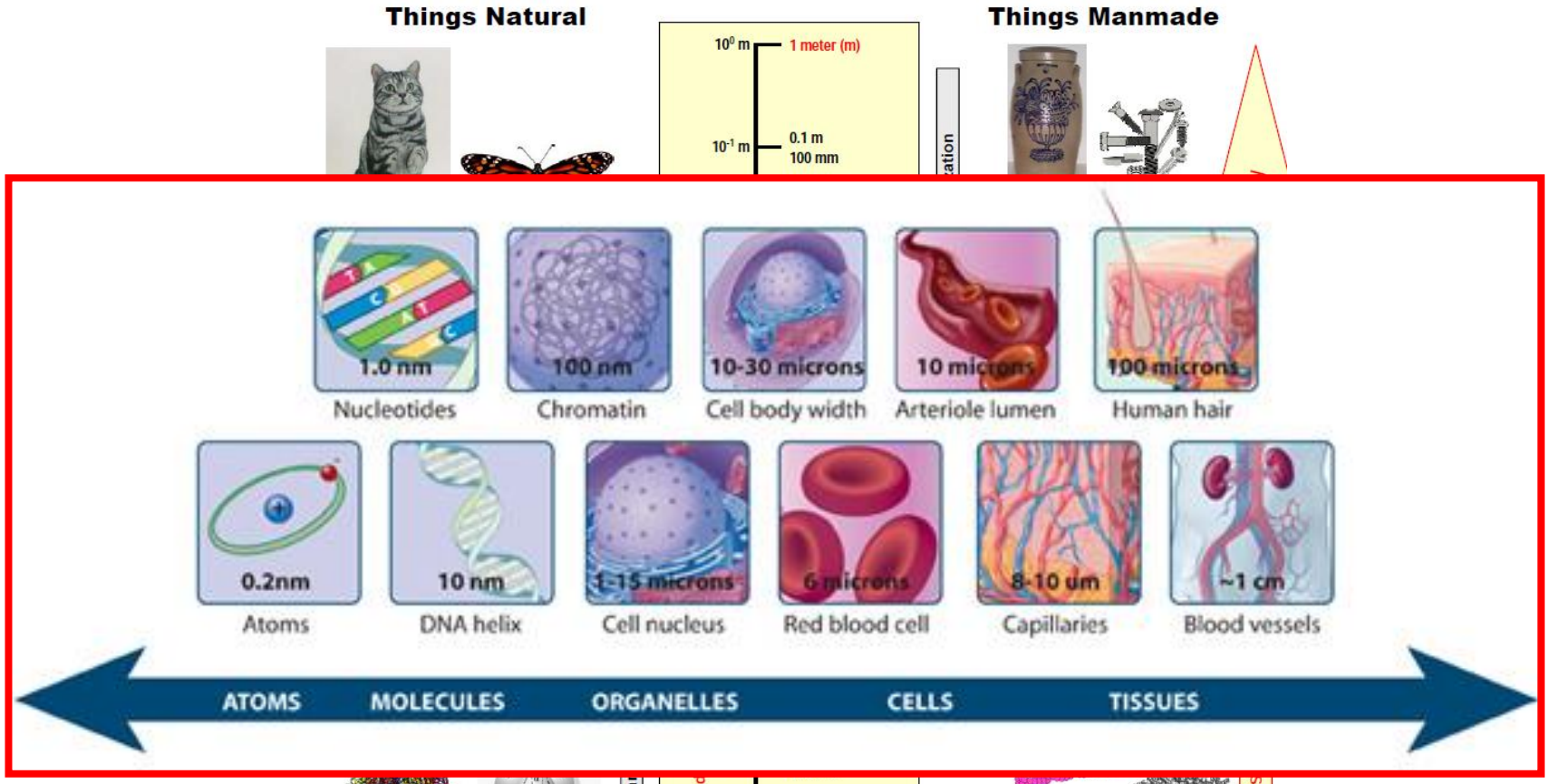
조직공학적 관점에서의 나노의학의 기본개념 및 최근 연구동향

융합과학기술대학원
이강원

The Scale of Things

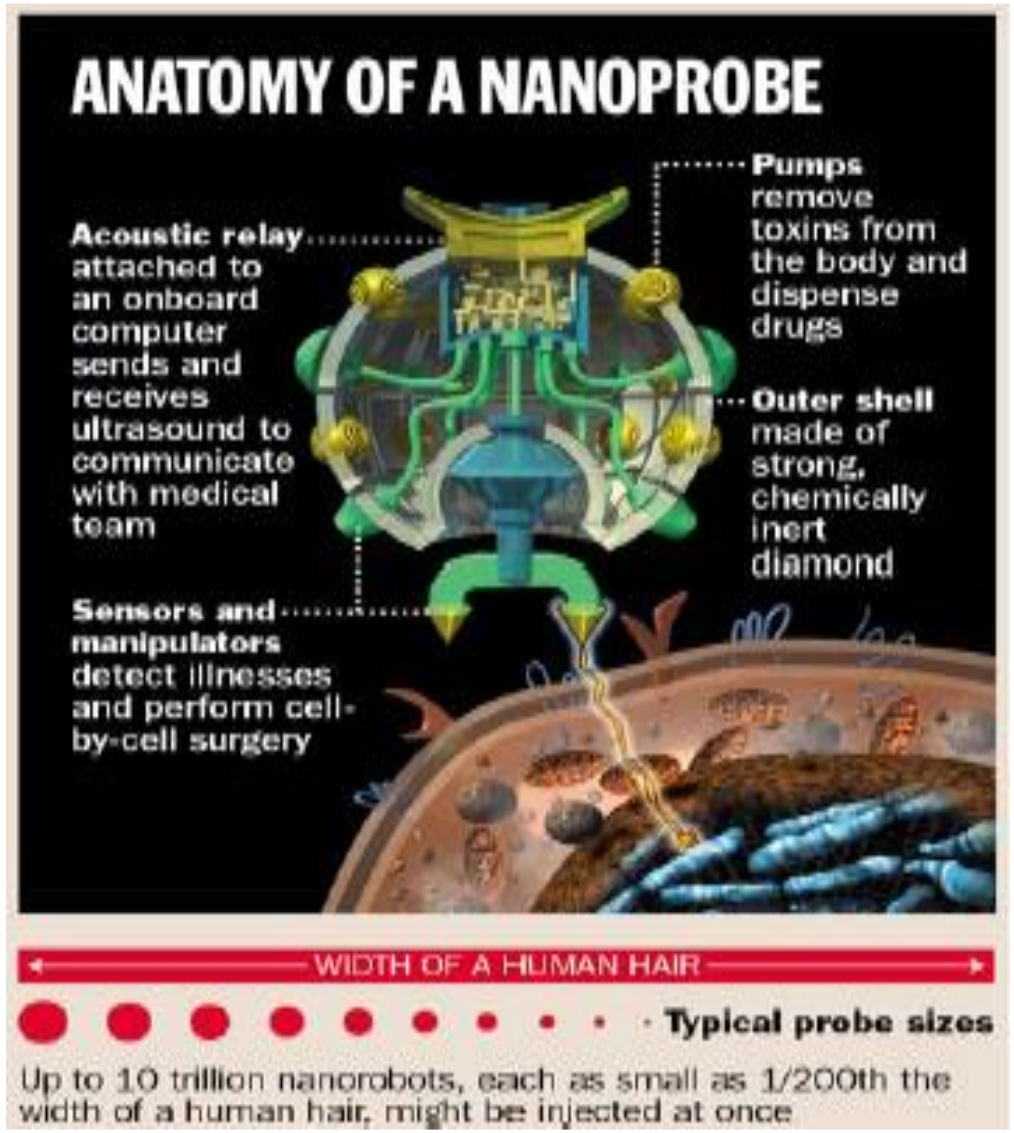
- Human hair 50,000 nm
- Bucky ball 1 nm
- Water molecule .2 nm
- Violet light 400 nm
- Cold virus 30 nm
- Bacteria 1-10 um
- Small protein 10 nm

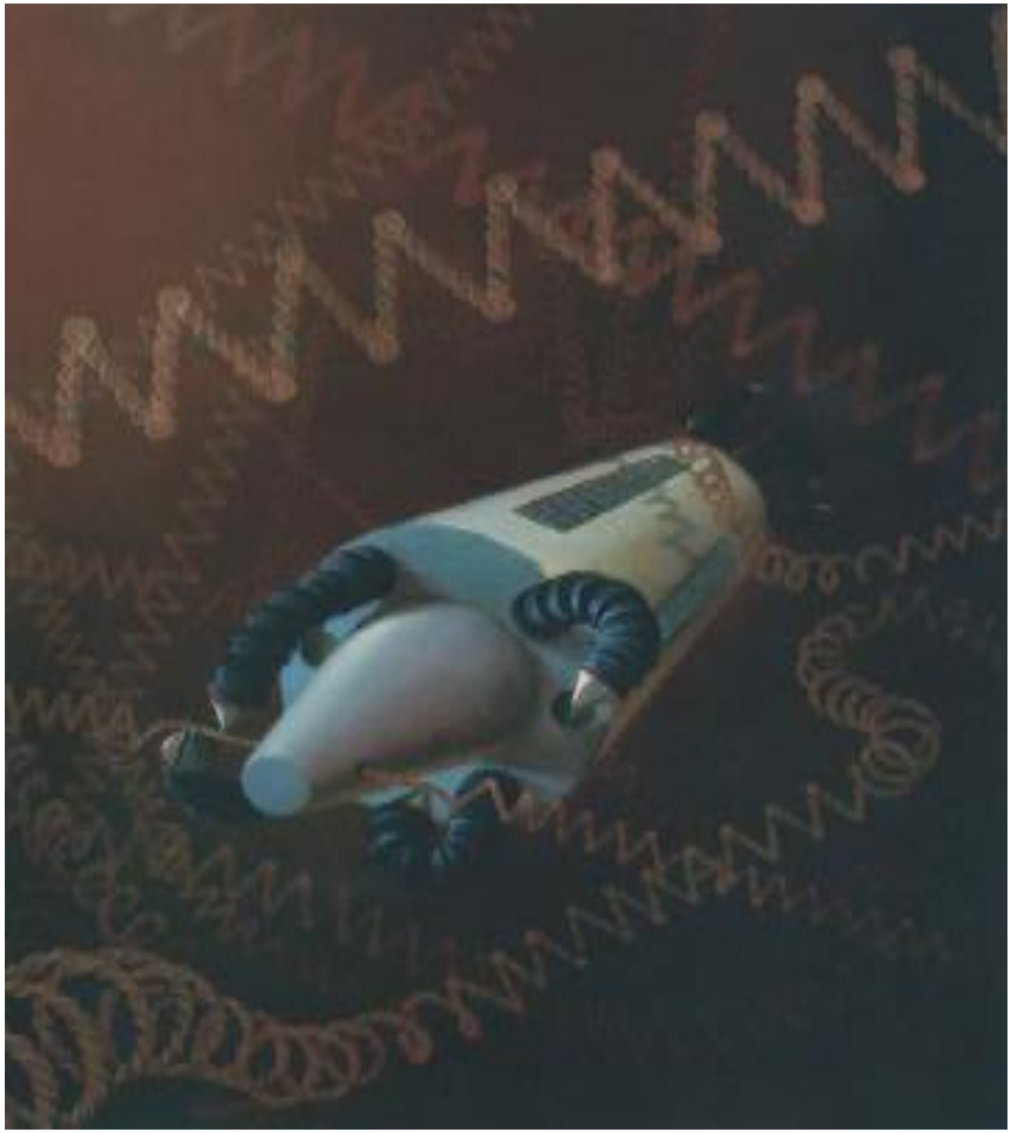


http://www.nature.com/scitable/topicpage/what-is-a-cell-14023083

Chart from http://www.sc.doe.gov/production/bes/scale_of_things.html

NanoBiotechnology & Nanomedicine

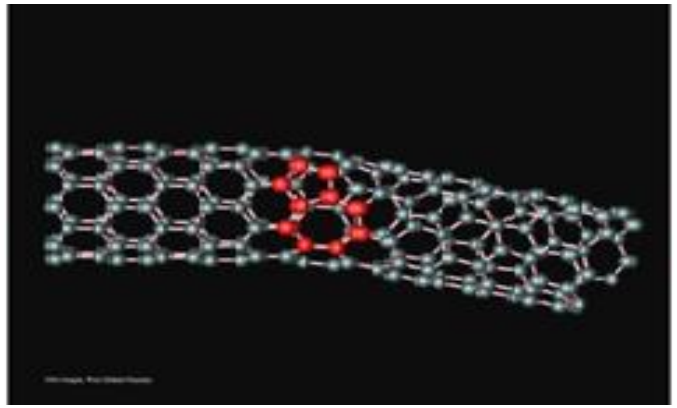




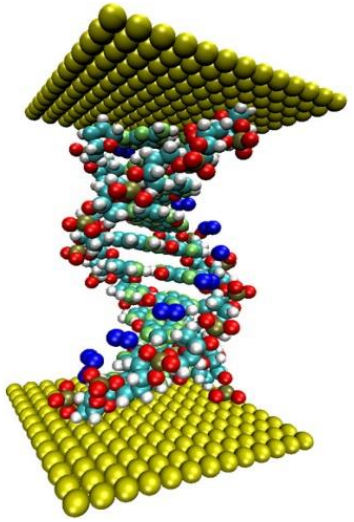
Human hair width: 30 – 100 um → 150 – 500 nm

What is Nanotechnology ?

Nanotechnology is the engineering of functional systems at the molecular scale. In its original sense, 'nanotechnology' refers to the projected ability to construct items from the bottom up, using techniques and tools being developed today to make complete, high performance products



Semiconducting metal junction formed by two carbon nanotubes



An engineered DNA strand

Nanotechnology

- Nanotechnology is the study of particles

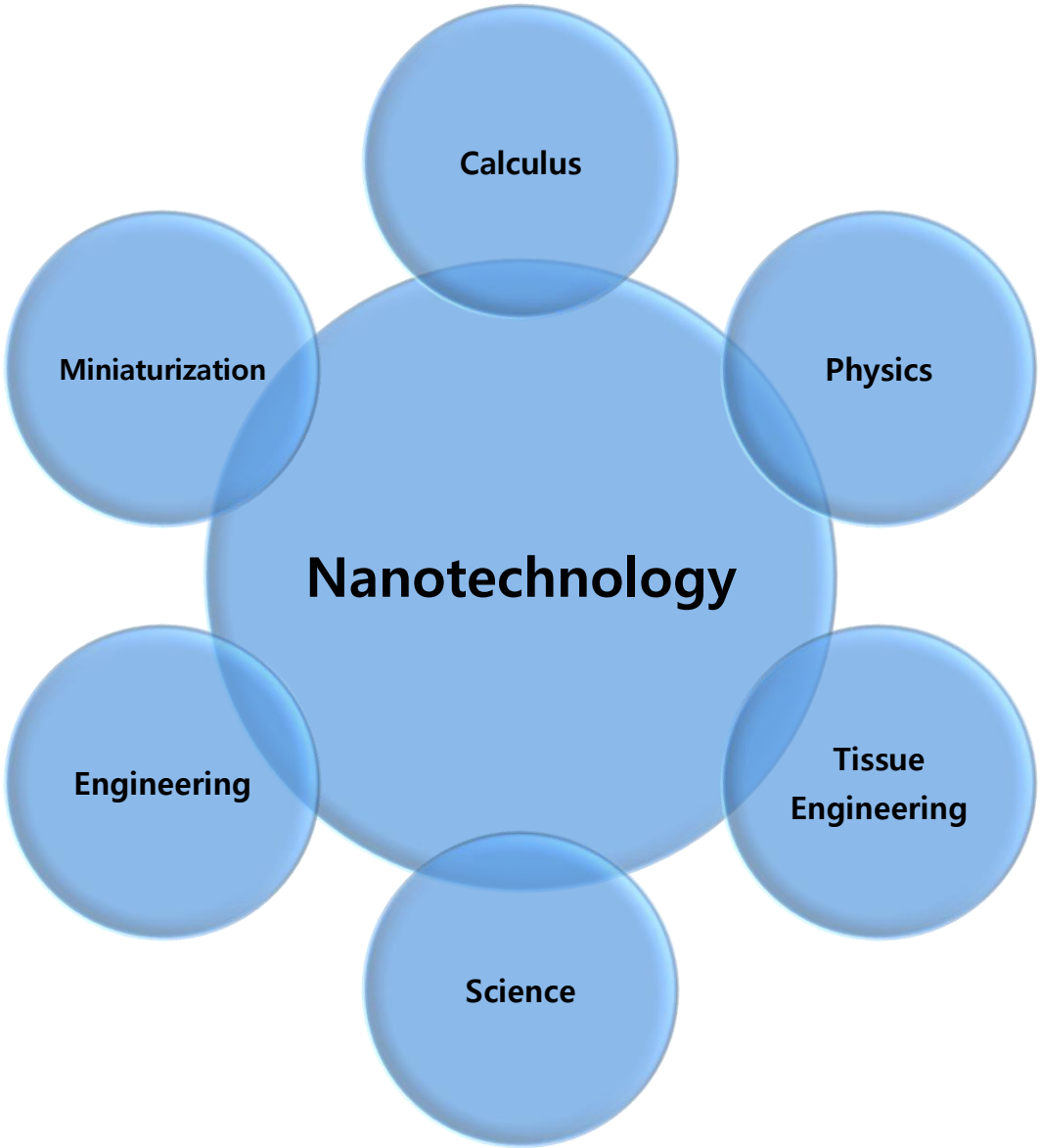
  $1x10^{-9}$ of a meter in size.
- Over the past decade the field has gained tremendous ground, including nanomedicine.
- Nanomedicine is the application of nanotechnology to do everything from precise delivery of drugs to cell repair.

Definition of Nanomedicine

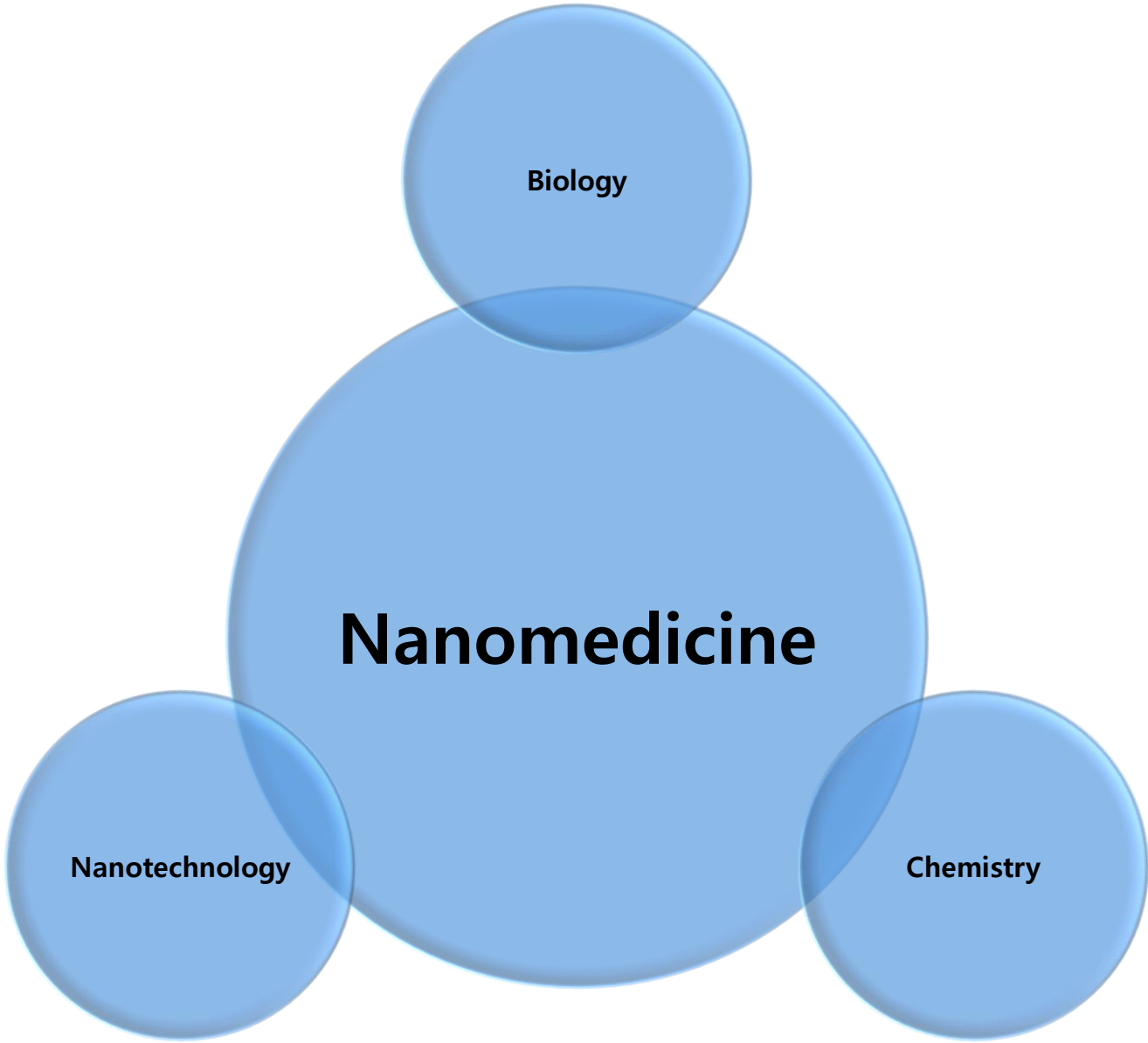
- Nanomedicine is defined as the monitoring, repair, cons truction and control of human biological systems at the molecular level, using engineered nanodevices and nan ostructures.
- The detection and controlled manipulation of human bi ological system at the molecular level via engineered na nodevices and/or nanostructures.

