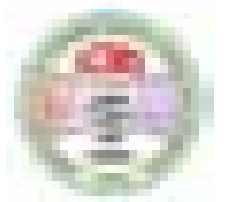
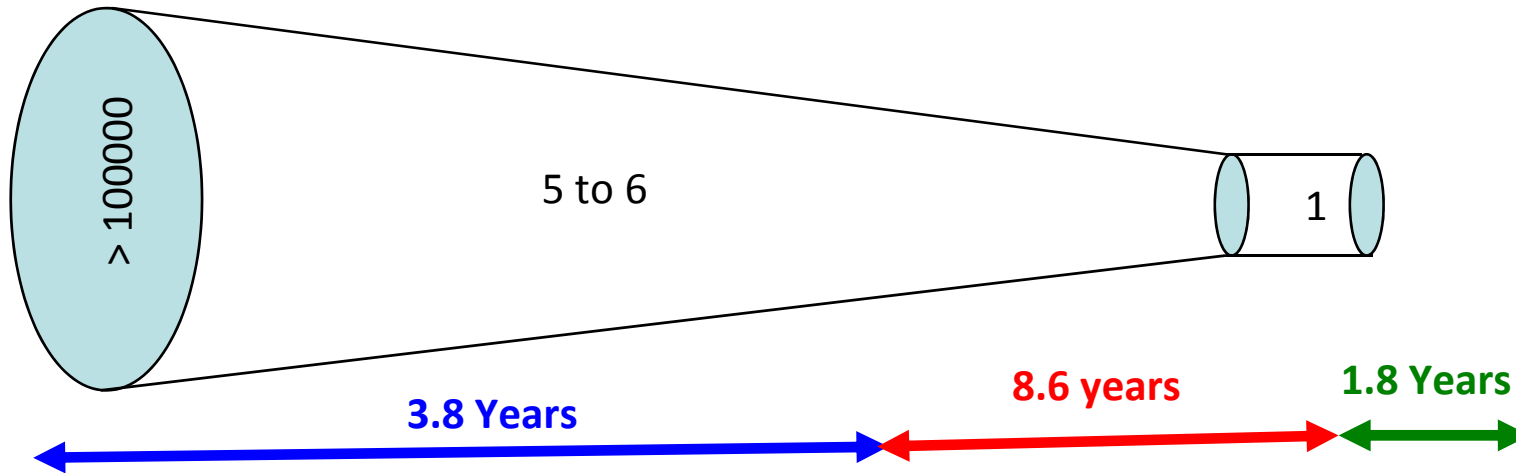
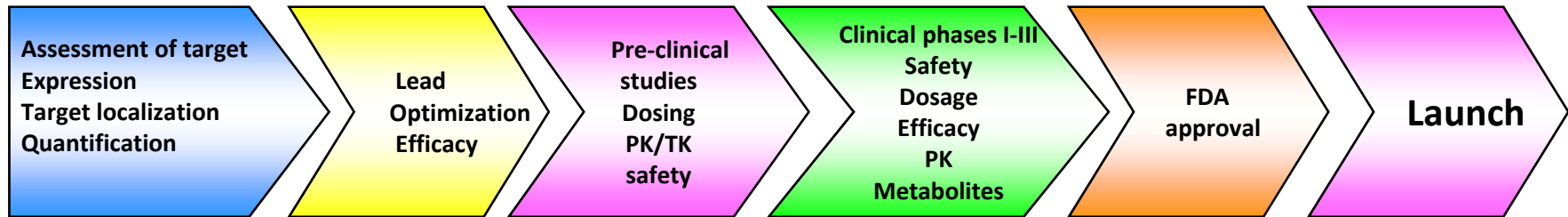


# Magnetic nanoparticles as carriers in drug delivery – Challenges & future opportunities

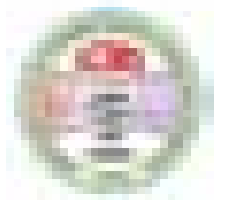
Venkat Manohar  
Indian Institute of Chromatography &  
Mass Spectrometry(IICMS)  
Chennai -32



# Drug discovery 'game' !!



Jurgen k. Willmann, et al., Nature Rev., 7, 2008, 591



# We shall discuss

- **Basic requirements of drug delivery**
- **Nanoparticles in drug delivery**
- **Magnetism & biology**
- **Magnetic nanoparticle and its role**
- **An example**
- **In future....**



## \* Drug synthesis & drug product

- ◆ Drug substance- API

- ◆ Drug product - bridge chemistry with biology

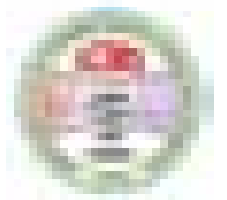
- ◆ Drug product - numerous aspects of formulation

  - Solubility

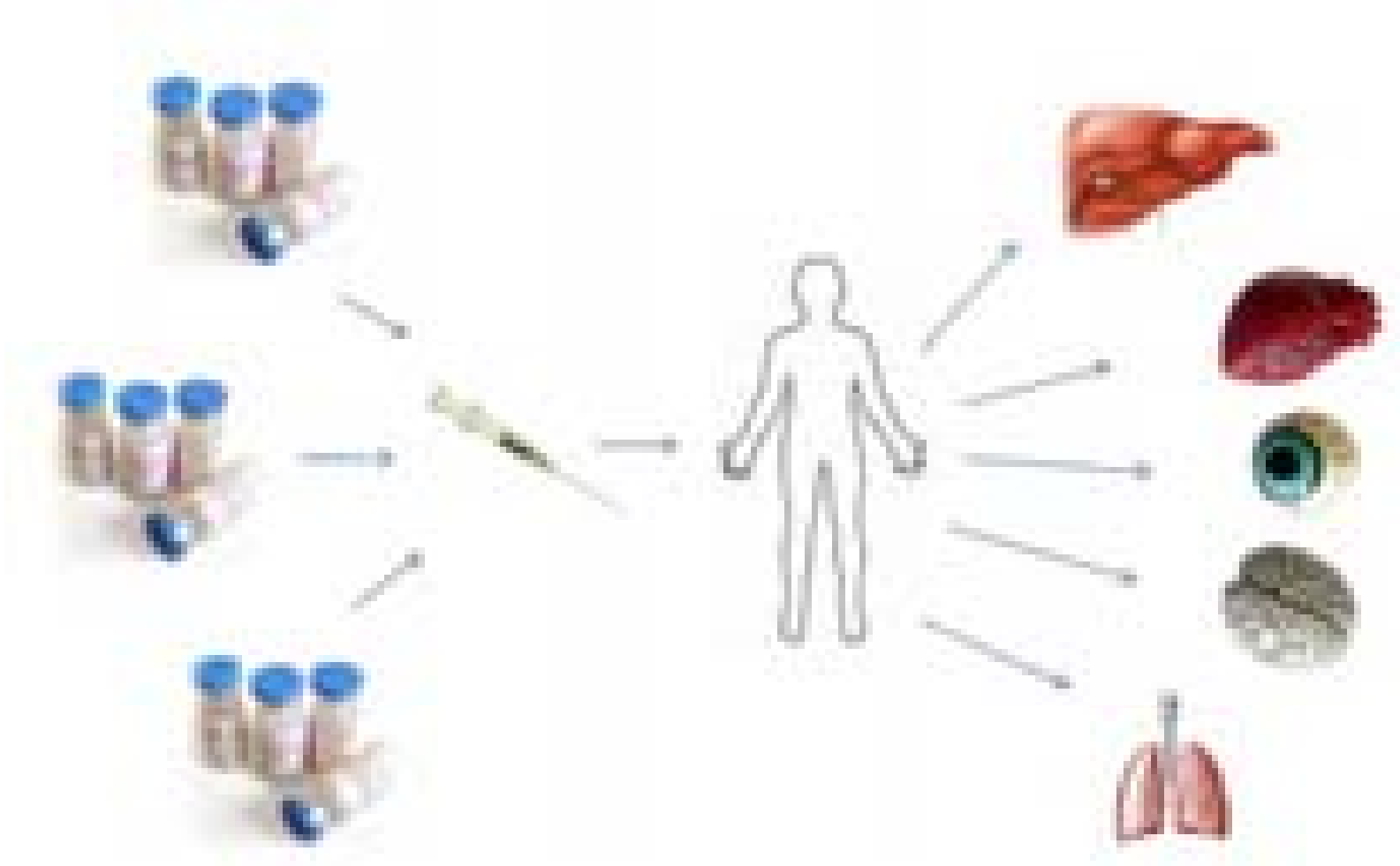
  - Delivery

  - Efficacy

  - No toxicity



# Ideal drug delivery process



**Formulation**

**Absorption**

**Distribution**

**Metabolism**

*\*Hovig Kouyoumdjian, Michigan State University, 2009*



# Ideal Mail delivery process



*\*Hovig Kouyoumdjian, Michigan State University, 2009*

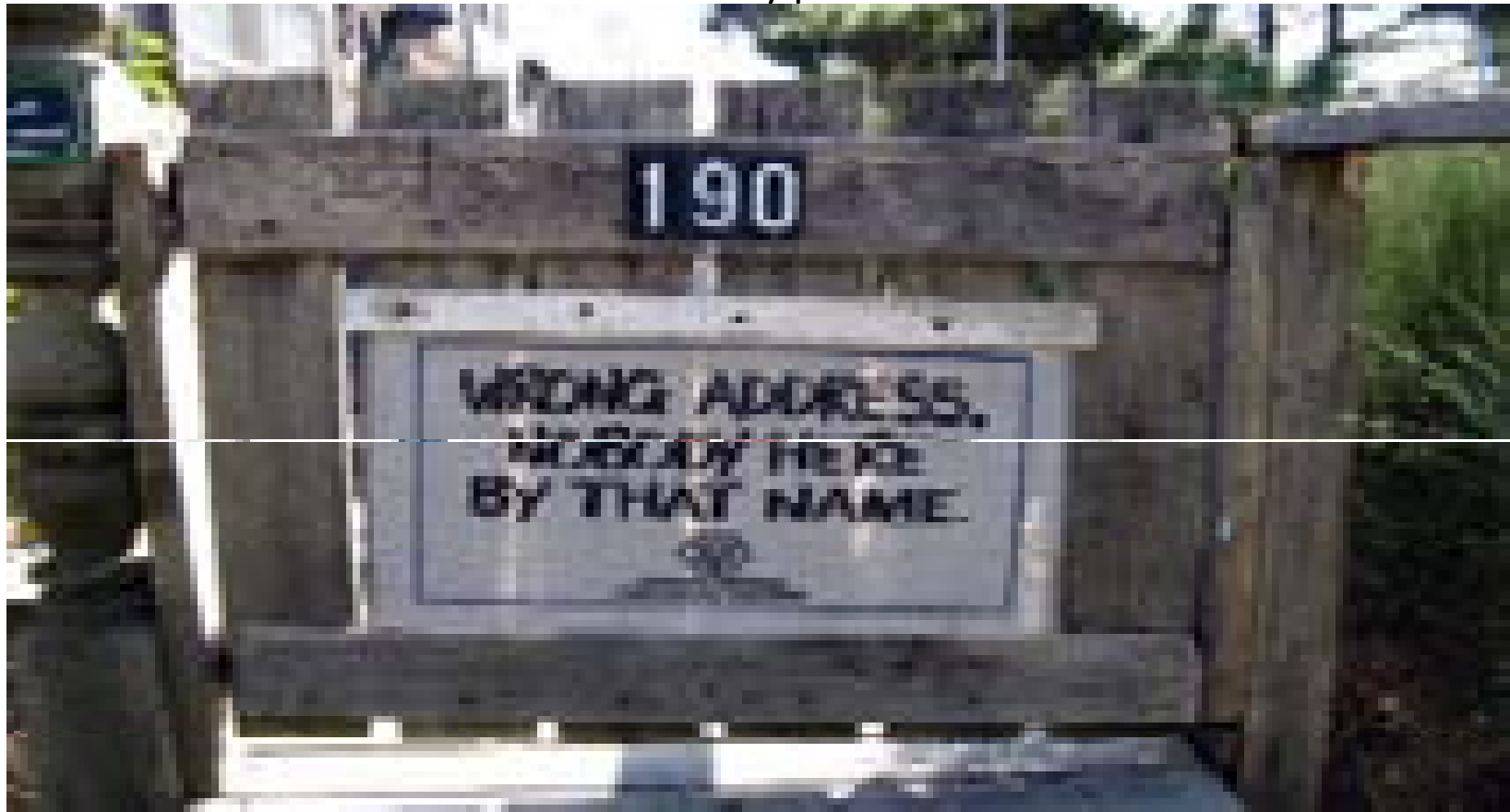




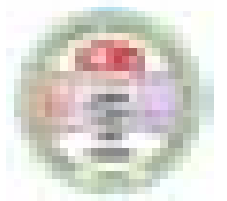
*\*Hovig Kouyoumdjian, Michigan State University, 2009*



