DEPARTMENT OF PHYSICS AND NANOTECHNOLOGY
SRM INSTITUTE OF SCIENCE AND TECHNOLOGY

INDUSTRIAL NANOTECHNOLOGY

Cancer Nanomedicine: Targeted Therapy

Cancer is considered as one of the most challenging health care problems.

Though there are many approved drugs that can be used for cancer therapy, drug resistance and delivery are among of the barriers of the treatment.

In addition, pathological characteristics of tumors and their abnormal blood vessel architecture and function also reduce the efficiency of the conventional cancer treatment.

NPs have many properties such as their small size, ability to load various drugs and large surface area, and ability to increase the absorption of conjugated.

Therefore, the **NPs have been considered as excellent tumor-targeting vehicles.**

The recent nanoscale vehicles include liposomes, polymeric nanoparticles, magnetic nanoparticles, dendrimers, and nanoshells; lipid-based NPs have been used as conjugates.

There are few examples of approved conjugated anticancer NPs including AmBisome (amphotericin B liposomal) and Doxil (liposomal doxorubicin).

There are several therapeutic methods that have been used to treat tumors and their surrounding environments.

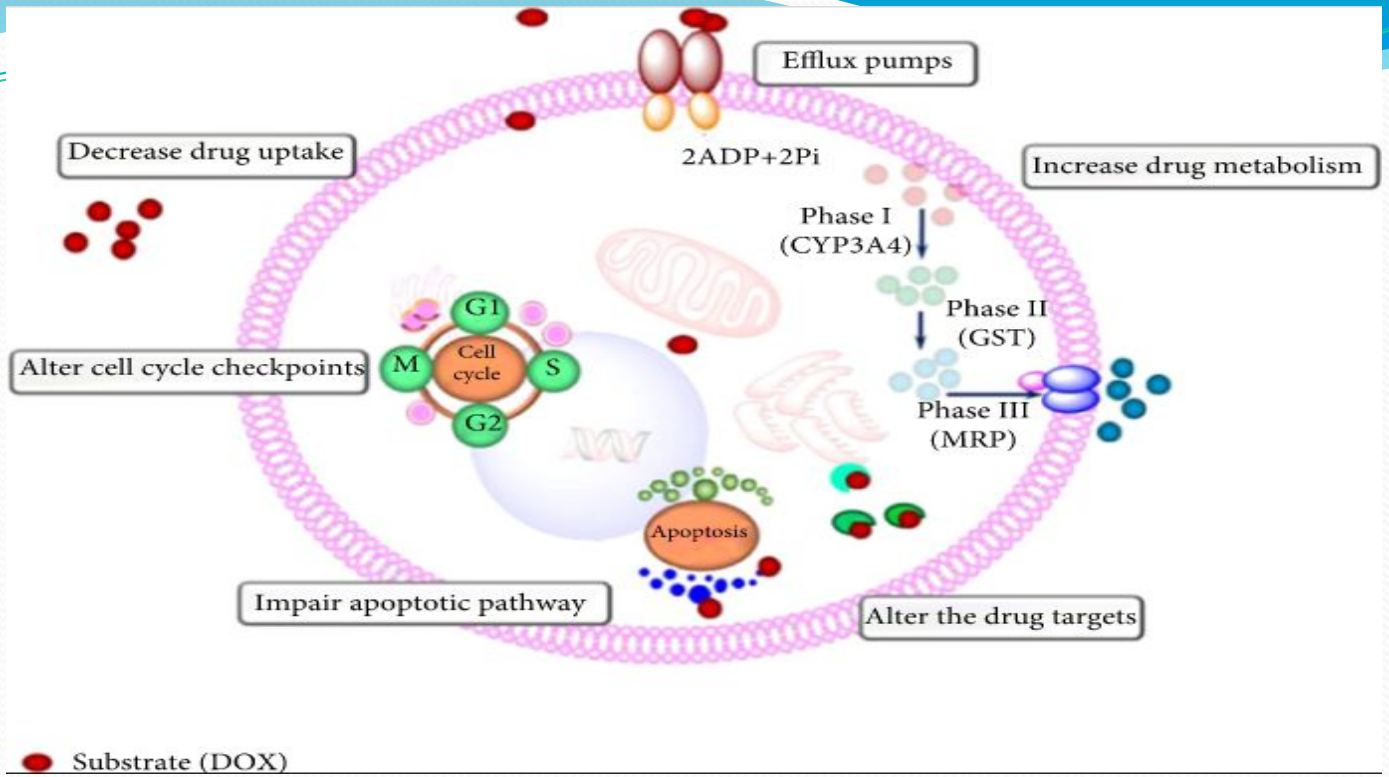
An example of these strategies is **chemotherapy, which was first tried in 1942** when Louis Goodman and his colleagues tested using nitrogen mustard in treatment of non-Hodgkin's lymphoma.