"An Engineered Nanodevice"

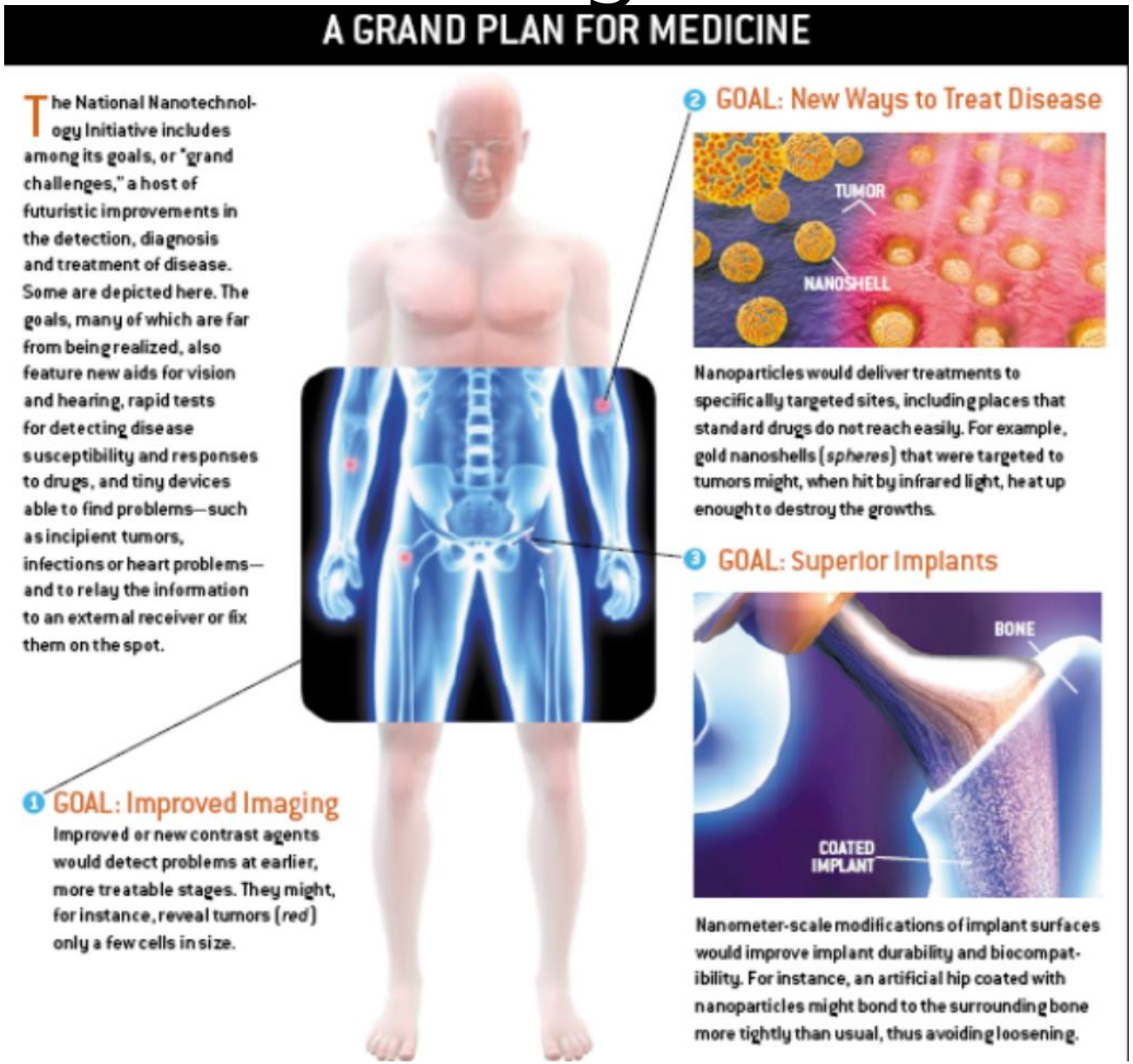
Nanotechnology



Nanomedicine



Nanomedicines for diagnosis and therapy



The Properties of Medical Nanodevices

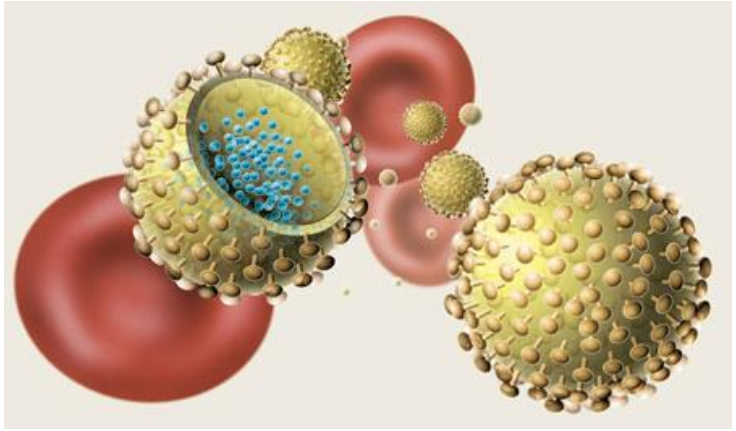
- Shape and size
- Biocompatibility
- Powering
- Communication
- Navigation

Potential applications of Nanomedicine

drug delivery	implant materials
improved imaging	artificial tissues
DNA analyses	improving brains
nanobarcode® technique	cleaning teeth, lungs and arteries

Drug Delivery

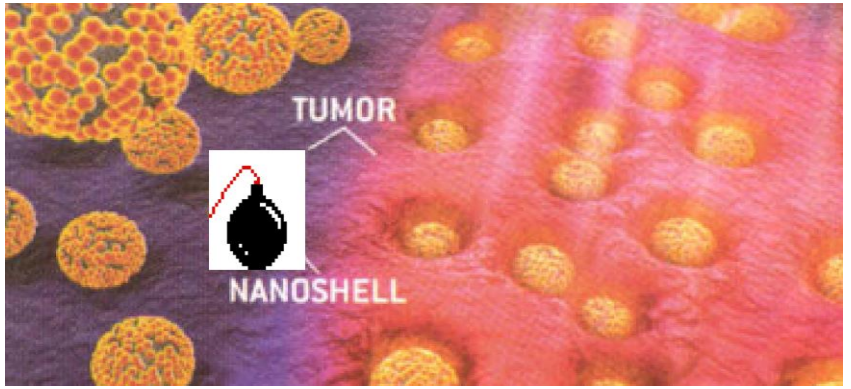
- Drugs normally injected can be injested.
- The rate at which the drug stays in the body can be manipulated. 약리학 (Pharmacokinetics, PK-약동학, Phamacodynamics, PD-약력학)
- Provides for lower doses needed.



Drug Delivery

1. Nanoparticles may deliver drugs in sophisticated ways, like target-specific and trigger-based drug dose. Target specific delivery may enable the use of lower doses, because the whole body will not be saturated with the drug. Side effects may be minimized, and it may be possible to use stronger drugs, which can not be used by conventional drug delivery.

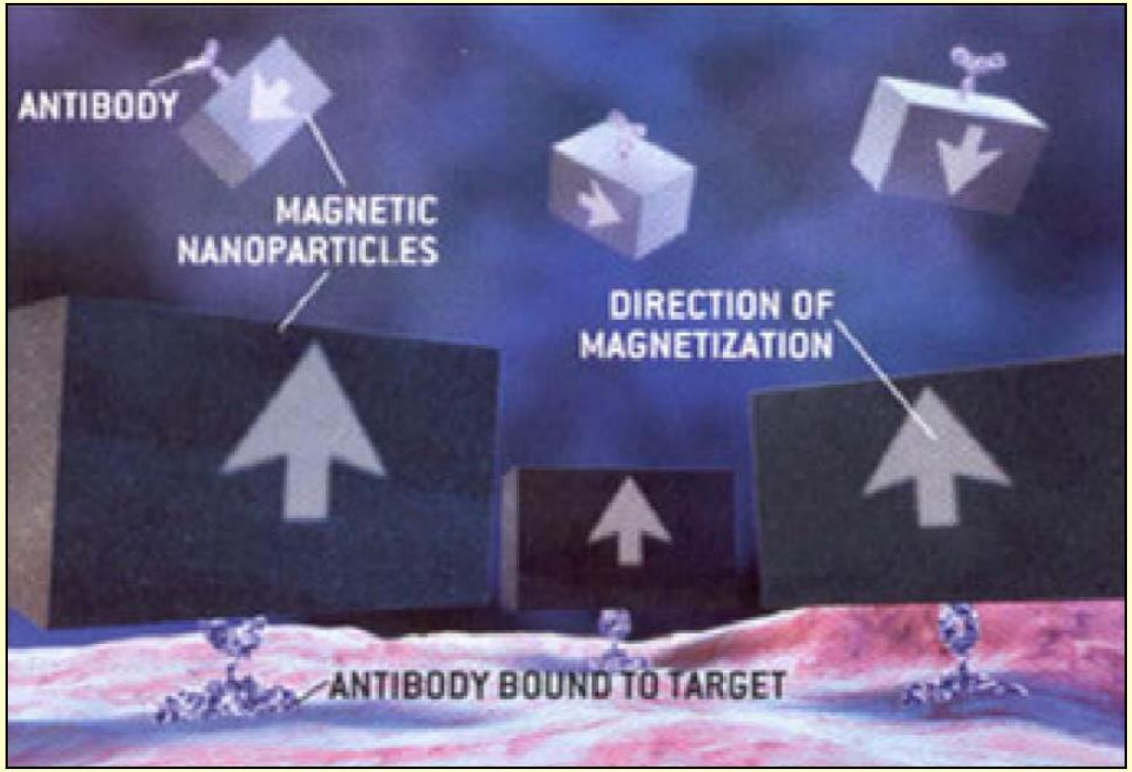
2. The use of particles in cancer healing is an example of target-specific action. Gold plated spheres will be linked to tumor cells. The nanoshells will be heated from the outside with an infrared source. Heating the shells will destroy the cancerous cells, leaving the surrounding tissue unharmed. (maybe???)



Methods of Drug Delivery

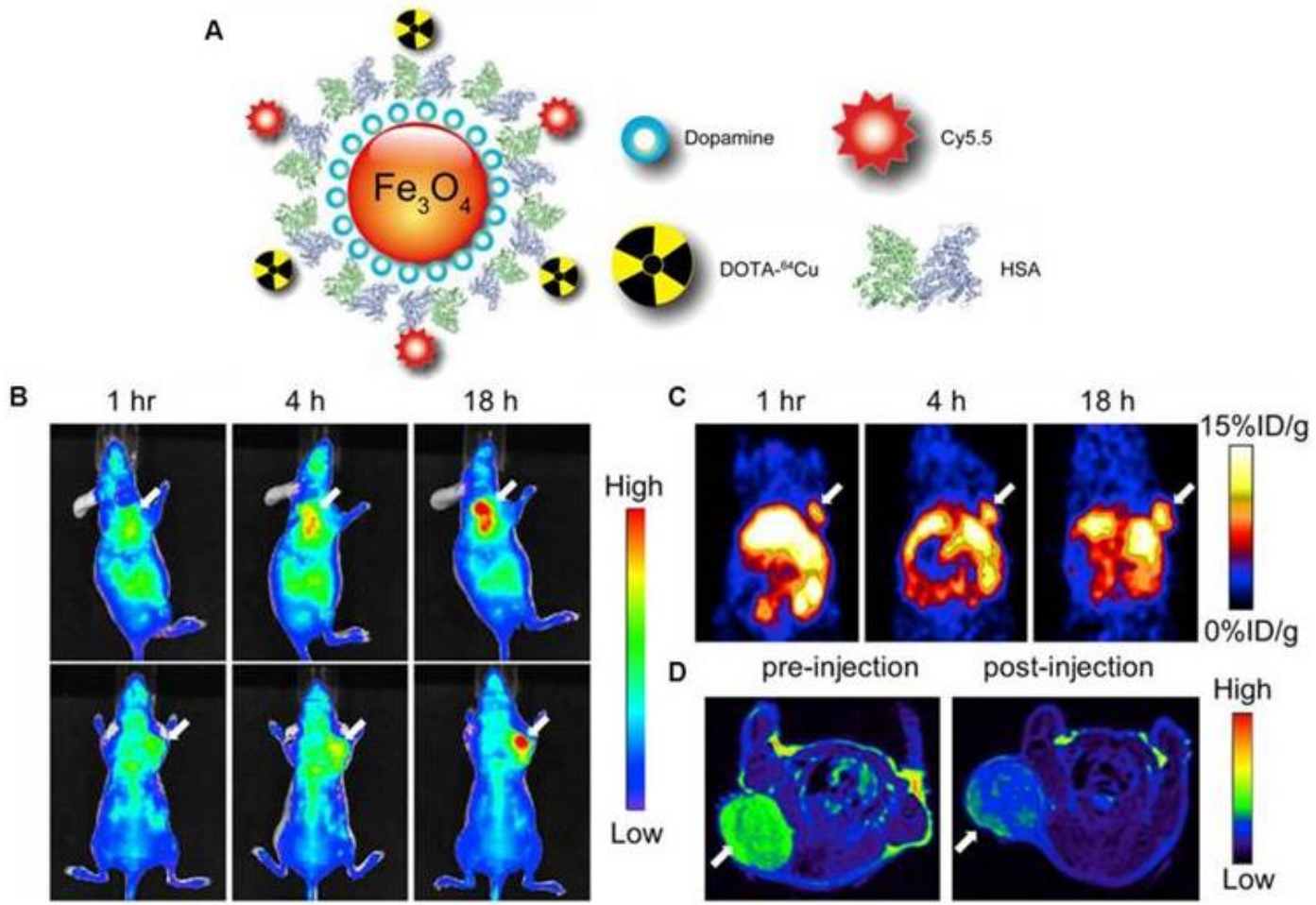
- Oral Delivery
- Inhalation
- Transdermal
- Implantation
- Injection

Improved Imaging and Diagnostics



Improved imaging with better contrast agents may help to diagnose diseases more sensitively. The method may enable the detection of very small tumors and other organisms which cause disease.

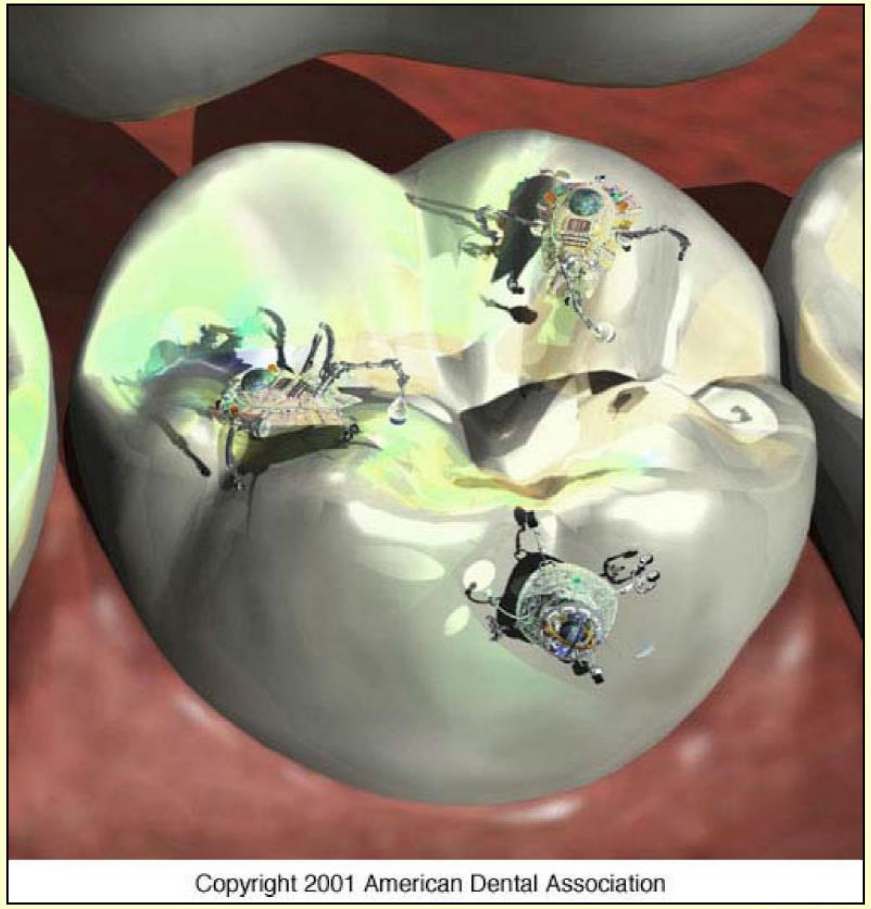
Multimodal Tumor imaging



(A) Schematic illustration of the multi-funtional HSA-IONPs. **(B)** Representative *in vivo* NIRF images of mouse injected with HSA-IONPs. Images were acquired 1 h, 4 h and 18 h post injection. **(C)** *In vivo* PET imaging results of mouse injected with HSA-IONPs. Images were acquired 1 h, 4 h and 18 h post injection. **(D)** MRI images acquired before and 18 h post injection.

Biomaterials, Xie et al., 2010.