Ideal Mail delivery process

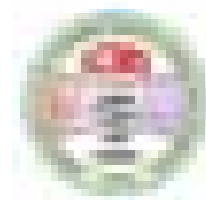


\*Hovig Kouyoumdjian, Michigan State University, 2009



# Aspects of Drug Delivery

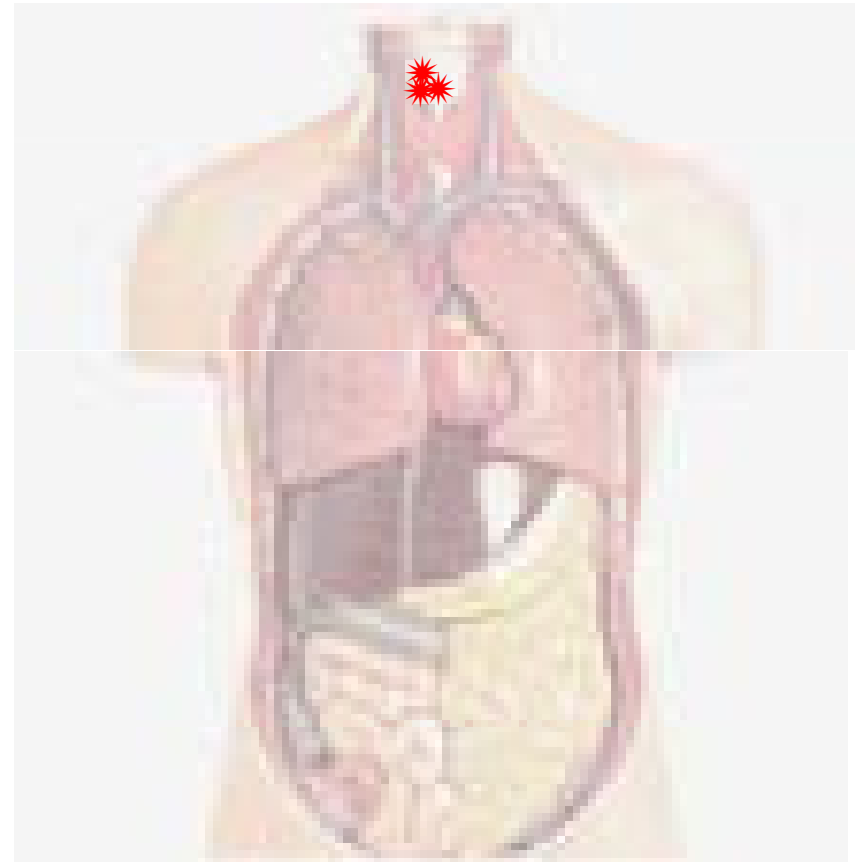
- **The ability to specifically formulate drugs to achieve better absorption, distribution, metabolism and excretion (ADME) properties**
- **Drugs can be delivered as free molecules or bound to carriers**
- **Pharmacologically active compounds usually delivered combined to a carrier**



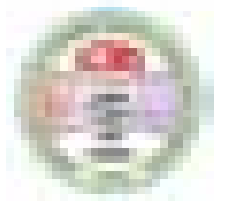
# Oral Delivery

**Oral delivery involves transport of drug via gastrointestinal tract**

**Drugs are readily metabolized in liver**



*\*Hovig Kouyoumdjian, Michigan State University, 2009*

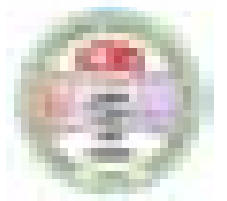
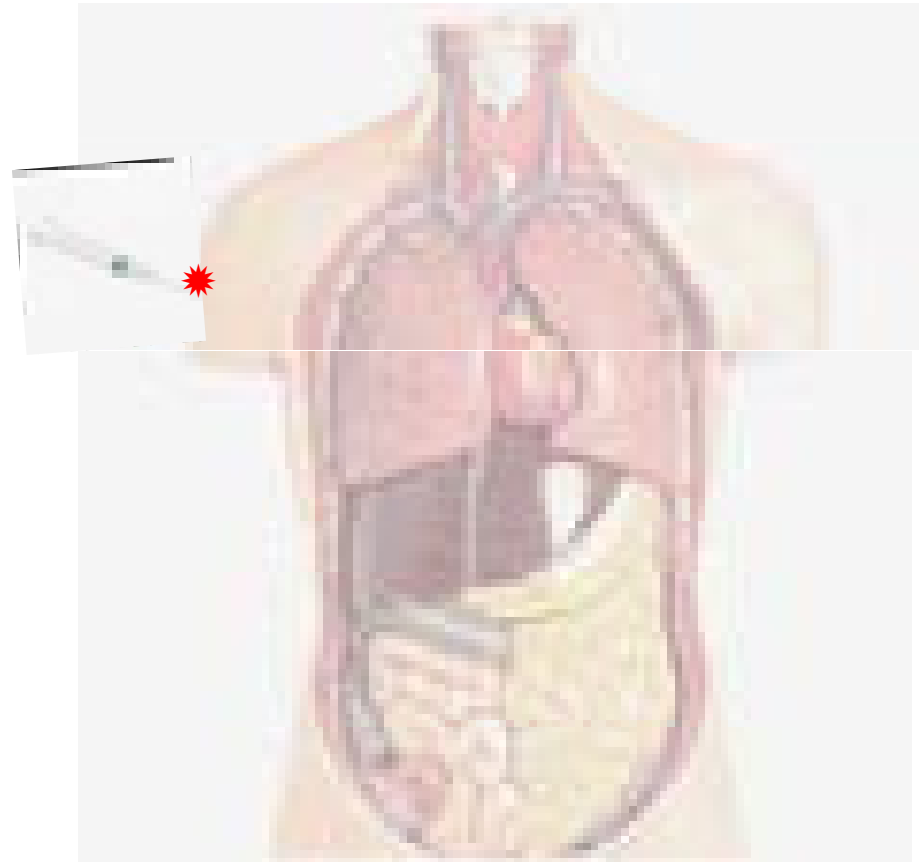


# Intravenous Delivery

Bioavailability a problem with intravenous delivery

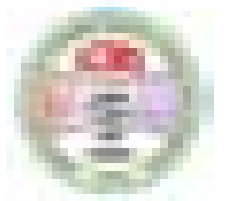
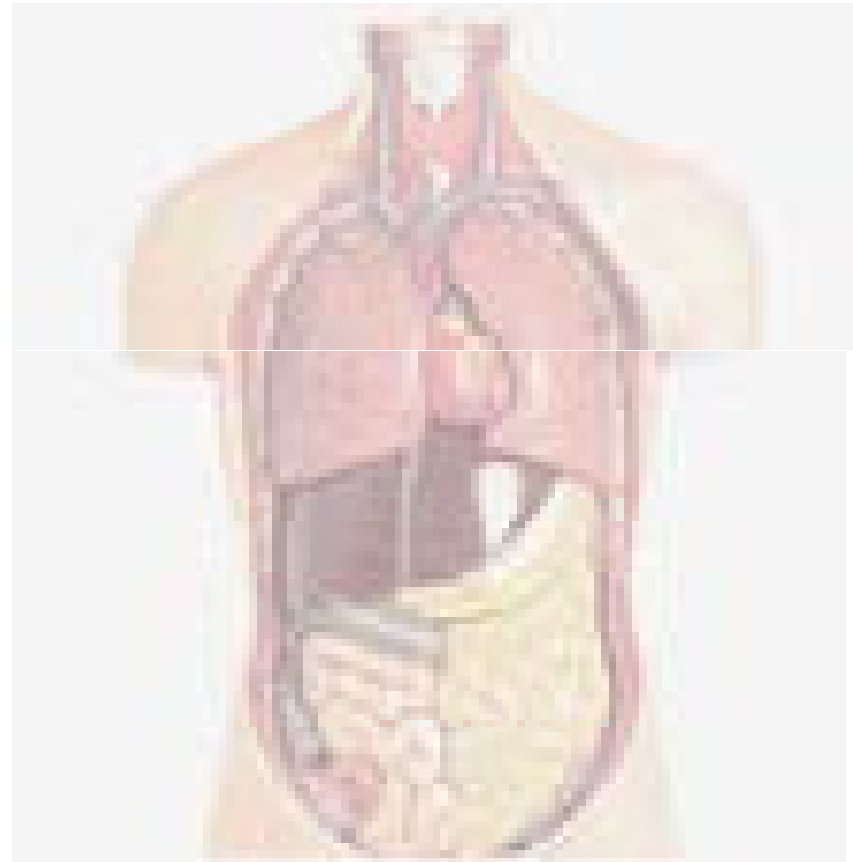
● Instability

● Toxicity



## Enhanced Bioavailability

- ✦ **Bioavailability is obtained when drug is resistant to liver metabolism**
- ✦ **Two conditions should be met for better delivery**
- ✦ **Site specificity**
- ✦ **Prolonged release**

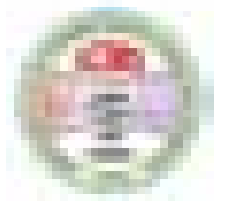


## An ideal drug delivery vehicle .....

Should also possess a trigger allowing for the rapid and complete release of free drug within the tissue

Should achieve high intravascular drug concentrations necessary for driving cellular drug uptake

Should increase drug penetration further from vessels



## Wonderful happenings in physics...

**Kamerlingh Onnes**

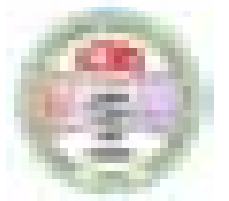
**Low temperature .... goes down and down...**

**Percy Bridgman**

**High pressure /vacuum ... goes higher and higher....**

**Feynmann**

**Controlling “things” on a small scale .... smaller and smaller....**



## Nanotechnology & drug delivery



Famous lecture of Dr Feynmann on December 29, 1959 on title, called “There’s plenty of room at the bottom”, American Physical Society, Caltech., USA.

