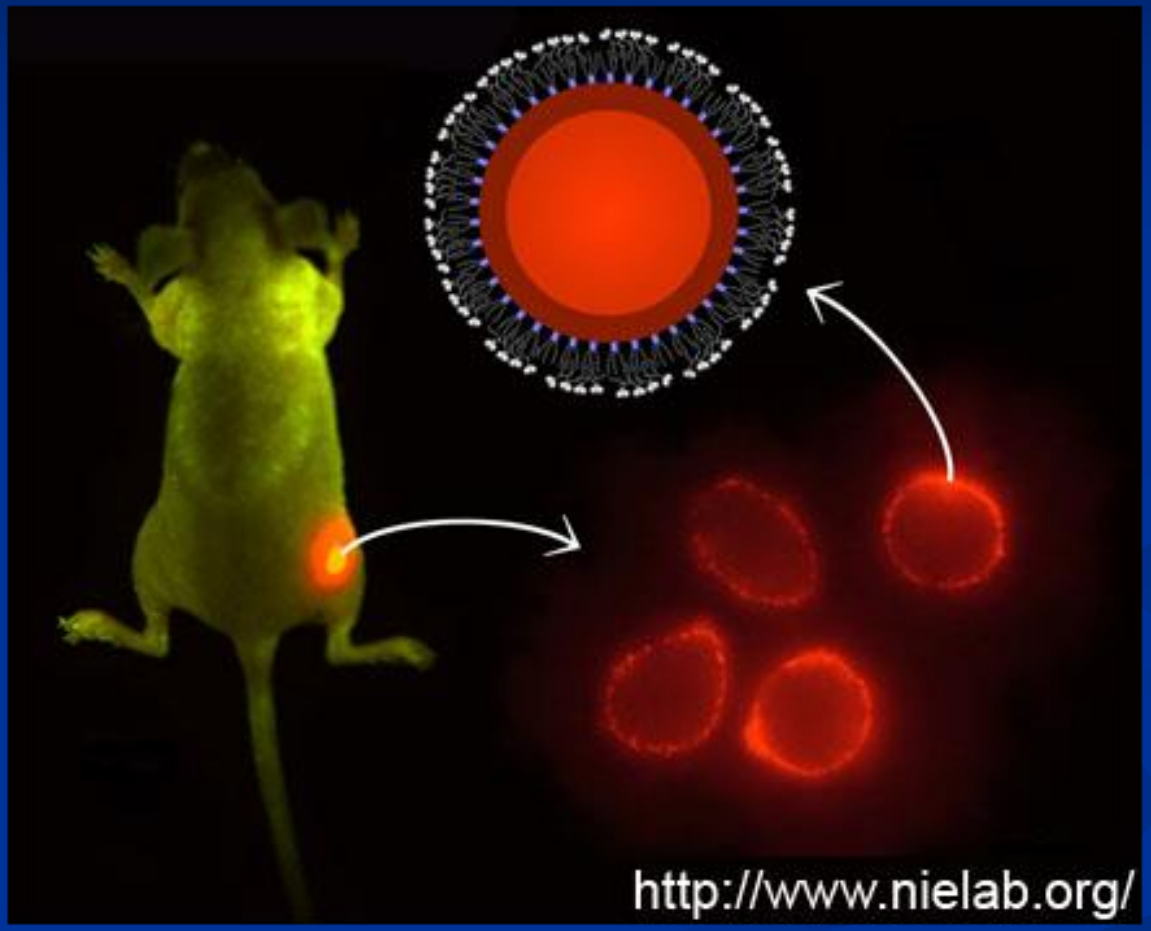
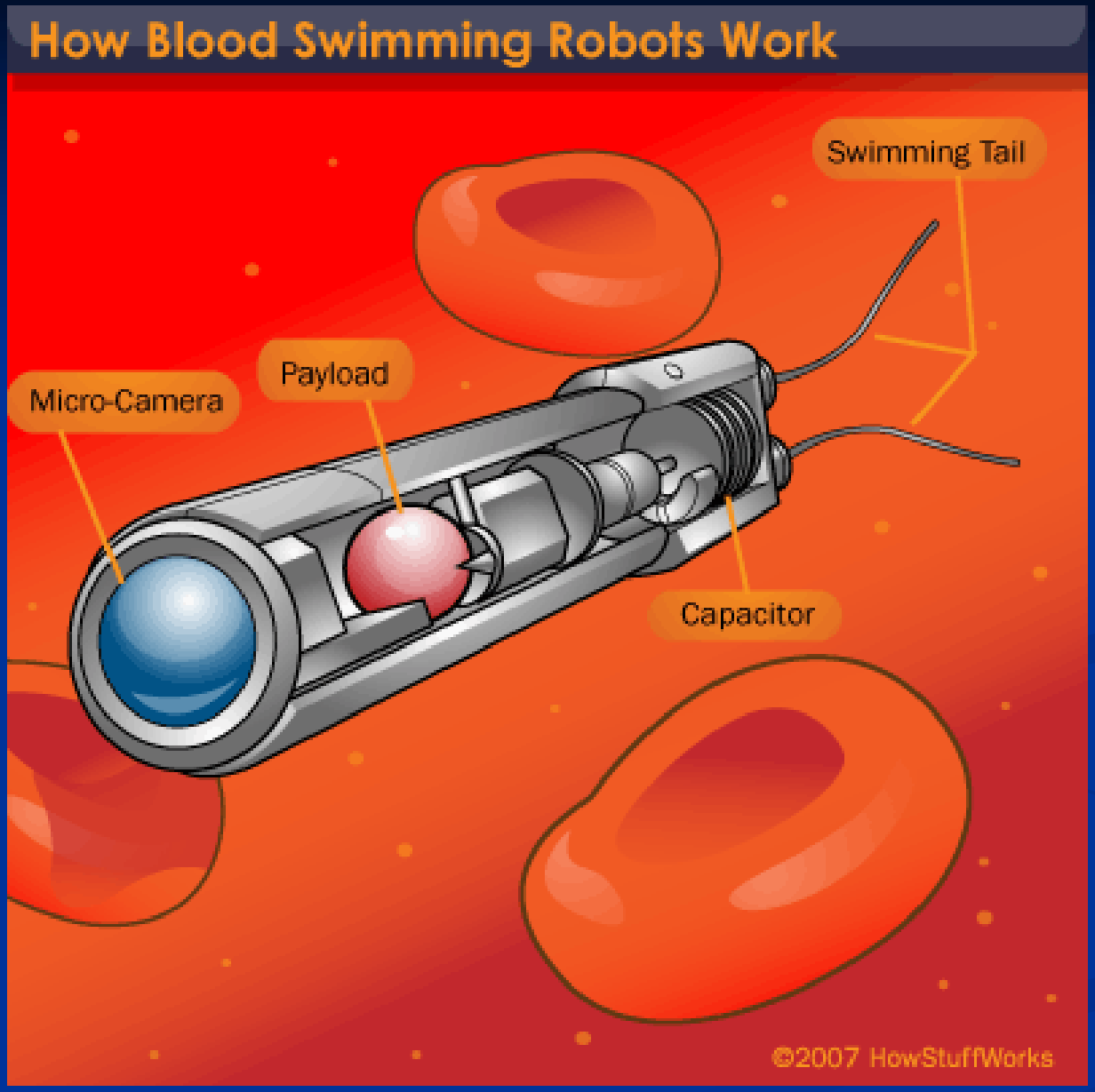
Nanotechnology & Nanomedicine



Hans Bluyssen, 23-11-2022

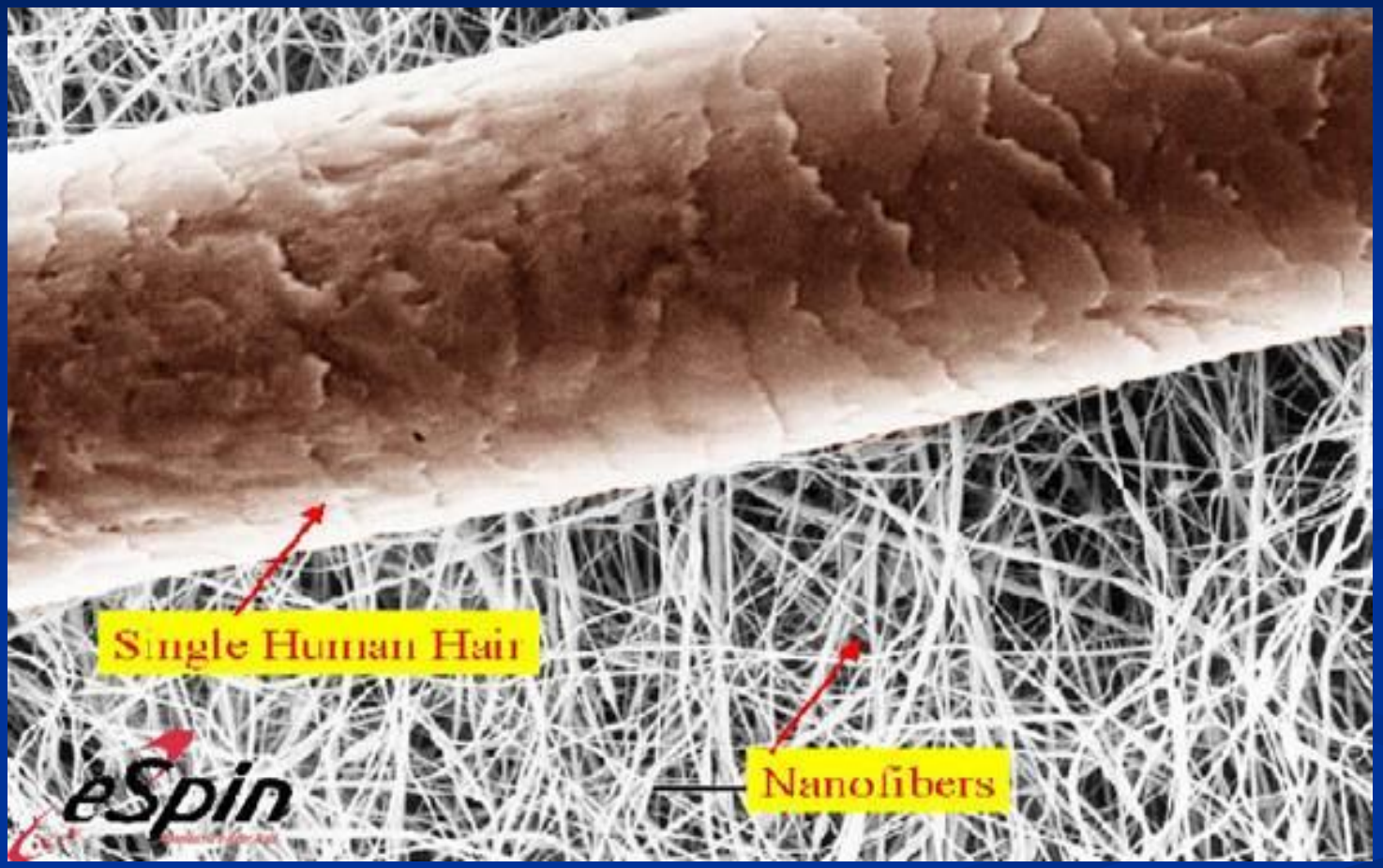


- Ability to enter cells and correct DNA or a deficiency.
- Repair cells, tissue, and even organs.
- Carry tiny cameras and be powered from the electrolytes in the blood.
- Break up blood clots or even kidney stones.

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

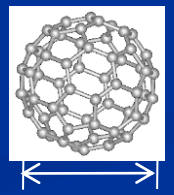
1.27 × 10^7 m

22 cm

0.22 m

Fullerenes

C_{60}



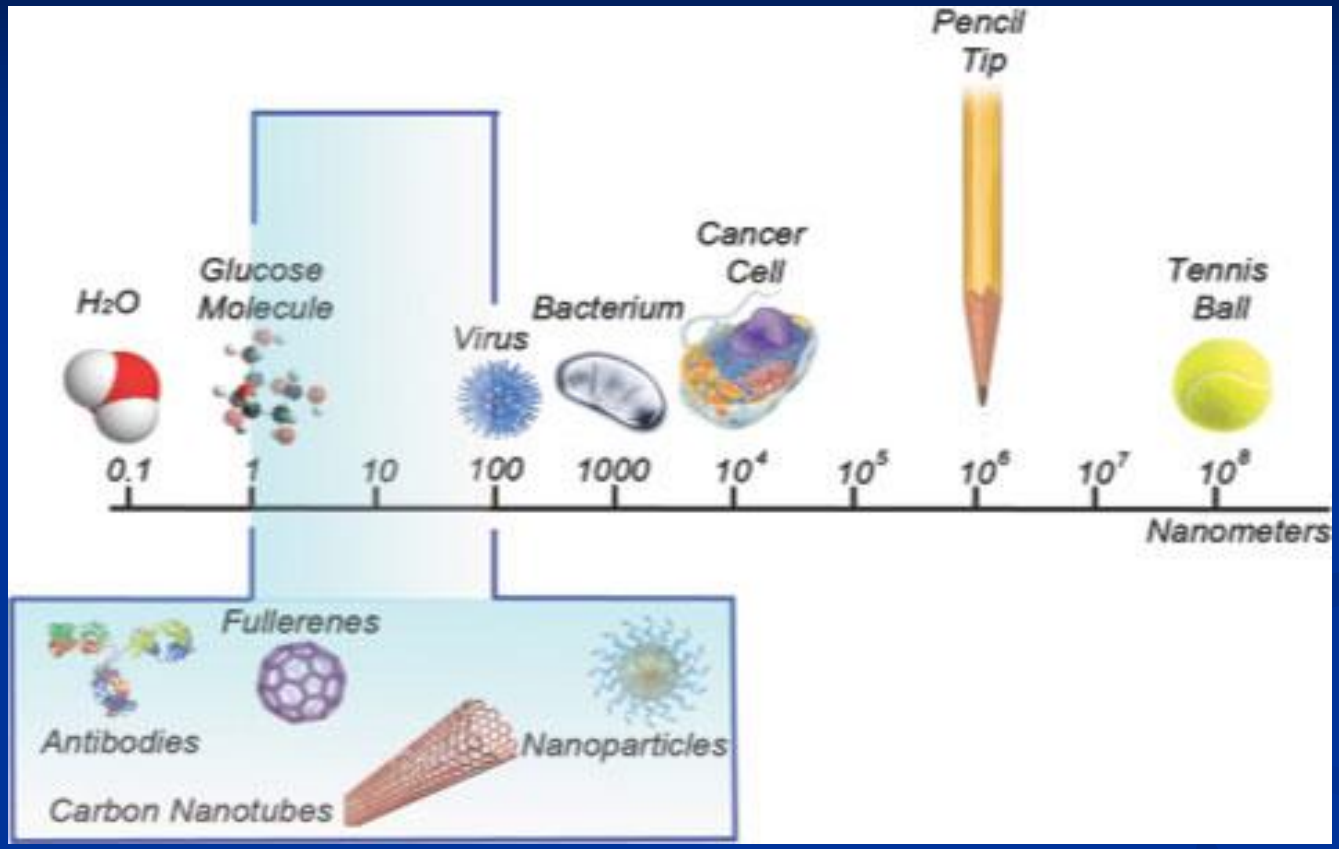
0.7 nm

0.7 × 10^{-9} m

10 millions times smaller

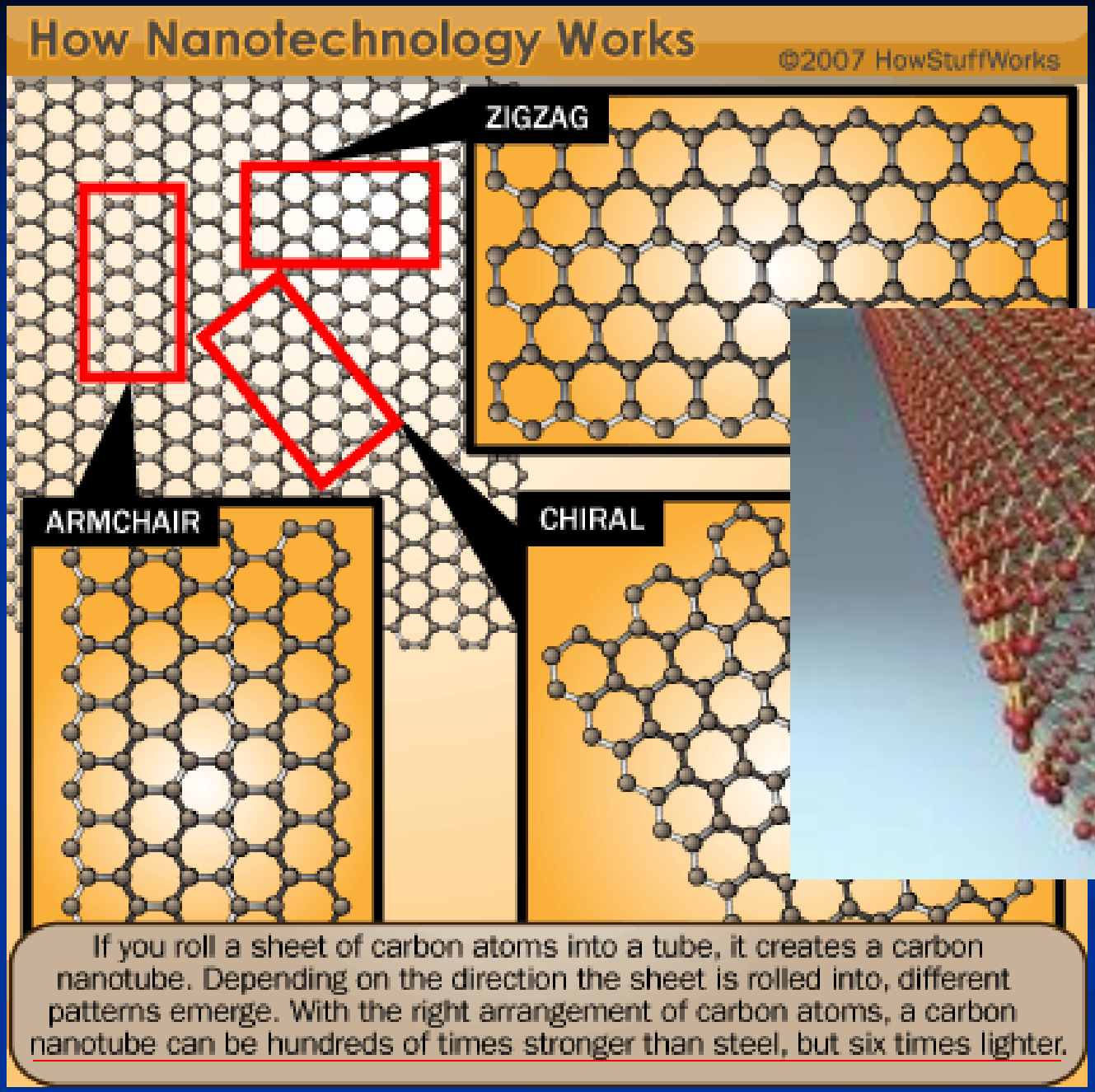
1 billion times smaller

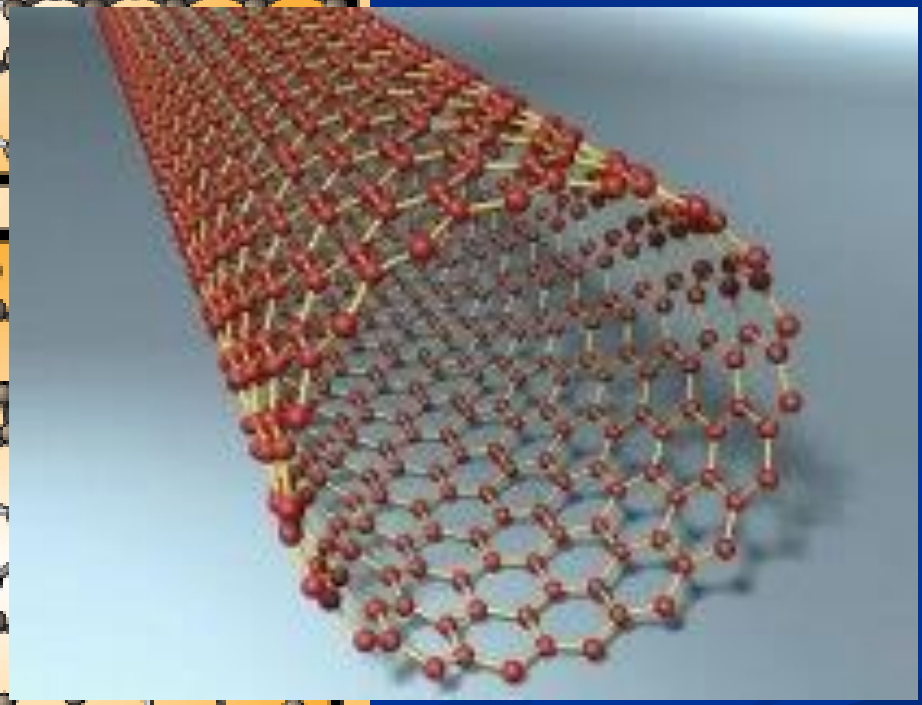
Nanotechnology



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

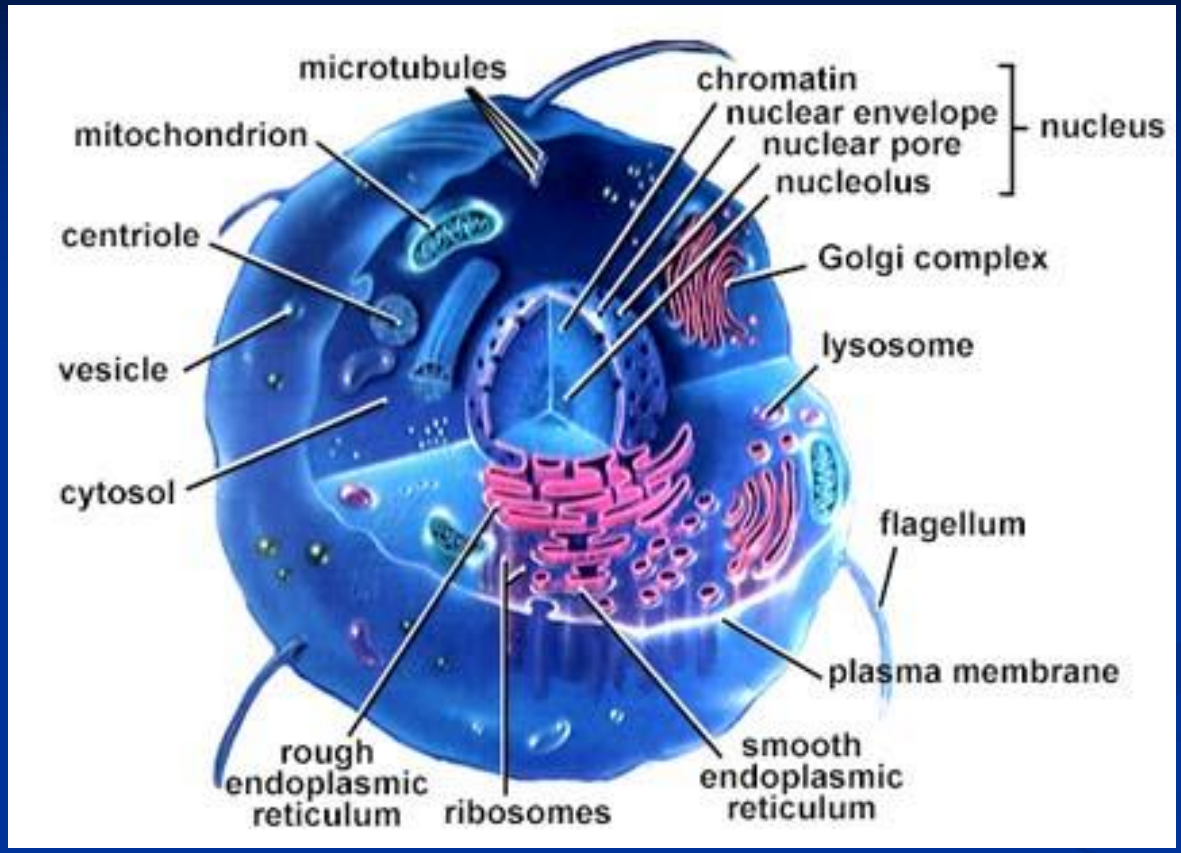
Carbon Nanotube





Fullerenes

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

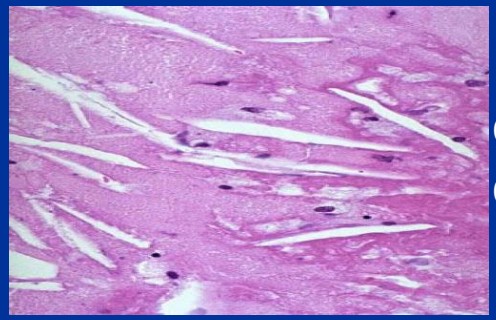


Cells themselves (organelles) are very complex and efficient nano-machines.

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

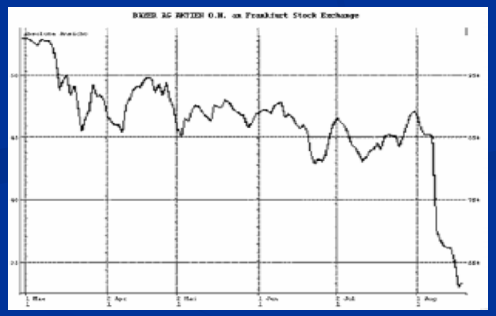


arterial plaque

some lipid lowering drugs:

