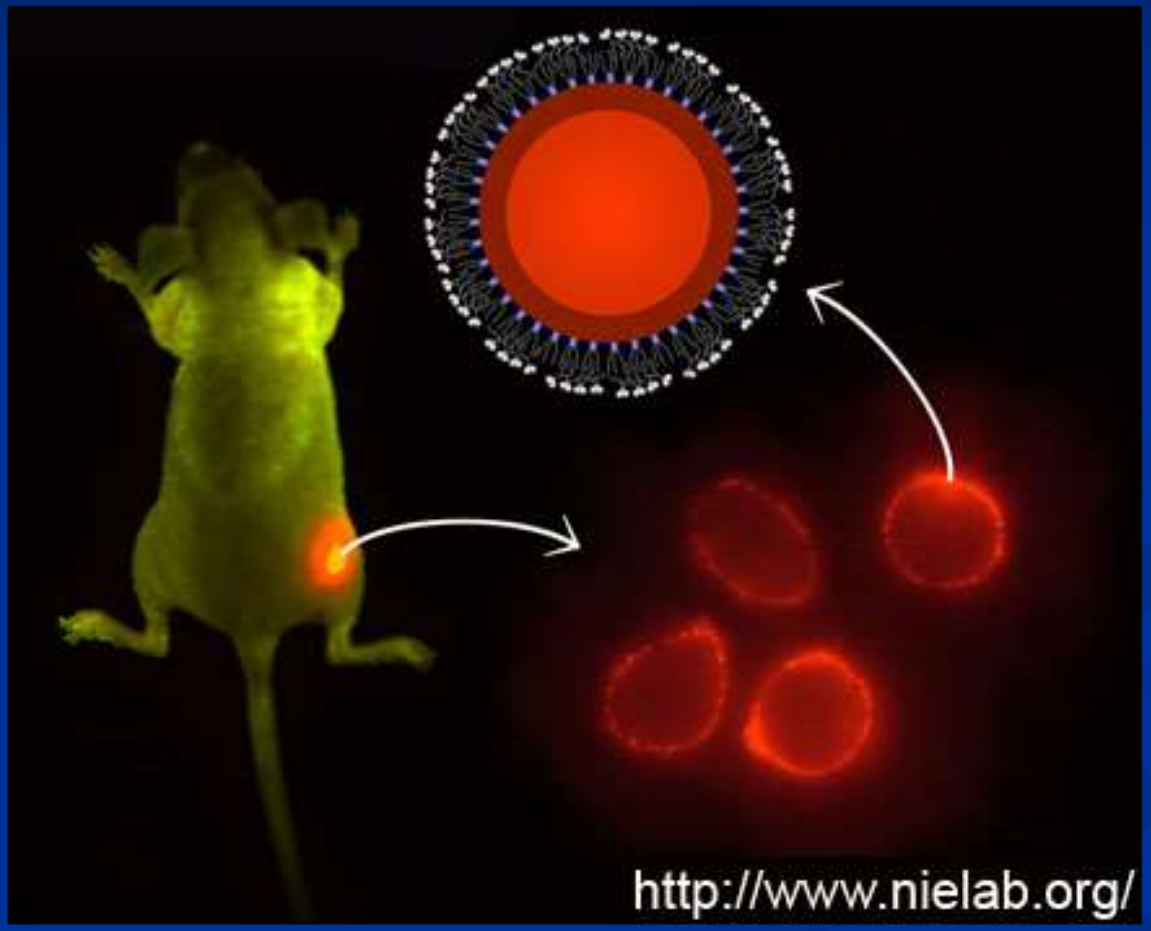
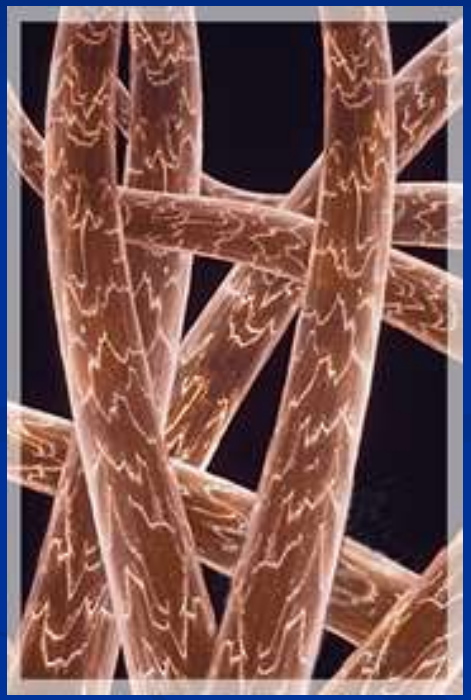
Nanotechnology & Nanomedicine



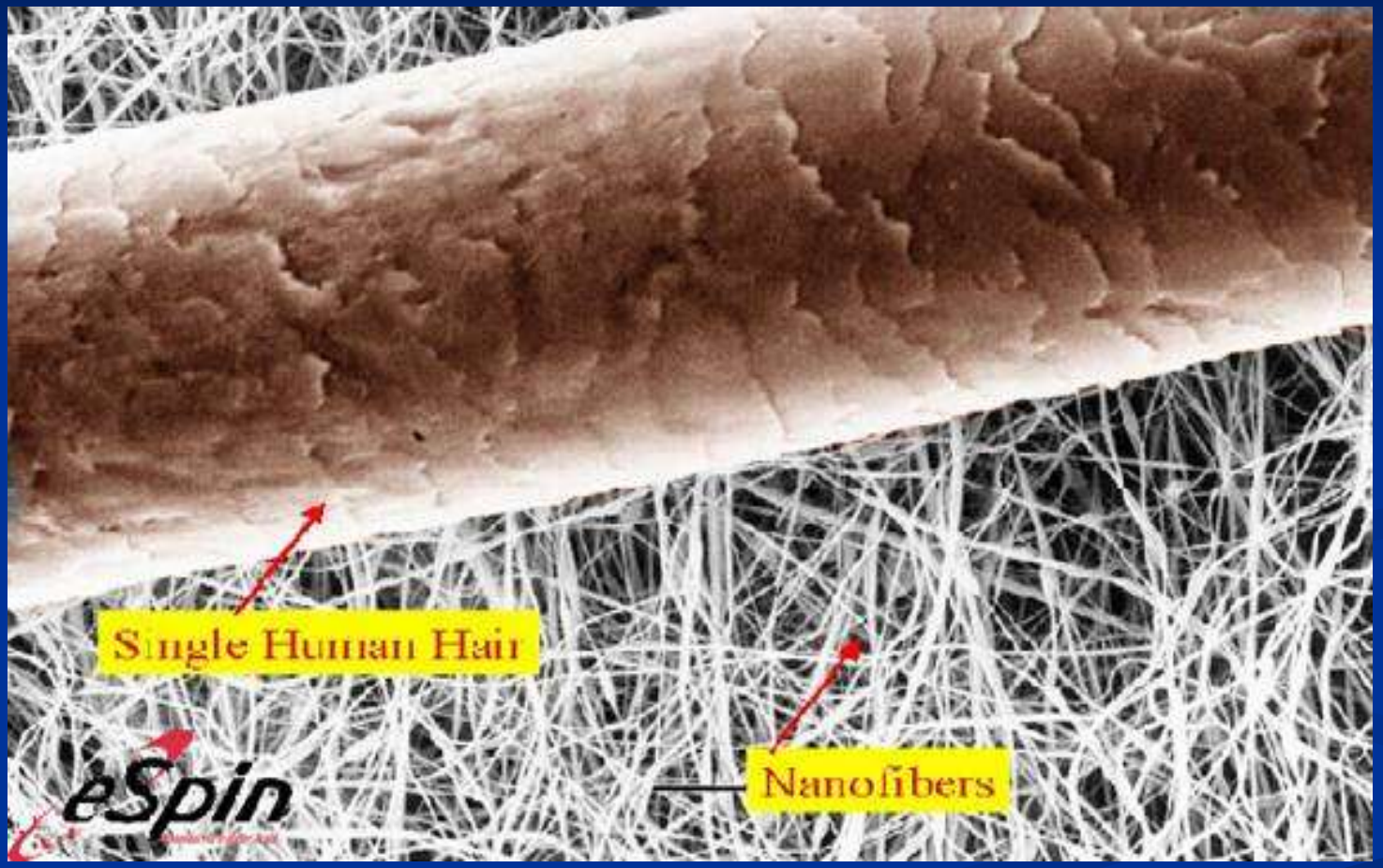
Hans Bluyssen, 04-11-2019

Nanotechnology

- Engineering and manufacturing at the scale of a nanometer or nanoscale (nanometer = 10^{-9} meter), a hundred-thousandth the width of a human hair.
- Examples of nano-substance are- Atom diameter 0.15 nm, diameter of double strand DNA 2 nm, and cell 10.000 nm.



Compared to Human Hair



A Human Hair is about 100,000nm wide

What is Nanoscale

12,756 Km

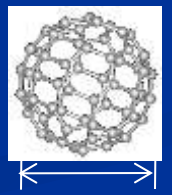
1.27×10^{7} m

22 cm

0.22 m

Fullerenes

C_{60}



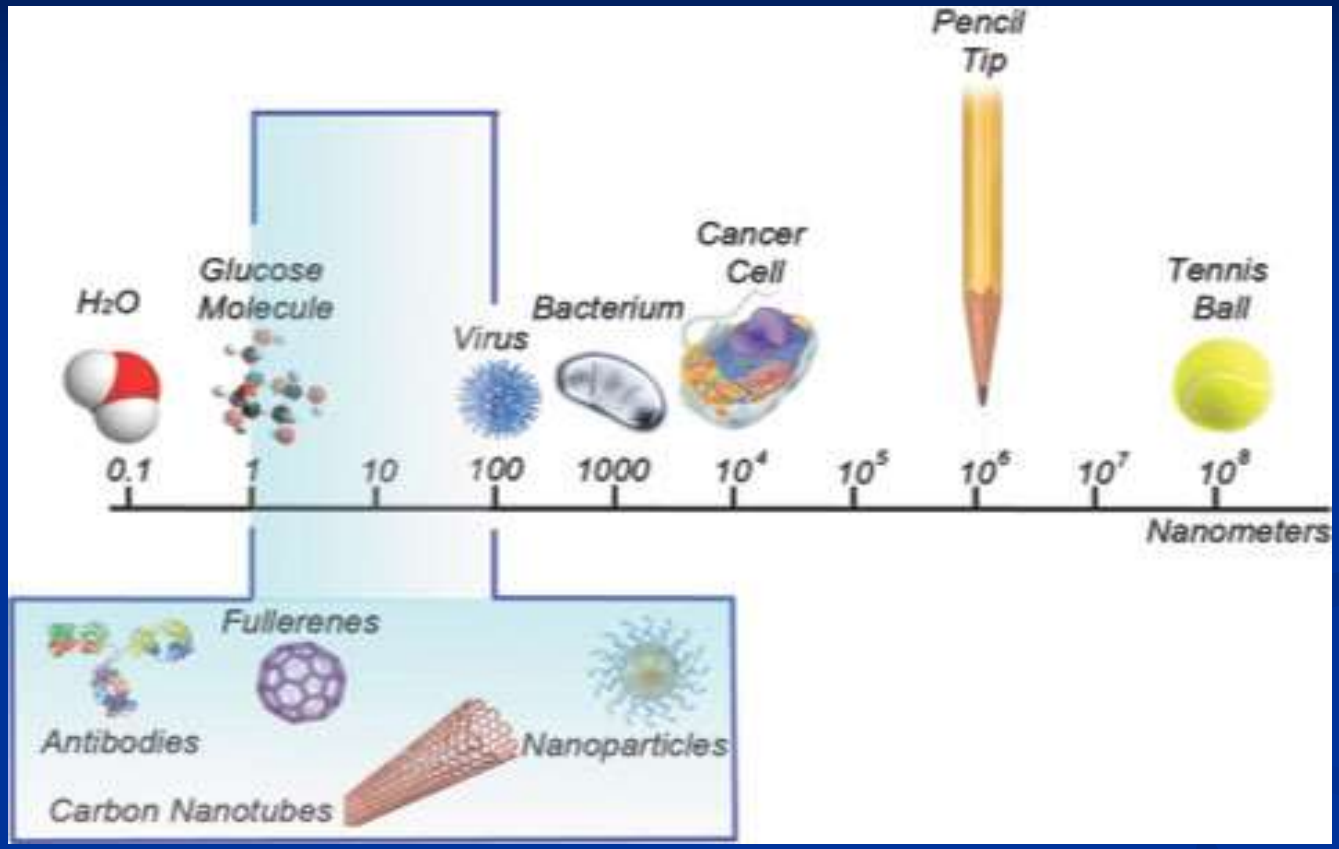
0.7 nm

0.7×10^{-9} m

10 millions times smaller

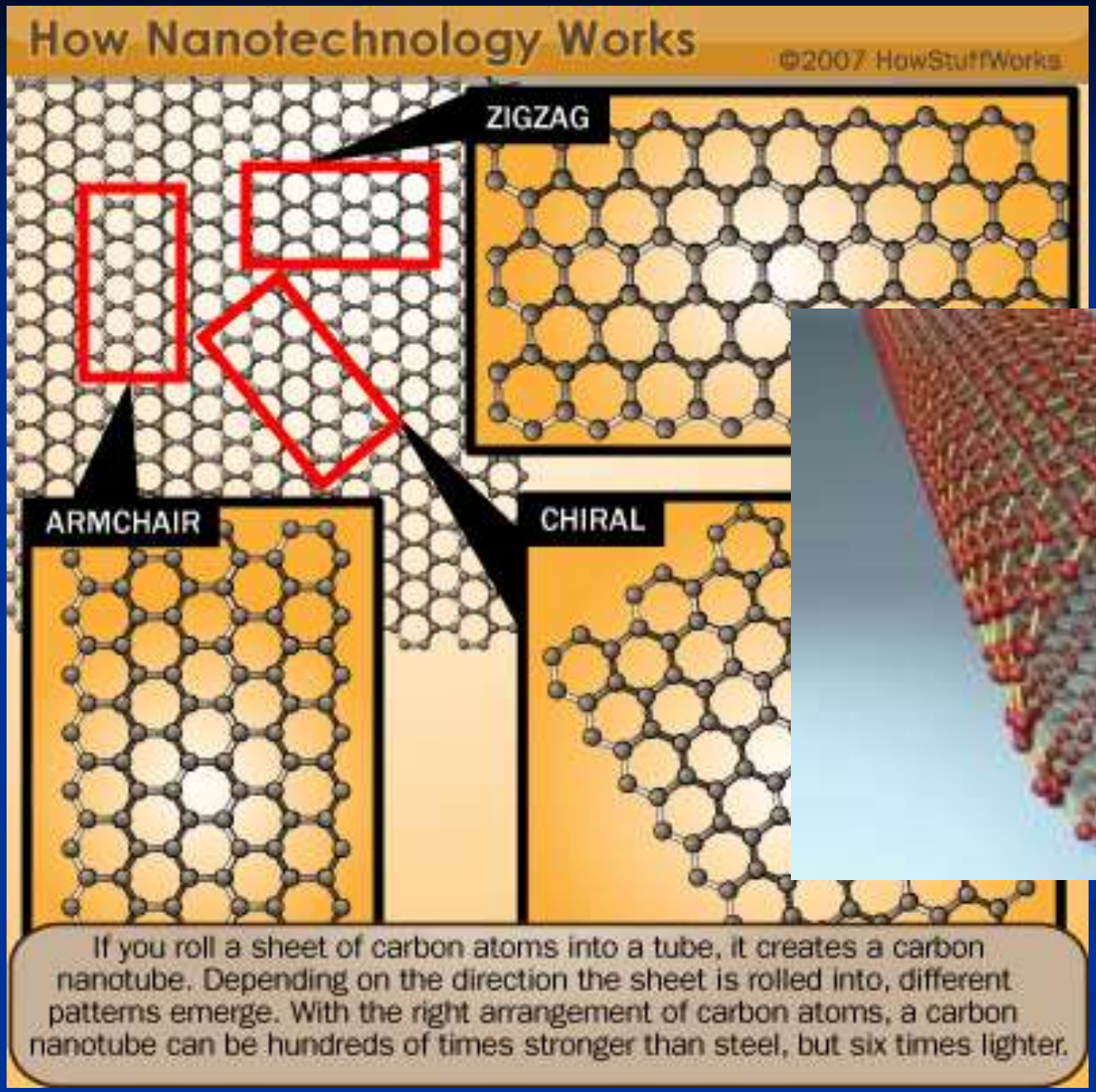
1 billion times smaller

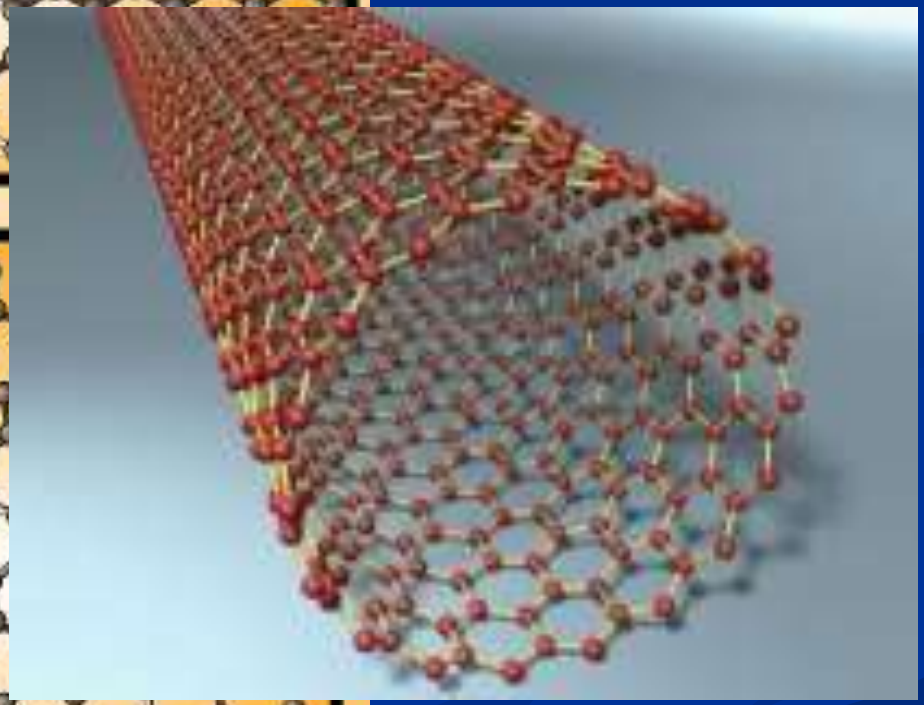
Nanotechnology



Creation of functional devices in the nanometre range and the exploitation of the unique properties of these devices in various fields

