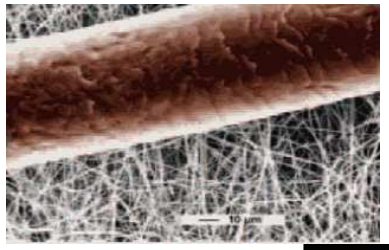
NANOPHARMACEUTICALS & DRUG DELIVERY SYSTEMS

MECHMOD

Sergei Vyboishchikov

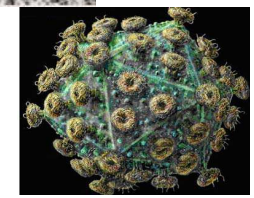
NANOSCALE



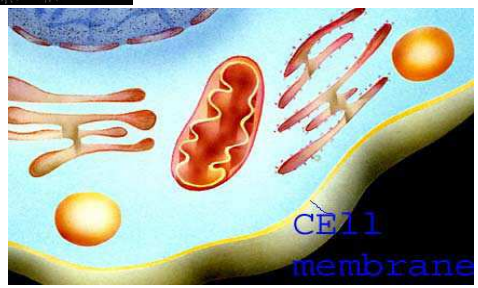
Human hair: 80 000 nm width

1 nm = 10^{-9} m = 10^{-6} mm =
= 10^{-3} μm = 10 ångström

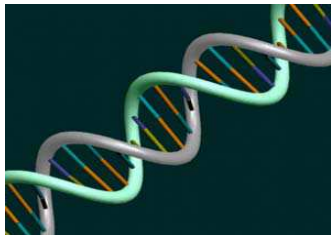
A nanometer is about the size of ten atoms in a row



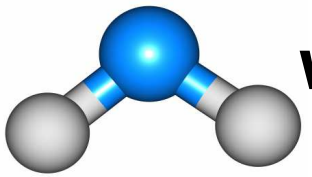
AIDS Virus: ~ 90 nm diameter



Cell membrane: 10 nm thick



DNA: 3 nm diameter



Water molecule – 0.3 nm width

Nanoparticle: 1 – 100 nm in diameter

ORIGINS OF NANOSCIENCE

- **Mesoscopic** physics:
  a sub-discipline of condensed matter physics which deals with materials that have a length scale of **between** the size of ***molecules*** and materials measuring ***microns***, typically 100–1000 nm.

- **Colloid** chemistry:
  A colloidal system = ***dispersed phase*** + ***continuous phase***
  Particle size: typically 5–200 nm
  Example: fat droplets in milk ~100–1000 nm

- **Minituarization** in technology (especially electronics):
  Example: integrated circuits;
  Chemical Vapor Deposition (CVD) and
  Physical Vapor Deposition (PVD) technologies

Visible light wave length = 380 to 750 nm

380 nm **750 nm**

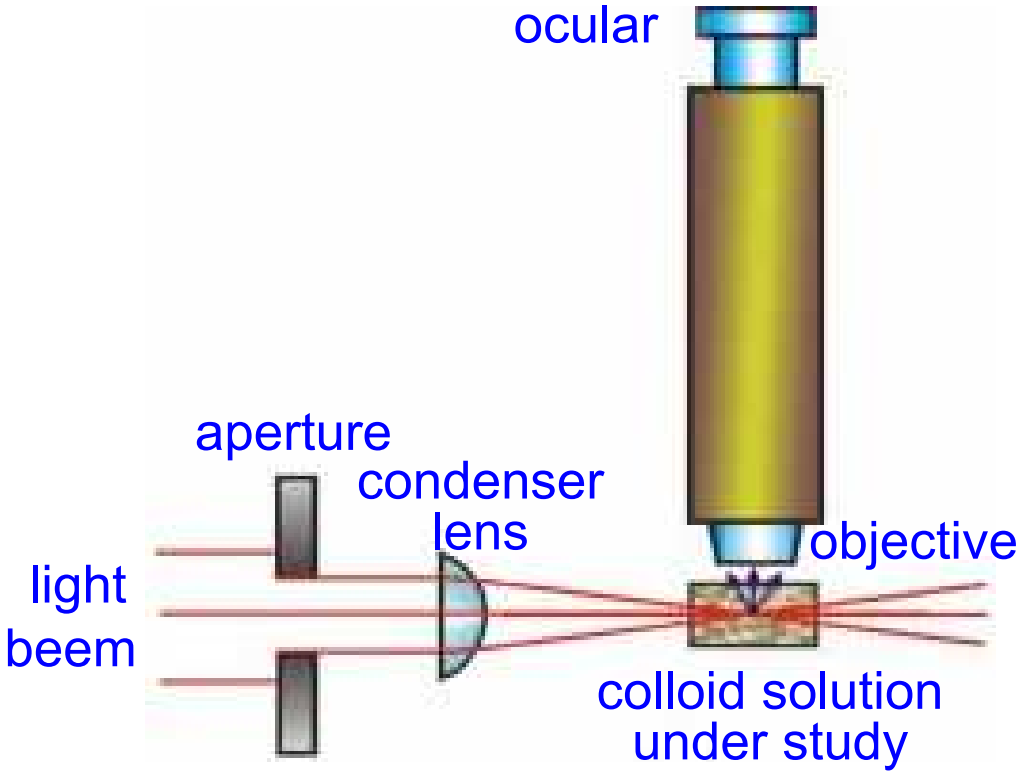
- Nanoparticles are smaller in size than the wave length of the visible light

  ⇒ light microscopy is of **no use** for stidying nanoparticles

- **Methods** available:
  - Ultramicroscopy
  - Scanning Electron Microscopy (SEM)
  - Scanning Electron Microscopy (SEM)
  - Atomic Force Microscopy (AFM)
  - Scanning Tunneling Microscope (STM)

ULTRAMICROSCOPE

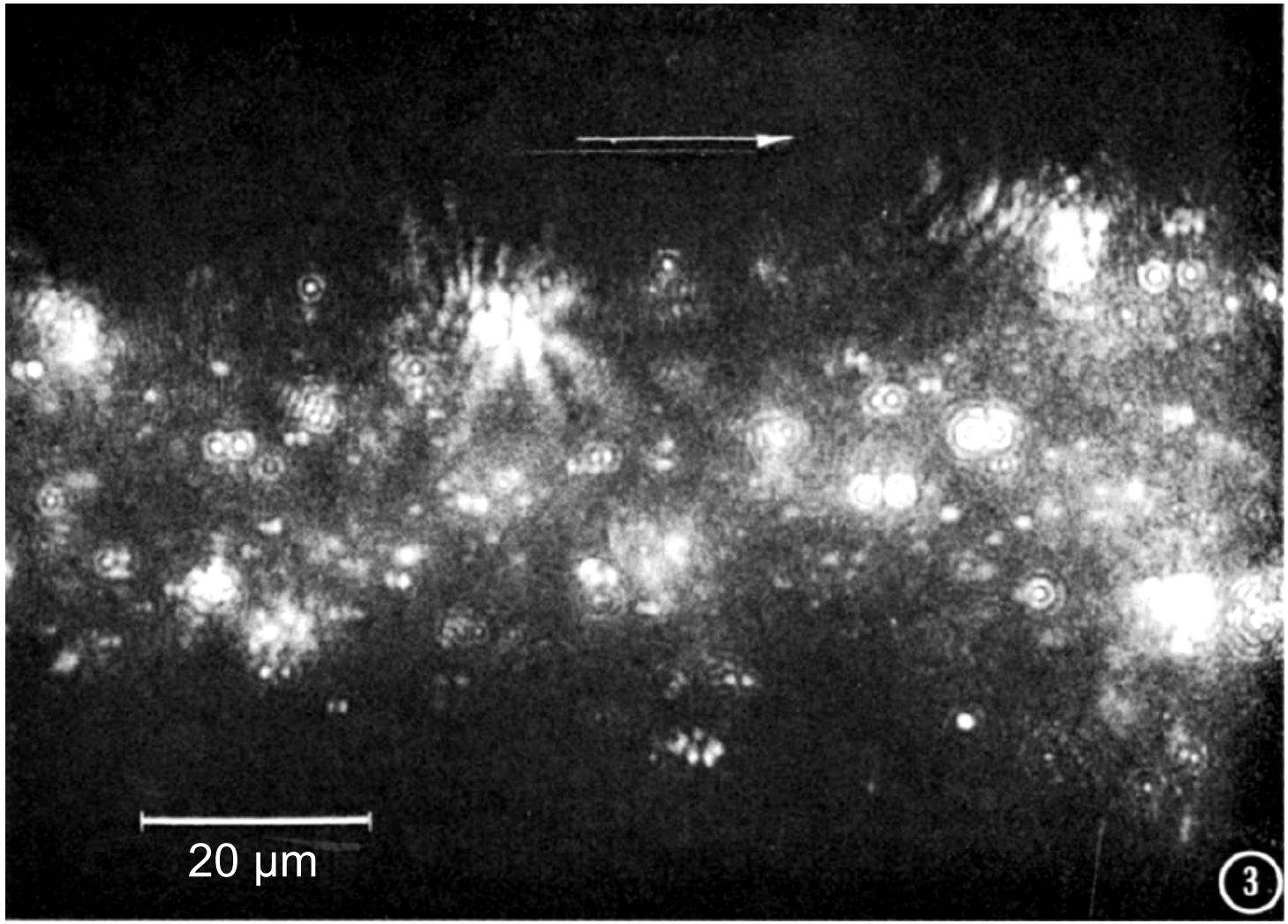
- Siedentopf & Zsigmondy **1903**; Nobel prize to Zsigmondy **1925**
- **Indivudual** colloidal particles can be observed and counted; usually their shape and size can **not** be determined
- The observation is done in direction perpendicular to the light beam **scattered** light (Tyndall effect)



ULTRAMICROSCOPE

- Typical image:

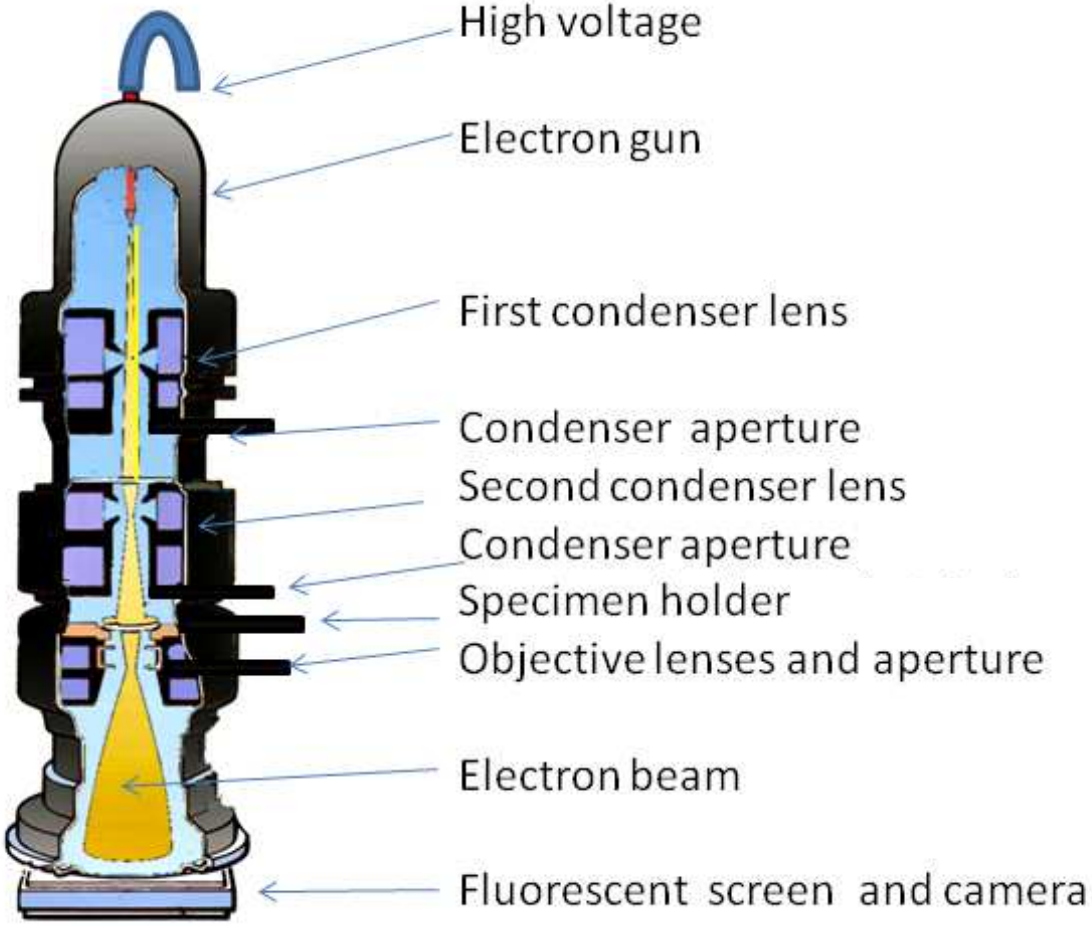
Colloidal myelin, illuminated by a laser beam. Paired bright points represent large vesicles. Single points are vesicles smaller than 1 μm. The bright illuminated areas represent large vesicles enclosing many smaller ones.



