Nano-Medicine in Pharmaceuticals

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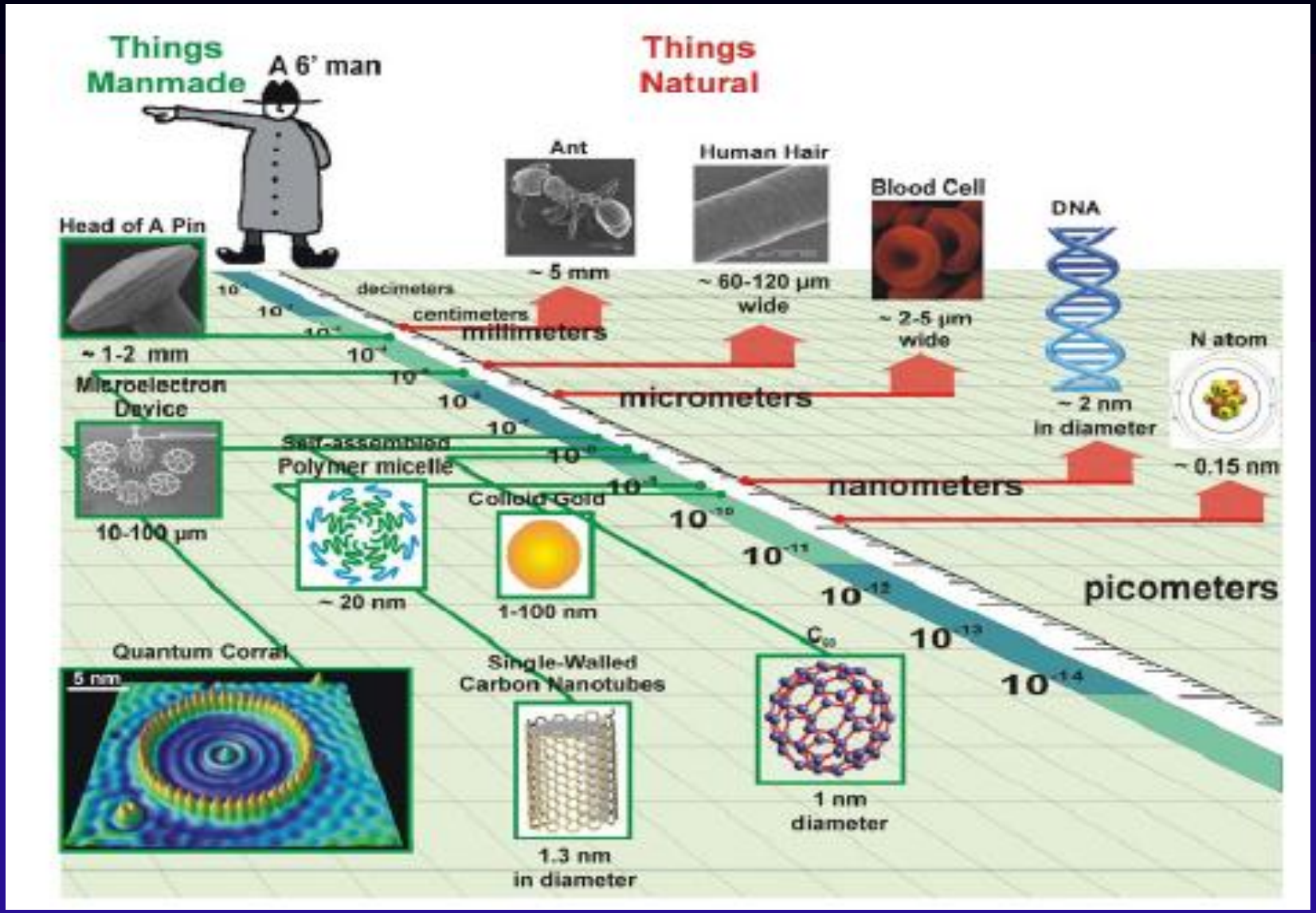
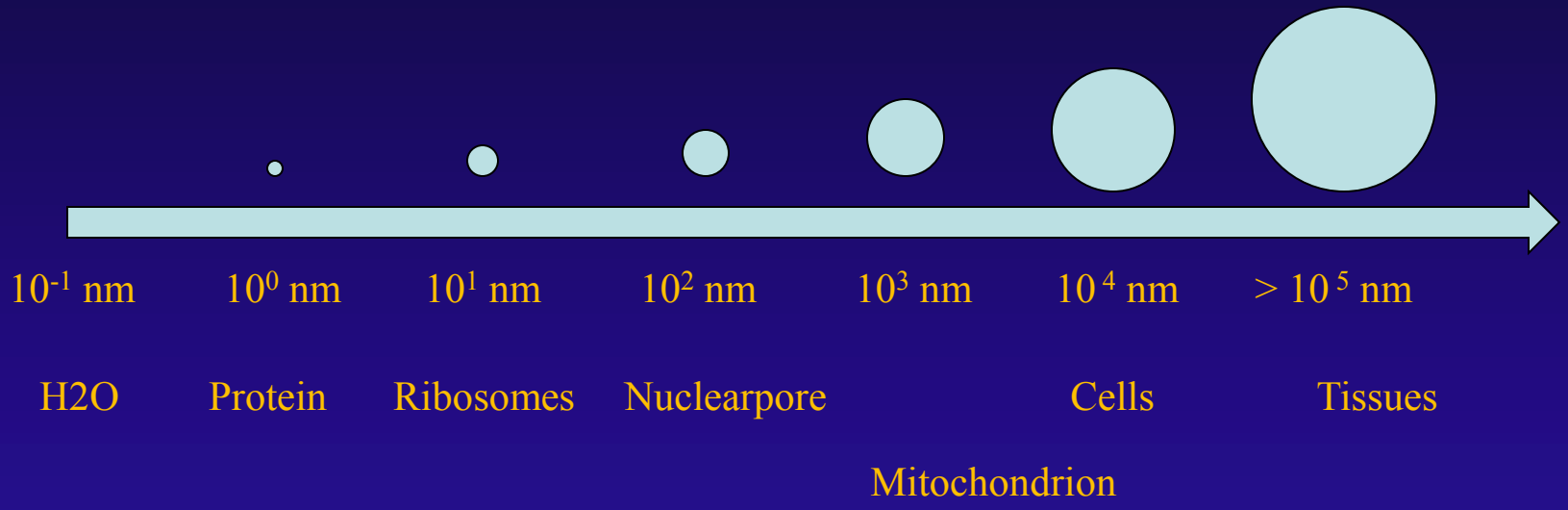


Comparisons of biological molecules found in nature with representative sizes of `small' object of man made Nanomaterials

Nanotechnology

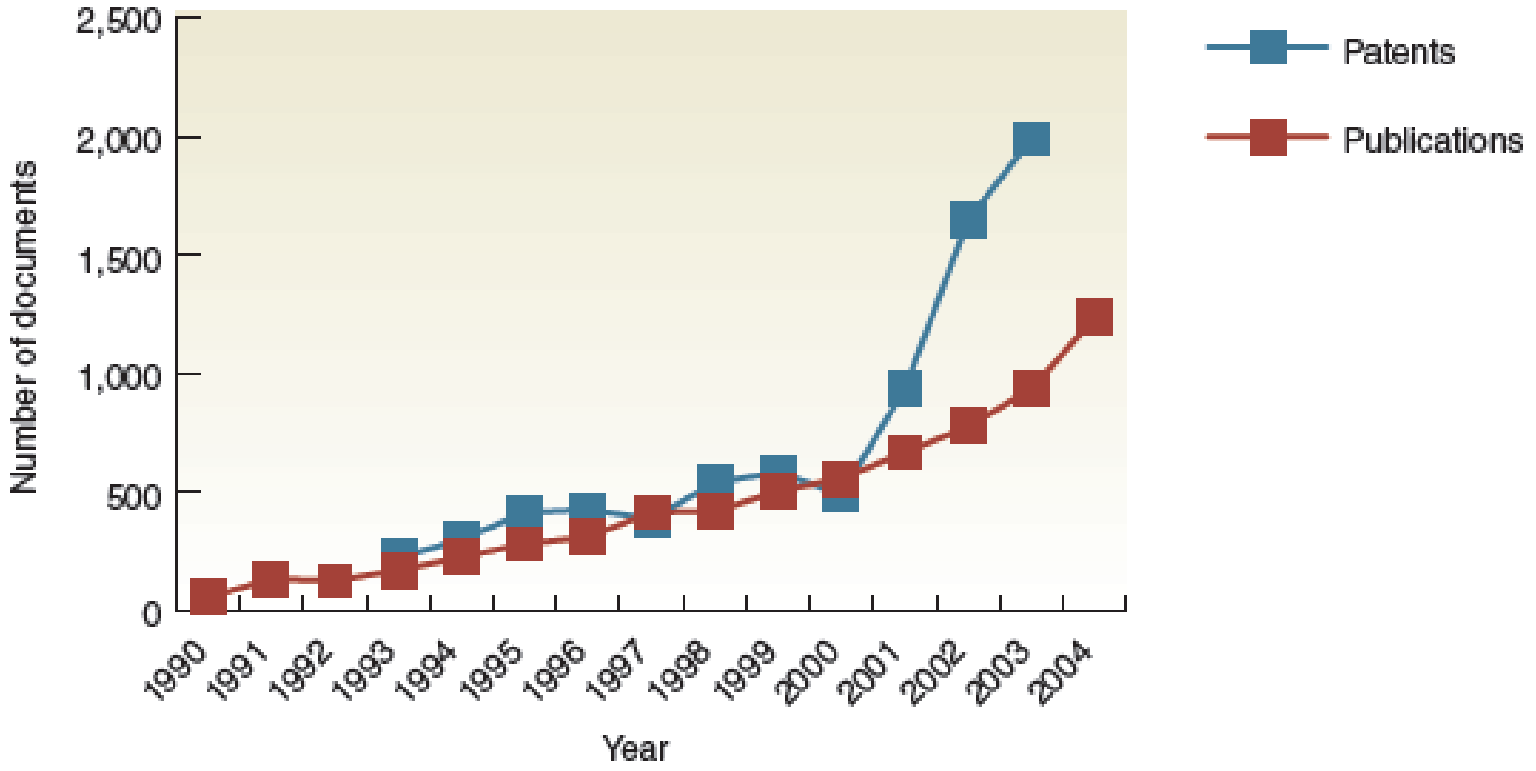
Nanotechnology involve research in the level of 100 nm and below in at least one dimension



Why Nanotechnology ?

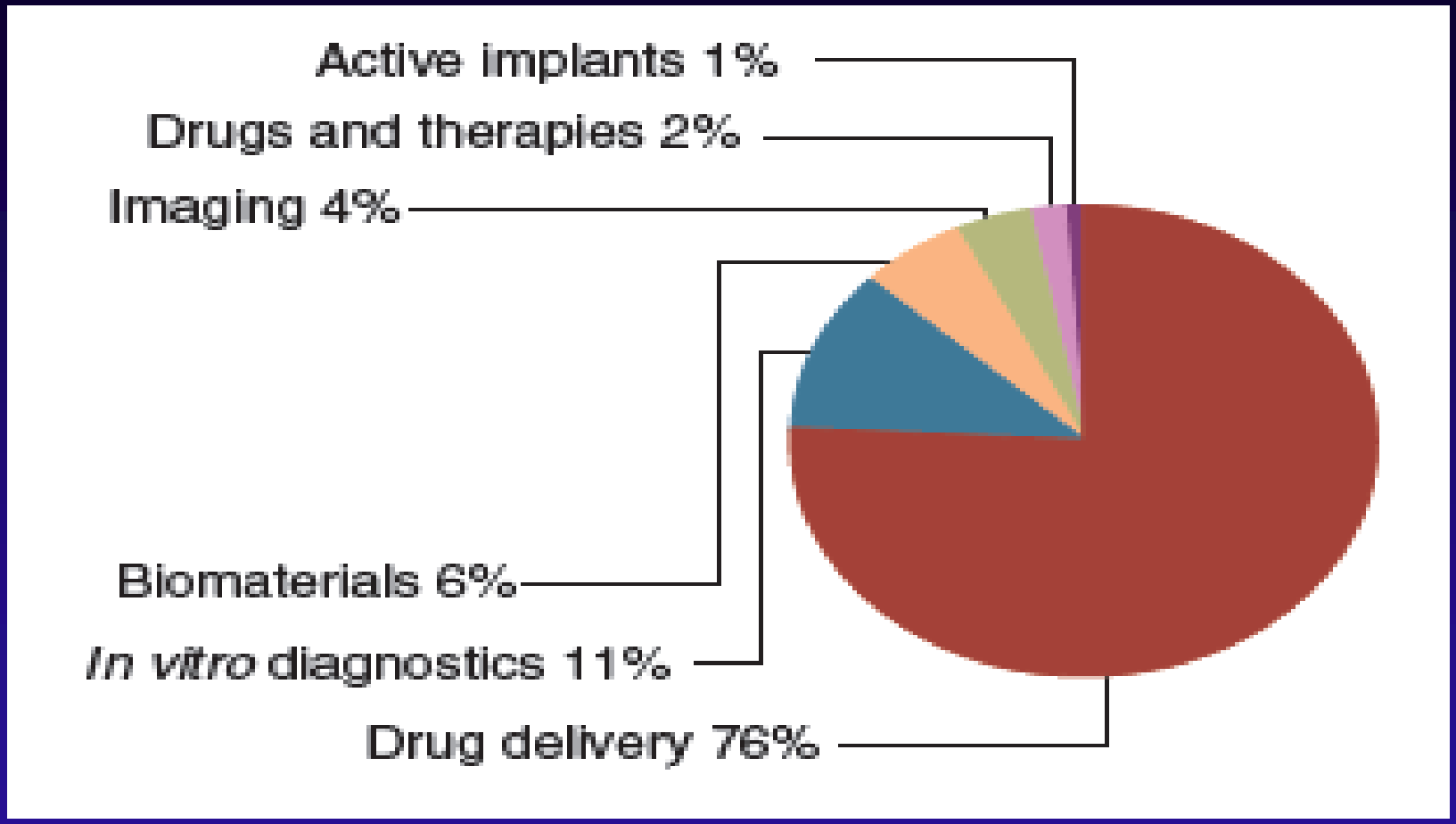
`..More than 2,000 publications in the last 2 years (4,000 papers since 2000; from ISI Web of Knowledge, 'nanoparticle and cell' hit)…' (Levy et al., 2010)