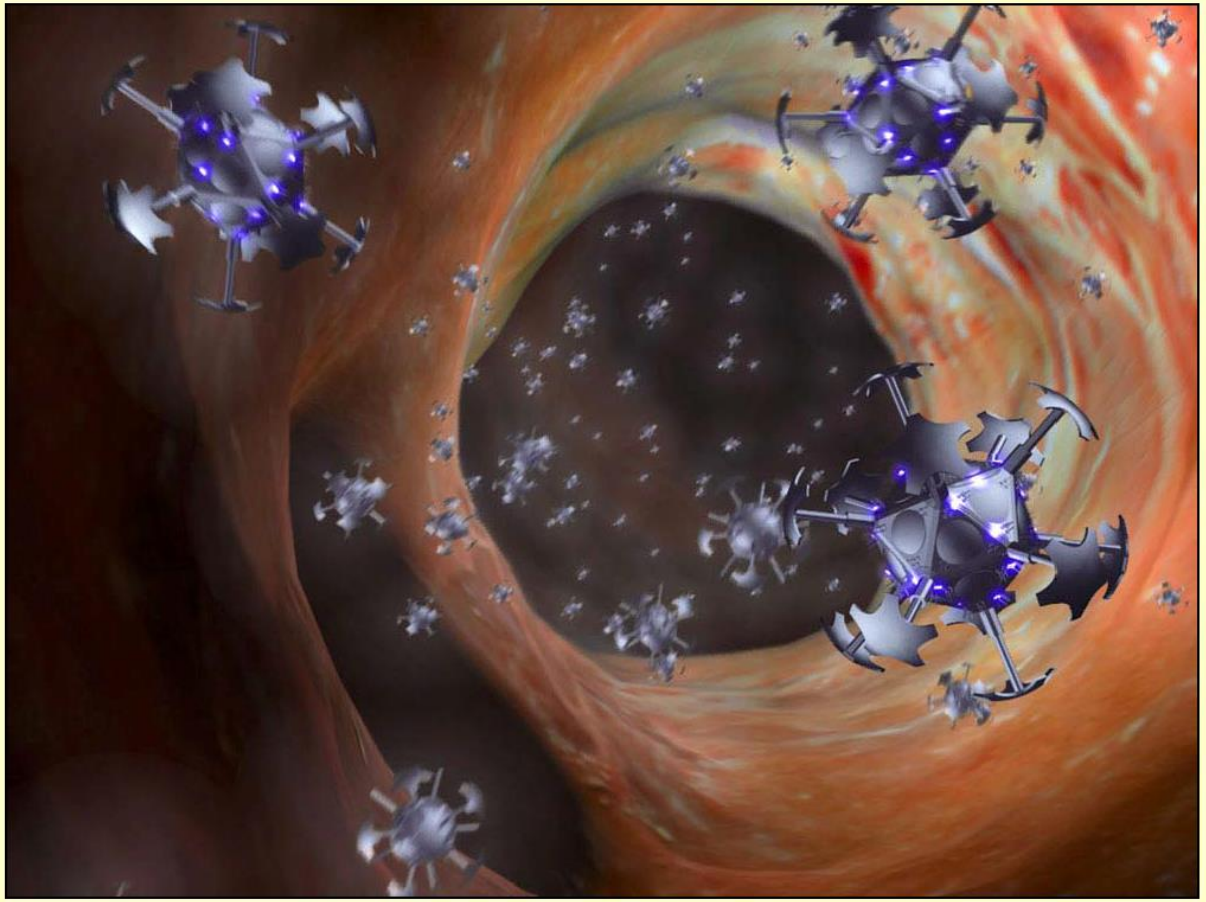
Tooth Cleaning Robots



Copyright 2001 American Dental Association

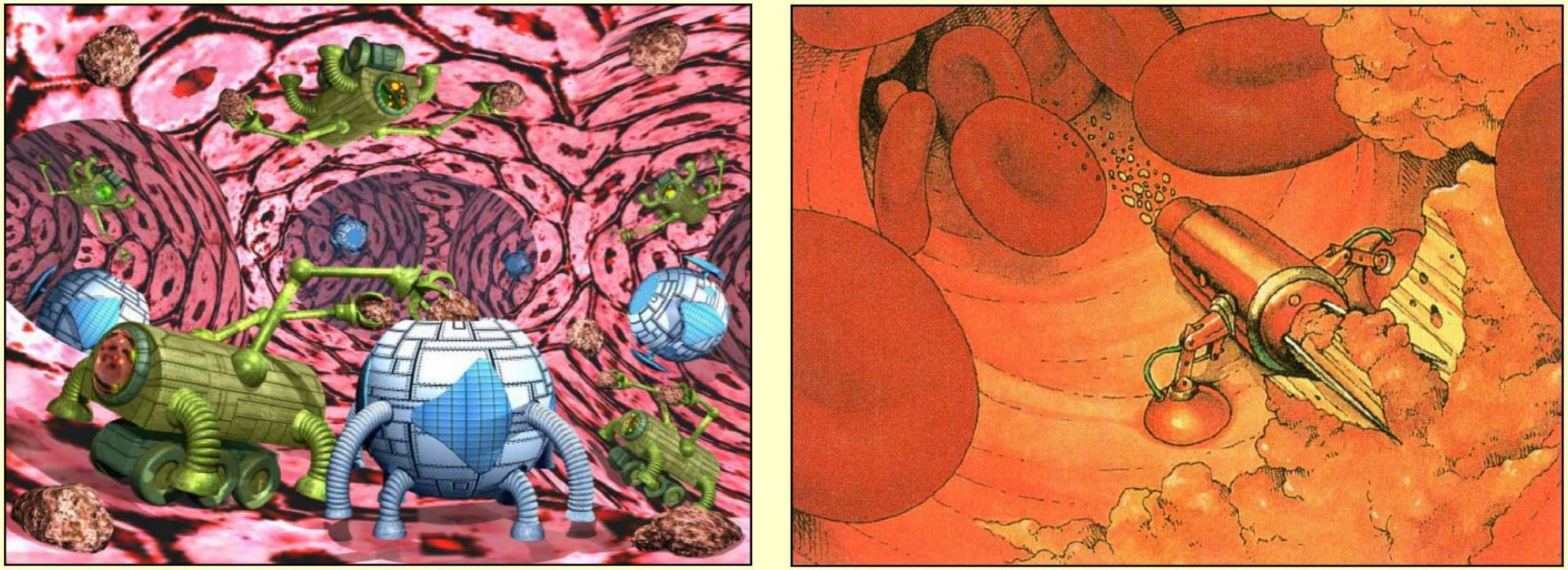
Teeth cleaning robots collect harmful bacteria from the mouth. (The robots are magnified x1000)

Lung Cleaning Robots



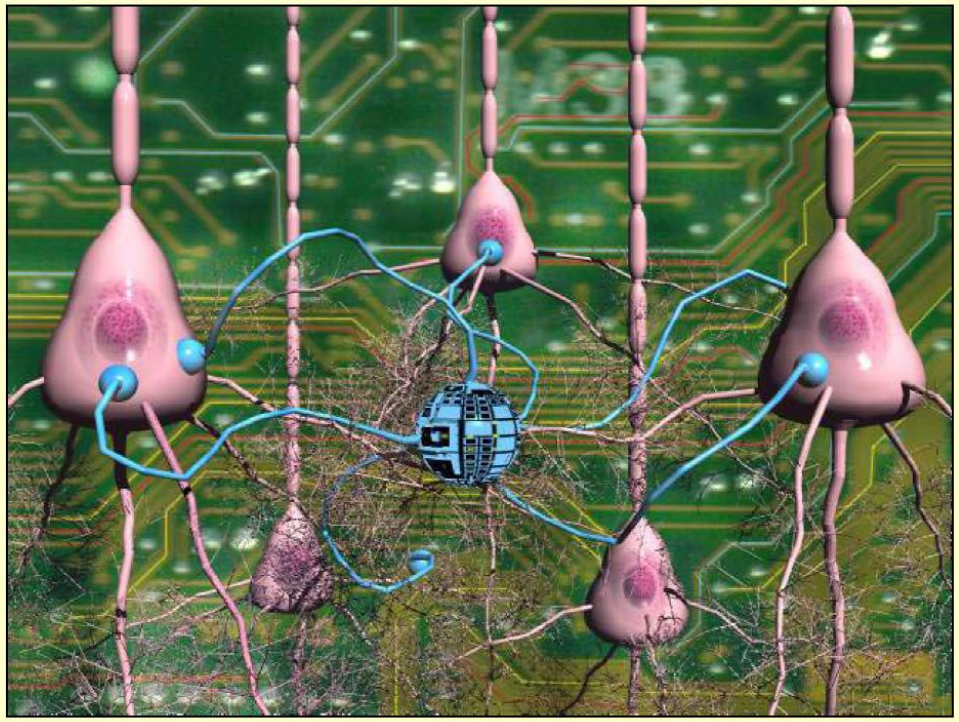
Similar cleaning robots can be used in lungs. We have natural macrophages in alveoli, but they are not able to metabolize foreign particles like fibers of asbestos and toxic effects of smoking from the lungs.

Artery Cleaning Robots



Extra fat can be removed from the arteries with cleaning robots. Mobile nanorobotic janitors (green) patrol the lungs, collecting inhaled debris and transporting it to recycling stations (blue-gray).

Improving the memory



A nanostructured data storage system may store a nanocomputer and an amount of information equivalent to an entire library. The spheres are in contact with the brain cells

Practical Application of Nanomedicine

- Diagnostic

  Imaging and identification

- Therapeutic

  Delivering medication to the exact location.

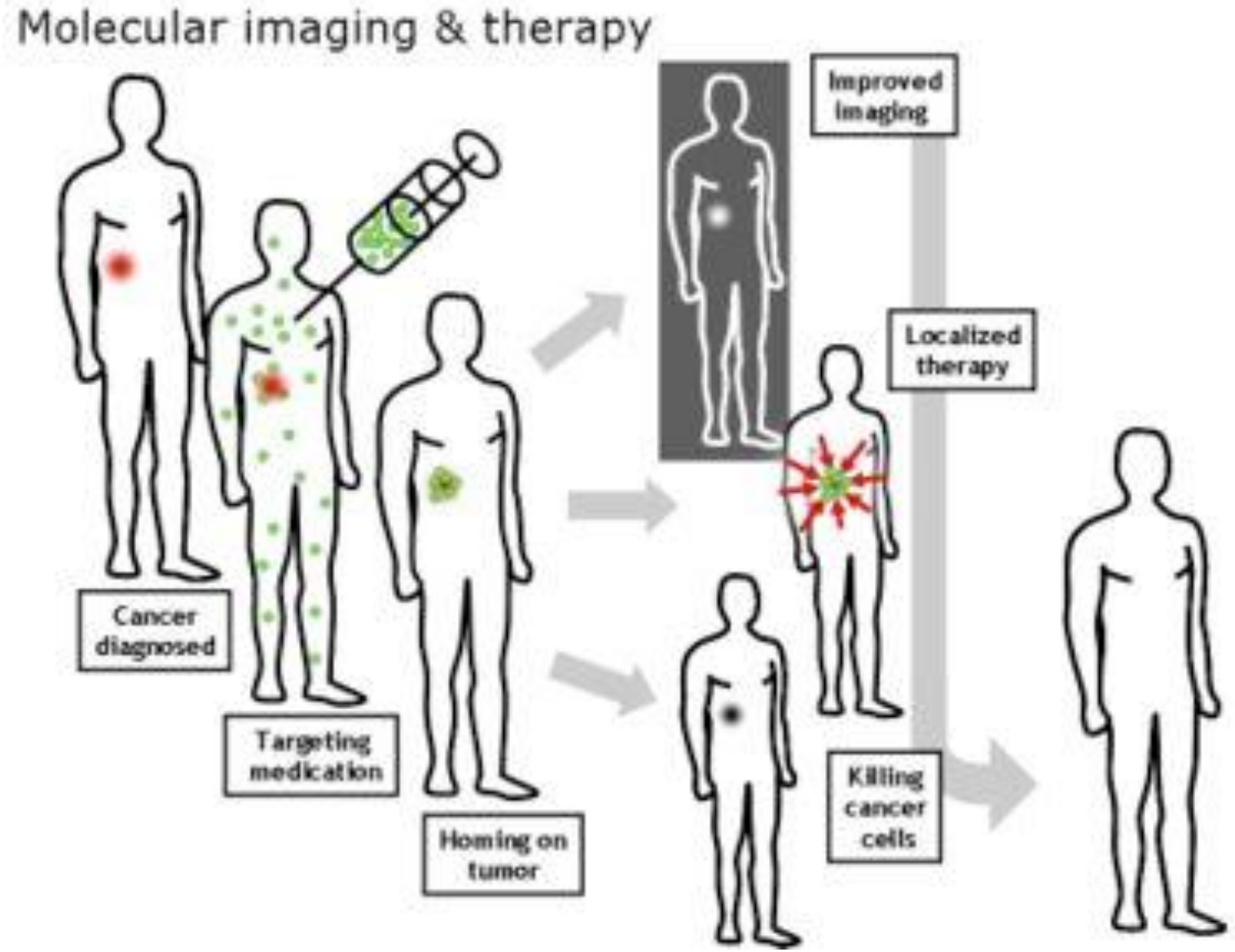
  Killing of bacteria, viruses & cancer cells

  Repair of damaged tissues.

Improved Imaging of Tumors

- Nanoparticles made of a metal such as magnesium oxide.
- Coated with antibodies found specifically in cancer cells.
- Nanoparticles localize around cancer.
- MRI done would should a more detailed image of where the cancer is.

Imaging


Molecular imaging & therapy
Cancer diagnosed
Targeting medication
Homing on tumor
Improved imaging
Localized therapy
Killing cancer cells

Advantages of nanomedicine

- Drug delivery to the exact location.
- Lesser side effects.
- Molecular targeting by nano engineered devices
- Detection is relatively easy.
- No surgery required.
- Diseases can be easily cured.

Disadvantages

- Nearly not practical yet.
- High cost.
- Implementation difficulties.

Nanodreams

1. Nanomedicine will (hopefully) eliminate virtually all common diseases, all medical pain and suffering => theoretically eternal life.

2. Extension of human capabilities.

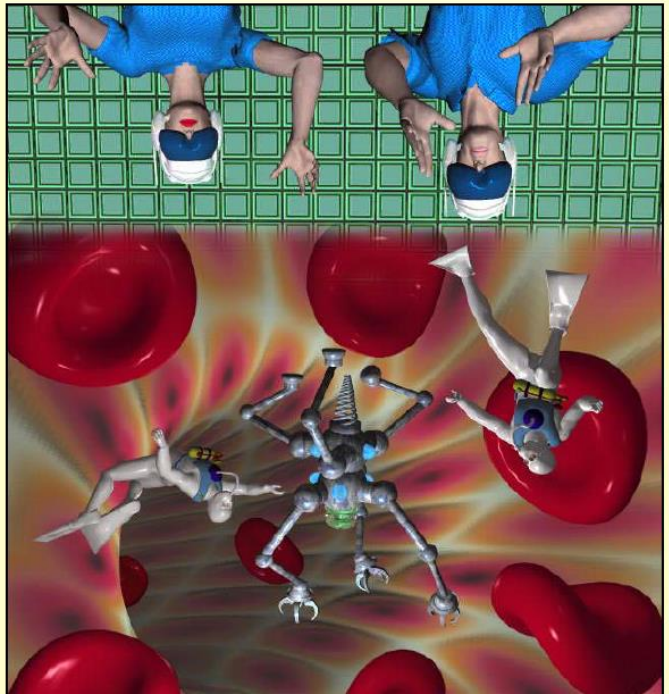
3. New era of peace. People who are well-fed, well-clothed, well-educated, healthy and happy will have little motivation to make war.

4. Pollution-free industry will guarantee the well-being of nature.

Nanohorrors

1. Self replicating nanorobots could become massive chemical and biological weapons.

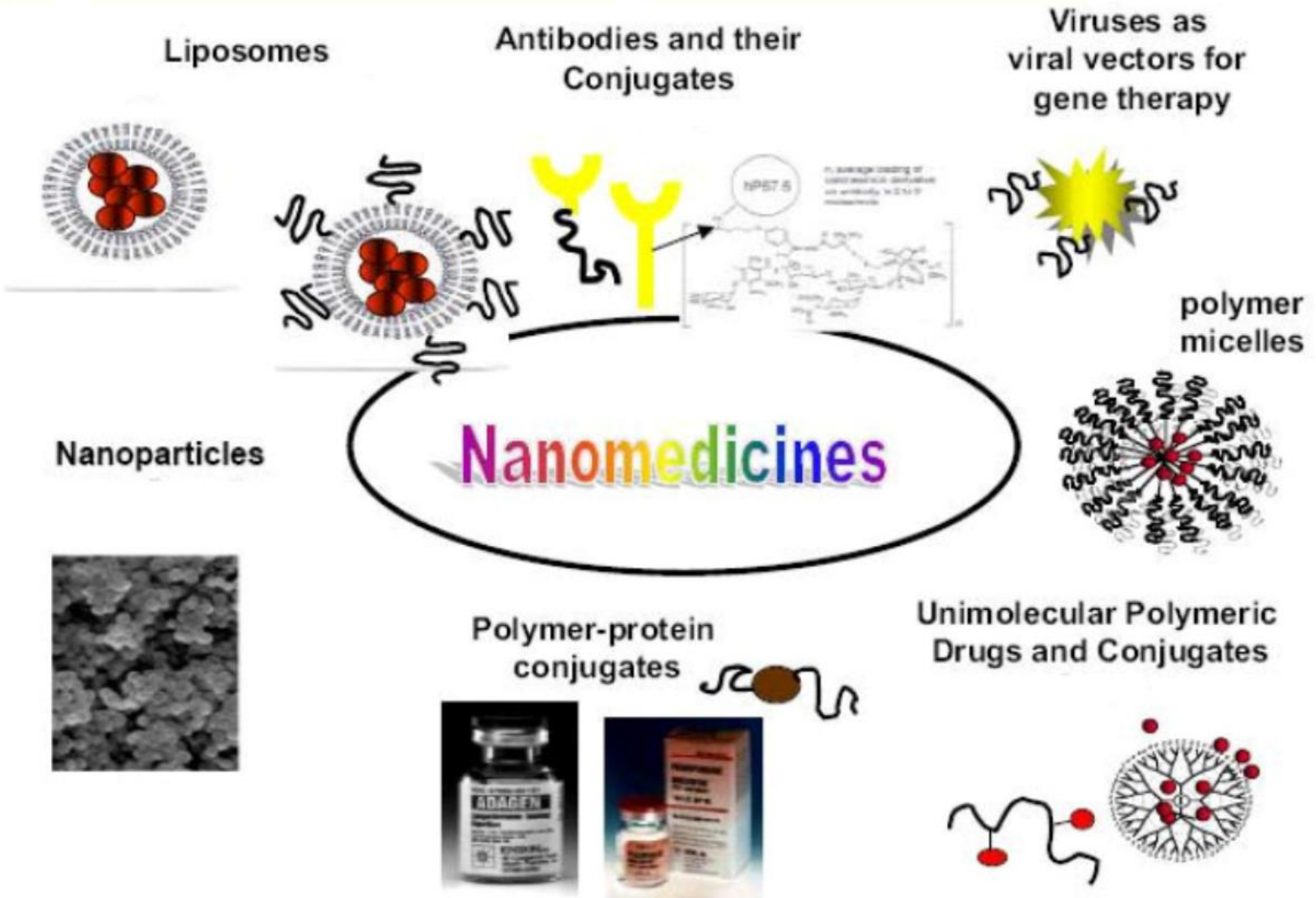
2. Changes to human properties, such as brains, respiration, muscles and DNA will be uncontrolled and may threat the existence of human being.



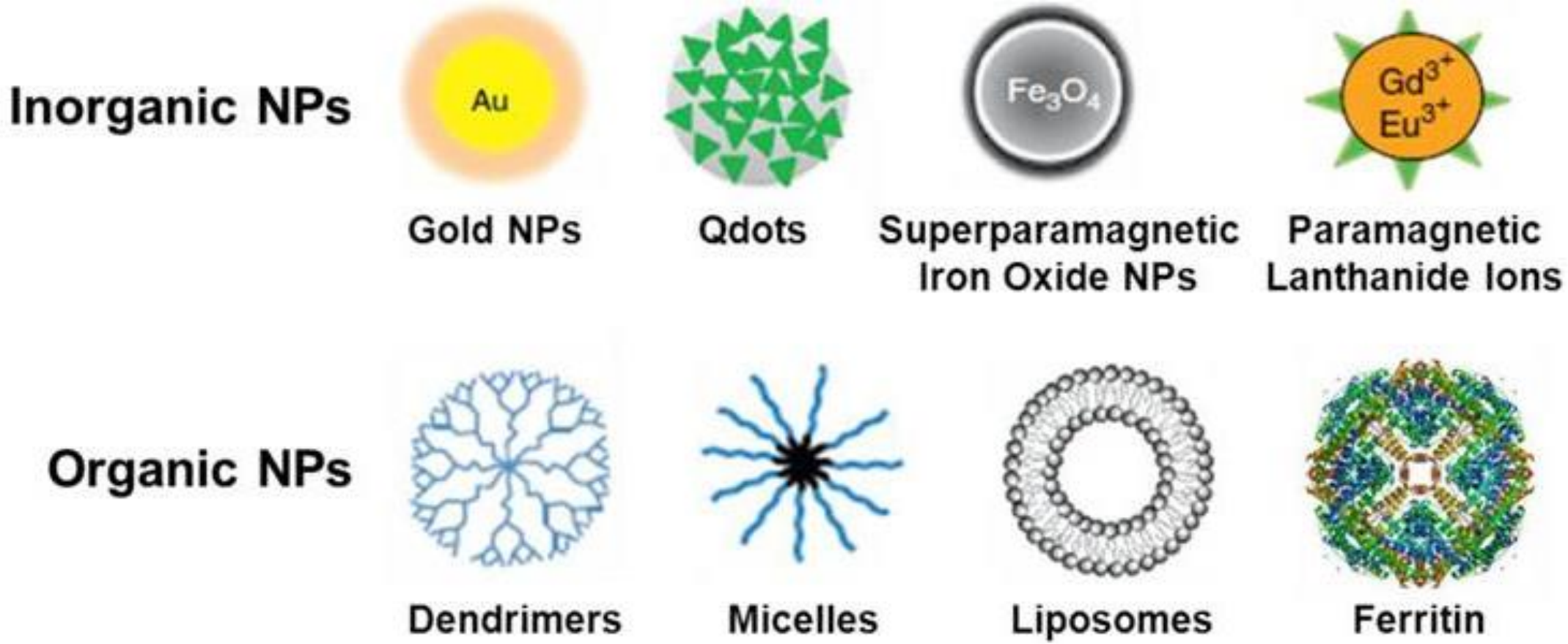
Summary

- Although realization of the full potential of nanomedicine may b e years or decades away, recent advances in nanotechnology-rela ted drug delivery, diagnosis, and drug development are beginnin g to change the landscape medicine.
- Nano therapies could, in the long term, be much more economic al, effective and safe and could greatly reduce the cost of current medical procedures.
- Therefore, Nanomedicine is future medicine.