However, chemotherapy has helped in the improvement of cancer therapy of patients; in most cases, cancer with a more progressive stage normally occurs, and usually, multidrug resistance takes place.

Targeting the surrounding environment of tumors also has been tried, since cancer cells depend primarily on oxygen and angiogenesis for survival and metastasis.

The failure of chemotherapy in the clinic is mainly due to different extents of multidrug resistance (MDR) results with approximately 90% of cancer patients died.

MDR occurs when tumor cells develop resistance to structurally and functionally unrelated classes of chemotherapeutic agents leading to drug inactivation and/or drug efflux from cancer cells leading to obstacle of the treatment.



There are several report hypotheses of the molecular mechanisms of MDR, mainly including increasing efflux of membrane transport proteins, detoxification by reducing the drug activation and potentiating drug metabolism, alteration in drug targeting by enhancing the DNA repair mechanism, blocking apoptosis, and alteration of cell cycle regulation.

All these mechanisms synergistically interact together to produce MDR.

Cancer Targeting with Conjugated Nanoparticles

They prevent the degradation of the conjugated drug. They also improve its absorption through the epithelial diffusion that ultimately results in reaching the optimum concentration in a short time.

NPs also alter the pharmacokinetic and distribution profile of the drug in the tissue and increase the intracellular efflux in cancer cells.

Enhancing the permeability and retention effects of anticancer drugs is considered as passive targeting of NPs to the tumors.

However, actively **targeted NPs can be designed based on tumor microenvironment- and ligand-directed targeting to the tumor cells.**

Therefore, as a unique inherent property of NPs to the solid tumors, the nanoparticle is considered as an excellent tumor-targeting vehicle.

This effect makes the accumulation of NPs preferable at the tumor site.

In addition, the multifunction of NPs allows targeting the tumor site that is directly connected to the main blood circulation.

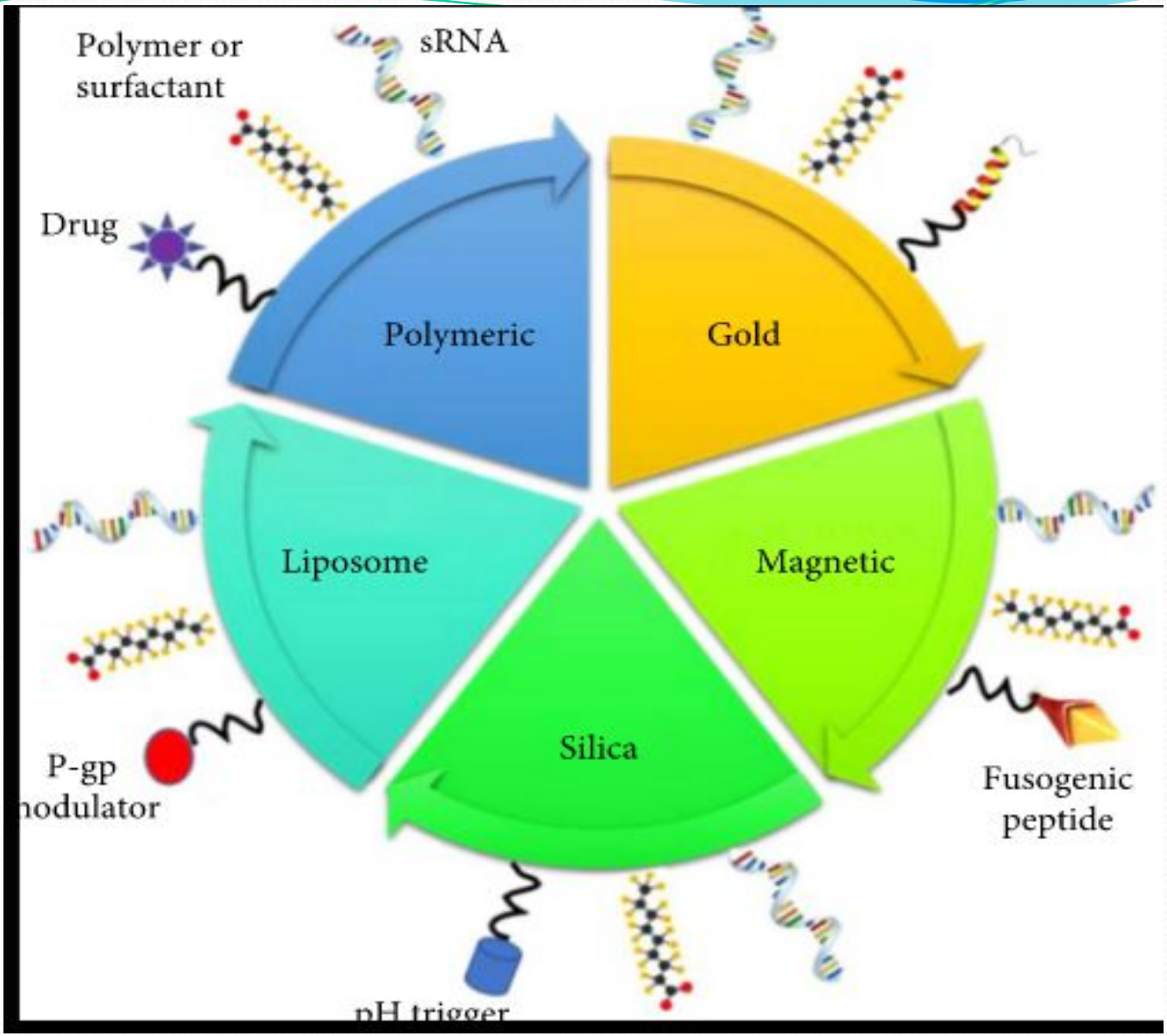
A nanoparticle–biomolecule conjugate is **a nanoparticle with biomolecules attached to its surface**