G. Albrecht-Bühler, E. D. Wachsmuth, *Trans. Am. Microscop. Soc.*, **1973**, *92*, 26–35

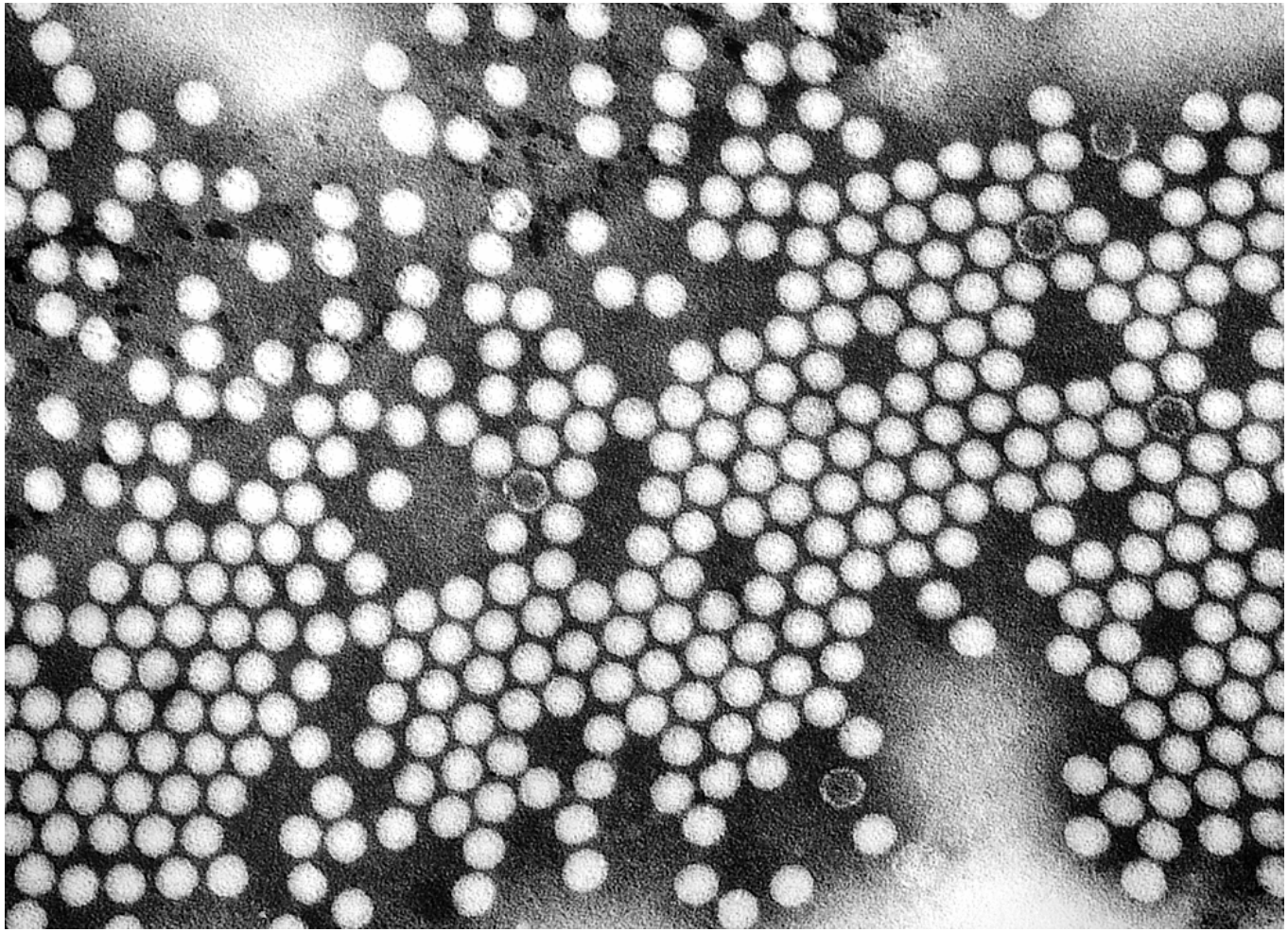
TRANSMISSION ELECTRON MICROSCOPE

- Use high-energy **electron beams** instead of light
- **Magnification** of up to 1 million times
- **De Broglie** wavelength of an electron can be much smaller than of a photon



TRANSMISSION ELECTRON MICROSCOPE

- Typical image:
  Polio virus (~30 nm in size)



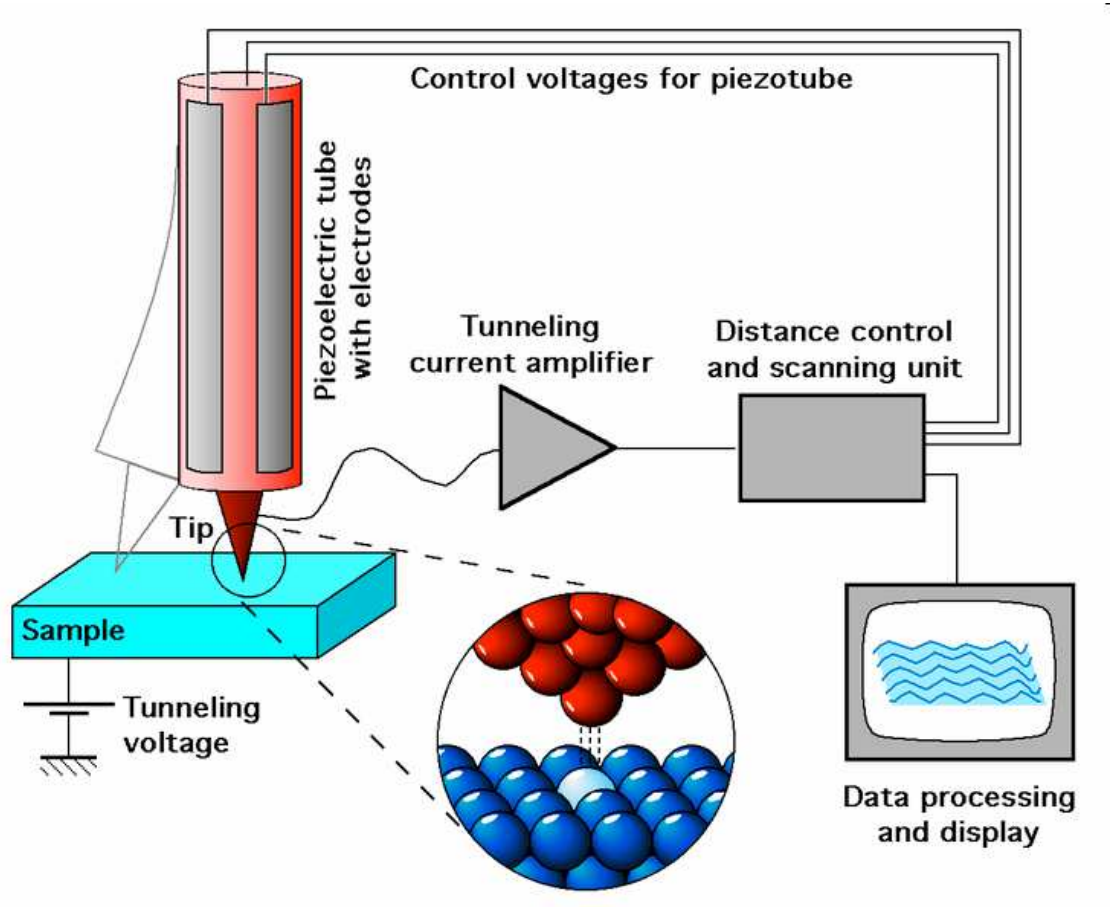
SCANNING ELECTRON MICROSCOPE

- Sample surface is scanned with an electron beam in a **raster scan** pattern
- **Magnification** of 10 to 500 000 times – about 10 nm resolution
- **3D images** are possible
- Sophisticated sample preparation
- Conducting surfaces are usually needed

SCANNING TUNNELING MICROSCOPE

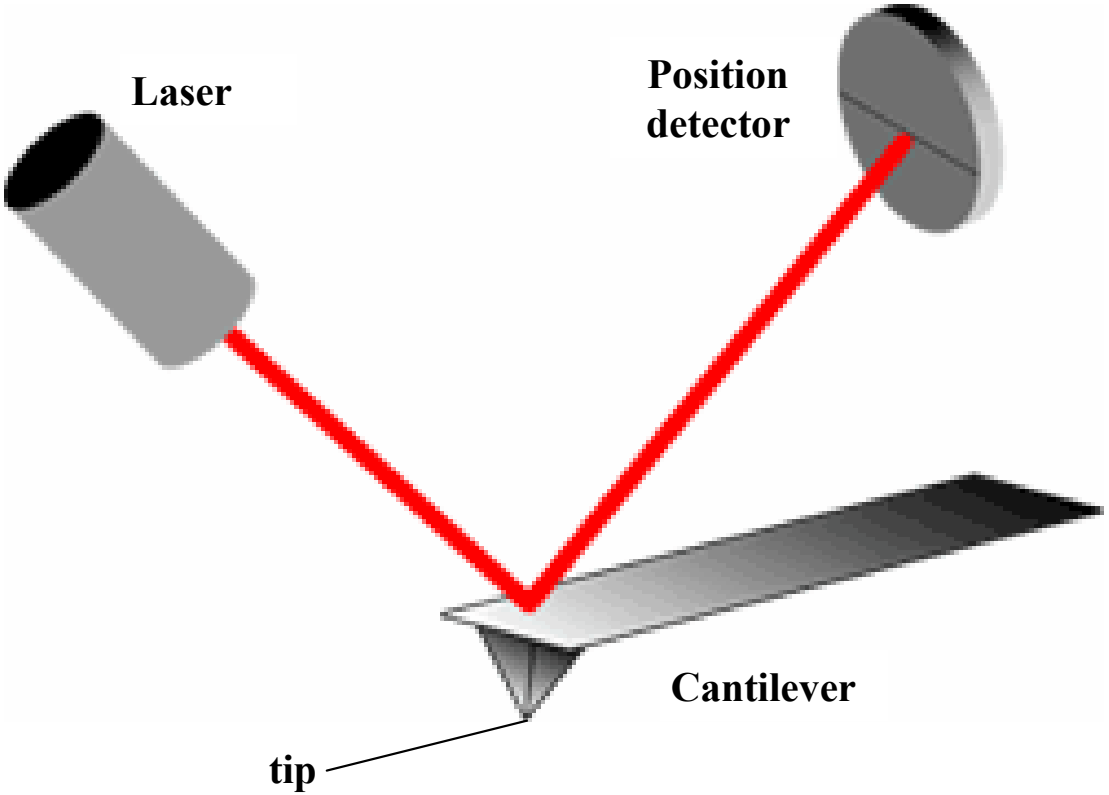
- A very fine conducting **tip** is move along the **surface** to be studied
- The **tunnel current** between the **tip** and the **surface** is measured

  ⇒ **conducting** surfaces only
- A **quantum effect**
- **Atomic** or nearly atomic **resolution**



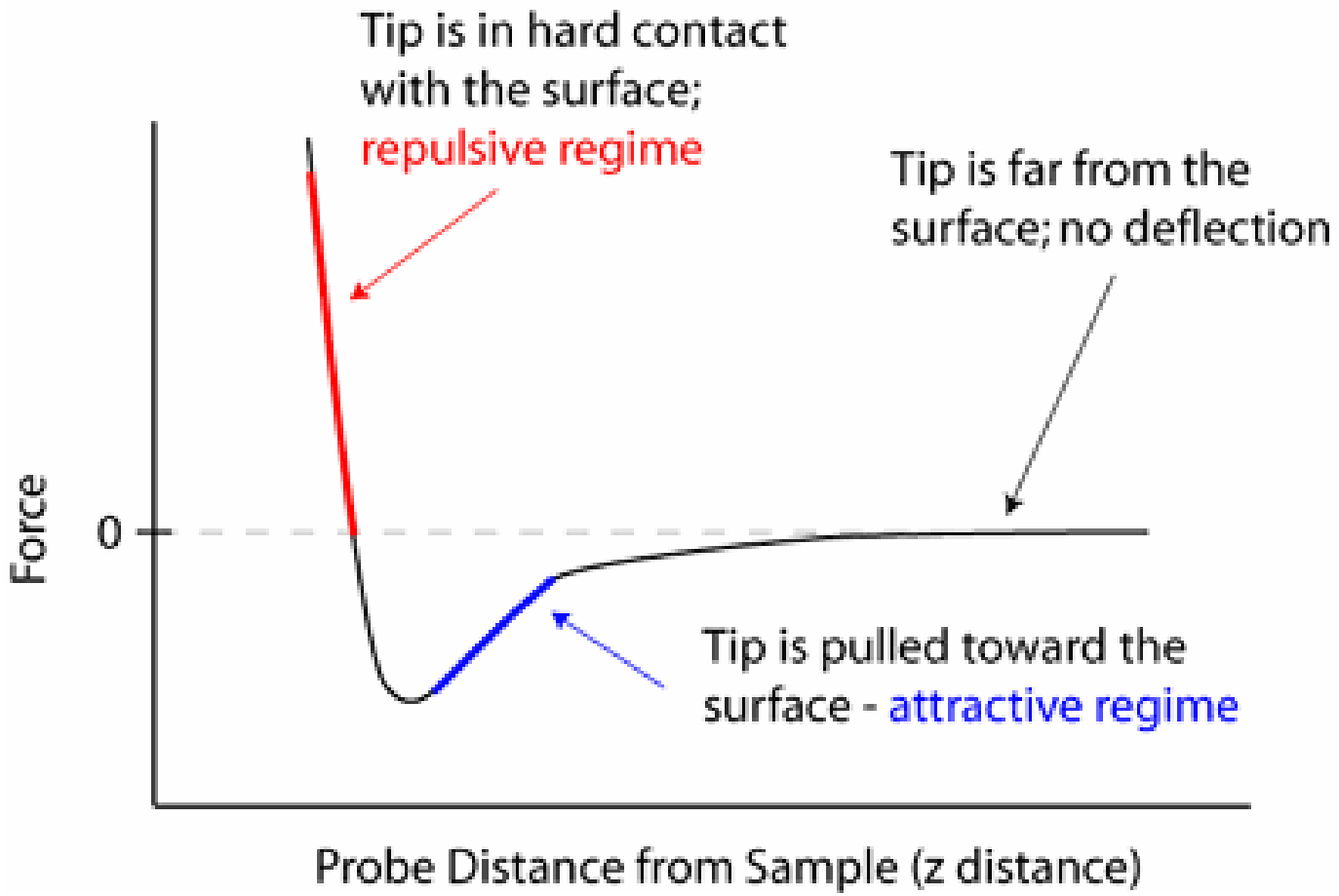
ATOMIC FORCE MICROSCOPY

- **AFM** can image almost any type of surface (polymers, ceramics, composites, glass, and biological samples)
- **Tips** and **cantilevers** are microfabricated of **Si** or **Si_3N_4**.