Types of nanomedicines

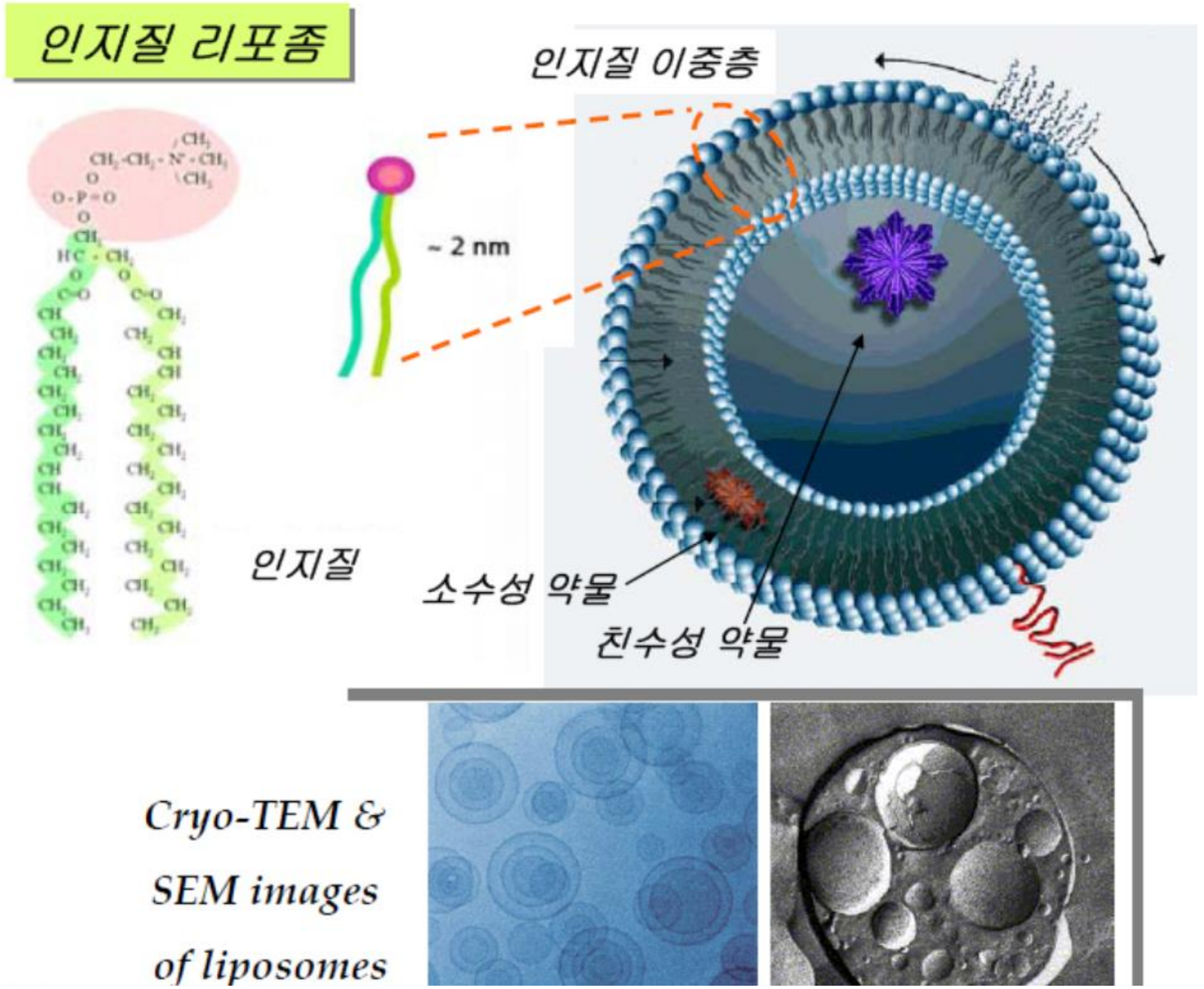


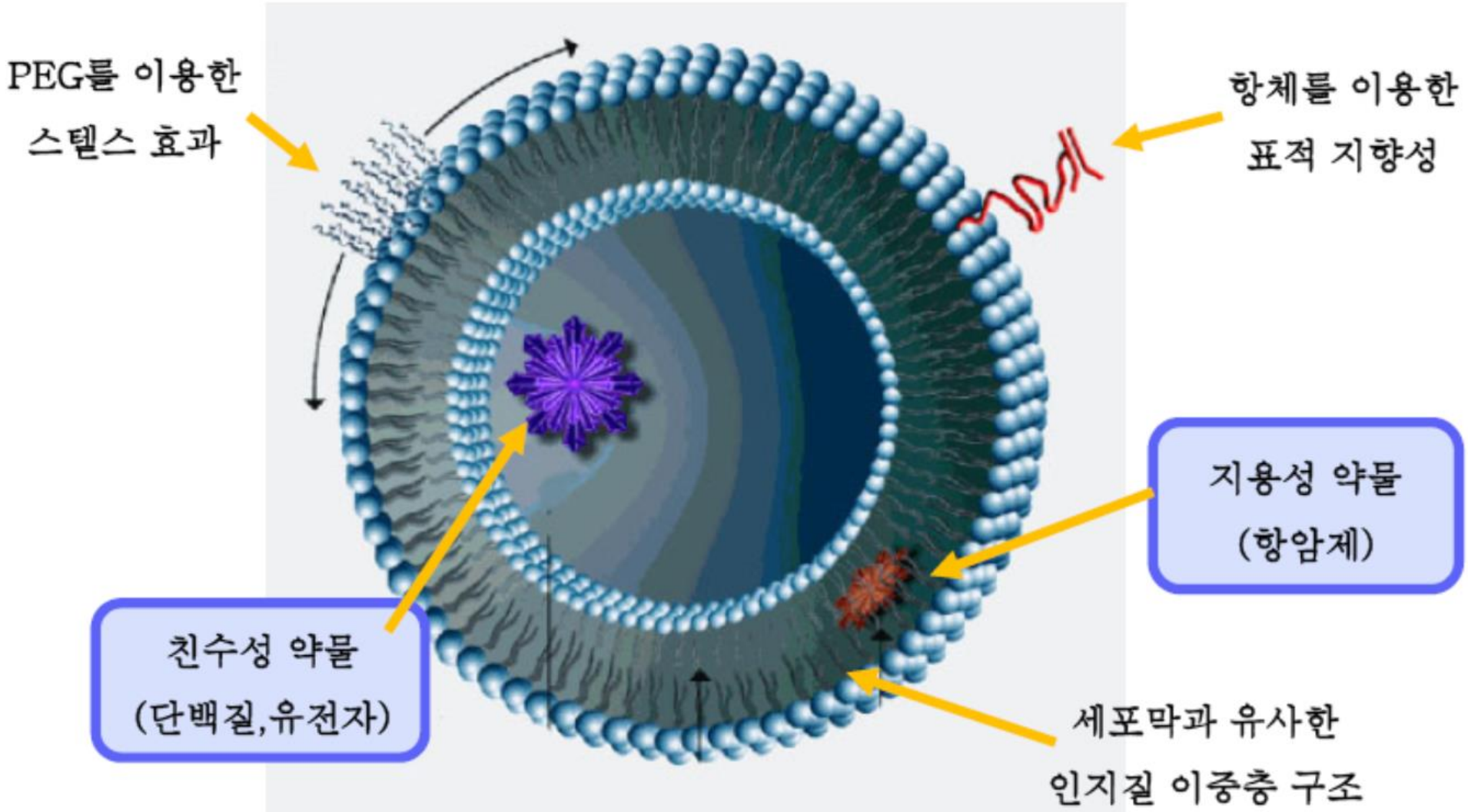
Schematic representation of typical nano cores for the construction of radiolabeled nanoparticles



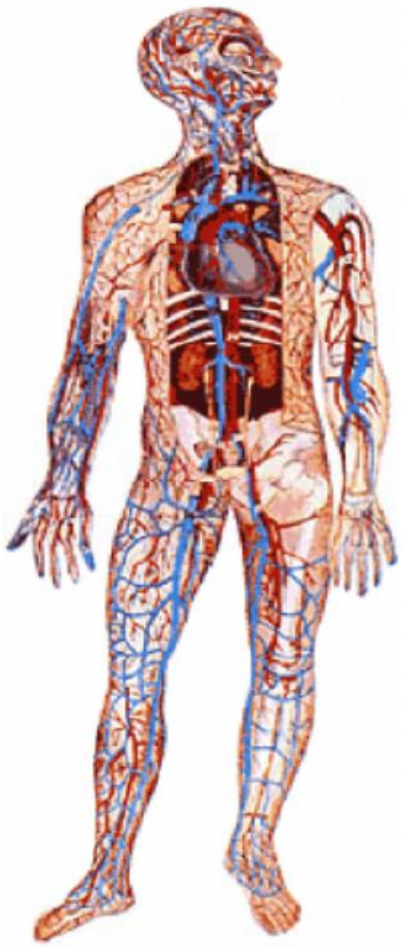
Theranostics 2014, 4, 290

Liposomes


인지질 리포좀
인지질 이중층
~ 2 nm
인지질
소수성 약물
친수성 약물
Cryo-TEM &
SEM images
of liposomes


PEG를 이용한
스텔스 효과
항체를 이용한
표적 지향성
지용성 약물
(항암제)
친수성 약물
(단백질,유전자)
세포막과 유사한
인지질 이중층 구조

Importance of nanomedicine for cancer treatments



In blood
(proteins, cells, salts, etc.)

Reticuloendothelial System
(RES)

within several minutes

Clearance by natural protection system
(ex. phagocytosis by macrophages)

Particle Size (μm)	Fate
<0.06	liver/several parts of body
0.06-0.4	liver
0.4-8.0	liver
>8.0	lung

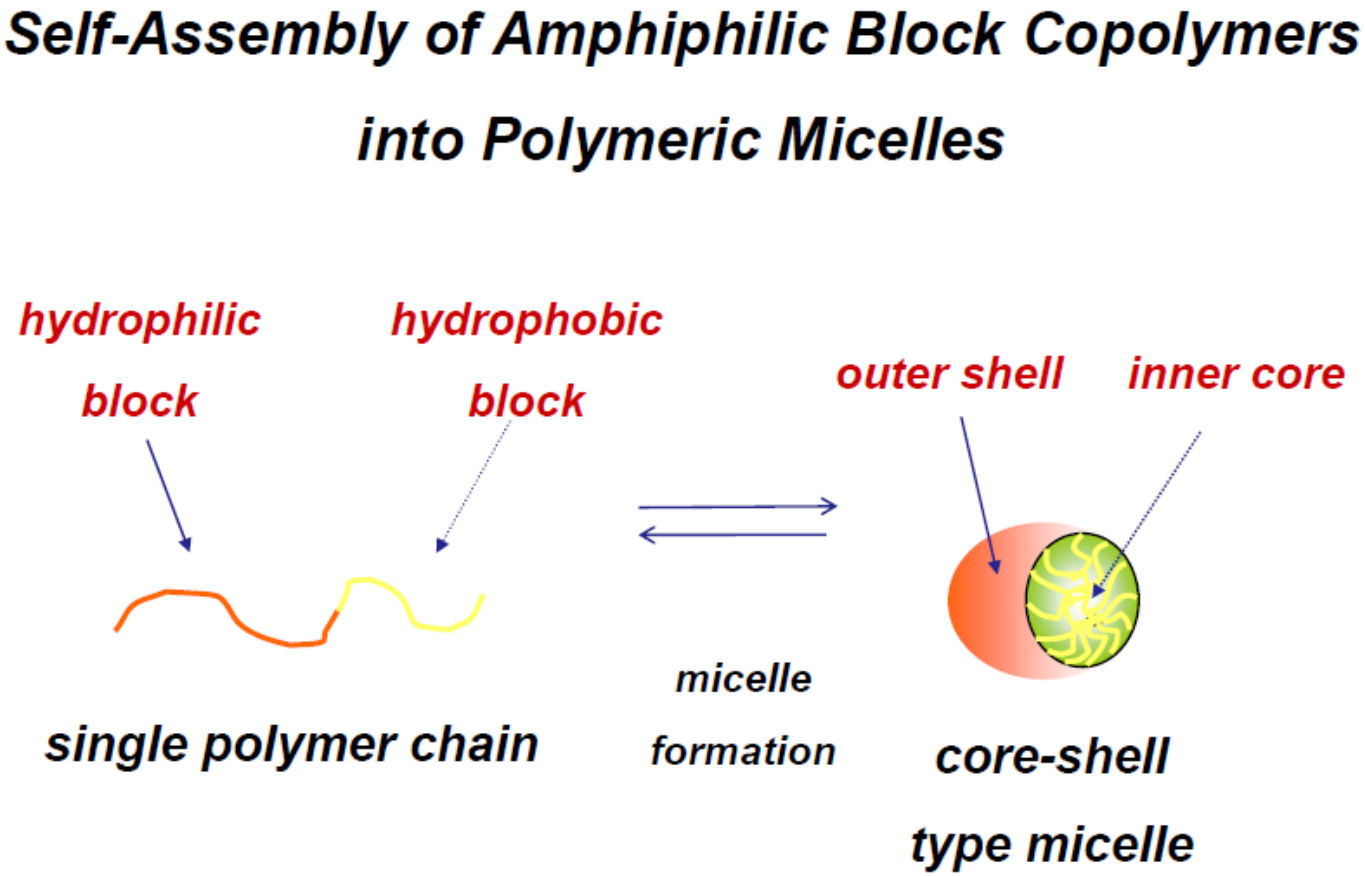
RES: An older term for the mononuclear phagocyte system

- A part of the immune system that consists of the phagocytic cells located in reticular connective tissue.
- The cells are primarily monocytes and macrophages, and they accumulate in lymph nodes and the spleen.

Video of Magnetic Separation of Magneto-Fluorescent Supernanoparticles (video from Bawendi Group, MIT)

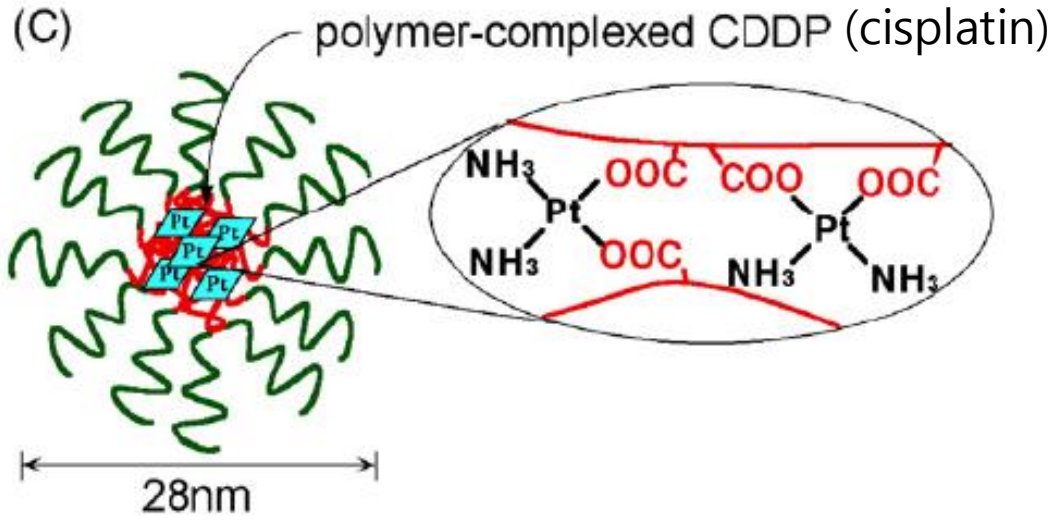
Magneto-fluorescent core-shell supernanoparticles (red emission) can be separated from non-magnetic silica-coated quantum dots (green emission) using a magnetic column.

Polymeric micelles

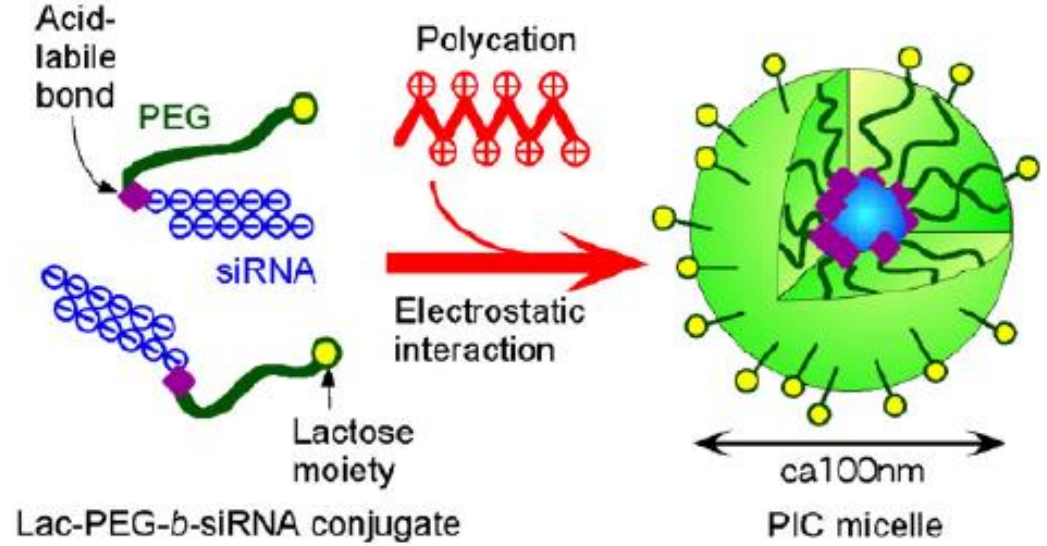


Other types of polymeric micelles

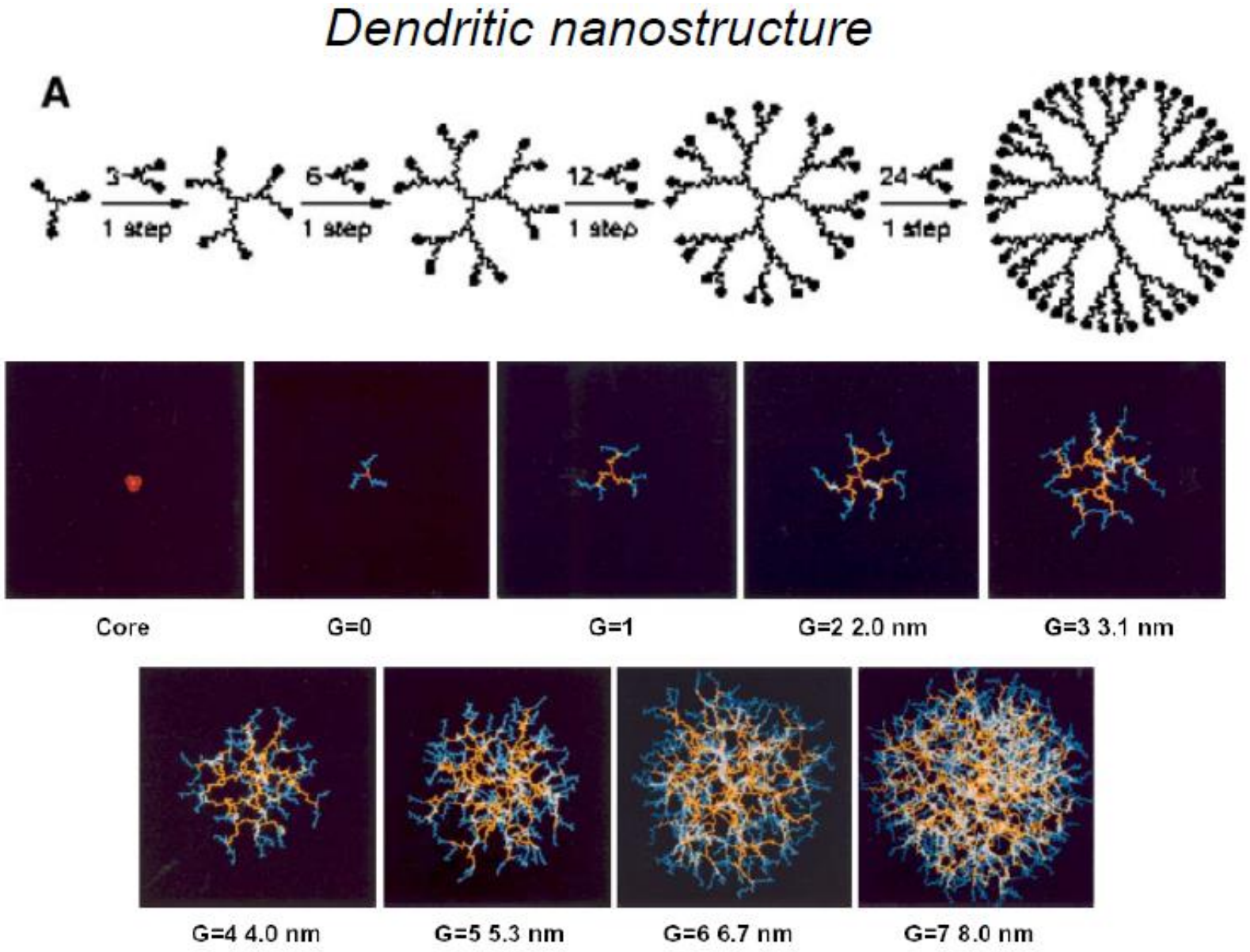
Polymer-metal complex micelles

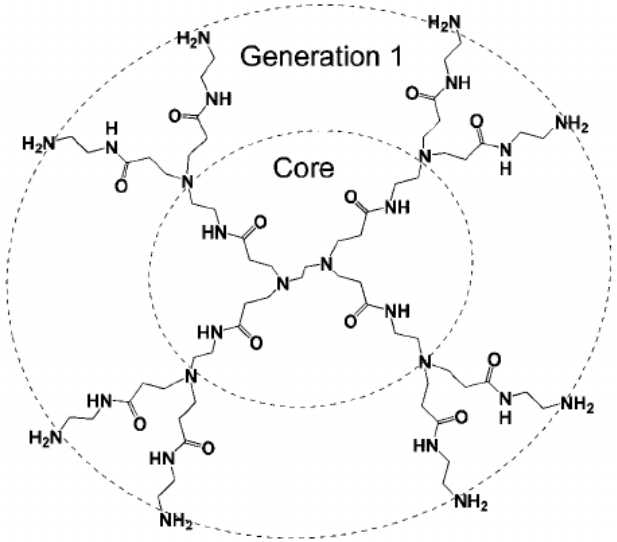


Polyion complex micelles



Dendrimers





Poly(amidoamine, PAMAM)