The **conjugation** of **nanoparticles** with antibodies combines the properties of the **nanoparticles** themselves with the specific and selective recognition ability

Nanoparticles

Antibody conjugated Nanoparticles (ACNPs)

An Overview of Conjugation Strategies for Clinical Implementation of Polymeric ACNPs


Polymer or surfactant
sRNA
Drug
Polymeric
Gold
Liposome
Magnetic
Silica
P-gp
nodulator
Fusogenic peptide
pH trigger

Barriers for the Treatment of Tumors Overcome by Nanoparticles

At the cellular level, the drug resistance is considered as a physiological barrier to the success of the anticancer drug.

The penetration of chemotherapy to the solid tumor is difficult due to the pathological characteristics of the solid tumors that include abnormal blood vessel architecture and function, interstitial hypertension, lack of lymphatics (a network of tissues, vessels and organs that work together to move a colorless, watery fluid called lymph back into your circulatory system), and dilated angiogenesis(the formation of new blood vessels)

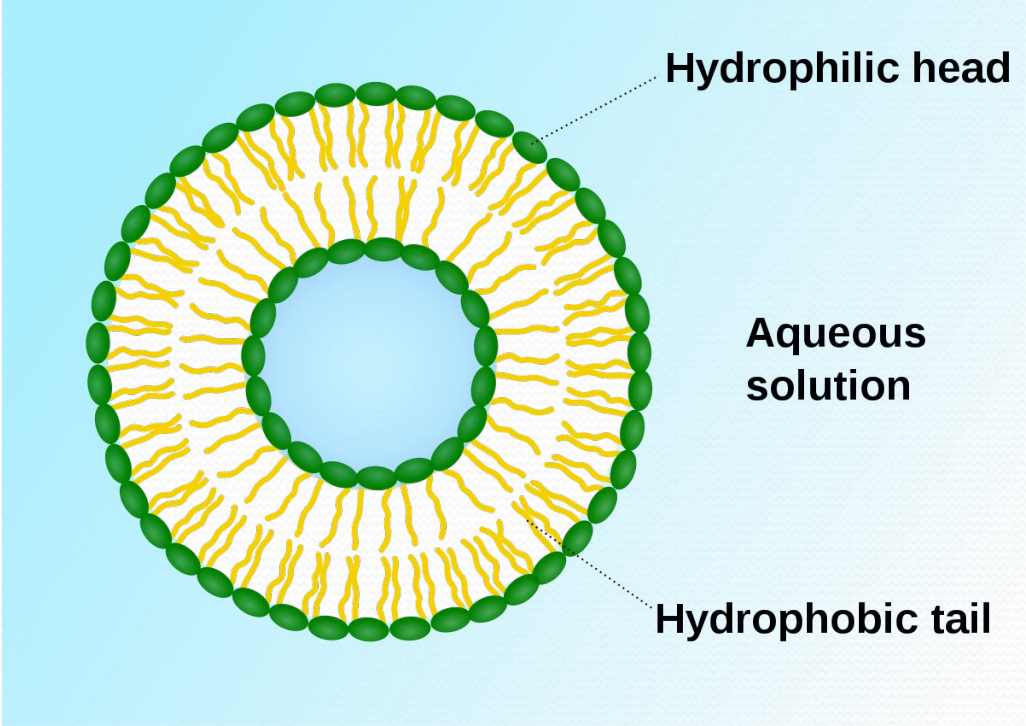
This microenvironment to certain extent contributes to the drug resistance results in decreasing in the drug accumulation and/or penetration to the solid tumor.