In ancient Greek, “Nano” means “dwarf”

The vision for nanotechnology laid as early as 1959..!!

Nanotechnology – creation and utilization of materials at nanometer length scale

Particles < 100.0 nm



**\*The term “nanotechnology”, used first by Dr Norio Taniguchi, University of Tokyo – engineering materials precisely at nanometer level.**

**Electronic industry: IBM, USA – electron beam lithography – nanostructure devices at a scale of 40 – 70 nm, 1970s**

**Huge investments in the development of nanotechnology ...**

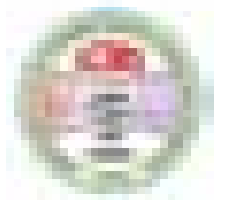
**In India, several institutes are engaged in nanotechnology**

**Synthesis & characterization – IITs, Universities**

**Applications – health care – Institute of life Sciences, so on...**

**Word of caution: Nanotechnology can not “fix” everything !!!!**

*\* S K Sahoo, et.al., Nanomedicine: Nanobiotechnology, Biology, and Medicine, 3, 2007, 20*



## **\*Nanotechnology in health care – drug delivery**

### **Nano-scale structure of nanoparticles**

- increase in surface area to volume ratio**
- responsible for different behavior compared to traditional micro-particles**

*\*Libo Wu, et.al., Advanced Drug Delivery Reviews, 63, 2011,456*



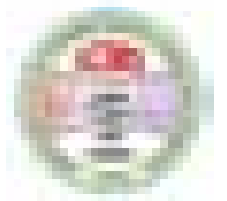
## \*Nanotechnology in health care – drug delivery

### Advantages

- Improved oral bioavailability
- Enhanced solubility of poorly water soluble drugs
- Enhanced dissolution rate
- Delivery of the drugs to site specific
- Injection formulation using nanosuspension of poorly water soluble drugs
- Reducing toxicity with increase in drug efficacy – better delivery
- Delivery of the drugs to brain overcoming blood-barrier

### Concerns

- ✦ Complex manufacturing
- ✦ Nanotoxicity
- ✦ Stability issues
- ✦ Storage and transport



## \*Nanoparticle size, toxicity, status and applications

Nanoparticle	Size	Toxicity	Status	Application
Liposome	100-200nm	low	Clinical use	Delivery
Small polymer	~200kDa	low	Research	Delivery
Dendrimer	2-6nm	variable	Phase I	Delivery
Virus	30-100nm	High	Phase II	Delivery
Metal core dendrimer	2-4 nm for gold	--	Research	Delivery
Nanoshells	60-400nm	Non-toxic	Research	Imaging, treatment
Quantum Dots	2-10nm	Toxic	commercial	Sensing, imaging
Carbon nanotubes	variable	---	Research	Delivery, sensing

\*Priya Pathak, et.al., <http://www.azonano.com/oars.asp>



## Why magnetic nanoparticles (MNPs)?

### Edge over other types of nanoparticles

- Ability to get directed & concentrated within the target tissue
- Ability to hold them at the target until the therapy is complete
- Ability to remove them once the therapy is complete

Close to a ideal drug delivery, called "pharmacyte"

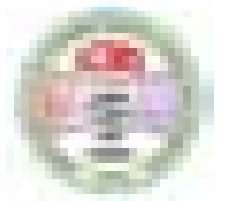


## \*Magnetism & Biology

Freeman and others say, “ If not with the marriage of magnetism and medicine, this paper is at least concerned with an affair between these two different fields.”

The famous two page paper by Freeman in 1960 was the basis of MNPs. He explained how these fine particles could be transported into vascular system and made them concentrated at a particular point in the body with the aid of magnetic field

*\*Freeman et.al., J. Appl Phys 31, 1960, 404S*



## \*Features of MNPs

Cell – 10-100 $\mu$ m

Virus – 20-450nm

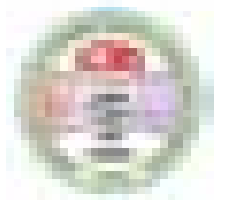
Protein – 5-50nm

Gene – 2nm wide & 10-100nm long

- ✱ **Controllable size – few nm to tens of nm**
- ✱ **Dimensions – “get close’ to biological scale**
- ✱ **Influence of external magnetic field gradient – “Action at a distance” (delivery of the drug at a target)**
- ✱ **Their usage as hyperthermia to enhance the drug effects (malignant cell destruction)**
- ✱ **Therapeutic agent – Early diagnosis & Therapy**

*\*Q A Pankhurst, et.al., J Phys. D: Applied Phys. 36, 2003, R167*

*\*Dongwon Yoo, et.al., Accounts in Chem. Research, 2011 (to appear)*

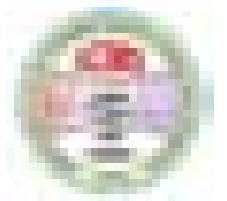


# What is a MNP?

## Basics of magnetism!

Paramagnetism      Paramagnetic materials - significant atomic magnetic moments, often due to unpaired valence electrons

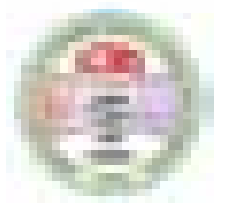
Diamagnetism      Diamagnetic materials - no atomic magnetic moments. undergo induced magnetization under an external magnetic field.

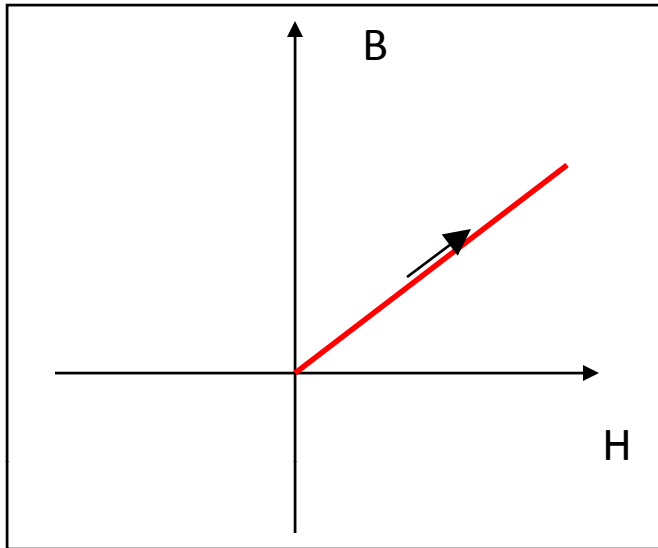


## Ferromagnetism

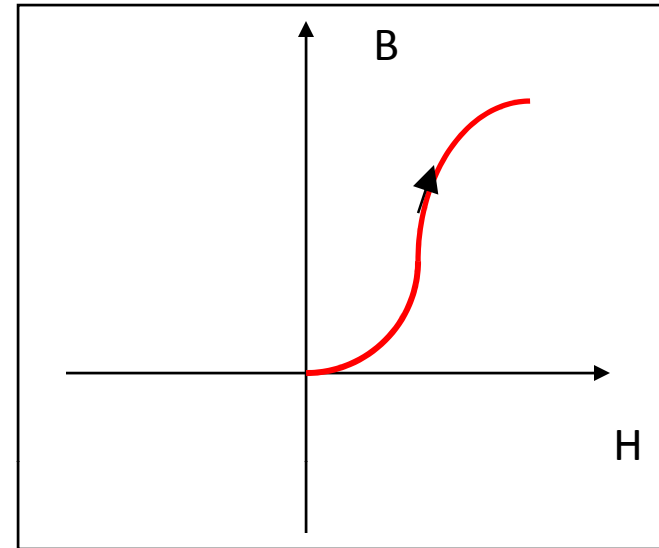
Ferromagnetic substances - significant magnetic moments. Unlike paramagnetic materials, they have a significant attraction to other magnetic materials

Ferromagnetic materials have other interesting features





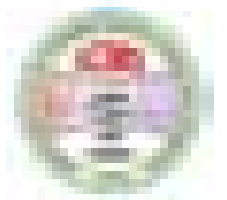
Paramagnetic

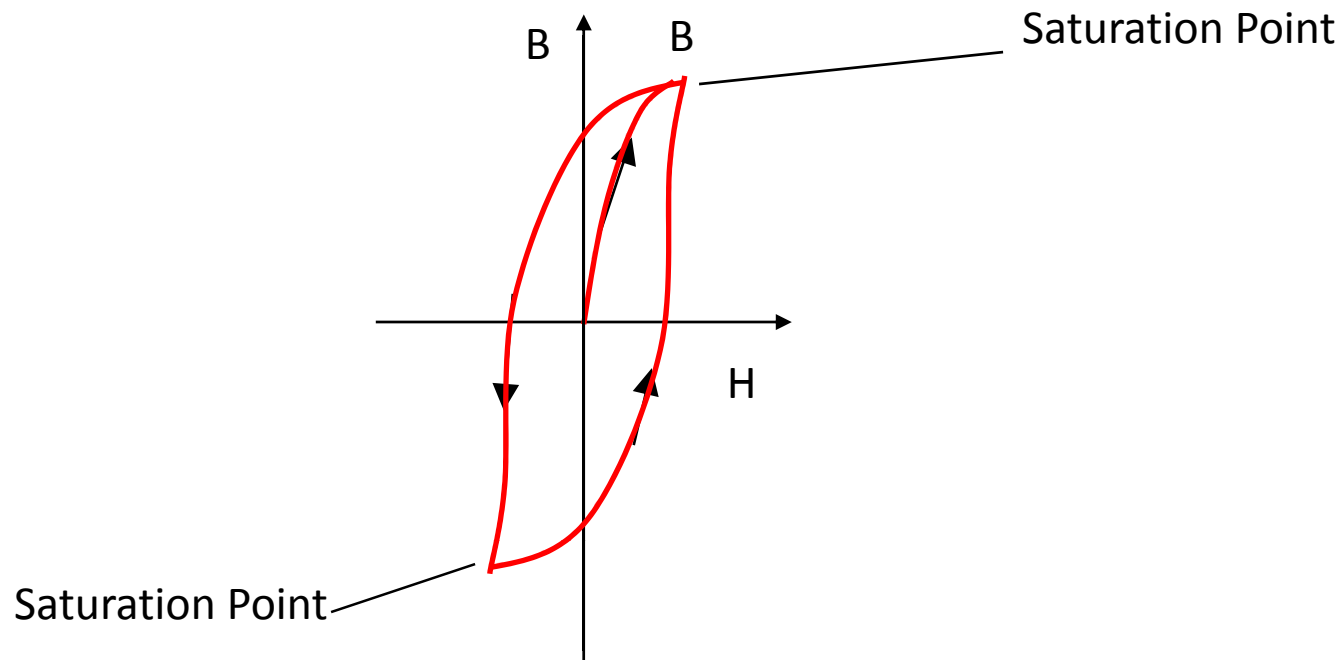


Ferromagnetic

Comparing the response of the total magnetic field to the applied Field Strength

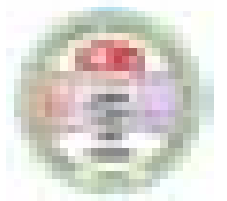
The relation for the Ferromagnetic curve is non-linear





The figure above shows a hysteresis curve between the two saturation points of a particular ferromagnetic material

The saturation point corresponds to the maximum magnetization that a material can achieve



# Magnetic Susceptibility and Permeability

$$\vec{M} = \chi \vec{H}$$

## Magnetic Susceptibility

The magnetic susceptibility relates the degree (and sense) of magnetization given an applied field strength. It is characteristic of the substance and it is very much temperature dependent.



