, once radionuclide is produced it is extracted out using an appropriate method and usually is in the form of a simple inorganic or simple organic compound. We call it the primary precursor. For example,  $^{11}\text{C}$  leaves the cyclotron in the chemical forms of carbon dioxide, carbon monoxide, methane, or ammonium cyanide. The  $^{13}\text{N}$  comes out as ammonia, while  $^{18}\text{F}$  can be extracted as  $\text{F}_2$  gas or fluoride ion. Precursors also can be various inorganic salts such as  $\text{Na}^{131}\text{I}$  or metallic ions in solution such as  $\text{TcO}_4^-$ .

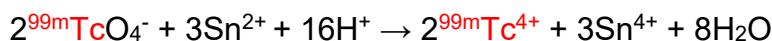
Primary precursors are often not chemically reactive enough for the labelling reactions: the immediate precursors used in labelling reactions should be chemically highly reactive and should very rapidly react with its substrates (vectors)! Hence, primary precursors are chemically processed into more reactive chemical forms, for example,  $^{11}\text{C}$  primary precursors are converted into methyl-iodide, formaldehyde, phosgene, methyl-lithium or methyl-triflate - all these are highly reactive chemicals:

- $^{11}\text{C}$  primary precursors:  $^{11}\text{CO}_2$ ,  $^{11}\text{CO}$ ,  $^{11}\text{CH}_4$ ,  $\text{NH}_4^{11}\text{CN}$
- $^{11}\text{C}$  secondary precursors:  $^{11}\text{CH}_3\text{I}$ ,  $^{11}\text{CH}_2\text{O}$ ,  $^{11}\text{COCl}_2$ ,  $^{11}\text{CH}_3\text{Li}$ ,  $^{11}\text{CH}_3\text{-SO}_3\text{CF}_3$

For example, typical conversion of  $^{11}\text{CO}_2$  into  $^{11}\text{CH}_3\text{I}$  could go as follows:



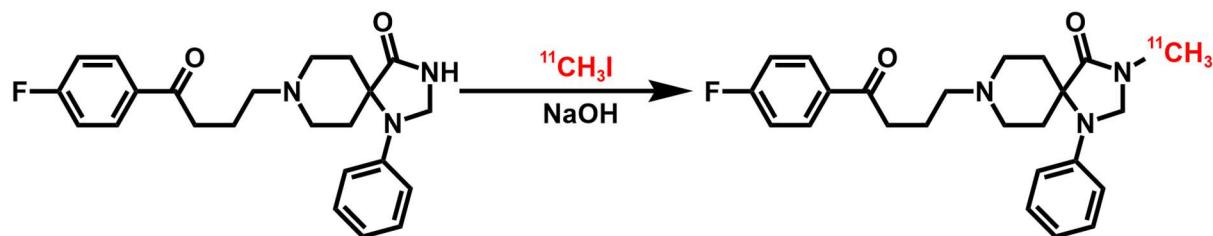
Another important radionuclide  $^{99m}\text{Tc}$ , is in the form of pertechnetate ion, which is quite inert, non-reactive. Therefore, to be complexed, it must be reduced for example into  $\text{Tc}^{4+}$  ion by using redox reaction with  $\text{Sn}^{2+}$  ion:



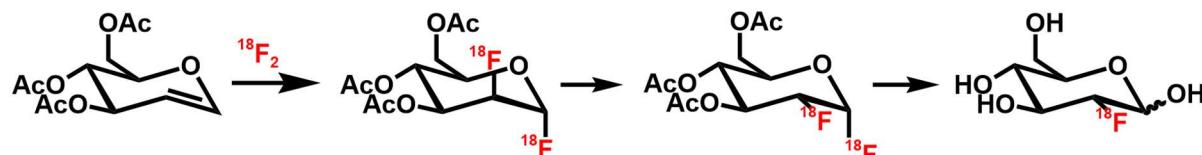
### Labelling reactions

Once the secondary, reactive precursor is ready the labelling reaction is undertaken. Labelling reaction is often the last reaction by which a vector molecule reacts with a secondary precursor and the radiopharmaceutical agent molecule is obtained. Most of these reactions are chemical, namely, metal complexations or organic synthetic reactions. Organic reactions are usually those that are very fast, rapid with proven high yields, reactions that make one product only. For example, these reactions can be simple ones (like substitutions, additions, condensations or coupling reactions) or can be some modern catalytic reactions with transition metals (Ni, Pd, Cu) or are sometimes so called “click reactions” like famous CuAAC cycloaddition reaction. Other methods are isotope exchange or sometimes biosynthetic methods. In general, quantities in these reactions are extremely small (nano or micro scale), so the special equipment and techniques are required.

Typical labelling reactions include simple chemical reactions of organic chemistry and is usually done as the last step when all precursors are ready, such as in this example of alkylation with  $^{11}\text{CH}_3\text{I}$ :

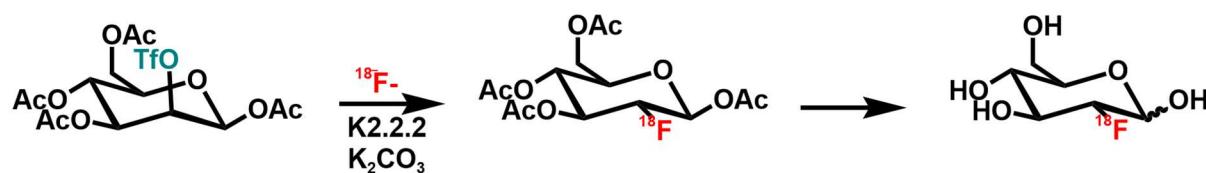


Yet, some labelling reactions cannot be accomplished in one single last step since covalent modification is needed such is the case when protecting groups are used. For example, the first labelling reaction used to make FDG was *via* electrophilic addition of  $^{18}\text{F}$  fluorine gas onto double bond moiety of a glucose derivative while all hydroxyl groups were protected with acetyl protecting group. After the labelling reaction protecting acetyl groups are removed by hydrolysis along with fluorine at the terminal hemiacetal carbon. This method is nowadays outdated and replaced with more efficient one that is using  $^{18}\text{F}^-$  ion instead of fluorine gas.



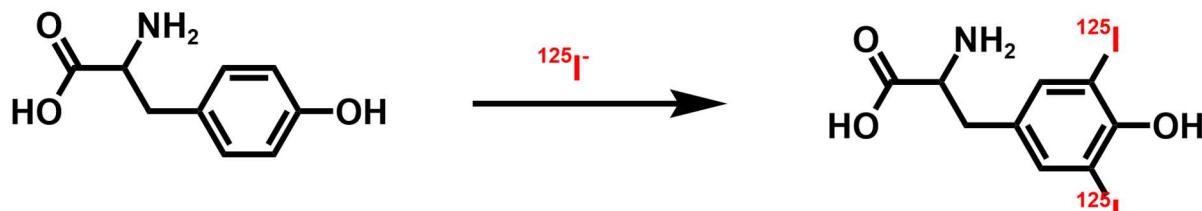
In general, labelling reactions should try to avoid post-labelling covalent modifications as much as possible. In other words, design of labelling reaction should be such to avoid additional reactions after the labelling reaction especially in the case of  $^{11}\text{C}$  whose half-life is much shorter than the one of  $^{18}\text{F}$ : every additional synthetic step will diminish radiochemical yield due to the fast decay of the radionuclide. Simply, there is no time for complicated synthetic pathways.

Another example of labelling reactions is the modern reaction to synthesize FDG by using nucleophilic substitution ( $\text{S}_{\text{N}}2$ ) in dry conditions. Fluorine-18 comes as  $^{18}\text{F}^-$  ion and takes place of triflate which is a reactive and good leaving group. The last step is deprotection of hydroxyl groups. This modern synthesis of FDG is one of the most used reactions in radiopharmaceutical chemistry and the central point in the production of PET imaging agents:



In fact, most of the labelling reactions by using organic reactions are nucleophilic substitutions and/or allylations.

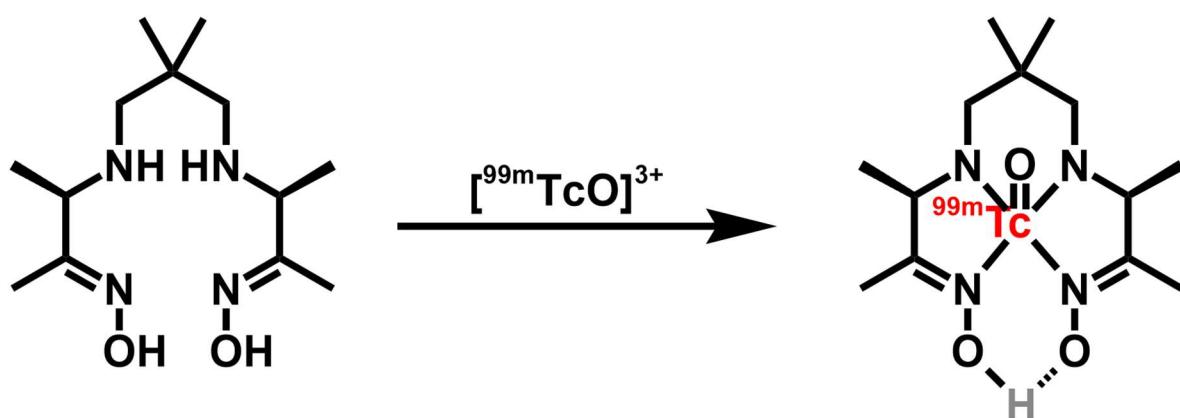
Another example of simple organic synthetic procedure is synthesis of  $^{125}\text{I}$ -labelled tyrosine, this is typical electrophilic substitution:



The next example is so called indirect labelling where firstly a coupling between a chelating ligand (like DTPA) and a monoclonal antibody is performed and only then radionuclide is added (in this case it is  $^{111}\text{In}$ ):



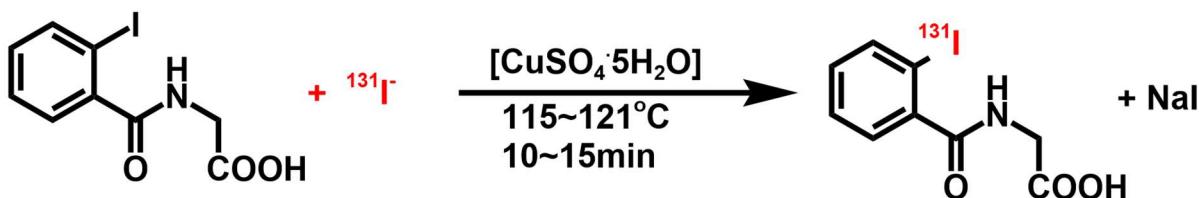
Metal complexation is another very important type of chemical labelling whereby a metal cation gets complexed with a ligand such as this chelating ligand. These chelate complexes are usually of very high stability. Here is an example, complexation of  $^{99\text{m}}\text{Tc}$  with chelating ligand to form  $^{99\text{m}}\text{Tc}$ -exametazime, agent for imaging of stroke (infarction of brain):



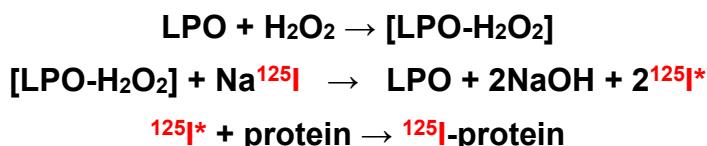
Another chemical labelling method is the isotope exchange. It was very popular before when the main imaging radionuclide was radioiodine but now it is less used. A substrate containing some stable isotope, call it X, reacts with a precursor containing a radioactive isotope X\* of the same element, and then the exchange happens:



This type of labelling was very popular for radioiodine agents, like in this case of labelling of ortho-iodomaluronic acid:



Biosynthetic methods are quite rare, but are getting more popular, for example the biochemical reactions facilitated and catalysed by some enzymes: in this case secondary precursor is formed by using an enzyme lactoperoxidase (LPO):



Biosynthetic methods can be very interesting because of their simplicity and use of live organisms (often genetically modified) such as bacteria, yeasts or cell cultures (Figure 85). This kind of use of live organism are called *in vivo* biosynthesis. For example, it is possible to make <sup>11</sup>C-labelled glucose by photosynthesis: plant cells are exposed to <sup>11</sup>CO<sub>2</sub> and strong light for few minutes and in this way <sup>11</sup>C-glucose can be obtained in a good yield in 30 minutes!



Figure 85: cultured plant cells can be used to make  $^{11}\text{C}$ -labelled glucose by photosynthesis

In general, labelling rate should be as high as possible. Also, labelling methods must be as fast as possible since there is no time to waste: time is running, and radionuclides are decaying.

Product of labelling should be chemically stable, labelling should be as selective as possible (without by-products), while the radiochemical purity should be acceptable.

Again, it is important to emphasise that labelling reactions are done with extremely tiny quantities of radiochemicals (trace quantities). Therefore, micro and ultra-micro techniques are used during the operation. For example, equipment and techniques similar to HPLC as well as microfluidics are standard. Also, high specific activity has to be obtained and introduction of unnecessary carriers should be avoided. Practically, these processes are fully automated, the whole instrumentation for the processing, labelling and synthesis is placed in a so-called “hot cell” and remotely controlled to ensure radiation protection. Before the actual labelling, the process of labelling is “validated”: this means it has to be tested many times with both non-radioactive (mock-up) and radioactive versions to ensure it really works well and always gives desired product in expected yields: this validation also has to be documented.

### Hot cells and automatic synthesis

Processing of medicinal radionuclides in a modern radiochemical facility is wholly performed in so-called “hot cells” (Figures 86-88).

What are the hot cells? Those are special chambers similar to glove-boxes, but with a very heavy lead shielding to ensure radiation protection and where all equipment for production of precursors, labelling and purifications is placed: whole process is automated, pre-programmed and remotely controlled by using a software.



**Figure 86:** An example of a “hot cell”, an isolated shielded cabinet where radiochemical and labelling reactions are done, and radiopharmaceuticals are automatically made out of their precursors.



Figure 87: A radiochemist working in an open cell, while automated equipment can be seen at right side.

In practice, “microchemical” apparatuses and systems for all precursor synthesis and labelling is fully automated and remotely controlled! Whole processing equipment is placed into hot cell and whole processing gets done automatically by programming it and controlling it *via* a computer (Figure 88).



Figure 88: processing equipment inside the hot cell and computer software controlling it remotely.

In the beginning radiochemists and other professionals were setting up their own systems and apparatuses (Figure 89) for the remotely controlled automatic micro-synthesis and purification of radiopharmaceuticals.

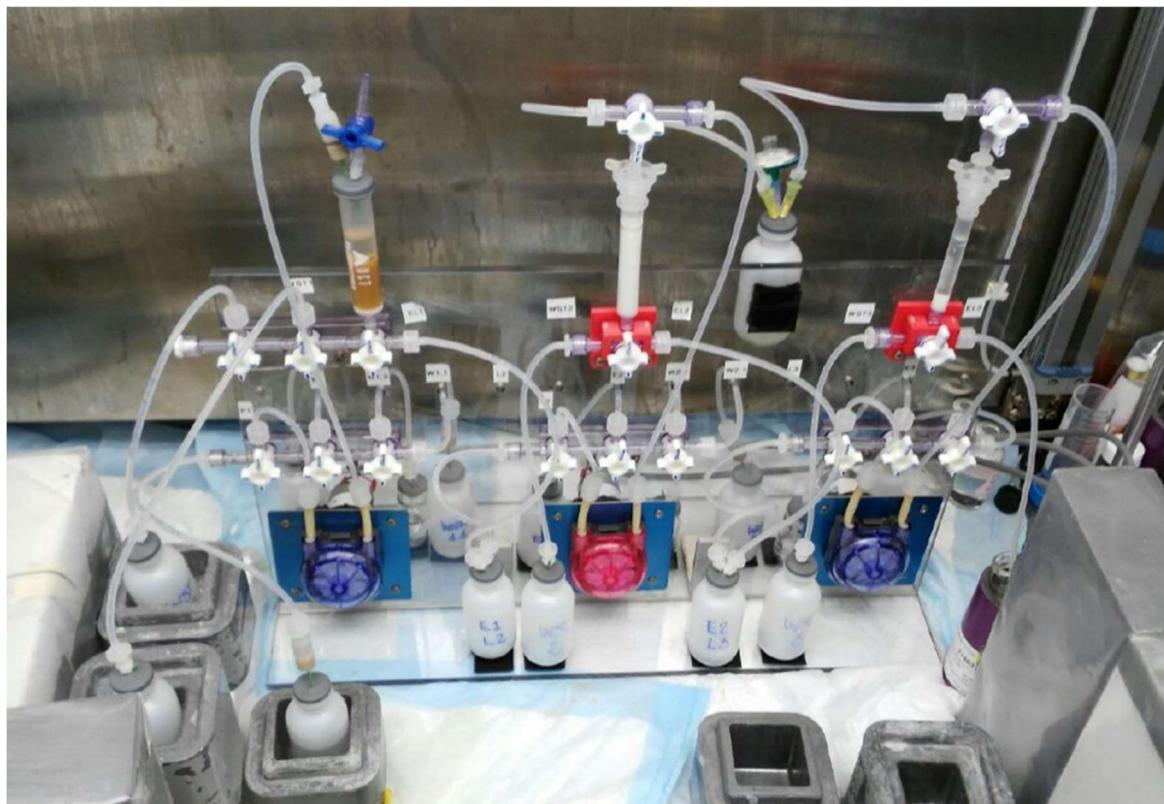


Figure 89: System for automatic synthesis and purification od radiopharmaceuticals (custom made)

Today many sophisticated, robotized and very powerful automatic synthesis systems are commercially available (Figures 90-91), especially for the typical routine day-to-day radiopharmaceutical preparations (such as is FDG).



Figure 90: Various commercially available instrumentation for the fast automatic micro-synthesis of radiopharmaceutical agents.



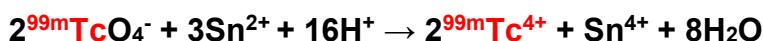
**Figure 91:** Various modern commercially available instrumentation for the fast automatic micro-synthesis of radiopharmaceutical agents.

These above-shown instruments are similar in their technological solutions to modern HPLC and other analytical instrumentation with heavy use of microfluidics technology, however they are used for syntheses, not analyses.

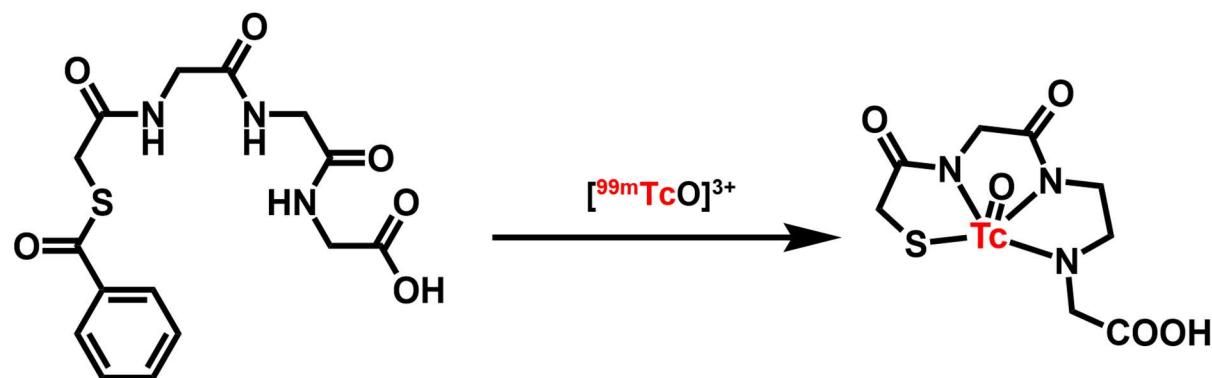
### Preparation of radiopharmaceuticals produced by radionuclide generators

As for  $^{99m}\text{Tc}$  today there are commercially available simple kits for the quick, rapid preparation of  $^{99m}\text{Tc}$  radiopharmaceuticals. A similar strategy is now adopted for another radionuclide obtained via radionuclide generator such as  $^{68}\text{Ga}$ , although use of  $^{68}\text{Ga}$  and other such radionuclides is far less frequent the use of  $^{99m}\text{Tc}$  radiopharmaceuticals that present a staple of nuclear medicine in most of the world.

These kits (Figure 92) are in a form of a vial and contain all the reagents needed for immediate preparation of desired radiopharmaceutical agent. They contain a reducing agent, a substance that quickly reduces  $^{99m}\text{TcO}_4^-$  ion into lower oxidation states, like  $^{99m}\text{Tc(V)}$ ,  $^{99m}\text{Tc(IV)}$  or even  $^{99m}\text{Tc(I)}$ . Usually it is tin-(II)-chloride.



Kits also contain chelators or other simple ligands that form a complex with Tc ion (as illustrated in the case of  $^{99m}\text{Tc}$ -mertiatide) and the desired radiopharmaceutical agent gets instantly formed:



Additionally, these commercial kits contain some other chemicals to ensure stability, proper pH and radiopharmaceutical quality. For every different radiopharmaceutical agent there is a different kind of kit containing a proper mix of reagents and everything a radiochemist has to do is to elute some fresh  $^{99m}\text{TcO}_4^-$  from a generator, filter it over special microporous filter into one of these kits, mix it, filter it again and that all, agent is ready for application. Usage of these kits is very simple, easy and can be routinely performed by any well-trained technician.



Figure 92: Example of a commercial kit for quick preparation of  $^{99m}\text{Tc}$ -mertiatide

## Radiochemical purity and purification methods

Once a radiopharmaceutical substance is prepared by the labelling process it needs to be either isolated or purified. In some cases, radiopharmaceutical needs an extraction operation in order to be extracted out from the reaction mixture. A synthetic labelling procedure often yields a product containing various impurities: it may contain unwanted by-products, precursors or unlabelled vectors. All these impurities need to be removed. Therefore, highly efficient, and very quick methods of isolation and purification need to be undertaken in order to obtain the desired radiopharmaceutical substance of proper radiochemical and chemical purity, reaching the required pharmaceutical standards of purity. However, these methods need to be appropriate for the micro/nano quantities.

Radiochemical purity (RCP) is defined as the proportion of the total radioactivity in the sample which is present as the desired radiolabelled species. In other words, radiochemical purity is telling us what is the percentage of activity that is coming from our desired radiopharmaceuticals substance. Radiochemical impurities are all other, different substances in the sample containing the same radionuclide as does the desired radiopharmaceutical substance. For example:

the imaging agent  $^{99m}\text{Tc}$ -sestamibi (Figure 93) may contain, apart from the substance itself, additional various impurities that also contain  $^{99m}\text{Tc}$ , such as free unreacted  $^{99m}\text{TcO}_4^-$ , particles of solid  $^{99m}\text{TcO}_2$  and some other  $^{99m}\text{Tc}$ -water soluble impurities. However, clinical requirement for  $^{99m}\text{Tc}$ -sestamibi radiochemical purity is

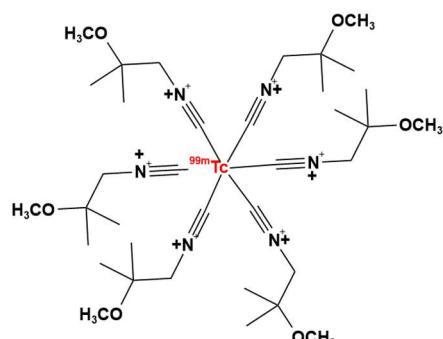


Figure 93:  $^{99m}\text{Tc}$ -sestamibi

calculated as presented and should be above 90%. This equation explains how radiochemical purity is calculated:

$$\text{R.C.P.} = \frac{A_{^{99m}\text{Tc}-\text{sestamibi}}}{A_{\text{total}(\text{}}^{99m}\text{Tc)\text{}}} \times 100\% > 90\%$$

### Purification methods:

To remove impurities either chemical or radiochemical proper purification methods has to be used. There are many methods but the most versatile and the most important is the chromatography. It is simple, quick, and efficient, giving very pure radiopharmaceuticals and can be suitable for very tiny quantities. There are different types of chromatography such as adsorption chromatography, ion exchange chromatography, gel chromatography, but all of them can be used in the technique of high-performance liquid chromatography (HPLC). HPLC is automated and very suitable for tiny quantities of substances and is used both for the purification and verification/testing of radiochemical purity. Other forms of chromatographic methods are paper chromatography or thin layer chromatography, but these are used mostly to test/verify purity, such as radio-TLC. There are other methods such as electrophoresis. Extraction, co-precipitation and distillation methods are purification methods usually used not for the final product, but for the precursors.

In practice, purification is performed using HPLC systems that is fully automated and integrated with other components of the synthesis and labelling system inside the hot cells. In general, for the production and preparation of PET imaging agents ( $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$  and some others) everything is automated, from cyclotron to the vial.



Figure 94: Instrumentation for the purification of radiopharmaceuticals

## Pharmaceutical formulation, purity and quality

At the very end, the pure radiopharmaceutical agent needs to be pharmaceutically formulated under sterile conditions before the application. In most of the cases it needs to be diluted to the desired activity with isotonic saline solution (solution of sodium chloride) to ensure so-called physiological tonicity. Being isotonic means that it has the same osmotic concentration as has 0.91% of saline. This is the appropriate concentration for parenteral application. Also, other ingredients and additives need to be added, to regulate pH and viscosity or preservatives.

The radiopharmaceutical preparation is usually filtered to the point of sterility and being non-allergenic and non-pyrogenic. This means that preparation is filtered through such fine filters that any particle larger than protein cannot pass: dead or alive viruses, bacteria, microscopic dust or any other particles such as debris of microorganisms and various endotoxins need to be removed to ensure that injection of such preparation will not infect patient nor cause any immunological (allergic or anaphylactic) reaction. In other words, preparation needs to be biologically completely safe for the patient.

Finally, the formulated agent is dispensed into sterile medical vials of standard volume. Activity of each vial is calibrated by using dose calibrators, date and time is recorded and vials are labelled with all necessary information. And all these steps, from cyclotron to the medicinal vial must be well documented and the records save so that it can be tracked if necessary. A radiopharmaceutical preparation needs to fulfil all the standards of pharmaceutical purity and quality, mainly for the parenteral preparations. It needs to:

- have acceptable radiochemical and chemical purity,
- be chemically stable,
- be isotonic,
- be sterile (which means there should be no detectable bacteria and viruses),
- be non-pyrogenic (should not cause any fever reaction)
- non-allergenic, which means it should not cause any allergic reaction in patients.



**Figure 95: Vial for application**

## Chapter VI - Equipment and instrumentation for radiopharmaceutical chemistry

Each step of the production process requires special sets of facilities, instruments, devices. The size of these devices ranges from huge reactors and cyclotrons to small instruments that can be worn on you coat. Radionuclide production requires large reactors and cyclotrons or small generators. Radiochemical synthesis and purification require large hot cells and very sophisticated automatic synthesis modules, preparative HPLCs and instruments for the formulation of ready-to-use radiopharmaceutical agents. Quality analysis and control (QC) is the final step before radiopharmaceutical agents are sent to be applied in patients, and it has to ensure and verify that they are of required quality. Finally, at the very end there are various instruments used to for nuclear imaging such as SPECT and PET scanners or their combination with classic imaging techniques such as CT and MRI. Yet, there is one group of equipment that is present in all these steps in all facilities and that is equipment for radiation protection.

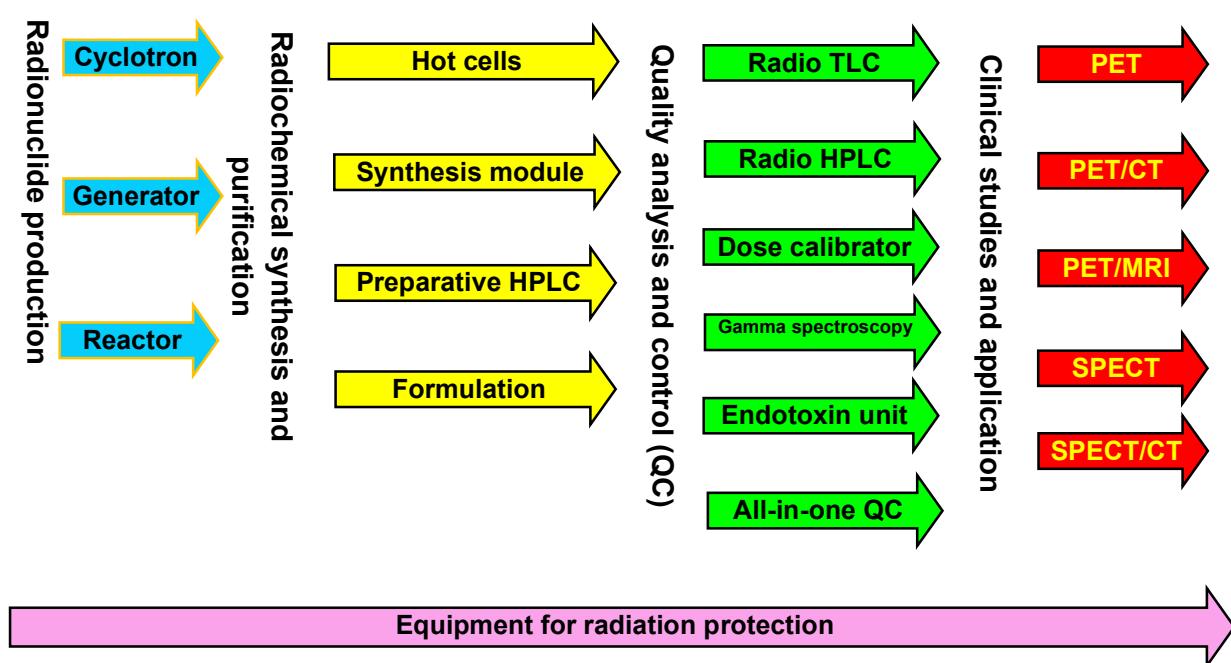


Figure 96: Essential instrumentation for radiopharmaceutical chemistry throughout production process

### Production of medical radionuclides

Production of medical radionuclides is achieved using some of these three types of instruments, ranging from huge reactor via smaller (but still large) cyclotrons to small radionuclide generators. These large instruments and facilities have been discussed in detail in the previous chapter and here will be mentioned just shortly and their main features re-iterated.

Reactors are large and important facilities; their main current importance comes from their ability to produce  $^{99}\text{Mo}$ , parent of  $^{99\text{m}}\text{Tc}$  which comprises 70-80% of all nuclear

medical procedures. However, possibility to make  $^{99m}\text{Tc}$  directly from cyclotrons is growing, hence importance of reactors for the production of medical radionuclides may decrease in future.

Cyclotrons are, on the other hand becoming more and more important facilities for the production of medical radionuclides. They are much smaller and cheaper than reactors while being the key instruments for the production of positron-emitting radionuclides for PET. In fact, each larger city (size of 1 million to 500 000) may afford to have at least one radiopharmaceutical facility with a middle size cyclotron (10-30 MeV) able to make most of medical radionuclides.

Cyclotrons will be getting more important and eventually, once being able to easily make beta/alpha emitting medical radionuclides could make reactors completely out of date.

In each radiopharmaceutical facility radiation protection is of paramount importance. When cyclotrons are working and are making new radionuclides *via* nuclear reactions huge intensity of ionising radiation ( $\gamma$  and X rays) is emitted out: any person standing next to a working cyclotron could quickly receive a fatal dose of radiation.

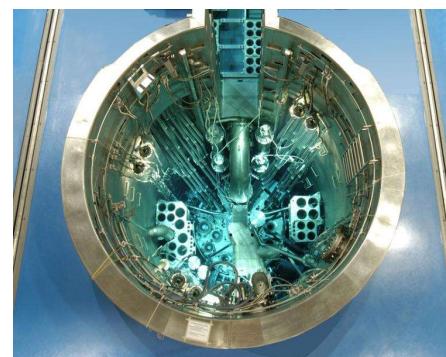


Figure 97: Reactor and cyclotron

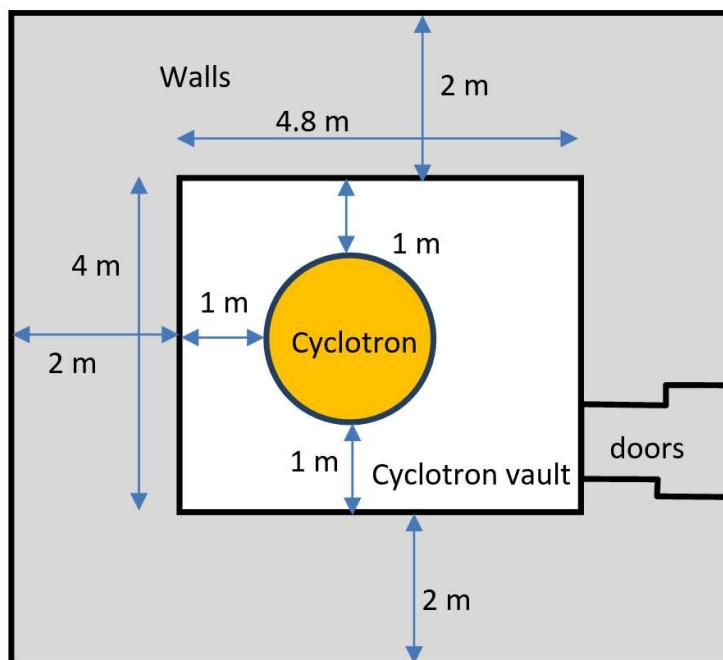


Figure 98: Layout of a typical cyclotron vault, where cyclotron is being placed inside a separate room with very thick concrete walls.



Therefore, in order to make them fully safe medical cyclotrons are placed into so-called “cyclotron vault” or “bunker”, separated from the rest of the facility by 2 m thick walls (made of special concrete with added barite gravel, barite is a mineral containing barium carbonate), while doors are closed with a concrete plug. Persons can enter only when cyclotron is “off”.

Generators are, as shown in the previous chapter, in fact an extended arm of the reactors and cyclotrons where these large machines are not present. They are very compact “boxes”, small and handy devices that can be used anywhere, including countryside hospitals. Their simplicity makes their used still the most prevalent, 80% of all nuclear imaging procedures start with milking out some  $^{99m}\text{Tc}$  from these generators.

## Synthesis of radiopharmaceuticals

In the previous chapter the synthesis of radiopharmaceutical active substances from simple radionuclide precursors was discussed, including the central reaction of labelling by which radionuclide gets attached to its pharmacological vehicle (also known as “vector”). As for PET radionuclides the light elements ( $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$ ) are automatically transported in a gas from *via* pipes by using an inert carrier gas from a cyclotron into hot cells where automatic micro-synthesis of reactive precursors and then the cascade of labelling micro-scale synthetic reactions are performed as well as purification and formulation. It is all done while being remotely controlled and directed *via* computer! Hot cells are heavily lead-shielded chambers, the central places of radiopharmaceutical chemistry where all radiochemistry happens. Also, internal area of hot cells is made very clean by injecting purified air to ensure the highest pharmaceutical purity and quality of produced radiopharmaceuticals.

The cascade of labelling synthetic reaction, by which radiopharmaceutical substances are made are sophisticated small systems that resemble tiny, miniature, compact chemical plants (factories). Sophisticated technologies, like microfluidics, robotics and sensors are used. Automated synthesis devices, including the purification modules are all placed in the hot cells. The process is validated and tried many times with non-radioactive version to ensure it always works and gives product of specified quality. The images of the hot cells and synthesis equipment are all shown the previous chapter, Figures 86 to 91.

## Quality control instruments for radiopharmaceuticals

Once the radiopharmaceutical agent is prepared it needs to be analysed and the quality assessed. There are several instruments used for analysis and quality control. The most common are Radio-Thin-Layer chromatography (Rad-TLC), Radio-HPLC, gamma spectrometer, dose calibrators and the automated QC instrument.

Thin-layer chromatography (or TLC) is a common, simple, fast and reliable method in any organic chemistry or medicinal chemistry laboratory to identify a compound

and test its purity. Detection is usually based on UV active groups or some other chemical method. Radio-Thin-Layer chromatography is the same as TLC, but detection is done by automatic screening with a radiation detector probe (a Geiger-Muller counter or scintillator). It can assess identity and purity (radionuclide and radiochemical) of any radiopharmaceutical agent. Nowadays there are radio-TLC readers/scanners that are able to visualize radioactive spots (Figure 99).

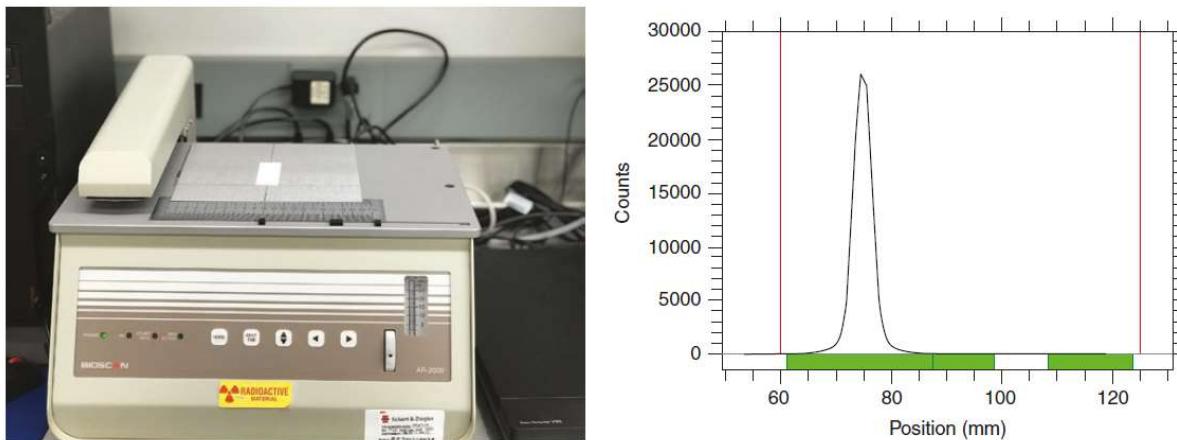


Figure 99: Radio-TLC scanner/reader (left) and its radiogram (right).

Much more useful, sensitive and accurate method is “Radio-HPLC” or Radio High Performance Liquid Chromatography (Figure 100). HPLC is an automated form of column chromatography, where a stationary phase in the form of very fine powder is very densely packed in a small metallic column and the eluent is pushed through the column under very high pressure (several MPa). Using this method, a small (micro) quantity of analysed samples are quickly and very efficiently separated. This HPLC is fully automatized, and it is the main analytical method in pharmaceutical industry.

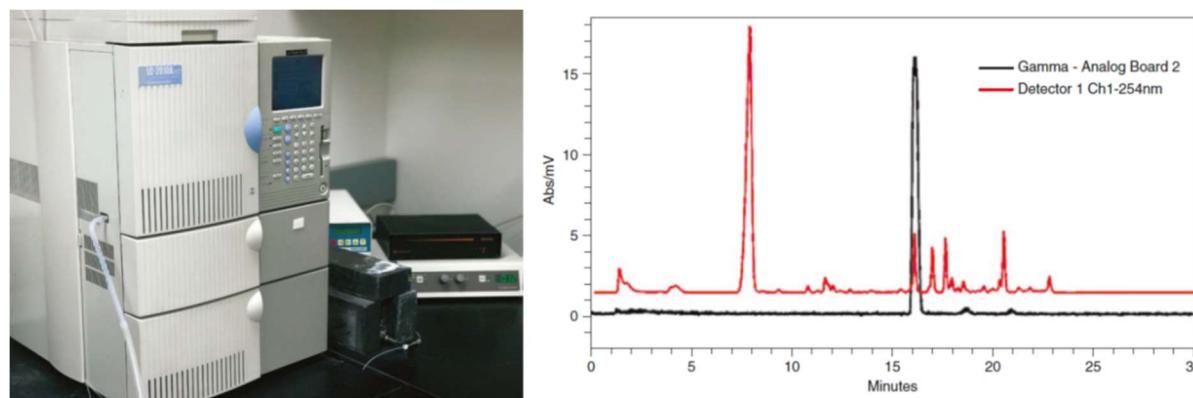


Figure 100: Radio-HPLC (left) and its chromatogram (right) showing radioactive (black) and UV channel (red).

Because HPLC is a micro-scale method usually it is used only for the analysis, but in radiopharmaceutical chemistry where the whole product is at the micro scale HPLC is very good for both preparative purification and for the analysis. Radio-HPLC usually has two detectors, a UV, and a radiation counter (mostly a scintillator) and

can “see” both the radioactive substances and impurities as well as non-radioactive UV active impurities.

Gamma spectroscopy (Figure 101) is a standard and very sensitive radioanalytical method, heavily used in radiochemistry. In radiopharmaceutical chemistry it is used to test and verify radionuclidic purity: it counts radioactivity of the sample while separating each emission based on its energy: it can distinguish between different radionuclides hence find out what is the radionuclidic purity of a sample.



Figure 101: Gamma spectrometer

Dose calibrators (Figure 102) are used for the quick measurement of the activity of a vial containing some radiopharmaceutical agent. This is in fact a calibrated counter with ionisation chamber – an operator needs to pick the radionuclide that will be measured.



**Figure 102: Dose calibrator**

Today there are commercially available sophisticated instruments that can measure and test many various critical parameters from a same small sample. These are one-in-all devices that can assess parameters such as colour, clarity, pH, residual compounds, bacterial endotoxins, residual solvents, radionuclide identity, radioactivity concentration, radiochemical purity. In general, one such instrument can cover most of the parameters needed to characterise a produced radiopharmaceutical agent and validate its quality.

### PET and SPECT scanners

After a radiopharmaceutical agent is produced it is given (usually in a parenteral form, such as injection) to a patient or a healthy volunteer. Then the treated person body is scanned by using either SPECT or PET scanner to produce images. Strictly speaking scanners are not radiochemical equipment and radiochemists are not involved in application of radiopharmaceuticals and interpretation of the images created by these instruments. This is completely in the realm of medical doctors (specialists in nuclear medicine). Yet, it is worthy for radiochemist to know and understand method of their work and how they create images.

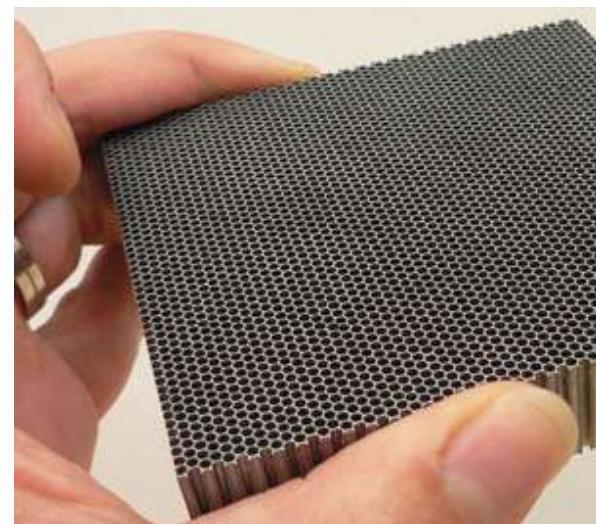
SPECT scanner (Figure 103) or “Single-photon emission computed tomography” scanner is also known as “gamma camera”. It is used to visualise gamma emission from the gamma emitting radiopharmaceuticals (containing  $^{99m}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{123}\text{I}$ ,  $^{67}\text{Ga}$ ). SPECT scanners are still the main instruments in the nuclear medicine, since 70-80% of all nuclear imaging is done by using  $^{99m}\text{Tc}$  and scanned by SPECT.



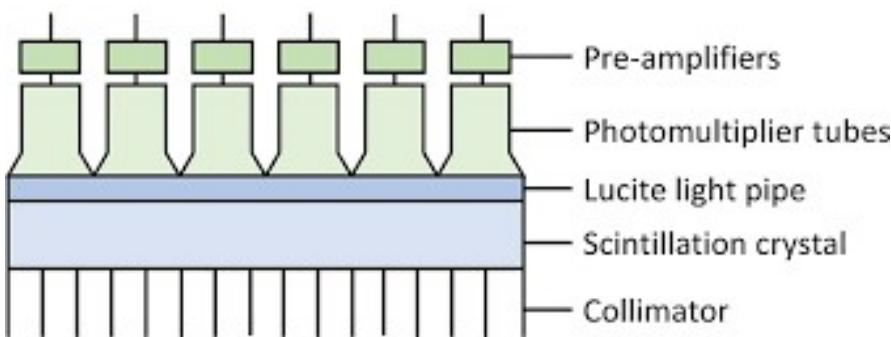
**Figure 103:** SPECT/CT instrument contains a SPECT scanner (also known as “gamma-camera”, just above the patient) and a CT scanner.

#### How SPECT scanner or gamma camera works?

In fact, it is a special array of scintillator detectors. There is an array of photo multiplier tubes and beneath is a large scintillator crystal or an array of crystals (Figure 105). The key part of this camera is the collimator (Figure 104). It is a large array of special tubes, in fact it looks like a very fine hexagonal prismatic honeycomb, but quite thick. It is usually made of lead, and it is used to collimated gamma photons: only photons immediately beneath each pore can pass. In this way, using collimator a pixelated image is created. Today, the photo multiplicator tube array is replaced with an array of fine micro elements made of semiconductors such as those in digital cameras. This semiconductor camera gives much better resolution.

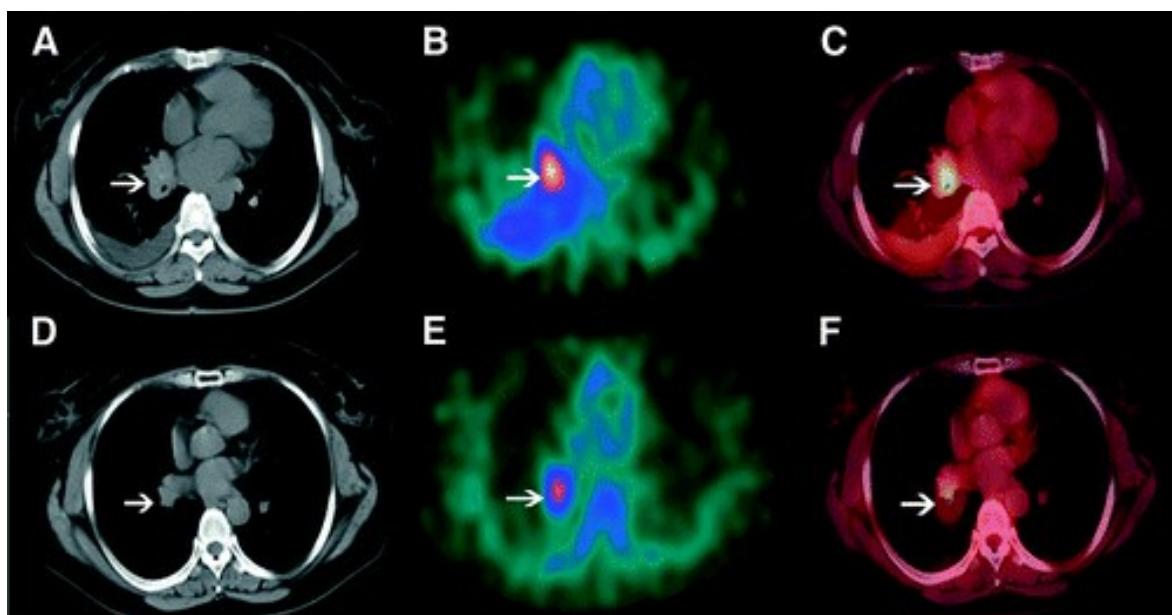


**Figure 104:** Collimator



**Figure 105:** Schematic diagram of gamma camera head (up), array of photo-multiplicator tubes (down)

How the image created by SPECT camera looks like? In the Figure 106B and 106E you can see how a SPECT image actually looks like.

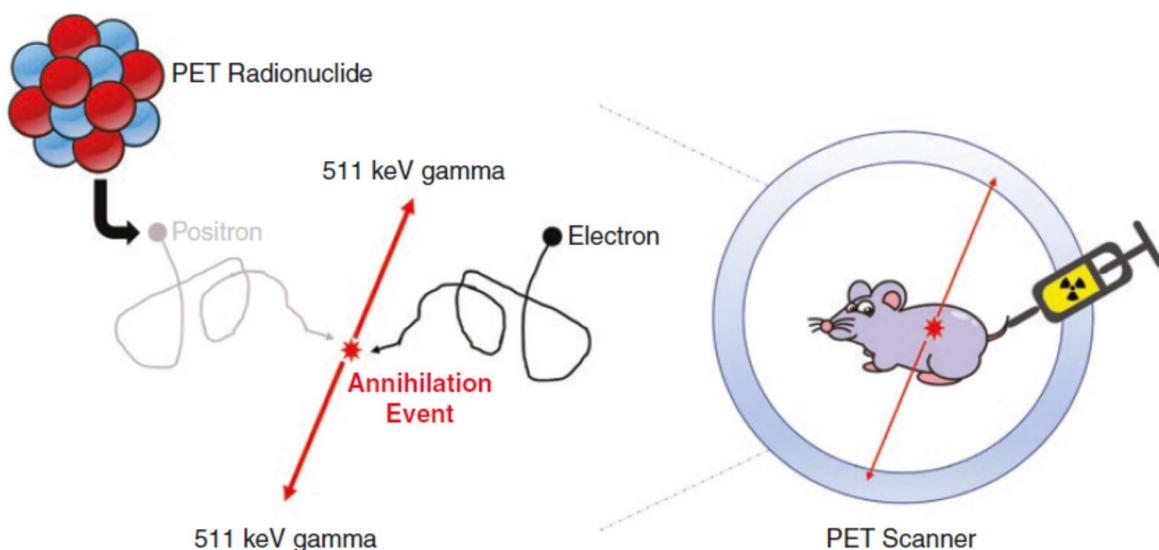


**Figure 106:** A and D are images created by CT, B and E are images created by SPECT and C and F are combined SPECT/CT images

SPECT doesn't really give clear images of high resolution and in some cases, it is not very clear from where in the body the signal is coming from, therefore it is usually combined with a computed tomography (CT) scan image to make anatomy clearer and then experts can identify location of the signal much better. Therefore, today we have combined SPECT/CT imaging instruments.

PET scanners are on the other hand bit more sophisticated instruments. "PET" is an acronym and stands for "Positron Emission Tomography". Tomography is a technique for displaying a representation of a cross section through a human body or other solid object usually using X-rays. However, in the case of PET imaging is achieved not with X-rays but with gamma rays.

PET imaging is based on detection of annihilation gamma ray coincidence. When the emitted positron encounters an electron, both particles are immediately annihilated and turned into two antiparallel photons of exactly 511 keV that are moving in opposite directions (Figure 107).

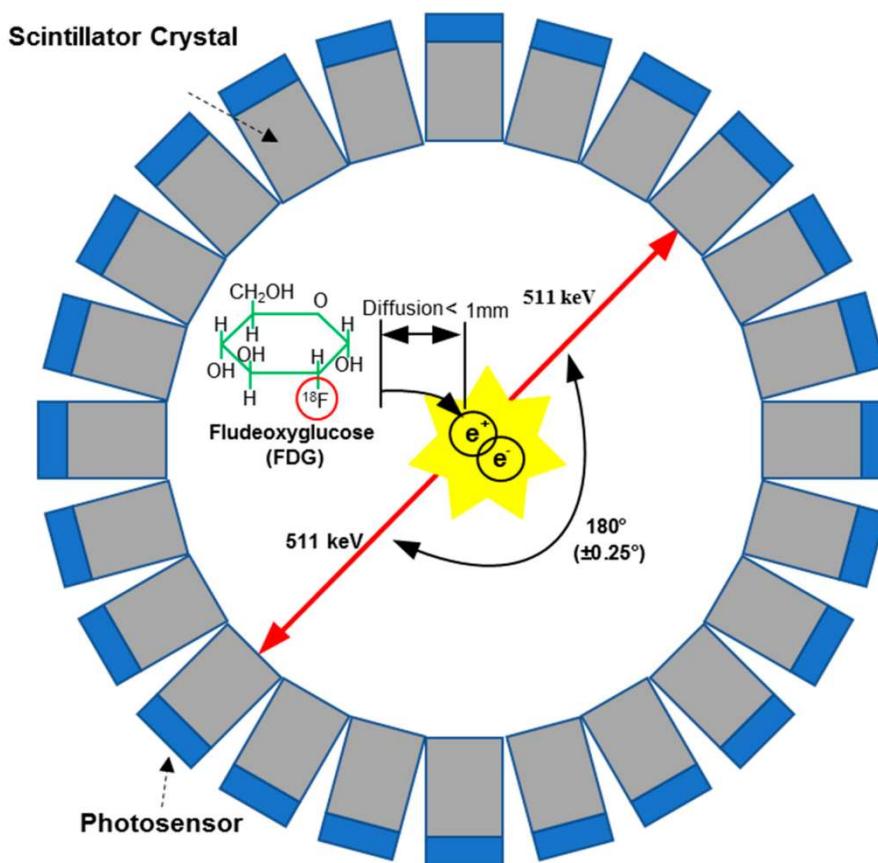


**Figure 107:** Detection of antiparallel annihilation photons created from the collision of a positron emitted from radionuclide and an electron from shrouding matter is the bases of positron emission tomography.

PET scanner has a ring of very sensitive scintillator detectors (Figure 108): these are arranged in a circle and are fully closing a ring. When two photons are emitted, each in opposite directions, PET scanner detectors detect these two coincidental photons, hence recording the exact location inside the ring from where the emission is sent. This is enough of information for the electronics of the scanner to construct a 3D image map in space of all locations from where photons are emitted.

A person is scanned by moving its body through the circular array of scintillator detectors (Figure 109). Resolution and sensitivity of PET scanners have improved considerably: scintillator detectors and the electronics is becoming much more sophisticated, location detection more accurate and precise. In the Figure 109 you

can see how quality and resolution of PET image have been hugely improved starting from the first PET scanners until the most modern ones.



**Figure 108:** Circular array of scintillation detectors can very accurately locate exact location of the annihilation event, the please where most probably is the tracer molecule. In this case 3D image map of the area where tracer molecules are is created



**Figure 109:** PET Scan instrument (left) and improvement of PET scanner resolution from the first scanners until the modern ones (right)

In the clinical practice today a sole PET scan is rarely used because just like in the case of SPECT imaging visualisation of anatomy of other organs and body structures is missing and as the consequence it is often not very clear where exactly tracer is located. Therefore, the context of image relating to the anatomy of other body structures is missing. To better determine the location of emission and visualise internal organs PET imaging is usually combined with the classic imaging methods such as CT (computed x-ray tomography) or MRI (magnetic resonance imaging) (Figures 110 and 111).



Figure 110: Combined PET/MRI scanner instrument

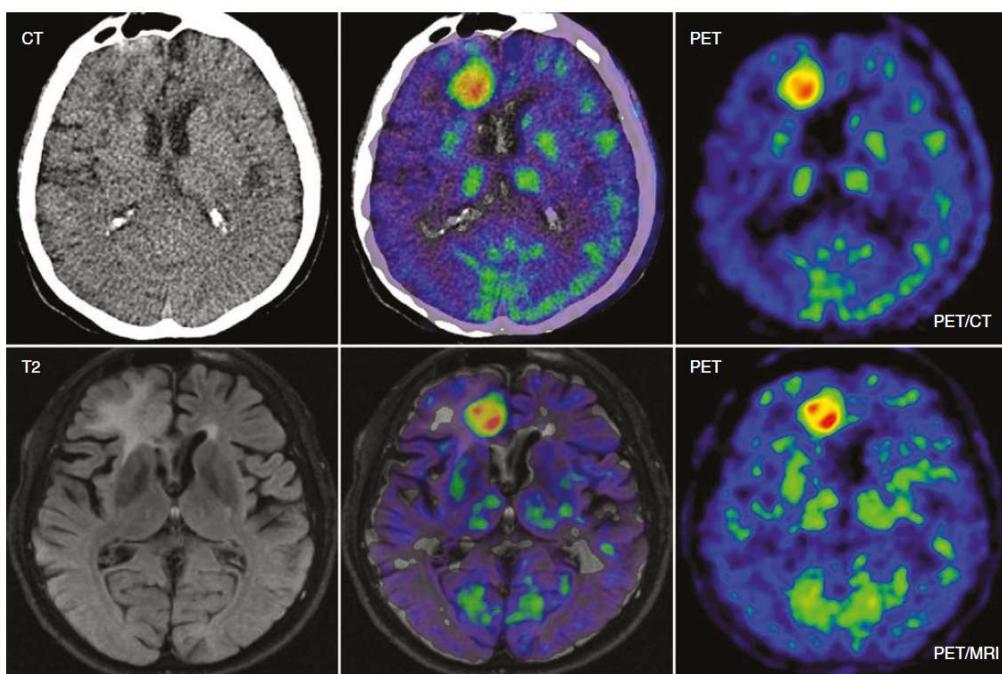


Figure 111: CT or MRI combined with PET gives very clear image of the tumour in brain and medical doctor can easily locate it in the context of general brain anatomy.



In the Figure 111 combinations of PET images that are able to locate the anatomical position of a brain tumour very well with classic CT image of the same brain and also an MRI image that looks even better. When superimposed these images are then telling the physician much more than each individual image can.

## Instrumentation used for radiation protection

In all the rooms of a radiopharmaceutical facility or any radiochemical facility, or nuclear medicine areas where radiopharmaceuticals are applied there are always instruments and devices used for radiation protection. The most basic ones are those worn personally by each radiation worker: so-called personal dosimeters. They measure personal dose of ionizing radiation received by each worker in each month: whole body dose, skin dose and quite often a finger dose. They are based on the phenomenon of thermoluminescence and are worn on lab coats or fingers. Apart from these, simple, thermoluminescence dosimeters (TLD, Figure 112 left) workers can wear larger personal electronic dosimeters (PED, Figure 112, right) that are miniature dose monitors, measuring dose rate in real time and give a sum of dose received in any time.



**Figure 112:** Thermoluminescence dosimeters (TLD) for fingers and for the whole body (left) and personal electronic dosimeter (PED) for whole body dose (right)

Ionisation chambers (Figure 113) are classic dose rate monitors, hand-held instruments used to occasionally measure ambient dose rate of ionizing radiation in laboratories and in proximity to equipment such as cyclotrons, hot cells or synthesis modules. Having accurate knowledge of dose rates allows radiation workers to make decisions about when to enter such spaces and/or service the equipment in a safe manner.

Contamination monitors (Figure 114) are usually simple hand-held counters that have a scintillator probe (rarely Geiger Mueller) or are proportional counters. They have large windows and are used for the contamination control: to check if any worker is contaminated, if laboratory workplace or tools are contaminated, or if solid waste is contaminated. Another type of instruments is so called “whole body

monitors”, large instruments based on proportional counters and are used to screen workers and visitors when exiting facilities (Figure 115, left): rule is that a worker is not allowed to leave the controlled area of a facility if do not check itself and ensure that there is no contamination on body, clothes, or shoes.



**Figure 113:** Ionisation chamber monitors for monitoring immediate radiation dose in close vicinity, handheld (left), stationary (right)



**Figure 114:** Modern hand-held contamination monitoring instruments.

Finally, the area monitoring system (Figure 115 right) is the most important radiation protection system in the radiopharmaceutical facility. It is a system of small static dose rate monitors that are located at the strategically important places and corners. They are monitoring ambient gamma dose rate all the time (24/7) like security cameras. System is recording the measured dose rate and takes into account natural background dose and identifies possible incidents in the real time: alarm switches on if the dose rate crosses certain threshold. It is used to detect weakness

in the radiation protection system and to ensure compliance with the safety rules: it gives statistical analysis of where spikes of gamma radiation usually are appearing and when.



**Figure 115:** Large whole-body contamination monitor (left), system for area monitoring (right)

### Organisation of a radiopharmaceutical facility

In the Figure 116 a floor plan of a typical radiopharmaceutical facility is shown. It consists of the office area that is not controlled nor supervised since it is highly unlikely that contamination will find its way to those areas. Actual work is done in the yellow area which is a radiation-controlled area and the green area that is the “clean” area. The workers are entering facility *via* “lock-room” or “barrier room” where every worker needs to dress up itself in proper clothing and then cross the barrier into supervised area.

The cyclotron is placed in a separate room surrounded with a very thick concrete wall to prevent any leakage of ionising radiation. Instead of a simple door the entrance into the room is plugged with a very thick and heavy concrete plug to prevent any leakage of radiation. Next to the cyclotron room is the service room where targets are being prepared, power supply room where electricity is fed into the facility and the control room from where cyclotron is controlled. Produced radionuclides are sent into the radiochemistry room *via* underground pipes by using an inert gas. The radiochemistry room, labelled green, is considered both controlled area and the clean area where highest pharmaceutical level of cleanliness and

hygiene needs to be achieved using specially filtered air. Inside the rooms are hot cells – the place where all production process happens. The materials are brought in and out of the radiochemical room *via* two hatches. When radiopharmaceutical agent is made and placed into a vial it is immediately taken for quality control into the quality control room and then exported to the clinic *via* special exit for goods.

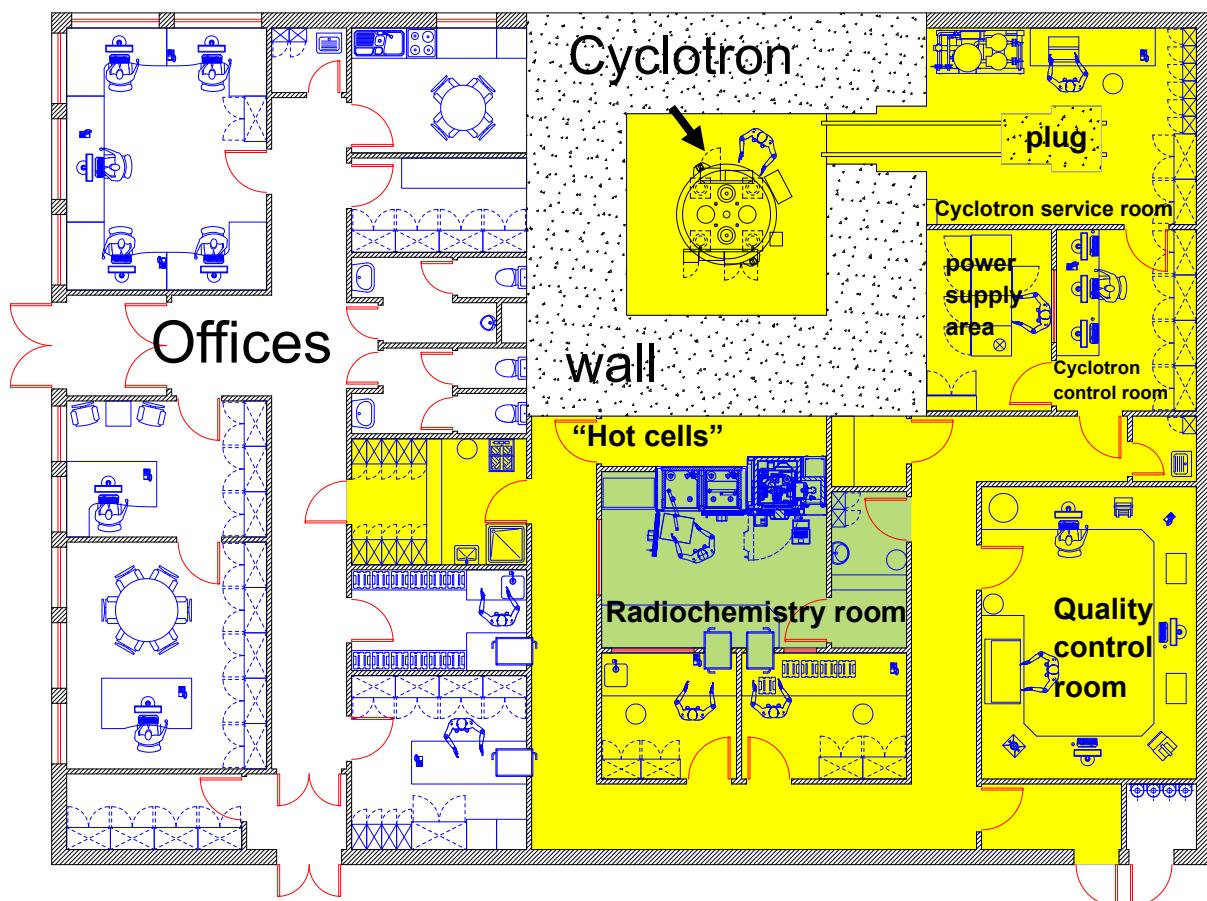


Figure 116: A floor plan of a typical radiopharmaceutical facility

These facilities are the standard in the area of PET radiopharmaceutical but can have additional rooms for preparation of other radiopharmaceuticals as well as additional laboratories for development of novel radiopharmaceuticals and experimental work. In many institutions nuclear medicine clinic with PET and SPECT scanners is usually located very next to such facility.

## Chapter VII - Radionuclides and their vectors

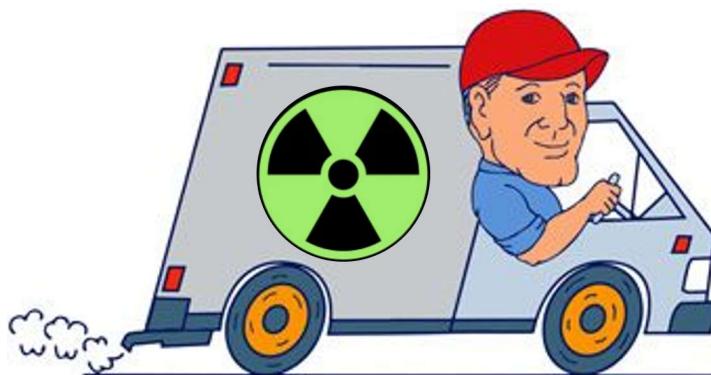
The main topic of this chapter is to explain and clarify what is a vector in radiopharmaceutical chemistry. It is a word from mathematics and physics, but in radiopharmaceutical chemistry, vector is something else: it is a molecule onto which a radionuclide is bound and is carried by: vector carries the radionuclide through the body and delivers it exactly where it should go! We can say that a typical molecule of a radiopharmaceutical agent consists of its radionuclide and its vector (Figure 117).



**Figure 117:** Radiopharmaceutical contains a radionuclide and a vector (yellow arrow) that is taking the radionuclide where it needs to arrive.

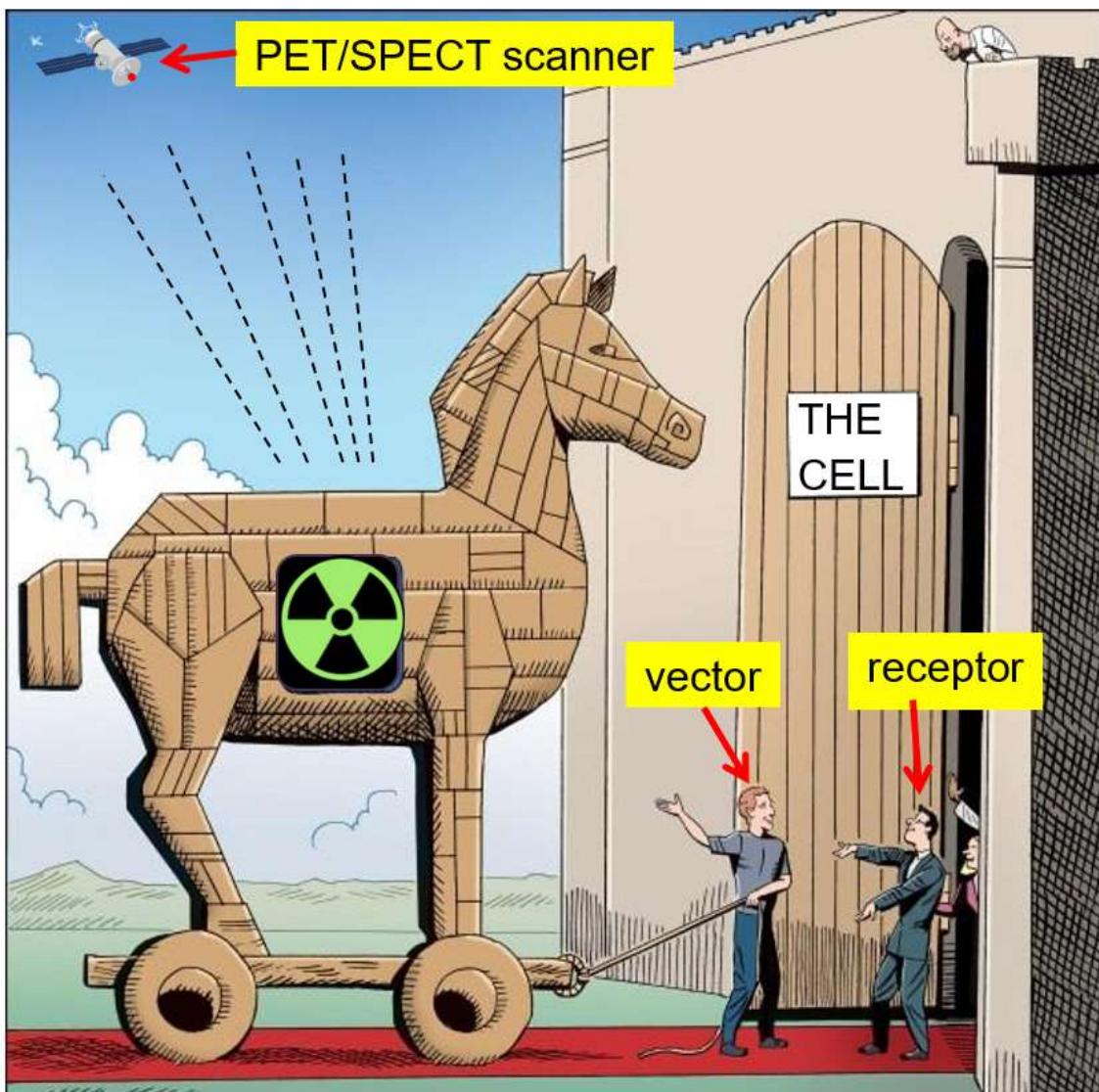
The vector is responsible for the physiological/pharmacological behaviour of the whole radiopharmaceutical molecule while radionuclide attached onto vector is the source of ionizing radiation responsible for imaging or radiotherapeutic effect.

A vector can be compared with a delivery service (Figure 118): vector is like a delivery person and his car: delivery person knows the address, where to deliver the goods and can even recognize the place of delivery (knows where should go and deliver it), while radionuclide is a cargo that has to be delivered (secretly).



**Figure 118:** Radiopharmaceutical as a delivery service with a radioactive cargo

In fact, we can use another funny allegory: in the case of radiopharmaceutical agent for imaging then radionuclide is like a secret cargo hidden in a Trojan horse (Figure 119). Vector is like a person who takes the horse to the fortress and recognises the receptor, person who recognises the vector and opens the gate is the receptor while the radioactive cargo is hidden inside the horse and the fortress (the cell) has no idea what is coming in. However, the radionuclide is emitting some invisible gamma ray signals that are then getting picked by a satellite on the sky (it is actually a PET or SPECT scanner) and the “satellite” locates the cell because the signals (rays emitted by radionuclide) are coming from that location.



**Figure 119: Trojan horse allegory**

Another allegory is with targeted radiotherapy: it can be compared with a guided cruising missile having a nuclear warhead (Figure 120). The missile is programmed to fly and seek the target; it has ability to recognize it. Once it finds it, rocket hits it and unleashes the deadly nuclear charge. Guided missile is like a vector and nuclear warhead is like the radionuclide carried by that vector.



**Figure 120: Guided missile allegory: in the case of targeted radiotherapy**



But what are those vectors, actually? In fact, sometimes vector is not needed because a plain radionuclide in its simple chemical form of salt can be good enough to find the target by itself. However, these radionuclides are limited to handful of cases such as radioiodine ( $^{123}\text{I}$ ,  $^{125}\text{I}$ , and  $^{131}\text{I}$ ) that specifically goes to the thyroid gland, radium ( $^{223}\text{Ra}$ ,  $^{225}\text{Ra}$ ) and strontium ( $^{89}\text{Sr}$ ) that specifically accumulate in bones, while pertechnetate ion ( $^{99\text{m}}\text{TcO}_4$ ) simply goes directly into kidneys only and gets filtered out into urine.

Most of other radiopharmaceuticals are in fact labelled small, drug-like molecules, peptides or monoclonal antibodies. Going along that direction, the vector become larger, but also molecular recognition is better, affinity is stronger, selectivity is better, vector is simply better. There is another type of vectors that are fairly new in medicine and those are nanoparticles.

What is the characteristic of a good vector? Firstly, it needs to be stable in tissues, should not hydrolyse once it is injected. Secondly, it should not possess any pharmacological, toxicological or any kind of harmful effects at the typical radiopharmaceutical doses. Next, it needs to be able to arrive to the target very fast and not to be changed while traveling to the target; in short it has to have good pharmacokinetic properties. And finally, the most important is that it needs to have excellent binding to the target. Namely, to have high affinity and high selectivity towards the target, and if possible, should specifically bind the target. In short, we say it needs to have good pharmacodynamic properties.

Yet, some radiopharmaceuticals do not bind any specific molecular target in the body, they do not bind any receptor but are still being accumulated in certain tissues and organs making imaging possible. This is the case in  $^{99\text{m}}\text{Tc}$  radiopharmaceuticals that are mostly complexes without ability to bind any specific receptors (key-in-the lock analogy). But they do have specific solubilities, lipophilicities  $\text{pKa}$ , shape and other properties that are directing particular pharmacokinetic behaviour and accumulation in certain organs or tissues upon proper application (oral or parenteral). This is the reason why we can still use them.

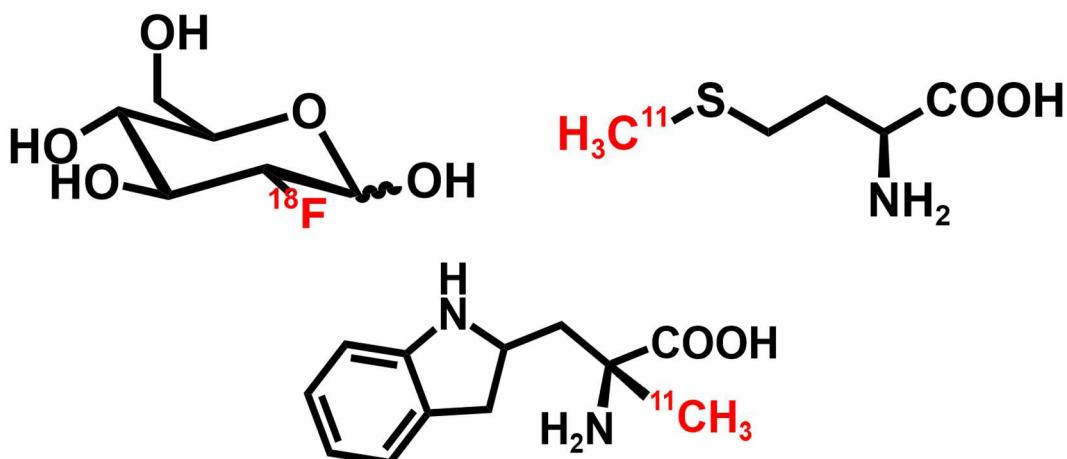
What kind of classes of compounds can be vectors? It can be:

- A small molecule (nutrient, hormone, xenobiotic such as drugs), usually has attached  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$  or radioiodine.
- A ligand that is complexing radiometal (such a ligands for  $^{99\text{m}}\text{Tc}$ )
- A peptide (such as oxytocin, octreotide, etc.)
- A protein (for example a monoclonal antibody)
- A nanoparticle

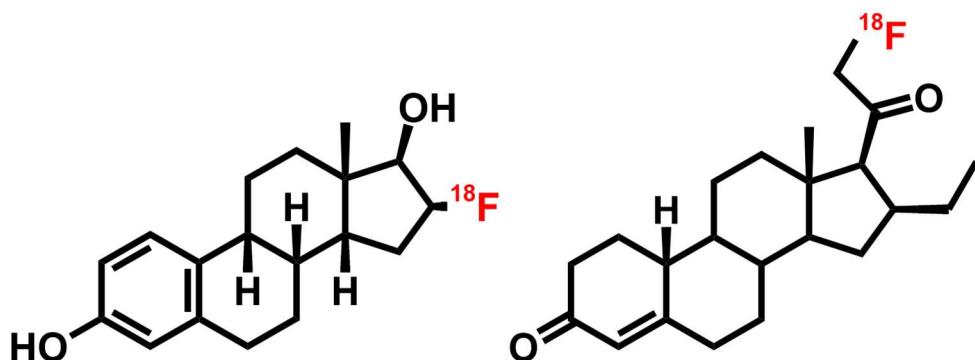
### **Small molecules**

Let us examine the first group, called small molecules that look like drugs or nutrients. We can categorise them based on their function.

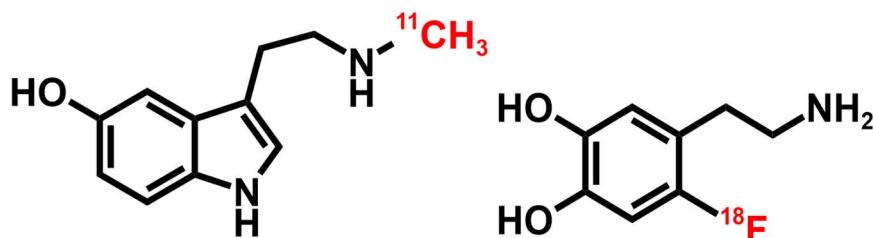
Firstly, there are nutrients and their analogues (such as like glucose, amino acids or vitamins). Although labelled with radionuclides they behave quite like the normal nutrients. A typical example is  $^{18}\text{FDG}$  a radiolabelled glucose (up left), or  $^{11}\text{C}$ -methionine (up right), radioactive amino acid, while  $\alpha\text{-}^{11}\text{CH}_3\text{-L-tryptophan}$  (down) is a radiolabelled analogue of an amino acid:



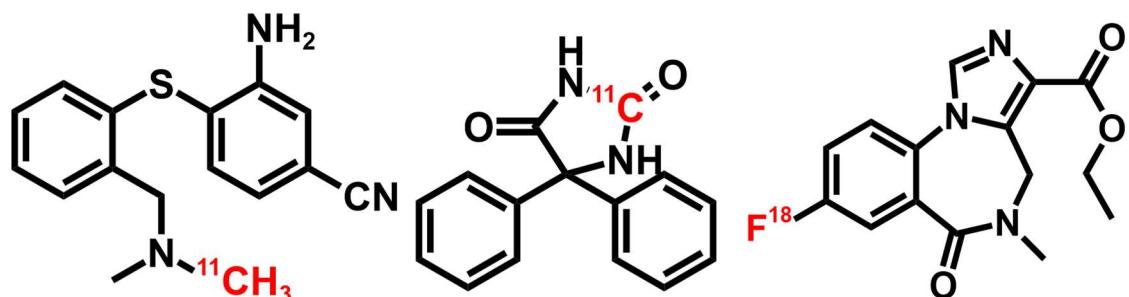
Then, the second group are small molecule hormones such as estrogen, thyroxin, testosterone, cortisol. Hormones are anyway active in very small quantities and have excellent binding affinity so once labelled they are very good vectors. Typical examples are  $^{18}\text{F}$ -fluorinated estrogen ( $^{18}\text{FES}$ , right) and progesterone ( $^{18}\text{FENP}$ , left):



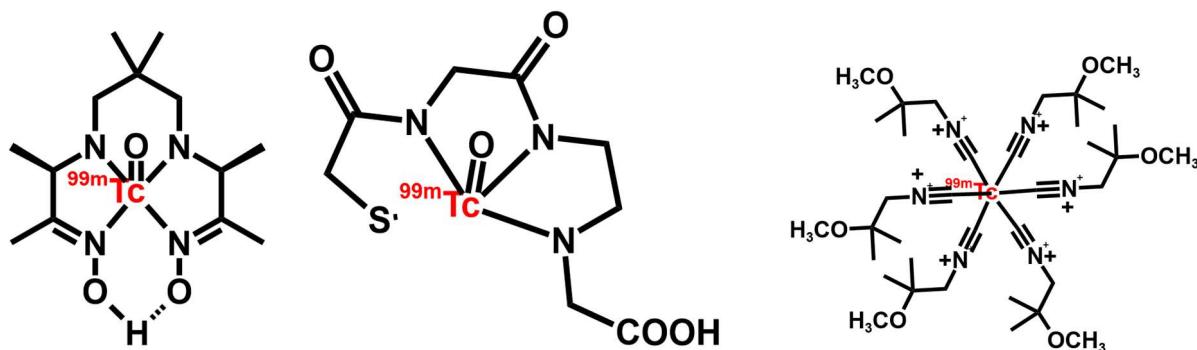
The third group are also natural compounds, neurotransmitters such as adrenaline, dopamine, serotonin, GABA. Labelled they are often useful for neurological imaging. For example, typical such radiolabelled neurotransmitters are  $^{11}\text{C}$ -labelled methylserotonin (left) and  $^{18}\text{F}$ -labelled dopamine (right):



One of the most important groups are xenobiotics that can be either drugs or other small molecules that look like drugs but are not officially recognised as drugs. They do act like drugs, bind some targets like receptors but since given in very tiny dose they do not have any physiological effects. Xenobiotic is a name for any substance that in normal occasion is not present in the body. Usually drugs, poisons and heavy metals are considered to be xenobiotics. However, the radiolabelled small xenobiotic molecules are usually radiolabelled drug, drug analogues of some other small molecules that failed to become drugs for various reasons (for example, their therapeutic window was too small, or they simply proved not useful in clinical scale) but can very strongly bind to certain receptors. Example is  $^{11}\text{C}$ -DASB (left), which is not a registered drug but strongly binds to serotonin-transporter protein in brain and is therefore a very important neuroimaging agent for various neurological and psychiatric diseases. There is also  $^{11}\text{C}$ -Labelled phenytoin (centre), otherwise known as drug for epilepsy, and  $^{18}\text{F}$ -labelled flumazenil (right), known as a sleeping pill but these are actually the real drugs just radiolabelled:



Finally, there are chelating and other ligands that form complexes with radioactive metallic ions. Taking into account importance of  $^{99\text{m}}\text{Tc}$  this is the most numerous groups of small molecule vectors. Chelating ligands form complexes with radionuclide ions that resemble “molecular envelopes” or “cages” that protect the body from radionuclide ion inside of it and are also directing its pharmacological behaviour. Chelating ligands can also serve as holders for metallic radionuclides such as  $^{111}\text{In}$  – the whole complex then can be conjugated (attached) to another, larger vector such as peptide or monoclonal antibody. Here are some examples of complexes formed by  $^{99\text{m}}\text{Tc}$  and certain ligands:



## Peptides

Another important group of vectors are peptides. What are peptides? They are small, short chains of amino acids linked via peptide bonds, just like proteins, but peptides are much shorter and smaller (Figure 121). They can serve many duties in cells or human body: they are hormones, signalling molecules, neurotransmitters, etc. Just like small molecules, they bind receptors and proteins in the body using molecular recognition; however, in the case of peptides the molecular recognition is much better and more specific than the one of small molecules.

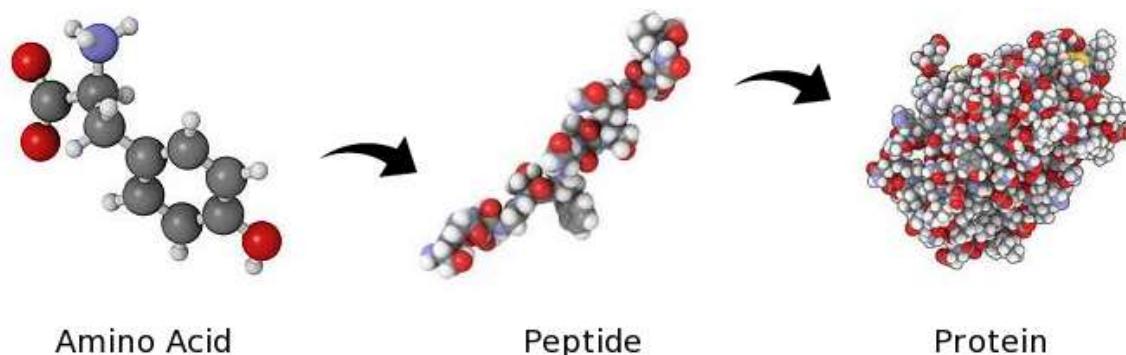


Figure 121: Peptides are made of few amino acids, while proteins are made of many amino acids.

For example, neuropeptides are neurohormones in brain, as well as oxytocin, so-called “hormone of happiness, love and trust”. Famous peptide is insulin, a hormone that regulates glucose in blood just like glucagon that also regulates glucose in blood but in opposite way than insulin. Also, there are endorphins, neuropeptides that bind opioid receptors in brain and alleviate pain. Some are general hormones like, somatotropin, also known as growth hormone, while octreotide (Figure 122) is an analogue of somatostatin that is growth hormone-inhibiting hormone, found in brain and digestive system.

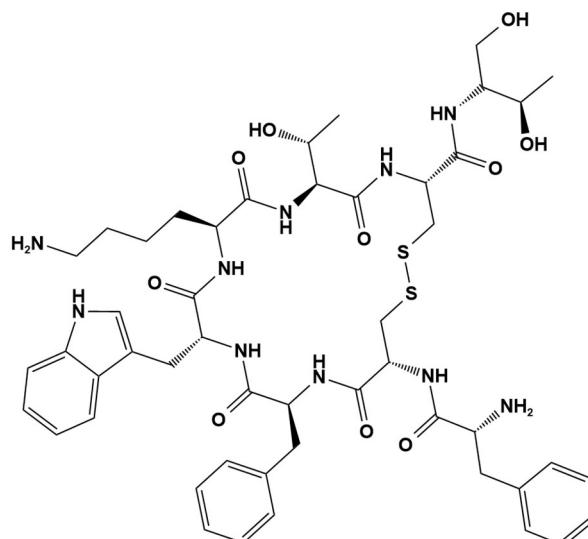
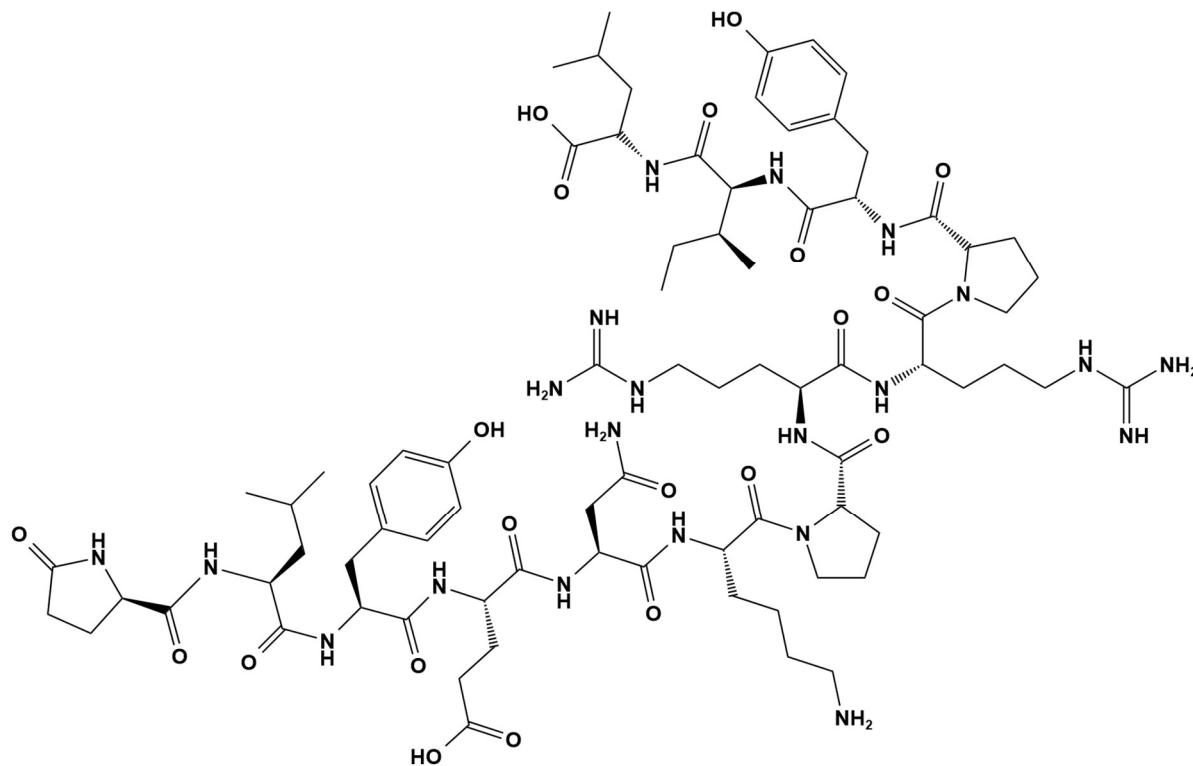


Figure 122: Octreotide, a cyclic peptide hormone that is made of 8 amino acids and cyclisation is achieved via S-S bonds of two methionine amino acids

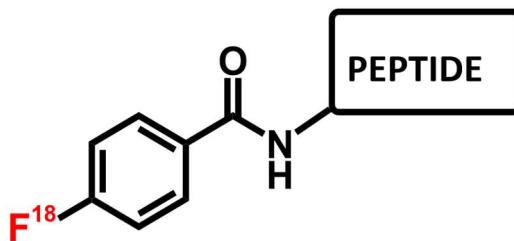
Finally, there are some hormones exclusively found in guts and digestive system such as cholecystokinin and gastrin. Peptides as large as shown in the case of

octreotide (Figure 122), that is an analogue of somatostatin, are very important in radiopharmaceutical chemistry. Even larger is neuropeptide (Figure 123) another peptide that can be radiolabelled.

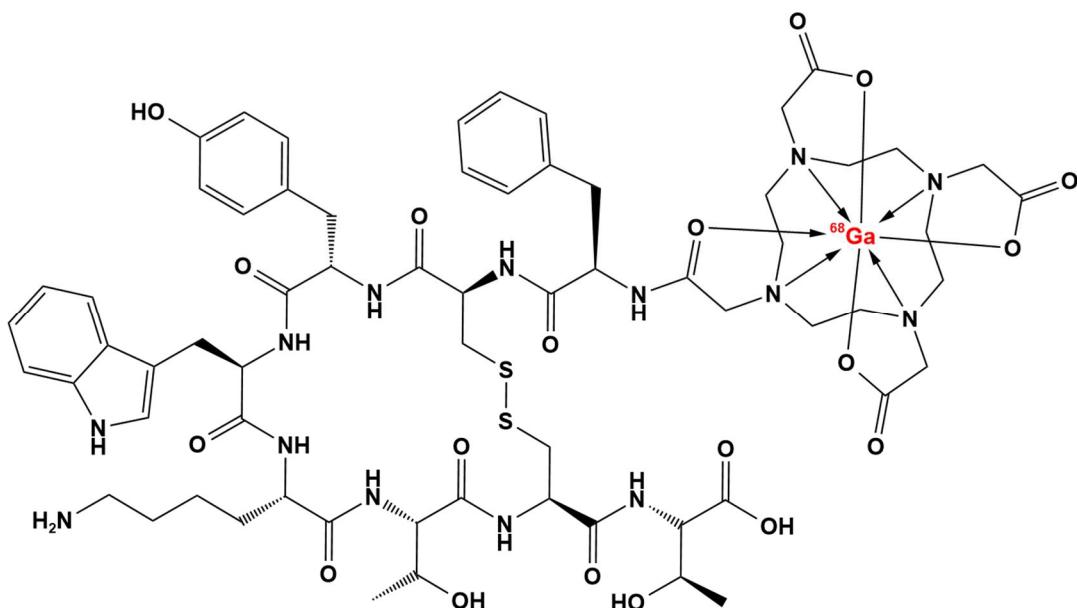


**Figure 123: Neurotensin, a 13 amino acid neuropeptide**

Labelling of peptides is rarely direct, but it usually goes *via* some secondary precursors, additional moiety that is bridging radionuclide and peptide. Sometimes it is another small molecule or simple moiety as this benzamide:



However, a chelating ligand also can be used for labelling of peptides: it is complexing a radioactive metallic ion, forms a “molecular cage” around it, and the whole complex is then attached onto a peptide. This peptide is the main vector. For example, this is the case of  $^{68}\text{Ga}$ -DOTA-octreotate ( $^{68}\text{Ga}$ -DOTA-TATE, Figure 124) where  $^{68}\text{Ga}^{3+}$  ion is chelated by DOTA chelating ligand and the whole Ga-DOTA complex is attached onto octreotide, a peptide that binds receptors in neuroendocrine tumours. This radiopharmaceutical agent is used for imaging of neuroendocrine tumorous.



**Figure 124:**  $^{68}\text{Ga}$ -DOTA-octreotate (also known as  $^{68}\text{Ga}$ -DOTA-TATE) where  $^{68}\text{Ga}$  is complexed in a DOTA ligand as in a cage and the linked with octreotate peptide molecule

Generally, radiolabelled peptides are not so common but are very powerful tool for imaging of rare diseases and cancer metastases. They are just being brought to market, and their time is yet to come. They are often used in scientific institutes as experimental agents on healthy volunteers or animals to investigate physiology and pathophysiology, to uncover secrets of diseases and how diseases form.

### Proteins as vectors – monoclonal antibodies

Our body is mostly made of proteins. Structural and functional elements of our cells are mostly proteins, as well as all enzymes, receptors, transporters, signal molecules and many other elements. Interaction of proteins with other proteins is the basis of many physiological functions, especially in immunological system. There are many proteins that can be used as vectors, but one type is especially useful and those are monoclonal antibodies. In fact, monoclonal antibodies are indeed a growing and increasingly important group of vectors for radiolabelling.

What are monoclonal antibodies? They are part of immunoglobulin family of proteins, created by our immunological system to fight various diseases. There are several classes of immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE. However here we are interested in IgG immunoglobulin type only. It is this group that are therapeutic monoclonal antibodies of our interest.

Today there are many artificially developed monoclonal antibodies (or “mABs”) made using methods of molecular biology, cell biology and biotechnology: genetic engineering, cloning and tissue culture. Monoclonal antibodies are like “magic bullets” or “guided cruising missiles”: they have superb molecular recognition ability against their specific targets (antigens). Their targets are usually some other protein structures often having oligosaccharide on it. Their selectivity is like of no other

vectors: they specifically bind only one target, only one type of cells, at very high affinity! Chemically speaking monoclonal antibodies are large proteins (Figure 125) shaped as a Latin letter Y. They consist of two heavier chains and two lighter chains. At the top of the “horns” there is an antigen binding site, or a place where antibody binds its target. That region is different in each type of antibody, depending on which target antibody binds. The rest of the molecule is always the same.