Publication & Patent Boom



Nanotechnology publications and patents worldwide. Source : Wagner et al., (2006). Nature Biotechnology Vol. 24, No. 10, pp 1211 - 1217

Healthcare & Pharmaceutical applications



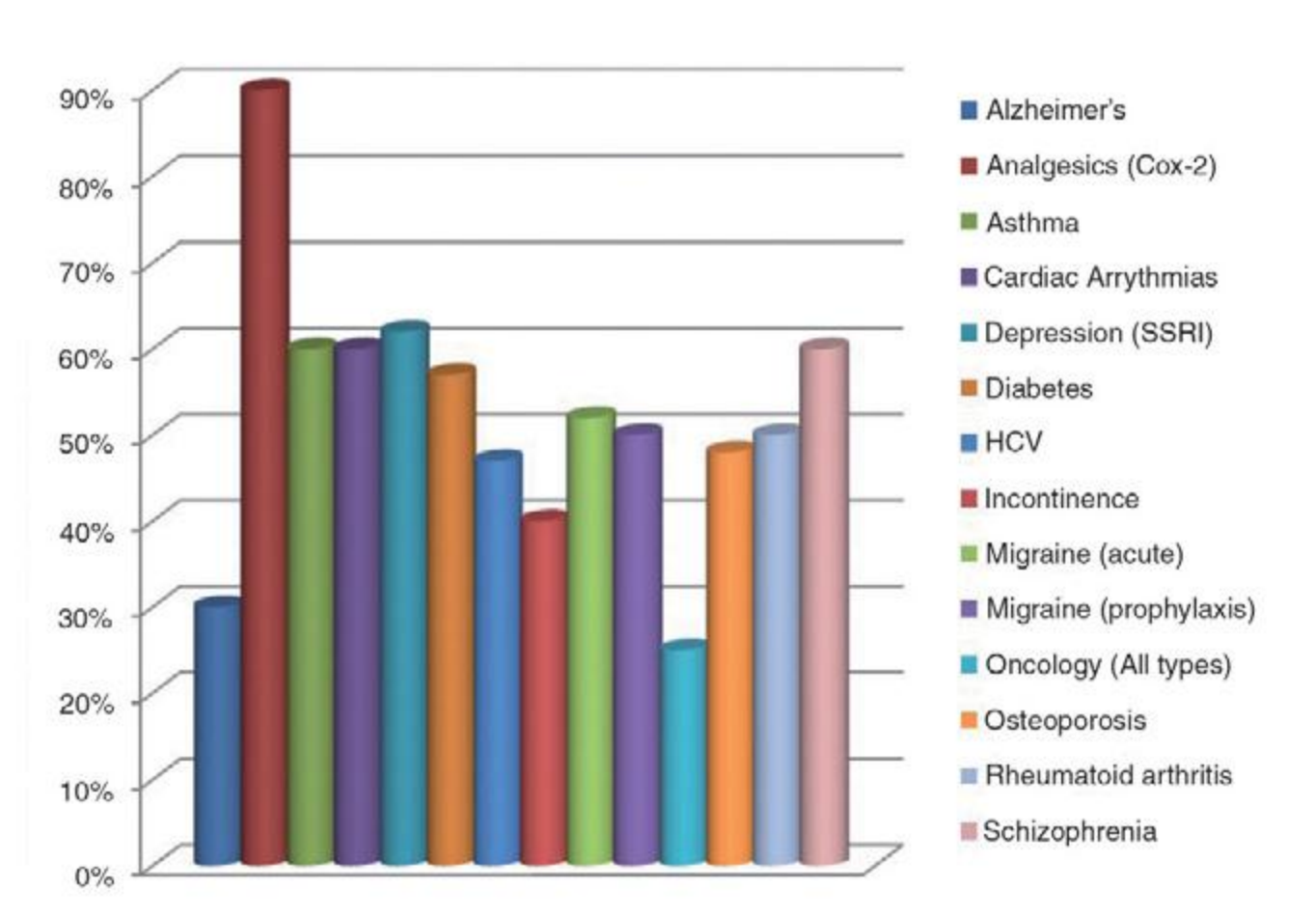
Sectorial breakdown of nanomedicine publications. Source : Wagner et al., (2006). Nature Biotechnology Vol. 24, No. 10, pp 1211 - 1217

Commercial effort ..

	Product pipeline				
Healthcare sector	Number of products	Sales ($ billions)	Total	Advanced stages[b]	Companies
Drug delivery	23	5.4	98	9	113
Biomaterials	9	0.07	9	6	32
In vivo imaging	3	0.02	8	2	13
In vitro diagnostics	2	0.78	30	4	35
Active implants	1	0.65	5	1	7
Drugs & therapy	0	0	7	1	7
Total	38	6.8	157	23	207

[a]Sales numbers of nanomedicines are estimates for the year 2004. [b]Drugs where the product is in clinical phase 2/3 or 3 and for all other products where market introduction is expected within two years.

Limitation of standard drug treatment (based on the response rates of the patients from selected group of therapeutics area).



(Source: TRENDS in Molecular Medicine, 2001).

Pharmaceutical R&D Landscape –

Development of a Model for Application of Nanotech Tools

Highly complex, costly, risky & time consuming

Traditional Model

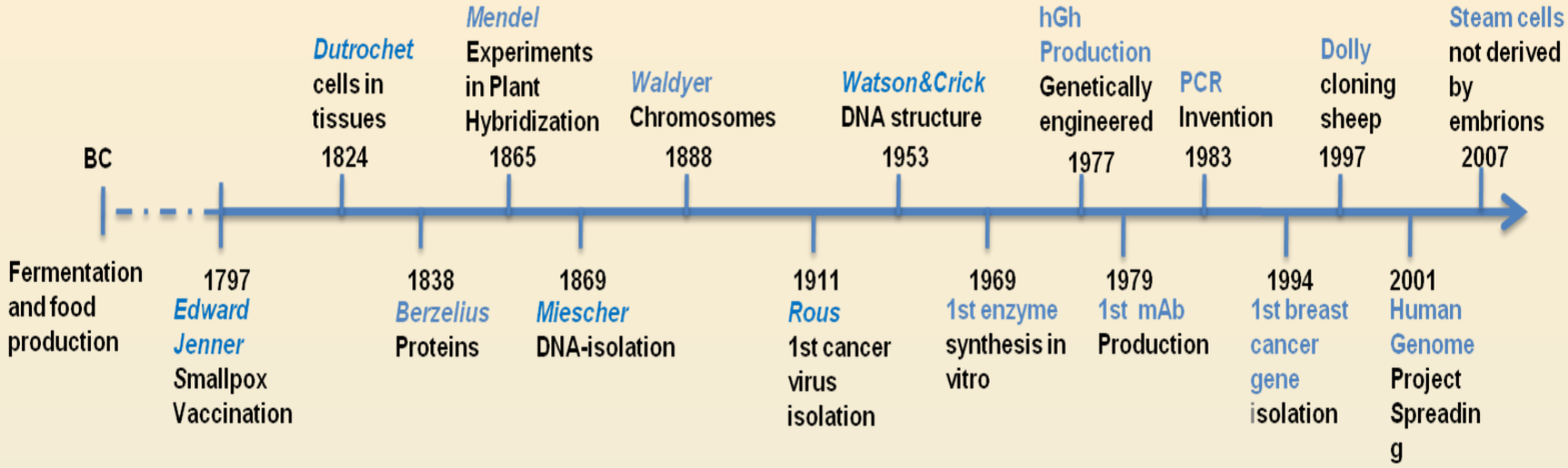
Highly complex, costly, risky & time consuming

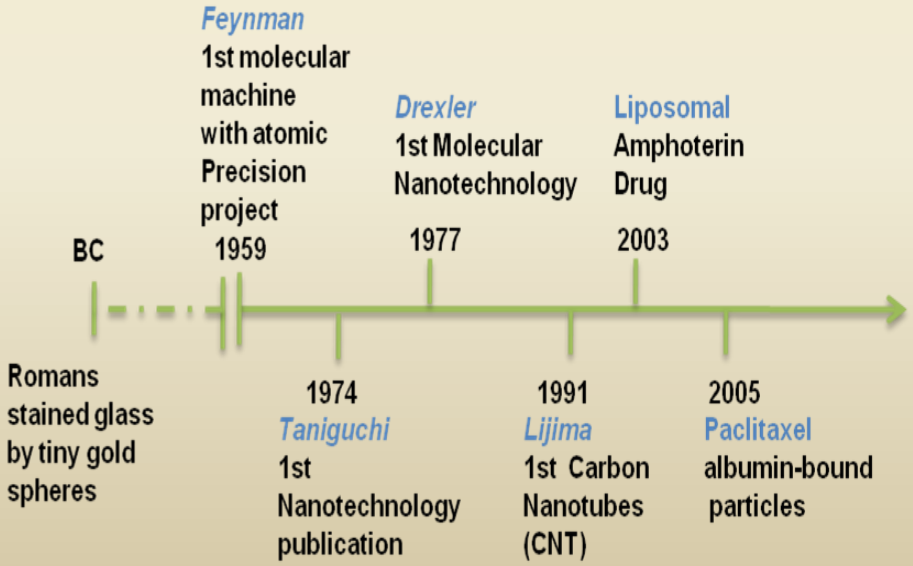
Less complex, costly, risky & time consuming

Alternate Model

Personalized Medicine	• Diagnostics & Drugs
Product Development	• Diagnostics • Drugs
Process Development	• Diagnostics • Drugs