**Some of the ferromagnetic materials are**

**Polymorphs of iron(III) oxide**

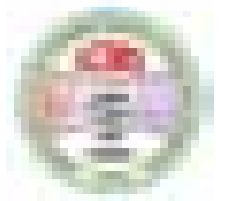
**-  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> as hematite**

**-  $\beta$  – Fe<sub>2</sub>O<sub>3</sub>**

**-  $\gamma$  – Fe<sub>2</sub>O<sub>3</sub> as maghemite wide applications**

**-  $\epsilon$  – Fe<sub>2</sub>O<sub>3</sub>**

**Fe<sub>2</sub>O<sub>4</sub> – magnetite – biocompatible & biodegradable**

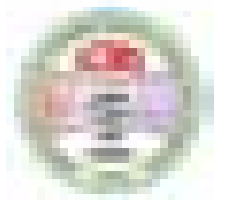


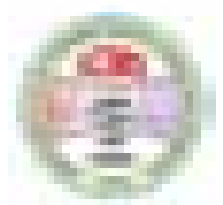
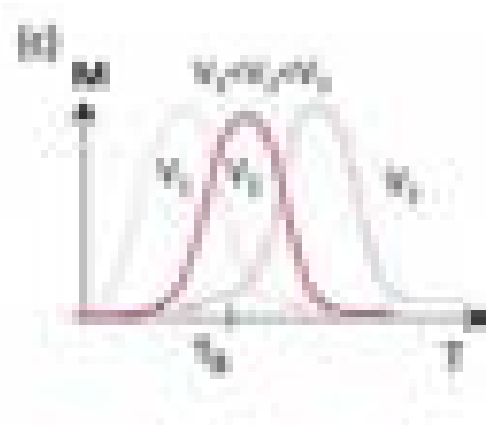
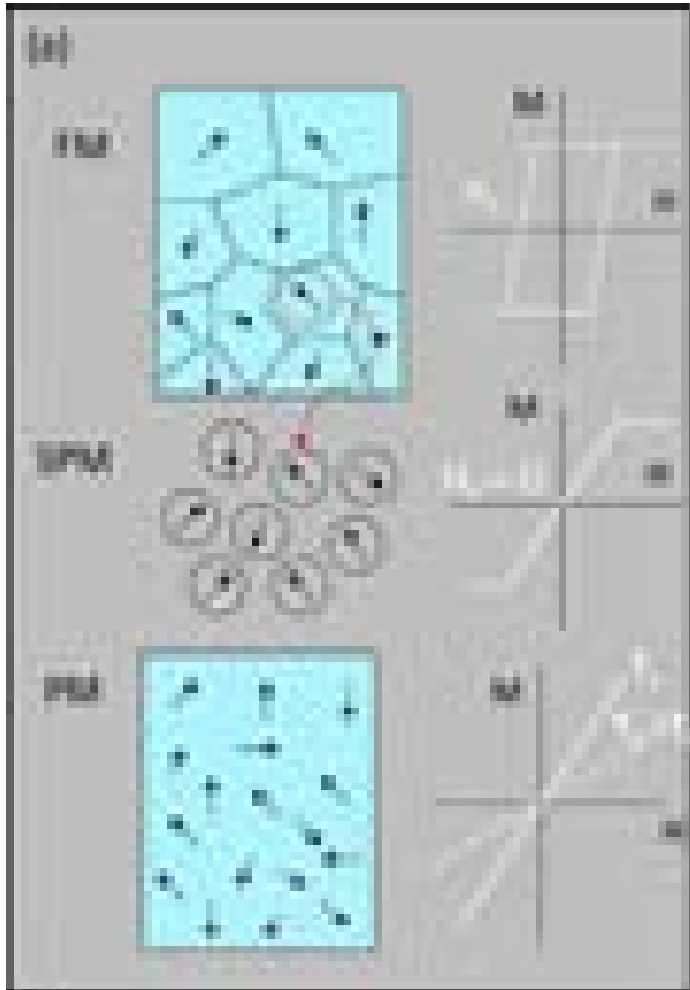
In nanoscale, the behavior these materials are different  
Size dependent magnetic behavior

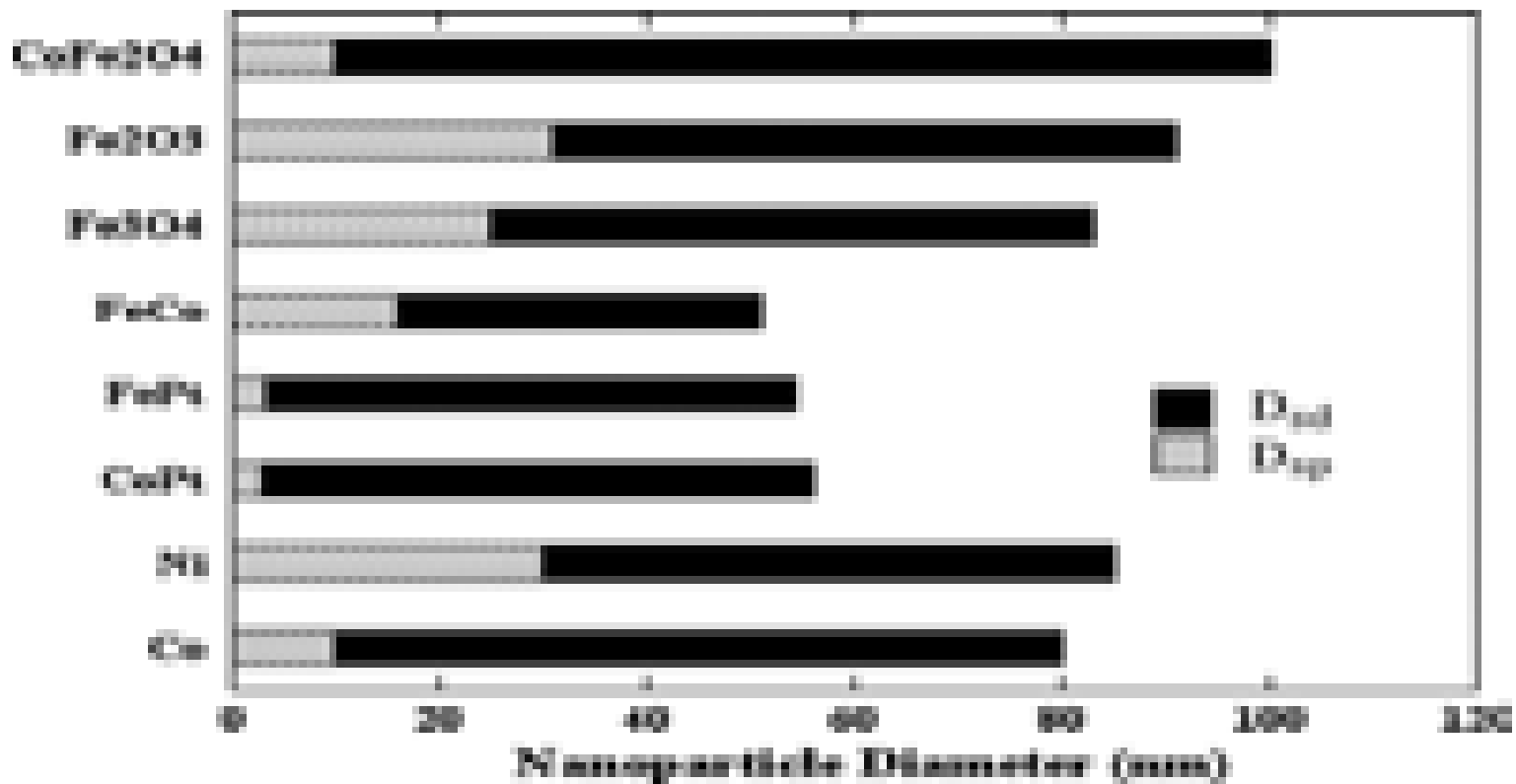
When these magnetic materials go to nanoscale  
domain wall becomes **single domain** in length scale  
Inter-particle exchange effects dominate

Ferromagnetic behavior ----- superparamagnetism state  
& temperature dependent behavior magnetic anisotropy

A domain is a group of spins whose magnetic moments are  
in the same direction. In the magnetisation procedure they  
act cooperatively. **In the bulk material, domains are  
separated by domain walls**





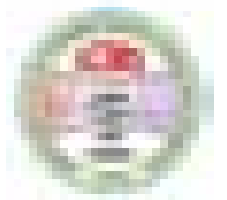


$D_{sd}$  Single domain size

Drug carrier

$D_{sp}$  A size defined by superparamagnetic effect

Drug carrier & therapy



# Design & fabrication of magnetic nanoparticles

Magnetic oxide nanoparticles

Well studied

Biocompatibility

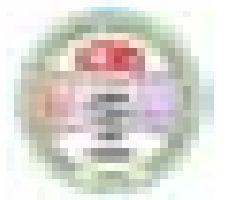
No hepatotoxicity (damage to liver)

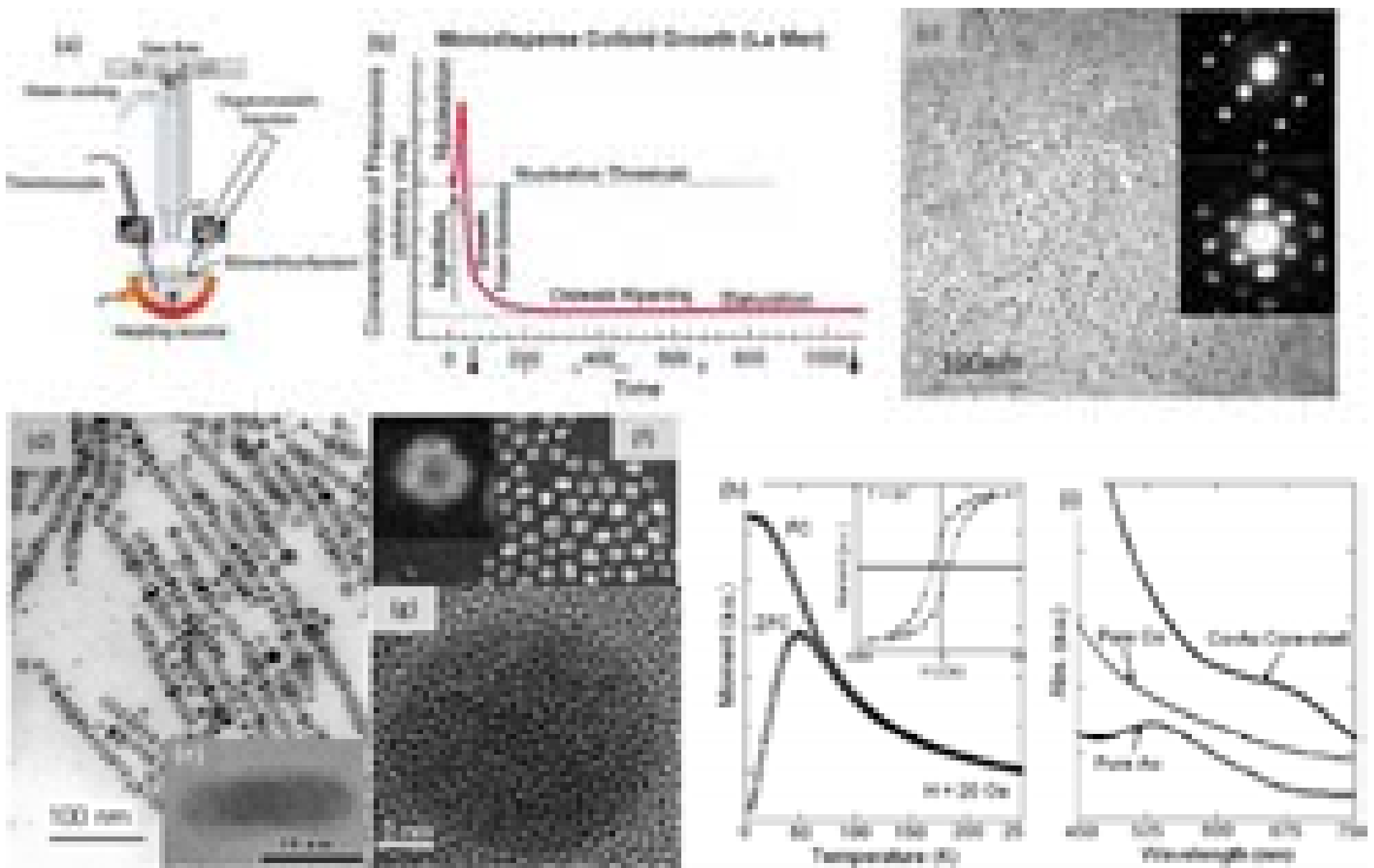
Commercially available

FDA approved (MRI contrast enhancers)