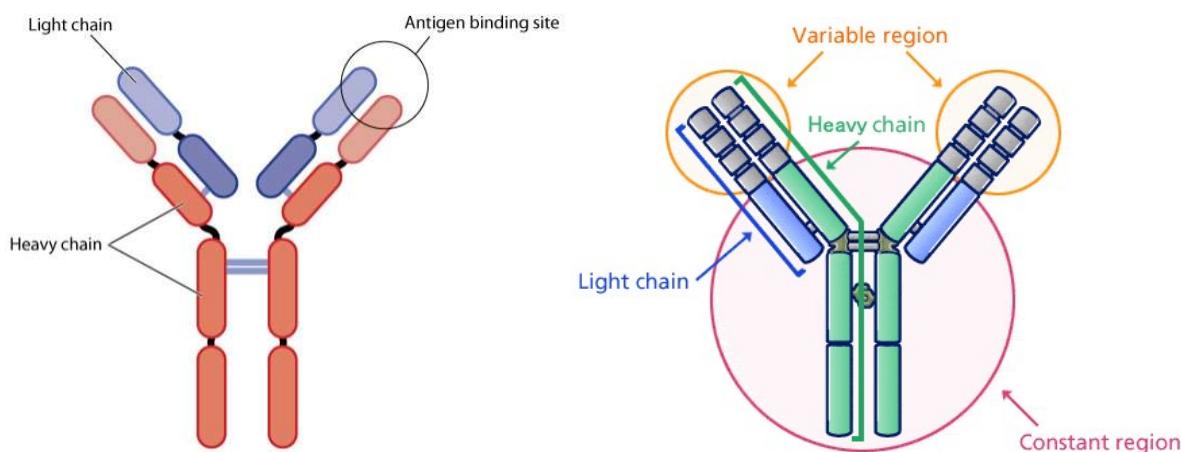


Figure 125: Structure of a monoclonal antibody – protein immunoglobulin IgG.

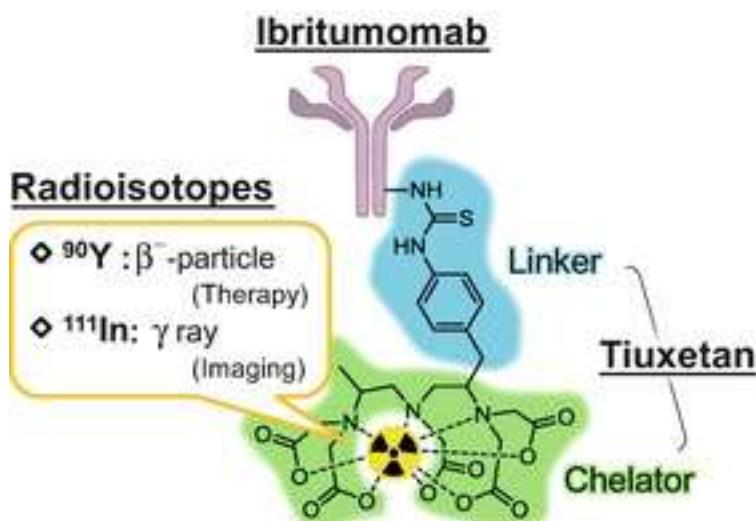


Figure 126: Ibritumomab-tiuxetan is a combination of a mAB ibritumomab and chelator linked via a linker:  $^{90}\text{Y}$  or  $^{111}\text{In}$  can be added.

Since monoclonal antibodies (mABs) are excellent vectors the strategy of radiopharmaceutical chemistry is to develop a mAB for a specific target and label it with a radionuclide. In practice this is achieved by conjugating a radionuclide (attached to a chelating agent or some other supporting moiety) with mAB through a linker. In this way a hybrid organo-metallo-protein molecular system is created with unmatched selectivity and affinity while carrying a signalling or deadly radionuclide.

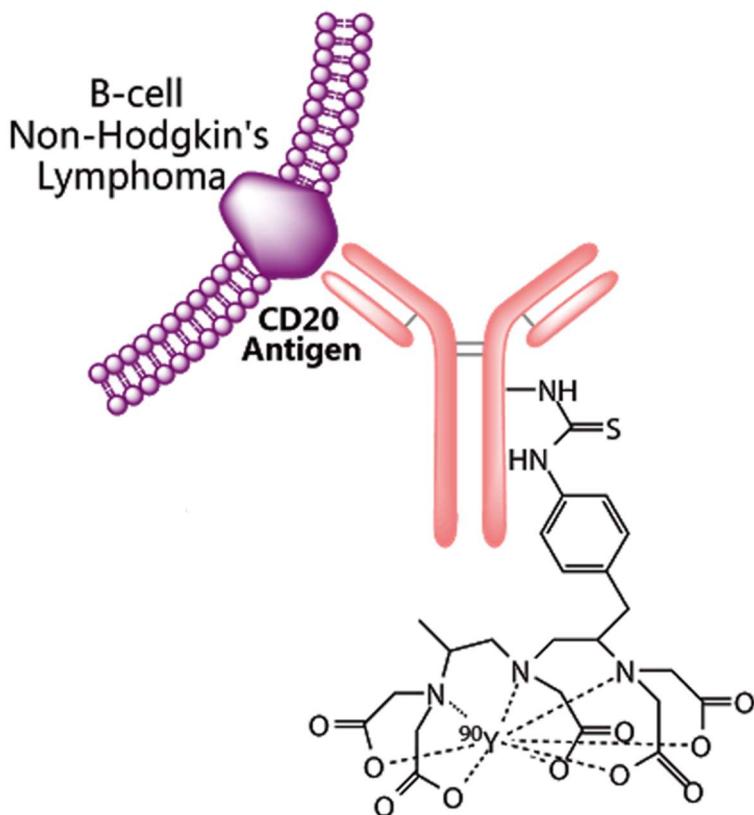


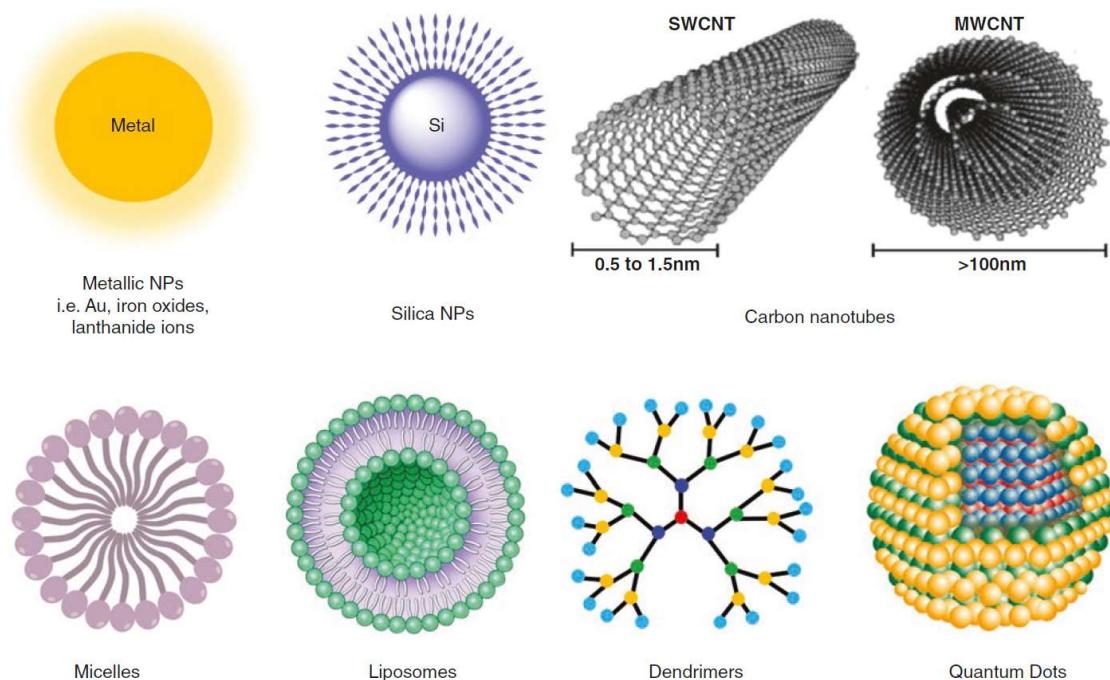
Figure 127: Ibritumomab tiuxetan with complexed  $^{90}\text{Y}$  binds CD20 antigen on malignant cells

This conjugate is called radioimmunoconjugate and can be used for nuclear imaging or targeted radiotherapy (radioimmunotherapy) or both in the same time. The most famous is ibritumomab that is conjugated with tiuxetan chelator and could have added  $^{111}\text{In}$  for imaging or  $^{90}\text{Y}$  for therapy. In the figure 127 it can be seen how the radioimmunoconjugate specifically binds its target, CD20 antigen that is specific for B-cells of lymphoma.

### Nanoparticles as vectors

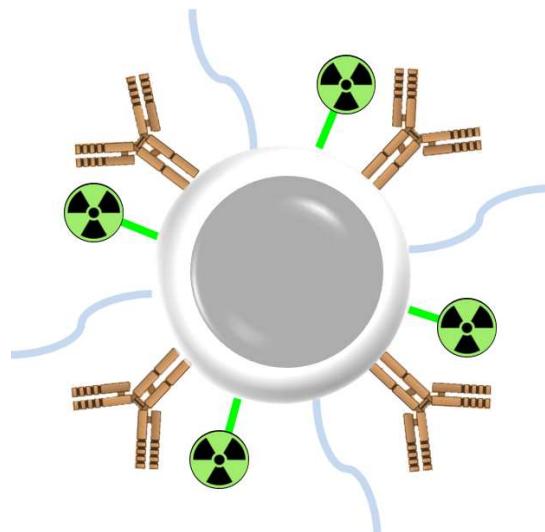
And finally, the last large group of vectors are nanoparticles. What are the nanoparticles?

They are very, very small particles of solid matter just like a powder, but so small and tiny that suspended in liquids they are behaving almost like solutions. In fact, nanoparticles have properties like no other form of matter, especially because of very large surface/weight ratio. How small they are? From 1 to 100 nm, this is the nanoscale. Nanoparticles are in fact size of smaller viruses such as HIV and coronavirus. Nanoparticles are used in many areas of technology but recently they are finding its way into medicine where they are used for drug delivery. They can be very effective vectors for the delivery of drugs and targeting of tissues since they are small enough to cross the walls of blood vessels and cell membranes. The pharmacological properties of nanoparticles can be tuned by choosing appropriate core materials and modifying their surface.

**Figure 129: Various types of nanoparticles**

In the Figure 129 one can see various types of nanoparticles: metallic, such as gold nanoparticles, nanoparticles made of silica ( $\text{SiO}_2$ ), then various carbon nanotubes, micelles, liposomes, dendrimers and finally quantum dots.

But how do we label nanoparticles? It is possible to make nanoparticles delivery vectors if we simultaneously functionalize surface of nanoparticles with some molecules that have ability of molecular recognition of targets such as mAB and with radio molecules. Keep in mind that the surface of nanoparticles is extremely large comparing to their mass and many molecules can be placed and concentrated on the surfaces of nanoparticles: it is that very large surface that makes nanoparticles so special comparing to all other forms of matter. The concept would be very useful in targeted radiation therapy; however, usage of nanoparticles in targeted radiation therapy is still experimental area. It is expected that in future nanoparticles will eventually play very important role in the targeted radiation therapy.

**Figure 129: Nanoparticle with attached radionuclides and mABs**

## Chapter VIII - Technetium-99m ( $^{99m}\text{Tc}$ )

Technetium is one of the most important radioactive elements in the area of nuclear medicine, and especial attention will be focused on it. Due to its huge importance, it will be the first radionuclide to examine in detail.

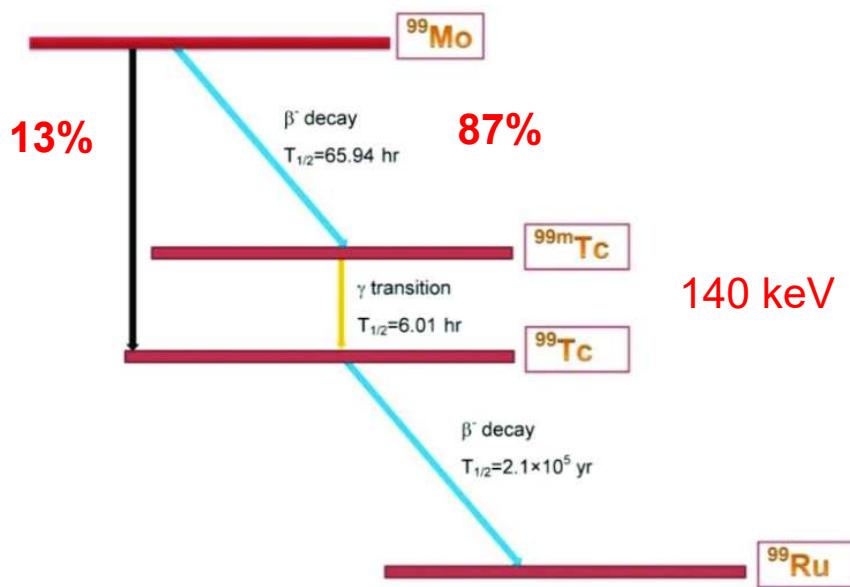
### Isotopes of technetium

It is the only element in the “d” block that has no stable isotopes. There are 51 isotopes of technetium, ranging from  $^{85}\text{Tc}$  to  $^{120}\text{Tc}$ , but only two are significantly important in radiochemistry:  $^{99}\text{Tc}$  and its meta-stable isomer,  $^{99m}\text{Tc}$ .  $^{99}\text{Tc}$  is a radionuclide from nuclear power plant reactors, part of the nuclear waste, by-product of nuclear reprocessing (namely, from the PUREX process). As such, it is considered as waste, burden that needs to be disposed or safeguarded.  $^{99}\text{Tc}$  decays by beta decay, emits soft beta particles (mean energy is 84 keV), has no gamma emission, and its daughter is stable isotope  $^{99}\text{Ru}$ . Its half-life is quite long, 211 100 years, therefore 1 µg of  $^{99}\text{Tc}$  has activity of only 627.9 Bq. Regarding the application there is no many of it, very few. It can be used as corrosion inhibitor in nuclear reactors and as a catalyst.

Another major isotope,  $^{99m}\text{Tc}$  is in fact a meta-stable nuclear isomer of  $^{99}\text{Tc}$ . It is an inter-product of  $^{99}\text{Mo}$  beta decay towards  $^{99}\text{Tc}$ . It decays into  $^{99}\text{Tc}$  by internal transition and emits a gamma photon only (energy is 140 keV). Its half-life, opposite to  $^{99}\text{Tc}$ , is only 6.007 hours which means that 1 µg of  $^{99m}\text{Tc}$  has activity of almost 200 GBq. And needless to say, it is the most important radionuclide in nuclear medicine!

### Nuclear properties of $^{99m}\text{Tc}$

This scheme summarizes nuclear properties of  $^{99m}\text{Tc}$  as well as its parent  $^{99}\text{Mo}$  and its daughter  $^{99}\text{Tc}$ :

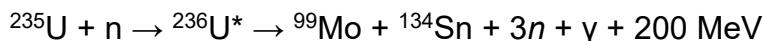




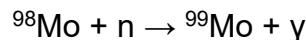
It is important to realize that  $^{99}\text{Mo}$  has half-life that is almost exactly 11 times larger than  $^{99\text{m}}\text{Tc}$ . However not all  $^{99}\text{Mo}$  decays into  $^{99\text{m}}\text{Tc}$ , but only 87% and this is something we have to take into account. The rest 13% goes directly into  $^{99}\text{Tc}$ . This ratio 0.87/0.13 is called the branching ratio. The gamma ray emitted by  $^{99\text{m}}\text{Tc}$  is 140 keV and is almost perfect in terms of energy for the excitation of scintillators (photomultiplication tubes). In total, nuclear properties of  $^{99\text{m}}\text{Tc}$  make it almost a perfect radionuclide for SPECT imaging.

### Production of $^{99\text{m}}\text{Tc}$

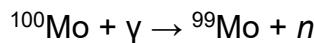
How is  $^{99\text{m}}\text{Tc}$  produced? This issue was mentioned in our earlier chapters, but here we will focus on  $^{99\text{m}}\text{Tc}$ . Currently the main overwhelming option is to firstly make  $^{99}\text{Mo}$  and then use it in a radionuclide generator to generate  $^{99\text{m}}\text{Tc}$  by a natural decay. Production of  $^{99}\text{Mo}$  can be achieved in the classic way by nuclear fission of  $^{235}\text{U}$  in nuclear reactors where it gets fissioned with thermal neutrons as described in this nuclear reaction equation:



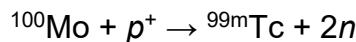
Another option to make  $^{99}\text{Mo}$  is irradiation of  $^{98}\text{Mo}$  with neutrons in nuclear reactors:



The third option (quite experimental and not used in practice) is irradiation of  $^{100}\text{Mo}$  with gamma photons using a linear accelerator:



Another, more experimental option is to make  $^{99\text{m}}\text{Tc}$  directly by using a cyclotron, and this is going to be the most probable future alternative for the production of  $^{99\text{m}}\text{Tc}$ . It could be made by irradiating  $^{98}\text{Mo}$  with protons in cyclotrons:



The main problem here will be the need to use enriched molybdenum that has to be almost 100% pure  $^{100}\text{Mo}$  metal.

### $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ radionuclide generators

The  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  radionuclide generators (explained in the Chapter IV) are the “extended arms” of nuclear reactor. In these devices  $^{99\text{m}}\text{Tc}$  is in so-called transient equilibrium with its parent radionuclide  $^{99}\text{Mo}$ . Molybdenum is slowly decaying over the course of two weeks constantly generating  $^{99\text{m}}\text{Tc}$  that is decaying 11 times faster than its parent,  $^{99}\text{Mo}$ . Even today these devices are the central in radiopharmaceutical chemistry.

The function of  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  radionuclide generator, how it works, its radiochemistry and use is extensively explained in the Chapter IV and will not be detailed here.

## The chemistry of technetium

For a radiochemist it is very important to understand chemistry of technetium. Tc is located in the middle of the periodic table (Figure 130), in the d-block, fifth period, seventh group. It is the only d-block transition metal that has no stable radionuclide and is very unique in that sense.

1	H																							2	He											
3	Li	4	Be																																	
11	Na	12	Mg																																	
19	K	20	Ca	21	Sc	22	Ti	23	V	24	Cr	25	Mn	26	Fe	27	Co	28	Ni	29	Cu	30	Zn	31	Ga	32	Ge	33	As	34	Se	35	Br	36	Kr	
37	Rb	38	Sr	39	Y	40	Zr	41	Nb	42	Mo	43	Tc	44	Ru	45	Rh	46	Pd	47	Ag	48	Cd	49	In	50	Sn	51	Sb	52	Te	53	I	54	Xe	
55	Cs	56	Ba	*	71	Lu	72	Hf	73	Ta	74	W	75	Re	76	Os	77	Ir	78	Pt	79	Au	80	Hg	81	Tl	82	Pb	83	Bi	84	Po	85	At	86	Rn
87	Fr	88	Ra	*	103	Lr	104	Rf	105	Db	106	Sg	107	Bh	108	Hs	109	Mt	110	Ds	111	Rg	112	Cn	113	Nh	114	Fl	115	Mc	116	Lv	117	Ts	118	Og
*				*	57	La	58	Ce	59	Pr	60	Nd	61	Pm	62	Sm	63	Eu	64	Gd	65	Tb	66	Dy	67	Ho	68	Er	69	Tm	70	Yb				
*				*	89	Ac	90	Th	91	Pa	92	U	93	Np	94	Pu	95	Am	96	Cm	97	Bk	98	Cf	99	Es	100	Fm	101	Md	102	No				

Figure 130: Place of Tc in the Periodic Table of elements.

Technetium (Tc) is in the same group with manganese (Mn) and rhenium (Re); therefore, chemical properties of Tc are similar to Re and Mn, but in fact chemical behaviour of Tc is more like Re than Mn. The most stable chemical form of Tc is pertechnetate anion ( $\text{TcO}_4^-$ ), in which Tc is in the oxidation state +7 (VII). It is an equivalent of permanganate ion ( $\text{MnO}_4^-$ ), however it is not as strong oxidation agent as is permanganate ion. Its structure is tetrahedral (Figure 131), where Tc atom is in the middle of a tetrahedron, and oxygen atoms are in the vertexes of the tetrahedron. It is very soluble in water, and it is hard to be immobilized. In such form it cannot form any complexes with ligands and cannot be used for any labelling. In order to be used in labelling it has to be reduced.

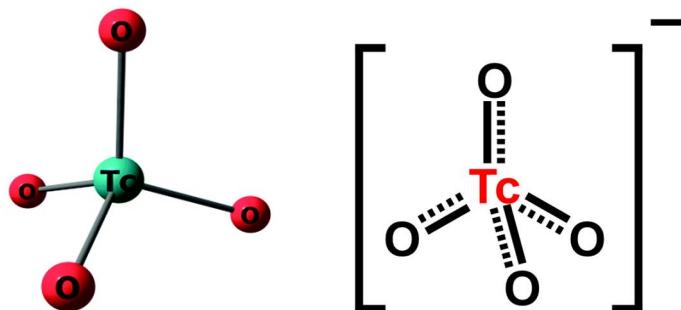


Figure 131: Tetrahedral shape of  $\text{TcO}_4^-$  ion

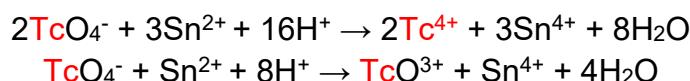


Apart from oxidation number +7 technetium can be reduced into oxidation states +6, +5, +4, +3, +2, +1, 0, and even -1. However, Tc(+5) and Tc(+4) are the most common and important, while the Tc(+4) state is the most stable low oxidation state. Oxidation states Tc(+6) and Tc(+5) like to disproportionate into Tc(+4) and Tc(+7). To stabilise Tc(+5) usually a complexation with a ligand is needed. Reduction strongly depends on media pH, “strength” of the reducing agent and the nature of the coordinating ligands. Oxidation states Tc(+4), Tc(+5) and Tc(+1) are typical for  $^{99m}\text{Tc}$  radiopharmaceuticals. In the Table 4 one can see all the oxidation states of technetium and examples of ions, compounds and complexes. Those coloured in red are actual radiopharmaceuticals.

Oxidation state	Example of compound (ion, complex, radiopharmaceutical)
Tc(+7)	$\text{TcO}_4^-$
Tc(+6)	$\text{TcO}_4^{2-}$
Tc(+5)	$[\text{TcO}]^{3+}$ , $[\text{TcO}_2]^+$ , $[\text{Tc}(\text{NCS})_6]$ , <b>Tc-MAG<sub>3</sub></b> , <b>Tc-BAT</b> , <b>Tc-Tetrofosmin</b>
Tc(+4)	$[\text{Tc}(\text{NCS})_6]^{2-}$ , $\text{TcCl}_4$ , $\text{TcO}_2$ , $[\text{TcO(OH)}\text{EDTA}]^{3-}$ <b>Tc-MDP</b>
Tc(+3)	<b>Teboroxime</b> , $[\text{TcCl}_3(\text{Et}_2\text{PhP})_3]$
Tc(+2)	$[\text{TcCl}_4]^{2-}$ , $[\text{Tc}(\text{NO})\text{Br}_4]^-$
Tc(+1)	$[\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ , <b>[Tc(MIBI)<sub>6</sub>]<sup>+</sup></b>
Tc(0)	$\text{Tc}_2(\text{CO})_{10}$
Tc(-1)	$[\text{Tc}(\text{CO})_5]^-$

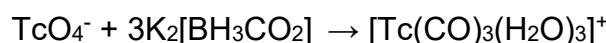
Table 4: The oxidation states of technetium and examples of ions, compounds and complexes

Therefore, to make radiopharmaceutical agent containing  $^{99m}\text{Tc}$  pertechnetate ion has to be reduced. A proper reducing agent needs to fulfil the following requirements: to be non-toxic (biocompatible), to have ability to rapidly, quickly reduce pertechnetate, and that is stable over a period of time at an appropriate pH range. In practice the most common reducing agent is tin(II) chloride, also known as stannous chloride ( $\text{SnCl}_2$ ):



It has ability to reduce pertechnetate ion to +5, +4 or even +3 oxidation states depending on the pH of the reaction medium. Therefore, reduction is achieved in a certain pH buffer that keeps a stable pH. For example, a citrate buffer at pH neutral gives exclusively Tc(+5), while acidic pH gives Tc(+4). In practice there is a huge access of  $\text{Sn}^{2+}$  in kits comparing to  $^{99m}\text{Tc}$ , about 1 million more!

Other reducing agents for pertechnetate ion are, for example, sodium borohydride and thiourea dioxides. One important secondary precursor for labelling is technetium tricarbonyl complex,  $[\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  where technetium is in  $\text{Tc}^{1+}$  oxidation state. It can be made using a special reagent called potassium boranocarbonate ( $\text{K}_2[\text{BH}_3\text{CO}_2]$ ):



It is a stable organometallic complex ion and is used as a precursor for many  $\text{Tc}^{1+}$  complexes. Generally, Tc in lower oxidation states ( $\text{Tc}^{3+}$ ,  $\text{Tc}^{4+}$ ,  $\text{Tc}^{5+}$ ...) tends to hydrolyse and form insoluble  $\text{TcO}_2$ . In order to prevent hydrolysis Tc cations, they need to be stabilised by complexation with proper ligands. In the same time complexation is also labelling since it gives technetium a particular pharmacological property. Overwhelming majority of  $^{99\text{m}}\text{Tc}$  radiopharmaceuticals are complexes.

### Coordination chemistry of $^{99\text{m}}\text{Tc}$

Due to numerous oxidation states and ability to form many complexes with various ligands technetium chemistry is very rich. It forms various coordination geometries depending on if it is monooxo or dioxo ion or is free from oxygen (Figure 132). Also, there are nitrido technetium ions that are becoming more popular. Some radiopharmaceuticals developed in China have nitrido technetium core. Usual ligands contain donor atoms such as O, N, S, or P. The best donor is oxygen and then nitrogen, sulphur, while phosphorus is the weakest.

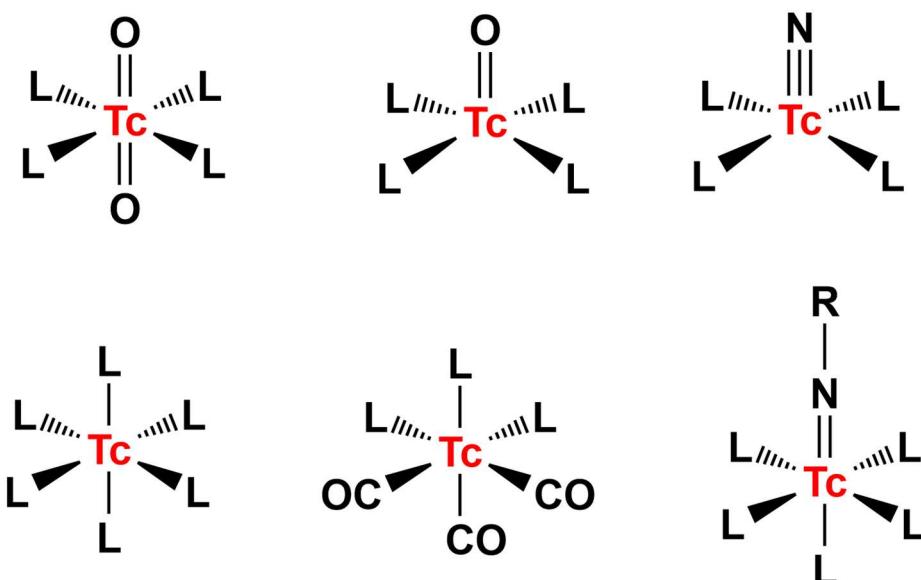
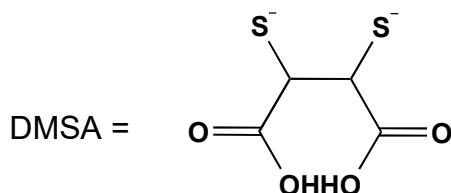
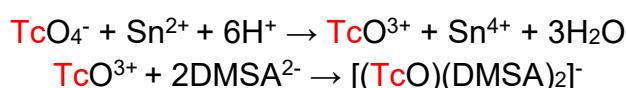


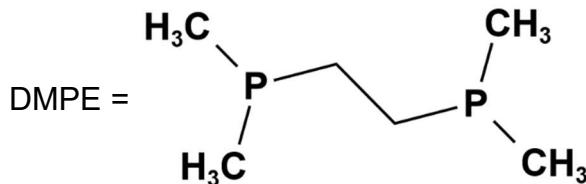
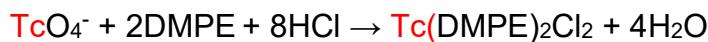
Figure 132: Various coordination geometries that Tc can adopt depending of oxidation state and form. L in the diagrams denotes any monodentate ligand.

There are many ways how coordination and formation of complexes can be achieved. Firstly, the most usual is the direct complexation upon reduction. This reaction is often simultaneous and happens in commercial kits: pertechnetate is reduced with  $\text{Sn}^{2+}$  and then immediately complexed with ligands such as DMSA (Dimercapto succinic acid):

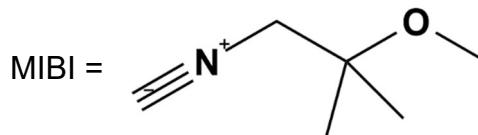
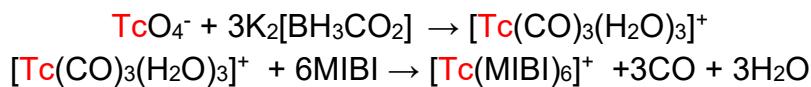




Another method is reductive complexation where a ligand reacts with pertechnetate and gets oxidized, while the pertechnetate ion is reduced. This is example with DMPE, a phosphino ligand:



The third method is the exchange of ligands. Firstly, a secondary precursor is made, for example technetium tricarbonyl complex, and then carbonyls water molecules are exchanged with six MIBI ligands to get this important radiopharmaceutical:



Every newly developed radiopharmaceutical agent needs to be chemically characterized, and this includes obtaining characterization data such as NMR, IR or mass spectra, data on electrochemical and optical properties and often solid-state crystal structure using x-ray diffraction. However, as we know,  $^{99m}\text{Tc}$  is very radioactive, for example 925 MBq of  $^{99m}\text{Tc}$  is just 4.7 ng. Therefore,  $^{99m}\text{Tc}$  cannot be used for chemical development of new  $^{99m}\text{Tc}$  complexes. We need to use its less radioactive isomer  $^{99}\text{Tc}$ . But sometimes it is not practical, so researchers often do another trick: they use rhenium instead of technetium. Rhenium has very similar chemistry to technetium: just like technetium rhenium forms  $\text{ReO}_4^-$ , a perrhenate ion, it has similar oxidation states and is stable, non-radioactive. Hence, we say that Re is a non-radioactive surrogate for Tc. The drawback is that rhenium requires different reagent and conditions, and Re complexes and compound tend to behave somewhat different.

### Current $^{99m}\text{Tc}$ radiopharmaceuticals: structure, synthesis, and clinical use

Despite fast development of PET imaging methods and radiopharmaceuticals  $^{99m}\text{Tc}$ -based imaging using SPECT scanners and  $^{99m}\text{Tc}$  radiopharmaceuticals still dominating nuclear medicine and will be dominating for the years to come, especially in the countries and areas where PET imaging is not available and/or very expensive.

Table 5 presents the main examples of radiopharmaceuticals that contain  $^{99m}\text{Tc}$ . On the left side is the therapeutic area and on the right side are typical agents used.

These are just few examples the real list is much longer, but we will not cover all of them. In fact, these are the most commonly used in nuclear medicine.

Therapeutic area	Radiopharmaceutical agents
Agents for cardiac perfusion imaging	$^{99m}\text{Tc}$ -Sestamibi, $^{99m}\text{Tc}$ -Tetrofosmin
Agents for bone imaging	$^{99m}\text{Tc}$ -MDP
Agents for renal imaging	$^{99m}\text{Tc}$ -DMSA, $^{99m}\text{Tc}$ -DTPA, $^{99m}\text{Tc}$ -MAG <sub>3</sub>
Agents for imaging of liver and gallbladder	$^{99m}\text{Tc}$ -Mebrofenin, $^{99m}\text{Tc}$ -Disofenin
Agents for imaging of brain	$^{99m}\text{Tc}$ -Exametazime, $^{99m}\text{Tc}$ -ECD
Other agents	$^{99m}\text{Tc}$ -Sulfur colloid

Table 5: The main examples of  $^{99m}\text{Tc}$ .

Nearly all  $^{99m}\text{Tc}$  radiopharmaceuticals are complexes of  $^{99m}\text{Tc}$  in certain form with some kind of organic ligands. One can raise a question: which or what kind of receptors are these radiopharmaceuticals targeting and binding?

Most of the time these complexes do not target any receptor! Their application in nuclear medicine imaging is based on their preferential accumulation in certain tissues due to their unique pharmacokinetics: their molecular structure is such to form certain solubility, lipophilicity, size and other properties that after particular application (oral or intravenous) direct them to certain tissues where they are being accumulated so imaging becomes possible even though they are not binding any specific receptor!



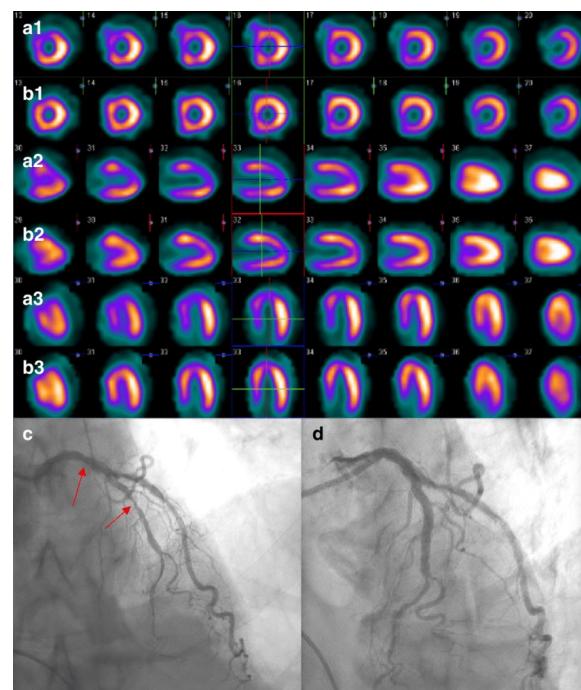
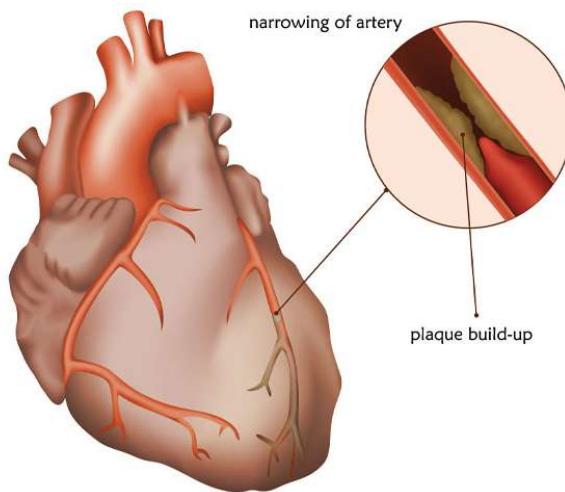
Figure 133: Patient is being scanned by using gamma camera for SPECT imaging.

### Agents for imaging of cardiac perfusion

What is cardiac perfusion? It is flow and supply of fresh oxygenated blood into the heart muscle. As we know there is a common disease called atherosclerosis when blood vessels are clogged with fat and then flow of blood gets worse and patient then suffers pain in the chest called angina pectoris, but if fully clogged and blocked

then this is called heart attack or infarction and usually kills. In order to see how good or bad blood flows through heart muscle nuclear imaging uses various radiopharmaceutical agents for imaging based on  $^{99m}\text{Tc}$ . The most important are  $^{99m}\text{Tc}$ -sestamibi and  $^{99m}\text{Tc}$ -tetrofosmin while there are also some others and some newer such as:

- $^{99m}\text{Tc}$ -sestamibi
- $^{99m}\text{Tc}$ -Tetrofosmin
- $^{99m}\text{Tc}-(\text{Et}_2\text{PhP})_3\text{Cl}_3$
- $^{99m}\text{Tc}-(\text{DMPE})_2\text{Cl}_2$
- $^{99m}\text{Tc}$ -TBI
- $^{99m}\text{Tc}$ -Teboroxime

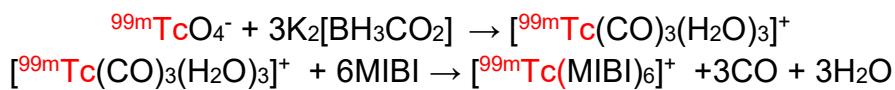


**Figure 134:** When a plaque (left) builds up in heart artery flow of blood in the heart muscle is compromised: this flow rate can be identified (right) by using  $^{99m}\text{Tc}$  imaging radiopharmaceuticals and SPECT imaging.

### $^{99m}\text{Tc}$ -sestamibi

The most famous radiopharmaceutical agent for heart disease is  $^{99m}\text{Tc}$ -Sestamibi. It is a cationic lipophilic  $^{99m}\text{Tc}^{1+}$  complex with 6 (“sesta” in Latin language) “mibi” ligands where “mibi” is a short name for the ligand methoxy-iso-butyl-isonitrile. Therefore, the name “sesta” which means “six” and “mibi” are the ligands.

It can be made using a ligand exchange method with versatile complex technetium tricarbonyl, where reaction with “mibis” yields the agent:



The formed complex ion is positively charged, however, because of its “mibi” lipophilic groups it can accumulate in blood vessels. This agent is used to identify both myocardial ischemia (lack of oxygen in heart muscle), necrosis in angina pectoris (damage of heart muscle) and infarction (complete clog of heart artery).

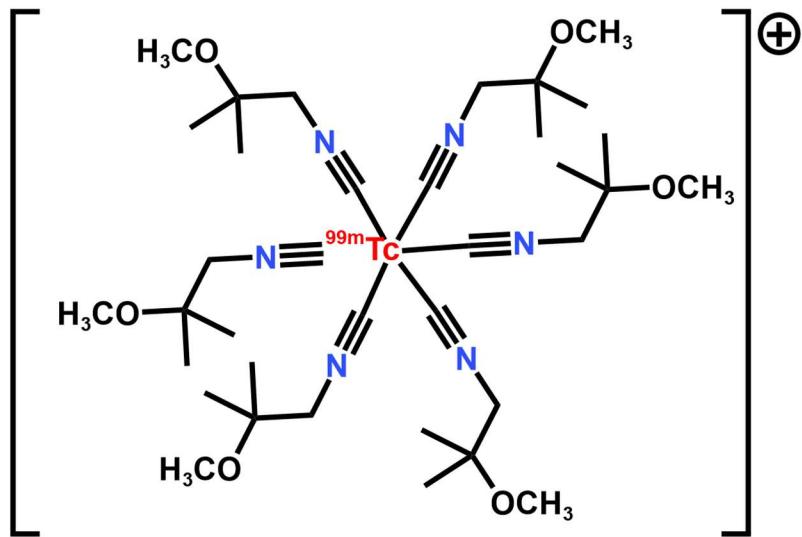


Figure 135: Structure of  $^{99m}\text{Tc}$ -Sestamibi

### $^{99m}\text{Tc}$ -Tetrofosmin

Another important agent is  $^{99m}\text{Tc}$ -tetrofosmin. It is a cationic, trans-dioxo-bis(diphosphine)-technetium(V) complex where this large phosphino ligand encircles the  $\text{TcO}_2^+$  core. It can be easily made using a high-yielding kit by combining the 1,2-bis[bis(2-ethoxyethyl)phosphino] ethane ligand with stannous chloride as the reducing agent. It is used to identify regions of myocardial ischemia (parts of heart muscle that doesn't get enough of oxygen) and infarct in patients with suspected heart disease. This imaging procedure is important when physicians are planning heart surgery.

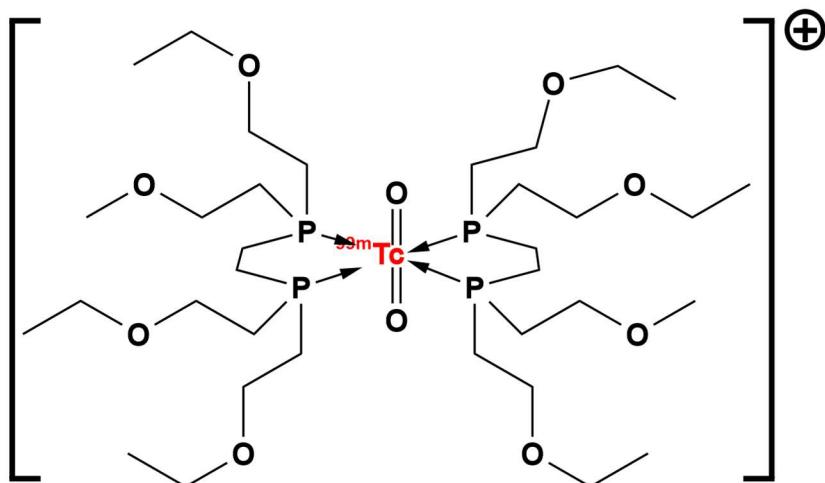
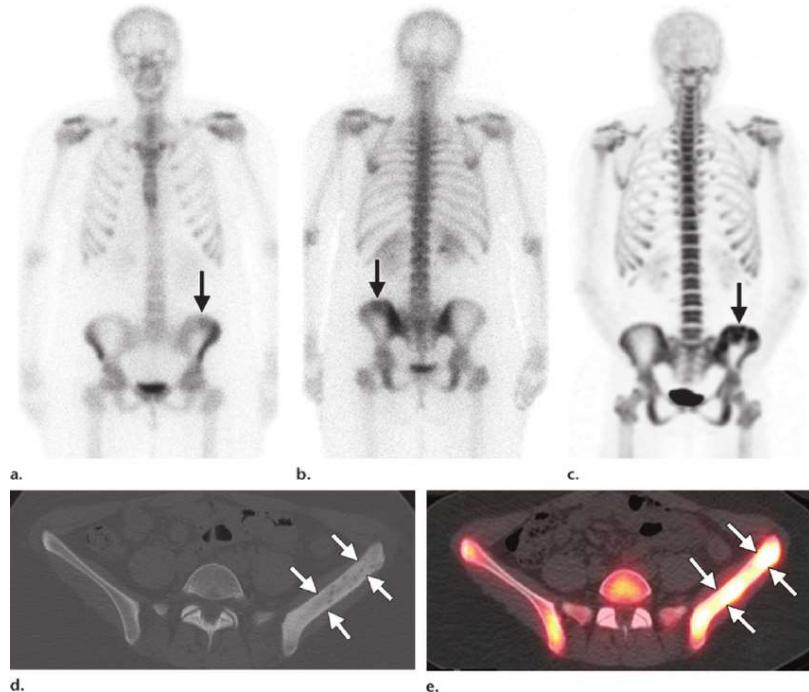


Figure 136: Structure of  $^{99m}\text{Tc}$ -Tetrofosmin

## Agents for bone imaging

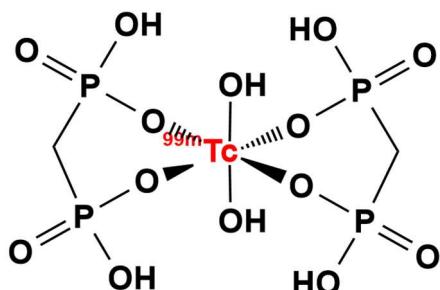
Agents for bone imaging are radiopharmaceuticals used to localize bone metastases as well as other diseases that can alter the natural turn-over in the bone by SPECT imaging. The most important agent here is  $^{99m}\text{Tc}$ -medronate or  $^{99m}\text{Tc}$ -MDP.



**Figure 137:** Bone imaging using SPECT: imaging identifies area of pathological bones formation, such a bone metastasis

### $^{99m}\text{Tc}$ -medronate or $^{99m}\text{Tc}$ -MDP

$^{99m}\text{Tc}$ -medronate is complex where  $^{99m}\text{Tc}$  is chelated by a methylene bisphosphonate also called medronic acid. It is prepared using a kit, by reacting  $^{99m}\text{TcO}_4^-$  with medronic acid, ascorbic acid, and tin(II) fluoride ( $\text{SnF}_2$ ). This complex is not charged like the previously described two but neutral. It works by binding to a bone mineral called hydroxyapatite and hence is able to accumulate at the sites of high calcium metabolism such as newly formed bones and bone cells. It is used in number of bone related diseases, such as bone metastases, skeletal tumours and other various bone diseases, but also in some other types of tumorous such as sarcomas and adenocarcinomas.



**Figure 138:** Structure of  $^{99m}\text{Tc}$ -Medronate

## Agents for renal imaging

Agents used for renal imaging are actually for the screen of kidney function. “Renal” means “of kidney”. These agents are used to check if kidneys are working well or not and if it is anything wrong with blood filtration and urine flow. They are used in the case of kidney failure, cancer, inflammation, necrosis, obstructions and other kidney diseases. Here we have three main agents,  $^{99m}\text{Tc}$ -DMSA,  $^{99m}\text{Tc}$ -DTPA, and  $^{99m}\text{Tc}$ -MAG<sub>3</sub>. Interestingly all renal agents are negatively charged complex ions.

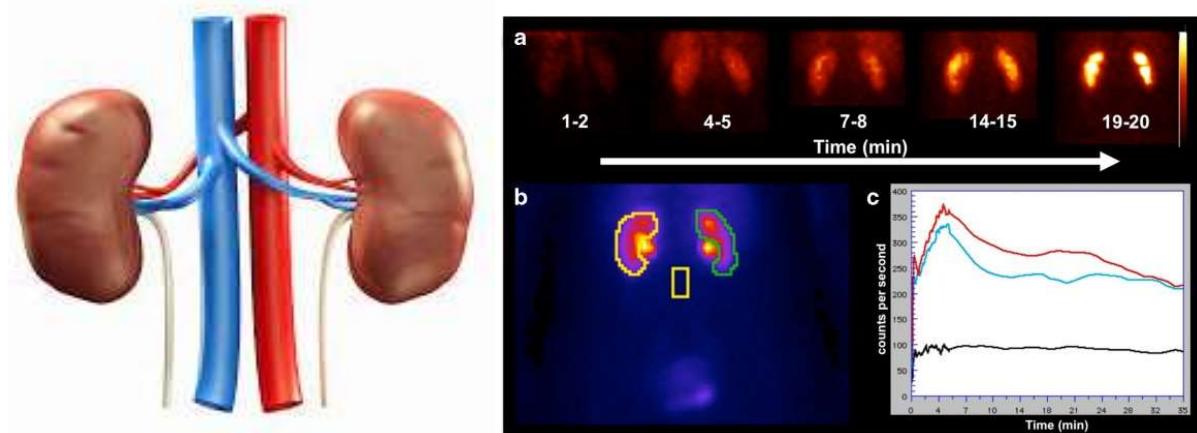


Figure 139: Kidney (left) imaging using SPECT: imaging identifies function of kidneys such as perfusion, filtration and urine formation

### $^{99m}\text{Tc}$ -DMSA

$^{99m}\text{Tc}$ -DMSA is a complex anion that consists of  $^{99m}\text{TcO}^{3+}$  (Tc oxidation state is +5) with a ligand named dimercaptosuccinic acid (DMSA). It is prepared by using a kit: freshly milked  $^{99m}\text{TcO}_4^-$  is mixed with DMSA,  $\text{SnCl}_2$  and ascorbic acid. Many these kits contain ascorbic acid; it is nothing but common vitamin C that works as a reducing agent and a stabiliser.  $^{99m}\text{Tc}$ -DMSA is used to evaluate kidney function; in patients with advanced failure of kidney function will show little presence of this agent in kidneys, while healthy kidney shows lots of agent present in kidneys.

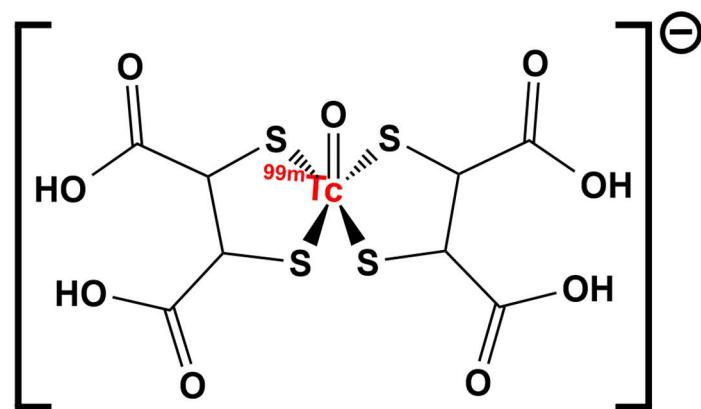


Figure 140: Structure of  $^{99m}\text{Tc}$ -DMSA ion

### ***<sup>99m</sup>Tc-DTPA***

Another radiopharmaceutical agent for the imaging of kidney function is  $^{99m}\text{Tc}$ -DTPA. It is a simple chelate complex of  $^{99m}\text{Tc}^{4+}$  with DTPA (also known as pentetic acid or diethylenetriaminepentaacetate). It also can be made using a kit and is used to assess quality of flow of blood through kidneys and how well kidneys are filtrating blood. It also has applications for brain imaging.

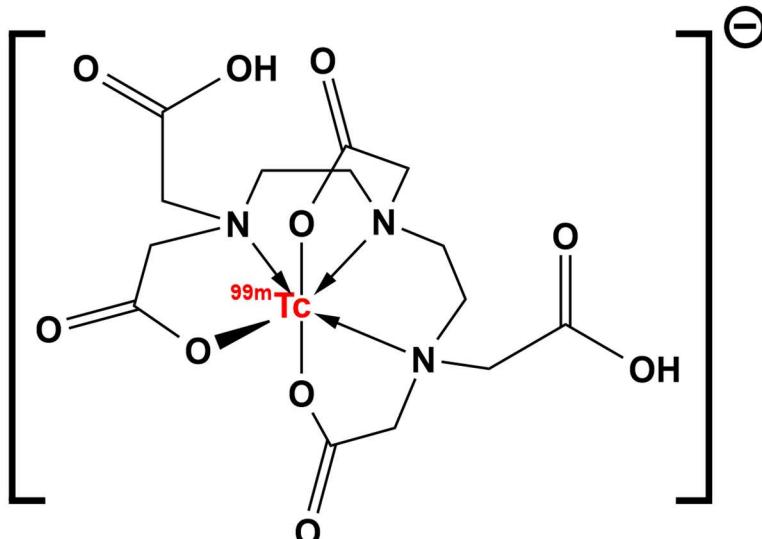


Figure 141: Structure of  $^{99m}\text{Tc}$ -DTPA ion

### ***<sup>99m</sup>Tc-mertiatide or <sup>99m</sup>Tc-MAG<sub>3</sub>***

The most famous agent for imaging of kidney function is  $^{99m}\text{Tc}$ -MAG<sub>3</sub> also known as  $^{99m}\text{Tc}$ -mertiatide. It is a tetradeятate anionic monooxo complex of  $^{99m}\text{TcO}^{3+}$  (oxidation state +5), and a ligand called mercaptoacetyltriglycine (MAG<sub>3</sub>). It is used to assess kidney function, notably renal failure and urinary tract obstruction. Also, it is used to test health of kidney of donors before kidney transplantations.

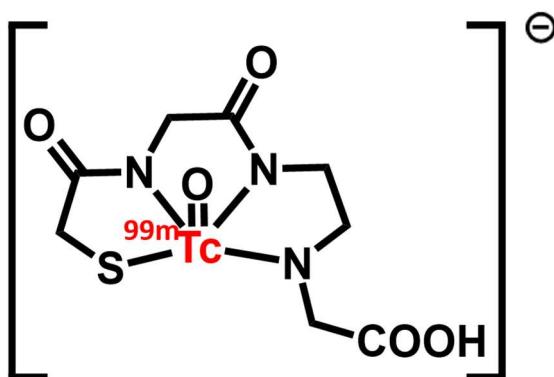


Figure 142: Structure of  $^{99m}\text{Tc}$ -MAG<sub>3</sub> ion

## Agents for imaging of liver and gallbladder

Agents for imaging of liver and gallbladder are used to test how good liver and gallbladder work, for diagnosis of gallbladder disease. There are two main agents here,  $^{99m}\text{Tc}$ -disofenin and  $^{99m}\text{Tc}$ -mebrofenin. There is also an older agent called  $^{99m}\text{Tc}$ -HIDA (“HIDA” ligands was dimethyl acetanilide iminodiacetic acid) that was very similar but is now obsolete, which means it is not used any more. However, even today everyone calls this kind of SPECT imaging of liver “HIDA” test even the actual HIDA agent is not used any more.

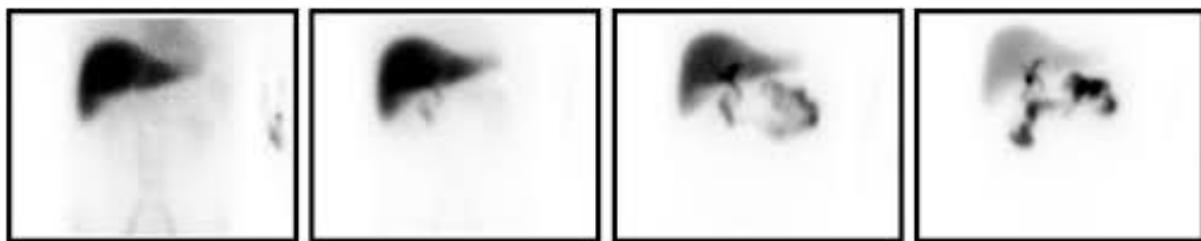


Figure 143: Liver and gallbladder imaging using SPECT: imaging identifies function of liver and gallbladder.

### $^{99m}\text{Tc}$ -disofenin

$^{99m}\text{Tc}$ -disofenin is also known as  $^{99m}\text{Tc}$ -DISIDA and it is a complex anion of  $^{99m}\text{Tc}^{4+}$  and two molecules of ligand named diisopropyl acetanilide iminodiacetic acid (acronym is “DISIDA”). It is a lipophilic agent and is not filtered by kidneys, but instead it goes into liver and then is very quickly removed into gallbladder. It is used for imaging and diagnosis of gallbladder disease – if there is a diseased it takes more than 1 hour to be seen in the gallbladder, while in healthy persons  $^{99m}\text{Tc}$  can be seen in gallbladder within one hour.

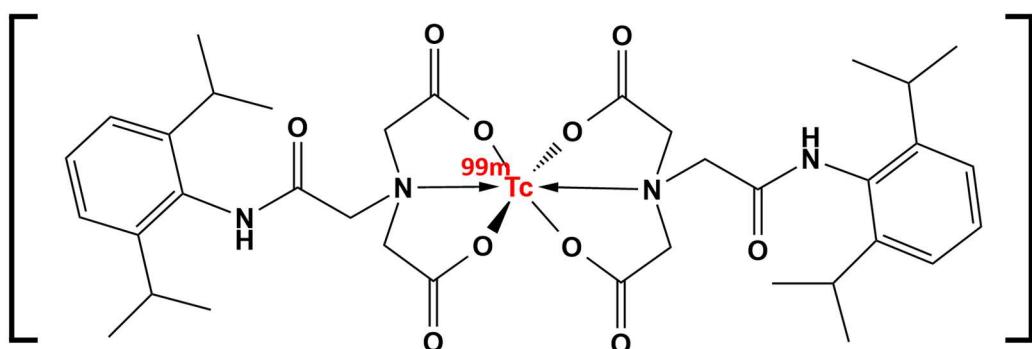


Figure 144: Structure of  $^{99m}\text{Tc}$ -disofenin

### $^{99m}\text{Tc}$ -mebrofenin

Another very similar agent is  $^{99m}\text{Tc}$ -mebrofenin and the only difference is the terminal side of the ligand with 3 methyl groups and a bromine substituent.

## Agents for imaging of brain

Agents for imaging of brain are usually mostly those that are imaging flow of blood through brain and also called cerebral perfusion agents. They need to be very lipophilic and neutral as molecules, not ions. This is required to enable them to cross so called blood-brain barrier, a functional barrier that allows only some substances to cross from bloodstream into the brain itself. They are used for imaging stroke and in various cerebrovascular diseases where blood flow through brain is affected.

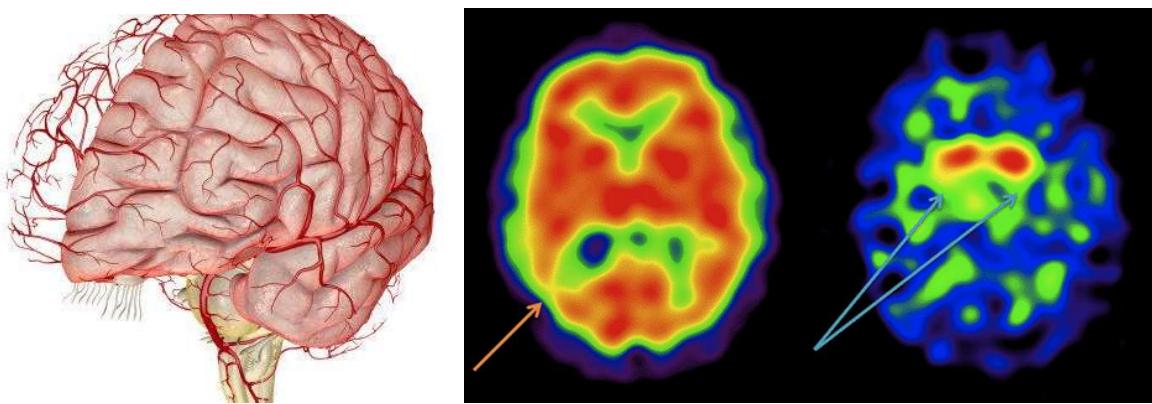


Figure 145: Brain imaging using SPECT: imaging identifies blood circulation in brain and its malfunction.

### $^{99m}\text{Tc}$ -Exametazime

$^{99m}\text{Tc}$ -Exametazime is a chelate complex of  $^{99m}\text{Tc}(\text{V})$  with a bisoxime ligand commonly referred to as hexamethyl propylene amine oxime (HMPAO). It is neutral (not ion) and lipophilic and passes from blood into brain where it is hydrolysed and then cannot leave the brain. Hence it accumulates in brain. It is used to test flow of blood through brain (also known as cerebral perfusion) in patients suffering from various cerebrovascular diseases.

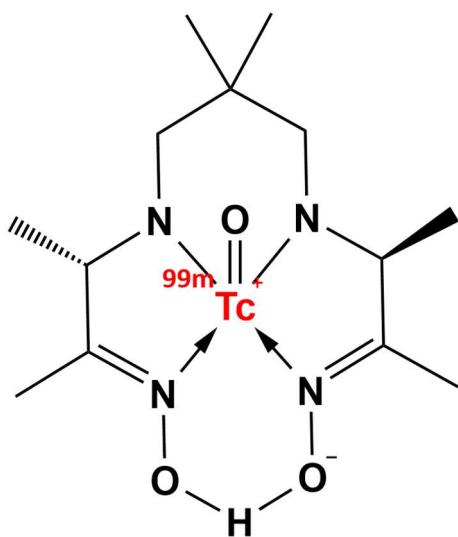


Figure 146: Structure of  $^{99m}\text{Tc}$ -Exametazime

### ***<sup>99m</sup>Tc-ECD***

Another similar agent is <sup>99m</sup>Tc-ECD. It is a neutral, square pyramidal complex containing a <sup>99m</sup>TcO<sup>3+</sup> core and a diaminedithiol ligand (ethylene cysteine dimer or ECD). Similar to previous agent it is a stable and lipophilic complex that can pass into brain *via* passive diffusion. It is used for brain imaging in patients that have had a stroke.

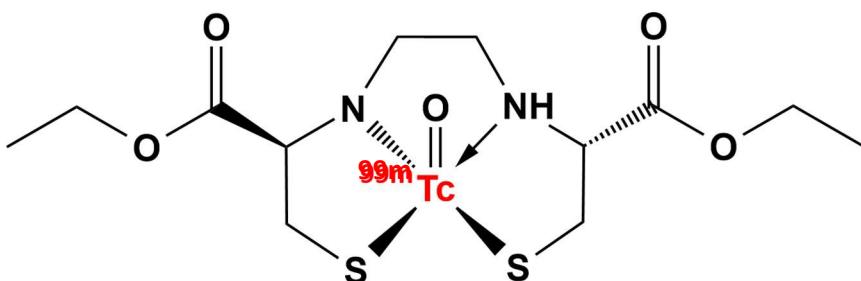


Figure 147: Structure of <sup>99m</sup>Tc-ECD

### **Other agents**

Finally, the last agent to mention is <sup>99m</sup>Tc-sulfur colloid. Opposite to all previous agents that are well defined molecular complexes, this one is not. It is a colloid adduct of <sup>99m</sup>Tc and sulphur molecules S<sub>8</sub>. They are colloid nanoparticles (optimal size range is 15–100 nm) but can be larger. They are obtained by mixing <sup>99m</sup>TcO<sub>4</sub><sup>-</sup> with anhydrous sodium thiosulfate, EDTA, and gelatine: adjusting the pH results in the formation of the <sup>99m</sup>Tc-sulphur colloid. It has many applications, but its pharmacokinetics (where in body it will go) depends on route of administration. It can be used for imaging of breast cancer metastases in lymph nodes, stomach spleen, bone marrow or liver.

### **Advanced <sup>99m</sup>Tc radiopharmaceuticals**

Technetium-99m is an excellent medical radionuclide, it is relatively cheap, and readily available by using <sup>99</sup>Mo/<sup>99m</sup>Tc radionuclide generators. It gives out only gammas rays of ideal energy for the SPECT imaging (140 keV). Moreover, SPECT scanners are cheap instruments, much cheaper than PET scanners and many hospitals can afford it. In total, it is much cheaper and easier to work with <sup>99m</sup>Tc than with positron emitting radionuclides. Unfortunately, classic <sup>99m</sup>Tc radiopharmaceutical complexes are not very sophisticated: they lack pharmacodynamic behaviour typical for PET tracers labelled with <sup>18</sup>F and <sup>11</sup>C, they cannot specifically target any desired receptors like most of <sup>18</sup>F and <sup>11</sup>C agents can. <sup>99m</sup>Tc is a metallic ion and cannot form covalent bonds with carbon, hence cannot be simply attached onto some organic small molecule.

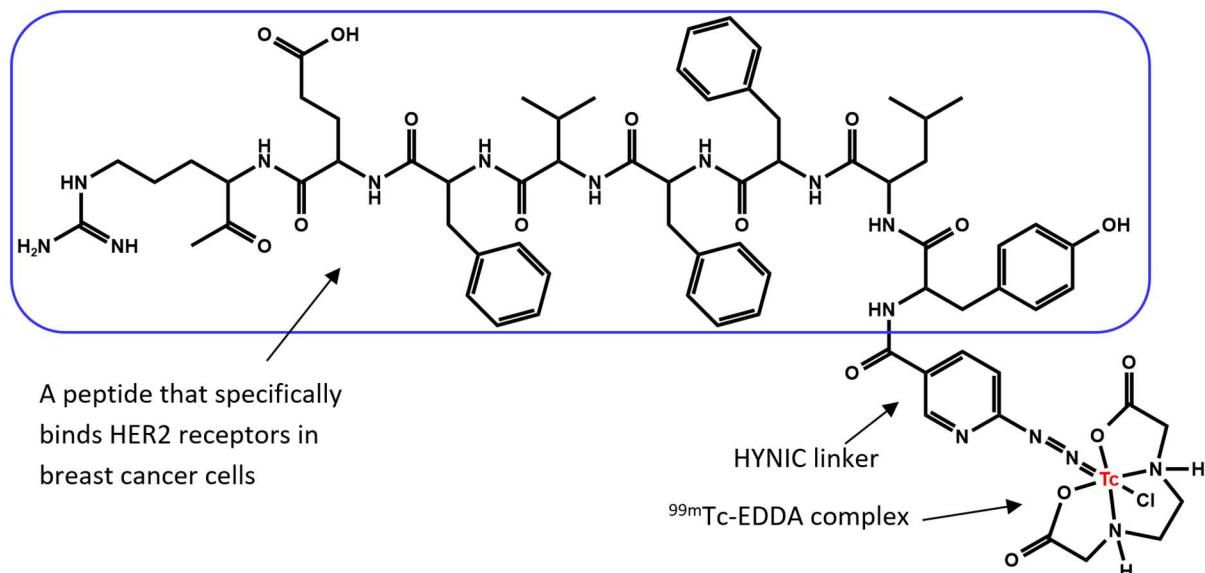
Therefore, it has been a long idea to somehow make vectors for <sup>99m</sup>Tc that will have ability to specifically target certain biological and physiological targets such as specific cancer cells or receptors. This can be achieved using so called

bioconjugates, combination of biomolecules and ligands for  $^{99m}\text{Tc}$  linked via a linker. By using them high level of sophistication can be achieved:  $^{99m}\text{Tc}$  is “caged” by using bisfunctional ligands such as HYNIC, some additional co-chelating ligand and linked onto a biomolecule such as peptide or monoclonal antibody (mAB) that serves as specific vectors with molecular recognition of various targets. These are neutral and molecules (not ions) and can enter cells and brain! We have made some allegories with delivery service and guided missiles in the previous chapters yet, when it comes to bioconjugated complexes of radionuclides such as is  $^{99m}\text{Tc}$  we can make another allegory, this time with a radioactive ion held in a cage and linked onto a pigeon *via* a chain. The pigeon is flying to its destination bringing the radioactive material with itself (Figure 148): the pigeon is a biomolecule searching for its receptor, while the cage with a chain is like a bisfunctional ligand holding  $^{99m}\text{Tc}$ .



**Figure 148: Allegory of a pigeon carrying a cage with a radioactive material in it.**

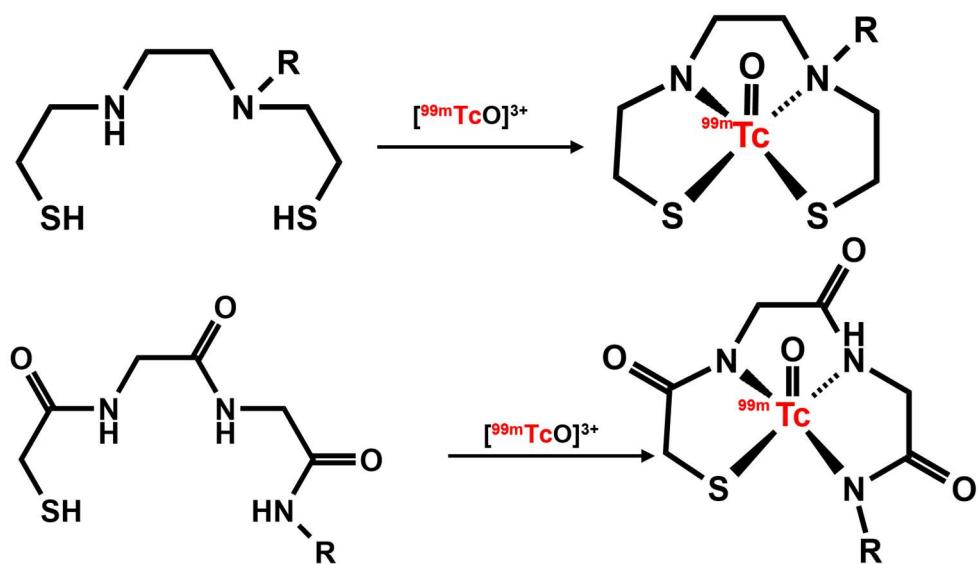
In recent years new, advanced  $^{99m}\text{Tc}$  radiopharmaceuticals are developed where  $^{99m}\text{Tc}$  is usually in Tc(V) or Tc(I) oxidation state and are chelated by advanced so-called bisfunctional ligands containing complexing linkers such as HYNIC and others. Bisfunctional ligands are those chelating  $^{99m}\text{Tc}$  and linking it to some biomolecules with molecular recognition for certain biological targets! This is illustrated on the example of a large  $^{99m}\text{Tc}$ -HYNIC-H6F bioconjugate molecule (Figure 149) that still has no proper name. In this molecule  $^{99m}\text{Tc}$  is complexed by a special HYNIC monodentate ligand, but also contains co-ligand EDDA that is actually a glycine dimer, and a chloride ion. Whole complex is linked *via* the HYNIC linker with a short peptide that contains eight amino-acids and has ability to specifically binds to called HER2 receptors, very typical and numerous on the surface of certain breast cancer cells. Hence, we could use this advanced agent for the for imaging of breast cancer and its metastases.



**Figure 149:**  $^{99m}\text{Tc}$ -HYNIC-H6F, advanced agent for imaging of breast cancer

## Chelators for $^{99m}\text{Tc(V)}$

As explained previously  $^{99m}\text{Tc(V)}$  can be easily made by reduction of pertechnetate by using  $\text{Sn}^{2+}$  and it can be in the form of  $\text{TcO}^{3+}$ ,  $\text{TcO}_2^{+}$ ,  $\text{TcN}^{3+}$ . Also, it can be in the form of a special complex with a ligand named HYNIC. There are many chelators for  $^{99m}\text{Tc(V)}$  but most of them are multidentate and contains more than one electron-donor atoms such as nitrogen or sulphur. For example, two typical are bis(aminoethanethiol) (or BAT), mercapto-acetyl-triglycine (or MAG<sub>3</sub>). These ligands can be conjugated to other molecules as shown and serve as "cages" and chelators to carry  $^{99m}\text{Tc(V)}$  and conjugate it to biomolecules. Another very important already mentioned ligand for  $^{99m}\text{Tc(V)}$  is hydrazinonicotinamide or HYNIC.



**Figure 150:** Complexation of BAT (up) or MAG<sub>3</sub> (down) with [<sup>99m</sup>TcO]<sup>3+</sup> ion

HYNIC (hydrazinonicotinamide) bifunctional ligand was created as a convenient method for radiolabelling biomolecules with  $^{99m}\text{Tc}$ . The reactive succinimide of

HYNIC can be readily conjugated to peptides and proteins, and the hydrazine donor moiety can form stable metal-nitrogen multiple bonds to complex  $^{99m}\text{Tc}$ . The succinimide part of the molecule is very reactive and quickly reacts with amino groups in peptides or proteins forming a bioconjugate. A wide range of biomolecules can be labelled using HYNIC ligand as a linker.

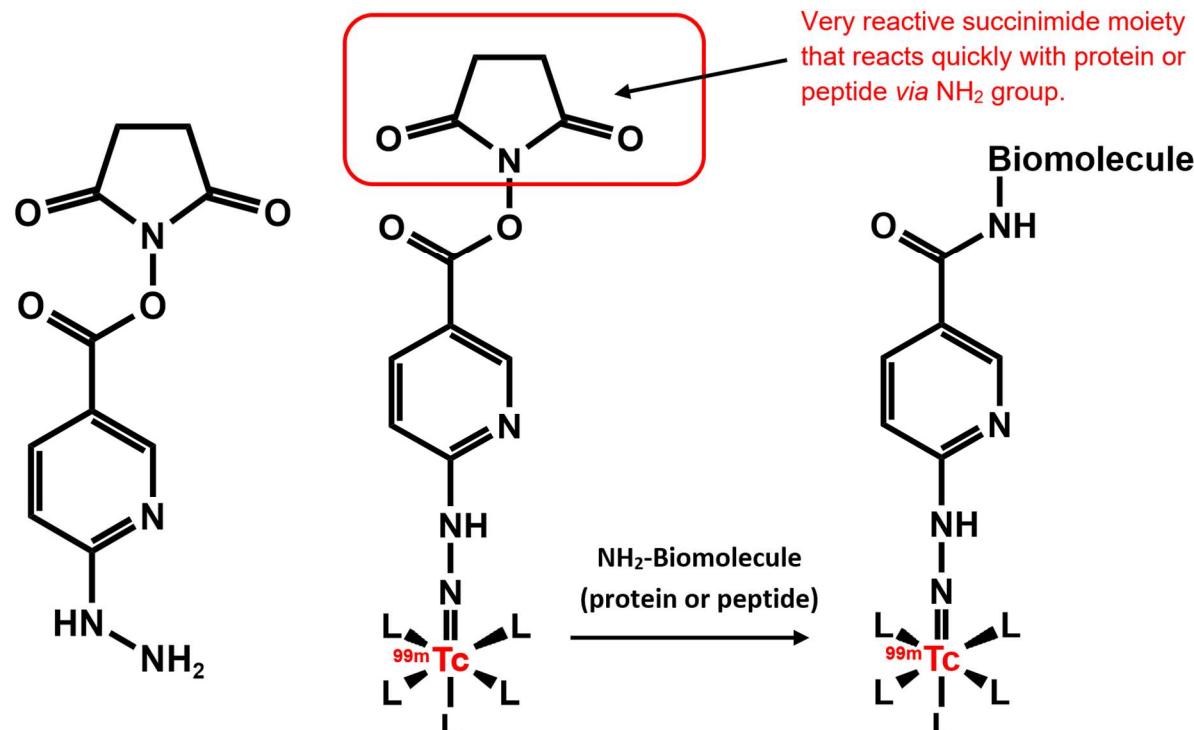


Figure 151: HYNIC linker (left) forms a complex with  $^{99m}\text{Tc}$  and a co-ligand and then its ester part reacts (right) with a biomolecule forming a bioconjugate.

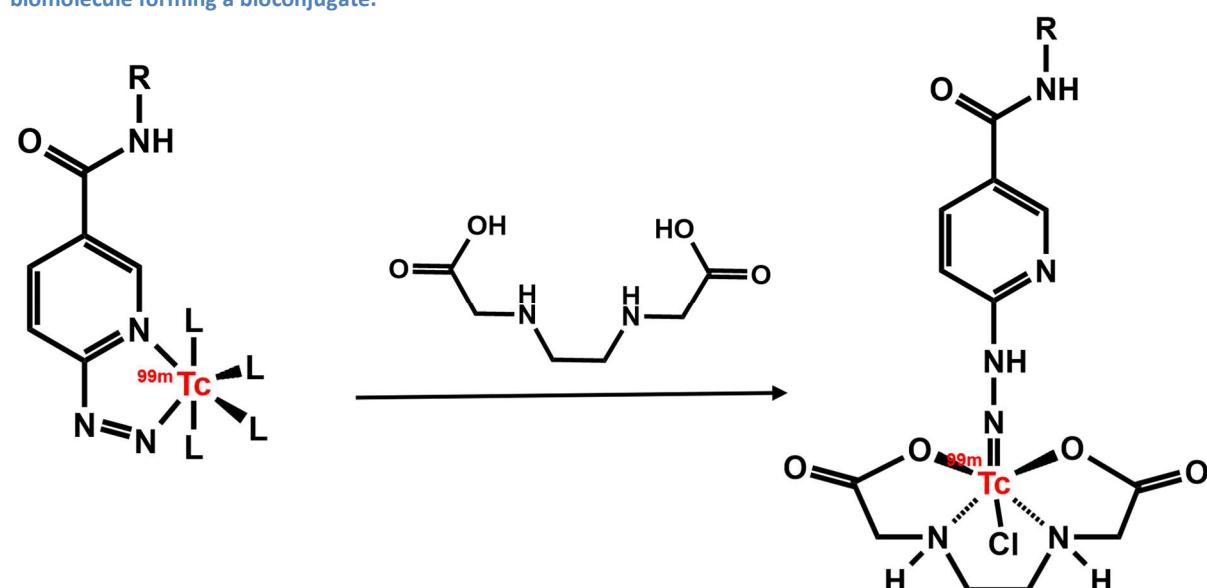


Figure 152: HYNIC linker (left) forms a complex with  $^{99m}\text{Tc}$  and a co-ligand EDDA

To occupy the remaining coordination sites, HYNIC, which can act as a monodentate ligand or a bidentate ligand with the added coordination of its pyridine nitrogen, requires co-ligands. These co-ligands create a convenient handle for fine-tuning the pharmacokinetic properties of the imaging agent by varying the polarity and charge

of the additional ligands. Some of the typical co-ligands are EDDA (ethylene-N,N'-diglycine), tricine, some phosphine ligands but also chloride ions and water can occupy the rest of the coordination space around technetium atom (Figure 152).

### Chelators and complexes of Tc(I)

Another important oxidation state of technetium used for the advanced, bioconjugated radiopharmaceuticals agents is Tc(I). Precisely, it is used in the form of  $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  ion that can easily release three water molecules and be chelated with various chelating linkers. Typical example is illustrated in the Figure 154 where  $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  ion reacts with a tridentate ligand-biomolecule conjugate and forms a mixed complex. Ligands used for the complexation of Tc(I) usually have at least one functional group that releases hydrogen and forms a neutral complex with Tc(I), while other electron-donors are electrically neutral. The three carbonyls usually remain on the complex (Figure 153).

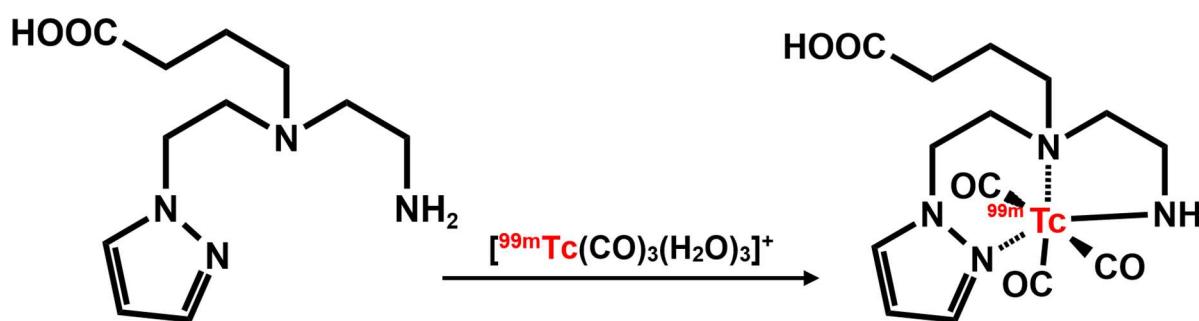


Figure 153:  $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  ion forms a complex with a tridentate ligand

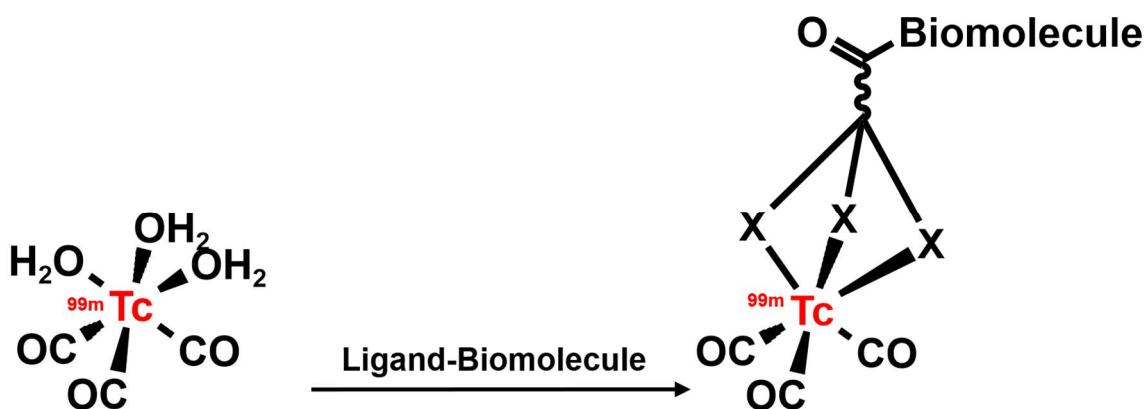
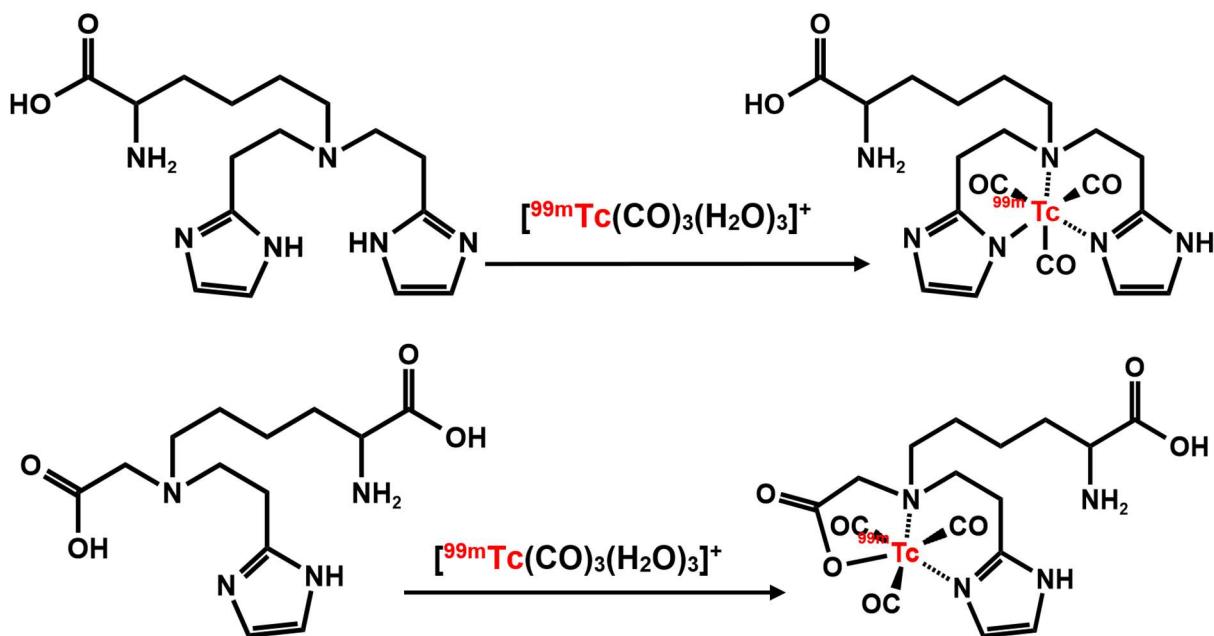
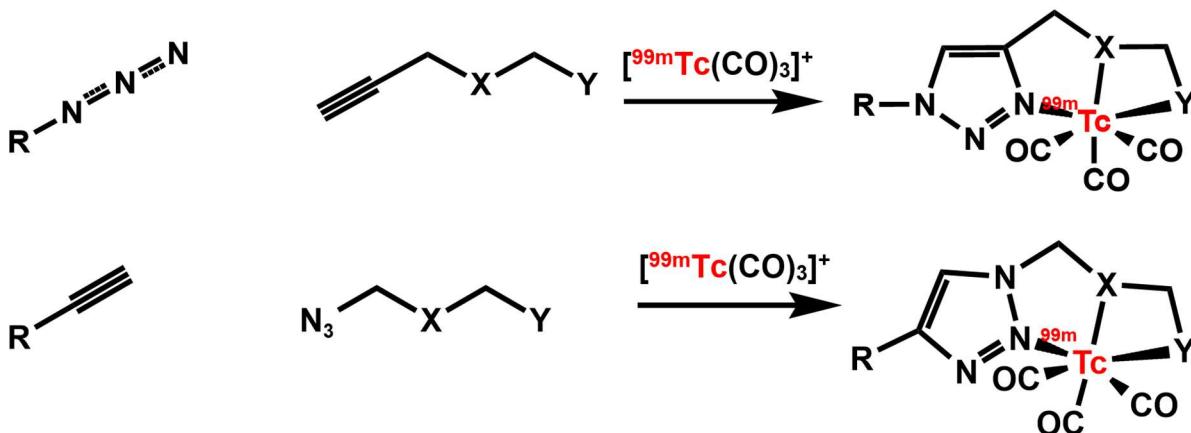


Figure 154:  $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  ion forms a complex with a tridentate ligand conjugated with a biomolecules; X can be N, O, S, or P atoms, while a biomolecule can be a protein or a peptide.

Figure 155 shows two more examples of chelating linker ligands for  $^{99m}\text{Tc}(\text{I})$ : one has two imidazole moieties whereby one hydrogen is gone, and nitrogen makes bond with technetium, and in another case one is imidazole, and another is carboxylic acid.

Figure 155:  $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  ion forms complexes with tridentate ligands

Another very interesting form of complexation is the direct complexation while assembling the ligand. It can be achieved using so called Click-reaction in presence of  $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ : the Click-reaction is a nickname for Huisgen cycloaddition reaction between azides and alkyne moieties (Figure 155b). It is catalysed by Cu(I) to form a triazole ring and is also known as copper-catalysed azide-alkyne cycloaddition or “CuAAC”. It can be so called normal click reaction or invert click-reaction.

Figure 155b:  $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$  ion forms complexes via Click reaction.

## Next generation of $^{99\text{m}}\text{Tc}$ radiopharmaceuticals

Three examples of new, “next-generation” radiopharmaceuticals will be shown. These new, sophisticated, and still experimental  $^{99\text{m}}\text{Tc}$ -radiopharmaceuticals are promising bioconjugates for cheap and specific imaging of cancers (metastases) and many other diseases. These will be an agent for imaging of prostate cancer cell, an agent for imaging of neuroendocrine tumours and, at the end, agent for imaging of angiogenesis.

### $^{99m}\text{Tc}$ -trofolastat

$^{99m}\text{Tc}$ -trofolastat (Figure 156) is a bioconjugate with  $^{99m}\text{Tc}$  for imaging of prostate cancer cells (including distant metastases). Here  $[^{99m}\text{Tc}(\text{CO})_3]^+$  ion is in complex with a bisimidazole chelating ligand, and the whole complex is then conjugated with small peptides that can specifically bind a protein called PSMA (prostate-specific membrane antigen) that can be specially found on prostate cancer cells. Here we can see the concept of caged radionuclide linked onto a vector that is taking it to a specific place.

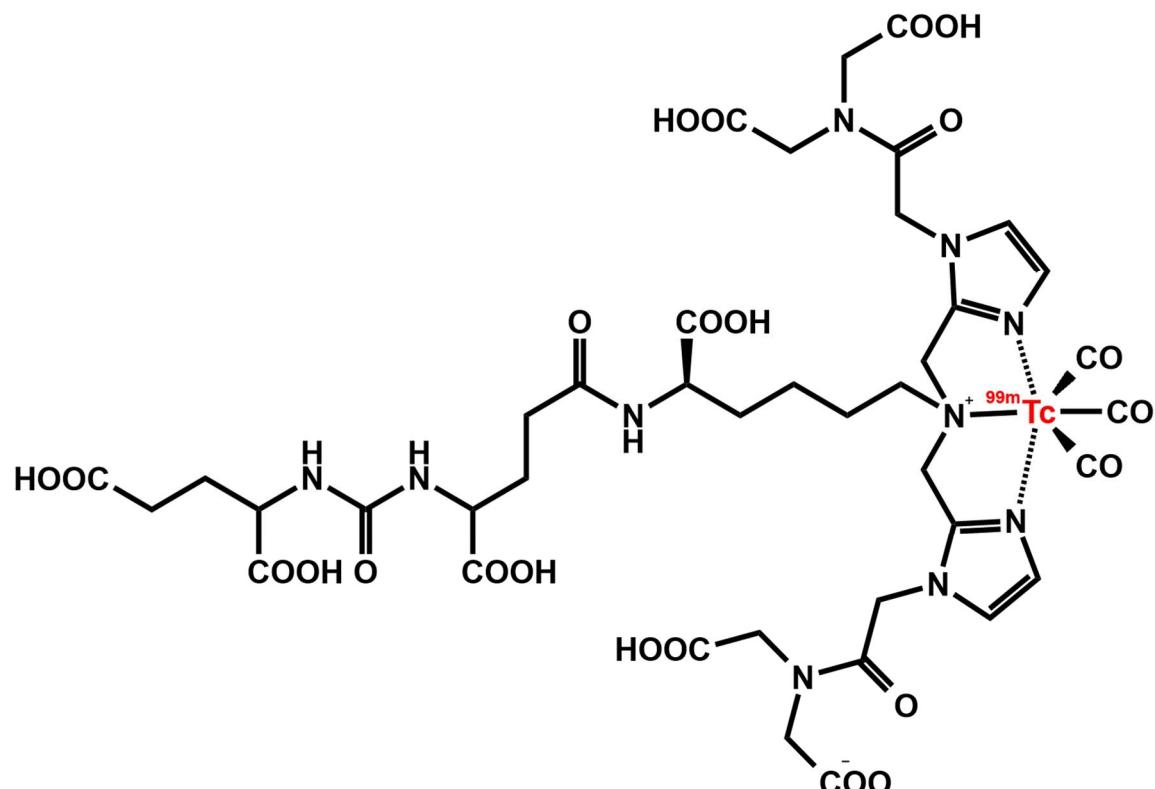
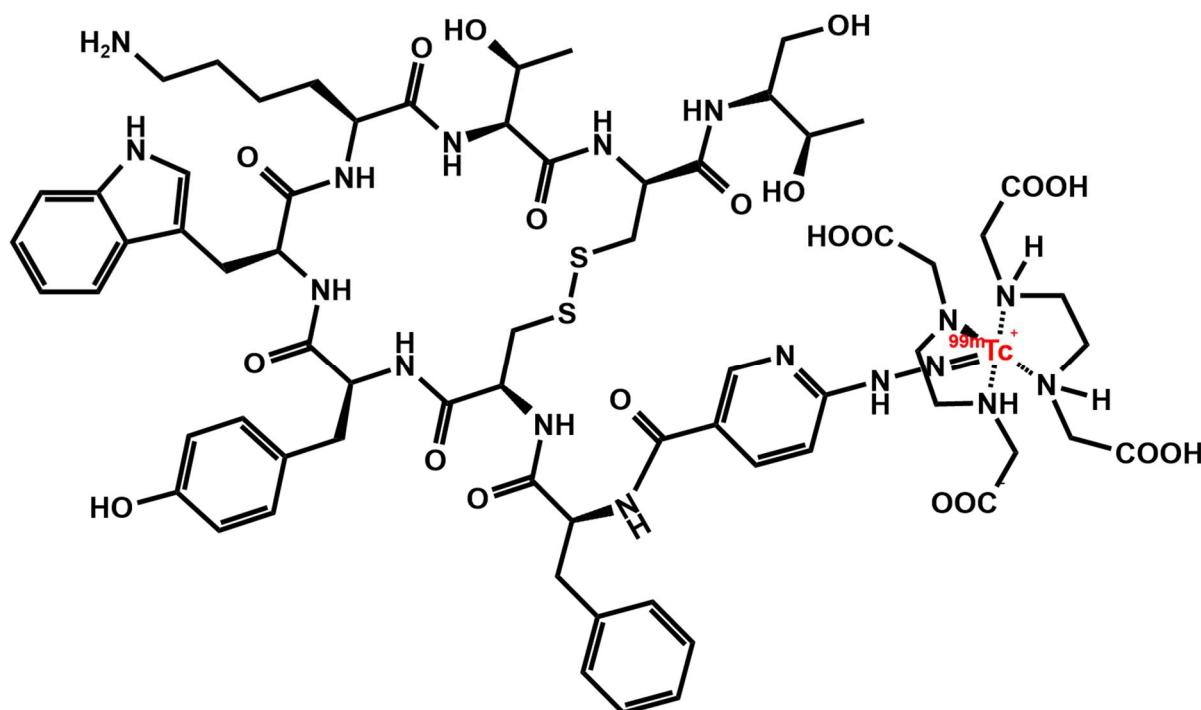


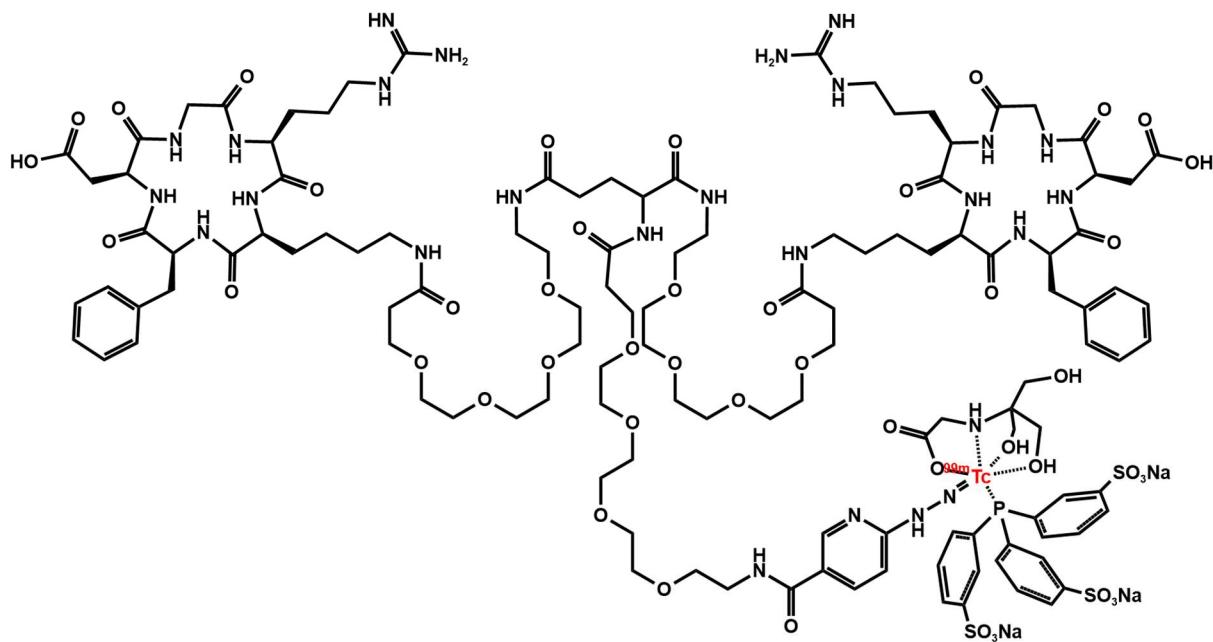
Figure 156: Structure of  $^{99m}\text{Tc}$ -trofolastat

### $^{99m}\text{Tc}$ -EDDA/HYNIC-TOC

Another example is a bioconjugate with  $^{99m}\text{Tc}$  for imaging of neuroendocrine tumours. Here we have  $^{99m}\text{Tc}$  in oxidation state (+5) complexes by HYNIC and two EDDA (ethylene diamine diacetic acid) co-ligands and then the whole big complex is linked with a cyclic peptide called tyrosine-octreotide (or TOC) that acts as analogue of somatostatin.

Figure 157: Structure of  $^{99\text{m}}\text{Tc}$ -EDDA/HYNIC-TOC **$^{99\text{m}}\text{Tc}$ -3PGRD<sub>2</sub>**

Finally, the last example is a very complicated molecule called  $^{99\text{m}}\text{Tc}$ -3PGRD<sub>2</sub>: a bioconjugates with  $^{99\text{m}}\text{Tc}$  for imaging of angiogenesis, formation of new blood vessels. It contains many elements one main ligand (HYNIC) two co-ligands and a very large vector 3PGRD<sub>2</sub>.

Figure 158: Structure of  $^{99\text{m}}\text{Tc}$ -3PGRD<sub>2</sub>

But what is angiogenesis and why is it important to make images of it? It is a process of growing small new blood vessels around newly formed tissues or in order to repair



inflamed tissues. But it is also typical for places where cancer grows, metastases and some other diseases like rheumatoid arthritis. Therefore, this agent can be used for cancer imaging and imaging of some other diseases like rheumatoid arthritis. As you can see it is a very complicated molecule and is still in clinical trials. As the image shows,  $^{99m}\text{Tc}$  is in a complex with HYNIC ligands, one tricine co-ligand, and one TPPTS, a phosphine-based organic ligand. This large complex is conjugated with a very long chain with two prongs, made mostly of three biocompatible polyethylene glycols. At the end of each prong are cyclic peptides. It is these two peptides that are vectors, binding into receptors typical for angiogenesis process.