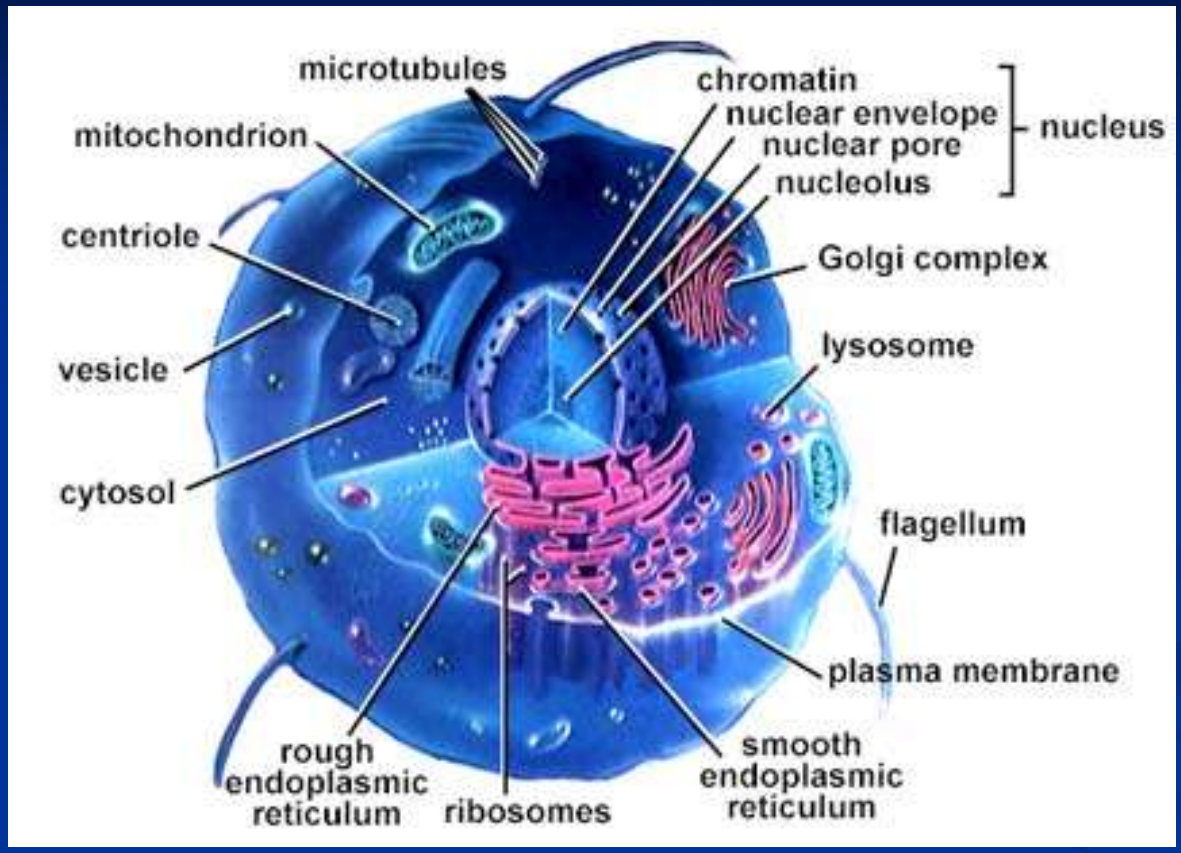
Carbon Nanotube





Fullerenes

Where all the action is: the cell (10-30 um) !

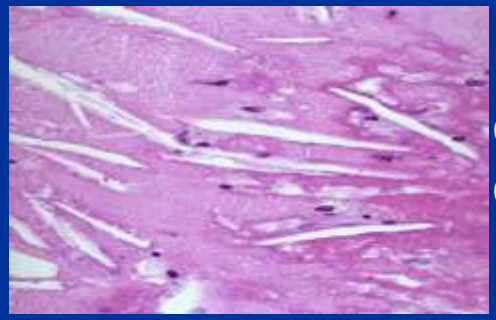


Cells themselves (organelles) are very complex and efficient nano-machines.

Most areas of nanoscience aim to learn from biological nanosystems.

Watering flowers or flooding the neighborhood?
treating atherosclerosis with lipid lowering drugs

arteriosclerosis:
- *begins at the cell*
- > *focal lesions* in the arteries
- leads to myocardial infarction and stroke

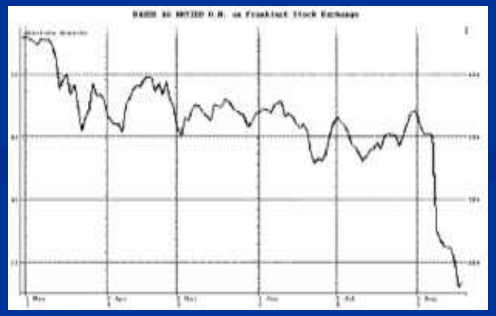


arterial plaque

some lipid lowering drugs:

effects on plaques
can save lives

effects on liver



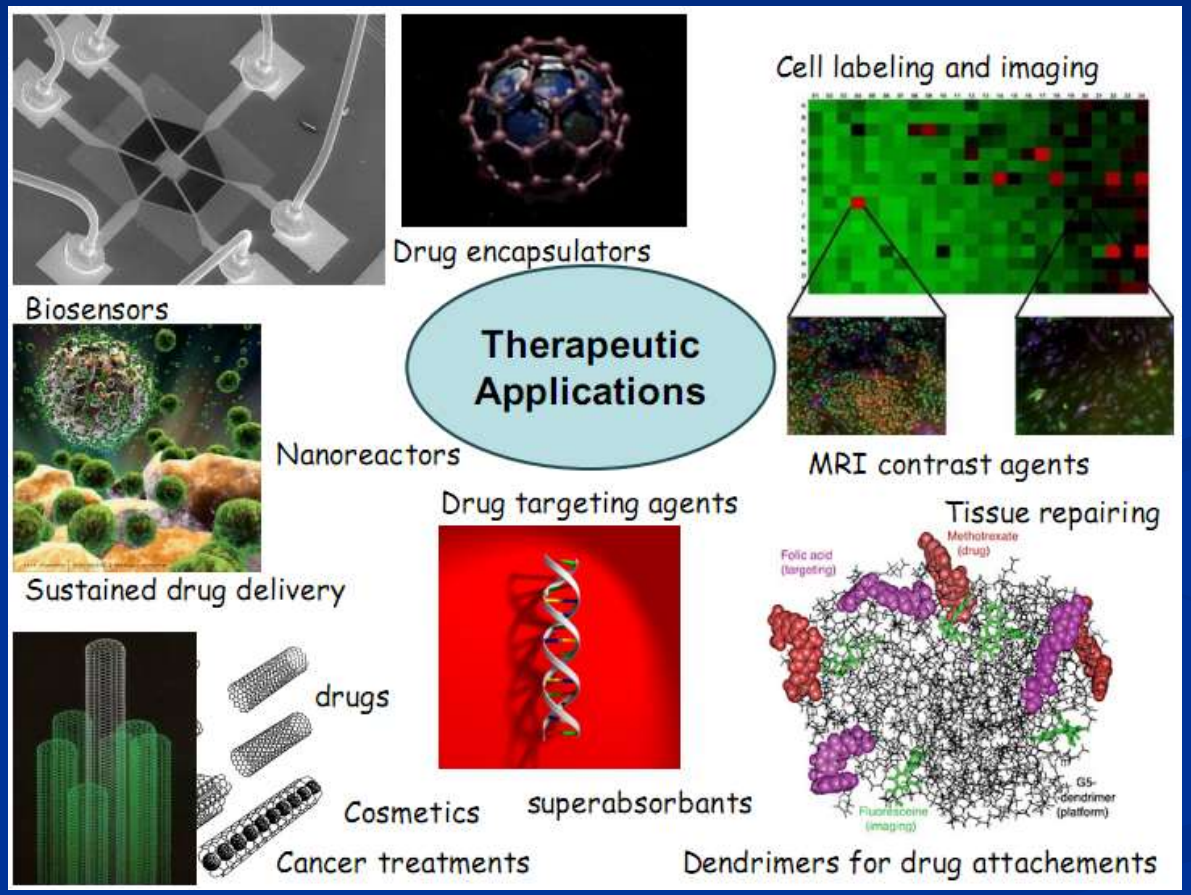
Can endanger large companies

effects on immune system

muscles:
can lead to cell death
can endanger human life

!!!!!Solution: Nanomedicine

Nanomedicine



The application of nanotechnology to

disease treatment,

diagnosis,

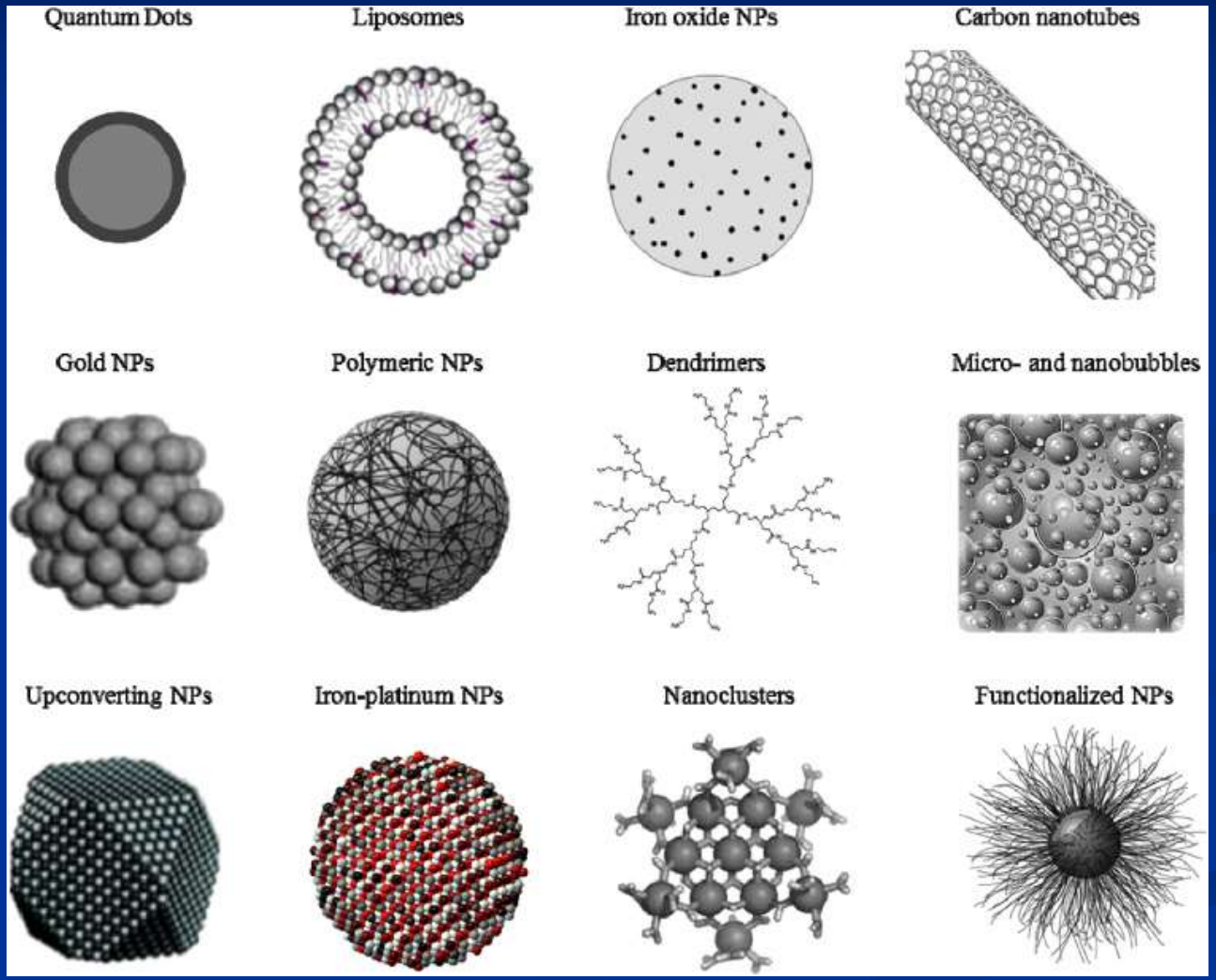
monitoring,

control of biological systems

NANOTECHNOLOGY TOOL BOX

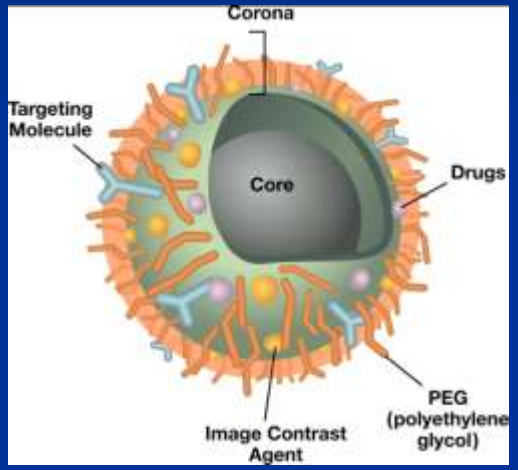
- NANOPORES
- NANOPARTICLES
- NANOFIBERS
- NANOELECTRONICS
- NANOCANTILEVERS
- NANORIBBONS
- NANOCOMPOSITES
- NANOTUBES
- NANOCRYSTALS
- NANOARRAYS
- NANOPROBES
- NANOSHELLS
- NANOCOATINGS
- BUCKYBALLS

Nanoparticles

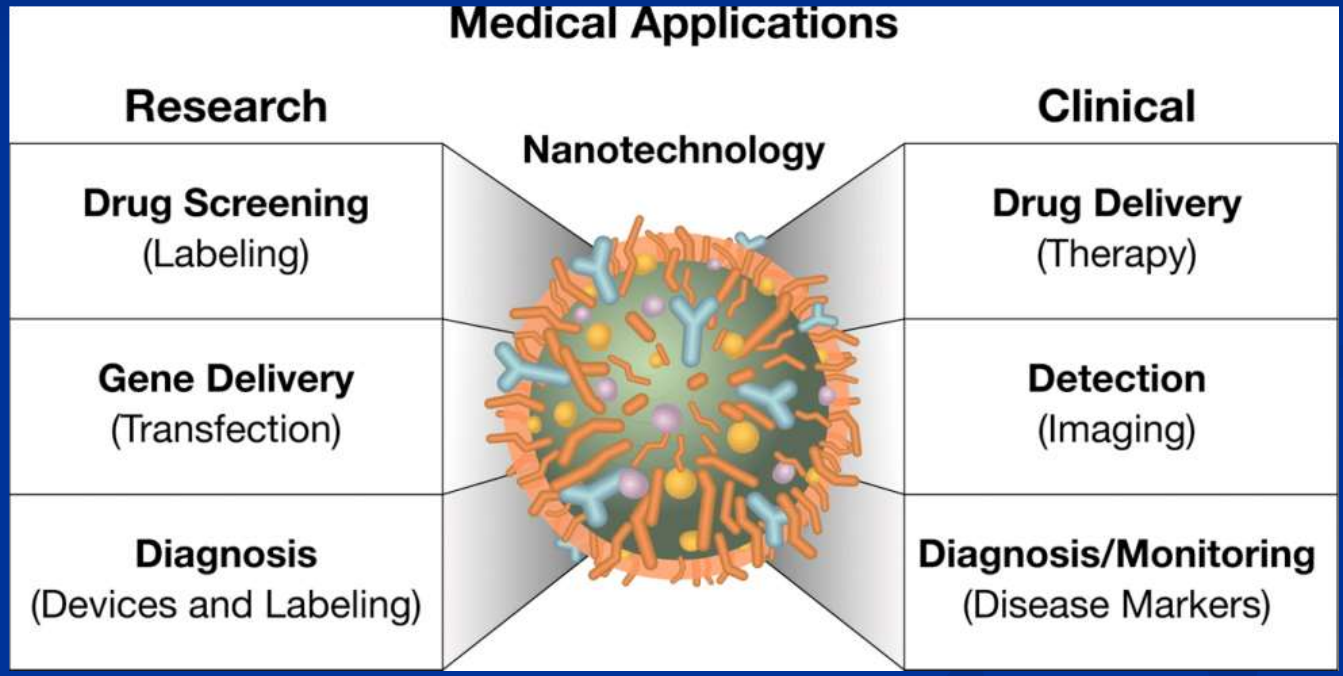


•[8]Re F, Moresco R, Masserini M. Nanoparticles for neuroimaging. Journal of Physics D: Applied Physics. 2012;45(7):073001.