## *Method of preparation*

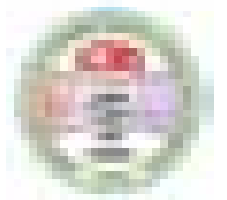
- ◆ Co-precipitation method, called Massart's co-precipitation method
- ◆ Thermal decomposition of a solution of  $\text{Fe}^{3+}$  chelate in the presence of hydrazine
- ◆ Sonochemical decomposition of hydrolyzed  $\text{Fe}^{2+}$  salts & heat treatment
- ◆ Oxidation of ferrous salts in the presence of surfactants
- ◆ Solution phase decomposition of iron precursors at elevated temperatures
- ◆ A two step process to synthesize  $\text{Fe}_2\text{O}_4$  : Nucleation with surfactant & oxidation

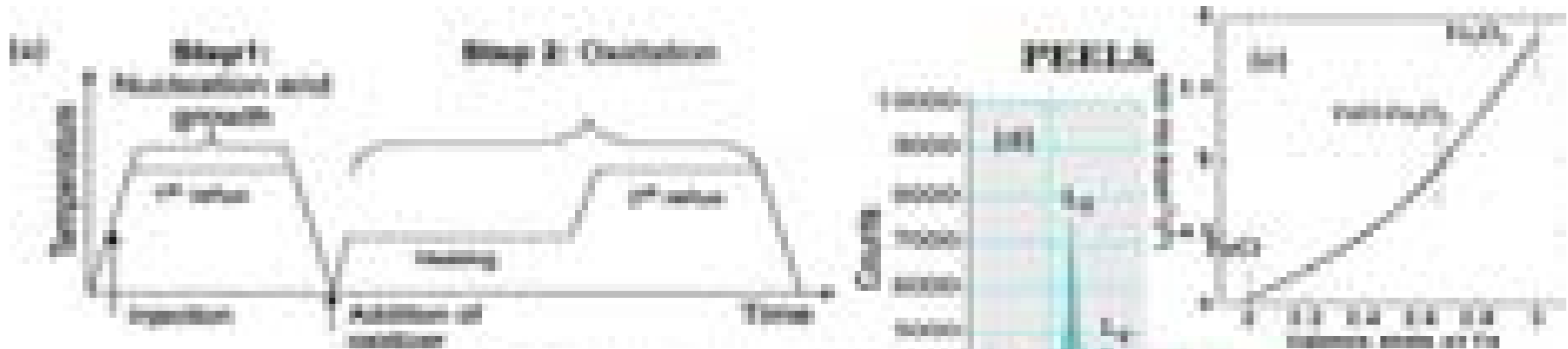




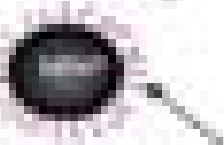
## Co-precipitation method

Kannan M Krishnan, *IEEE Transactions on Magnetics*, 46, 2010, 2523

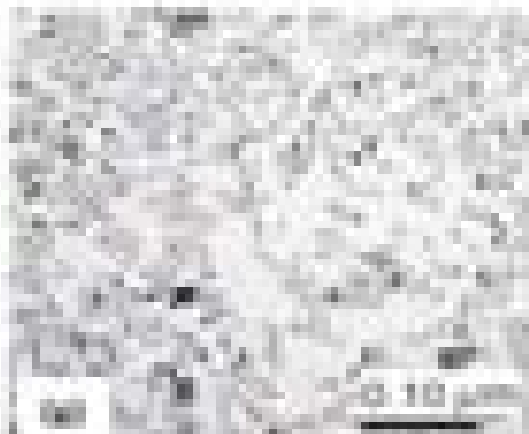
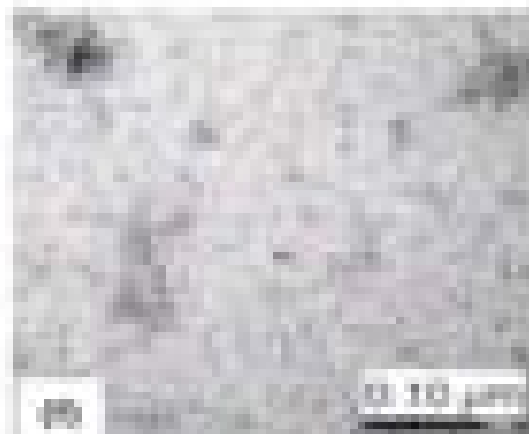
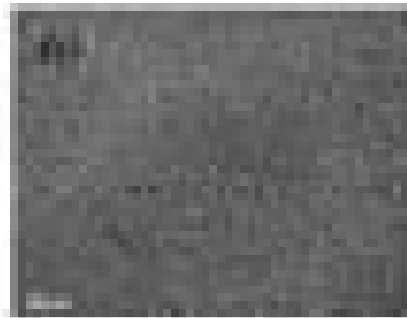




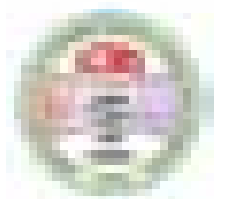
110 An agglomerated particle



Hydrophobic core with Fe<sub>2</sub>O<sub>3</sub>



A two step process to synthesize Fe<sub>2</sub>O<sub>4</sub> : Nucleation with surfactant & oxidation



## Biocompatibility & functionality

To ensure that the formulation using MNPs has the ability to overcome the biological barriers



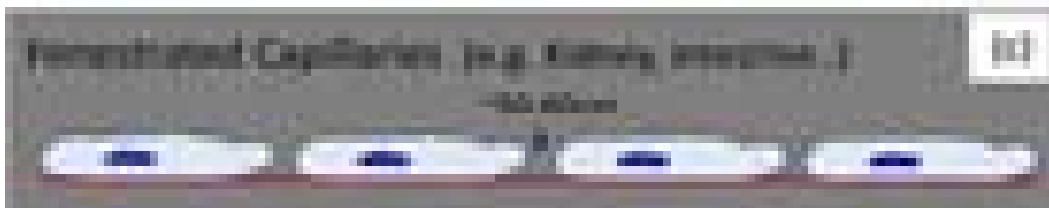
# Different classes of blood capillaries