Timeline comparison : Life Science vs Nanotech

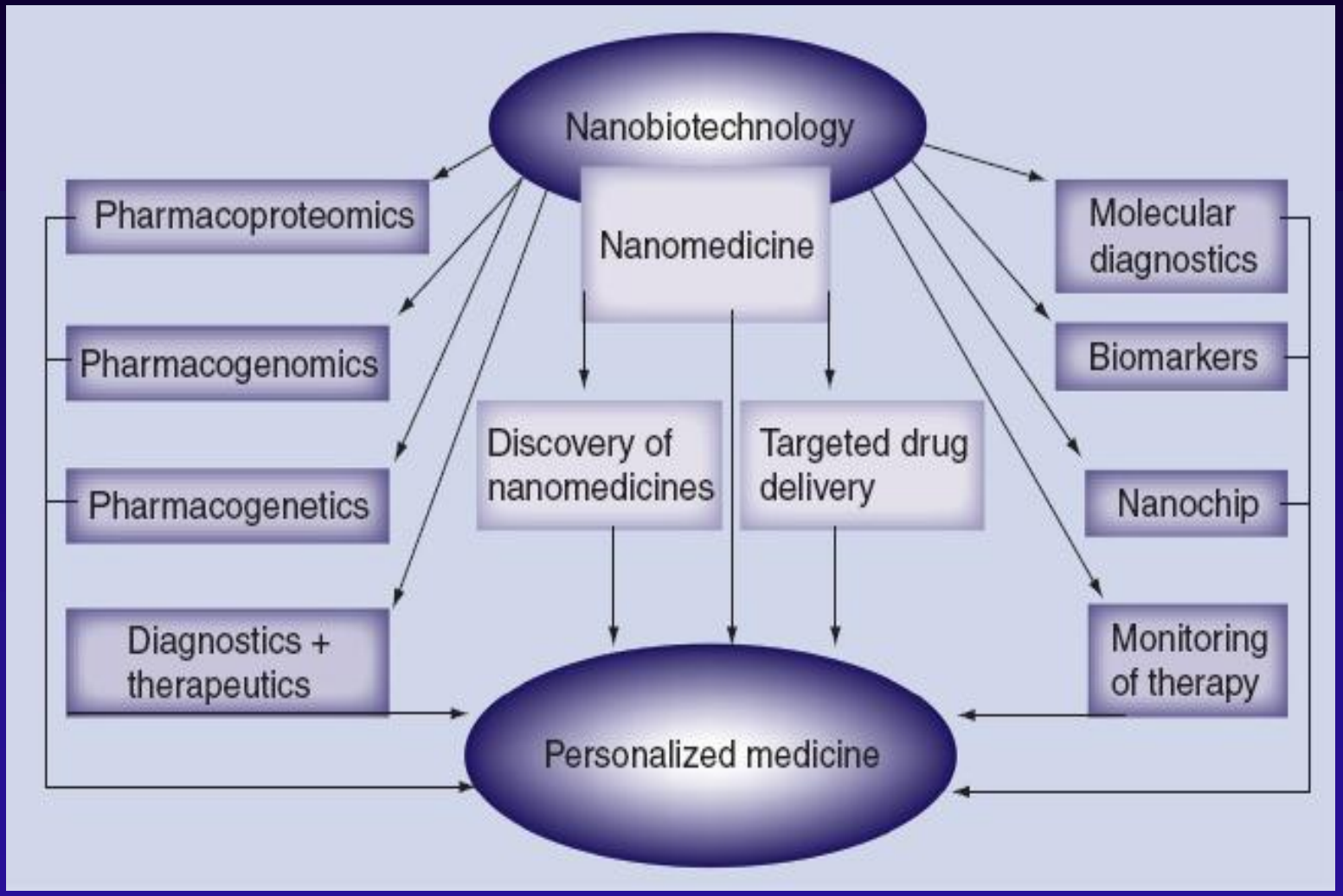




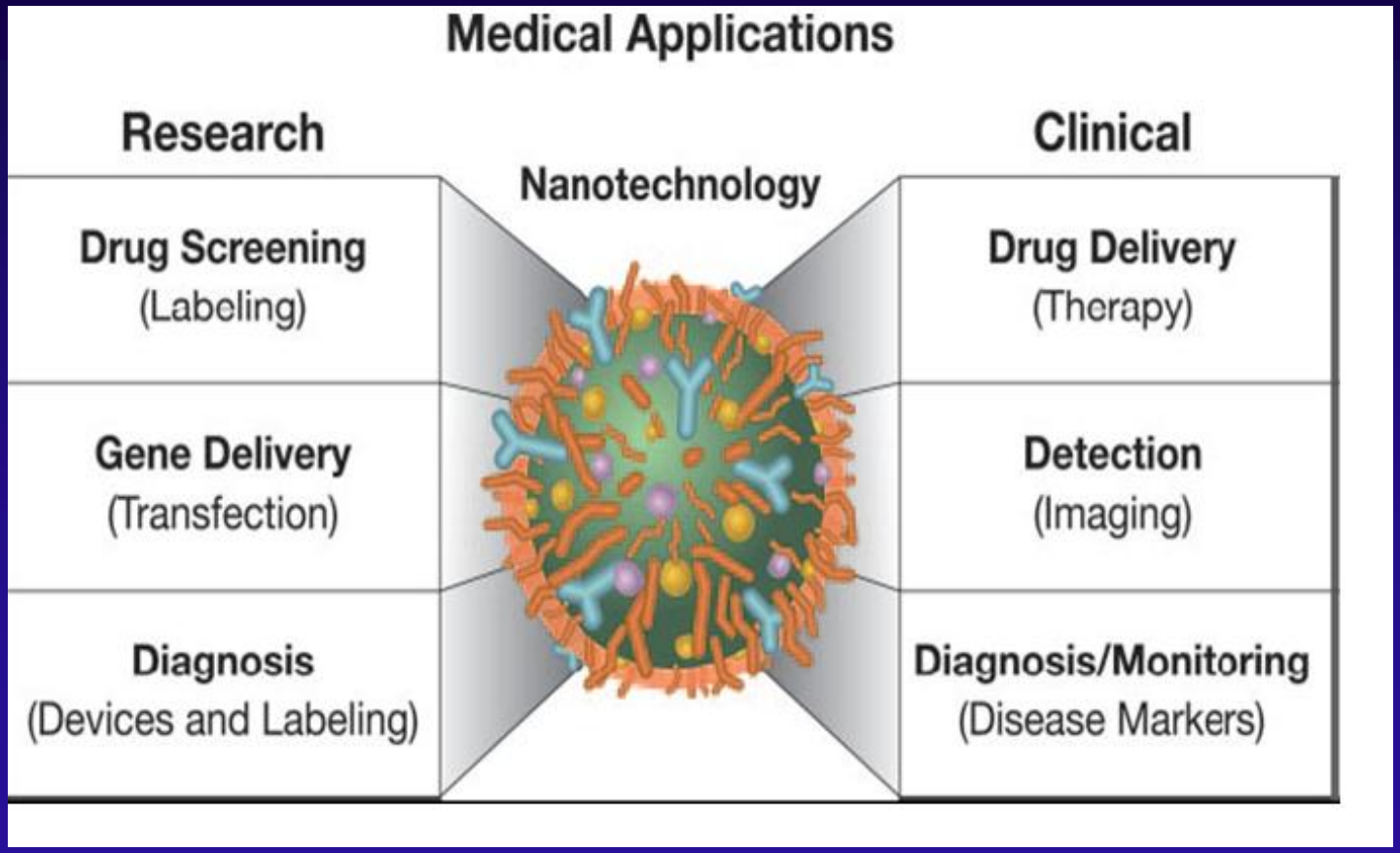
The use of Nanoparticle in Biomedicine - Seven challenges (Sanhai et al., 2008)

1. Determination of the distribution of NP in the body following systemic administration
2. Development of imaging modalities for visualizing the distribution over time
3. Understanding of mass transport across compartment boundaries in the body (how NP negotiate with biological barriers)
4. The need to predict the risk of NP (will be discuss in next slide)
5. The need to predict the benefit of NP
6. Establishment of standard/reference material & consensus protocol that can provide benchmark for the development of novel classes of materials
7. Realization of an analytical tool kit for Nanopharmaceutical manufacturing + specs sheet of toxicology, safety & biodistribution properties obtained via standardized, validated methods

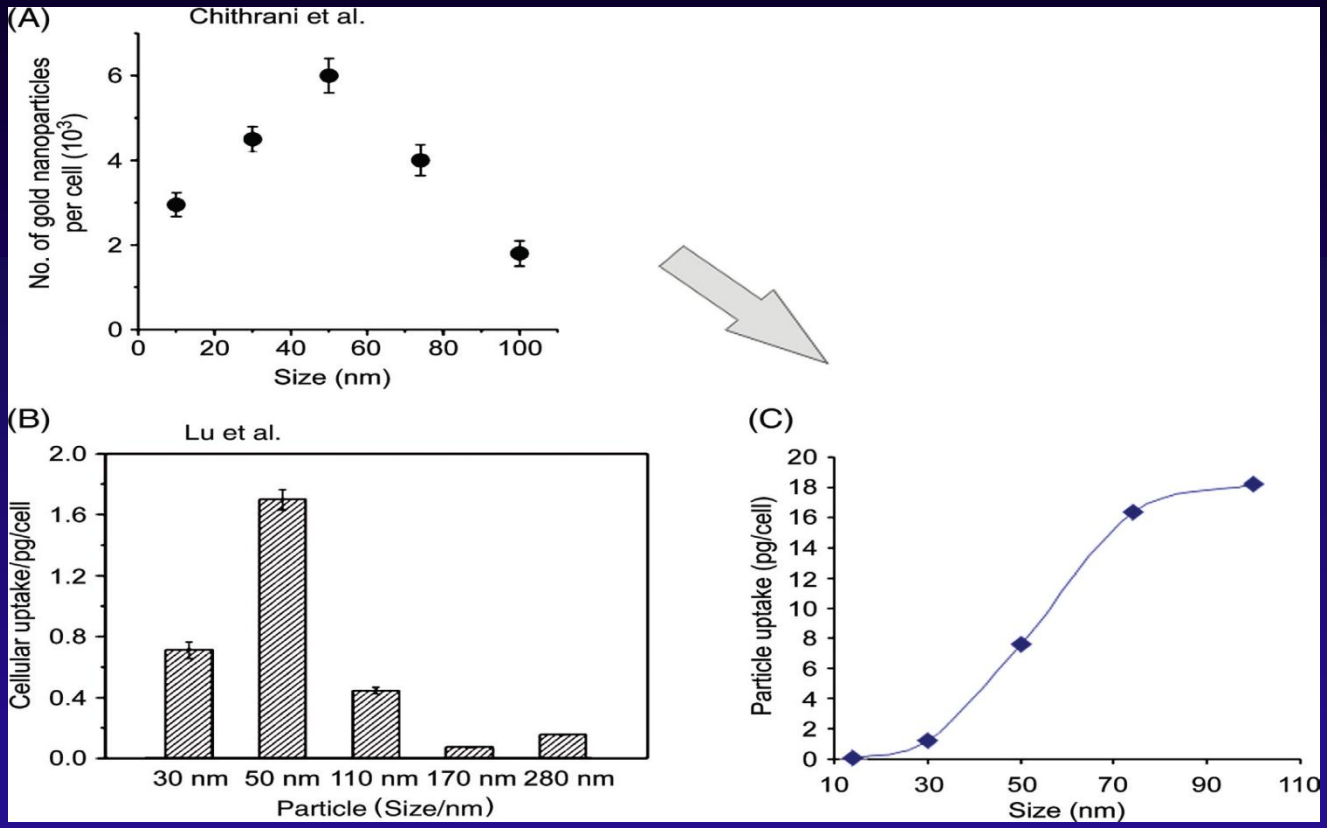
How Nanotechnology can fit in Medicine & Healthcare



Nanotechnology in biomedical application



Nanoparticles uptake by the cells



Comparison of nanoparticle uptake as a function of size reported by (A) Chithrani et al. (2006) and (B) Lu et al. (2009). (C) The results of Chithrani et al. (2006) are re-plotted with the particle uptake expressed in pg/cell, instead of number of particles per cell

Chithrani et.al.,. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett 2006; 6: 6628.

Lu F, Wu SH, Hung Y, Mou CY. Size effect on cell uptake in well-suspended, uniform mesoporous silica nanoparticles. Small 2009; 5: 1408-13.

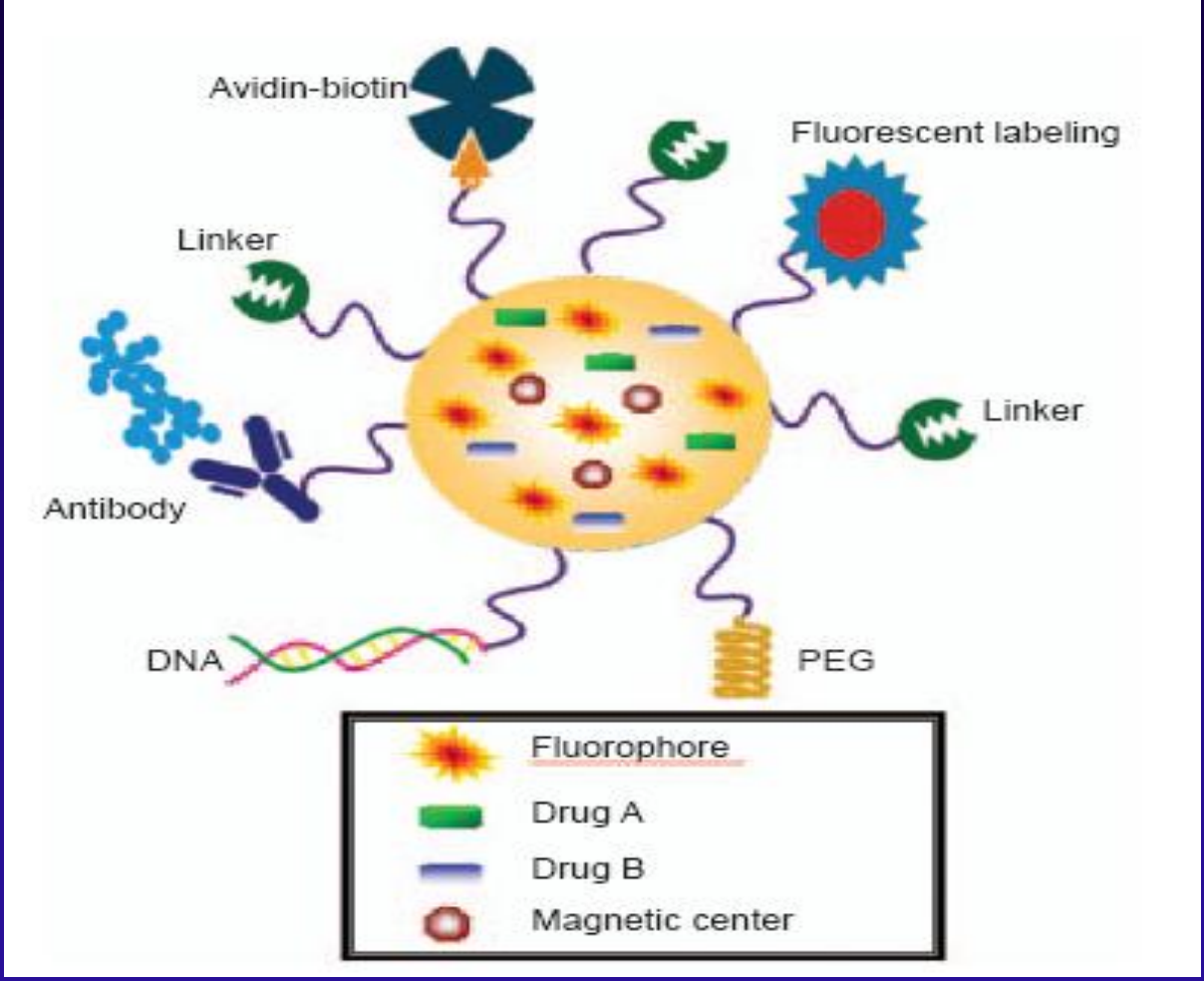
Nanocarriers

- Made from a material that is biocompatible,
- Well characterized, and easily functionalized;
- Exhibit high differential uptake efficiency in the target cells
- Soluble or colloidal under aqueous conditions for increased effectiveness
- Have an extended circulating half-life,
- A low rate of aggregation
- A long shelf life.

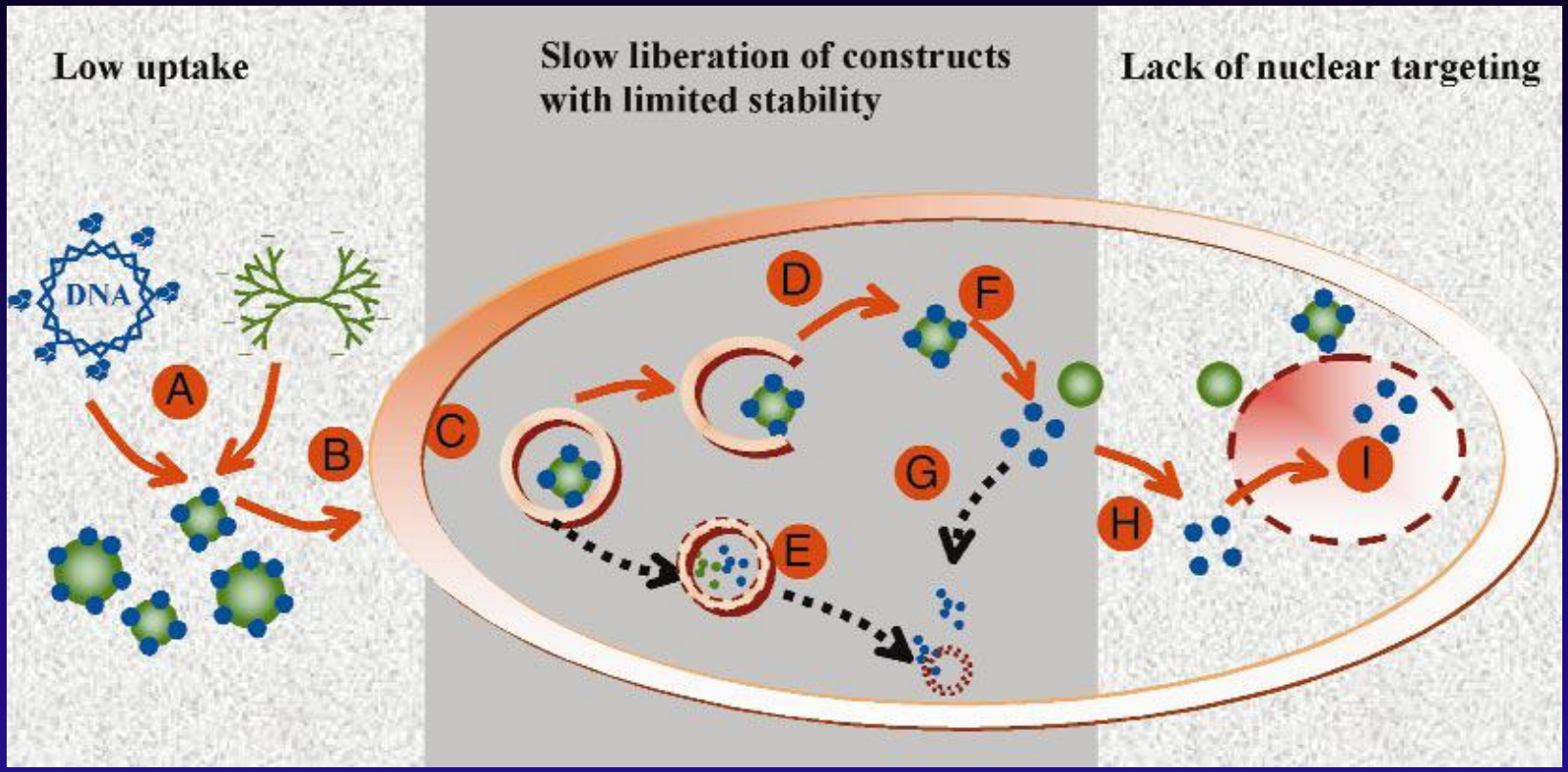
Nanocarrier advantages

- Protect the drug from premature degradation;
- Prevent drugs from prematurely interacting with the biological environment
- Enhance absorption of the drugs into a selected tissue (for example, solid tumour);
- Control the pharmacokinetic and drug tissue distribution profile;
- Improve intracellular penetration.