Nevertheless, chemotherapy encounters another major barrier, i.e., **multidrug resistance** even after its penetration to the tumors.

Pharmaceutical history of the development of nanoparticle started with the first discovery of liposome

this ultimately allows **increasing the specificity of effective drugs and overcoming the resistance of tumors.**

(Liposomes are **small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids**. Due to their size and hydrophobic and hydrophilic character(besides biocompatibility), liposomes are promising systems for drug delivery.



The therapeutic index of NPs has improved the potential of commonly used drugs through increasing the efficacy and decreasing the toxicity of the drug and keeping its concentration in the steady state over a long period of time.

Thus, drug-coated NPs should have long half-life to give the maximum effect.

The targeting of active sites of transporters or receptors is the main character of NPs because of their flexible surface chemistry that allows for potential conjugation of targeting ligands.

On the other hand, several **anticancer agents exhibit low specificity** towards cancer cells.

Therefore, the delivery of drugs to the solid tumors is still a difficult approach.

The reticuloendothelial system (RES) that known as “mononuclear phagocytes” is the major defense system in the bloodstream of the body that rapidly removes NPs from the blood.

RES recognizes the NPs as foreign bodies. Hydrophilic and flexible polymers can coat the NPs from the opsonins hence avoid the uptake of NPs by the RES

Biomarkers

A biomarker is an indicator of a biological state of disease. It is characteristic of a specific state and therefore can be used as a marker for a target disease.

These biomarkers can be used to study cellular processes, and monitor or recognize disruption or alterations in the cellular processes of cancer cells.

A biomarker can be a protein, a fragment of a protein, DNA, or RNA-based. Biomarkers, specifically cancer biomarkers, are **an indication of cancer** and by detecting them the existence of that specific cancer can be verified.

Alongside the development of proteomic technologies, many protein biomarkers have been discovered for many types of cancer.

As well, with DNA methylation analysis researchers have also been able to discover DNA biomarkers for some of the widely spread cancers.

Biomarkers in relation with nanotechnology and biosensors have opened up a new era of early cancer diagnosis and precise drug delivery.


BIOMARKERS

Gold Nanoparticles

Gold nanoparticles (GNPs) have been in the bio-imaging spotlight due to their special optical properties.

GNPs with strong surface-plasmon-enhanced absorption and scattering have allowed them to emerge as powerful imaging labels and contrast agents.

Furthermore, GNPs have been proven to be **more biocompatible, less cytotoxic, and resistant to photobleaching.**

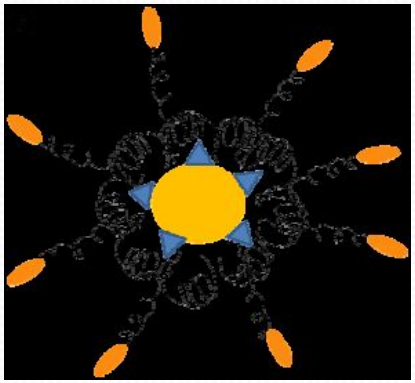
According to their size and shape, GNPs can absorb and scatter light from the visible to near-infrared (NIR) region.