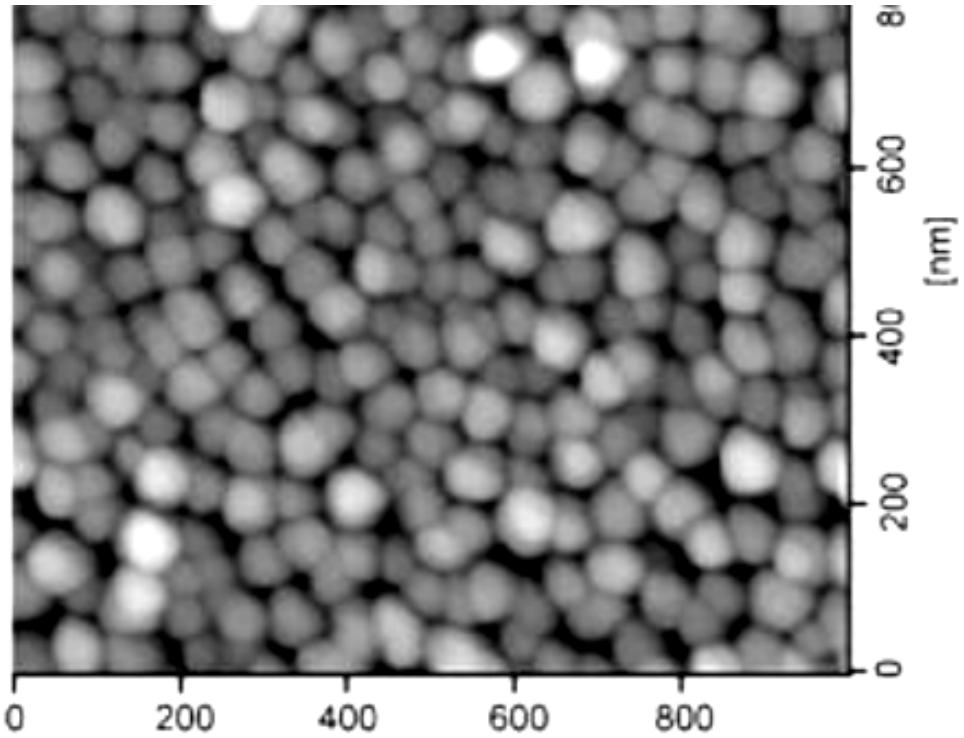
ATOMIC FORCE MICROSCOPY

- Force measurement

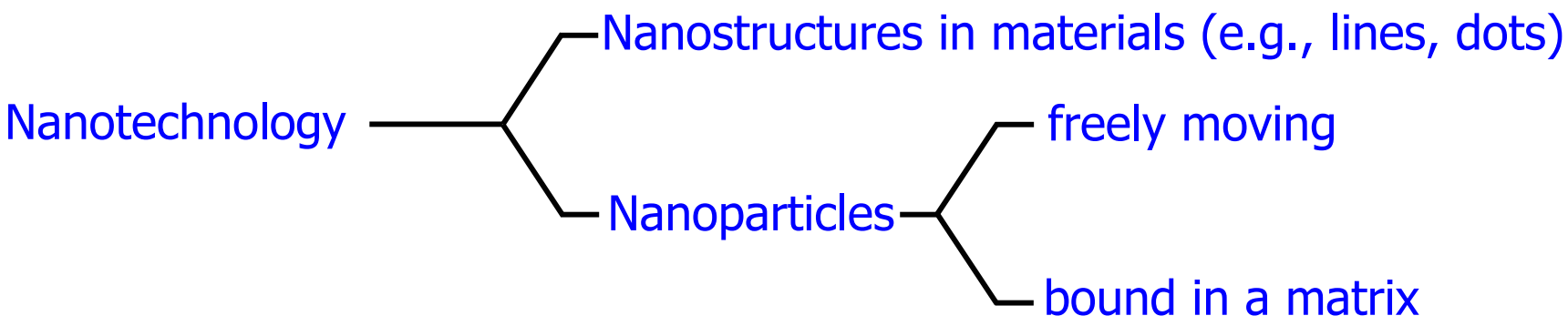


ATOMIC FORCE MICROSCOPY

- Typical image:
  Gold nanoparticles



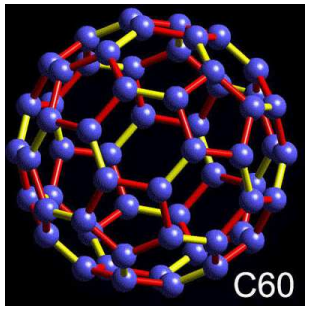
NANOTECHNOLOGY



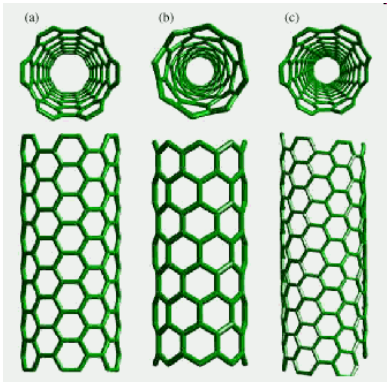
Nanostructures can be nanosized in:

- two dimensions (nanotubes, nanowires)
- three dimensions (nanoparticles)

NANOPARTICLES



C_{60} – fullerene



a) armchair (b) zigzag (c) chiral

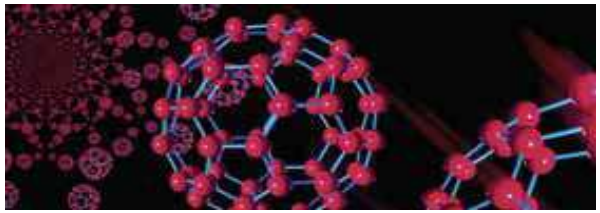
Carbon nanotubes

Technological applications:

- AFM (atomic force microscope) probe tips
- Flat panel display screens ("NanoEmissive Display")
- Nanocomposite materials
- Hydrogen storage
- Nanoscale electronics

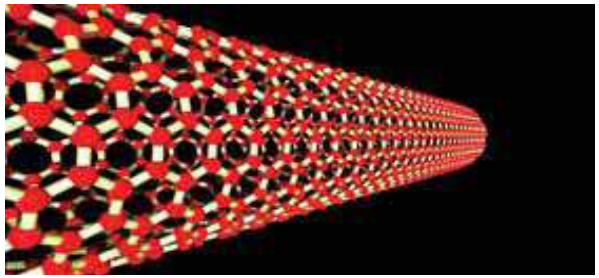
NANOPARTICLES

- C_{60} – **buckminsterfullerene**
  - – Strong and light

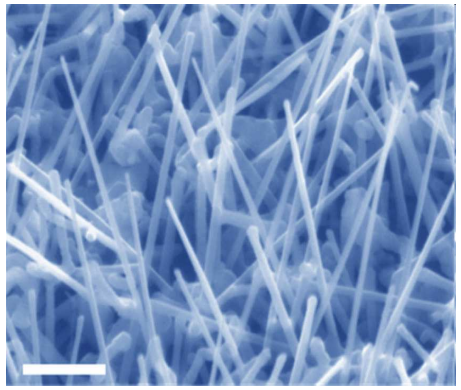


- Carbon **nanotubes**

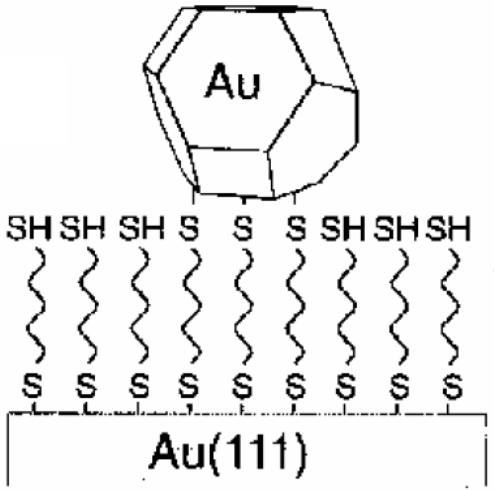
– Electronic, magnetic, and mechanical properties



- **Nanowires**
  - – Narrow, electrical devices, biosensors



- **Self-assembled** nanostructures:
  - – thiols on gold suface



HYDROGEN STORAGE

- Carbon nanotubes are able to store hydrogen and could provide a safe, efficient, and cost-effective way to do so.

- Hydrogen atoms bond to the carbon atoms of the nanotube, and can be later released with slight changes in temperature and pressure.

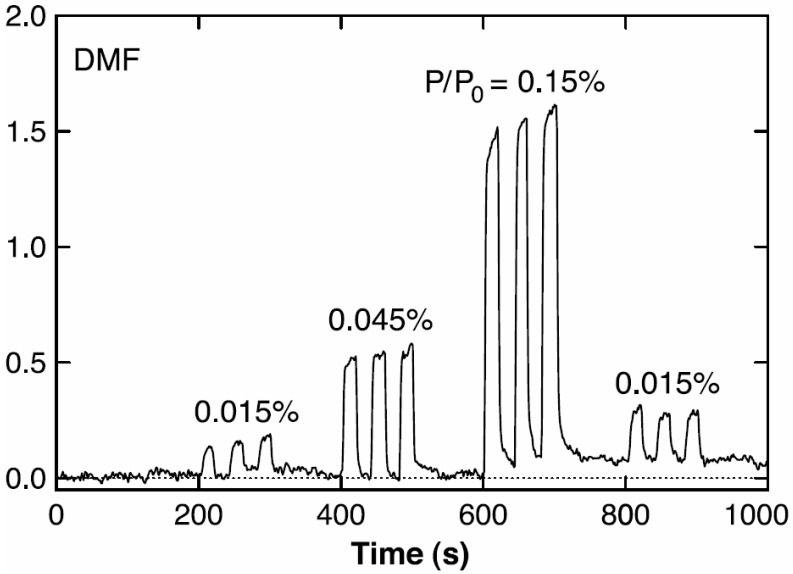
Dillon, A.C. *et al*. Science, 286, 1127 (1999)

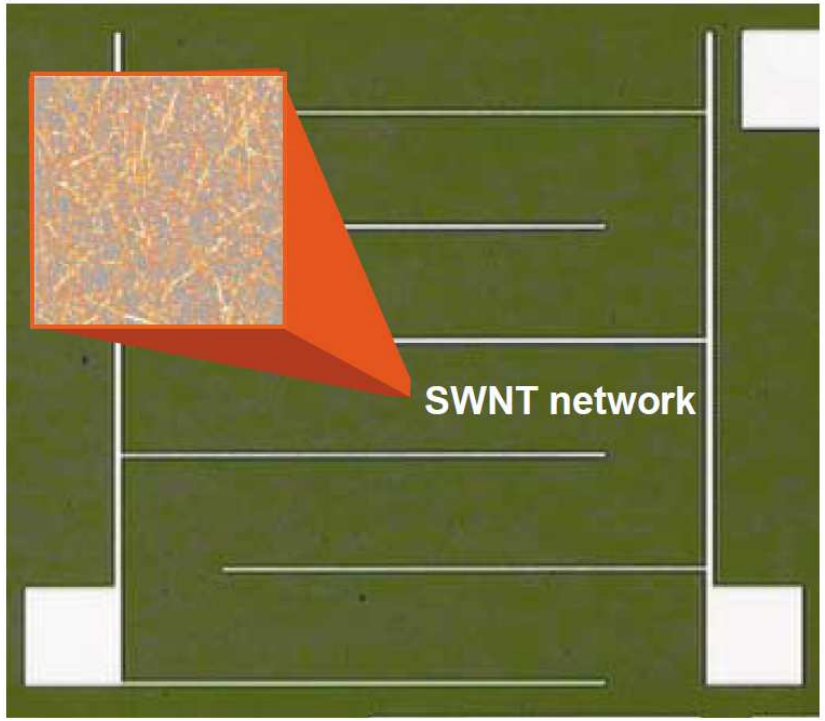
CHEMICAL SENSORS

Semiconducting carbon nanotubes display a large change in capacitance (i.e., ability to store charge) in the presence of certain gases (e.g., NO_2 and NH_3). Also detecting chemical weapons (sarin)

- smaller size
- higher sensitivity
- faster response

Use for security
and environmental applications.