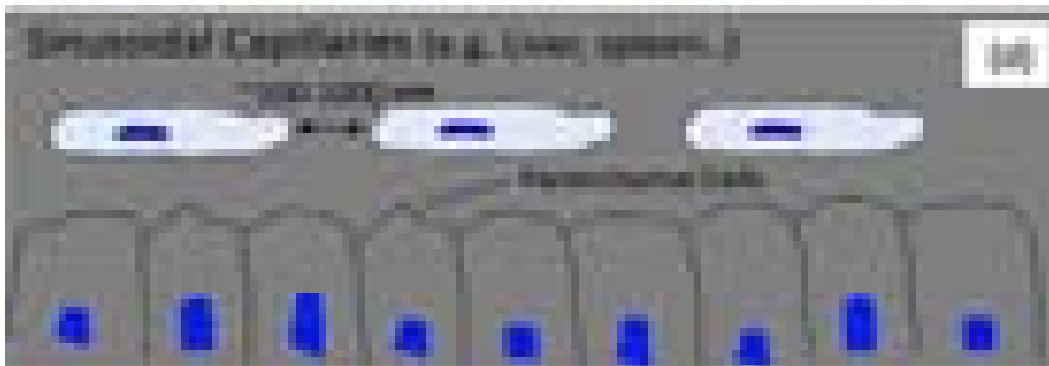
Blood-brain barrier - ~2 nm



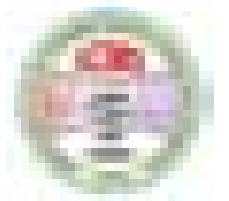
Continuous capillaries - ~6 nm



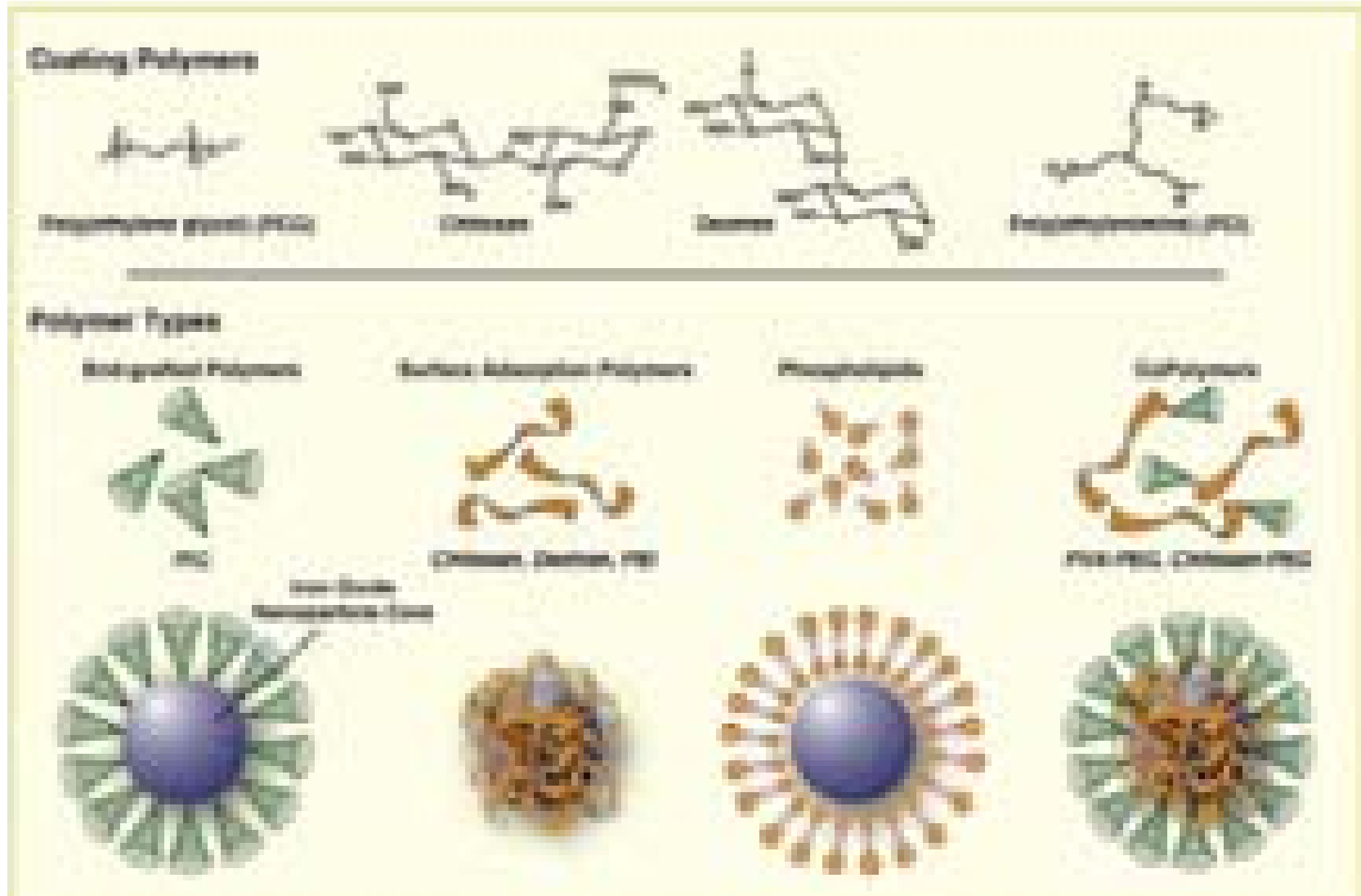
Kidney, intestine – 50 – 60 nm



Sinusoidal capillaries –  
Liver, Spleen ~100 nm –  
1000nm



## Process of making MNPs biocompatible



Depicting the assembly of polymers onto the surface of magnetic nanoparticle cores.

*O. Veisheh, et al., Advanced Drug Delivery Reviews (2009), doi:10.1016/j.addr.2009.11.002*



**MNPs can be used in two ways**

## **Magnetic Nanoparticles in Biomedicine**

***In Vitro***

**Diagnostics**

**Magnetic  
Separation**

- *Cell-sorting*
- *Immunoassays*
- *Lab on a Chip*

**Relaxometry**

- Relaxation (M)  
Neel and  
Brownian motion
- *Immunoassays*



# *In Vivo*

## Diagnostics

### Imaging

MRI, MPI & Molecular Imaging

Contrast enhancement

Change in relaxation dynamics

- *Imaging*
- *Molecular Probes*

## Therapeutics

### Drug & Gene Delivery

Localized Delivery

Magnetically

directed particles

• *Cancer Treatment etc.*

### Hyperthermia

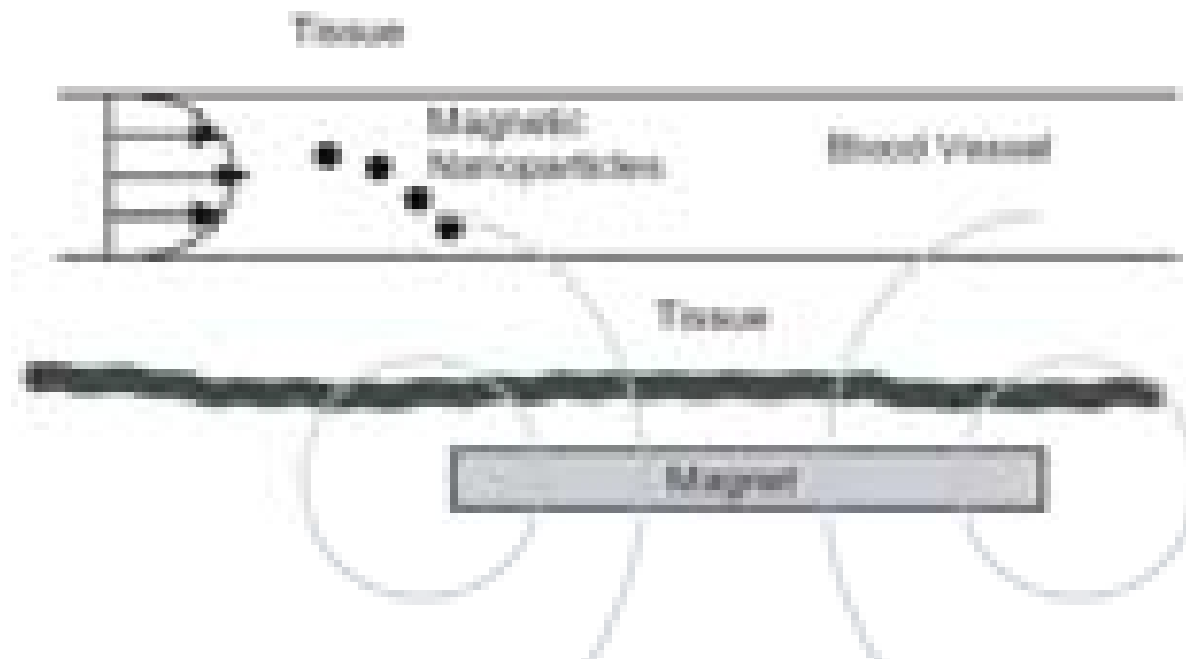
Local Heating

Magnetic losses

in a/c magnetic field

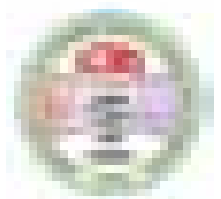
• *Cancer Treatment*



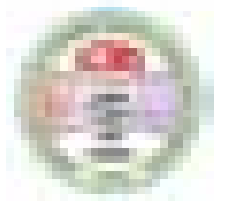


A hypothetical magnetic drug delivery system shown in cross-section: a magnet is placed outside the body in order that its magnetic field gradient might capture magnetic carriers flowing in the circulatory system.

$$F_m = V_m \Delta \chi \nabla \left( \frac{1}{2} B \cdot H \right)$$



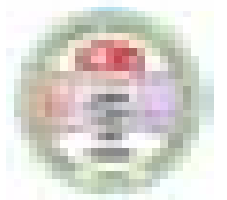
## A typical example of MNP based drug delivery



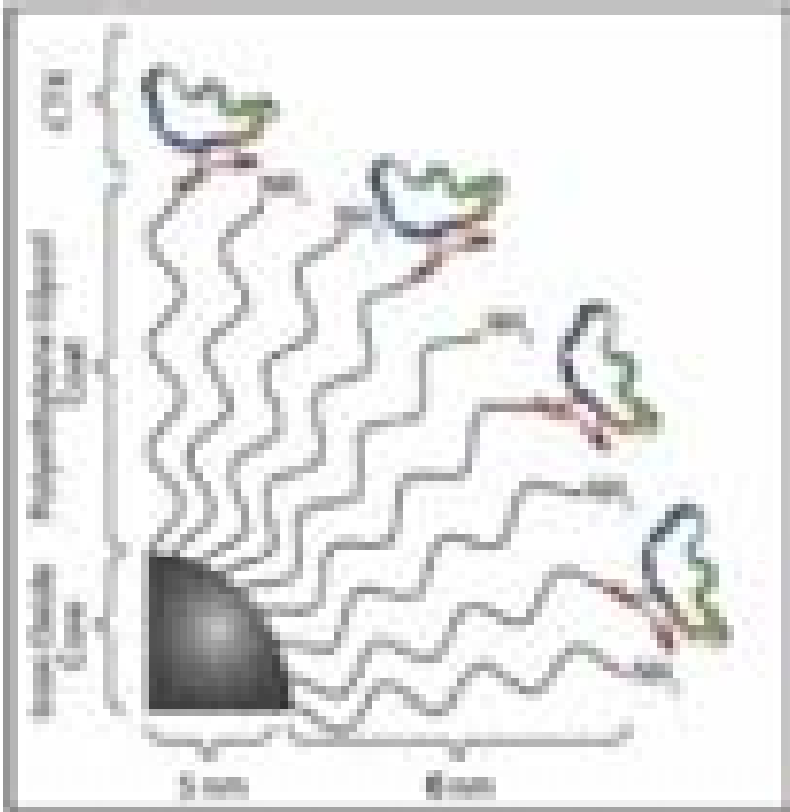
## Chlorotoxin (CTP)

Inhibition of tumor cell invasion - Chlorotoxin (CTP), a peptide derived from **vemon** of the Giant Israeli scorpion *Leiurus q. hebraeus*. It is a recombinantly produced peptide containing 36 amino acids, 8 cysteine residues, 4 disulfide cross-links.

This 4 kDa peptide has demonstrated **high specificity** and affinity for the vast majority of **brain tumors** and recent research has further shown that CTX specifically binds to **prostate cancer, sarcoma, and intestinal cancer**. This peptide has broad implications as a cancer cell targeting ligand, demonstrating excellent targeting in previous *in vitro* and *in vivo* **nanoparticle studies**



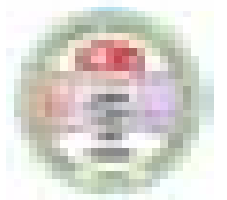
## CTX conjugated to MNP



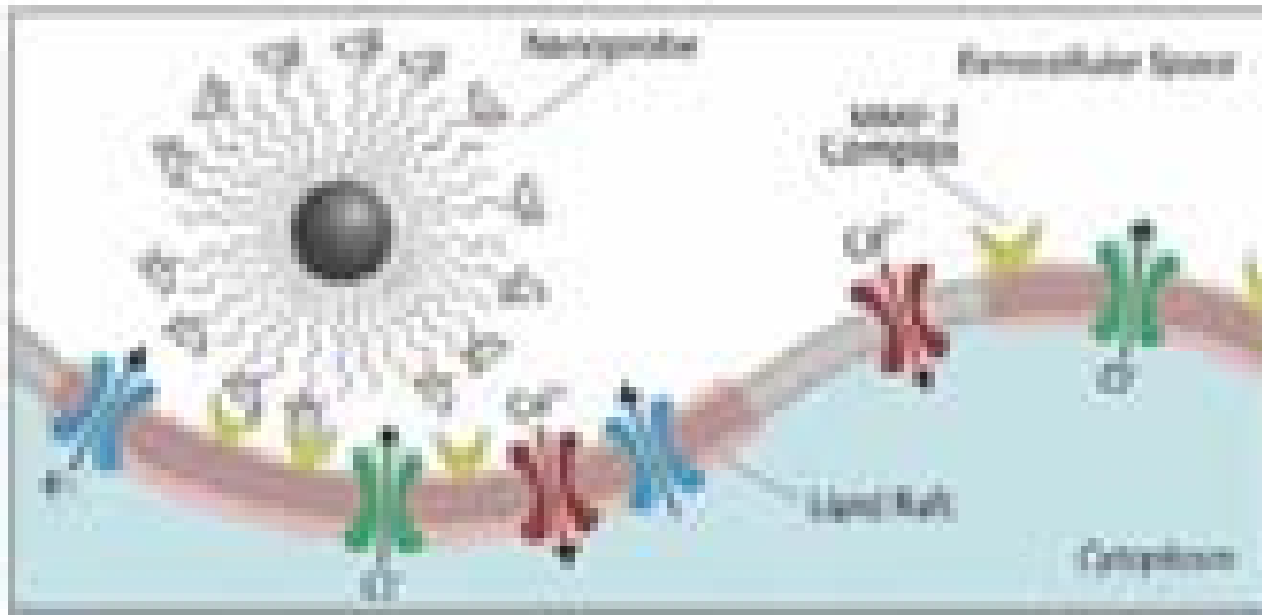
**Iron oxide nanoparticle core,  
coated with an amine-  
functionalized PEG silane**