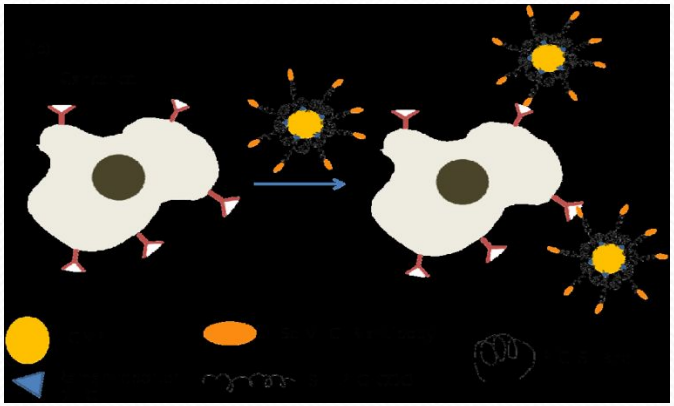
GNPs have been extensively studied, especially in the medical area, and have been used as colorimetric biosensors, cancer imaging, cancer therapy, and drug delivery.

They have been found to amplify the efficiency of Raman scattering and thus have been proposed as a novel tag.

A class of nontoxic nanoparticles for in vivo tumor targeting based on **pegylated** colloidal gold (colloidal gold coated with a protective layer of polyethylene glycol) and surface-enhanced Raman scattering (SERS) has been reported.

These gold nanoparticles have been encoded with Raman reporters and conjugated with ScFv antibody for in vitro and in vivo tumor targeting, recognizing the epidermal growth factor receptor (EGFR) which is a popular biomarker used in cancer targeting



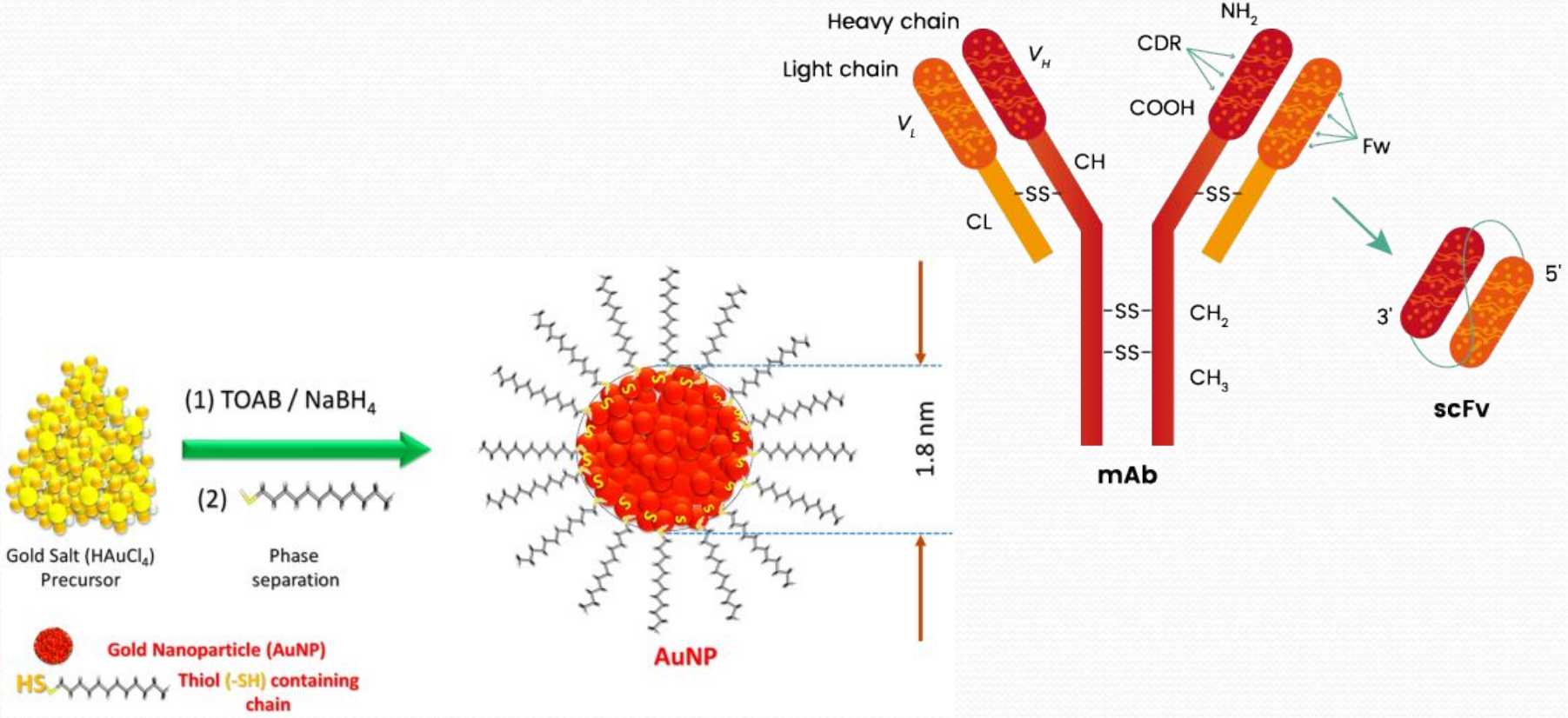


These SERS GNPs with ScFv antibody to target EGFR were more than 200 times brighter than NIR emitting quantum dots, and allowed spectroscopic detection of small tumors (0.03 cm3) at penetration depth of 1–2 cm.