effects on plaques
can save lives

effects on liver



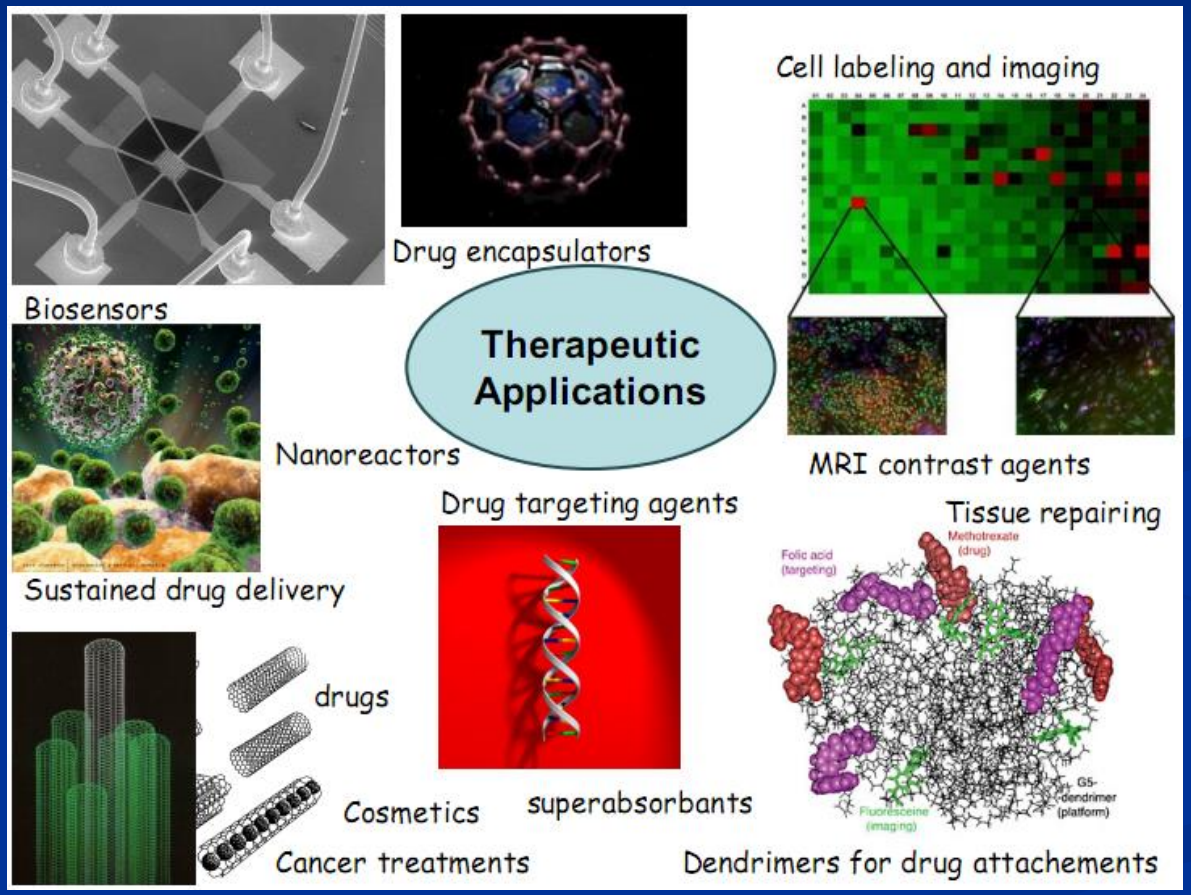
Can endanger large companies

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,

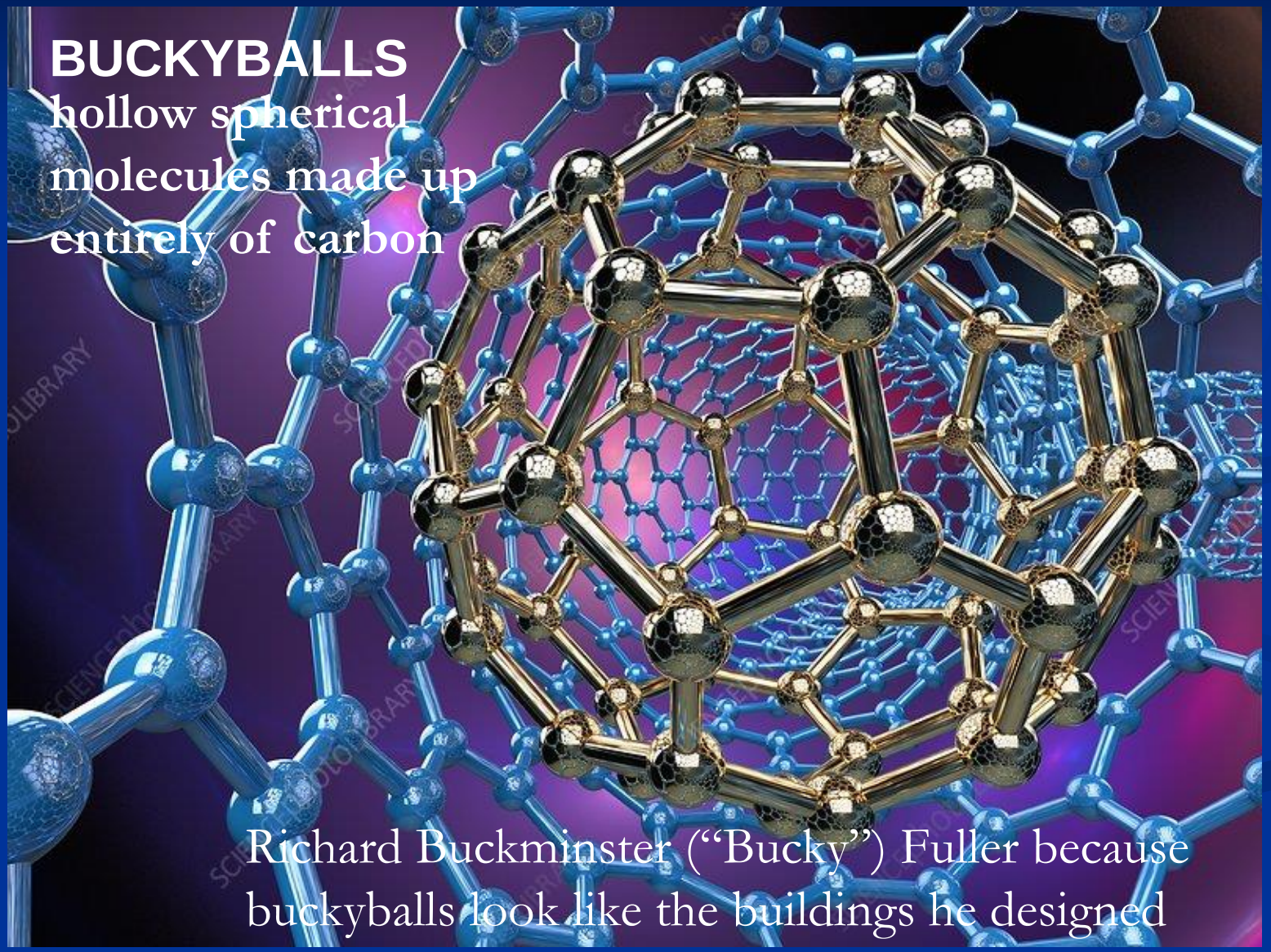
monitoring,

control of biological systems

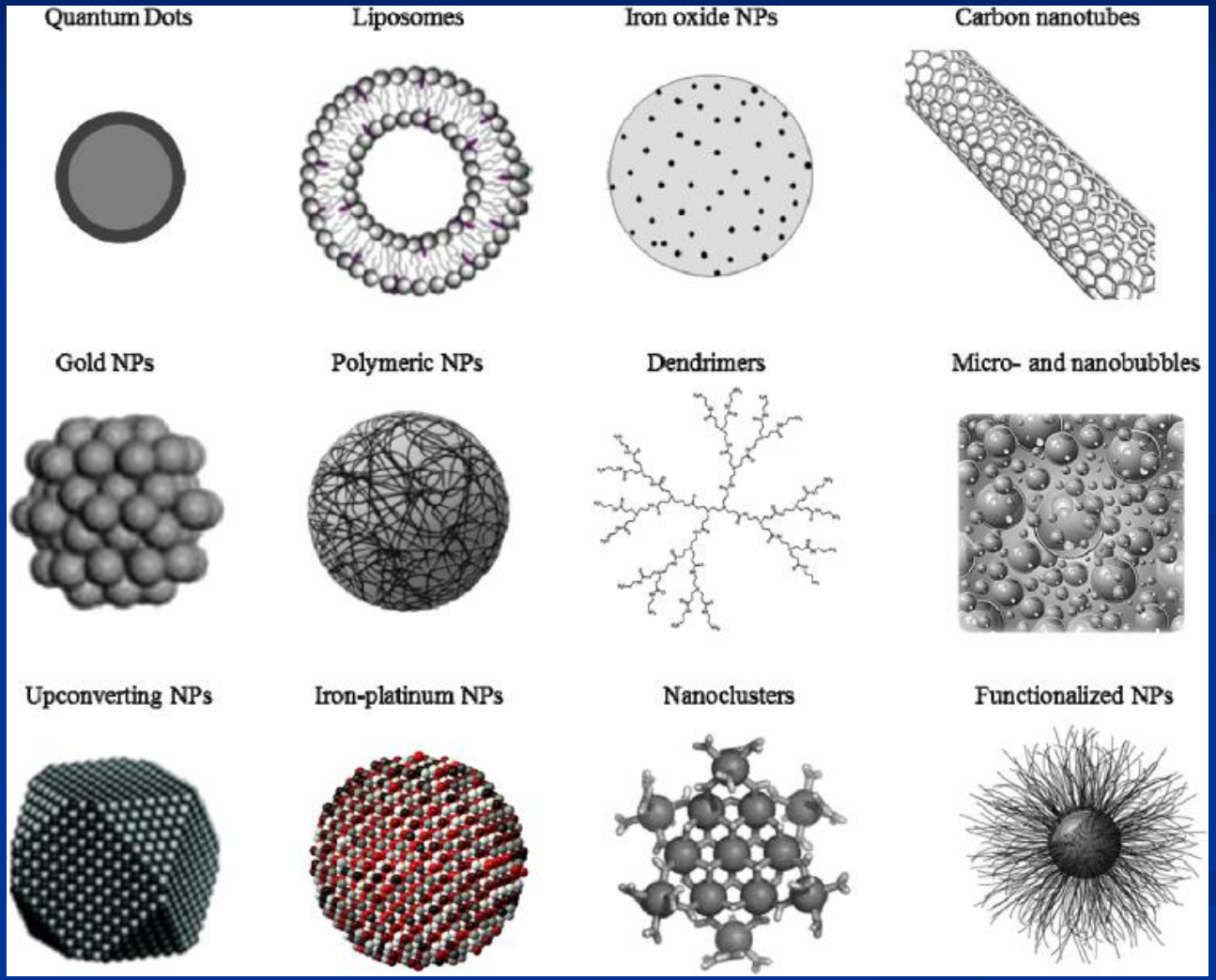
NANOTECHNOLOGY TOOL BOX

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

NANOTECHNOLOGY TOOL BOX

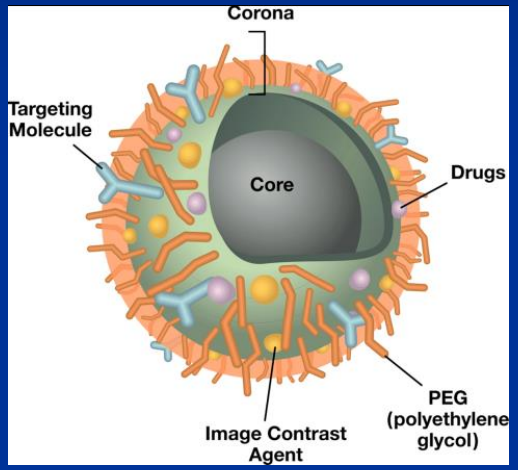


Nanoparticles

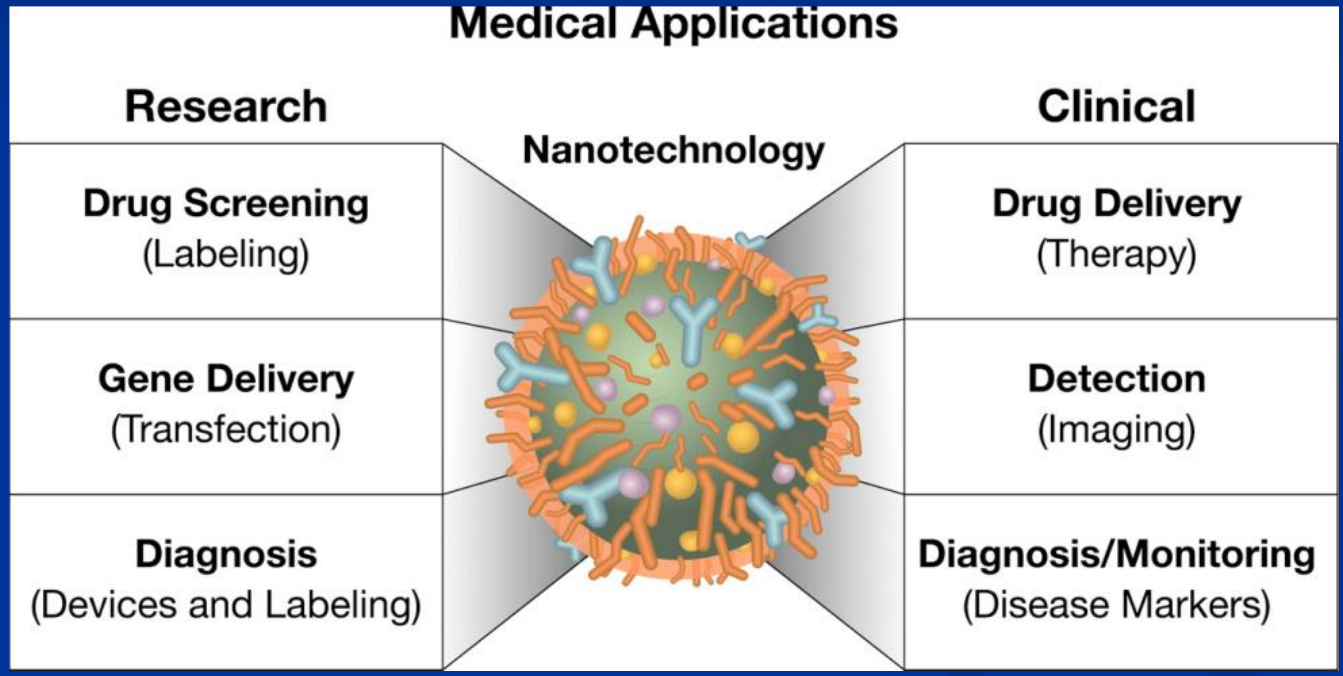


•[8]Re F, Moresco R, Masserini M. Nanoparticles for neuroimaging. Journal of Physics D: Applied Physics. 2012;45(7):073001.

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



Functionalized nanoparticles

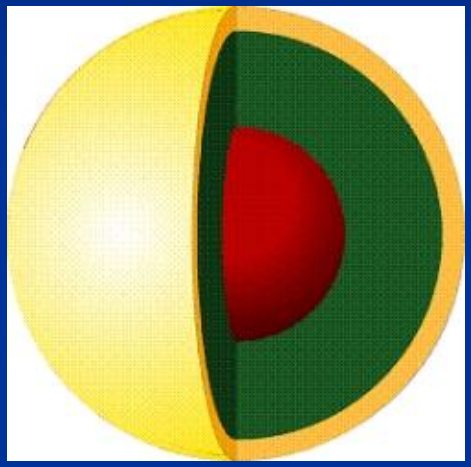


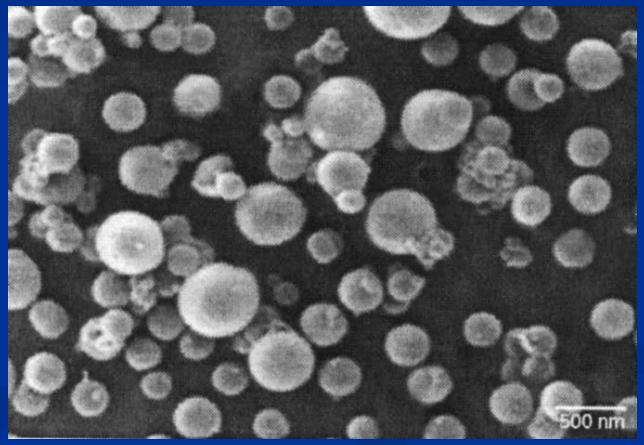
•McNeil SE. *J Leukoc Biol*, 2005. **78**(3): p. 585-94.
•doi:10.1189/jlb.0205074

Nanomedicine

A vehicle for delivery of therapeutics into the body

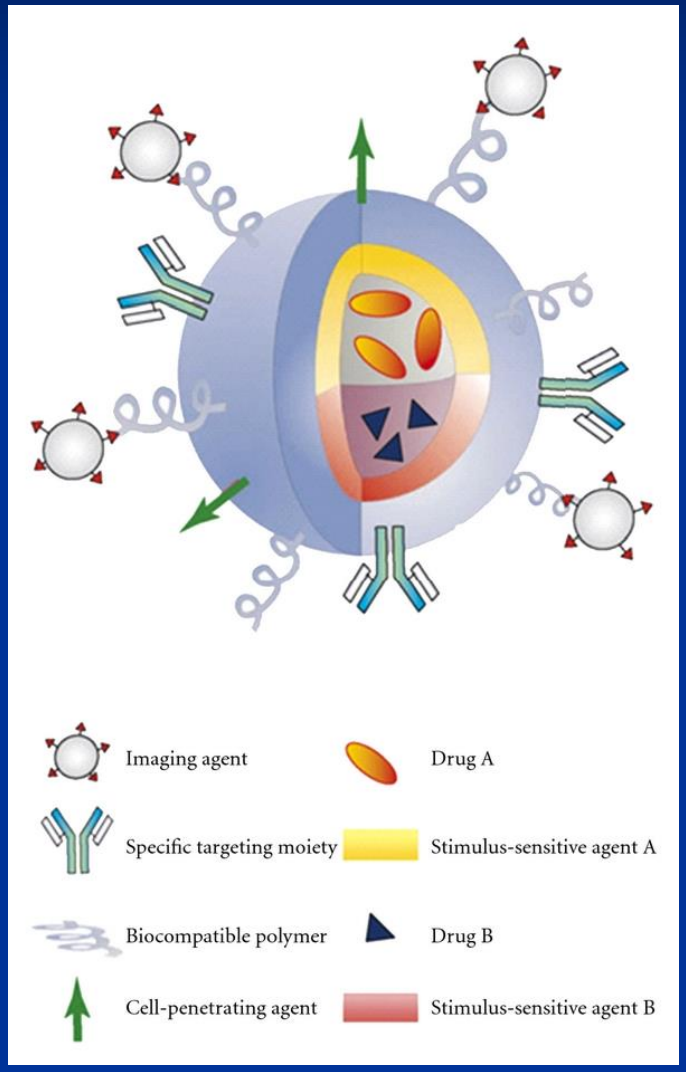
Functionalized nanoparticles

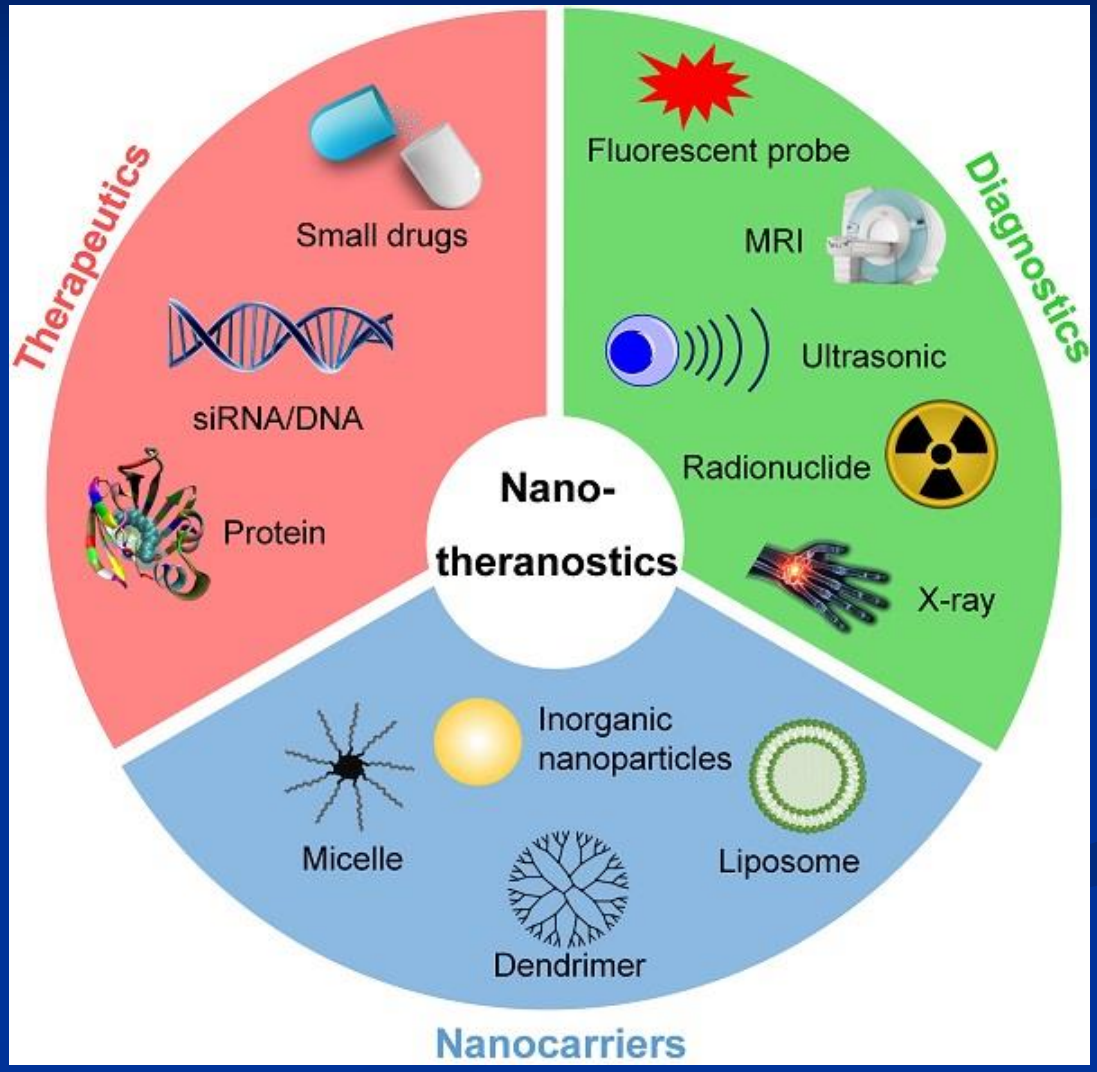




- Small molecule drug compounds, DNA/genes, proteins, vaccines, etc.
- Administration routes to reach systemic circulation or infected organs and cells: oral, intravenous, inhalation, ocular, topical

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





Functionalizednanoparticles

Nanoparticles and Drug delivery

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

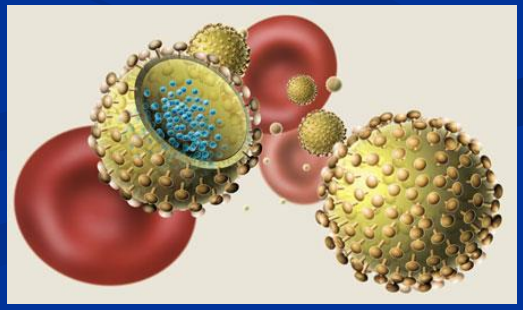
-Ingested vs injected

-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.

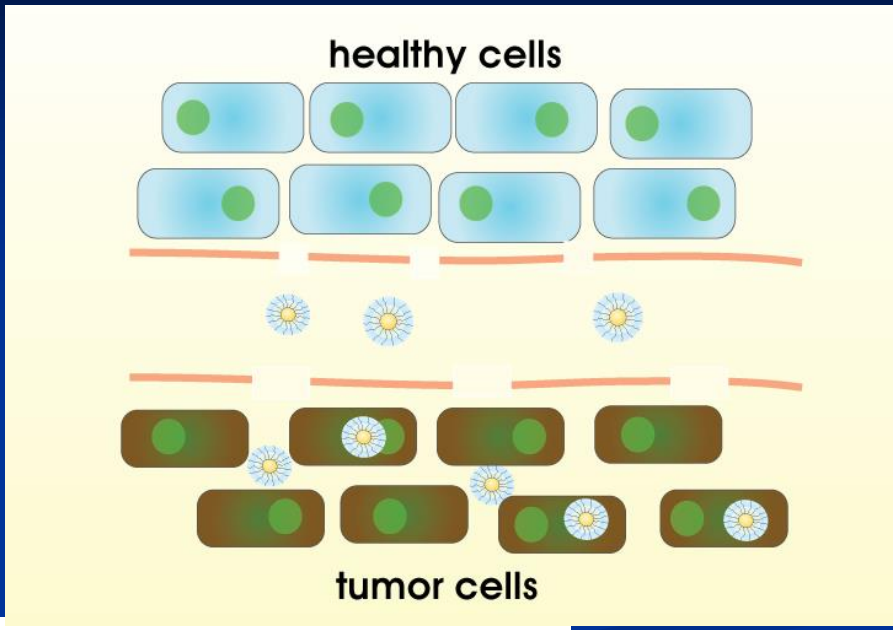


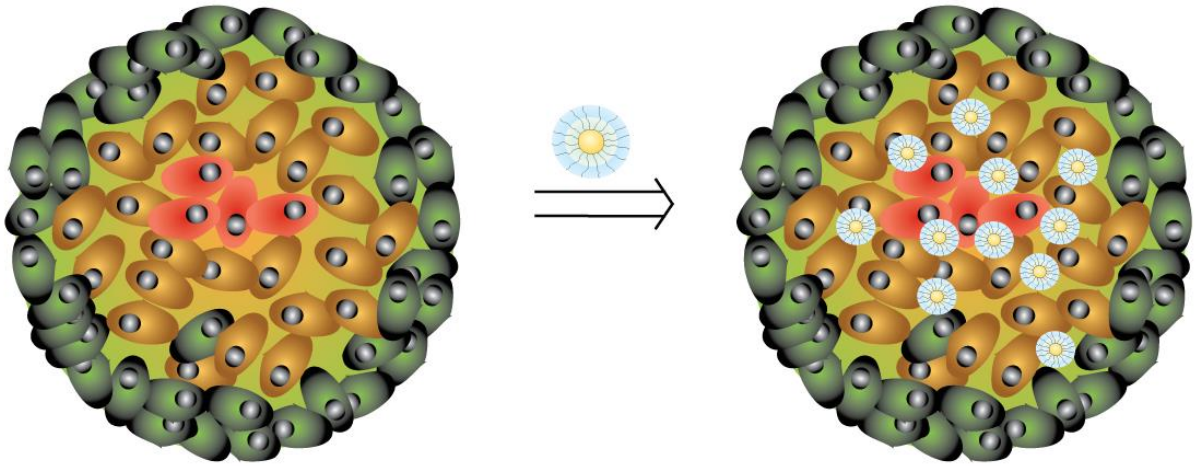
Nanoparticles and Drug delivery

- 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.

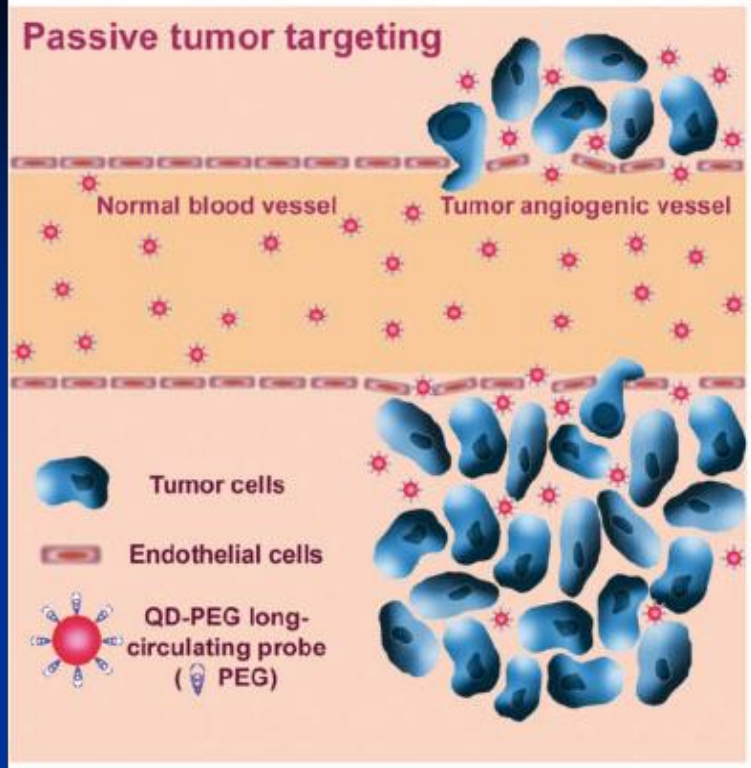
Nanoparticle use in Cancer Treatments

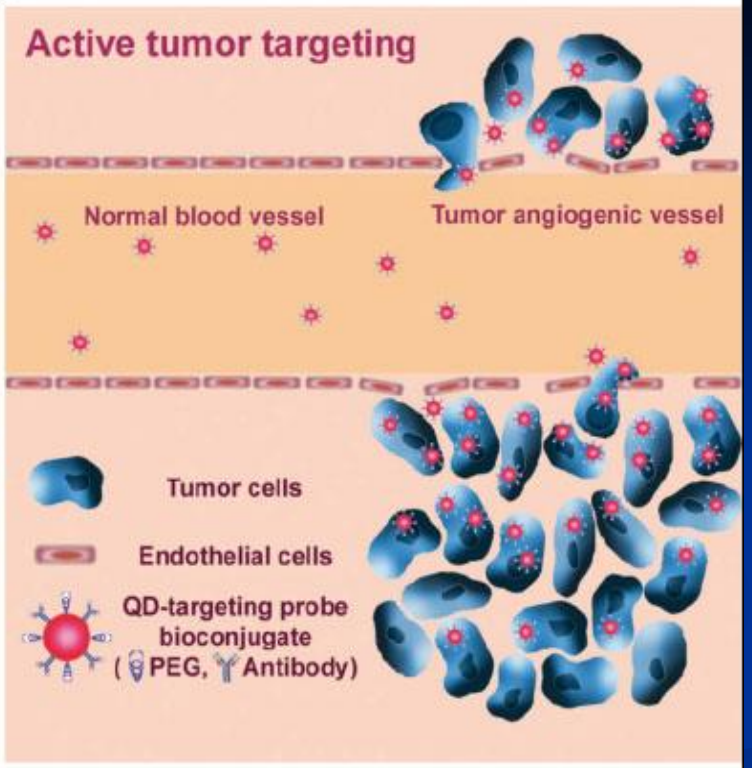
- Because of their small size, nanoparticles can pass through interstitial spaces between necrotic and quiescent cells.
- Tumor cells typically have larger interstitial spaces than healthy cells
- Particles collect in center bringing therapeutics to kill the tumor from inside out.



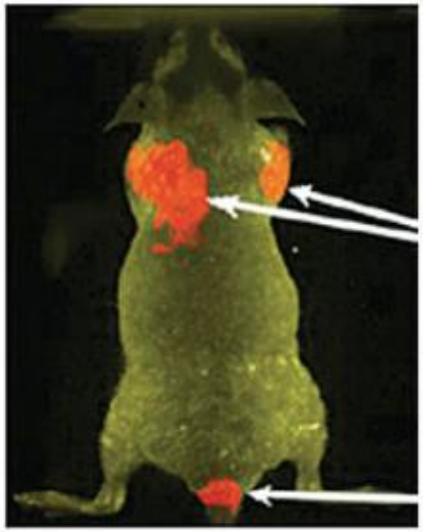


(*a*)


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)

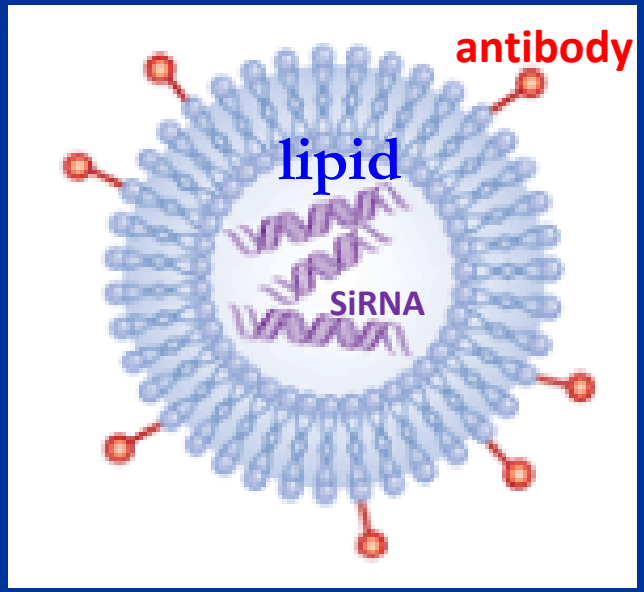
(*b*)


Tumors
Injection site

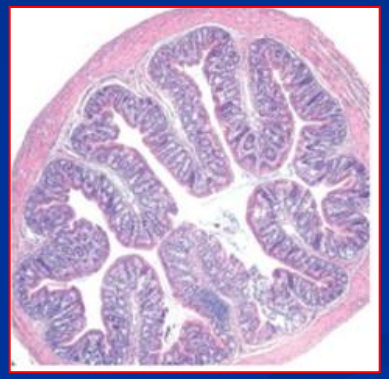
A Drug Delivery Nanoparticle

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)

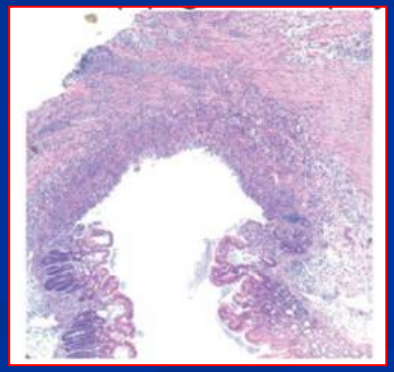
B.