The goal of nanomedicine is to develop safer and more effective therapeutic and diagnostic modalities



Functionalized nanoparticles

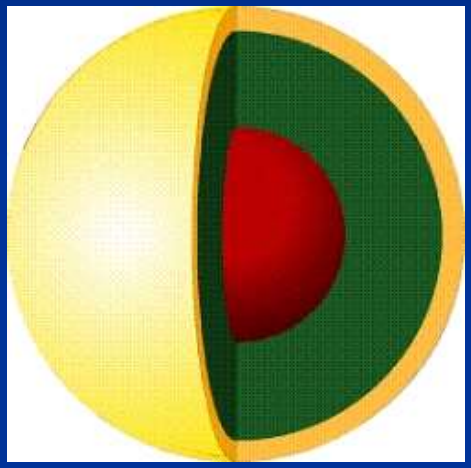


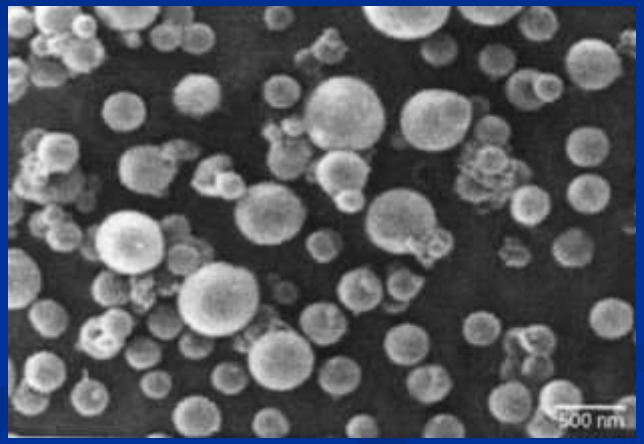
•McNeil SE. *J Leukoc Biol*, 2005. **78**(3): p. 585-94.
•doi:10.1189/jlb.0205074

Nanomedicine

A vehicle for delivery of therapeutics into the body

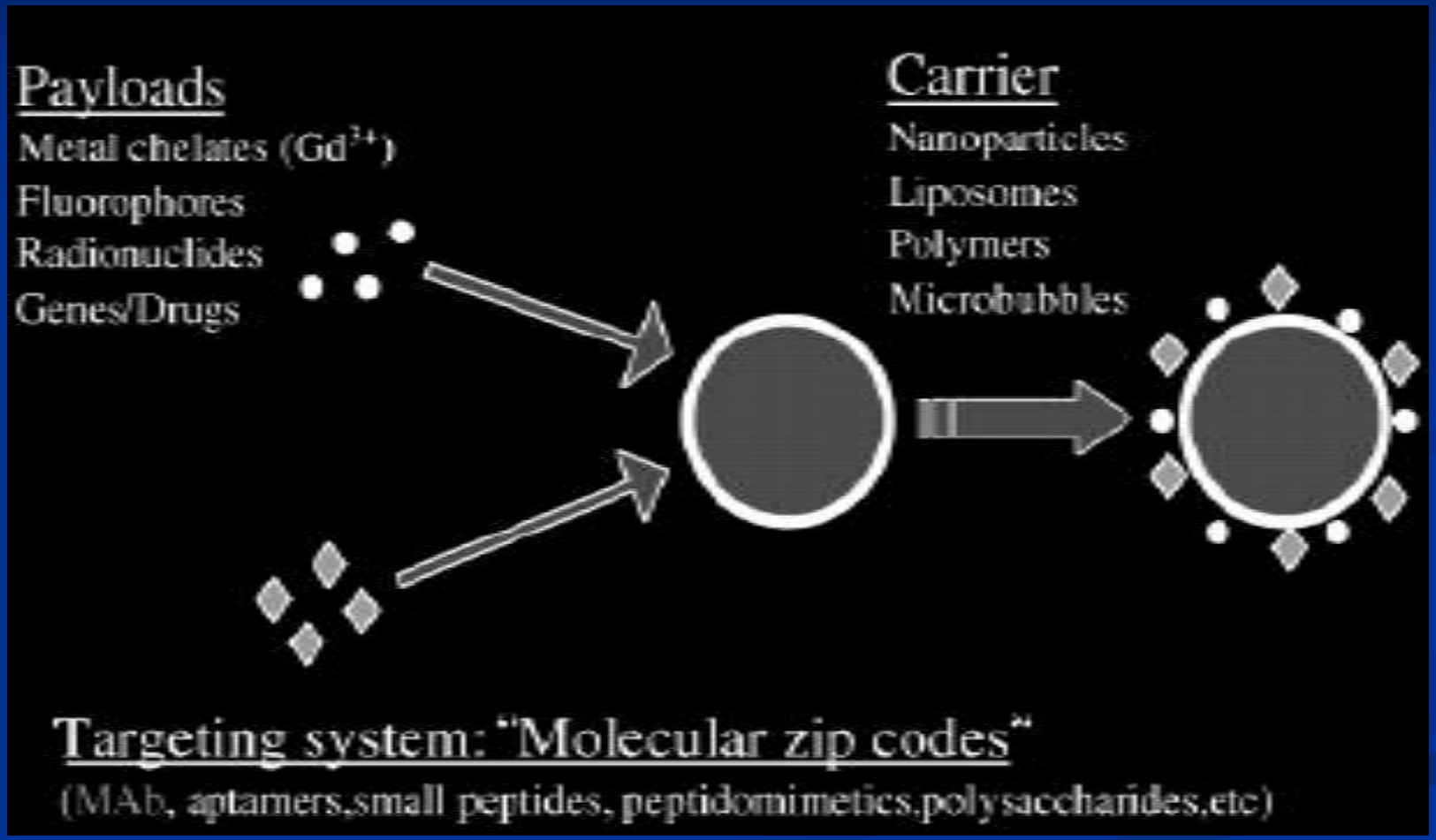
Functionalized nanoparticles





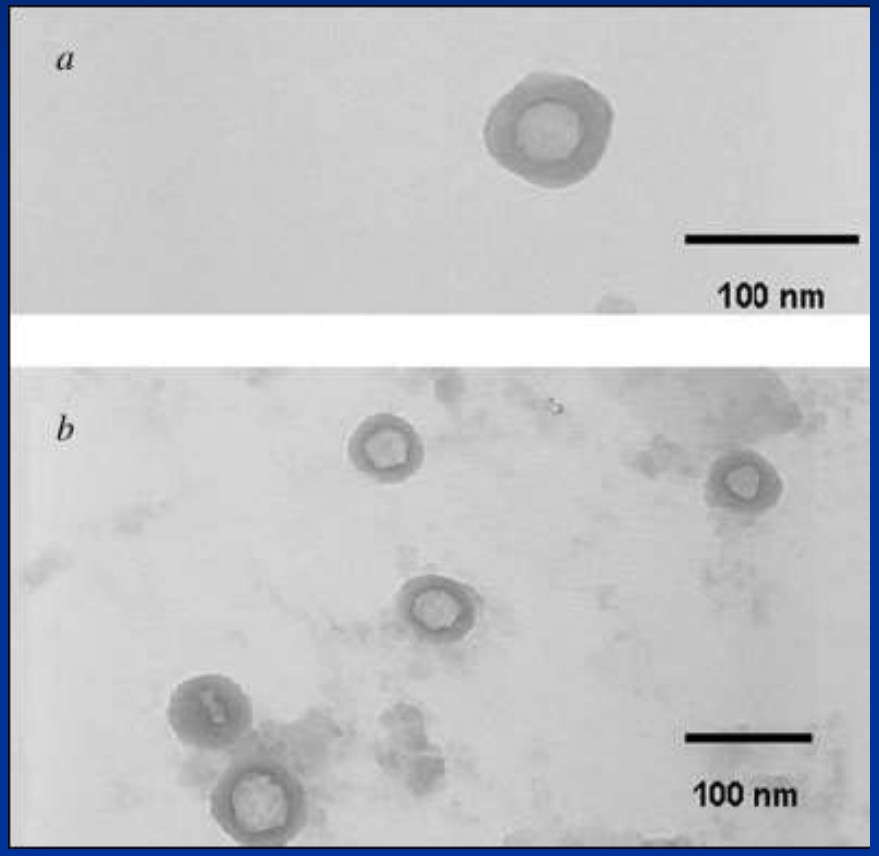
- Small molecule drug compounds, DNA/genes, proteins, vaccines, etc.
- Administration routes to reach systemic circulation or infected organs and cells: oral, intravenous, inhalation, ocular, topical

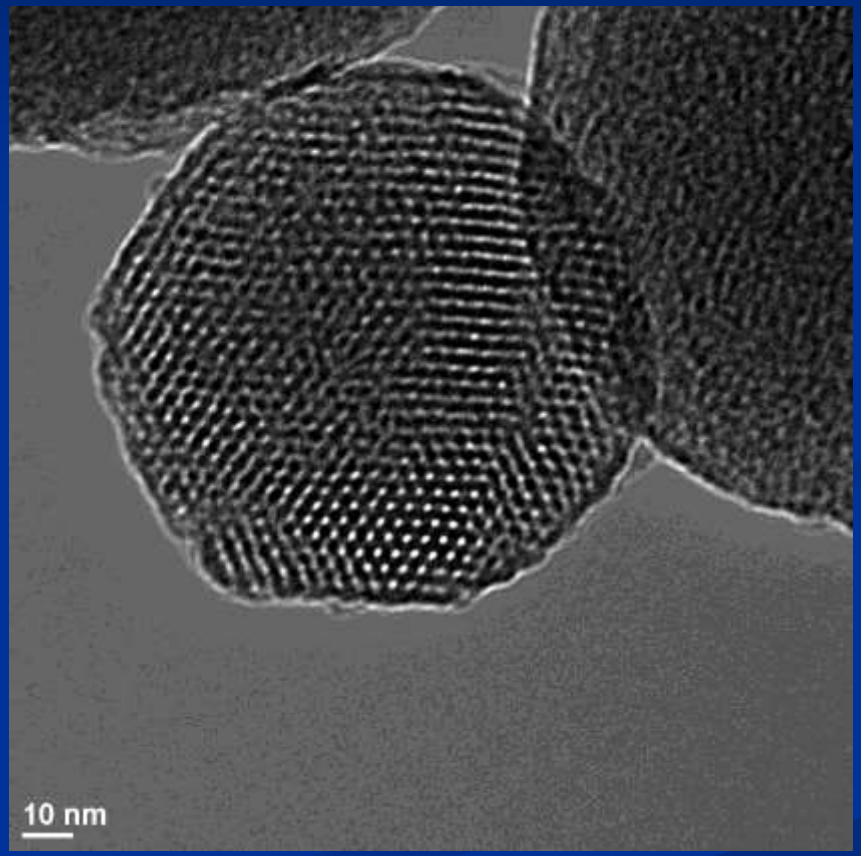
Nanoparticle as delivery system for drugs or genes for tissue and cell



Functionalized nanoparticles

Silica nanocapsules

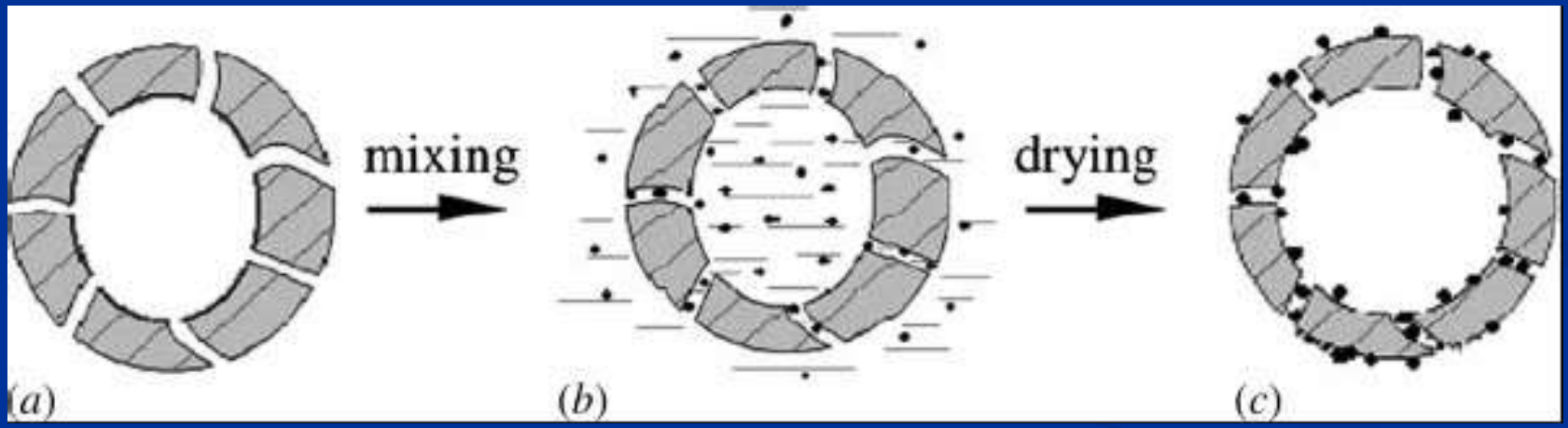




Porous hollow silica nanocapsules

Nanoparticles/nanocapsules as drug delivery carriers to cancer

Functionalized nanoparticles



Porous hollow silica nanocapsules for

Cefradine delivery (antibacterial chemical)

Nanoparticles and Drug delivery

Drug targeting by nanoparticles or nanocapsules offers the following enormous advantages:

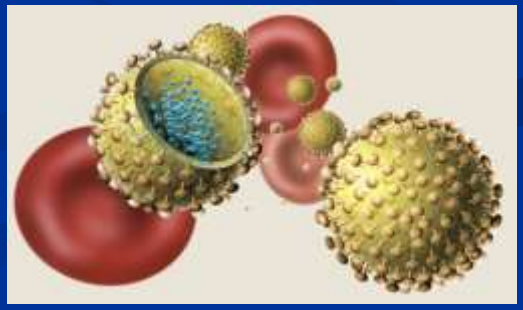
-Ingested vs injected

-reduces dosage, ensures the pharmaceutical effects, and minimizes side-effects;

-protects drugs against degradation and enhances drug stability.

Nanoparticles can penetrate through small capillaries and are taken up by cells, which allows efficient drug accumulation at target sites.

A sustained and controlled release of drugs At target sites over a period of days or even weeks is possible.

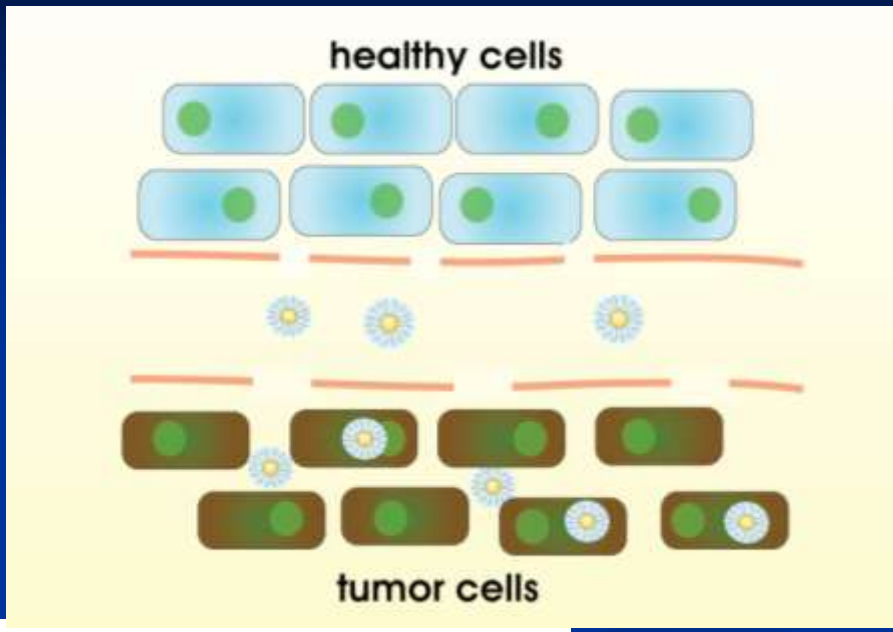


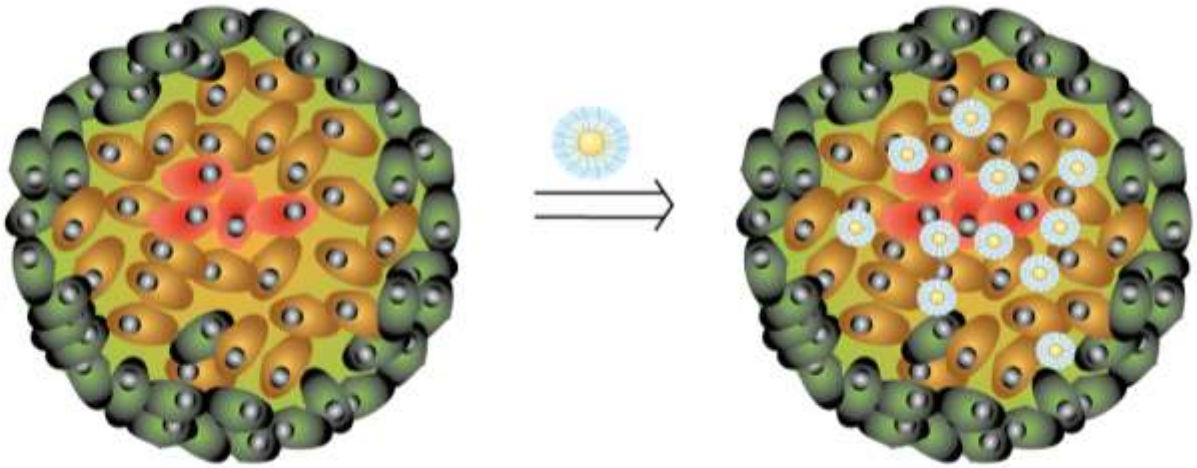
Nanoparticles and Drug delivery

- Nanoparticles with diameter less than 200nm are not screened out of circulation by liver and spleen.
- Nanotech based drug delivery is less toxic as well as inexpensive.

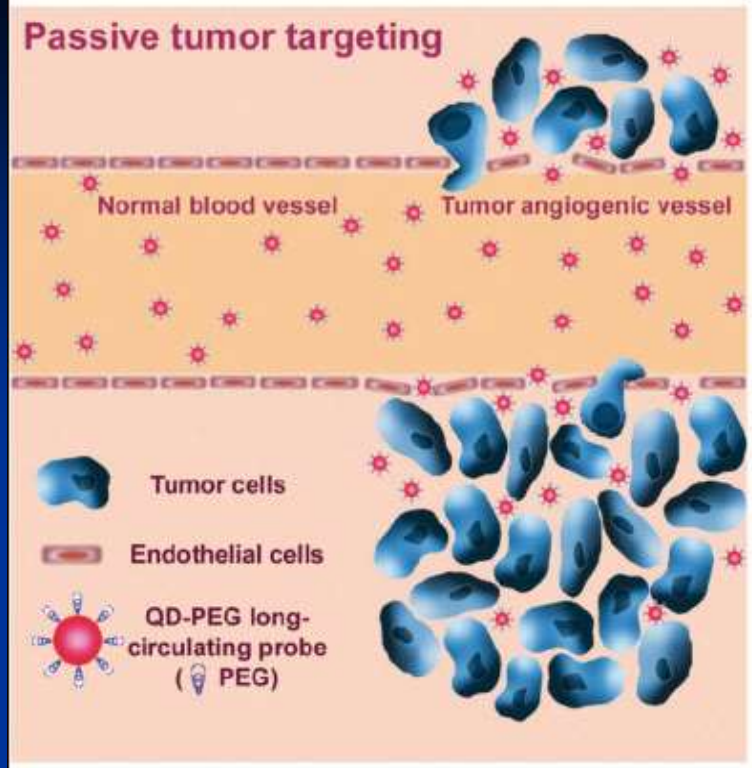
Nanoparticle use in Cancer Treatments

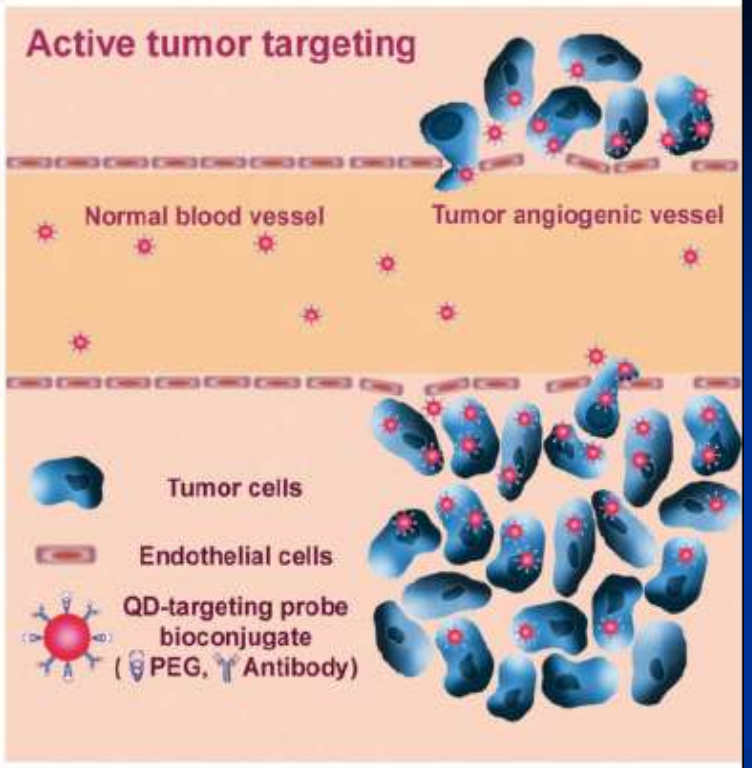
- Because of their small size, nanoparticles can pass through interstitial spaces between necrotic and quiescent cells.
- Tumor cells typically have larger interstitial spaces than healthy cells
- Particles collect in center bringing therapeutics to kill the tumor from inside out.