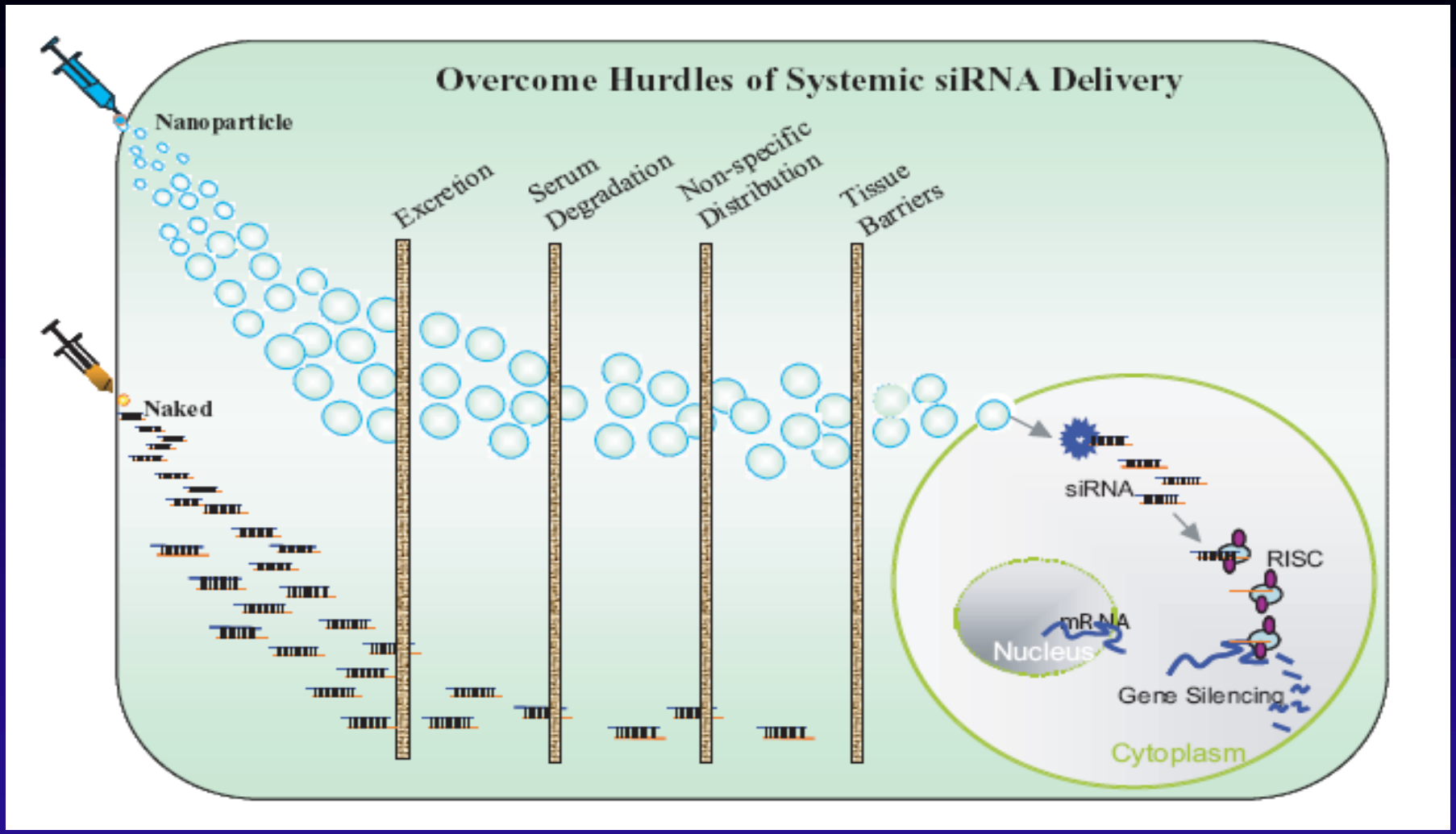
How Nanoparticles works as a delivery agent/(s)



Why NP are needed ?

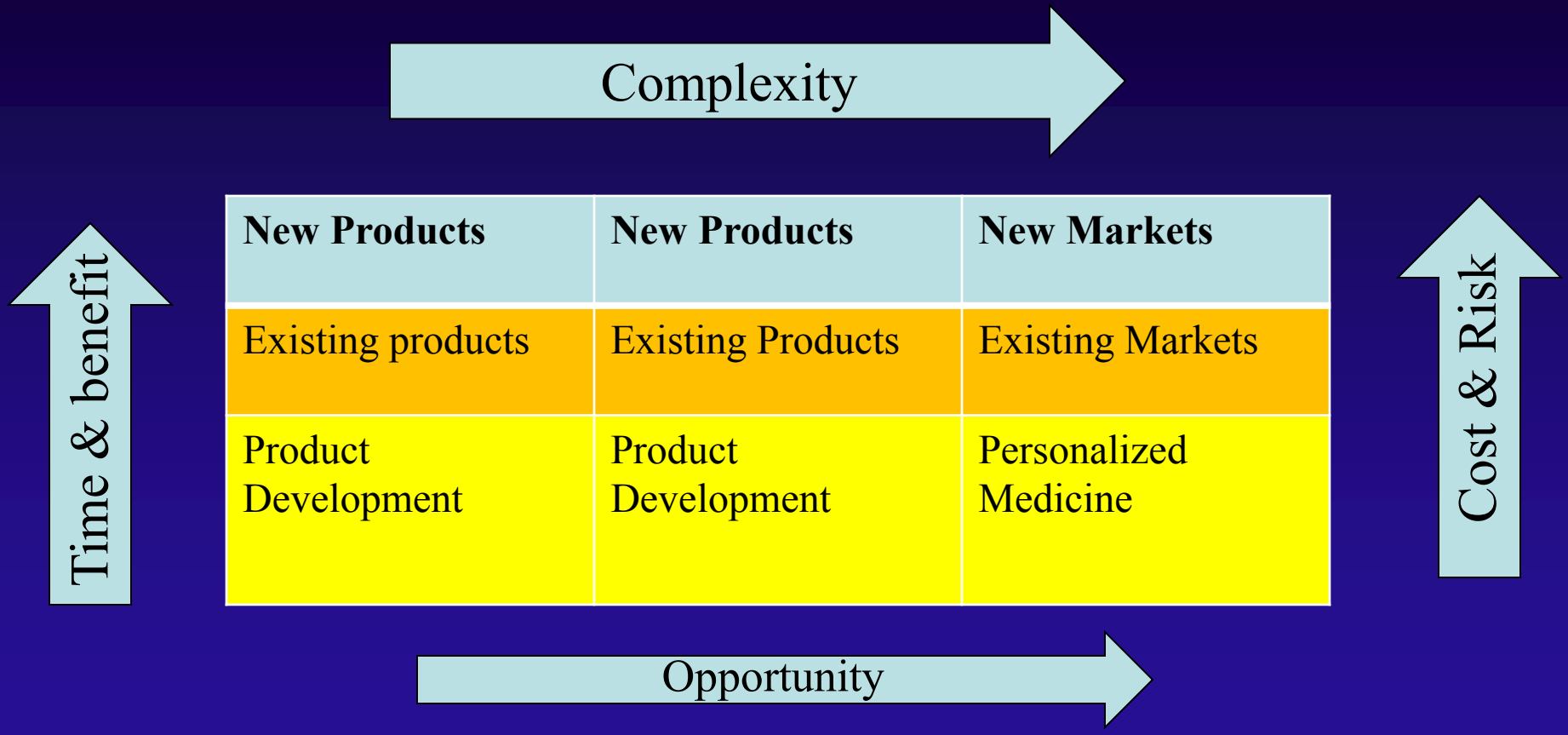


Schematic drawing of biomolecule delivery pathways with **three major barriers**: low uptake across the plasma membrane, inadequate release of molecules with limited stability, and lack of nuclear targeting. (A) biomolecule–complex formation. (B) Uptake. (C) Endocytosis (endosome). (D) Escape from endosome. (E) Degradation (edosome). (F) Intracellular release. (G) Degradation (cytosol). (H) Nuclear targeting. (I) Nuclear entry and expression



Examples of normal siRNA delivery compare to NP-assisted targetting

Innovation for implementation of Nanotechnology



New Products	**New Products**	**New Markets**
Existing products	Existing Products	Existing Markets
Product Development	Product Development	Personalized Medicine

NanoSynTest - platform

Micro Tech., 30(2004), 281-292.

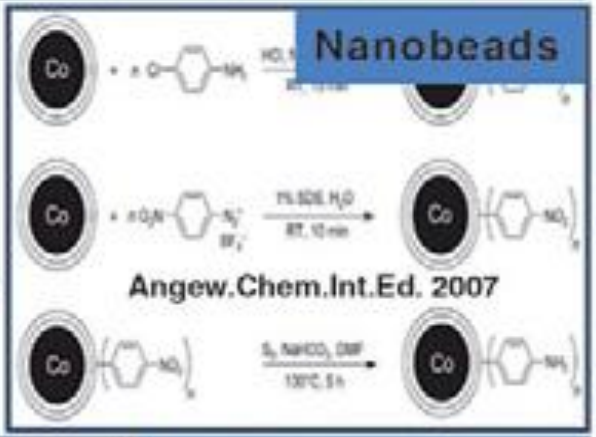
Miniaturization in High Throughput synthesis

The X-Cube™

Ultra-fast EM

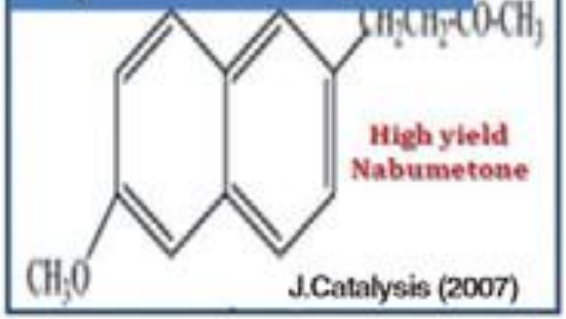
Nanotech Tools for Process Development

Nanobeads



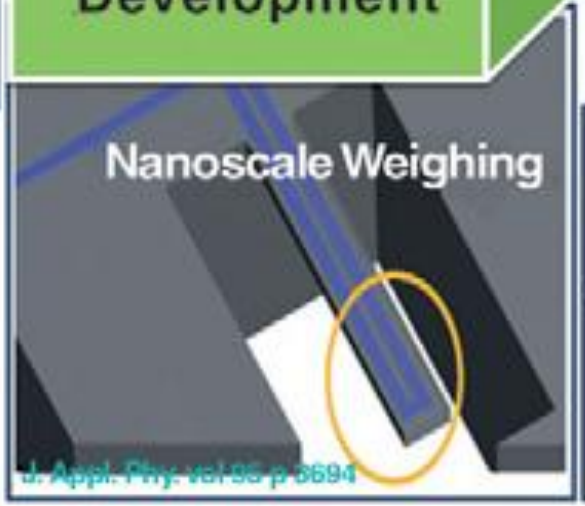
Angew.Chem.Int.Ed. 2007

MgO Nanocatalysts



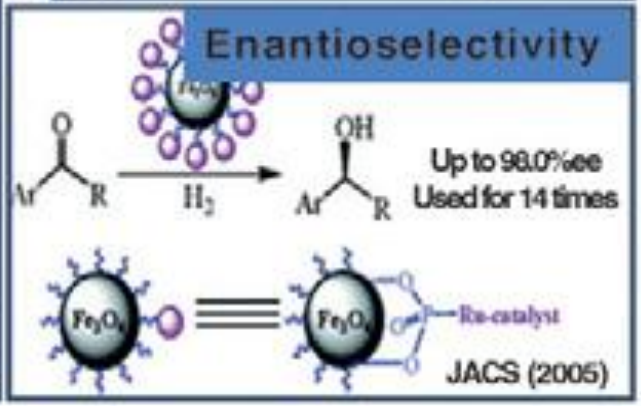
J.Catalysis (2007)

Nanoscale Weighing



J. Appl. Phy. vol 95 p 8694

Enantioselectivity



JACS (2005)