### **$^{99m}\text{Tc}$ – the “medical radionuclide”**

It is important to remember that 70-80% of all nuclear medicine procedures in the world is done with  $^{99m}\text{Tc}$ . The reasons are not only historical, but because it is very simple and easy to get  $^{99m}\text{Tc}$  by milking a radionuclide generator and to do labelling using a kit, while cyclotrons are not so available and expensive to operate. However, as cyclotrons are becoming more available that may change. There are many advantages of  $^{99m}\text{Tc}$  in nuclear medicine. Firstly,  $^{99m}\text{Tc}$  has gamma emission only, there is no radiation damage from alpha or beta particles. Secondly, those gamma photons are of 140 keV and this energy is an ideal for scintillator detectors. Next, technetium has a good and rich chemistry, and finally, usage of  $^{99m}\text{Tc}$  from radionuclide generators is very easy and practical.  $^{99}\text{Mo}$  produced in reactors is processed and packed into radionuclide generators and sent to many smaller hospitals, while hospitals can cheaply buy them and have simple and convenient source of  $^{99m}\text{Tc}$  for 2 weeks. On the other hand, there are some disadvantages: after all, technetium is a transition metal and cannot make very stable covalent bonds, cannot be so elegantly attached to most vectors like  $^{18}\text{F}$  or  $^{11}\text{C}$  can, it needs a chelation “cage”. Complexes with  $^{99m}\text{Tc}$  are not very “drug-like” and for sophisticated targeting they require a secondary vector (for example a peptide). The  $^{18}\text{F}$  and  $^{11}\text{C}$  radiopharmaceuticals are much more versatile and have much better targeting than those of  $^{99m}\text{Tc}$ .

## Chapter IX - Fluorine-18 ( $^{18}\text{F}$ )

Due to its favourable nuclear decay properties, the positron-emitting fluorine-18 ( $^{18}\text{F}$ ) is the most important radionuclide for radiopharmaceuticals used for positron emission tomography (PET) and along with  $^{99\text{m}}\text{Tc}$  is one of the most important radionuclides in nuclear medicine. Most of PET scans are undertaken with some kind of  $^{18}\text{F}$  radiotracer and in vast number of cases it is  $^{18}\text{F}$ Fluorodeoxyglucose or  $^{18}\text{FDG}$ .

### Isotopes of fluorine

Fluorine itself has 18 known isotopes ranging from  $^{13}\text{F}$  to  $^{31}\text{F}$ , but only  $^{19}\text{F}$  is stable. The isotopes having  $A > 19$  are mostly beta ( $\beta^-$ ) emitting, and are very short-lived; the longest lived has half-life just few seconds. On the other hand,  $^{17}\text{F}$  and  $^{18}\text{F}$  are positron ( $\beta^+$ ) emitting, but only  $^{18}\text{F}$  has the half-life in minutes, long enough to be practical for nuclear medicine. It is interesting to note that  $^{16}\text{F}$  and  $^{15}\text{F}$  are emitting protons, while isotopes  $^{28}\text{F}$  and  $^{30}\text{F}$  are emitting neutrons! Nevertheless, only  $^{19}\text{F}$  is natural, while all the other fluorine isotopes are artificial (Figure 159).

$^{15}\text{F}$	$^{16}\text{F}$	$^{17}\text{F}$	$^{18}\text{F}$	$^{19}\text{F}$	$^{20}\text{F}$	$^{21}\text{F}$	$^{22}\text{F}$	$^{23}\text{F}$	$^{24}\text{F}$	$^{28}\text{F}$	$^{30}\text{F}$
Emit protons	Emit positrons	Stable			Emit betas (electrons)					Emit neutrons	

Figure 159: Selected isotopes of fluorine

### Nuclear properties of $^{18}\text{F}$

$^{18}\text{F}$  is the longest-lived radioisotope of fluorine; its half-life is 109.739 minutes, which means 1 hour and 50 minutes. Some people like to roughly round the half-life of  $^{18}\text{F}$  as two hours. It overwhelmingly decays (Figure 160) to  $^{18}\text{O}$  by positron emission ( $\beta^+$ , 96.9%) and just in very small percentage by electron capture (3.14%).

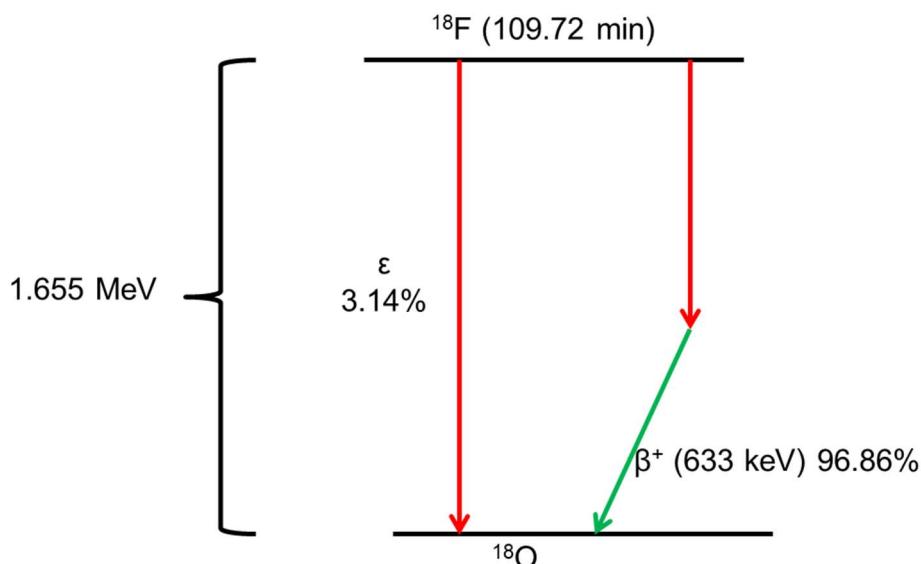
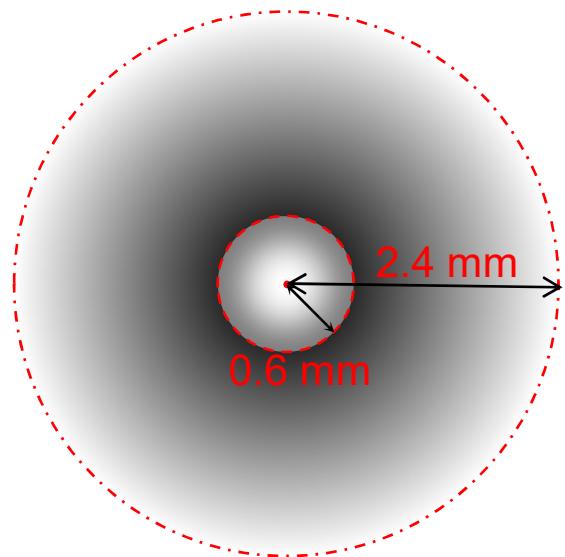


Figure 160: Decay of  $^{18}\text{F}$

The emitted positrons ( $\beta^+$ ) are behaving just like beta ( $\beta^-$ ) particles but are of positive charge: they do not have distinct energy but have rather spectrum of energies where the mean energy ( $E_{\text{mean}}$ ) is 250 keV and only minimal number of positrons have maximal energy ( $E_{\text{max}}$ ) of 634 keV. These energies can be extrapolated into a distance (in cm) the positrons are traveling (in any direction) from the point of emission: most of positrons can fly in any random direction in water or human tissue 0.6 mm, and this is the mean distance, while only the most energetic positrons fly as far as 2.4 mm (Figure 161). In general, these emitting characteristics are very favourable meaning that the uncertainty of emission location is low. This is why positrons emitted by  $^{18}\text{F}$  are able to give high-resolution PET images. In general, lower the energy of positrons extrapolates into shorter the mean flying distance of the positron, lower uncertainty, and higher resolution of PET images.



**Figure 161:** Sphere of probability of how long a positron emitted by  $^{18}\text{F}$  will travel until it encounters an electron and undergoes annihilation. At the same time it represents the sphere of probability where annihilation photon will be emitted comparing to the location of  $^{18}\text{F}$  nucleus.

Therefore, the most of positrons will be flying into tissue 0.6 mm, and by then will most probably encounter an electron. Electron and positron are the particle-antiparticle pair. Once they meet, they undergo annihilation (Figure 162) whereby both are converted into two gamma photons each emitted in exactly opposite directions. The photons are exactly of 511 keV and all annihilation photons are always of 511 keV, this is very typical for annihilation photons no matter what was the radionuclide that emitted positrons and what was the energy of positrons. The detected coincidence of these photons is the base of PET imaging: instrument is able to calculate the exact position from where photons are emitted but not the position from where positron was emitted: this is uncertainty. However in most of the cases it is somewhere in the radius of 0.6 mm from where annihilation happened and photons were emitted.

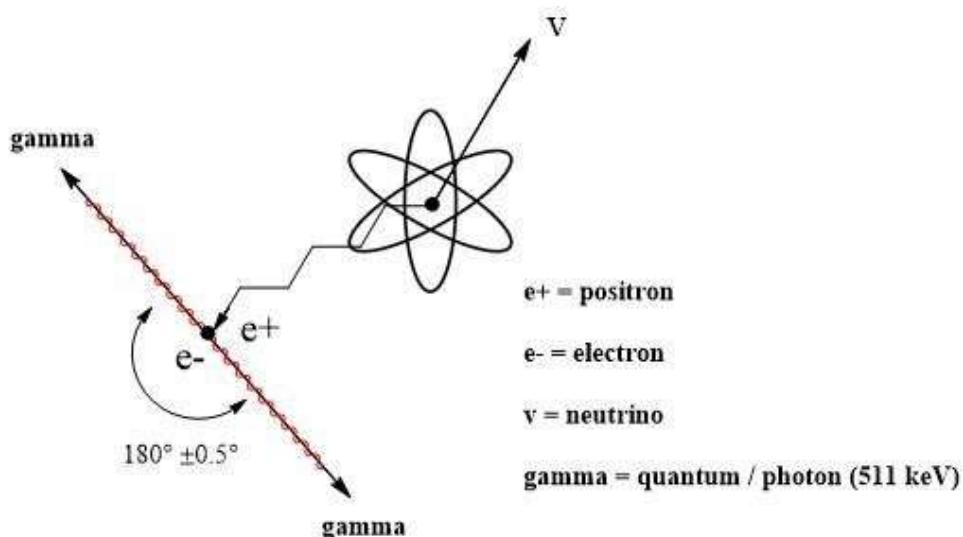


Figure 162: Annihilation process creates two antiparallel gamma rays

## Production of $^{18}\text{F}$

How  $^{18}\text{F}$  is produced? It is produced in cyclotrons, by bombarding targets (gas or liquid) with protons. In fact,  $^{18}\text{F}$  is the most important and most common radionuclide made by medical cyclotrons. There are small medical cyclotrons that are optimised just for the production of  $^{18}\text{F}$ . There are two main forms for produced  $^{18}\text{F}$ , elemental gas in the tandem with stable the  $^{19}\text{F}$  (**molecule  $^{18}\text{F}-\text{F}$** ) or the free fluoride ion ( $^{18}\text{F}^-$ ).

When elementary form,  $^{18}\text{F}-^{19}\text{F}$  gas is made,  $^{18}\text{F}$  is in a molecule with a stable  $^{19}\text{F}$  atom. The stable fluorine serves as a carrier of the radioactive one, therefore the specific activity is halved, 50% of fluorine is the stable, non-radioactive fluorine. This form of  $^{18}\text{F}$  could be made by bombarding  $^{20}\text{Ne}$  with deuterons ( $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$ ) and the target is therefore also a gas. This method was the first one  $^{18}\text{F}$  was ever made. However, it is considered as not so efficient way of making  $^{18}\text{F}$ . Another option is bombardment of highly enriched  $^{18}\text{O}_2$  gas in a gas target chamber with protons ( $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ ). This is a bit more efficient way of making  $^{18}\text{F}$ . In these methods small amounts if non-radioactive  $\text{F}_2$  are added and then  $^{18}\text{F}-^{19}\text{F}$  molecules are obtained. The problem with this method is that it gives product of quite low specific activity (0.05-0.5 GBq/ $\mu\text{mol}$ ).

Another and much more common (the main) way of making  $^{18}\text{F}$  is to make free fluoride ion ( $^{18}\text{F}^-$ ), i.e. free from any other fluorine ("no carrier added", or n.c.a.  $^{18}\text{F}$ ). This is achieved by bombarding liquid water enriched with  $^{18}\text{O}$  with protons. In this way very high specific activity of  $^{18}\text{F}$  can be obtained (5000 GBq/ $\mu\text{mol}$ ) For example, production of up to 500 GBq of  $^{18}\text{F}^-$  fluoride can be achieved using irradiation for 120 min at 18 MeV with a beam current of 145  $\mu\text{A}$ . This is in fact the most common nuclear reaction in the medical cyclotrons.

## Chemical properties of fluorine

When it comes to the synthesis of radiopharmaceuticals the chemical properties of a radionuclide are in fact even more important for radiochemistry. Different from  $^{99m}\text{Tc}$  that is a transition metal, fluorine is a non-metal, a halogen gas in the topmost position in the periodic table (Figure 164). It possesses some extreme properties: it is the most electronegative element and has the highest oxidation potential. It is very corrosive and reactive when in elemental form. Because of its high electronegativity, introduction of a fluorine atom into a molecule can have significant effects on the physicochemical properties of the compound: for example, the presence of fluorine can shift the  $\text{pK}_\text{a}$  values of nearby acidic and basic functional groups by several log units. Moreover, it can also change the pharmacodynamic and pharmacokinetic properties of the molecule: it can change the way molecules behaves in the body.

1 H															2 He		
3 Li	4 Be																
11 Na	12 Mg																
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og
57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb				
89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No				

Figure 164: Place of fluorine in the Periodic table.

Fluorine is a monovalent halogen element, and it forms a covalent and stable bond with carbon atoms (C-F). When covalently bound to carbon, fluorine atom has van der Waals radius of 1.47 Å. This is larger than that of a hydrogen atom (1.2 Å), but smaller than methyl, amino, or hydroxyl groups. It can form hydrogen bonding (Figure 165) with hydrogen (F...H-O). It was observed that the replacement of a hydrogen atom with fluorine can reduce the basicity of the compound, leading to increased lipophilicity and modifying its physiological behaviour. Fluorine can enhance the binding affinity of a compound for its target or even improve the metabolic stability by blocking metabolically labile sites. Fluorine is never present on any natural biochemical compounds. Amino acids, sugars, neurotransmitters, hormones or fatty acids do not contain fluorine: therefore, the introduction of  $^{18}\text{F}$  may significantly affect their physiological behaviour.

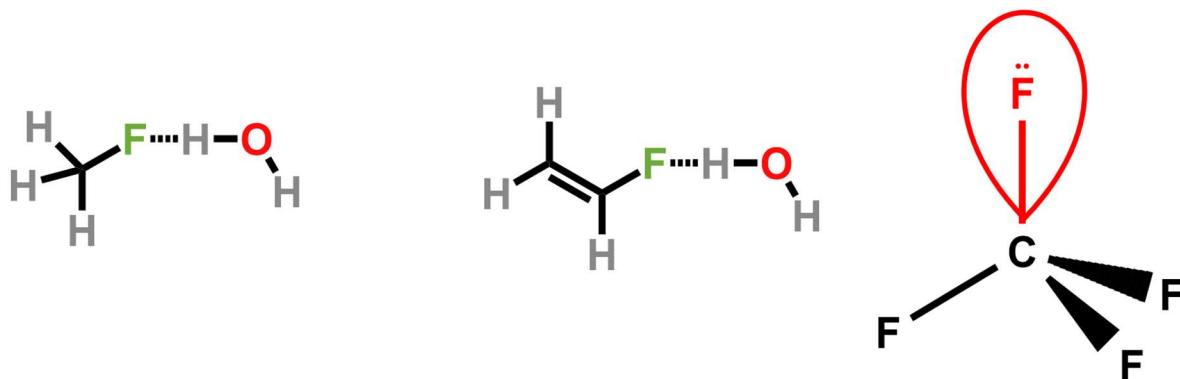


Figure 165: Fluorine forms strong hydrogen bonds

Labelling of any molecule with fluorine is called fluorination while in the case of  $^{18}\text{F}$  it is called or **radiofluorination**. Generally, there are two main types of labelling reactions for  $^{18}\text{F}$ . The first are nucleophilic substitutions (or nucleophilic radiofluorinations). Those are done at electron-depleted carbon atoms and are usually performed using free, non-carrier-added  $^{18}\text{F}^-$  (radiofluoride ion) made in cyclotrons using the liquid target. Another type are electrophilic substitutions (or electrophilic fluorinations), done at electron-rich carbon atoms. For these reactions one needs to use gas  $^{18}\text{F}-\text{F}$ , and due to low specific activity of  $^{18}\text{F}-\text{F}$  these are challenging labelling synthetic procedures.

### $^{18}\text{F}$ pre-processing

Before nucleophilic radiofluorinations are done  $^{18}\text{F}^-$  ion made from water needs to be pre-processed! The reactions with fluoride ions are challenging, and fluoride ion is not very good for nucleophilic substitutions. While in water fluoride ion is highly hydrated due to its high charge density and cannot react (Figure 166). Moreover, it can be easily protonated with hydrogen ion (in fact proton) to make hydrofluoric acid (HF): water or proton-donating functional groups such as -OH, -COOH, -NH<sub>2</sub>, -SH will give their hydrogen to form HF, although some tertiary alcohols may not cause troubles. As the rule, hydrofluoric acid or hydrated fluoride ions will not react in nucleophilic substitution reactions.

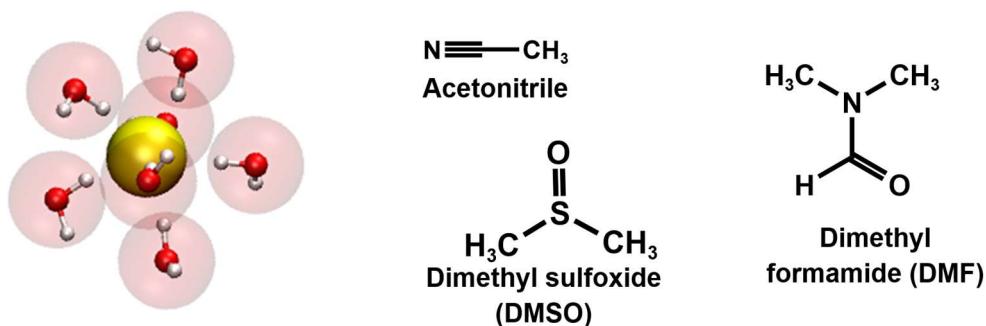


Figure 166: Fluoride ion (yellow) surrounded with water molecules (left), polar, aprotic organic solvents suitable for fluorination reactions, acetonitrile, DMSO and DMF (right)

Therefore, fluoride ion needs to be liberated from aqueous (water) medium and transferred into some very dry, polar “aprotic” solvents such as DMF, DMSO or acetonitrile (Figure 166 right). But here is a problem! Fluoride ion generally is not very soluble in such solvents and tends to precipitate unless it is somehow solubilised. It needs so-called “phase transfer agent”, a molecule that will make it soluble and it can be some tetraalkylammonium salt but the best phase transfer agent is a large macrocyclic compound, a cryptand called “Kryptofix 2.2.2.” or “K.2.2.2”: it can complex potassium counter ion and help dissolve fluoride in polar aprotic solvents (Figure 167).

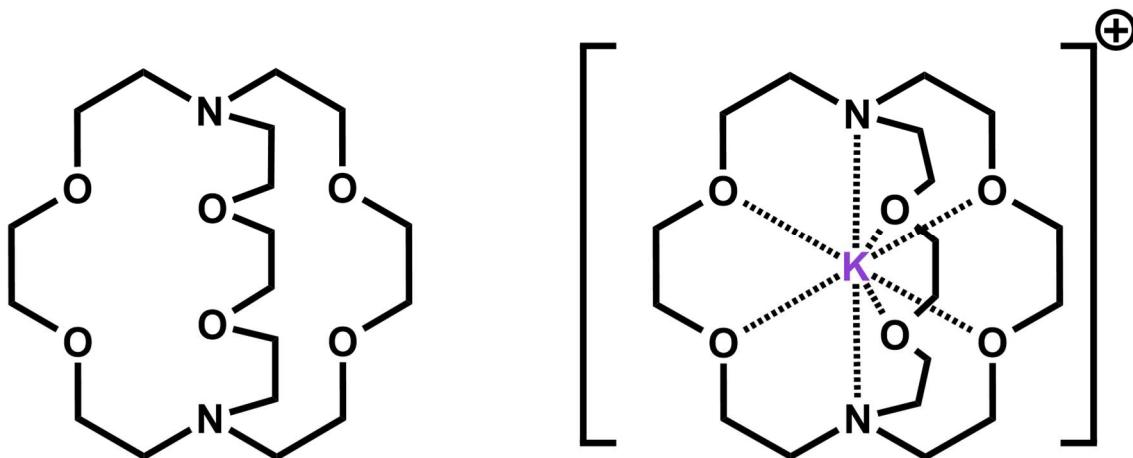


Figure 167: Structure of a macrocyclic molecule (cryptand) named Kryptofix 2.2.2 without (left), and with complexed potassium ion (right)

In practice, fluoride ion from cyclotron is firstly adsorbed onto some ion-exchange column, then eluted out using solvents such as acetonitrile that contains dissolved base (such as  $\text{K}_2\text{CO}_3$ ) with a phase transfer agent Kryptofix 2.2.2 (Figure 167) or sometimes denoted as K2.2.2. Solution is then dried to remove all the remaining water. The result is “naked” fluoride ion solubilised in acetonitrile and able to undergo nucleophilic substitutions. Any presence of free proton-donor functional group ( $-\text{OH}$ ,  $-\text{NH}_2$  or  $-\text{COOH}$ ) in the substrate may protonate  $^{18}\text{F}^-$ , form HF, spoil the nucleophilic properties of fluoride and diminish the yield.

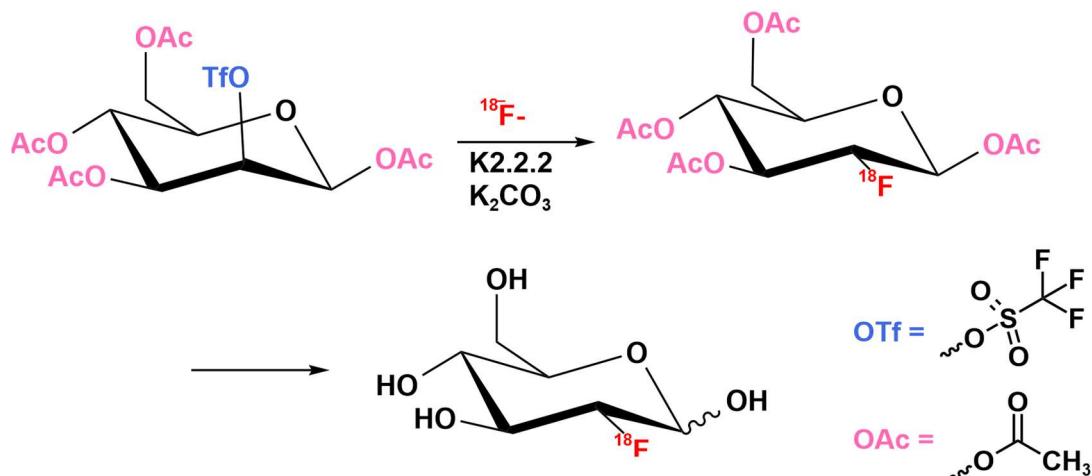
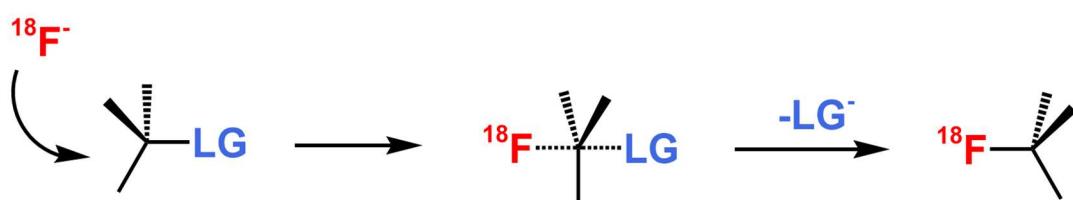


Figure 168: Synthesis of  $^{18}\text{FDG}$

Therefore, these groups, if present, need to be protected using some appropriate protecting group (*tert*-butyloxycarbonyl or BOC, acetyl, etc.) and later needs to be removed. This is best illustrated in the synthesis of  $^{18}\text{FDG}$  with  $^{18}\text{F}$  fluoride and in presence of K.2.2.2 and potassium carbonate (Figure 168): all –OH groups in the substrate (glucose derivative) are protected with the acetyl group and after the labelling reactions acetyl groups are removed and FDG is made.

### Aliphatic nucleophilic $^{18}\text{F}$ -substitutions

Aliphatic nucleophilic  $^{18}\text{F}$ -substitutions are the most common radiofluorinations. They can be  $\text{S}_{\text{N}}1$  or  $\text{S}_{\text{N}}2$  mechanism but  $\text{S}_{\text{N}}2$  is more common. During  $\text{S}_{\text{N}}2$  mechanism fluoride attacks the electron-depleted carbon atom and the leaving group (LG) leaves the compound:



During this mechanism inversion of configuration (or Walden inversion) happens, molecule changes chirality, from S into R (L into D and vice versa). However, this reaction needs a good leaving group (LG). It can be some other halogen such as Cl, Br, I, but mostly and the most efficient are some types of sulfonyl, like tosyl, nosyl, mesyl, or triflate (Figure 169). Aliphatic nucleophilic substitutions are usually performed at higher temperatures ( $\sim 80\text{--}100\text{ }^{\circ}\text{C}$ ) in polar aprotic solvents like acetonitrile, DMF, DMSO, but addition of *tert*-butanol may work also and actually proved to improve yields.

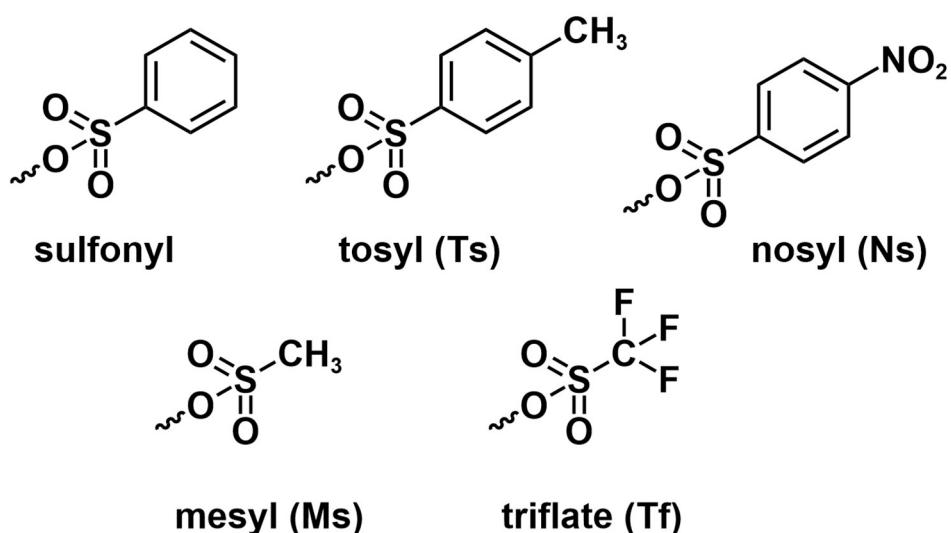


Figure 169: Typical good leaving group used in substrates to be exchanged with  $^{18}\text{F}$  during radiofluorinations

An excellent example of such reaction is the synthesis of  $^{18}\text{FDG}$  ( $^{18}\text{Fluorodeoxyglucose}$ , Figure 168). This is the most important and the most common labelling reaction not only in the area of radiofluorine but also in the whole area of PET tracers, and quite probably the most important in the whole radiopharmaceutical chemistry! The “Ac” in this reaction is the acetyl protecting group capping every oxygen on the molecule, while “OTf” is the triflate or trifluoromethanesulfonate ( $\text{CF}_3\text{SO}_3^-$ ) group, one type of the sulfonyls: it is a standard good leaving group. The first step, labelling reaction, is performed with cryptand “Krypofix 2.2.2.” that helps to dissolve fluoride. This labelling reaction is very quick and efficient, and with high yielding. Immediately after in the next step the de-protection reaction removes acetyl protecting group and radiofluorine-labelled glucose is made.  $^{18}\text{FDG}$  is synonymous with  $^{18}\text{F}$  and is the most important  $^{18}\text{F}$  radiopharmaceutical! It is daily made in all PET radiopharmaceutical centres in the world routinely. In fact, some radiopharmaceutical centres make only FDG! It is used for PET imaging of all tissue that have high glucose metabolism, but mainly cancer cells: cancer cells especially metabolize glucose and are always filled with glucose. Therefore, presence of  $^{18}\text{FDG}$  make cancer cells glow with gamma radiation.

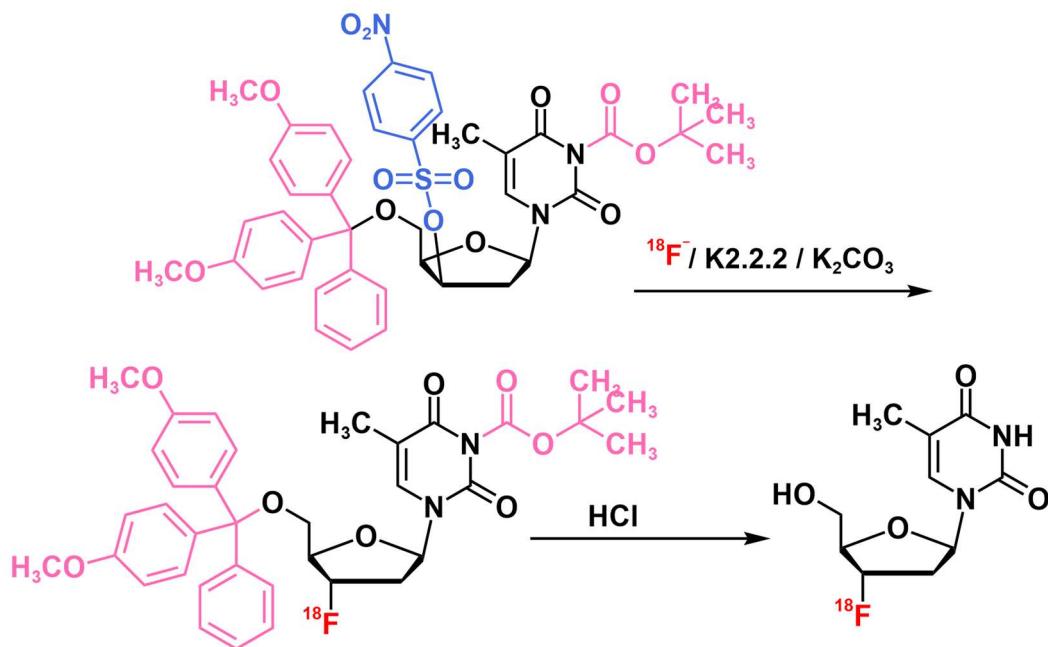


Figure 170: Synthesis of  $^{18}\text{Fluorothymidine}$

The next example is the synthesis of 3'-deoxy-3'- $^{18}\text{Fluorothymidine}$  ( $^{18}\text{FLT}$ , Figure 170). This is, in fact, a radiofluorine-labelled thymidine. Originally, thymidine is one of the ATCG nucleotide bases of DNA, and it is naturally present in our cells, but more in those that are multiplying a lot. Here one can see that the good leaving group is nosyl ( $\text{NO}_2\text{-Ph-SO}_3^-$ ), while all the rose groups are just protecting groups: BOC or tert-butyloxycarbonyl group and DMTr-protecting group. The reactions are very similar as in the previous example: in the labelling reaction the fluoride ion reacts and removes nosyl, while in the next step addition of hydrochloric acid causes de-protection and  $^{18}\text{Fluorothymidine}$  molecule is synthesised. It has similar conditions

like the previous reaction, the only difference is the starting substrate for the fluorination step.  $^{18}\text{F}$ Fluorothymidine is used as the radiotracer for imaging of cancer cells: thymidine is a natural DNA nucleotide and there are usually plenty of it in the cells that multiply a lot, especially cancer cells. Therefore,  $^{18}\text{F}$ -labelled thymidine or  $^{18}\text{F}$ -fluorothymidine is used to monitor cancer, to find how tumours respond to therapy.

Another example is the synthesis of  $^{18}\text{FP-CIT}$  (also known as  $^{18}\text{F}$ Fluoropropyl-CIT). Here the good leaving group is tosyl – just like nosyl and triflate, tosyl is preferred leaving group in aliphatic nucleophilic  $^{18}\text{F}$ -substitutions. However, the medium, solvent here is mixture of acetonitrile and *tert*-butyl alcohol. This goes against the main rule for nucleophilic radiofluorinations that says no free –OH group should be present. However, in this particular case presence of *tert*-butyl alcohol not only makes no trouble, just the opposite, it improves the radiochemical yield. This phenomenon is not yet fully explained, but it works very well in practice.

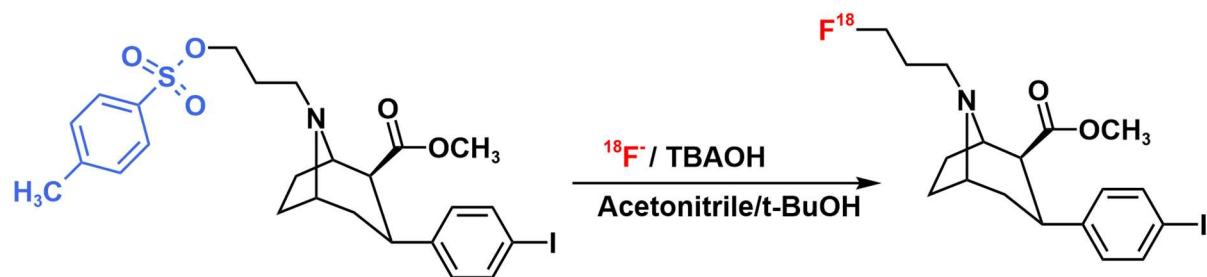


Figure 171: Synthesis of  $^{18}\text{FP-CIT}$

On the other hand, this particular case does not override the general rule that says: no hydroxyl groups should be present in nucleophilic radiofluorinations!  $^{18}\text{F}$ Fluoropropyl-CIT binds a protein in brain called dopamine transporter. This protein is usually located between two neurons in a formation called synaptic cleft. It is interesting to note that the same protein, dopamine transporter, is the target for cocaine.  $^{18}\text{F}$ Fluoropropyl-CIT is in fact an analogue of cocaine. It is also used for imaging of Parkinson's disease (a form of degeneration of neural function).

### Aromatic nucleophilic $^{18}\text{F}$ -substitutions ( $\text{S}_{\text{N}}\text{Ar}$ )

The aromatic nucleophilic  $^{18}\text{F}$ -substitutions are usually done *via* so called  $\text{S}_{\text{N}}\text{Ar}$  (addition-elimination) mechanism. There is one important difference comparing to aliphatic nucleophilic substitutions:  $\text{S}_{\text{N}}\text{Ar}$  mechanism needs not only good leaving group (LG), but also a good electron-withdrawing group on aromatic ring (-R), preferably in the *ortho*- or *para*-position relative to the leaving group.

This electron-withdrawing group pulls the electrons through the aromatic system and creates deficiency of electrons in the aromatic ring (it can be a benzene ring but also some other aromatic rings that contain nitrogen or sulphur such as pyridine). Typical good leaving group for aromatic nucleophilic fluorinations are nitro ( $-\text{NO}_2$ ) group and

positively charged trimethylammonium ( $-[\text{N}(\text{Me})_3]^+$ ) group. These reactions require high temperature and high-boiling point solvents like DMSO, DMF, or *N,N*-dimethylacetamide (DMA).

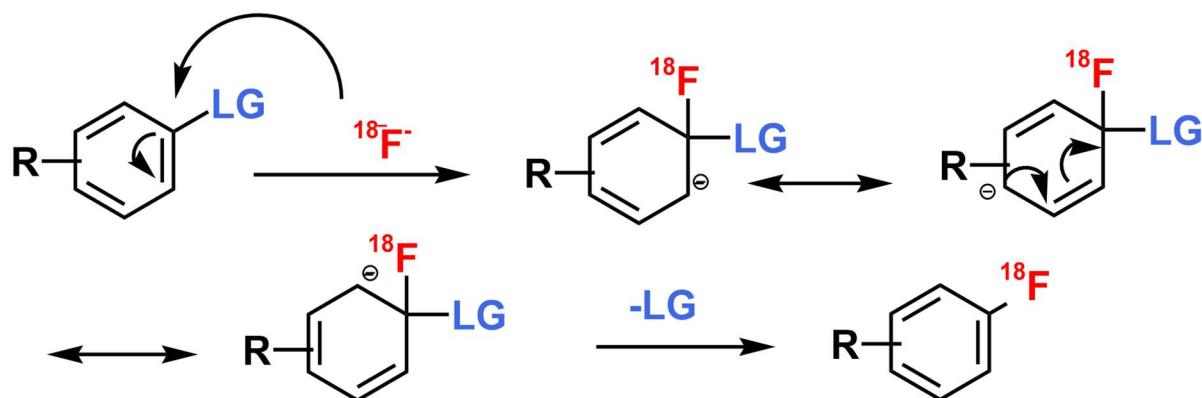


Figure 172:  $\text{S}_{\text{N}}\text{Ar}$  (addition-elimination) mechanism

The first example of such reactions is synthesis of  $^{18}\text{F}$ -labelled altanserin (Figure 173). Here the nitro group is the good leaving group, while the “keto” group in the para-position relative to the leaving group is the good electron-withdrawing group. Therefore, this reaction works very well, and conditions are similar as in aliphatic nucleophilic radiofluorinations.

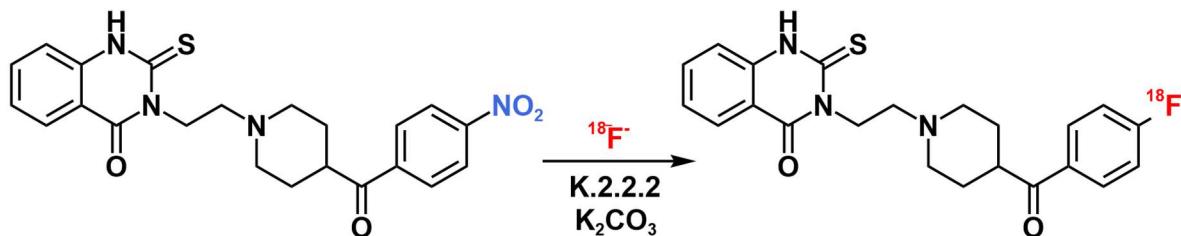
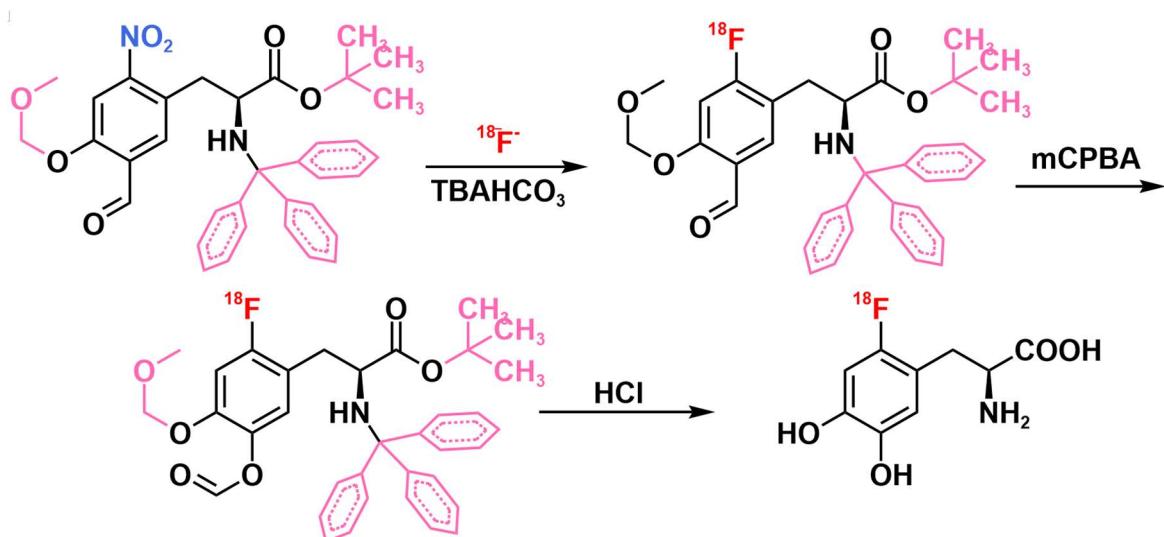


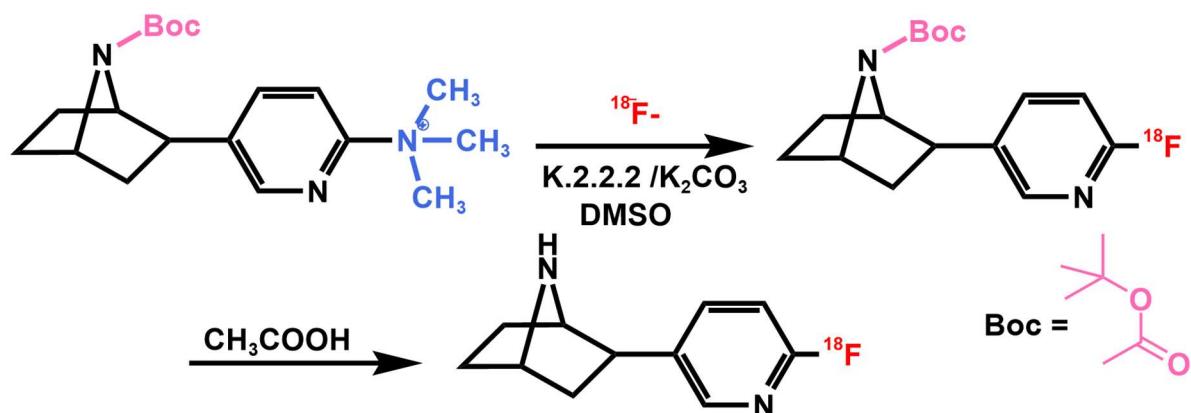
Figure 173: Synthesis of  $^{18}\text{F}$ -labelled altanserin

This was one of the first radiofluorinations *via*  $\text{S}_{\text{N}}\text{Ar}$  reaction mechanism and gives very good yields. This PET radiotracer,  $^{18}\text{F}$ -Altanserin binds co-called serotonin 5-HT<sub>2A</sub> receptors in brain, and it is used for the imaging of numerous neurological and psychiatric conditions and diseases. Interestingly, its non-radioactive analogue fluoroaltanserin doesn't have any pharmacological applications, it is used only with radiofluorine as PET radiotracer.

Another example is telling us how nucleophilic substitutions on aromatic rings are not always easy and straight-forward. For example, in the synthesis of  $^{18}\text{F}$ -labelled DOPA (Figure 174) we use nitro group as the good leaving group but having just hydroxyl groups on the other side is not that good since it is not withdrawing electrons as much as it is needed. Instead, the synthesis starts with a heavily protected substrate that has carbonyl group in the para position opposite to the nitro group.

Figure 174: Synthesis of  $^{18}\text{F}$ -labelled DOPA

DOPA is a molecule that has lots of proton-donating groups, therefore all these groups need protection. Labelling reaction brings  $^{18}\text{F}$  into the molecule and nitro group leaves. After the labelling the good electron-withdrawing group (carbonyl) is removed by converting it into another that has oxygen attached to benzene ring using a special oxidizing reagent called *meta*-Chloroperoxybenzoic acid (mCPBA) that contains peroxide. De-protection with HCl then removes all the protecting groups and the result is  $^{18}\text{F}$ -labelled DOPA. Itself it is a drug used to treat Parkinson's disease, a degenerative disease of neurons that usually affects old people. It is a precursor of the neurotransmitter dopamine. Its radiolabelled version,  $^{18}\text{F}$ -labelled DOPA binds dopamine receptors in brain, and is used to view dopamine receptors in brain, diagnose and monitor patients with Parkinson's disease.

Figure 175: Synthesis of  $^{18}\text{F}$ -fluoroepibatidine

What if instead of benzene ring one tries to perform aromatic nucleophilic radiofluorination on heteroaromatic ring, for example, pyridine that has nitrogen? We can look into this example, synthesis of  $^{18}\text{F}$ -fluoroepibatidine (Figure 175). The good leaving group is positively charged trimethylammonium group and it leaves very easily, even without any electron-withdrawing group in the *para*-position. This is because presence of nitrogen atoms in the aromatic ring in fact withdraws electrons

by itself, especially if nitrogen is in the *ortho*-position (position 2) or in the *para*-position (position 4)! This  $^{18}\text{F}$ -labelled PET tracer  $^{18}\text{F}$ luoroepibatidine binds to acetylcholine receptors in brain.

Sometimes direct aromatic nucleophilic radiofluorinations are very impossible and some other synthetic tricks need to be done. One of these tricks is so called indirect aromatic nucleophilic radiofluorinations: here we firstly label a building block with  $^{18}\text{F}$  such as the reaction shown in the Figure 176 where fluoride reacts with very exotic reagent called diaryliodinium and gives a secondary precursor, a building block (or synthon) called  $^{18}\text{F}$ -fluoroiodobenzene. This aromatic nucleophilic radiofluorination reaction is very quick and high yielding. Then, fluoroiodobenzene building block is used as a reagent in another high yielding reaction, modern palladium-catalysed C-C cross-coupling called Stille coupling. The result is  $^{18}\text{F}$ -labelled rofecoxib or  $^{18}\text{F}$ -fluororofecoxib. It binds to enzyme cyclooxygenase (COX) and it is used for the PET imaging of inflammations and rheumatoid diseases.

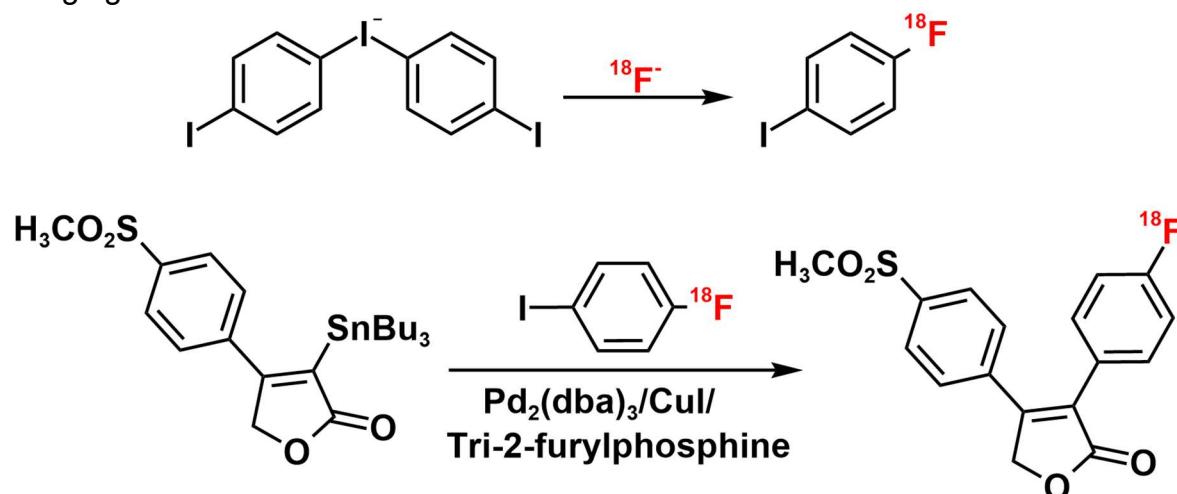


Figure 176: Synthesis of  $^{18}\text{F}$ -rofecoxib

Here we summarise some key rules for performing nucleophilic  $^{18}\text{F}$ -fluorinations. Firstly, traces of water can substantially diminish the nucleophilicity of radiofluoride ion and can lead to reduced yields. In light of this, nucleophilic radiofluorinations should be carried out under absolutely anhydrous conditions. Solvents, carrier gases, and lab equipment should always be dry. Secondly, number of synthetic steps should be reduced to minimum: not more than 3 steps, all reactions should be quick and high yielding! Third, in the case of more demanding radiofluorinations, radiolabelling can be substantially hampered by the adsorption of  $^{18}\text{F}^-$  onto the walls of the reaction vessel (up to >50%). And finally, under acidic conditions,  $^{18}\text{F}^-$  forms volatile HF. It is crucial to make sure that this gas is trapped by appropriate equipment.

## Electrophilic $^{18}\text{F}$ -substitutions

Opposite to nucleophilic substitutions the electrophilic substitutions (also called electrophilic radiofluorinations) are chemical reactions between an electrophilic  $^{18}\text{F}$  source, denoted as  $\{\text{F}^+\}$ , and electron-rich reactants, such as alkenes, aromatic rings, or carbanions (Figure 177).

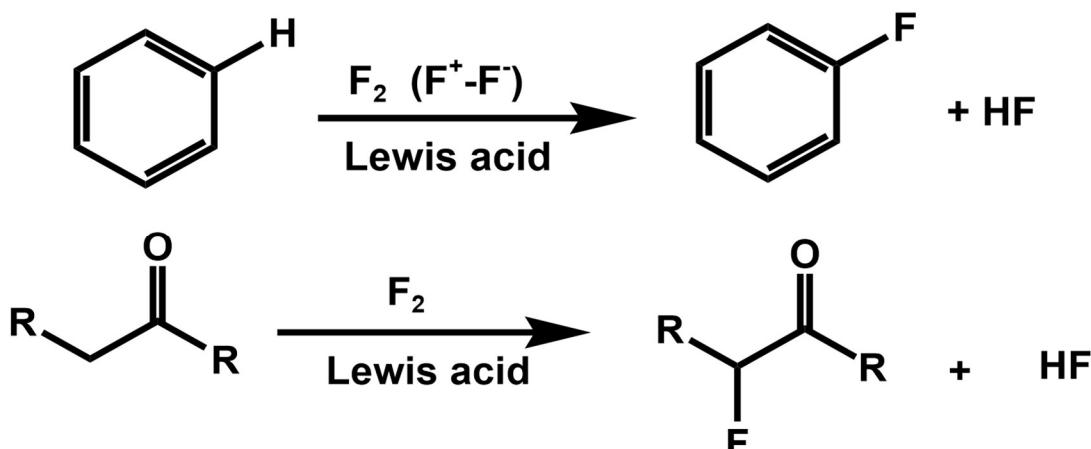


Figure 177: Electrophilic fluorinations by using  $\text{F}_2$  gas

One must keep in mind and be aware that electrophilic fluorine,  $\{\text{F}^+\}$  in fact is not a free molecular entity but is carried by some other molecule.

Historically the first  $^{18}\text{F}$ -labelled PET radiotracers were made using electrophilic radiofluorinations. In practice elemental fluorine gas is the primary source of electrophilic fluorine where one atom is  $^{18}\text{F}$  and another is the stable F ( $^{18}\text{F}-\text{F}$  gas). The labelling reactions can be done directly with  $^{18}\text{F}-\text{F}$  gas or with some  $^{18}\text{F}$ -labelled electrophilic reagents (usually obtained from cyclotron-produced  $^{18}\text{F}-\text{F}$  fluorine gas).

However, there are significant problems with electrophilic  $^{18}\text{F}-\text{F}$  gas that pose certain challenges and limitations:

1. The chemical nature of elemental fluorine gas: it is a highly (overly) reactive and poorly selective electrophilic radiofluorination agent: reactions often do not go the way it should.
2.  $^{18}\text{F}-\text{F}$  gas has inherent limitation: for electrophilic radiofluorinations  $^{18}\text{F}-\text{F}$  gas achieves maximal theoretical radiochemical yield of just 50% since half of molecule is non-radioactive fluorine.
3. Produced  $^{18}\text{F}-\text{F}$  gas is generally of very low specific activity (only 0.05–0.5 GBq/ $\mu\text{mol}$ ) and the final yield of the produced radiopharmaceutical agents is even lower.

What this actually means? Although the chemical yield can be, in fact, fairly good, the radiochemical yield is very poor: there are plenty of non-radioactive, hence useless molecules of the tracer containing the stable instead of radioactive isotope and very few molecules do contain the radioactive isotope (Figure 178). The non-radioactive molecules bind the target as much as the radioactive ones (affinity is the

same) and are occupying the vast majority the target places (receptors). The result is very poor imaging signal and not so clear image. On the other hand, if majority of imaging agent molecules do contain radionuclide, then most of the target places are occupied by functional radiotracer and will give a good imaging signal. This problem is much more pronounced when the radiopharmaceutical agent molecules need to bind certain target molecule in the body, for example, receptors. And most of  $^{18}\text{F}$ -labelled PET tracers do bind some receptors.

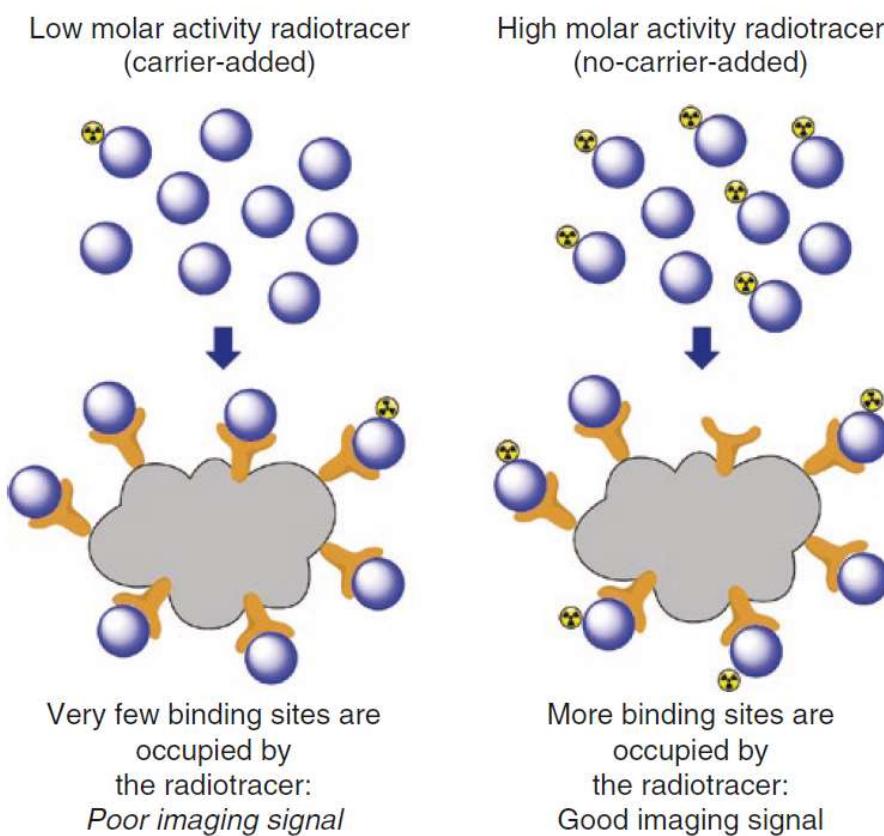


Figure 178: Difference between tracers effect if tracer is made from carrier-added (left) and non-carrier-added radionuclide.

Therefore, all these problems need somehow to be mitigated. Problem of overly high and non-selective reactivity is easier to address; too reactive  $^{18}\text{F}$ -F gas is often converted into some less reactive molecule, but more selective and easier to handle  $^{18}\text{F}$ -labeled electrophilic fluorination reagents, sources of electrophilic  $\{\text{F}^+\}$ . Problem of low specific activity or low-molarity of  $^{18}\text{F}$ -F gas is more challenging to address. Firstly, special protocol of irradiation is conducted, so-called “two-shot” where gas is irradiated twice. This increases molarity a bit, however not decisively. The most promising solution so far is in fact to make  $^{18}\text{F}$ - $^{18}\text{F}$  gas ( $^{18}\text{F}_2$ ) where both atoms in the fluorine gas molecule are radioactive by using nucleophilic, high-molarity radiofluoride ion ( $^{18}\text{F}^-$ ). This can be achieved using various novel procedures.

## Secondary labelling precursors and building blocks for electrophilic radiofluorinations

Secondary labelling precursors and building blocks for electrophilic radiofluorinations are made usually from  $^{18}\text{F}$ -F gas. This gas is converted into less reactive, but more selective and easier to handle secondary precursors. There are many various secondary precursors in the routine or experimental use, such as acetyl hypofluoride, perchloryl fluoride, trifluoromethyl hypofluorite, Selectfluor, fluoropyridinium salts, fluoropyridines, fluorosulfonamides such as NFSI (N-fluorobenzenesulfonimide) and others (Figure 179).

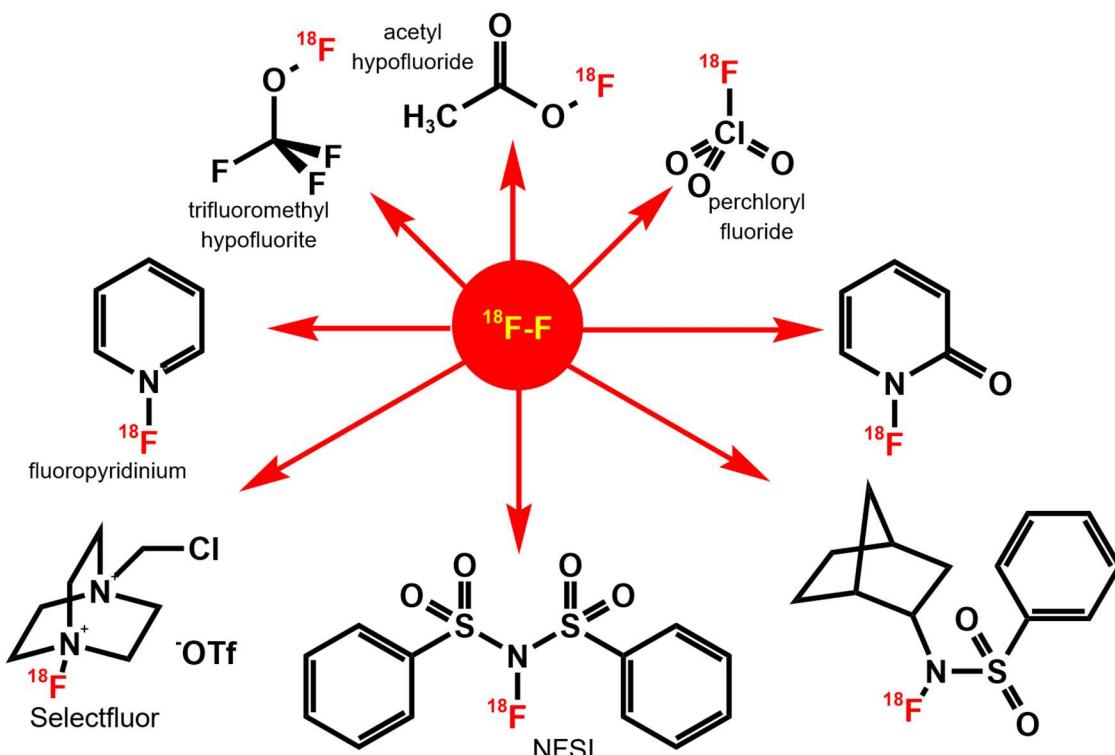


Figure 179: Various secondary reactive precursors that can be made from  $^{18}\text{F}$ -F gas

In all these secondary labelling precursors the fluorine is bound to elements with slightly lower electronegativity (like O, N), hence forming bonds F-O, F-N, F-NSO or F-Cl. In this way the fluorine is turned into electropositive, electrophilic  $\{{}^{18}\text{F}^+\}$  synthon. This synthon is not in a free form like a fluoride ion, but it is rather bound to some other electronegative element.

### Selectfluor

The most important among secondary precursors is the reagent called Selectfluor. It has a complicated chemical name, chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(triflate), however name Selectfluor is commonly used. It is a powerful secondary precursor, carrier of electrophilic fluorine and is used for quick and efficient electrophilic fluorinations.

It is made by reacting  $^{18}\text{F}-\text{F}$  or  $^{18}\text{F}_2$  gas with the substrate chloromethyl-1,4-diazoniabicyclo[2.2.2] octane triflate (Figure 180). This reaction is achieved in dry acetonitrile in presence of lithium triflate at low temperature.

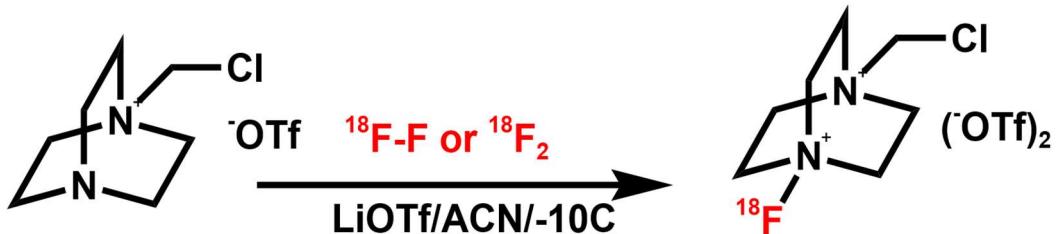


Figure 180: Synthesis of Selectflour reagent.

The reactions (Figure 181) with Selectflour usually involve reactants with some organotin or organoborate functional groups on the aromatic ring. These groups are in fact good leaving groups in electrophilic substitution; Selectfluor donates its radiofluorine that gets substituted with organometallic group.

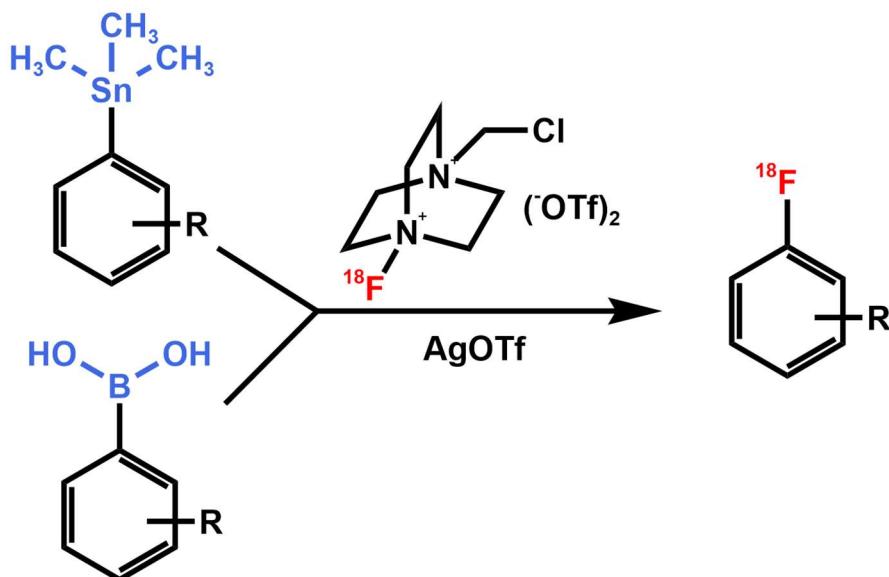


Figure 181: Radiofluorination with Selectflour as carrier of electrophilic  $^{18}\text{F}$

### Direct electrophilic radiofluorinations vs. fluoro-demetallation reactions

Generally, there are two main types of electrophilic radiofluorinations, the direct ones and those made by using certain reactive organometallic reagents. The direct one is very simple and looks straight-forward, but the problem is its poor selectivity: fluorine can go to the various positions on the benzene ring. For example, direct electrophilic radiofluorination of DOPA (Figure 182) is done by a simple reaction in HF, however various unwanted isomers are formed.

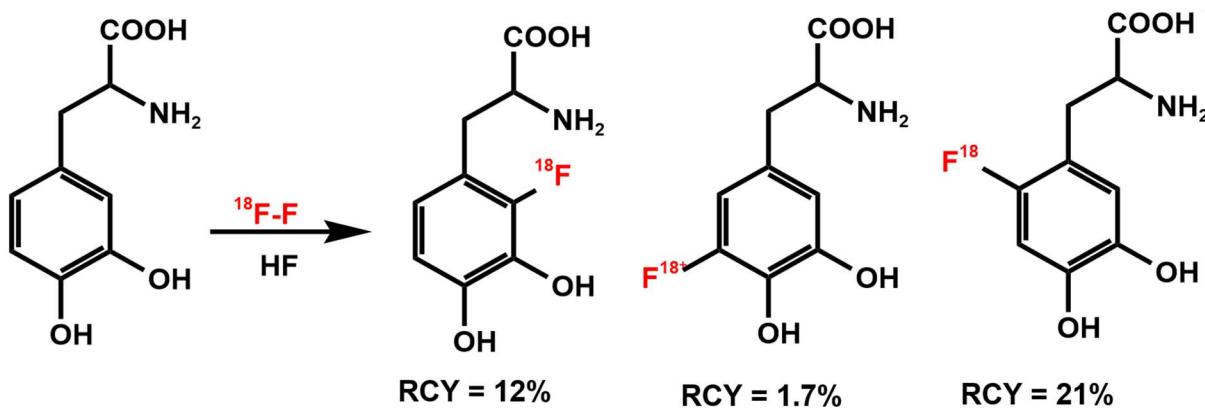


Figure 182: Direct electrophilic radiofluorination of DOPA by using  $^{18}\text{F}$ -F gas, RCY means radiochemical yield.

In order to address this problem a special organometallic reagent is used. This reagent has organic (trimethyl) tin, silicon or germanium bound onto its benzene ring. These organometallic groups are good leaving groups and can be quickly exchanged with electrophilic fluorine from a secondary precursor, such as acetyl hypofluoride ( $^{18}\text{FOAc}$ ), like in the figure 183:

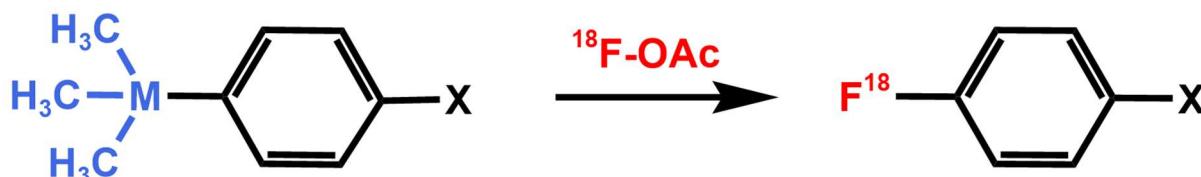


Figure 183: Fluoro-demetallation reaction, where M = Si Ge or Sn while X = Br, F,  $\text{CF}_3$ ,  $\text{CH}_3$ , H,  $\text{OCH}_3$

Hence, the position of the organometallic group on the ring defines the place where electrophilic radiofluorination will occur. In this way, for example,  $^{18}\text{F}$ -DOPA can be made by reacting a secondary radiofluorine precursor (like  $^{18}\text{FOAc}$ ) with the substrate that has trimethyl-tin moiety at the position 6. The radiochemical yield in this case is much better and only one isomer is made. Also, all proton donating groups in the organometallic reagent are capped with some protecting groups such as *tert*-butoxycarbonyl (also known as BOC).

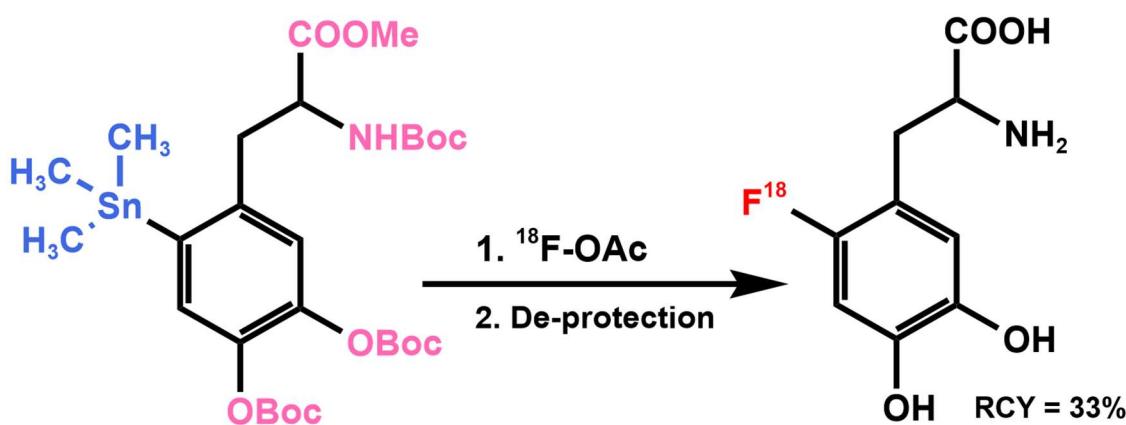


Figure 184: Synthesis of  $^{18}\text{F}$ -DOPA via fluoro-demetallation gives higher radiochemical yield (RCY)



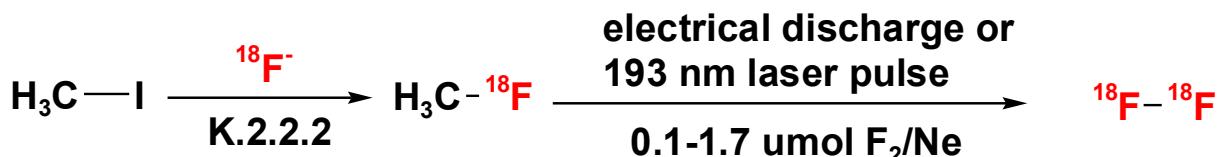
## Preparation and use of high molar activity electrophilic synthons from $^{18}\text{F}$ -fluoride

The problem of low molar activity (specific activity) of  $^{18}\text{F}$ -F gas or secondary precursors derived from  $^{18}\text{F}$ -F gas is the major issue and the main limitations for the use of electrophilic radiofluorination. It needs to be addressed, since the radiopharmaceuticals derived from such low molar activity source cannot be used for the imaging of “saturable” binding sites like receptors. In order to overcome this challenge some methods for making electrophilic  $\{^{18}\text{F}^+\}$  from highly abundant (high-molar activity)  $^{18}\text{F}$ -fluoride ions are developed. The  $^{18}\text{F}^-$  ion can be made using liquid target much more efficiently and can achieve huge specific activity of 5000 GBq/ $\mu\text{mol}$  comparing to 0.05–0.5 GBq/ $\mu\text{mol}$  of  $^{18}\text{F}$ -F gas. These methods are based on concept of reversing the polarity (“*umpolung*”) of nucleophilic  $^{18}\text{F}$  fluoride ion. It can be achieved using three recently developed methods:

1. Homolysis of  $^{18}\text{F}$ -labelled methyl-fluoride ( $\text{CH}_3\text{F}$ ) in the presence of tiny amounts of  $\text{F}_2$  gas.
2. Formation of electrophilic radiofluorination agents through the reaction of  $^{18}\text{F}^-$  with strong oxidizing agents.
3. Use of  $^{18}\text{F}^-$  with transition metal complexes

### Homolysis of $^{18}\text{F}$ -labelled methyl-fluoride

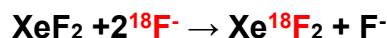
In the first method homolytic cleavage of  $^{18}\text{F}$ -labelled  $\text{CH}_3\text{F}$  in an electrical discharge chamber or *via* the use of UV laser pulses at constant power used to make  $^{18}\text{F}_2$  gas. Firstly, high molar activity  $^{18}\text{F}^-$  is produced in a cyclotron using liquid target and  $^{18}\text{O}(p,n)^{18}\text{F}$  reaction. Then, radiofluoride is converted into  $^{18}\text{F}$ -labelled  $\text{CH}_3\text{F}$  in the presence of methyl iodide (by nucleophilic substitution). Finally, the mixture of  $^{18}\text{F}$ - $\text{CH}_3\text{F}$  and tiny amounts of  $\text{F}_2$  gas are atomized using an electric discharge or a UV laser pulse to form  $^{18}\text{F}_2$ :



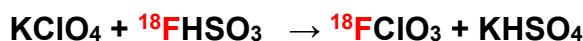
By using this method under optimal conditions, the molar activities of  $^{18}\text{F}_2$  gas were up to 55 GBq/ $\mu\text{mol}$ . This strategy has been used to produce radiotracers with molar activities exceeding 15 GBq/ $\mu\text{mol}$  in sufficient amounts for PET imaging (400–800 MBq).

### Reaction of $^{18}\text{F}^-$ with strong oxidizing agents

The second option is more chemical method: turning the nucleophile  $^{18}\text{F}^-$  into the electrophile  $\{^{18}\text{F}^+\}$  by some chemical reactions. The first option is isotope exchange method by using an exotic  $\text{XeF}_2$  reagent, the result is  $^{18}\text{F}$ -labelled  $\text{XeF}_2$ :



Another option is synthesis of perchloryl fluoride ( $^{18}\text{FCIO}_3$ ) by using sulphur trioxide ( $\text{SO}_3$ ) in sulphuric acid (mixture of  $\text{SO}_3$  in  $\text{H}_2\text{SO}_4$  is often called “oleum”) and then reacting the product with potassium perchlorate ( $\text{KClO}_4$ ). Unfortunately, this reaction proved not to be very reproducible.



On the other hand, synthesis of an exotic electrophilic radiofluorination agent called  $^{18}\text{F}$ -fluoro-benziodoxole by reaction of  $^{18}\text{F}^-$  with hypervalent iodine tosylate proved to be much more efficient and it can achieve excellent radiochemical yield, giving specific activity of up to 400 GBq/ $\mu\text{mol}$ !

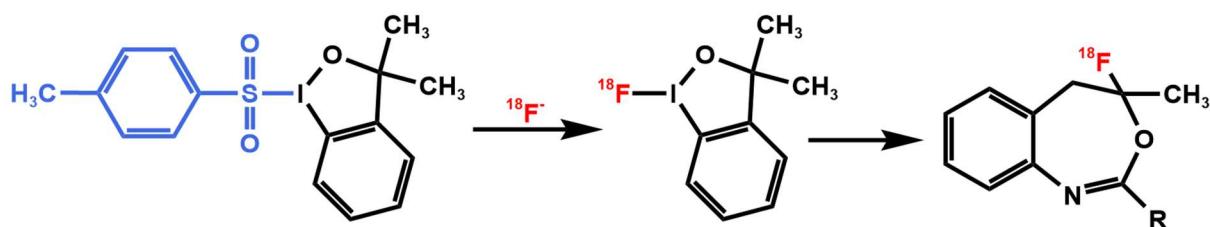


Figure 185: Electrophilic radiofluorination *via*  $^{18}\text{F}$ -fluoro-benziodoxole

### Use of $^{18}\text{F}^-$ with transition metal complexes

Finally, the third method is using organometallic reagents and catalyst with transition metals (Figure 186). For example, copper can be used as catalyst, while very exotic complex of palladium, organic ligands and radiofluorine proved to be able to achieve electrophilic radiofluorination upon reacting with organometallic reagents.

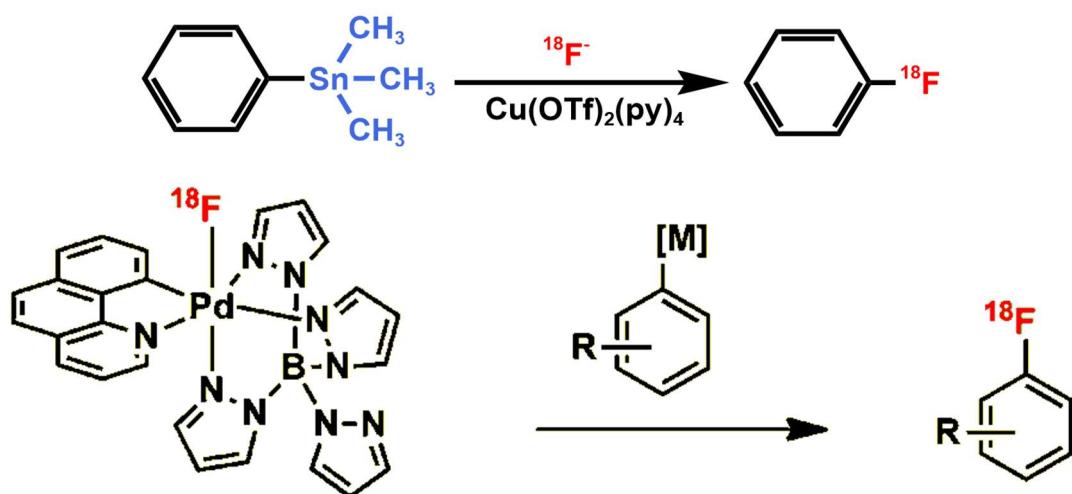


Figure 186: Electrophilic radiofluorination *via* Cu or Pd catalyst.

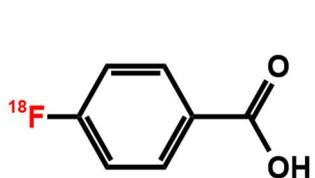
## Radiofluorinations of proteins and peptides

Fluorine forms stable F-C bonds, but at very harsh conditions (high temperature, anhydrous conditions, base or acids, etc.). Biomolecules (such as proteins, peptides or mABs) cannot withstand those conditions: under these conditions proteins and peptides get quickly denatured and completely lose their physiological functions. Labelling of biomolecules with  $^{18}\text{F}$  needs another strategy. How can biomolecules (having specific targeting ability) be labelled with  $^{18}\text{F}$ ? There are three strategies to label biomolecules:

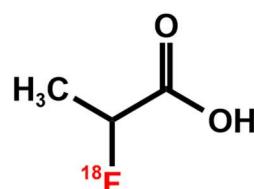
1. The first one is based on usage of a  $^{18}\text{F}$ -labeled prosthetic group (a functional group that serves as a carrier of  $^{18}\text{F}$ ) and conjugate it with a biomolecule.
2. In the second strategy  $^{18}\text{F}$  reacts with  $\text{Al}^{3+}$  and forms a complex  $[\text{Al}^{18}\text{F}]^{2+}$  ion. This complex ion behaves like a like similar metallic ions (for example like  $^{99\text{m}}\text{TcO}^{3+}$ ) and can form further complexes with chelating ligands conjugated with biomolecules. This is similar strategy like in  $^{99\text{m}}\text{Tc}$ .
3. The third strategy is based on ability of boron to form multiple stable bonds with fluorine, so called “trifluoroborate” group, to conjugate it with biomolecule and even to undergo isotope exchange with  $^{18}\text{F}$ .

### Radiofluorination by using a prosthetic group

Prosthetic group is a moiety, functional group, that gets attached onto a protein (usually via R-NH<sub>2</sub> terminal or lysine residue) using some simple fast reaction. The procedure is usually to label a prosthetic group with  $^{18}\text{F}$  using any suitable labelling chemical reaction (either nucleophilic or electrophilic substitution). Then, formed  $^{18}\text{F}$ -labelled prosthetic group is conjugated with a protein using a second, usually a mild, but a quick and high-yielding reaction (there is no time to waste in labelling procedures). Typical  $^{18}\text{F}$ -labelled prosthetic groups are *para*-fluorobenzoic acid, 2-fluoropropanoic acid or 5-fluoro-pentyne:



**4-(fluoro- $^{18}\text{F}$ )benzoic acid**

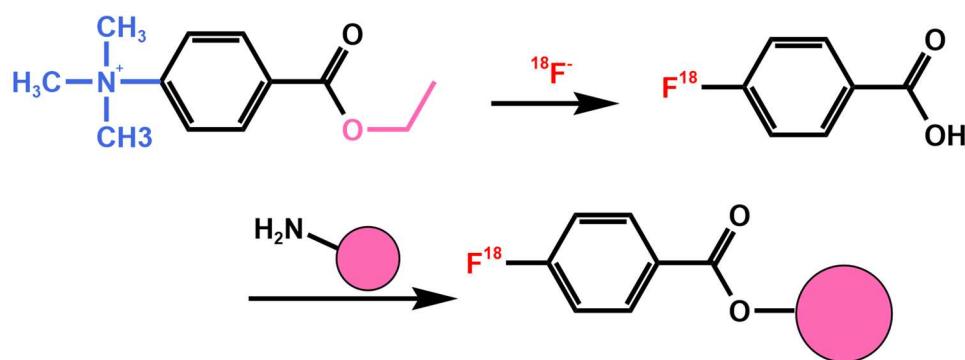


**2-(fluoro- $^{18}\text{F}$ )propanoic acid**

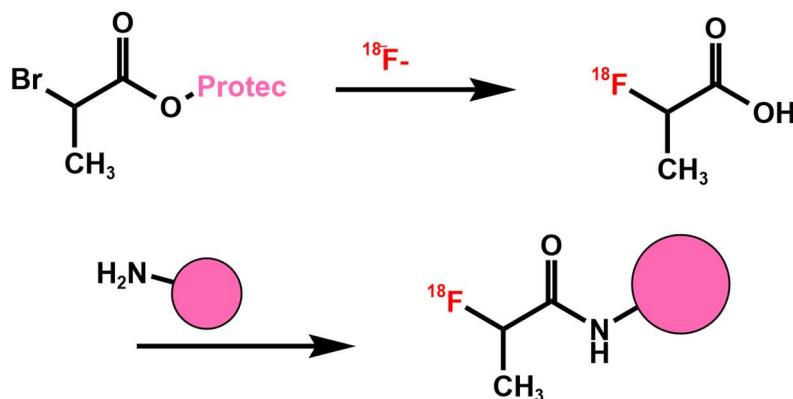


**5-(fluoro- $^{18}\text{F}$ )pentyne**

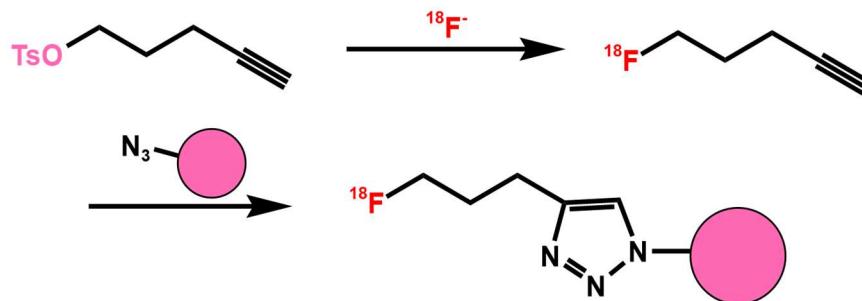
For example, 4-fluorobenzoic acid (also known as *para*-fluorobenzoic acid) is made by using aromatic nucleophilic substitution reaction where trimethylammonium group is a good leaving group in the “*para*” position from carboxylic group (protected with ethyl) that is also a good electron-withdrawing group. In the next step, the *para*- $^{18}\text{F}$ fluorobenzoic acid is conjugated onto a protein *via* any amine group on the protein:



In the second case, 2-bromopropionic acid (also having some kind of protecting group) reacts with nucleophilic  $^{18}\text{F}^-$  ion in a nucleophilic substitution reaction, and then 2-fluoro-propionic acid is formed. Similar as in the previous example, it is then reacted with a protein to form a  $^{18}\text{F}$ -labelled protein:



In the third example, nucleophilic  $^{18}\text{F}^-$  ion reacts with an tosyl-pentyne (tosyl is a good leaving group, and this molecule also has an ethynyl group with a triple “ $\text{C}\equiv\text{C}$ ” bond) forming a  $^{18}\text{F}$ -labelled fluoropentyne and then a protein labelled with an azide group reacts with the ethynyl group in a so called copper-catalysed azide-alkyne cycloaddition reaction (CuAAC or “click” reaction) and forms a  $^{18}\text{F}$ -labelled protein where triazolyl ring serves as a linker:



### Formation of $[\text{Al}^{18}\text{F}]^{2+}$ complex ion.

The second strategy involves a bit exotic ion  $[\text{Al}^{18}\text{F}]^{2+}$  ion. Radiofluoride ( $^{18}\text{F}^-$  ion) forms a strong and stable complex with the metallic aluminium  $\text{Al}^{3+}$  cation. The Al-F bond is quite stable, its energy in the  $[\text{Al}^{18}\text{F}]^{2+}$  complex is 675 kJ/mol, and this is

higher than that of any other Al-halogen bond. What is excellent is that Al-F bond is also very stable even in human body, across various conditions, however, the free  $[Al^{18}F]^{2+}$  complex tends to accumulate in bones. It is interesting to note that this complex can be formed only at pH between pH 4.0 and 5.5. It makes complexes just like  $[^{99m}TcO]^{3+}$  and can be complexed by bifunctional ligands (chelators conjugated with proteins or peptides). However, proper ligands for  $[Al^{18}F]^{2+}$  complex ion are different comparing to those for  $[^{99m}TcO]^{3+}$  ion. For  $[Al^{18}F]^{2+}$  complex ion preferred are pentadentate ligands.

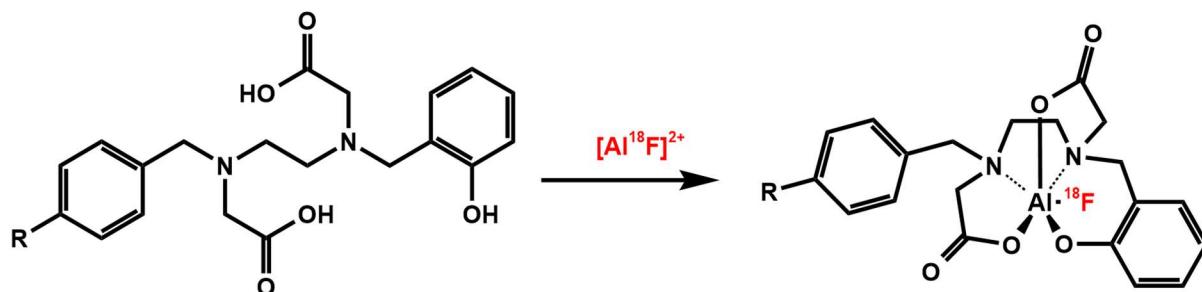


Figure 187: Complex ion  $[Al^{18}F]^{2+}$  forms a complex with pentadentate ligand

The best chelators for  $[Al^{18}F]^{2+}$  are small macrocyclic ligands based on three ethylene-amine-acetic-acid segments joined in a macrocycle, called NOTA or NODA. These macrocycles are perfect for aluminium and are linked with some protein *via* appropriate linker. Simple complexation reaction makes whole big molecular system labelled with radiofluorine in a quick complexation reaction.

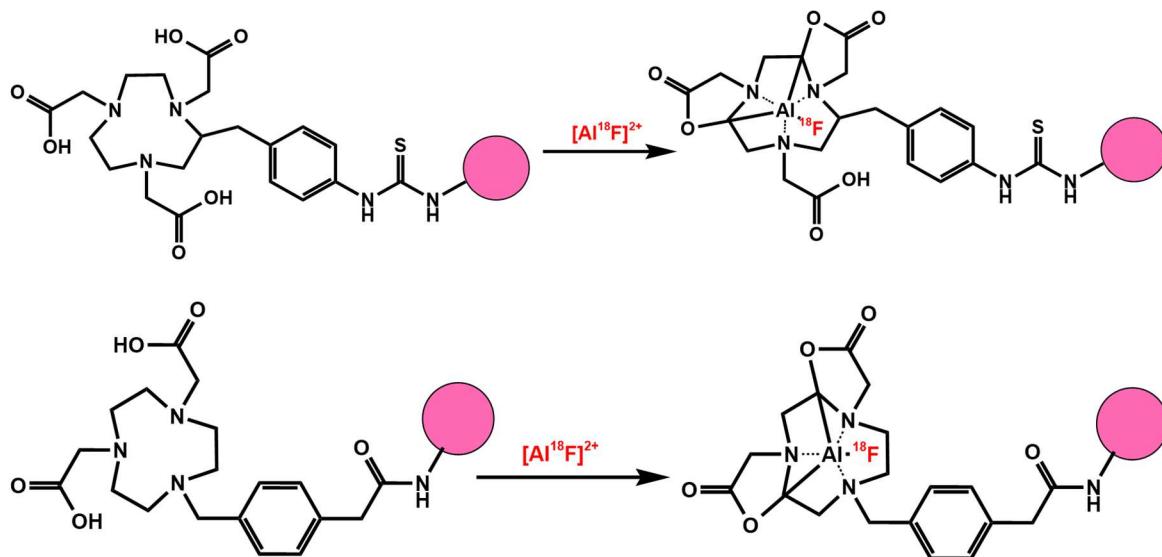


Figure 188: Formation of complexes with NOTA-based bioconjugate (up) or NODA-based bioconjugate (down).

A nice example is neurotensin peptide labelled with  $[Al^{18}F]^{2+}$  by using the NOTA chelating ligand (Figure 189). The labelling reaction is quick and high yielding, but yet it is achieved at 100°C, and not all proteins can withstand such high temperature for 10 minutes. Neurotensin is a peptide from brain that takes part in the regulation of hormones and has significant interaction with some other mechanisms in brain.

However, its receptors are sometimes very numerous in certain types of tumours, so  $^{18}\text{F}$ -labelled neurotensin, a complicated radiopharmaceutical agent is used in imaging of some types of cancer.

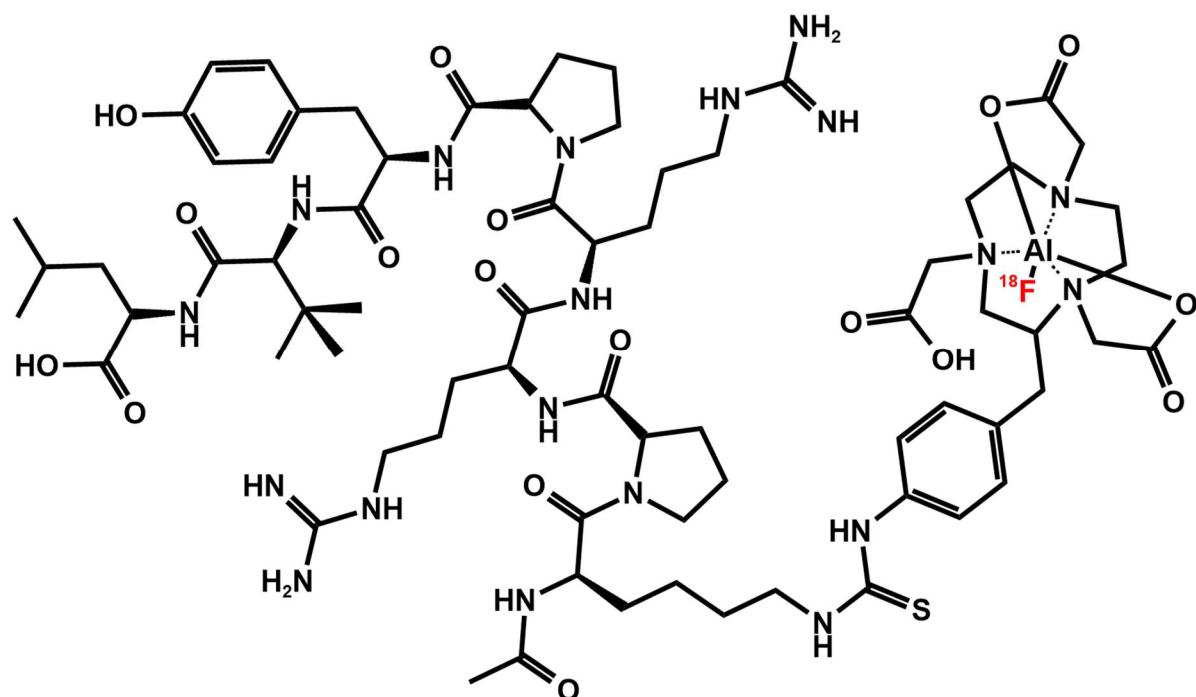
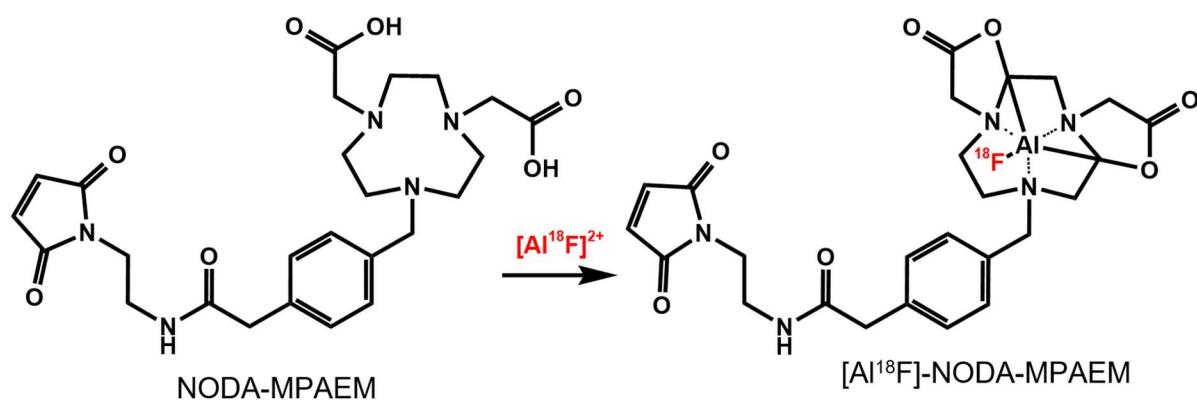


Figure 189: Neurotensin labelled with NODA-[ $\text{Al}^{18}\text{F}$ ] complex

Since not all peptides and protein are able to withstand high temperatures required for labelling reactions a special bifunctional ligand was developed for the quick radiolabelling of proteins with  $[\text{Al}^{18}\text{F}]^{2+}$  complex ion at room temperature. It is called NODA-MPAEM and it consists of three parts: NODA chelating macrocycles, a linker and a very reactive moiety called maleimide. The maleimide is a reactive group that reacts quickly with any sulphydryl groups (-SH) in a biomolecule at room temperature. The truth is that there are usually many sulphydryl groups in proteins that are from cysteine amino acid.



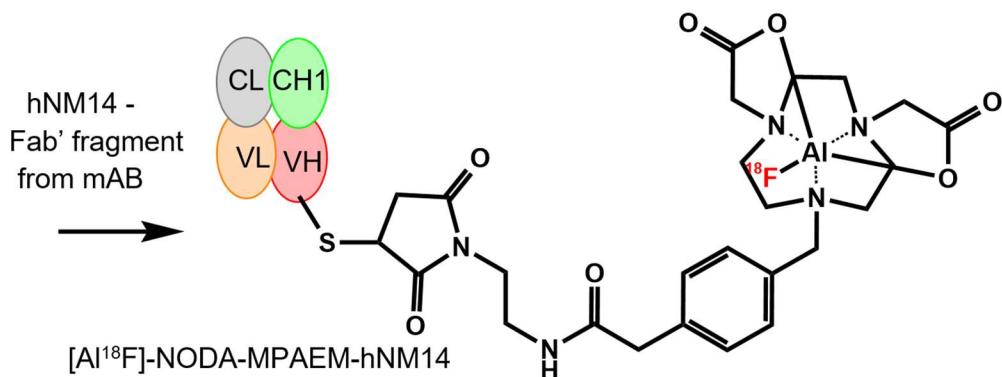


Figure 190: Synthesis of  $\text{Al}^{18}\text{F}$ -labelled Fab' fragment by using NODA-MPAEM

Therefore, NODA-MPAEM firstly reacts with the  $[\text{Al}^{18}\text{F}]^{2+}$  complex ion at room temperature and then with a protein, for example with a Fab' fragment of mAB (part of monoclonal antibody responsible for molecular recognition of a target). This kind of special bioconjugate is shown in the Figure 190. This is also achieved at room temperature allowing labelling of very sensitive molecules that would be otherwise degraded (denatured) at higher temperature and their molecular recognition ability would be damaged.