Advantages of Dendrimers

-Well-controllable architectural design

->Size, Shape, length/density, Functionality

-Incorporation of Bioactive agents

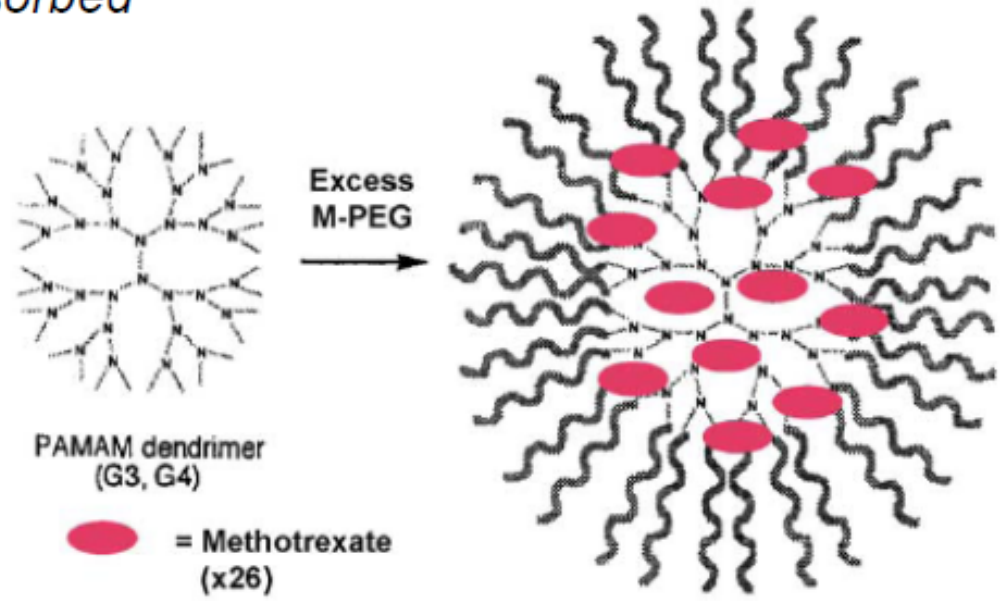
->Chemically attached, Physically adsorbed

-Delivery of Anti-cancer drugs

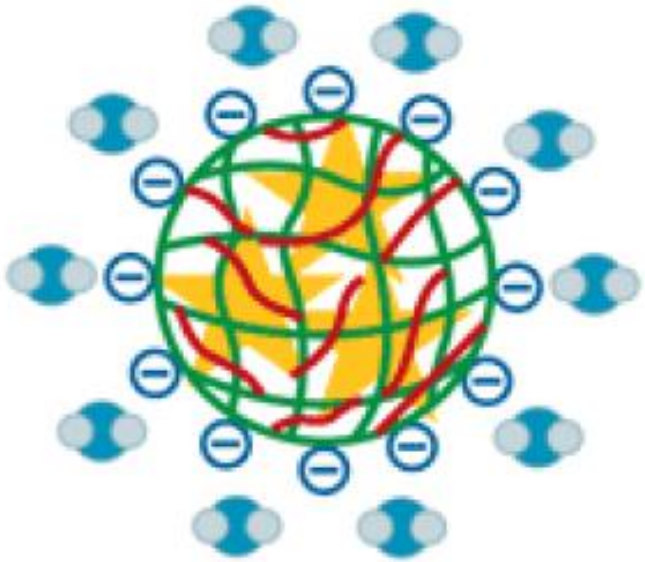
->Slower release

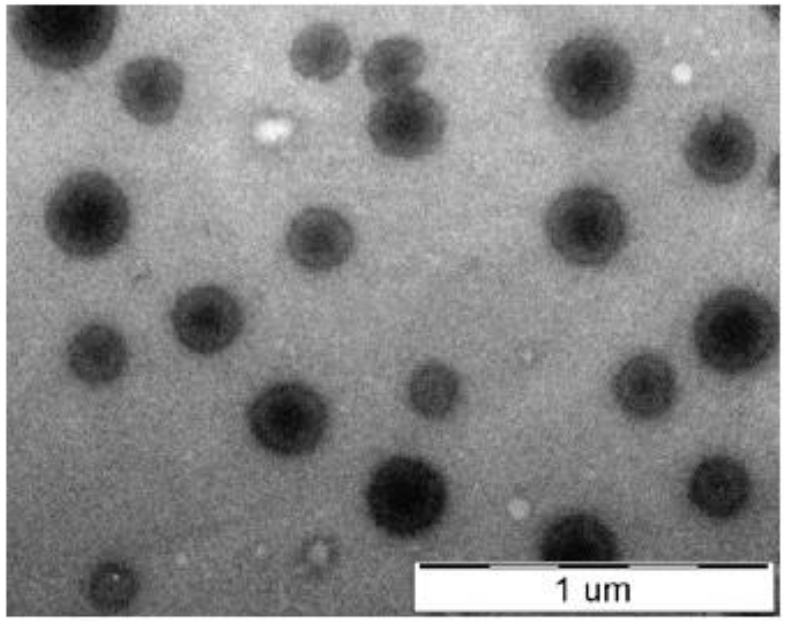
->High accumulation in solid tumor

->Lower toxicity



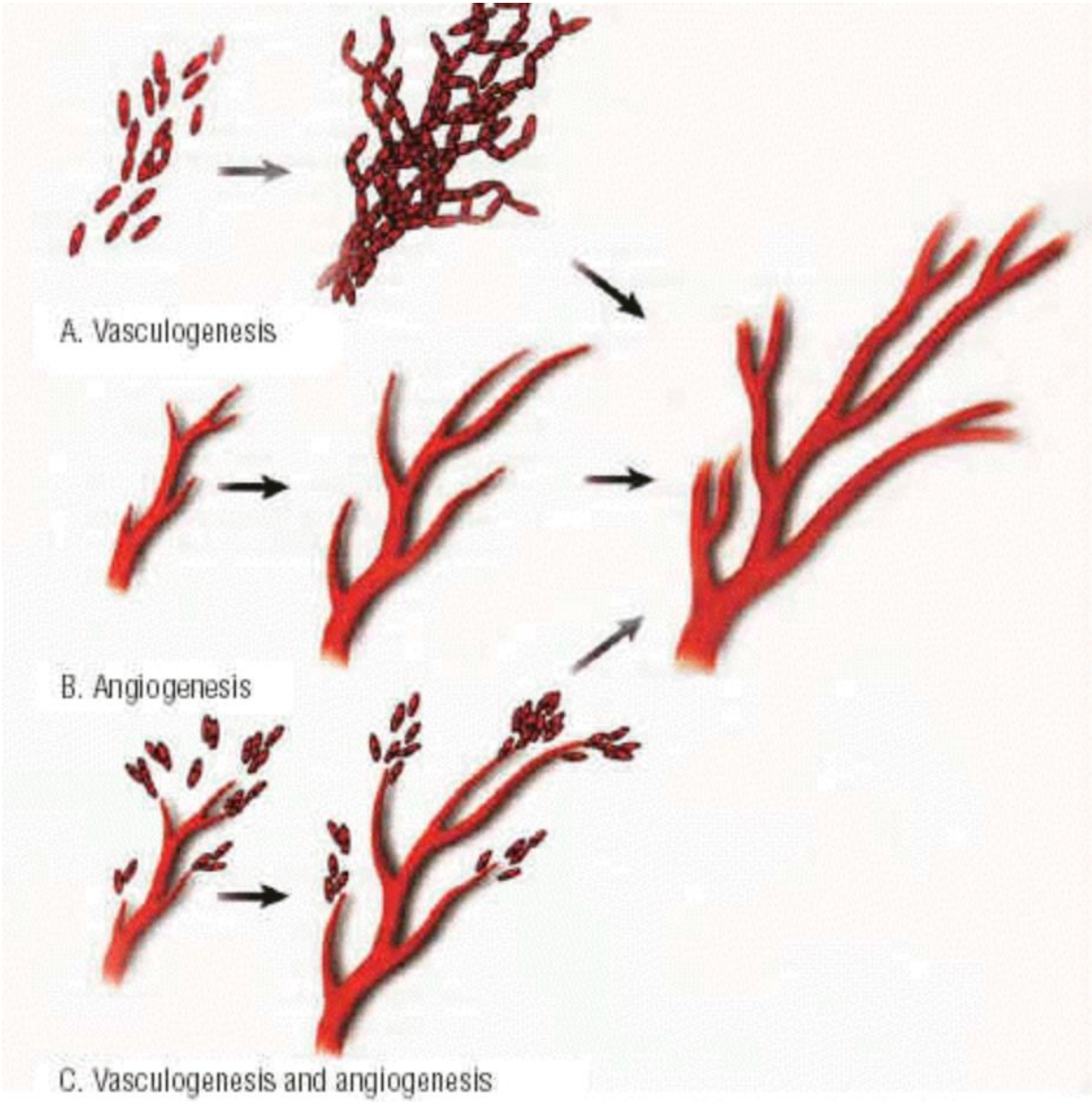
Hydrogels Nanoparticles



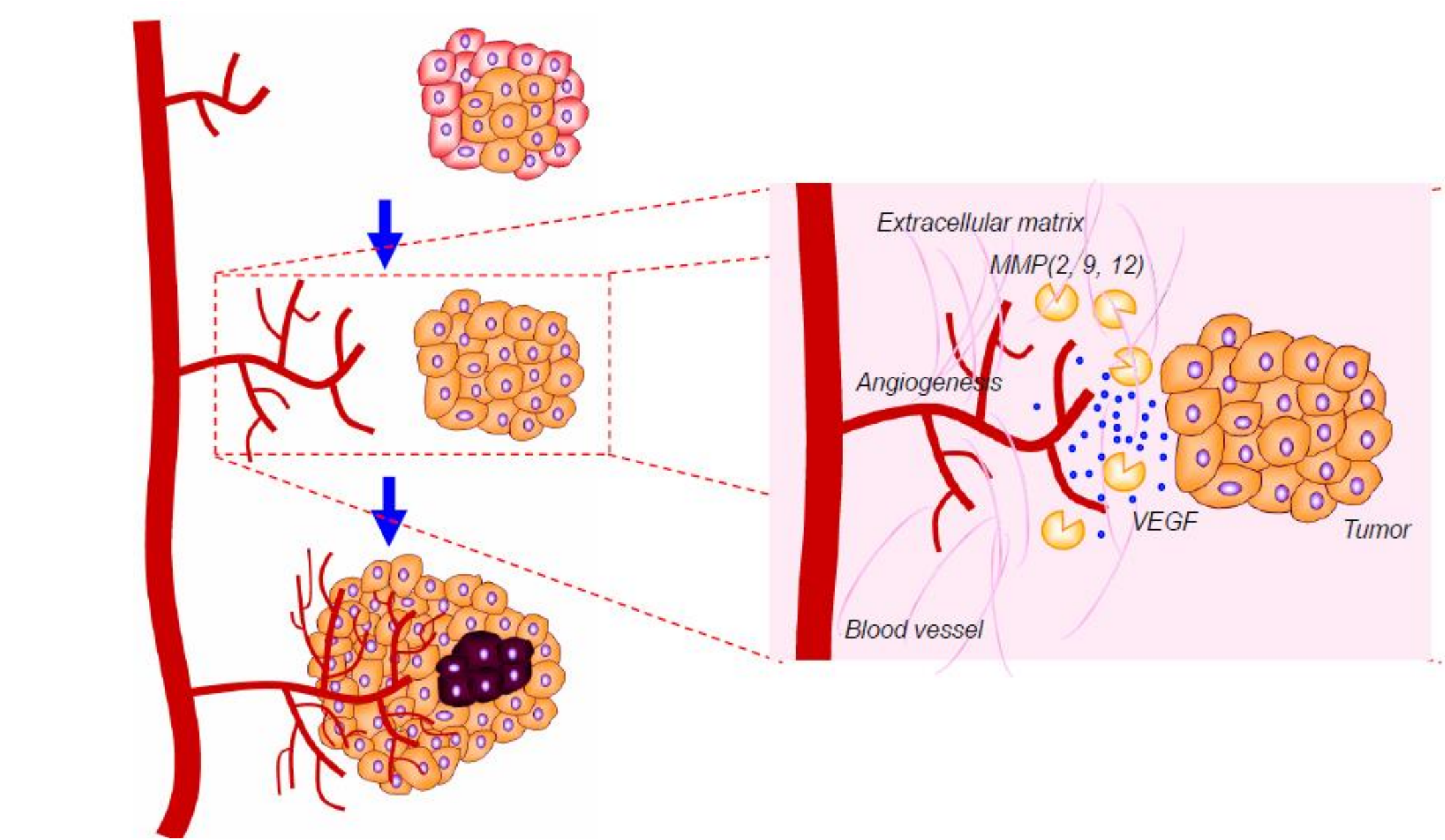


- *Nanoscale hydrophilic polymer networks*
- *Encapsulation of hydrophilic drugs (protein, DNA etc.)*
- *Diverse biomedical applications, including drug delivery, gene delivery, and molecular imaging*

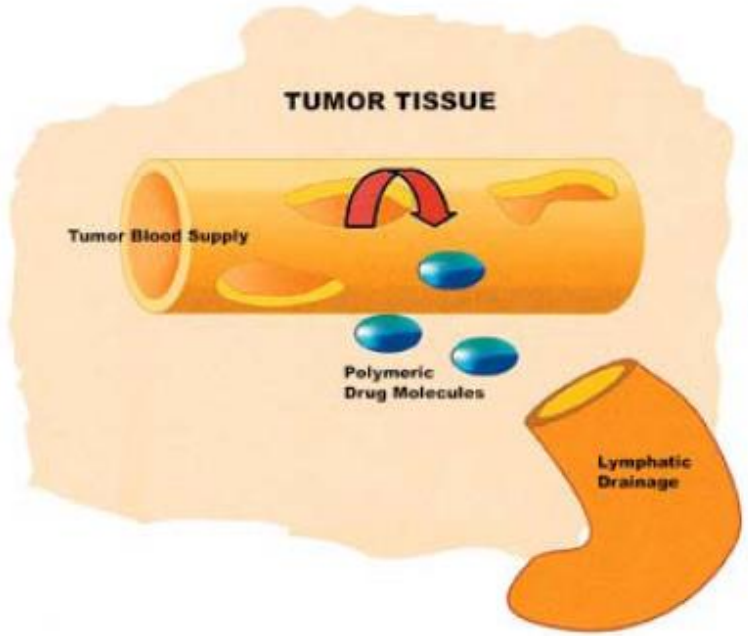
Vasculogenesis and Angiogenesis



Angiogenesis in cancer



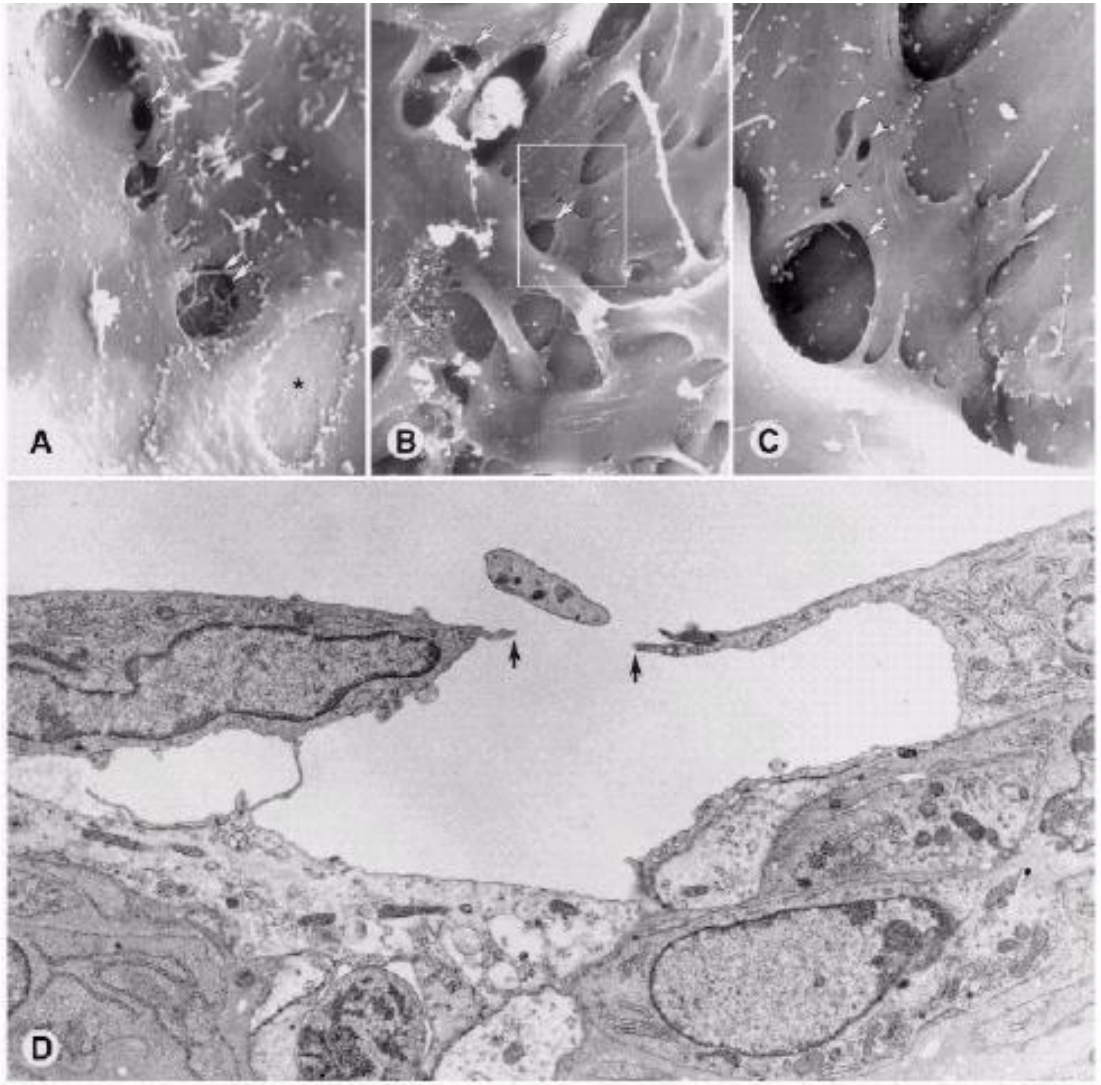
Enhanced Permeability and Retention Effect