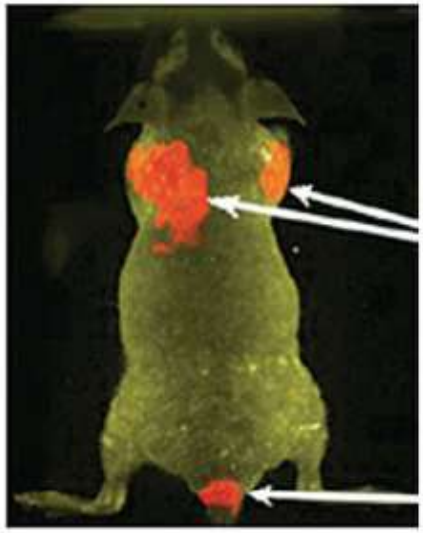


(a)


Passive tumor targeting
Normal blood vessel
Tumor angiogenic vessel
Tumor cells
Endothelial cells
QD-PEG long-circulating probe
(PEG)


Active tumor targeting
Normal blood vessel
Tumor angiogenic vessel
Tumor cells
Endothelial cells
QD-targeting probe bioconjugate
(PEG, Antibody)

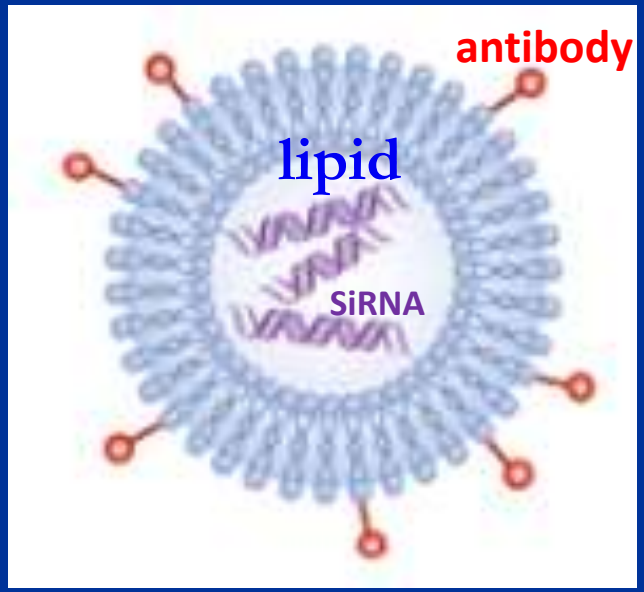
(b)


Tumors
Injection site

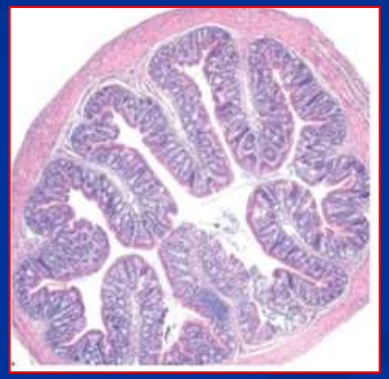
A Drug Delivery Nanoparticle

A. Nanoparticles for drug delivery can be metal-, polymer-, or lipid-based. Below (left) an example of the latter, containing SiRNA encapsulated, and functionalized with a specific antibody. SiRNA can control often lethal inflammatory body responses, as shown in the microscopic images below (right)

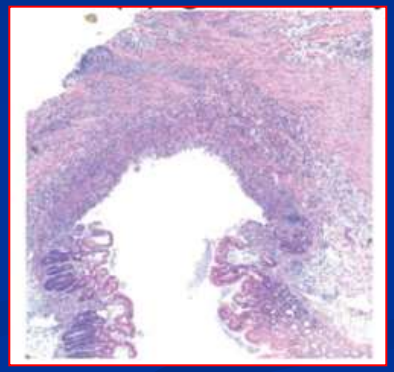
B.



C.



Healthy tissue



Sick tissue treated with non-targeted nanoparticles

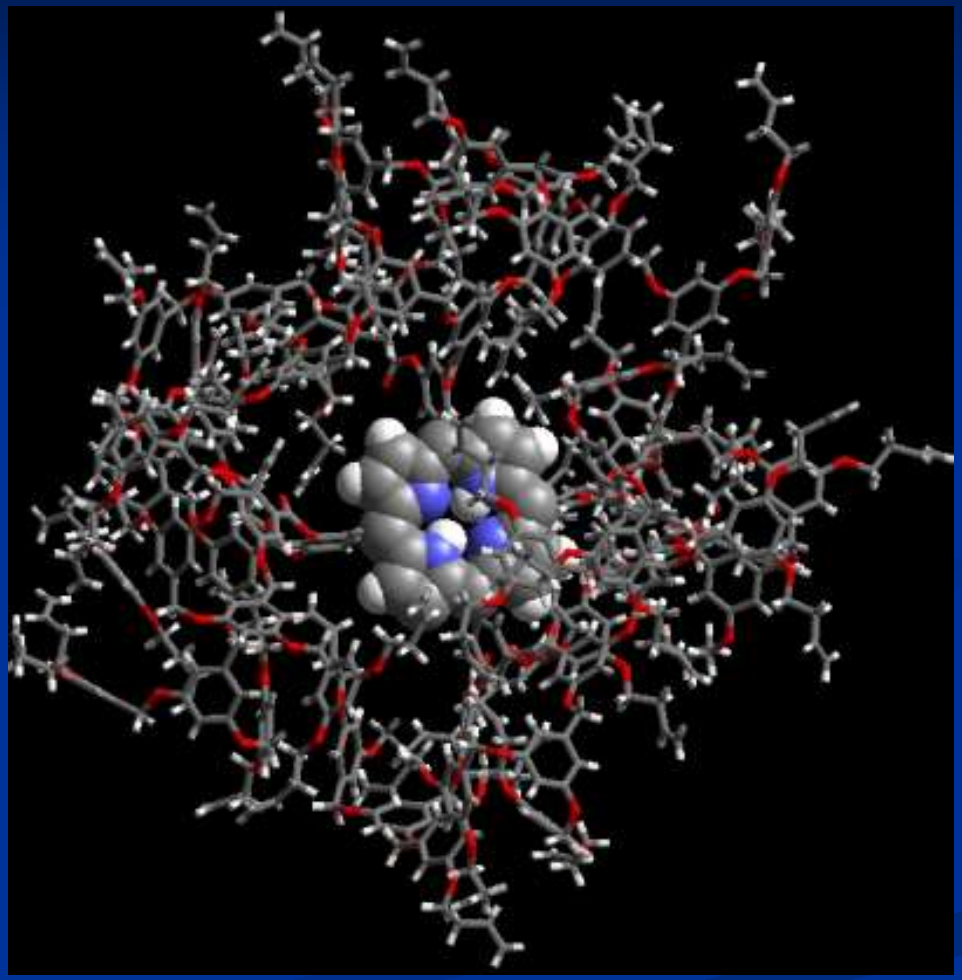
Sick tissue treated with targeted nanoparticles

Science 2008, Vol. 316, pp 627-630

Dendrimers

Dendritic polymers = Dendrimers

Polyamidoamine (PAMAM)
phosphorous-based, **Polylysine**



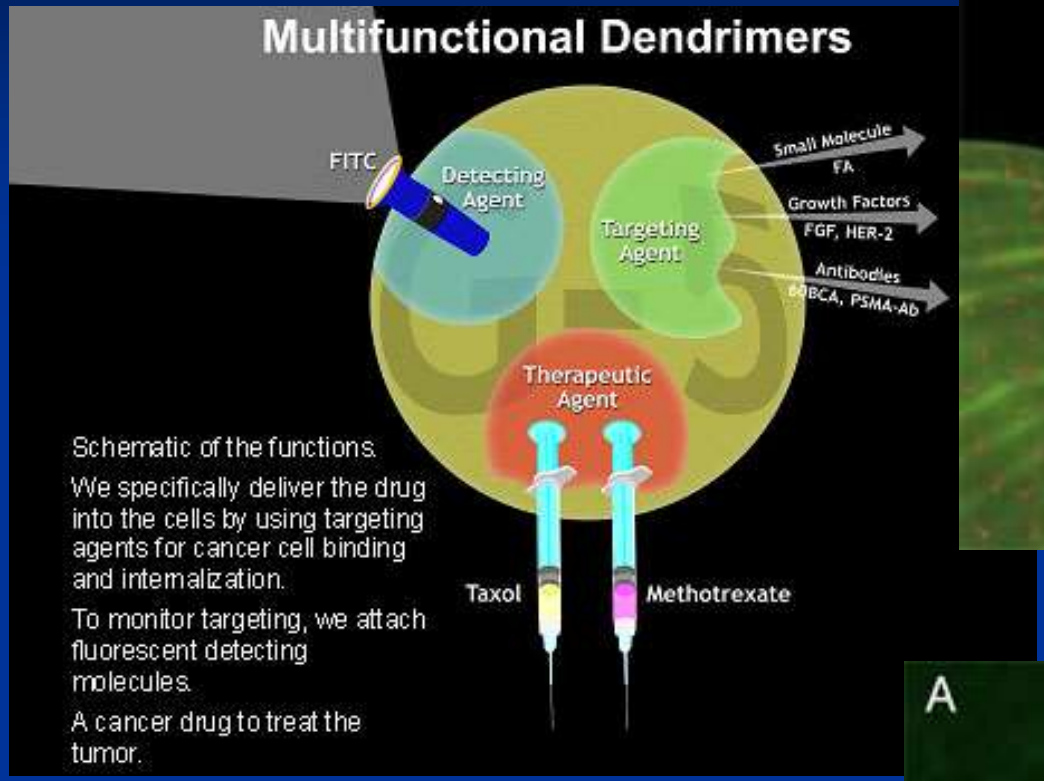
Highly branched structures –

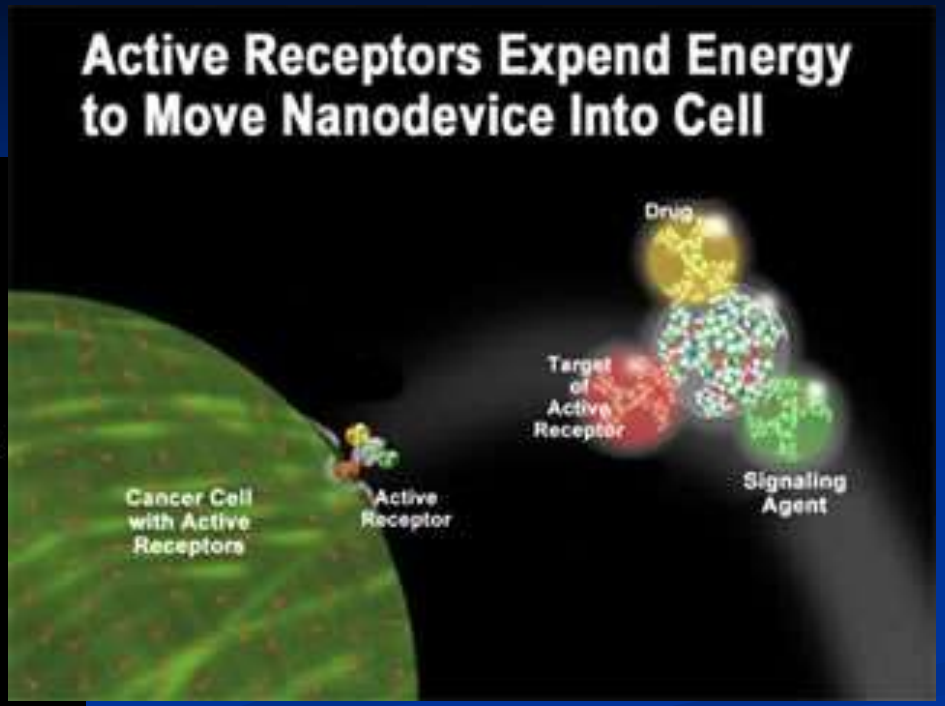
Molecular "hooks" – to attach

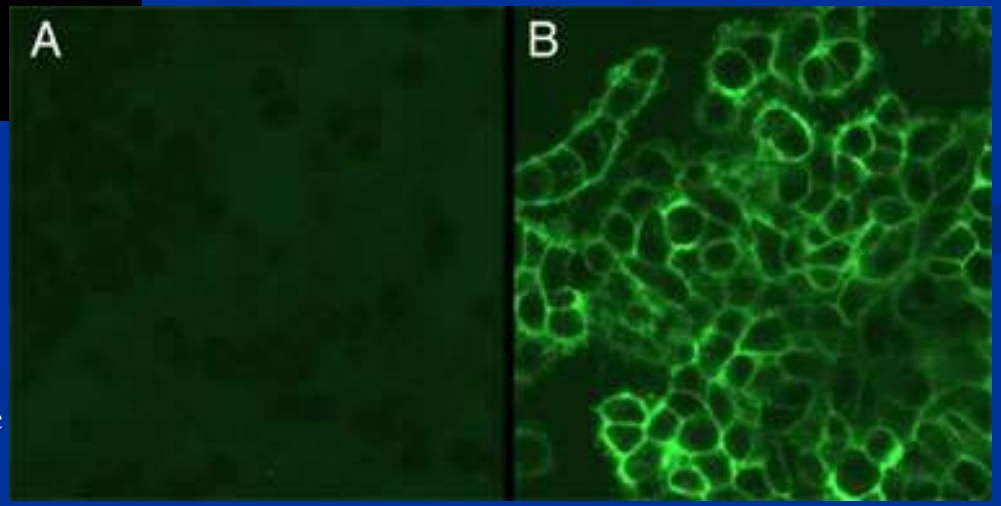
Cell identification tags,
fluorescent dyes, enzymes

ideal building blocks in nanochemistry for the creation of more complex three-dimensional structures.

Dendrimers & Therapeutics

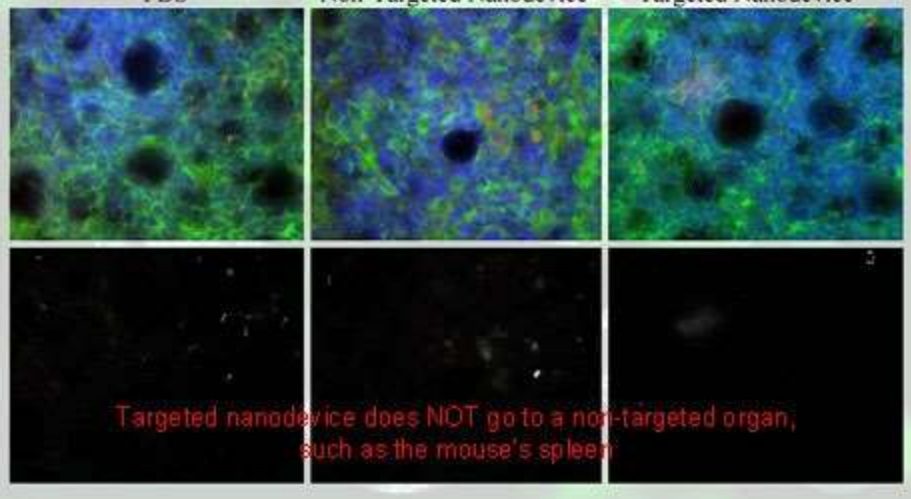




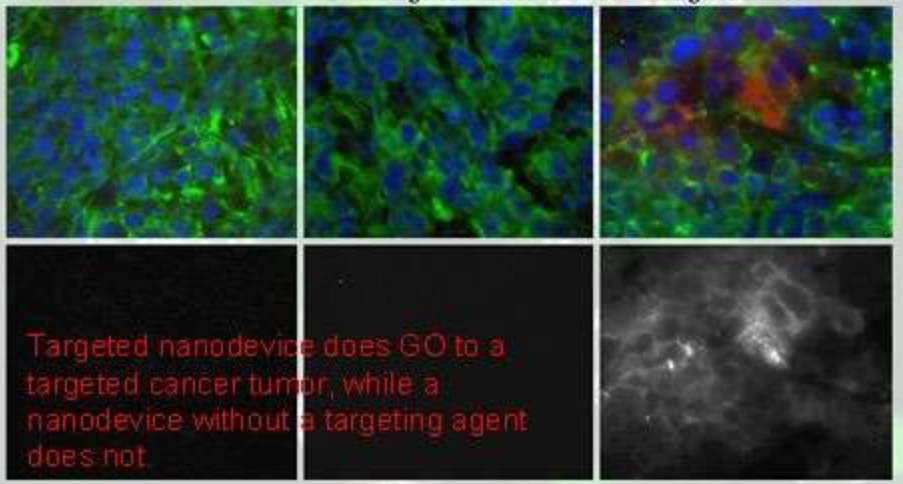


The Michigan Nanotechnology Institute for Medicine and Biological Sciences

Spleen
(a non-targeted organ)