Nanotech Tools for Product Development

Nanoparticulate Drugs

NanoCrystal® Technology

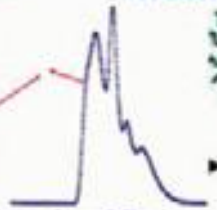
Nanotemplate Engineering™

SkyePharma

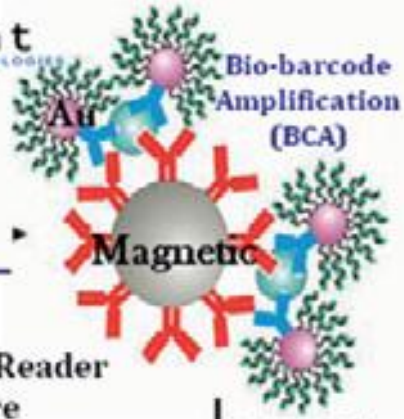
Nanomed Pharamceuticals®

Ultrasensitive Bioassays

invitrogen evident TECHNOLOGIES



Multiplex Analysis using QDs

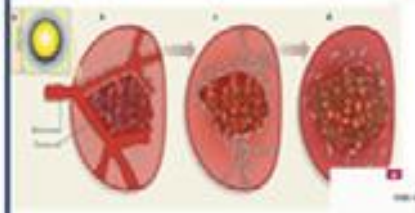


Verigene® Reader
Nanosphere

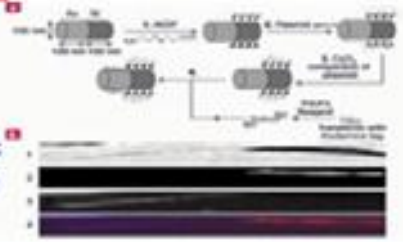
JACS (2005)

Detection of biomarker for Alzheimer's disease (PNAS 2005)

Controlled Release & Delivery



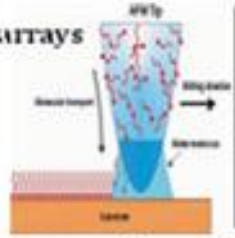
Polymer@lipid NPs for Anti-angiogenesis. (Nature 2006)

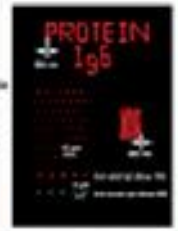


Multifunctional Nanorods for Gene Therapy (Leong)

High-throughput Synthesis & Screening

Nanoarrays





Dip Pen Lithography (C.Mirkin)

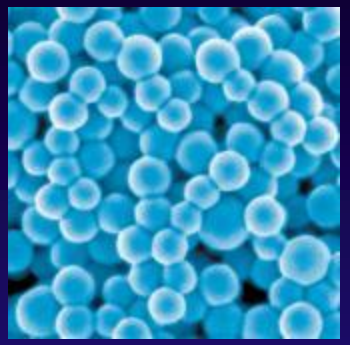
BioTrove

NANOSTREAM

Type of Nanoparticles

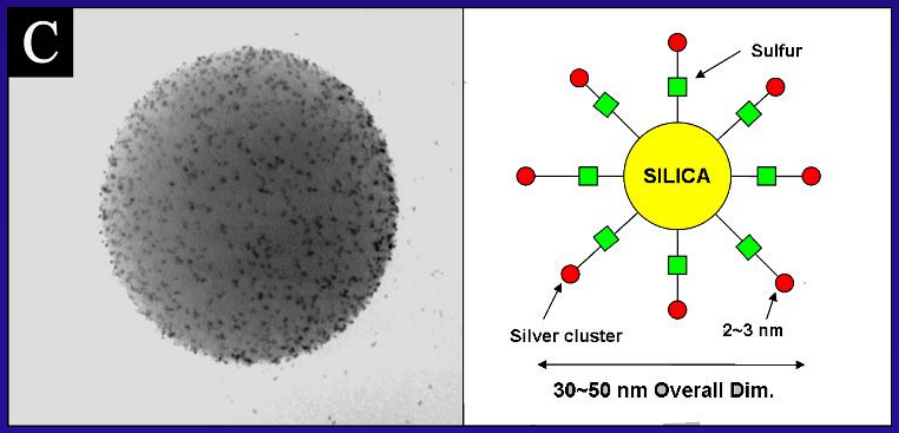
Nanosilica
Nanomagnetic
NanoGold
Liposomes
Polymers

Nanosilica (18nm – 200 nm)

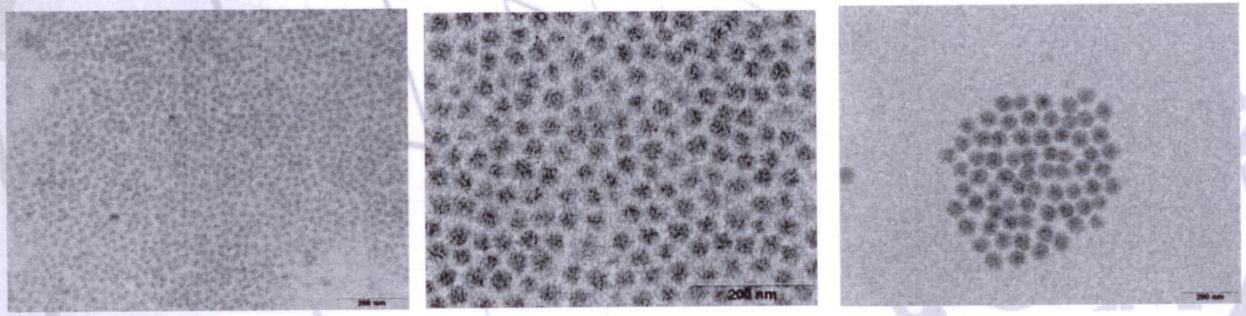


Potential application :

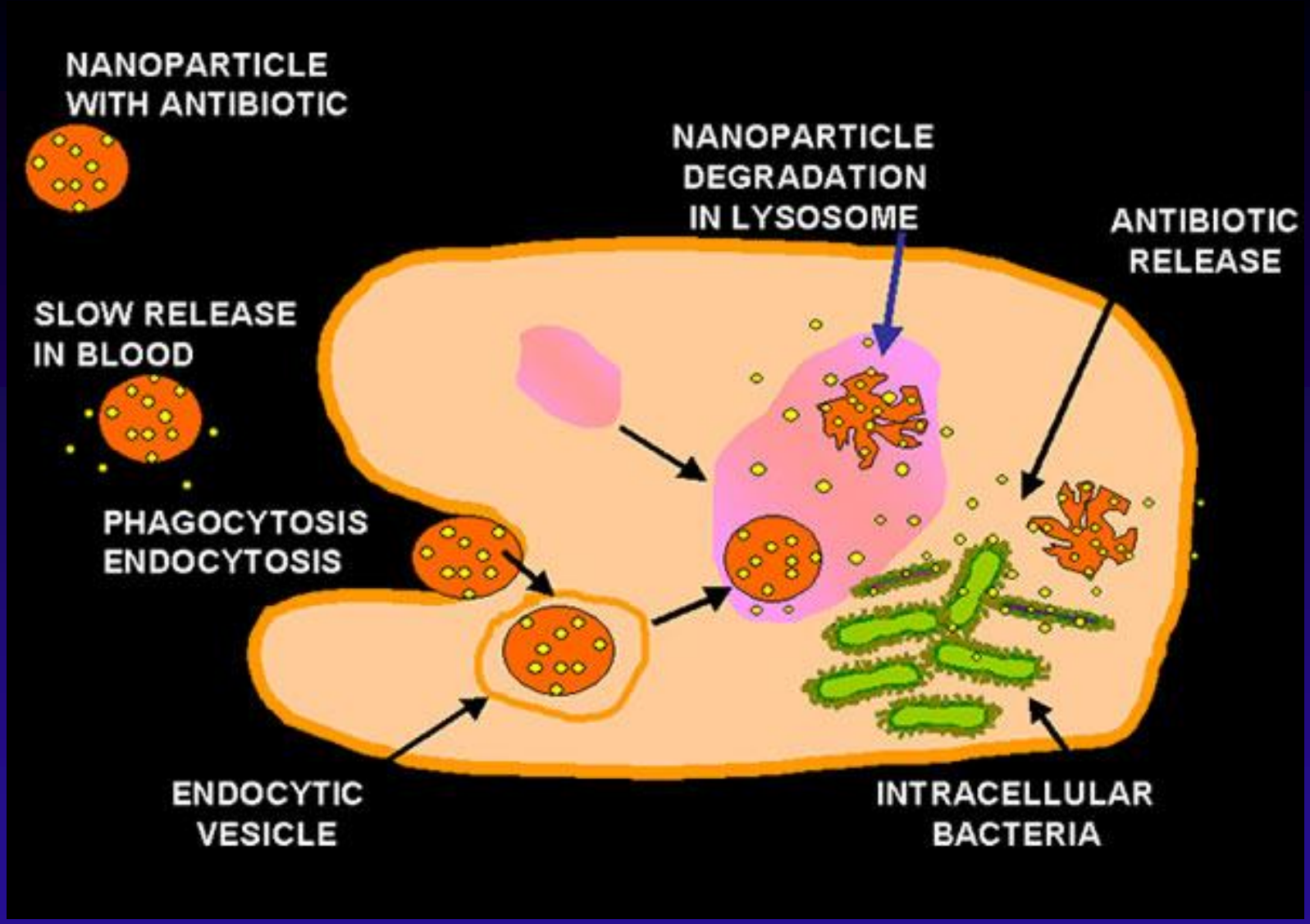
Drug Delivery System
Molecular carrier for biomolecules (siRNA, DNA, protein, antibodies etc)



TEM micrographs of nanoSilica



Controllable size by changing synthesis parameters

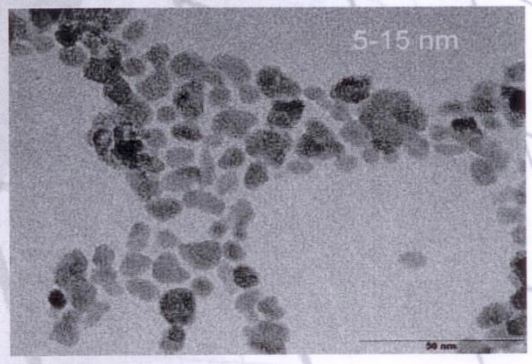


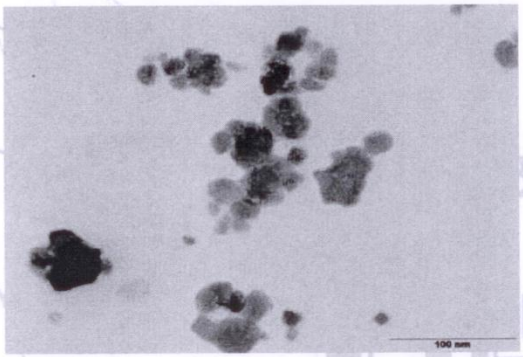
Mode of action : Nanosilica as a Drug Delivery System

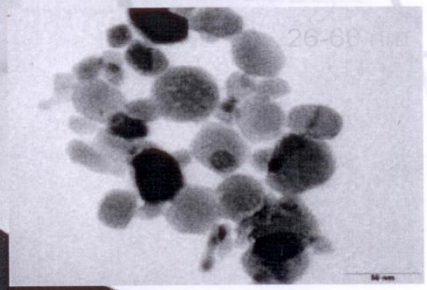
Nanomagnetic (5 nm – 70 nm)

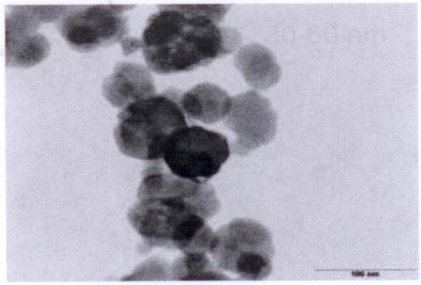
Nanomaterials that can respond in some way in the present of magnetic field (Video !)