- *Macromolecules passively accumulate in solid tumor more than the low molecular weight anticancer agents do.*

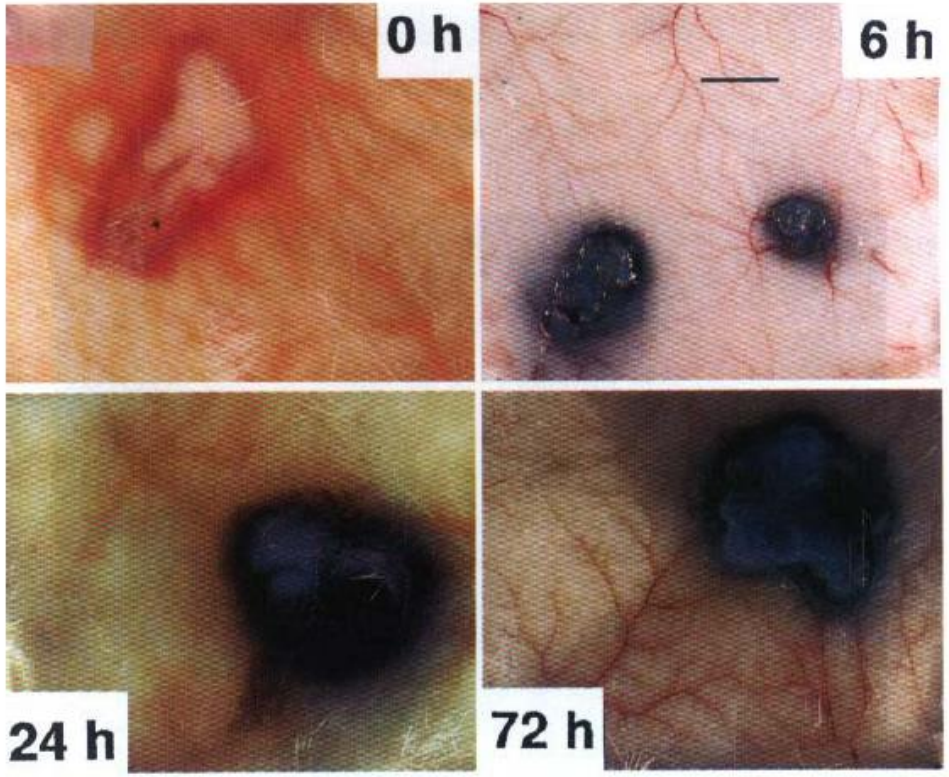
- *Unique biological properties of tumor*
  - *High level angiogenesis / hypervasculature*
  - *Defective vascular architecture*
  - *Deficient lymphatic drainage from tumor tissue*

Leaky blood vessels in tumors



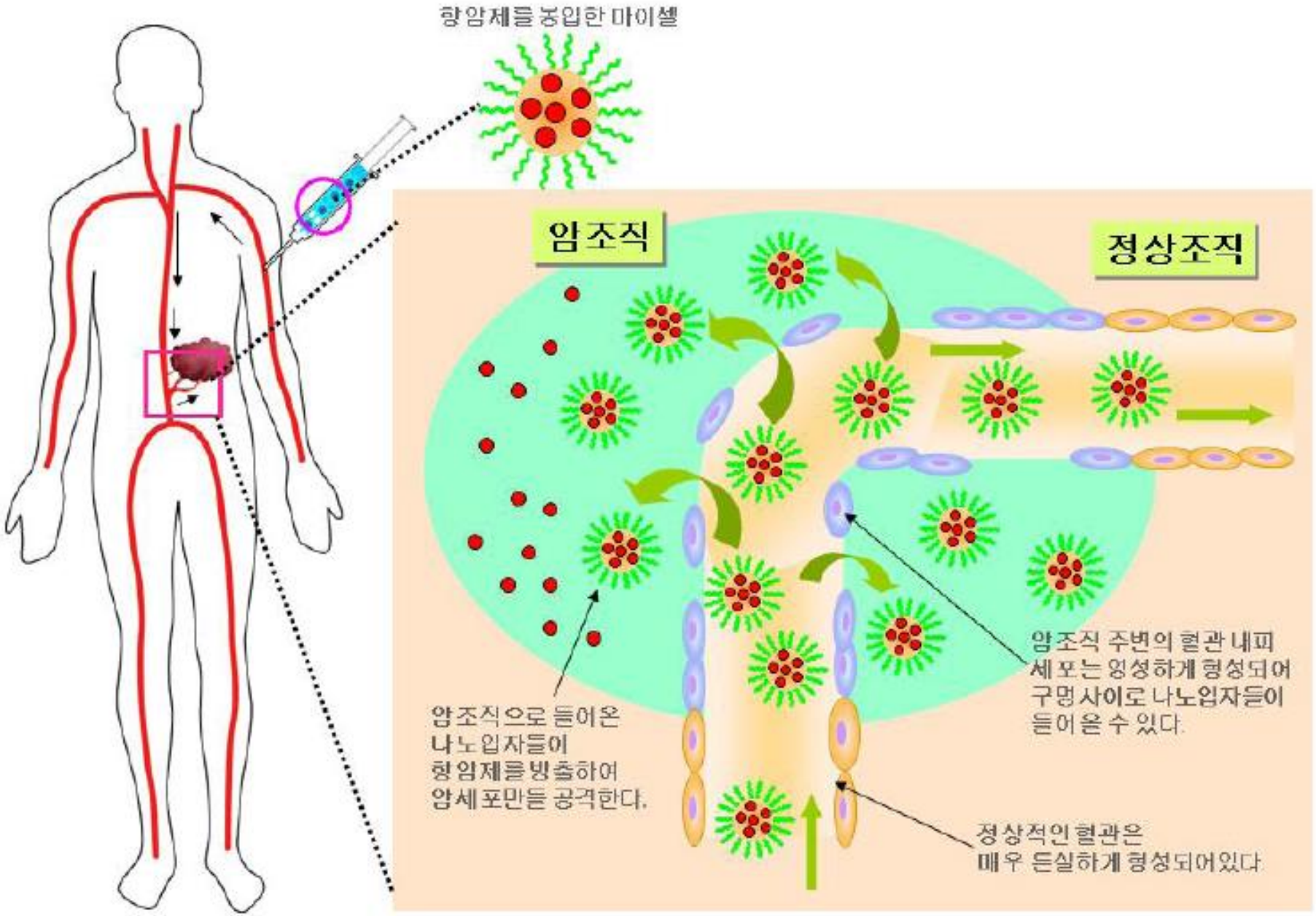
Hashizume et. al., American Journal of Pathology, 156(4), 1365-1380 (2000)

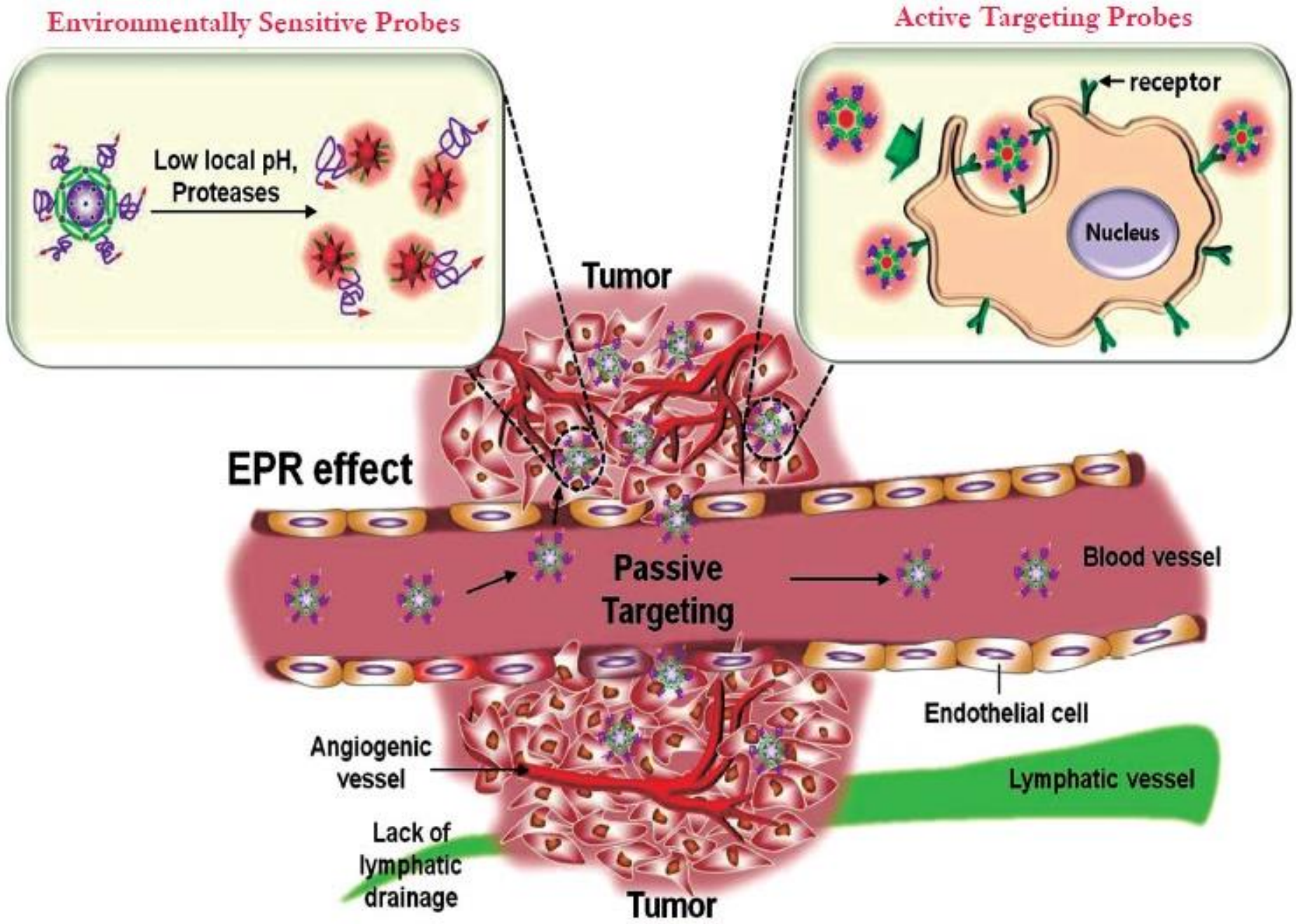
Passive tumor targeting by EPR effect



Accumulation of Evans blue-albumin complex in tumor tissue and normal skin in tumor-bearing mice. Tumor S-180 was injected into the skin.

Tumor targeting in nanomedicine




Environmentally Sensitive Probes
Low local pH,
Proteases
Active Targeting Probes
receptor
Nucleus
Tumor
EPR effect
Passive
Targeting
Blood vessel
Endothelial cell
Angiogenic
vessel
Lymphatic vessel
Lack of
lymphatic
drainage
Tumor

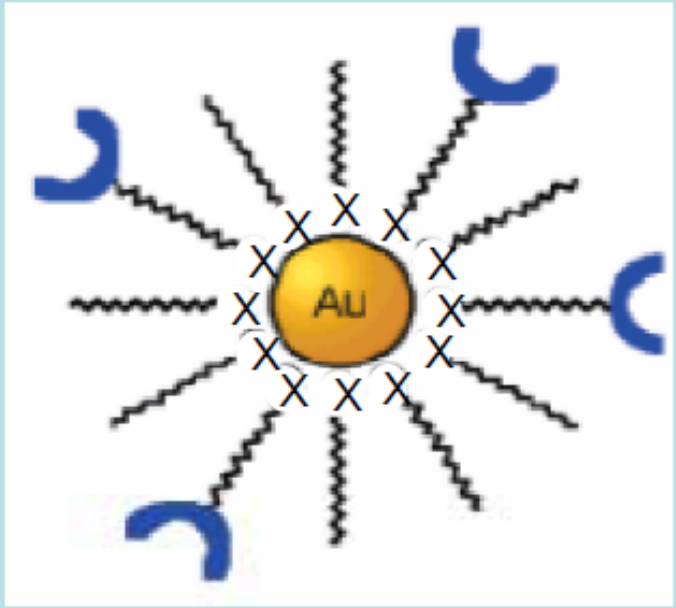
Synthesis and fabrication of inorganic nanomaterials

Growth of colloidal nanocrystals by reduction in the presence of surfactants

General Strategy

- Metal precursor solution
- Reducing Agent
- Capping (stabilizing agent)
- Solvent (heated)

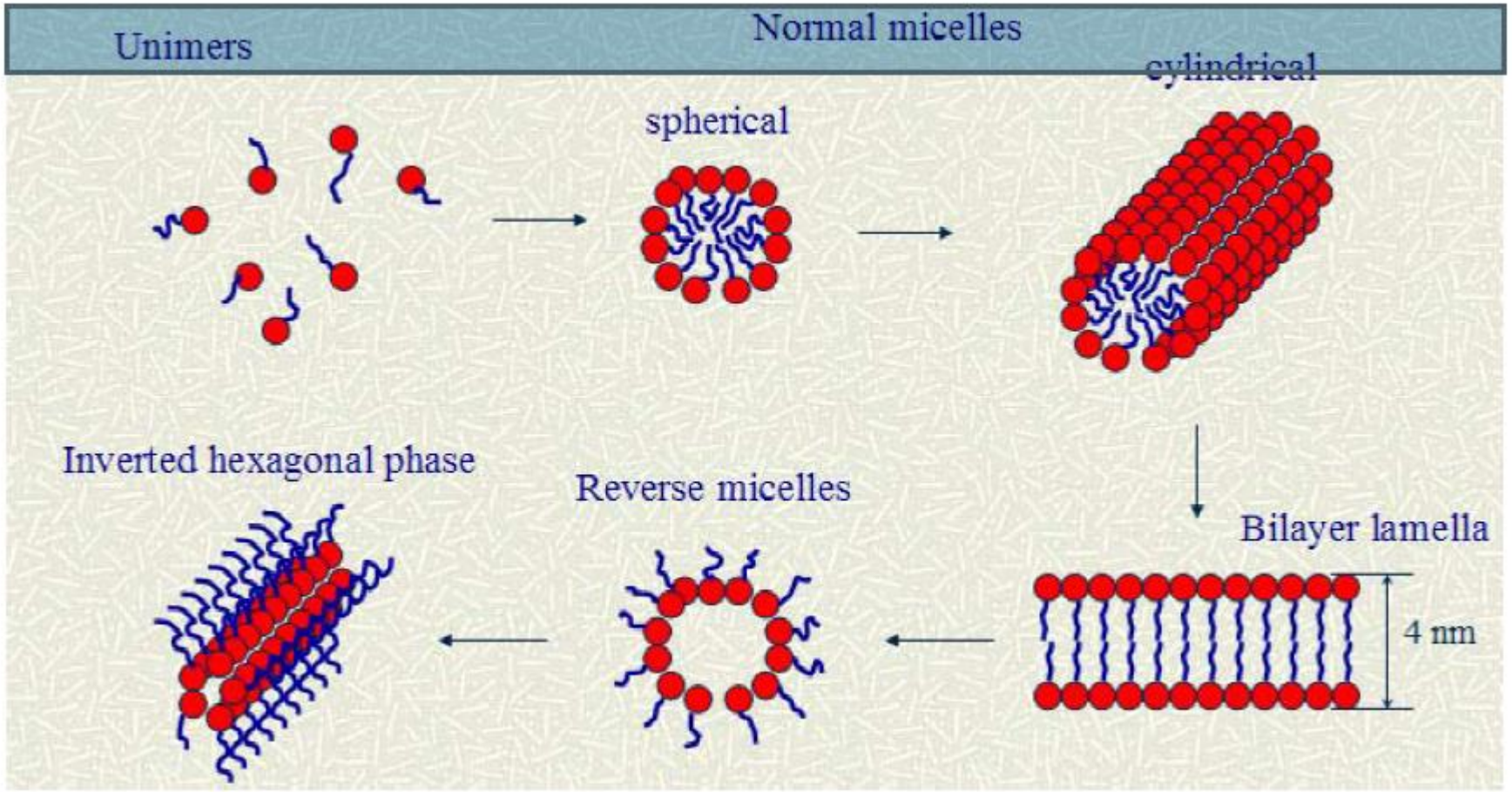
Example: Gold nanocrystals



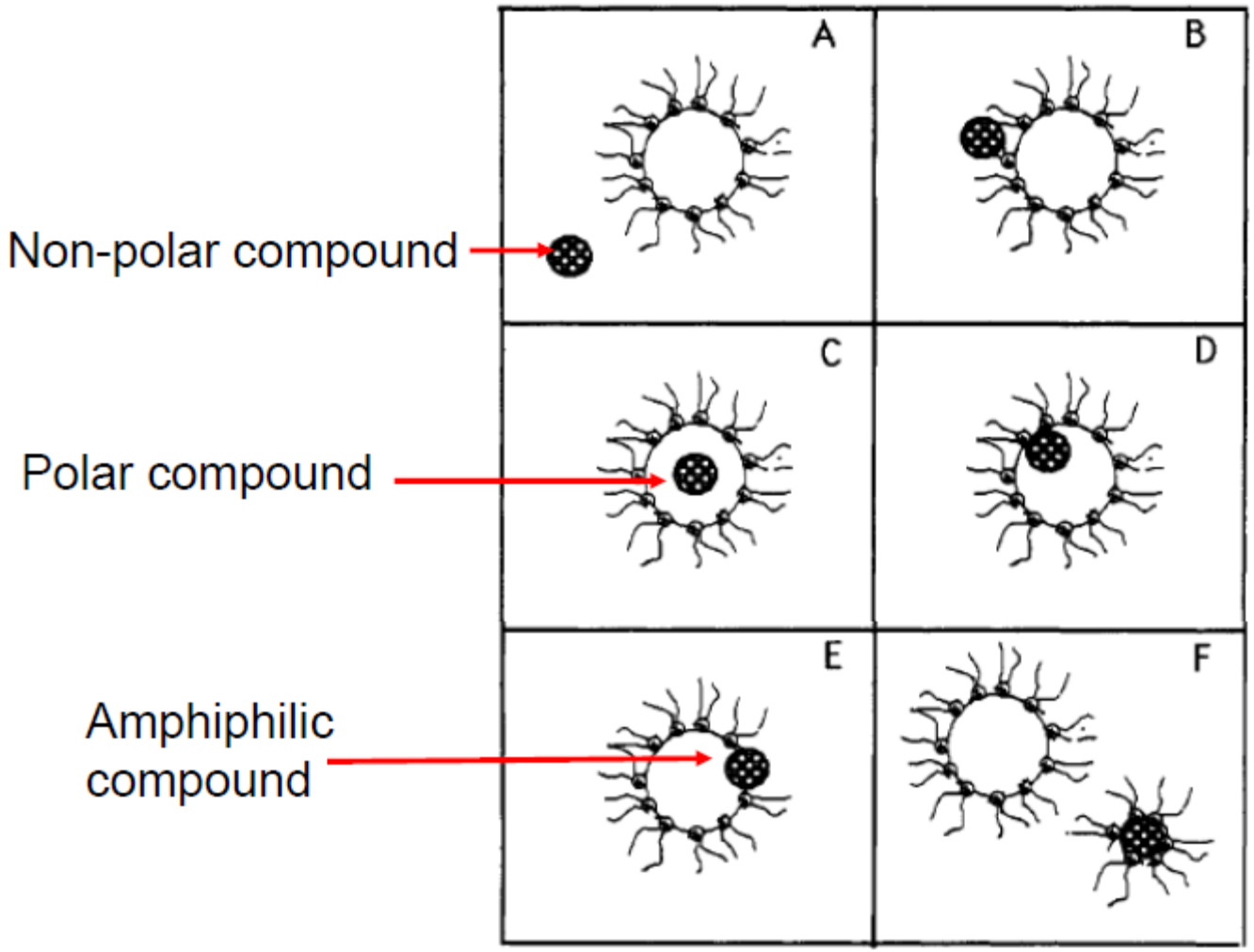
tetrachloroauric acid ($HAuCl_4$)
boil with
Trisodium citrate

Frens, G.. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions, Nature (1973), 241(105), 20-2. J. Turkevich, et. al. Coagulation of Colloidal Gold. *Discussions Faraday Soc.* No. 11, 58 (1951).