For GNPs as stable and versatile molecular imaging agents, a complementary oligonucleotide-based approach has been proposed.

A 5'-thiol-modified and 3'-NH2-modified oligonucleotide was coated onto the nanoparticles and subsequently conjugated with anti-EGFR proteins through DNA-DNA hybridization.

A single-chain variable fragment (scFv) is not actually a fragment of an antibody, but instead is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids.



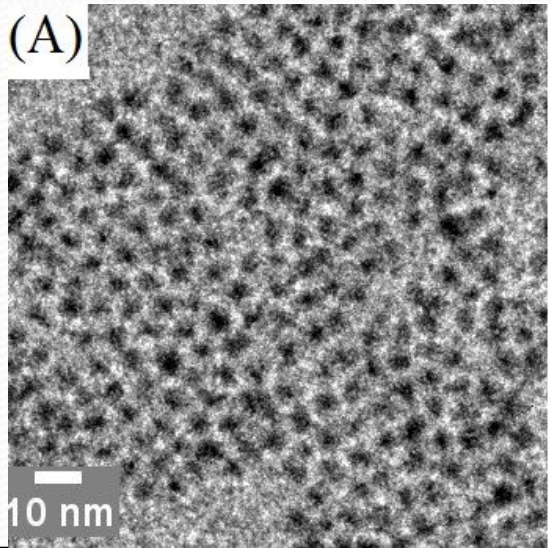
Quantum dots (QDs)

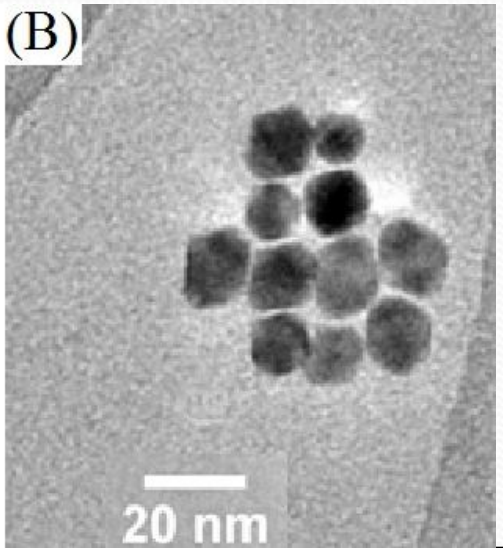
QDs are an exciting material to work with due to their unique optical properties compared to traditional organic fluorescent labels.

Organic fluorescent dyes have several drawbacks that have limited their usefulness as molecular imaging tags.

Their low photobleaching threshold and broad absorption/emission peak width have hindered their use in long term imaging and multiplexing (detecting multiple labels simultaneously).