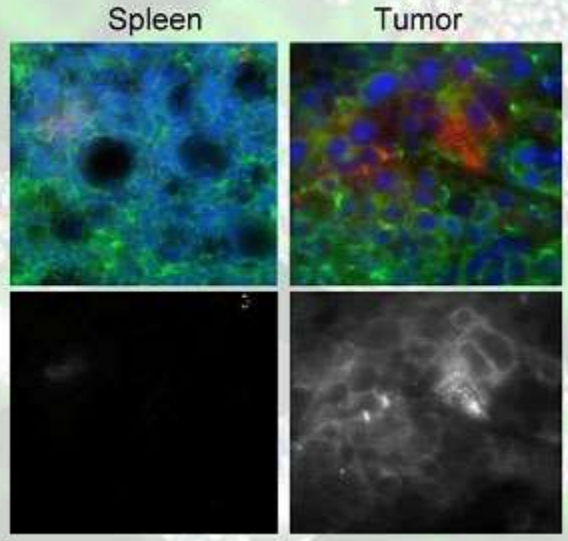
Cancer Tumor
(targeted)



In Vivo Study: drug study in animals

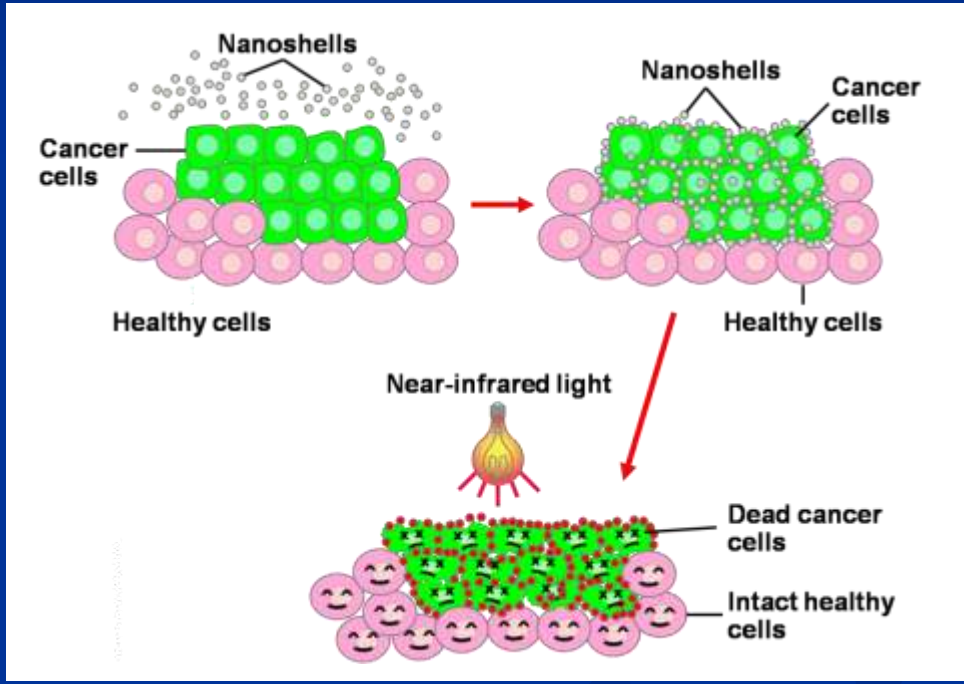
- **Mice that received conventional drug: Free MTX**
  - Lost hair (shutdown of protein synthesis)
  - Lost weight (general toxicity)
  - Non-necrotic tumors, no tumor reduction unless high dosis: **drug ineffective**
- **Mice that received drug in targeted Nanodevice**
  - Retained hair
  - No weight loss (non-toxic)
  - Necrotic tumors, reduction in size with low dose of drug: **drug effective**

Targeting Works



Nanotechnology in Health Care

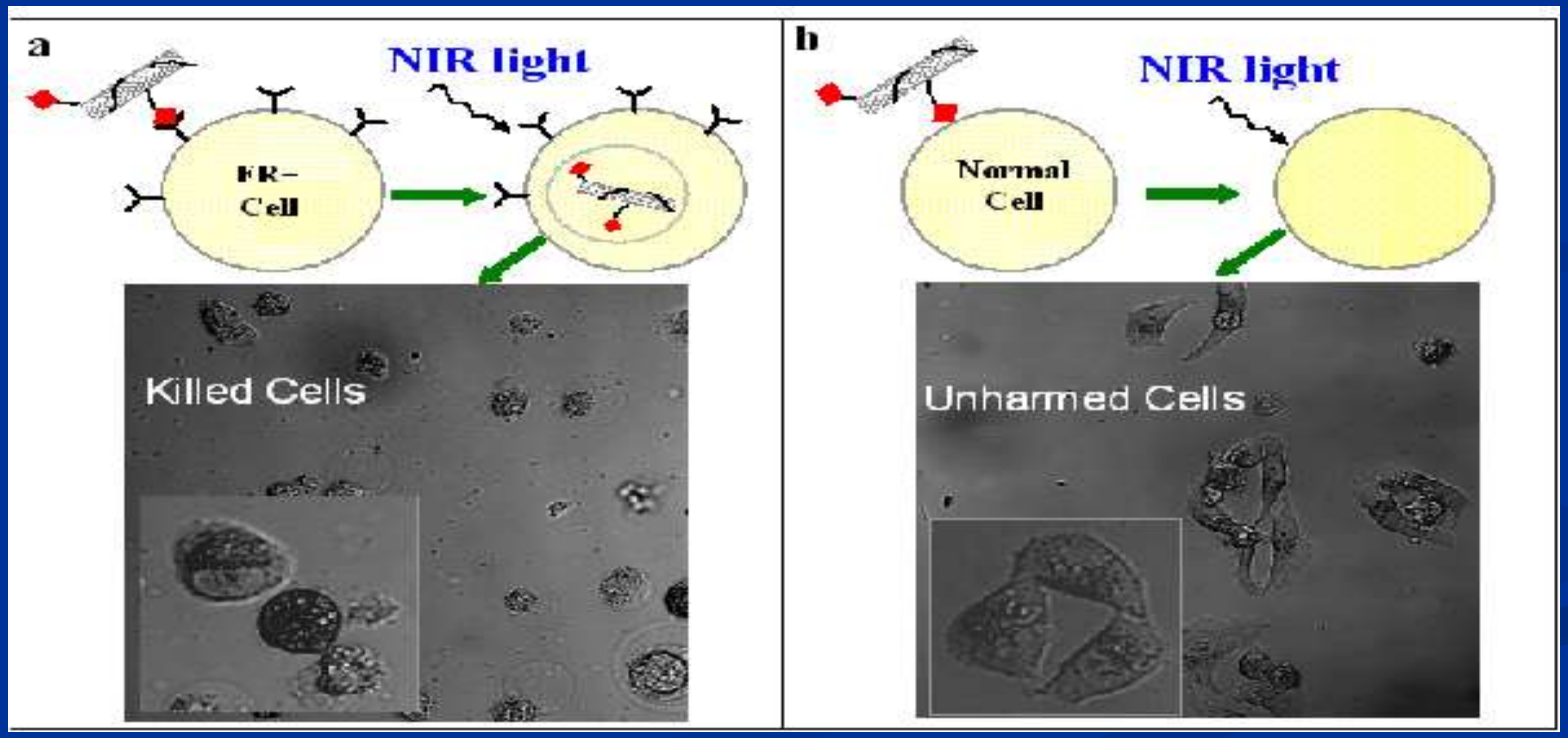
- Thermal ablation of cancer cells
  - Nanoshells have metallic outer layer and silica core
  - Selectively targeted to cancer cells
  - The nanoshells are heated with an external energy source killing the cancer cells



Thermal ablation of cancer cells assisted by nanoshells coated with metallic layer and an external energy source – *National Cancer Institute*

Photodynamic Therapy

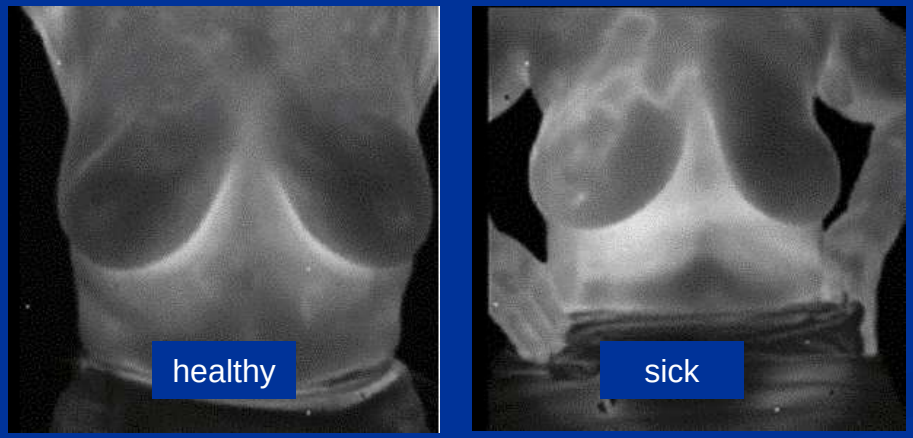
- Unlike chemotherapy it does not leave a "toxic trail"



Diagnosis using Nanothermometers

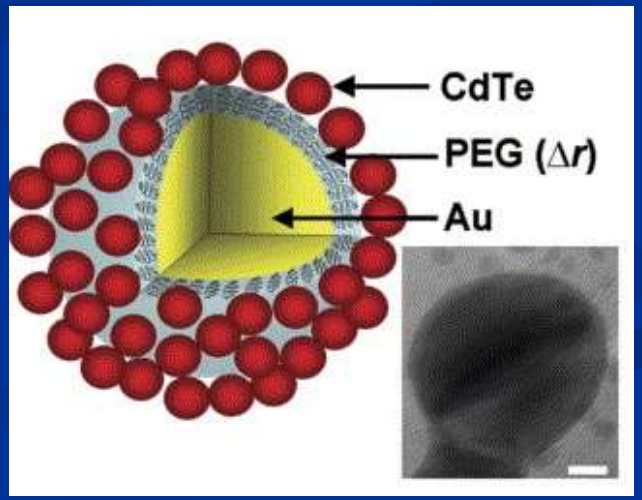
Cancer cells appears to have a more elevated temperature than normal cells. Therefore, a local temperature mapping can be used to determine the spread of a tumor

A gold nanoparticle is functionalized with a PEG coating, which itself is assembled to a layer of smaller QD's. The emission properties of the nanoparticle change with temperature due to the stretching/contraction of the PEG



Thermal image of a healthy and cancerous breast

•Source: 9th European Congress of Thermology, Krakow, Poland

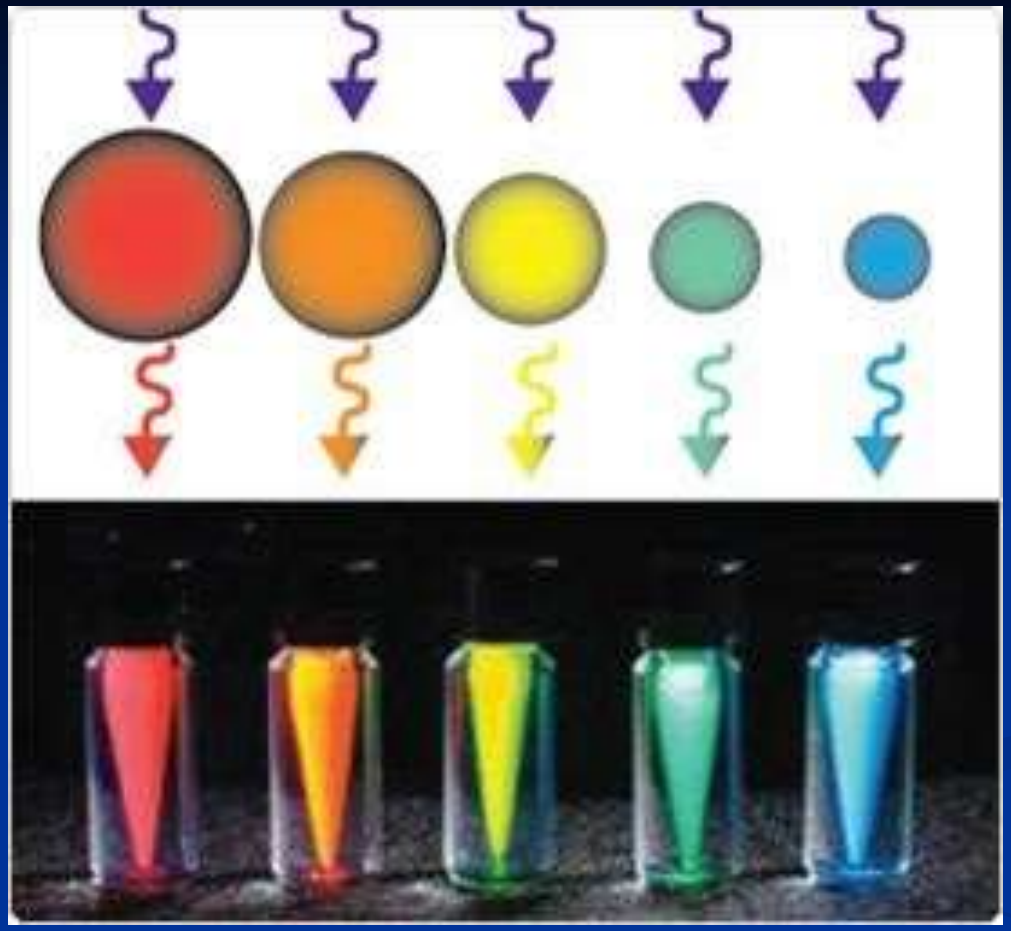


Angew. Chem. Int. Ed. 2005, Vol. 44, 7439 –7442

What are Quantum Dots?

- **Quantum dots** are tiny particles or **nanocrystals** of a semiconducting material with diameters in the range of 2-10 nanometers (10-50 atoms). The most apparent result of this is fluorescence, wherein the **nanocrystals** can produce distinctive colors determined by the size of the particles.

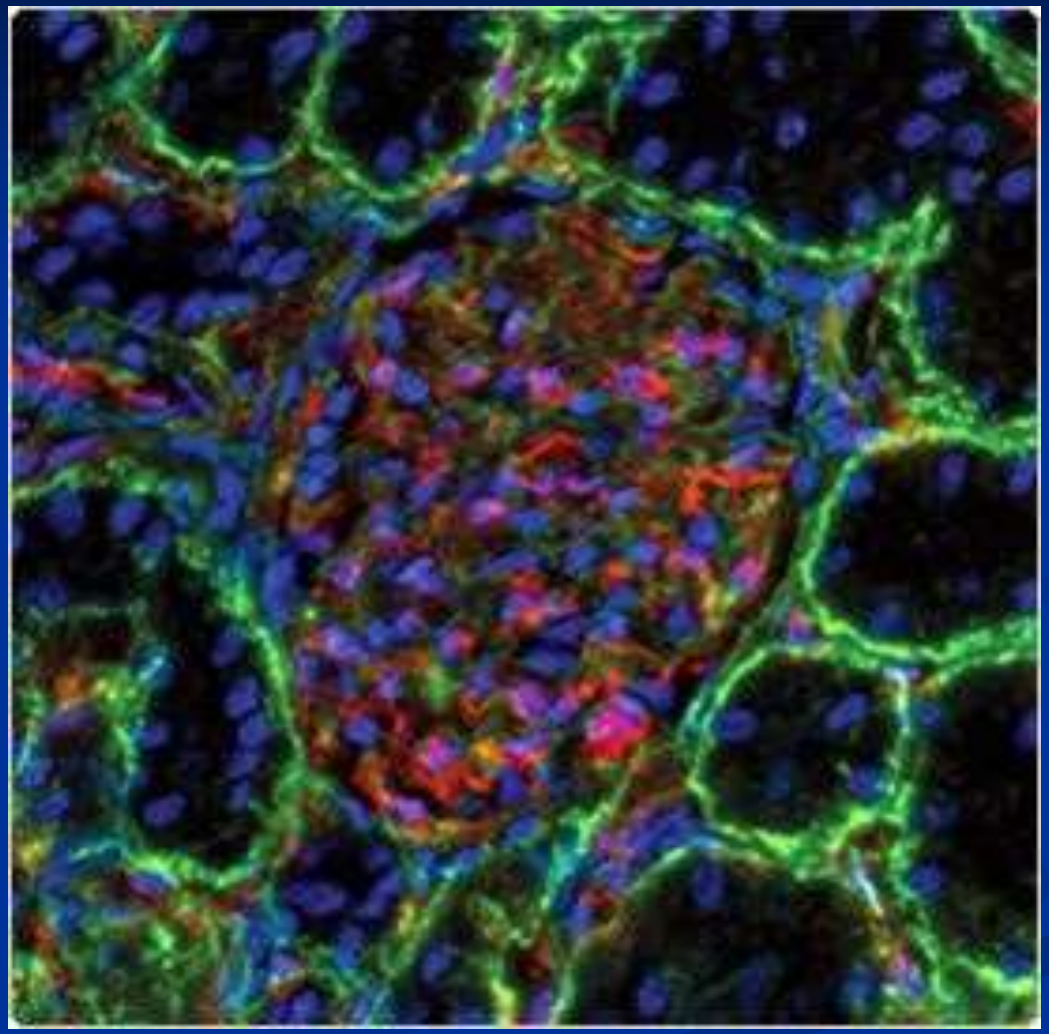
Enables long-term imaging experiments.



widely exploited in the development of multicolor assays

Tuneability of Qdot® nanocrystals. Five different nanocrystal solutions are shown excited with the same long-wavelength UV lamp; the size of the nanocrystal determines the color.

Laminin in a mouse kidney section was labeled with an anti-laminin primary antibody and visualized using green-fluorescent Qdot® 565 IgG.
PECAM (platelet/endothelial cell adhesion molecule; CD31) was labeled with an anti–PECAM-1 primary antibody and visualized using red-fluorescent Qdot® 655 IgG.
Nuclei were stained with blue-fluorescent Hoechst 33342.