**The Alexa Fluor 680 (AF680)  
fluorochrome and CTX -  
conjugated to the amino  
terminal groups of the  
nanoparticle-bound PEG**

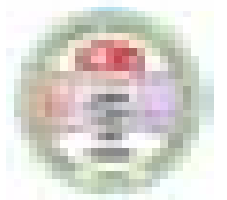
**via successive reactions with *N*-  
succinimidyl- *S*-acetylthioacetate  
(SATA) and succinimidyl  
iodoacetate (SIA)**



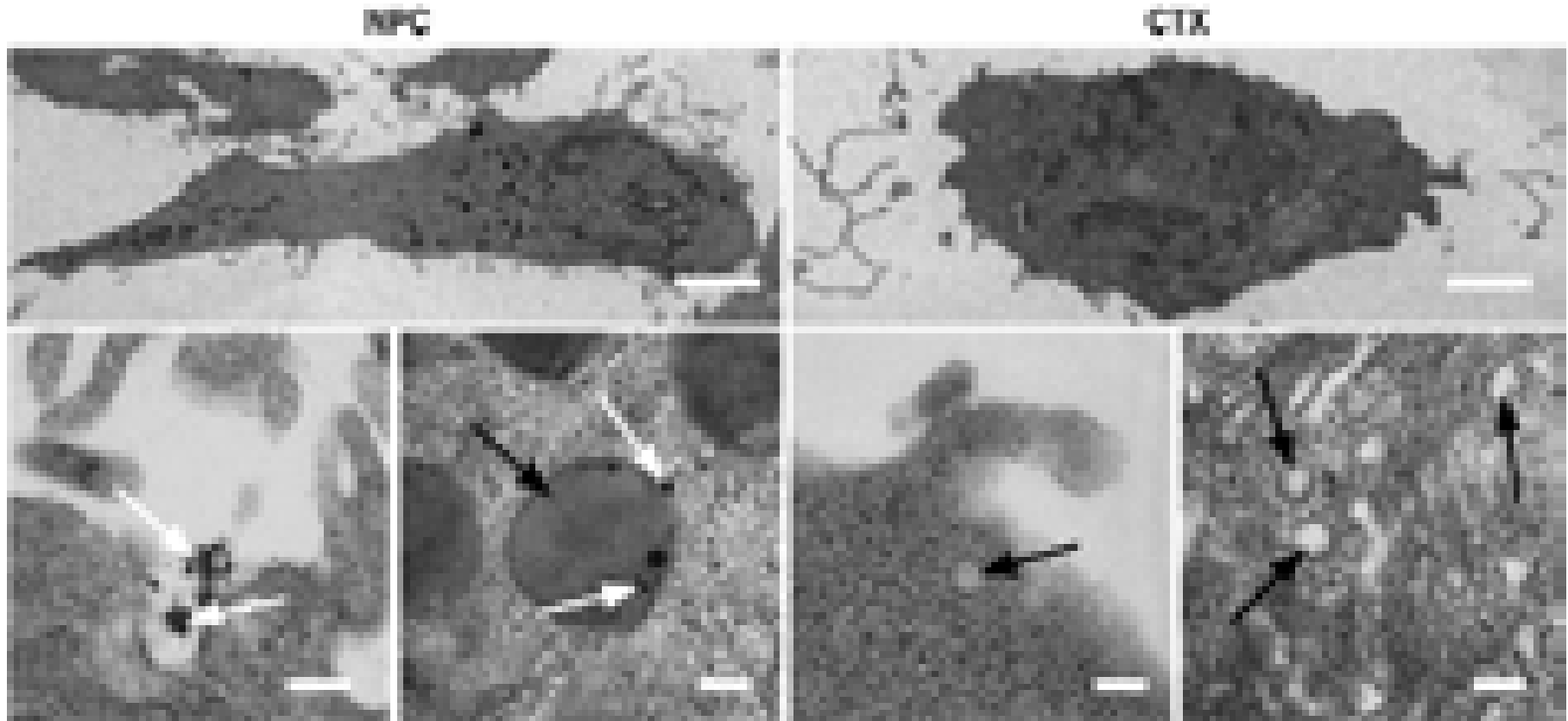
## Schematic showing anchoring of the drug specific to site



The MNP was designed to bind and **inhibit the activity of the MMP-2 endopeptidase**, and to induce endocytosis of the lipid rafts, subsequently **limiting** invasive cell activities

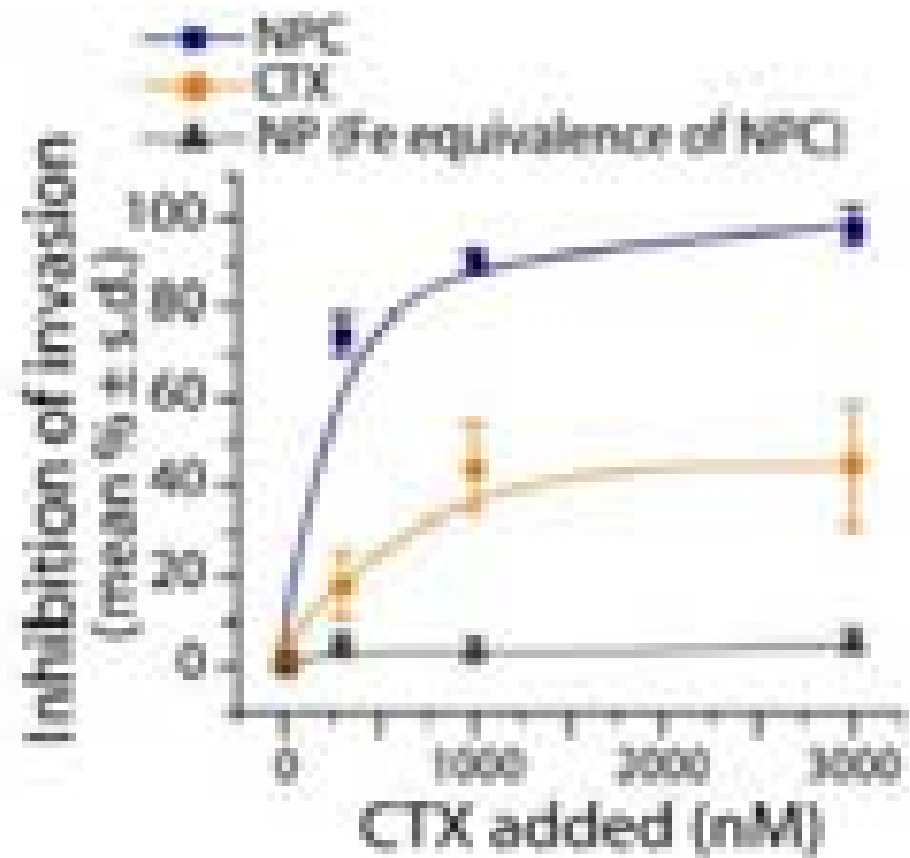


## TEM image showing the invasion of MNP into the cell



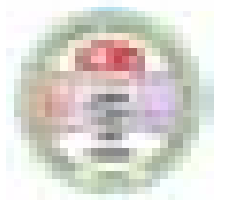
TEM images showing increased membrane uptake subsequent to NP-CTX (NPC in figure) binding. Scale bars represent 5 mm for whole cell images (first row) and 200 nm for high magnification images (second row). White and black arrows identify NP-CTX and endosomes, respectively.



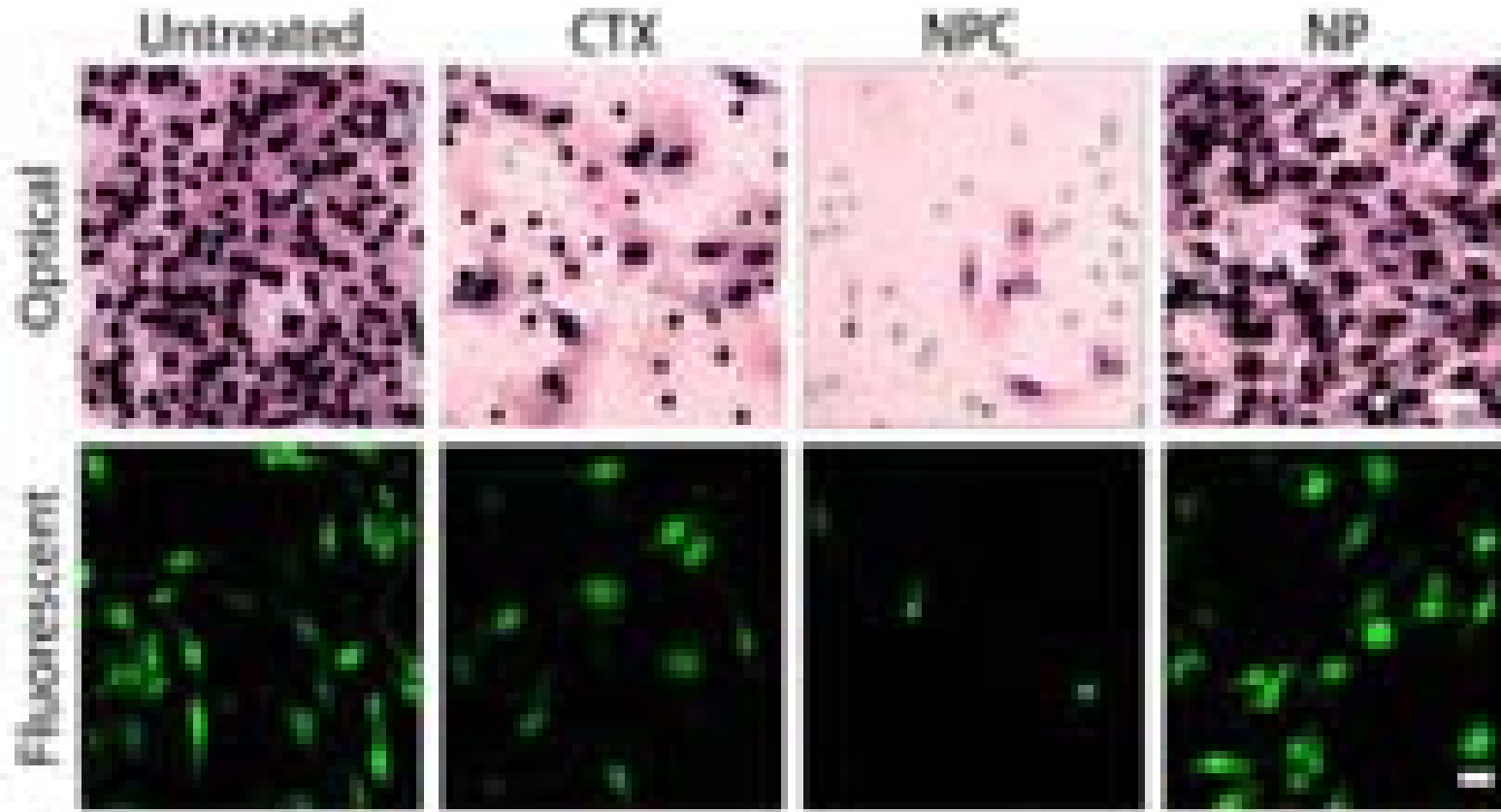


**Illustrates that NP-CTX is more efficient at limiting glioma cell invasion**

*Omid Veisheh, et al., Small. 2009 February ; 5(2): 256–264. doi:10.1002/smll.200800646*