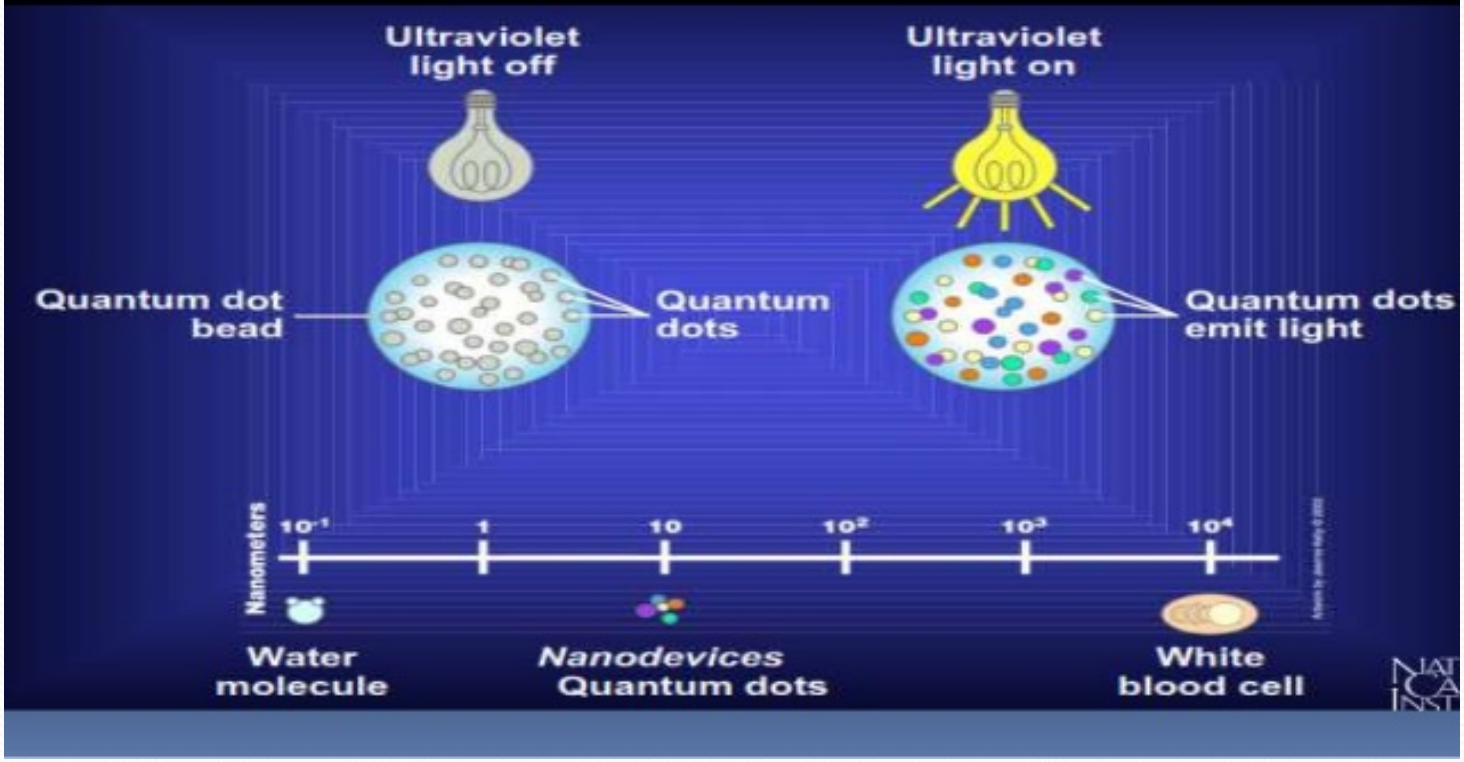
QDs have properties that overcome these limitations of the organic fluorescent dyes including high resistance to photobleaching, broad-band absorption with narrow emission bands ranging from UV to NIR, and size tunable emission bands.





Quantum Dots

Another minuscule molecule that will be used to detect cancer is a quantum dot. Quantum dots are tiny crystals that glow when they are stimulated by ultraviolet light.



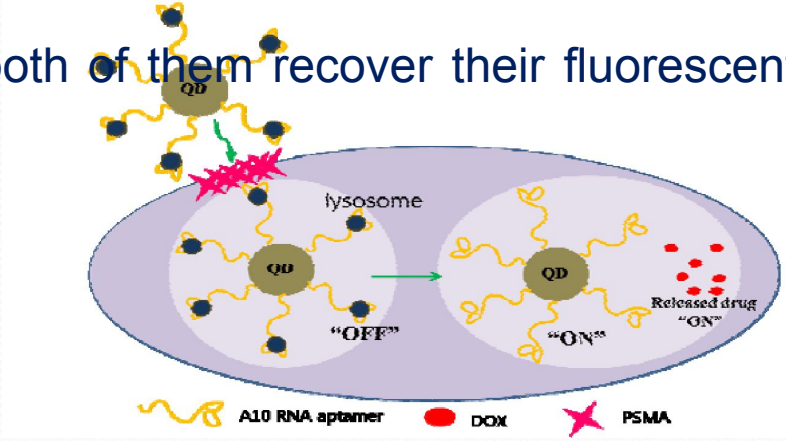
A10 RNA, aptamer that recognizes prostate specific membrane antigen (PSMA), was conjugated to the QD to target cancer cells.

Doxorubicin (DOX), a well known anthracycline (anthracyclines are **widely used chemotherapy drugs derived from certain types of Streptomyces bacteria)** drug with fluorescent properties, was intercalated in the conjugate. This conjugate offers an exciting method of imaging cancer cells.

The intercalated DOX within A10 RNA conjugated to the QD quenches the fluorescence of both DOX and QD.