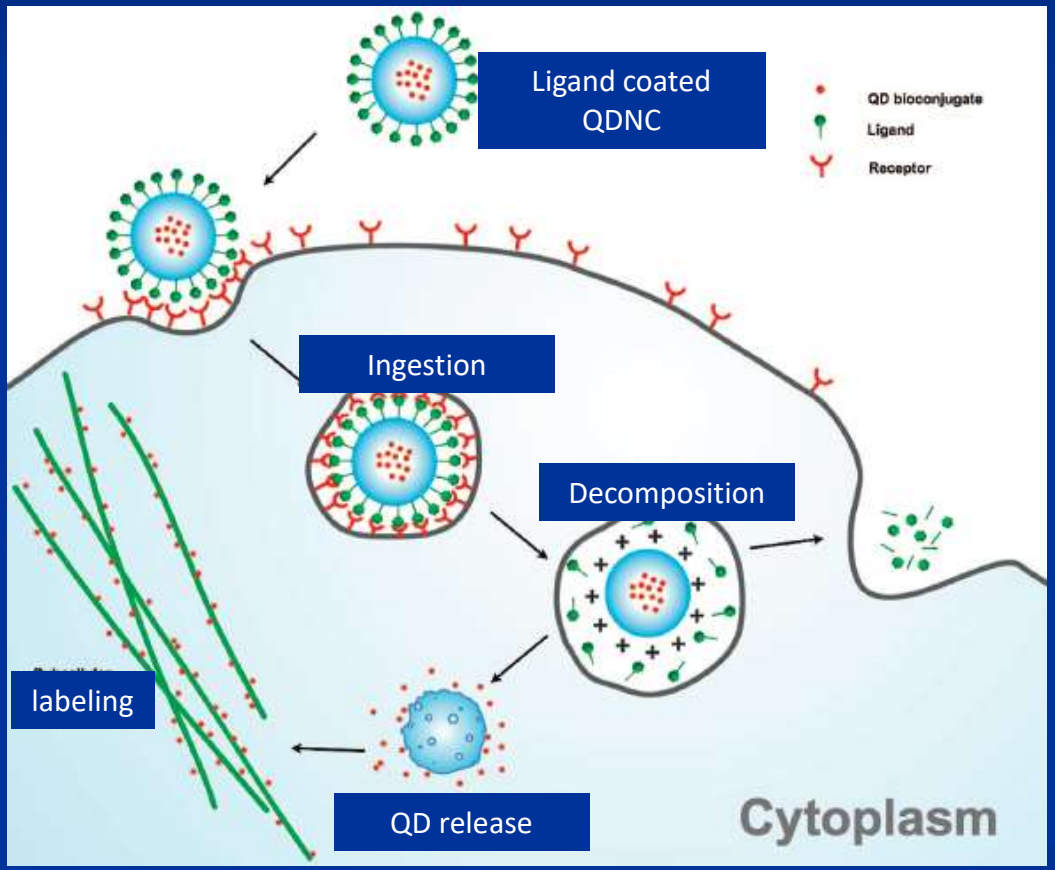


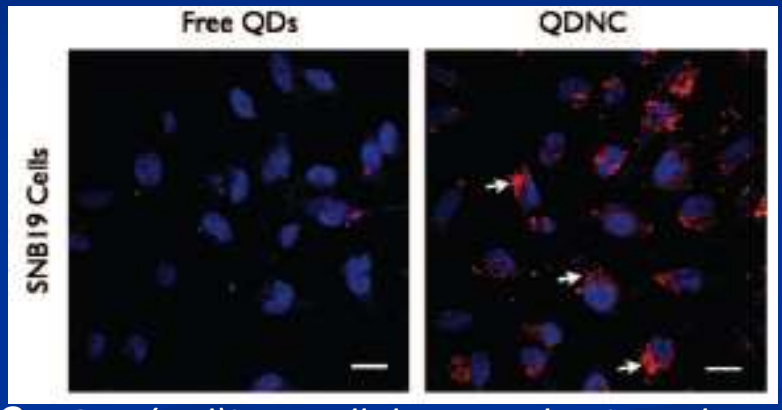
Quantum Dot Bioconjugate

Qdot® bioconjugate is a generic term to describe Qdot® nanocrystals coupled to proteins, oligonucleotides, small molecules, etc., which are used to direct binding of the quantum dots to targets of interest.

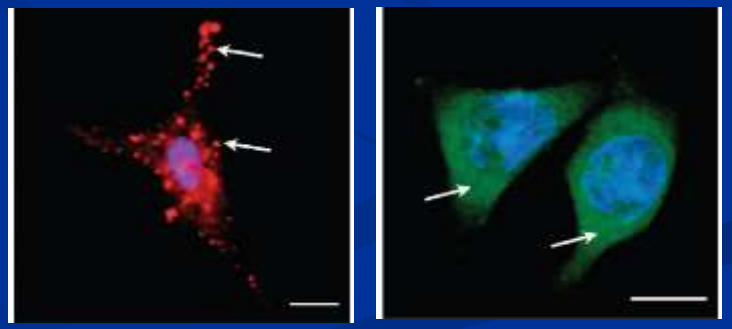
Targeting QD's for intracellular imaging

A. Using a drug-delivery-like mechanism, a targeted lipid-based nanoparticle (TNP) encapsulating QD's specifically 'attacks' a cell having the receptors that pair with its ligand coating. Upon ingestion and destruction of the TNP, the QD's are set free and accumulate on intracellular structures



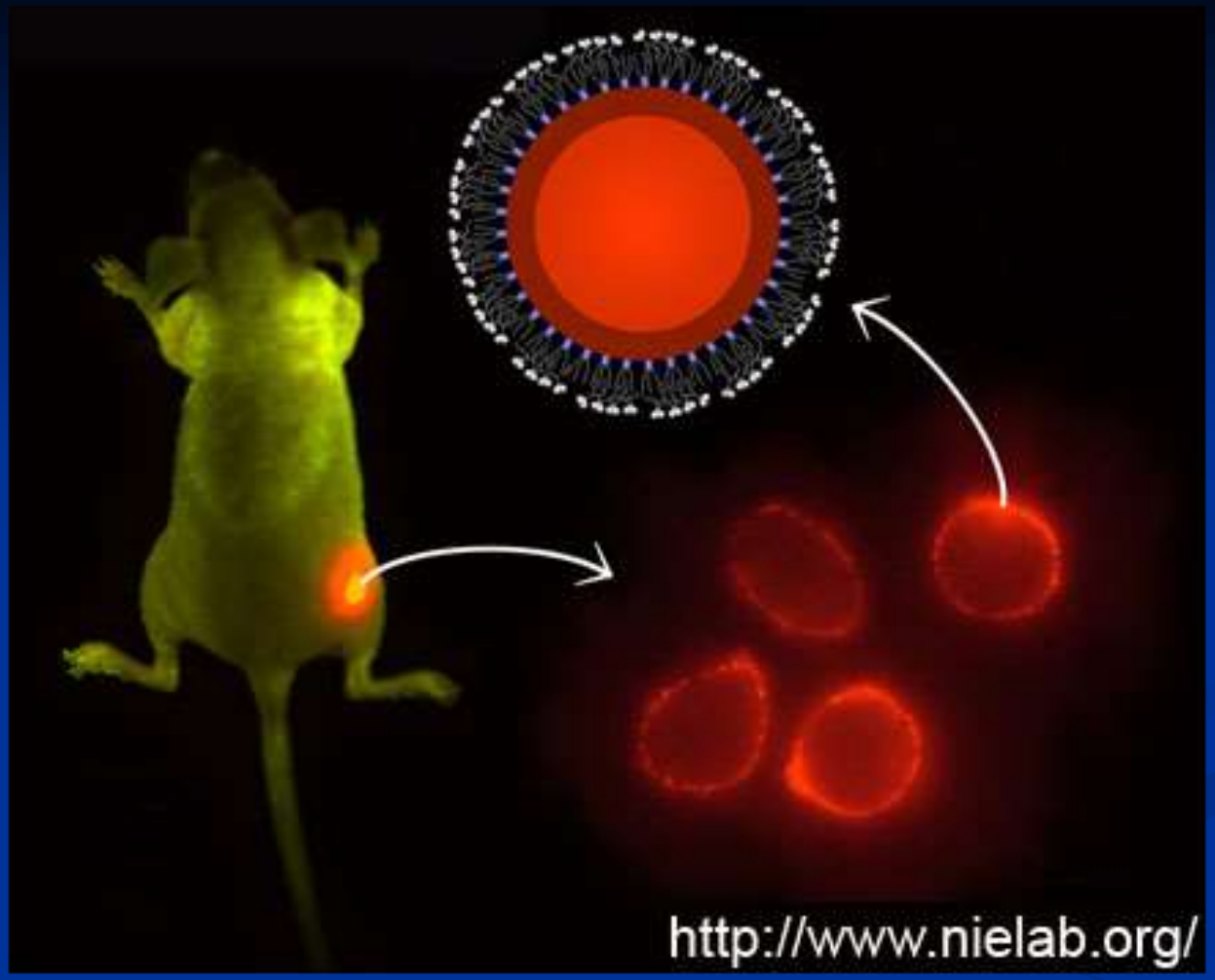


C. *QD (red)intracellular uptake is enhanced when using the QDNC instead of the free QD's*



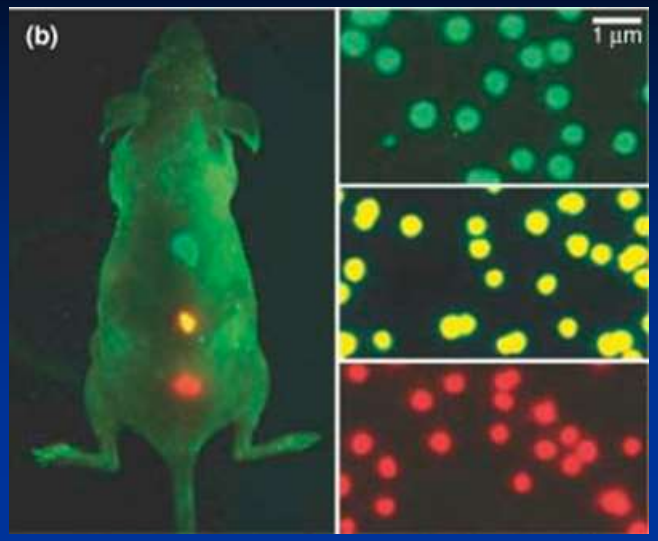
D. *Imaging of nucleus (blue) and cytoplasm (other) after 30 min (left) and 3 hours after uptake*

Nano Letters 2008., Vol. 8, pp3887-3892



Water soluble quantum dots to image sentinel lymph nodes which are used for diagnosing breast cancer.

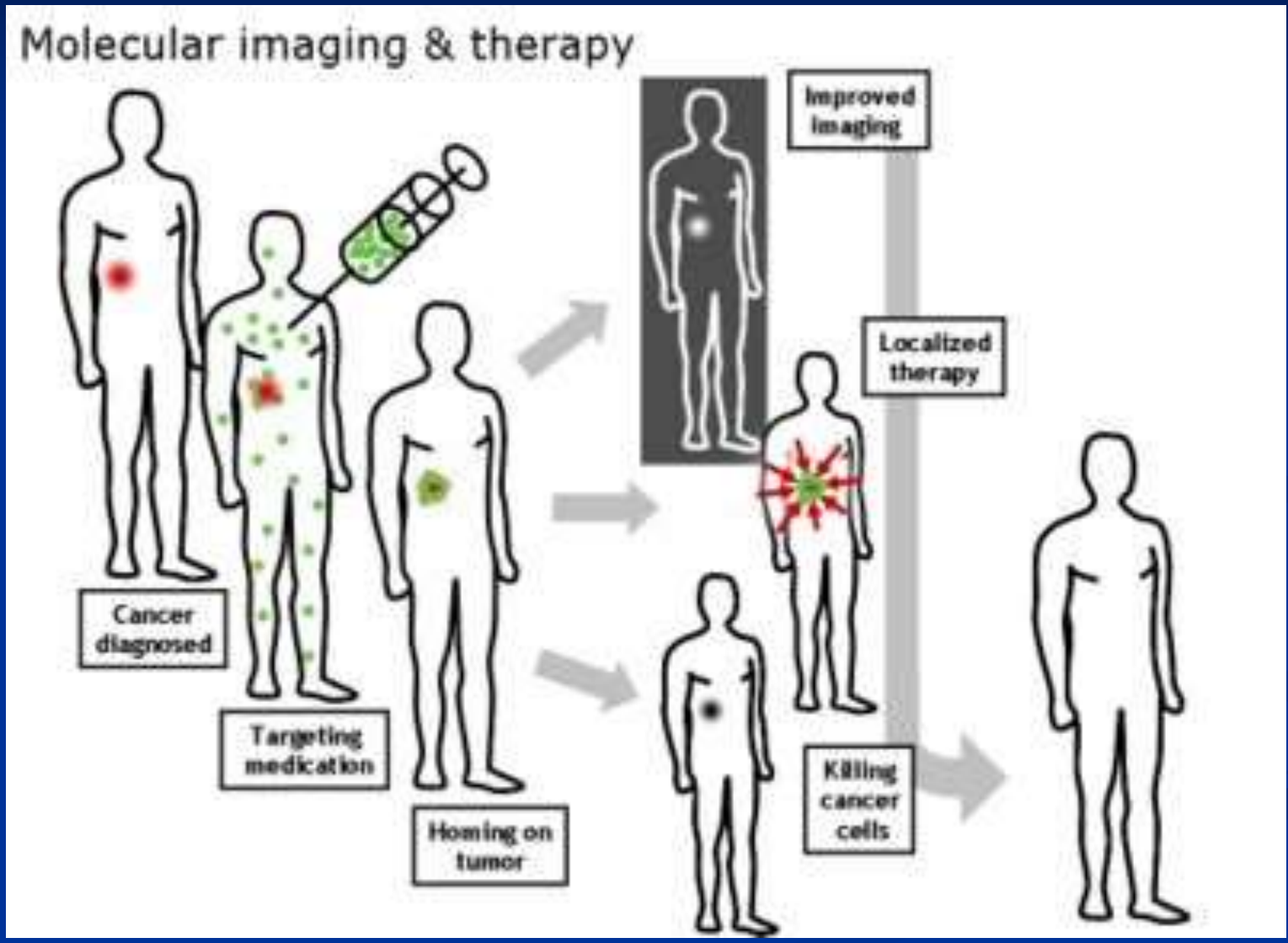
Antibody-Modified Quantum dots for the sensitive imaging of the tumour tissue on a tumour-bearing mouse.



Nanotech in Disease Imaging & Therapeutic Monitoring

in vivo imaging system including the relationship between metastasis of cancer and the onset of angiogenesis and the efficiency of anticancer drugs.

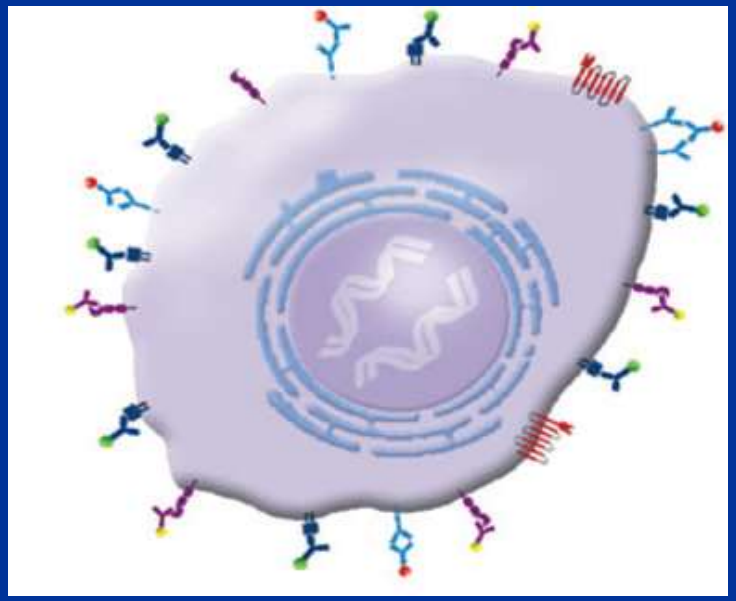
Imaging


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

Diagnosis

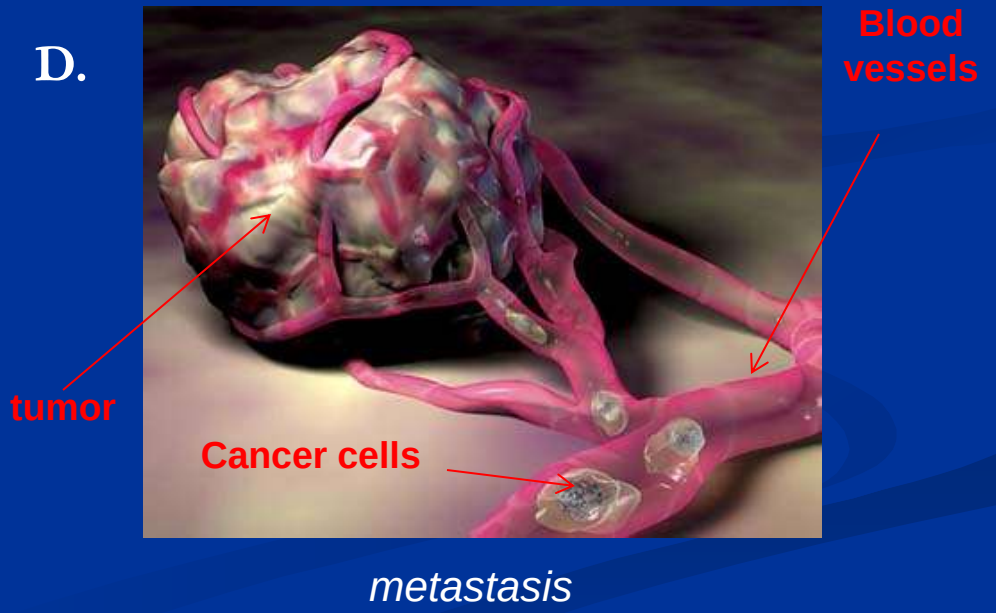
A. Detection of multiple biomarkers simultaneously

B. A specific phenotype of cancer cells has a particular combination of biomarkers on its membrane



C. Different phenotypes show different aggressiveness on their metastatic behavior

D.