Capacitance change $\Delta C/C$ of a SWNT chemicapacitor in response to repeated doses of DMF

NANOMEDICINE

- **Nanomedicine** is medical application of nanotechnology.

- It covers areas such as nanoparticle drug delivery and possible future applications of molecular nanotechnology and nanovaccinology.

- Nanomedicine uses nano-sized tools for diagnosis, prevention, and treatment of diseases

- Design of nano-sized **multifunctional therapeutics**

- Design of **drug delivery systems**

- **Medical research**: using analytical tools and devices to achieve a better understanding of the molecular basis of disease

NANOMEDICINE

- Transfer of nanomedicine into routine clinical practice requires a multi-disciplinary approach and relies upon careful consideration of clinical, ethical and societal perception

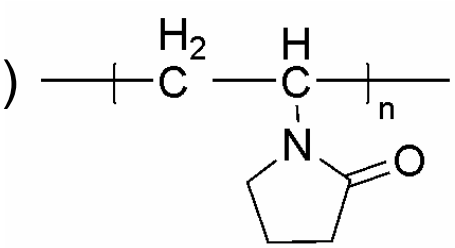
- Multidisciplinary areas:
  - Materials science
  - Device fabrication
  - Safety and toxicological issues in respect of environmental impact and manufacturing procedures

NANOPARTICLE-BASED DRUG DELIVERY

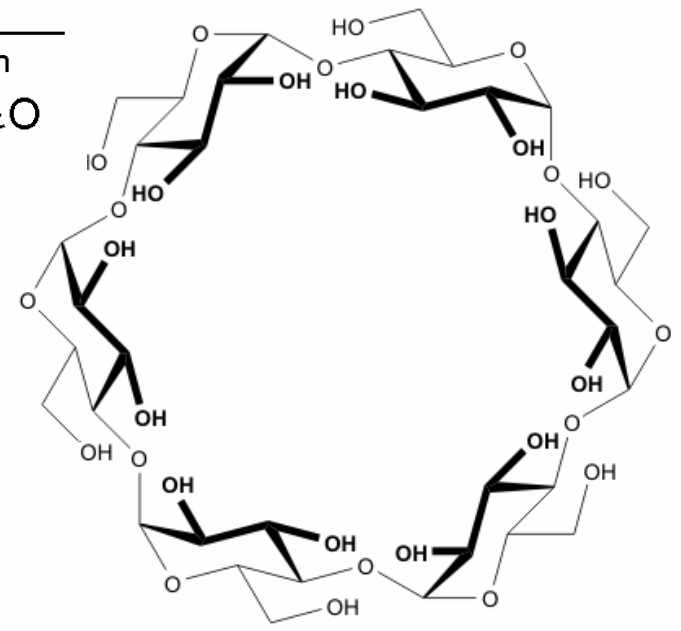
- **Drug Delivery** at macroscopic level: process of administering a drug to achieve a therapeutic effect.
  - **Non-invasive**: topical (skin), nasal, buccal, sublingual, vaginal, ocular, rectal; inhalation;
  - **Injection**: intramuscular, intravenous
- **Miniaturization** of carrier particles
- Suitability depends on **size**
- Various **routes of delivery** (e.g. intravenously)
- Main challenge: increasing half-life time of the drug
- Slower degradation for **hydrophilic** drugs (to avoid that they decompose too rapidly)
- Sustained release formulation for **hydrophobic** drugs (to avoid that they release too slowly)

NANOMEDICINE with FULLERENES

- **Toxicity** considerations
- **Solubility** issues: C_{60} is water insoluble
- Biological use of fullerenes require water **solubility** and **no aggregation**
- **Complexation** with water-soluble (supra)molecules:
  - Surfactants
  - Polyvinylpyrrolidone (PVP)



  - Cyclodextrines



DRUG DELIVERY WITH FULLERENES

- Attaching drug molecules to a fullerene.
- Then, possibly, the medicine-loaded fullerene can then be attached to an antibody.
- Example: **taxol** is an anti-cancer drug used to treat lung, ovarian, breast cancer, Kaposi's sarcoma, etc.

– **C_{60}-Taxol conjugate** was coupling from Taxol succinate and a fullerene aminoderivative

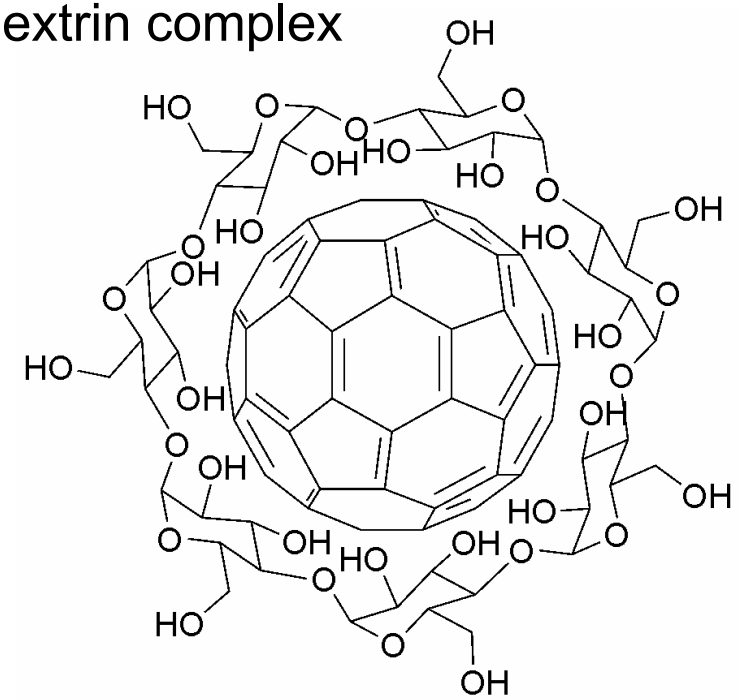
– releases Taxol in (bovine) blood plasma with a hydrolysis half-life ~80 min

TOXICITY CONSIDERATIONS for FULLERENES

- Studies examining the **toxicity** of fullerenes on human systems are the subject of much debate.
- Studies using well-characterized, **non-aggregated**, single-species fullerenes suggest fullerenes are **not toxic** at physiologically relevant doses.
- C_{60} is **toxic** to fish

SOLUBILIZATION of FULLERENES

- Supramolecular **non-covalent** solubilization
- ***Example***: C_{60} in toluene mixed with PVP in chloroform; solvents evaporated; residue dissolved in water
- ***Example***: Cyclodextrin complex

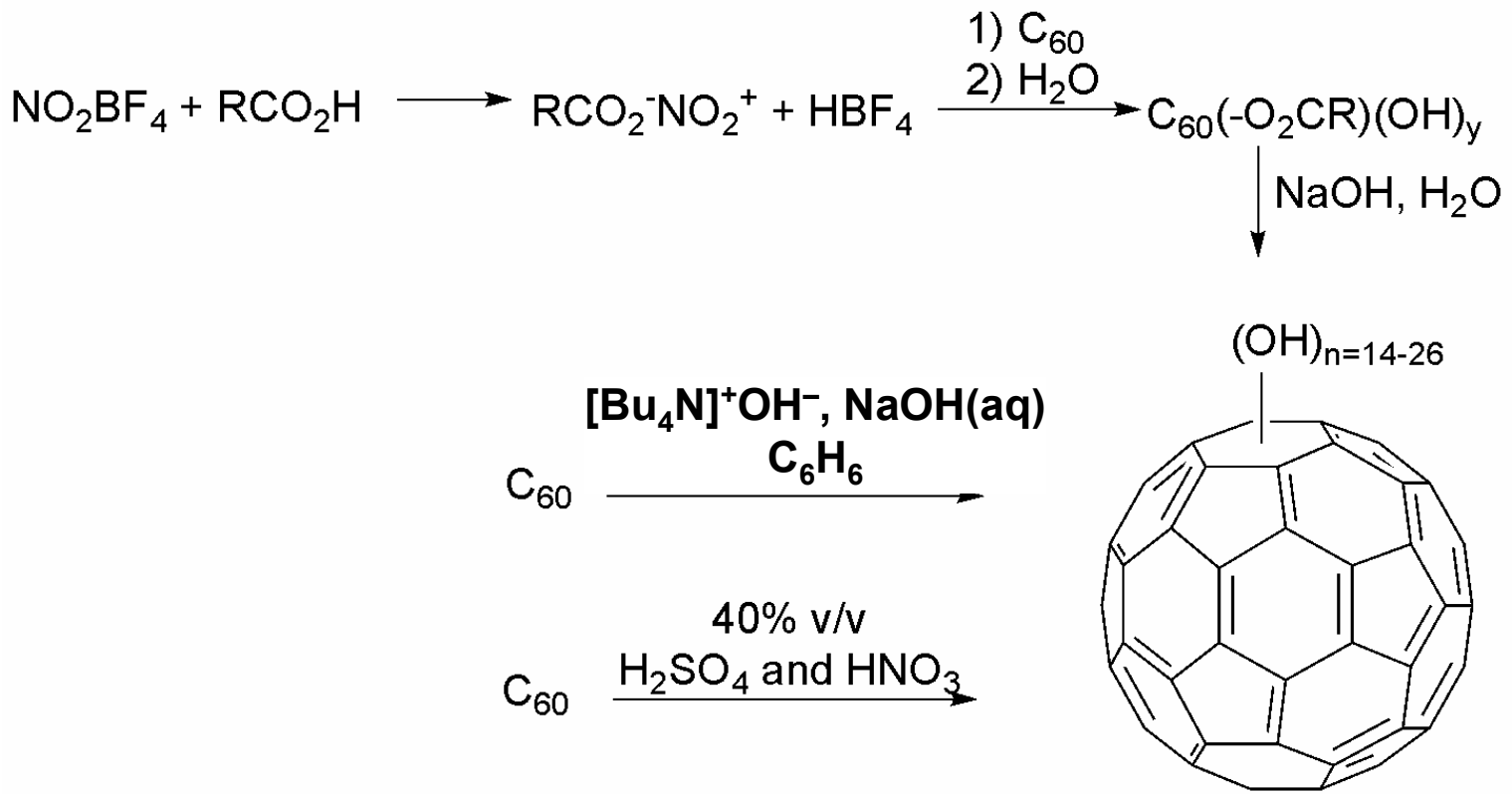


- Aggregation phenomena occur with 1:1 complexes

SOLUBILIZATION of FULLERENES

- **Covalent** approaches: expoiting the rich chemistry of fullerenes.