### Radiolabelling with $[\text{R-Ar-BF}_2^{18}\text{F}]^-$

The third strategy is to employ negatively charged trifluoroborate group  $[\text{R-BF}_3]^-$  as a carrier of  $^{18}\text{F}$ . The boron-fluorine (B-F) bond is one of the strongest known, with a bond dissociation energy is quite high (732 kJ/mol). Aryl-boronic acids can be fluorinated with  $\text{KHF}_2$  in acidic pH ( $\text{pH}>7$ ) to yield aryltrifluoroborate salts  $[\text{R-Ar-BF}_3]^-$  with high yields (Figure 191).

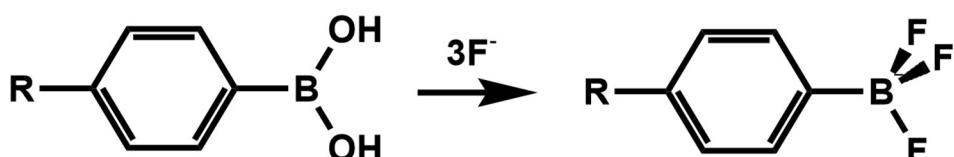


Figure 191: Formation of trifluoroborate group in presence of fluorine ions

Therefore, if there is a mix of stable fluorine and radiofluorine (in ratio 2:1) then the product is radiolabelled  $[\text{R-Ar-BF}_2^{18}\text{F}]^-$  as shown below in the Figure 192:

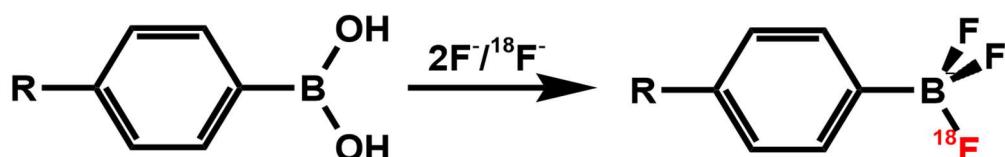
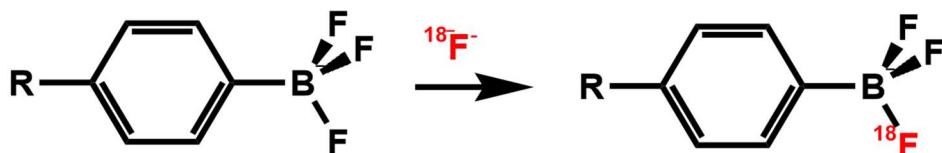
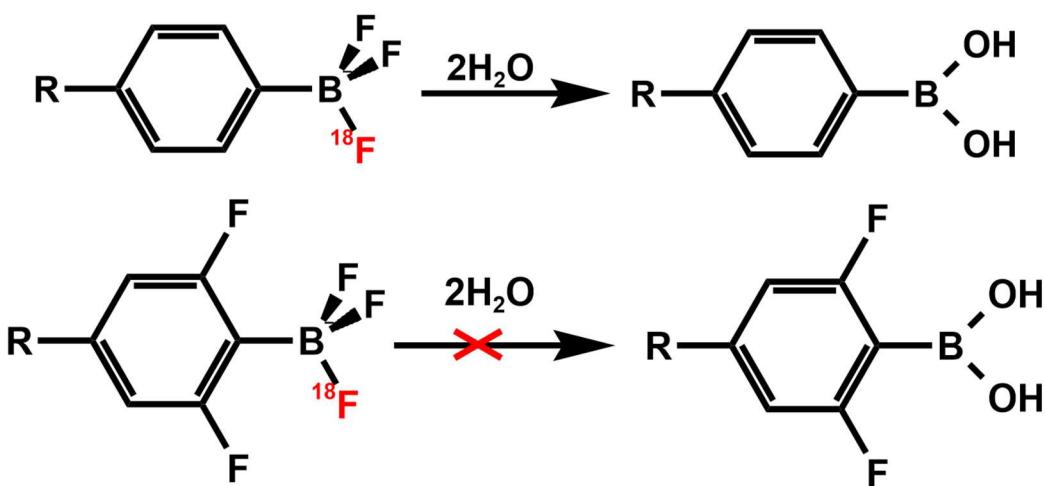


Figure 192: Formation of radiolabelled  $[\text{R-Ar-BF}_2^{18}\text{F}]^-$  ion

The non-radioactive aryltrifluoroborate salts  $[\text{R-Ar-BF}_3]^-$  can, at the right conditions, undergo isotopic exchange: stable F is replaced with radioactive  $^{18}\text{F}$  and labelling is achieved in very elegant way (Figure 193).

Figure 193: Isotopic exchange of F with  $^{18}\text{F}$ 

Unfortunately, trifluoroborate group is stable in acidic aqueous media, but not so stable at basic pH ( $\text{pH} < 7$ ). It tends to hydrolyse back into boric acid by releasing fluoride ions (Figure 194 up). However, this problem can be mitigated and minimized: trifluoroborate group is more stable if there is a fluorine atom at the benzene ring at the position 2 (“*ortho*” position), as shown in the Figure 194, down.

Figure 194: In presence of water trifluoroborate group will hydrolyse but it will not if there are fluorine atoms on the phenyl ring in the *ortho* position.

Very important and useful second precursor for the labelling of biomolecules using trifluoroborate group is  $^{18}\text{F}$ -labelled alkyl-ammonio-methyl-trifluoroborate or AMBF (Figure 195).

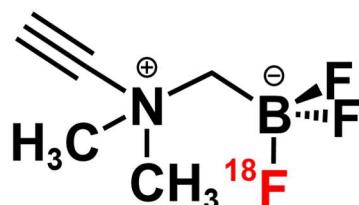


Figure 195: Alkyl-ammonio-methyl-trifluoroborate or AMBF

It is a small molecule that contains two charged groups of opposite charge: negatively charged trifluoroborate and positively charged alkyl-ammonio group. These positive and negative charges are neutralising each other, so in total the molecule is neutral. These kinds of molecules that contain both charges are called “ambiphilic” and usually are very soluble in water. It was found that a positive charge next to the negative trifluoroborate is actually very stabilising and hydrolysis of trifluoroborate is negligible.

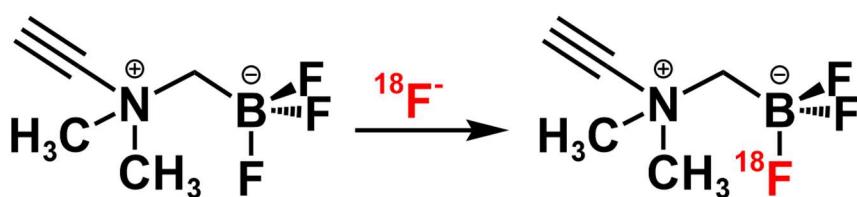
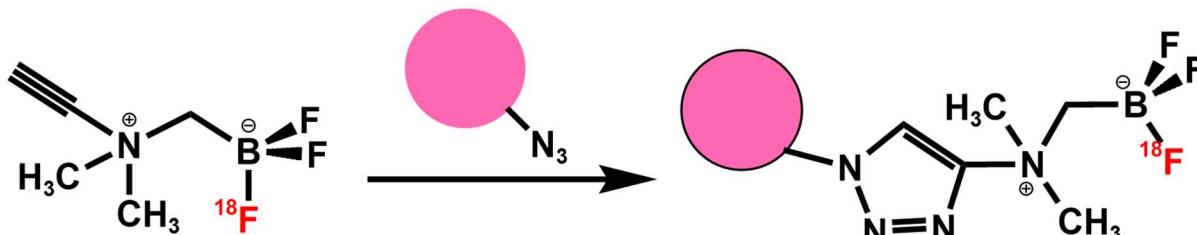


Figure 196: Isotope exchange in AMBF

It also contains the ethynyl “ $\text{C}\equiv\text{C}$ ” group so some biological molecules can be labelled in two steps using the “CuAAC” (click) reaction. In the first step, isotopic exchange labels AMBF molecule with radiofluorine (Figure 196) and then the click reaction conjugated  $^{18}\text{F}$ -labelled AMBF onto any protein (Figure 197).

Figure 197:  $^{18}\text{F}$ -labelled AMBF reacts in CuAAC “Click” reaction with azide-labelled protein and forms  $^{18}\text{F}$ -labelled protein.

## Overview of $^{18}\text{F}$ radiopharmaceuticals in clinical and experiments practice

Apart from  $^{18}\text{FDG}$  and other important  $^{18}\text{F}$ -labelled PET tracers presented earlier ( $^{18}\text{F}$ -Fluorothymidin,  $^{18}\text{F}$ -DOPA,  $^{18}\text{FP-CIT}$ ,  $^{18}\text{F}$ -Altanserin,  $^{18}\text{F}$ -Fluoroepibatidine and  $^{18}\text{F}$ -Fluororofecoxib) there are plenty of other developed  $^{18}\text{F}$ -labelled tracers. Some of them are used in routine clinical practice to diagnose and evaluate various diseases and some of them are just experimental PET probes used in scientific research of human physiology and pathophysiology. Thanks to the fact that  $^{18}\text{F}$  radionuclide can be quickly and efficiently inserted into literally any organic molecule  $^{18}\text{F}$ -labelled PET radiotracers are usually small “drug-like” molecules. Some of them are  $^{18}\text{F}$ -labelled drug, others are derived from similar drugs in clinical or experimental practice. Most of them are designed to and able to selectively bind certain cellular structures such as receptors. Most of them are neutral and lipophilic and can pass into brain. Therefore, they are ideal for imaging and visualisation of various neural receptors in brain or receptors in other tissues. We can differentiate between four main medical areas for which  $^{18}\text{F}$  PET radiotracers are used:

- Neurology (to visualise areas of brain rich in certain neural receptors and diagnose neurological, psychological, and cognitive function, diseases, and conditions; many of them are  $^{18}\text{F}$ -labelled clinical or experimental drugs or drug analogues),
- Oncology (to accurately identify and locate cancer cell, distant cancer cell colonies such as metastases and to probe resistant cells),
- Cardiology (to diagnose and test function and conditions of heart and blood vessels)
- Other areas (all other  $^{18}\text{F}$ -labelled PET radiotracers).

## **<sup>18</sup>F-labelled PET radiotracers in neurology, psychiatry, and psychology**

<sup>18</sup>F-labelled radiotracers for imaging of neurological and psychiatric conditions and diseases as well as for the studies in neuropsychology are the most numerous PET tracers. Neurology is the medical branch dealing with the function and disorders of brain where behaviour of a patient is not affected, for example with diseases such as epilepsy, Parkinson disease, sleeping disorders, etc. Psychiatry is branch of medicine dealing with mental disorders such as neuroses, schizophrenia, depression, mania, addictions, etc. Neuropsychology is the branch of psychology that is looking for a link between the biochemistry and physiology of brain and human behaviour. The nature of fluorine attached to small molecule is such that it makes them very good and useful for imaging of brain. These are mostly lipophilic compounds that bind to certain receptors or neurotransmitter transporter proteins. Besides some shown previously 13 additional <sup>18</sup>F-labelled PET tracers will be presented herein.

### **<sup>18</sup>F-Raclopride**

<sup>18</sup>F-Raclopride (Figure 198 left) is a selective antagonist (or blocker) of so-called D<sub>2</sub> dopamine receptors in brain. It is used for the diagnosis of various diseases such as movement disorders, Huntington's disease, personality disorders and many others.

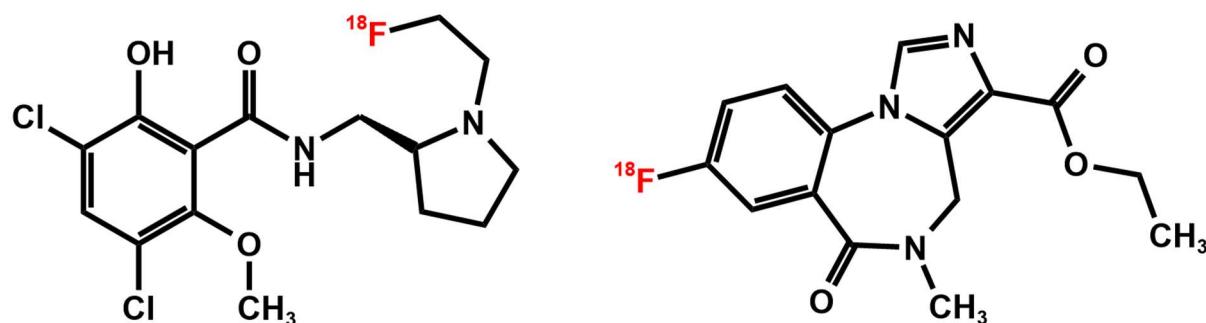


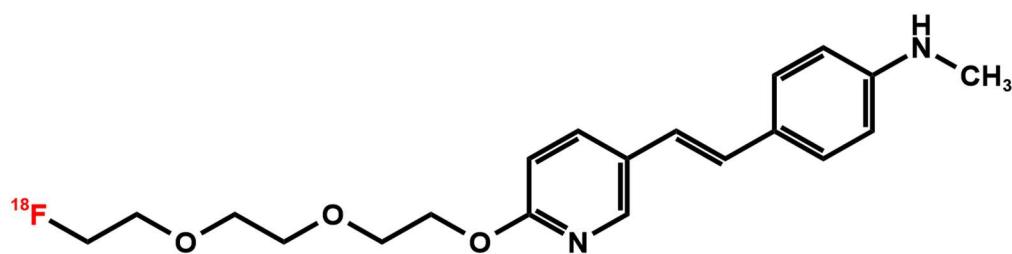
Figure 198: Structures of <sup>18</sup>F-Raclopride (left) and <sup>18</sup>F-Flumazenil (right)

### **<sup>18</sup>F-Flumazenil**

<sup>18</sup>F-Flumazenil (Figure 198 right) is a selective GABA<sub>A</sub> receptor antagonist; it is in fact a radioactive version of non-radioactive drug and is used for the localization of drug-resistant temporal lobe epilepsy.

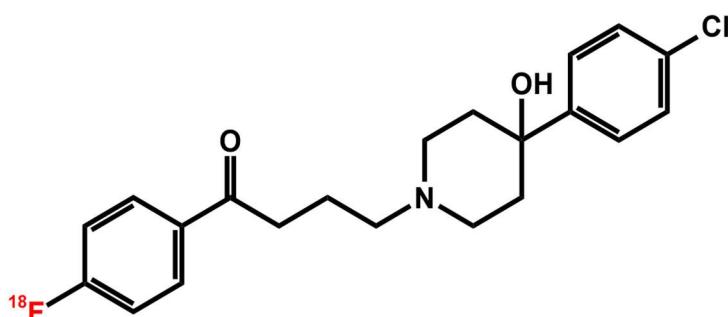
### **<sup>18</sup>F-Florbetapir**

<sup>18</sup>F-Florbetapir (Figure 199) binds pathologic deposition of amyloid β (Aβ) protein in brain and therefore is used for PET imaging of dementia and Alzheimer disease.

Figure 199: Structure of <sup>18</sup>F-Florbetapir

### **<sup>18</sup>F-Haloperidol**

<sup>18</sup>F-Haloperidol (Figure 200) is radioactive version of a famous antipsychotic drug haloperidol, widely used in clinical practice for treating schizophrenia. It binds dopamine D<sub>2</sub> receptors (therefore it is called D<sub>2</sub> blocker). It is used for mapping of D<sub>2</sub> receptors and investigations of psychiatric disorders.

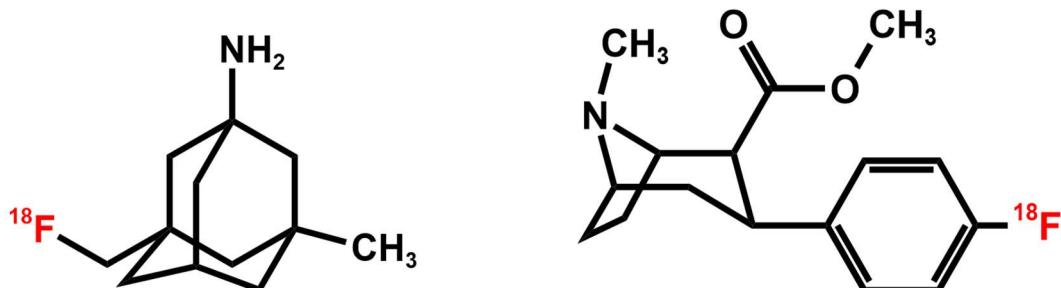
Figure 200: Structure of <sup>18</sup>F-Haloperidol

### **<sup>18</sup>F-Memantine**

<sup>18</sup>F-Memantine is derivative of adamantane (Figure 201, left), and it is <sup>18</sup>F-labelled drug called memantine. It is NMDA-receptor blocker and is used for mapping of the NMDA-receptor complexes in brain and elsewhere, and this is useful for the investigations of cognitive functions (memory, learning, etc.).

### **<sup>18</sup>F-CFT**

<sup>18</sup>F-CFT is derivative of tropane and is an analogue of cocaine. It binds monoamine transporter protein in brain and is used for PET studies of the dopamine transporter system in humans.

Figure 201: Structure of <sup>18</sup>F-Memantine and <sup>18</sup>F-CFT

### ***<sup>18</sup>F-Diazepam***

<sup>18</sup>F-Diazepam (Figure 202, left) is the <sup>18</sup>F-labelled version of the famous tranquilizer diazepam also known as Valium. It binds GABA<sub>A</sub> receptors and can be made by electrophilic substitution using <sup>18</sup>F-F gas.

### ***<sup>18</sup>F-Fluoro-oxoquazepam***

<sup>18</sup>F-Fluoro-oxoquazepam (Figure 202, right) is another <sup>18</sup>F-benzodiazepine derivative, also binds GABA<sub>A</sub> receptors in brain, but better than diazepam, and is used for similar PET investigations.

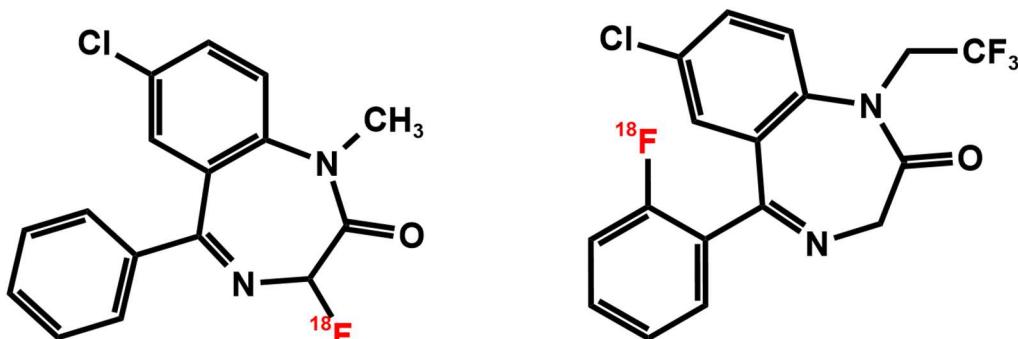


Figure 202: Structure of <sup>18</sup>F- Diazepam (left) and <sup>18</sup>F-Fluoro-oxoquazepam (right)

### ***<sup>18</sup>F-Fluoroatipamezol***

<sup>18</sup>F-Fluoroatipamezol (Figure 202) binds so called  $\alpha_2$ -adrenergic receptors (neurotransmitters called adrenalin or noradrenalin usually bind these receptors) in brain and is therefore used for mapping adrenergic innervation in the human brain. This mapping has importance in neuroscience, to reveal physiology and pathophysiology of brain tissue and ultimately help in various neurological and psychological diseases and abnormal states.

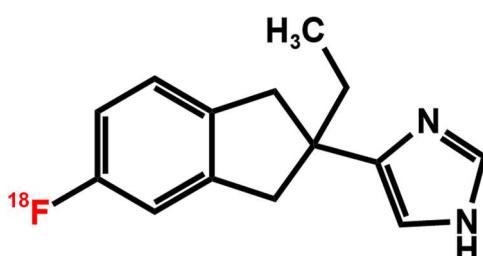
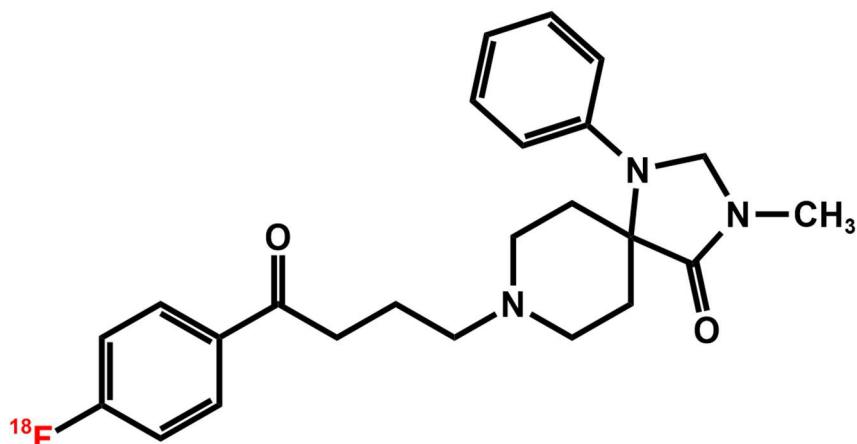


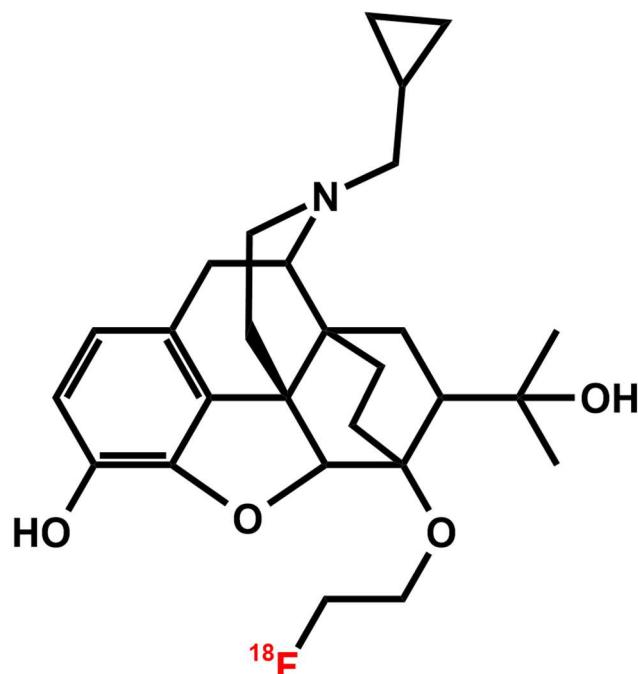
Figure 202: Structure of <sup>18</sup>F- Fluoroatipamezol

### ***<sup>18</sup>F-NMSP***

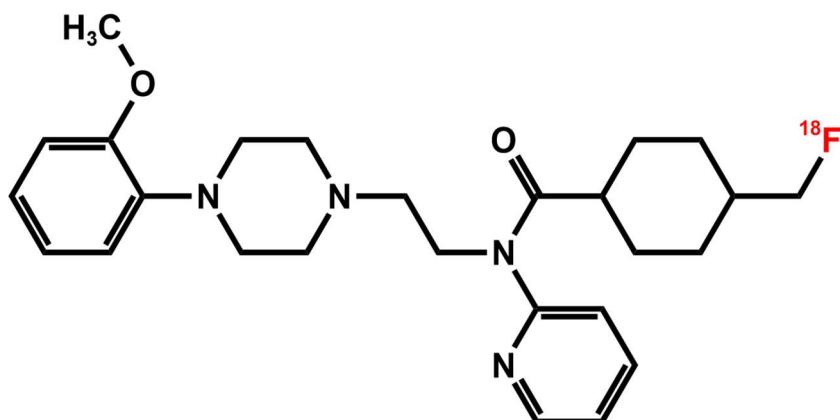
<sup>18</sup>F-N-methylspiperone or <sup>18</sup>F-NMSP (Figure 203) is an analogue of the neuroleptic spiroperidol, binds dopamine receptors and is used for the studies of dopamine receptor in humans and conditions related to dopamine receptors such as schizophrenia.

Figure 203: Structure of  $^{18}\text{F}$ - NMSP **$^{18}\text{F}$ -FDPN**

$^{18}\text{F}$ -fluoroethyl-diprenorphine or  $^{18}\text{F}$ -FDPN (Figure 204) binds opioid receptors and is a PET tool for studies of the system related to opioid receptors. In this way neuroscientists, psychiatrists and psychologists are able to uncover mechanisms and processes involving opioid receptors such as problem of pain and alleviation of pain, but also problems with addiction, and overdoses.

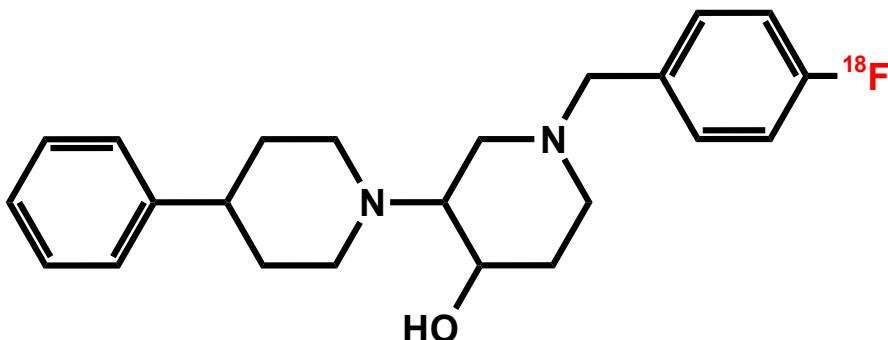
Figure 204: Structure of  $^{18}\text{F}$ - FDPM **$^{18}\text{F}$ -Mefway**

$^{18}\text{F}$ -Mefway (Figure 205) is a 5-HT<sub>1A</sub> receptor antagonist (binds and blocks so called 5-HT<sub>1A</sub> receptors in brain that usually bind neurotransmitter called serotonin). It is used in medical research to quantify distribution of serotonin 5-HT<sub>1A</sub> receptors in some regions of brain in order to study of various central nervous system disorders.

Figure 205: Structure of  $^{18}\text{F}$ -Mefway

### $^{18}\text{F}$ -FBT

$^{18}\text{F}$ -fluorobenzyltrozamicol (also known as  $^{18}\text{F}$ -FBT, Figure 206) binds “vesicular acetylcholine transporter” protein (VAcChT) in neurons and is used in experimental physiology and pharmacology to study neurological topics.

Figure 206: Structure of  $^{18}\text{F}$ - FBT

### $^{18}\text{F}$ -labelled PET radiotracers in oncology

Oncology is a medical branch dealing with treatment of tumours (cancers) either solid ones (lungs, breasts, prostate, colon, brain etc.) or those of blood cells (leukaemia, lymphomas, myelomas). As such it is one of the most demanding and invested medical branches due to the high mortality from cancer. The most important  $^{18}\text{F}$ -labelled PET radiotracer in oncology is, for sure,  $^{18}\text{FDG}$ . Besides it there are many other radiotracers, and some are already presented earlier such as  $^{18}\text{Fluorothymidine}$ . Herein five more  $^{18}\text{F}$ -labelled tracers will be presented.

### $^{18}\text{F}$ -Fluorouracil

$^{18}\text{F}$ -Fluorouracil (Figure 207 left) is the  $^{18}\text{F}$ -labelled version of old anticancer drug fluorouracil. The only difference is the radioactivity of the fluorine atom. Fluorouracil binds and inhibits enzyme “thymidylate synthase” required for DNA replication. It targets cancer cells and is used for differentiation of malignant tumours from inflammation using PET scan.

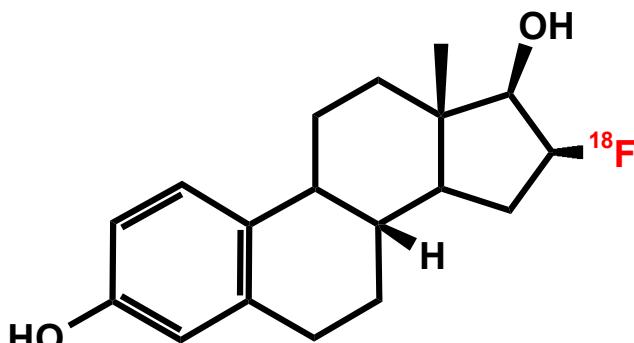
Figure 207: Structures of <sup>18</sup>F-Fluorouracil (left) and <sup>18</sup>F-Fluciclovine (right)

### <sup>18</sup>F-Fluciclovine

<sup>18</sup>F-Fluciclovine (Figure 207 right) is the <sup>18</sup>F-labelled synthetic analogue of amino acid L-leucine. Due to their increased metabolic needs, it is taken up by cancer cells much more than healthy cell. Also, unlike naturally occurring amino acids, it cannot be metabolised, and it accumulates in tumour cells. Therefore, it can be used for imaging of tumours.

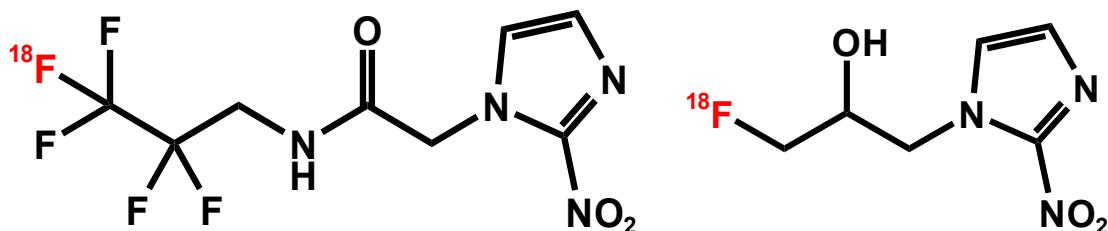
### <sup>18</sup>F-Fluoroestradiol

<sup>18</sup>F-Fluoroestradiol (<sup>18</sup>FES, Figure 208) is an analogue of female hormone estrogen: it specifically binds to estrogen receptors. Some breast cancer cells are rich in these receptors and this radiotracer is used to detect and locate breast cancer cells.

Figure 208: Structure of <sup>18</sup>F-fluoroestradiol

### <sup>18</sup>F-EF5 and <sup>18</sup>F-MISO

<sup>18</sup>F-EF5 (Figure 209 left) is used for PET imaging of hypoxia (lack of oxygen) in cancer cells. This condition is characteristic for cells that have higher immunity against radiation (this is called radioresistance). This PET tracer is used to detect radioresistant cancer cells: this can help show how a tumour will respond to treatment. Its smaller analogue, <sup>18</sup>F-fluoromisonidazol (<sup>18</sup>F-MISO, Figure 209 right) is used for the same purpose.

Figure 209: Structure of <sup>18</sup>F-EF5 (left) and <sup>18</sup>F-MISO (right)

### **<sup>18</sup>F-labelled PET radiotracers in cardiology**

Cardiology is the medical branch dealing with heart diseases. These diseases are very common and often deadly: cardiomyopathy, atherosclerosis, angina pectoris, heart infarction, arrhythmias are disease affecting millions of people around the world, therefore this branch of medicine is very important. However, the <sup>18</sup>F-labelled PET radiotracers in cardiology are not so common, and here only two will be presented.

<sup>18</sup>F-Fluorometaraminol (Figure 210 left) is an analogue of neurotransmitter and hormone adrenaline, and it binds adrenalin receptors in heart. It is used for mapping of adrenergic nerves of the heart, PET imaging of nerve integrity in heart in patients with heart arrhythmia. <sup>18</sup>F-Fluorocarazolol (Figure 210, right) is another interesting <sup>18</sup>F-labelled radiotracer for cardiology; it binds and blocks  $\beta_2$ -receptors that usually bind adrenalin. It is used for imaging of  $\beta_2$ -receptors in heart.

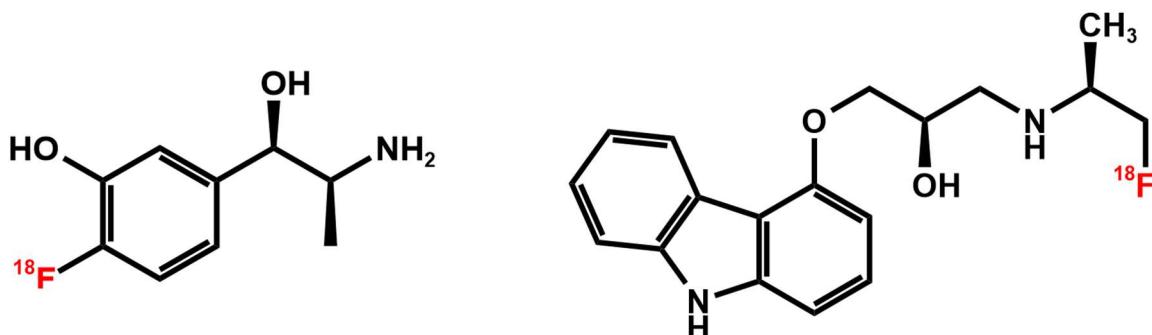


Figure 210: Structure of <sup>18</sup>F-Fluorometaraminol (left) and <sup>18</sup>F-fluorocarazolol (right)

### **Other <sup>18</sup>F-labelled PET radiotracers**

There are many other important <sup>18</sup>F-labelled PET tracers but here we will show only two. <sup>18</sup>F-Fluoroganciclovir (Figure 211) is <sup>18</sup>F-labelled antiviral drug ganciclovir. It targets thymidine kinase enzyme in herpes simplex virus 1 and is used to identify and assess infection of liver with adenovirus. Also, it is used in experimental medicine. <sup>18</sup>F-CFB (Cholestryl-*p*-<sup>18</sup>Fluorobenzoate, Figure 211) is derivative of cholesterol and selectively accumulates in the adrenal cortex (core of adrenal glands). Therefore, it is a radiopharmaceutical agent for PET imaging of various adrenal disorders (disorders of adrenal gland).

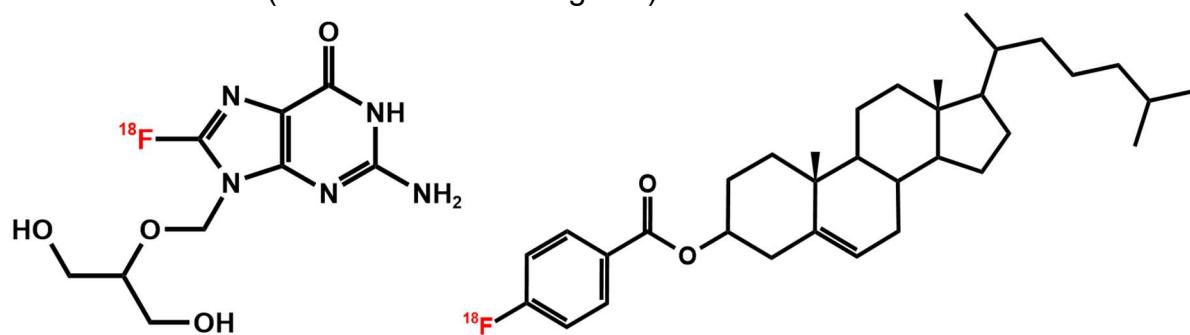


Figure 211: Structure of <sup>18</sup>F-Fluoroganciclovir (left) and <sup>18</sup>F-CFB (right)

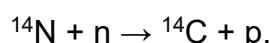


## Chapter X - Carbon-11

Carbon-11 is the second most important radionuclides for PET imaging. Due to its unique properties, it is slowly becoming more important and has a great potential to become key part of nuclear medicine.

### Isotopes of carbon

Carbon is a common non-metal element, basis for the organic world. It has 13 isotopes; however, only  $^{12}\text{C}$  and  $^{13}\text{C}$  are natural.  $^{12}\text{C}$  presents overwhelming part of natural carbon comprising 98.93% of all carbon atoms, while  $^{13}\text{C}$  makes just 1.07%. This 1% of  $^{13}\text{C}$  is the basis for so called C-13 NMR spectroscopy. The longest-lived radioisotope of carbon is  $^{14}\text{C}$ , with its half-life of 5730 years. This is also the only carbon radioisotope found in nature: just trace quantities are formed in atmosphere cosmogenically by the reaction:



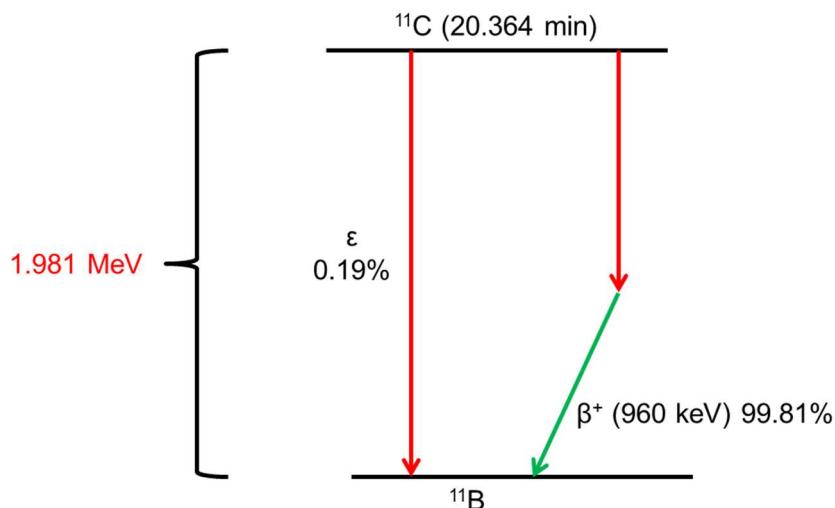
$^{14}\text{C}$  emits just soft beta particles and cannot be used in nuclear medicine, however it is the basis for so called C-14 dating method widely used in archaeology, forensics and palaeontology. Also,  $^{14}\text{C}$  is often made in graphite moderated nuclear reactors when carbon captures neutrons. In addition,  $^{14}\text{C}$  can be used in medical biochemical laboratory tests (so called “*in vitro*” application which means “*in glass*”) but cannot be used on live humans (so called “*in vivo*”, which means “*in live*”) since it emits soft beta particles only and these particles cannot be seen in live body. Therefore, the only carbon isotope that can be used in nuclear medicine is  $^{11}\text{C}$ . Just like  $^{18}\text{F}$  it is emitting positrons and has sufficient although quite short half-life (20 minutes). All other carbon radioisotopes have half-life too short (few seconds or much less), impractical for nuclear medicine.

$^8\text{C}$	$^9\text{C}$	$^{10}\text{C}$	$^{11}\text{C}$	$^{12}\text{C}$	$^{13}\text{C}$	$^{14}\text{C}$	$^{15}\text{C}$	$^{16}\text{C}$	$^{17}\text{C}$	$^{18}\text{C}$	$^{19}\text{C}$	$^{20}\text{C}$
Emit positrons				Stable	Emit betas (electrons)							

Figure 212: Selected isotopes of carbon

### Nuclear properties of $^{11}\text{C}$

The half-life of  $^{11}\text{C}$  is 20.364 minutes. This half-life is sufficiently long to make PET tracers, but it is too short for transportation, and this presents a major obstacle for  $^{11}\text{C}$  to be more widely used in nuclear medicine.  $^{11}\text{C}$  decays to  $^{11}\text{B}$  by positron emission ( $\beta^+$ , 99.81%) and just in very tiny, insignificant percentage by electron capture (0.91%) as shown in the Figure 213. The positrons ( $\beta^+$ ) emitted by  $^{11}\text{C}$  have mean energy of 384 keV (that is higher than that of  $^{18}\text{F}$  which is 250 keV) while the maximal energy is 960 keV (for  $^{18}\text{F}$  is 634 keV).

Figure 213: Decay of  $^{11}\text{C}$ 

When translated into flying distance (range) how far positrons emitted by  $^{11}\text{C}$  will fly in water or tissue before they get annihilated by electrons then the average range is 1.2 mm while the maximum range is 4.3 mm (Figure 214). This is larger than the average, mean and maximal distances for  $^{18}\text{F}$ . In theory, this produces twice larger uncertainty, but in practice this is not a grave drawback, in fact  $^{11}\text{C}$  gives fairly good PET images.

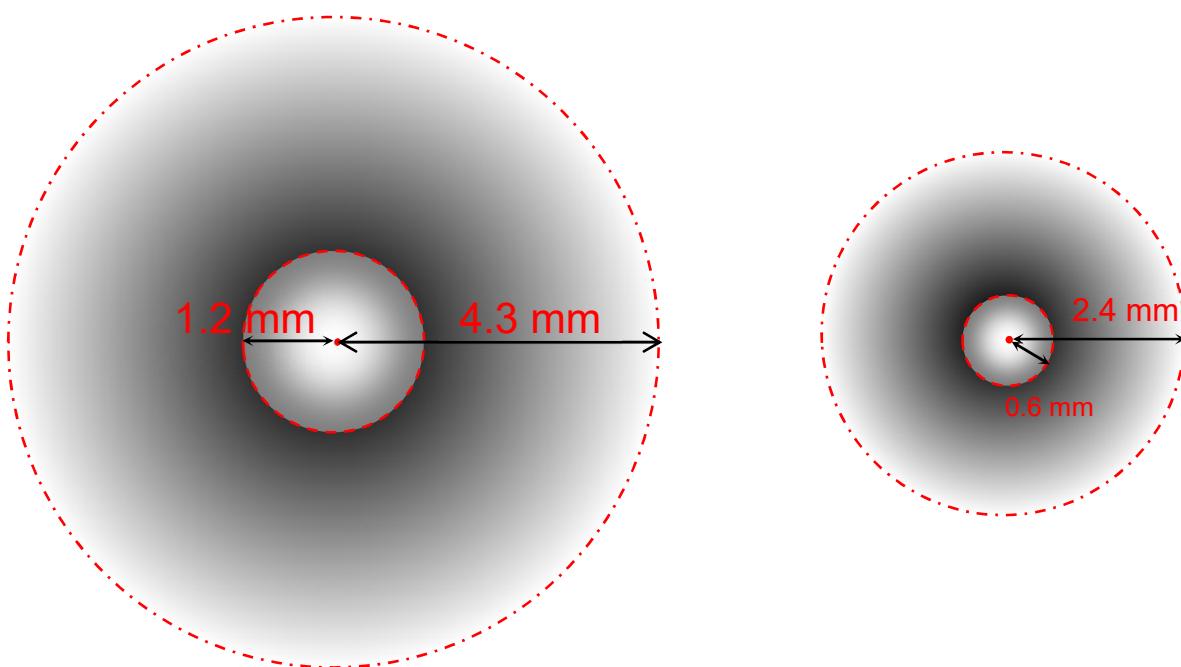
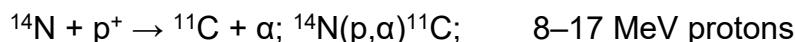


Figure 214: Spheres of probability of positron range of  $^{11}\text{C}$  (left) and  $^{18}\text{F}$  (right). The red dot in the middle of the cycles represents decaying nucleus while dark cloud around it represents possible zone of flying range of positron before it encounters electron and gets annihilated into two photons. The most probable distance between decaying nucleus and positron firing photon will be anywhere 1.2 mm away in the case of  $^{11}\text{C}$  and 0.6 mm in the case of  $^{18}\text{F}$ . The maximal distance will be 4.3 mm for  $^{11}\text{C}$  and 2.4 mm for  $^{18}\text{F}$ . This means that  $^{18}\text{F}$  inherently makes images of better resolution since there is less uncertainty where location of tracer actually is.

## Production of $^{11}\text{C}$

$^{11}\text{C}$  is produced in cyclotrons and, together with  $^{18}\text{F}$ , is the most important medical radionuclide produced by medical cyclotrons. Generally, it can be made by small power cyclotrons. It is made by bombarding natural ( $^{14}\text{N}$ ) nitrogen gas with protons of 8–17 MeV:



In this nuclear reaction the nitrogen atom absorbs a proton, and one alpha particle is released to give  $^{11}\text{C}$ . It is a typical gas phase target bombardment using a gas target chamber.

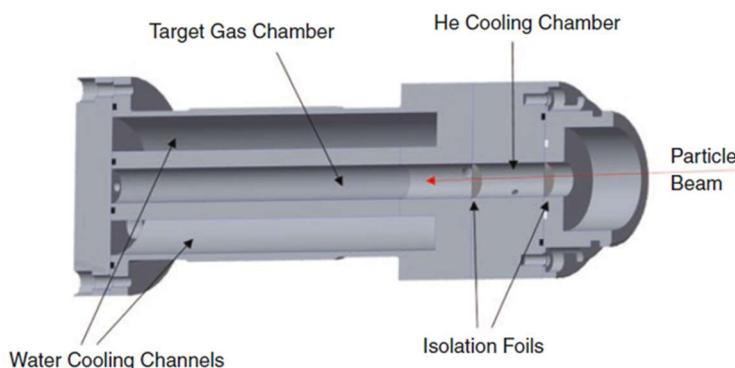


Figure 215: Gas target chamber for the production of  $^{11}\text{C}$

It is important that nitrogen gas used as a target is of high purity. In general carbon comes in the form of a solid, but we need a gas, therefore a reagent is added into the gas target chamber. If 0.5 to 1% of oxygen gas is added, then the result is  $^{11}\text{C}$ -labelled carbon dioxide ( $^{11}\text{CO}_2$ ). On the other hand, if 5-10% of hydrogen gas is added then the result is  $^{11}\text{C}$ -labelled methane ( $^{11}\text{CH}_4$ ). Which gas will be made depends on the radiopharmaceutical agent that needs to be synthesised and method of its synthesis. However, in practice,  $^{11}\text{CH}_4$  gas is more useful. Thus obtained gases,  $^{11}\text{CO}_2$  and  $^{11}\text{CH}_4$  are called the primary precursors or “in-target” precursors.

## Chemical aspects of $^{11}\text{C}$ comparing to $^{18}\text{F}$

$^{11}\text{C}$  carbon has one big advantage comparing to  $^{18}\text{F}$ : opposite to fluorine carbon is a biogenic element. This means it is normally present in biomolecules. In fact, it is the major backbone of all organic and biological matter. Together with H, O, N, S and P carbon makes all matter of the living world and biosphere. Other elements that can be found in biological realm are Cl, K, Na, Mg, Ca, Fe, Zn, and I, but these are of less importance in biology. A PET tracer labelled with  $^{11}\text{C}$  cannot be distinguished (chemically, biologically) from non-labelled versions;  $^{11}\text{C}$ -labelled radiopharmaceuticals behave exactly the same as their non-labelled equivalents: no difference whatsoever. Therefore,  $^{11}\text{C}$ -labelled radiopharmaceuticals are the perfect de Hevesy tracers, body cannot see any difference between artificial  $^{11}\text{C}$ -labelled molecule and the same one with natural  $^{12}\text{C}$ . This is a stark difference comparing to fluorine because the fluorine attached onto organic molecules is foreign to organism



and labelling organic molecule with fluorine usually changes its properties and behaviour. Another great advantage of  $^{11}\text{C}$  is that its chemistry is incredibly rich and  $^{11}\text{C}$  can be inserted into literally any organic molecule. That makes number of possible PET tracers tremendously huge.

## Major drawbacks of $^{11}\text{C}$

Unfortunately, the major drawback of  $^{11}\text{C}$  is its very short half-life of just 20 minutes. Compared with 109 minutes of  $^{18}\text{F}$  it is more than 5 times shorter. A rule of thumb says that the time span from production in a synchrotron to “ready-to-use” vial with a purified pharmaceutical grade  $^{11}\text{C}$ -labelled PET tracer should not be longer than three half-lives. In the case of  $^{11}\text{C}$  it is 1 hour. This means that the work with  $^{11}\text{C}$  is much less practical and convenient comparing to  $^{18}\text{F}$ . Preparation of  $^{11}\text{C}$  PET tracers requires much more fast/rapid labelling chemistry and purification procedures. Transportation of produced radiopharmaceutical agent containing  $^{11}\text{C}$  from one facility to another is less likely, half-life is just too short for any significant transportation (although one can imagine ultra-fast drones being able to fly vials several kilometres away to another facility). Therefore,  $^{11}\text{C}$ -labelled tracers are usually applied in the same facility where they are produced. In total, all these points make  $^{11}\text{C}$  much less attractive despite being the perfect De Hevesy tracer.

## Primary and secondary precursors for $^{11}\text{C}$ -labelling

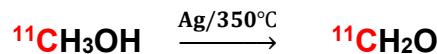
As mentioned before, the two main primary precursors coming from cyclotron are  $^{11}\text{CO}_2$  and  $^{11}\text{CH}_4$  whereby methane is more preferred, since it is made with higher specific activity, practical for labelling procedures.

However, these gases, the primary precursors are rarely directly used for labelling due to their low reactivity (although  $^{11}\text{C}$ -labeled acetates and some fatty acids can be easily prepared directly from  $^{11}\text{CO}_2$ ). Therefore secondary, more reactive precursors are made from  $^{11}\text{CO}_2$  or  $^{11}\text{CH}_4$ :

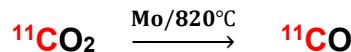
- $^{11}\text{C}$ -labelled methanol ( $^{11}\text{CH}_3\text{OH}$ ) can be made by the reaction of  $^{11}\text{CO}_2$  and reducing agent lithium aluminium hydride ( $\text{LiAlH}_4$ ):



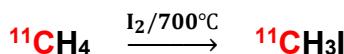
- Formaldehyde ( $^{11}\text{CH}_2\text{O}$ ) is prepared from  $^{11}\text{C}$ -labelled methanol by heating it on silver at  $350^\circ\text{C}$ :



Also, carbon monoxide ( $^{11}\text{CO}$ ) can be made from carbon dioxide by heating it in the presence of molybdenum metal at  $820^\circ\text{C}$ :



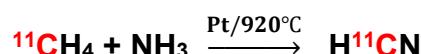
- Methyl iodide ( $^{11}\text{CH}_3\text{I}$ ), the most important and the most versatile secondary precursor is made by heating  $^{11}\text{C}$ -labelled methane in the presence of iodine gas ( $\text{I}_2$ ) at  $700^\circ\text{C}$ :



- Even more reactive precursor, methyl-triflate ( $^{11}\text{CH}_3\text{OTf}$ ) is made if  $^{11}\text{C}$ -labelled methyl iodide is reacted with silver-triflate at  $200^\circ\text{C}$ :



- Hydrogen cyanide ( $\text{H}^{11}\text{CN}$ ), very toxic, but very reactive gas can be made from methane by reacting it with ammonia ( $\text{NH}_3$ ) at  $920^\circ\text{C}$  in presence of platinum metal catalyst:



- Phosgene ( $^{11}\text{COCl}_2$ ), famous warfare agent and very reactive chemical can be made by reacting methane with copper chloride ( $\text{CuCl}_2$ ) at  $380^\circ\text{C}$  to obtain radiocarbon tetrachloride ( $^{11}\text{CCl}_4$ ) and then it is oxidised with oxygen in presence of iron metal.



- Carbon disulfide ( $^{11}\text{CS}_2$ ) is made when methyl iodide is reacted with elemental sulphur ( $\text{S}_8$ ) on sand at  $500^\circ\text{C}$ .

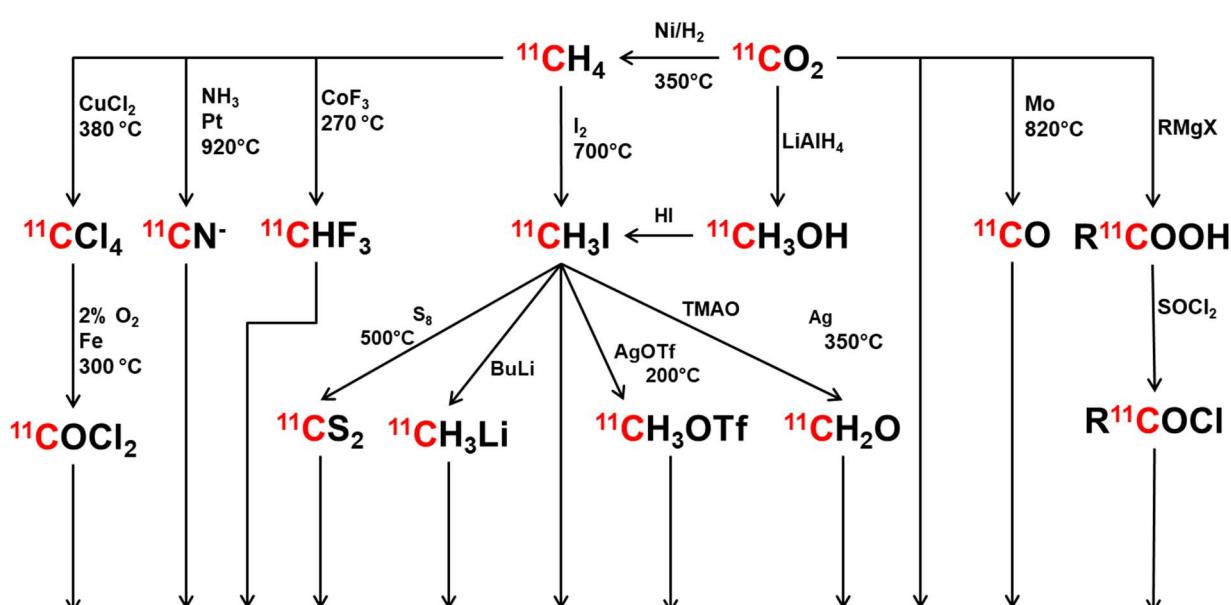


Figure 216: Scheme summarising reactions for making secondary precursor from primary ones ( $^{11}\text{CH}_4$  and  $^{11}\text{CO}_2$ )

This scheme summarises all the  $^{11}\text{C}$  chemistry of primary and secondary precursors and how to obtain each one of them from primary precursors. The first row are primary precursors  $^{11}\text{CH}_4$  and  $^{11}\text{CO}_2$ . It is interesting to note that  $^{11}\text{CO}_2$  can be turned into  $^{11}\text{CH}_4$  by reacting it with hydrogen gas ( $\text{H}_2$ ) in presence of nickel metal (Ni) as a catalyst at  $350^\circ\text{C}$ . The second row are secondary precursors obtained from the primary ones,  $^{11}\text{CCl}_4$ ,  $\text{H}^{11}\text{CN}$ ,  $^{11}\text{CHF}_3$ ,  $^{11}\text{CH}_3\text{I}$ ,  $^{11}\text{CH}_3\text{OH}$ ,  $^{11}\text{CO}$  and  $\text{R}^{11}\text{COOH}$  ( $^{11}\text{C}$ -labelled carboxylic acids). Finally, the third row are those tertiary precursors obtained

from the secondary ones:  $^{11}\text{COCl}_2$  (phosgene),  $^{11}\text{CS}_2$  (carbon disulfide),  $^{11}\text{CH}_3\text{Li}$  (methyl lithium),  $^{11}\text{CH}_3\text{OTf}$  (methyl triflate),  $^{11}\text{CH}_2\text{O}$  (formaldehyde) and  $\text{R}^{11}\text{COCl}$  (acyl chlorides).

What is interesting to note is the centrality of  $^{11}\text{C}$ -labelled methyl iodide ( $^{11}\text{CH}_3\text{I}$ ) in the world of  $^{11}\text{C}$ -labelling. This precursor can be used for labelling directly or indirectly, to make other powerful building blocks and reagents. It is also interesting to note that  $^{11}\text{CH}_3\text{I}$  can be made using two synthetic pathways as well as formaldehyde. All these secondary and tertiary precursors are used in huge number of labelling reactions to make many various  $^{11}\text{C}$ -labelled PET tracers in form of small drug-like molecules (radiopharmaceuticals). Another interesting thing to note is that all these reactions involve usually one  $^{11}\text{C}$  atom and are done at very high temperatures in the presence of some inorganic catalyst. These kind of high temperature, metal-catalysed reactions are in fact very efficient and rapid, allowing very fast transformations in very high radiochemical yields.

Here is another funny graphic showing what we have seen on previous page: in the root of all the  $^{11}\text{C}$ -labelling tree is the cyclotron reaction and the stem are  $^{11}\text{CO}_2$  and  $^{11}\text{CH}_4$ . Then all the branches and fruits are all other precursors that can be made out of these two primary precursors.

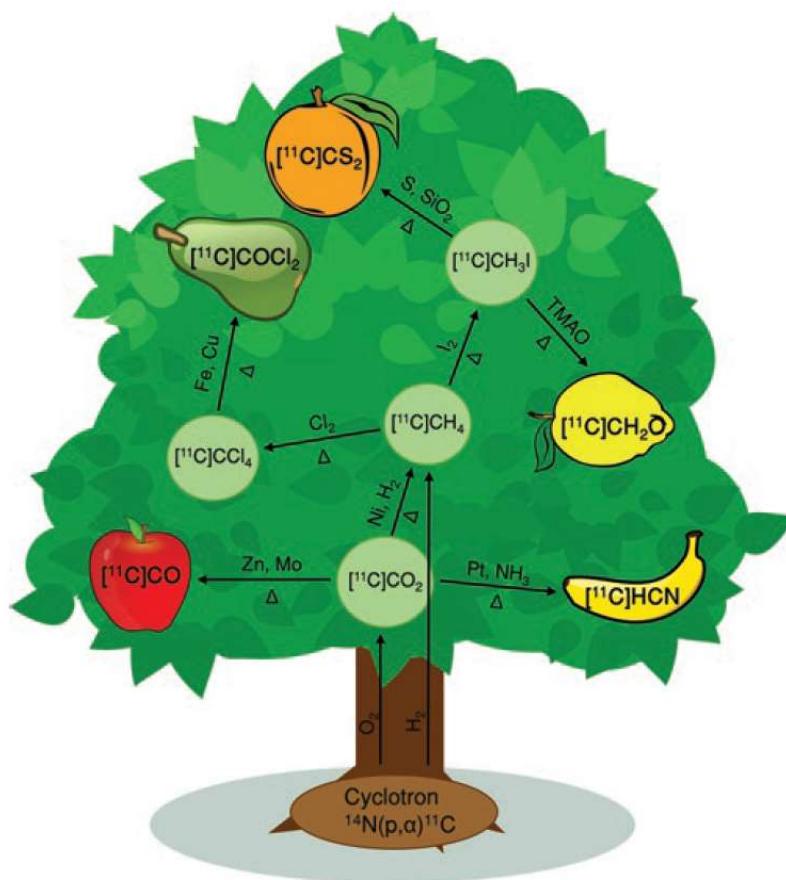


Figure 217: Tree of primary and secondary  $^{11}\text{C}$  precursors



## General aspects of $^{11}\text{C}$ -labelling

Labelling with  $^{11}\text{C}$  is in fact quite similar like labelling with  $^{18}\text{F}$ , but there are some specific things regarding the  $^{11}\text{C}$ -labelling.

The labelling reaction, i.e. introduction of  $^{11}\text{C}$  into the tracer molecule should be as late as possible in the reaction sequence: it should be the last reaction if possible. Also, the time of the labelling reaction should be as short as possible to optimize radiochemical yield and molar activity. Therefore, some slow reactions cannot be used, even if they are high yielding, while the use of catalysis (metal or enzyme-based) is strongly encouraged to speed up the reaction and improve yields. In fact, shortening time of reaction has priority over improving the yield since too long reaction time diminishes the radiochemical yield.

Reactions used for  $^{11}\text{C}$ -labelling are very numerous: number, diversity and variety of reactions used for  $^{11}\text{C}$  labelling are far more numerous than in the case of  $^{18}\text{F}$ -labelling reactions and covers almost all areas and reaction of organic chemistry. Due to the huge diversity of reactions with carbon  $^{11}\text{C}$  can be inserted into nearly any organic molecule.

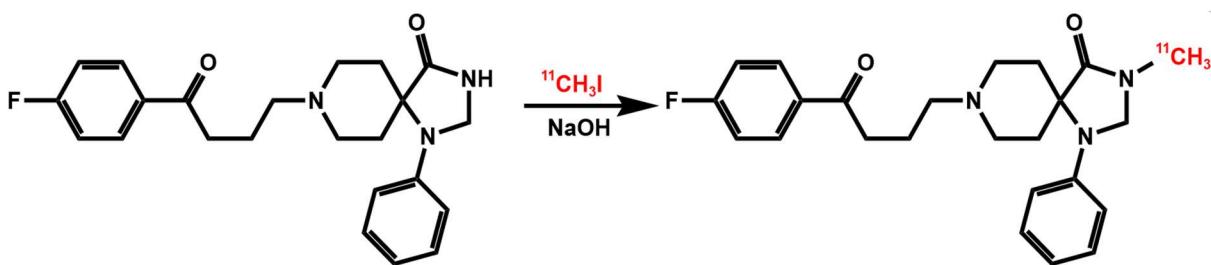
Possibilities of various PET tracers are endless, for sure if time allows. Some of the typical reactions used for  $^{11}\text{C}$ -labelling are;

- methylations (usually with  $^{11}\text{CH}_3\text{I}$  or  $^{11}\text{CH}_3\text{OTf}$ ),
- carbonylations (with  $^{11}\text{CO}$ ),
- Grignard reactions (with  $^{11}\text{CO}_2$ ),
- $^{11}\text{CO}_2$  fixation reactions,
- various substitutions (nucleophilic and electrophilic),
- transition metal-catalysed reactions (with  $^{11}\text{CH}_3\text{I}$  or  $^{11}\text{CO}$ )
- enzymatic catalysis reactions.

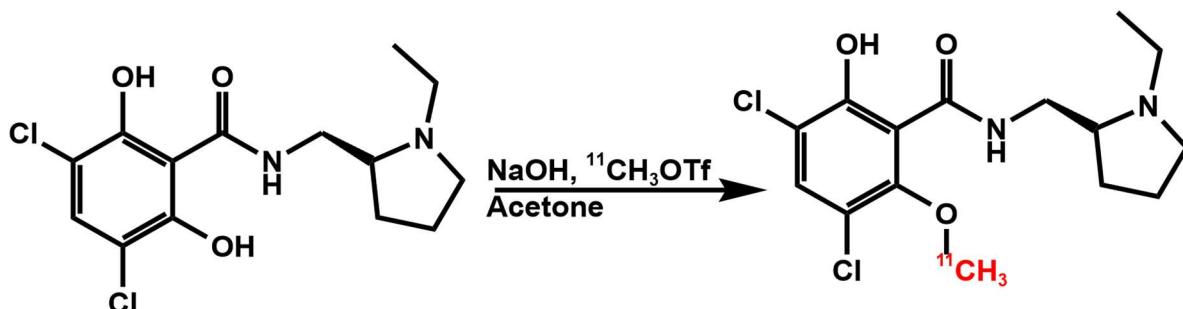
## $^{11}\text{C}$ -labelling with $^{11}\text{CH}_3\text{I}$

The  $^{11}\text{CH}_3\text{I}$  or  $^{11}\text{C}$ -labelled methyl iodide is the central and the most useful precursor or reagent for the  $^{11}\text{C}$ -labelling. It is widely used for “methylations”, where molecules with proton-donor functional groups (for example some alcohols or amines) are labelled or “tagged” with  $^{11}\text{C}$ -labelled methyl group  $^{11}\text{CH}_3$ .

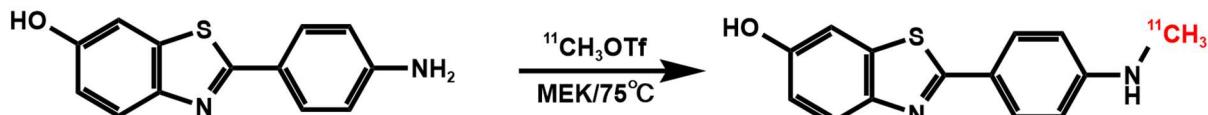
For example,  $^{11}\text{CH}_3\text{I}$  is used in a simple reaction with a base such as sodium hydroxide ( $\text{NaOH}$ ) to “tag” or label spiroperone, a famous drug (Figure 218). Also,  $^{11}\text{CH}_3\text{I}$  is used for the quick synthesis of other, tertiary precursors, such as is very reactive  $^{11}\text{C}$ -labelled methyl triflate ( $^{11}\text{CH}_3\text{OTf}$  or  $^{11}\text{CH}_3\text{SO}_2\text{CF}_3$ ). This tertiary precursor is way more reactive than the parent  $^{11}\text{CH}_3\text{I}$ . Also, other precursors such as  $^{11}\text{CS}_2$ ,  $^{11}\text{CH}_3\text{Li}$ ,  $^{11}\text{CH}_3\text{NO}_2$ ,  $^{11}\text{CH}_3\text{NCO}$  can be made from  $^{11}\text{CH}_3\text{I}$ .

Figure 218: Synthesis of  $^{11}\text{CH}_3$ -labelled spiroperone

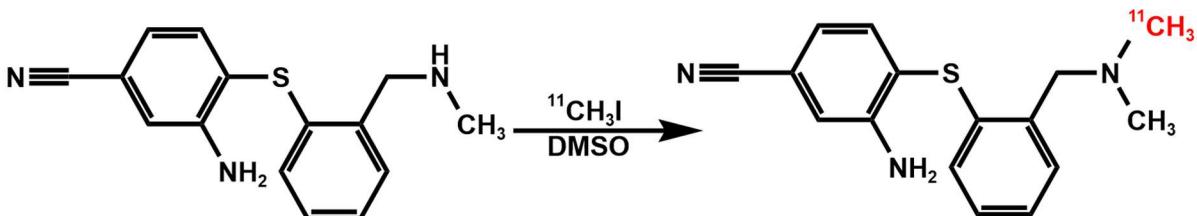
In another example (Figure 219)  $^{11}\text{C}$ -raclopride, a PET tracer for neuroimaging is made by using  $^{11}\text{CH}_3\text{OTf}$  in the presence of a base and solvent: it just gets added onto hydroxyl group (OH).

Figure 219: Synthesis of  $^{11}\text{C}$ -labelled raclopride

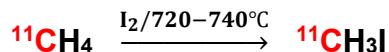
Some other examples of  $^{11}\text{C}$  labelling or “tagging” by methylation will be shown. The first example is labelling of  $^{11}\text{C}$ -PiB (Figure 220), also known as Pittsburgh compound B, PET tracer for imaging of Alzheimer disease by using methyl-triflate:

Figure 220: Synthesis of  $^{11}\text{C}$ -PiB

Another example is synthesis of  $^{11}\text{C}$ -DASB (Figure 221), very important neuroimaging agent for various neurological and psychiatric diseases and conditions: it goes very easy and efficient in DMSO and with no other additive:

Figure 221: Synthesis of  $^{11}\text{C}$ -DASB

$^{11}\text{C}$ -labeled methyl iodide,  $^{11}\text{CH}_3\text{I}$  can be made using a two synthetic methods or pathways. The first is so called “gas phase process”, here we can use  $^{11}\text{CH}_4$  and react is with iodine gas at 720 to 740°C:



Another option is to use  $^{11}\text{CO}_2$  where it is firstly reduced by hydrogen gas in the presence of nickel metal catalyst and then is used in the reaction identical to the first one. This method is in fact more preferred:



The second option is so called “wet process” that happens at low temperature. Carbon dioxide is firstly reacted with the reducing agent called lithium aluminium hydride and then with water to obtain methanol. Methanol is then reacted with hydrogen iodide to obtain methyl iodide. This wet process is in fact not as efficient as the gas phase process:



Except in methylations where methyl group is added onto heteroatoms such as N, O or S  $^{11}\text{C}$ -labelled methyl iodide can be used for C-C coupling reactions where methyl radical is added onto C atom, even directly onto the benzene ring. These are advanced, modern palladium-catalysed C-C coupling reactions such as Stille coupling and Suzuki-Miyaura coupling. In the first example  $^{11}\text{CH}_3$  is used as a reagent in Stille coupling (Figure 222) to make  $^{11}\text{C}$ -labelled acetophenone:

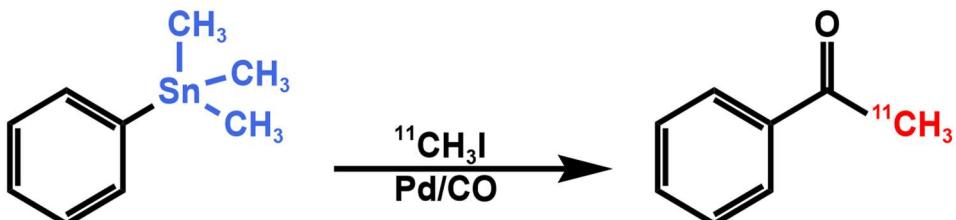


Figure 222: Synthesis of  $^{11}\text{C}$ -acetophenone by using Stille coupling.

Another example is Suzuki-Miyaura coupling, synthesis of PET tracer  $^{11}\text{C}$ -cetrozole: a large boric acid ester group is substituted with an  $^{11}\text{C}$ -methyl group by using palladium-based catalysts (Figure 223).

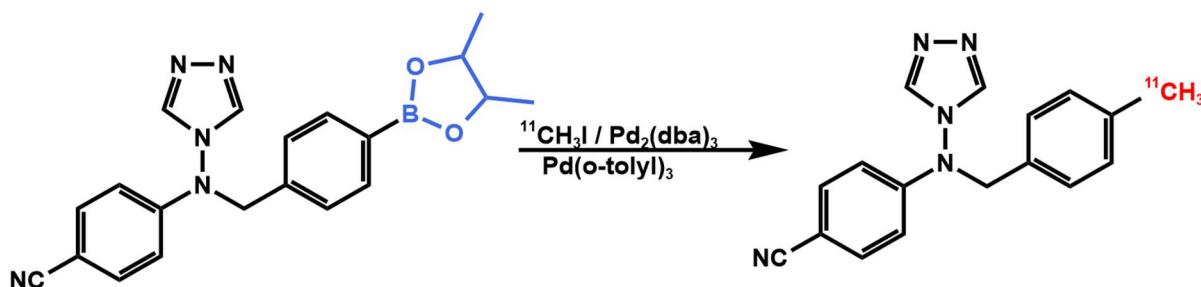


Figure 223: Synthesis of  $^{11}\text{C}$ -cetrozole by using Suzuki-Miyaura coupling.

Generally, transition metal-catalysed, C-C bond formation reactions have been found to significantly expand the synthetic opportunities with  $^{11}\text{C}$ .

### $^{11}\text{C}$ -labelling with $\text{CH}_3\text{Li}$

The C-C coupling can be also achieved by using tertiary  $^{11}\text{C}$ -labelled precursor methyl lithium. Generally, organolithium chemistry is one of the most important methods for C-C coupling: using organolithium reagents it is possible to make huge range of various organic compounds. Typical organolithium reagents are *tert*-butyl-lithium (*t*-BuLi) or *n*-butyl-lithium (*n*-BuLi).  $^{11}\text{CH}_3\text{Li}$  is  $^{11}\text{C}$ -labelled organolithium reagent, and these C-C coupling reactions are usually aided by a palladium containing catalyst. Here is an example where any benzene aromatic ring with bromine leaving group can be substituted with  $^{11}\text{C}$ -labelled methyl by using  $^{11}\text{CH}_3\text{Li}$  and a palladium-based catalyst in presence of oxygen (Figure 224).

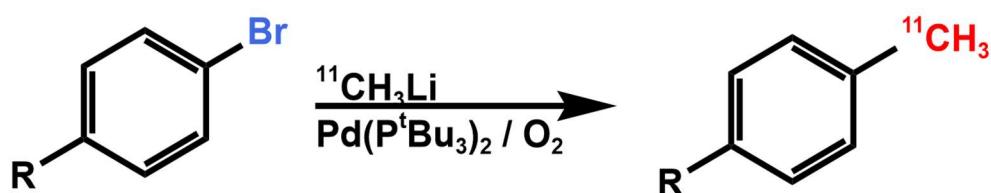
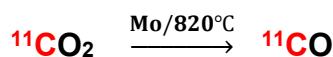


Figure 224: Adding  $^{11}\text{CH}_3$ - group by using  $^{11}\text{CH}_3\text{Li}$  and a palladium-based catalyst in presence of oxygen

### $^{11}\text{C}$ -labelling with CO: $^{11}\text{C}$ -carbonylations

Another important group of  $^{11}\text{C}$ -labelling reactions are  $^{11}\text{C}$ -labelling carbonylations. This term “carbonylation” refers to the introduction of carbon monoxide into organic molecules.  $^{11}\text{CO}$  can be produced from  $^{11}\text{CO}_2$  by using a reaction with molybdenum metal at  $820^\circ\text{C}$ :



However, CO is generally very inert gas, but transition metals are able to activate it. Therefore,  $^{11}\text{C}$ -carbonylations are typically performed using transition metal catalysts, reacting nucleophilic and electrophilic reagents.

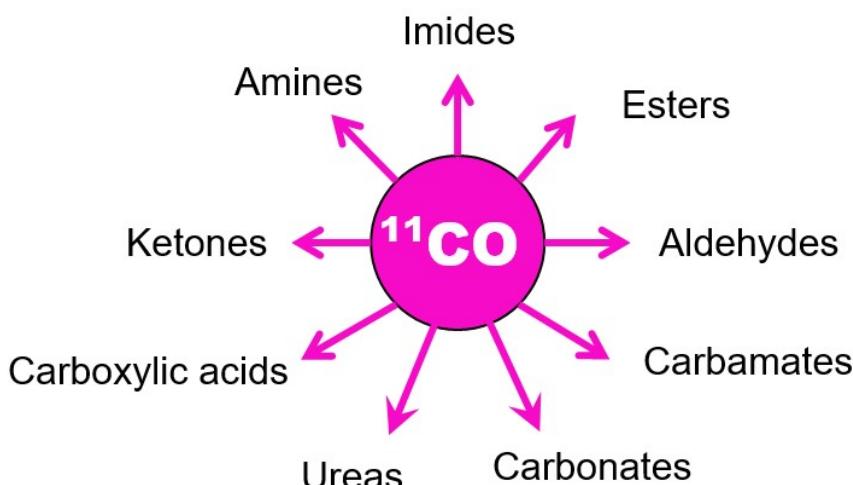


Figure 225:  $^{11}\text{CO}$  can be used in carbonylations of many types of compounds

These  $^{11}\text{C}$ -carbonylations can be good option to make many different kinds of  $^{11}\text{C}$ -labelled tracers and these include imides, esters, amines, ketones, aldehydes, carboxylic acids, ureas, carbonates, carbamates and many other (Figure 225).

Here three examples of these  $^{11}\text{C}$ -carbonylations will be shown. The first is a general synthesis of  $^{11}\text{C}$ -acetophenone (Figure 226) by using a Stille reagent and palladium catalyst: radioactive carbon is inserted into a “keto” position (compare this reaction with Stille coupling on the Figure 222 two pages up!).

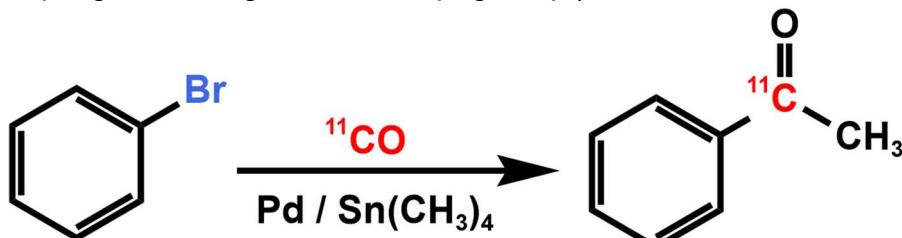


Figure 226:  $^{11}\text{C}$ -carbonylation of bromobenzene by using tetramethyl-tin and palladium catalyst

The second example (Figure 227) is the synthesis of  $^{11}\text{C}$ -labelled phenytoin (a famous old antiepileptic drug) using rhodium (Rh) based catalyst.

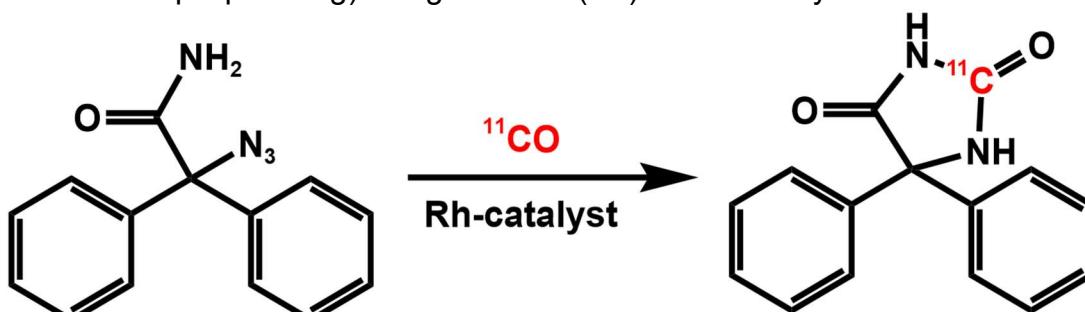


Figure 227: Synthesis of  $^{11}\text{C}$ -phenytoin via  $^{11}\text{C}$ -carbonylation

And finally, the third example (Figure 228) is the synthesis of  $^{11}\text{C}$ -labeled zolmitriptan (a drug for migraine) using a selenium reagent:

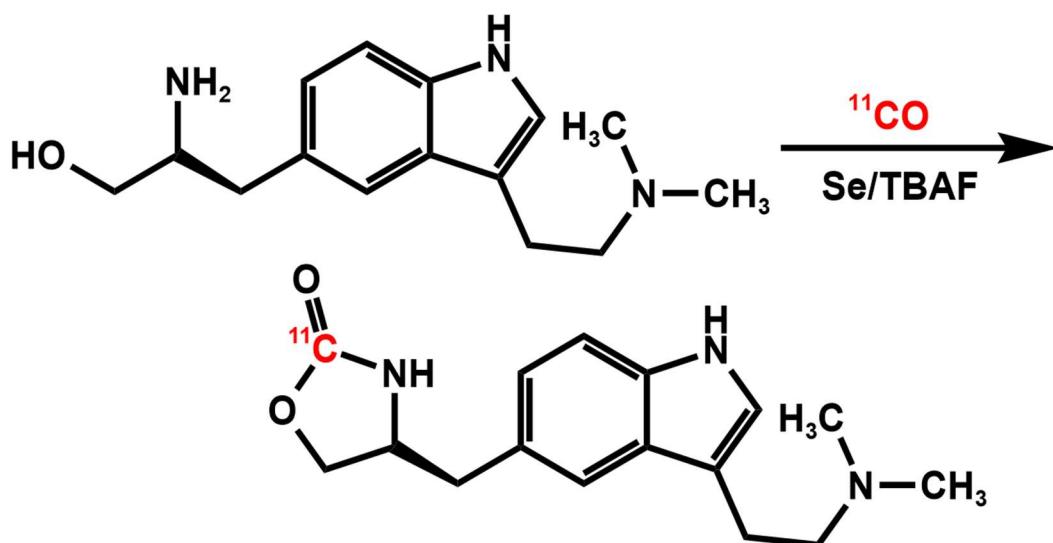
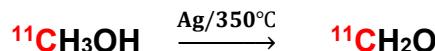


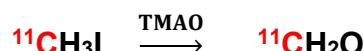
Figure 228: Synthesis of zolmitriptan via  $^{11}\text{C}$ -carbonylation

### <sup>11</sup>C-labelling with formaldehyde (<sup>11</sup>CH<sub>2</sub>O)

<sup>11</sup>C-labelled formaldehyde is another useful secondary precursor. It can be used for reductive aminations (synthesis of amines), for various ring closure reactions, for electrophilic aromatic substitution, and for many other labelling reactions. <sup>11</sup>C-labelled formaldehyde can be made in two ways. In the first one <sup>11</sup>C-labelled methanol reacts with silver metal at 350°C:



In an alternative method <sup>11</sup>C-labelled methyl iodide reacts with trimethylamine-N-oxide (TMAO) and gives <sup>11</sup>C-labelled formaldehyde:



An example of use of <sup>11</sup>C-labelled formaldehyde in labelling of serotonin to produce <sup>11</sup>C-N-methylserotonin, an analogue of neurotransmitter serotonin used for PET imaging of brain. Reaction goes at 90°C and takes only 5 minutes.

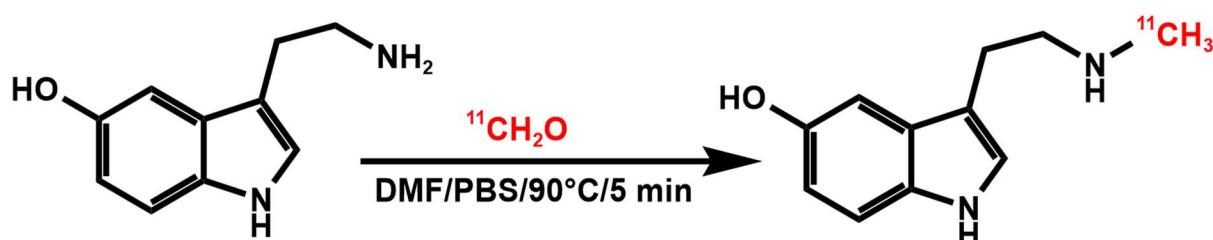


Figure 228: Synthesis of <sup>11</sup>C-N-methylserotonin by using <sup>11</sup>C-formaldehyde

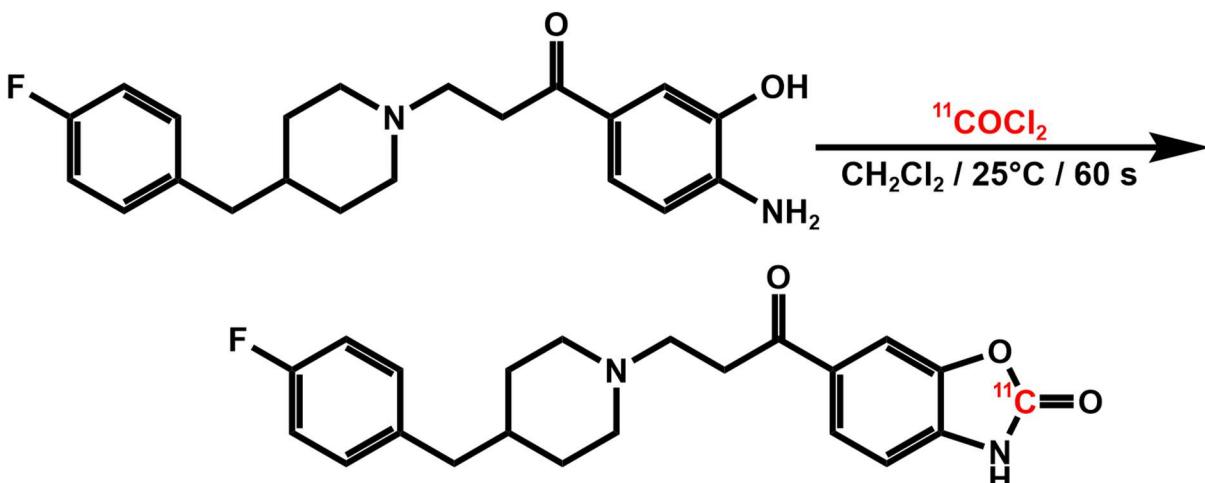
### <sup>11</sup>C-labelling with <sup>11</sup>C-phosgene (<sup>11</sup>COCl<sub>2</sub>)

Important precursor for <sup>11</sup>C-labelling is <sup>11</sup>C-phosgene (<sup>11</sup>COCl<sub>2</sub>). Generally, phosgene is a very toxic and reactive gas: in World War I in Europe it was used as chemical warfare agent. However, in radiopharmaceutical chemistry it is used in very tiny quantities, hence, actual risk of poisoning is negligible. It can be used for the <sup>11</sup>C labelling of amides, carbamates, ureas, and uric acids.

Synthesis of <sup>11</sup>C-labelled phosgene is a bit challenging and has two steps. In the first one <sup>11</sup>C labelled methane reacts with copper (II) chloride at 380°C and gives <sup>11</sup>C labelled tetrachloride carbon. This compound then gets oxidised with oxygen in the presence of iron metal at 300°C to yield <sup>11</sup>C labelled phosgene:

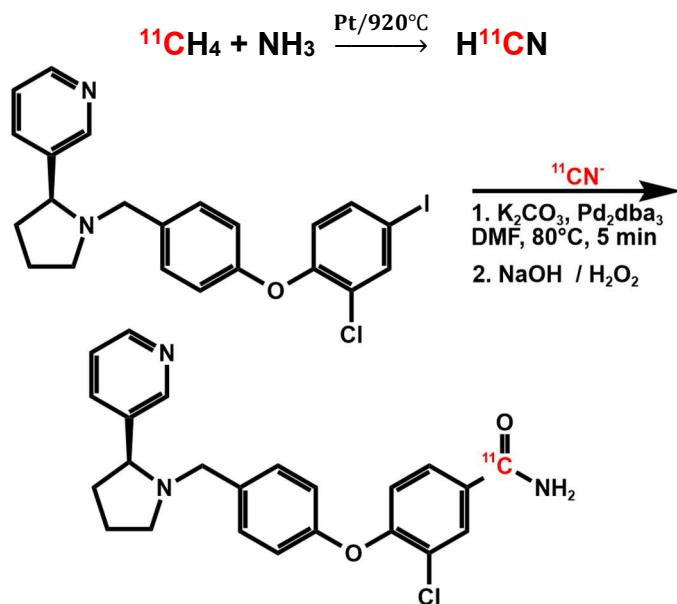


The example of use of <sup>11</sup>C labelled phosgene is the synthesis of <sup>11</sup>C-labelled EMD-95885, a PET tracer for imaging of brain. It reacts with amino and hydroxyl groups to form imido-ester moiety at room temperature in dichloromethane and reaction is finished in just 60 seconds.

Figure 229: Synthesis of  $^{11}\text{C}$ - EMD-95885 by using  $^{11}\text{C}$ -phosgene

### $^{11}\text{C}$ -labelling with $^{11}\text{C}$ -hydrogen cyanide ( $\text{H}^{11}\text{CN}$ )

Hydrogen cyanide is another very toxic and reactive gas, famous for its deadliness. There were numerous cases of poisoning with cyanide throughout history. Even very experienced synthetic chemists will try to avoid using hydrogen cyanide. As is the case with phosgene, radiochemists can work with  $^{11}\text{C}$ -labelled  $\text{H}^{11}\text{CN}$  without fear, since the amount used lies far below that which is dangerous. It can be used for the synthesis of nitriles, amides, carboxylic acids, and amines. It can be synthesised in a one step process where  $^{11}\text{C}$ -labelled methane reacts with ammonia at  $920^\circ\text{C}$  in presence of platinum (serves as a metallic catalyst):

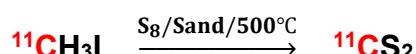
Figure 230: Synthesis of  $^{11}\text{C}$ - LY2795050 by using  $^{11}\text{C}$ -HCN

Example of its use in labelling is synthesis of  $^{11}\text{C}$ -labelled LY2795050 (Figure 230), another neuro-imaging PET tracer: cyanide ion reacts with the substrate in the presence of base ( $\text{K}_2\text{CO}_3$ ), palladium-based catalyst in DMF at  $80^\circ\text{C}$  and the

reaction is done in 5 minutes. Formed nitrile is then oxidised by hydrogen-peroxide ( $\text{H}_2\text{O}_2$ ) to give the desired tracer.

### $^{11}\text{C}$ -labelling with $^{11}\text{C}$ -carbon disulphide ( $^{11}\text{CS}_2$ )

The last precursor to be reviewed is  $^{11}\text{C}$ -labelled carbon disulphide ( $^{11}\text{CS}_2$ ). It looks like  $\text{CO}_2$  but is more reactive. Can be used to make large variety of sulphur-containing  $^{11}\text{C}$ -labelled compounds such as thiocarbonyl-thioureas, S-alkylated thioureas, and many other. It is obtained from  $^{11}\text{C}$ -labelled methyl iodide where it reacts with elementary sulphur ( $\text{S}_8$ ) on sand at  $500^\circ\text{C}$ :



An example of its use is the synthesis of  $^{11}\text{C}$ -tanaproget (used for imaging of progesterone receptors).  $^{11}\text{C}$ -labelled carbon disulphide reacts with amino and adjacent hydroxyl groups and closes a ring. This reaction is done without any catalyst in DMSO at  $150^\circ\text{C}$  and takes only 10 minutes.

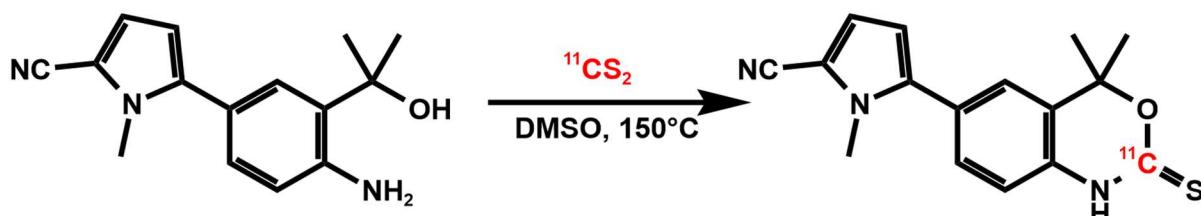


Figure 231: Synthesis of  $^{11}\text{C}$ - tanaproget by using  $^{11}\text{C}$ - $\text{CS}_2$

### $^{11}\text{C}$ -labelling via Grignard reaction – $\text{CO}_2$ fixation

The primary precursor  $^{11}\text{C}$  labelled carbon dioxide ( $^{11}\text{CO}_2$ ) is generally quite inert and non-reactive gas. However, using methods of  $\text{CO}_2$  fixations it can be directly activated and inserted into variously PET tracers.  $^{11}\text{C}$ -labelled  $\text{CO}_2$  is typically activated by reaction with organo-metallic reagents such as Grignard reagents or organolithium compounds. The most typical is the Grignard reaction with  $^{11}\text{CO}_2$ . The Grignard reagent is in fact organo-magnesium bromide compound that contains the organic substrate. It reacts with  $^{11}\text{C}$ -labelled carbon dioxide and forms an adduct ( $\text{R}'-\text{^{11}COOMgBr}$ ). This adduct is then hydrolysed with water to give corresponding  $^{11}\text{C}$ -labelled carboxylic acid where  $^{11}\text{C}$  atom is the one of carboxylic group (Figure 232).

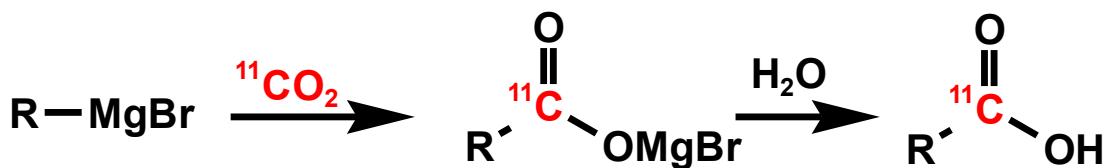
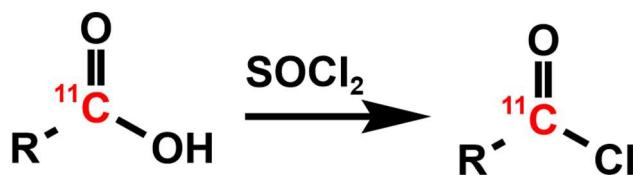
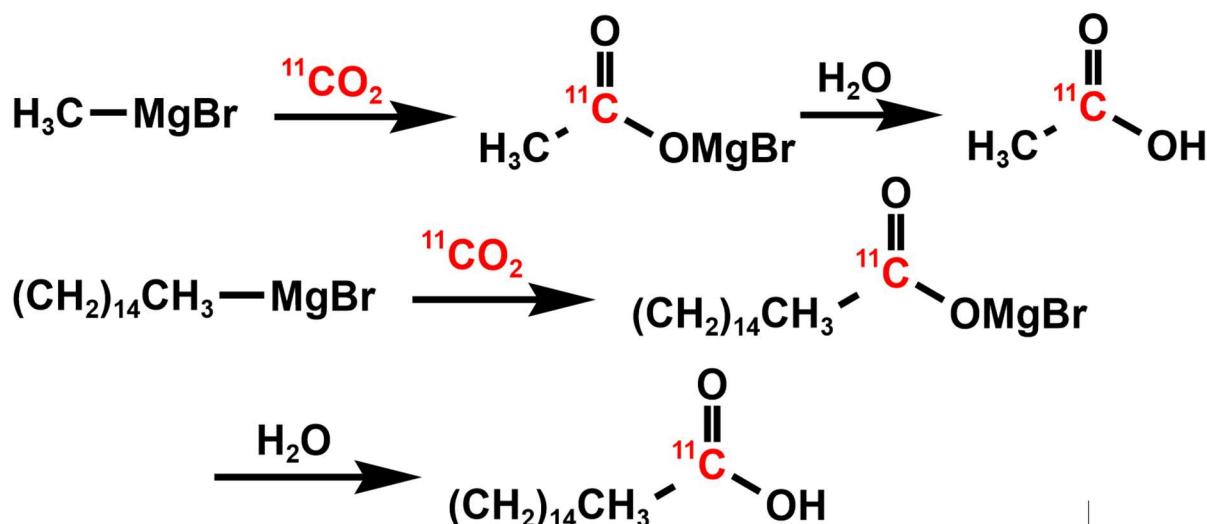


Figure 232: Synthesis of  $^{11}\text{C}$ -labeled carboxylic acid via  $\text{CO}_2$  fixation by using Grignard reagent

Furthermore, this  $^{11}\text{C}$ -labelled carboxylic acid can be converted into  $^{11}\text{C}$ -labelled acyl chloride after reaction with thionyl chloride ( $\text{SOCl}_2$ ) as shown below in the Figure 233.

Figure 233: Synthesis of  $^{11}\text{C}$ -labelled acyl chloride by using  $\text{SOCl}_2$  reagent

The  $^{11}\text{C}$ -labelled acyl chloride also very useful precursors for many PET tracers. A typical and the simplest example of  $^{11}\text{CO}_2$  fixation by using Grignard reaction is the synthesis of  $^{11}\text{C}$ -acetic acid or  $^{11}\text{C}$ -palmitic acid where methyl magnesium bromide and palmityl magnesium bromide are used as Grignard reagents.

Figure 234: Synthesis of  $^{11}\text{C}$ -acetic acid (up) or  $^{11}\text{C}$ -palmitic acid (down) by using Grignard reagents.