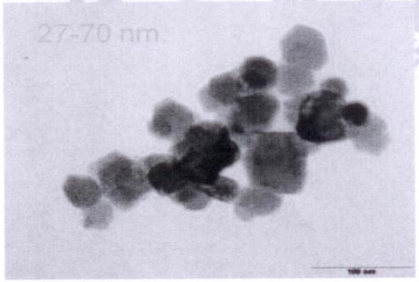
TEM micrographs of NanoMag











Changeable size, shape and properties by tuning synthesis parameters

Potential application of NanoMag

Therapy

Drug delivery

Hyperthermia/
Thermal ablation

Radiotherapy
combined MRI

Musculoskeletal system
associated diseases

Anemia chronic kidney
disease

Diagnosis

In vitro

in vivo

Sensing

MRI

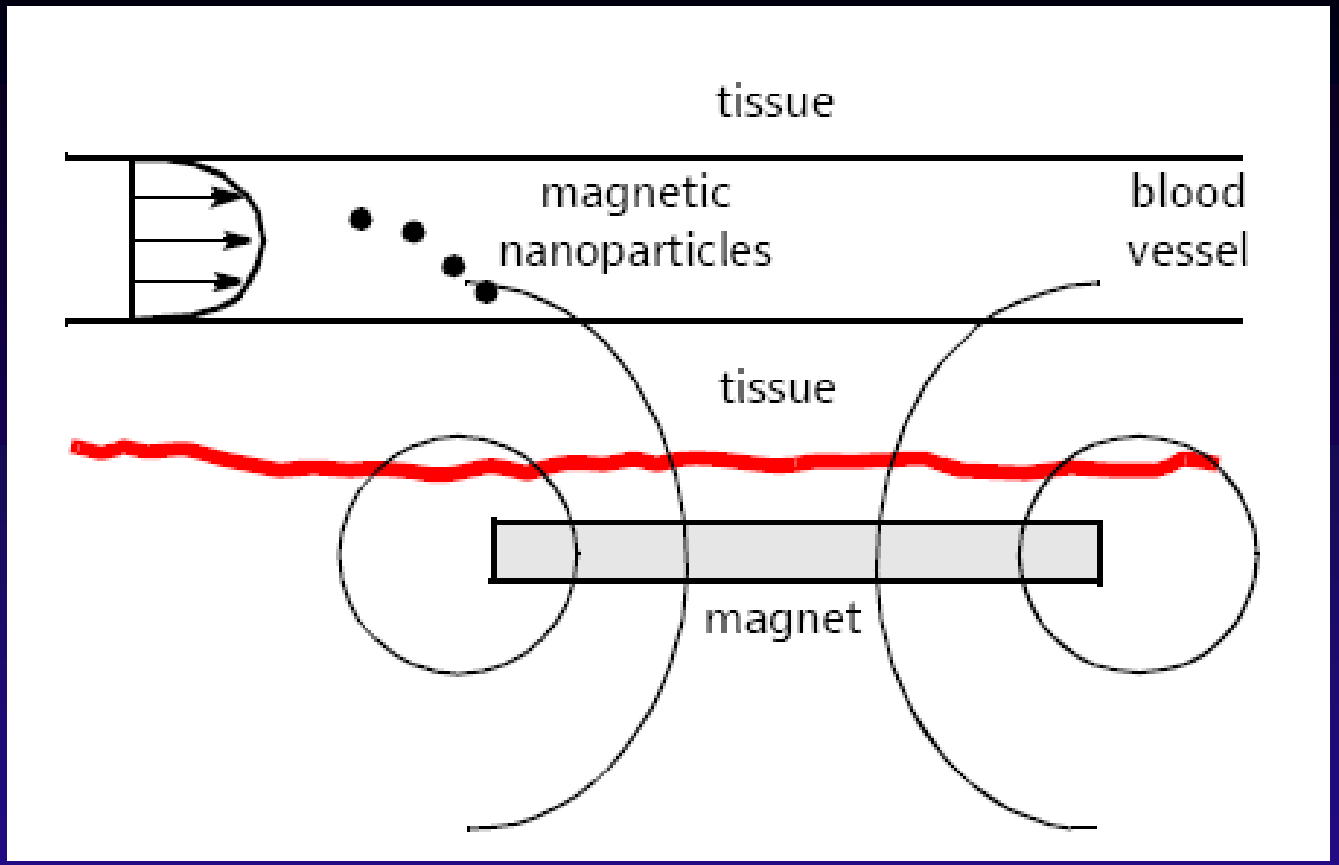
Cell Sorting

Bioseparation

Enzyme immobilization &
immunoassays

Transfection

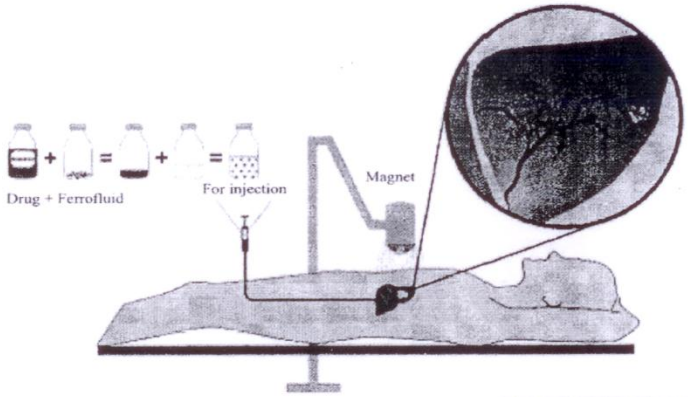
Purification



A hypothetical magnetic drug delivery system shown in cross-section : a magnet is placed outside the body in order that its magnetic field gradient might capture magnetic carriers flowing in the circulatory system

examples

Magnetic-field guided drug delivery with magnetic aerosols



Magnetic drug targeting

Particles size dependent of NanoMag in vivo

- 300 nm~3.5 um : Useful of imaging of Gastrointestinal Tract

- 60 nm~150 nm : Effective to be taken up by RES that lead to rapid uptake in liver & spleen

-10 nm~100 nm : Optimal for IV injection & have the most prolonged blood circulation

* Small enough to evade the RES of the body as well as to penetrate small capillaries of the tissues

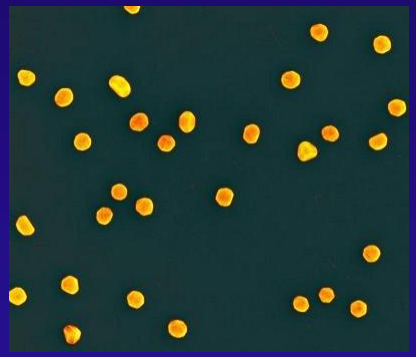
- 10 nm~40 nm : Optimal for prolonged blood circulation

* these particles can cross capillary wall and often phagocytosed by macrophages which traffic to lymph nodes & bone marrow

Video Clip Here !!!

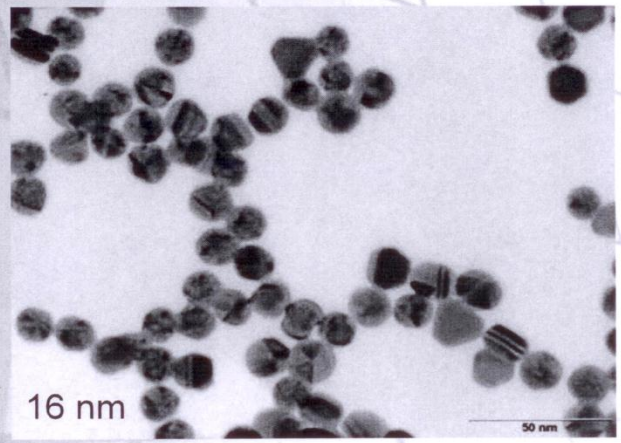
Nanogold (10nm – 30nm)

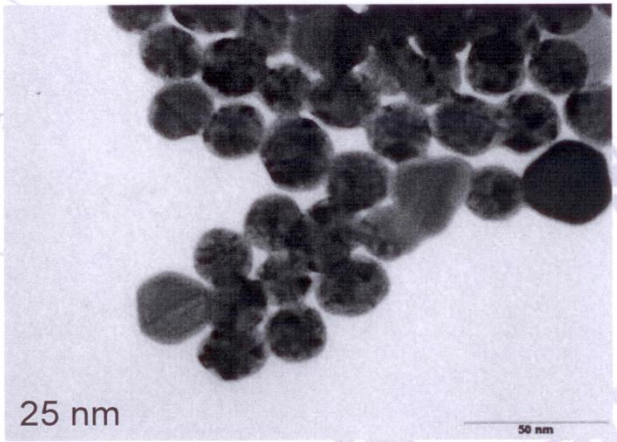
Is a suspension (or colloid) of nanometer-sized particles of gold in a fluid — usually water. The liquid is usually either an intense red colour (for particles less than 100 nm), or a dirty yellowish colour (for larger particles).



Most applications : Biomolecules tagging & Detect
System

TEM micrographs of AuNP





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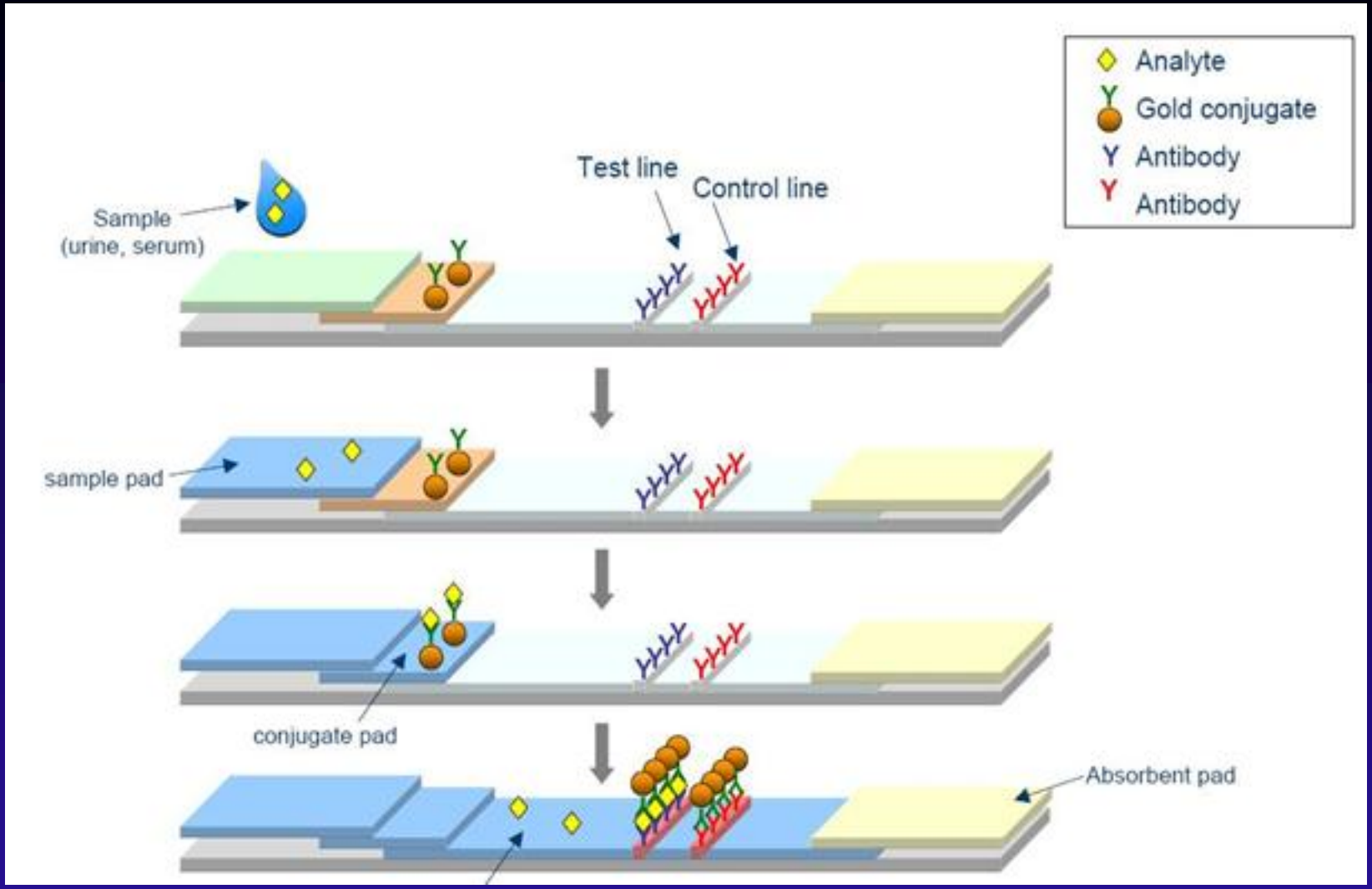
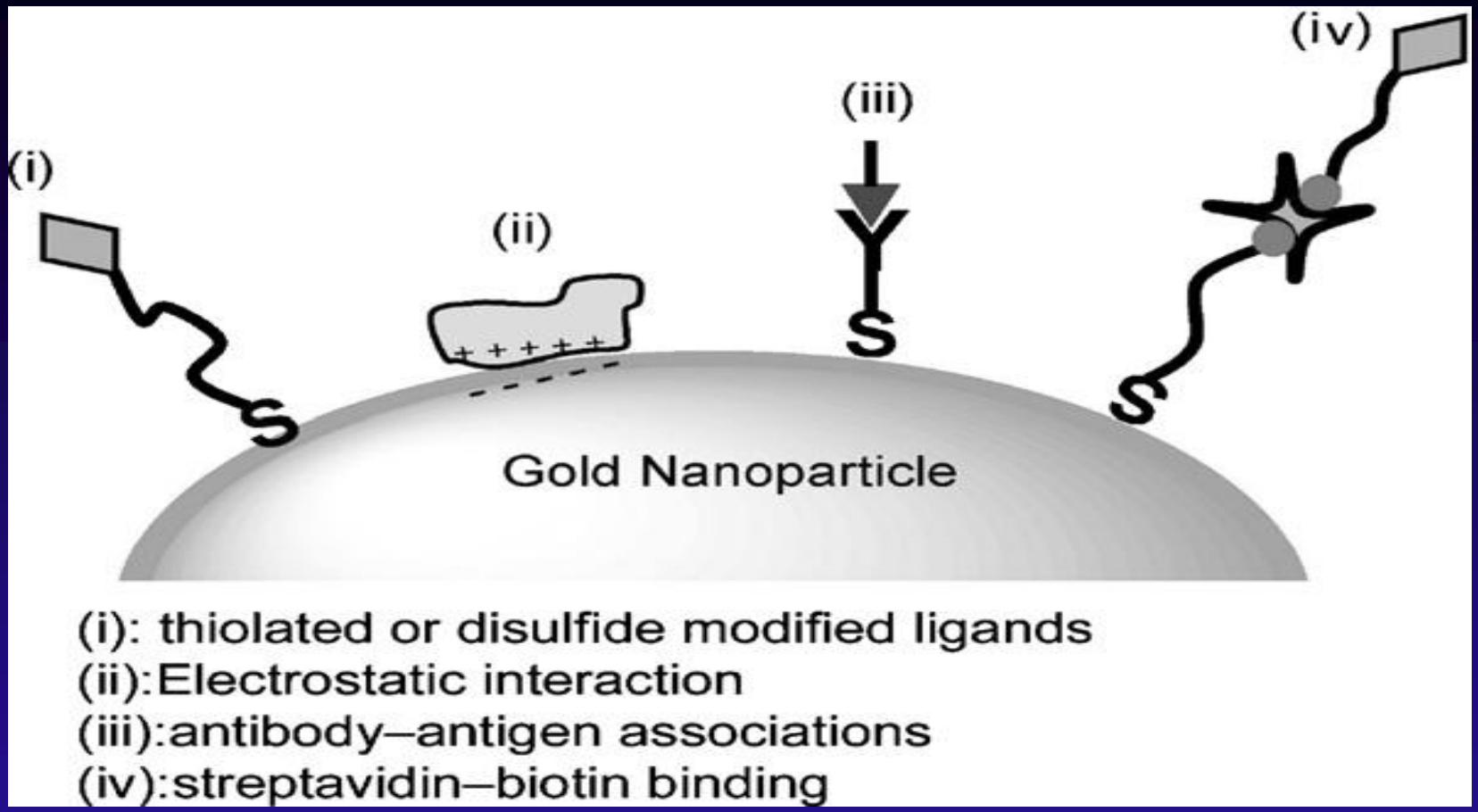