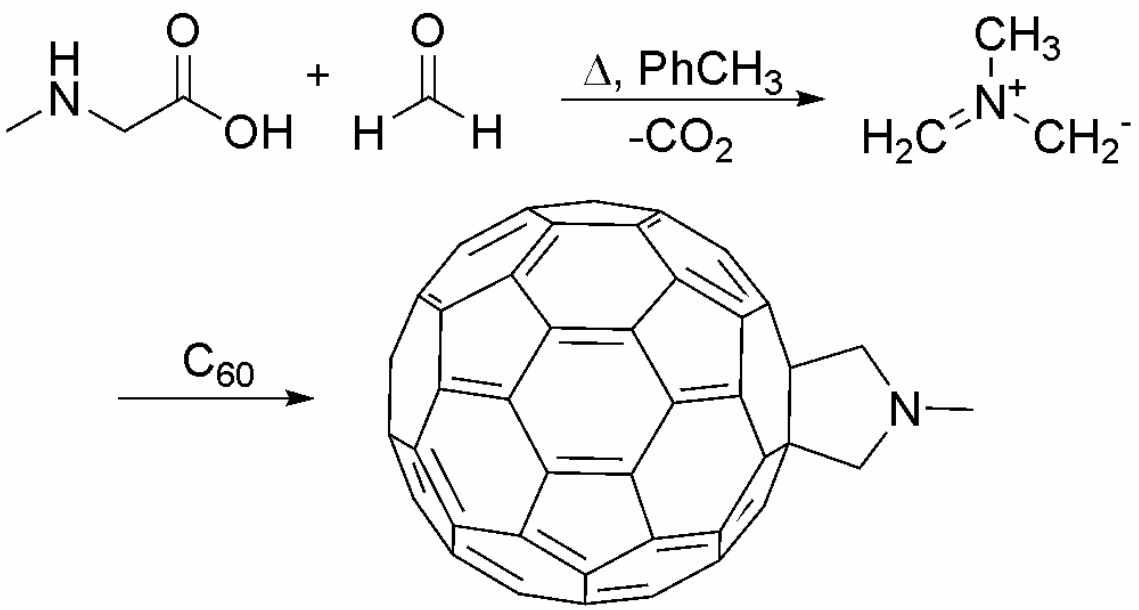
Example: water-soluble, non-aggregating C_{60} derivatives:



- Structure remains ill-defined and number of hydroxyls added is variant

SOLUBILIZATION of FULLERENES

- **Covalent** approaches: Prato's reaction



- Well-defined structure
- Addition of up to nine pyrrolidines is possible

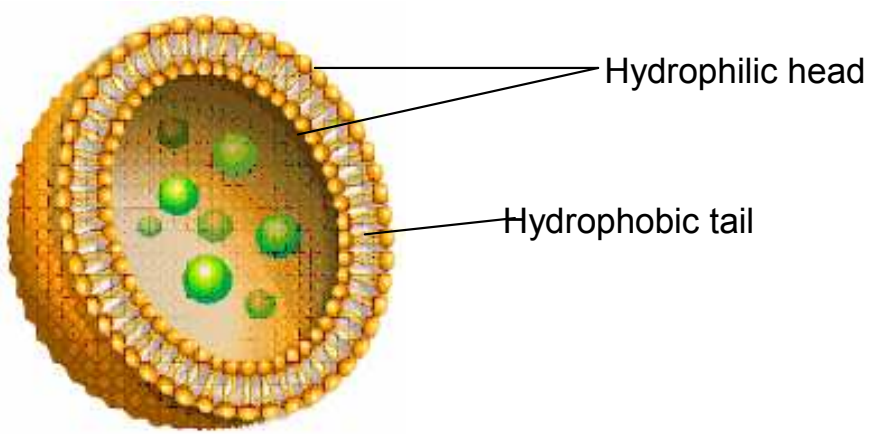
FULLERENES for MEDICAL DIAGNOSTICS

- **NMR Imaging**: usually a **paramagnetic contrast agent** (**gadolinium** compound) is used
- Problem: Gd is **toxic**
- Solution: **encapsulation** of Gd into a fullerene (**metallofullerene**)

  $Gd_2@C_{92}$

NANOPARTICLES IN MEDICINE: THERAPEUTIC APPLICATIONS AND DEVELOPMENTS

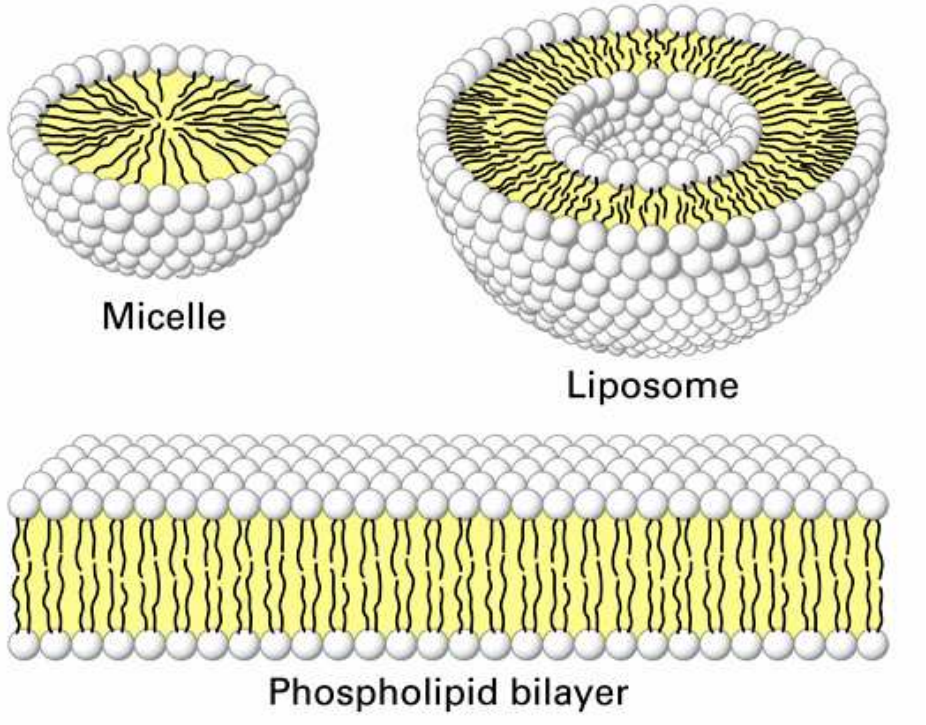
- In 2006 more than 150 companies are developing nanoscale therapeutics.
- **Liposomes** and **polymer-drug conjugates** (>80%)



L Zhang *et al. Clinical pharmacology & therapeutics*, 83, 5 (2008)

LIPOSOMES

- **Liposomes** are spherical lipid **vesicles** with a bilayered membrane



- The membrane consists of a (phospho)lipid bilayer that works as a surfactant, with a hydrophilic head toward an aqueous solution.
- The aqueous solution is both outside and inside the liposome.
- On the contrary, there is no aqueous solution inside a **micelle**.

LIPOSOMES:

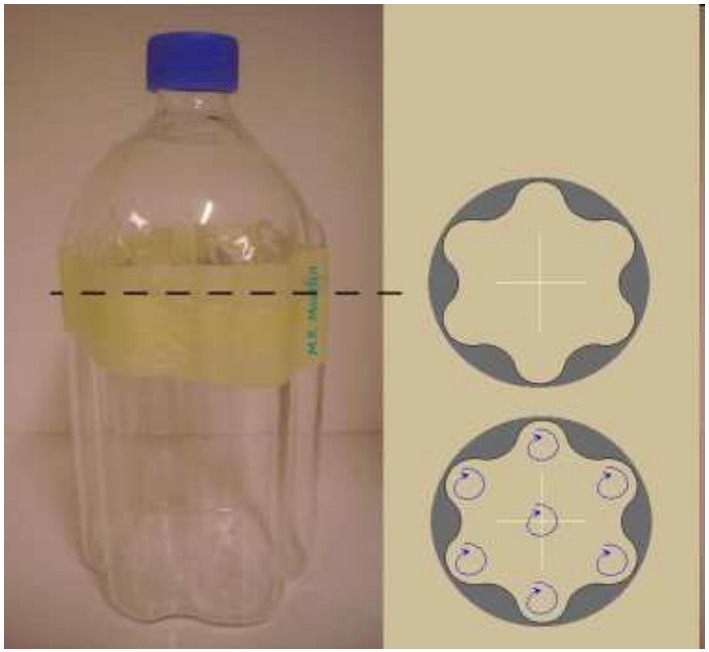
- **Multilamellar liposomes** : with several membranes and layers of aqueous solution, *onion*-like structure (Size: 2000–5000 nm)
- **Large unilamellar liposomes** : with one membrane and one vesicle of aqueous solution, *see above*.(Size: 100–1000 nm).
- **Small unilamellar liposomes** : with one membrane and one vesicle of aqueous solution, *see above* (Size: 20–100 nm).

PREPARATION OF LIPOSOMES

- Usually, liposomes are **not** formed spontaneously.
- Some lipid vesicles, but not necessarily liposomes, are formed when a phospholipid (e.g., lecithin) is placed into an aqueous soluction, upon some stirring.
- Typically, **sonication** (ultrasound) is applied. High *shear rates* (=high intensity) is needed to obrain unilamellar liposomes.
- **Sonication** is considered a "gross" method, as it can damage the drug to be incorporated into the liposome.
- More advanced methods: extrusion and Mozafari's heating method.

MOZAFARI METHOD

example: nisin encapsulation



- Heating liposomal ingredients with nisin and glycerol at 60°C while stirring by a magnetic stirrer 45–60 min under N_2 atmosphere.
- A bottle with a baffled wall of the bottle is used to create multiple turbulences.

DRUG DELIVERY USING LIPOSOMES

General advantages:

- A liposome encapsulates an aqueous solution inside a hydrophobic membrane; hydrophilic molecules cannot easily pass through the membrane.

  $\Rightarrow$ the solute *inside* (the **drug**) will not be lost;

  $\Rightarrow$ hydrophilic molecules from *outside* will not enter $\Rightarrow$ no damage to the **drug**

- A liposome can carry both **hydrophobic** (inside the membrane) and **hydrophilic** (inside the bubble) molecules

DRUG DELIVERY USING LIPOSOMES

Several delivery approaches:

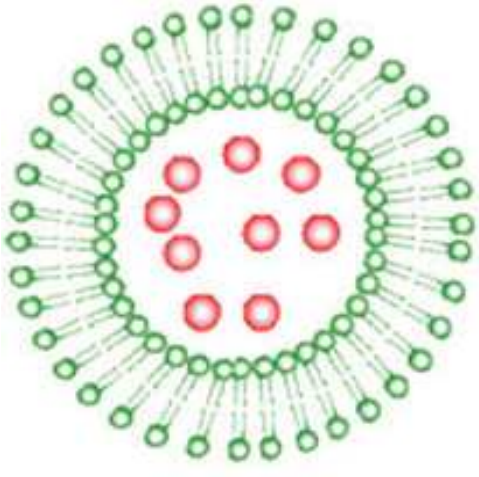
- **Fusion** of the liposome membrane with the cell membrane $\Rightarrow$ the liposome content is delivered to the cell.
- pH neutralization inside the liposome neutralizes the drug, thus allowing it to penetrate through membranes (both the liposomal and cellular) by **diffusion**.
- **Endocytosis**: engulfing liposomes with the liposomal membrane by a target cell.
- **Phagocytosis** (a particular case of endocytosis): the cell ingests the liposome. The membrane invaginates the liposome in a pocket, closes the pocket, and the liposome is sealed off into a large vacuole (*phagosome*).

LIPOSOMES AGAINST CANCER

- **Liposomes** (and some macromolecules) accumulate in tumor tissue much more than they do in normal tissues.
- Even very small tumors (~0.2 mm) depend on the blood supply and thus stimulate the production of blood vessel (angiogenesis).
- These blood vessels are abnormal – their endothelial cells are leaky, and nano-sized molecules and particles can enter the tumor cell – **Enhanced Permeability and Retention Effect** (**EPR**).
- Tumors lack a well-defined lymphatic system $\Rightarrow$ no lymphatic drainage.
- **EPR** allows for selectively targeting cancer cell, while normal cell would not be penetrated into.
- Liposomes of a size ≤400nm can rapidly enter a tumor sites from the blood, but are kept in the bloodstream by the endothelial wall in healthy tissue vasculature.
- **Examples**: Doxorubicin (known from 1950), Camptothecin (natural alkaloid discovered in 1966) and Daunorubicin