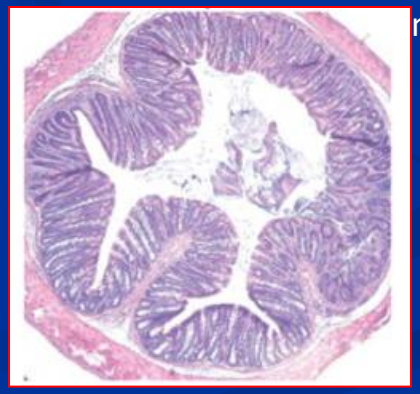
C.



Healthy tissue



Sick tissue treated with non-targeted nanoparticles



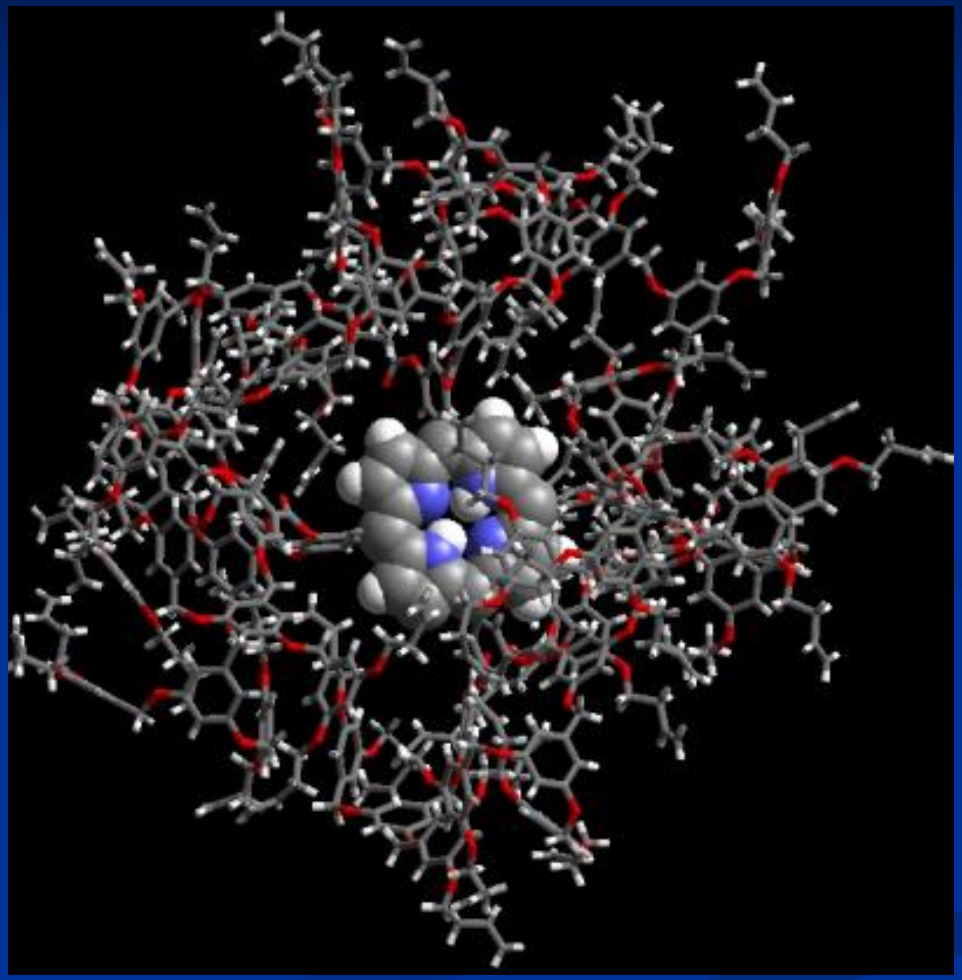
Sick tissue treated with targeted nanoparticles

Science 2008, Vol. 316, pp 627-630

Dendrimers

Dendritic polymers = Dendrimers

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



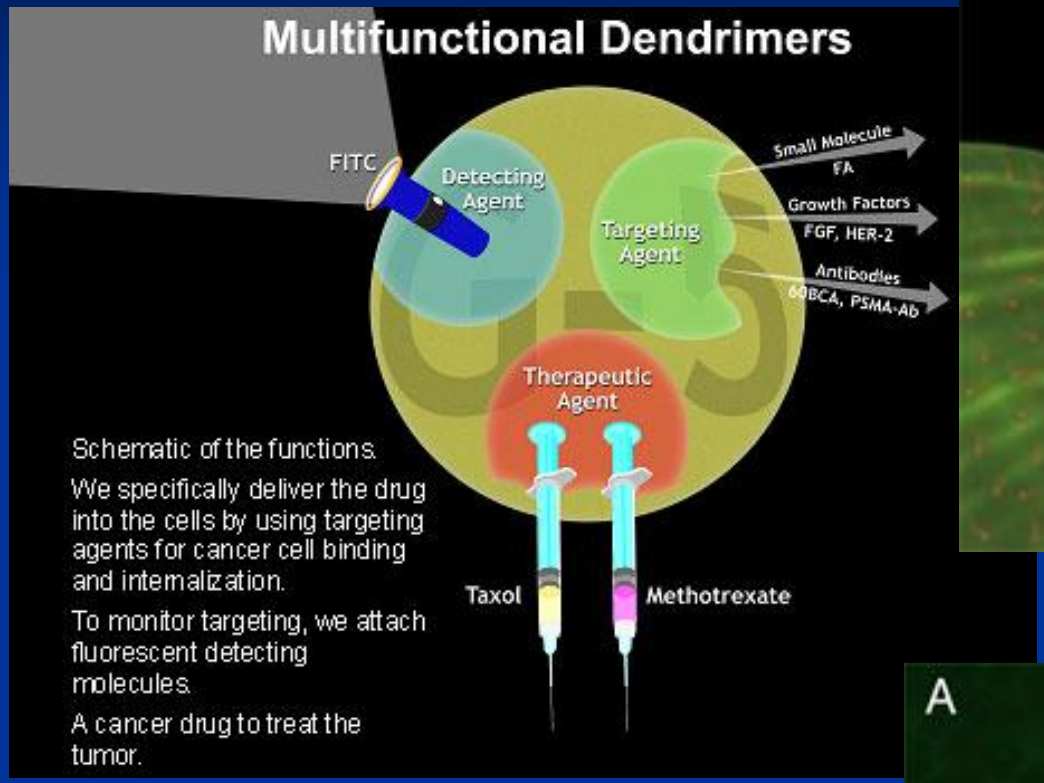
Highly branched structures –

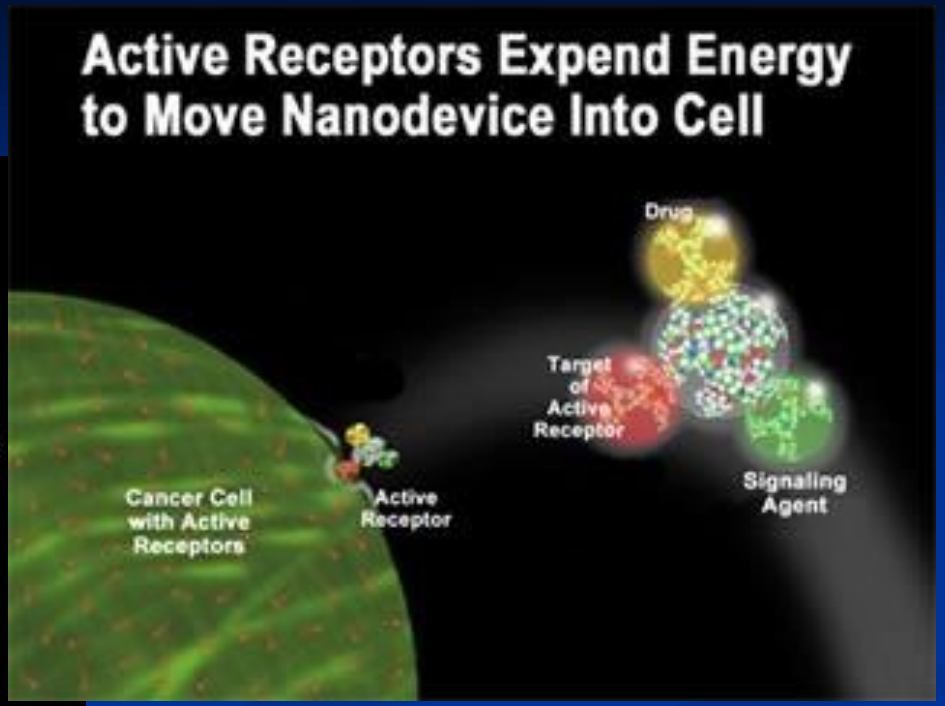
Molecular "hooks" – to attach

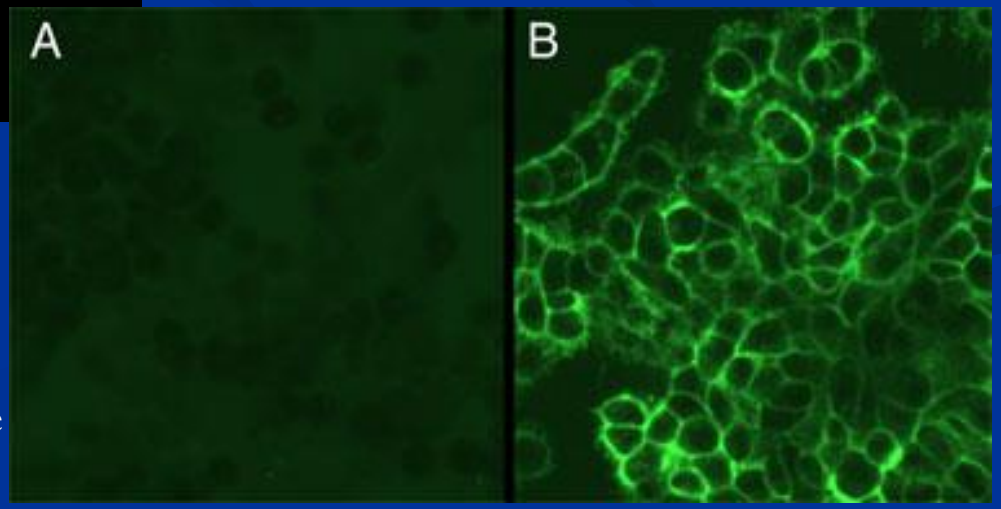
Cell identification tags, fluorescent dyes, enzymes

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

Dendrimers & Therapeutics

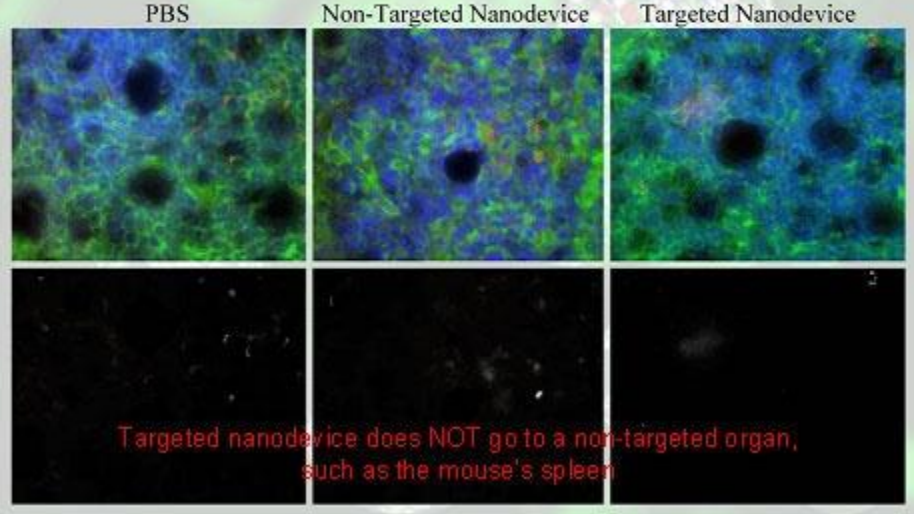




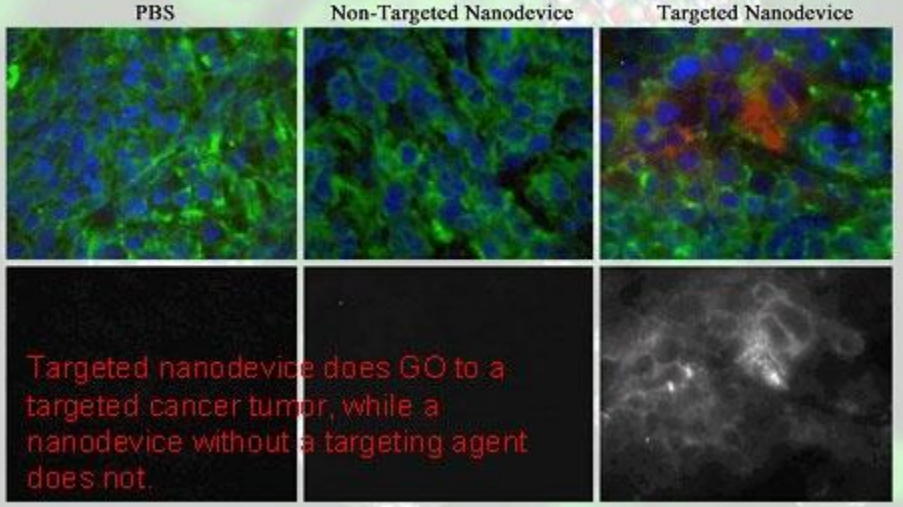


The Michigan Nanotechnology Institute for Medicine and Biological Sciences

Spleen
(a non-targeted organ)



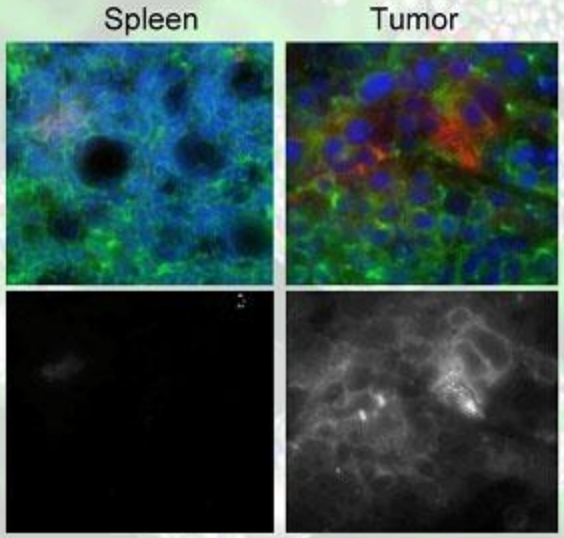
Cancer Tumor
(targeted)



In Vivo Study: drug study in animals

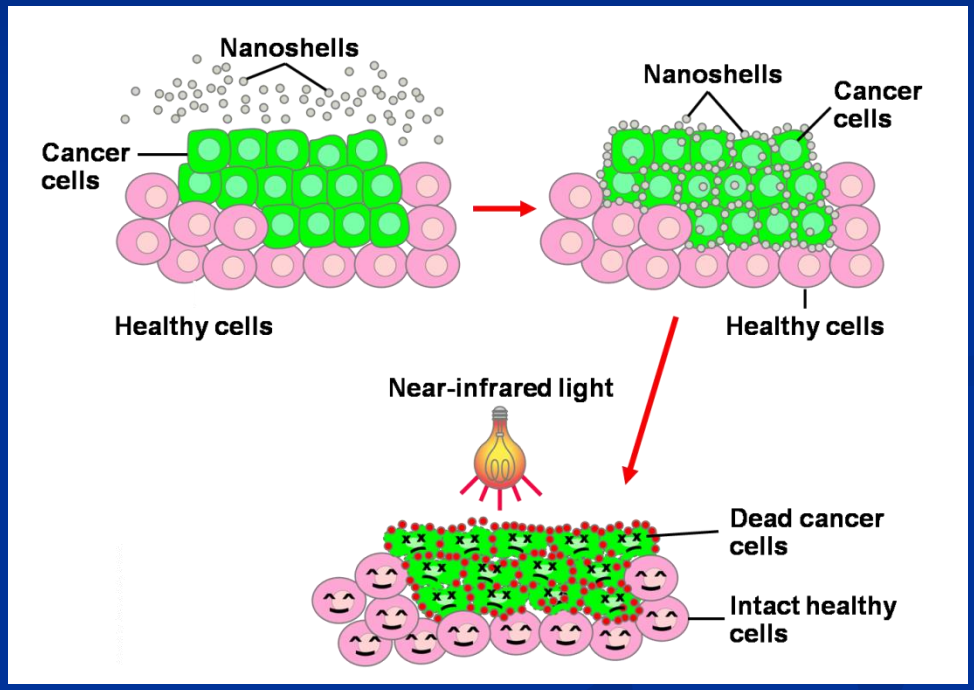
- **Mice that received conventional drug: Free MTX**
  - Lost hair (shutdown of protein synthesis)
  - Lost weight (general toxicity)
  - Non-necrotic tumors, no tumor reduction unless high dosis: **drug ineffective**
- **Mice that received drug in targeted Nanodevice**
  - Retained hair
  - No weight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

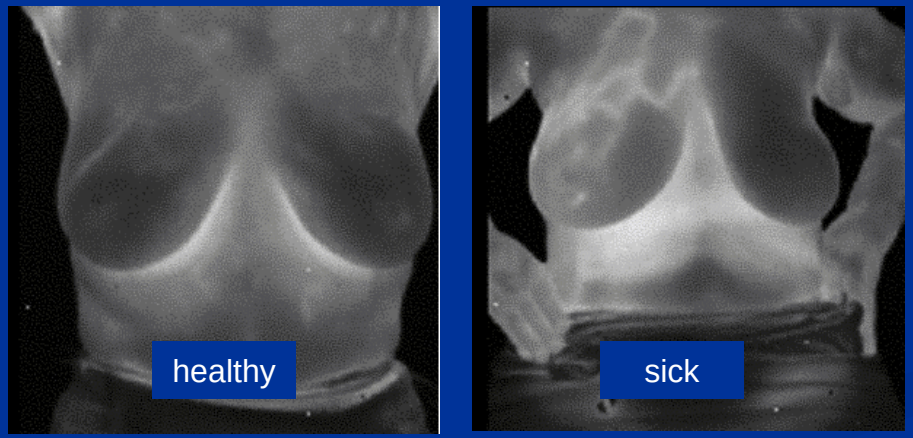


Thermal ablation of cancer cells assisted by nanoshells coated with metallic layer and an external energy source – *National Cancer Institute*

Diagnosis using Nanothermometers

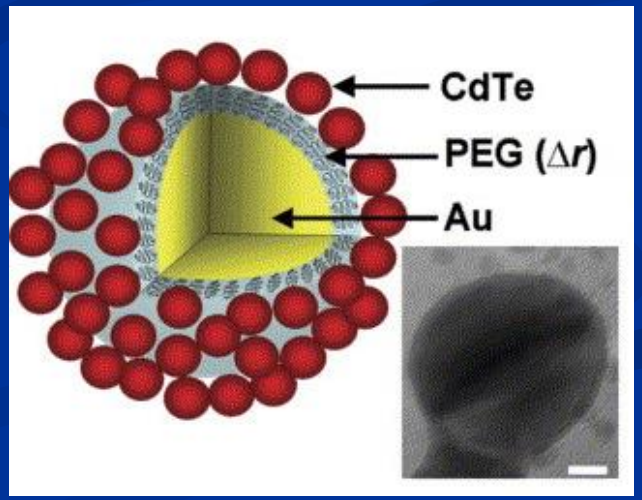
Cancer cells appears to have a more elevated temperature than normal cells. Therefore, a local temperature mapping can be used to determine the spread of a tumor

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



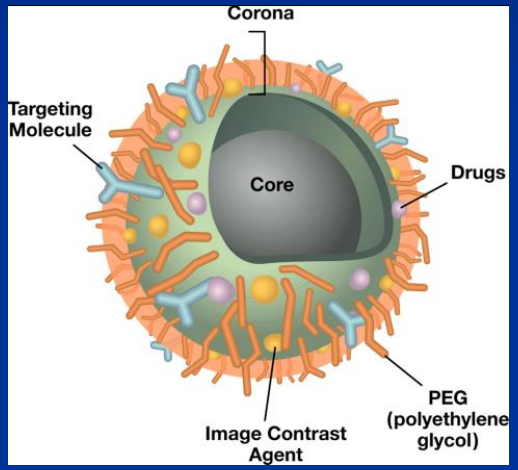
Thermal image of a healthy and cancerous breast

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

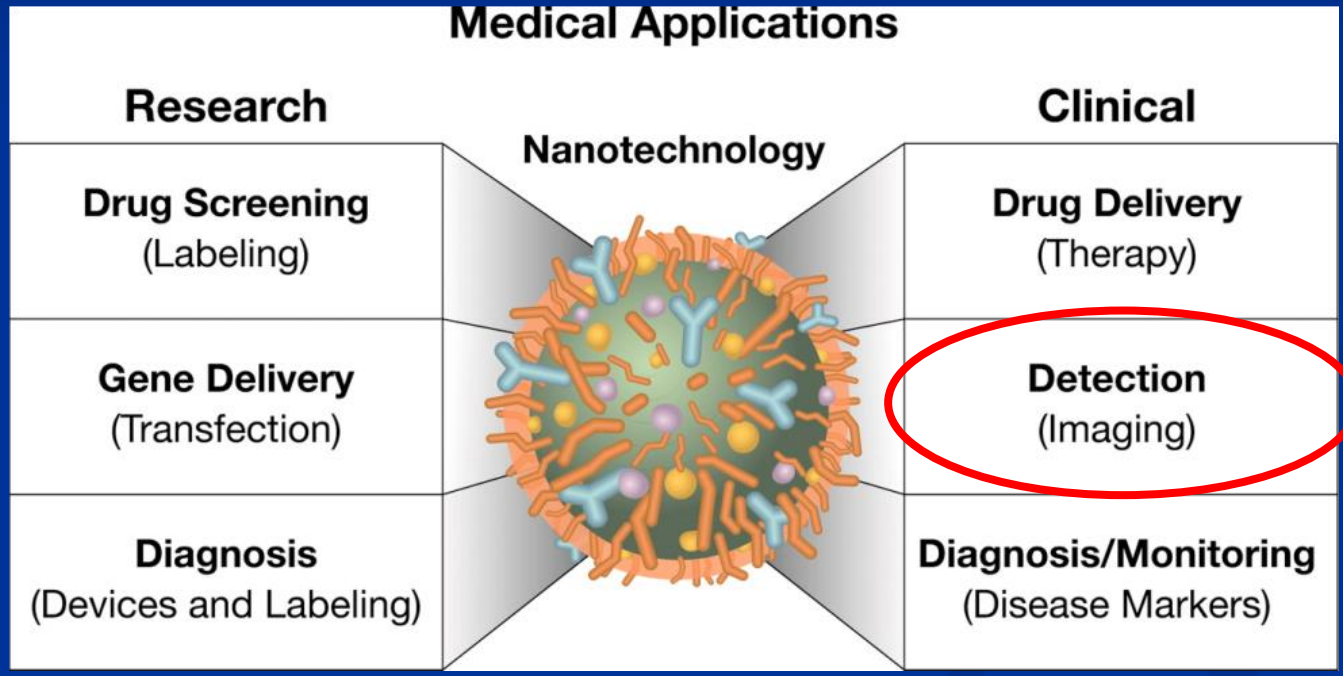


Angew. Chem. Int. Ed. 2005, Vol. 44, 7439 –7442

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



Functionalized nanoparticles

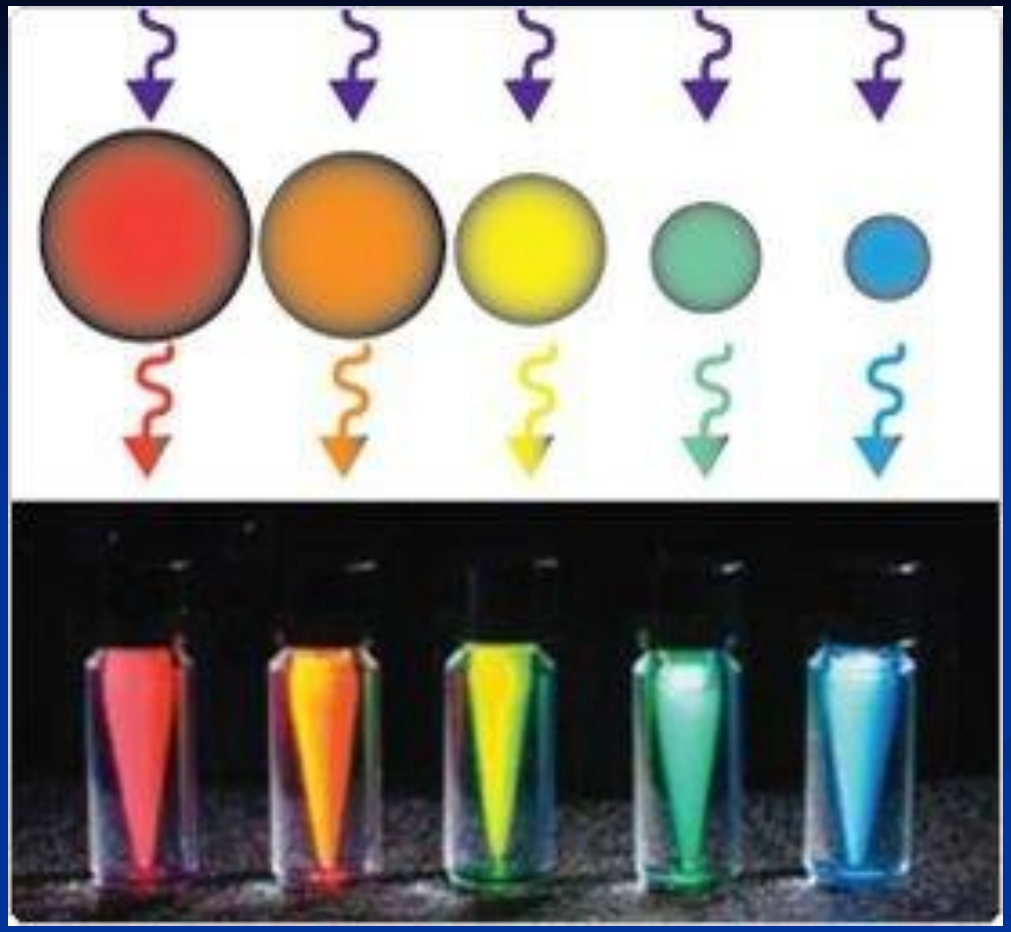


•McNeil SE. *J Leukoc Biol*, 2005. **78**(3): p. 585-94.
•doi:10.1189/jlb.0205074

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).
- **Nanocrystals** can produce distinctive colors determined by the size of the particles: **Fluorescence**.

Enables long-term imaging experiments.



Excitation

Emission

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.

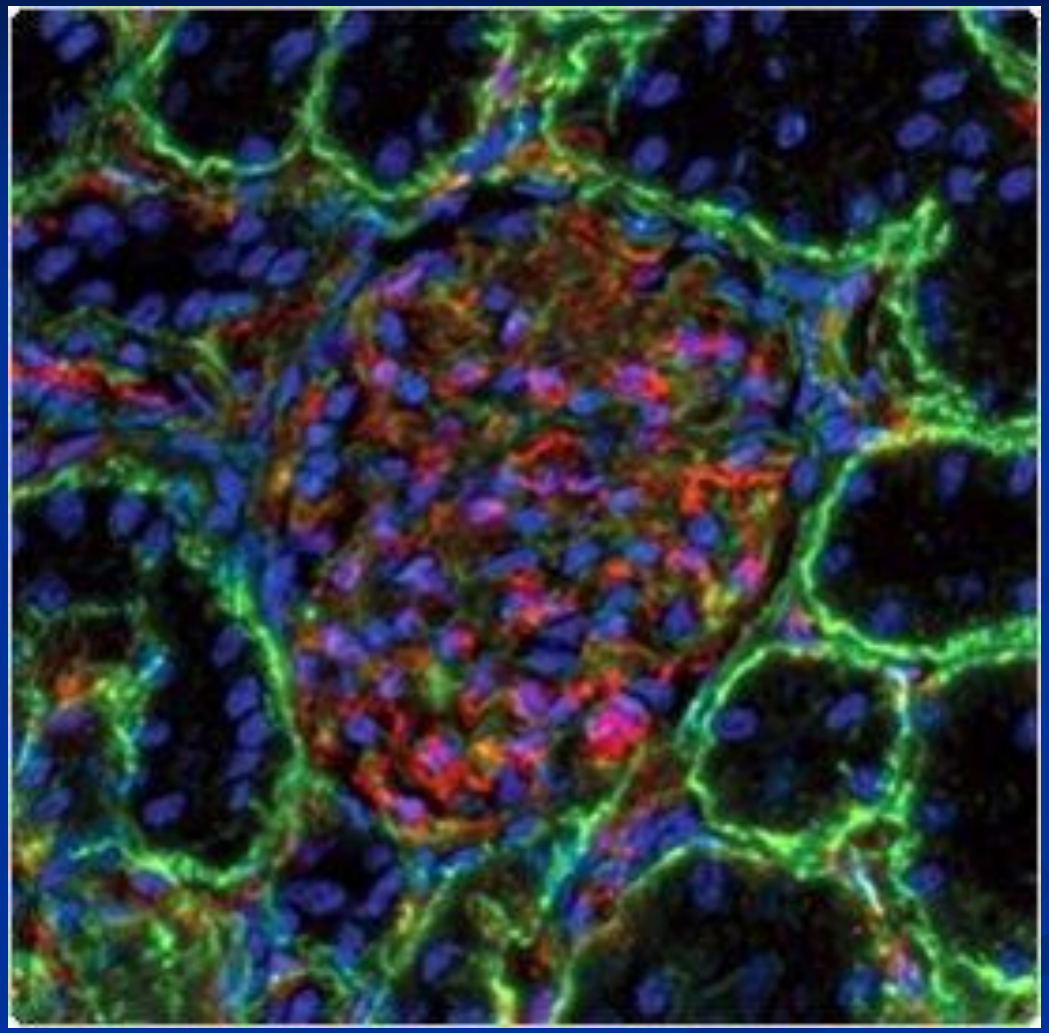
Quantum Dot Bioconjugate

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.