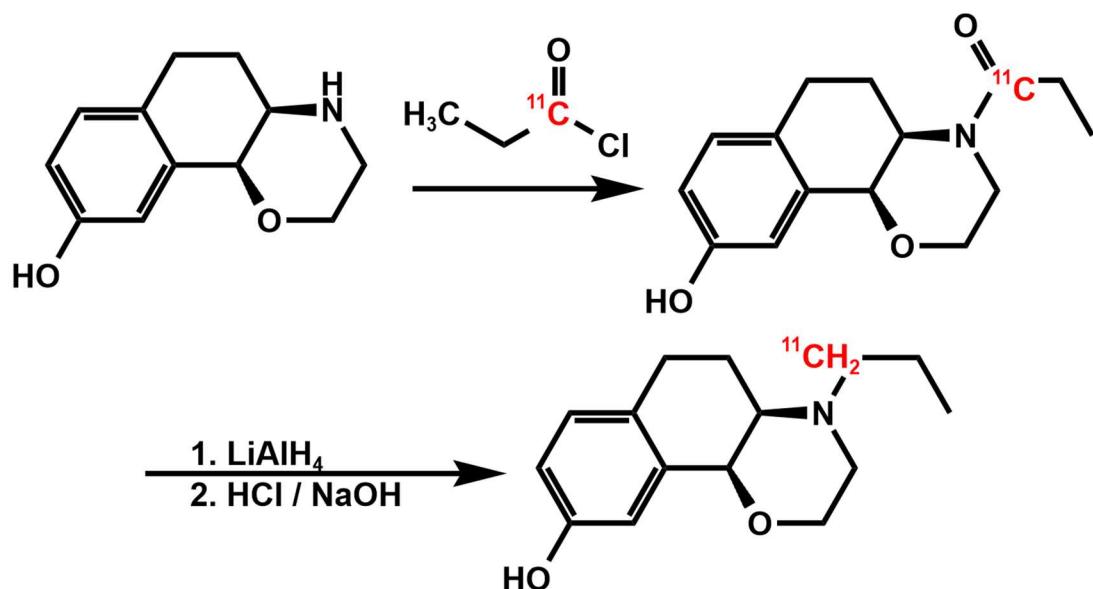
### $^{11}\text{C}$ -labelling with acyl chlorides ( $\text{R}^{11}\text{COCl}$ )

As mentioned before,  $^{11}\text{C}$ -labelled acyl chlorides ( $\text{R}^{11}\text{COCl}$ ) can be made from  $^{11}\text{C}$ -labelled carboxylic acids by reacting them with thionyl chloride. They are very reactive and can react with amines, alcohols or thiols to form amides, imides esters, thioesters and many other groups that then can be further functionalised to obtain large variety of PET tracers.

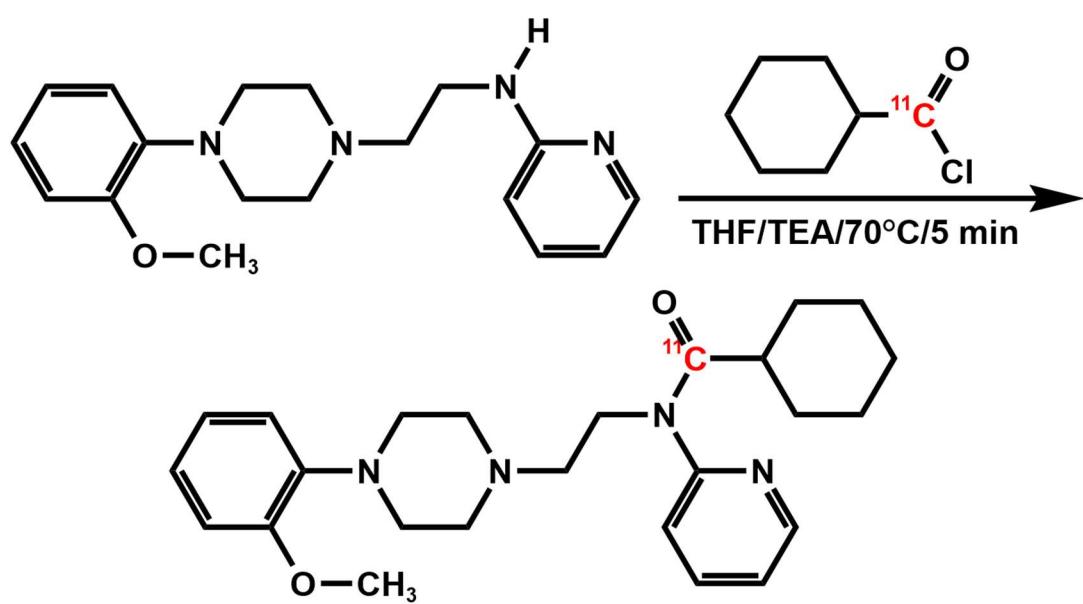
There are two nice examples of using  $^{11}\text{C}$ -labelled acyl chlorides, synthesis of (+)- $^{11}\text{C}$ -PHNO, and the synthesis of  $^{11}\text{C}$ -labelled WAY-100635, both PET tracers for brain imaging.

In the first example (Figure 235)  $^{11}\text{C}$ -labelled ethanoyl chloride reacts with the secondary amine group in the substrate and gives an amide. This amide is then converted into amine after reduction with lithium aluminium hydride and the final product is (+)- $^{11}\text{C}$ -PHNO.

In the second example  $^{11}\text{C}$ -labelled cyclohexanecarbonyl chloride also reacts with the secondary amine group in the substrate and forms an amide,  $^{11}\text{C}$ -WAY-100635 (Figure 236)



**Figure 235: Synthesis of (+)-<sup>11</sup>C-PHNO**



**Figure 236: Synthesis of  $^{11}\text{C}$ -WAY-100635**



## Overview of common $^{11}\text{C}$ -labelled PET imaging agents

Number of PET radiotracers based on  $^{11}\text{C}$  developed so far is enormous, somewhat even larger than those containing  $^{18}\text{F}$ . It is not possible to review all of them, it would take another book to achieve this. Here several of them will be mentioned, mostly those already presented in this or previous chapter, mainly in the synthetic examples. Medical branches where  $^{11}\text{C}$  radiotracers are used are mainly used in the area of oncology and neurology (but also including psychology and psychiatry)

### $^{11}\text{C}$ -labelled amino acids and derivatives in oncology

The main use of  $^{11}\text{C}$ -labelled radiotracers is in the area of oncology, precisely in the diagnostics of cancer. Oncology is the area where the cost of diagnostic procedure matters less than a need to assess suspicion on tumours and metastases therefore many tracers were developed. The most important group of  $^{11}\text{C}$  radiotracers in oncology are  $^{11}\text{C}$ -labelled amino acids: namely what is  $^{18}\text{FDG}$  in the area of  $^{18}\text{F}$ -labelled PET tracers those are the  $^{11}\text{C}$ -labelled amino acids for the area of  $^{11}\text{C}$ -labelled PET tracers.

The  $^{18}\text{FDG}$  has one problematic drawback: it shows in high concentration not only in cancer cells, but also in (healthy) brain tissue. Therefore, this makes brain blanket-covered in gamma-emission and any presence of tumour in brain is blurred and cannot be seen. Another area in the body where  $^{18}\text{FDG}$  is concentrated is urine bladder: the consequent gamma emission is making any precise diagnostic of neighbouring tissues problematic, especially this is in the case of prostate and attempts to visualise the prostate cancer.

However, amino acids are especially highly accumulated in brain cancers tissues and cells, but not in a healthy brain (presence of  $^{11}\text{C}$ -labelled amino acids in the healthy brain is low). In addition,  $^{11}\text{C}$ -labelled amino acids behave exactly the same as natural amino acids. Therefore,  $^{11}\text{C}$ -labelled amino acids are excellent radiotracers for imaging, visualisation and diagnostics of brain tumours using PET. The most important and the most used  $^{11}\text{C}$ -labelled tracer for imaging brain tumours as well as in whole nuclear medicine is L- $^{11}\text{C}$ -methionine ( $^{11}\text{C}$ -MET). When it comes to clinical importance  $^{11}\text{C}$ -MET is the  $^{11}\text{C}$  equivalent of  $^{18}\text{FDG}$ .

### L- $^{11}\text{CH}_3$ -Methionine ( $^{11}\text{C}$ -MET)

L- $^{11}\text{CH}_3$ -Methionine ( $^{11}\text{C}$ -MET, Figure 237) is **the most important  $^{11}\text{C}$ -labelled PET tracer** and is used for the diagnostics and staging of brain tumours. Usefulness of  $^{11}\text{C}$ -MET radiotracer can be illustrated in the Figure 238: on the left side (A) is an image of a brain of a patient having a brain tumour called glioblastoma. It is visualised by using  $^{18}\text{FDG}$  and presence of the tracer is everywhere: physician cannot clearly identify where the

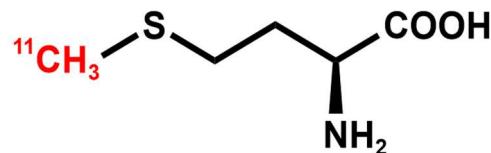


Figure 237:  $^{11}\text{C}$ -Methionine

tumour actually is located. However, on the right image is the same patient but instead of  $^{18}\text{FDG}$  it is given  $^{11}\text{C}$ -MET. Position of the tumour is clearly visible!

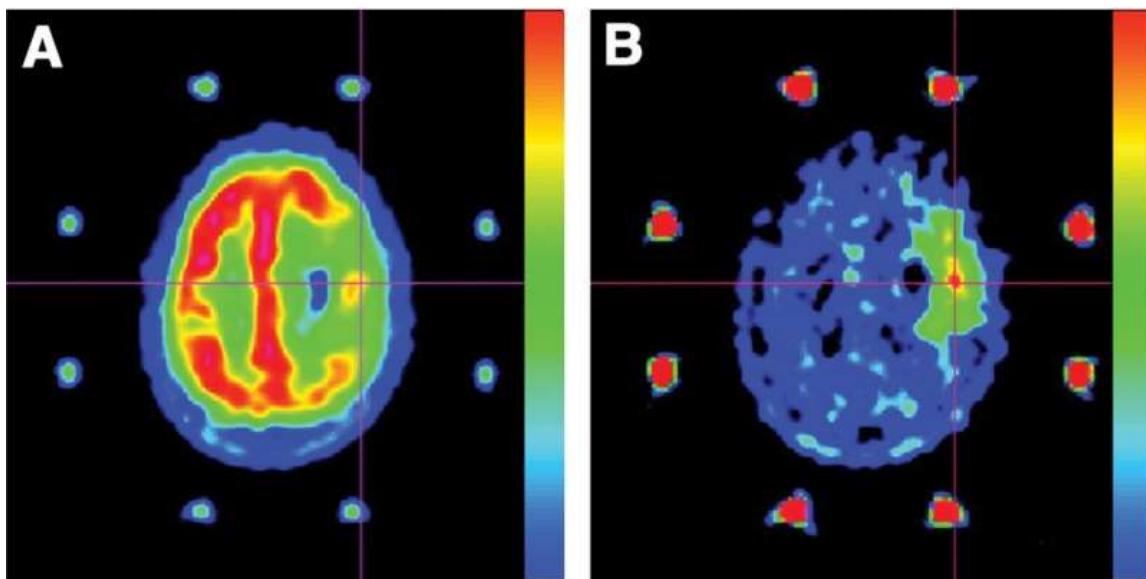


Figure 238: PET scan of a patient's brain with tumour by using  $^{18}\text{FDG}$  (left) and  $^{11}\text{C}$ -MET (right)

Although several  $^{11}\text{C}$ -labeled amino acids have been used in brain tumour imaging,  $^{11}\text{C}$ -MET is indisputably the most often employed due to its fast, convenient, and high-yield radiochemical synthesis which enables scans for two to three consecutive patients with the same production batch. Its synthesis (Figure 239) is very simple and cheap and is accomplished by methylation of homocysteine (its sulphydryl group) with  $^{11}\text{C}$ -labelled methyl iodide in the presence of a base and in ethanol.

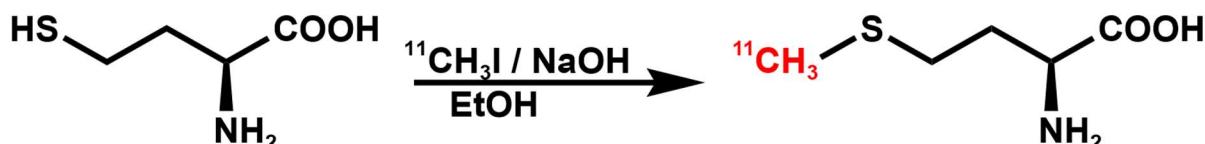


Figure 239: Synthesis of  $^{11}\text{C}$ -MET

### *Alpha- $^{11}\text{CH}_3$ -L-Tryptophan ( $^{11}\text{C}$ -AMT)*

Another important  $^{11}\text{C}$ -labelled amino acid for the imaging of tumours is alpha- $^{11}\text{CH}_3$ -L-tryptophan ( $^{11}\text{C}$ -AMT, Figure 240). It is used not only in the imaging of brain tumours, but also in the imaging of other tumours, such as breast and lung cancers. Its synthesis (Figure 241), opposite to  $^{11}\text{C}$ -MET is troublesome and complicated. It starts with heavily protected tricyclic precursor that is deprotonated at its alpha position using very strong base called lithium diisopropylamide (LDA) at very low temperature in tetrahydrofuran (THF) solvent. Formed carbanion quickly reacts with the  $^{11}\text{C}$ -labelled methyl iodide ( $^{11}\text{CH}_3\text{I}$ ) and then formed compound is treated firstly

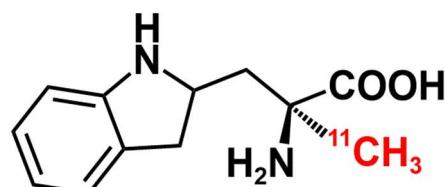


Figure 240: Alpha- $^{11}\text{CH}_3$ -L-tryptophan

with trifluoroacetic acid (TFA) to break the ring and then is de-protected with potassium hydroxide at 160°C and neutralised with hydrochloric acid (HCl) to give the final product,  $^{11}\text{C}$ -AMT

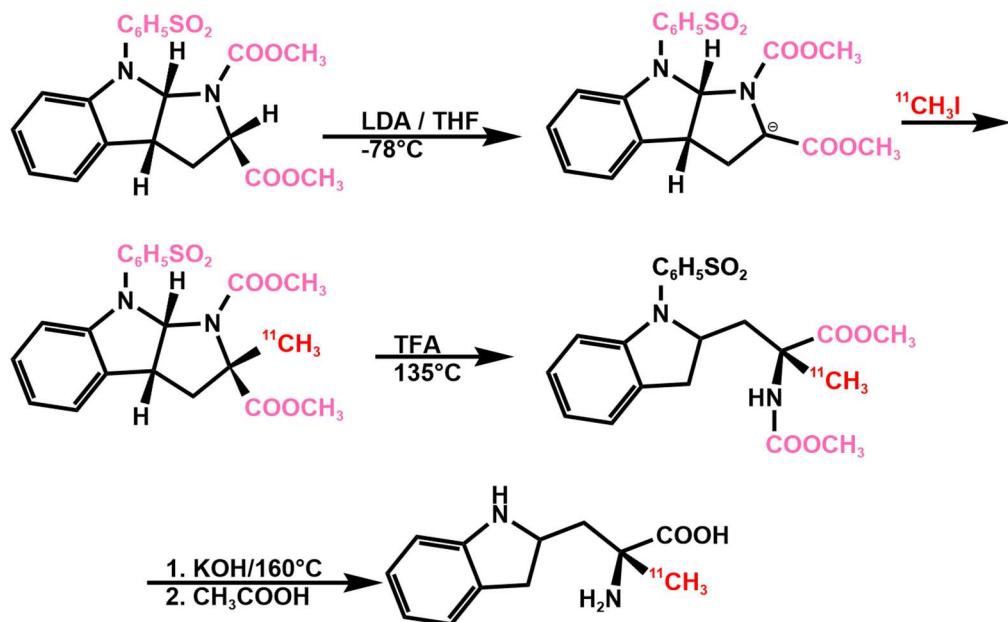


Figure 241: Synthesis of alpha- $^{11}\text{C}$ -L-triptophan ( $^{11}\text{C}$ -AMT)

### L- $^{11}\text{C}$ -Choline ( $^{11}\text{C}$ -CHO)

As mentioned before, use of  $^{18}\text{FDG}$  in the diagnosis of prostate cancer is limited and challenging due to its high concentration in the adjacent urinary bladder (just like in brain). Because of high gamma emission from bladder image of prostate is unclear and images are often ambiguous. However,  $^{11}\text{C}$ -choline (Figure 242, left) is used in this case: it targets the biosynthesis of phospholipids, process that is increased in prostate cancer cells and makes good images of prostate and prostate tissue (Figure 242 right).

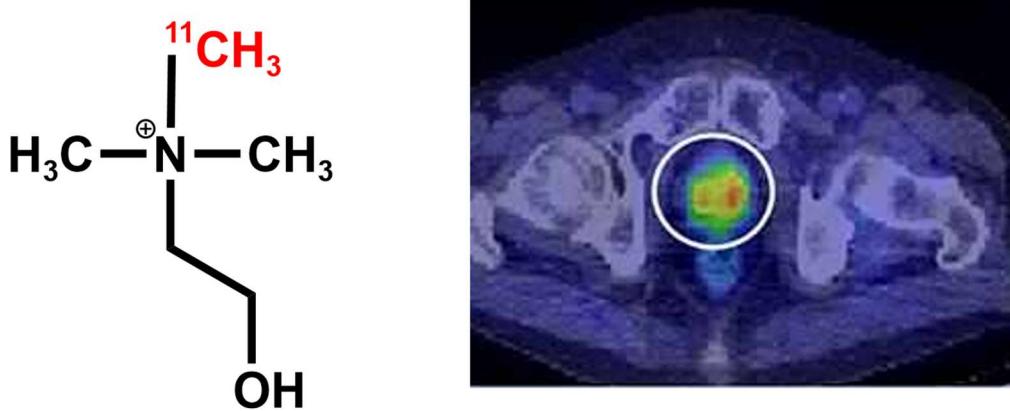
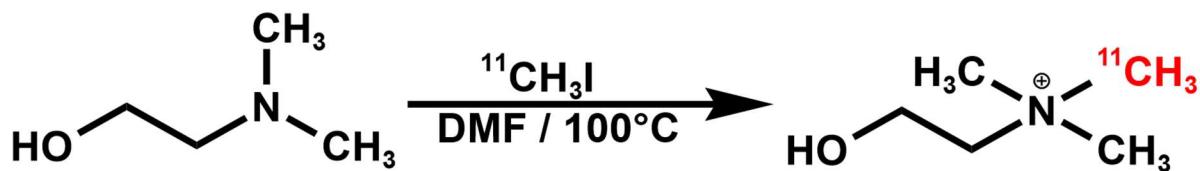


Figure 242: Structure of  $^{11}\text{C}$ -Choline (left), prostate cancer imaging using  $^{11}\text{C}$ -CHO (right)

Choline is not an amino acid, but a derivative of amino acid. Synthesis of  $^{11}\text{C}$ -choline (Figure 243) by methylation is quite easy: dimethyl-aminoethanol reacts with  $^{11}\text{C}$ -labelled methyl iodide in DMF at 100°C and the tracer is ready.

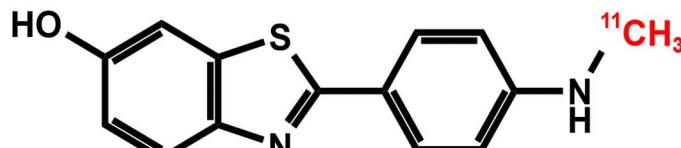
Figure 243: Synthesis of  $^{11}\text{CH}_3$ -Choline ( $^{11}\text{C}$ -CHO)

### $^{11}\text{C}$ -labelled PET tracers for brain imaging

Neurology and neuroscience are the areas where uniqueness of the tissues and inability to test function by using any other method makes PET imaging very useful since imaging of interference of PET radiotracers with synaptic neurotransmission can reveal us functional secrets of how our brain works, how do we create emotions, thoughts, memory, and many other neural functions as well as human behaviour. The fact that we can uncover secrets of human emotions and behaviour by using PET imaging makes research on  $^{11}\text{C}$  PET radiotracers very attractive: for many reasons  $^{11}\text{C}$  PET radiotracers are more appropriate for neuroimaging than  $^{18}\text{F}$ . Synthesis of most of these radiotracers have been already shown in previous pages.

### $^{11}\text{C}$ -Pittsburgh Compound B ( $^{11}\text{C}$ -PiB)

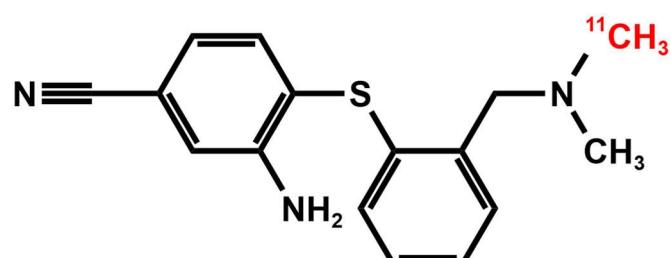
$^{11}\text{C}$ -Pittsburgh Compound B ( $^{11}\text{C}$ -PiB) is a  $^{11}\text{C}$  PET tracer used in investigational studies and diagnosis of Alzheimer's disease. It is a serious neurodegenerative disease that

Figure 245: Structure of  $^{11}\text{C}$ -Pittsburgh Compound B

affects millions of elderly people and is a significant social burden. Finding the mechanism of this disease and an efficient therapy is therefore urgent.  $^{11}\text{C}$ -PiB binds beta-amyloid plaques in brain tissue and is the most applied PET tracer for the imaging beta-amyloid plaques and Alzheimer's disease. Synthesis of  $^{11}\text{C}$ -PiB is accomplished by using alkylation with  $^{11}\text{CH}_3\text{I}$  as it is shown in the Figure 220.

### $^{11}\text{C}$ -DASB

$^{11}\text{C}$ -DASB is  $^{11}\text{C}$ -labelled 3-Amino-4-(2-Dimethyl-aminomethyl-phenyl sulfanyl)-benzonitrile where  $^{11}\text{C}$  atom is located on aminomethyl group (Figure 246). It is one of the most important  $^{11}\text{C}$  PET tracers for brain imaging. It binds serotonin transporter protein (SERT) in brain and is used for the assessment of SERT expression and density. This can enhance analysis of various neuropsychiatric disorders, such as depression, anxiety, bipolar disorder, obsessive

Figure 246: Structure of  $^{11}\text{C}$ -DASB

compulsive disorder, alcoholism, Parkinson's disease, and drug addiction. Synthesis of  $^{11}\text{C}$ -DASB is achieved by methylation with  $^{11}\text{CH}_3\text{I}$  as described in the Figure 221.

### $^{11}\text{C}$ -Raclopride

$^{11}\text{C}$ -Raclopride is very similar to  $^{11}\text{F}$ -raclopride but is in fact more appropriate for the brain imaging. It highly selectively binds dopamine ( $\text{D}_2/\text{D}_3$ ) receptor and is used for the imaging of movement disorders such as Parkinson's and Huntington's diseases, but also for imaging of personality disorders, example, detachment personality trait, as well as for patients with severe major depressive episodes. Synthesis of  $^{11}\text{C}$ -Raclopride is accomplished by methylation with  $^{11}\text{CH}_3\text{I}$  as shown in the figure 219.

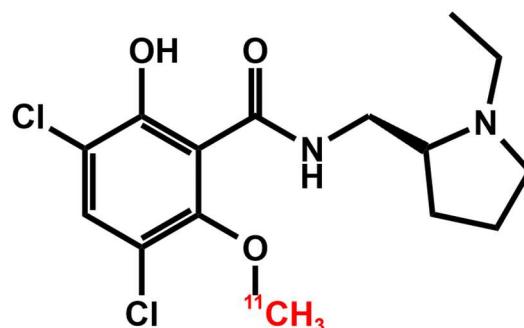


Figure 246: Structure of  $^{11}\text{C}$ -Raclopride

### $^{11}\text{C}$ -Harmine

$^{11}\text{C}$ -Harmine ( $^{11}\text{C}$ -HAR, Figure 247) is an inhibitor of an enzyme called monoamine oxidase A (MAO-A) in brain and is used for the imaging of MAO-A enzyme density in brain. This has application in studies of mood disorders and brain function such as seasonal mood changes. Also, it is used to study various behaviour disorders such as behaviour of impulsive and violent male offenders with antisocial personality disorder and high psychopathic traits. Its synthesis is based on quick methylation of hydroxyl group as shown below in the Figure 247.

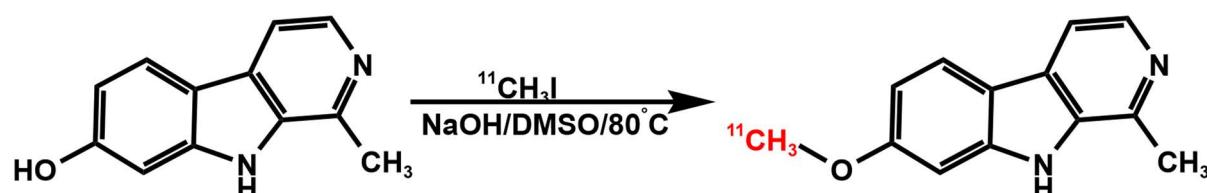


Figure 247: Synthesis and structure of  $^{11}\text{C}$ -Harmine

### (+)- $^{11}\text{C}$ -PHNO

(+)- $^{11}\text{C}$ -PHNO (Figure 248) is another important brain imaging PET tracer that targets dopamine  $\text{D}_2$  and  $\text{D}_3$  receptors in brain but has preferential affinity for the  $\text{D}_3$  over the  $\text{D}_2$  receptors. It is used for the imaging of mental disorders such as depression or schizophrenia as well as imaging of Parkinson's disease. Its synthesis is described in the Figure 235.

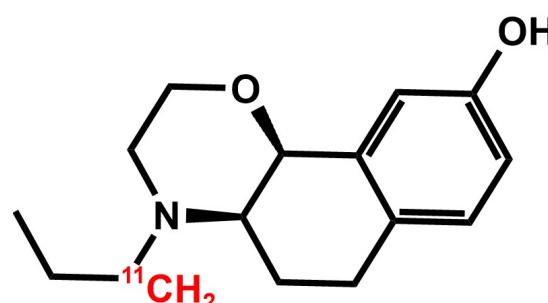


Figure 248: Structure of (+)- $^{11}\text{C}$ -PHNO

### **<sup>11</sup>C-WAY**

<sup>11</sup>C-WAY-100635 (Figure 249) binds serotonin-1A receptors ( $5\text{-HT}_{1A}$ ) and dopamine D<sub>4</sub> receptors. It is used for imaging of depression, panic disorder and Alzheimer disease.

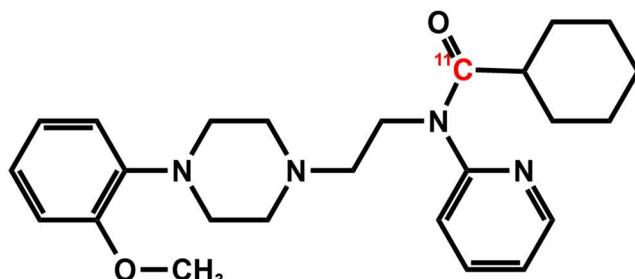


Figure 249: Structure of <sup>11</sup>C-WAY

### **<sup>11</sup>C-EMD-95885**

<sup>11</sup>C-labelled EMD-95885 (Figure 250) is a selective antagonist for the NMDA receptors in brain but binds just NR2B subunit of those NMDA receptors. It is used for the imaging of brain damage and recovery progress after stroke and is also used for the imaging of higher brain functions such as mental and cognitive functions (for example memory, learning, IQ).

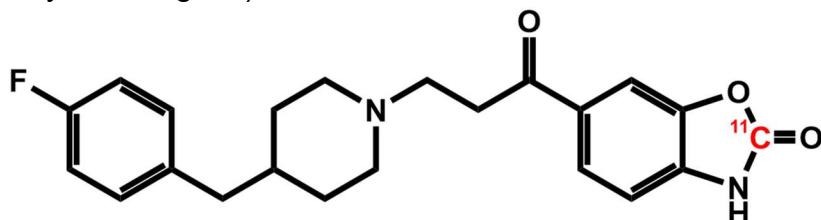


Figure 250: Structure of <sup>11</sup>C- EMD-95885

### **<sup>11</sup>C-LY2795050**

<sup>11</sup>C-labelled LY2795050 is an  $\kappa$ -opioid receptor antagonist and is regarded as a promising PET tracer for the investigation of all the conditions linked to these receptors such as depression, anxiety disorders, drug abuse, and alcoholism.

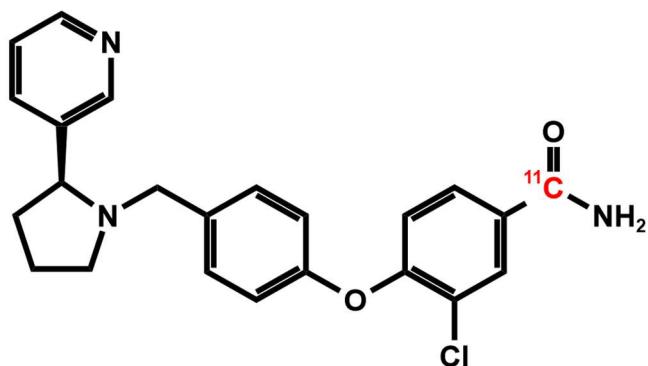


Figure 251: Structure of <sup>11</sup>C-LY2795050

## Chapter XI - Nitrogen-13 and Oxygen 15

Nitrogen 13 ( $^{13}\text{N}$ ) and Oxygen 15 ( $^{15}\text{O}$ ) are positron emitting radionuclides of PET imaging that are very rarely used in clinical or experimental practice. Yet, despite their exotic nature of being very short-lived they are worthy to be reviewed.

### Isotopes of Nitrogen

Nitrogen has 15 isotopes (Figure 252), but only two are stable,  $^{14}\text{N}$  and  $^{15}\text{N}$ . In total,  $^{14}\text{N}$  makes most of natural nitrogen (99.6%) in the atmosphere and elsewhere, while only 0.4% of all nitrogen is  $^{15}\text{N}$ . This minor isotope,  $^{15}\text{N}$  is basis for relatively rare  $^{15}\text{N}$  NMR spectroscopy. Isotopes from  $^{16}\text{N}$  onwards are beta-emitting and have very short half-life, while  $^{16}\text{N}$  has half-life only 7 seconds. On the other side,  $^{11}\text{N}$  and  $^{10}\text{N}$  emit protons, while  $^{12}\text{N}$  emits positron, but has extremely short half-life, and therefore cannot be used in nuclear medicine. The only isotope of nitrogen that could be used in medicine is  $^{13}\text{N}$ . It emits positrons and has half-life of 9.965 minutes (approximately 10 minutes).  $^{13}\text{N}$  is sometimes created in the atmosphere due to cosmic radiation when gamma radiation hits  $^{14}\text{N}$  and it loses one neutron and becomes  $^{13}\text{N}$ .

$^{10}\text{N}$	$^{11}\text{N}$	$^{12}\text{N}$	$^{13}\text{N}$	$^{14}\text{N}$	$^{15}\text{N}$	$^{16}\text{N}$	$^{17}\text{N}$	$^{18}\text{N}$	$^{19}\text{N}$	$^{20}\text{N}$	$^{21}\text{N}$	$^{20}\text{N}$
Emit protons	Emit positrons			Stable				Emit betas (electrons)				

Figure 252: Selected isotopes of nitrogen

### Nuclear properties of $^{13}\text{N}$

The half-life of  $^{13}\text{N}$  is just 10 minutes, and that is too short: very impractical and limiting, but still some radiochemistry and PET scans can be accomplished. It decays to  $^{13}\text{C}$  by positron emission that presents 99.8% of all decay events (Figure 253).

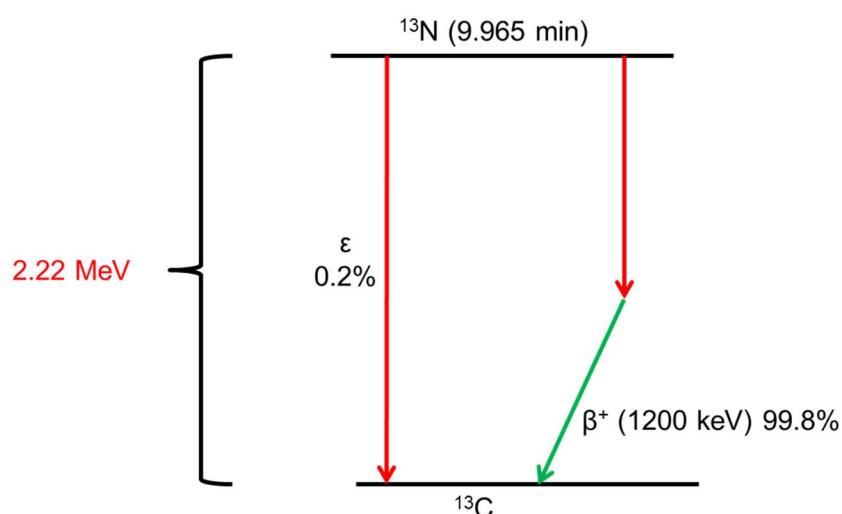


Figure 253: Decay of  $^{13}\text{N}$

The positrons ( $\beta^+$ ) of  $^{13}\text{N}$  are on average bit stronger than those of  $^{11}\text{C}$  and  $^{18}\text{F}$ : the mean energy of 491 keV, while the maximal energy is 1200 keV. If we look at the flying distances then positrons emitted by  $^{13}\text{N}$  fly in water or tissue usually 1.8 mm, while the maximum range is 5.5 mm. As it can be seen these distances are more than distances for  $^{11}\text{C}$  and  $^{18}\text{F}$ . And this is now an important limitation and drawback because PET images made by  $^{13}\text{N}$  are not as clear as those made by  $^{11}\text{C}$  and  $^{18}\text{F}$  PET tracers (Figure 254).

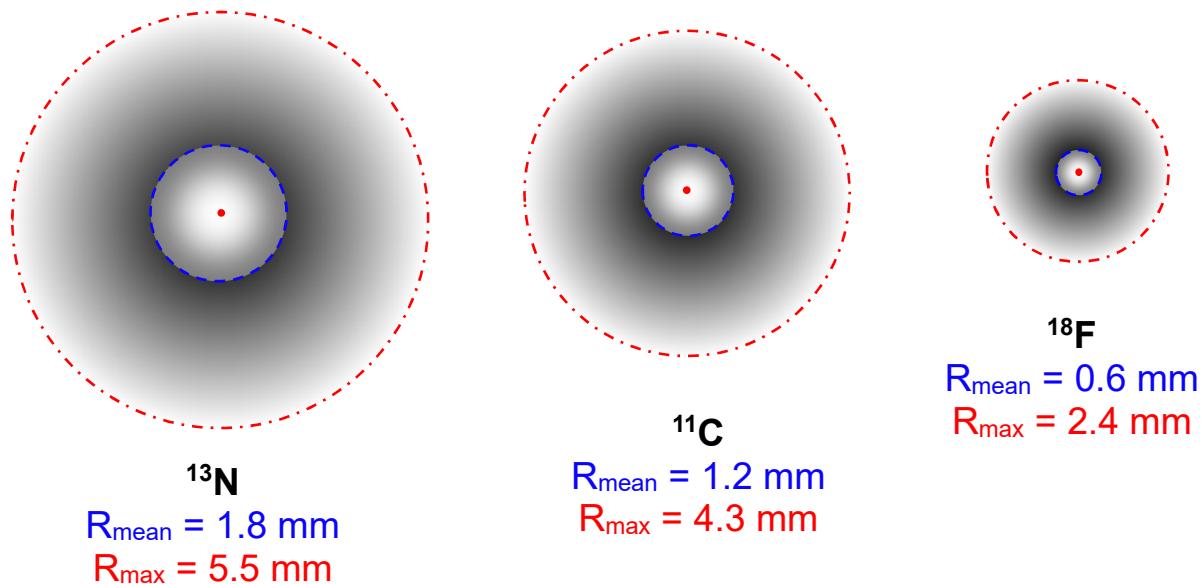
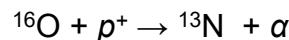


Figure 254: Comparison of positron fly distances of  $^{13}\text{N}$ ,  $^{11}\text{C}$  and  $^{18}\text{F}$ . What is obvious that  $^{13}\text{N}$  has the largest fly distance sphere and therefore gives the poorest PET image resolution and clarity, large the sphere poorer the PET image resolution and clarity.

## Production of $^{13}\text{N}$

$^{13}\text{N}$  can be produced in cyclotrons, by bombardment of targets with protons. There are three options for production of  $^{13}\text{N}$ .

In the first one, the most common oxygen isotope is bombarded with protons, and the product are one alpha particle and one  $^{13}\text{N}$ :



The target in this reaction is actually common liquid water that has some added ethanol, while desired “in-target” product is  $^{13}\text{N}$ -labelled ammonia ( $^{13}\text{NH}_3$ ). This is in fact the most common way to make  $^{13}\text{NH}_3$ . Addition of ethanol prevents excessive formation of nitrites and nitrates instead of  $^{13}\text{NH}_3$ . However, if no ethanol is added then the product is mostly  $^{13}\text{N}$ -labelled nitrate ion ( $^{13}\text{NO}_3^-$ ).

Figure 225 shows the system for the production of  $^{13}\text{N}$  using the reaction  $^{16}\text{O}(p,\alpha)^{13}\text{N}$ . Water is injected from the technical room remotely into the target chamber where is irradiated with accelerated protons. The whole target chamber is heavily cooled with

both water and helium. Once irradiated enough the 6-port valve is switched and helium takes the water with produced  $^{13}\text{NH}_3$  into the hot cells.

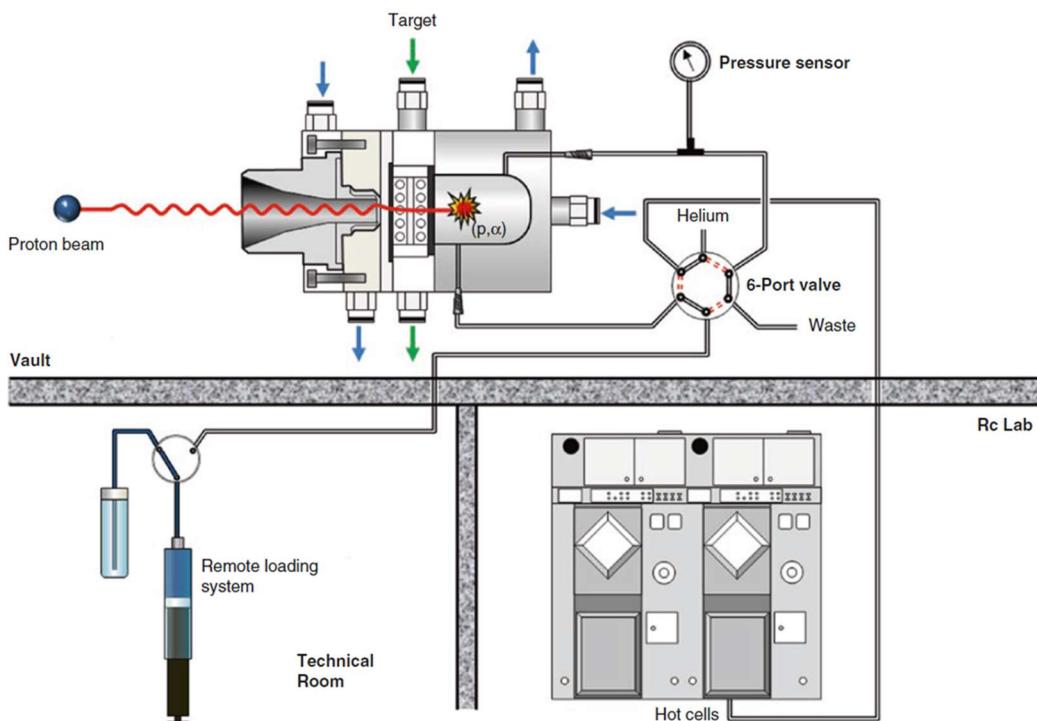


Figure 255: Production of  $^{13}\text{N}$  in cyclotrons by using water as target (option I)

The second option is bombardment of  $^{12}\text{C}$  ("normal" carbon) with deuterons where  $^{13}\text{N}$  is produced along with one neutron ejected:

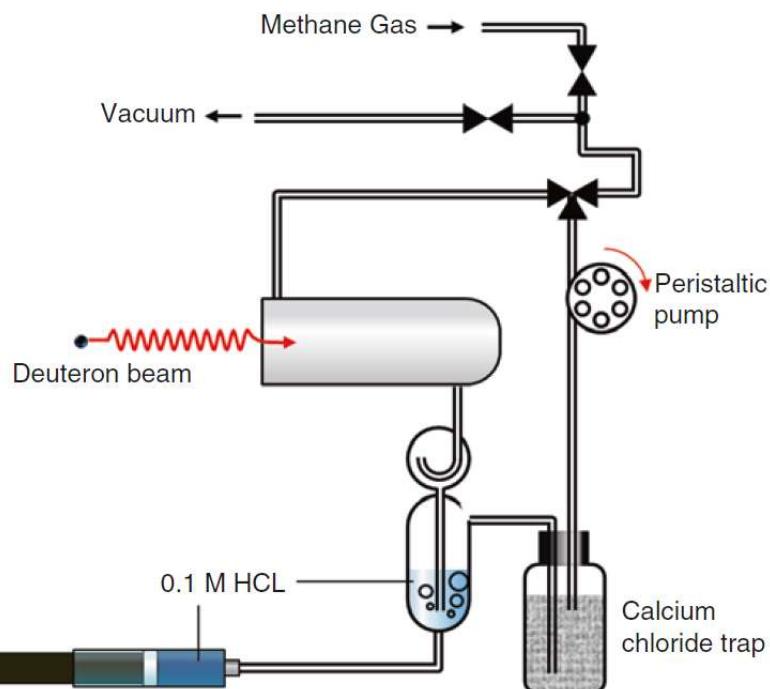
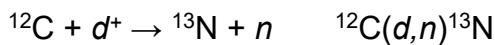
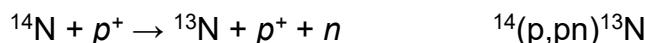


Figure 256: Production of  $^{13}\text{N}$  in cyclotrons by using  $\text{CH}_4$  as target (option II)

In this case the target is methane gas, and the “in-target” product is also  $^{13}\text{N}$ -labelled ammonia ( $^{13}\text{NH}_3$ ). The figure 256 shows production of  $^{13}\text{NH}_3$  by using the second option, bombardment of methane. It comes into the gas chamber where it is irradiated and the resulting gas goes into an aqueous solution of hydrochloric acid where  $^{13}\text{NH}_3$  gets absorbed while the rest of unreacted methane goes into calcium chloride trap where water is removed and then again into the same irradiation chamber for more irradiation. Once there is enough of  $^{13}\text{NH}_3$  activity in the HCl trap irradiation may stop.

In the third option common nitrogen ( $^{14}\text{N}$ ) is bombarded with protons and the result are one neutron, one proton and  $^{13}\text{N}$ . The target is  $\text{N}_2$  gas with some  $\text{O}_2$ :



However, this is the same reaction used for production of  $^{11}\text{CO}_2$ . In fact, during the production of  $^{11}\text{CO}_2$  some  $^{13}\text{N}$  is also made as the by-product. This by-reaction is less probable than the one yielding  $^{11}\text{C}$ , but still can be employed to make  $^{13}\text{N}$ .

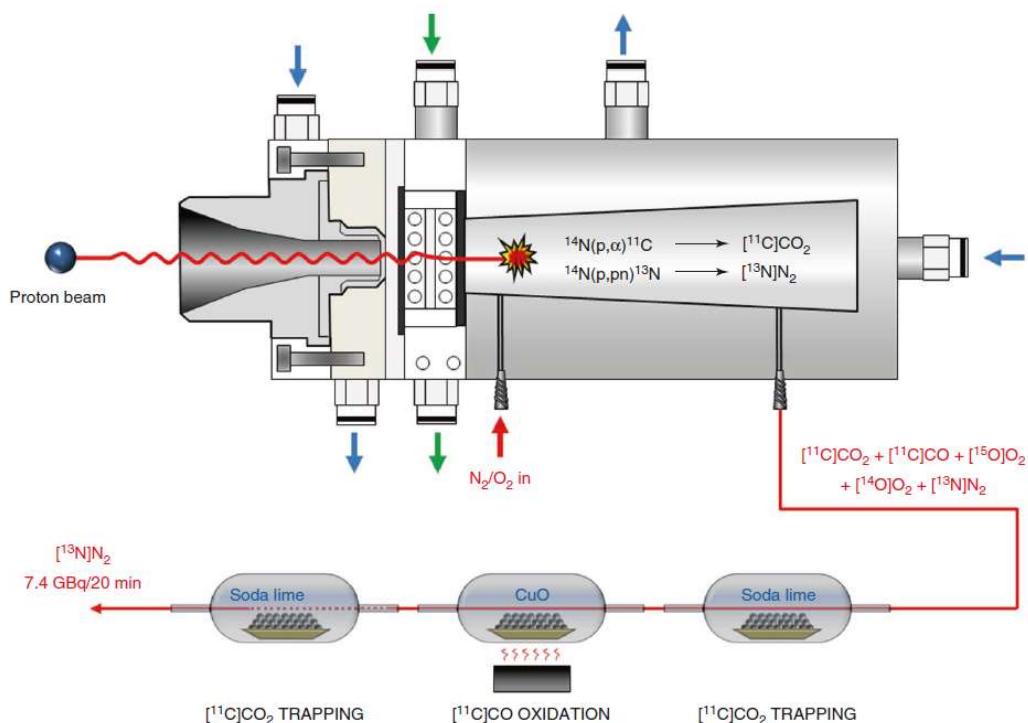


Figure 257: Production of  $^{13}\text{N}$  in cyclotrons by using  $\text{N}_2$  as target (option III)

The third option of making  $^{13}\text{NH}_3$  as the by-product of  $^{11}\text{CO}_2$  production is shown in the Figure 257: It starts the same as production of  $^{11}\text{CO}_2$  with irradiation of  $^{14}\text{N}_2$  with accelerated protons and several reactions are happening and many products are made. However, then gas is scrubbed with soda-lime (mixture of  $\text{CaO}$  and  $\text{NaOH}$ ) and this removes  $^{11}\text{CO}_2$ , then it goes through heated  $\text{CuO}$  catalyst that is oxidising  $^{11}\text{CO}$  into  $^{11}\text{CO}_2$  with present  $\text{O}_2$  and then again  $^{11}\text{CO}_2$  is removed with soda lime. The resulting gas contains only radioactivity from  $^{13}\text{N}$ .

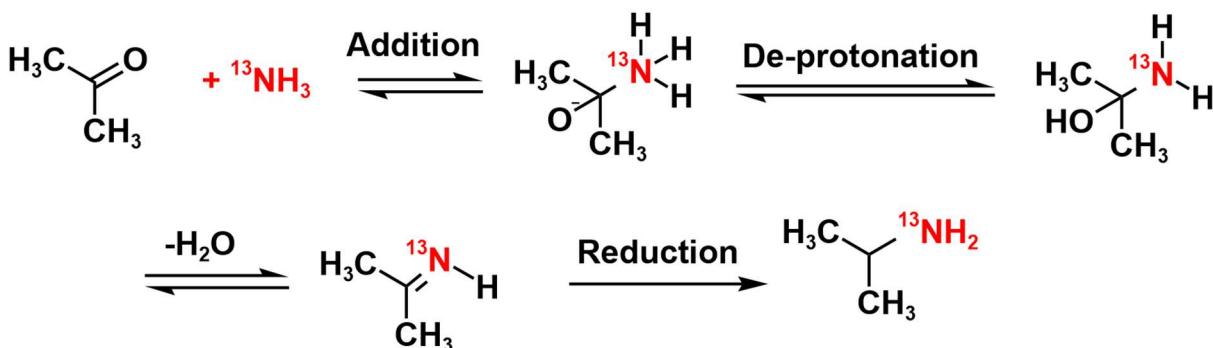
## Advantages and drawbacks/limitations of $^{13}\text{N}$

What are advantages of  $^{13}\text{N}$  as PET tracer? Firstly,  $^{13}\text{N}$ , just like  $^{11}\text{C}$  is an isotope of a biogenic element and  $^{13}\text{N}$ -labelled PET tracer cannot be distinguished from natural biomolecules. Secondly, nitrogen, just like  $^{11}\text{C}$  has very rich chemistry.

Drawbacks are, on the other hand stemming mainly from its too short half-life of just 10 minutes that is almost exactly half of those of  $^{11}\text{C}$ . Therefore, all synthesis must be accomplished within 30 minutes, and this is very challenging and limiting. Fortunately, modern sophisticated automation in radiochemistry helps a lot and it is not impossible. Otherwise very short half-life causes very low radiochemical yield. For the same reason transportation out of radiopharmaceutical site to other medical facilities is out of question, it is not feasible: produced radiopharmaceutical agent has to be applied on the site where it is made. Also, fly distances of  $^{13}\text{N}$  positrons is a bit large and this inherent drawback makes more blurred images of lower quality, but this drawback is in fact not so serious as too short half-life. Therefore,  $^{13}\text{N}$  PET scans are largely limited to scientific research, they are quite unattractive for the routine clinical PET scans. The main  $^{13}\text{N}$ -labelled radiopharmaceutical is therefore nothing but  $^{13}\text{N}$ -labelled ammonia. In addition,  $^{13}\text{N}$  is also quite problematic for radiochemistry and produced activity is often not enough. Radiochemistry of  $^{13}\text{N}$  is still domain of research, not clinical routine.

## Radiochemistry of $^{13}\text{N}$

As obvious the primary precursor of  $^{13}\text{N}$  is  $^{13}\text{N}$ -labelled ammonia ( $^{13}\text{NH}_3$ ). Also, in the same time it is the main radiopharmaceutical of  $^{13}\text{N}$ , it used for imaging of blood flow in heart. The  $^{13}\text{NH}_3$  can be used to prepare amines, amides, ureas, and carbamates. The most common reaction is so called “reductive amination” reaction. There a carbonyl compound such as ketone or aldehyde reacts with ammonia and addition makes an adduct that undergoes deprotonating and dehydration to form an imide, ketimides or aldimides (so called “Schiff bases”). These imides can be then reduced into amines:



An example of such reductive amination is synthesis of  $^{13}\text{N}$ -labeled amphetamine where ketone reacts with  $^{13}\text{N}$ -labelled ammonia in presence of aluminium amalgam

(Al/HgCl<sub>2</sub>) in ethanol to form aldimide and in the next step this aldimide is reduced into <sup>13</sup>N-labelled amphetamine:

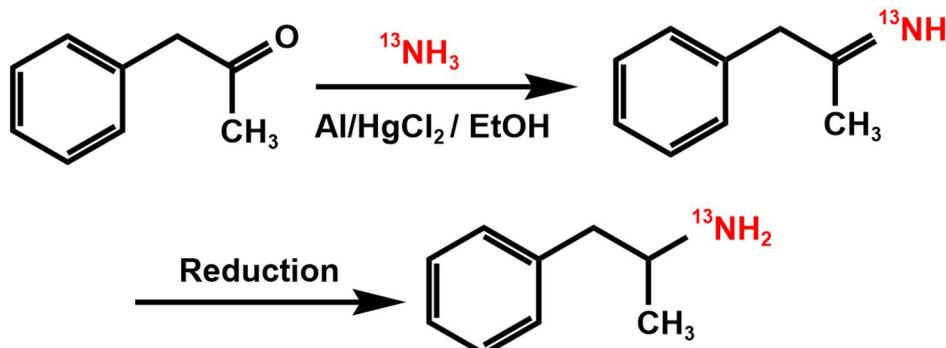


Figure 258: Synthesis of <sup>13</sup>N-labelled amphetamine

Another set of <sup>13</sup>N-labelling examples involves preparation of very reactive starting precursors for labelling by using a special reagent named triphosgene. It is a safer substitute for toxic phosgene, and it reacts with amines, phenols or alcohols to make isocyanates, carbamic chlorides or carbonochloridates (Figure 259). These very reactive compounds react very quickly with <sup>13</sup>N-labelled ammonia and produce <sup>13</sup>N-labelled ureas or carbamates:

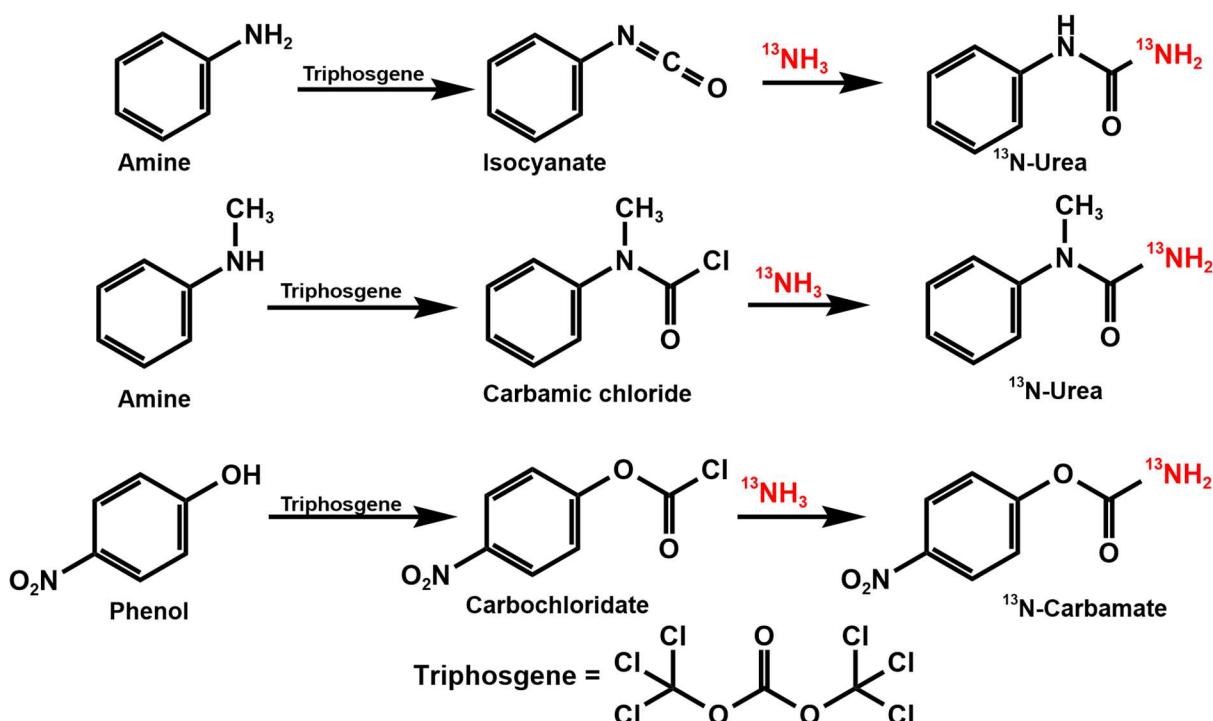
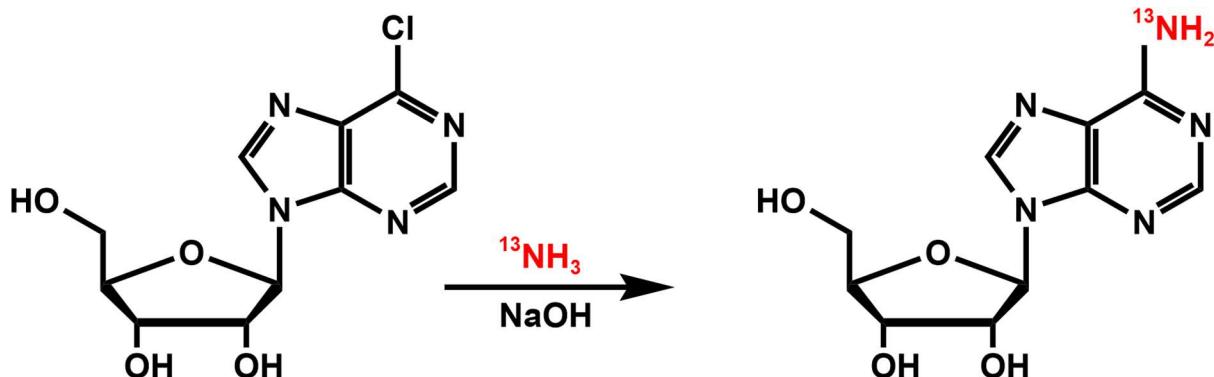
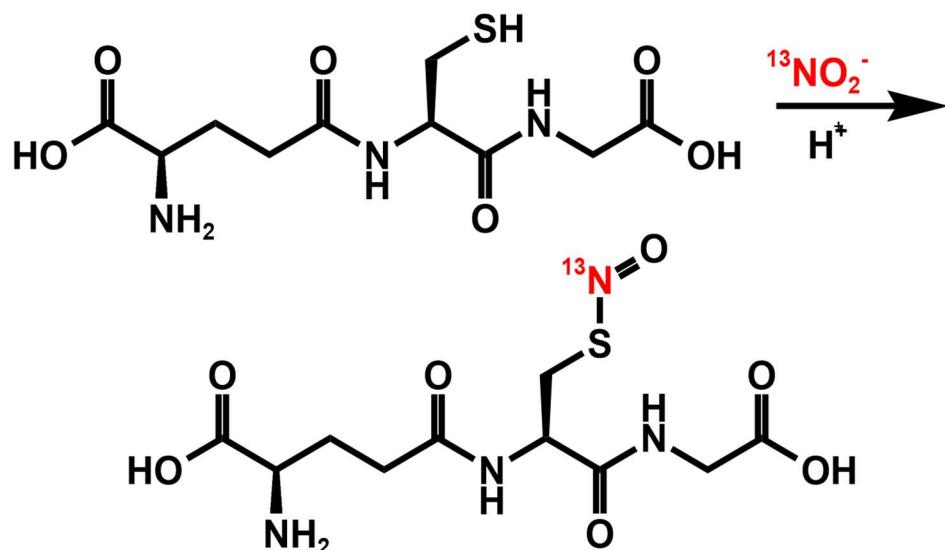


Figure 259: Use of triphosgene for making precursors for various <sup>13</sup>N-labelled compounds

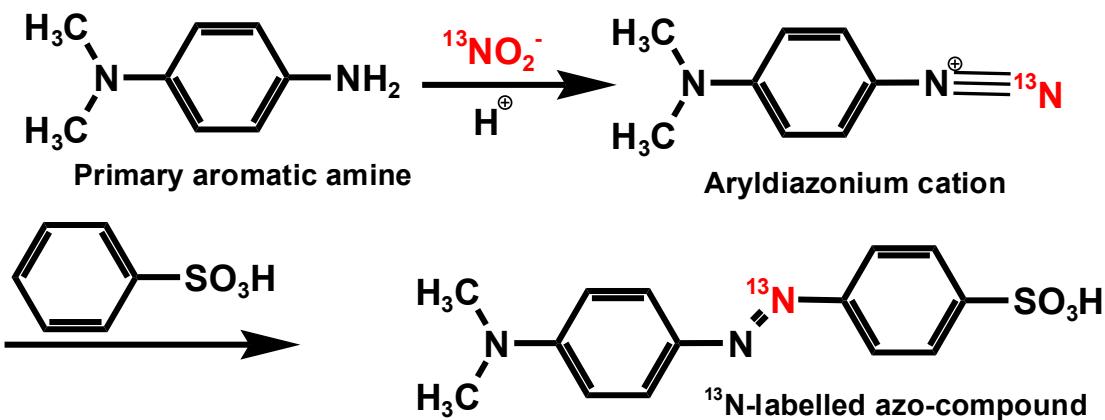
Important example of <sup>13</sup>N-labelling by using <sup>13</sup>NH<sub>3</sub> is ammonolysis, a form of nucleophilic substitution involving ammonia. Chlorinated version of purine nucleoside reacts with ammonia in presence of sodium hydroxide and forms <sup>13</sup>N-labelled adenine, a DNA base (Figure 260).

Figure 260: Synthesis of  $^{13}\text{N}$ -labelled adenosine

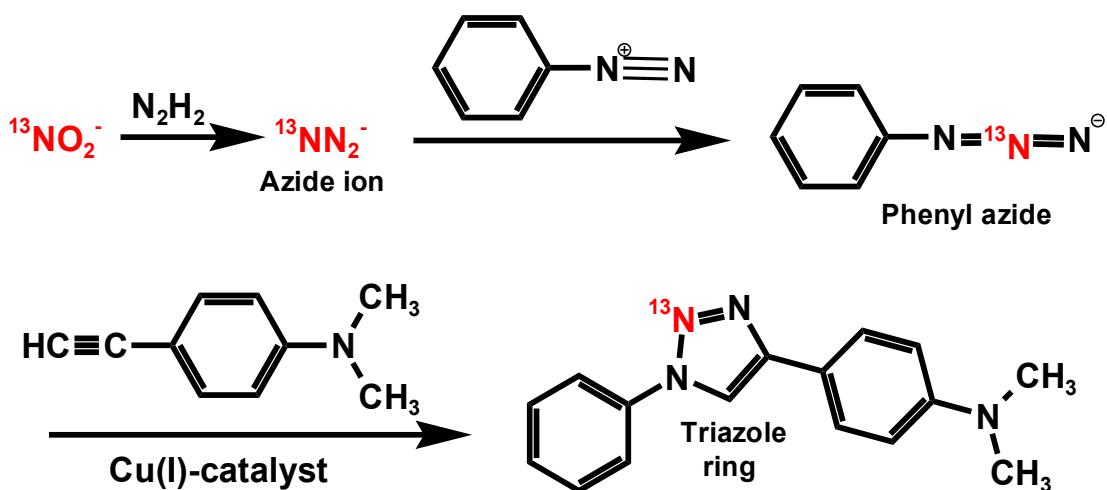
Another option for  $^{13}\text{N}$ -labelling is not to use  $^{13}\text{N}$ -labelled ammonia, but instead to use  $^{13}\text{N}$ -labelled nitrate ion:  $^{13}\text{NO}_3^-$ . This is the primary  $^{13}\text{N}$ -labelled product that comes from cyclotron if we do not add any ethanol into water irradiated in cyclotron target rig. The  $^{13}\text{NO}_3^-$  can be reduced into a secondary precursor, nitrite ion ( $^{13}\text{NO}_2^-$ ) that can be used for the synthesis of various tracers such as S-nitrosothiols, N-nitrosamines, azo-compounds, and azides. The azides can be subsequently used for the preparation of  $^{13}\text{N}$ -labeled triazoles. An example of  $^{13}\text{N}$ -labelling with  $^{13}\text{NO}_2^-$  is synthesis of S- $^{13}\text{N}$ -nitrosoglutathione (Figure 261) where nitrite ion quickly reacts with glutathione in presence of an acid.

Figure 261: Synthesis of  $^{13}\text{N}$ -labelled adenosine

Another example of using  $^{13}\text{NO}_2^-$  is the synthesis of  $^{13}\text{N}$ -labelled compounds in so called diazo-coupling reaction (Figure 262). In this electrophilic aromatic substitution reaction, the aryl diazonium cation is the electrophile and the activated arene is a nucleophile. In most cases, including this example, the diazonium compound is also aromatic. Primary aromatic amine reacts with  $^{13}\text{N}$ -labelled nitrite ion in acidic media and aryl diazonium cation is formed. Then the cation reacts with phenyl-sulphonate to couple into a  $^{13}\text{N}$ -labelled azo-compound.

Figure 262: Synthesis of  $^{13}\text{N}$ -labelled azo-compound

The last example of  $^{13}\text{N}$  radiochemistry is the use of  $^{13}\text{NO}_2^-$  for the synthesis of  $^{13}\text{N}$ -labelled triazoles (Figure 263). This is a bit complicated labelling synthesis in three steps, and they yield might be very low. However, it works and could be employed.  $^{13}\text{N}$ -labelled nitrite ion reacts with the hydrazine and forms a  $^{13}\text{N}$ -labelled azide ion. It then reacts with some diazo-reagent and forms phenyl-azide. The last step is copper catalysed CuAAC cycloaddition, or called the “click” reaction where ethynyl group reacts with azide and copper (I) ion makes this reaction fast and complete. The result is a radiopharmaceutical agent with  $^{13}\text{N}$ -labelled triazole ring.

Figure 263: Synthesis of  $^{13}\text{N}$ -labelled triazole

## Isotopes of Oxygen

Oxygen has 15 isotopes and three of them,  $^{16}\text{O}$ ,  $^{17}\text{O}$  and  $^{18}\text{O}$  are stable and natural. The overwhelming majority of all oxygen on Earth (atmospheric oxygen and water, rivers, lakes, oceans, soil, stones, and biosphere) is  $^{16}\text{O}$  that makes 99.76% of all stable oxygen, then  $^{18}\text{O}$  makes small amount of 0.2%, while  $^{17}\text{O}$  is in traces, just 0.04%. All its isotopes with mass number more than 19 are beta emitting but decay very quickly, the longest half-life is just 27 seconds. The isotopes  $^{14}\text{O}$  and  $^{15}\text{O}$  are positron emitting isotopes, with half-lives of 70 seconds for  $^{14}\text{O}$  and 122 seconds for  $^{15}\text{O}$ . Although these 122 seconds is very, very short timeframe  $^{15}\text{O}$  radionuclide can be and is used in PET diagnostics, but very rarely and very limited. Its use in PET imaging is, in fact, quite exotic and unusual.

$^{12}\text{O}$	$^{12}\text{O}$	$^{13}\text{O}$	$^{14}\text{O}$	$^{15}\text{O}$	$^{16}\text{O}$	$^{17}\text{O}$	$^{18}\text{O}$	$^{19}\text{O}$	$^{20}\text{O}$	$^{21}\text{O}$	$^{22}\text{O}$	$^{23}\text{O}$
Emit protons	Emit positrons	Stable				Emit betas (electrons)						

Figure 264: Selected isotopes of nitrogen

## Nuclear properties of $^{15}\text{O}$

The half-life of  $^{15}\text{O}$  is only 122 s, and this is approximately just 2 minutes, and this is very impractical and very limiting (Figure 265). Just compare it with other PET radionuclides that have several times and orders of magnitude longer half-life. Therefore,  $^{15}\text{O}$  needs to be produced and applied in just 6 minutes. This makes no space for too much radiochemistry and  $^{15}\text{O}$  radiopharmaceuticals boils down to two radiopharmaceuticals,  $^{15}\text{O}$ -labelled water, and butanol. It decays to  $^{15}\text{N}$  by almost exclusively positron emission.

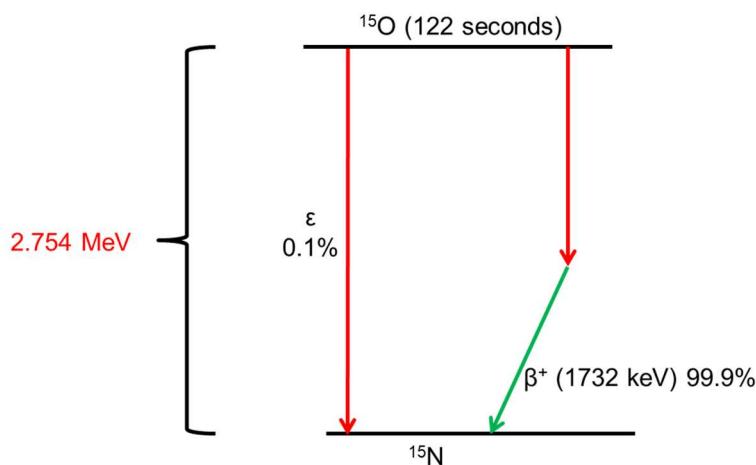


Figure 265: Decay of  $^{15}\text{O}$

Another significant drawback of  $^{15}\text{O}$  is its energy that is, comparing with  $^{18}\text{F}$ , very high. The  $^{15}\text{O}$ -emitted positrons have mean energy of 735 keV, and maximal energy of 1732 keV. Translated into fly distances or ranges in tissue it is average 3 mm and maximum range of 8.4 mm (Figure 266). This makes uncertainty of decay location

much larger and PET images are more blurred, even more than it is the case in  $^{13}\text{N}$ , not so clear. This is an important drawback of  $^{15}\text{O}$ , but still not as dramatic and debilitating as its very short half-life.

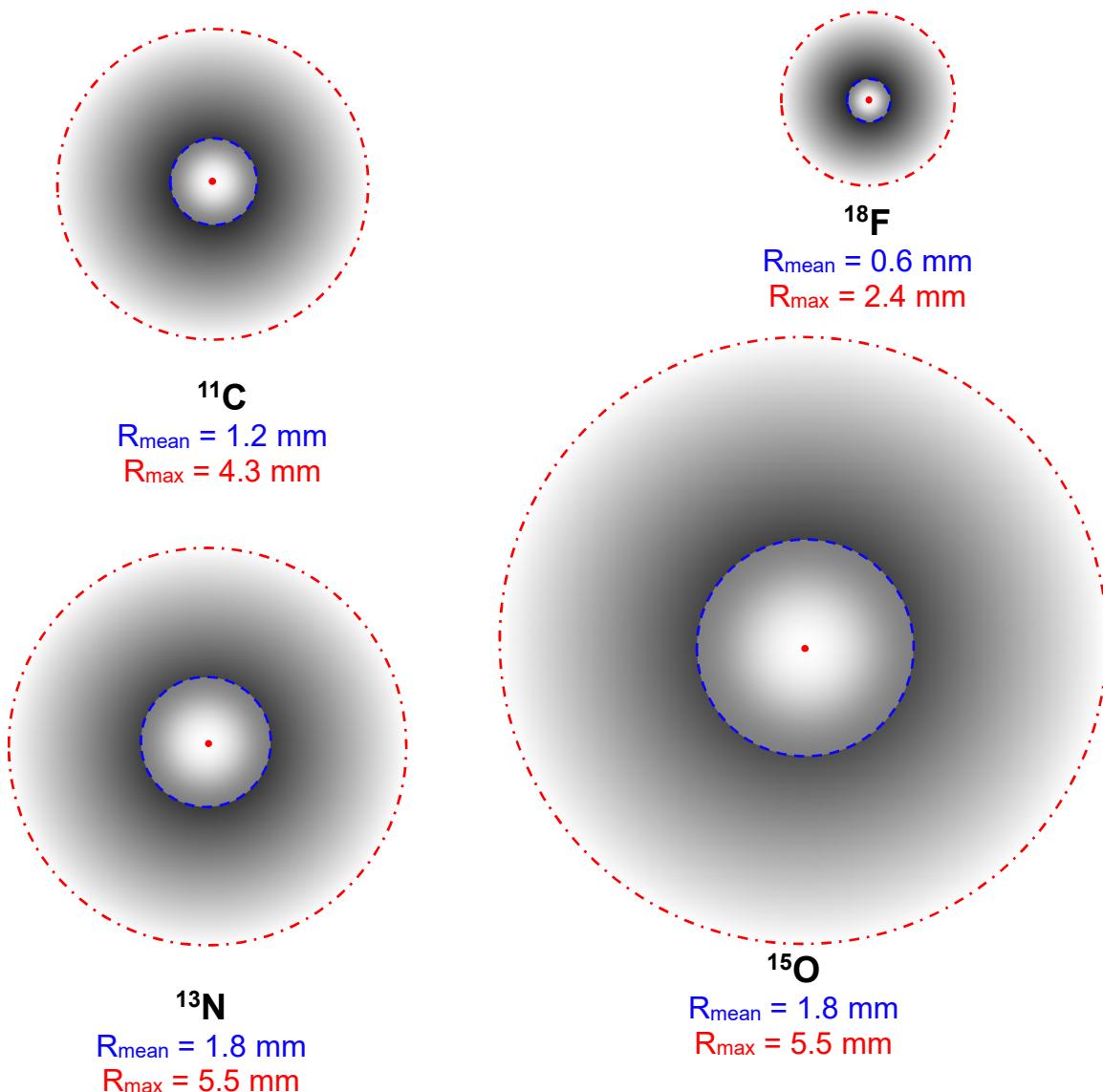
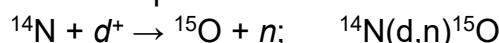


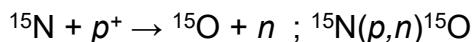
Figure 266: Comparison of  $^{15}\text{O}$  positron flying distances with those of  $^{13}\text{N}$ ,  $^{11}\text{C}$  and  $^{18}\text{F}$ . It is clear that average and maximal distances are much higher in the case of  $^{15}\text{O}$  and this is the reason why PET images are much less clear than those of other light positron emitting radionuclides.

## Production of $^{15}\text{O}$

$^{15}\text{O}$  could be produced in cyclotrons by two ways. In the first one natural  $^{14}\text{N}_2$  gas is bombarded with accelerated deuterons and  $^{15}\text{O}$  is produced while one neutron is released. The target is nitrogen gas while the “in-target” product is  $^{15}\text{O}$ -labelled water ( $\text{H}_2^{15}\text{O}$ ). To achieve this reaction deuterons are accelerated to 3-9 MeV and this can be accomplished by even the least powerful accelerators:



In another option isotopically enriched  $^{15}\text{N}_2$  gas is used and bombarded with protons. The result is the same as in the first one,  $^{15}\text{O}$ -labelled water ( $\text{H}_2^{15}\text{O}$ ). In the first case we use isotopically enriched projectile and in another isotopically enriched target:



The system and the apparatus is bit different: the syringe pushes nitrogen (especially if it is  $^{15}\text{N}_2$  that is very expensive since it has to be isotopically enriched) into the target gas chamber, gets bombarded and once it is irradiated enough the gas is pushed back through the vortex tube in a cold trap where produced  $^{15}\text{O}$ -labelled water is solidified into ice and trapped while the rest of gas is going back. Then 6-port valve changes its position and helium can push the water vapours from the vortex tube into radiochemical cell.

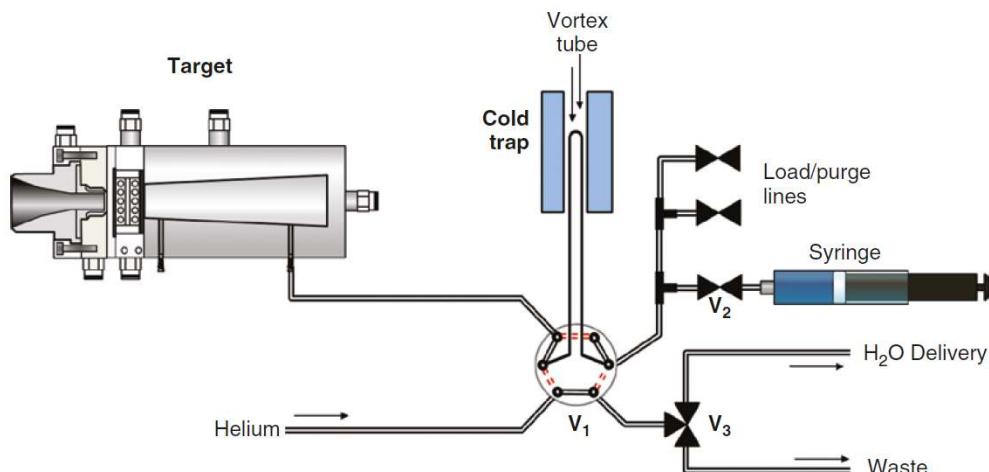


Figure 267: Production of  $^{15}\text{O}$

## Radiochemistry of $^{15}\text{O}$

When it comes to radiochemistry of  $^{15}\text{O}$ , it is definitely the poorest of all. Due to its fatal main drawback, very short half-life, the  $^{15}\text{O}$  radiochemistry and PET imaging with are vanishingly limited and rare. The usage of  $^{15}\text{O}$  comes down to two radiopharmaceuticals, out of which one is the actual in-target product,  $^{15}\text{O}$ -labelled water. It can be used for the ventilations imaging studies and diagnostics on lungs. A patient inhales and exhales air containing  $\text{H}_2^{15}\text{O}$  vapour while being on a PET scanner to test the lung function. Another possible use of  $\text{H}_2^{15}\text{O}$  is for the imaging of blood flow in the heart, brain and tumours. The second  $^{15}\text{O}$ -labelled radiopharmaceutical known is  $^{15}\text{O}$ -labelled butanol ( $\text{C}_4\text{H}_9^{15}\text{OH}_2$ ). It can be made in a quick hydrolysis reaction between tri-butyl-borane and  $^{15}\text{O}$ -labelled water (Figure 268). The application of it is for the PET imaging of blood flow in brain.

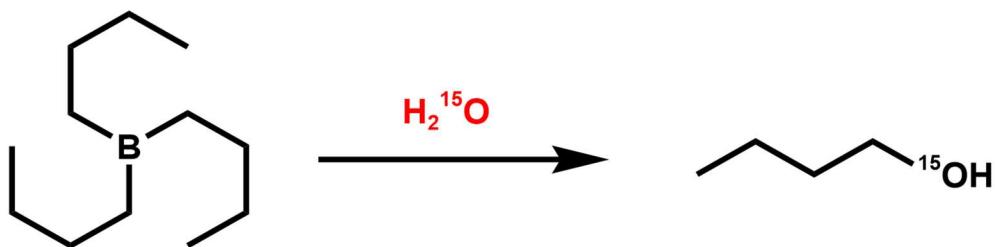


Figure 268: Synthesis of  $^{15}\text{O}$ -labelled butanol

## Chapter XII – Radioiodine

Radioiodine is the common name for the group of four radioactive isotopes of iodine. Radioactive iodine is one of the oldest radioelements used in medicine and it is still used and quite useful despite of arrival of other radionuclides. It has a place in both therapy and diagnostics as well as basic research in biochemistry and life sciences. From 1960 to 2000s radioiodine and  $^{99m}\text{Tc}$  were the key radionuclides used in SPECT imaging, backbone of nuclear medicine. Recent development of PET imaging as well as  $^{18}\text{F}$  and  $^{11}\text{C}$  radiopharmaceuticals has diminished importance of SPECT radiopharmaceuticals based on  $^{123}\text{I}$  and  $^{124}\text{I}$ . However,  $^{131}\text{I}$  is very important radionuclide for radiotherapy and still will be for the years to come.

### Isotopes of iodine

There are 37 known isotopes of iodine going from  $^{108}\text{I}$  to  $^{144}\text{I}$ . All these are radioactive and artificial isotopes except  $^{127}\text{I}$ , which is the only one natural and stable. Out of all these 36 radioactive isotopes only four of them are attractive for nuclear medicine. The first is  $^{123}\text{I}$ , which is the most important and the most used radioactive isotope of iodine in nuclear medicine, it decays by electron capture (EC), emits gamma rays ( $\gamma$ ), and is used in SPECT imaging. The second is  $^{124}\text{I}$  which decays by both EC and positron emission and is rarely used in PET imaging. Then, the third is  $^{125}\text{I}$  which decays by electron capture (EC), emits gammas ( $\gamma$ ), and is sometimes used in SPECT imaging but much more in “*in vitro*” research and diagnostic techniques such as radioimmunoassay. The fourth is  $^{131}\text{I}$ , which decays by beta-decay, emits both betas and gammas and is used both in radiotherapy and SPECT imaging. All these four iodine radioisotopes are called commonly radioiodine and can be generally represented as  $\text{I}^*$ .

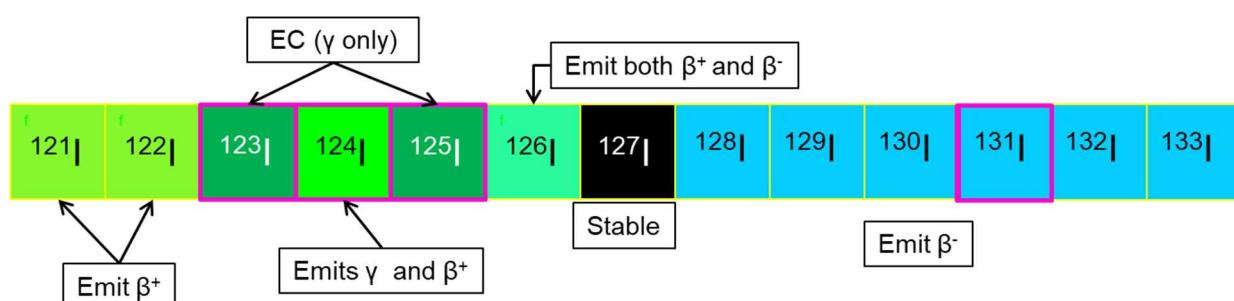


Figure 269: Selected isotopes of iodine

### Nuclear properties of $^{123}\text{I}$

Iodine-123 ( $^{123}\text{I}$ ) decays by electron capture (EC or  $\epsilon$ ) and emits gamma radiation of 159 keV. This energy of gamma photon is very good and appropriate for SPECT imaging, just like the one emitted by  $^{99m}\text{Tc}$ . Its half-life is 13.2 hours which is very good taking into account that it can be made by cyclotron and therefore there is plenty of time for radiochemistry. It ultimately decays into stable tellurium isotope  $^{123}\text{Te}$ . This radioiodine is heavily used in radioiodinations of many small molecules or

even peptides or proteins by which radiopharmaceutical agents are made for SPECT imaging. However, its importance is fading since 1990s due to development of  $^{18}\text{F}$  and  $^{11}\text{C}$  PET tracers and PET scanners getting cheaper.

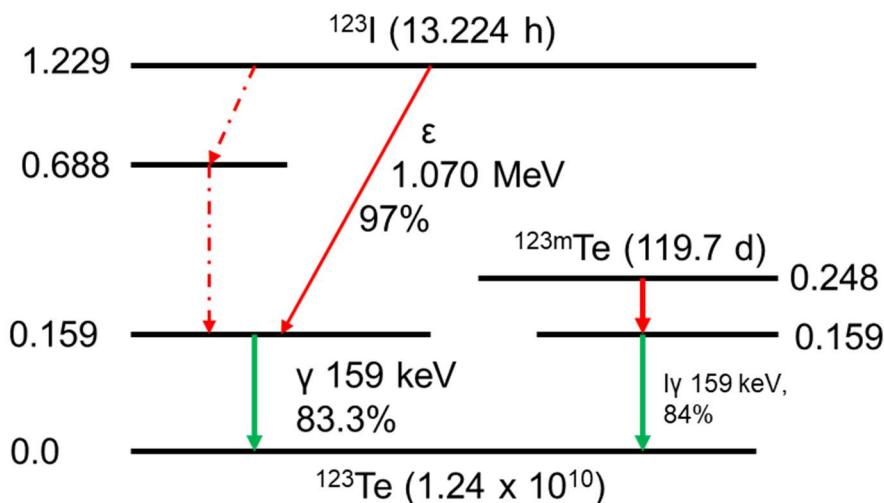
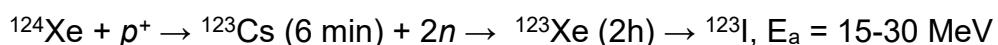
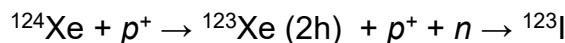


Figure 270: Decay diagram of  $^{123}\text{I}$

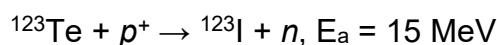
### Production of $^{123}\text{I}$

$^{123}\text{I}$  is produced by cyclotrons by irradiation of  $^{124}\text{Xe}$  with protons.  $^{124}\text{Xe}$  nucleus captures accelerated proton and becomes  $^{123}\text{Xe}$  and then quickly decays giving out its daughter  $^{123}\text{I}$ . It also can be converted into  $^{123}\text{Cs}$  which then decays again into  $^{123}\text{Xe}$  and again into  $^{123}\text{I}$ :



This transmutation requires more powerful protons of 15-30 MeV, and these have to be accelerated using a powerful cyclotron.

Another option is to bombard solid a target, tellurium isotope  $^{123}\text{Te}$  with protons of 15 MeV and the product is  $^{123}\text{I}$ :



However, this transmutation needs a solid target made of isotopically enriched  $^{123}\text{Te}$ . Produced  $^{123}\text{I}$  is in the form of elementary iodine solid ( $^{123}\text{I}_2$ ) and gets trapped onto the inner wall of the irradiation capsule under refrigeration and is then eluted with sodium hydroxide (NaOH). Sodium hydroxide turns solid elementary iodine into soluble iodide ( $\text{I}^-$ ) and hypoiodite ( $\text{IO}^-$ ). This is so called reaction of disproportionation of iodine, and it happens in alkaline solution.

## Nuclear properties of $^{124}\text{I}$

Iodine-124 ( $^{124}\text{I}$ ) decays both by electron capture (74.4%) and positron decay (25.6%). Its half-life is 4.18 days which is much, much better than  $^{18}\text{F}$ . Unfortunately, there is a huge, major drawback of its positrons, these are too energetic: mean energy is 819 keV, while maximal energy is 2138 keV. This is too much, and resolution is worse than in the case of  $^{15}\text{O}$ . Also, there are additional single photon gamma emissions of 604 keV.  $^{124}\text{I}$  ultimately decays into  $^{124}\text{Te}$ . It can be made by cyclotron, and in theory could be simultaneously used both for PET and SPECT imaging! But reality is that it is rarely used, the major reason is not so good PET image resolution due to large positron energy.

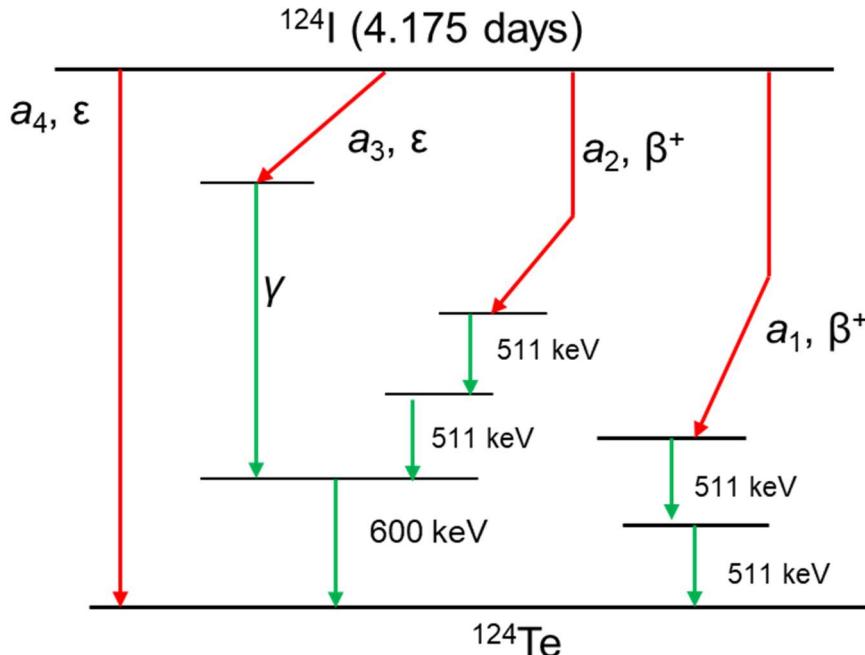
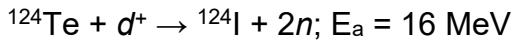


Figure 271: Decay diagram of  $^{124}\text{I}$

## Production of $^{124}\text{I}$

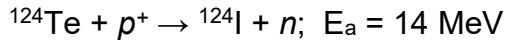
For the production of  $^{124}\text{I}$  by cyclotrons one can use three options.

In the first option solid target  $^{124}\text{Te}$  is bombarded with deuterons and gives  $^{124}\text{I}$  after two neutrons are released:



This reaction is high yielding in activity, but product is contaminated with unwanted  $^{125}\text{I}$  isotope. The target is solid  $\text{TeO}_2$  or elementary tellurium metalloid enriched in  $^{124}\text{Te}$  isotope. For this reaction a cyclotron of weaker power is enough.

The second option is bombardment of  $^{124}\text{Te}$  target with protons. It gives low yields, but much better radionuclidian purity;  $^{125}\text{I}$  isotope is absent:





The target is also  $\text{TeO}_2$  or elementary tellurium enriched in  $^{124}\text{Te}$  as in the previous case. This is the most common way of making  $^{124}\text{I}$ .

The third option is bombardment of  $^{125}\text{Te}$  with protons with very energetic protons (20 MeV and more). This is the most demanding option but gives both the high radionuclidian purity and high yield of activity:



For this method one needs special tellurium enriched in  $^{125}\text{Te}$  and a stronger cyclotron device.

### Nuclear properties of $^{125}\text{I}$

Iodine-125 ( $^{125}\text{I}$ ) decays by electron capture (EC,  $\epsilon$ , 100%) and by that it emits gamma radiation of 35 keV. This energy of gamma photons is not good for SPECT imaging it is too weak but is good generally for simple gamma scintillators found in gamma counters. The half-life is 59.49 days which is more than generous and comfortable. It decays into  $^{125}\text{Te}$ . Contrary to previous two radioiodines  $^{125}\text{I}$  isotope is made in nuclear reactors. Due to its weak gamma energy, it is not very attractive for nuclear medicine and imaging by SPECT but is very attractive (due to very long half-life) for the usage in “*in-vitro*” diagnostics and scientific research in the area of biomedicine and general life science. There are numerous  $^{125}\text{I}$ -labelled reagents and tracers developed and produced for scientific research on laboratory animals or cell culture or in microbiology and other life sciences. In fact, many important discoveries in biochemistry and life sciences were achieved by using  $^{125}\text{I}$ -labelled reagents.

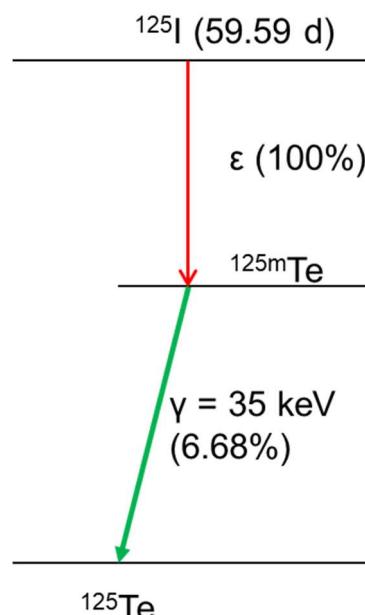
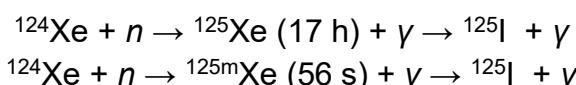


Figure 272: Decay diagram of  $^{125}\text{I}$

### Production of $^{125}\text{I}$

Isotope  $^{125}\text{I}$  is produced in large quantities using nuclear research reactors from xenon isotope  $^{124}\text{Xe}$ .  $^{124}\text{Xe}$  captures thermal neutron and becomes either  $^{125\text{m}}\text{Xe}$  or  $^{125\text{g}}\text{Xe}$ . In both cases these xenon isotopes decay by releasing gamma photons and are transmuting into  $^{125}\text{I}$ .



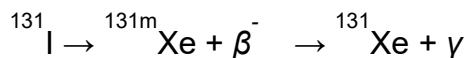
The irradiation target is the natural xenon gas (it contains only 0.0965 % of desired  $^{124}\text{Xe}$ ) loaded under pressure into special capsules made from zirconium alloy that is transparent to thermal neutrons.



After irradiation of few days several radioisotopes of xenon are produced, but only  $^{125}\text{Xe}$  leads to  $^{125}\text{I}$ , while the other xenon radioisotopes decay quickly either to stable xenon, or to various Cs isotopes including those radioactive such as  $^{135}\text{Cs}$  and  $^{137}\text{Cs}$ . Therefore, irradiated gas is allowed to decay for 3 or 4 days to eliminate short-lived unwanted radioisotopes, and to allow the newly created  $^{125}\text{Xe}$  to decay into  $^{125}\text{I}$ . The irradiated capsule is cooled to a low temperature and free  $^{125}\text{I}_2$  iodine gas solidifies into iodine solid on the capsule inner wall. Then this  $^{125}\text{I}_2$  iodine solid is extracted with NaOH solution which turns iodine into soluble iodide ( $\text{I}^-$ ) and hypoiodite ( $\text{IO}^-$ ) salts. This is disproportionation reaction. The long-lived  $^{135}\text{Cs}$  and  $^{137}\text{Cs}$  radioisotopes that may be present in product are usually removed by ion-exchange column.  $^{125}\text{I}$  remains in the solution in the form of  $\text{Na}^{125}\text{I}$  (sodium iodide) or  $\text{Na}^{125}\text{IO}$  (sodium hypoiodite).

### Nuclear properties of $^{131}\text{I}$

The fourth important radioiodine is  $^{131}\text{I}$  radioisotope. It decays by beta decay into  $^{131}\text{Xe}$ . It emits beta-particles ( $\beta^-$ ) with the mean energy of 191 keV, and maximal energy of 606 keV. These beta-particles ( $\beta^-$ ) are regarded as strong or hard beta-particles and penetrate into tissues from 0.6 to 2 mm and can give tissue a significant radiation dose. Its intermediate daughter  $^{131\text{m}}\text{Xe}$  turns into  $^{131}\text{Xe}$  by releasing gamma photons with energy of 382 keV.



Half-life is 8.05 days and is made in nuclear reactors. Since it emits both energetic betas and not so energetic gammas it can be used in both targeted radiotherapy and for the SPECT imaging (actually in the same time) which makes it very special in nuclear medicine (the isotope itself is a theranostic). Nevertheless,  $^{131}\text{I}$  is carcinogenic and is causing cell death and mutations if taken internally: it was the major cause of radiation hazard immediately after the Chernobyl accident. Also except in the medicine it is also used as an industrial radiotracer in “fracking” (extraction of natural gas by hydraulic fracturing of underground rocks).

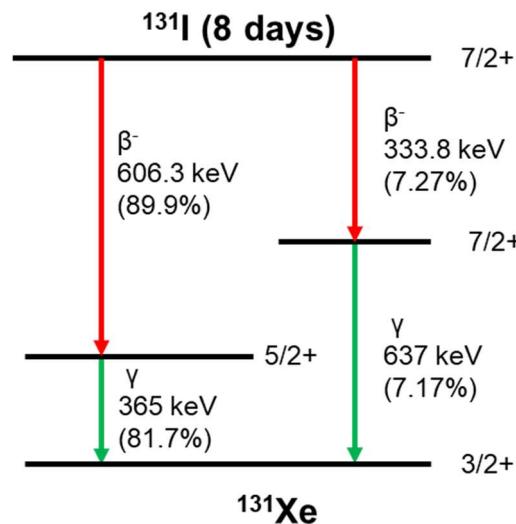


Figure 273: Decay diagram of  $^{131}\text{I}$

### Production of $^{131}\text{I}$

Isotope  $^{131}\text{I}$  is produced in the research nuclear reactors by irradiation of stable  $^{130}\text{Te}$  with thermal neutrons.  $^{130}\text{Te}$  captures thermal neutron and becomes  $^{131}\text{Te}$ , but then decays by beta decay into  $^{131}\text{I}$ :



The target is natural elemental tellurium powder. There are other by-products created by neutron capture, but these are either stable isotopes of tellurium, iodine or xenon, and these isotopes decay very quickly leaving only  $^{131}\text{I}$  as a radioactive product. After the irradiation  $^{131}\text{I}$  product is extracted out by dry distillation giving off  $^{131}\text{I}_2$  gas. It is then converted into the  $\text{Na}^{131}\text{I}$  salt, the primary precursor for  $^{131}\text{I}$ -labelling.

$^{131}\text{I}$  is also made by fission of  $^{235}\text{U}$  in the nuclear power plant thermal reactors but is not extracted from this source. Sometimes it can be released in small amounts from these thermal reactors, but also in large quantities in the case of nasty incident such as Chernobyl and Fukushima incidents were.