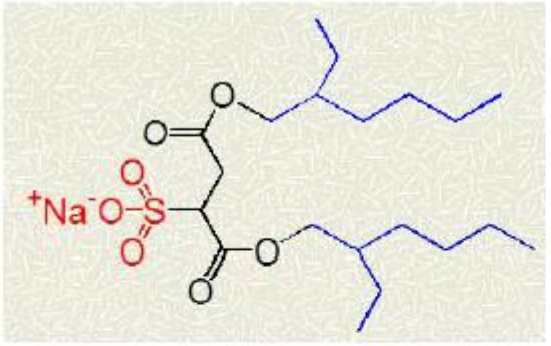
Molecular surfactant template growth of nanoparticles

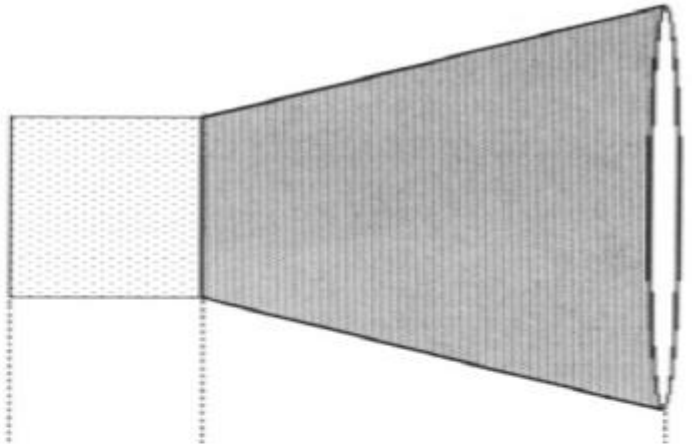


Different solute interaction possibilities can occur



Synthesis of nanoparticles using reverse micelles



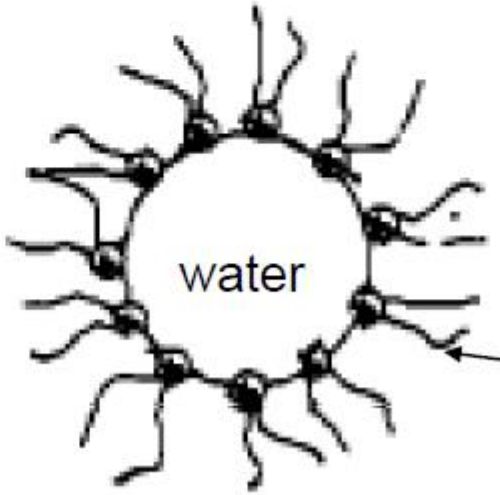


AOT: Sodium
bis (2-ethylhexyl)suflosuccinate)

Isooctane

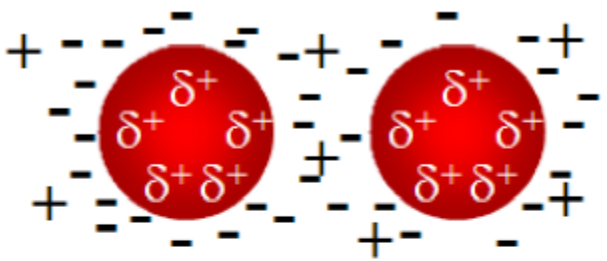
Radius of micelle (nm) = 0.15 [water] / [AOT]

Molar ratio

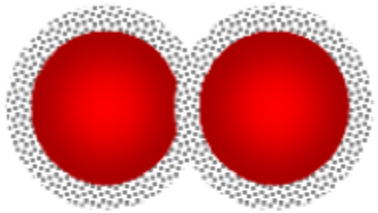


AOT molecules

Nanoparticles stabilization against aggregation

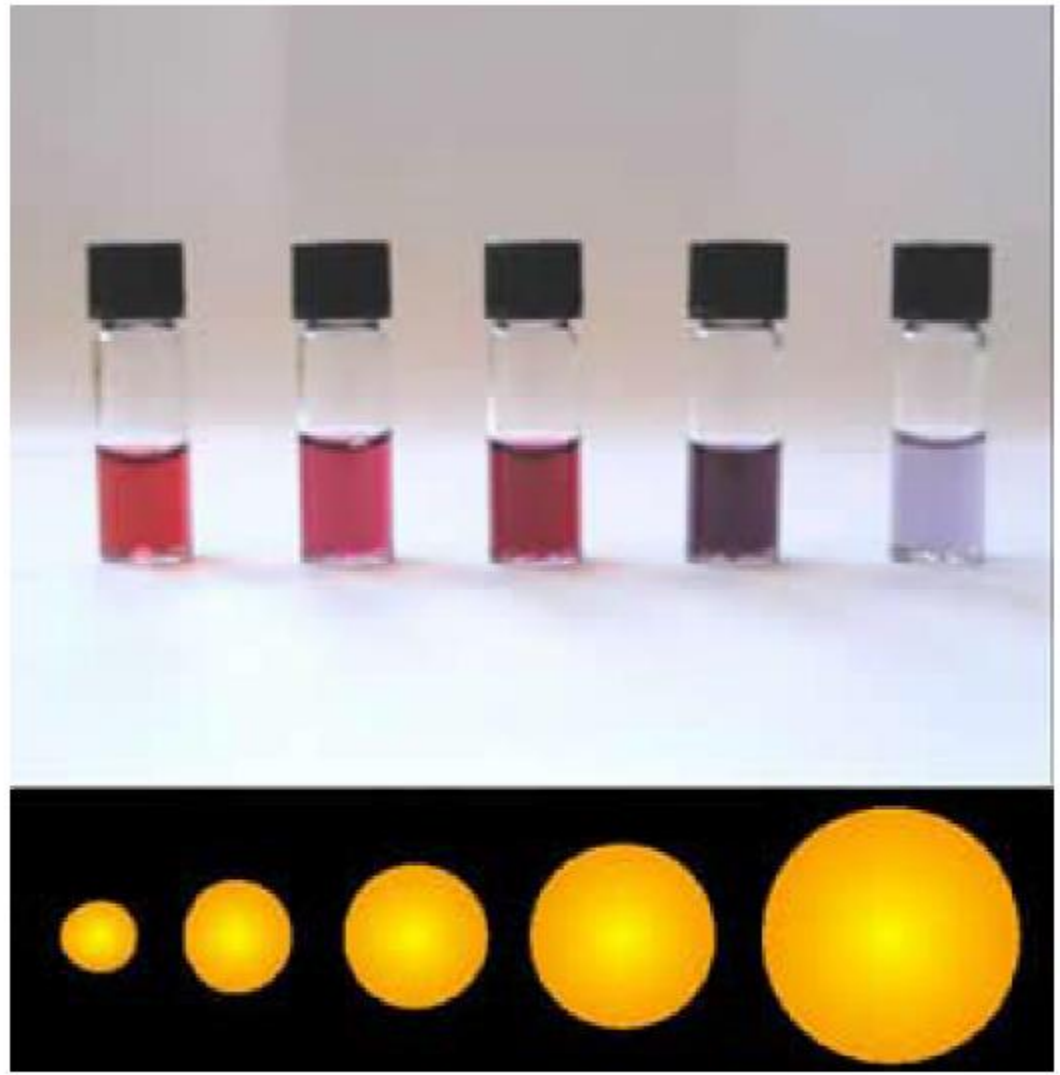


Electrostatic
(adsorption of ions)



Coat particles with
Organic molecules / polymers

Capping (stabilizing agent)
e.g. Trioctylphosphine oxide (TOPO)



The diameter of gold nanoparticles determines the wavelengths of light absorbed. The colors in this diagram illustrate this effect.

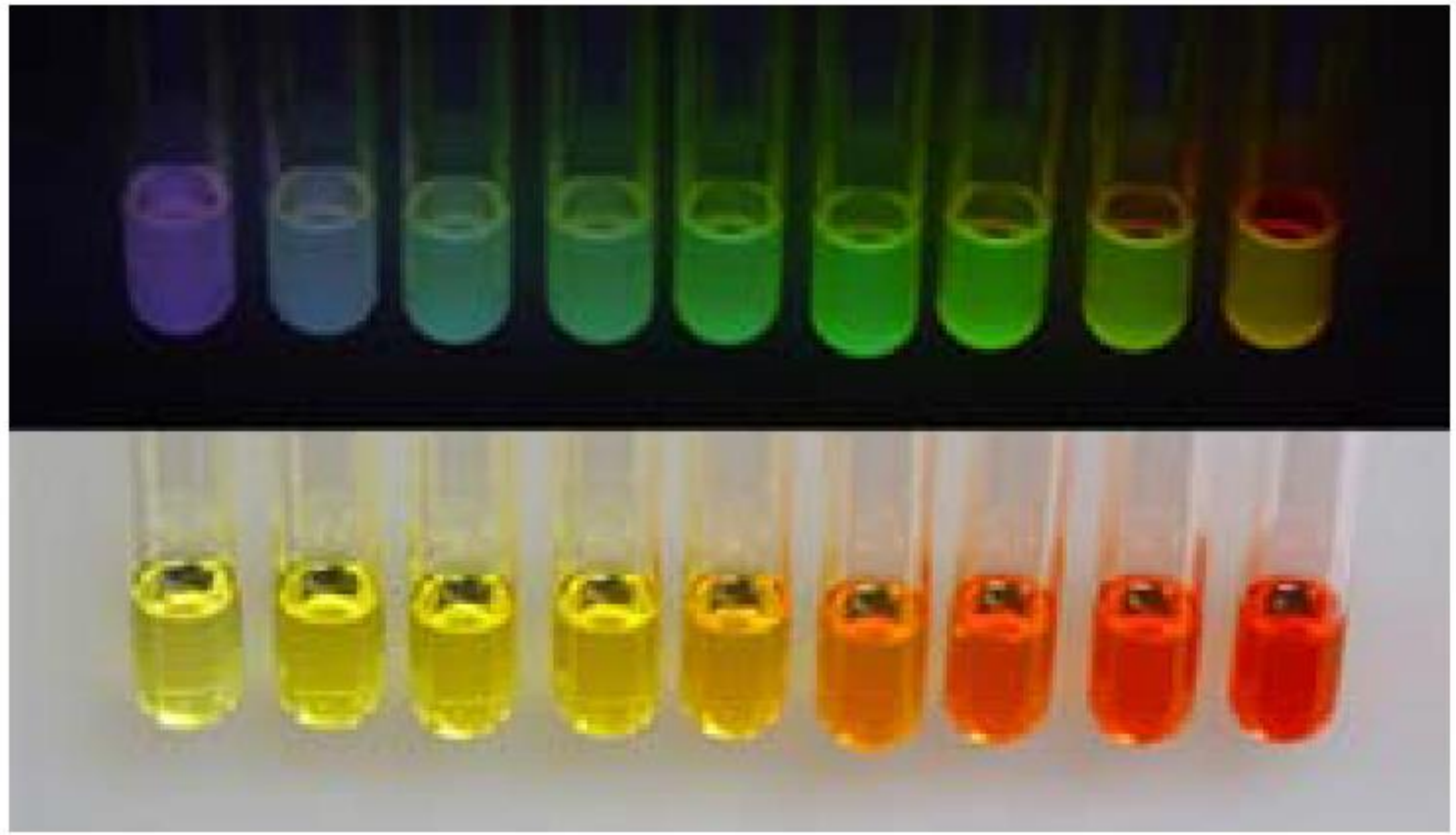
Quantum dots (QDs)

Fluorescence induced by exposure to ultraviolet light in vials containing various sized Cadmium selenide (CdSe) quantum dots.

A **quantum dot** is a semiconductor nanostructure that confines the motion of conduction band electrons, valence band holes, or excitons (pairs of conduction band electrons and valence band holes) in all three spatial directions.

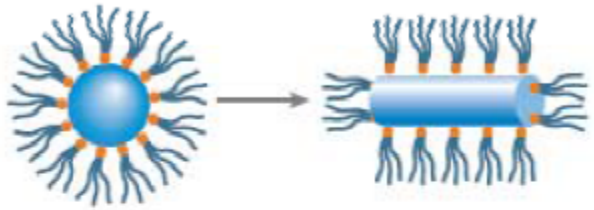
Video of Nanocrystal Synthesis (video from Bawendi group at MIT)

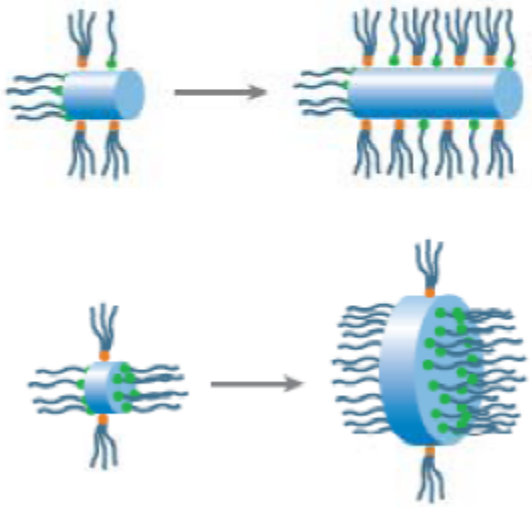
Synthesis of CdSe nanocrystals by injection of cadmium and selenium precursors into a high boiling point coordinating solvent at 360 degrees C. Intense color results from the quantum confinement effect on the electron as a result of the nanocrystal size being less than the Bohr radius of the exciton. Thus, larger particle size results in a red-shift in the emission. A size series was generated by allowing the crystals to grow at 280 degrees C. Over a period of 2 minutes, the fluorescence spectrum from 515 nm to 635 nm was covered.



Different sized quantum dot nanoparticles are shown above, first in ultraviolet light and then in ambient light. The length of the synthesis reaction determines particle size for CdSe, increasing from left to right. In colloidal suspension, this semiconductor behaves in the same way as a metal.

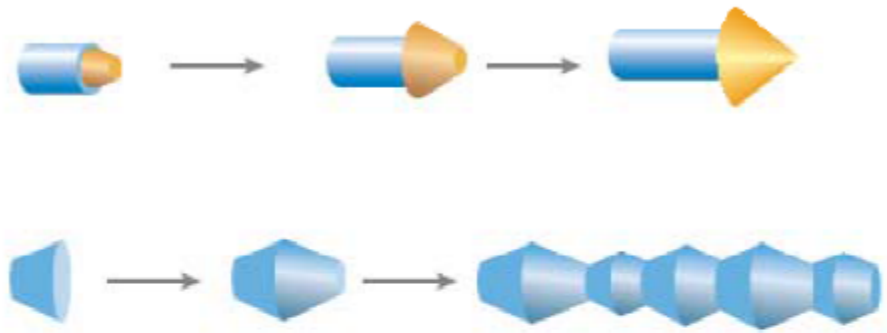
A. Kinetic control: High energy facets grow faster than low energy facets

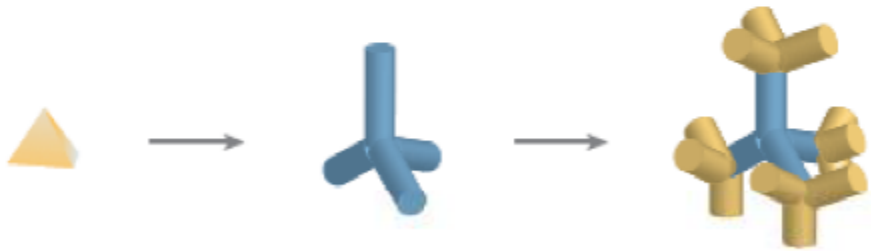




B. Kinetic shape control through selective adhesion

C. Intermediate energy facet eliminates high energy facet.





D. Controlled branching of nanocrystals

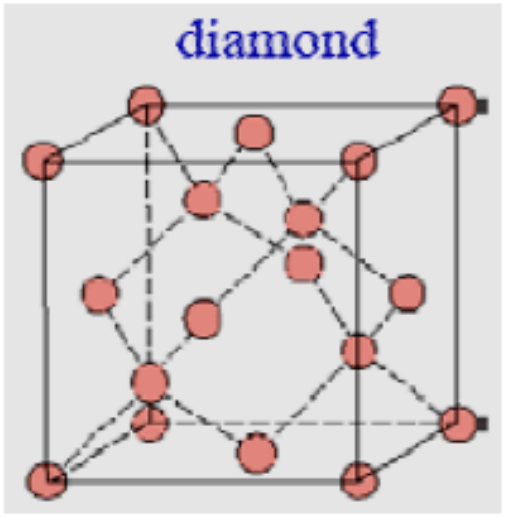
Y. Yin and A. P. Alivisatos, Nature, (2005) 664-670

Fullerene: A form of carbon

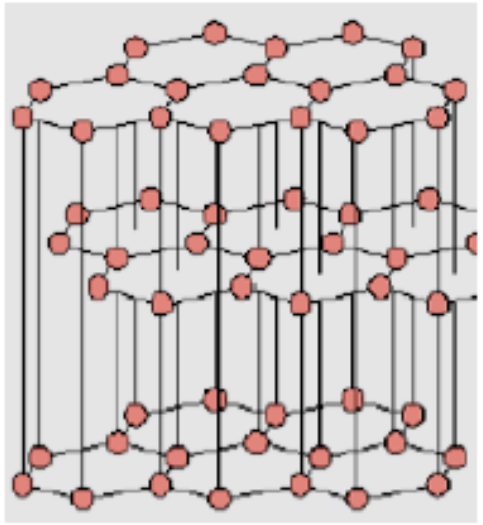
A fullerene is a molecule of carbone in the form of a hollow sphere, ellipsoid, tube, and many other shapes. Spherical fullerenes are also called Buckminsterfullerenes (buckyballs)

(metastable)

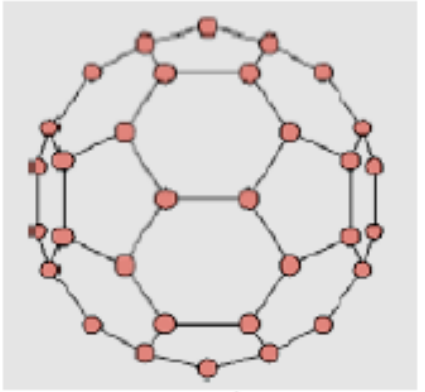
diamond



Graphite (stable)

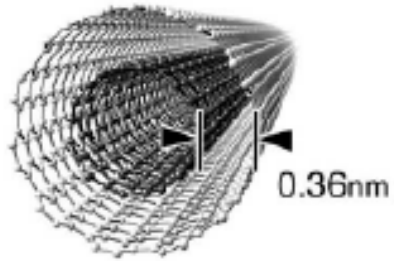


Bucky ball
(metastable)



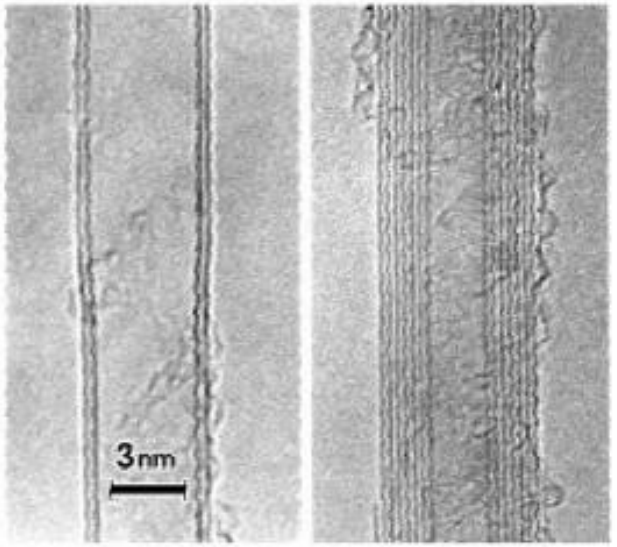
Smalley & Kroto, 1997 Nobel

Nanotube = rolled sheet of graphite



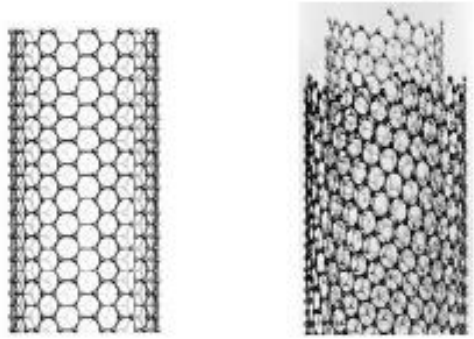
Helical Microtubules of graphitic Carbon, Ijima, S, Nature, 354 (6348): 56-58 NOV 7 1991
Cited: >4000 times

Different types of nanotubes



Up to cm long

SWNT
(single
Wall nanotube)
Diameter ~ 1.4 nm



MWNT
(multiwall)
Diameter 10-20 nm

Must read Paper:
Iijima, Sumio. Carbon nanotubes: past, present, and future. Physica B: Condensed Matter (2002), 323, 1-5.