Rapid Detection via Immuno-Chromatography (eg. Pregnancy test dipstick)

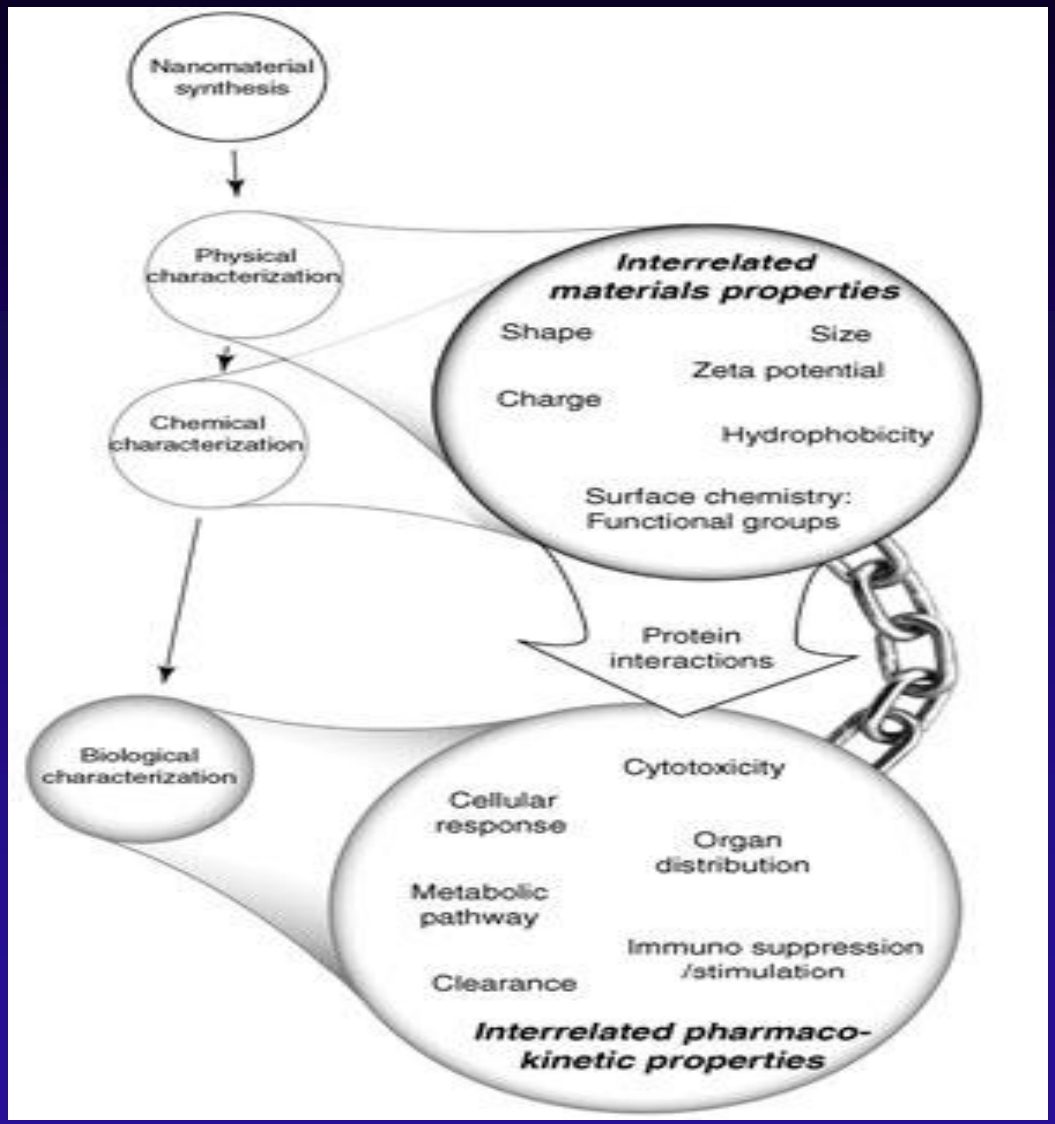


Nanogold can be conjugate to biomolecules using different methods (established)

Biomolecules & drugs

- Peptides, Polypeptides
- mAb
- siRNA, Aptamer
- DNA, Oligonucleotides
- Drugs (anti-TB drugs etc)

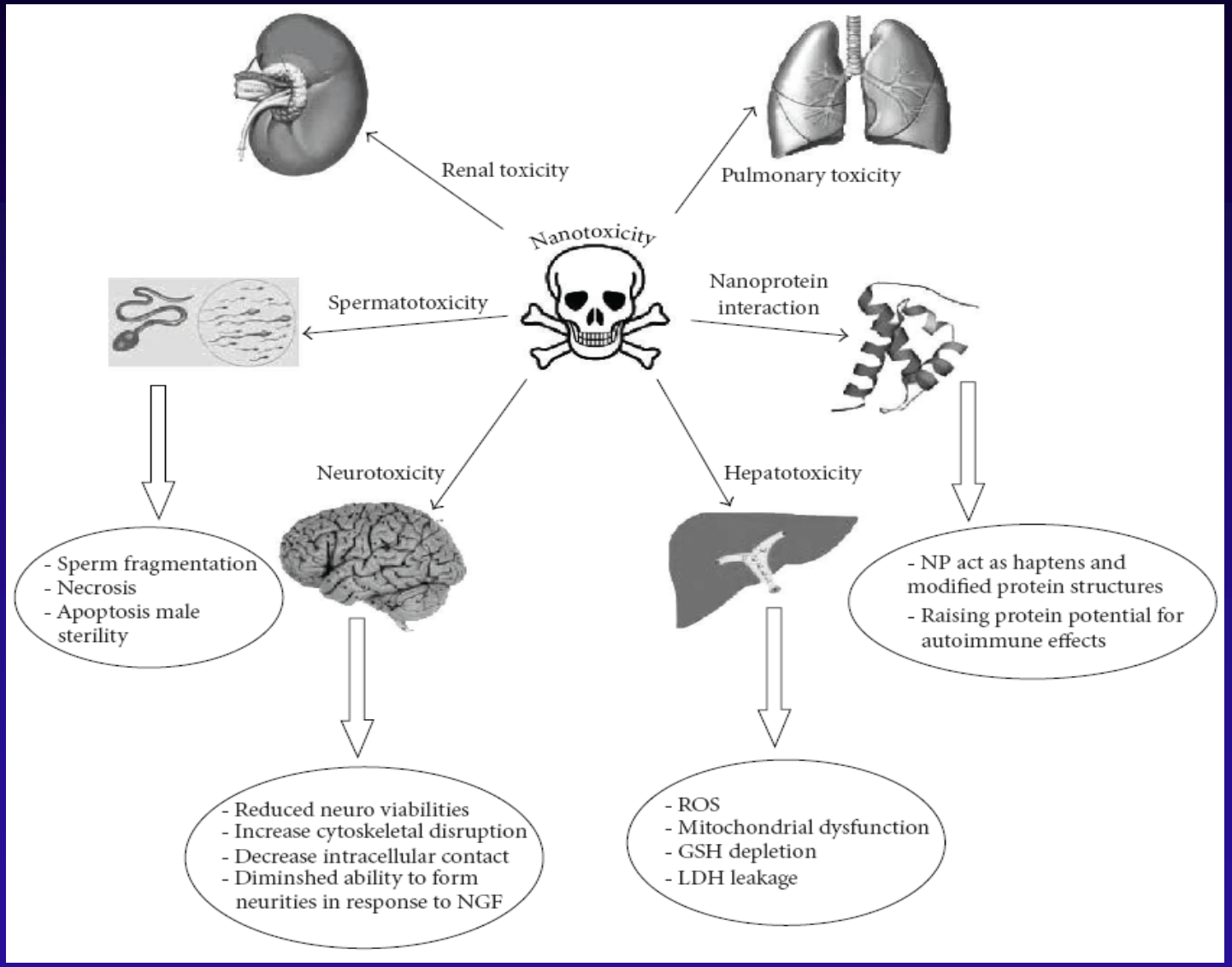
Pathway for a Nanoparticle before it can be use for Cellular & Life Science Application



Potential targets include:

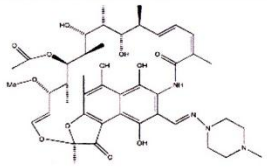
- Liver and organs of the reticuloendothelial system (RES)
- Kidney (e.g.: possibility of urolithiasis, tubular lesions),
- Central nervous system (thru BBB)
- Reproductive organs
- Cardiovascular system (e.g.: formation of aggregates),
- Development of inflammatory reactions, which appear to constitute a major risk for the respiratory tract, related to the formation of agglomerates

Nanotoxicity impact on human health (cont.)



The use of Nanosilica for Anti-TB Drug Delivery System @ NanoBRI USM

Box A : Flow Chart of NanoSilica Encapsulated Rifampicin



Rifampicin - $C_{43}H_{58}N_4O_{12}$; MW 822.9) is a red-brown crystalline
powder poorly soluble in water; it is dissolved in methyl alcohol and can be stored at room temperature protected

Why Rifampicin ? It is a drug with Multifunction (against TB, *Staph. aureus, N. meningiococci* & other Gram –ve bacteria)

Synthesis (Si + Rifampicin)

Silica Encapsulated Rifampicin (RS)

Dialysis

Concentration (AMICON)

(RS)

(RS)

Excipient Addition

(RS)

(RS)

Sterilization using 0.22 µm filter (Formulation : 1xPBS/5% Glucose)

What kind of material : Biocompatible, None/Low side effect, usable with appropriate regiment, % of surfactant <5%

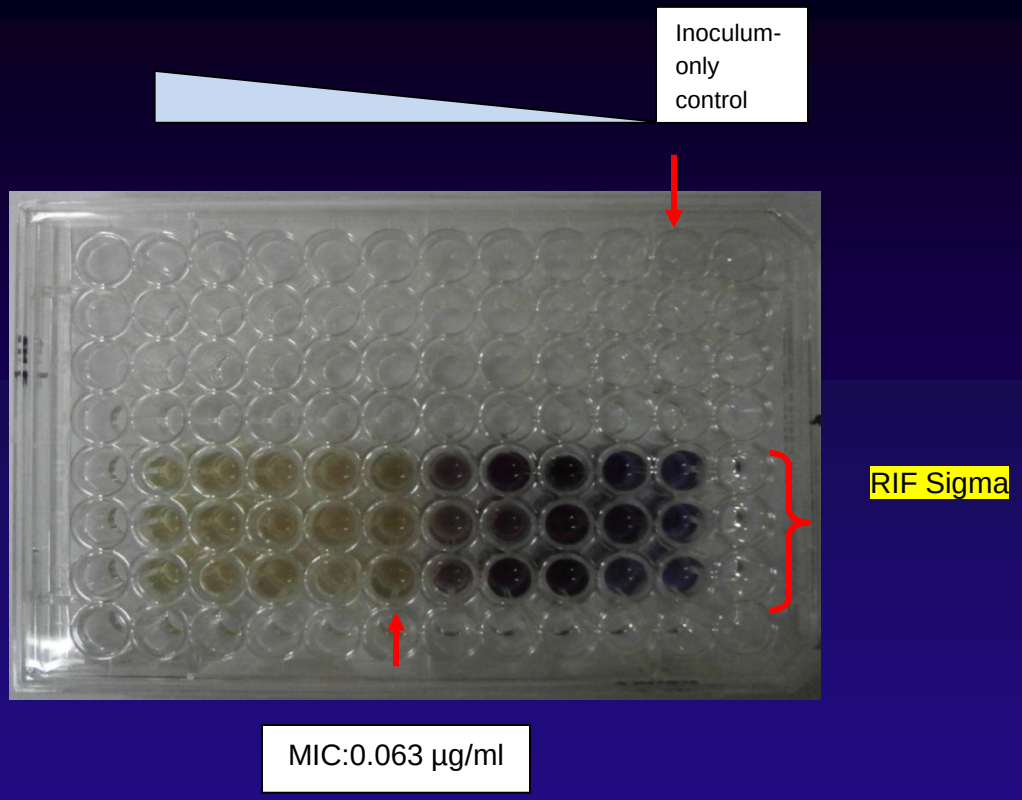
Rifampicin (Pharmacokinetics)
This drug is readily absorbed from the gastrointestinal tract (food may delay or decrease RIF absorption); within 2 to 4 hours after ingestion of a dose of 600 mg, peak plasma concentrations may reach 7-10 mg/L. It also can be given intravenously. In blood, RIF is bound to plasma proteins, and distributes into body tissues
and fluids, including cerebrospinal fluid and breast milk, and crosses the placenta. The half-life of RIF ranges from 2 to 5 hours. RIF is metabolized in the liver, and excreted in the bile, feces and urine.