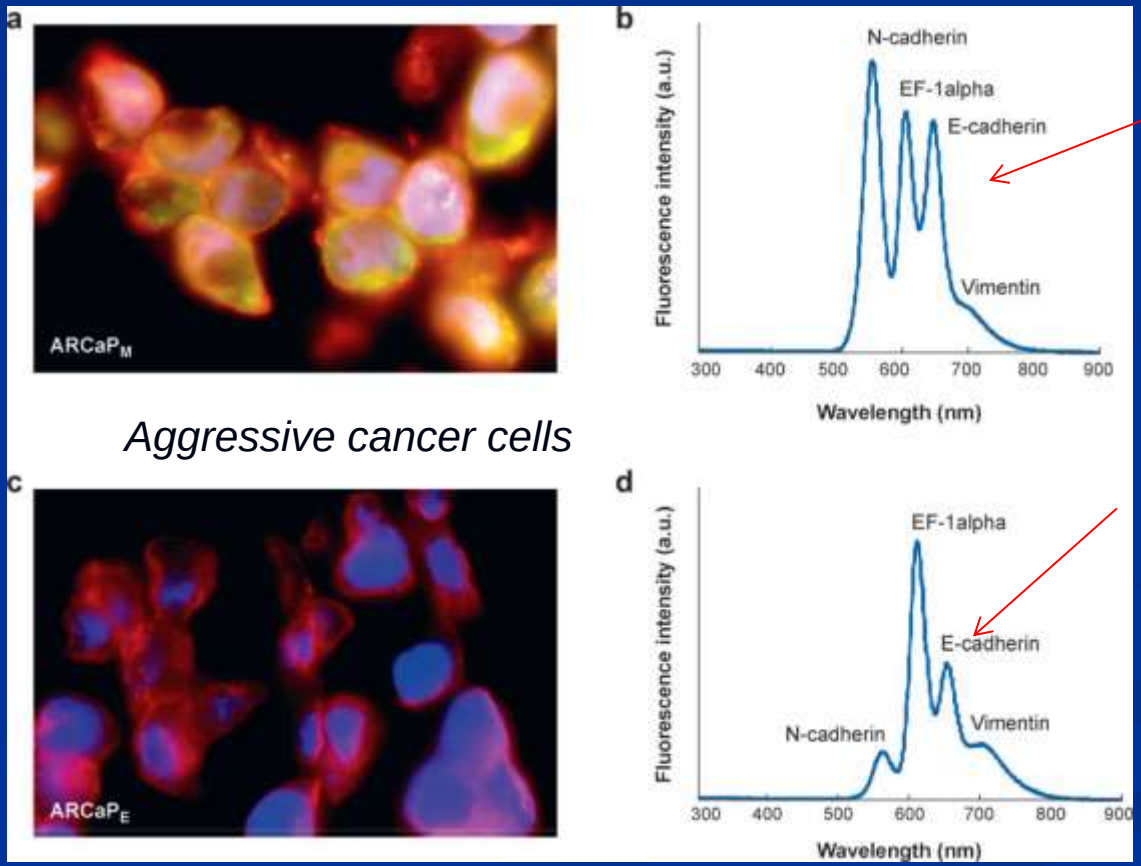
metastasis

Source: www.cancernews.com

Multiplex Diagnosis

A. Four quantum dots of different diameter (i.e. different color) are respectively functionalized with four different antigens. Allowing for the distinction of two distinct phenotypes

As a result cancer cells of different phenotype are colored differently



Aggressive cancer cells

Mild cancer cells

The peak intensity correlates to the concentration of a specific QD

Each peak correspond to the emission of a specific QD/antigen

•Nature Protocols 2007. Vol. 2, pp. 1-15

The Opportunity

Problem: For many cancers, response rates of patients treated surgically first, followed by chemotherapy and/or radiation are poor

- Surgery alone does not cure most patients of cancer
- Following surgery, many patients present with metastatic disease

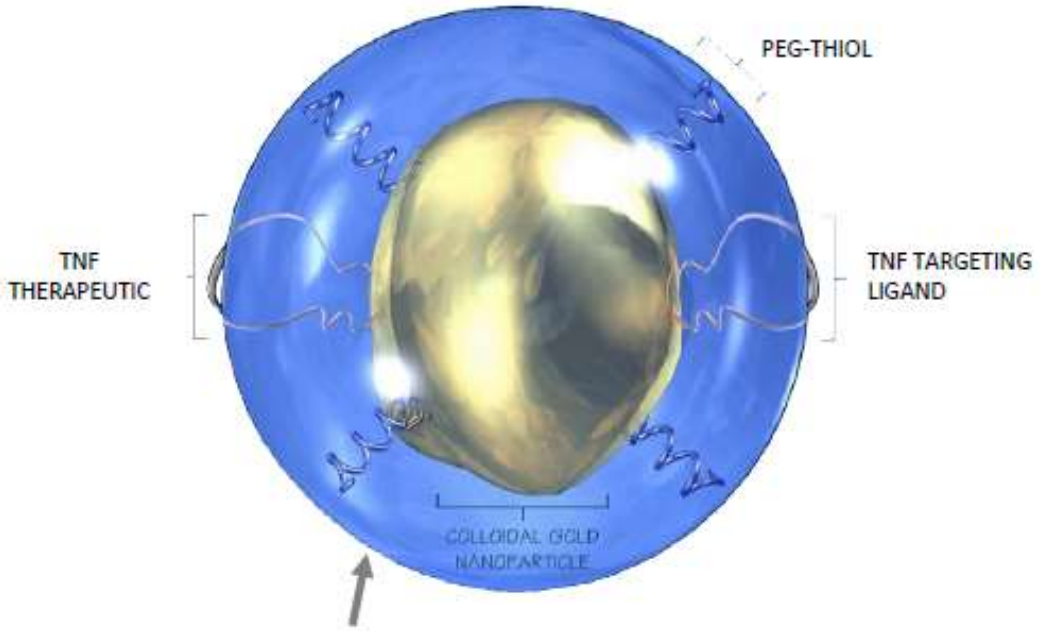
Need: Improve efficacy and safety, and minimize recurrent disease

- Targeting tumors
- Limit exposure of healthy tissues and organs to cytotoxics

Solution: Use nanotechnology-based therapeutics, first

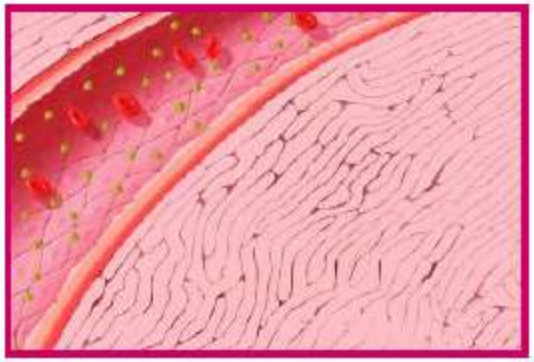
- First treat patients medically to reduce tumors, use surgery only if needed
  - May lead to improved tumor regression, reduced side effects, and reduced recurrent disease

Design of CYT-6091 (Aurimune®):

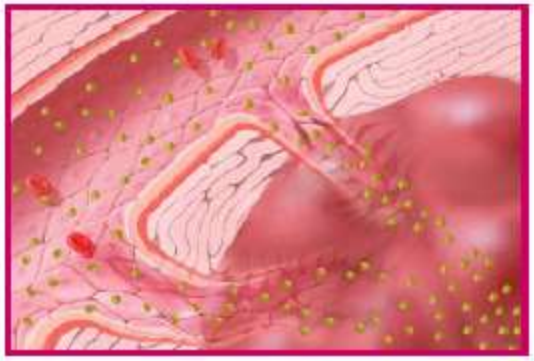


CYTIMMUNE

Safe, Targeted Delivery: Size Matters



Too Large for Toxic Side Effects. CYT-6091 is small enough to safely travel through healthy blood vessels, but too large to pass through blood vessel walls into healthy tissues and organs, resulting in reduced toxicity.



Small Enough to Exit Tumor Vessels. All solid tumors are fueled by new, "leaky" blood vessels that have gaps in their walls. When CYT-6091 reaches these "leaky" vessels, the nanoparticles are small enough to pass through these walls into their target, the tumor.

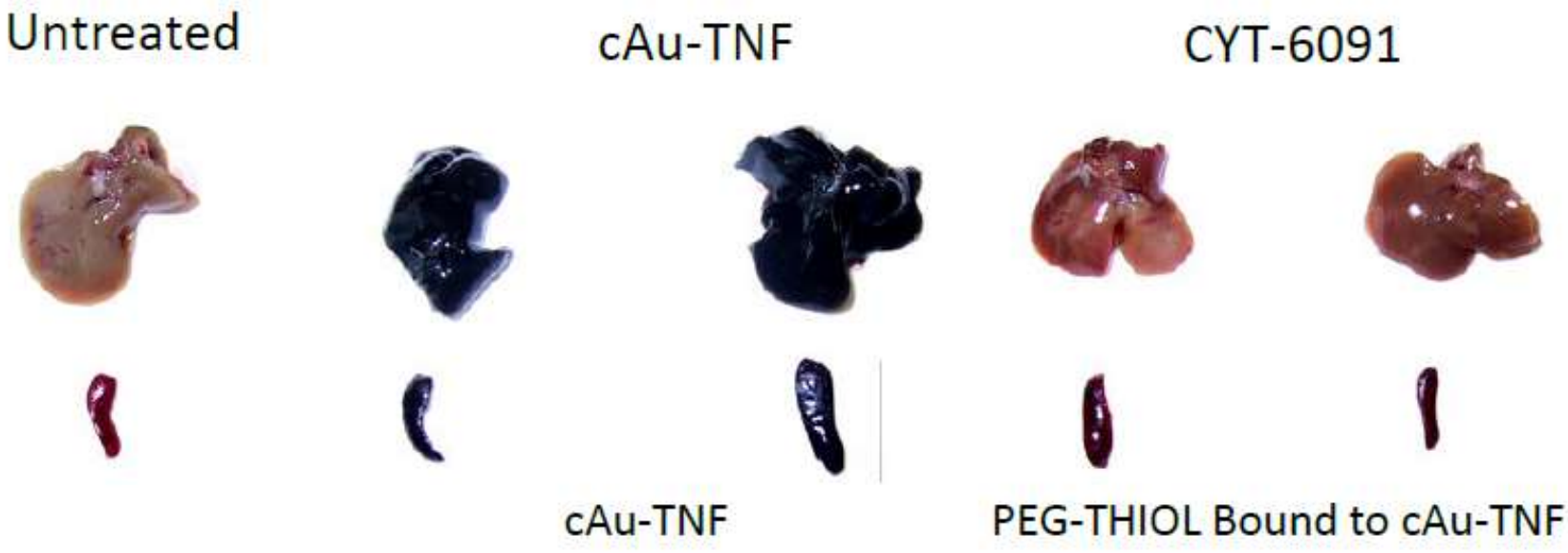
Due to its engineered nanometer size and targeted capabilities, CYT-6091 is able to reduce toxicity and increase efficacy.

CYTIMMUNE

CYT-6091: Avoids Immune Recognition and Uptake

PEG bound to gold nanoparticles prevents uptake by the liver and spleen, major organs of the MPS, (black color is aggregated gold particles)

- Uncoated nanoparticles may be safe, but do not reach tumor target

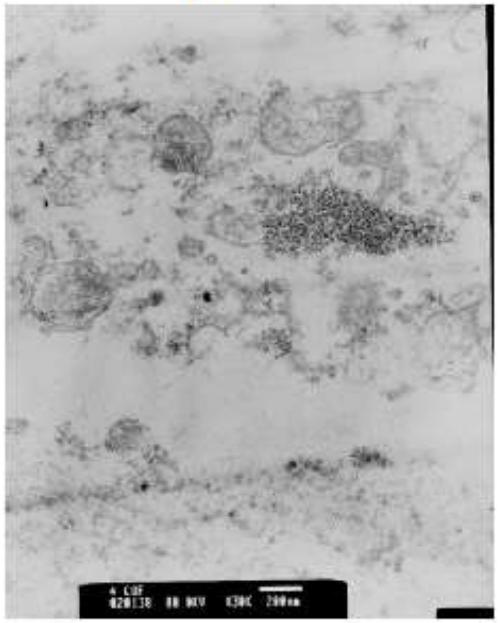


CYTIMMUNE

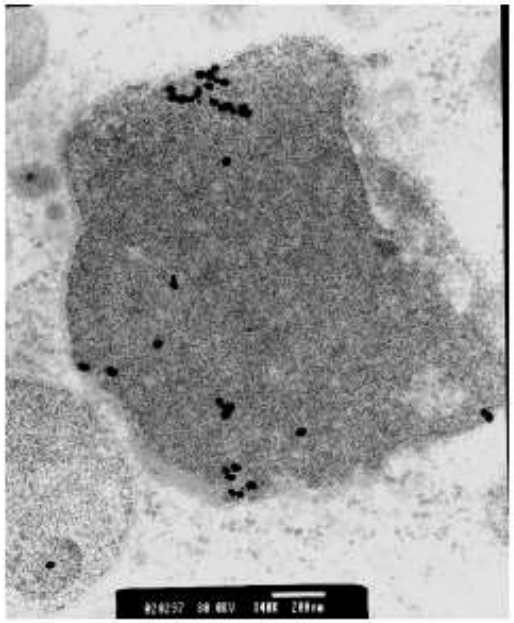
Differential Uptake of CYT-6091 in Mouse Model

Electron micrographs comparing tumor and healthy tissue

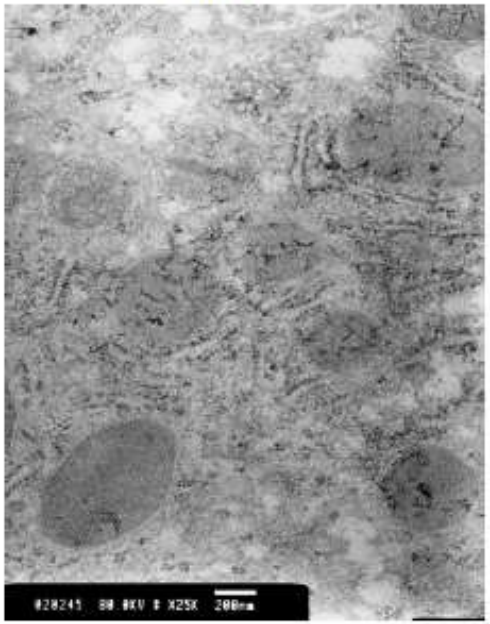
Spleen



Tumor



Liver



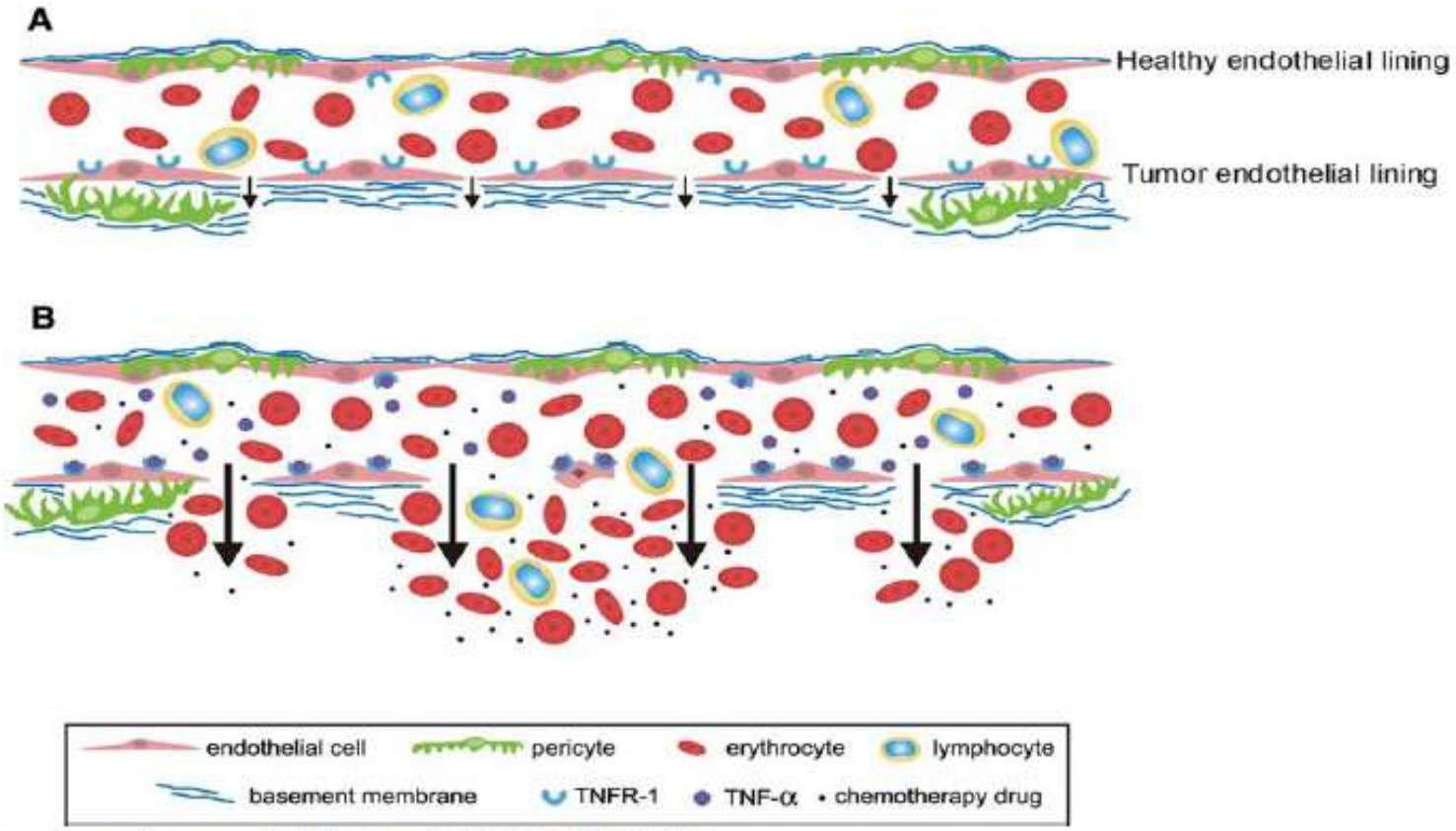
Bar at bottom = 200 nm

CYTIMMUNE

Effect of Systemically Administered CYT-6091 on Tumor Vasculature

By delivering TNF to the tumor vasculature CYT-6091 causes vascular breakdown

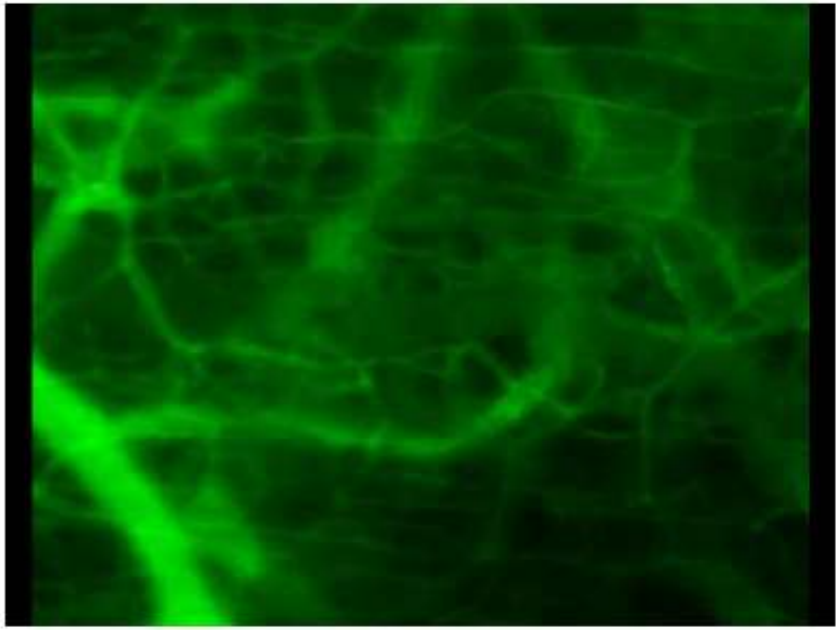
- Massive vascular leak destroys high intra-tumor pressure