## Chemical properties of iodine

Iodine is, just like fluorine a halogen element, but much less reactive, much less electronegative than fluorine. It is in fact a metalloid with slight metallic properties. Iodine exists in several oxidation states whereby the most common is “-1” ( $\text{I}^-$ , iodide ion), then “+1” ( $\text{IO}^-$ , hypoidite ion), the “+3” ( $\text{IO}_2^-$ , iodite), “+5”, ( $\text{IO}_3^-$ , iodate ion), and finally the most oxidised is “+7” ( $\text{IO}_4^-$ , periodate ion). In organic molecules carbon-iodine bond (C-I) is a weak one when the carbon is  $\text{sp}^3$  hybridized (in aliphatic compounds). In this case iodo-organic compounds are susceptible to decomposition by hydrolysis and  $\beta$ -elimination but also some enzymes in the body can facilitate removal of iodine from organic molecules. Therefore, radioiodine is almost exclusively introduced onto  $\text{sp}^2$  carbons (for example onto aromatic rings), although some radioiodinated tracers with the iodine on an aliphatic carbon (such as iodo-fatty acids) have been developed and are in use. The most important precursor for  $\text{I}^*$ -labelling (radioiodination) is radioiodide ion ( $\text{I}^-$ ) in the form of sodium iodide salt ( $\text{NaI}^*$ ). Radioiodine can be attached onto small molecules as well as on peptides and proteins.

## Radioiodine in nuclear medicine

Historically radioiodine was one of the first radionuclides used in nuclear medicine, namely  $^{131}\text{I}$ . For decades  $^{125}\text{I}$  and  $^{131}\text{I}$  radioiodines, along with radium and  $^{99\text{m}}\text{Tc}$  were the main radionuclides in the nuclear medicine and imaging: it was impossible to imagine nuclear medicine without radioiodines. Hundreds of radioiodine-labelled radiopharmaceuticals and other radioiodine reagents (especially for radioimmunoassay kits) were developed and produced over the previous decades. However, the importance of radioiodines is fading due to the arrival of  $^{18}\text{F}$  and  $^{11}\text{C}$  radiopharmaceuticals and PET tracers; cyclotrons and PET scanners are also being more available and affordable. It is expected that importance of radioiodine will continue to fade but will not be fully diminished.

## **NaI\*, the simplest radiopharmaceutical of iodine**

Historically sodium iodide ( $\text{NaI}^*$ ), the primary precursor for radioiodinations is also the most important radioiodine radiopharmaceutical. Out of all simple radionuclides it has a unique property that it specifically targets only one organ, thyroid gland without any vector needed. Iodine in the form of iodide ion naturally gets accumulated in thyroid gland where it is used for the biosynthesis of hormones thyroxin ( $\text{T}_4$ , Figure 274) and triiodothyronine ( $\text{T}_3$ ); these hormones are essential in the control of metabolism. The thyroid cells have special mechanism to capture iodide and bring it into cells where it is inserted into  $\text{T}_4$  and  $\text{T}_3$  hormones. Therefore,  $^{123}\text{I}$ ,  $^{124}\text{I}$  and  $^{131}\text{I}$  can be used for the imaging of thyroid gland using PET or SPECT (Figure 275);  $^{131}\text{I}$  can be also used for both imaging and treatment of thyroid diseases: hyperthyroidism (overactive thyroid gland) and some cases of thyroid cancer.

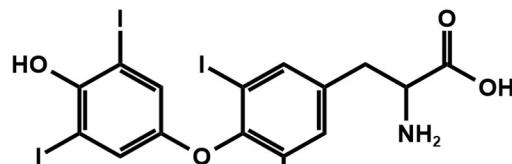


Figure 274: Hormone thyroxine ( $\text{T}_4$ )

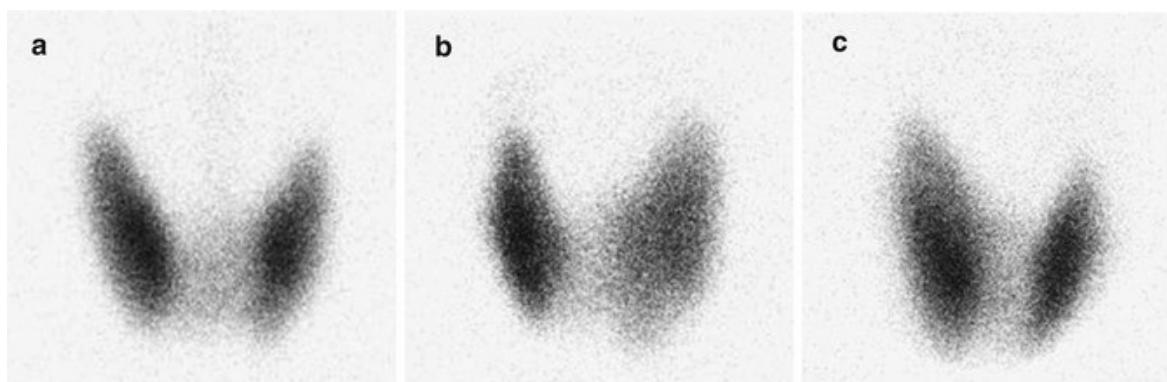


Figure 275: SPECT of thyroid gland by using  $\text{Na}^{123}\text{I}$

## **Radioiodination of small molecules**

Labelling any vector molecules, either small molecule or peptide with any radioiodine ( $\text{I}^*$ ) is called radioiodination. Radioiodination is usually achieved on aromatic rings. The typical methods for  $\text{I}^*$ -labelling (radioiodinations) are:

- electrophilic substitutions,
- nucleophilic substitutions,
- additions on unsaturated bonds
- isotope exchange methods.

### **Radioiodination by electrophilic substitution**

Electrophilic substitutions are performed with radioiodide ion ( $\text{I}^{*-}$ ) that is the standard primary precursor. Radioiodide is not an electrophile, but a nucleophile, therefore

some kind of an oxidizing agent is needed to convert iodide to an electropositive form. Electrophilic radioiodination (Figure 276) could be achieved with various aromatic rings such as phenols, anilines, imidazole, indole, benzene, etc.

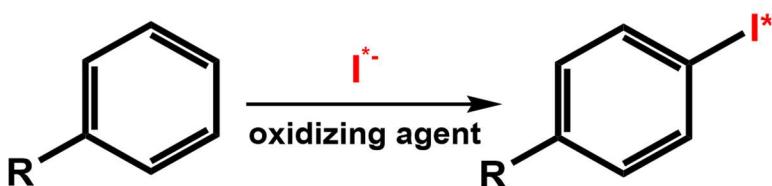


Figure 276: Typical electrophilic iodination reaction

There are many oxidizing agents (Figure 277) for electrophilic radioiodinations, but there are some of those that are often used: chloramine T, iodogen, *N*-chlorosuccinimide, *tert*-butyl-hydroperoxide, hydrogen peroxide and peracetic acid.

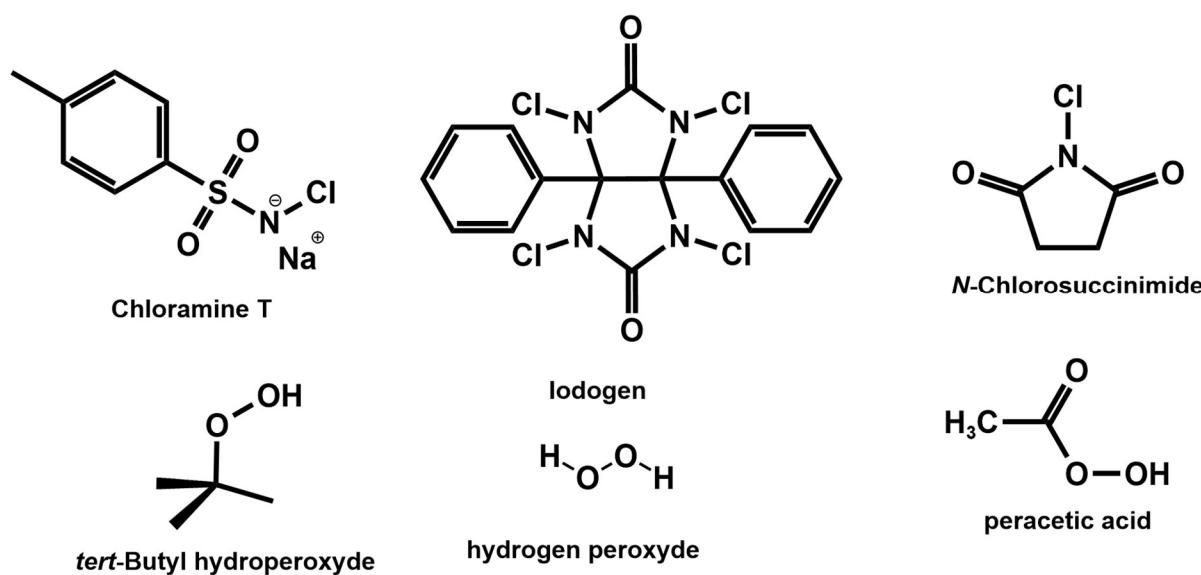


Figure 277: Oxidizing agents for electrophilic radioiodinations

The typical electrophilic iodination goes with *N*-chlorosuccinimide that reacts with radioiodide and forms very reactive compound where radioiodine is bonded onto nitrogen (Figure 278). In the next step iodine is immediately transferred onto benzene ring of a substrate. The problem with this reaction is that it is not very regiospecific, iodine can be attached onto either *meta*- or *para*- position of the aromatic ring. This is not very desirable situation.

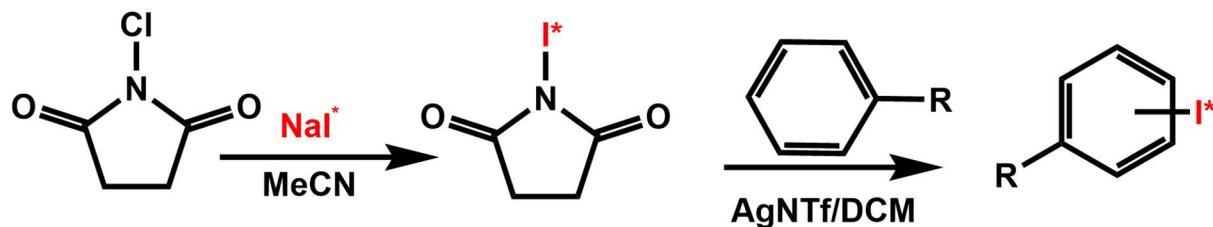


Figure 278: Typical electrophilic iodination with *N*-chlorosuccinimide

Therefore, in order to address this problem more sophisticated reaction is usually undertaken for radioiodination, so called iododemetalations. This is reaction between activated electrophilic radioiodine ( $\{I^{*+}\}$ ) and some organometallic substrate (Figure 279). In most of the cases those are iododestannylation where metal on aromatic ring is tin (Sn). However other metals can be used, such as silicon (Si), mercury (Hg), boron (B), germanium (Ge), and thallium (Tl). The key advantage of iododemetalations is the faster kinetics, higher radiochemical yield and radioiodine gets inserted exactly where it should be. These reactions, especially with tin as the metal centre, are the most common types of radioiodinations.

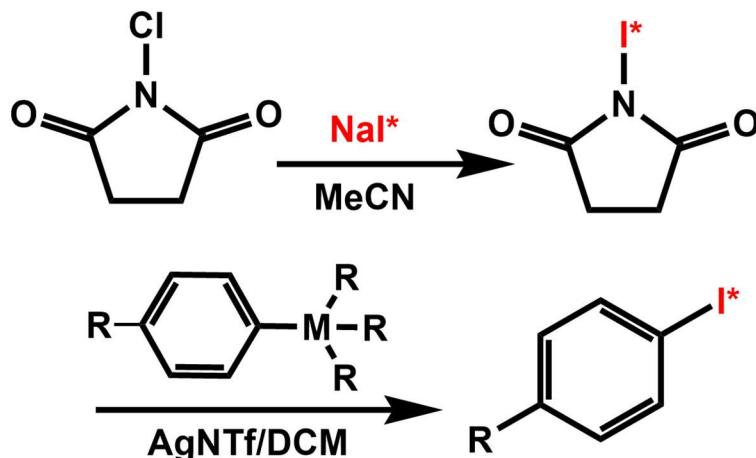


Figure 289: Electrophilic iodination via iododemetalations

### Radioiodination by nucleophilic substitution

Except by electrophilic radioiodinations the radioiodines can be introduced into aromatic rings or aliphatic chains by nucleophilic substitutions. In general, these are very similar as nucleophilic radiofluorinations, predominantly carried out for iodoalkanes and iodoarenes by  $S_N2$  and  $S_NAr$  reactions, respectively. As source of iodine, plain nucleophile radioiodide ion ( $I^{*-}$ ) is used. The good leaving groups here are various sulfonates (such as triflate and tosylate) or diazonium groups. In addition, boronic esters can be good leaving groups if the substitution is catalysed by copper catalyst. As was the case in nucleophilic radiofluorinations  $S_NAr$  reactions are facilitated by electron-withdrawing groups in *ortho*- or *para*- position. For example, boron-pinacoline ester reacts with  $^{123}I^-$  ion in the presence of copper-(I) catalyst and substitutions is quickly achieved (Figure 290). In this case boron-pinacoline ester is a good leaving group.

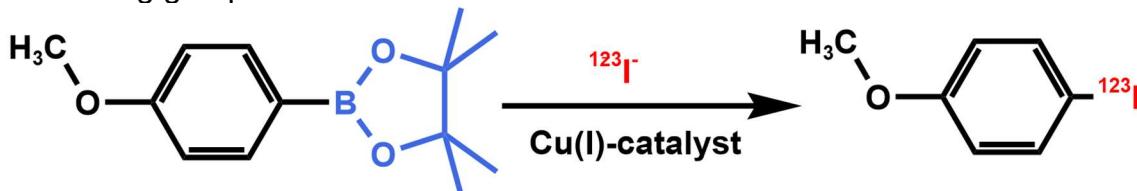
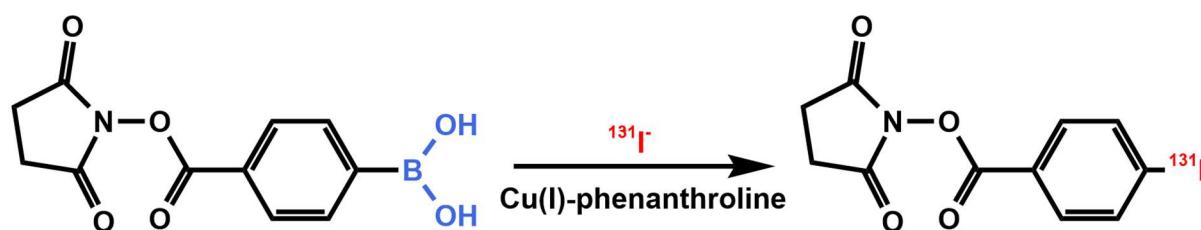
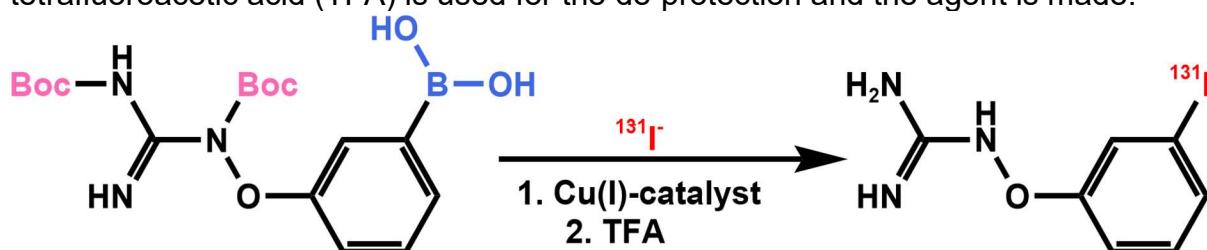


Figure 290: Nucleophilic iodination by using copper catalyst.

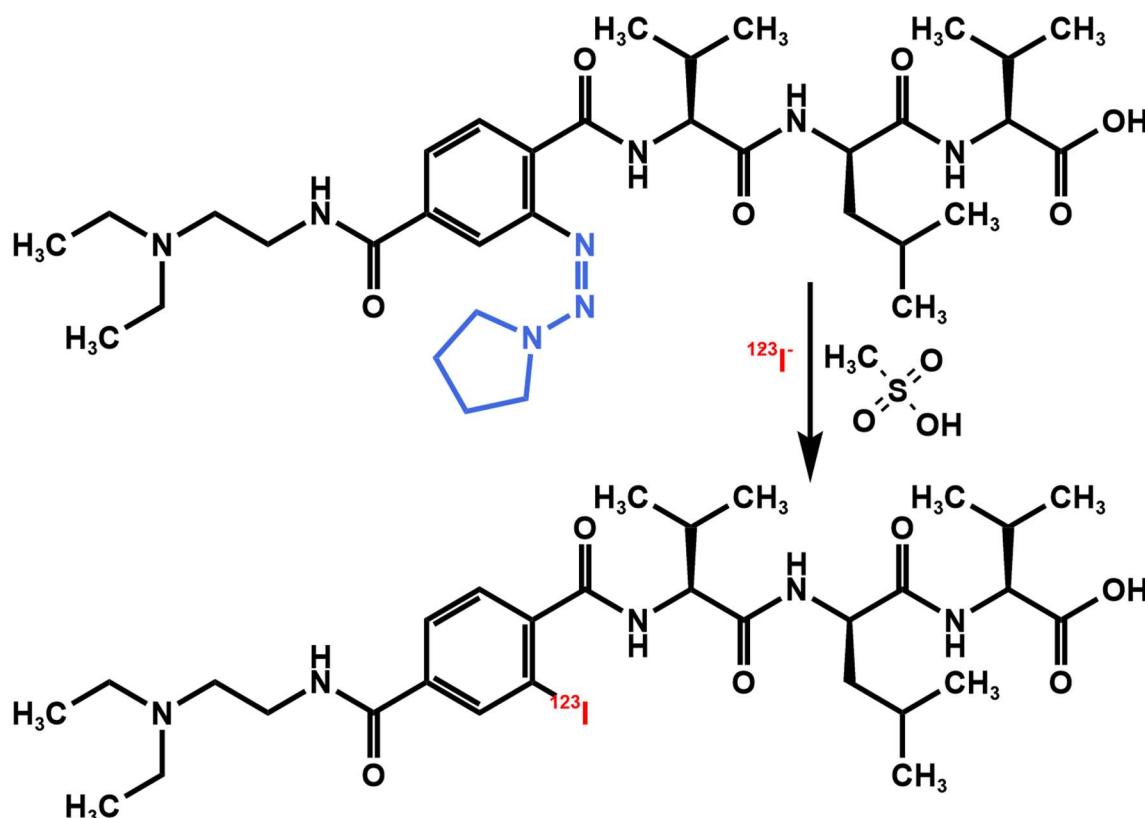
Another example is the synthesis of important labelling agent for  $^{131}I$ -labelling of peptides and proteins, *para*- $^{131}I$ -SIB (Figure 290). The boronic acid reacts with  $^{123}I^-$  ion in presence of copper catalyst and the reagent is ready.

Figure 291: Synthesis of *para*-<sup>131</sup>I-SIB

Next, there is also a synthesis of *meta*-<sup>131</sup>IBG, an important radiopharmaceutical for therapy and imaging (Figure 292). BOC-protected substrate with boronic acid in the *meta*- position reacts with <sup>123</sup>I- ion in the presence of copper catalyst. In the next step tetrafluoroacetic acid (TFA) is used for the de-protection and the agent is made.

Figure 292: Synthesis of *meta*-<sup>131</sup>I-BG

In the final example of nucleophilic radioiodinations a triazene group, a more stable form of diazo group is used as a good leaving group for radioiodination in presence of methane sulphonic acid. The result is <sup>123</sup>I-labelled proteasome inhibitor peptide (Figure 293). This is an example how radioiodination can be achieved directly on a peptide, without using any linker or prosthetic group.

Figure 293: Synthesis of <sup>123</sup>I-labelled proteasome inhibitor peptide

## Radioiodination by isotope exchange method

Important group of reactions for radioiodination are isotope exchange methods, although their importance is fading due to development of better radioiodinations. Radioiodine reacts with a substrate functionalized with non-radioactive iodine or bromine and halogens get exchanged! This reaction can be facilitated by high temperature or Cu(I) ion, or both at the same time. For example, Figure 294 presents quick radioiodination of I\*-labelled *ortho*-iodohippuric acid.

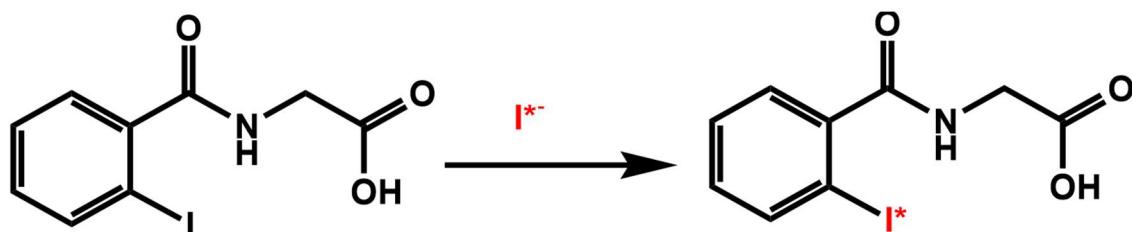


Figure 294: Synthesis of I\*-labelled *ortho*-iodohippuric acid.

## Overview of the most common radioiodine SPECT tracers

### *ortho*-Iodohippuric acid

The radiopharmaceutical agent *ortho*-iodohippuric acid (Figure 294 and 295), also known as “IOH” is usually labelled with  $^{123}\text{I}$  or  $^{131}\text{I}$  and can be made by isotope exchange methods using a kit. It also can be in the form of a sodium salt. It is used for the imaging of effective plasma flow through kidneys (diagnostic test known as “renography”) by using SPECT imaging. Labelled with  $^{131}\text{I}$  (firstly used in 1960) it was for many years the only radiopharmaceutical agent available for renography. Its use has dramatically decreased due to development of other, better radiopharmaceutical agents for renal imaging.

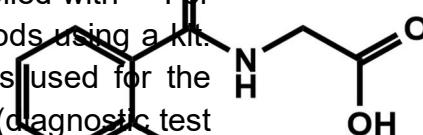


Figure 295: of I\*-*ortho*-iodohippuric acid

### I\*-cholesterol

Radioiodine-labelled cholesterol usually labelled with  $^{125}\text{I}$  or  $^{131}\text{I}$ . Interestingly here radioiodine is attached onto aliphatic carbon. It is used for SPECT imaging of adrenal cortex in patients suspected of having Cushing's syndrome, hyperaldosteronism, or pheochromocytoma.

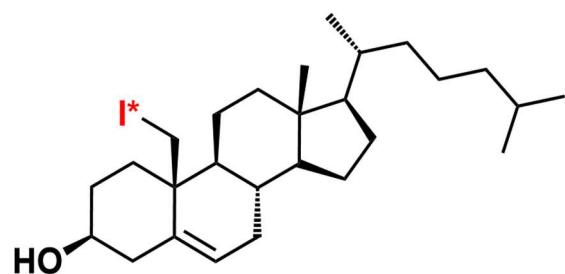
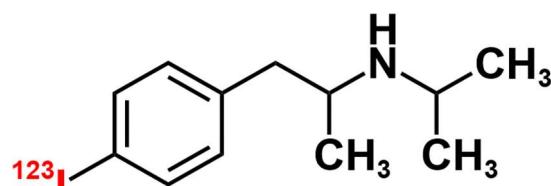


Figure 296: Structure of I\* cholesterol

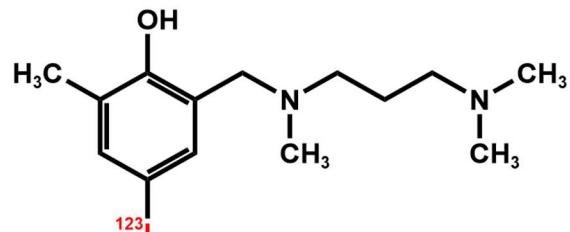
## Iofetamine ( $^{123}\text{I}$ -IMP)

Another important radioiodine agent is iofetamine ( $^{123}\text{I}$ -IMP) is *N*-isopropyl-*p*-iodoamphetamine, a radioiodinated version of amphetamine, famous drug. It binds serotonin and noradrenalin transporter proteins in the brain and is used in imaging of blood flow in brain, as well as for the diagnostics of epilepsy and Alzheimer disease using SPECT.

Figure 297: Structure of  $^{123}\text{I}$ -Iofetamine

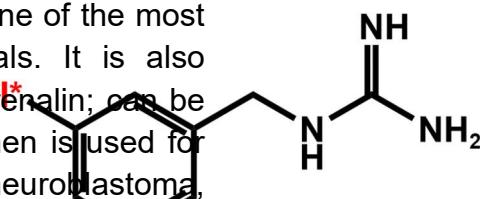
## $\text{I}^*$ -HIPDM

$\text{I}^*$ -HIPDM is an old imaging agent today usually labelled with  $^{123}\text{I}$ . It is used in SPECT imaging of blood flow in brain in patients who suffered stroke and have dementia, but also in the imaging of blood flow in lungs, precisely, for the detection of localized lung diseases.

Figure 298: Structure of  $\text{I}^*$ -HIPDM

## Meta-iodobenzylguanidine ( $\text{I}^*$ -*m*-IBG)

Meta-iodobenzylguanidine ( $\text{I}^*$ -*m*-IBG or MIBG) is one of the most important radioiodine-labelled radiopharmaceuticals. It is also known as “lobenguane” and is an analogue of adrenalin; can be labelled with  $^{123}\text{I}$ ,  $^{124}\text{I}$  or  $^{131}\text{I}$ . If labelled with  $^{123}\text{I}$  then is used for the imaging of neuroendocrine tumours (neuroblastoma, paraganglioma, and pheochromocytoma) by SPECT scanner. If labelled with  $^{124}\text{I}$  it is used for the PET imaging of these tumours. But if labelled with  $^{131}\text{I}$  then is used for both imaging and treatment of neuroendocrine tumours.

Figure 300: Structure of  $\text{I}^*$ -*m*-IBG

## Ioflupane

Ioflupane, also known as  $^{123}\text{I}$ -FP-CIT is a  $^{123}\text{I}$ -labelled analogue of cocaine and has a high binding affinity for the presynaptic dopamine transporters (DAT) in the brain. It is used for the SPECT imaging of brain: precisely for the diagnosis of Parkinson's disease.

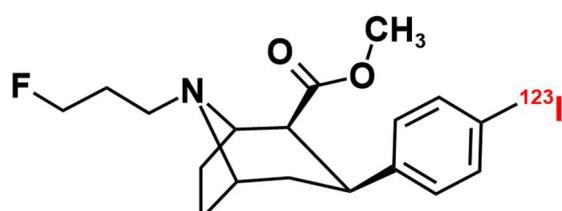


Figure 301: Structure of Ioflupane

## Iomazenil

Another important radioiodine-labelled radiopharmaceutical agent for the SPECT imaging of brain is  $^{123}\text{I}$ -radioiodinated benzodiazepine called iomazenil. It is an analogue of flumazenil and binds benzodiazepine receptors ( $\text{GABA}_\text{A}$  receptors) in the brain. It is used for the SPECT imaging of epilepsy: to identify in the brain from where epileptic seizures start. It can be used for the imaging of schizophrenia.

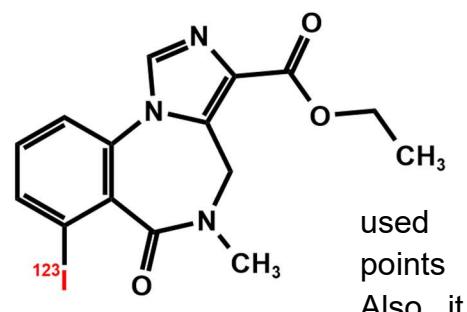


Figure 302: Structure of Iomazenil

used  
points  
Also, it

## $^{123}\text{I}$ -fatty acids

The  $^{123}\text{I}$ -labelled fatty acids are used for the SPECT imaging of heart in patients who recently had heart infarction, and to detect abnormal fatty acid metabolism in ischemic heart disease. It is possible to foresee how their recovery will progress. These are  $^{123}\text{I}$ -iodoheptadecanoic acid (HDA), which is the most common one, then  $^{123}\text{I}$ -iodo-*p*-phenylpentadecanoic acid (*p*-IPPA) and  $^{123}\text{I}$ - $\beta$ -methyl-*p*-iodophenylpentadecanoic acid (BMIPP).  $^{123}\text{I}$ -HDA is the one most commonly used, but has a drawback: it tends to lose  $^{123}\text{I}$  due to hydrolysis and enzyme activity. Therefore, improved artificial  $^{123}\text{I}$ -labelled fatty acids were introduced;  $^{123}\text{I}$ -*p*-IPPA and  $^{123}\text{I}$ -BMIPP where  $^{123}\text{I}$  is bound onto the terminal phenyl group and is much less prone of hydrolysis.

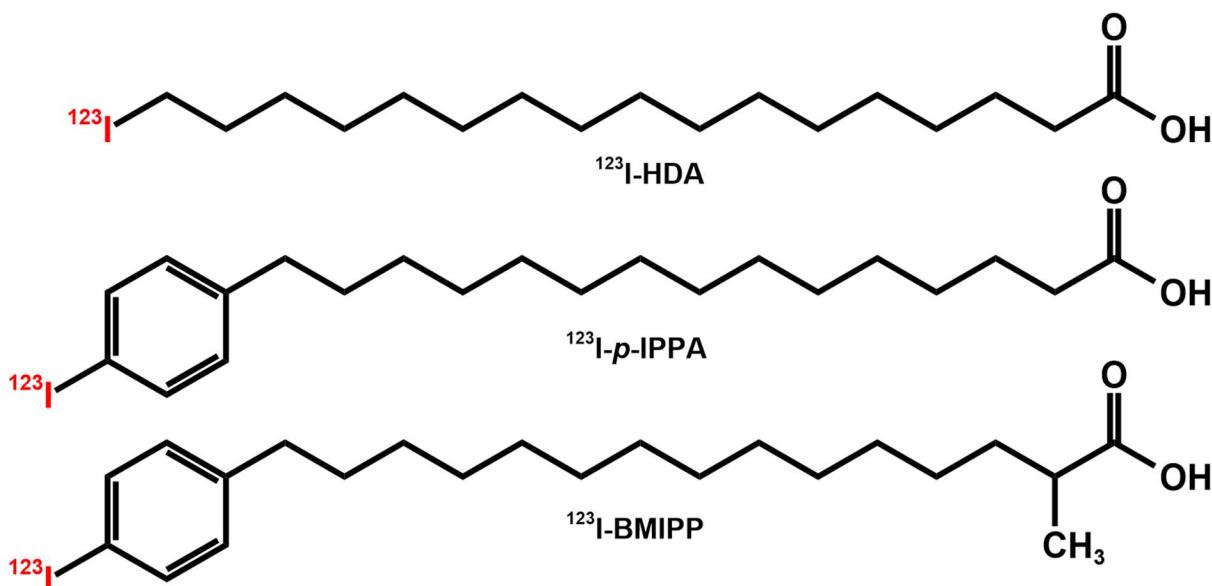


Figure 302: Structure of  $^{123}\text{I}$ -fatty acids

## Kit methods for radioiodination

To simplify and speed up the day-to-day routine work on preparation of radioiodine-labelled radiopharmaceuticals, shorten the time needed for preparation, ensure radiochemical purity of the prepared agents special kits for radioiodination were introduced. These kits are in practical sense very similar to  $^{99m}\text{Tc}$  kits and can be used for the quick preparations of radioiodine radiopharmaceutical agents by well-trained technicians or pharmacists. However, they work bit differently. Here the precursor is a reactive molecule bound onto supporting polymer (Figure 303) and is removed from the polymer only in the presence of activated oxidised radioiodine. Typical reactive molecule is an organo-tin derivative of the tracer molecule: only those reacting with radioiodine will be removed from the polymer and dissolved in solution, while the other molecules will stay bound onto the polymer. These kits are simple and quick to use and ensure high radiochemical yield and high radiochemical purity.

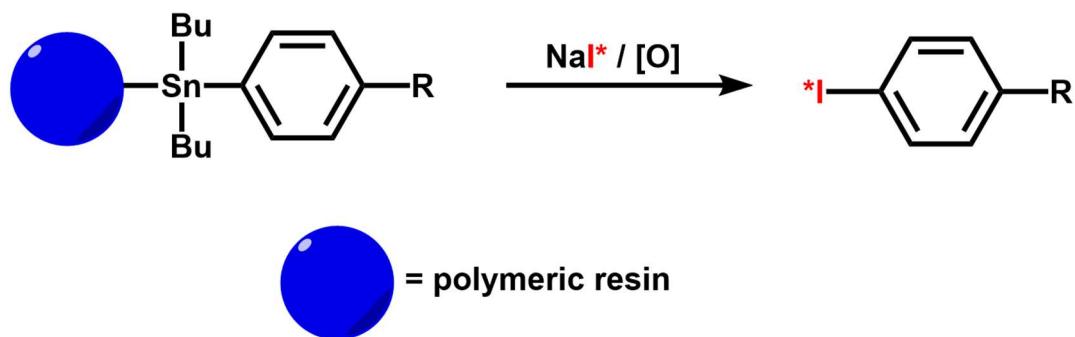


Figure 303: Concept of radioiodination on polymeric resin, used for kits for instant radioiodinations

## Radioiodination of Peptides and Proteins

Peptides and proteins are much better vectors than small molecules, since they possess superb ability to specifically bind targets *via* molecular recognition. Just like in the case of  $^{18}\text{F}$  it is desirable to attach radioiodine onto peptides and proteins. The method for the bioconjugation of radioiodine and biomolecules are fairly similar to those used for  $^{18}\text{F}$ , however opposite to radiofluorination direct radioiodinations of peptides are possible.

Methods for radioiodination of peptides and proteins are

- direct radioiodination
- use of pre-labelled prosthetic groups
- preconjugation of precursor moiety
- click chemistry

## Direct radioiodination of proteins and peptides

Opposite to radiofluorinations, direct radioiodinations are possible because electrophilic radioiodination do not ask for harsh conditions as fluorine does. Many peptides containing amino acid tyrosine residues have been radioiodinated by the direct electrophilic substitution. Reactions are performed in aqueous medium, at the room temperature for 5–10 min at a pH of around 7.0–7.5 and using oxidants such as chloramine-T or iodogen (Figure 304).

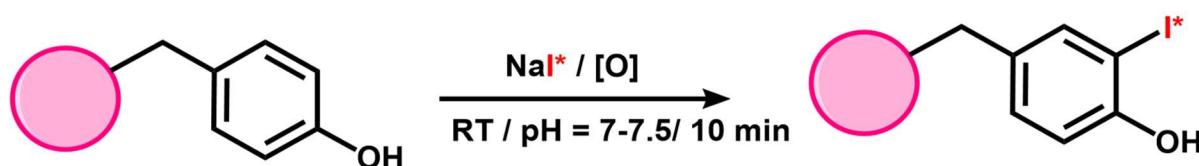


Figure 304: Direct iodination of tyrosine aromatic moiety on a protein or a peptide

## Radioiodination of proteins and peptides via pre-labelled prosthetic group

Another, quite common method is the use of pre-labelled prosthetic groups. In this method, a radioiodine-labelled reagent (prosthetic group) is attached onto a peptide or protein under mild conditions. In this way peptides and proteins are not subjected to any potentially harmful oxidants, and this is an excellent option for very sensitive proteins such as mABs. Also, the radiolabelled peptide will be stable toward *in vivo* deiodination.

The typical prosthetic groups are *meta*- or *para*-<sup>\*</sup>I-SIB (Figure 305 up), Bolton-Hunter reagent or radioiodine-labelled maleimido-reagents (Figure 305 down). This method usually consist of two steps, the first step is the formation of radioiodine-labelled reagent by some common radioiodination reaction, while the second step is the bioconjugation, reaction of the reagent and protein or peptide.

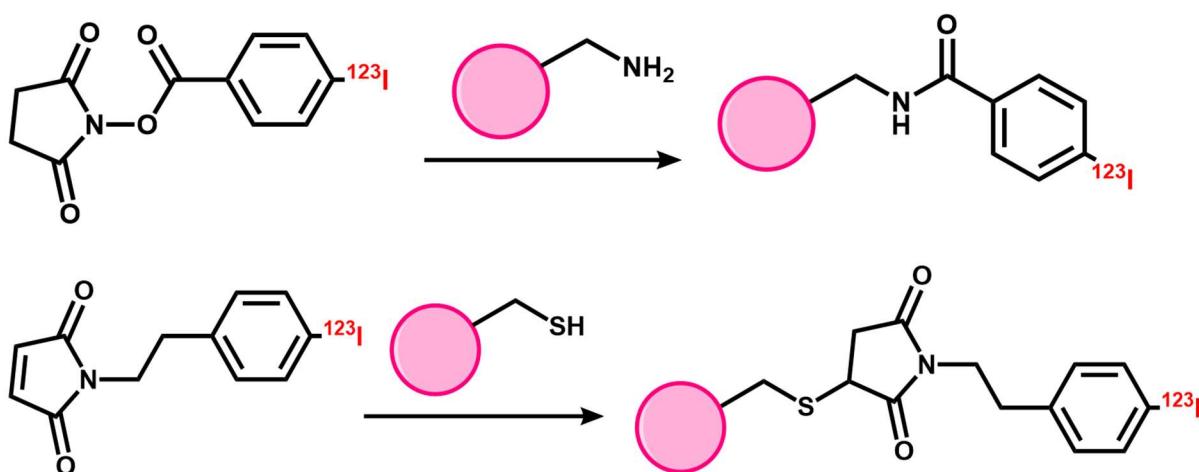


Figure 305: Radioiodination of protein or a peptide by using para-<sup>123</sup>I-SIB (up) or para-<sup>123</sup>I-maleimido-reagent (down)

## Preconjugation of precursor moiety

Preconjugation of precursor moiety is the opposite method comparing the previous. In order to save some time prosthetic group is firstly bioconjugated onto a peptide or a protein and only then the radioiodination step is performed. Typically, an organotin or another organometallic leaving group is attached onto the peptide by using a suitable reactive moiety, and the radioiodination is performed by using nucleophilic S<sub>N</sub>Ar substitution (Figure 306).

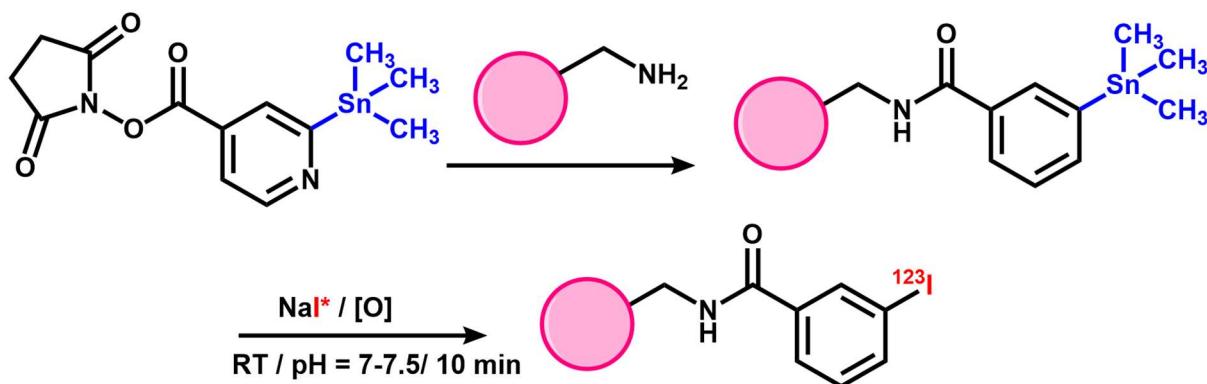


Figure 306: Radioiodination of protein or a peptide bioconjugate with organotin reagent: the last reaction is radioiodination

## Radioiodinations of peptides and proteins by using click chemistry

Finally the some powerful coupling reaction from the arsenal of click chemistry can be employed and in this particular case it is the so-called inverse electron demand Diels-Alder reactions (IEDDA, Figure 307). It is a very fast and reliable reaction between cyclooctene and 1,2,4,5-tetrazine. This reaction needs no catalyst and can be accomplished even in cells. The first step is radioiodination of 1,2,4,5-tetrazine-bearing reagent *via* electrophilic substitution reaction of organotin leaving group with <sup>123</sup>I. The next step is IEDDA click reaction between I<sup>\*</sup>-tetrazine reagent and cyclooctene-protein bioconjugate.

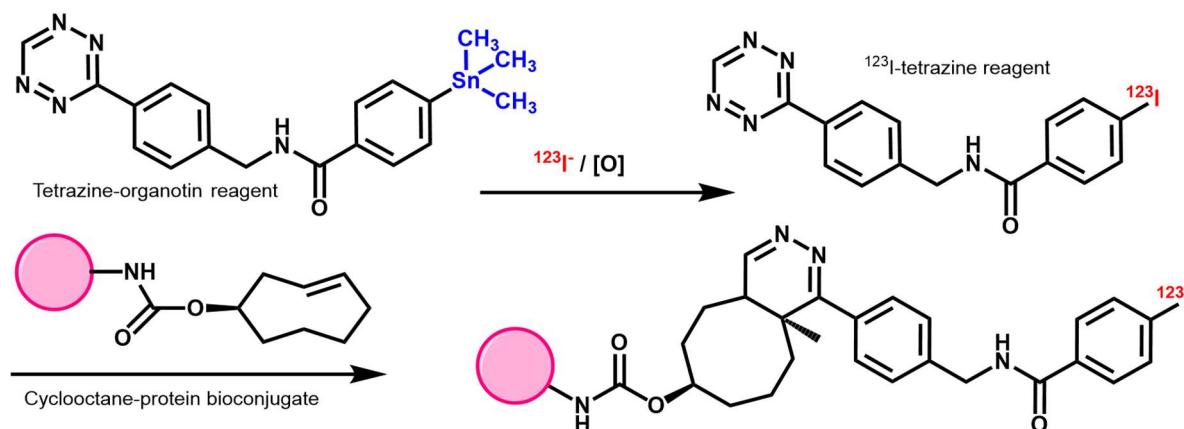
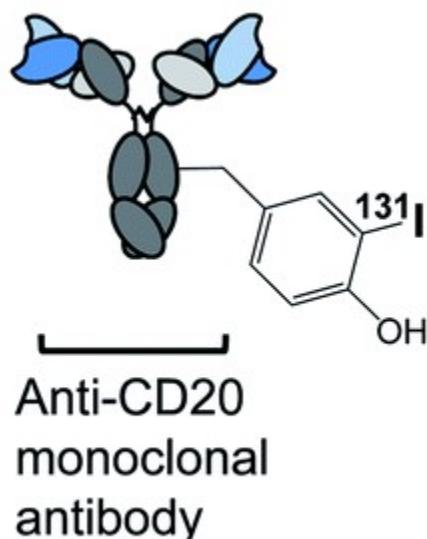


Figure 307: Radioiodination of protein or a peptide via inverse electron demand Diels-Alder click reaction (IEDDA)

## Tositumomab

The most famous radioiodine-labelled protein is tositumomab. It is a  $^{131}\text{I}$  labelled monoclonal antibody for the targeted radiotherapy (TRT). Here  $^{131}\text{I}$  is labelled using direct electrophilic substitution onto the phenyl residue of a tyrosine amino acid. It targets the CD20 antigens that are very numerous on B-lymphocyte cancerous cells of the non-Hodgkin lymphoma: the high dose of tositumomab kills almost all the cancerous cells. However, tositumomab is in fact a theranostic, a combination of therapeutic and diagnostic since  $^{131}\text{I}$  emits both beta particles and gamma photon. Beta particles are responsible for its therapeutic effect (they kill cancerous B lymphocytes of the lymphoma), while the gamma photons can be detected by SPECT imaging. Hence, the cancer cells, usually accumulated in lymph nodes can be monitored in real time to locate them and to verify their disappearance.

Tositumomab was present on the market of Western countries under trade name Bexxar. Unfortunately, unreliable supply of  $^{131}\text{I}$  and very high price rendered its use undesirable by hospitals and gradually it was discontinued from the market.



## Chapter XII - Gallium and indium

Gallium and indium are two p-block metals whose isotopes are being increasingly used in clinical SPECT and PET imaging, although their isotopes are still mainly experimental and developmental isotopes whose time is yet to come.

### Isotopes of gallium

Gallium has 34 isotopes (Figure 308), two are stable and natural ( $^{69}\text{Ga}$  and  $^{71}\text{Ga}$ ) and all others are radioactive and artificial.  $^{70}\text{Ga}$  is between the two stable isotopes, but is radioactive and emits beta particles ( $\beta^-$ ) and gamma rays ( $\gamma$ ). Isotopes from  $^{72}\text{Ga}$  onwards are beta-emitting ( $\beta^-$ ) isotopes, and are not suitable for the medical applications.  $^{68}\text{Ga}$  emits positrons ( $\beta^+$ ) and is used in nuclear medicine for the PET imaging.  $^{67}\text{Ga}$  decays by electron capture (EC,  $\epsilon$ ) and emits only gamma ( $\gamma$ ) photons; it is used (though very rarely) for the SPECT imaging. The isotopes from  $^{66}\text{Ga}$  downwards are all positron emitting but its half-life is too short. Therefore, gallium isotopes that are used in nuclear medicine are  $^{67}\text{Ga}$  and  $^{68}\text{Ga}$ .

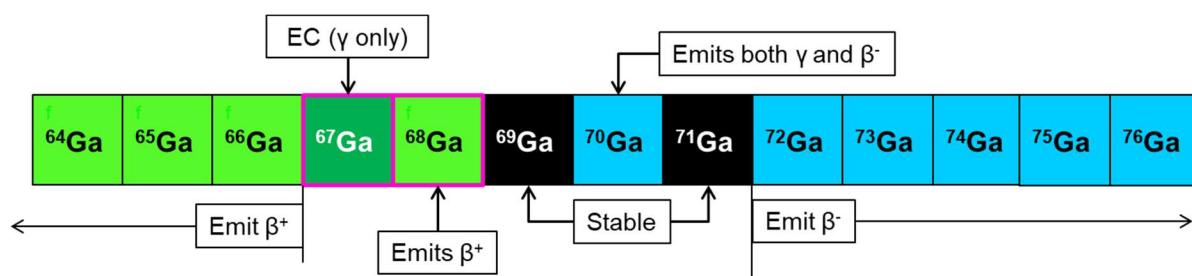


Figure 308: Isotopes of gallium

### Nuclear properties of $^{67}\text{Ga}$

Gallium-67 ( $^{67}\text{Ga}$ ) decays by electron capture (Figure 309) and emits only gamma ( $\gamma$ ). Total emitted gamma energy is 159 keV, however, there are several emitted gamma ray (complex gamma spectrum, 93.3 keV, 184.6 keV, and 300.2 keV) which is not ideal for the SPECT imaging.

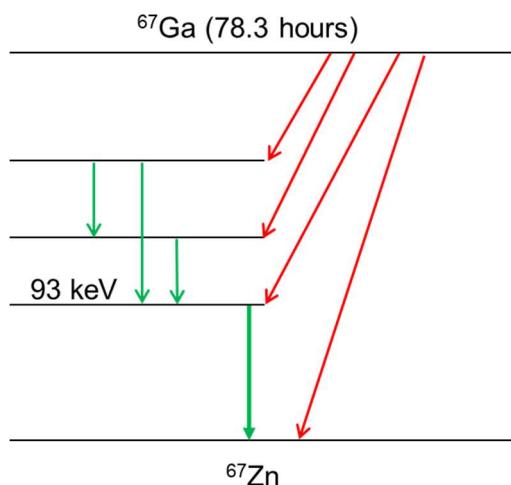


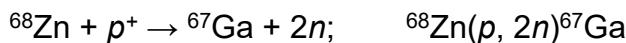
Figure 309: Decay diagram of  $^{67}\text{Ga}$



The half-life of  $^{67}\text{Ga}$  is 3.26 days (78.3 hours), which is quite convenient. It decays into  $^{67}\text{Zn}$ . Because of the long half-life it is used in those application where pharmacokinetics are slow, however it is rarely used, although its potential used is much larger than it is currently used.

## Production of $^{67}\text{Ga}$

$^{67}\text{Ga}$  is produced using cyclotrons by bombarding a solid target made of zinc-68 ( $^{68}\text{Zn}$ ) with proton of ideal energy around 11 MeV:



The target is isotopically enriched  $^{68}\text{Zn}$  metal plated onto copper holder. After the irradiation, the target is dissolved in concentrated hydrochloric acid (HCl) and the separation of  $^{67}\text{Ga}$  from  $^{68}\text{Zn}$  is achieved either by ion exchange column or by solvent/solvent extraction method using di-isopropyl-ether as solvent.

## Nuclear properties of $^{68}\text{Ga}$

Gallium-68 ( $^{68}\text{Ga}$ ), on the other hand decays mainly (88%, see Figure 310) by  $\beta^+$  decay, hence emits positrons and is used in PET imaging. These positrons are unfortunately much more energetic than those from  $^{18}\text{F}$  or  $^{11}\text{C}$  with mean energy of 890 keV, and the maximal energy of 1920 keV. This means low resolution of PET images. The half-life is 67.71 minutes which is a bit more than half of  $^{18}\text{F}$ . It decays into a stable  $^{68}\text{Zn}$ . As for now,  $^{68}\text{Ga}$  is not very used but its potential is significant and it is becoming more and more important. Some are saying that it has potential to replace  $^{99\text{m}}\text{Tc}$ . Another similarity with  $^{99\text{m}}\text{Tc}$  is that is obtained by radionuclide generator using its parent, germanium-68 ( $^{68}\text{Ge}$ ).

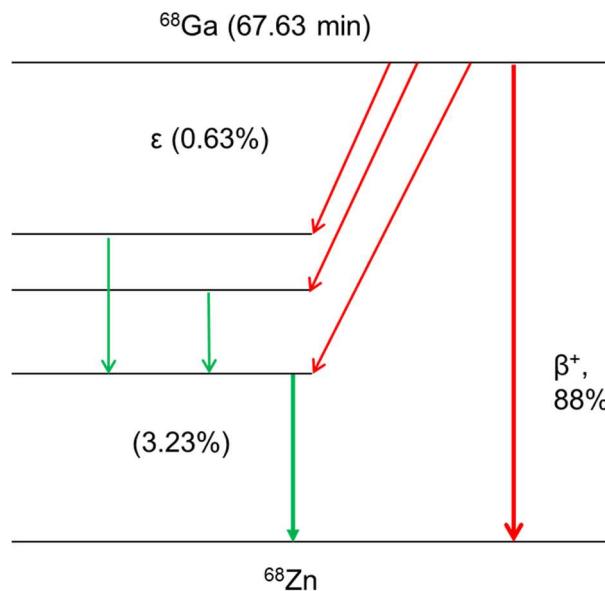


Figure 310: Decay diagram of  $^{68}\text{Ga}$

## Production of $^{68}\text{Ga}$ ( $^{68}\text{Ge}$ )

$^{68}\text{Ga}$  is produced from its parent  $^{68}\text{Ge}$  by using  $^{68}\text{Ge}/^{68}\text{Ga}$  radionuclide generator (as similar to  $^{99\text{m}}\text{Tc}$ ).  $^{68}\text{Ge}$  decays by electron capture and is emitting only very weak gammas. Its half-life is 271 days (6504 hours):



$^{68}\text{Ge}$  can be produced by irradiation of natural gallium with highly energetic protons:



This nuclear reaction require protons of 23 MeV which is quite high: powerful cyclotrons are needed to achieve this particle acceleration and also high proton currents to ensure good yield of activity. After the irradiation the target is dissolved in hydrochloric acid (HCl) and the separation of  $^{68}\text{Ge}$  from  $^{65}\text{Zn}$  and gallium is based on the extraction of the germanium chloride ( $^{68}\text{GeCl}_4$ ) into organic solvents such as toluene, carbon tetrachloride and benzene.  $^{68}\text{Ge}$  is then back-extracted twice from the organic phase into small volumes of diluted hydrochloric acid.

## $^{68}\text{Ge}/^{68}\text{Ga}$ Radionuclide generators

$^{68}\text{Ge}/^{68}\text{Ga}$  generator works in very similar principle as  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ , however it differs in the lifetime of use, dynamics of daughter generation and milking, as well as and chemistry of adsorption and elution. The half-life of  $^{68}\text{Ge}$  is much longer than for  $^{99}\text{Mo}$ , it is 271 days or more than 6500 hours, while the half-life of  $^{68}\text{Ga}$  is 1.13 hour. With such huge difference the two radionuclides are in secular equilibrium (in difference to transient equilibrium of  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  system).

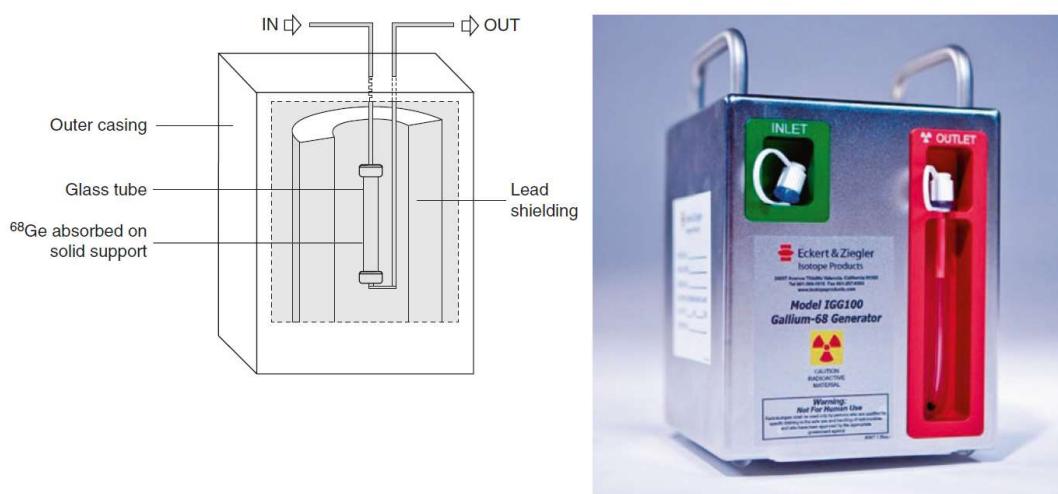


Figure 311:  $^{68}\text{Ge}/^{68}\text{Ga}$  radionuclide generator, scheme inside (left) and the actual appearance (right)

This means that  $^{68}\text{Ge}/^{68}\text{Ga}$  generator lasts much longer than  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator and is made much more robust. Inside the generator is a tube with  $^{68}\text{Ge}$ , which is absorbed into titanium-dioxide or tin-dioxide powder. In some modern generators



special composite material is used that consist of silica gel modified with dodecyl 3,4,5-trihydroxybenzoate. Elution of  $^{68}\text{Ga}^{3+}$  ions is achieved by using HCl solution.

Due to the favourable decay dynamics the milking of this “German cow” (this is the nickname of this radionuclide generator: as the one for  $^{99\text{m}}\text{Tc}$  is called “Moly cow” since it contains molybdenum, Moly is also a name for a girl in English language) can be done several times per day. The main drawback of these  $^{68}\text{Ge}/^{68}\text{Ga}$  radionuclide generators is that eluted  $^{68}\text{Ga}^{3+}$  usually is not pure, but there are also impurities of titanium (Ti), zinc (Zn) and germanium (Ge). In other words, radionuclidic purity is not perfect. However, this problem can be addressed and the purity improved by ion-exchange purification methods.

## Isotopes of indium

Indium has 41 isotopes, and only  $^{113}\text{In}$  is stable. However,  $^{113}\text{In}$  makes only 4.3% of all natural indium. The rest is an extremely weakly radioactive and natural (primordial)  $^{115}\text{In}$  with the half-life of  $4.41 \times 10^{14}$  years. This means that all natural indium metal is in fact very slightly radioactive (this is similar to natural bismuth,  $^{209}\text{Bi}$  that is also radioactive, but that one is the most stable form of bismuth). Isotopes  $^{112}\text{In}$  and  $^{114}\text{In}$ , interestingly, decay by both  $\beta^-$  and  $\beta^+$  decays. Isotope  $^{111}\text{In}$  decays by electron capture, while the isotopes from  $^{110}\text{In}$  downwards decay by positron emission ( $\beta^+$  decay). Nevertheless, due to favourable half-life and gamma emission only  $^{111}\text{In}$  is used in nuclear medicine.

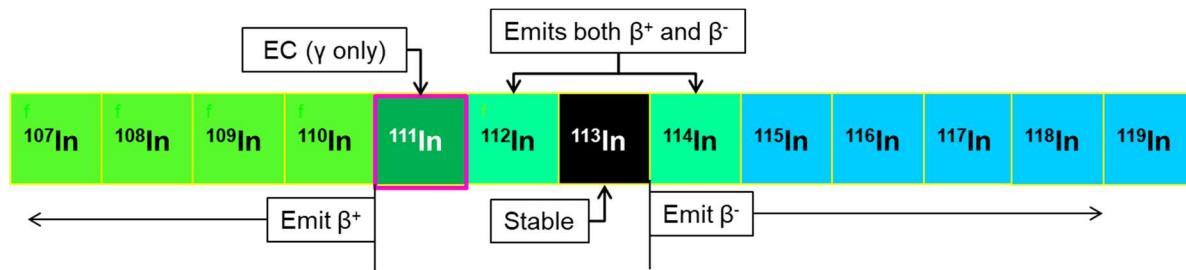


Figure 312: Isotopes of indium

## Nuclear properties of $^{111}\text{In}$

Indium-111 ( $^{111}\text{In}$ ) decays by electron capture (EC,  $\epsilon$ ) and is emitting only gamma rays. There are two prominent gamma rays, one at 171.3 keV and another at 245.4 keV (total of 405 keV). Half-life of  $^{111}\text{In}$  is 2.80 days (67.31 hours) which is quite convenient and therefore  $^{111}\text{In}$  can be used in applications where pharmacokinetics are slow. The decay product is  $^{111}\text{Cd}$ . It is used in form of complexes for the SPECT imaging.

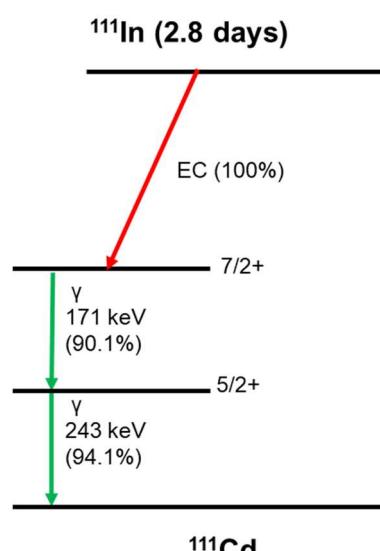


Figure 313: Decay diagram of  $^{111}\text{In}$

## Production of $^{111}\text{In}$

$^{111}\text{In}$  can be produced in cyclotrons by bombarding solid cadmium target with protons:

- Option 1:  $^{111}\text{Cd} + p^+ \rightarrow ^{111}\text{In} + n$
- Option 2:  $^{112}\text{Cd} + p^+ \rightarrow ^{111}\text{In} + 2n$

In the first option target is an  $^{111}\text{Cd}$  and in the other  $^{112}\text{Cd}$ . For this purpose natural or isotopically enriched solid cadmium metal plated onto solid support can be used, while the protons need to be accelerated to 20 MeV. This can be achieved in more powerful cyclotrons.

After irradiation, target is dissolved in hydrobromic acid (HBr) and can be purified by either ion-exchange chromatography or solvent/solvent extraction with diisopropyl-ether.

## Chemical and biological properties of gallium and indium

Both gallium and indium are so called “p-block” metals, both neighbours from the 13<sup>th</sup> group; they are in the same group as the light metal aluminium and also metalloid boron (Figure 314). Gallium and indium are chemically very similar although there are some differences. Both metals form 3+ ions,  $\text{Ga}^{3+}$  and  $\text{In}^{3+}$ .

1 H	2 He																
3 Li	4 Be	5 B	6 C	7 N	8 O	9 F	10 Ne										
11 Na	12 Mg	13 Al	14 Si	15 P	16 S	17 Cl	18 Ar										
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og
57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb				
89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No				

Figure 314: Position of gallium and indium in the Periodic table of elements

Gallium and indium have fully filled so called *d*-orbitals, and hence do not behave as the *d*-block metals (Fe, Tc, Cu, Ni...); they exhibit no ligand field or crystal field stabilization energies, and their coordination geometries are governed by the steric requirements of their ligands.  $\text{Ga}^{3+}$  and  $\text{In}^{3+}$  are both relatively “hard” Lewis acid metal ions, with high charge density and a preference for oxygen and nitrogen donor atoms. Their bonding is highly ionic, requires well-designed chelating ligands to

achieve adequate coordination stability.  $\text{Ga}^{3+}$  ion radius is significantly smaller than the one of  $\text{In}^{3+}$ . Therefore,  $\text{Ga}^{3+}$  forms complexes with up to six donor atoms and frequently adopts octahedral geometries similar to  $\text{Fe}^{3+}$ , while  $\text{In}^{3+}$  forms stable complexes with up to nine donor atoms in its coordinating sphere. Even in the weak acidic media  $\text{Ga}^{3+}$  and  $\text{In}^{3+}$  like to form insoluble hydroxides,  $\text{Ga}(\text{OH})_3$  and  $\text{In}(\text{OH})_3$ . Therefore, chelating agents need to be added to prevent this precipitation. Stabilizing ligands such as citrate, acetate and oxalate are often used.

Due to its coordination similarities with iron(III) the free  $\text{Ga}^{3+}$  ion behaves similar as iron in human body. Gallium in serum is primarily bound to so-called transferrin protein. It is a protein that normally binds and carries iron in the blood. Imaging studies using  $^{67}\text{Ga}$  show the accumulation of  $^{67}\text{Ga}^{3+}$  in the bone, liver, spleen, kidneys, intestine, but especially in tumours, particularly lymphoma cells. This phenomenon is used for the imaging of tumours and lymphomas. However, the uptake of  $^{67}\text{Ga}^{3+}$  into cancer cells is slow, but  $^{67}\text{Ga}$  has long half-life.  $^{67}\text{Ga}$  was also found to accumulate in sites of infection and inflammation, an observation that has been exploited for the SPECT imaging of infections and inflammations. Therefore,  $^{67}\text{Ga}^{3+}$  in the form of citrate is used as radiopharmaceutical agent for the SPECT imaging of lymphoma cells, inflammation and infection, especially hidden inflammation.

### Radiolabeling with $^{68}\text{Ga}$

At the first radiopharmaceutical chemists **where** trying to emulate the success of  $^{99\text{m}}\text{Tc}$ , so that  $^{68}\text{Ga}^{3+}$  becomes PET version of  $^{99\text{m}}\text{Tc}$ , however, the development of  $^{68}\text{Ga}$  radiopharmaceuticals took its own, more sophisticated way. In fact, the development of  $^{68}\text{Ga}$  radiopharmaceuticals is still on going, and very few radiopharmaceutical agents are in use. However, it is foreseen that  $^{68}\text{Ga}$  will become much more important radionuclide in nuclear medicine. The main concept of  $^{68}\text{Ga}$  radiopharmaceuticals is the use of modern sophisticated bifunctional chelating agents, bioconjugated with biomolecule that serve as vectors with molecular recognition (Figure 315).

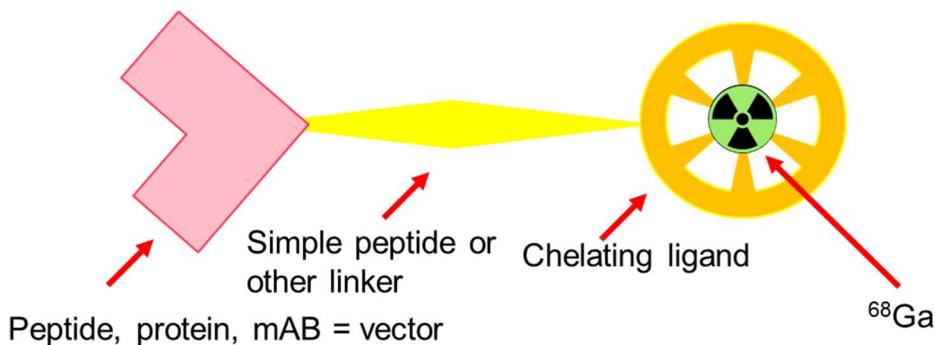


Figure 315: All  $^{68}\text{Ga}$  radiopharmaceuticals are all bioconjugates with proteins or peptides and all require some ligand to keep them well caged.

## Ligands for the complexation of $^{68}\text{Ga}^{3+}$

Ligands for  $^{68}\text{Ga}^{3+}$  need to be fit for  $\text{Ga}^{3+}$ , with high stability and rapid complexation kinetics.  $\text{Ga}^{3+}$  usually gets coordinated with 6 donors, just like transition metals such as Fe. The ligands for  $\text{Ga}^{3+}$  can be macrocyclic (form a closed ring, Figure 316), acyclic (not forming a ring, but rather open chain, Figure 317) or bifunctional (having a peptide/protein attached via a linker). Some of the macrocyclic ligands are DOTA, NOTA, or NODAGA which contain nitrogen or oxygen as electron-donors or macrocycles with phosphorus-containing side chains such as NOTP and TRAP.

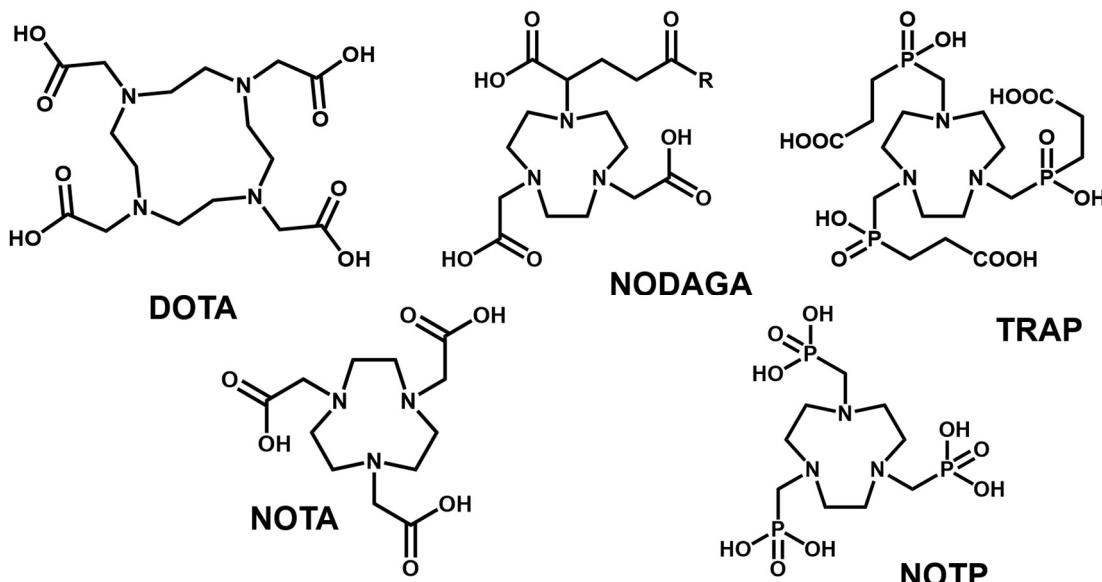


Figure 316: Macro cyclic ligands for  $^{68}\text{Ga}^{3+}$  ions

Acyclic ligands are opened long and large such as HBED, DFO, THP and DATA that bend around the metallic ion forming loops.

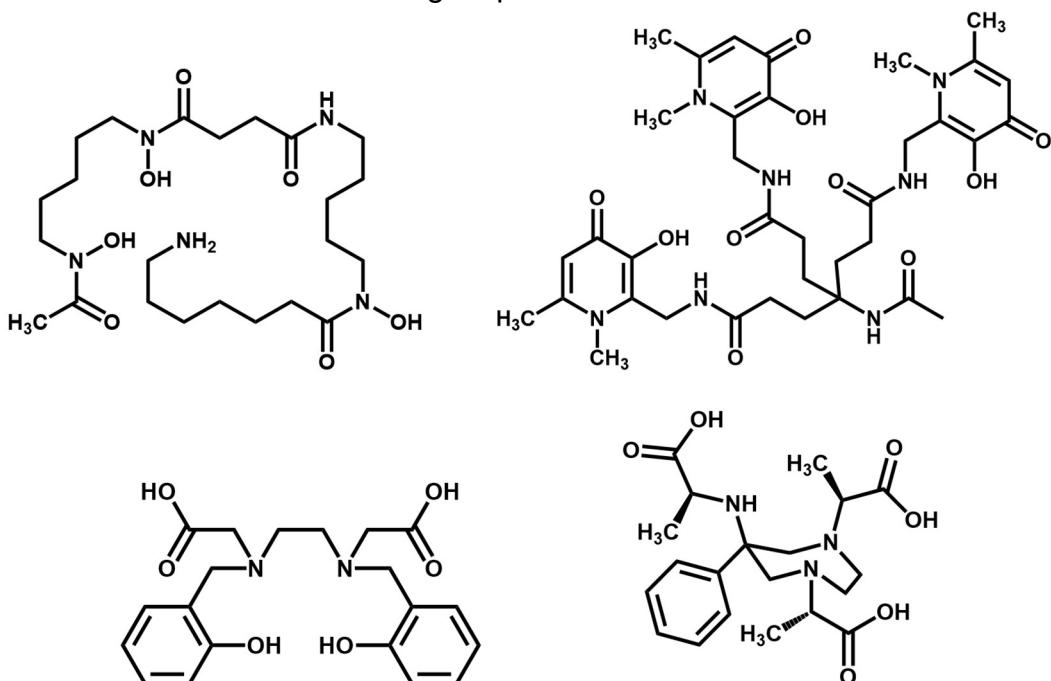


Figure 317: Acyclic ligands for  $^{68}\text{Ga}^{3+}$  ions

## Radiopharmaceuticals of $^{68}\text{Ga}$

All radiopharmaceuticals of  $^{68}\text{Ga}$  share the same feature of bioconjugates and are considered to be sophisticated. They are still evolving and improving and it is expected that these radiopharmaceuticals will become more important. Here some of them will be presented.

### $^{68}\text{Ga}$ -DOTA-Octreotide ( $^{68}\text{Ga}$ -DOTA-TOC)

$^{68}\text{Ga}$ -DOTA-Octreotide ( $^{68}\text{Ga}$ -DOTA-TOC) is an advanced, complex radiopharmaceutical agent where  $^{68}\text{Ga}$  is complexed by DOTA macrocyclic ligand and the whole complex is linked onto a cyclic peptide called octreotide that contains eight amino acids and is cyclised via disulfide ( $-\text{S}-\text{S}-$ ) bond by its two cysteine amino acids. This peptide is an analogue of natural hormone called somatostatin and can bind to somatostatin receptors very well. It is used for PET imaging of neuroendocrine tumours or lung cancer in which somatostatin receptors are overexpressed or overactive.

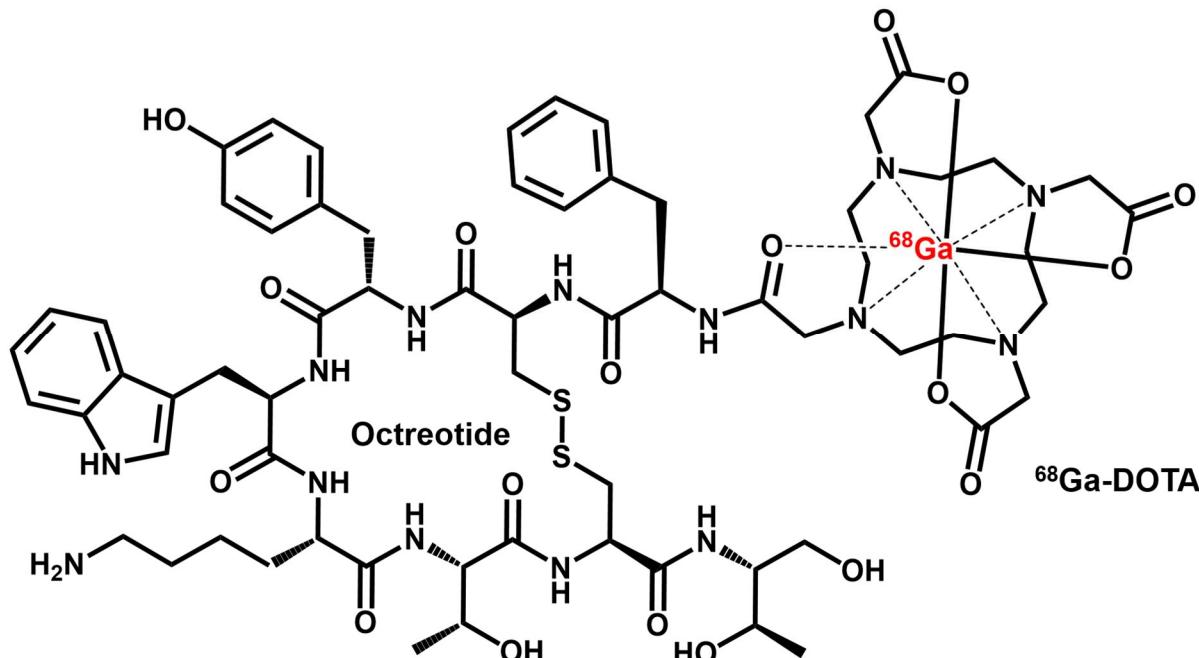


Figure 318: Structure of  $^{68}\text{Ga}$ -DOTA-Octreotide

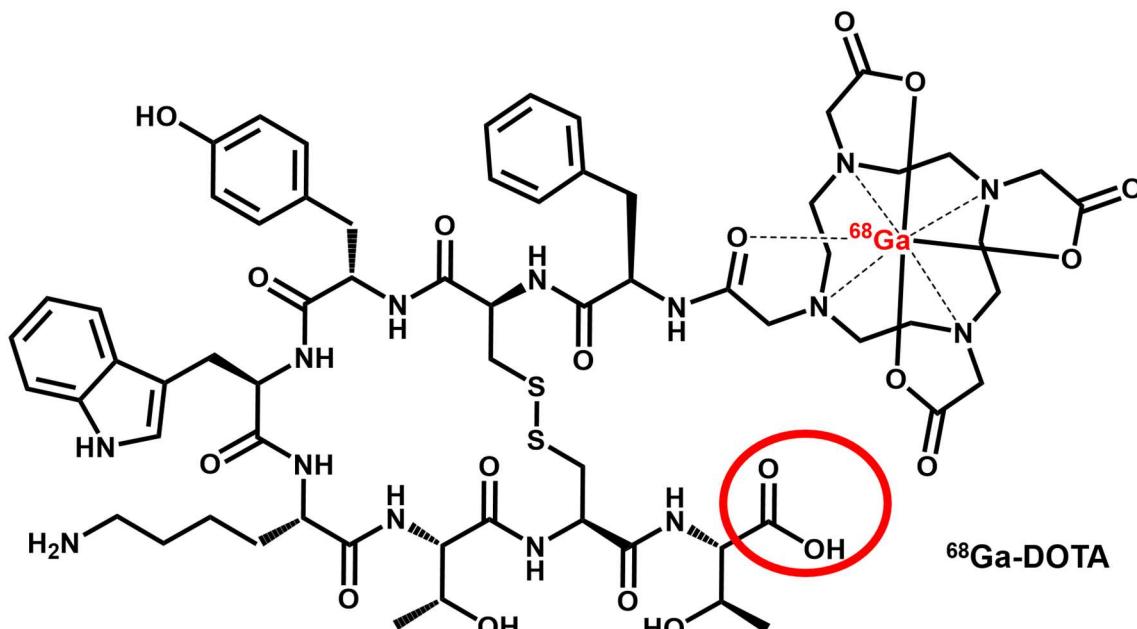
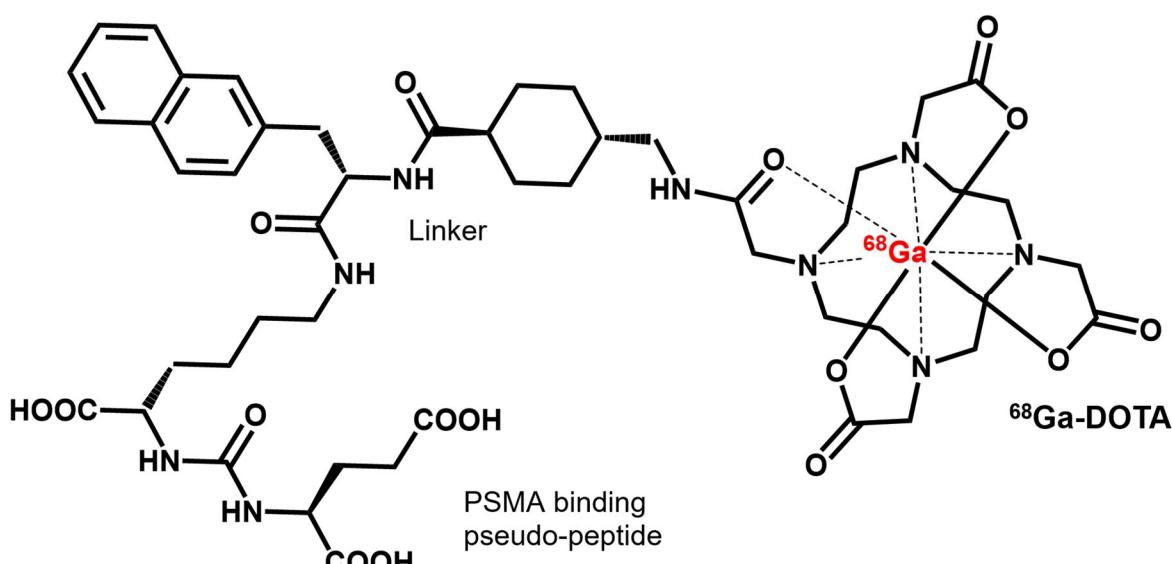
### $^{68}\text{Ga}$ -DOTA-Octreotate ( $^{68}\text{Ga}$ -DOTA-TATE)

Another variant of the same radiopharmaceutical is  $^{68}\text{Ga}$ -DOTA-Octreotate or  $^{68}\text{Ga}$ -DOTA-TATE. The only tiny difference is additional carbonyl group on the octreotide (see figure 319, encircled with red circle), so it is not octreotide, but oxidised form, octreotate.

### $^{68}\text{Ga}$ -DOTA-PSMA 617

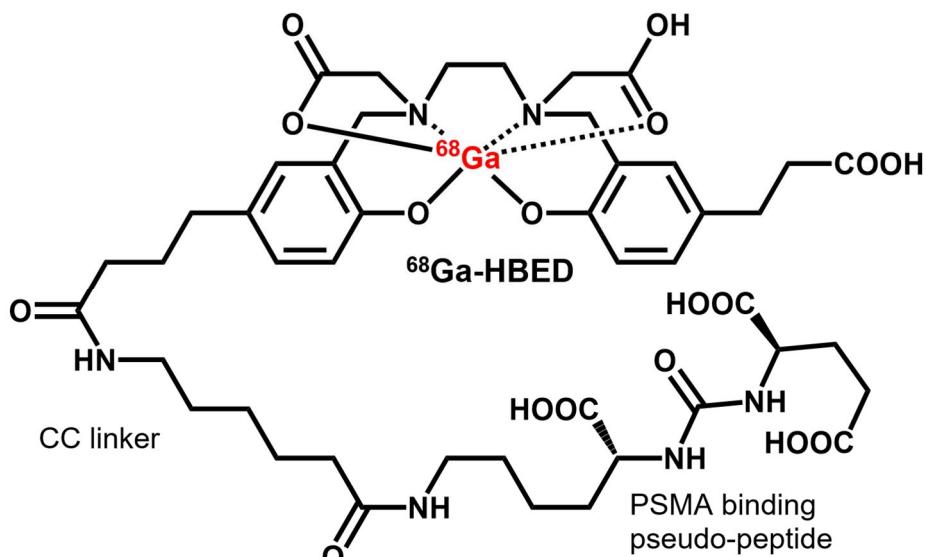
Another important advanced radiopharmaceutical agent with  $^{68}\text{Ga}$  is  $^{68}\text{Ga}$ -DOTA-PSMA 617 (Figure 320). Here  $^{68}\text{Ga}^{3+}$  ion is also chelated by DOTA chelating ligand

and the Ga-DOTA complex is attached, *via* a linker, onto a small pseudo-peptide that binds so-called prostate-specific membrane antigen (PSMA), hence specifically targeting prostate cancer cells. This radiopharmaceutical agent is used for the PET imaging of prostate cancer cells.

Figure 319: Structure of  $^{68}\text{Ga}$ -DOTA-TATEFigure 320: Structure of  $^{68}\text{Ga}$ -DOTA-PSMA 617

### $^{68}\text{Ga}$ -HBED-CC-PSMA

$^{68}\text{Ga}$ -HBED-CC-PSMA is another where  $^{68}\text{Ga}^{3+}$  ion is chelated by HBED acyclic chelating ligand. The complex is linked (via a CC linker) with PSMA-binding pseudo peptide that binds PSMA on the prostate cancer cells. This radiopharmaceutical agent is also used for the imaging of prostate cancer.

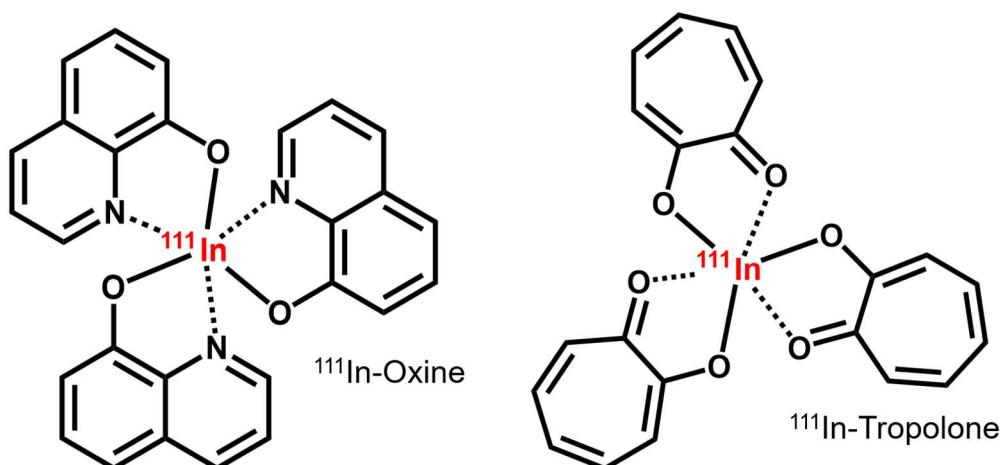
Figure 321: Structure of  $^{68}\text{Ga}$ - HBED-CC-PSMA

### Radiolabelling with $^{111}\text{In}$

As was the case with gallium isotopes, at first it was expected that radiopharmaceuticals of  $^{111}\text{In}$  will be developed equivalently to  $^{99\text{m}}\text{Tc}$ . However this has not happened, only few radiopharmaceuticals were developed that resemble  $^{99\text{m}}\text{Tc}$  complexes while more radiopharmaceuticals are being developed by using concept of complexation with protein-linker-chelate bioconjugate. However,  $^{111}\text{In}$  became important in one unique area and that is cell tracking of white blood cells.

#### Cell tracking with $^{111}\text{In}$

For quite long time the main application of  $^{111}\text{In}$  in the nuclear medicine was for the radiolabelling of white blood cells and platelets. For example,  $^{111}\text{In}$ -oxine complex of  $^{111}\text{In}^{3+}$  with three 8-hydroxyquinolinate ligands became commercially available even in the 1980s. This complex is neutral and very lipophilic and is able to cross cell membrane.

Figure 322: Radiopharmaceutical complexes with  $^{111}\text{In}$  used for cell tracking

Once it is inside of cell  $^{111}\text{In}^{3+}$  leaves the complex and binds some cell structures, therefore stays trapped in cells. Another similar complex with the same application is  $^{111}\text{In}$ -tropolone. These radiopharmaceuticals are used for the tracking of white blood cells by SPECT: this tracking of immune cells can help physicians to detect hidden inflammation sites in the patient's body.

### Ligands for the complexation of $^{111}\text{In}$

$\text{In}^{3+}$  is an ion that is larger than  $\text{Ga}^{3+}$  and therefore requires larger, more complex ligands with more O and N donors. The main ligand for  $^{111}\text{In}^{3+}$  is a branched molecule called pentetic acid or diethylene-triamine-pentaacetic acid (DTPA). It is in fact a larger version of the famous EDTA complexing agent. Another two ligands for  $^{111}\text{In}^{3+}$  are actually just derivatives of DTPA: 1B3M-DTPA that was later got a name "Tiuxetan" and CHX-A-DTPA. Both ligands are made with *para*-SCN-phenyl side functional group that has ability to quickly react with any protein and peptide achieving instant bioconjugation (see Figure 323, encircled red).

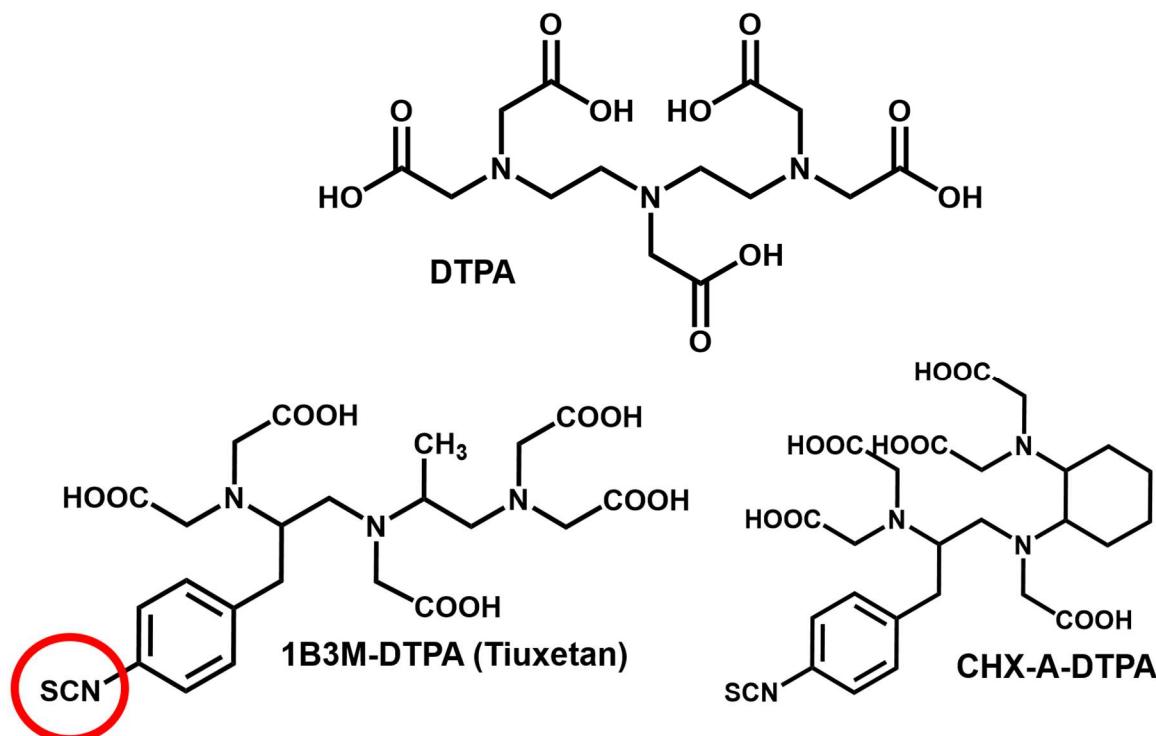


Figure 323: Ligands for  $^{111}\text{In}^{3+}$  ions

### Radiopharmaceuticals of $^{111}\text{In}$

Number of radiopharmaceuticals in clinical practice that contain  $^{111}\text{In}$  is, unfortunately not so numerous. Here three of them will be presented.

#### $^{111}\text{In}$ -Octreotide

Widely used radiopharmaceutical agent that contains  $^{111}\text{In}$  is  $^{111}\text{In}$ -Octreotide. It is an agent where  $^{111}\text{In}$  is complexed by DTPA and the complex is linked onto octreotide (Figure 324). This agent is basically very similar to the  $^{68}\text{Ga}$  agents seen before;

however, this  $^{111}\text{In}$  version was developed much earlier than the  $^{68}\text{Ga}$  version. Just like the  $^{68}\text{Ga}$  version it is used for the imaging of neuroendocrine tumours, but using SPECT instead of PET. Nevertheless, the  $^{68}\text{Ga}$  variant proved in the clinical practice to be much better and superior agent for imaging comparing to this  $^{111}\text{In}$  version.

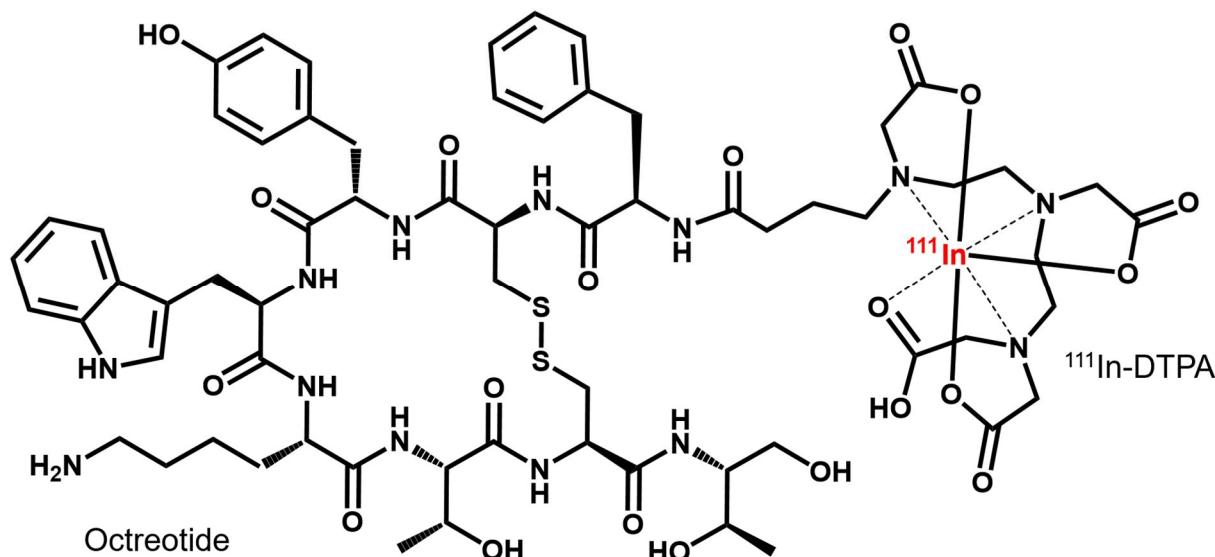


Figure 324: Structure of  $^{111}\text{In}$ -Octreotide

### $^{111}\text{In}$ -Capromab pendetide

$^{111}\text{In}$ -capromab pendetide is an  $^{111}\text{In}$  containing radioimmunoconjugate agent used for the SPECT imaging of prostate cancer (Figure 325). Here pendetide is the DTPA chelating agent with a tripeptide linker. It acts as a chelating agent for  $^{111}\text{In}$ , and the whole complex is attached onto capromab, a mouse monoclonal antibody which recognizes a protein found on both prostate cancer cells and normal prostate tissue.

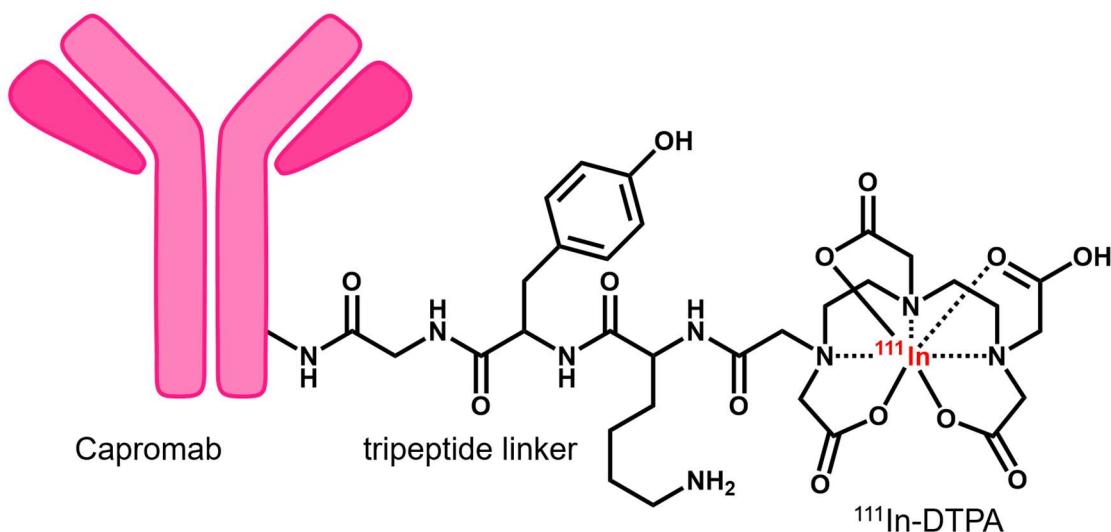


Figure 325: Structure of  $^{111}\text{In}$ -Capromab pendetide

### **$^{111}\text{In}$ - Ibritumomab tiuxetan**

$^{111}\text{In}$  is also used as an imaging radionuclide in ibritumomab tiuxetan radioimmunoconjugate (Figure 326). Tiuxetan is a derivative of DTPA ligand: it can complex either  $^{90}\text{Y}$  or  $^{111}\text{In}$ .  $^{90}\text{Y}$  is used for the  $\beta^-$ -particle radiotherapy of non-Hodgkin B-lymphocyte lymphoma, while  $^{111}\text{In}$  is used for the SPECT imaging of lymph nodes infested with those cancerous cells. It is attached to ibritumomab, a monoclonal antibody for the specific targeting of CD20 antigen on B-lymphocyte lymphoma cells.  $^{111}\text{In}$ -ibritumomab tiuxetan is used for the imaging, while  $^{90}\text{Y}$ -Ibritumomab tiuxetan is used for the radiotherapy: in the clinical practice these are used in tandem.

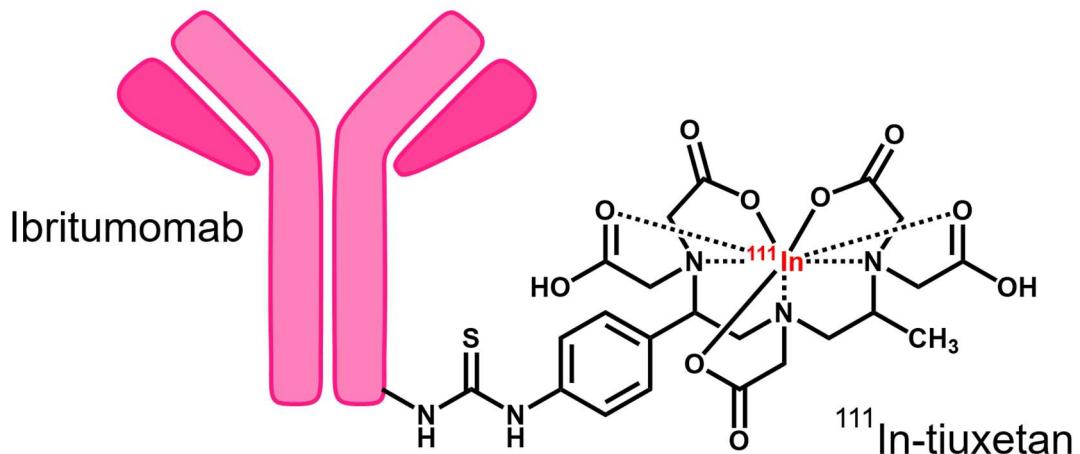


Figure 326: Structure of  $^{111}\text{In}$ -Ibritumomab-tiuxetan

## Chapter XIII - Copper and Zirconium

Copper and zirconium are both transitional metals (“d-block”) elements. Their medical radioisotopes are  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$  and  $^{89}\text{Zr}$ , however they are so rarely used that we should count them mostly as experimental and developmental radionuclides whose time is yet to come.