Laminin in a mouse kidney section was labeled with an anti-laminin primary antibody and visualized using green-fluorescent Qdot® 565 IgG.

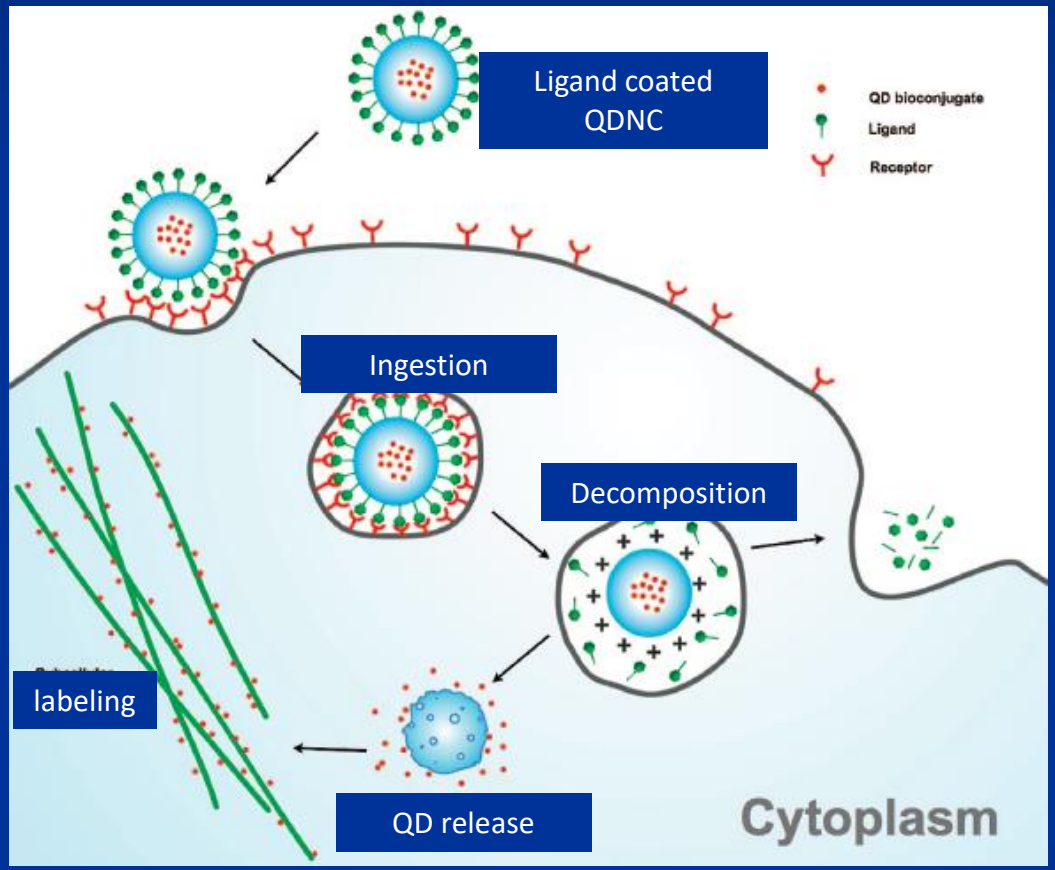
PECAM (platelet/endothelial cell adhesion molecule; CD31) was labeled with an anti–PECAM-1 primary antibody and visualized using red-fluorescent Qdot® 655 IgG.

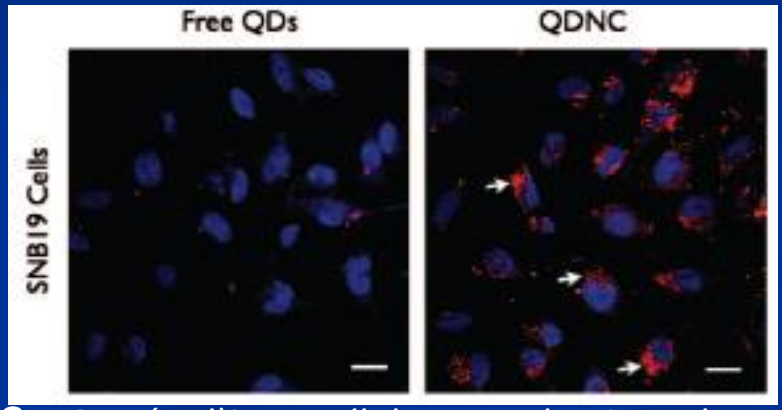
Nuclei were stained with blue-fluorescent Hoechst 33342.



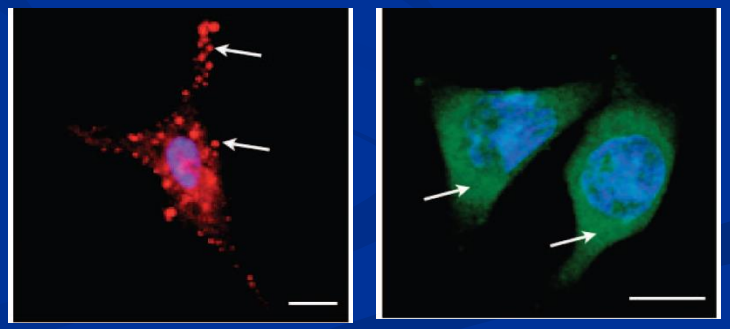
Targeting QD's for intracellular imaging

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



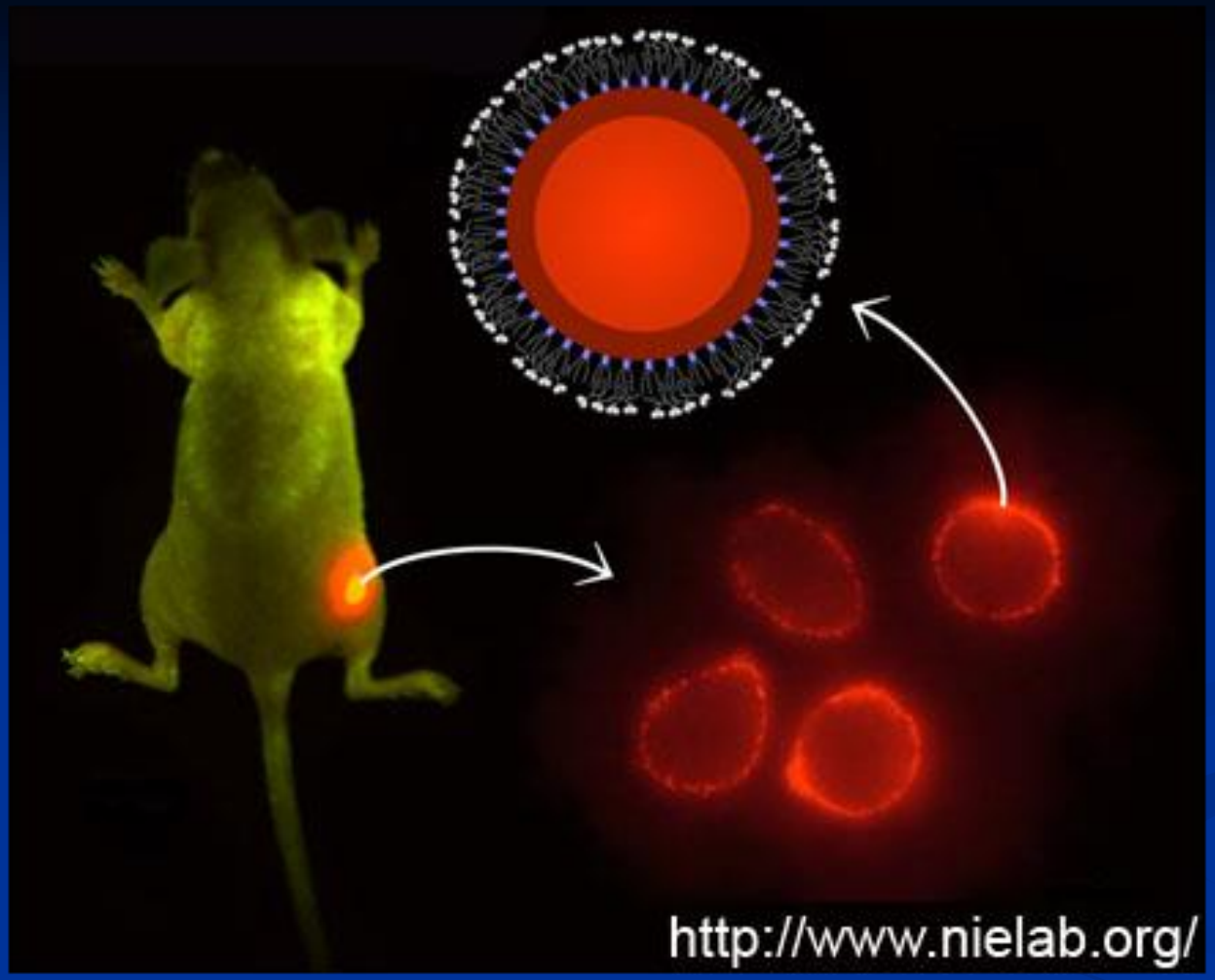


C. *QD (red)intracellular uptake is enhanced when using the QDNC instead of the free QD's*



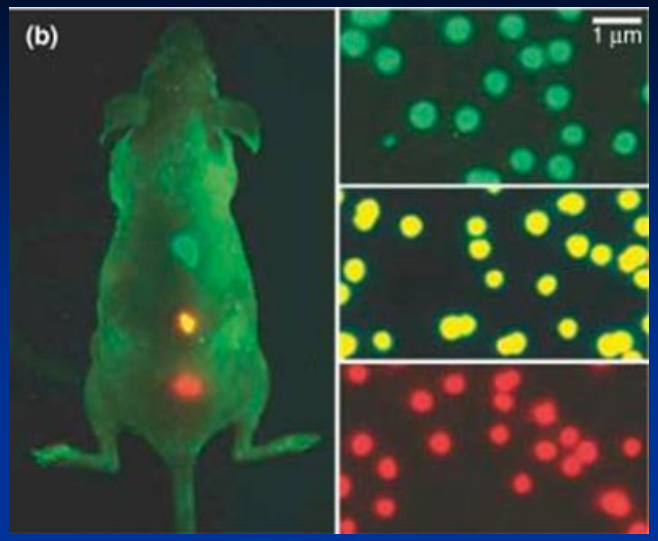
D. *Imaging of nucleus (blue) and cytoplasm (other) after 30 min (left) and 3 hours after uptake*

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



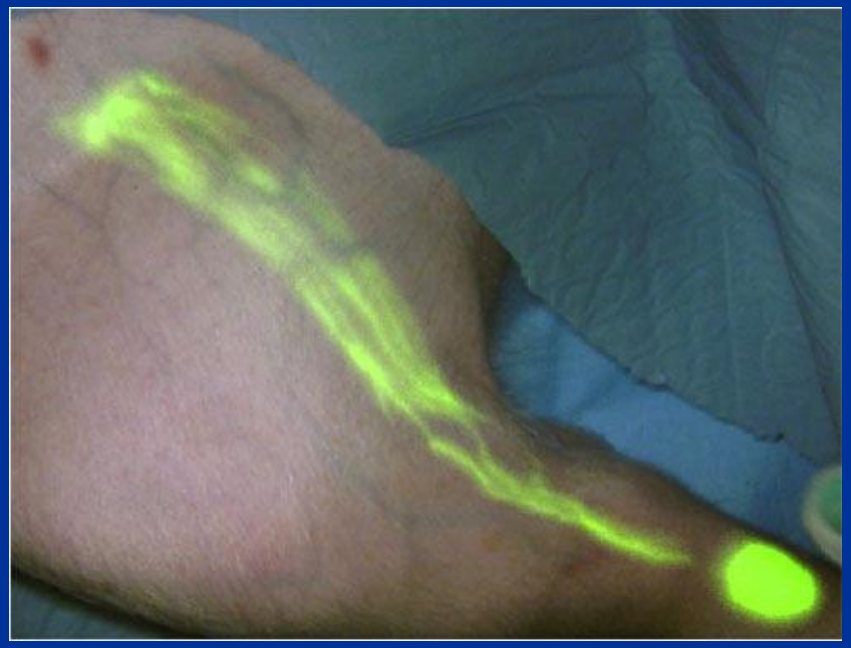
Water soluble quantum dots to image sentinel lymph nodes which are used for diagnosing breast cancer.

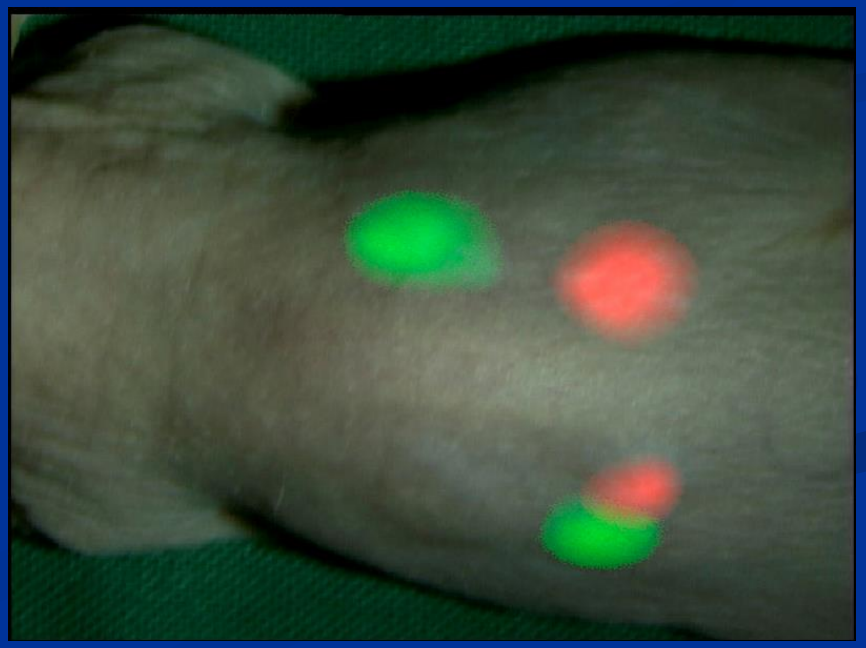
Antibody-Modified Quantum dots for the sensitive imaging of the tumour tissue on a tumour-bearing mouse.



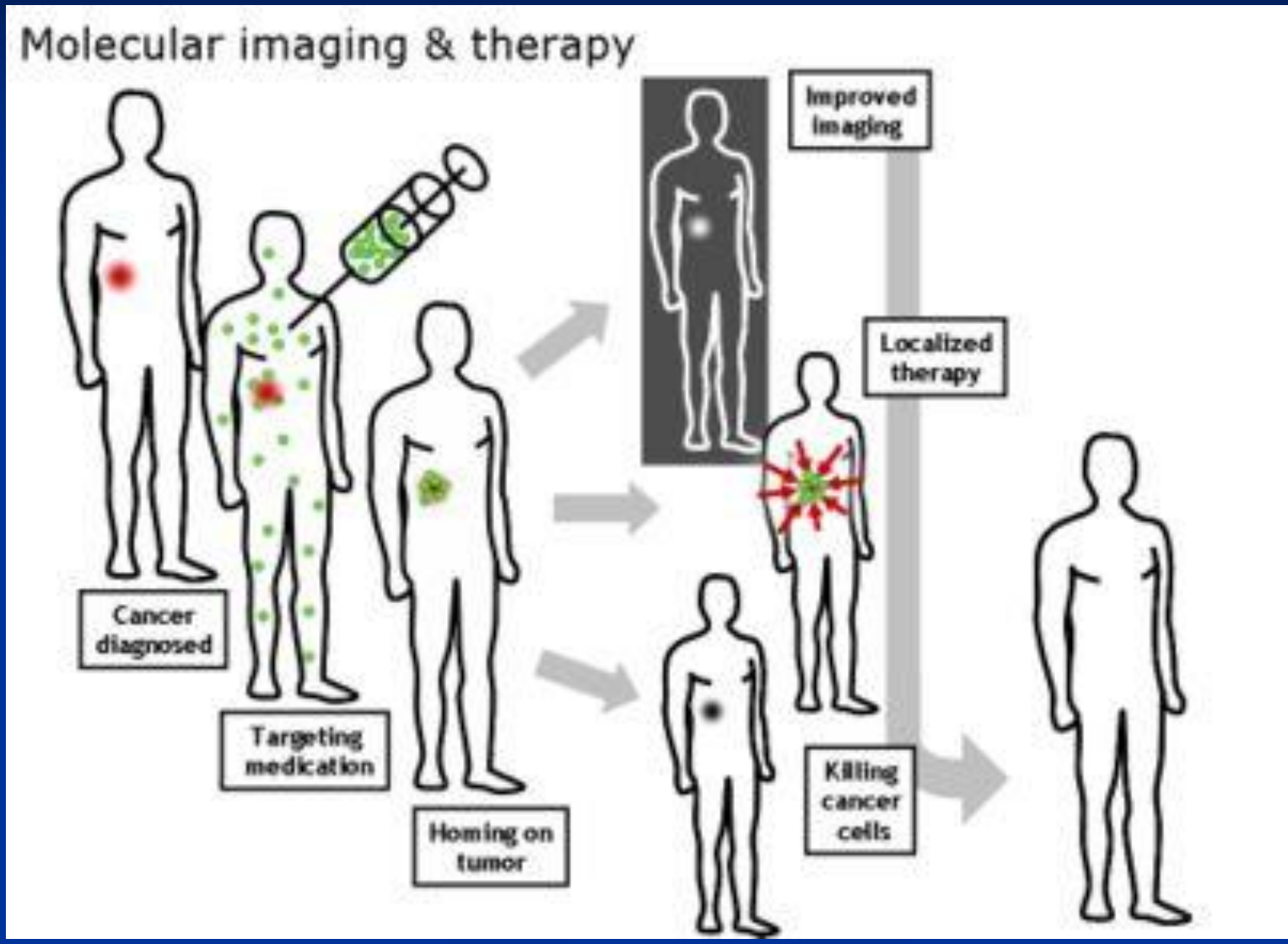
Nanotech in Disease Imaging & Therapeutic Monitoring

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

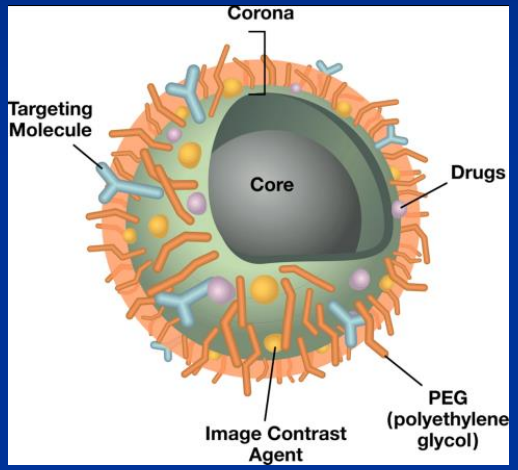




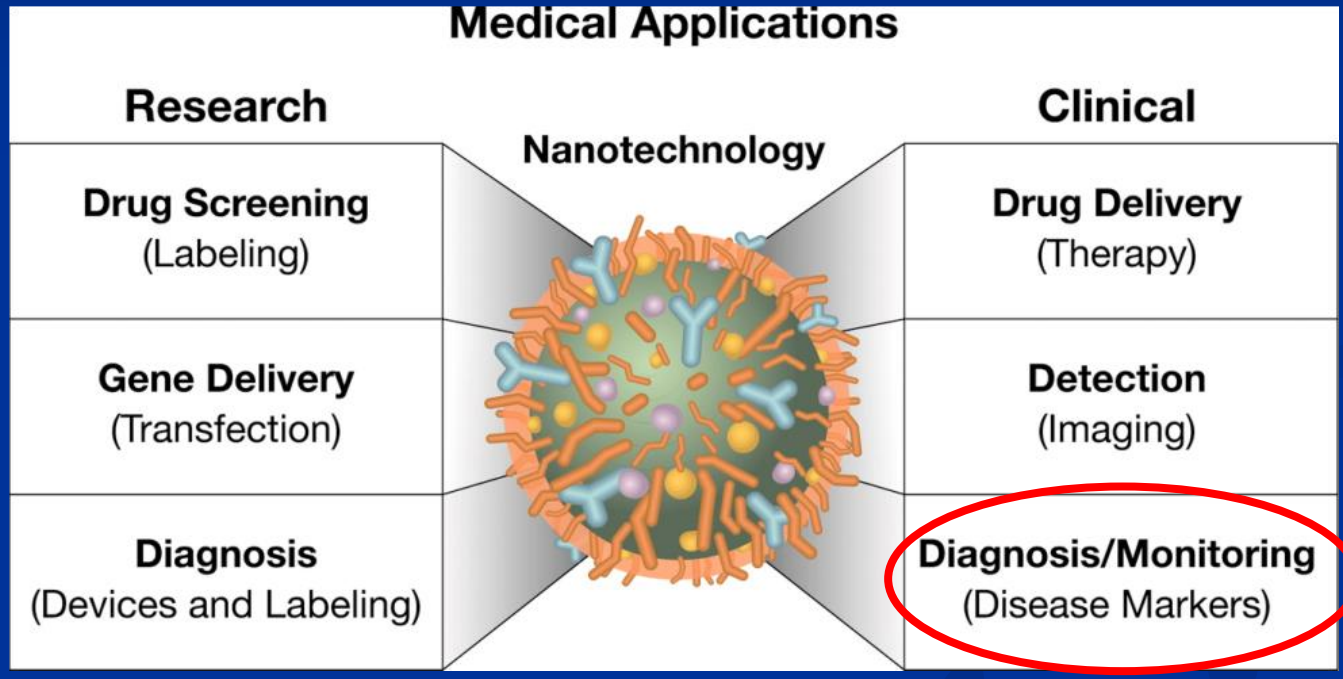
Imaging


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

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



Functionalized nanoparticles

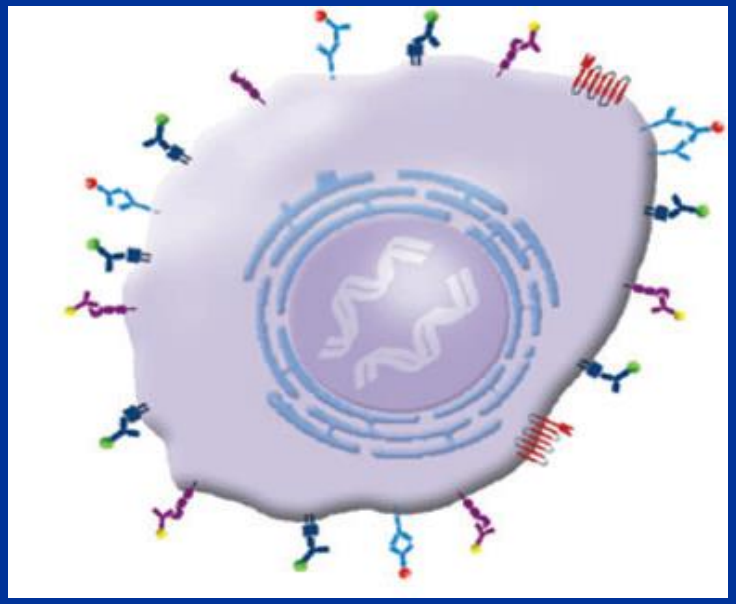


•McNeil SE. *J Leukoc Biol*, 2005. **78**(3): p. 585-94.
•doi:10.1189/jlb.0205074

Diagnosis

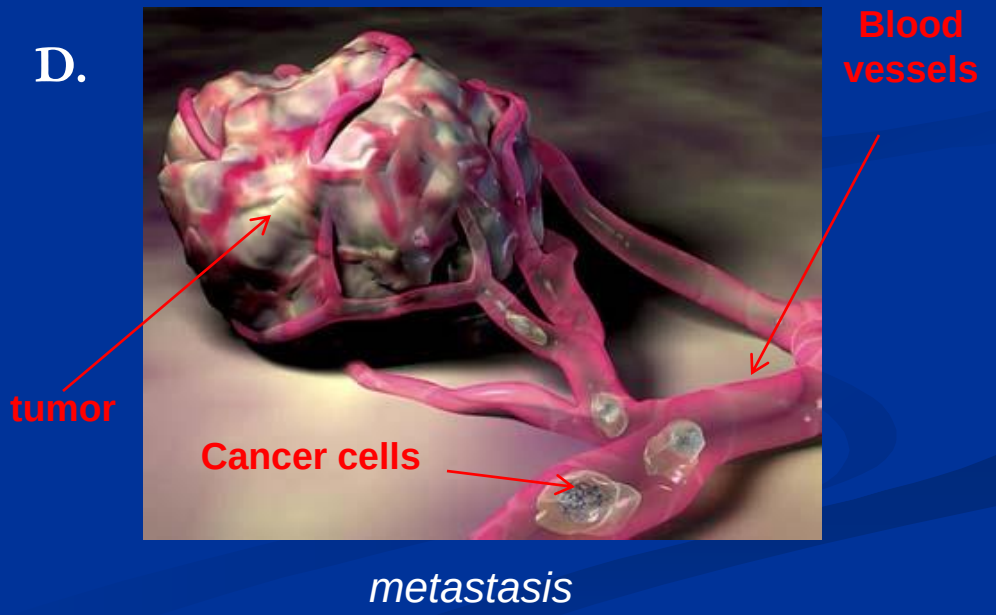
A. Detection of multiple biomarkers simultaneously

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.