Rifampicin Daily Dose : Adult (10mg/kg), children (10-20mg/kg) ; Max. 300mg

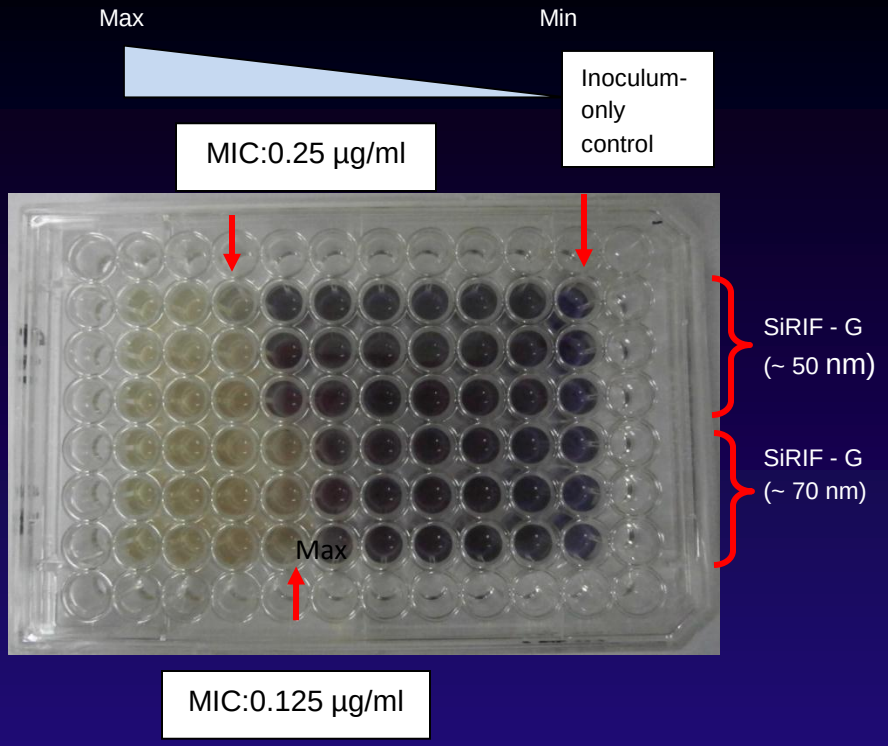
Action of Rifampicin :

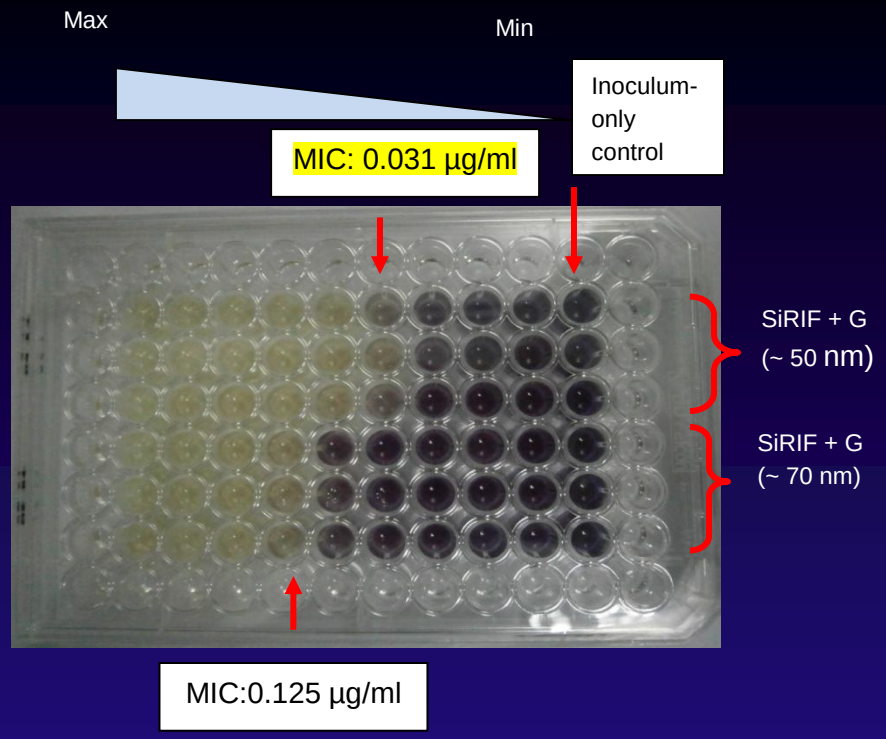
RIF inhibits gene transcription, by interacting with the beta subunit of the ribonucleic acid (RNA) polymerase enzyme. It is bactericidal against dividing mycobacteria and also has some activity against non-dividing bacilli. *M. tuberculosis* strains are normally susceptible to 0.1-2 mg/L. The introduction of RIF, thus, allowed reduction of the duration of standard antituberculosis treatments from one year to nine months. This was later reduced to six months after incorporation of PZA. RIF is also active against a wide range of microorganisms, including staphylococci, *Neisseria* spp. *Haemophilus influenzae* and *Legionella* spp.

Question answered : 1. Nanoparticle size between 30nm – 50 nm & 100 nm (suitability will be determined by cytotoxicity and other appropriate experiments. 2. Good amount of drug : will be decided by in vitro cytotoxicity assay and previous reported dose

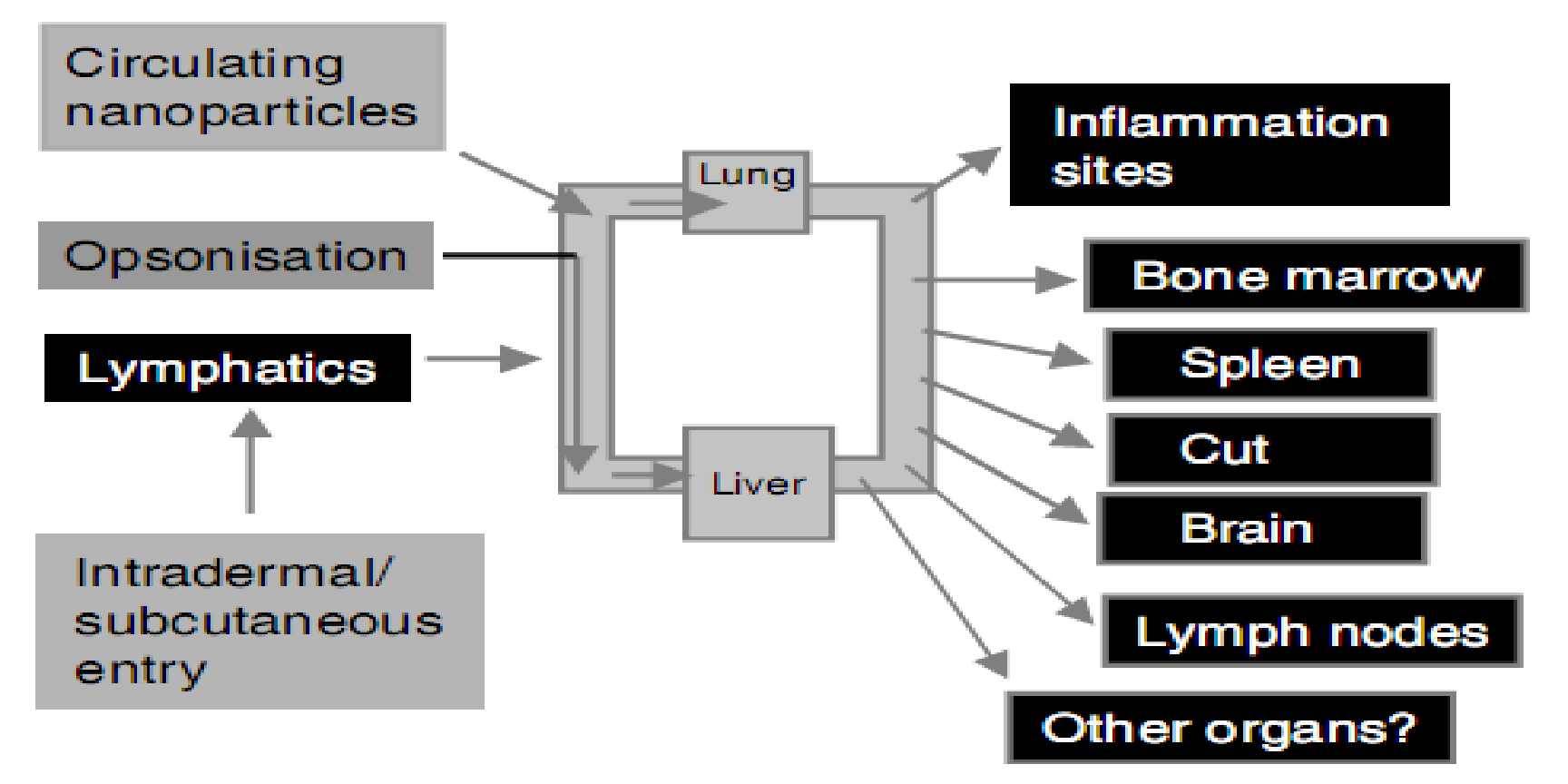


TEMA (MIC experiment) on *M. Bovis*BCG using Rifampicin Sigma (2 µg/ml)



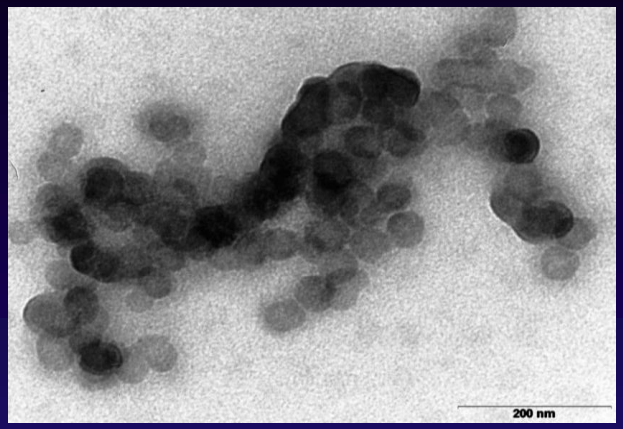


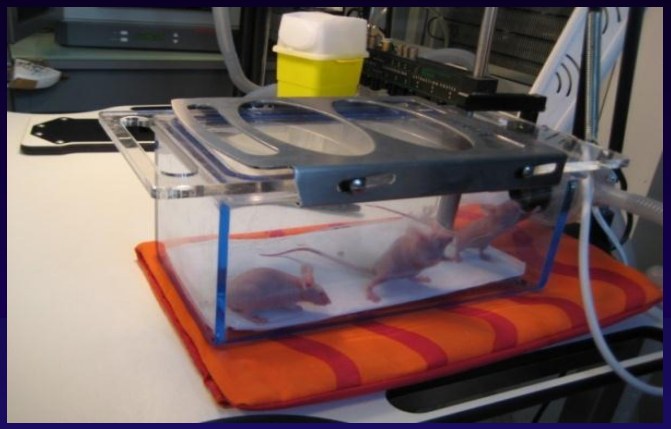
TEMA (MIC experiment) on *M. Bovis* BCG using Silica-Rifampicin with glucose (as excepient) & without Glucose : samples designed at different of sizes; ~50 nm and ~70 nm.



Summary diagrams showing the possible areas of localization of nanoparticles to various tissues from the blood circulation.

Biodistribution analysis of Nanosilica (50 nm) loaded with Rifampicin & DiD

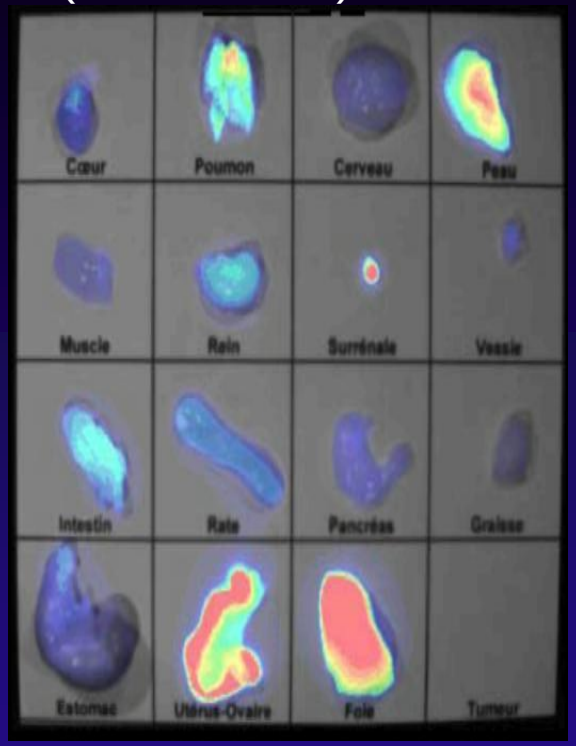




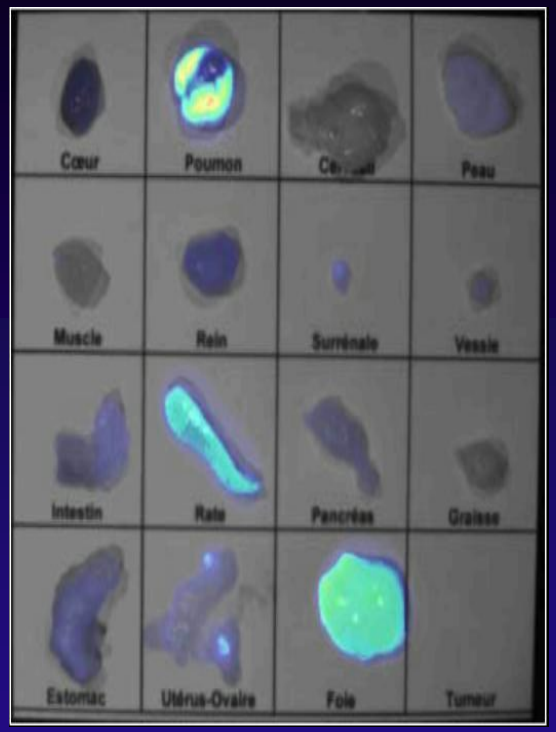
Sacrifice & Analysis

Normal Light

Fluorescence (NP+Rif+DiD)



Fluorescence (DiD only)



Several parts of mice were taken for imaging purposes. Read from Left to Right (1st row: heart, **lungs**, brain, **skin;** 2nd row: muscle, **kidney**, adrenal, bladder; 3rd row: **intestine,** spleen, pancreas, fat; 4th row: stomach, **uterus-ovary**, **liver**, tumor (not applicable)

Nanoparticles into the cells : Difficulties

1. Very few comprehensive quantitative studies of the kinetics of interaction and uptake of nanomaterials into the cells – Time for saturation of equilibrium, size/shape dependent ?

2. How to know exactly which species are taken up by the cell. Is it single nanoparticles or aggregates formed prior to, or during interaction with the membrane? Does functionalization of surface play important role ?

3. Passive uptake of nanomaterials always results in endosomal localization. This is a stringent limitation for biological and biomedical applications and therefore considerable efforts need to be done to escape/bypass the endocytotic pathway

Other `Do-able' Applications

- Diagnostics tool
- Biomolecule vehicle
- Purification
- Transfection tools (LMGT)
- Imaging medical devices

NanoBiotech Research & Innovation (NanoBRI), Institute for Research in Molecular Medicine (INFORMM), USM

Services :

Production of Nanosilica, NanoMag and Nanogold

Characterization of Nanomaterial

QC of conjugation quality, solution absorbtion & transmission, compound concentration

Cytotoxicity test of Nanomaterial/Nanobased cosmetic on cells

Contract Research

Training of scientist in Nanotechnology, Nanomedicine & Nanobiotechnology

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Thank You