### Isotopes of copper

Famous metal copper has total of 29 isotopes. Two,  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  are stable and natural. The isotope between them,  $^{64}\text{Cu}$  is very unique among medical radionuclides: it decays by electron capture (44%),  $\beta^+$  emission (17%), but also by  $\beta^-$  emission (39%). Therefore, this radionuclide emits in the same time  $\beta^+$ ,  $\beta^-$  and  $\gamma$ . It is the main copper medical radioisotope.  $^{67}\text{Cu}$  is, on the other hand, a pure beta emitter with a reasonably long half-life (62 hours) and could be used in targeted radiotherapy (TRT).  $^{62}\text{Cu}$  decays by positron emission and has half-life of 9.7 min: it could be used in PET imaging but is very rarely used. There are other two radioisotopes that decay by positron emission and are very rarely used in PET imaging, in fact these are mostly used in scientific research. One is  $^{61}\text{Cu}$  with its half-life of 3.33 hours and another is  $^{60}\text{Cu}$  with the half-life of 23.7 minutes. However, the most important copper radioisotopes for nuclear medicine are  $^{64}\text{Cu}$  and  $^{67}\text{Cu}$ . Nevertheless, comparing to  $^{11}\text{C}$  and  $^{18}\text{F}$ , these copper isotopes are in fact very rarely used and could be considered as exotic medical radionuclides.

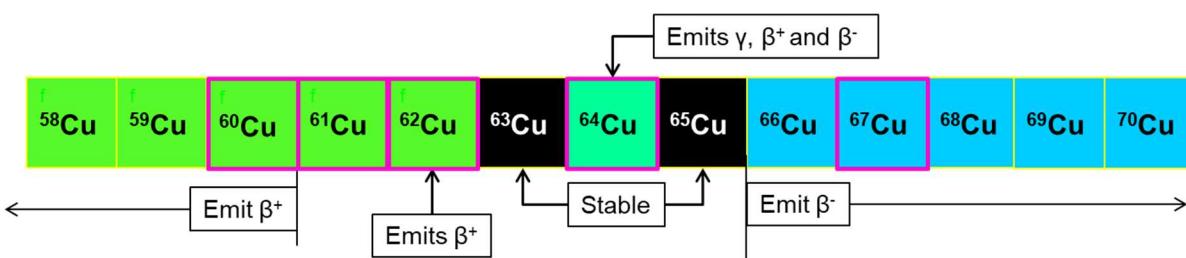


Figure 327: Isotopes of copper

### Nuclear properties and production of $^{67}\text{Cu}$

Copper-67 ( $^{67}\text{Cu}$ ) has half-life of 61.8 hours which is quite long. It decays into by beta-decay, therefore emitting  $\beta^-$  particles of 484 keV and 395 keV. It also emits also some gamma rays (115 keV) which are appropriate for SPECT imaging. Its nuclear properties make it nearly ideal for the targeted radiotherapy (TRT), but it can also be used in the SPECT imaging. For example  $^{67}\text{Cu}$ -citrate was used for the imaging of patients with lung cancers or tuberculosis using SPECT imaging.

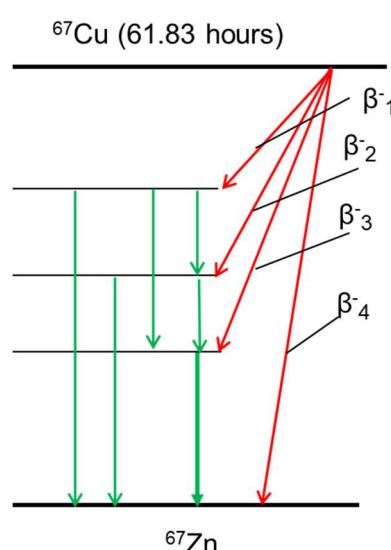
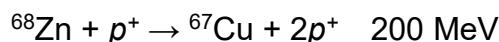


Figure 328: Decay diagram of  $^{67}\text{Cu}$

$^{67}\text{Cu}$  can be made by bombarding  $^{68}\text{Zn}$  in the form of enriched zinc metal or zinc oxide targets with 200 MeV protons:



Due to the very large energy of protons required for this nuclear transformation it is achieved using large powerful linear accelerators (LINAC), not cyclotrons. Therefore, this production needs to be done in special scientific organisations that have such powerful LINAC, and not many of them exist in the world. This is the biggest limitation of this radionuclide. In addition, the product obtained is a mixture of radionuclides ( $^{61}\text{Cu}$ ,  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ , Co, Zn, Ni...). Non-copper radionuclides (Co, Zn, Ni) are removed using ion exchange chromatography, while  $^{61}\text{Cu}$  and  $^{64}\text{Cu}$  decay quickly leaving only  $^{67}\text{Cu}$ .

### Nuclear properties and production of $^{64}\text{Cu}$

Copper-64 ( $^{64}\text{Cu}$ ) is a unique radionuclide among the medical radionuclides since it decays by electron capture (44%), positron emission (17%) and  $\beta^-$  emission (39%). It has half-life of 12.7 hours. When it comes to energy of the particles, the positrons have pretty good energies for PET imaging, 278 keV in average and the maximal is 653 keV. The beta-particles are a bit less energetic, 190 keV in average and maximal 578 keV. Its decay products depend on the decay mode: when decaying by electron capture or positron emission then it decays into  $^{64}\text{Ni}$ . If decay mode is by the beta-decay then the daughter is  $^{64}\text{Zn}$ . Also there are some gamma emitted, but these are weak. This radionuclide is mostly used in PET imaging, but could be also used in targeted radiotherapy.

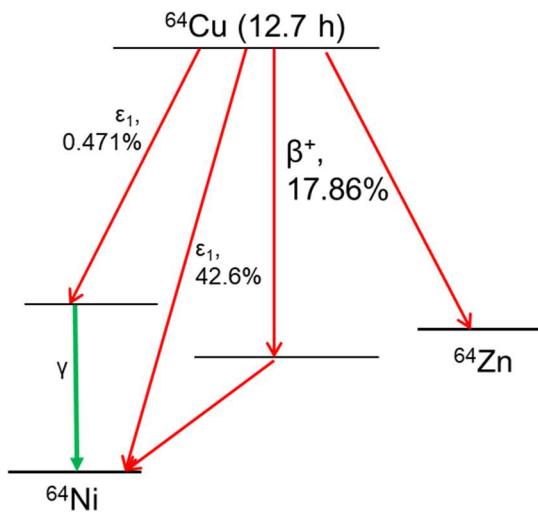
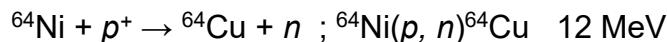


Figure 329: Decay diagram of  $^{64}\text{Cu}$

$^{64}\text{Cu}$  is made by bombarding highly enriched  $^{64}\text{Ni}$  target with protons of 12 MeV:



Since protons do not have to be very energetic, a medium power medical cyclotron can be used for this transformation. Usually, by using this method very high specific

activities can be produced (370 TBq/mmol) and radionuclidic purity of the obtained product is excellent (99%): after irradiation the solid targets are dissolved in HCl and  $^{64}\text{Cu}$  is isolated by using ion exchange chromatography. Generally, production of  $^{64}\text{Cu}$  is much more convenient and easy than production of  $^{67}\text{Cu}$ !

## Coordination and chelation chemistry of Cu

Copper, is a common and quite ubiquitous metal around us: chemically it is an important transition metal with very rich coordination chemistry. It usually forms  $\text{Cu}^+$  and  $\text{Cu}^{2+}$  ions, and tends to accept electrons from various electron donors, such as oxygen, nitrogen, phosphorus or sulphur. It forms stable complexes with simple electron donors, for example with water, ammonia, cyanide ion, pyridine and many other simple ligands. However, the complex stability is better with bidentate ligands such as ethylene-diamine than with monodentate. More donors mean more stability: polydentate ligands (chelators) make the most stable complexes.

1	H
3	Li
4	Be
11	Na
12	Mg
19	K
20	Ca
21	Sc
22	Ti
23	V
24	Cr
25	Mn
26	Fe
27	Co
28	Ni
29	Cu
30	Zn
31	Ga
32	Ge
33	N
34	O
35	F
36	Ne
5	B
6	C
7	Si
8	P
9	S
10	Cl
13	Al
14	Si
15	As
16	Se
17	Br
18	Ar
37	Rb
38	Sr
39	Y
40	Zr
41	Nb
42	Mo
43	Tc
44	Ru
45	Rh
46	Pd
47	Ag
48	Cd
49	In
50	Sn
51	Sb
52	Te
53	I
54	Xe
55	Cs
56	Ba
71	Lu
72	Hf
73	Ta
74	W
75	Re
76	Os
77	Ir
78	Pt
79	Au
80	Hg
81	Tl
82	Pb
83	Bi
84	Po
85	At
86	Rn
87	Fr
88	Ra
103	Lr
104	Rf
105	Db
106	Sg
107	Bh
108	Hs
109	Mt
110	Ds
111	Rg
112	Cn
113	Nh
114	Fl
115	Mc
116	Lv
117	Ts
118	Og
57	La
58	Ce
59	Pr
60	Nd
61	Pm
62	Sm
63	Eu
64	Gd
65	Tb
66	Dy
67	Ho
68	Er
69	Tm
70	Yb
89	Ac
90	Th
91	Pa
92	U
93	Np
94	Pu
95	Am
96	Cm
97	Bk
98	Cf
99	Es
100	Fm
101	Md
102	No

Figure 330: Position of copper in the Periodic table of elements

## Copper-bis-thiosemicarbazones (BTS)

All radiopharmaceutical agents of copper are complexes with some polydentate ligands. The most used are those simple acyclic, tetradentate (with four donor atoms), called bis-thiosemicarbazones or BTS ligands. These ligands make quite stable complexes with  $^{64}\text{Cu}$  and are used for the PET imaging of various tumours and other tissues: precisely, these are used for PET imaging of hypoxia in tumour cells. This can help physicians to reveal the grade of tumour and how aggressive they are and how well it can be cured. Also some of these can be used for the PET imaging of heart blood perfusion. In some sense  $^{64}\text{Cu}$ -BTS complexes resemble  $^{99\text{m}}\text{Tc}$  complexes due to their pharmacology directed by their pharmacokinetics.

However, there is a problem with these simple acyclic ligands and that is as follows: once in the body Cu<sup>2+</sup> ion on this complex can be reduced into Cu<sup>1+</sup>, stability of the complex decreases and Cu can leave the complex. This is very unwanted situation and therefore better ligands are needed.

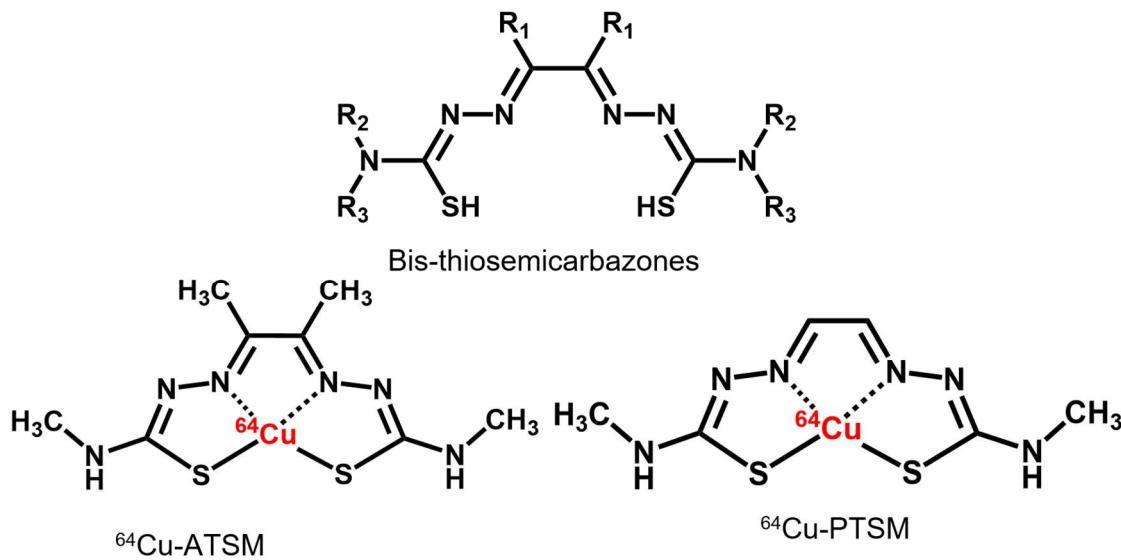


Figure 331: General structure of bis-thiosemicarbazones (up), and two complexes with <sup>64</sup>Cu (down)

### Macrocyclic chelator ligands for <sup>64</sup>Cu

Fortunately, copper is very good in making complexes and there are plenty of macrocyclic ligands that copper likes. Macrocyclic ligands are, in general, always more stable than acyclic and stability of complex inside the body is crucial: radionuclide should never “fall off” from the ligand while in the body being either Cu<sup>1+</sup> or Cu<sup>2+</sup>. Due to the macrocyclic effect, macrocyclic chelator ligands offer superb stability. There are several families of macrocyclic chelator ligands for Cu:

- Traditional macrocyclic chelator ligands (such as NOTA, DOTA, TETA),
- Sarcophagines
- Cross-bridged macrocyclic chelators.

### Traditional macrocyclic chelators

Classic macrocyclic ligands are NOTA, DOTA and TETA (Figure 332), all in fact derivatives of ethylene-diamine acetic acid elements, either trimeric like NOTA or tetrameric like DOTA. TETA is very similar to DOTA but has one more CH<sub>2</sub> residue in the monomer.

### Sarcophagines

Sarcophagines are more advanced and more complicated macrocyclic chelating ligands for copper. They resemble TETA ligands, but these have another branch across the ring. The copper ion moves inside of the cleft created between the branches and gets fully caged inside of this 3D ligand (Figure 333). The name

comes from a Latin word “sarcophagus” which is a large stone coffin used for the burial of ancient Egyptians and Romans. Hence, copper ion gets buried inside of this ligand. Such ligand is, for example, diamino-sarcophagine or DiamSar.

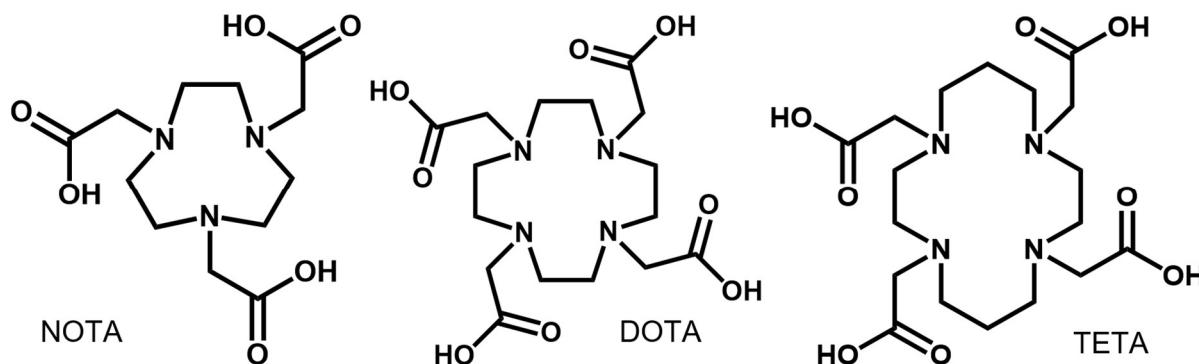


Figure 332: Traditional macrocyclic chelators, NOTA, DOTA and TETA

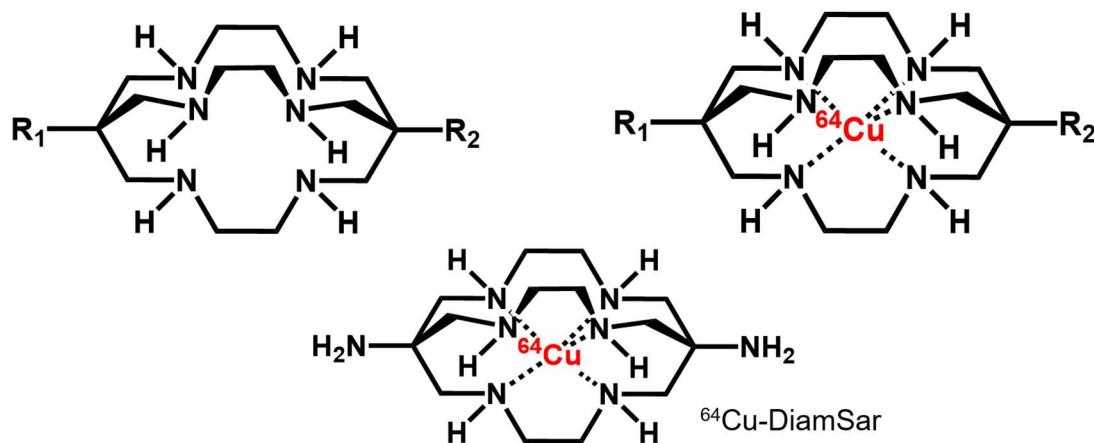


Figure 333: Sarcophagines: empty (up left), with  $^{64}\text{Cu}$  (up right) and DiamSar with  $^{64}\text{Cu}$  (down)

### Cross-bridged macrocyclic chelators

The last important group of macrocyclic ligands for  $^{64}\text{Cu}$  are so called cross bridged macrocyclic chelators (Figure 334). They are similar to TETA ligands but have one bridge across the macrocyclic cleft. Some of these actually have one carboxylic group and one phosphonate group. They bind copper ions (Figure 335) much more efficient: the molecule forms a pit where the copper ion sits and gets complexes. These complexes are very stable.

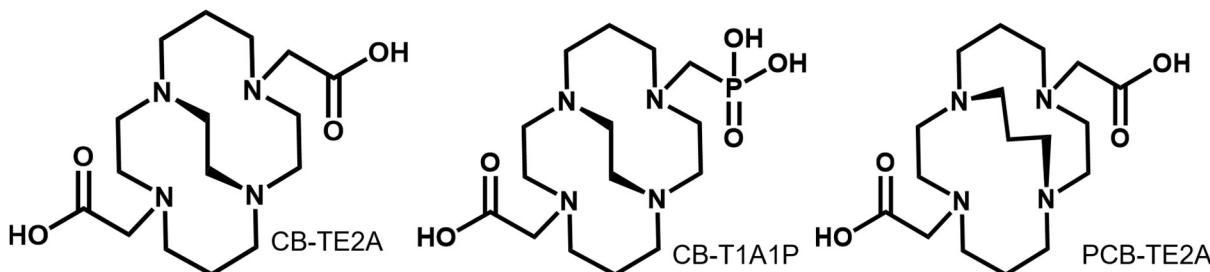
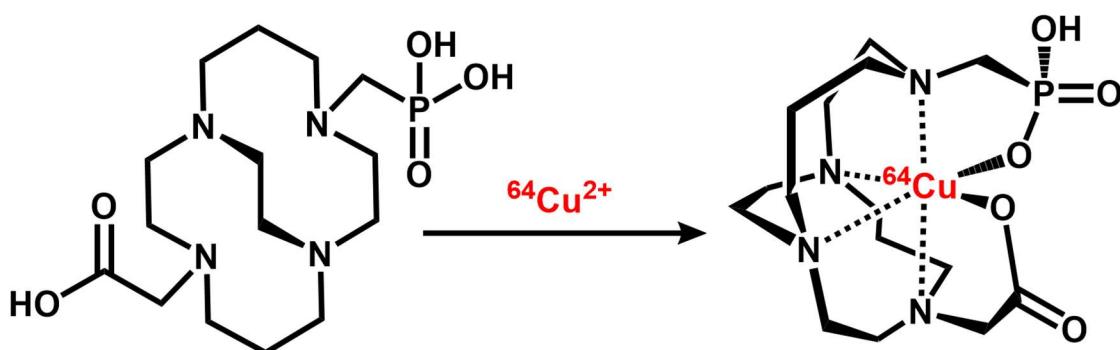
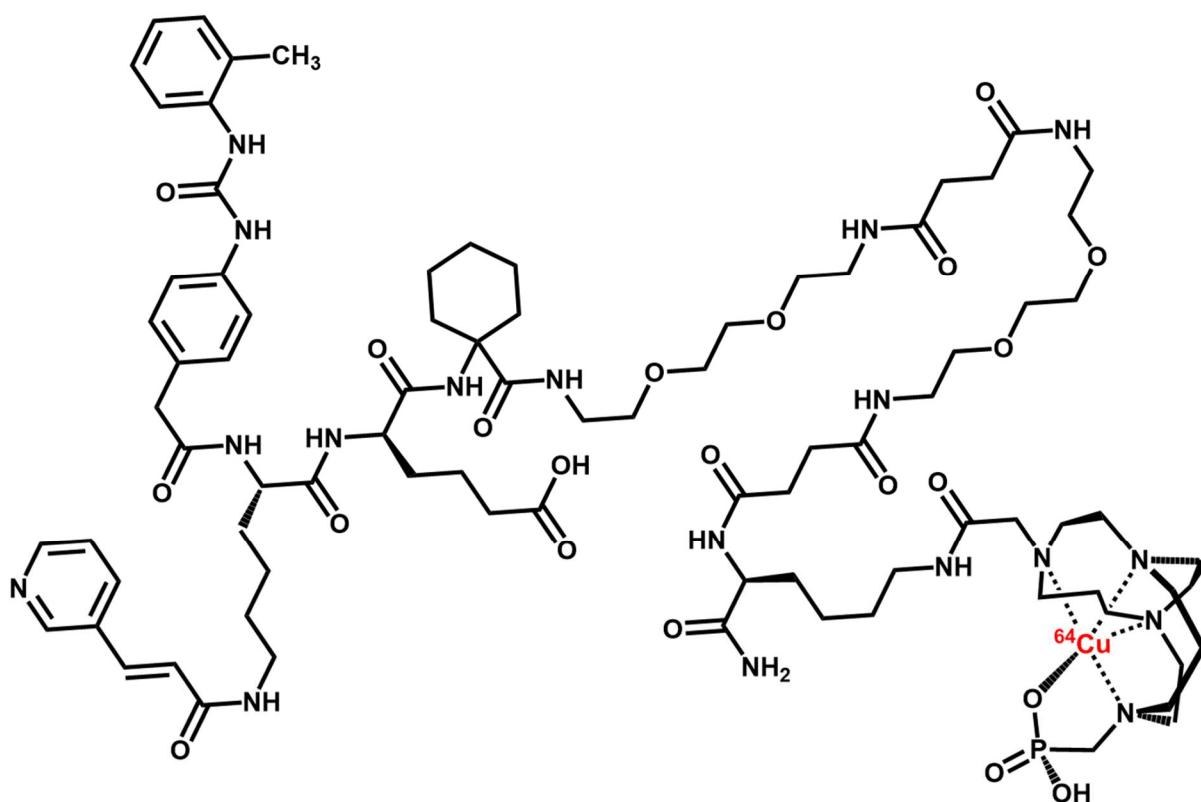


Figure 334: Cross-bridged macrocyclic chelators, CB-TE2A, CB-T1A1P and PCB-TE2A

Figure 335: Cross-bridged macrocyclic chelator CB-T1A1P binds  $^{64}\text{Cu}^{2+}$  ion

### $^{64}\text{Cu}$ -bioconjugates

Most of  $^{64}\text{Cu}$  radiopharmaceuticals are bioconjugates containing some very strong ligand such as those presented above and some biomolecule or a macromolecule that binds onto some biological receptor or structure. An example of a copper-based radiopharmaceutical is  $^{64}\text{Cu}$ -bioconjugate where  $^{64}\text{Cu}$  is complexes by a cross-bridged macrocyclic ligand with phosphonate moiety and then the complex is attached onto LLPA (Figure 336). It is called  $^{64}\text{Cu}$ -CB-TE1A1P-LLP2A. This molecule targets and binds so-called “Very Late Antigen-4” (also known as “ $\alpha 4\beta 1$  Integrin”), specifically present on the multiple myeloma cells and is used for the PET imaging of multiple myeloma.

Figure 336: Bioconjugate  $^{64}\text{Cu}$ -CB-TE1A1P-LLP2A.

## Isotopes of zirconium

Another important metallic radionuclide that is getting more and more important in the area of radiopharmaceutical chemistry is zirconium. It has 38 isotopes out of which four are stable and natural. However, there is another natural isotope,  $^{96}\text{Zr}$  but that one it is a radioactive, primordial isotope with astronomically long half-life of  $2.0 \times 10^{19}$  years!  $^{93}\text{Zr}$  is a product of nuclear fission of  $^{235}\text{U}$  and is found in the nuclear high level waste (so called PUREX raffinate) and has very long half-life (1.53 million years). The only zirconium isotope used in nuclear medicine is  $^{89}\text{Zr}$ . It decays by positron decay, emits positrons and is used for the PET imaging.

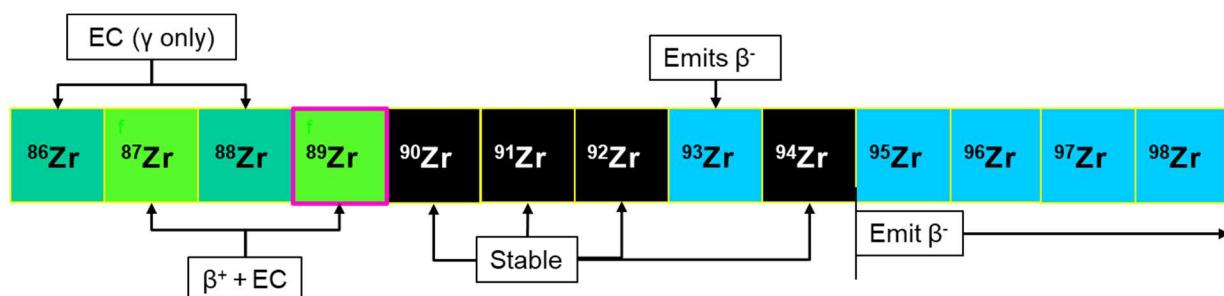
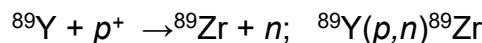


Figure 337: Isotopes of zirconium, only  $^{89}\text{Zr}$  (in purple frame) is used in nuclear medicine

## Nuclear properties and production of $^{89}\text{Zr}$

Zirconium-89 ( $^{89}\text{Zr}$ ) has a relatively long half-life: 3.3 days, which is the same as 78.4 hours. It decays into stable  $^{89}\text{Y}$  23% of time by positron emission ( $\beta^+$ ) and 77% of time by electron capture (EC) (Figure 338). The emitted positrons have average energy of 395.5 keV and the maximum energy of 902 keV. It also emits gamma rays of 909 keV. The energy of positrons is such that they fly into tissue 1.23 to 3.8 mm. This is the twice the size of  $^{18}\text{F}$  range, but still make good images.  $^{89}\text{Zr}$  have just relatively recently became very interesting for PET imaging. Because it has very long half-life it has ability to facilitate the tracking of targeting vectors that require lots of time to achieve optimal target-to-background contrast ratios.

$^{89}\text{Zr}$  is commonly produced in cyclotrons by bombarding solid target, foil of  $^{89}\text{Y}$  metal with protons of 12-15 MeV:



The target is a natural yttrium metal foil and obtained yield and radionuclidic purity are very good! After the irradiation the target is dissolved in HCl, and the produced

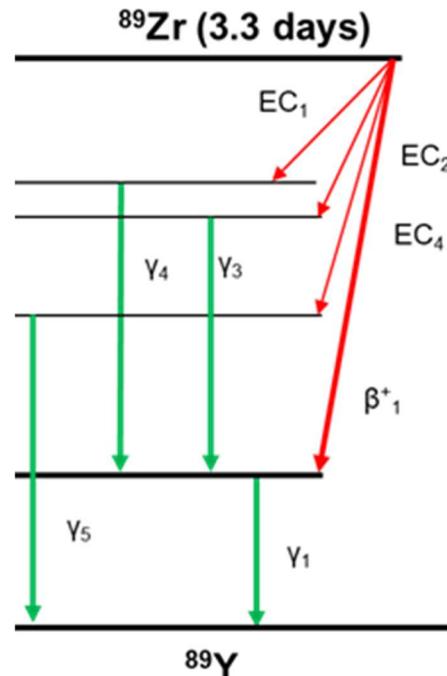


Figure 338: Decay diagram of  $^{89}\text{Zr}$

$^{89}\text{Zr}$  is separated by extraction using a hydroxamate-functionalized resin. Hydroxamate is a ligand with a high affinity for  $^{89}\text{Zr}$ , while the rest of yttrium can be washed away from the resin with HCl.

## Chemical and coordination properties of zirconium

Just like copper, technetium, yttrium, zirconium is also a transitional (“d-block”) metal. It is located in the fourth group, just below titanium. And similar to titanium, zirconium makes  $\text{Zr}^{4+}$  ions. Zirconium ion is a bit larger than copper it can form complexes with coordination number up to 8. Generally, it has coordination chemistry similar to Fe. The main ligand for  $\text{Zr}^{4+}$  is a natural compound called desferrioxamine (DFO). This ligand is currently the gold standard for the coordination of  $^{89}\text{Zr}^{4+}$ . Except DFO there are other ligands for the complexation (coordination) of  $\text{Zr}^{4+}$ :

- Desferrioxamine★ (extended DFO)
- DFO-HOPO
- 3,4,3-(L1-1,2-HOPO)
- BPDET-LysH22,2-3-HOPO
- Macroyclic hydroxamates (Fusarinine)

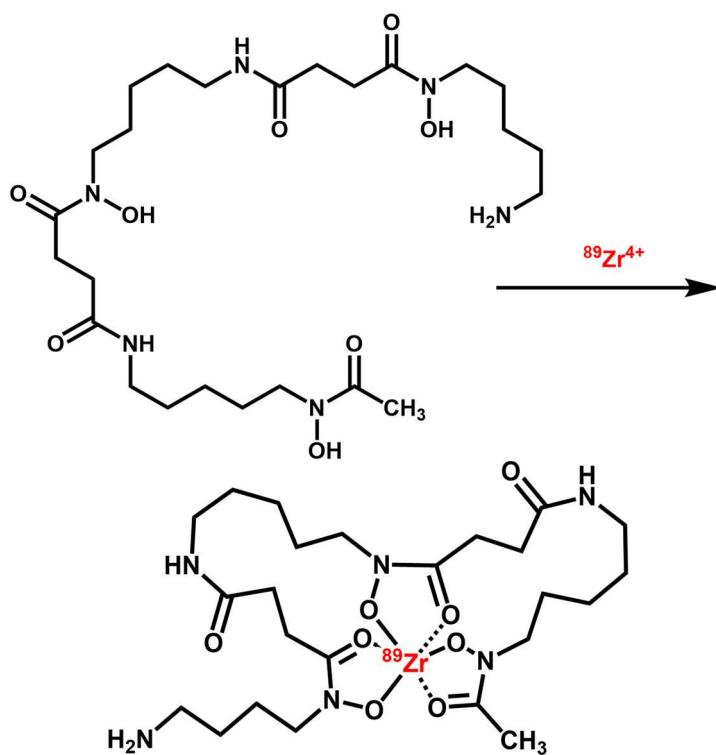


Figure 339: Natural product, desferrioxamine (up) is a linear molecule and it can strongly chelate  $^{89}\text{Zr}$

### Desferrioxamine (DFO)

Desferrioxamine (or DFO, see figure 339) is a linear molecule that encircles and coordinates  $\text{Zr}^{4+}$ . It is a natural product, firstly isolated from a bacterium called

*Streptomyces pilosus* and it was found to excellently binds iron. These kinds of natural products that have ability to coordinate and chelate iron are called siderophores: DFO is one of them. However, as it binds iron DFO likes also to bind zirconium. Coordinated complex  $^{89}\text{Zr}$ -DFO is usually bioconjugated with some proteins or peptides that serve as vectors.

### Radiopharmaceuticals of $^{89}\text{Zr}$ - conjugation of DFO to Antibodies

Therefore a complex  $^{89}\text{Zr}$ -DFO can be bioconjugated with numerous biomolecules such as antibodies and hence creating a radiopharmaceutical agent with  $^{89}\text{Zr}$  for PET imaging (Figure 340). Application of such radiopharmaceutical is for PET imaging of any cells, tissue or structures whom biomolecular vector (such as MAb) can target. As mentioned before half-life of  $^{89}\text{Zr}$  is 3.3 days and this means that targeted cells or tissues can be tracked and identified in the body much longer than it is the case with any other radionuclide. This is a unique characteristic of  $^{89}\text{Zr}$ .

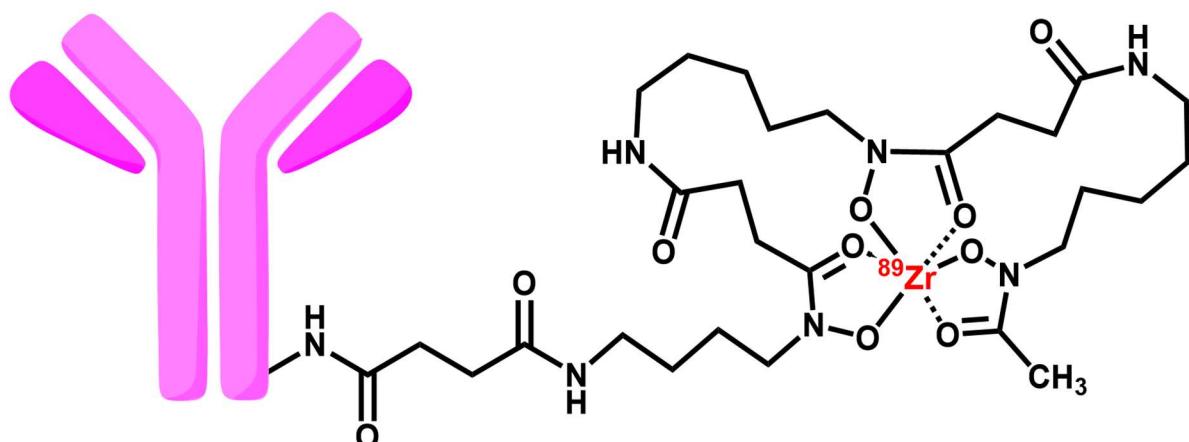


Figure 340: Bioconjugate that contains mAb linked via diamide linker with an  $^{89}\text{Zr}$ -DFO chelate complex.

## Chapter XIV - Yttrium and Lutetium

Another two important metallic radionuclides are yttrium-86, yttrium-90 ( $^{86}\text{Y}$  and  $^{90}\text{Y}$ ) and lutetium-177 ( $^{177}\text{Lu}$ ). Yttrium is a transitional metal but behaves very much like lanthanides to whom lutetium belongs.  $^{86}\text{Y}$  is very rarely used for imaging while  $^{90}\text{Y}$  is one of the most important radionuclides for targeted radiotherapy.  $^{177}\text{Lu}$  is on the other hand used for both imaging and therapy at the same time; hence we call this radionuclide a theranostic (**therapeutic + diagnostic**)

### Isotopes of Yttrium

Yttrium has 36 isotopes but only  $^{89}\text{Y}$  is the stable and natural isotope of yttrium. All yttrium isotopes with  $A < 89$  are emitting positrons ( $\beta^+$ ), while the isotopes with  $A > 89$  are  $\beta^-$  emitting.  $^{86}\text{Y}$  is a positron emitting yttrium radioisotope and can be used for PET imaging.  $^{90}\text{Y}$  emits very energetic  $\beta^-$  particles and is used for the targeted radiation therapy (radiotherapy, TRT).

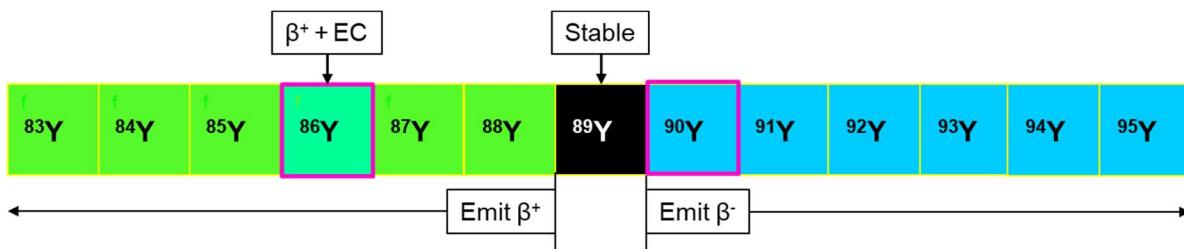


Figure 341: Isotopes of yttrium,  $^{86}\text{Y}$  and  $^{90}\text{Y}$  (in purple frame) are used in nuclear medicine

### Nuclear properties and production of $^{86}\text{Y}$

Yttrium-86 ( $^{86}\text{Y}$ ) decays by both positron decay and electron capture (EC) into  $^{86}\text{Sr}$ ; it emits positrons ( $\beta^+$ ), but just 33% of the time (Figure 342). Average energy of these positrons is 535 keV. The half-life is 14.7 hours. The major drawback of  $^{86}\text{Y}$  is that it emits 102 different gamma rays with energies ranging from 139 to 4900 keV. These gammas interfere with the annihilation co-incident gamma ray of 511 keV.  $^{86}\text{Y}$  is very rarely used for the PET imaging, usually as a pre-therapy diagnostic test using chelates bioconjugated with mABs or peptides. It could be used before the targeted radiotherapy with  $^{90}\text{Y}$  but in practice  $^{111}\text{In}$  is used. It is important to emphasize that in clinical practice PET imaging is preferred over SPECT imaging due to the better images it gives.

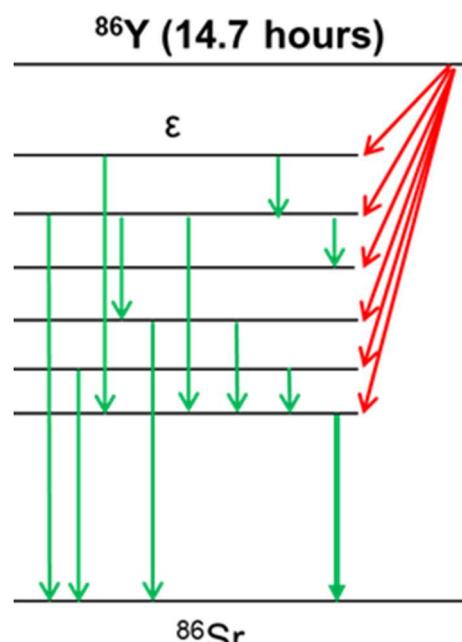
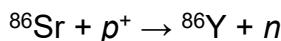


Figure 342: Decay diagram of  $^{86}\text{Y}$

$^{86}\text{Y}$  is made using cyclotrons by bombarding

strontium-86 ( $^{86}\text{Sr}$ ) target with protons:

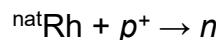


The target is enriched  $^{86}\text{Sr}$  in the form of a solid target made of strontium carbonate. Protons for this nuclear transformation have to be accelerated to 22 MeV. After the irradiation the irradiated target is dissolved in an acid and the product is co-precipitated using lanthanum as the carrier and then purified on ion-exchange column.

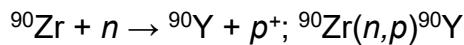
### Nuclear properties and production of $^{90}\text{Y}$

On the other hand, yttrium-90 ( $^{90}\text{Y}$ ) decays purely by  $\beta^-$  decay into  $^{90}\text{Zr}$  with the half-life of 64.1 hours, which is quite long (Figure 343). The emitted beta particles are very energetic: average energy is 934 keV, while the maximal energy is 2280 keV. This means that the mean free path length of  $\beta^-$  particles emitted by  $^{90}\text{Y}$  is approximately 12 mm. This is more than one centimetre. Due to the long half-life and very energetic  $\beta^-$  particles emitted,  $^{90}\text{Y}$  radioisotope is an excellent radionuclide for the targeted radiotherapy (TRT) of any tumour or malignant disease. It is especially good for larger metastases with lots of thick tissue since long traveling beta-particles can reach them.

$^{90}\text{Y}$  radionuclide can be produced by cyclotron spallation: firstly, energetic neutrons are made by bombarding natural rhodium metal foil with highly energetic protons, and this bombardment ejects highly energetic neutrons. This kind of process of creating neutrons from accelerated protons is called spallation:



Then the obtained energetic neutrons immediately bombard a solid  $^{90}\text{Zr}$  target:



Another option is to use the natural decay of  $^{90}\text{Sr}$  which gives  $^{90}\text{Y}$ : parent  $^{90}\text{Sr}$  decays with the quite long half-life of 29 years by the beta-decay and gives its daughter  $^{90}\text{Y}$ :



Since the half-life of  $^{90}\text{Y}$  is much shorter than the half-life of  $^{90}\text{Sr}$  this pair of parent-daughter can exist together in a secular equilibrium for many years.  $^{90}\text{Sr}$  is found in quite large quantities in the nuclear fuel high-level waste (PUREX raffinate), from

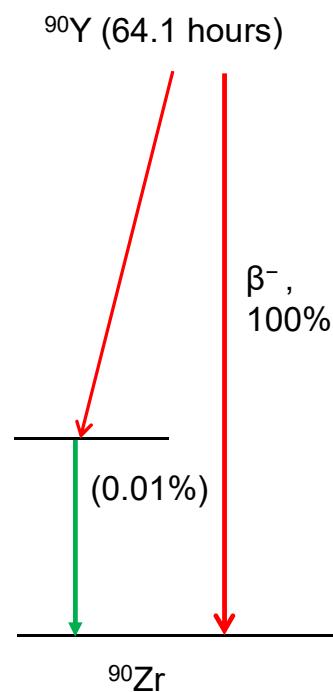


Figure 343: Decay diagram of  $^{90}\text{Y}$

where it can be extracted and purified. Therefore,  $^{90}\text{Sr}/^{90}\text{Y}$  radionuclide generators can be made and could provide stable long-term source of  $^{90}\text{Y}$  for years. The only problem is that  $^{90}\text{Sr}/^{90}\text{Y}$  radionuclide generators are not routine and simple devices, and more work is needed to develop practical radionuclide generators that will be giving  $^{90}\text{Y}$  samples of high activity and high radionuclidian and radiochemical purity. Current drawback is that  $^{90}\text{Y}$  obtained from  $^{90}\text{Sr}/^{90}\text{Y}$  radionuclide generators contains radionuclidian impurity of some residual  $^{90}\text{Sr}$  which is radiotoxic and should be strictly avoided.

## Isotopes of lutetium

Lutetium is one of two lanthanides used in nuclear medicine. It has 39 isotopes, and the only stable one is  $^{175}\text{Lu}$  that makes 97.41% of natural lutetium: the rest 2.59% is  $^{176}\text{Lu}$ , very weakly radioactive, it has very long half-life ( $3.78 \times 10^{10}$  years) and therefore is called primordial radioisotope.  $^{173}\text{Lu}$  decays by EC only. In nuclear medicine only  $^{177}\text{Lu}$  is used: recently it was found that it can be very useful for the targeted radiation therapy!

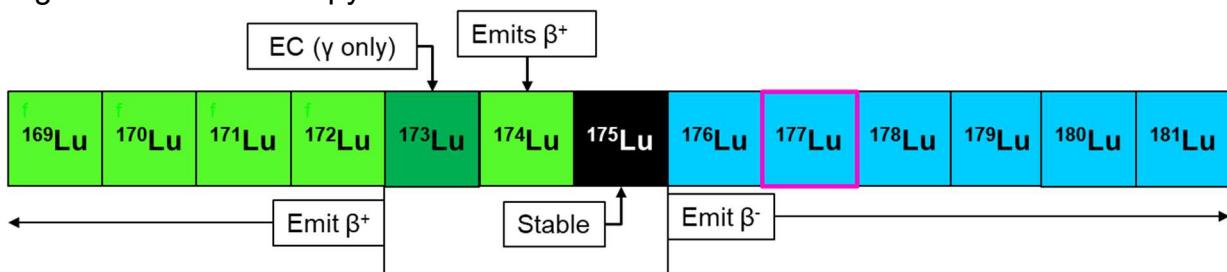


Figure 344: Isotopes of zirconium, only  $^{89}\text{Zr}$  (in purple frame) is used in nuclear medicine

## Nuclear properties and production of $^{177}\text{Lu}$

Lutetium-177 ( $^{177}\text{Lu}$ ) decays into Hf (hafnium) by beta-decay and has half-life is 6.64 days which is excellent for targeted radiation therapy. It emits weak  $\beta^-$  particles; average energy is 133.6 keV, while the maximal energy is 497 keV. The energy of  $\beta^-$  particles is such to penetrate tissue only 1.6 mm, an order of magnitude weaker than  $^{90}\text{Y}$ .  $^{177}\text{Lu}$  also emits some weak gamma rays (33 keV).  $^{177}\text{Lu}$  is used for the targeted radiotherapy (TRT), but also can be used for the SPECT imaging at the same time. Therefore, it is considered to be a true theranostic.

$^{177}\text{Lu}$  can be produced in nuclear research reactors by bombarding  $^{176}\text{Lu}$  target with thermal neutrons. After the radiochemical purification resulting  $^{177}\text{Lu}$  is “carrier added” radionuclide. However, this is not very acceptable for the targeted radiotherapy. Another option is to irradiate stable

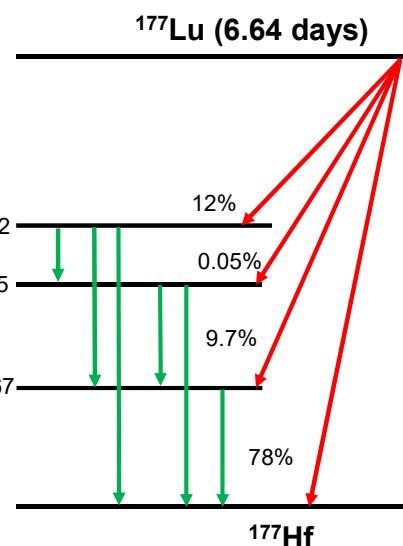


Figure 345: Decay diagram of  $^{86}\text{Y}$

ytterbium-176 ( $^{176}\text{Yb}$ ) with neutrons in the nuclear reactor. This nuclear reaction forms  $^{177}\text{Yb}$  that has a short half-life of just 115 min and decays by beta-decay into  $^{177}\text{Lu}$ . Produced  $^{177}\text{Lu}$  then can be separated from its parent ytterbium (Yb) and purified to acceptable radionuclidic and radiochemical purity.

## Chemical properties of yttrium and lutetium

Just like gallium and indium, yttrium and lutetium are chemically similar. Yttrium is in the 3<sup>rd</sup> group (just below scandium) while lutetium just formally also belongs to the 3<sup>rd</sup> group. However, lutetium is actually a lanthanide! Both yttrium and lutetium form trivalent ( $\text{M}^{3+}$ ) ions ( $\text{Y}^{3+}$  and  $\text{Lu}^{3+}$ ).

Although yttrium is not part of the lanthanide group, it behaves very similar to lanthanides. Therefore, it is often treated as a “pseudo-lanthanide”. Radiolabelling conditions and chelator selectivity are effectively the same for  $^{177}\text{Lu}^{3+}$  as well  $^{86}\text{Y}^{3+}$  or  $^{90}\text{Y}^{3+}$  ions. Both metal ions  $\text{Y}^{3+}$  and  $\text{Lu}^{3+}$  have a tendency to form insoluble  $[\text{M}(\text{OH})_3]$  precipitates in the aqueous solutions above pH 3. Therefore, chelators are needed to keep  $\text{Y}^{3+}$  and  $\text{Lu}^{3+}$  ions in solution. Very strong chelators are also needed to ensure  $^{177}\text{Lu}^{3+}$  as well as  $^{86}\text{Y}^{3+}$  or  $^{90}\text{Y}^{3+}$  ions will not “fall off” their vectors once in the body.

### Bifunctional chelators for $\text{Lu}^{3+}$ and $\text{Y}^{3+}$

There are several types of chelating ligands for Lu and Y. Some of them are macrocyclic, based on NETA or DOTA macrocycles open chain such as the one based on DTPA or H4OCTAPA. But all of them have *para*-isothiocyanato-benzyl group (“*p*-SCN-Bn”) that is used for the bioconjugation with proteins and peptides.

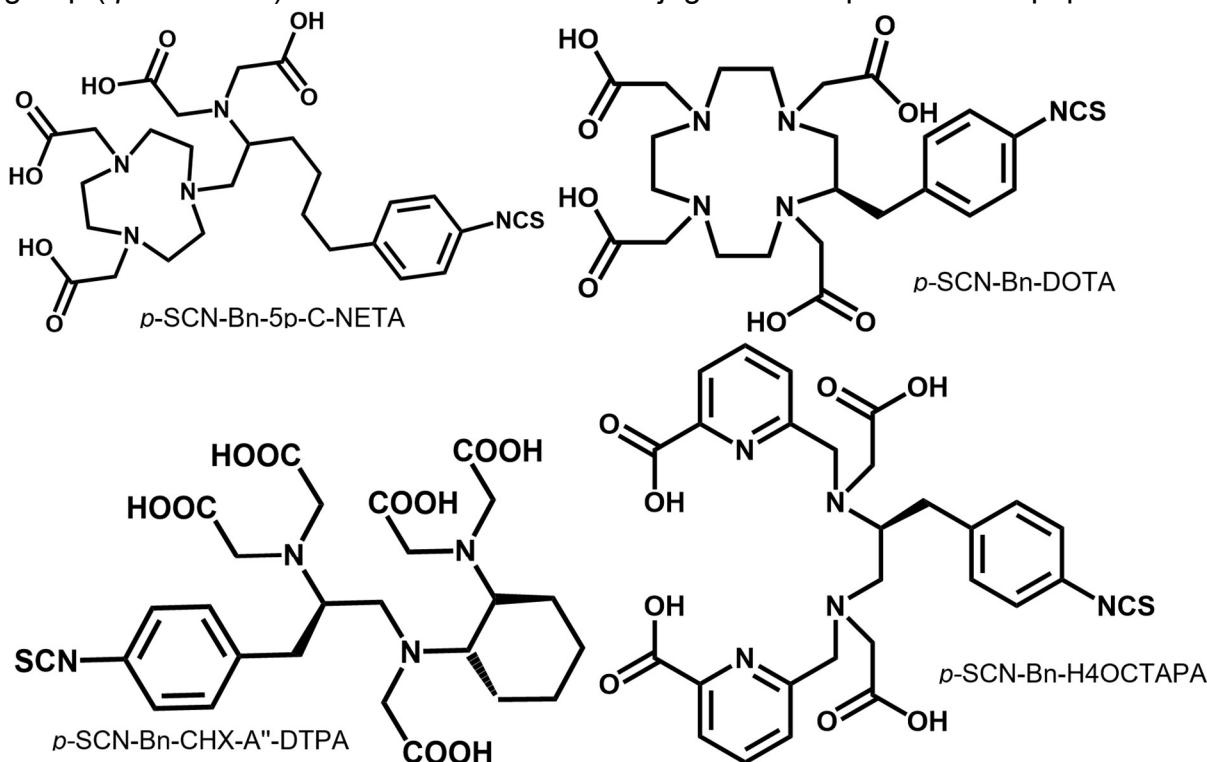


Figure 346: Bifunctional chelators for  $\text{Lu}^{3+}$  and  $\text{Y}^{3+}$

## Radiopharmaceuticals of yttrium and lutetium

Except the  $^{86}\text{Y}$  that is (rarely) used for the PET imaging, the major role of  $^{90}\text{Y}$  and  $^{177}\text{Lu}$  radionuclides is the targeted radiotherapy (TRT). Use of these radionuclides in TRT is still in the development phase, with more and more new radiotherapeutics being approved recently. All these  $^{90}\text{Y}$  and  $^{177}\text{Lu}$  radiotherapeutics are based on bioconjugates between a strong chelating agent (strong chelation is the key for the efficient radiation delivery) and a protein or peptide that specifically binds onto a certain type of cells. Currently all the  $^{90}\text{Y}$  and  $^{177}\text{Lu}$  radiotherapeutics are used to treat some forms of malignant disease (also called neoplasms): cancer, leukaemia, or lymphoma. Lots of them are radioimmunoconjugates that contain monoclonal antibodies as vectors for the specific molecular recognition and cancer cell targeting.

### $^{90}\text{Y}$ -Ibritumomab tiuxetan

The best known radiotherapeutic that contains  $^{90}\text{Y}$  is  $^{90}\text{Y}$ -Ibritumomab tiuxetan, also known under its trade name Zevalin. It is used for the  $\beta^-$ -radiotherapy of non-Hodgkin B-lymphocyte lymphoma.  $^{90}\text{Y}$ -tiuxetan complex is attached to ibritumomab, a monoclonal antibody for the specific targeting of CD20 antigen on B-lymphocyte lymphoma cells. Hence, this radiotherapeutic is a radioimmunoconjugate. While  $^{90}\text{Y}$ -Ibritumomab tiuxetan is used for the targeted radiotherapy, its  $^{111}\text{In}$  version,  $^{111}\text{In}$ -Ibritumomab tiuxetan is used for the SPECT imaging of lymphoma cells; in the clinical practice these are used in tandem. However, it is a trend to use  $^{86}\text{Y}$  instead of  $^{111}\text{In}$  since PET imaging gives much better images than SPECT imaging.

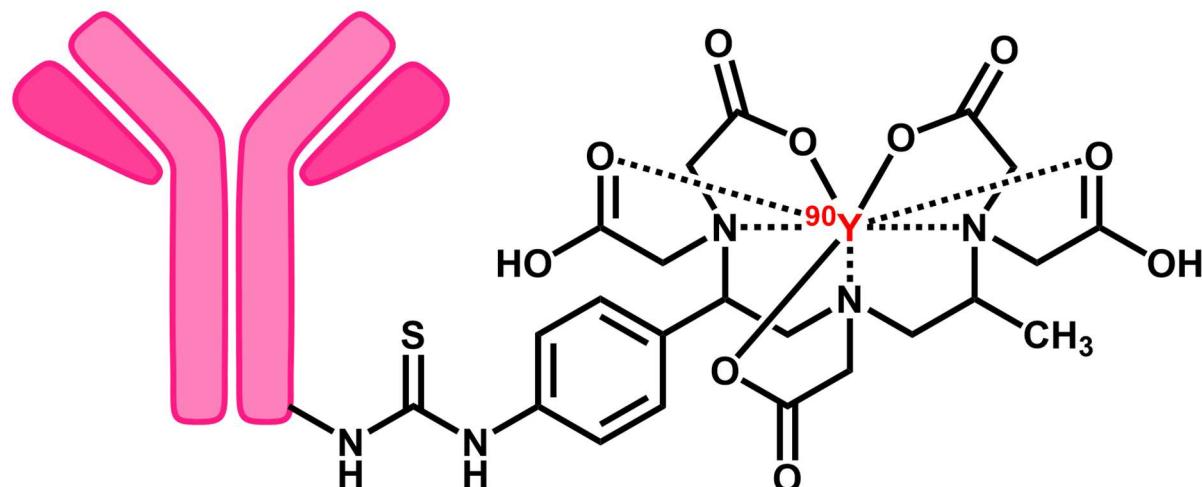
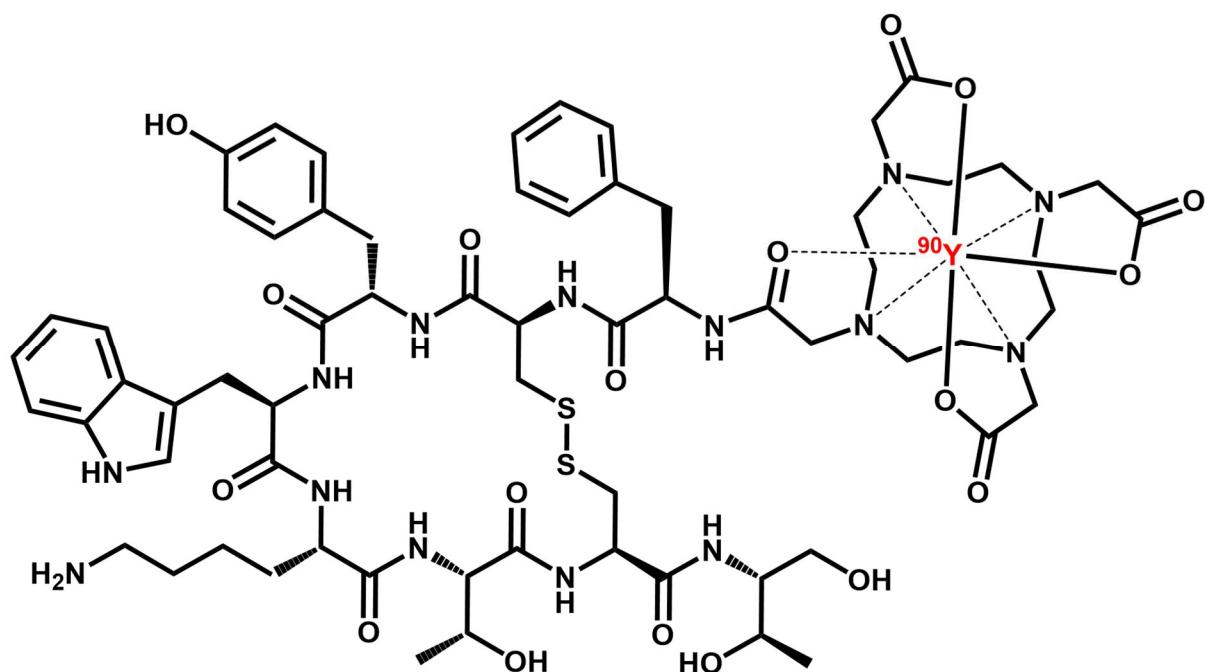


Figure 347: Structure of  $^{90}\text{Y}$ -Ibritumomab tiuxetan

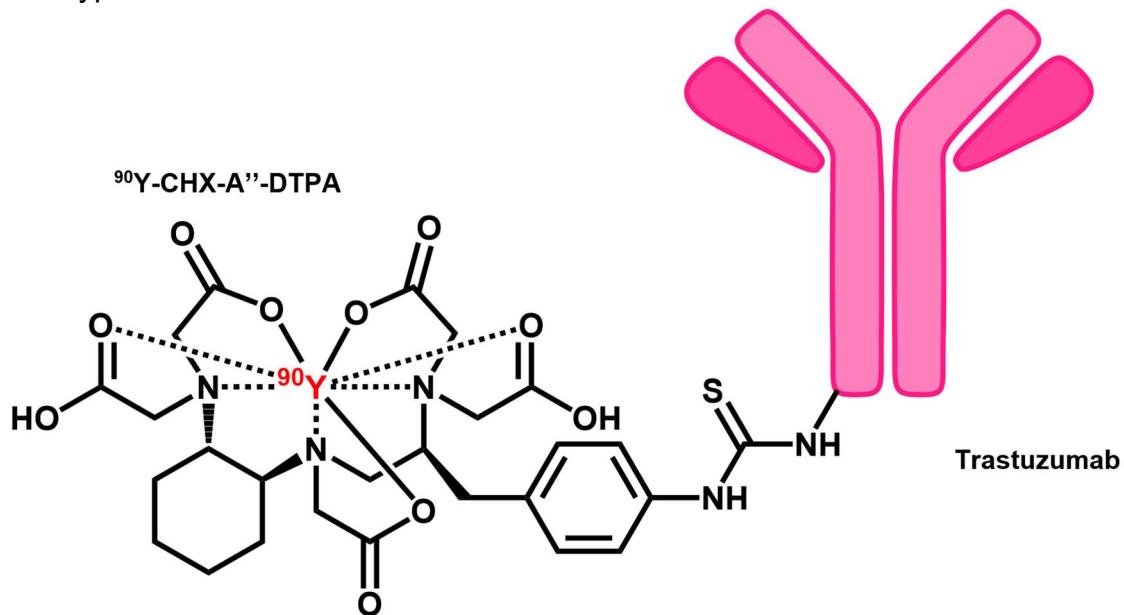
### $^{90}\text{Y}$ -DOTATOC

$^{90}\text{Y}$ -DOTATOC (Figure 348) is a  $^{90}\text{Y}$ -containing version of DOTA-octreotide bioconjugate. While the  $^{68}\text{Ga}$ -containing version is used for the PET imaging of neuroendocrine tumours or lung cancer,  $^{90}\text{Y}$ -containing version is used for the targeted radiotherapy of these tumours.

Figure 348: Structure of  $^{90}\text{Y}$ -DOTATOC

### $^{90}\text{Y}$ -CHX-A''-DTPA-Trastuzumab

And another example of radioimmunoconjugates with  $^{90}\text{Y}$  is  $^{90}\text{Y}$ -CHX-A''-DTPA-Trastuzumab (Figure 349). In this case the  $^{90}\text{Y}$  radionuclide is chelated by CHX-A''-DTPA chelating ligand and the whole complex ( $^{90}\text{Y}$ -CHX-A''-DTPA) is then bioconjugated to trastuzumab, a monoclonal antibody that binds to HER2 receptors. The HER2 receptors are very numerous on some types of breast cancer, and therefore this radiopharmaceutical agent can be used for targeted radiotherapy of certain types of breast cancers.

Figure 349: Structure of  $^{90}\text{Y}$ -CHX-A''-DTPA-Trastuzumab

## **<sup>90</sup>Y-microspheres**

Very different type of radiopharmaceutical agent is <sup>90</sup>Y-microspheres (Figure 350). These microspheres are in fact little particles made of resin or glass that contain <sup>90</sup>Y. Their size is very small, 32-25  $\mu\text{m}$ . Each particle may contain from 2500 to 50 Bq, but millions of particles are administrated, so the total dose is about 3 GBq to 5 GBq. Microspheres are injected into the liver artery and are causing “radio-embolization”: they get stuck in the vicinity of cancer cells and deliver large dose of ionizing radiation to cancer cells causing their death. These microspheres are used for the treatment of hepatocellular carcinoma (cancer of liver), but also for any other cancer type with metastases in liver.



Figure 350: Action of <sup>90</sup>Y-microspheres in liver (left) and vial of <sup>90</sup>Y-microspheres (right)

## **<sup>177</sup>Lu-DOTATATE**

<sup>177</sup>Lu is relatively new radiotherapeutic radionuclide and there are not many examples of radiopharmaceutical agents that use <sup>177</sup>Lu. <sup>177</sup>Lu-DOTATATE (Figure 351) is one of them. It is a bioconjugate of <sup>177</sup>Lu-DOTA complex and octreotide peptide. It is just like the <sup>68</sup>Ga version; however, it is not used for the imaging, but for the targeted radiotherapy of neuroendocrine tumours.

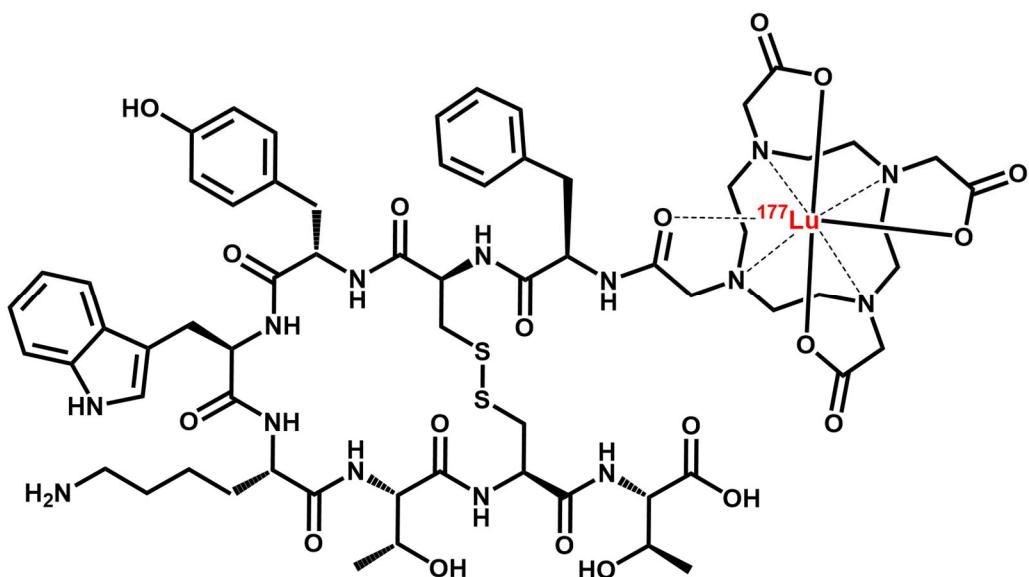


Figure 351: Structure of <sup>177</sup>Lu-DOTATATE



## Chapter XV - Alpha-emitting radionuclides

The alpha emitting radionuclides are those that decay by alpha decay and are emitting alpha particles. In general, these are all heavy metals and some heavy non-metal elements. Roughly speaking these are the elements beyond thallium: actinides, transactinides, 7s-elements such as francium (Fr) and radium (Ra), then 6p-elements and their radionuclides such as lead (Pb), bismuth (Bi), polonium (Po), astatine (At), radon (Rn), then some exotic lanthanide radionuclides such as neodymium-144 ( $^{144}\text{Nd}$ ), terbium-149 ( $^{149}\text{Tb}$ ), samarium-148 ( $^{148}\text{Sm}$ ), gadolinium-152 ( $^{152}\text{Gd}$ ), europium-151 ( $^{151}\text{Eu}$ ), and even some heavy *d*-block radionuclides such as tungsten-183 ( $^{183}\text{W}$ ) or osmium-186 ( $^{186}\text{Os}$ ). In general, after lead (Pb) there is no more stable radionuclide, all are radioactive and most of them emit alpha particles.

Figure 352: Periodic table of radioactive elements and radioisotopes emitting alpha particles: actinides (green), transuranic elements (light blue), some p-block elements and some their radionuclides (full yellow), francium and radium (pink), some lanthanide radionuclides (orange) and some radioisotopes of osmium and tungsten

However, only ten (10) radionuclides of all those alpha-emitting radionuclides can qualify to be considered for the use in nuclear medicine. Their primary use is not imaging but radiotherapy, precisely, targeted radiotherapy of cancer and similar neoplasm diseases (leukaemia, lymphomas). Some scientists are advocating that these radionuclides should be also considered to be used in treatment of microbes, bacteria and viruses.

What qualifies an alpha-emitting radionuclide to be selected as medical radionuclide? The main parameter is the half-life; it cannot be very long, should be from 30 minutes to 2-3 weeks. Secondly, it has to have abundance of  $\alpha$  emissions and energy of the particles should be high. In addition, it should not be radiotoxic, but should be able to form very stable complexes with macrocyclic ligands, and finally it should be easy to produce and not so expensive. Very few radionuclides fit these strict criteria, and these are terbium-149 ( $^{149}\text{Tb}$ ), bismuth-212 ( $^{212}\text{Bi}$ ), bismuth-213 ( $^{213}\text{Bi}$ ), astatine-211



( $^{211}\text{At}$ ), radium-223 ( $^{223}\text{Ra}$ ), radium-224 ( $^{224}\text{Ra}$ ), actinium-225 ( $^{225}\text{Ac}$ ), thorium-226 ( $^{226}\text{Th}$ ), thorium-227 ( $^{227}\text{Th}$ ) and fermium-255 ( $^{255}\text{Fm}$ ). All the radionuclides are listed in the Table 6.

Radionuclide	Half life	Chemical nature
<b>Terbium-149 (<math>^{149}\text{Tb}</math>)</b>	4.12 hours	Lanthanide metal
<b>Bismuth-212 (<math>^{212}\text{Bi}</math>)</b>	60.55 minutes	6p metal
<b>Bismuth-213 (<math>^{213}\text{Bi}</math>)</b>	45.61 minutes	6p metal
<b>Astatine-211 (<math>^{211}\text{At}</math>)</b>	7.21 hours	6p non-metal (halogen)
<b>Radium-223 (<math>^{223}\text{Ra}</math>)</b>	11.43 days	7s alkali earth metal
<b>Radium-224 (<math>^{224}\text{Ra}</math>)</b>	3.63 days	7s alkali earth metal
<b>Actinium-225 (<math>^{225}\text{Ac}</math>)</b>	10.0 days	Actinide metal
<b>Thorium-226 (<math>^{226}\text{Th}</math>)</b>	30.57 min	Actinide metal
<b>Thorium-227 (<math>^{227}\text{Th}</math>)</b>	18.70 days	Actinide metal
<b>Fermium-255 (<math>^{255}\text{Fm}</math>)</b>	20.07 hours	Transuranic synthetic actinide m.

Table 6: Alpha-emitting radionuclides eligible for medical use.

### Historical reminder: $^{226}\text{Ra}$ was a radiotherapeutic

In the Chapter II history of radiopharmaceuticals was extensively review, but here we will recall that it was alpha-emitting  $^{226}\text{Ra}$  that was the first radiotherapeutic and radiopharmaceutical agent. The idea of using radioactive materials for the treatment of cancer is as old as is our knowledge of radioactivity. Even few years after being discovered radium was suggested to be used for therapy of cancer! At that time  $^{226}\text{Ra}$  was the only available radioactive material of high activity.

It is fair to say that there were some successes in using  $^{226}\text{Ra}$  in treating skin diseases and skin cancer at that moment, however, due to very long half-life  $^{226}\text{Ra}$  (it is 1600 years) it stays in the body, goes to the bones, and stays in the bones forever. Therefore,  $^{226}\text{Ra}$  is very radiotoxic and can cause cancer. It was used not only for skin cancer, but for many other things, even in non-medical applications, uncontrolled and as quackery. Because of uncritical and uncontrolled use, it has caused lots of toxicity and tragedies, and finally its use was stopped in 1930s. However, recently interest for radiotherapy with radium is back, this time it is not  $^{226}\text{Ra}$ , but  $^{223}\text{Ra}$  and  $^{224}\text{Ra}$ . For example,  $^{223}\text{RaCl}_2$  (trade name is Xofigo) is used for treatment of cancer metastases in bones.  $^{224}\text{Ra}$ -labelled  $\text{CaCO}_3$  micro-particles are also used for the treatment of cancer metastases in bones, while  $^{224}\text{RaCl}_2$  was used for the treatment of ankylosing spondylitis.

## Radiobiological effects of $\alpha$ -emission

What are alpha particles and why these particles are interesting for radiotherapy? As we know alpha particles are emitted from nucleus upon alpha decay and are fast moving, very energetic helium nuclei (consisting of two protons and two neutrons), with charge 2+. Their energies are between 3 and 8 MeV and are discrete, exactly typical for each radioisotope. Also, they have significantly larger mass. If we compare characteristics of alpha particles and beta particles (Table 7) we can see some striking differences.

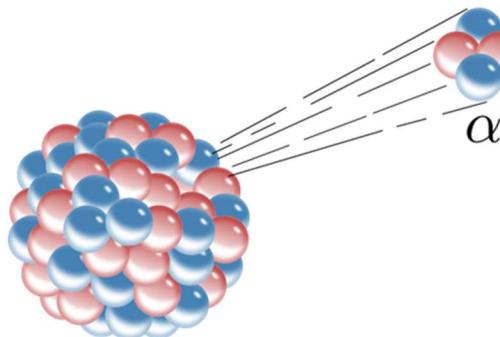


Figure 353: Nucleus emits alpha particle

Type	Mass (emu)	Velocity (km/s)	Charge	Energy range (keV)	Range in tissue ( $\mu\text{m}$ )	LET (keV/ $\mu\text{m}$ )
$\alpha$	4.0012	6000	+2	3000-8000	16-75	100-200
$\beta^-$	0.000549	285 000	-1	500-3000	1500-19000	0.15-0.3

Table 7: Comparison of alpha and beta particles

Alpha particles are many times heavier than beta particles (7000 times, imagine difference between 8 g bullet versus 60 kg artillery projectile), yet velocity of alpha particles is much smaller, while beta particles are almost at the speed of light (95% if it). Energies of alpha particles are an order of magnitude larger than are the ones of beta particles. However, due to the larger mass and charge the flying ranges in tissue are much shorter for alpha particles than for beta particles.

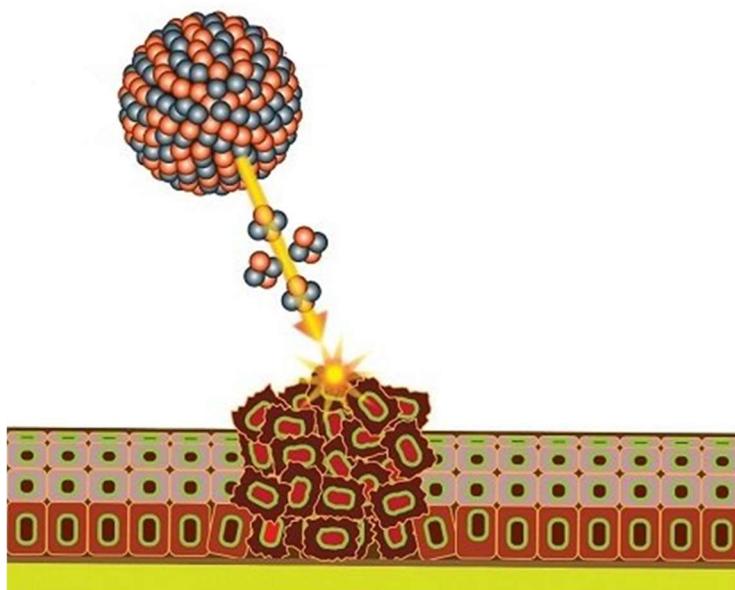


Figure 354: Alpha-particles bombard cells much more heavily and energetically than beta-particles

This means that alpha particles deposit much larger energy per unit of path in tissue than beta particles. This is called Linear Energy Transfer (LET in keV/ $\mu\text{m}$ ) and is in fact an equivalent of force! Therefore, alpha particles deliver onto cells much larger radiation dose per Bq than beta particles. The total effect is that alpha particles are much more deadly for cells than beta particles. Even a single alpha particle may kill a cell, while on average 400 beta particles are needed to kill one cell (Figure 354). We can make allegory: shooting cells with beta particles is like shooting a car with an automatic rifle of few millimetres, while shooting cells with alpha particles is like shooting the same car with an artillery projectile of 155 mm calibre.

### Drawback of medical $\alpha$ -emitting radionuclides

Despite much more efficient killing ability there are some drawbacks of alpha-emitting radiotherapeutics we need to consider and have in mind. While beta-emitting radiotherapeutics are decaying into stable, non-radioactive daughters, alphas are usually decaying into another radioactive daughters: alpha-emitting radionuclides usually have a decay-chain (several successive radioactive daughters) until they reach a stable isotope, just like the one of  $^{225}\text{Ac}$  that has five more radioactive daughters before it reaches relatively stable  $^{209}\text{Bi}$ :

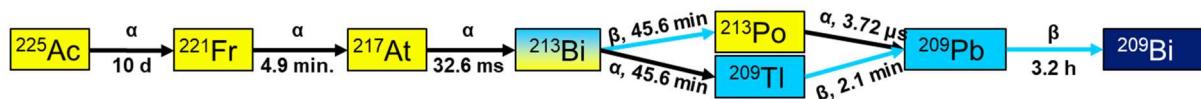


Figure 355: Decay chain of  $^{225}\text{Ac}$

Yet, this could be a good thing, but usually is a problem. If the daughter decays quicker than the parent (shorter half-life) then it may stay in the vicinity of the cell and give the cell multiple shots of alpha particles, and it is a good, useful thing. However, if the daughter decays slower than the parent (has longer half-life) then it may “wander off” into non-targeted tissue. This is problem, especially when radon (Rn) is created: inert gas atom just “goes away” and decays somewhere else. However, it is highly improbable that after the decay and emission of alpha particle daughter will stay complexed inside the ligand (and vector bioconjugate) for two reasons. Firstly, due to the recoiling daughter tends to be ejected out of any molecule including a stable complex. Secondly, the daughter is usually chemically different than the parent and this change of chemical nature often makes complex less stable, and sometimes impossible. In general, targeted therapy with alpha-emitters is still an experimental area, and not a routine. The first therapeutic options (except  $^{223}\text{RaCl}_2$ ) are still in the clinical trials and are yet to be approved.

### Production of $\alpha$ -emitting radionuclides

When it comes to the production of alpha emitting radionuclides typically it was produced by using powerful accelerators (cyclotrons and linear accelerators). However, there are certain challenges associated with the production using cyclotrons. Firstly, this production must be done in specialised institutions only (since

very powerful accelerators are needed). Secondly, currently these radionuclides can be obtained only in small quantities, while medical community needs giga-becquerels of quantity per production session. Thirds, it is often very hard to purify, and isolate needed radionuclide in sufficient purity, therefore more work needs to be done on separation methods. And finally, those radionuclides with half-lives less than 1 day are challenging for transport. Fortunately, most of alpha-emitting radionuclides could be obtained from their parents using come radionuclide generators. These generators face often the major challenge: lack of quick separation of various parents/daughters. Possible radionuclide generator pairs are:

- $^{221}\text{Rn}/^{221}\text{At}$
- $^{229}\text{Th}/^{225}\text{Ra}/^{225}\text{Ac}$
- $^{225}\text{Ac}/^{213}\text{Bi}$
- $^{230}\text{U}/^{226}\text{Th}$
- $^{255}\text{Es}/^{255}\text{Fm}$
- $^{228}\text{Th}/^{224}\text{Ra}$
- $^{212}\text{Pb}/^{212}\text{Bi}$
- $^{227}\text{Ac}/^{227}\text{Th}/^{223}\text{Ra}$

### Vectors and ligands for alpha-emitting radionuclides

As we know, the alpha-emitting medical radionuclides (except the At) are all heavy metals and to be used in the targeted radiotherapy needs to be chelated by using a proper chelating ligand (usually a macrocyclic) and bioconjugated with a protein (like monoclonal antibody) or a peptide that serves as a vector for targeting a cancer cell. Only sometimes a free metal ion is fine, and this is limited to the case of  $^{224}\text{Ra}$  or  $^{223}\text{Ra}$  for bone cancer treatment only. There are two paths (Figure 356) to the radiotherapeutic agent: a ligand with a proper linking and bioconjugation moiety can be firstly complexed (chelated) with a radionuclide ion, and then bioconjugated with a protein to give the radiotherapeutic. Another way is to first perform bioconjugation and just then label the obtained vector with a radionuclide ion.

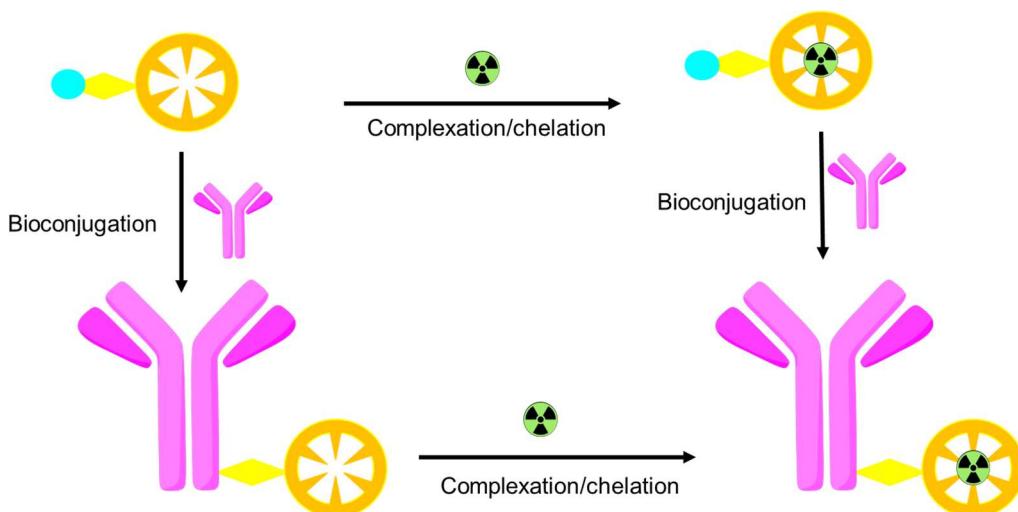
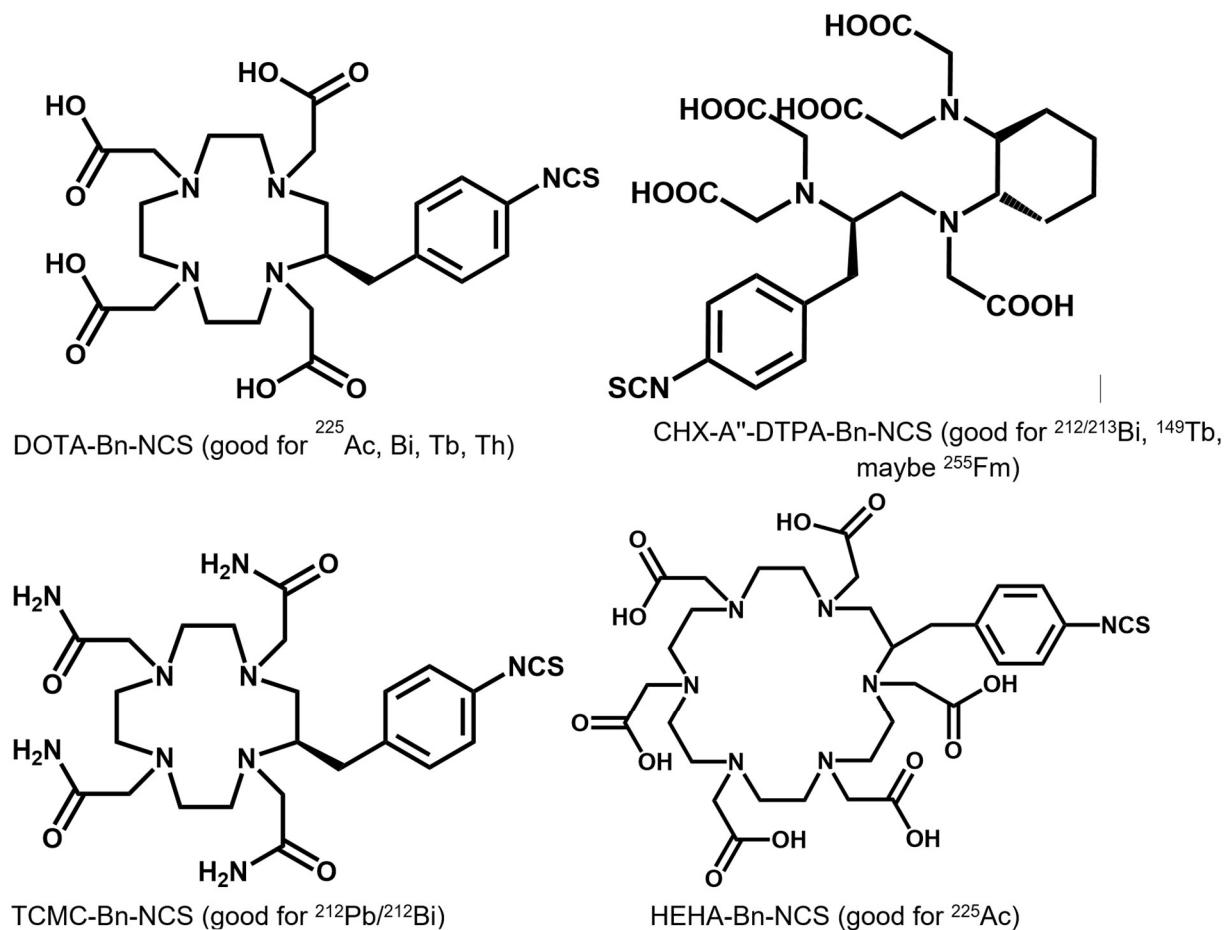
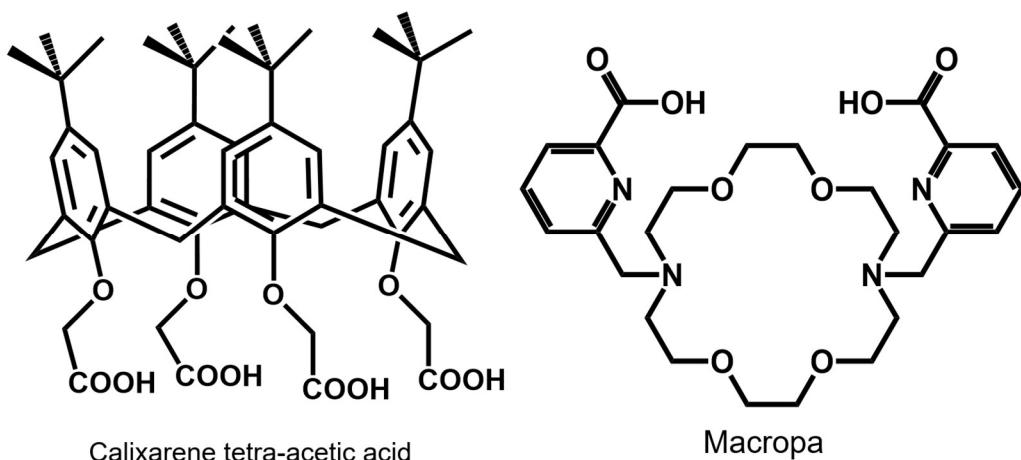


Figure 356: Two pathways to bioconjugate



Ligands for alpha-emitting medical radionuclides are generally similar like those for other radio-metals: the most prevalent is DOTA which is good for most if the metals,  $^{225}\text{Ac}$ , Bi, Tb, Th. The second option is CHX-A''-DTPA-Bn-NCS which is good for  $^{212}/^{213}\text{Bi}$ ,  $^{149}\text{Tb}$ , and maybe could also complex  $^{255}\text{Fm}$ . TCMC-Bn-NCS, amide derivative of DOTA is good for  $^{212}\text{Pb}/^{212}\text{Bi}$  radiotherapeutic pair. HEHA-Bn-NCS (which is in fact a six-fold version of DOTA) was found to be good for  $^{225}\text{Ac}$ . Ligand called ( $\text{Me}-2,3\text{-HOPO}$ )<sub>4</sub>-Bn-NCS or just “HOPO” is good for  $^{227}\text{Th}$  and  $^{226}\text{Th}$  while H<sub>4</sub>Py4Pa-Bn-NCS proved to offer much better complex stability for  $^{225}\text{Ac}$ .





Calixarene tetra-acetic acid was tested as a ligand for  $^{223}\text{Ra}$  and  $^{224}\text{Ra}$  and showed somewhat good properties but eventually failed to prove to be sufficiently stable in the human body.

### Terbium-149 ( $^{149}\text{Tb}$ )

Terbium-149 ( $^{149}\text{Tb}$ ) is the only lanthanide metal among the alpha-emitting radionuclides. Its half-life is 4.12 hours, and it decays in two different ways, by alpha decay (16.7%) into  $^{145}\text{Eu}$ , by  $\beta^+$  and EC (83.3%) into  $^{149}\text{Gd}$  also emitting some gamma photons:

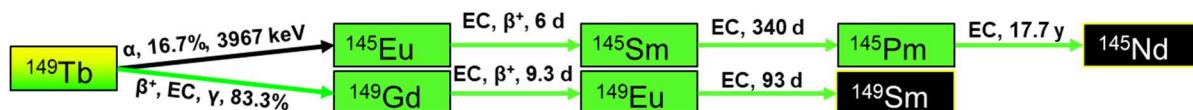


Figure 356: Decay chain of  $^{149}\text{Tb}$

Energy of its alpha particles is 3967 keV. Its daughters are then decaying by decay chains into stable  $^{149}\text{Sm}$  and  $^{145}\text{Nd}$ . All the radionuclides in the decay chain are lanthanides and are decaying by electron capture or positron decay.

Due to its alpha emission, it might be used in targeted radiotherapy, while due to its positron and gamma emission it could be also used for PET or SPECT. Therefore,  $^{149}\text{Tb}$  could be a theranostic. Its production is very hard, and this is the main drawback of this radionuclide: it is obtained by bombarding certain lanthanides with some heavy ions (N, O, C). Currently no radiopharmaceutical agent with  $^{149}\text{Tb}$  is in the clinical practice or in clinical trials, although some preclinical tests are conducted on animals.

### Bismuth-212 ( $^{212}\text{Bi}$ )

Bismuth-212 ( $^{212}\text{Bi}$ ) is an alpha-emitting radionuclide of a *p*-block element bismuth. Its half-life is 60.55 minutes (approximately one hour) and it decays in two ways: by alpha decay (35.9%) into  $^{208}\text{TI}$  (which decays by  $\beta^-$  decay into the stable  $^{208}\text{Pb}$ , half-life is 3 min), and by  $\beta^-$  decay (64.1%) into  $^{212}\text{Po}$  (which then simultaneously decays by alpha decay into stable  $^{208}\text{Pb}$ , half-life is just 300 ns).

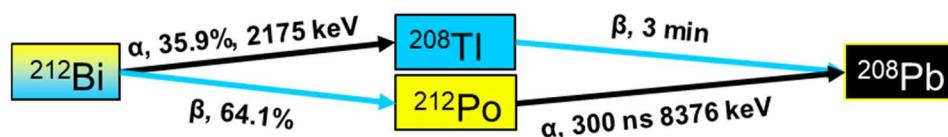
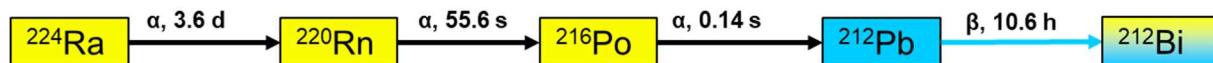


Figure 357: Decay chain of  $^{212}\text{Bi}$

Therefore, decay of  $^{212}\text{Bi}$  results in additional alpha and beta shots of its daughters that quickly decay in the vicinity of the target. The alpha particles energies range from 6051 to 8785 keV (including those from  $^{212}\text{Po}$ ).  $^{212}\text{Bi}$  can be obtained by a radionuclide generator that contains  $^{224}\text{Ra}$ :

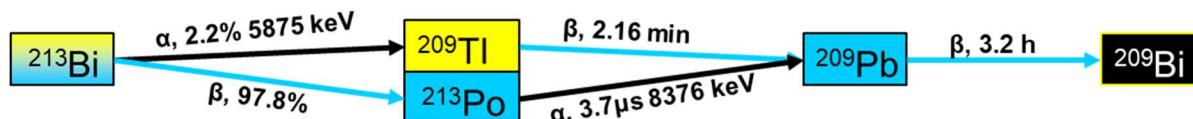
Figure 358: Generation of  $^{212}\text{Bi}$  via  $^{224}\text{Ra}$  decay chain

Quick decay chain produces  $^{212}\text{Bi}$  from  $^{224}\text{Ra}$ . The  $^{224}\text{Ra}$  is originally obtained by radionuclide generator containing  $^{228}\text{Th}$ , while  $^{228}\text{Th}$  is obtained by nuclear bombardment of  $^{226}\text{Ra}$  with neutrons in a nuclear reactor.

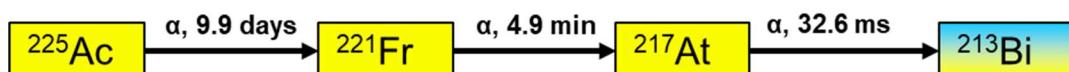
Another option is to use the immediate parent,  $^{212}\text{Pb}$  as an “internal radionuclide generator”. The idea is to label a TCMC-mAB bioconjugate with  $^{212}\text{Pb}$  (which has the half-life of 10.62 hours, and decays by beta decay):  $^{212}\text{Pb}$  decays into  $^{212}\text{Bi}$  while being held at the target and the target is then targeted by particles from both  $^{212}\text{Pb}$  and its daughter  $^{212}\text{Bi}$  (and also the daughters of  $^{212}\text{Bi}$ ). Based on this concept a clinical trial was performed using  $^{212}\text{Pb}$ -TCMC-trastuzumab radioimmunoconjugate for the treatment of HER2 positive breast cancer.

### Bismuth-213 ( $^{213}\text{Bi}$ )

Bismuth-213 ( $^{213}\text{Bi}$ ) is another important medical radionuclide of bismuth:

Figure 359: Decay chain of  $^{213}\text{Bi}$ 

Its half-life is 45.6 minutes (slightly shorter than  $^{212}\text{Bi}$ ), and it decays by alpha decay (only 2.2%) into  $^{209}\text{TI}$ , which then decays by  $\beta^-$  emission into  $^{209}\text{Pb}$  (half-life is 2.16 min). But  $^{213}\text{Bi}$  decays mostly by  $\beta^-$  decay (97.8%) into  $^{213}\text{Po}$ . That  $^{213}\text{Po}$  then almost simultaneously decays (since half-life is just 3.7  $\mu\text{s}$ ) by very strong alpha emission (alphas of 8376 keV) also into  $^{209}\text{Pb}$ . It also emits gamma of 440 keV, and therefore could be used for SPECT imaging. Hence it is theranostic.  $^{213}\text{Bi}$  could be obtained using a radionuclide generator containing  $^{225}\text{Ac}$ ; its daughters quickly decay into  $^{213}\text{Bi}$ :

Figure 360: Generation of  $^{213}\text{Bi}$  via  $^{225}\text{Ac}$  decay chain

The best-known chelator for  $^{213}\text{Bi}$  is CHX-A''-DTPA. There is not many radiotherapeutics based on  $^{213}\text{Bi}$ , however there is a radiotherapeutic agent where  $^{213}\text{Bi}$  was tried in clinical research,  $^{213}\text{Bi}$ -labeled DOTATOC.