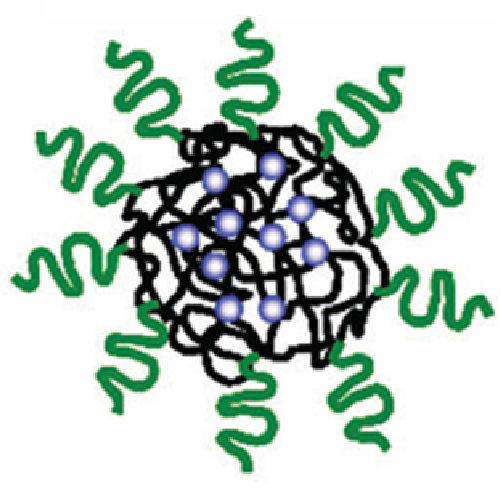
THERAPEUTIC NANOPARTICLE PLATFORMS IN PRECLINICAL DEVELOPMENT

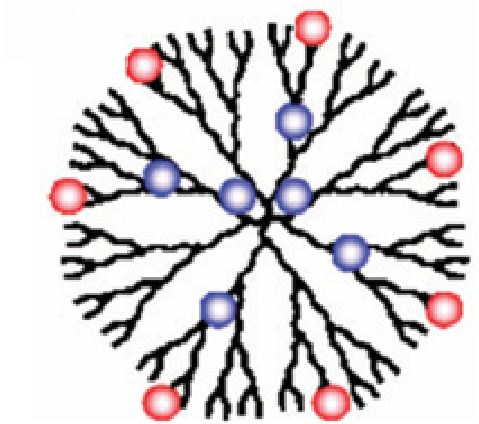
Liposome



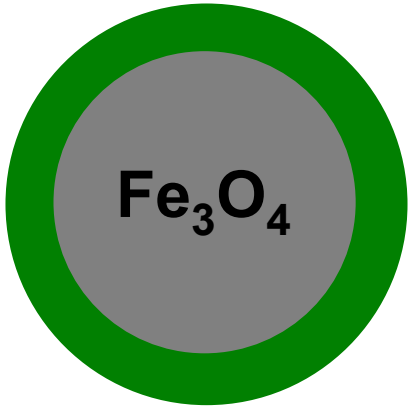
Polymer-drug conjugate

Polymeric nano-particle





Dendrimer



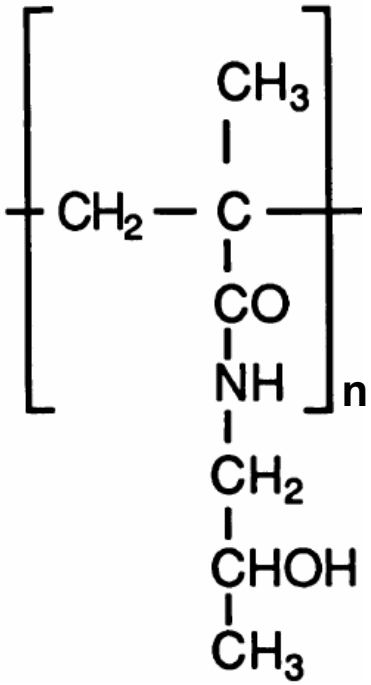
Iron oxide nanoparticle

hydrophilic drug

hydrophobic drug

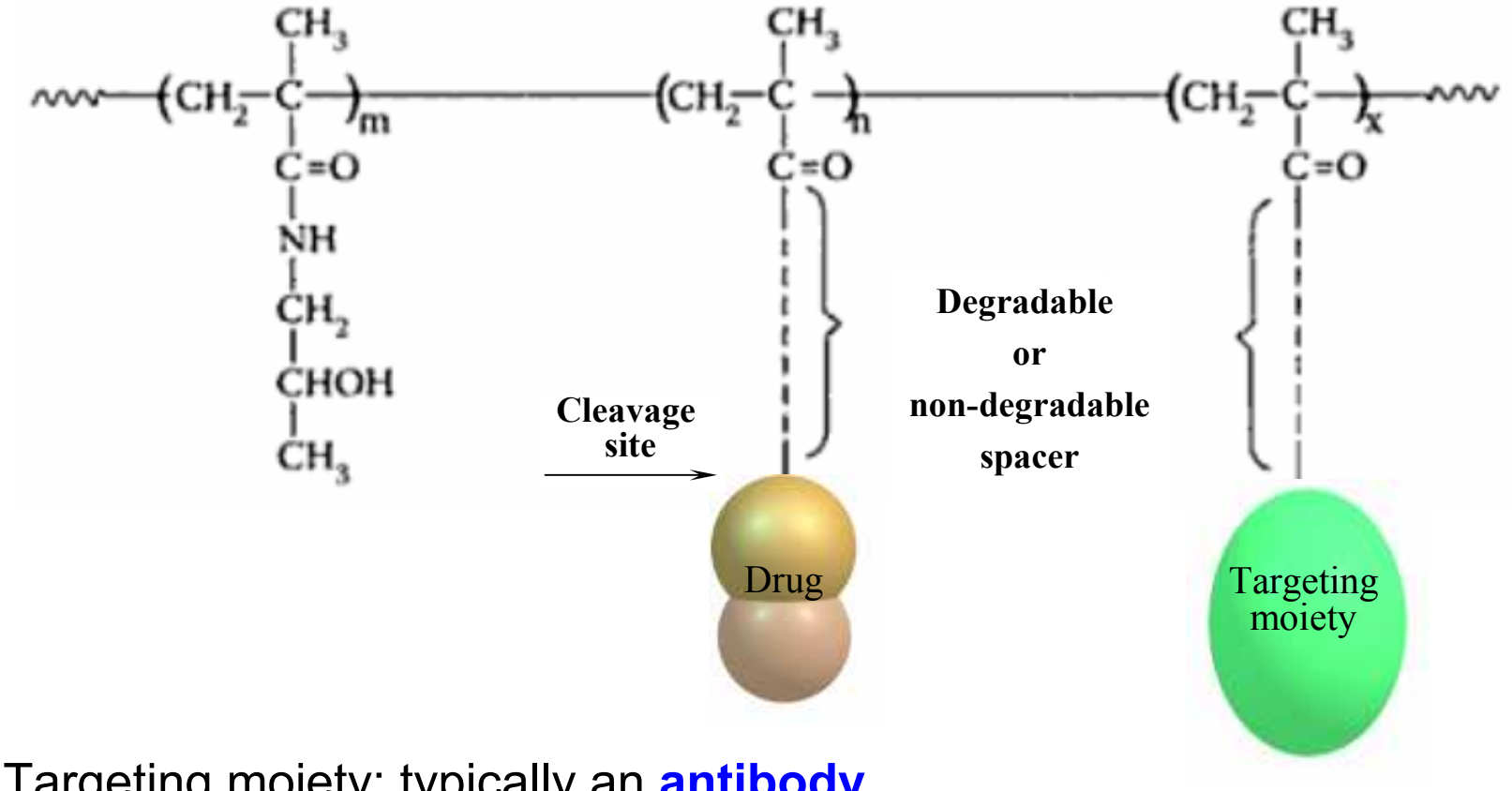
POLYMER-DRUG CONJUGATES

- **Polymers used for conjugation** must be:
  - hydrophilic
  - non-immunogenic
  - bio-compatible and bio-degradable
- Allows tuning molecular weight
- Examples of polymers used:
  - **polyethylene glycol (PEG):** $(–O–CH_2–CH_2–O–)_n$
  - ***N*-(2-hydroxypropyl) methacrylamide (HPMA)**
  - various **copolymers**



POLYMER-DRUG CONJUGATES

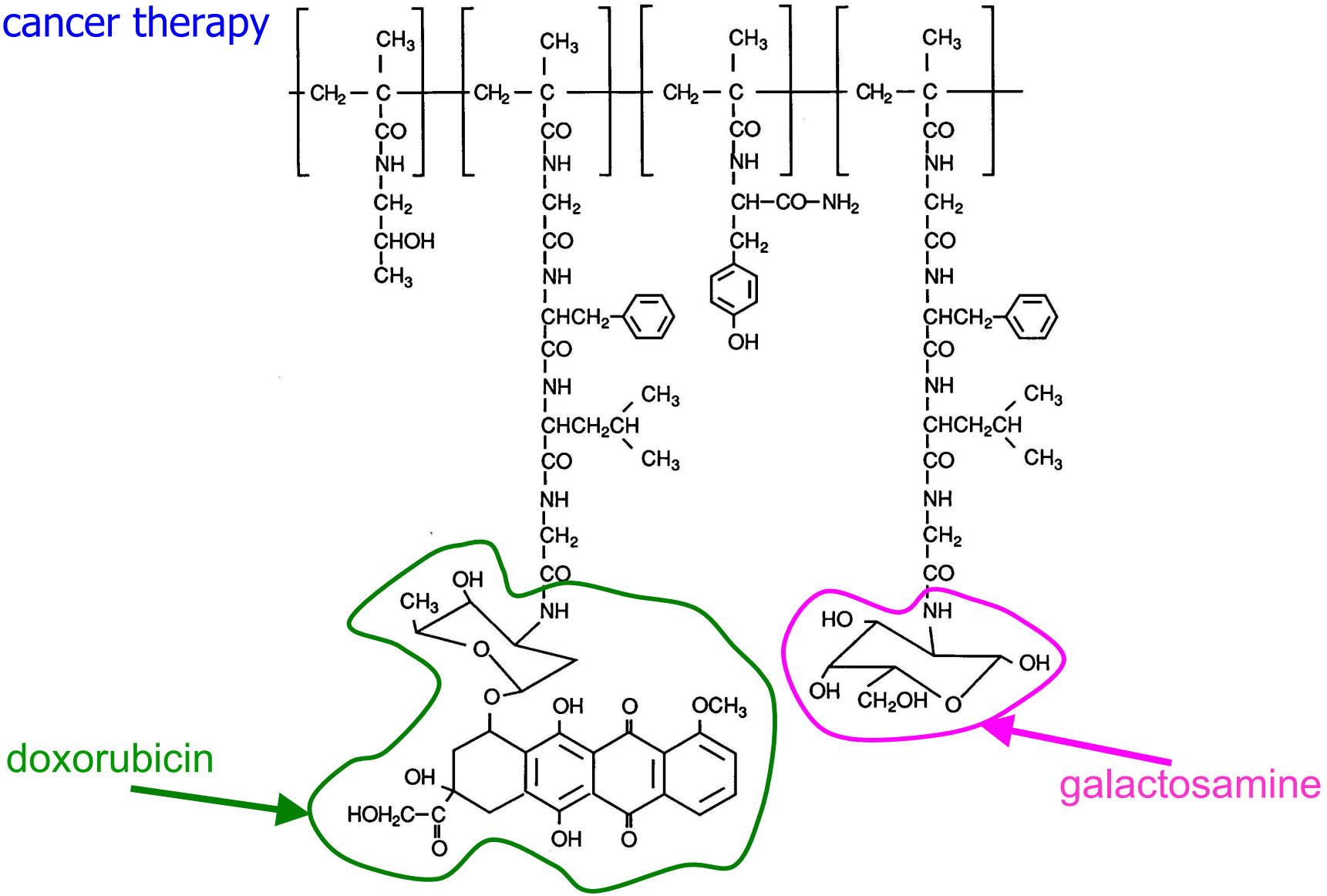
- **Drug delivery system with an HPMA backbone**:



Targeting moiety: typically an **antibody**

POLYMER-DRUG CONJUGATES

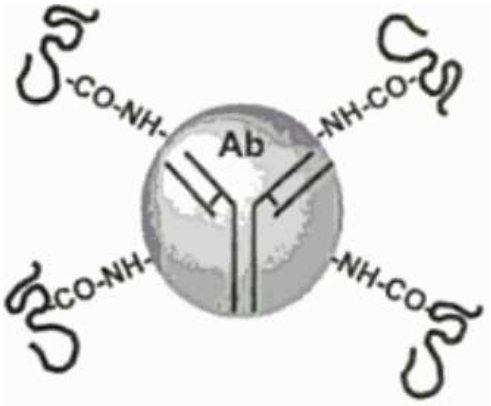
- **Example of HPMA usage:** HPMA copolymer containing doxorubicin for liver cancer therapy



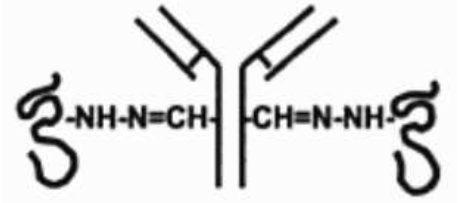
Seymour, Ulbrich, Wedge, Hume, Strohalm, Duncan, *Br. J. Cancer* (1991), 63, 859

POLYMER-DRUG CONJUGATES:

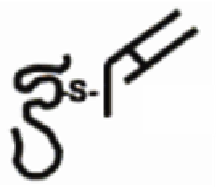
- Linking **polymer** to the **antibody**:



Random attachment by **amide** linkage



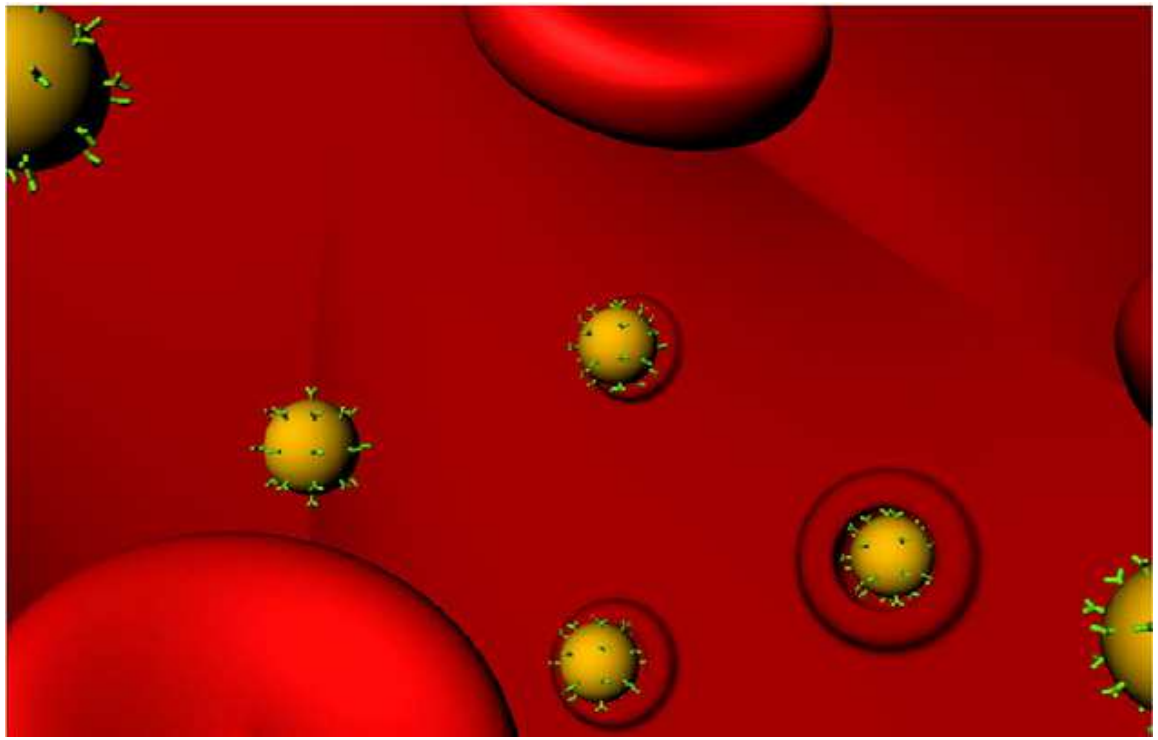
site-specific attachment by **hydrazone** linkage (via sugar chains)



site-specific attachment by **thioether** linkage (via sulfhydril groups)

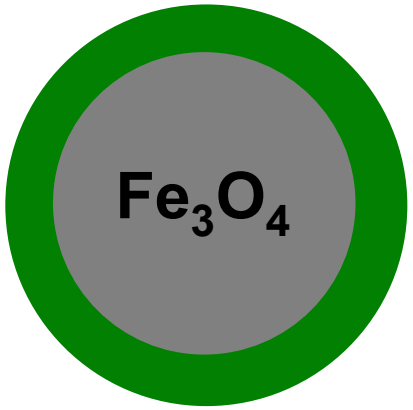
POLYMER-DRUG CONJUGATES:

- **Example:** antibody-coated nanoparticles for the active targeting of endothelial cells.