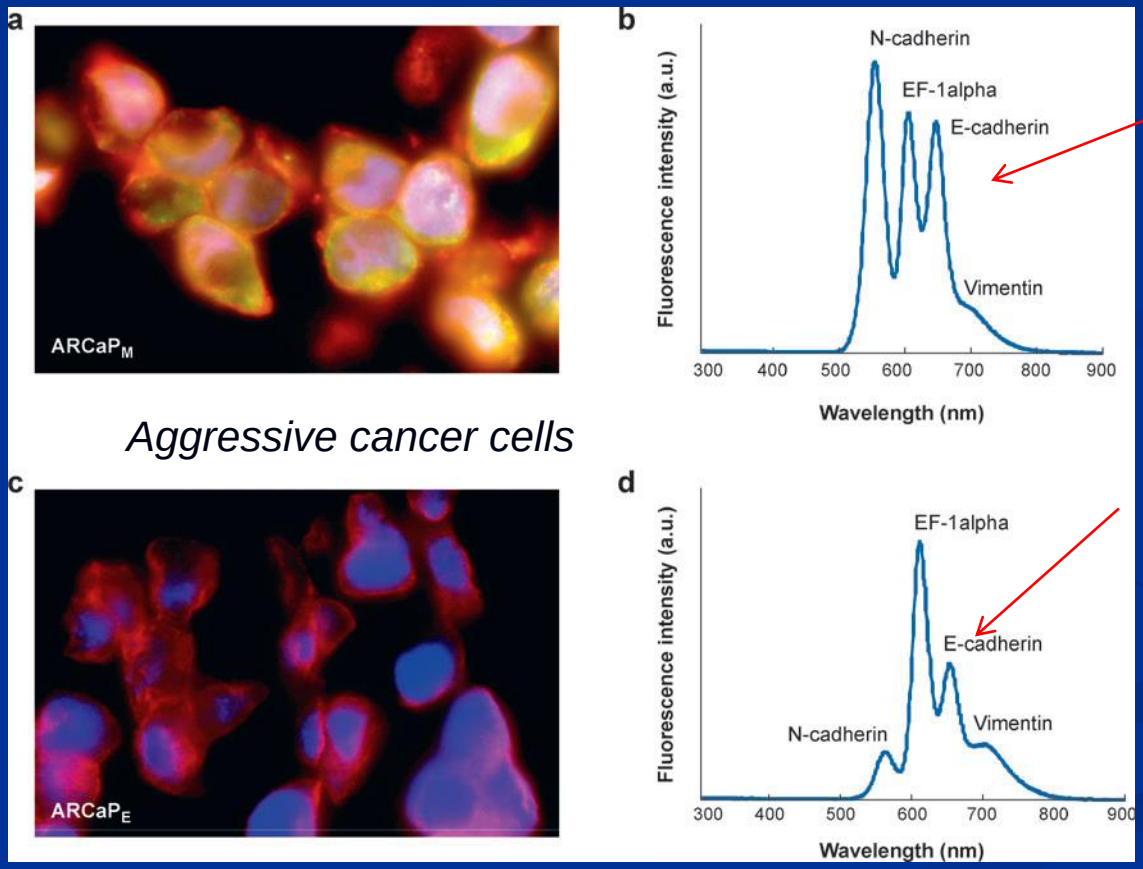
metastasis

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

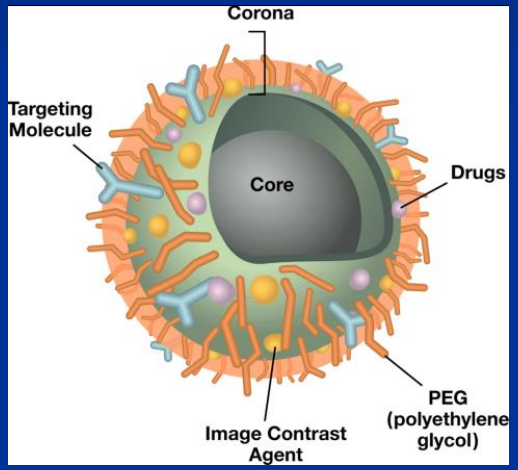
Mild cancer cells

The peak intensity correlates to the concentration of a specific QD

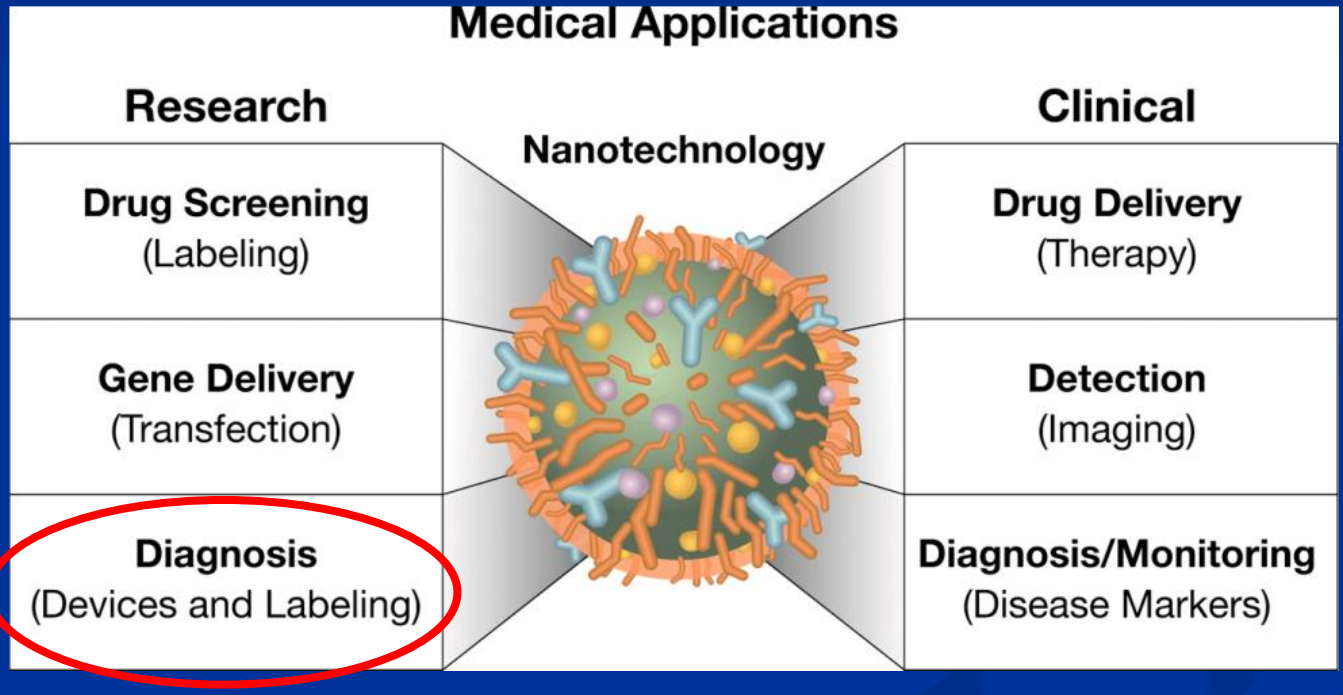
Each peak correspond to the emission of a specific QD/antigen

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

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



Functionalized nanoparticles

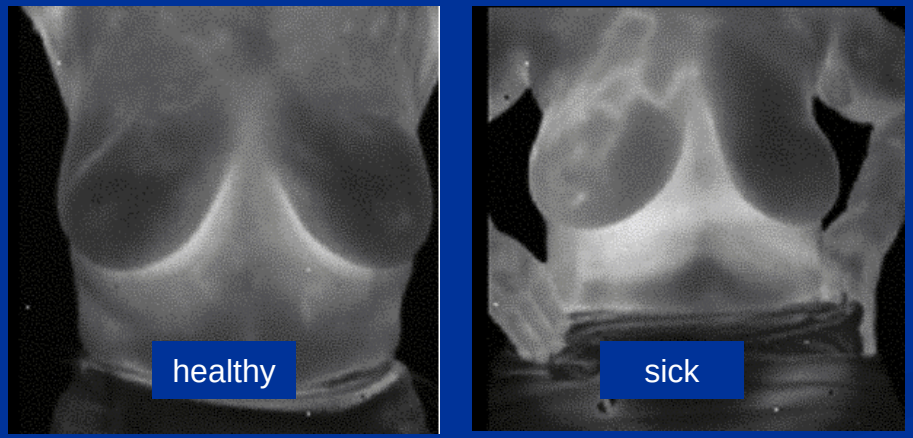


•McNeil SE. *J Leukoc Biol*, 2005. **78**(3): p. 585-94.
•doi:10.1189/jlb.0205074

Diagnosis using Nanothermometers

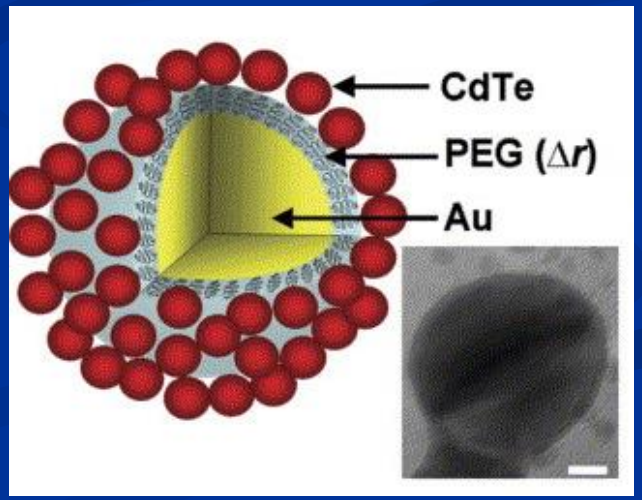
Cancer cells appears to have a more elevated temperature than normal cells. Therefore, a local temperature mapping can be used to determine the spread of a tumor

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

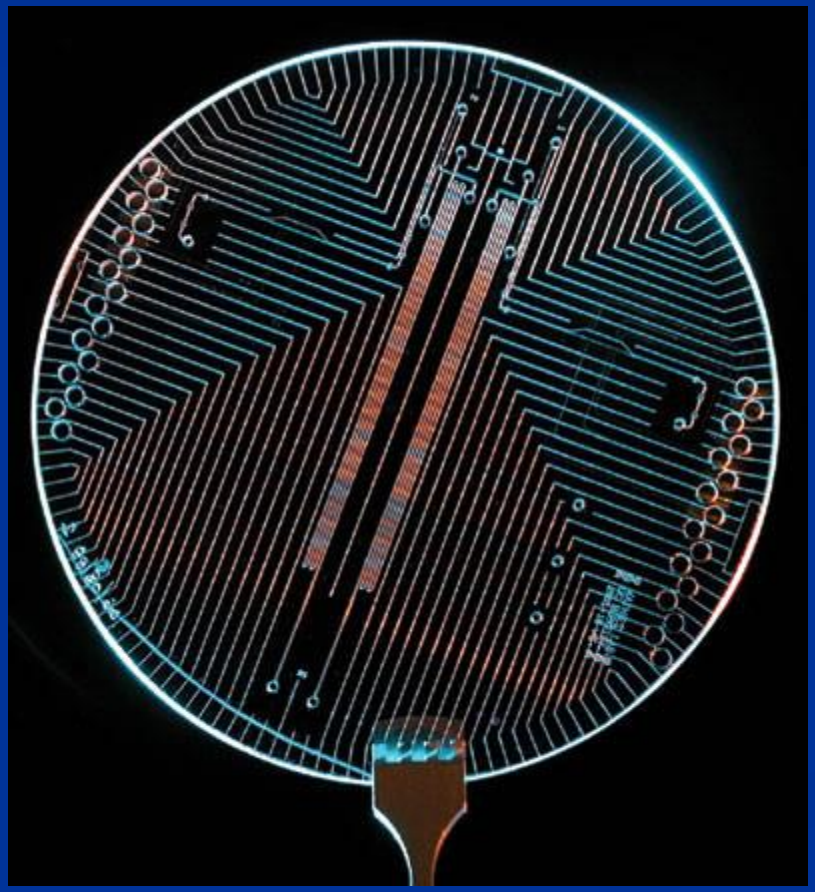
•Source: 9th European Congress of Thermology, Krakow, Poland

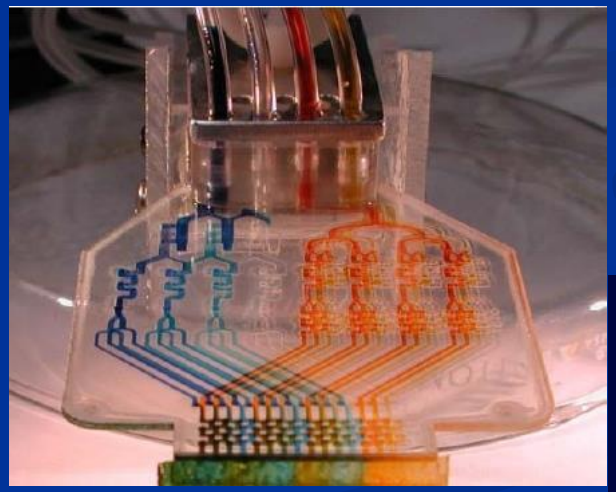


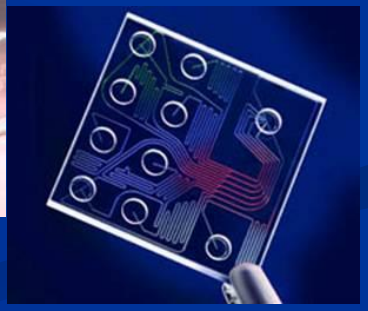
Angew. Chem. Int. Ed. 2005, Vol. 44, 7439 –7442

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



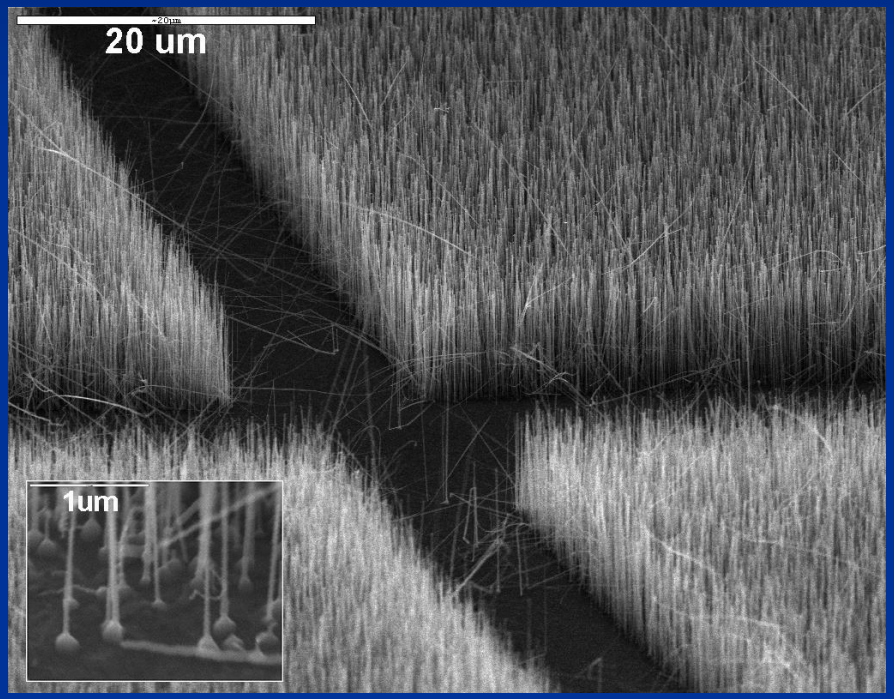




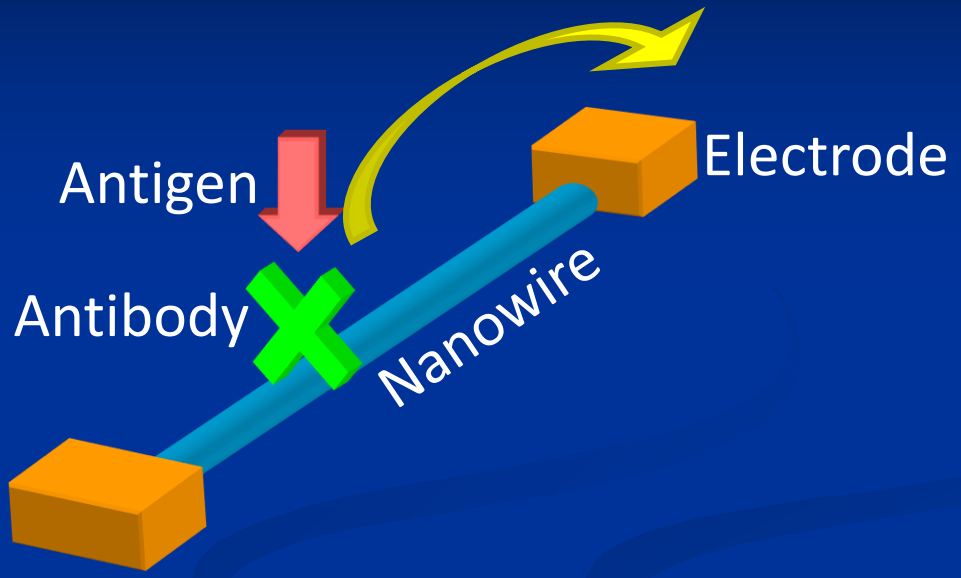
- Credits: Mathies Lab, UC-Berkeley
- Quake Lab, Stanford
- Agilent, Inc.

Diagnostics – Biosensors

Novel Materials



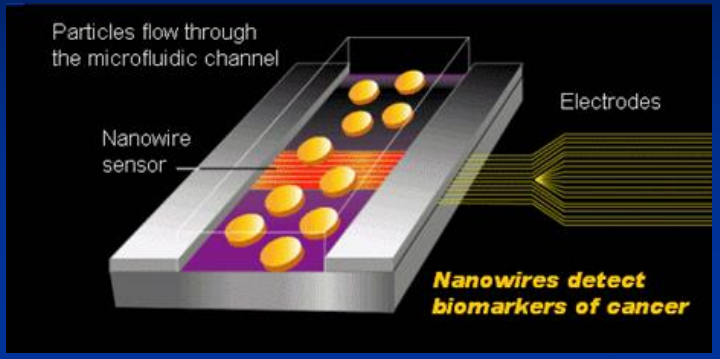
ZnO nanowires



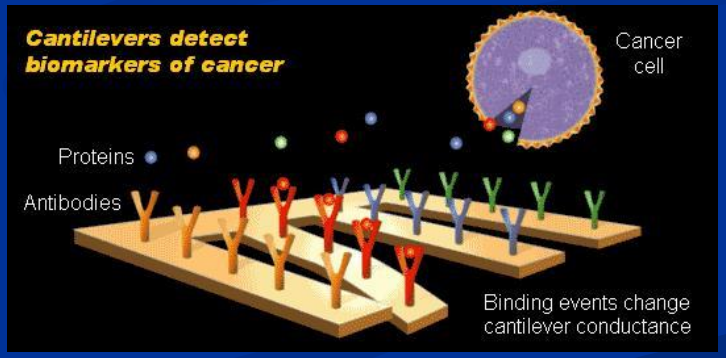
Ultra-sensitive biosensor for the detection of bio-markers using bio-compatible ZnO nanowires.

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

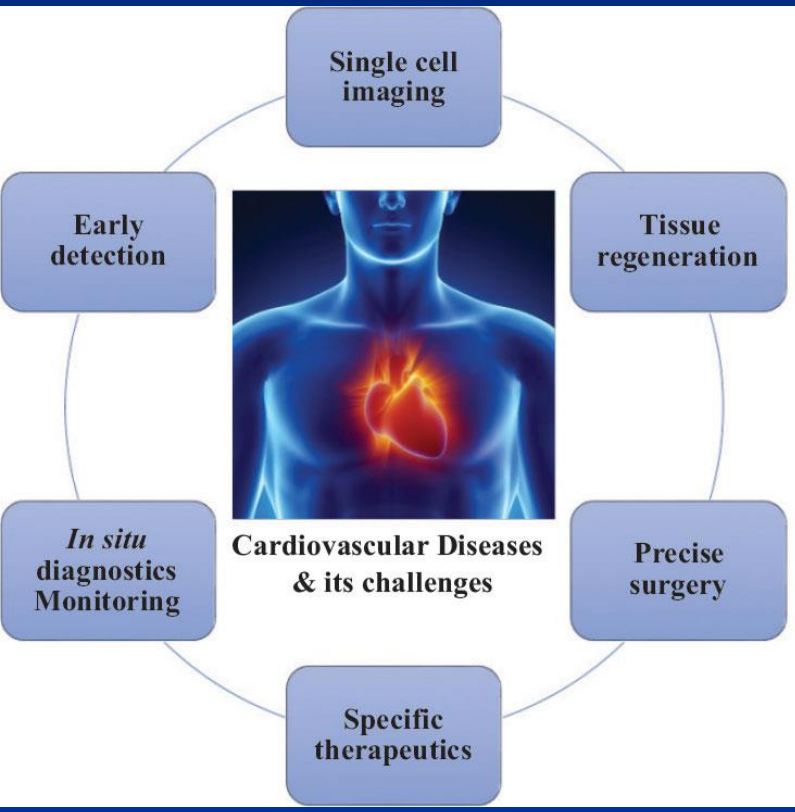


The microfluidic channel with nanowire sensor can detect the presence of altered genes associated with cancer *– J. Heath, Cali. Insti. of Technology*

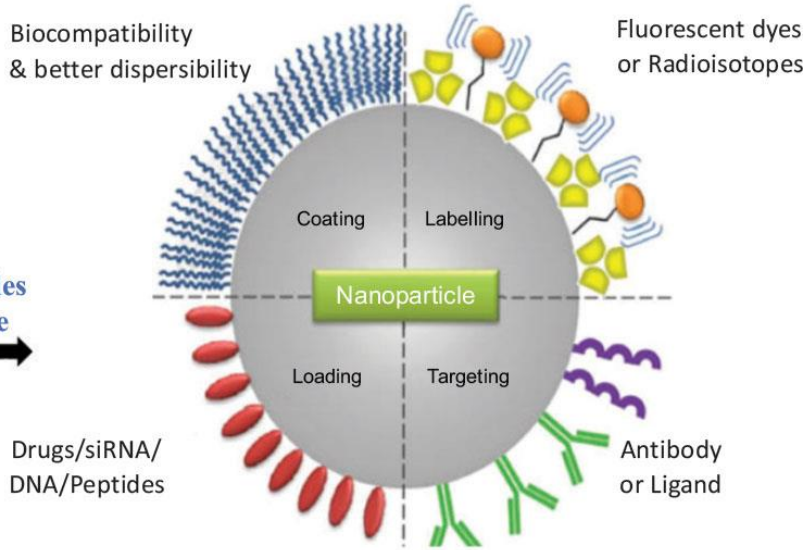


The nanoscale cantilever detects the presence and concentration of various molecular expressions of a cancer cell
– A. Majumdar, Univ. of Cal. at Berkeley

Nanomedicine in Atherosclerosis

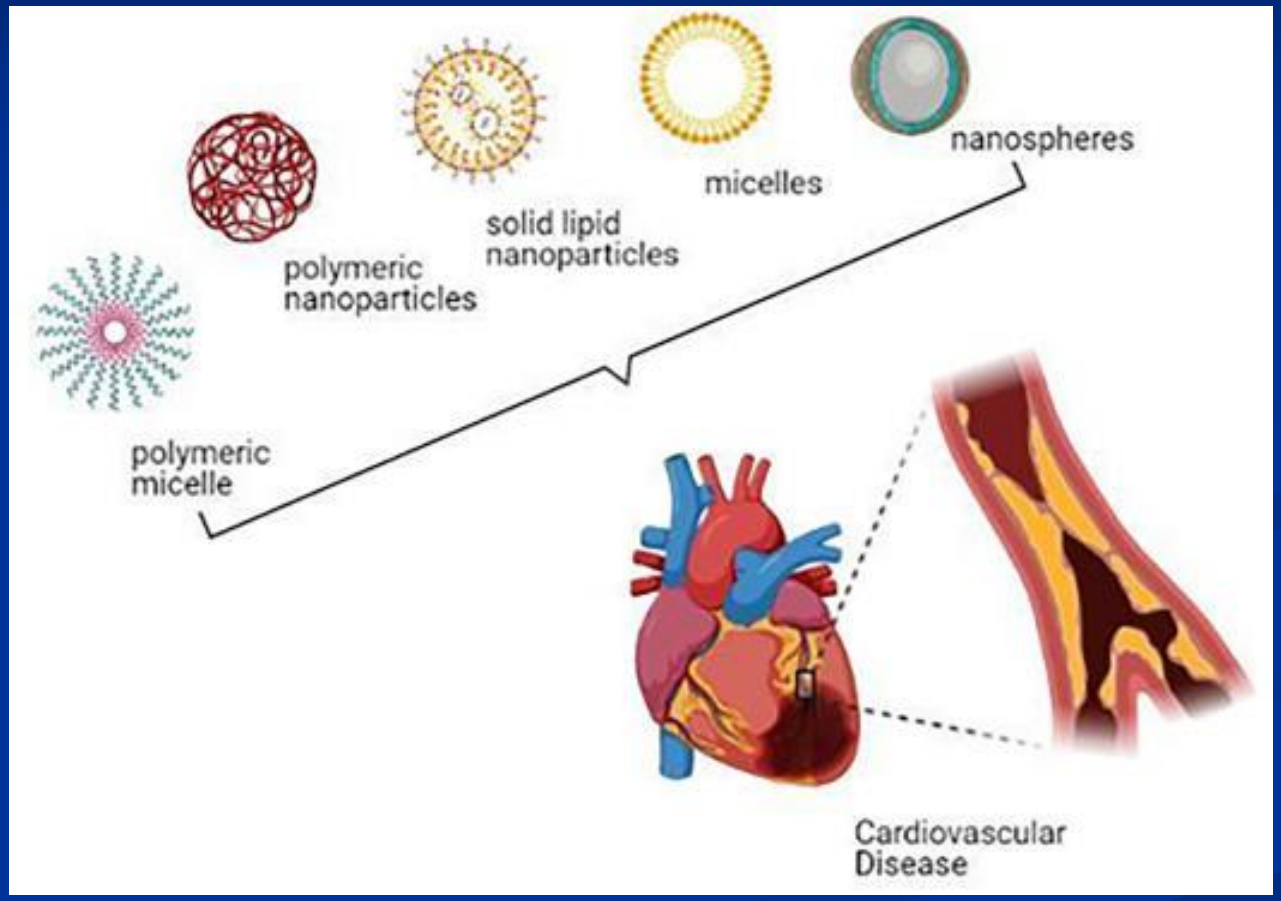


Treatment possibilities with Nanomedicine



Surface modified Nanoparticles with different geometry, size and functional property

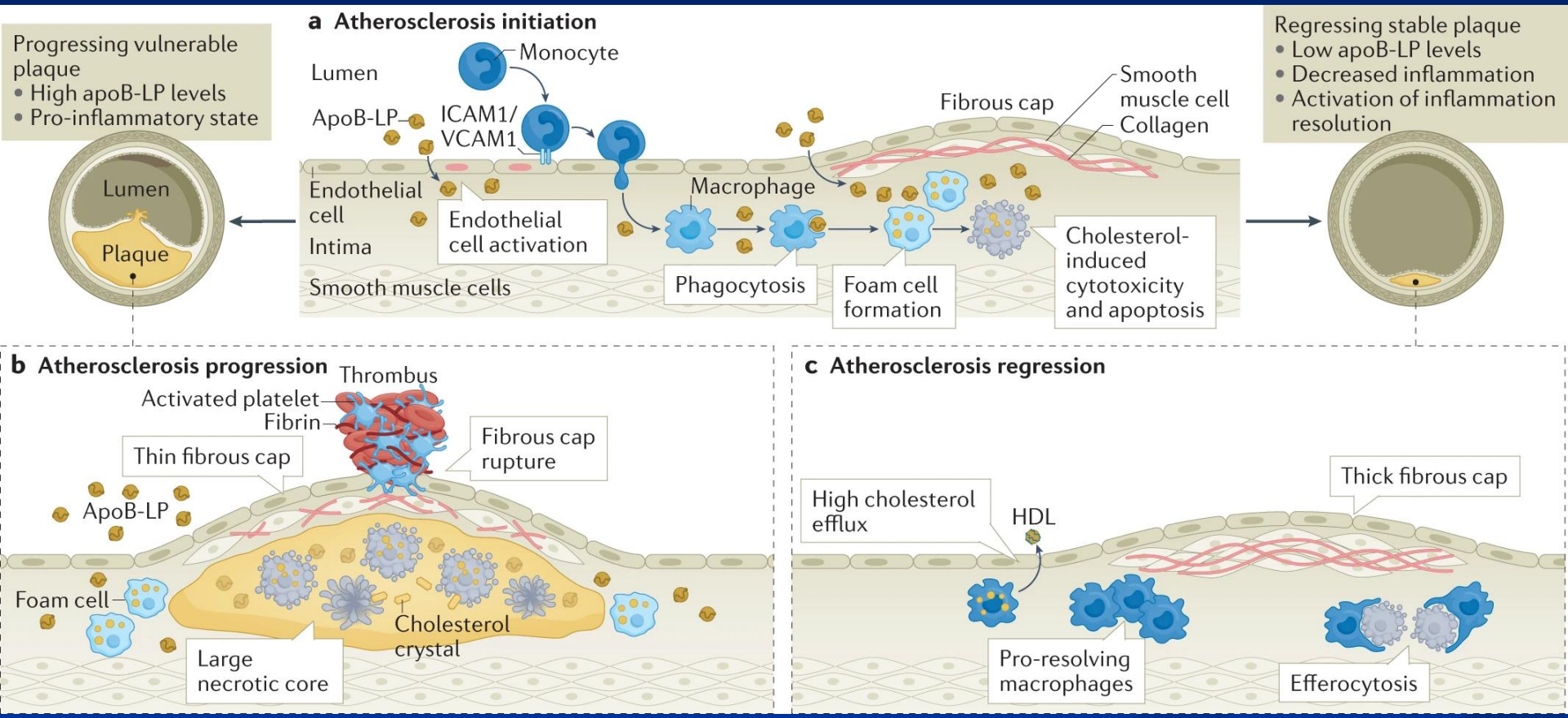
Nanomedicine in Atherosclerosis



Macrophage-targeted nanomedicine for the diagnosis and treatment of atherosclerosis

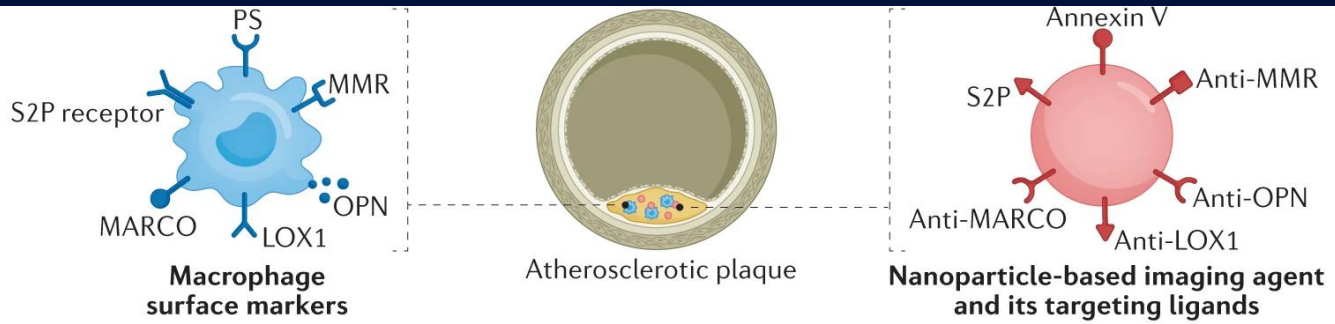
Wei Chen[1], *Maaike Schilperoort*[2,3,4], *Yihai Cao*[5], *Jinjun Shi*[1] ✉, *Ira Tabas*[2,3,4] ✉ *and Wei Tao*[1] ✉

Atherosclerosis & Macrophages

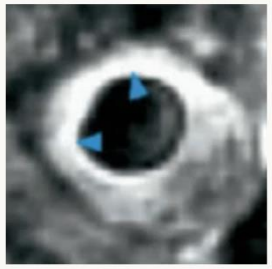
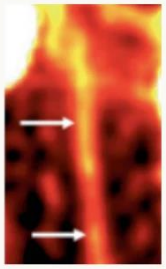
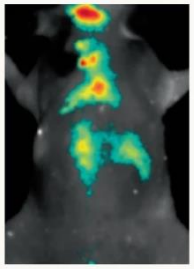
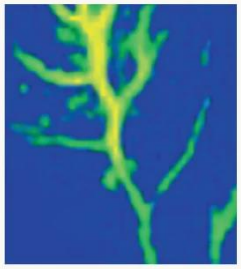


Non-invasive bioimaging technologies facilitate the visualization of high-risk atherosclerotic plaques with high spatiotemporal resolution.