Common methods to characterize biomaterials surfaces

TABLE 1 Common Methods to Characterize Biomaterial Surfaces

Method	Principle	Depth analyzed	Spatial resolution	Analytical sensitivity	Cost[c]
Contact angles	Liquid wetting of surfaces is used to estimate the energy of surfaces	3–20 Å	1 mm	Low or high depending on the chemistry	$
ESCA (XPS)	X-rays induce the emission of electrons of characteristic energy	10–250 Å	10–150 μm	0.1 at%	$$$
Auger electron spectroscopy[a]	A focused electron beam stimulates the emission of Auger electrons	50–100 Å	100 Å	0.1 atom%	$$$
SIMS	Ion bombardment sputters secondary ions from the surface	10 Å–1 μm[b]	100 Å	Very high	$$$
FTIR-ATR	IR radiation is adsorbed and excites molecular vibrations	1–5 μm	10 μm	1 mol%	$$
STM	Measurement of the quantum tunneling current between a metal tip and a conductive surface	5 Å	1 Å	Single atoms	$$
SEM	Secondary electron emission induced by a focused electron beam is spatially imaged	5 Å	40 Å, typically	High, but not quantitative	$$

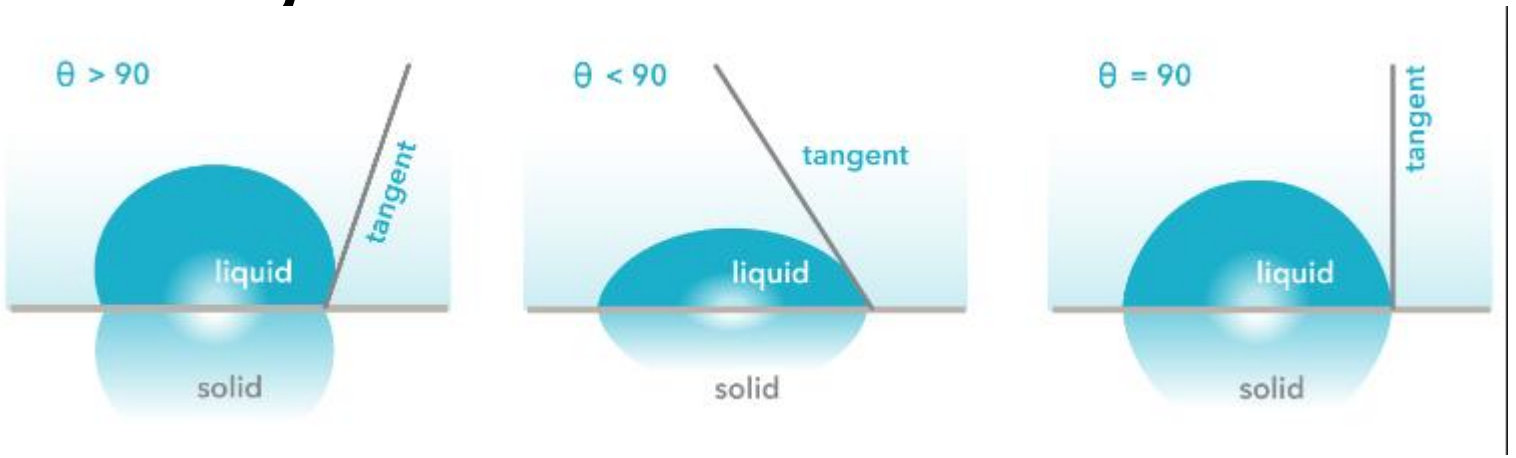
[a] Auger electron spectroscopy is damaging to organic materials and is best used for inorganics.
[b] Static SIMS ≈ 10 Å, dynamic SIMS to 1 μm
[c] $, up to $5000; $$, $5000–$100,000; $$$, >$100,000.

Other techniques for surfaces

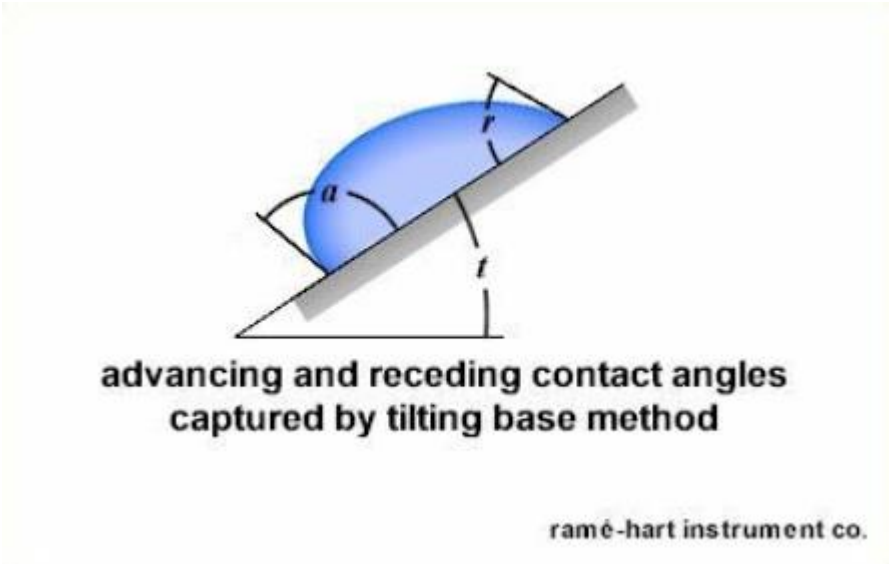
TABLE 7 Methods that may have Applicability for the Surface Characterization of Biomaterials

Method	Information obtained
Second-harmonic generation (SHG)	Detect submolayer amounts of adsorbate at any light-accessible interface (air–liquid, solid–liquid, solid–gas)
Surface-enhanced Raman spectroscopy (SERS)	High-sensitivity Raman at rough metal interfaces
Ion scattering spectroscopy (ISS)	Elastically reflected ions probe only the outermost atomic layer
Laser desorption mass spectrometry (LDMS)	Mass spectra of adsorbates at surfaces
Matrix assisted laser desorption ionization (MALDI)	Though generally a bulk mass spectrometry method, MALDI has been used to analyze large adsorbed proteins
IR photoacoustic spectroscopy (IR-PAS)	IR spectra of surfaces with no sample preparation based on wavelength-dependent thermal response
High-resolution electron energy loss spectroscopy (HREELS)	Vibrational spectroscopy of a highly surface-localized region, under ultrahigh vacuum
X-ray reflection	Structural information about order at surfaces and interfaces
Neutron reflection	Thickness and refractive index information about interfaces from scattered neutrons—where H and D are used, unique information on interface organization can be obtained
Extended X-ray absorption fine structure (EXAFS)	Atomic-level chemical and nearest-neighbor (morphological) information
Scanning Auger microprobe (SAM)	Spatially defined Auger analysis at the nanometer scale
Surface plasmon resonance (SPR)	Study aqueous adsorption events in real time by monitoring changes in surface refractive index
Rutherford backscattering spectroscopy (RBS)	Depth profiling of complex, multiplayer interfacial systems

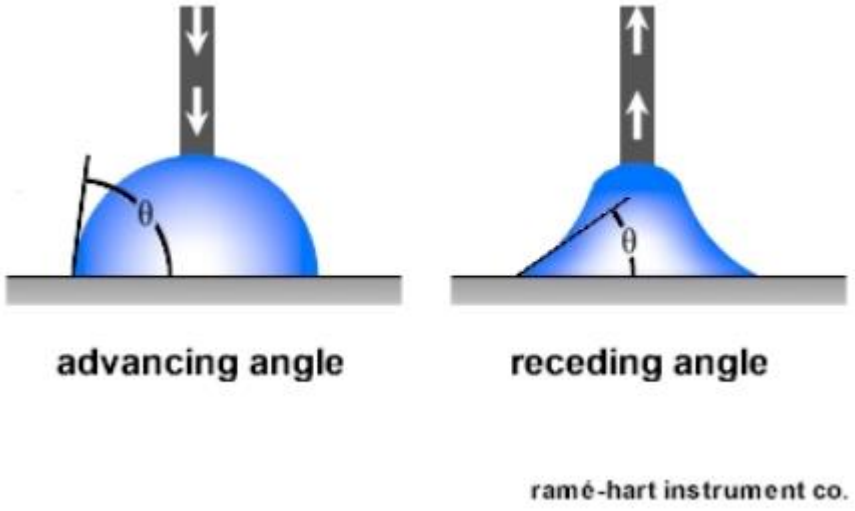
Contact angle methods



Tilting plate method



Add and remove volume method



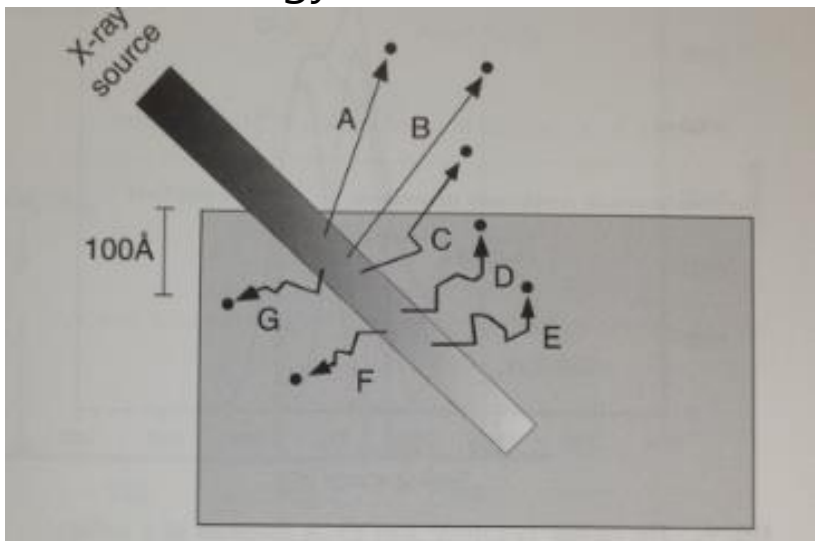
Electron spectroscopy for chemical analysis (ESCA)

ESCA (also known as X-ray photoelectron spectroscopy, XPS) is based on the photoelectron effect. A high energy X-ray photon can ionize an atom, producing an ejected free electron with kinetic energy KE:

$$KE = hv - BE$$

hv **=photon energy (e.g., for Al Kalpha, hv = 1486.6 eV)**

BE=energy necessary to remove a specific electron from an atom. BE orbital energy



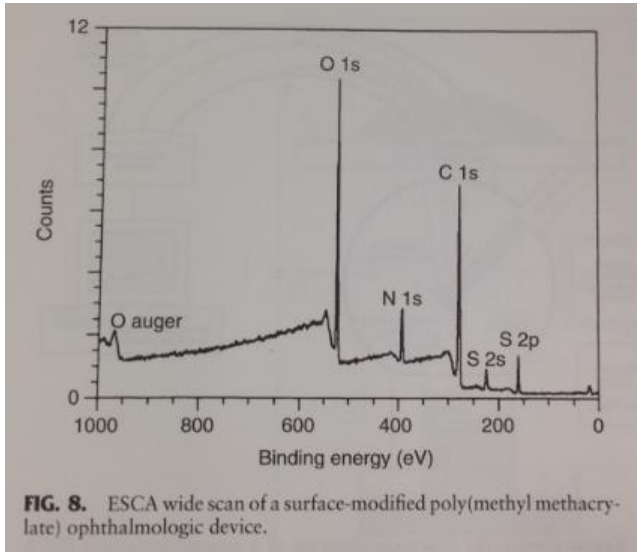


FIG. 8. ESCA wide scan of a surface-modified poly(methyl methacrylate) ophthalmologic device.

Depth profiles: 0.5-10 nm, AES. 0.5-5 nm, Secondary Ion Mass Spectroscopy (SIMS): 0.1-1 nm

AES, XPS, TOF-SIMS

	AES	XPS	TOF-SIMS
Probe beam Analysis beam	Electrons Electrons	Photons Electrons	Ions Ions
Sampling Depth	5-50 Å	5-50 Å	1-10 Å
Detection Limits	1×10^{-3}	1×10^{-4}	1×10^{-6}
Information	Elemental, SEM	Elemental, Chemical	Elemental, Chemical, Molecular
Spatial Resolution	~100 A^{o}	~10 μm	~1000 A^{o}
Restriction	Inorganics (e-beam damage of organics a major problem)	Few	Quantification Standards Required

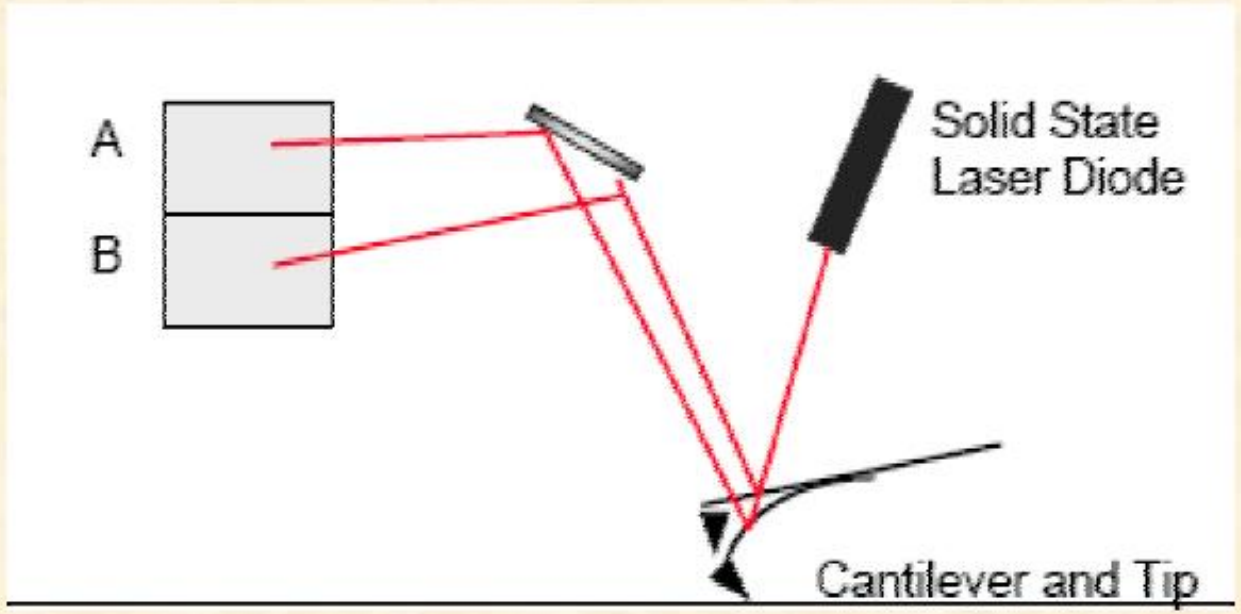
Atomic Force Microscopes (AFM)

An AFM scanning head from Digital Instruments.

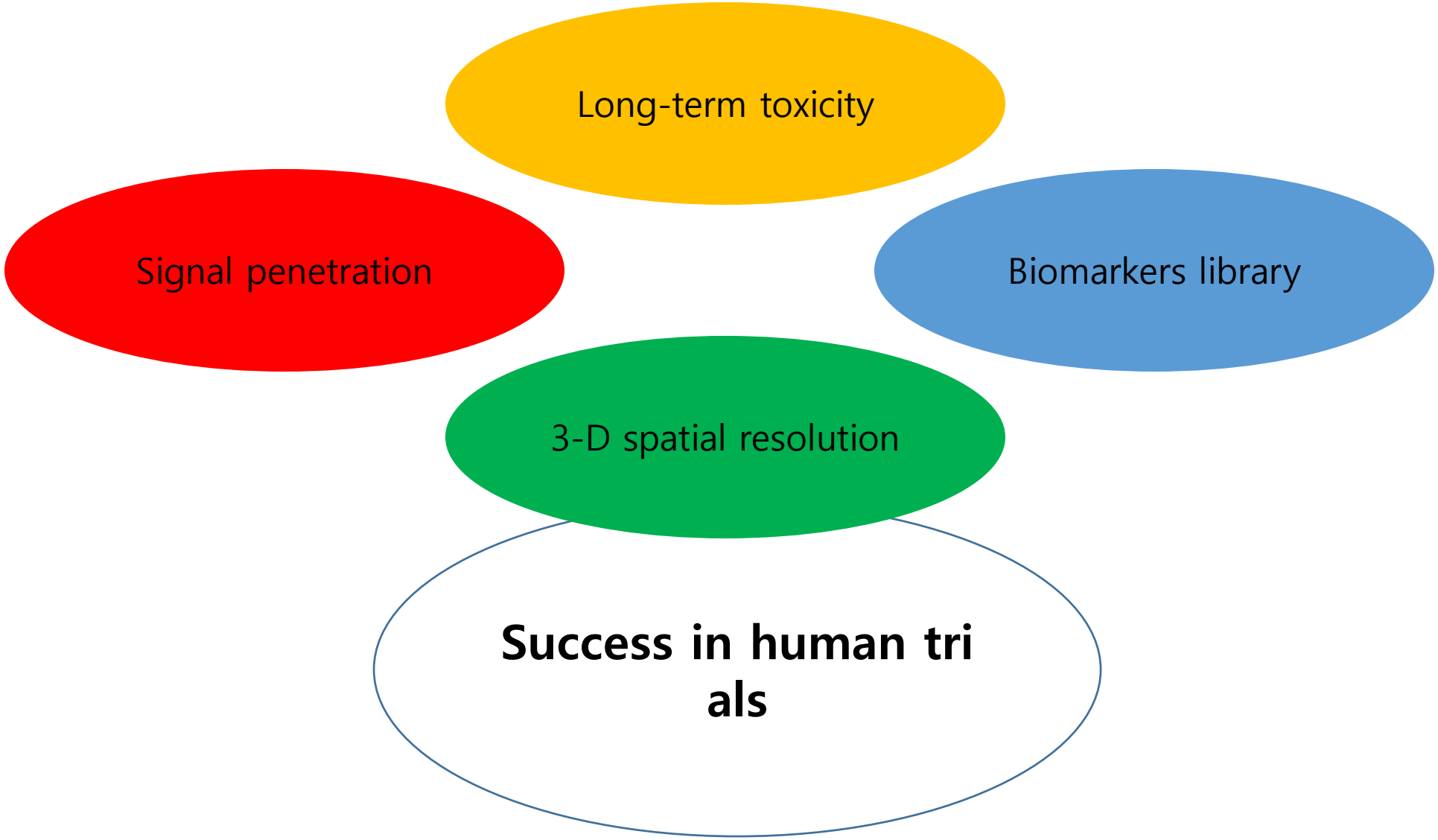
The Atomic Force Microscope was developed to overcome a basic drawback with STM - that it can only image conducting or semiconducting surfaces.
The AFM, however, has the advantage of imaging almost any type of surface, including polymers, ceramics, composites, glass, and biological samples.

Modes of operation

- Used for Contact Mode, Non-contact and TappingMode AFM
-Laser light from a solid state diode is reflected off the back of the cantilever and collected by a position sensitive detector (PSD). This consists of two closely spaced photodiodes. The output is then collected by a differential amplifier
- Angular displacement of the cantilever results in one photodiode collecting more light than the other. The resulting output signal is proportional to the deflection of the cantilever.
- Detects cantilever deflection <1A



Unsolved issues



Challenges

- Multiple modality and functional nanoparticles
- Fight against the tendency of nanoparticles to be adsorbed by reticuloendothelial system
- Avoid aggregation of nanoparticles for in vivo viability
- Improve retention times of the nanoparticles inside the body to allow the therapeutic effect
- Substitute potentially toxic elements

Challenges

- Compromise between coating and hydrodynamic radius
- Eliminate the inflammatory and immune response triggered by some polymer coatings
- Avoid undesired degradation exposing toxic elements (QD) or untimely delivering cargo
- Increase contrast for human medical imaging (tissues are naturally fluorescent)