MAGNETIC NANOPARTICLES

- Mostly used are **Fe_3O_4** nanoparticles
- Also used are **Co**, **Fe**, and **FeCo** nanoparticles
- Only **coated** magnetic are useful in bio-systems to avoid aggregation and achieve bio-compatibility
  - **SiO_2**-coating by reverse microemulsion synthesis
  - **surfactant** coating by reverse microemulsion synthesis
  - **polysaccharide** coating
  - **...**



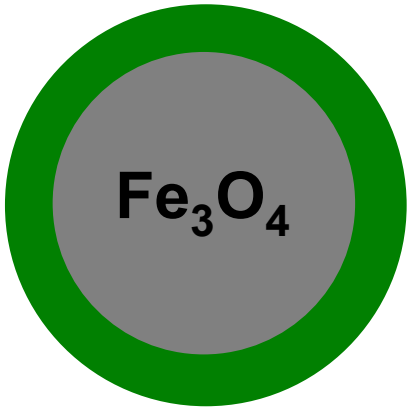
Iron oxide nanoparticle

MAGNETIC NANOPARTICLES

- **Synthesis** of PEG-coated **Fe_3O_4**

  Co-precipitation method:

  - $FeCl_3$ (aq)+ $FeCl_2$ (aq) + $NH_4OH \rightarrow Fe_3O_4$
  - Stirring Fe_3O_4 with PEG (polyethylene glycol) and sodium oleate
  - Centrifugating and drying
  - Separation and fractioning in the magnetic field



Iron oxide nanoparticle

MAGNETIC NANOPARTICLES

- **Usage: Magnetic hyperthermia**

  – (Superpara)magnetic nanoparticles produce heat in alternating magnetic field

  – If put inside a tumor and the patient placed in the magnetic the tumor is heated and eventually killed (t> 45 °C)

  – Can be combined with chimiotherapy (t~ 42 °C)

- **Usage: NMR Imaging**: (superpara)magnetic nanoparticles as a **paramagnetic contrast agent**

QUANTUM DOTS

- **Quantum dot**: a **semiconductor** whose electrons and holes are **confined** in all three spatial dimensions
  - a very small piece of a semiconductor material

- **Quantum behavior** is observed when the QD is sufficiently **small**:

$$\frac{\hbar^2}{2m_e R^2} \sim k_B T$$

R is the radius of the QD, m_e is the electron mass

- The **quantum behavior** manifests itself in **discrete (quantized) energy levels**:

$$E_n = \frac{\hbar^2 n^2}{2m_e R^2}$$

n is the quantum number

- The **band gap** is **size-dependent**:

$$\Delta E = \Delta E_{bulk} + \frac{\hbar^2 n}{2m_e R^2}$$

QUANTUM DOTS

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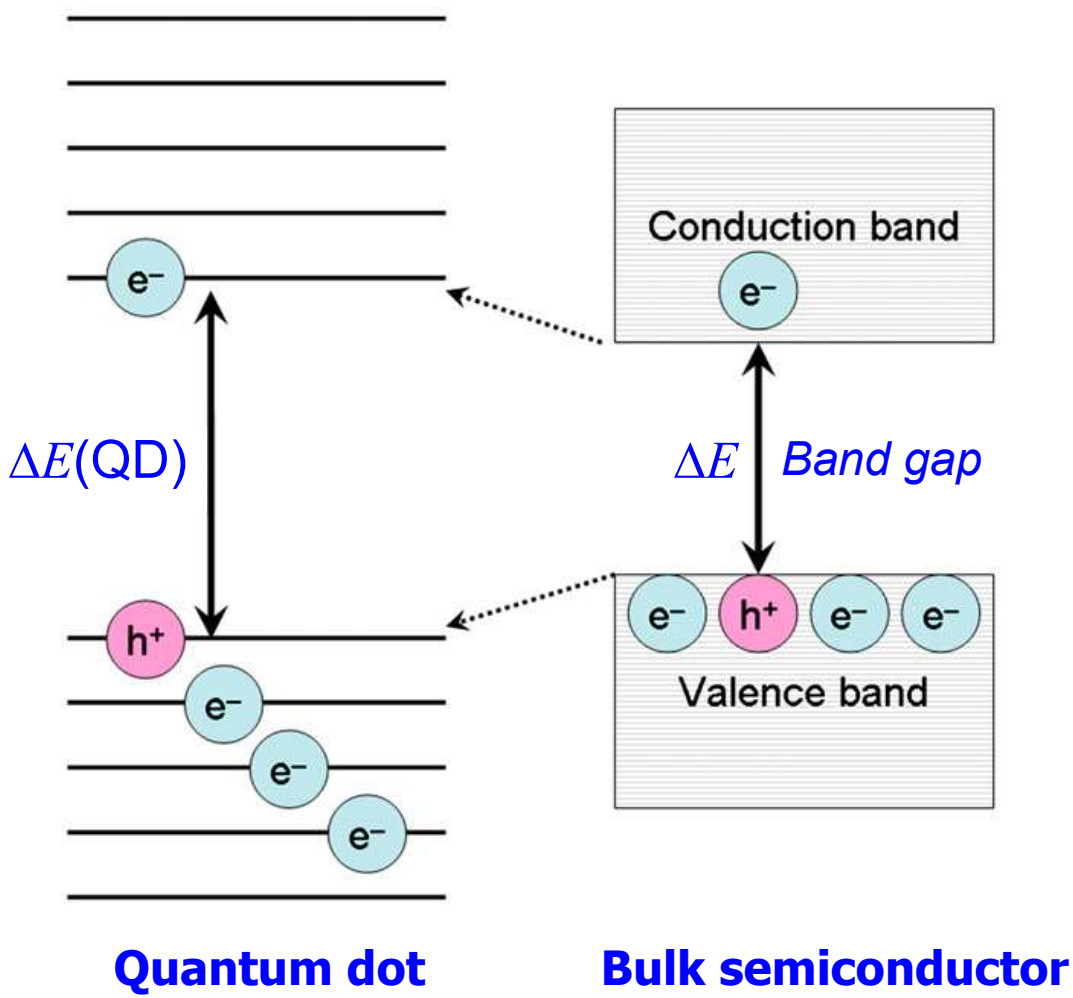


QUANTUM DOTS

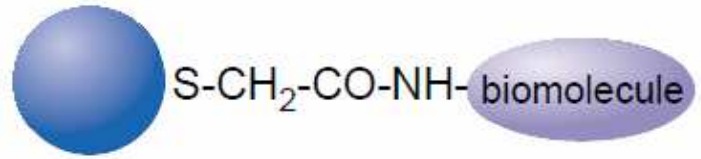
- Main application of the QDs are in LEDs **(light-emitting devices/diodes)**

- **Example**:
  - CdSe, CdS, InAs, InP
  - Size: 100–100 000 atoms ≈ diameter of 10–50 atoms ≈ diameter of 2–10 nm

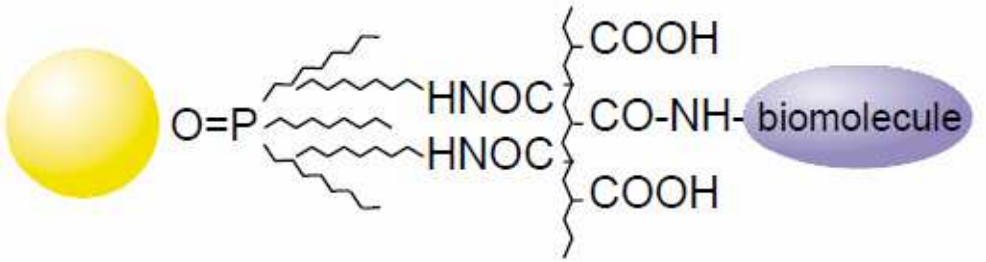
- **Preparation**:
  - Mostly colloidal synthesis

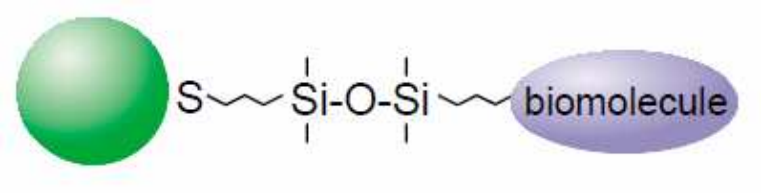
QUANTUM DOT BIOCONJUGATION

bifunctional linkage
(Use of a bifunctional ligand
e.g., mercaptoacetic acid)

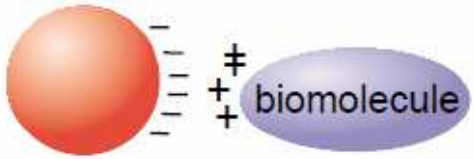


hydrophopic attraction
TOPO-capped QDs bound to acrylic polymer





Silanization
solubilization and bioconjugation
using mercaptosilane



Electrostatic attraction

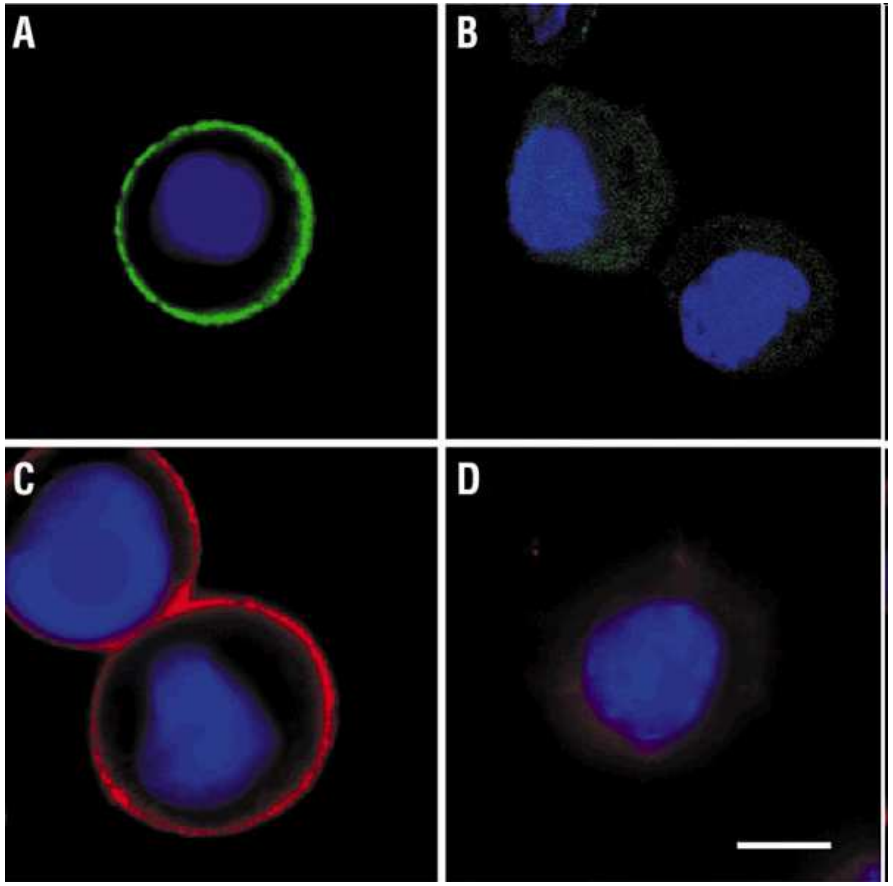
QUANTUM DOTS for CANCER DIAGNOSTICS

- **Example**:

– QDs coated with polyacrylate and covalently linked to **antibodies** or **streptavidin.**