### Astatine-211 ( $^{211}\text{At}$ )

Half-life of astatine-211 ( $^{211}\text{At}$ ) is 7.21 hours, and partially decays by alpha decay (42%) into  $^{207}\text{Bi}$  (alpha particle energy is 5870 keV), while partially decays by

electron capture (58%) into  $^{211}\text{Po}$ . That  $^{211}\text{Po}$  then decays very quickly (half-life is just 0.5 seconds) into  $^{207}\text{Pb}$ , emitting a powerful alpha particle of 7450 keV:

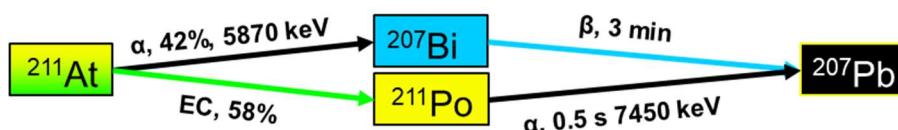
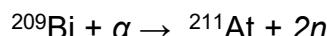


Figure 361: Decay chain of  $^{211}\text{At}$

$^{211}\text{At}$  can be made in cyclotrons by bombarding natural bismuth ( $^{209}\text{Bi}$ ) with accelerated alpha particles:



Astatine is a halogen metalloid just like iodine and cannot be labelled onto vectors using metal-ligand interactions like other alpha-emitters. Instead, nucleophilic and electrophilic substitutions need to be used like for iodine (Figure 362). For example, a SIB, prosthetic reagent that contains an organotin group could be reacted with  $^{211}\text{At}^-$  ion and it could form  $^{211}\text{At}$ -labeled SIB, which then could react very quickly with any amino group on a protein or peptide to give  $^{211}\text{At}$ -labelled protein *via* a benzamide linker:

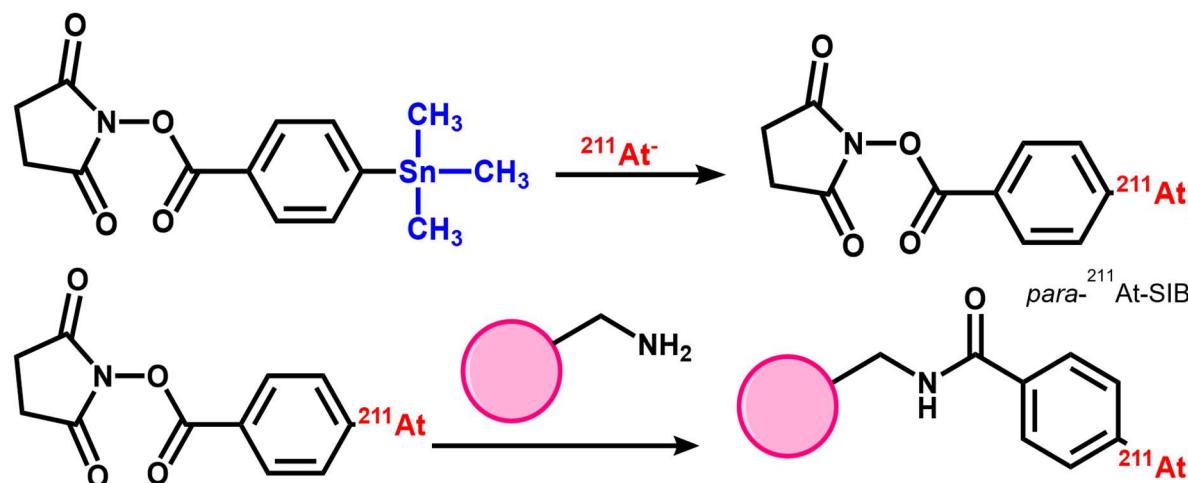


Figure 362: Labelling molecules and proteins with  $^{211}\text{At}$

## Radium-223 ( $^{223}\text{Ra}$ )

$^{223}\text{Ra}$  has half-life of 11.43 days and it decays by alpha decay into  $^{219}\text{Rn}$  which then quickly decays by the decay chain that goes through  $^{215}\text{Po}$ ,  $^{211}\text{Pb}$ ,  $^{207}\text{Tl}$  to reach stable  $^{207}\text{Pb}$ :

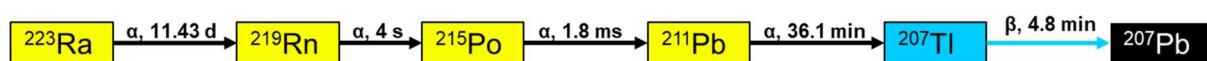


Figure 363: Decay chain of  $^{223}\text{Ra}$



This is good since the daughters are providing additional showers of alpha and beta particles in the vicinity of  $^{223}\text{Ra}$  parent, multiplying its effect. The energies of alpha particles are ranging from 5540 keV to 5747 keV.  $^{223}\text{Ra}$  can be obtained via  $^{227}\text{Ac}/^{223}\text{Ra}$  generator that makes  $^{223}\text{Ra}$  by the decay chain that goes through  $^{227}\text{Th}$ :

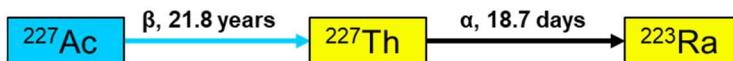


Figure 364: Formation of  $^{223}\text{Ra}$  from decay of  $^{227}\text{Ac}$

The parent for this radionuclide generator,  $^{227}\text{Ac}$  can be obtained by irradiation of natural  $^{226}\text{Ra}$  with neutrons in nuclear reactors.

Radium is an alkali earth metal, and it is located in the second group of the periodic table with other chemically similar metals, Be, Mg, Ca, Sr, Ba. Due to the chemical similarity to calcium radium in body behaves just like calcium; it has natural ability to target bones and bone cells! Therefore, it can be used without any vector, it is a vector by itself just like iodine. While iodine target tissue is the thyroid gland, for radium it is bone.  $^{223}\text{RaCl}_2$  is approved radiopharmaceutical agent (Xofigo™) for cancer metastases in bones. Due to its good availability  $^{223}\text{Ra}$  is very attractive to be used in targeted radiotherapy. However, no ligands have been found for use with radium that provides high enough stability for clinical applications. Attempts with DTPA, DOTA, and calixarene tetraacetic acid have shown that the calixarene tetraacetic acid provides the most stable complexes, but that stability was not sufficient for the clinical use. Macropa is another molecule that is being investigated as possible ligands for  $^{223}\text{Ra}$ . As an alternative to chelation, incorporation of radium isotopes into nanoparticles may provide an approach that is successful for clinical applications.

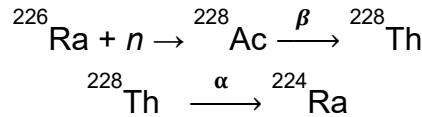
### Radium-224 ( $^{224}\text{Ra}$ )

Radium-224 ( $^{224}\text{Ra}$ ) is another medical radionuclide of radium. It has half-life of 3.63 days, it decays into  $^{220}\text{Rn}$  emitting an alpha particle of 5673 keV. The decay then goes by the decay chain that goes through  $^{220}\text{Rn}$ ,  $^{216}\text{Po}$ ,  $^{212}\text{Pb}$ , and  $^{212}\text{Bi}$ :



Figure 365: Decay chain of  $^{224}\text{Ra}$

$^{224}\text{Ra}$  can be obtained by radionuclide generator containing  $^{228}\text{Th}$ . This  $^{228}\text{Th}$  is obtained by nuclear bombardment of  $^{226}\text{Ra}$  with neutrons in a nuclear reactor:



$^{224}\text{RaCl}_2$  was used for the treatment of a rheumatic disease of bones called ankylosing spondylitis, a rare type of arthritis that causes pain and stiffness in spine.



Also,  $^{224}\text{Ra}$ -labeled  $\text{CaCO}_3$  microparticles could be used for the treatment of cancer metastases in bones.

## Actinium-225 ( $^{225}\text{Ac}$ )

Actinium is currently the most promising alpha-emitting medical radionuclide. Its half-life is approximately 10 days, and it decays into  $^{221}\text{Fr}$  by alpha emission (5776 keV), but then the decay chain goes on, includes  $^{217}\text{At}$ ,  $^{213}\text{Bi}$ ,  $^{213}\text{Po}$ ,  $^{209}\text{TI}$ ,  $^{209}\text{Pb}$ , and finally reach almost stable  $^{209}\text{Bi}$ :

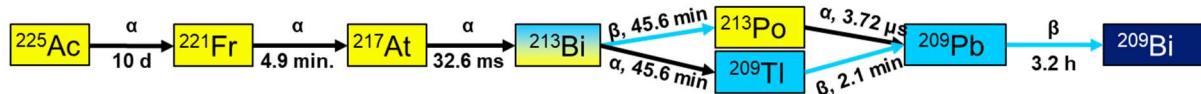
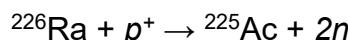


Figure 366: Decay chain of  $^{225}\text{Ac}$

The formation of  $^{213}\text{Bi}$  could be problematic, since it has a longer half-life (45.6 min) and can “wander off” into non-targeting tissues, and there give healthy cells unwanted radiation dose.  $^{225}\text{Ac}$  belongs to the neptunium-237 ( $^{237}\text{Np}$ ) decay chain, and could be extracted from high-level nuclear waste, but the quantity extracted is too little for the medical use. Another option is production by cyclotron:  $^{225}\text{Ac}$  can be made by using powerful cyclotrons, bombarding  $^{226}\text{Ra}$  with protons of 16.8 MeV:



Although  $^{225}\text{Ac}$  is very promising medical radionuclide, all the current methods for its production yield just small activities of  $^{225}\text{Ac}$ . The problem of production needs to be solved: the current production of  $^{225}\text{Ac}$  is not enough for the widespread use in targeted radiotherapy. When it comes to ligands suitable for  $^{225}\text{Ac}$  the typical ligands are DOTA and HEHA, but new one, H4Py4PA proved to offer much better complex stability.

The most promising radiopharmaceutical agent so far is  $^{225}\text{Ac}$ -DOTA-PSMA-617, where the vector is a short peptide, and it is proved powerful for the treatment of metastatic prostate cancer.

In addition, there are many  $^{225}\text{Ac}$ -containing radiotherapeutics (mostly radioimmunoconjugates) that are in clinical trials:



Fig. 367: Glowing sample of  $^{225}\text{Ac}$

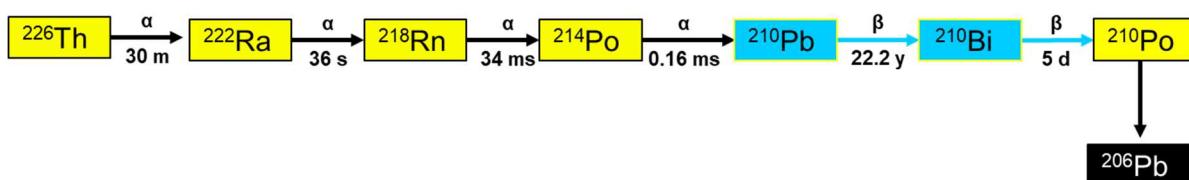
- $^{225}\text{Ac}$ -DOTA-J591, where the vector is a mAB is being tested also for the prostate cancer.
- $^{225}\text{Ac}$ -DOTA-Lintuzumab, (lintuzumab is a CD33-targeting mAB) is being tested for the treatment of acute myeloid leukaemia.
- $^{225}\text{Ac}$ -DOTA-Daratumumab is another radioimmunoconjugate, being tested for multiple myeloma.
- $^{225}\text{Ac}$ -H4Py4PA-trastuzumab is tested for the treatment of breast cancer,



- $^{225}\text{Ac}$ -DOTATATE for neuroendocrine tumours,
- $^{225}\text{Ac}$ -HEHA-201B for lung cancer
- $^{225}\text{Ac}$ -DOTA-Girentuximab for kidney cancer.

## Thorium-226 ( $^{226}\text{Th}$ )

Thorium-226 ( $^{226}\text{Th}$ ) is one of two important thorium medical radionuclides. Its half-life is just 30.6 minutes, decays by alpha emission into  $^{222}\text{Ra}$ , and the energy of the alpha particles is 6306 keV. The decay chain then goes to the stable  $^{206}\text{Pb}$ , via  $^{218}\text{Rn}$ ,  $^{214}\text{Po}$ ,  $^{210}\text{Bi}$ ,  $^{210}\text{Po}$  until it reaches stable  $^{206}\text{Pb}$ :



Very short half-lives of the three consecutive daughters and their alpha emissions make  $^{226}\text{Th}$  a powerful and potentially promising radiotherapeutic.  $^{210}\text{Pb}$  has half-life of 22.2 years but is not very radiotoxic therefore does not present a problem.  $^{226}\text{Th}$  can be obtained by using  $^{230}\text{U}/^{226}\text{Th}$  radionuclide generator:

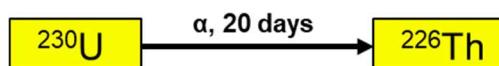


Figure 368:  $^{230}\text{U}/^{226}\text{Th}$  radionuclide generator

The  $^{230}\text{U}$  itself can be obtained from  $^{230}\text{Pa}$  which can be made by bombarding natural thorium ( $^{232}\text{Th}$ ) with protons:



## Thorium-227 ( $^{227}\text{Th}$ )

Another important thorium medical radionuclide is thorium-227 ( $^{227}\text{Th}$ ). It has half-life of 18.7 days and decays by alpha emission into  $^{223}\text{Ra}$ .  $^{223}\text{Ra}$  has a bit shorter half-life of 11.43 days and therefore can be redistributed after leaves the ligand (it can “wander-off” into a non-targeted tissue and give healthy cells radiation dose). However, since radiotoxicity profile of  $^{223}\text{Ra}$  is very well known, and it goes into bones, it is not such a danger.



Figure 369:  $^{230}\text{U}/^{227}\text{Th}$  radionuclide generator

$^{227}\text{Th}$  can be obtained by using  $^{227}\text{Ac}/^{227}\text{Th}$  generator, the same one that makes  $^{223}\text{Ra}$ . But in this case  $^{227}\text{Th}$  is “milked out” before it decays into  $^{223}\text{Ra}$ . The ligands of choice for  $^{227}\text{Th}$  and  $^{226}\text{Th}$  are DOTA and (Me-2,3-HOPO)<sub>4</sub>. There are some  $^{227}\text{Th}$ -based radiotherapeutics in the clinical trials. For example, there is  $^{227}\text{Th}$ -DOTA-



Trastuzumab, aimed at treatment of breast cancer, and  $^{227}\text{Th}$ -DOTA-rituximab that could be used for the treatment of lymphoma. Additionally, there are many other various types of cancer that could be targeted with  $^{227}\text{Th}$ .

## Fermium-255 ( $^{255}\text{Fm}$ )

Fermium-255 ( $^{255}\text{Fm}$ ) is the only transuranic element among the medical radionuclides and is extremely exotic. This radionuclide is in this list because its nuclear properties make it a good candidate for medical radiotherapeutic, however, nobody ever made enough of it for any experiment even just pre-clinical with cell cultures.

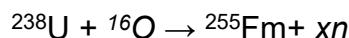
Nevertheless, if one day scientists are able to make it in large quantities it could be used in targeted radiotherapy. Its half-life is 20 hours, and it decays by alpha emission (energy of the alpha particles is 7054 keV) into californium-251 ( $^{251}\text{Cf}$ ). This daughter,  $^{251}\text{Cf}$  has quite long half-life of almost 900 years, therefore, is not of great radiotoxicity concern.

Obtaining  $^{255}\text{Fm}$  is a significant obstacle. It could be obtained by using  $^{255}\text{Es}/^{255}\text{Fm}$  radionuclide generator:

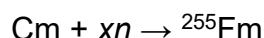


**Figure 370:**  $^{255}\text{Es}/^{255}\text{Fm}$  radionuclide generator

Einsteinium-255 ( $^{255}\text{Es}$ ) could be made by bombarding  $^{252}\text{Cf}$  with many neutrons. Another option could be bombardment of natural uranium isotope,  $^{238}\text{U}$  with heavy ions of oxygen:



The third option could be bombardment of curium (Cm) with multiple neutrons in a high flux nuclear reactor:



Unfortunately, currently nobody has enough of  $^{255}\text{Fm}$  for radiopharmaceutical experiments. If the production of  $^{255}\text{Fm}$  is solved, and if it could be obtained in GBq quantities then  $^{255}\text{Fm}$  could make a good medical radionuclide for targeted radiotherapy.

## Chapter XVI - The rest: Seldom used medical radionuclides

So far, we have reviewed radiochemistry, labelling and use of the most important medical radionuclides. These include the key radionuclides used in SPECT imaging, PET imaging and for the targeted radiotherapy (TRT):

- SPECT:**  $^{99m}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{123}\text{I}$ ,  $^{124}\text{I}$
- PET:**  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$ ,  $^{15}\text{O}$ ,  $^{89}\text{Zr}$ ,  $^{123}\text{I}$ ,  $^{68}\text{Ga}$ ,  $^{86}\text{Y}$
- TRT:**  $^{131}\text{I}$ ,  $^{90}\text{Y}$ ,  $^{177}\text{Lu}$ ,  $^{149}\text{Tb}$ ,  $^{212}\text{Pb}$ ,  $^{212/213}\text{Bi}$ ,  $^{211}\text{At}$ ,  $^{223/224}\text{Ra}$ ,  $^{225}\text{Ac}$ ,  $^{226/227}\text{Th}$ ,  $^{255}\text{Fm}$

The Periodic table is shown where these radionuclides are painted based on the role they have in nuclear medicine: some of them are for SPECT only like,  $^{99m}\text{Tc}$  and  $^{111}\text{In}$ , some of them are just for PET, some are for therapy only and some are mixed (one isotope is for one role and another is for another). For example,  $^{123}\text{I}$  is for SPECT only while  $^{131}\text{I}$  is mostly for the targeted therapy.

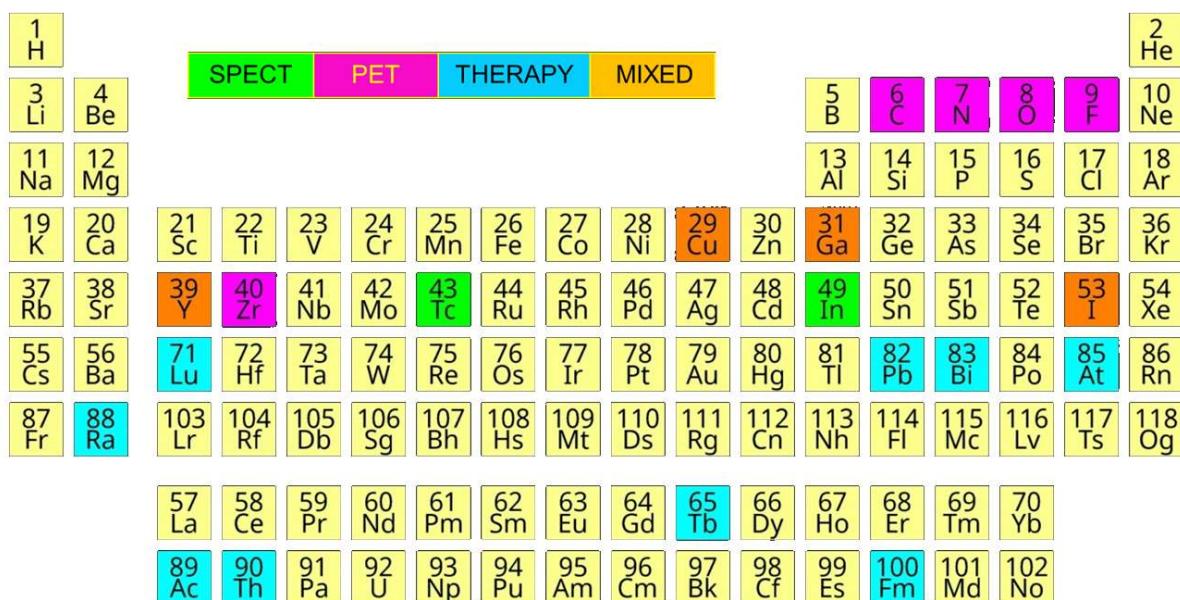


Figure 371: Medical radionuclides we reviewed so far

So, why not review all of them? Some radionuclides were once heavily used but are now obsolete (are not used any more). Some radionuclides are used rarely, have only one radiopharmaceutical agent. Some are very rarely used even in experimental imaging (pure exotic). We have been focusing on those currently used as well as those promising for the future. However, some are worthy of (honourable) mentioning!



## S-Block Elements (alkali and alkali earth metals)

The S-block elements are those belonging to the first and the second group of the periodic table and these are alkali (the first) and alkali earth metals (second). There are four medical radionuclides worthy of mentioning, potassium-38 ( $^{38}\text{K}$ ), rubidium-82 ( $^{82}\text{Rb}$ ), rubidium-82m ( $^{82\text{m}}\text{Rb}$ ) and strontium-89 ( $^{89}\text{Sr}$ ).

$^{38}\text{K}$  has half-life of just 7.64 minutes, it is a positron emitter and can be used in the form of  $^{38}\text{KCl}$  for PET imaging of myocardial perfusion (flow of blood through heart blood vessels).  $^{82}\text{Rb}$  and  $^{82\text{m}}\text{Rb}$  have half-life of 1.26 min and 50.56 days, respectively. They are positron emitters and are used in the form of their rubidium chloride salt in PET imaging of myocardial perfusion and ischemia (deficit of oxygen in heart).  $^{89}\text{Sr}$  has half-life of 32.41 hours, is a beta-emitter, and is used in radiotherapy, for the palliative treatment of bone metastases.

## P-Block Elements

P-block elements are those from the 13<sup>th</sup> to the 17<sup>th</sup> group, including those that are non-metals and metalloids. Here, interesting medical radionuclides worthy of mentioning are tellurium-201 ( $^{201}\text{TI}$ ), phosphorous-32 ( $^{32}\text{P}$ ), selenium-73 ( $^{73}\text{Se}$ ) and bromine-76 ( $^{76}\text{Br}$ ).  $^{201}\text{TI}$  has half-life of 3 days, it decays by electron capture, hence is gamma only emitter and was previously heavily used in SPECT imaging of myocardial perfusion. However, today its role is mostly marginal.  $^{32}\text{P}$  has half-life of 14.3 days, it is a pure beta-emitter, and in the form of sodium phosphate it is sometimes used for the radiotherapy of polycythemia vera (this is a type of blood cancer) and for the therapy of essential thrombocythaemia (it is a rare blood disorder). Additionally, it can be used for the palliative treatment of bone metastases. Today these applications are mostly obsolete. However,  $^{32}\text{P}$  is still today very much used in biology, molecular biology, plant science, and genetics and in medicine as a tracer for scientific research, usually in the form of nucleotides.  $^{73}\text{Se}$  has half-life of 7.15 hours, is a positron emitter, and as L- $^{73}\text{Se}$ -selenomethionine it was used for the PET imaging of amino acid metabolism. Finally,  $^{76}\text{Br}$  has half-life of 16.2 hours, is a positron emitter, and could be used for the PET imaging.

## Noble gases

Noble gasses are considered to be part of p-block elements but in this case, we will consider them separately. There we have krypton-81m ( $^{81\text{m}}\text{Kr}$ ) and xenon-133 ( $^{133}\text{Xe}$ ).  $^{81\text{m}}\text{Kr}$  has half-life of just 13 seconds; it decays by internal conversion and is gamma emitter. It was used in SPECT imaging of lung ventilation studies.  $^{133}\text{Xe}$  on the other hand has much longer half-life, 5.25 days, it is beta-emitter, and can be used in the SPECT imaging in lung perfusion studies (how well blood flows through lungs).



## D-Block Elements (Transition metals)

The D-block elements are in fact transition metals. Here we have many of those, but we will select several for this review. There are scandium-43 ( $^{43}\text{Sc}$ ), scandium-44 ( $^{44}\text{Sc}$ ), zinc-62 ( $^{62}\text{Zn}$ ) ruthenium-97 ( $^{97}\text{Ru}$ ), rhenium-186 ( $^{186}\text{Re}$ ) and rhenium-188 ( $^{188}\text{Re}$ ), iridium-192 ( $^{192}\text{Ir}$ ) and gold-198 ( $^{198}\text{Au}$ ).  $^{43}\text{Sc}$  and  $^{44}\text{Sc}$  have very similar half-lives of 3.89 hours and 3.97 hours, they are both positron emitters, and are used for PET imaging. Another positron emitting radionuclide for PET imaging is  $^{62}\text{Zn}$  who has half-life of 9.2 h.  $^{97}\text{Ru}$  has half-life of 2.8 days, decays by EC, emits gamma rays only, and is used for SPECT imaging. The two isotopes of rhenium,  $^{186}\text{Re}$  and  $^{188}\text{Re}$  have different half-lives; the first has 3.72 days and the second one 17.00 hours but are both beta-emitters and are used for radiotherapy. Their chemistry is almost identical to the one of  $^{99\text{m}}\text{Tc}$ , so the labelling methods could be the same. Both could be used in radiotherapy.  $^{192}\text{Ir}$  has half-life of 73.83 days, is another beta-emitter for radiotherapy.  $^{198}\text{Au}$  has half-life of 2.7 days, it is also a beta-emitter, but its use in radiotherapy would be a bit different: it was proposed that  $^{198}\text{Au}$ -doped gold nanoparticles bearing some protein vectors (such as mABs) could be used for the therapy of cancer.

## Lanthanides

And the last group to consider are lanthanides. Here we have samarium-153 ( $^{153}\text{Sm}$ ), holmium-166 ( $^{166}\text{Ho}$ ), and erbium-165 ( $^{165}\text{Er}$ ).  $^{153}\text{Sm}$  has half-life of 46.5 hours, it is a beta-emitter, but also emits gamma rays. It is used in SPECT imaging as well as radiotherapy in the form of  $^{153}\text{Sm}$ -lexidronam (a complex of  $^{153}\text{Sm}$  and a ligand ethylene diamine tetramethylene phosphonate, EDTMP) for the palliative treatment of pain associated with bone metastases.  $^{166}\text{Ho}$  has half-life of 26.8 hours, it is a high energy beta-emitter, and is used in radiotherapy in the form of a radioimmunoconjugate  $^{166}\text{Ho}$ -DOTA-bevacizumab for the treatment of tumours expressing vascular endothelial growth factor A (VEGF-A). Also,  $^{166}\text{Ho}$ -radiolabeled chitosan nanoparticles have also been explored for potential radiotherapeutic applications. Finally,  $^{165}\text{Er}$  has half-life of 10.4 hours, decays by electron capture, emits gamma rays only, and is used for SPECT imaging.



At the end we can have a look into Figure 272 showing special Periodic table of medical radionuclides. Yet, even in this chapter not all are shown: there are more potential radionuclides as well as those that have been tried at least once, at least in experiments:

**Figure 372: Periodic Table of medical radionuclides**

## Chapter XVII - Radiation Protection in Radiopharmaceutical Facilities

### Radiation dose

What is the radiation dose? It is an energy deposited in an irradiated medium by ionising radiation per unit of mass. Radiation dose can be expressed with a formula used to calculate a radiation dose if all these parameters are known. This equation is valid and simple only for gammas and X-rays:

$$D(\gamma, X) = \Gamma \times \frac{A}{d^2} \times t$$

For betas and alphas calculation is more complicated and the values are not calculated that easy. D stands for radiation dose from gammas or X-rays (Gy)  $\Gamma$  (Greek capital G) is radiation energy, gamma radiation coefficient and this is a constant typical for each radionuclide, is in  $\text{Sv.m}^2/\text{Bq.hour}$ . "A" stands for activity of radiation source in Becquerels (Bq),  $d$  for distance from the radiation source in meters (m) and  $t$  is the time of irradiation in hours. If we leave the time term then it is an equation not for dose but for dose per hour, and this is a dose rate usually expressed in  $\text{Sv/hour}$ :

$$\dot{D} = \Gamma \times \frac{A}{d^2}$$

$\text{Sv/hour}$  is a usual unit we measure in front of any radiation source using a dosimeter instrument.

Radionuclide	Gamma radiation coefficient ( $\Gamma$ ) ( $\mu\text{Sv.m}^2/\text{h. MBq}$ )	Contact with 5 ml syringe 1 MBq ( $\mu\text{Sv / minute}$ )
<sup>11</sup> C	<b>0.1596</b>	<b>49</b>
<sup>13</sup> N	<b>0.1596</b>	<b>49</b>
<sup>15</sup> O	<b>0.1596</b>	<b>49</b>
<sup>18</sup> F	<b>0.1547</b>	<b>48</b>
<sup>67</sup> Ga	<b>0.019</b>	<b>7</b>
<sup>68</sup> Ga	<b>0.1789</b>	<b>58</b>
<sup>90</sup> Y	<b>N/A</b>	<b>725</b>
<sup>99m</sup> Tc	<b>0.0211</b>	<b>6</b>
<sup>111</sup> In	<b>0.0867</b>	<b>20</b>
<sup>123</sup> I	<b>0.044</b>	<b>10</b>
<sup>131</sup> I	<b>0.0613</b>	<b>19</b>
<sup>177</sup> Lu	<b>0.0076</b>	<b>1.3</b>
<sup>223</sup> Ra (and progeny)	<b>0.0534</b>	<b>12.5</b>

Table 7: The major medical radionuclides and their gamma radiation constants

The Table 7 shows some major medical radionuclides and their gamma radiation constants. On the very right is the column depicting what dose of radiation our hand will receive if we hold a 5 ml syringe with 1 MBq of each radionuclide during one minute of work. What we can see is that some radionuclides present higher some lower risk, but it seems that <sup>90</sup>Y that emits only beta particles are in fact the highest risk.



## “Types “of radiation doses

There are three main “types” of doses:

1. **Absorbed dose**, D, is the actual physical dose of radiation deposited and its unit is grey, or more often miligrays and micrograys. Absorbed dose in greys are usually used to describe radiation dose absorbed by non-live items (like when food or medical products are irradiated to sterilize them).
2. **Equivalent dose**, H, is, on the other hand, absorbed dose where biological effectiveness of the radiation is considered, which depends on radiation type and energy. It means radiation will have different effect on biological tissues depending on is it alpha, beta, gamma, or neutron radiation. To make it right we must multiply absorbed radiation with factor  $W_R$ . This factor  $W_R$  is 1 for gammas, betas, and x-rays so we can make equivalence between D and H: therefore, for betas and gammas absorbed dose is the same as the equivalent dose. On the other hand,  $W_R$  for alpha particles is 20, but this has sense for internal irradiation and radiotherapy only, however  $W_R$  factor for neutrons depend on their energy and goes from 5-20. Different to absorbed dose, the unit is Sievert, Sv, but much more are used millisieverts and microsieverts.

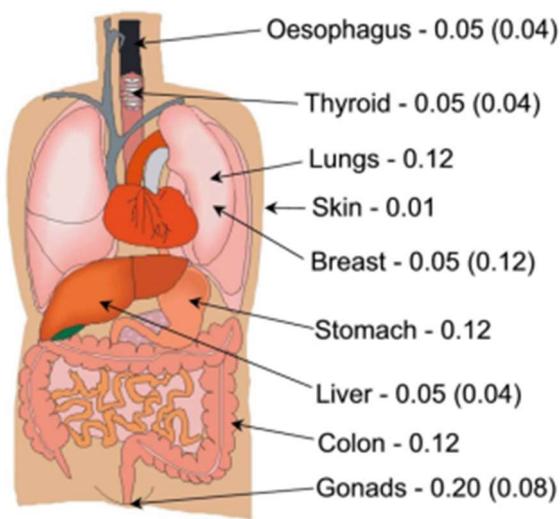
$$H = D \times w_R$$

3. **Effective dose**, E is on the other hand is equivalent dose that is tissue weighted with another factor  $W_T$ . The absorbed dose is the physical quantity while equivalent and effective dose are calculated quantities. We also have something that is called operative doses, and those are doses that are actually measured by dosimeters in Sv. So, we have ambient dose equivalent, personal dose equivalent while the badges and stalls are measuring whole body dose, skin dose, finger dose and eye dose.

$$E = H \times w_T$$

The effective dose (E) is the equivalent dose (H) weighted (multiplied) with the tissue weighting factor,  $W_T$ . Each organ has its  $W_T$ , and it depends on how sensitive the organ is (organ is more sensitive if its cells are multiplying more often). The  $W_T$  for the whole-body dose is 1 which means if the whole body was equally irradiated than we can say that effective dose is the same and the equivalent dose. But various organs have been affected differently with the visceral organs being mostly affected and the most sensitive to ionising radiation. This is the reason why if workers that work with significantly high dose rates of ionising radiation wear special lead aprons to cover their trunks and necks while extremities and head are allowed to stay opened.

Organ	$W_T$
Gonads	0.08
Red Bone Marrow	0.12
Colon	0.12
Lung	0.12
Stomach	0.12
Breasts	0.12
Bladder	0.04
Liver	0.04
Oesophagus	0.04
Thyroid gland	0.04
Skin	0.01
Bone surface	0.01
Salivary glands	0.01
Brain	0.01
Remainder of body	0.12

Table 8 and Figure 373:  $W_T$  factors of various body organs

The Figure 374 below summarises sources of external radiation giving out some radiation – it can be monitored using some instruments where we get operational quantities via instrument calibration, on the other hand physical quantities are used to calculate protection quantities that are then compared with operational quantities.

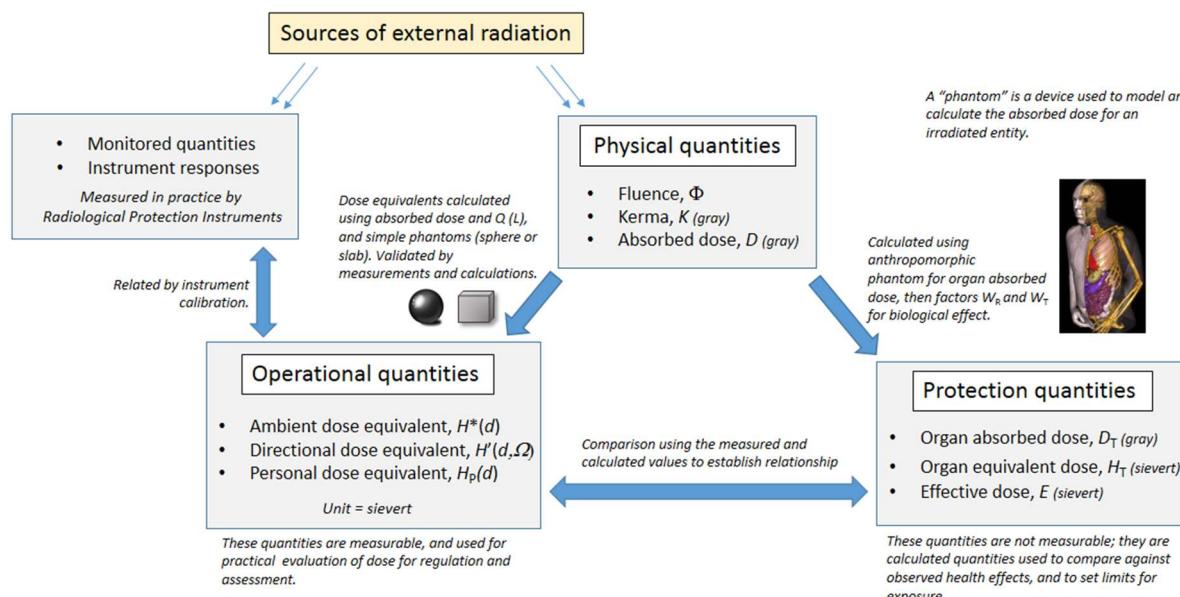


Figure 374: Dose quantities in SI units for external radioprotection

Figure 375 shows: absorbed dose in greys (Gy) that is joule per kilogram is the energy absorbed by irradiated sample of matter, and if we multiply this dose with  $W_R$  dimensionless factor we get the equivalent dose in Sieverts, which is again joule per kg, and it reflects biological effects of radiation R with weighing factor  $W_R$ . And finally, if we then multiply equivalent dose with the tissue weighting factor then we get the effective dose: this is either a whole-body dose to all tissues or dose for specific organs and this is also in Sieverts meaning joule per kg.

Quantity	Absorbed dose $D_T$	$W_R$	Equivalent dose $H_T$	Effective dose $E$	
	gray (Gy)		sievert (Sv)	Whole body dose to all tissue $= E$	Organ dose to tissue $T_1$ Organ dose to tissue $T_2$ Organ dose to tissue $T_3$ $= E$
Derivation	joule/kg	Dimensionless factor	joule/kg	Dimensionless factor	joule/kg
Meaning	Energy absorbed by irradiated sample of matter - a physical quantity.		Biological effect of radiation type R with weighting factor $W_R$ . Multiple radiation types require calculation for each, which are then summated.		Biological effect on tissue type T having weighting factor $W_T$ Partial irradiation Effective dose = summation of organ doses to those parts irradiated Complete (uniform) irradiation If whole body irradiated uniformly, the weightings $W_T$ summate to 1. Therefore, Effective dose = Whole body Equivalent dose

Figure 375: Protection dose quantities in SI units

## Protection from radiation

How do we protect ourselves, others, and the public from radiation?

There are two types of radiation risk from which we must protect ourselves, others, and the public environment; one is external irradiation which means protection from long distance gamma and x-rays coming out of radioactive substances and cyclotrons. Another is protection from contamination with radioactive substances and a risk of getting them inside of our body, and this is done by contamination control. Protection from radiation and extent of measures we must take to protect ourselves is based on the principle we call ALARA. ALARA is an acronym from a phrase:

### As Least As Reasonably Achievable

It says that the dose received by a worker should be as least as reasonably achievable. It sounds good but was often led to overkill in radiation protection measures. The new principle (ALARP) was recently introduced, is very similar and goes:

### As Least As Reasonably Practical

This means that dose received should be as least as reasonably practical. You can notice the key difference: something is achievable but is not practical. Now we tend to be more practical. And some are also adding this disclaimer: taking into consideration economic and social context. This means that we must adjust the radiation protection measured based on the risk we are encountering. The first thing we do is to assess the risk; we have to get idea what is it we are working with, what is the risk and how to mitigate the risk.

## Protection from external irradiation

How do ensure protection from external irradiation from gammas and x-rays?

When some think of radiation protection, the first thought is about shielding. But shielding comes secondary. The first is to use the parameters and factors contributing to overall dose, those that are part of the dose equation:

$$D(\gamma, X) = \Gamma \times \frac{A}{d^2} \times t$$

If we can modify **distance** from the source of ionising radiation, **time** spent being exposed, **activity** we are working with, or **radiation energy**, then these should be the first options to reduce the dose.

Distance is the most important: dose is falling with the distance squared and simple advice is to stay away from the source as much as it is possible. And when it is not possible try to use tools: tongs, tweezers or remote manipulators. Regarding the time a worker should work with radioactive sources as less time as possible, do not expose himself if there is no need, be quick, but careful, rehears technique if necessary. When it comes to activity and radiation energy, advice is often to use the least quantity of radioactive materials, work with smaller batches, if possible. But when preparing radiopharmaceutical agents this is not often possible: radioactivity one encounters in nuclear medicine and radiopharmaceutical chemistry could be much larger than in many other areas of radiochemistry (we are talking in GBq of radioactivity).

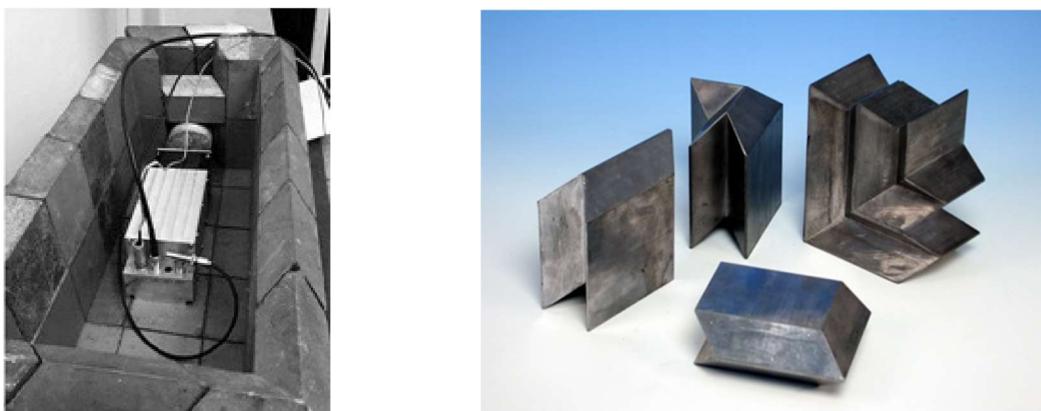
### *Shielding and shielding materials*

Using the distance, time, and other simple measures is sometimes enough to make your work safer. But in most of the cases is not enough, therefore some shielding must be used. If we are about to use a radionuclide that emits only beta particles, then the best shielding material is the dense acrylic polymer (also known as Perspex or Plexiglas)

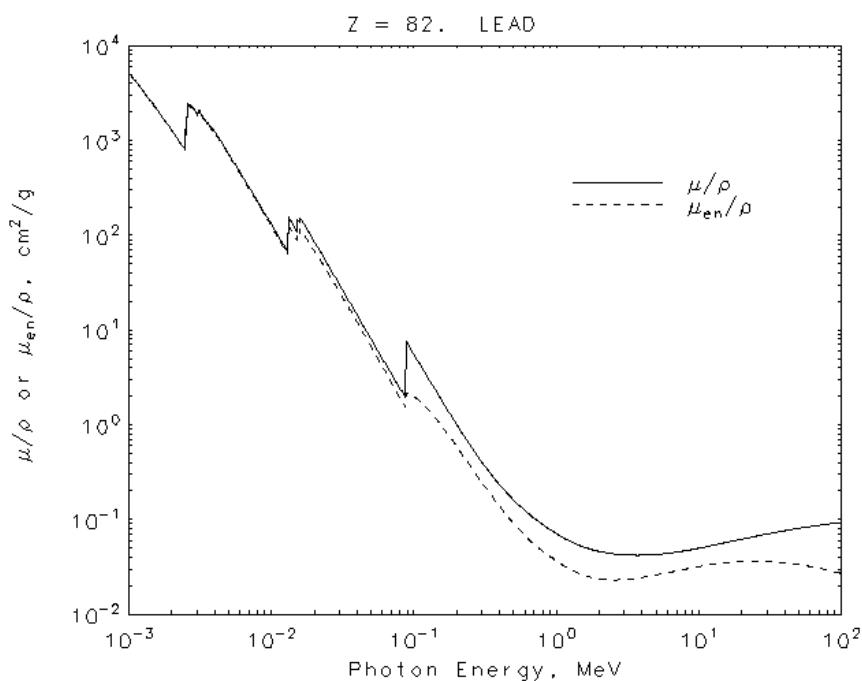


Figure 376: Shields, cabinets and boxes made of 1 cm thick acrylic polymer

Many acrylic shields and cabinets (Figure 376) are useful for shielding against energetic beta particle such the ones emitted by  $^{90}\text{Y}$  or  $^{177}\text{Lu}$ . An acrylic shield of 1 cm thickness blocks most of beta particles, while 2 cm stops all beta particles. Although metals have better stopping power, it is not good to directly use a metal for shielding against energetic beta particles: when they hit metals bremsstrahlung X-rays are formed, and these are much harder to be stopped. If we have mixed beta and gamma radiation the best option would be firstly to use 1 cm of acrylic for betas and then lead for gammas. Acrylic is the material from which not only shields are made, but also many other items for the work with betas.



**Figure 377:** Lead bricks in form of chevrons (to ensure overlapping and prevent ray leakage) are used to make “lead castles” to enclose sources of gamma radiation.



**Figure 378:** Attenuation coefficient of lead dependent on the wavelength of the gamma radiation: more photon is energetic lower is the attenuation coefficient. This means that the shielding is the most efficient for the photons less than 100 keV

When we have a strong gamma ray source, for example  $^{18}\text{F}$ , then acrylic is not efficient at all. We need something better: a very dense metal lead is the most



famous material for the shielding against gamma radiation. It has high attenuation coefficient (Figure 378), and the required shielding thickness can be easily calculated.

For most of the cases 4 cm of lead thickness (Figure 377) is enough to block radiation and significantly decrease the risk to acceptable levels. However, if isotopes with very penetrating radiation are used, then more lead is needed. Lead is used to make chevron bricks (Figure 377) from which we can then build so called “lead castles”. Also, lead is used to make pots, sheets, beads, and other items for shielding.

Shielding can be calculated, for example we can calculate what will be the dose rate of there is a layer of shielding material such as lead (Pb) that has known value of linear attenuation coefficient ( $\mu_{Pb}$ ) and known thickness ( $x_{Pb}$ ).

$$\dot{D} = \Gamma \times \frac{A}{d^2} \times (B \times e^{-\mu_{Pb} x_{Pb}})$$

Linear attenuation coefficient is a unique characteristic of every material, and it says how well it attenuates (absorbs) photons per unit of thickness. This characteristic depends on the material itself, it is an inherent property of every material, including water and air: each material has different  $\mu$  for different photon wavelength (the actual values can be found in handbooks, for lead see Figure 378). B is a dimensionless number that adds correction to the shielding value and makes it more realistic. It takes into account realistic geometries and additional complicated physical phenomena regarding the interaction of photons with matter. It is a different value for each different material, different gamma-ray wavelength, and thickness (values can be found in handbooks). However much more often we use so called Half Value Layer (HLV): it says how thick a layer of lead has to be used to decrease the dose rate by half and can be easily calculated from  $0.692/\mu$ ; in fact, is a constant for a given material and photon wavelength:

$$HLV = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$$

Radionuclide	Major photon energies (keV)	HVL Pb (mm)
$^{11}\text{C}$ , $^{13}\text{N}$ , $^{15}\text{O}$	511 (200%)	5.5
$^{18}\text{F}$	511 (194%)	5.5
$^{67}\text{Ga}$	93 (38%), 184, (21%), 300 (17%)	0.86
$^{99m}\text{Tc}$	140 (89%)	0.23
$^{111}\text{In}$	23 (68%), 171 (91%), 245 (94%)	0.257
$^{123}\text{I}$	27 (71%), 159 (83%)	0.067
$^{125}\text{I}$	~27–35	0.021
$^{131}\text{I}$	364 (81%)	3.0

Table 9: Half Value Layer (HLV) of lead in mm for stopping gamma radiation from various medical radionuclides

Table 9 shows, for each medical radionuclide having some gamma emission, either direct or indirect (from positrons) what should be the half-value layer (HVL) for a given set of various photon energies emitted by medical radionuclides. Taking into account that any material, no matter how dense it was, shields only by exponential function ( $e^x$ ), one must understand that lead or any other material can never completely and fully block the gamma radiation, but can rather decrease it to the acceptable and safe level.

There are many items in radiopharmaceutical chemistry and nuclear medicine that are made of lead, for example, pots, vials, and syringes are lead-shielded (Figure 379). Very special vials and syringes are used for radiopharmaceuticals (for storing, transferring, transporting, and applying), however in many cases shielding material of choice for syringes is not lead but another very dense metal and that is tungsten (W) as shown in the Table 10.

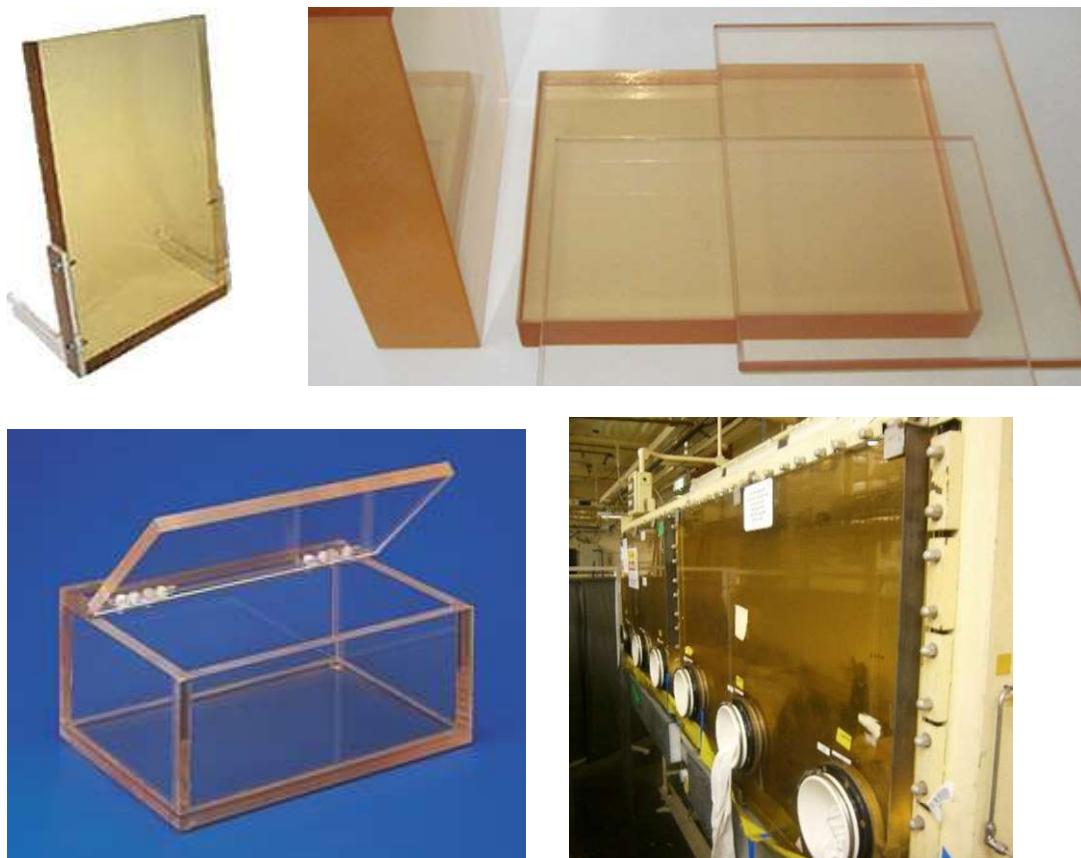


Figure 379: Various pots and syringes made with lead shielding for the storage and application of radiopharmaceuticals

Radionuclide	Syringe shielding	Vial shielding
$^{99m}\text{Tc}$	2 mm tungsten	7 mm lead
$^{18}\text{F}$	8 mm tungsten	25 mm lead
$^{90}\text{Y}$	10 mm plastic or 5 mm tungsten (to reduce associated bremsstrahlung)	10 mm plastic or 5 mm tungsten (to reduce associated bremsstrahlung)

Table 10: Comparison of tungsten and lead shielding efficiency: tungsten shields better and more efficiently therefore less is needed for shielding

There is also a material called leaded acrylic (Figure 380). It is a combination of two previous: acrylic that contains lead and it is translucent though has a dark yellow tinge. It works very well for weak x-rays and low energy gamma rays. However, more energetic photons cannot be stopped by this material, since it cannot contain very significant concentrations of lead, and usually has equivalent of no more than 0.4 mm of lead per cm of shield thickness. It is excellent for weak gammas, like from  $^{125}\text{I}$ , and can be used in nuclear chemistry for shielding against various actinides with a weak gamma emission. Gamma rays from positrons are on the other hand stronger and harder to stop, another material is needed.

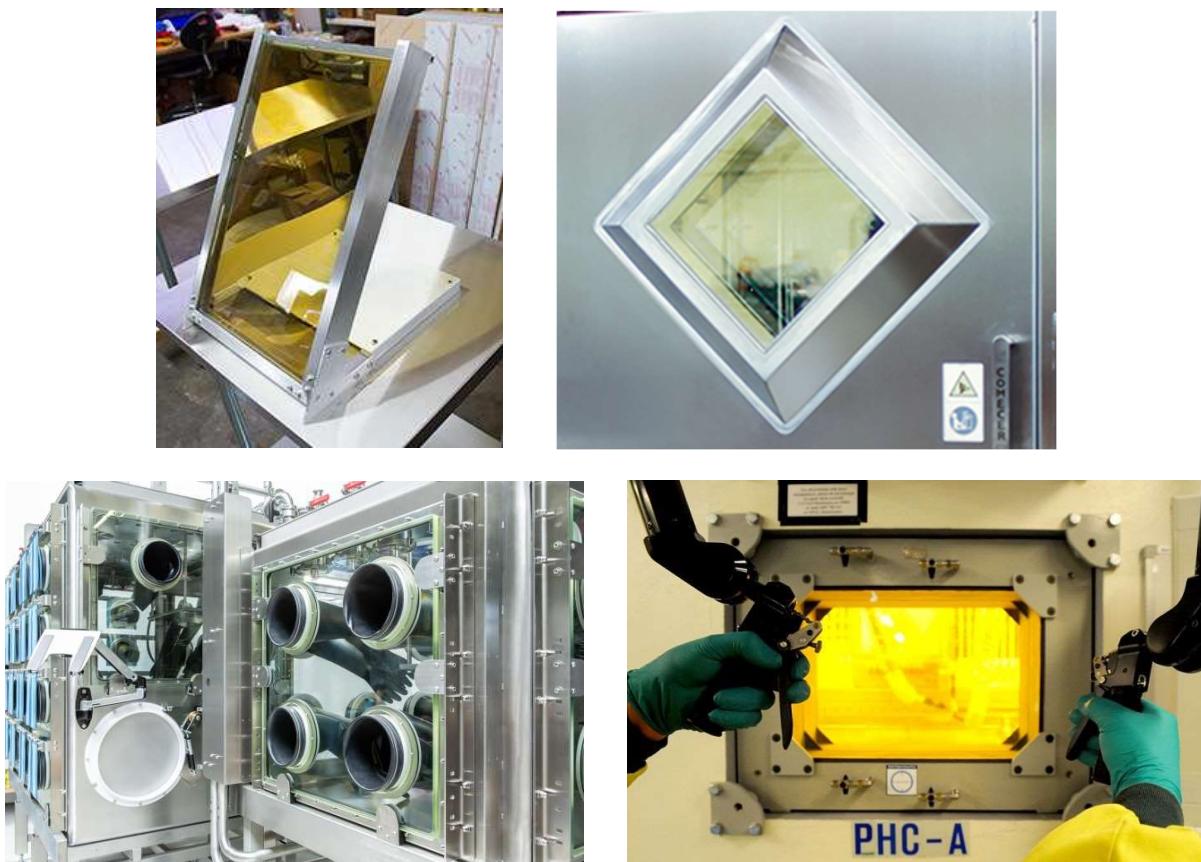


**Figure 380: Leaded acrylic as the material of choice for both beta radiation and weak gamma**

For stronger gamma emitters a better transparent shielding is needed and there we used leaded glass (Figure 381). It contains much more lead, approximately 3 mm of lead equivalent per cm of glass thickness. This is good enough for many demanding shielding, and therefore it is used in high-end facilities to make transparent windows in the nuclear industry, research, for gloveboxes, but also make windows of a hot cell in the radiopharmaceutical facility.

Concrete is another very good material for shielding, has much lower linear attenuation coefficient, but layer is much thicker (and also has structural role). In addition, the lower linear attenuation coefficient of concrete can be significantly improved if minerals and stones containing heavy metals such as barium (in the form of mineral baryte) or bismuth or are added. It is usually used for shielding against

gammas in large installation. For example, it is used to make thick, 2-meter shielding around cyclotrons in the form of cyclotron vault/bunker: while working cyclotron emits very intense gamma rays that could immediately give person a deadly dose! Therefore, cyclotrons are always surrounded with very thick wall of concrete (2 m) and even layers of lead!



**Figure 381:** Windows and shielding made of leaded glass, special gloveboxes and hot cells have windows made of leaded glass.

### ***Shielding and protection against external radiation in a radiopharmaceutical facility***

In modern radiopharmaceutical facilities all radiochemical and radiopharmaceutical operations and reactions, from pre-processing of radioactive precursors to the final purification of ready-made radiopharmaceutical agent are performed in as specially designed and constructed hot cell (see Figure 86-88). These are special forms of cabinets that are very heavily shielded with lots of lead and other shielding materials, they are usually made of stainless steel and all equipment is placed insides. Hot cells are usually also very clean inside, they are constantly ventilated with purified air to secure sterile conditions, while all operations are guided and controlled remotely by a computer. There hot cells are made to shield from very intense gamma radiation and are therefore the key radiation protection equipment for the work with radioactive materials in any radiopharmaceutical facility.

Hot cells are often equipped with special devices called remote manipulators (Figure 382). They are used when radiation is so intense that hands would receive too much radiation dose over the course of routine working time. There remote manipulators can be completely manual or can be aided by pneumatic power systems. Today, robotic arms can be remotely controlled by operator in order to perform all highly risky tasks.



**Figure 382:** Red arrow shows remote manipulators that are allowing handing of items inside the hot cell

The area monitoring system (see Figure 115 right) is the most important radiation protection system in the radiopharmaceutical facility. It is a system of small static dose rate monitors that are located at the strategically important places and corners. They are monitoring ambient gamma dose rate all the time (24/7) like security cameras. System is recording the measured dose rate and considers natural background dose and identifies possible incidents in the real time: alarm switches on if the dose rate crosses certain threshold. Is used to detect weakness in the radiation protection system and to ensure compliance with the safety rules: it gives statistical analysis of where spikes of gamma radiation usually are appearing and when.

Despite all the shielding and staying away and all other measures, radiation workers, especially in radiopharmaceutical facilities inevitably get some dose of ionising radiation, especially the dose into hand and finger. This dose must be strictly monitored, because there is an annual limit how much of ionising radiation a radiation worker is allowed to get over one year. It is 20 mSv of whole-body dose and 500 mSv of the dose into hand and fingers. It is very important that all of radiation workers when working with radioactive materials are wearing some kind of



personal dosimeters (see Figure 112) that record dose received during certain period of time, usually one month. The simplest are the film badges: they are designed to measure the whole-body dose and the skin dose. Wearing them won't prevent worker from getting the dose but will measure dose worker gets. Other types are finger stalls and rings for those workers who are likely to receive significant finger or hand dose. Workers in radiopharmaceutical chemistry usually have to wear those finger stalls. Another type is a personal electronic dosimeter that instantaneously measures, and records dose rates and total dose received. They are very useful when monthly personal dosimeter is not good enough and we need to measure the dose received in the real time.

### Control of contamination

Contamination is the unwanted spread of radiochemicals (for example radiopharmaceutical agents or their precursors) and other spreadable radioactive materials outside the designated containment or contained area.

It can be self-contamination, where we contaminate ourselves and this is then a risk for us. But also, we may contaminate our working place. This happens due to tiny splashes, spillages, powder spillage or with dirty gloves. This creates a risk not only for us, but also for our colleagues, and everyone in our working place. Once contamination starts spreading, we can find it almost everywhere. Contamination by using dirty gloves or cross-contaminations can be especially problematic since it spreads *via* typical common points everyone touches such as taps, switches, knobs, etc.

Finally, there can be contamination of public areas, and this becomes environmental risk, and a risk for the public. It happens in the case of severe incidents, or even in the case of a minor incident, the contamination may spread *via* footwear. But for a larger contamination of a public area to happen, the most probable scenario is an accident during transportation, or an accident involving radioactive waste.

In some cases, radioactive contamination may find a way to enter the human body (Figure 383). It can happen through skin, usually with splashes, although most of radioactive materials have very limited ability to pass through our skin. Yet, absorption is more probable through wounds or burns. Ingestion happens less often, accidentally, through mouth. Although one can think of it as a purely environmental issue, there are cases of accidental ingestions of radiochemicals. Some radiochemicals, such as actinides, have a very poor absorption. On the other hand, inhalation is way more dangerous. Radiochemicals are usually much more radiotoxic if they enter our body through the lungs, because once in the lungs, they stay longer period of time and are more probable of entering the bloodstream. Some radiochemicals can accidentally become volatile, like radioactive iodine,  $^{11}\text{CO}_2$ , hydrofluoric acid, while other radiochemicals can be airborne due to an accident, such as boiling accident, explosion or if fine powder particles are suspended in air.

Punctures are often very nasty way radioactive material can enter the body. They usually happen with needles, sharps, or broken glass.

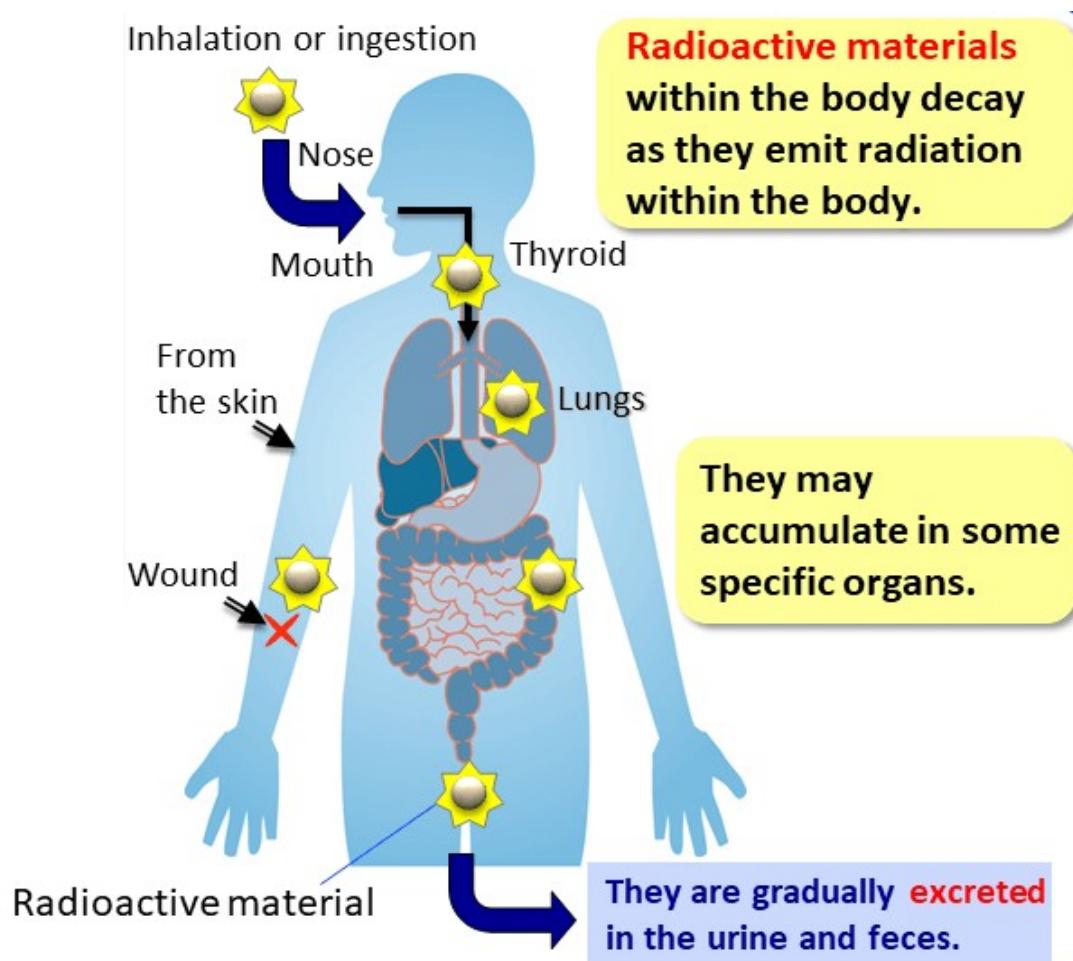


Figure 383: Typical routes by which radioactive contamination can enter human body

At day-to-day work it is never possible to fully out-rule some contamination being created or even spread. Everyone who works in radiochemistry knows that contaminations do happen. However, it is our duty to follow certain rules and methods, and to put a system in place that will minimise the risk of contamination, and control or prevent its spread. This system is called the control of contamination. It starts with well-designed and well-engineered working areas such as laboratories (Figure 384 left): workplaces where ionising radiation or radioactive materials are used are usually purposely designed and built for this specific type of work, they have special surfaces, edges, flooring, have proper ventilation with filters, proper hot cells, and fume hoods for radioactive work. Ventilation equipped with special filters prevents any leak of contamination outside the workplace and this is the most important part of the design. System of work prevents contamination of being spread while special care is given to radioactive waste disposal!

However, besides well-designed workplaces and expensive high-end equipment, human behaviour is the second factor that contributes to the risk: it is important to keep the workplace tidy and neat (Figure 384 right)! Lack of diligence, mess and

untidiness heavily contribute to the risk of incidents and contamination. It is very important to approach to radiation work seriously: it is not a joke. Personal responsibility, honesty, thoroughness, diligence, and vigilance can prevent and avoid many incidents.



**Figure 384:** Any control of contamination starts with well designed as well as neat and tidy laboratories and facilities.

Personal protective equipment must be worn. In 99% of the cases, it is standard laboratory personal protective equipment that includes good and suitable coat, goggles, and proper gloves made of latex or much better, nitrile (Figure 385). A responsible radiochemist should always wear personal protective equipment, and will change its gloves as often as practical, because this habit decreases the risk of cross-contaminations. Face respirators basically should not be worn because any air-borne fumes or particles that could be inhaled should be contained by specialised enclosed cabinets such as fume hoods, gloveboxes and hot cells: ventilation and filtration system associated with that equipment having highly efficient “HEPA” filters are there to prevent any leakage of any air-born radioactivity. Yet, some face respirators with highly efficient filters should be kept just in the case of a severe incident.





**Figure 385: Responsible and careful (radio)chemist always wears laboratory coat, goggles and nitrile gloves and gives other an example of personal responsibility**

What is a typical contamination scenario and what are the risks? A typical scenario is when a splash with radioactive material happens, and some drops of radioactive liquid contaminate worker's skin. Dose received in that case depends on half-life, activity in on contaminated area, skin area affected, and skin dose coefficient (Table 11). In this case special decontamination may be needed, while absorption through skin depends on lipophilicity of the radiochemical. Fortunately, in most of the cases it is negligible.

Radionuclide	Half-life	Skin dose coefficient in mGy.cm <sup>2</sup> /min.MBq
<sup>11</sup> C	20 minutes	38.1
<sup>13</sup> N	10 minutes	41.2
<sup>15</sup> O	2 minutes	48.2
<sup>18</sup> F	110 minutes	34.2
<sup>67</sup> Ga	78.3 hours	5.0
<sup>68</sup> Ga	68 min	36.1
<sup>89</sup> Sr	50 days	38.1
<sup>90</sup> Y	2.7 days	40.0
<sup>99m</sup> Tc	6.0 hours	31.7
<sup>111</sup> In	2.81 days	6.3
<sup>123</sup> I	13.2 hours	6.1
<sup>125</sup> I	60 days	2.5
<sup>131</sup> I	8.02 days	28.5
<sup>177</sup> Lu	6.73 days	23.5
<sup>201</sup> Tl	73 hours	4.4
<sup>223</sup> Ra	11.4 days	10.5

**Table 11: skin dose coefficient for typical medical radionuclides**

### Instrumentation for contamination control

Contamination monitors are usually simple hand-held radiation counters that have a scintillator probes (rarely Geiger Mueller) or are proportional counters (see Figure 114). They have large windows and are used for the contamination control: to check if any worker is contaminated, if laboratory workplace or tools are contaminated, or if solid waste is contaminated.

Another type of instruments is so called “whole body monitors”, large instruments based on proportional counters and are used to screen workers and visitors when exiting facilities (see Figure 115 left): usually one cannot leave the controlled area of a facility if do not check itself and ensure that there is no contamination on body, clothes, or shoes. Workplaces (bench, fume hood, etc.) needs to be monitored for eventual contamination before, during and after the work. Surfaces needs to be monitored even if there is a spec of a suspicion of contamination! Routine periodical

monitoring of all surfaces is usually part of the radiation safety strategy, but there is no need for high diligence if only very short-lived radionuclides are used and if all radioactivity is gone overnight. One should monitor equipment and yourself after work and should take whole body monitors when exiting the facilities. The monitoring instruments should be always calibrated to avoid false positives or false negatives. Also, the monitoring findings (values) should be always recorded.



**Figure 386:** Properly labeled source of ionising radiation (left), “radioactive” tape for labeling radioactive materials and sources (right)

Another important task is labelling of radioactive samples. All radioactive samples have to be properly labelled. Label should contain some ID number or code by which one is able to track it and trace it, but also there should be stated: isotope, initial activity, reference date, word “radioactive” and a trefoil symbol. Everything that is radioactive either stocks, samples, subsamples, or waste no matter how tiny have to be labelled at least with the word radioactive and trefoil symbol, including the reaction flasks: the best is to use so called “radioactive” tape to label all items that are radioactive.

### 13 commandments of radiation protection in laboratories and facilities

The system of radioprotection for radiopharmaceutical facilities is often complicated and demanding and it consists of many aspects and layers. But the first is a good and safe design (safe by design) of facilities and the labs at the very beginning. Then, the system of rules for the behavior of workers (that includes personal responsibility of each worker) is second most important. Finally, there are some rules, methods, and techniques for the safe routine work, and these are part of our radiochemical good skills. We can summarize the system and rules of radioprotection by the following 13 commandments:

1. Workplaces need to be well and safely designed at the very beginning and ventilation needs to work properly!
2. System of rules for the behavior of workers needs to be enforced including the personal responsibilities
3. Rules, methods, and techniques that form good radiochemical practice for the safe routine work needs to be known and strictly followed.
4. Workplace and facilities must be neat, clean, and tidy.

5. Dosimeters (personal and areal) needs to be worn and records of doses kept.
6. Workers and their health need to be periodically controlled.
7. Risk of all routine and non-routine operations need to be properly assessed and these assessments documented.
8. System of contamination monitoring and control (including designation of areas) needs to be in the place and routinely followed.
9. System for safe waste disposal needs to be in the place and functional.
10. All sources of ionizing radiation including samples needs to be controlled, well kept, and properly labeled, and radio-accountancy needs to be done on time.
11. Workers and radiation protection supervisors needs to be properly trained.
12. Inspections (internal and external) must be periodically performed to ensure that system is in the place and rules are respected.
13. Proper documentation needs to be kept and updated: local rules, system of work and standard operative procedures, records etc.



Figure 387: Vintage posters say to young radiochemists: Radiation is not to be feared but it must command your respect!



## Dictionary

English	Chinese (中文)
Radiopharmaceutical chemistry	放射药物化学
Nuclear medicine	核医学
Radiotracers	放射示踪剂
Radiolabelled molecules	放射性标记分子
Diagnostic	诊断性
Therapeutic	治疗性的
Radiotherapeutic	放射性治疗
Radioisotopes	放射性同位素
Ionising radiation	电离辐射
Nuclear imaging	核成像
Nuclear therapy or targeted radiotherapy (TRT)	核治疗
Positron Emission Tomography (PET)	正电子发射型断层扫描(PET)
Single-photon emission computed tomography	单光子发射计算机断层扫描术
Estrogen	雌激素
Receptor	受体蛋白
Physiological	生理
Somatostatin	生长抑素
Carcinoid	類癌
Alzheimer's dementia	阿尔茨海默氏症
X-ray scan	X射线扫描
Computed Tomography X-ray scan (CT scan)	计算机断层X射线扫描 (CT扫描)
Nuclear Magnetic Resonance (MRI)	核磁共振 (MRI)
Ultrasound	超声波
External beam of ionising radiation	外部电离辐射束
Brachytherapy	近距离治疗
Targeted radiotherapy	靶向放射治疗
Vector	向量
Endocrine disorders	内分泌失调
Chemotherapy	化疗
Radiotoxicity	放射毒性
Hyperthyroidism	甲状腺功能亢进症
Toxic nodular goitre	毒性结节性甲状腺肿
Metastases	遠端轉移
Neuroblastoma	成神经细胞瘤
Pheochromocytoma	嗜铬细胞瘤
Neuroendocrine	神经内分泌
Radioimmunotherapy	放射免疫疗法
Monoclonal antibody (mAB)	单克隆抗体
Chelator	螯合剂
B-cell lymphoma	B细胞淋巴瘤
Prostate cancer	腺癌



English	Chinese (中文)
X-rays	X 射线
Fluorescent	荧光
Cathode rays	阴极射线
Cathode tube	阴极管
Radioactivity	放射性活度
Inflammation	炎症
Quackery	假药
Impotence	阳痿
Golfer	高尔夫球手
Placebo	心理安慰剂
Jaw	下巴
Aplastic anaemia	再生障碍性贫血
Artificial radioactivity	人工放射性
Cyclotron	回旋加速器
Deuteron	氘核
Graphite	石墨
Carbohydrates	碳水化合物
Nitrogen fixation	固氮
Tooth enamel	牙釉质
Metabolism	新陈代谢
Tracer principle	示踪原则
Scintillator	闪烁器
Geiger-Mueller counter	G-M 计数器
Scintigraphy	闪烁显像
Glucose	葡萄糖
Yield	产量
Oncology	肿瘤学
Cardiology	心脏科
Neurology	神经学
Infection	感染
Nanoscience	纳米科学
Supramolecular chemistry	超分子化学



English	Chinese (中文)
Purity	纯度
Emission	发射
Pharmacological group	药理组
Therapeutic group	治疗组
Clinical use	临床使用
Neurotransmitter	神经递质
Peptide	肽类
Neurotensin	神经紧张素
Endocrine disorders	内分泌失调
Cardiovascular disorders	心血管疾病
Neurological disorders	神经系统疾病
Psychological disorders	心理障碍
Excretory organs	排泄器官
Kidney	肾脏
Trauma	创伤
Infection	感染
Pharmacology	药理学
Biochemistry	生物化学
Cell biology	细胞生物学
Physiology	生理学
Absorption	吸收
Distribution	分布
Metabolism	新陈代谢
Excretion	排泄物
Clinical pharmacology	临床药理
Indications	适应症
Contraindications	禁忌症
Side-effects	副作用
Pharmacodynamics	药效学
Pharmacokinetics	药物代谢动力学
Mechanisms of action	作用机制
Enzyme	酶
Tablets	片剂
Capsules	胶囊
Oral	口服
Stomach	胃部
Intestines	肠子
Liver	肝脏
Bloodstream	血流
Needle	针头
Parenteral	肠外治疗
Injection	注射
Intravenous	静脉注射



Artery	动脉
Intra-arterial	动脉注射
Intramuscular	肌肉注射
Subcutaneous	皮下注射
Infusion	输液
Inhalation	吸入式注射
Rectum	直肠
Rectal application	直肠应用注射
Sublingual	舌下含服注射
Transdermal	经皮注射
Lipophilic	亲脂性
Potent	烈性
Fentanyl	芬太尼
Target	靶子
Ligand	配体
Protein	蛋白质
Antibiotic	抗生素
Antiviriotics	抗病毒药
Inhibitor	抑制剂
Ion channels	离子通道
Transporter protein molecule	转运蛋白分子
Signal transducer	信号传分子
Molecular recognition	分子识别
Acetylsalicylic acid	乙酰水杨酸
Aspirin	阿司匹林
Ibuprofen	布洛芬
Paracetamol	扑热息痛
Cyclooxygenases	环氧化酶
Painkiller	止痛药
Morphine	吗啡
Tramadol	曲马多
Methadone	美沙酮
Opioid receptors	阿片受体
Agonist	激动剂
Activator	激活剂
Euphoria	欣快感
Sedation	镇静剂
Addiction	瘾
Atenolol	阿替洛尔
$\beta$ -receptors	$\beta$ -受体
Antagonist	拮抗剂
Blocker	封锁者
Adrenalin	肾上腺素
Haloperidol	氟哌啶醇



Dopamine	多巴胺
Schizophrenia	精神分裂症
Antipsychotic	抗精神病药
Atropine	阿托品
Acetylcholine	乙酰胆碱
Bradycardia	心动过缓
Topology	拓扑学
Cell membrane	细胞膜
Affinity	亲和力
Potency	药效
Poison	毒药
Radiolysis	辐射分解
Free radicals	自由基
Toxicology	毒理学
Radiotoxicology	放射性毒理学
Toxicity	毒性
Transmutation	核嬗变
Projectile	发射体
Bombardment	轰击
Beam	粒子束
Generator	发生器
Pool	冷却池
Flux	通量
Immersed	沉浸式
Fission	裂变
Irradiation	辐照
Abundant	丰富的
Precursor	前体
Capture	捕捉
Yield	产量
Tritium	氚
Cross section	截面
Decay	衰变
Rate	速度
Saturation	饱和度
Ampoule	安瓿
Quartz	石英
On-spot	当场
Bottleneck	瓶颈
Obstacle	障碍物
Accelerator	加速器
Spiral	螺线
Trajectory	轨道
Hydride	氢化物



Collide	碰撞
Isochronous	等时
Orthogonal	正交
Alternating	交替的
Orbit	轨道
Radius	半径
Vacuum	真空
Deflector	偏转器
Foil	箔纸
Electroplated	电镀
Tantalum	钽
Isotopically enriched	同位素富集
Impurities	杂质
Commercially available	市售
Apparatus	仪器
Immobilized	静止的
Elution	洗脱液
Adsorbed	吸附的
Alumina	氧化铝
Gel	凝胶
Zirconium	锆
Molybdenum	钼
Saline solution	生理盐水
Membrane	膜
Meta-stable	亚稳态
Radioisomer	放射性异构体
Transient	瞬态
Equilibrium	平衡
Shielding	屏蔽
Secular equilibrium	长期平衡
Gallium	镓
Germanium	锗
Titanium	钛
Tin	锡
Labelling	贴标签
Purification	纯化
Preparation	准备
Standardization	标准化
Assessment	评估
Carrier	载体
Purity	纯度
Vector	矢量
Physiological	生理学
Carbon dioxide	二氧化碳 ( $\text{CO}_2$ )



Carbon monoxide	一氧化碳 (CO)
Methane	甲烷 (CH <sub>4</sub> )
ammonium cyanide	氰化铵 (NH <sub>4</sub> CN)
Ammonia	氨 (NH <sub>3</sub> )
Reactive	有反应性
Methyl iodide	甲基碘 (CH <sub>3</sub> I)
Formaldehyde	甲醛 (O=CH <sub>2</sub> )
Phosgene	光气 (COCl <sub>2</sub> )
Methyl lithium	甲基锂 (CH <sub>3</sub> Li)
Methyl triflate	甲三氟甲磺酸酯 (CH <sub>3</sub> SO <sub>3</sub> CF <sub>3</sub> )
Redox (reaction)	氧化还原 反应
Complexation (reaction)	络合反应
Substitution (reaction)	取代反应
Addition (reaction)	加成反应
Condensation (reaction)	缩合反应
Coupling (reaction)	催化偶联反应
Catalytic (reaction)	催化反应
Cycloaddition	环加成反应
Biosynthetic	生物合成
Electrophilic addition	亲电加成反应
Electrophilic substitution	亲电取代反应
Nucleophilic substitution	亲核取代反应
Thyroxin	甲状腺素
Triflate	三氟甲磺酸酯
Chelating ligand	螯合配体
Photosynthesis	光合作用
Green algae	绿藻
Selective	选择性的
By-product	副产品
Microfluidics	微流体
English	中文
Automated	自动化
Instrumentation	仪表
Validation	验证
Validated	已验证
Mock	模拟
Documented	记录
Chamber	分庭
Glove-box	手套箱
Sophisticated	精巧的
Robotised	机器人化的
Kit	配套元件
Vial	小瓶



Reagent	试剂
Oxidation	氧化作用
Microporous	微孔
Extraction	萃取
Chromatography	色谱法
Myocardial perfusion	心肌灌注
Adsorption	吸附
High-performance	高性能化
HPLC	高效液相色谱法
Verification	验证
Electrophoresis	电泳
Precipitation	沉淀
Distillation	蒸馏
Integrated	集成的
Sterile	无菌的
Isotonic	等渗的
Tonicity	张力
Osmotic	渗透性
Additive	添加剂
pH	pH 值
Viscosity	黏度
Preservatives	防腐剂
Dispensed	分配的
Pyrogenic	热原物质 (发热的)
Fever	发烧
Allergenic	过敏性
Allergic reaction	过敏反应
Facility	设施
Radiopharmaceutical agent	放射性药剂
Paramount	最重要的
Vault	拱顶
Bunker	掩体
Concrete	混凝土
Barite	重晶石
Gravel	碎石
Plug	插头
Cascade	瀑布状
Miniature	迷你型
Thin-layer chromatography	薄层色谱法
Spectrometer	光谱仪
Calibrator	校准器
UV	紫外线
Column	柱子
Eluent	洗脱液



Preparative purification	准备性净化
Spectroscopy	光谱学
Clarity	清晰度
Endotoxin	内毒素 (脂多糖, 革兰氏阴性细菌外膜的主要组成部分)
Volunteer	志愿者
Array	阵列
Collimator	准直器
Hexagonal	六角形的
Prismatic	棱镜的
Honeycomb	蜂窝状的
Pixelated image	像素化图像
Semiconductor	半导体
Annihilation	湮灭
Coincidence	巧合
Antiparallel	反平行
Circular	圆
Resolution	分辨率
Superimposed	叠加
Radiation protection	辐射防护
Dosimeter	剂量计
Phenomenon	现象
Thermoluminescence	热释光
Ambient	周围环境
Proximity	接近
English	中文
Proportional	正比 (计数器)
Contamination	污染
Background	背景, 本底
Alarm	警报
Threshold	临界点
Compliance	遵守
Statistical analysis	统计分析
Spike	长钉
Trojan horse	特洛伊木马
Fortress	堡垒
Cruising missile	巡航导弹
Warhead	弹头
Peptides	肽
Monoclonal antibodies	单克隆抗体
Nanoparticles	纳米颗粒
Hydrolysis	水解
Nutrients	营养物质
Vitamins	维生素



Testosterone	睾丸激素
Cortisol	皮质醇
Adrenaline	肾上腺素
Dopamine	多巴胺
Serotonin	血清素
GABA	$\gamma$ -氨基丁酸
Xenobiotic	异型生物质（药物、杀虫剂、致癌物等）
Holder	持有者
Conjugated	共轭的
Amino acid	氨基酸
Neurohormone	神经激素
Oxytocin	催产素
Insulin	胰岛素
Glucagon	胰高血糖素
Endorphins	内啡肽
Somatotropin	生长激素
Somatostatin	体抑素
Digestive system	消化系统
Guts	内脏
Cholecystokinin	胆囊收缩素
Gastrin	胃泌素
Moiety	部分
Neuroendocrine tumour	神经内分泌肿瘤
Cancer metastases	癌症转移
Immunoglobulin	免疫球蛋白
Immune system	免疫系统
Molecular biology	分子生物学
Cell biology	细胞生物学
Biotechnology	生物技术
Genetic engineering	基因工程
Cloning	克隆
Tissue culture	组织培养
English	中文
Antigen	抗原
Horn	喇叭
Radioimmunoconjugate	放射免疫交联物
Lymphoma	淋巴瘤
Nanoscale	纳米级
Silica	二氧化硅
Micelles	胶束
Liposomes	脂质体
Dendrimers	树形聚合物
Quantum dots	量子点
Nuclear reprocessing	乏燃料后处理



Corrosion inhibitor	缓蚀剂
Enriched	富集的, 浓缩的
Periodic table	元素周期表
Manganese	锰
Rhenium	铼
Anion	阴离子
Oxidation state	氧化态
Permanganate	高锰酸盐
Tetrahedron	四面体
Vertex	頂點
Disproportionate	歧化反应
Tin	锡
Citrate	柠檬酸盐
Buffer	缓冲
Sodium borohydride	硼氢化钠
Thiourea	硫脲
Carbonyl	羰
Potassium	钾
Organometallic	有机金属
Hydrolysis	水解
Cation	阳离子
NMR spectroscopy	核磁共振波谱法
IR spectroscopy	红外光谱学
Mass spectroscopy	质谱法
Electrochemical	电化学的
X-ray diffraction	X 射线晶体学
Surrogate	代孕 - 化学类似物
Cardiac perfusion	心脏灌注
Oxygenated blood	含氧血液
Atherosclerosis	动脉粥样硬化
Angina pectoris	心绞痛
Infarction	心肌梗死
Myocardial ischemia	心肌缺血
Necrosis	坏死
Surgery	外科手术
Hydroxyapatite	羟基磷灰石
Calcium	钙
Skeletal	骨骼的
Sarcoma	肉瘤
Adenocarcinoma	腺癌
Renal	肾的
Urinary tract	尿路
Obstruction	梗阻
Kidney transplantations	肾脏移植



Gallbladder	胆囊
Obsolete	过时的
Bromine	溴
Cerebral perfusion	脑灌注
Stroke	中風
Cerebrovascular diseases	脑血管疾病
Pyramidal	金字塔形
Diffusion	扩散
Colloid	胶体
Adduct	加合物
Anhydrous	无水的
Sodium thiosulfate	硫代硫酸钠
Gelatine	明胶
Lymph nodes	淋巴結
Spleen	脾脏
Disintegration	崩解 (衰变)
Avogadro's number	阿伏伽德罗常数
Incubation	孵化
Ascorbic acid	抗坏血酸
Stickiness	粘性
Adhesion	粘着
Bisfunctional	双功能
Bioconjugate	生物共轭物
Monodentate ligand	单齿配体
Glycine	甘氨酸
Dimer	二聚体
Multidentate ligand	多齿配体
Conjugate	共轭
Hydrazine	肼
Pyridine	吡啶
Fine-tuning	微调
Tricine	三(羟甲基)甲基甘氨酸
Coordination	配位
Tridentate ligand	三齿配体
Electron-donor	给电子体
Imidazole	咪唑
Carboxylic acid	羧酸
Cycloaddition	环加成反应
Azide	叠氮化合物
Alkyne	炔烃
Triazole	三唑
Prostate	前列腺
Angiogenesis	血管新生
Tyrosine	酪氨酸



Rheumatoid arthritis	类风湿性关节炎
Prong	叉
Biocompatible	生物相容性
Polyethylene glycol	聚乙二醇
Extrapolated	外推的
Halogen	卤素
Electronegative	电负性
Corrosive	腐蚀性
Monovalent	单价的
Covalent	共价的
Van der Waals radius	范德华 半径
Methyl group	甲基
Amino group	氨基
Hydroxyl group	羟基
Hydrogen bonding	氢键
Basicity	基本性
Fluorination	氟化
Nucleophilic substitution	亲核取代反应
Electrophilic substitution	亲电取代反应
Aliphatic	脂肪族
Aromatic	芳香的
Benzene	苯
Heterocyclic	芳杂环类
Pyridine	吡啶
Hydrated	水合的
Charge density	电荷密度
Protonated	质子化的
Hydrofluoric acid	氢氟酸
Tertiary alcohol	叔醇
Fluoride ion	氟离子
Polar solvent	极性溶剂
Aprotic solvent	非质子溶剂
Tetraalkylammonium ion	四烷基铵离子
Macrocyclic compound	大环化合物
Cryptand	穴醚
Acetonitrile	乙腈
Proton-donor	质子供体
Protecting group	保护基团
Acetyl group	乙酰基
Inversion	倒置
Chirality	手性
Tert-butanol	叔丁醇
Thymidine	胸苷
Nucleotide base	核苷酸碱基



Hydrochloric acid	盐酸
Synapse	突触
English	中文
Synaptic cleft	突触裂
Neuron	神经元
Parkinson's disease	帕金森氏症
Degeneration	堕落
Peroxide	过氧化物
Heteroaromatic	芳杂环类
Acetylcholine	乙酰胆碱
Synthon	合成子
Palladium	钯
Rheumatoid disease	类风湿性疾病
Synonymous	同义的
Cocaine	可卡因
Analogue	模拟
Neurology	神经学
Psychiatry	精神病学
Nucleophilicity	亲核性
Anhydrous	无水的
Acidic conditions	酸性条件
Alkenes	烯烃
Carbanion	碳负离子
Mitigated	缓解的
Electropositive	电正性
Organometallic	有机金属
Selectivity	选择性
Obstacle	障碍
Silicon	硅
Germanium	锗
Homolysis	均裂
Oxidizing agent	氧化剂
Electrical discharge	放电
UV (Ultraviolet)	紫外线
Laser pulse	激光脉冲
Sulphur trioxide	三氧化硫
Sulphuric acid	硫酸
Potassium perchlorate	高氯酸钾
Hypervalent	超价分子
Copper	铜
Pioneered	先驱
Cognitive function	认知功能
Oncology	肿瘤科
Dopamine	多巴胺



Huntington's disease	亨廷顿氏病
Personality disorders	人格障碍
Amyloid protein	类淀粉蛋白
Dementia	痴呆
Alzheimer disease	阿兹海默症
Temporal lobe epilepsy	颞叶癫痫
Schizophrenia	精神分裂症
Adrenergic innervation	肾上腺能
Neuroleptic	抗精神病药
Differentiation	差异化
Malignant tumour	恶性肿瘤
Hypoxia	缺氧
Radio-resistance	耐辐射性
Adrenalin	肾上腺素
Heart arrhythmia	心律不齐
Antiviral	抗病毒药
Herpes simplex virus 1	单纯疱疹病毒
Adenovirus	腺病毒
Cholesterol	膽固醇
Adrenal cortex	肾上腺皮质
Adrenal glands	肾上腺
NMR spectroscopy	核磁共振波谱法
Cosmogenically	宇宙产生的
Archaeology	考古学
Forensics	司法科学
Palaeontology	古生物学
Carbon dioxide	二氧化碳
Methane	甲烷
Biogenic element	生源的元素
Biosphere	生物圈
Methanol	甲醇
Lithium aluminium hydride	氢化铝锂
Formaldehyde	甲醛
Carbon monoxide	一氧化碳
Methyl iodide	碘甲烷
Methyl-triflate	三氟甲磺酸甲酯
Hydrogen cyanide	氰化氢
Ammonia	氨
Platinum	铂
Copper chloride	氯化铜
Carbon tetrachloride	四氯化碳
Carbon disulphide	二硫化碳
Nickel	镍
Carboxylic acids	羧酸



Phosgene	光气
Acyl chlorides	酰氯
Methyl lithium	甲基锂
Methylation	甲基化
Carbonylation	羰基化
Grignard reaction	格氏反应
Fixation	固定
Sodium hydroxide	氢氧化钠
Acetophenone	苯乙酮
Boric acid	硼酸
Ester	酯
Organolithium reagent	有机锂试剂
tert-Butyllithium	叔丁基锂
n-Butyllithium	正丁基锂
Imide	酰亚胺
Ketone	酮
Aldehyde	醛
Carbamate	氨基甲酸酯
Urea	尿素
Phenytoin	苯妥英
Rhodium	铑
Selenium	硒
Chemical warfare agent	化学武器
Uric acid	尿酸
Dichloromethane	二氯甲烷
Deadliness	致命性
Nitrile	腈
Hydrogen-peroxide	过氧化氢
Thiourea	硫脲
Progesterone	孕酮
Magnesium	镁
Adduct	加合物
Thionyl chloride	氯化亚砜
Thiol	硫醇
Thioester	硫酯
Trifluoroacetic acid	三氟乙酸
Methionine	甲硫氨酸
Tryptophan	色氨酸
Potassium hydroxide	氢氧化钾
Hydrochloric acid	盐酸
Urinary bladder	膀胱
Glioblastoma	胶质母细胞瘤
Homocysteine	高半胱氨酸
Tetrahydrofuran	四氢呋喃



Ambiguous	模糊的
Phospholipid	磷脂
Choline	胆碱
Anxiety	焦虑
Bipolar disorder	躁郁症
Obsessive compulsive disorder	强迫症
Alcoholism	酗酒
Drug addiction	毒瘾
Personality disorder	人格障碍
Behaviour disorders	行为障碍
Offender	罪犯
Antisocial	反社会的
Psychopathic	精神病
Panic disorder	恐慌症
Nitrite	亚硝酸盐
Nitrate	硝酸盐
By-product	副产品
By-reaction	副反应
Soda lime	碱石灰
Amalgam	汞合金
Amphetamine	安非他命
Triphosgene	三光气
Phenol	苯酚
Isocyanates	异氰酸酯
Ammonolysis	氨解
Purine	嘌呤
Nucleoside	核苷
Adenosine	腺苷
Nitrosamine	亚硝胺
Azo compound	偶氮化物
Azide	叠氮化合物
Triazole	三唑
Glutathione	谷胱甘肽
Diazonium ion	重氮盐
Diazo-coupling	重氮偶合反应
Hydrazine	肼
Butanol	丁醇
Syringe	注射器
Vortex	涡流
Vanishingly	消失的
Borane	硼烷
Electron capture	电子俘获
Radioimmunoassay	放射免疫分析
Tellurium	碲



Hypoiodite ion	次碘酸盐
Disproportionation reaction	歧化反应
Metalloid	类金属
Microbiology	微生物学
Zirconium	锆
Fracking (hydraulic fracturing)	水力压裂
Iodite ion	亚碘酸盐
Iodate ion	碘酸盐
Periodate ion	高碘酸盐
Thyroxin	甲状腺素
Triiodothyronine	三碘甲状腺素
Phenol	苯酚
Aniline	苯胺
Imidazole	咪唑
Indole	吲哚
Hydrogen peroxide	过氧化氢
Peracetic acid	过氧乙酸
Regiospecific	区域特异性
Mercury	汞
Germanium	锗
Thallium	铊
Cushing's syndrome	库兴氏症候群
Hyperaldosteronism	醛固酮增多症
Pheochromocytoma	嗜铬细胞瘤
Epilepsy	癫痫
Neuroblastoma	神经母细胞瘤
Paraganglioma	副神经节瘤
Benzodiazepine	苯二氮草类
Epileptic seizure	癫痫发作
Heart infarction	心肌梗死
Fatty acid	脂肪酸
Ischemic heart disease	冠状动脉疾病
Diels-Alder reaction	狄尔斯-阿尔德反应
Lymphocyte	淋巴细胞
Non-Hodgkin lymphoma	非霍奇金氏淋巴瘤
Lymph node	淋巴结
Zinc	锌
Ion exchange	离子交换
Toluene	甲苯
Titanium dioxide	二氧化钛
Primordial	原始的
Cadmium	镉
Hydrobromic acid	氢溴酸
Chromatography	色谱法



Ligand field	配位场
Crystal field	晶体场
Stabilization energy	稳定能量
Lewis acid	路易斯酸
Octahedral	八面体
Coordinating sphere	配位场
Oxalate	草酸盐
Serum	血清
Transferrin protein	转铁蛋白
Spleen	脾脏
Intestine	肠
Infection	感染
Citrate	柠檬酸
Macrocyclic ligand	大环配体
Acylic ligand	无环配体
Pseudo-peptide	假肽
White blood cells	白血球
Platelets	血小板
Inflammation	炎症
Tandem	串联
Ubiquitous	无处不在
Monomer	单体
Cleft	裂口
Sarcophagus	石棺
Pit	坑
Multiple myeloma	多发性骨髓瘤
Astronomically long	天文长
Yttrium	钇
Zirconium	锆
Titanium	钛
Siderophore	铁载体
Strontium	锶
Lanthanum	镧
Spallation	散裂
Rhodium	铑
Lutetium	镥
Lanthanides	镧系元素
Hafnium	铪
Ytterbium	镱
Liver	肝脏
Artery	动脉
Embolization	栓塞
Microsphere	微球
Hepatocellular carcinoma	肝细胞癌



Thallium	铊
Actinides	锕系元素
Transactinides	超重元素
Francium	钫
Lead	铅
Bismuth	铋
Polonium	钋
Astatine	砹
Radon	氡
Neodymium	钕
Terbium	铽
Samarium	钐
Gadolinium	钆
Europium	铕
Tungsten	钨
Osmium	锇
Neoplasm	赘生物
Actinium	锕
Thorium	钍
Fermium	镄
Ankylosing spondylitis	强直性脊柱炎
Recoiling	后座力
Calixarene	杯芳烃
Rheumatic disease	风湿
Neptunium	镎
Acute myeloid leukaemia	急性骨髓性白血病
Californium	锎
Einsteinium	锿
Curium	锔
Rubidium	铷
Palliative treatment	姑息治疗
Selenium	硒
Krypton	氪
Scandium	钪
Ruthenium	钌
Iridium	铱
Holmium	钬
Erbium	铒
Vascular endothelial growth factor	血管内皮生长因子
Chitosan	壳聚糖
Coefficient	系数
Dose rate	剂量率
Dosimeter	剂量计
Visceral organs	内脏器官



Lead aprons	铅围裙
Calibration	校准
Shielding	屏蔽
Acrylic polymer	丙烯酸聚合物
Bremsstrahlung	轫致辐射
Attenuation	衰减
Chevron	雪佛龙
Dimensionless	无量纲
Syringe	注射器
Tinge	色调
Glove-box	手套箱
Film badge	电影徽章
Stall	手指摊
Splash	溅
Spillage	溢出
Cross-contamination	交叉感染
Knob	旋钮
Diligence	勤勉
Untidiness	不整洁
Thoroughness	彻底性
Vigilance	警觉
Goggles	风镜
Face respirator	面罩
Negligible	微不足道