a

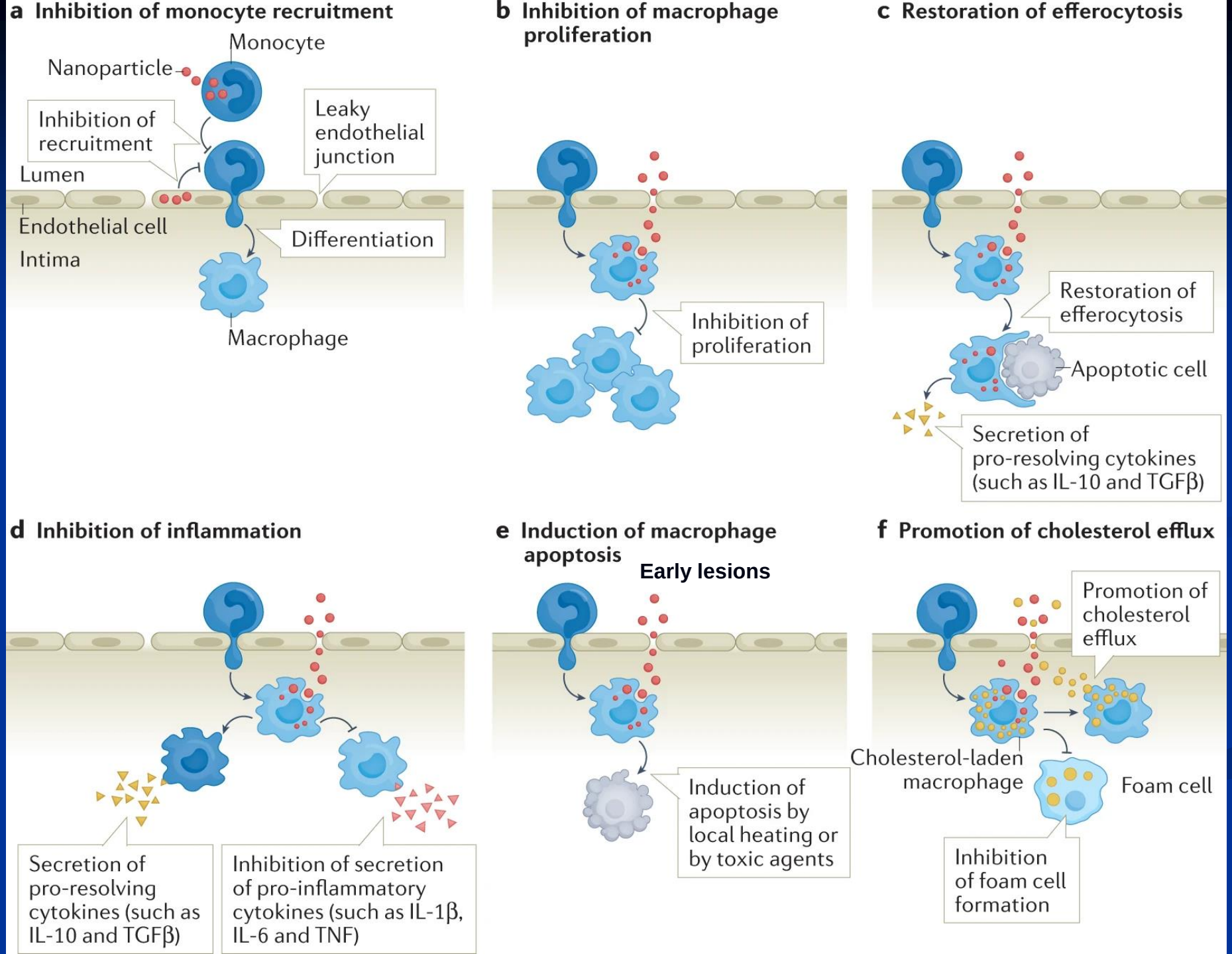


b

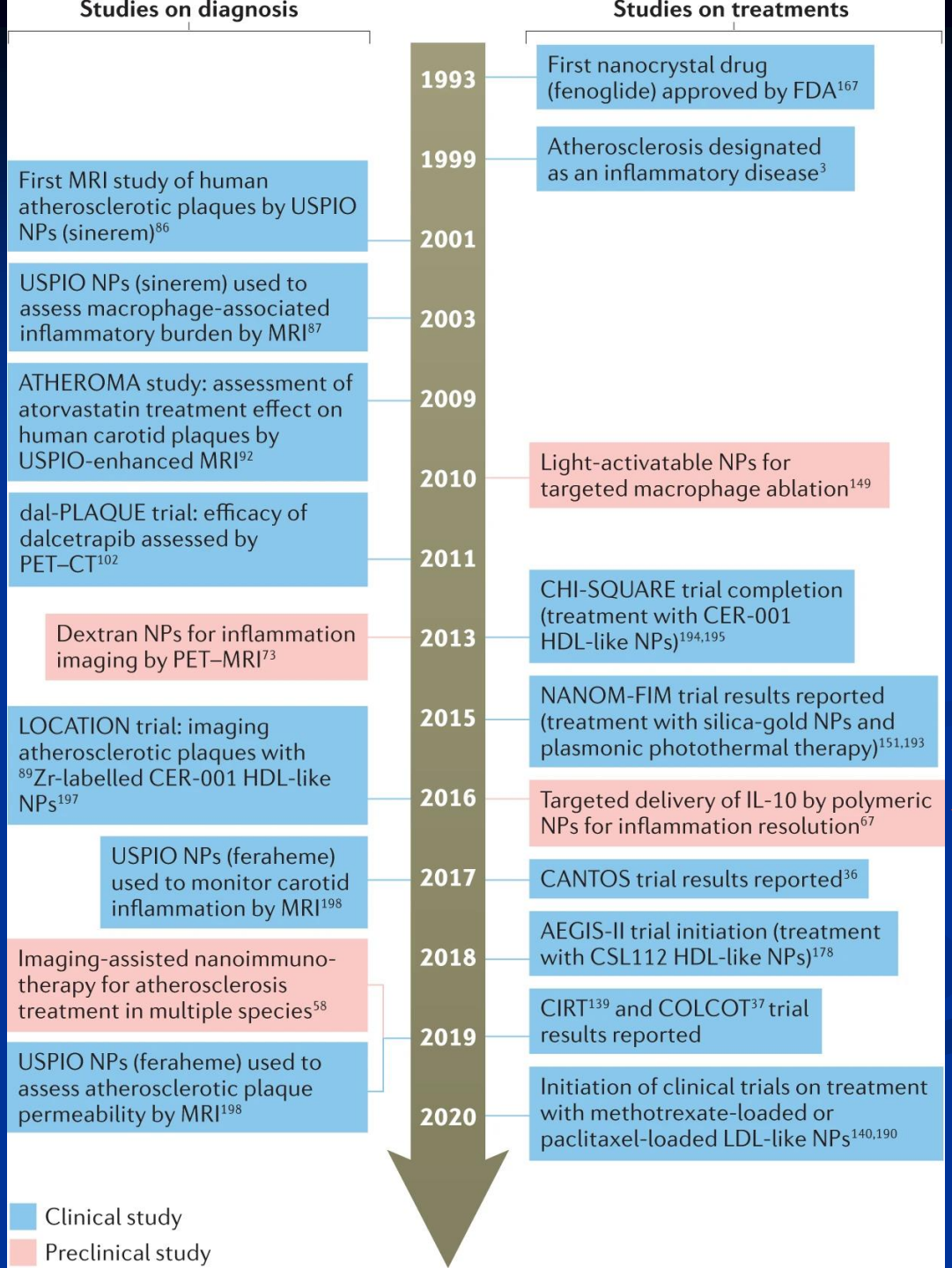
	MRI	CT	PET	FI	PAI
Advantages	• High spatial resolution (μm) • Excellent soft-tissue contrast	• High spatial resolution (μm) • Short scan time (5–15 min)	• Extremely high sensitivity (pmol/l)	• High spatial resolution (μm) • Short scan time (1–5 min)	• Multiple wavelength scan • Short scan time (1–5 min)
Disadvantages	• Long scan time (30–60 min)	• Low sensitivity (mmol/l)	• Low spatial resolution (mm) • Lack of anatomical reference	• Limited tissue penetration depth (μm–mm)	• Limited tissue penetration depth (μm–mm)
Imaging agents	• Iron oxide nanoparticles • Gd-containing nanoparticles	• Iodinated nanoparticles • Au nanoparticles	• ^{18}F, ^{89}Zr or ^{68}Ga-labelled nanoparticles	• Upconversion nanoparticles • Quantum dots	• ICG-containing nanoparticles • Au nanoparticles
Representative image	 Human left common carotid artery	 Rabbit aorta	 Rabbit aorta	 Mouse	 Mouse aorta

Applications

- Visualization of high-risk atherosclerotic plaques
- Quantification of plaque burden
- Evaluation of novel therapies


a Inhibition of monocyte recruitment
Monocyte
Nanoparticle
Inhibition of recruitment
Leaky endothelial junction
Lumen
Endothelial cell
Intima
Differentiation
Macrophage
b Inhibition of macrophage proliferation
Inhibition of proliferation
c Restoration of efferocytosis
Restoration of efferocytosis
Apoptotic cell
Secretion of pro-resolving cytokines (such as IL-10 and TGFβ)
d Inhibition of inflammation
Secretion of pro-resolving cytokines (such as IL-10 and TGFβ)
Inhibition of secretion of pro-inflammatory cytokines (such as IL-1β, IL-6 and TNF)
e Induction of macrophage apoptosis
Early lesions
Induction of apoptosis by local heating or by toxic agents
f Promotion of cholesterol efflux
Promotion of cholesterol efflux
Cholesterol-laden macrophage
Foam cell
Inhibition of foam cell formation

Milestones in the development of nanoparticle-based imaging contrast agents and therapeutics for atherosclerosis diagnosis and treatment. NP, nanoparticle; USPIO, ultrasmall superparamagnetic iron oxide.



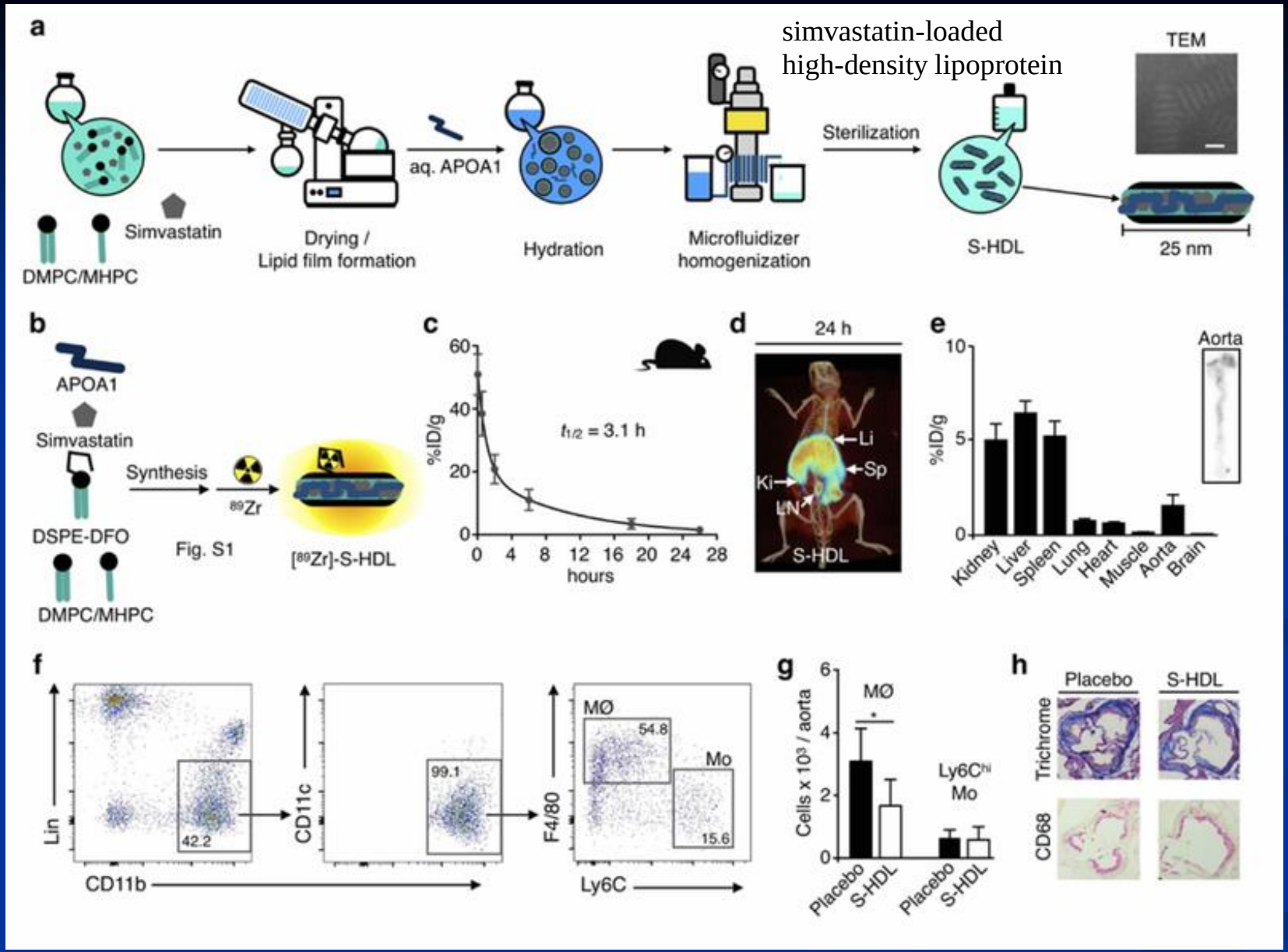
Imaging-assisted nanoimmunotherapy for atherosclerosis in multiple species

A simvastatin-loaded high-density lipoprotein (S-HDL) nanotherapeutic that we successfully applied in atherosclerosis mouse models to treat vessel wall inflammation.
Designed and implemented a high-pressure homogenization process, including innovative purification methods, to scale up S-HDL production, and generate the required amounts.

In this study, we report the escalation of a nanoimmunotherapy from mouse to large rabbit and porcine atherosclerosis models. Specifically, we integrated translational imaging readouts within the workflow to both analyze the nanoimmunotherapeutic's in vivo behavior and assess treatment response in larger animals.

We observed our nanoimmunotherapeutic's anti-inflammatory efficacy in mice as well as rabbits and pigs. Importantly, in the larger animal models, nanoimmunotherapeutically reduced inflammation halted plaque progression, underlining the approach's translatability and potential to acutely treat atherosclerosis.

Sci Transl Med. 2019 August 21; 11(506)



lipid-lowering drug

Fig. 1. Nanoimmunotherapy production scale-up and evaluation in Apoe−/− mice.

Sci Transl Med. 2019 August 21; 11(506)

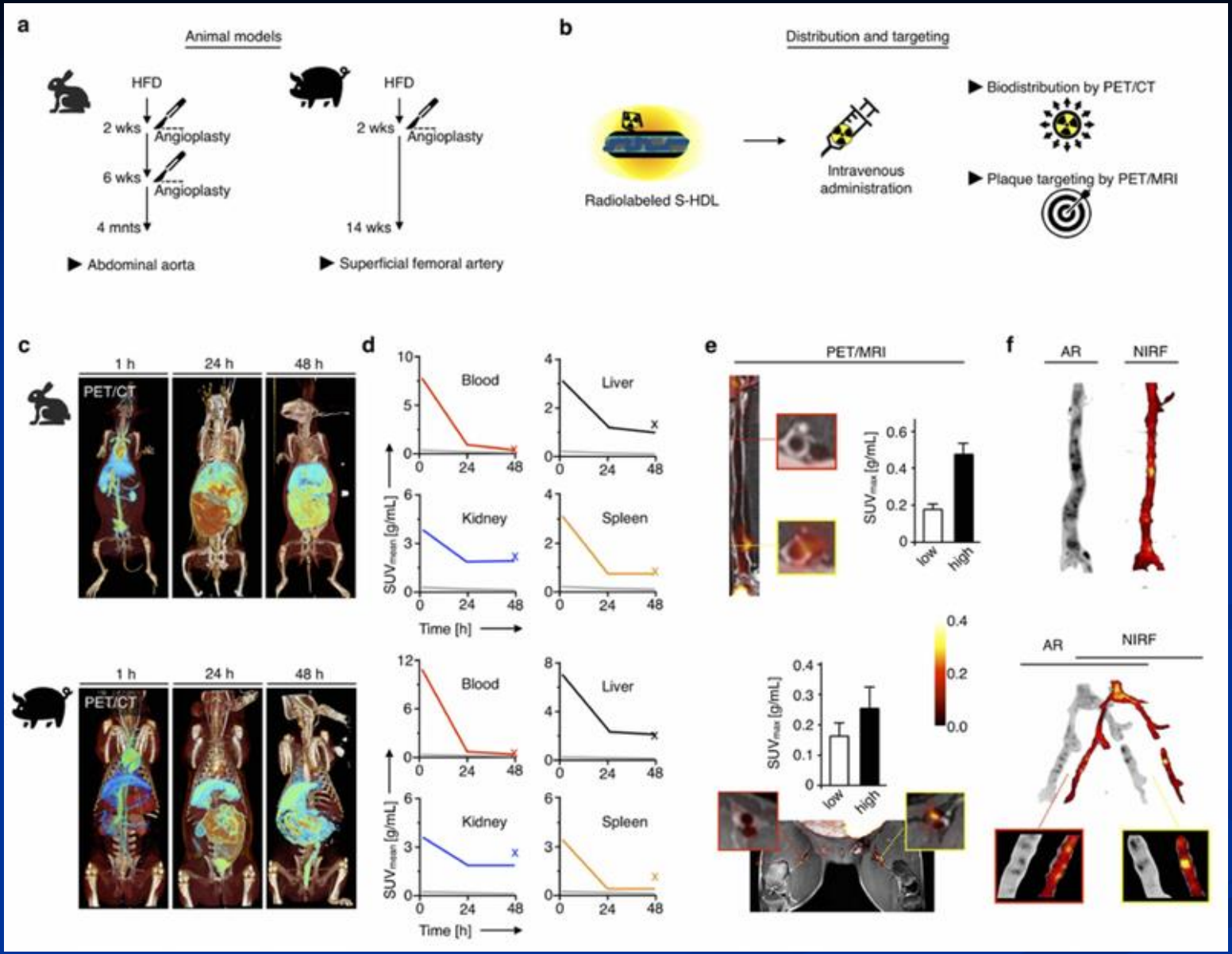


Fig. 2. S-HDL nanoimmunotherapy in vivo evaluation by non-invasive imaging in rabbits and pigs.

Sci Transl Med. 2019 August 21; 11(506)

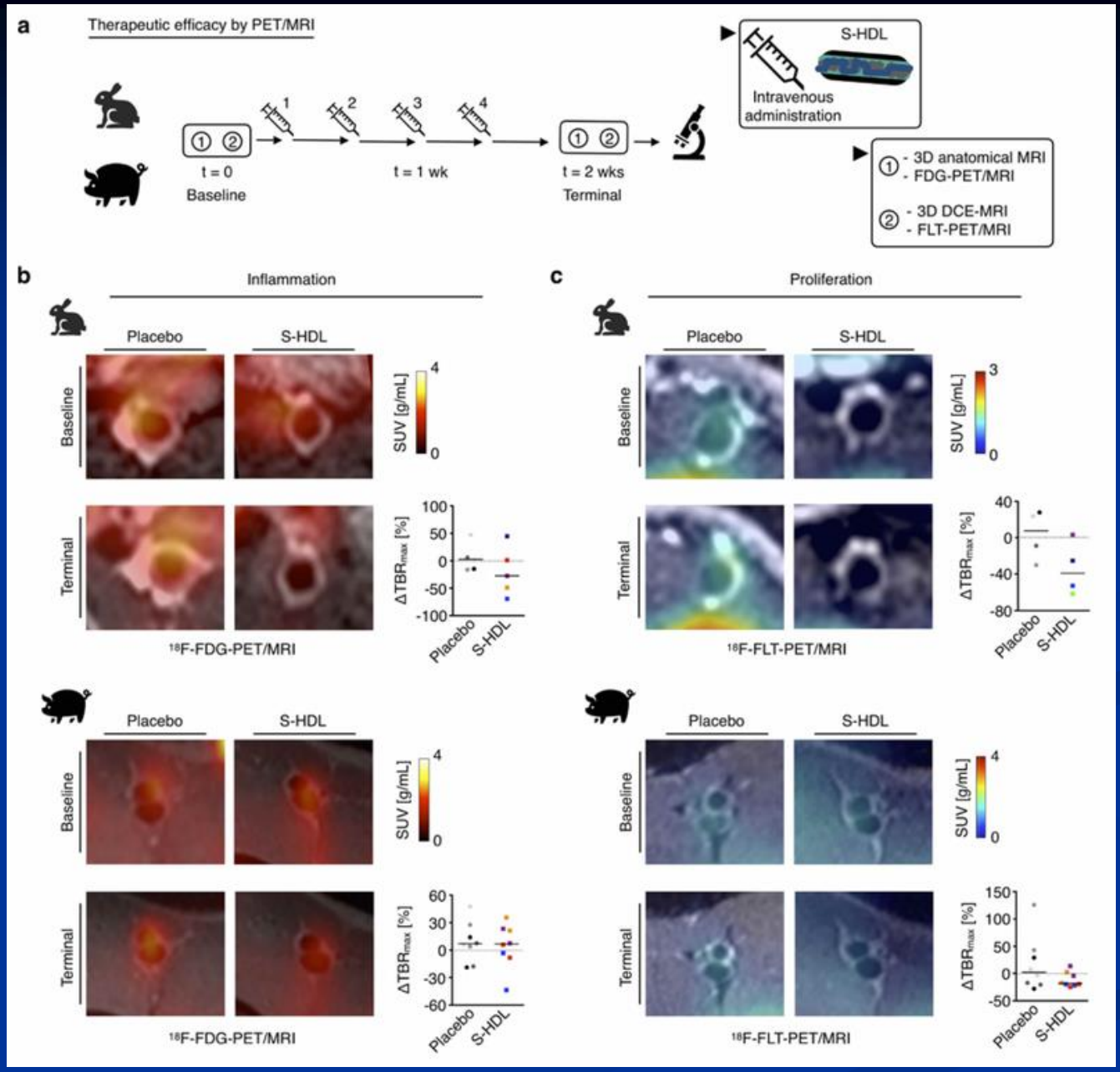


Fig. 3. Imaging-guided S-HDL nanoimmunotherapy in rabbits and pigs. PET-based readouts.

Sci Transl Med. 2019 August 21; 11(506)

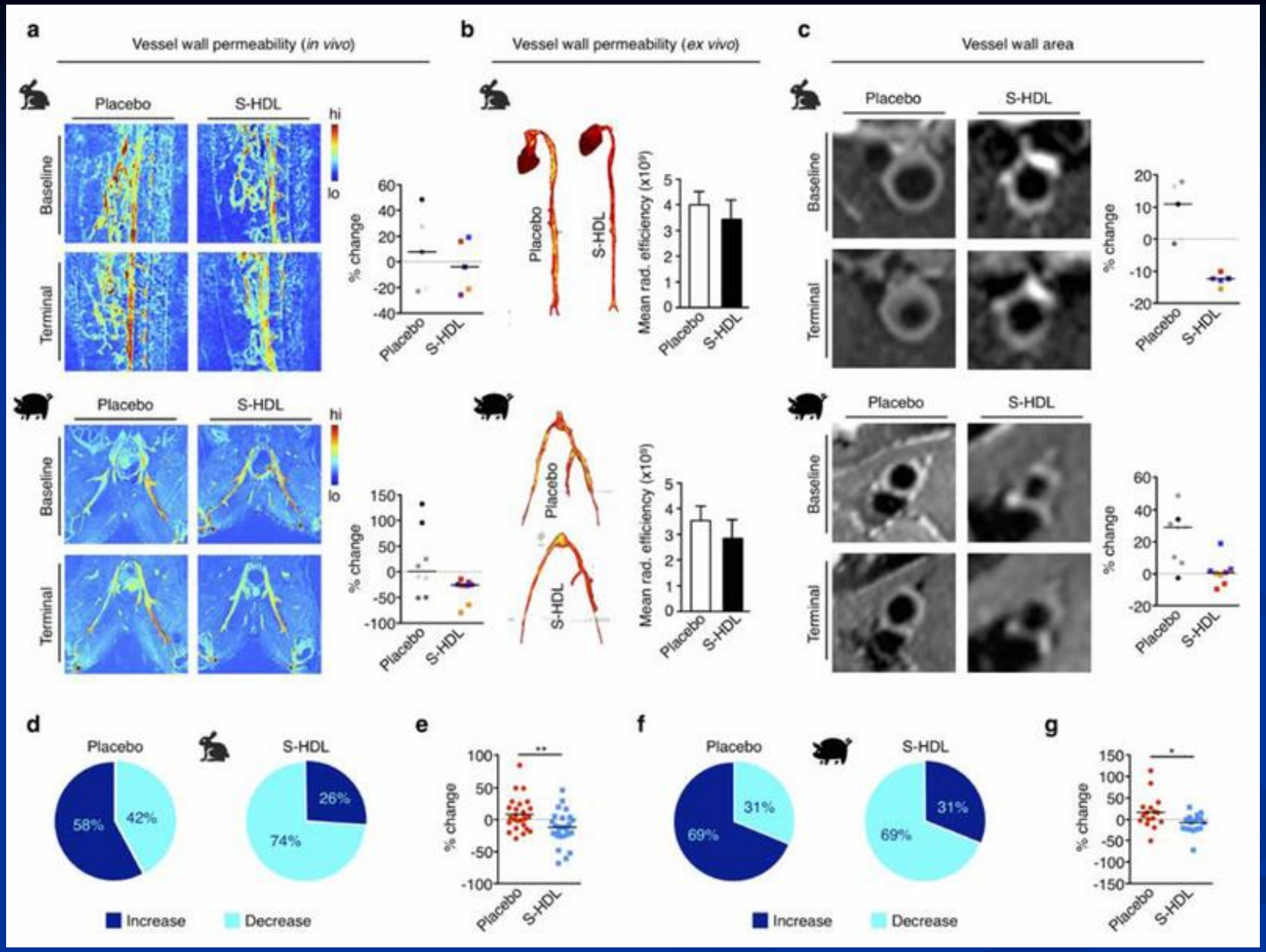


Fig. 4. Imaging-guided S-HDL nanoimmunotherapy in rabbits and pigs. MRI-based readouts

Sci Transl Med. 2019 August 21; 11(506)

Targeted Interleukin-10 Nanotherapeutics Developed with a Microfluidic Chip Enhance Resolution of Inflammation in Advanced Atherosclerosis

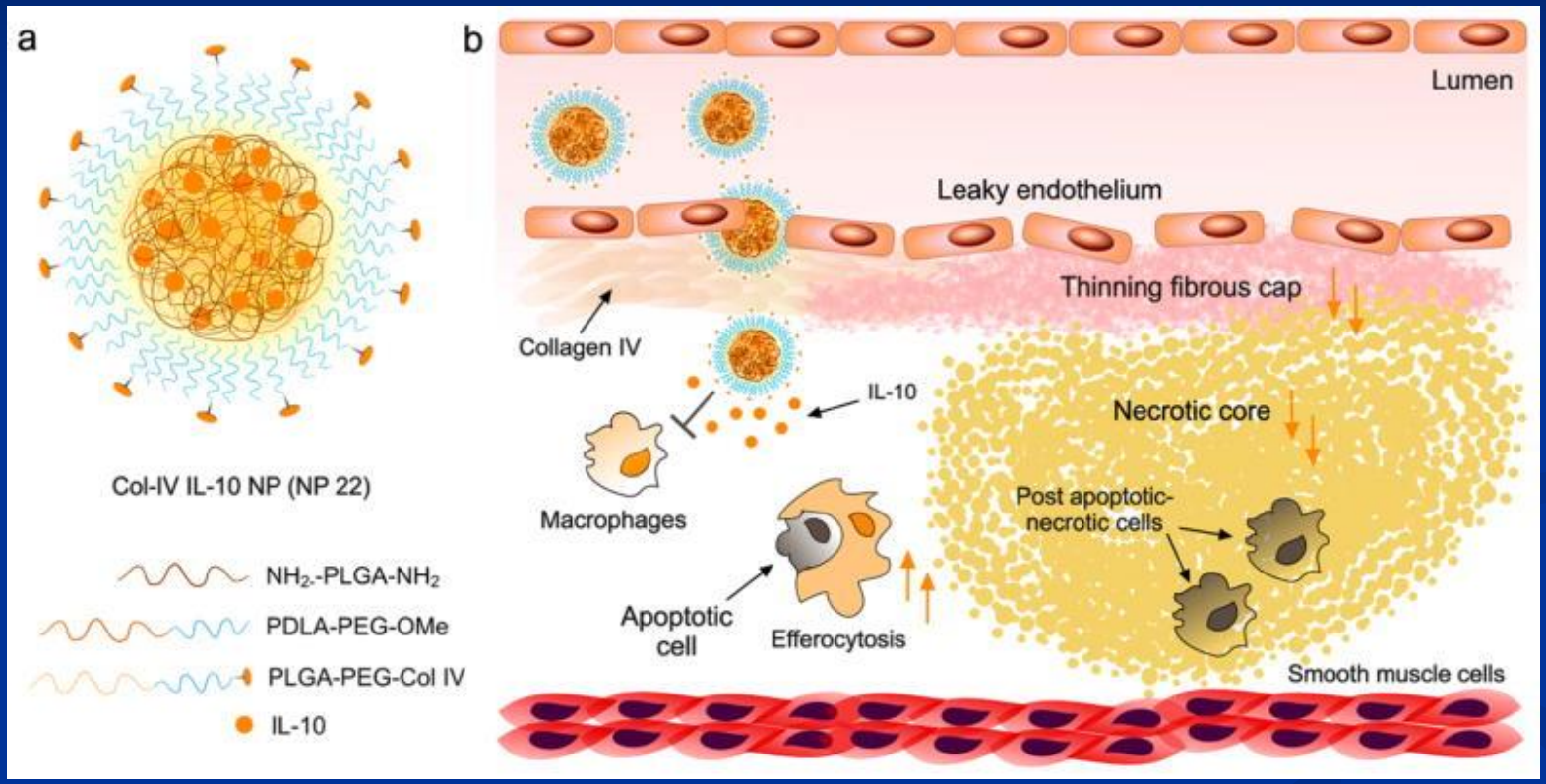
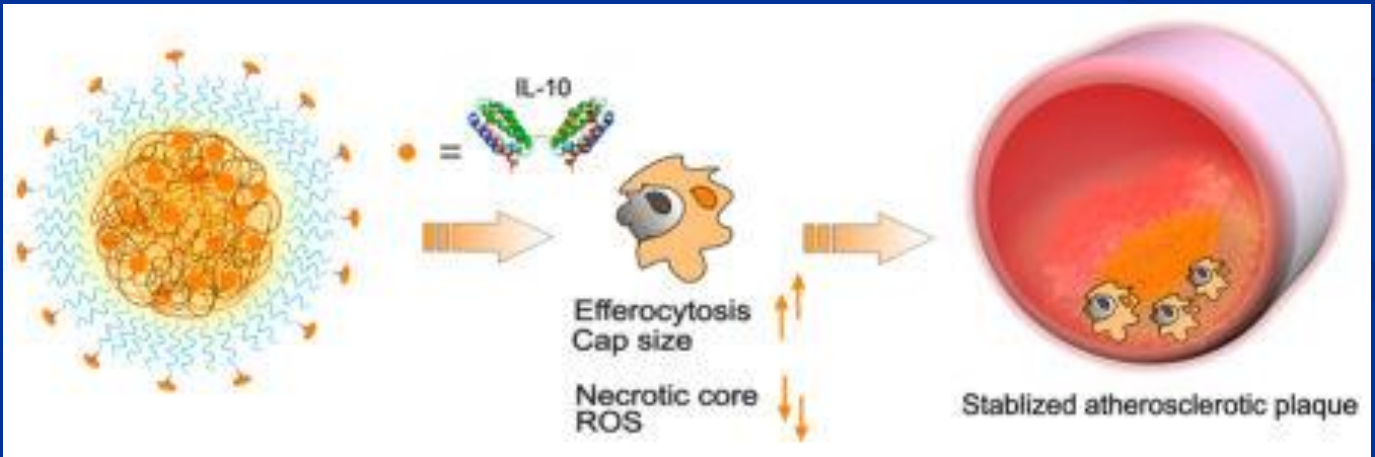


Figure 1 Targeted anti-inflammatory NP design and application to resolution of inflammation in atherosclerotic plaques.