When the QD-aptamer (DOX) conjugate finds the target cancer biomarker, it is taken into the cancerous cell through endocytosis. (**Endocytosis** is a cellular process in which substances are brought into the cell.)

When DOX is released from the conjugate, both of them recover their fluorescent properties and thus can be imaged.



QDs can be used as **signal amplifying agents** in ultrasensitive cancer biomarker detection . A recent study has been conducted with QD functionalized nanoparticles in immunoassays, targeting alpha-fetoproteins (AFPs).

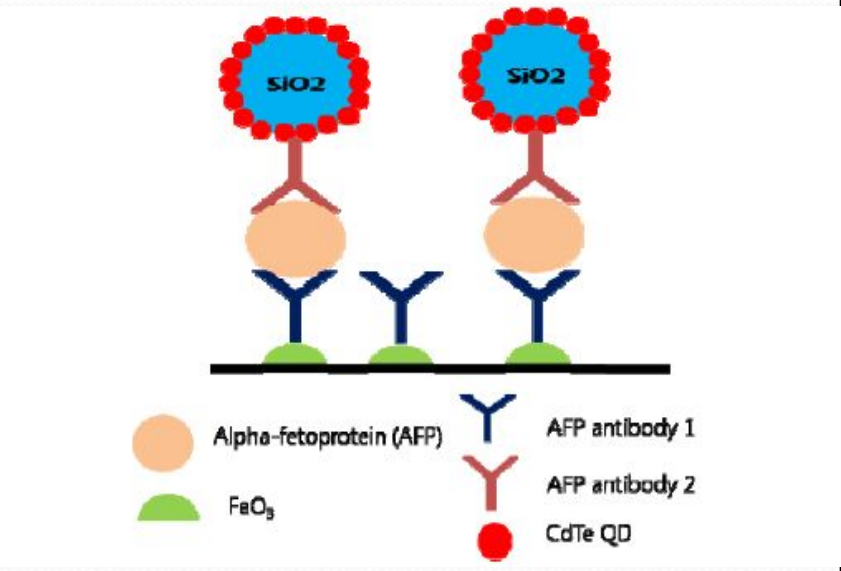
CdTe QDs have been coated on SiO2 particles and through anodic stripping. Si/QD/antibody showed increased oxidation current of Cd2+ proving its signal amplifying ability.

Increased amount of QDs per biomarker make the detection more sensitive, thus enabling detection even at low concentration

Magnetic particles have also been functionalized with QDs for cancer targeting, separation and imaging.

A high fluorescent multi-labeling could be achieved with this conjugation, providing both magnetic manipulation and multicolor fluorescent images.

By immobilizing anti-epithelial cell adhesion molecule (EpCAM) antibody, this conjugate targeted tumor cells circulating tumor cells.



QDs have also been integrated into nano-bio-chips (NBCs) for detecting multiple cancer biomarkers.

QD-labeled antibodies were used for multiple-color-fluorescence transduction signaling NBCs in combination with antigen capture by a microporous agarose bead array held in microfluidics.

Cancer biomarkers of interest were carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), and Her-2/*neu* in serum and saliva samples.

This type of miniaturized chip proved to be superior to traditional enzyme-linked immunosorbent assay (ELISA), reducing the detection limit by nearly two orders of magnitude.

Silica nanoparticles doped with fluorescence resonance energy transfer (FRET) dyes have been investigated as simultaneous and multiplexed detection.

Recently, a study has been conducted using these FRET nanoparticles to monitor cancer cells.

By modifying the nanoparticles with aptamers targeting T-cell leukemia and B-cell lymphoma and by changing doping ratio of the dyes trapped inside the silica shell, a variety of fluorescent emission spectra could be obtained with a single excitation wavelength.

By using the principle of decrease in transverse relaxation time due to aggregation of magnetic nanoparticles in presence of target molecules, concentration of cancer biomarkers could be measured.

This device allowed *in vivo*, local environment monitoring for cancer biomarkers and could be left implanted after tumor surgery.

A similar approached has also been used in the past to develop a biosensor to detect cancer biomarkers in turbid samples (blood, urine, and sputum).

When magnetic particles aggregate through affinity ligands to the molecular target, a decrease in the bulk spin-spin relaxation time of surrounding water molecules occurs.

This could be used as a chip-based nuclear magnetic resonance (NMR) system, and with miniaturization and multiplexing, detection of various biomarkers within a small sample volume could be achieved