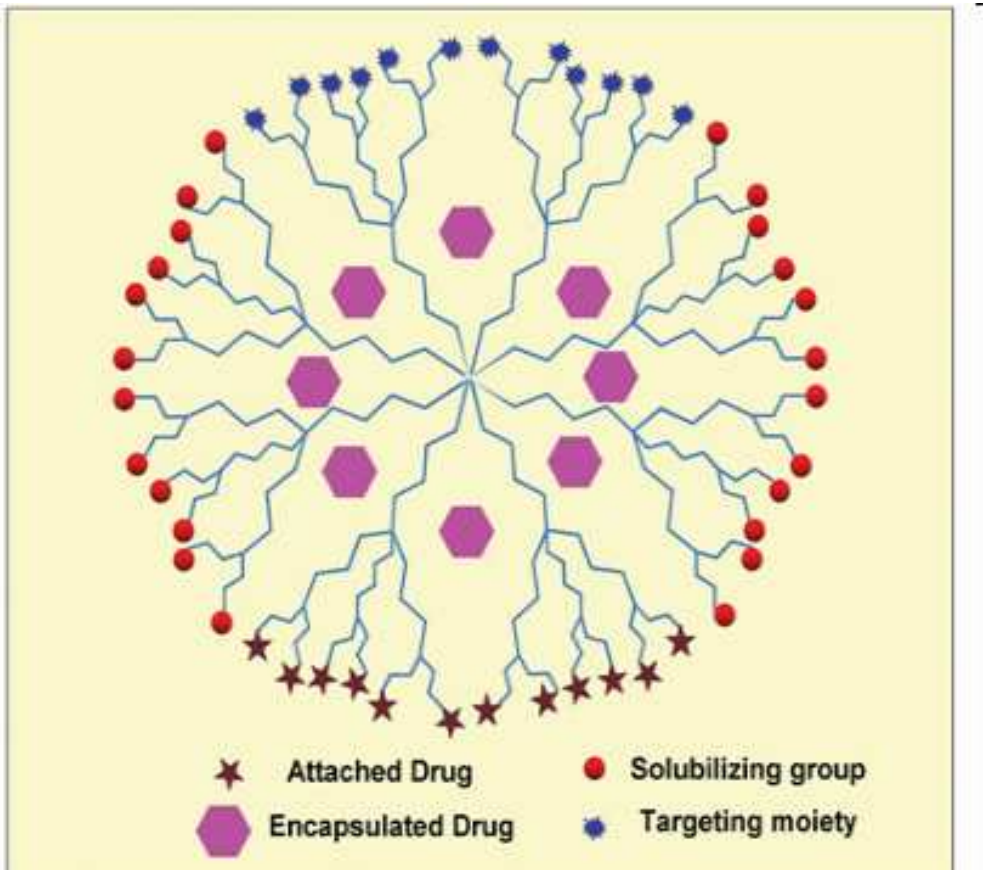
– Immunofluorescent labeling of breast cancer marker **HER2 (***Human Epidermal Growth Factor Receptor 2*) on the surface of cancer cells.

– The fluorescence is **specific** for the target and **brighter** and more **photostable** than comparable organic dyes.



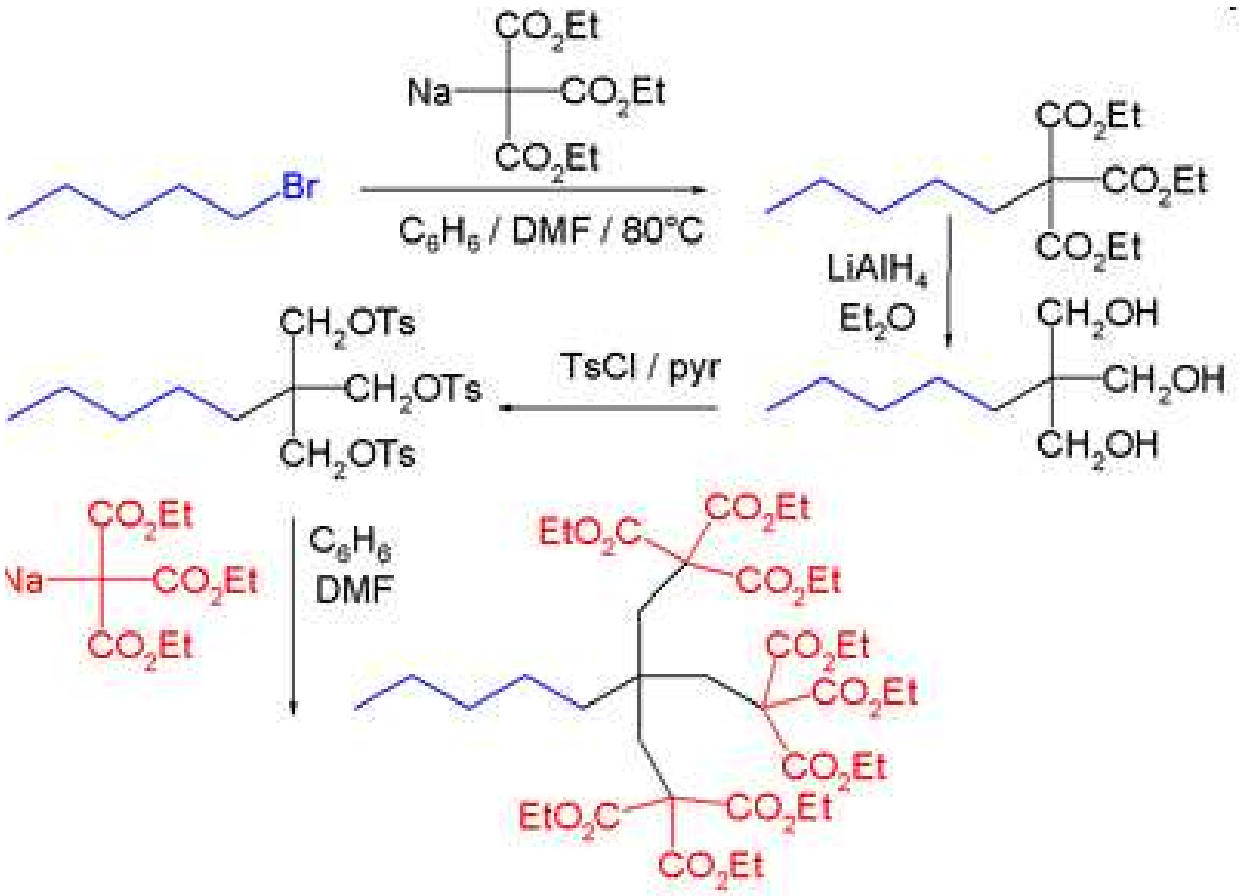
DENDRIMERS

- **Dendrimers** are tree-like macromolecules with branching reach out from central core.
- **Advantages**:
  - Tailored and uniform size
  - high degree of molecular uniformity
  - narrow molecular weight distribution
  - highly- functionalized terminal surface
  - Non-immunogenic
  - Possibly biodegradable



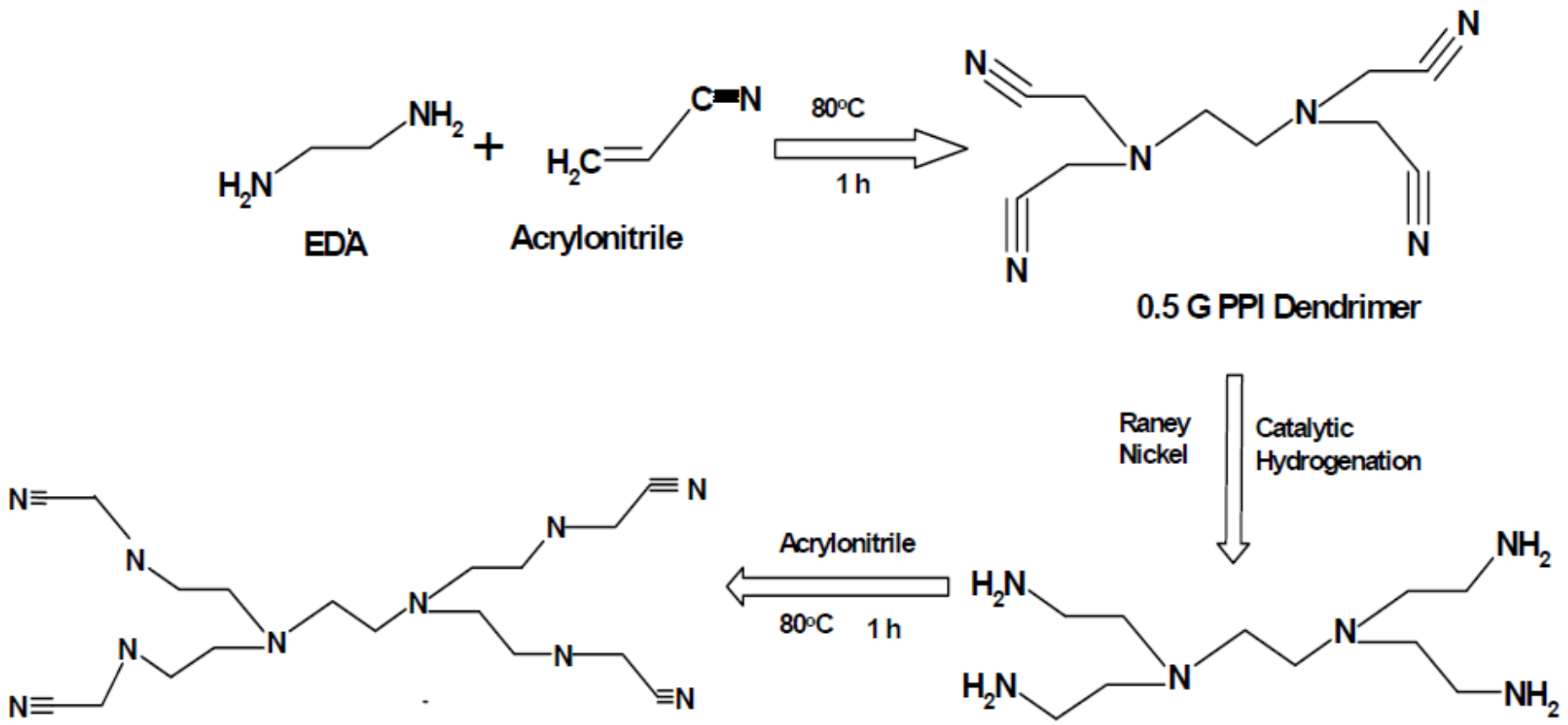
DENDRIMERS

- Dendrimer **synthesis:** **repetitive steps** starting with a central initiator core



DENDRIMERS

- Dendrimer **synthesis:** another example: **polypropyleneimine dendrimer**



DENDRIMERS

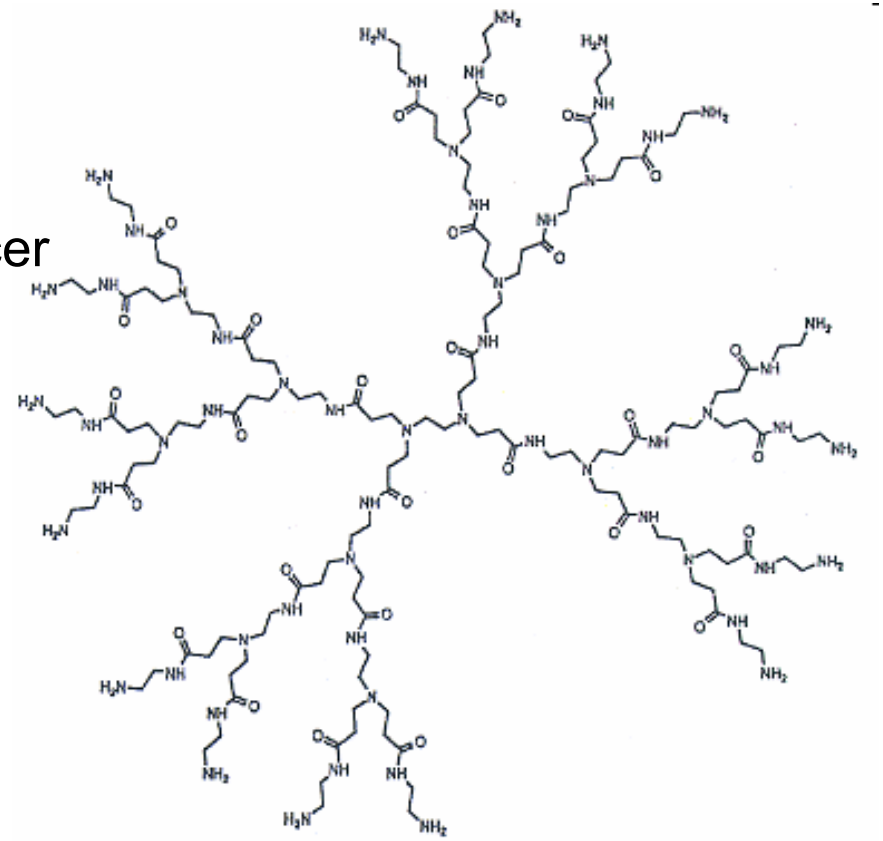
- Dendrimer **usage:** VivaGel® (Starpharma): poly-L-lysine dendrimer-based pharmaceutical currently in clinical trial for **antiviral protection** from genital herpes and HIV infection

- Other examples:

  – Folic acid–PAMAM (Poly-amido amine) dendrimers with **methotrexate**: epithelial cancer

  – PEG-poly-L-lysine dendrimers with **Chloroquine phosphate**: malaria and, possibly, HIV infection

  – Polypropyleneimine dendrimers with **Efavirenz**: HIV infection



NANOVACCINES

- **Nanoparticles** coated by or conjugated to **antigen** can provide targeted immune response with little or no side-effects.
- Antigen covalently conjugated to solid core nano-particle of a defined size.

- Polysterene-conjugated **antigen** proposed as nano-vaccine against tumors
  - Antigen covalently conjugated to solid core nano-beads of narrowly defined size (0.04–0.05 μm) causes high interferon and antibody production (in mice).