ACS Nano. 2016 May 24; 10(5): 5280–5292

Targeted Interleukin-10 Nanotherapeutics Developed with a Microfluidic Chip Enhance Resolution of Inflammation in Advanced Atherosclerosis

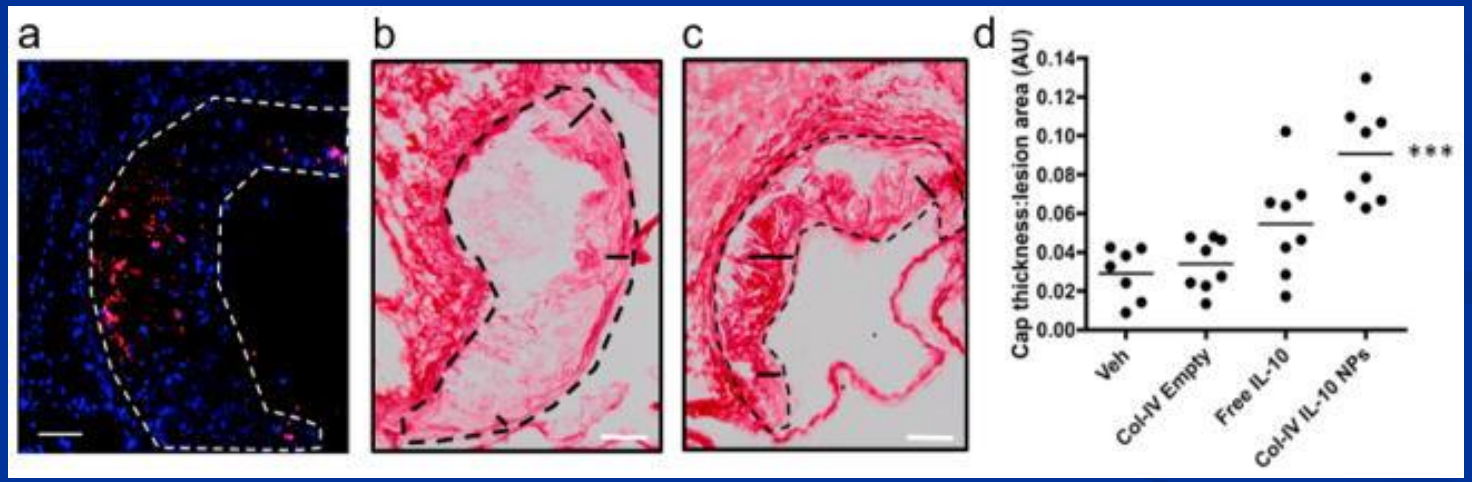
Here we present the development and efficacy investigations of controlled-release polymeric nanoparticles incorporating the anti-inflammatory cytokine interleukin 10 (IL-10) for targeted delivery to atherosclerotic plaques. Nanoparticles were nanoengineered via self-assembly of biodegradable polyester polymers by nanoprecipitation using a rapid micromixer chip capable of producing nanoparticles with retained IL-10 bioactivity post-exposure to organic solvent. A systematic combinatorial approach was taken to screen nanoparticles, resulting in an optimal bioactive formulation from in vitro and ex vivo studies. The most potent nanoparticle termed Col-IV IL-10 NP22 significantly tempered acute inflammation in a self-limited peritonitis model and was shown to be more potent than native IL-10. Furthermore, the Col-IV IL-10 nanoparticles prevented vulnerable plaque formation by increasing fibrous cap thickness and decreasing necrotic cores in advanced lesions of high fat-fed LDLr−/− mice. These results demonstrate the efficacy and pro-resolving potential of this engineered nanoparticle for controlled delivery of the potent IL-10 cytokine for the treatment of atherosclerosis.



ACS Nano. 2016 May 24; 10(5): 5280–5292

Figure 8 Col IV-IL-10 NPs increase subendothelial collagen in *Ldlr*$^{-/-}$ mice with established atherosclerosis.

Western diet for 12 wks

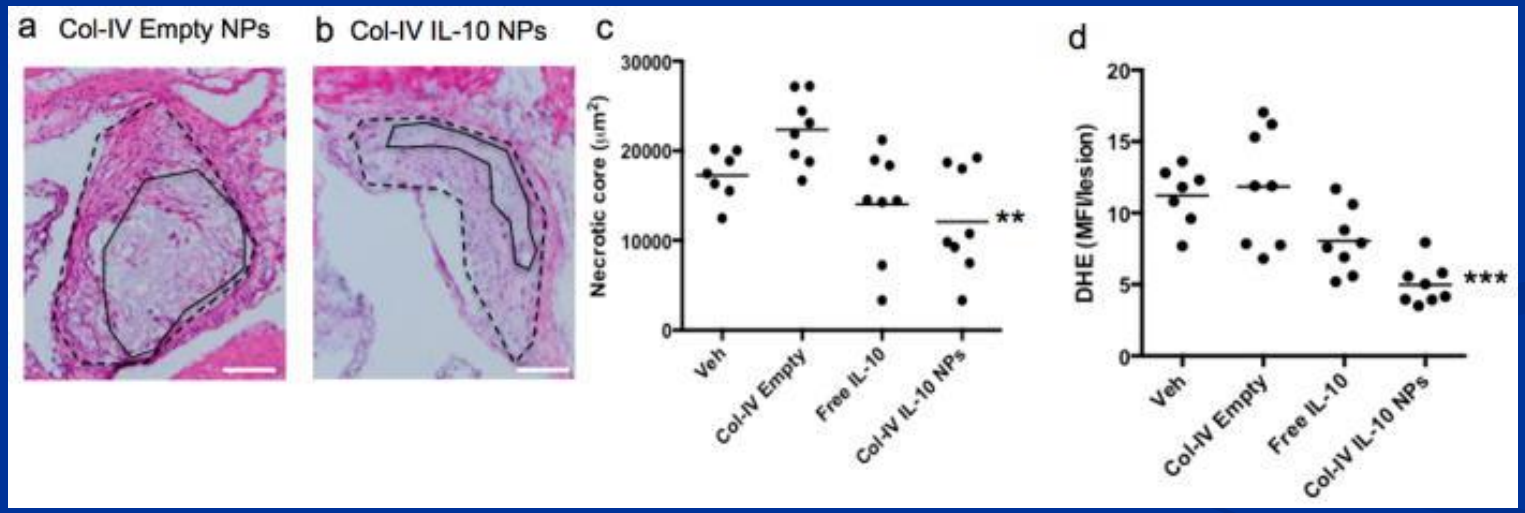


Decrease in cap thickness

ACS Nano. 2016 May 24; 10(5): 5280–5292

Figure 9 Col IV-IL-10 NPs decrease necrosis in *Ldlr*$^{-/-}$ mice with established atherosclerosis.

Western diet for 12 wks



Decrease in necrotic core

ACS Nano. 2016 May 24; 10(5): 5280–5292

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

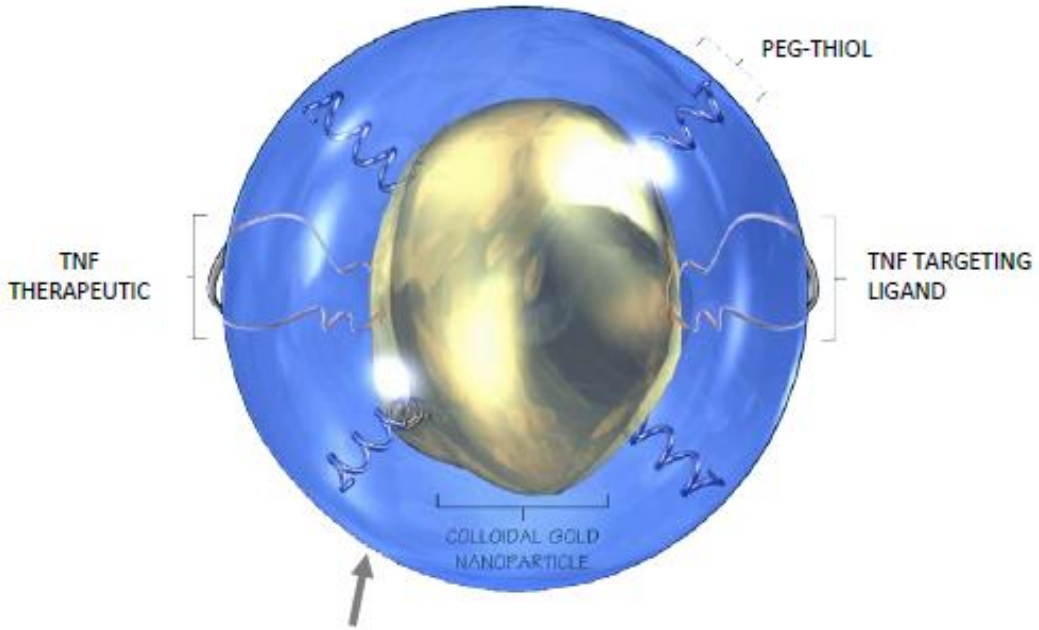
Need: Improve efficacy and safety, and minimize recurrent disease

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

Solution: Use nanotechnology-based therapeutics, first

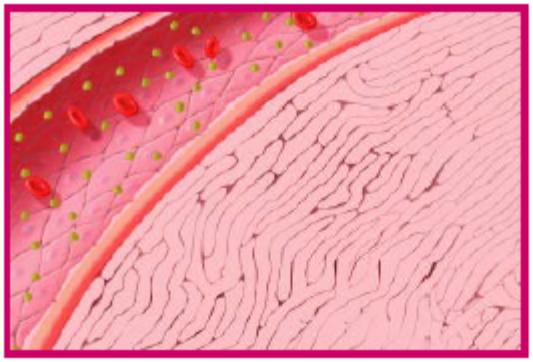
- First treat patients medically to reduce tumors, use surgery only if needed
  - May lead to improved tumor regression, reduced side effects, and reduced recurrent disease

Design of CYT-6091 (Aurimune®):

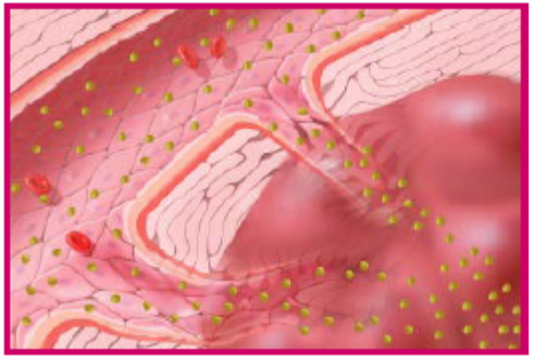


CYTIMMUNE

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.

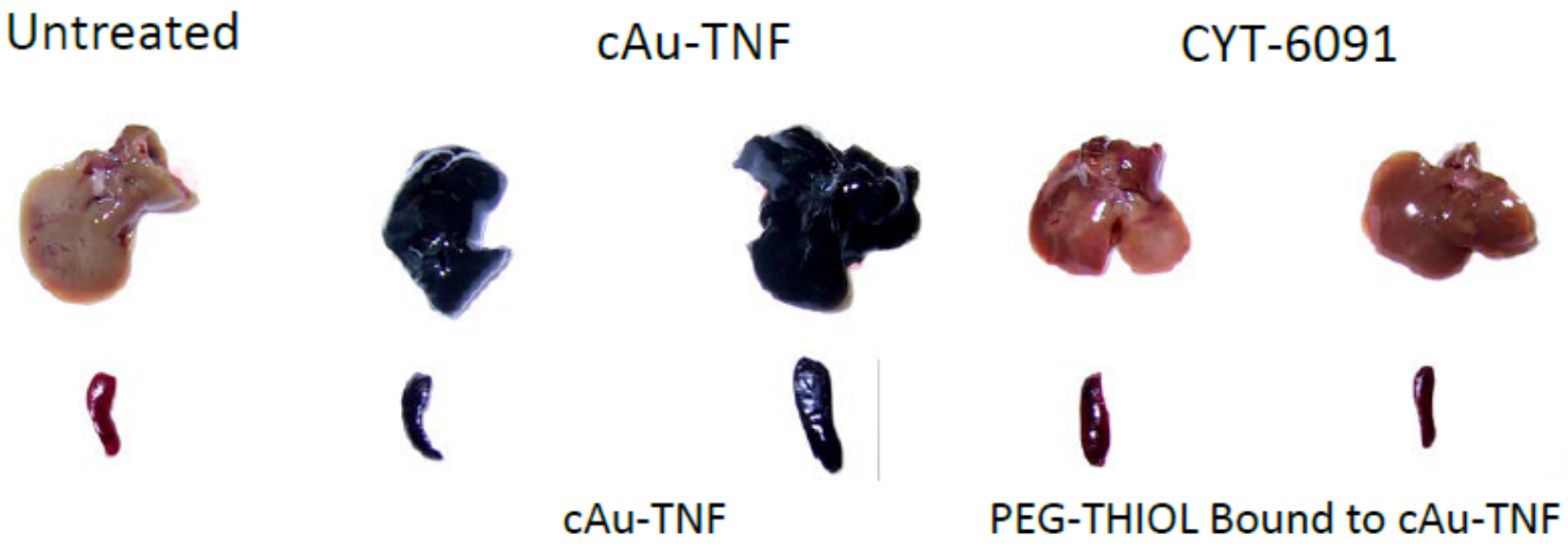
Due to its engineered nanometer size and targeted capabilities, CYT-6091 is able to reduce toxicity and increase efficacy.

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

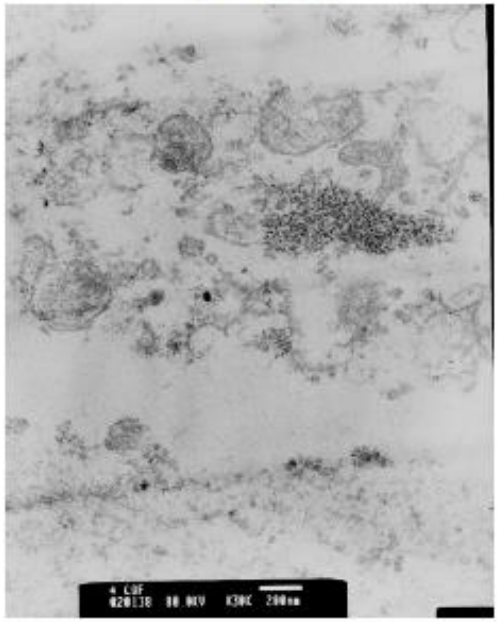


CYTIMMUNE

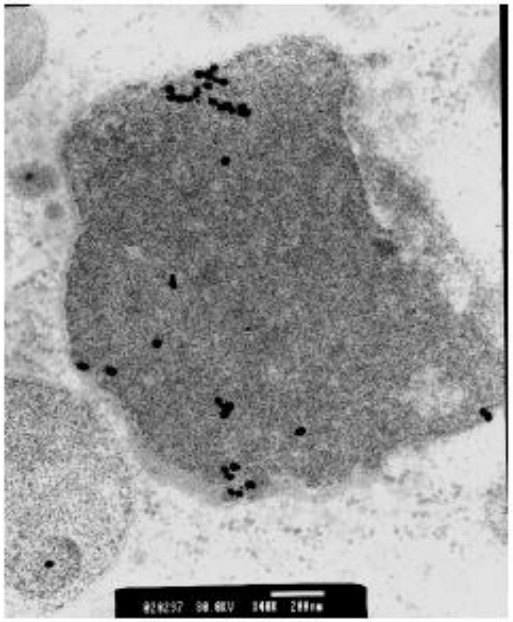
Differential Uptake of CYT-6091 in Mouse Model

Electron micrographs comparing tumor and healthy tissue

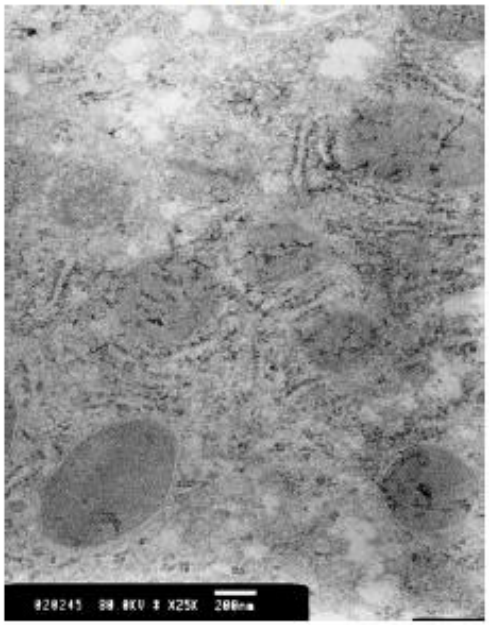
Spleen



Tumor



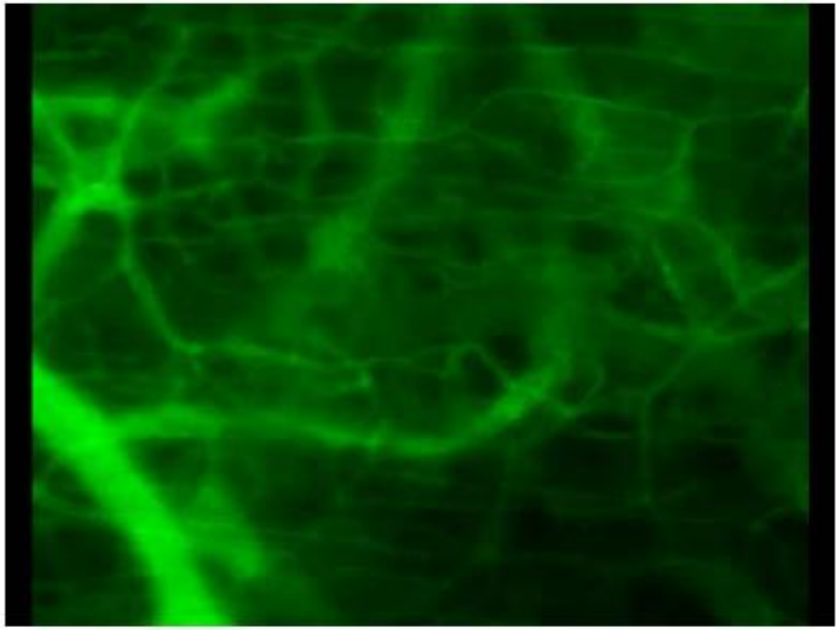
Liver



Bar at bottom = 200 nm

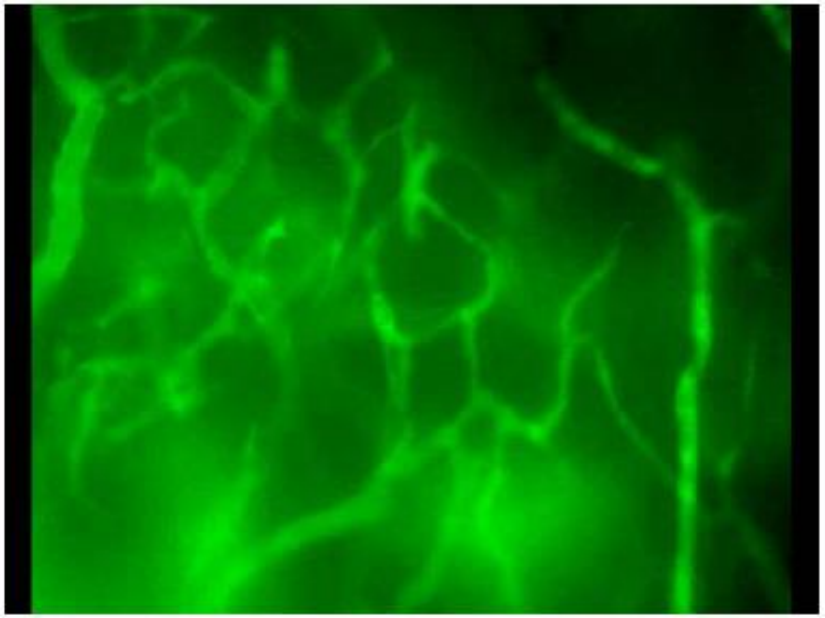
CYTIMMUNE

Selective Induction of Vascular Leak by CYT-6091



Normal Vasculature
No Vascular Leak

Albumin



Tumor Neovasculature
Vascular Leak

CYT-6091 + Albumin

CYTIMMUNE

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

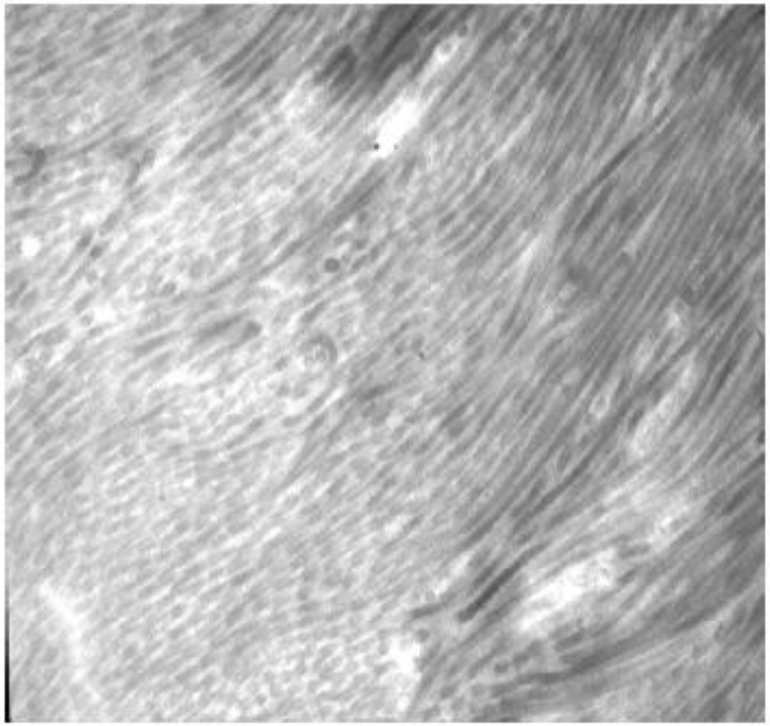
Not Antigenic. No antibody response

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

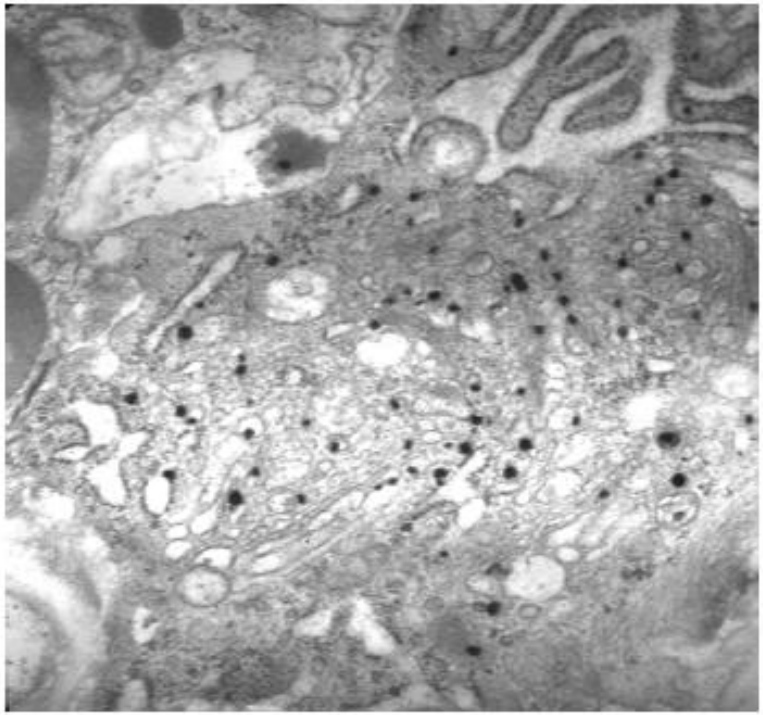
Electron Micrographs* of a Patient's Biopsies

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

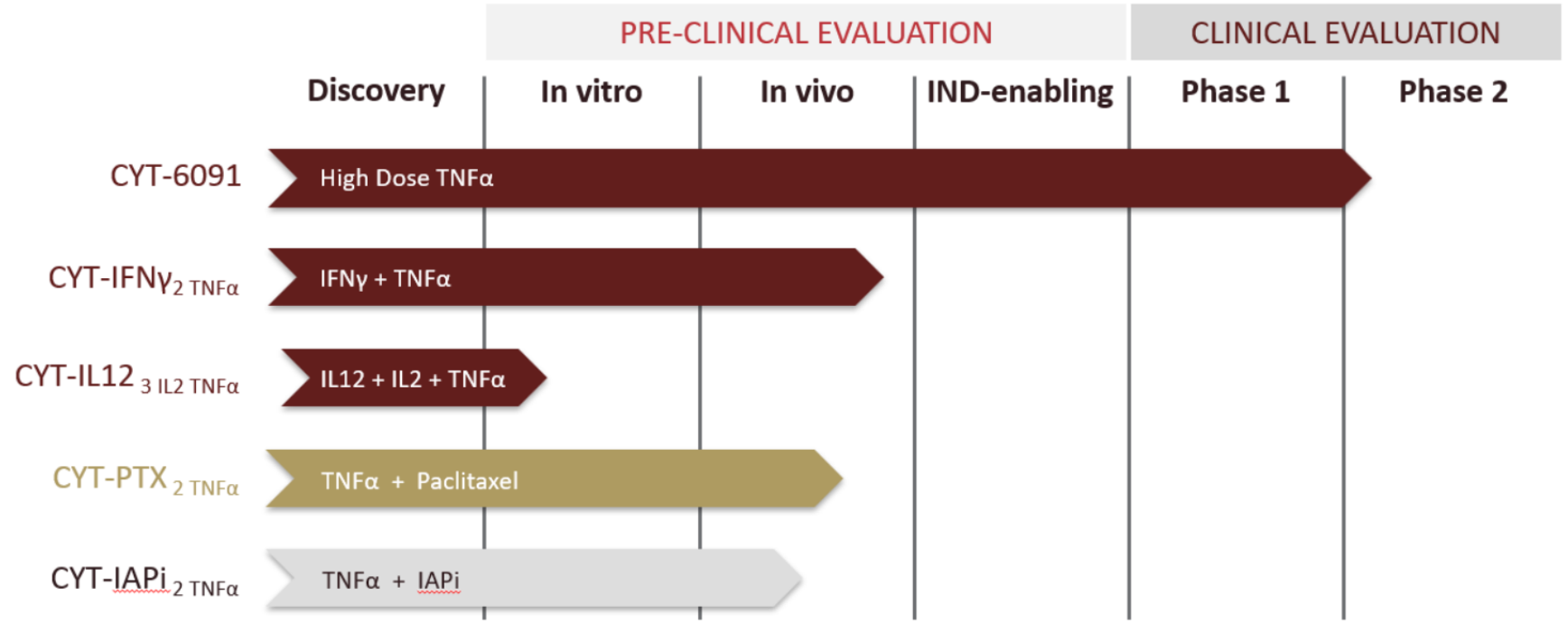
Avoids MPS Uptake

Manufactured to Defined Specifications

CYTIMMUNE

Cytimmune

A Diverse Pipeline of IO+ Therapeutics



ELSEVIER

Journal of Controlled Release
Volume 330, 10 February 2021, Pages 372-397

Review article

Nanomedicines accessible in the market for clinical interventions

Vedant Gadekar, Yogeshwari Borade, Suraj Kannaujia, Kuldeep Rajpoot, Neelima Anup, Vishakha Tambe, Kiran Kalia , Rakesh K. Tekade

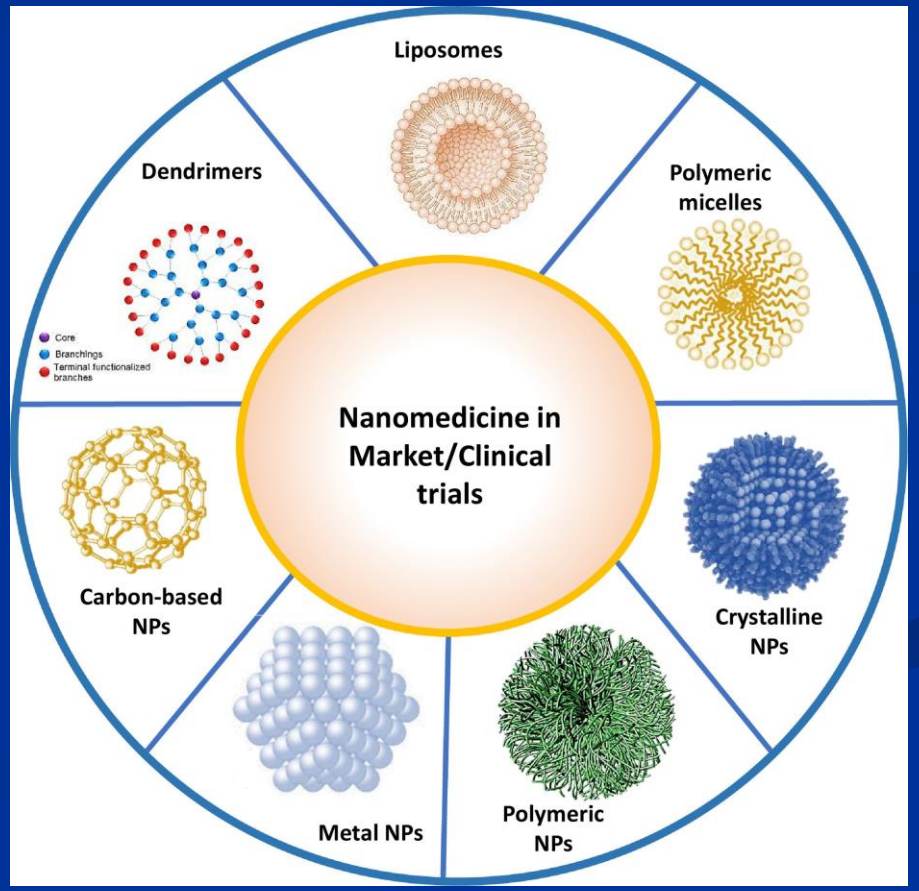


Fig. 1. Diagram illustrating various nanomedicines, which are currently in the market or under clinical trial.