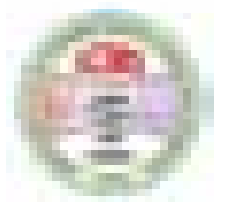


**NMP significantly limits cellular mobility compared to NP and CTX treatments.**



**Optical images of C6 cells (scale bar: 50  $\mu\text{m}$ ) and fluorescence images of EGFP-expressing C6 cells (scale bar: 20  $\mu\text{m}$ ) that have crossed through the pores of a matrigel invasion chamber**

*Omid Veisheh, et al., Small. 2009 February ; 5(2): 256–264. doi:10.1002/sml.200800646*



## Comparison of different biomedical imaging methods

Technique	Radiation Used	Spatial Resolution	Temporal Resolution	Sensitivity	Quantity of contrast agent used	Summary / Comments
Positron Emission Tomography (PET)	High Energy γ-rays	1-2 mm	1 Day to minutes	$10^{-11}$ - $10^{-12}$ Mole/L	Nanograms	Sensitive Quantitative Needs cyclotron
Single Photon Emission Tomography	Low Energy γ-rays	1-2 mm	minutes	$10^{-11}$ - $10^{-12}$ Mole/L	Nanograms	Many available probes
Computed Tomography	X-rays	50-200 μm	minutes	Not well characterized	Not Applicable	Good for bone, tumor but not for soft tissues
Magnetic Resonance Imaging (MRI)	Radio waves	25-100 μm	Minutes to hours	$10^{-4}$ - $10^{-6}$ Mole/L	Micrograms to Milligrams	Highest resolution Morphological and functional imaging Lower sensitivity Slow
Magnetic Particle Imaging (MPI)	Radio waves	200-500 μm	Seconds to minutes	$10^{-11}$ - $10^{-12}$ Mole/L	Nanograms	Quantitative Good sensitivity Fast Good resolution No tissue contrast

# Superparamagnetism & hyperthermia

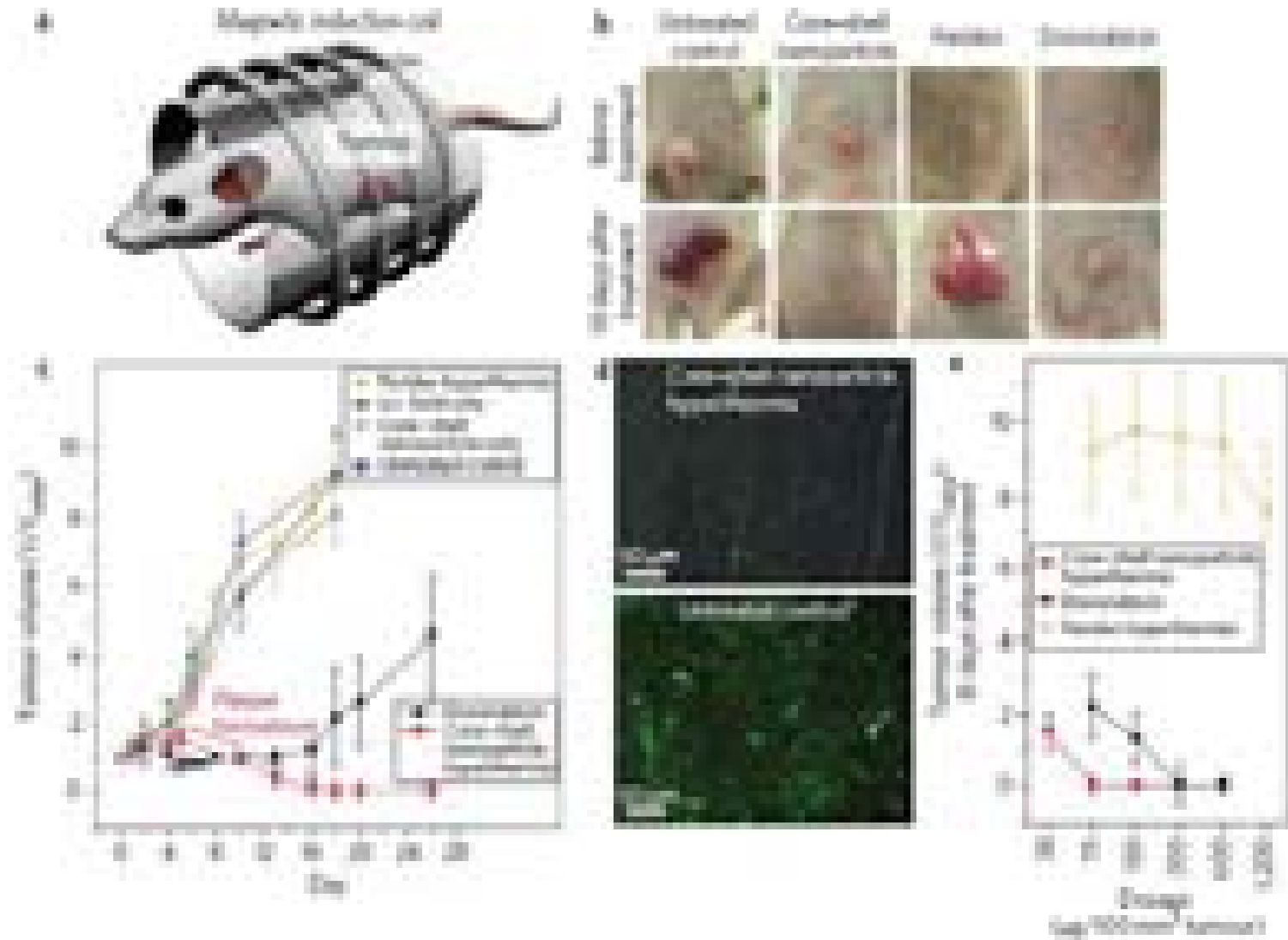
## Theranostic magnetic nanoparticles



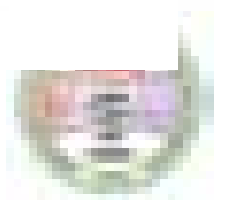
*Dongwon Yoo, et.al., Accounts in Chemical Research, 2011 (to appear)*



# Hyperthermia

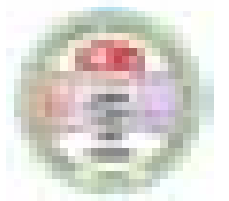


Jae-Hyun Lee, et.al., Nature nanotechnology, 6, July 2011, 418



## Challenges.....

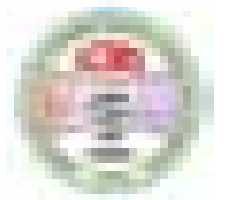
- **Coordinated multidiscipline approach**
- **Methodologies – imaging**
- **Toxicity**
- **Particokinetics**
- **Pharmacological studies**

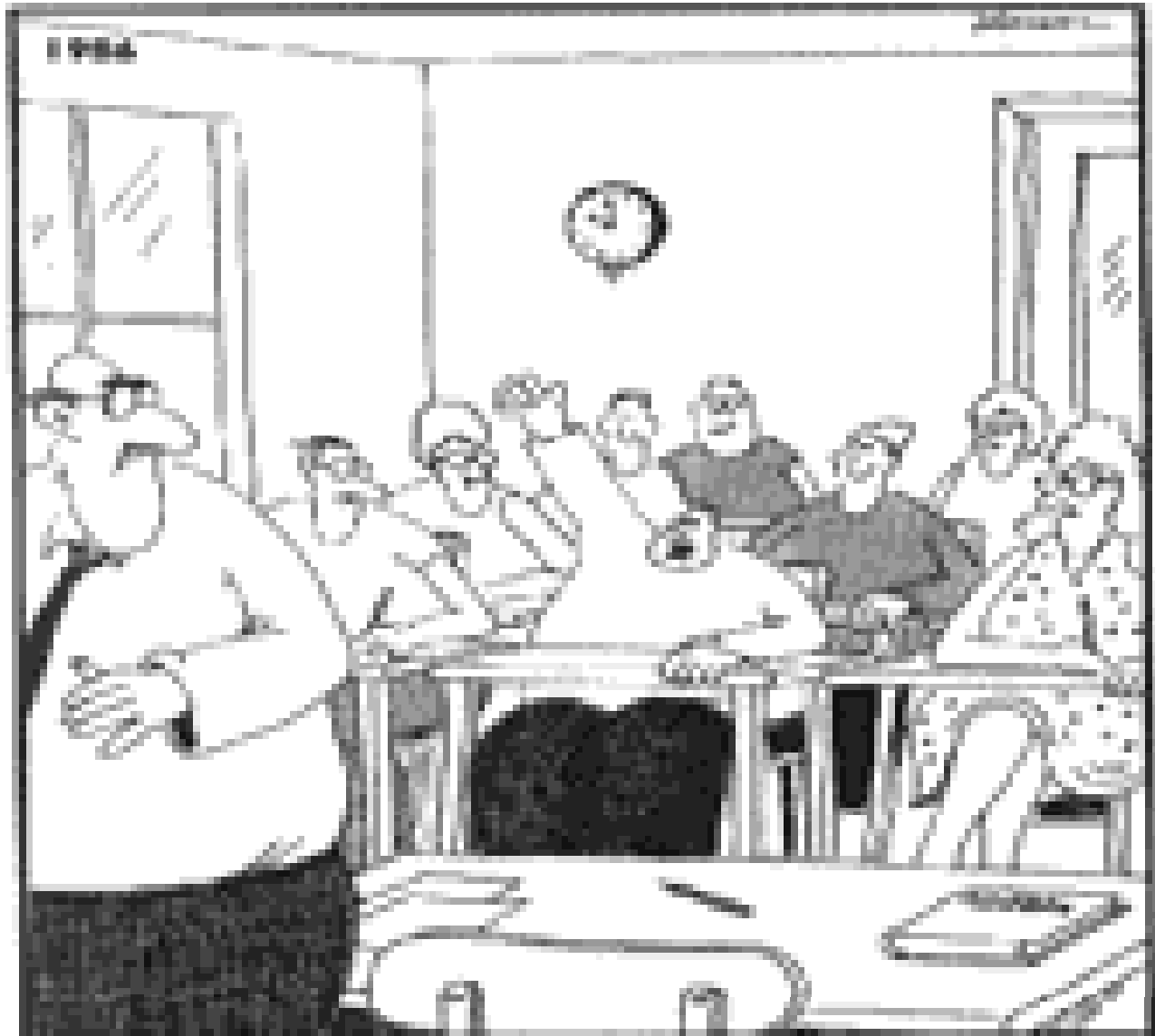


## In future.....

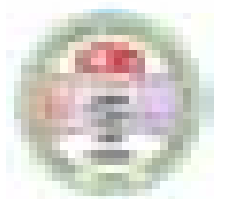
**Magnetic nanoparticle based drug delivery system  
moves to next phase**

- **Development of quantitative in vivo imaging**
- **Targeted and triggered drug release**
- **Image guided therapy**
- **Shape anisotropy and its role**
- **Surface functionalisation**
- **Development biocompatible molecules anchored to MNPs**
- **Localized physics of heating - hyperthermia**





"Mr. Osborne, may I be excused? My brain is full."



**Thank you all!**