From: R. van Horssen el al, The Oncologist 2006;11:397-408

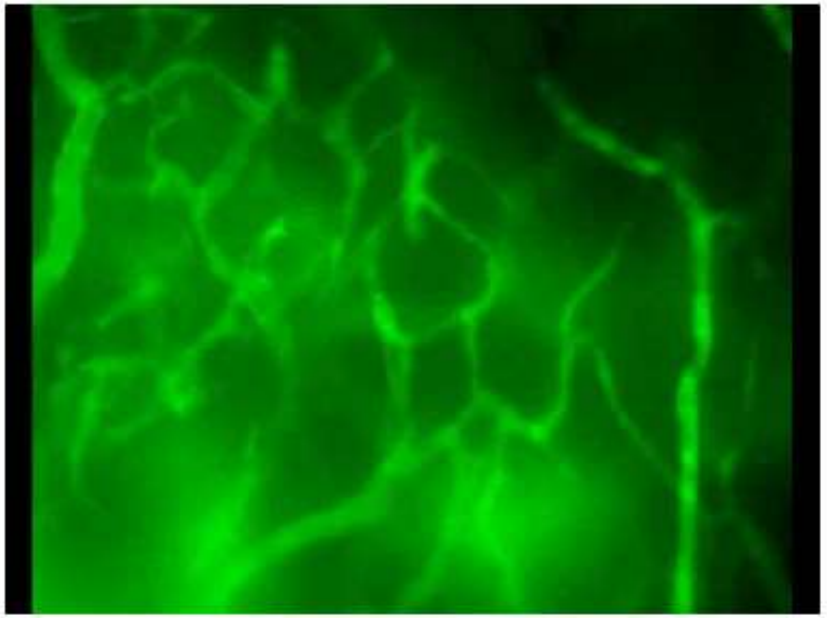
CYTIMMUNE

Selective Induction of Vascular Leak by CYT-6091



Normal Vasculature
No Vascular Leak

Albumin



Tumor Neovasculature
Vascular Leak

CYT-6091 + Albumin

CYTIMMUNE

https://cytimmune.blog/2017/07/12/watch-cyt-6091-in-action/

Killing Tumors: CYT-6091 Pre-Clinical Mouse Data

Stealthy. PEG-Thiol bound to colloidal gold nanoparticles avoids immune detection by the MPS

Targeted. CYT-6091 delivers TNF to solid tumors:

- *Passively* by extravasating from the tumor vasculature
- *Actively* by binding to TNF receptors on tumor endothelial cells

Accumulation. CYT-6091 accumulates TNF in TNF sensitive and insensitive tumors

- For TNF sensitive tumors:
  - One treatment induces potent anti-tumor responses at lower doses
- For TNF insensitive tumors:
  - One treatment induces transient anti-tumor response
  - Multiple doses causes cytostasis
  - Combination with doxorubicin is additive

Clinical Grade CYT-6091

Current production capacity scaled 10-fold from Phase I to Phase II

- Solved manufacturing challenge for a nanomedicine
- Process is robust, reproducible and cost effective
- 3-year shelf life as a freeze-dried product

CYTIMMUNE

CYT-6091 Phase I Trial: Clinical Observations

Safe, systemic delivery. Delivered 1.2 mg of TNF with no dose limiting toxicity

- No Hypotension, the dose-limiting toxicity associated with TNF use in man
- No Serious Adverse Events that were unexpected and related to treatment

Tumor targeted. Drug accumulation at tumor sites

- Gold particles seen in tumors but few if any in healthy tissues

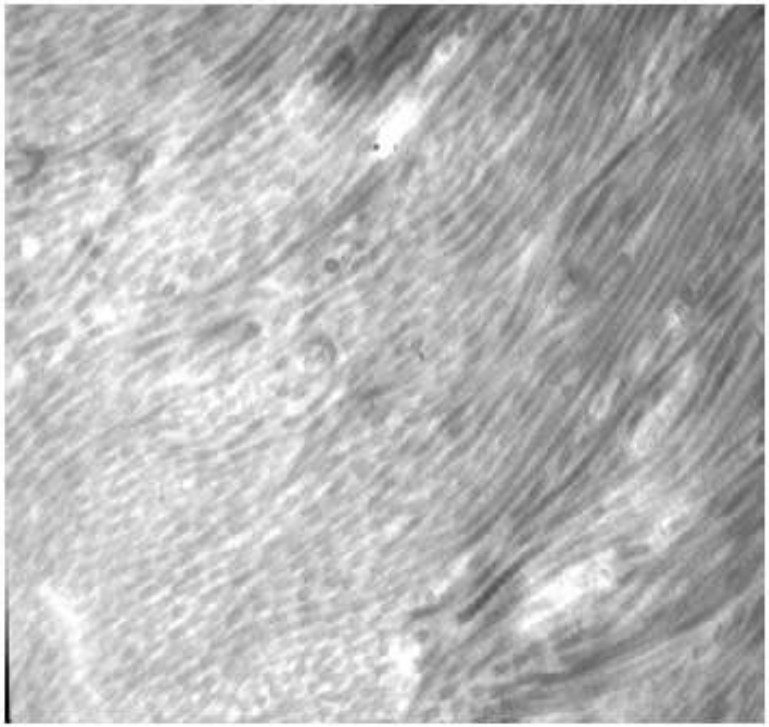
Not Antigenic. No antibody response

- Titer checks after CYT-6091 treatments show no anti-TNF antibodies

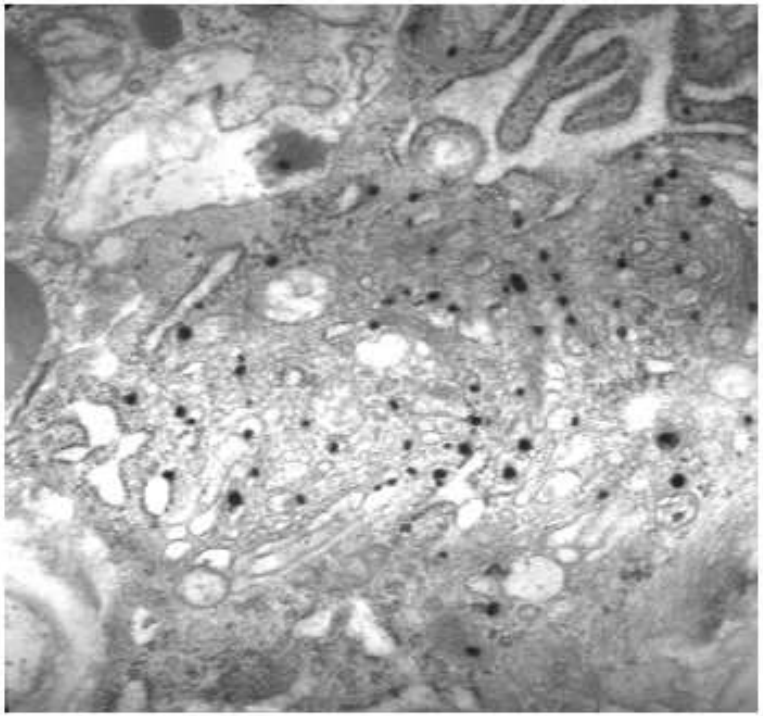
Electron Micrographs* of a Patient's Biopsies

Patient diagnosed with inoperable breast cancer

- Patient had no prior treatment; samples taken 24h after treatment
- Drug accumulated in tumor, not in healthy breast tissue



Healthy Breast



Tumor

*Magnification = 20,000x

CYTIMMUNE

CYT-6091: An Ideal Cancer Nanomedicine

Designed to meet critical requirements for tumor targeted therapy

- Not picked-up by liver and spleen
- Targets tumor endothelial cells
- Manufacturing process robust, reproducible and cost-effective

Targets Tumors

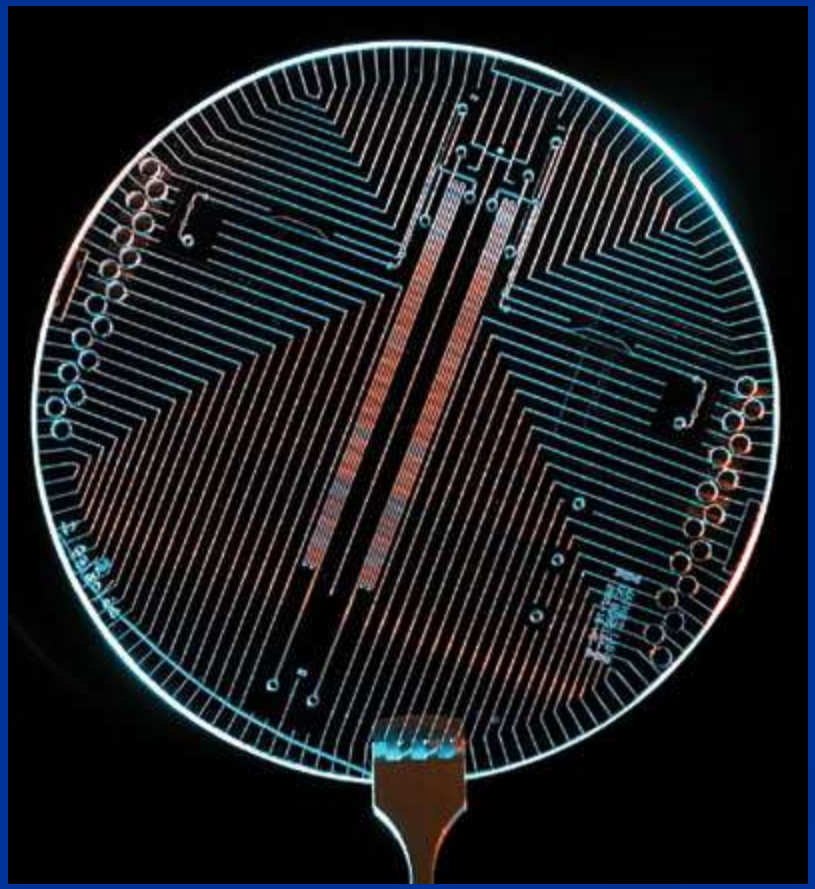
Avoids MPS Uptake

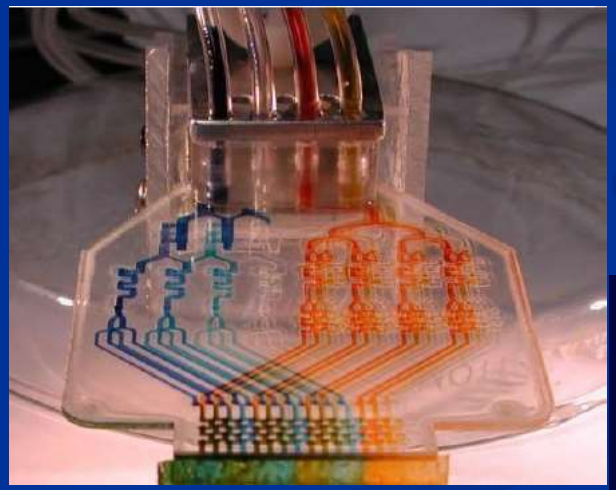
Manufactured to Defined Specifications

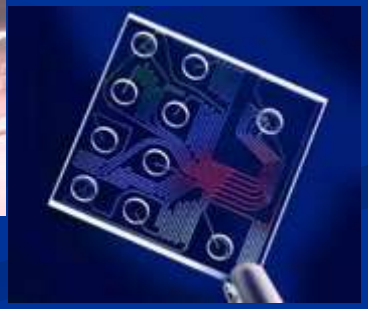
CYTIMMUNE

Lab-on-a-Chip

One of the more promising areas of nanofluidics is its potential for integration into microfluidic systems, i.e. MicroTotal Analytical Systems or Lab-on-a-chip structures



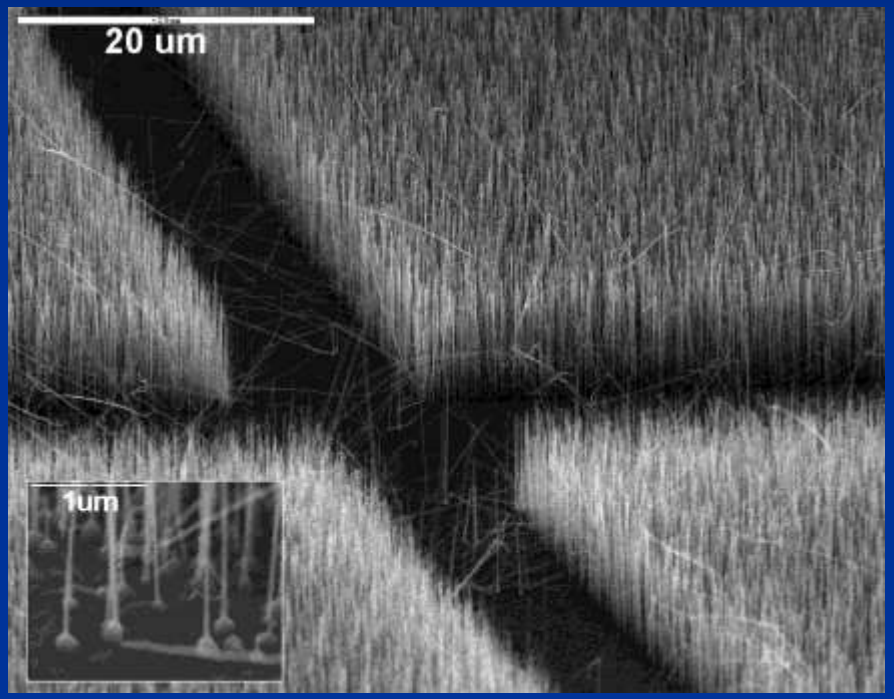




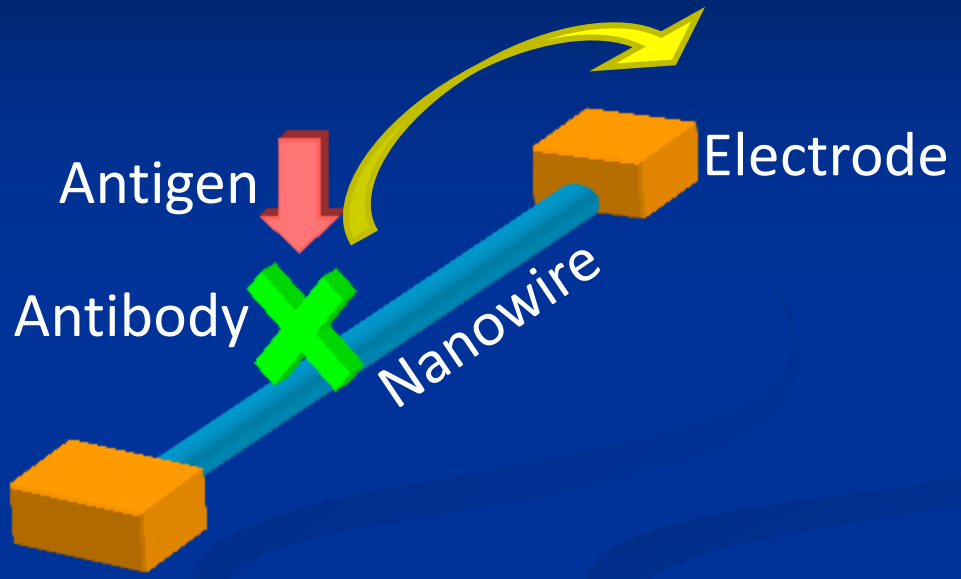
- Credits: Mathies Lab, UC-Berkeley
- Quake Lab, Stanford
- Agilent, Inc.

Diagnostics – Biosensors

Novel Materials



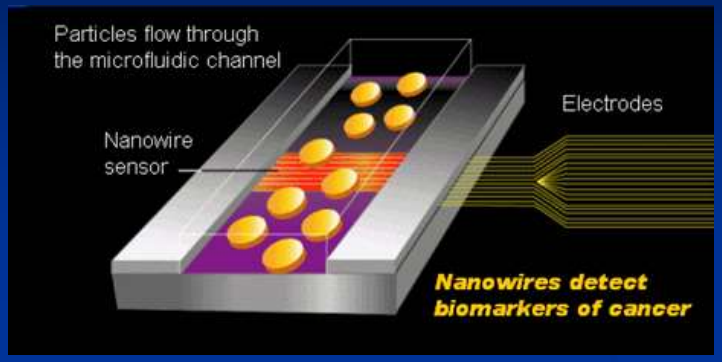
ZnO nanowires



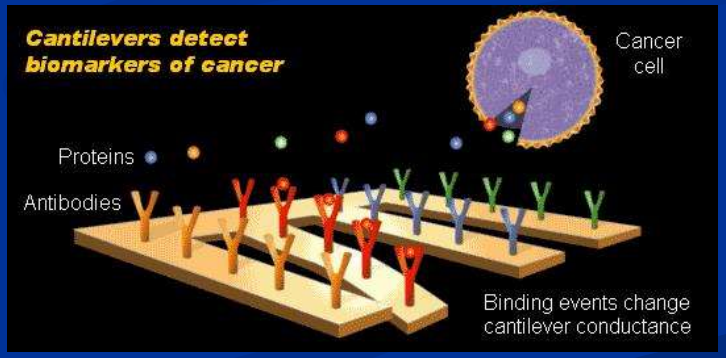
Ultra-sensitive biosensor for the detection of bio-markers using bio-compatible ZnO nanowires.

Lab-on-Chip in Health Care

- Detection and Diagnosis
- Lab on chips help detection and diagnosis of diseases more efficiently
- Nanowire and cantilever lab on chips help in early detection of cancer biomarkers



The microfluidic channel with nanowire sensor can detect the presence of altered genes associated with cancer *– J. Heath, Cali. Insti. of Technology*



The nanoscale cantilever detects the presence and concentration of various molecular expressions of a cancer cell *– A. Majumdar, Univ. of Cal. at Berkeley*

http://www.biomedox.com/media/nanotechnology-documentary-revolutionizing-medicine-and-healthcare_ebeb2ce1e.html

https://www.youtube.com/watch?v=Rs43PZyU8zU

http://www.britishsocietynanomedicine.org/nano-movies/


NANOTECHNOLOGY LAB