Fig. 2. The trend of different NPs in the market.

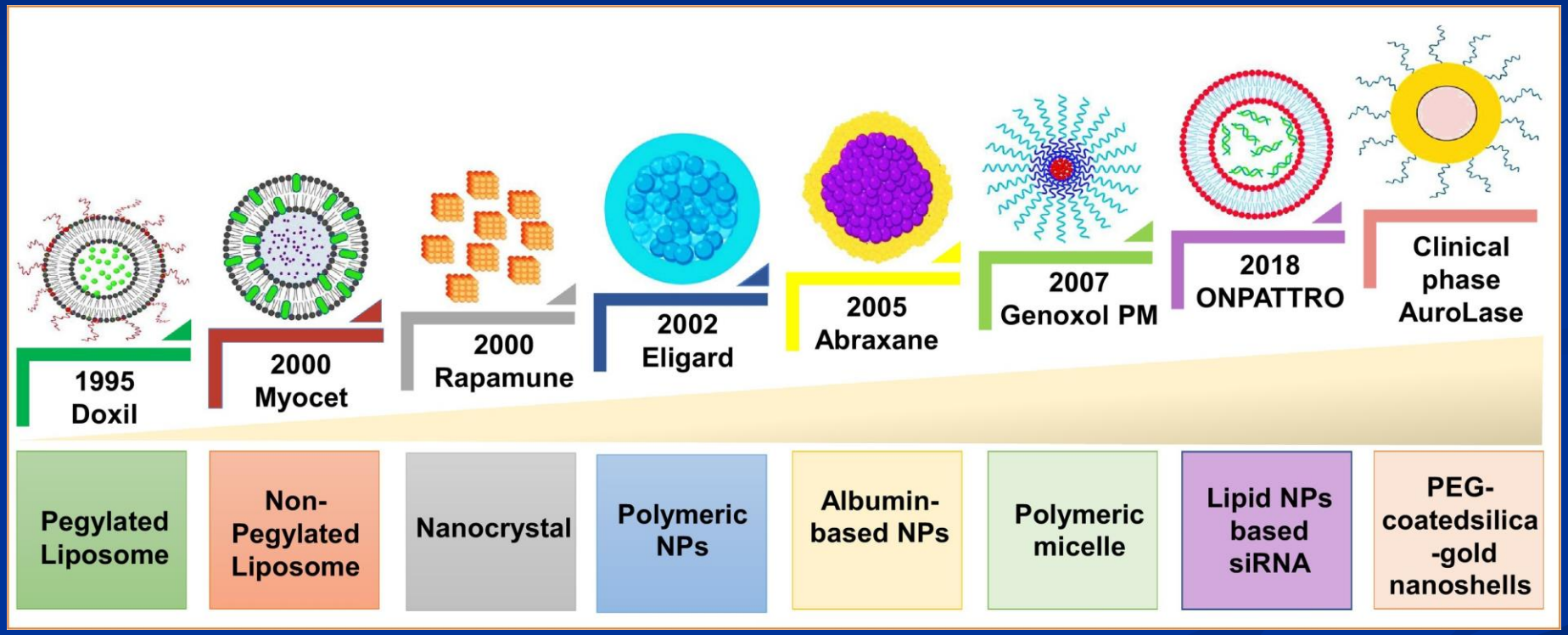


Fig. 3. Classification of NPs-based drug delivery systems for clinical applications.

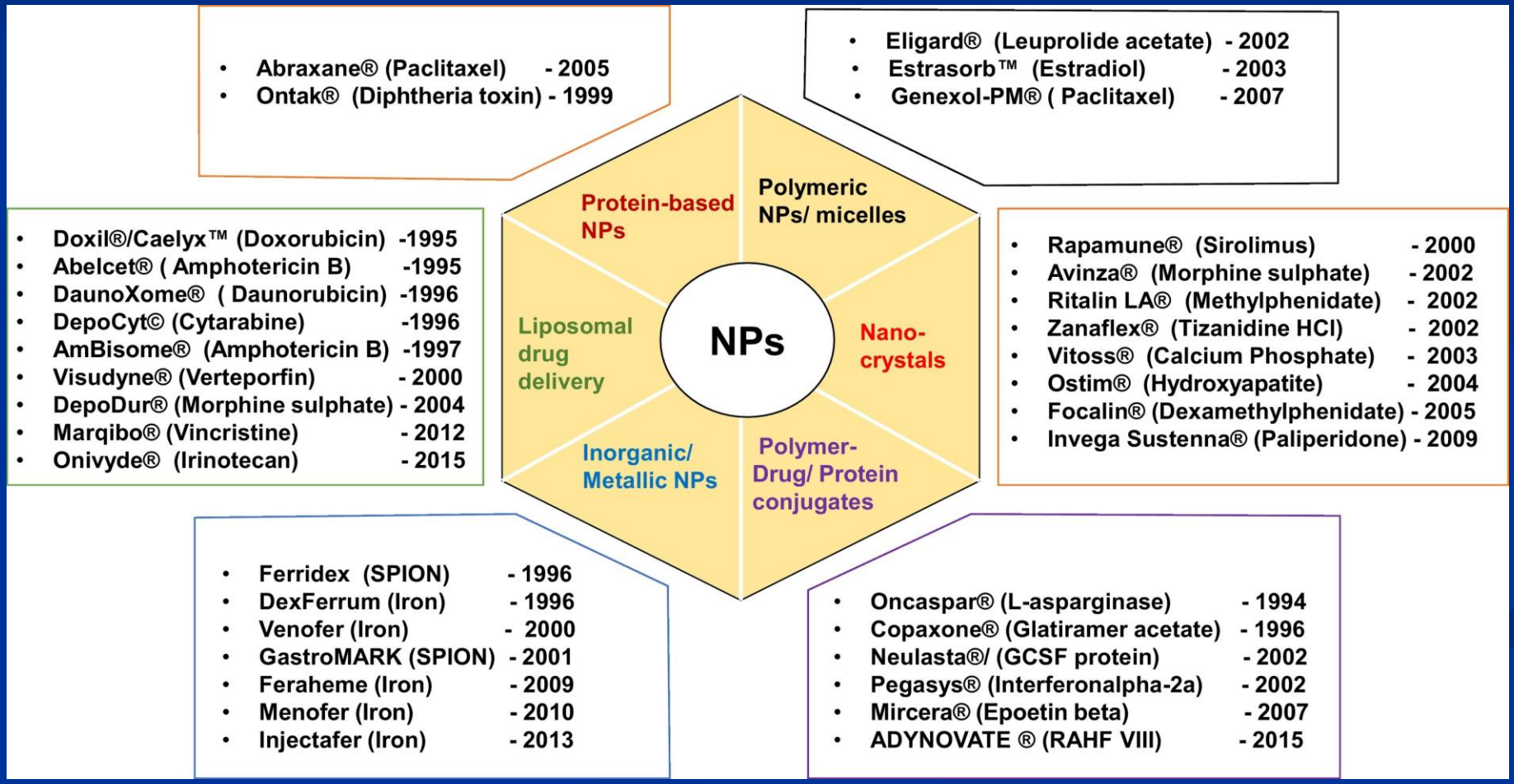


Fig. 4. Market trend, as well as the market size of the nanomedicines over the years.

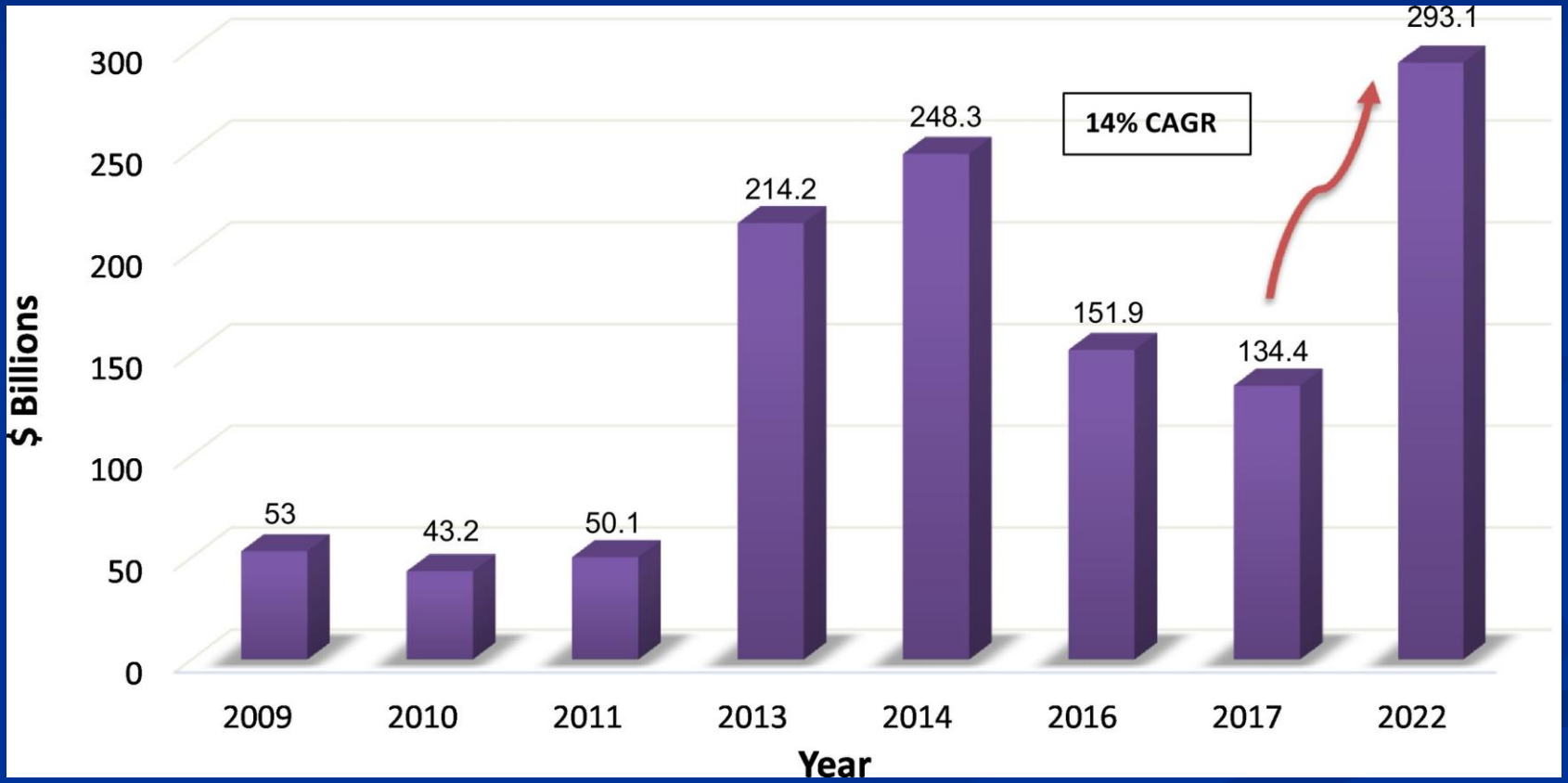


Table 1
Summary of various nanomedicine-based marketed products to treat multiple diseases.

Drug agent	Formulation type	Trade name	Clinical applications	Company	First introduced in the year
Cancers					
Doxorubicin	Liposome	Doxil®	Ovarian cancer, AIDS-related Kaposi's Sarcoma	Ben Venue Laboratories, TTY Biopharm	1995
Doxorubicin	Pegylated liposome	Lipo Dox®	Acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma	Sun Pharma	2013
Doxorubicin	Non-pegylated liposome	Myocet®	Metastatic breast cancer	Enzon Pharmaceutical; Elan Pharmaceuticals	2000
Daunorubicin citrate	Liposome	DaunoXom®	Acute myeloid leukemia	Gilead Sciences	1996
Paclitaxel	Albumin based NP's	Abraxane®	Breast cancer	Abraxis BioScience	2005
Irinotecan	Liposome	Onivyde®	Metastatic adenocarcinoma	Merrimack Pharmaceuticals	2015
Denileukin diftitox	Protein-based formulation	Ontak®	Cutaneous T-cell lymphoma therapy	Eisai	1999
Cytarabine	Liposome	DepoCyte®	Neoplastic meningitis	SkyePharma, Enzon Pharmaceuticals; Pacira Pharmaceuticals	1999
Vincristine sulfate	Liposome	Marqibo®	Philadelphia chromosome-negative acute lymphoblastic leukemia (ALL)	Talon Therapeutics; Acrotech Biopharma	2012
Paclitaxel	Lyophilised polymeric micelles	Genexol-PM®	Breast cancer and non-small cell lung cancer (NSCLC)	Samyang Corporation	2007
L-asparaginase enzyme	Pegylated enzyme	Oncaspar®	Acute lymphocytic leukemia	Enzon Pharmaceuticals; Baxter BioScience	1994
Leuprolide acetate	Sustained-release depot formulation	Eligard®	Prostate cancer	Tolmar Pharmaceuticals	2002
Fungal infections					
Amphotericin B (AmB)	Lyophilised formulation	Fungizone®	–	Ben Venue Laboratories; E.R. Squibb & Sons, LLC	1966
AmB	Lipid containing NP's	Abelcet®	Invasive fungal infection treatment	Leadiant Biosciences	1995
AmB	Liposome	AmBisome®	Systemic fungal infection	Gilead Sciences	1997
AmB	Parenteral freeze-dried	Amphotec®	Fungal infection	Ben Venue Laboratories	1996
Neurological disorders					
Morphine sulfate	Nanocrystal	Avinza®	Psychostimulant	Pfizer	2002
Methylphenidate hydrochloride	Nanocrystal	Ritalin LA®	Attention-deficit/hyperactivity disorder (ADHD) in children	Novartis	2002
Paliperidone palmitate	Nanocrystalline injectable	Invega Sustenna®	Schizophrenia	Janssen Pharmaceuticals	2009
small interfering ribonucleic acid (siRNA)	Lipid complex siRNA	Onpattro™	Hereditary transthyretin amyloidosis (hATTR) induced polyneuropathies	Alnylam Pharmaceuticals	2018
Ocular disorders					
Verteporfin	Liposome	Visudyne®	Choroidal neovascularization	QLT Phototherapeutics	2001
Cyclosporin	Nanoemulsion	Restasis®	Severe keratitis in dry eye patient	Allergan	2002
Cardiovascular diseases					
Fenofibrate	Nanocrystalline tablet	Tricor®	Primary hypercholesterolemia or mixed dyslipidemia as an adjunctive treatment with lipid-lowering diet	Abbott Laboratories	2004
Fenofibrate	Nanocrystalline tablet	Triglide®		Skyepharma	2005
Blood disorders					
Ferumoxytol	Semi-synthetic iron oxide NPs	Feraheme™	Anemia related to chronic kidney disease (CKD)	AMAG Pharmaceuticals	2009
Polynuclear iron (III)-oxyhydroxide	Iron NP's	Injectafer®	Iron deficiency anemia	Vifor Int.	2013
Iron molecule with unbranched carbohydrate	Iron NP's	Monofer®	Iron deficiency anemia	Pharmacosmos	2010
Methoxy polyethylene glycol (MPG)-epoetin beta	Polymer protein conjugate	Mircera®	CKD associated anemia	Hoffman-LaRoche	2007
Recombinant anti-hemophilic factor VIII	Polymer-protein conjugate	Adynovate®	Hemophilia A	Baxalta	2015
Recombinant human Granulocyte-colony stimulating factor (G-CSF)	Polymer-protein conjugate	Neulasta®	Febrile neutropenia	Amgen	2002
Liver diseases					
Interferon alfa-2a	Pegylated NP's	Pegasys®	Hepatitis B and C therapy	Genentech biotechnology	2002
Interferon alfa-2b	Pegylated NP's	Pegintron®	Hepatitis C	Merck	2001
Bone injuries					
Beta-tricalcium phosphate	Nanocrystal	Vitoss®	Bone growth and bone formation	Orthovita	2003
Nanocrystalline hydroxyapatite	Nanocrystal	Ostim®	Vascularization, cell migration, and bone formation	AAP biomaterials	2004
Dermatological diseases					
Silver	Silver NP's	PolyMem®	Chronic diabetic foot ulcers	Ferris MFG. Corp. (Burr Ridge, IL, US)	–

Fig. 5. Summary of marketed products available for treating several illnesses.

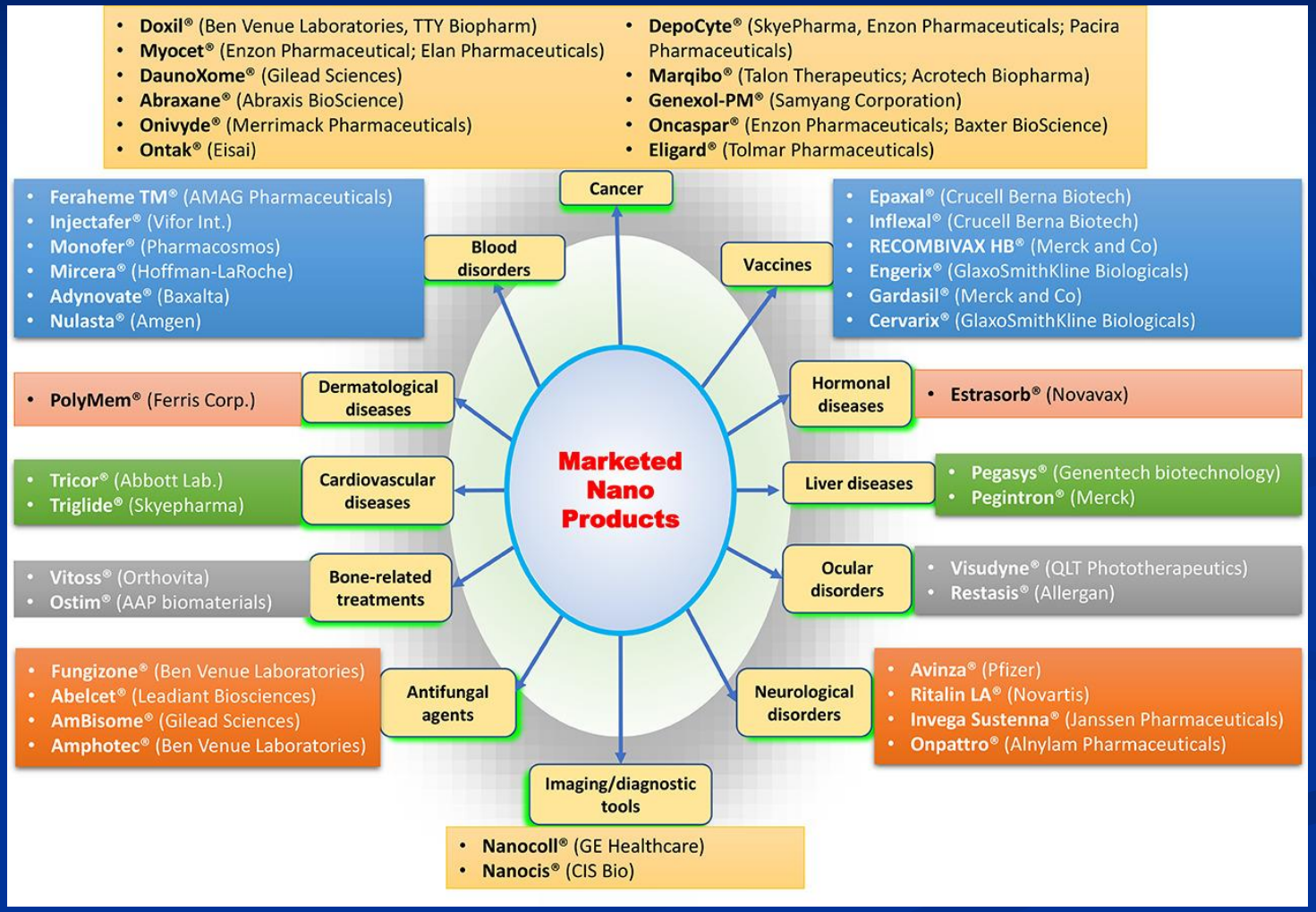


Fig. 6. Summary of various nanoplatforms available in the market.

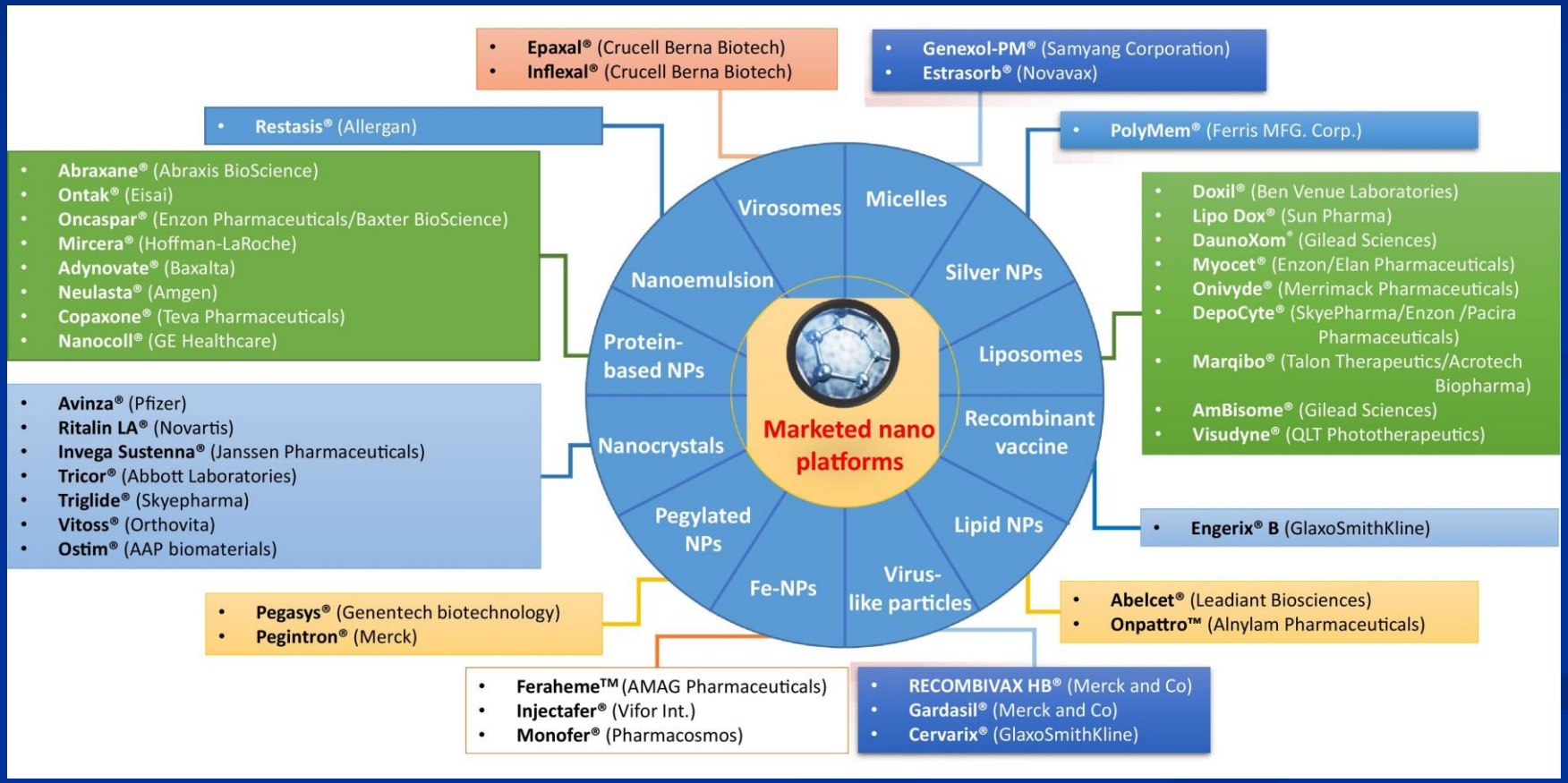


Table 4
Some essential nanotechnology-based formulations under clinical trials for the treatment of various illnesses.

Drug agent	Product name	Formulation type	Inventor/Company	Outcome	Clinical Status	References
Cancers						
Cisplatin	LiPlaCis	Liposome	LiPlasome Pharma	Stimuli-responsive targeted delivery for solid tumors	Phase I	[196]
Doxorubicin	Thermodox	Lyso-thermosensitive liposomes	Celsion Corporation, USA	Targeted delivery for hepatocellular carcinoma, refractory solid tumors, liver tumors, breast cancer	Phase I, II, and III	[119]
siRNA	TKM-080301	Lipid NPs	Arbutus Biopharm	Treatment of hepatocellular carcinoma and has completed phase I/II trials.	Completed phase I/II trials	[68]
Paclitaxel	Paclical	Polymeric micelles	Oasmia Pharmaceutical AB	Ovarian cancer	Completed phase III	[197]
Docetaxel	BIND-014	polymeric micelles	BIND Therapeutics	Targeted therapy for various cancers like prostate, non-small cell lung, cervical, metastatic, KRAS positive lung cancer	Completed phase II trial	[198]
Paclitaxel	NK105	polymeric micelles	Nippon Kayaku	Better tolerability an efficacy in gastric cancer	Completed phase III	[199]
Rapamycin	ABI-009	albumin-based NPs	Aadi with Celgene	Increase safety margin for the treatment of various cancers like neuroendocrine tumors, bladder cancer, colorectal cancer, perivascular epithelioid cell carcinoma, and glioblastoma	Phase I and II trials	[200]
Hafnium oxide	NBTXR3	Crystalline NPs	Nanobiotix	Enhanced tumor destruction and for an indication of locally advanced squamous cell carcinoma, prostate, rectum, liver, head and neck cancer	Phase I and II trials	[201]
Liver diseases						
siRNA	ND-L02-s0201	Lipid NPs	Nitto Denko/Bristol-Myers Squibb	Targeted therapy for advanced liver fibrosis	completed Ib/II clinical trial	[202,203]
siRNA	ARB-001467	Lipid NPs	Arbutus (Tekmira) Pharmaceuticals	Hepatitis B	Completed clinical phase II studies	[204]
Inflammatory disorders						
Pegsiticase and rapamycin	SEL-212	Polymeric NPs	Selecta Biosciences	Improved management of uric acid level in case of gout	Phase II	[205,206]
Imaging applications						
Gadolinium	AGuIX	Inorganic NPs	NH TherAguix	MRI contrast agent for brain metastases and gynecologic cancer	Phase I study	[207]
Indocyanine green	ONM-100	polymeric micelles	OncoNano Medicine	Imaging agent in intraoperative cancer	Phase I	[208]
Cy5(organic dye) and radiolabel ^{124}I	Cornell dots	Surface functionalized silica NPs	Ulrich Wiesner	Hybrid PET-optical tumor imaging of various cancer like brain, breast, colorectal, head and neck cancers	Phase I/II study	[209]
Vaccines						
mRNA-1944	Lipid NPs	Chikungunya infection	Moderna	Capable of eliciting chikungunya virus neutralizing antibodies	Phase I	[210]


NANOTECHNOLOGY LAB