Engineering Nanoparticles for Biomedical Applications

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Engineering Nanoparticles for Biomedical Applications

1.Magnetic Nanoparticles

- SPION for MRI
- Thermally blocked NP for biodiagnostics

2.Nanoparticles for Drug Delivery

- Multifunctional NP
- Thermosensetive NP

3.Ferrogel for drug delivery

Definition

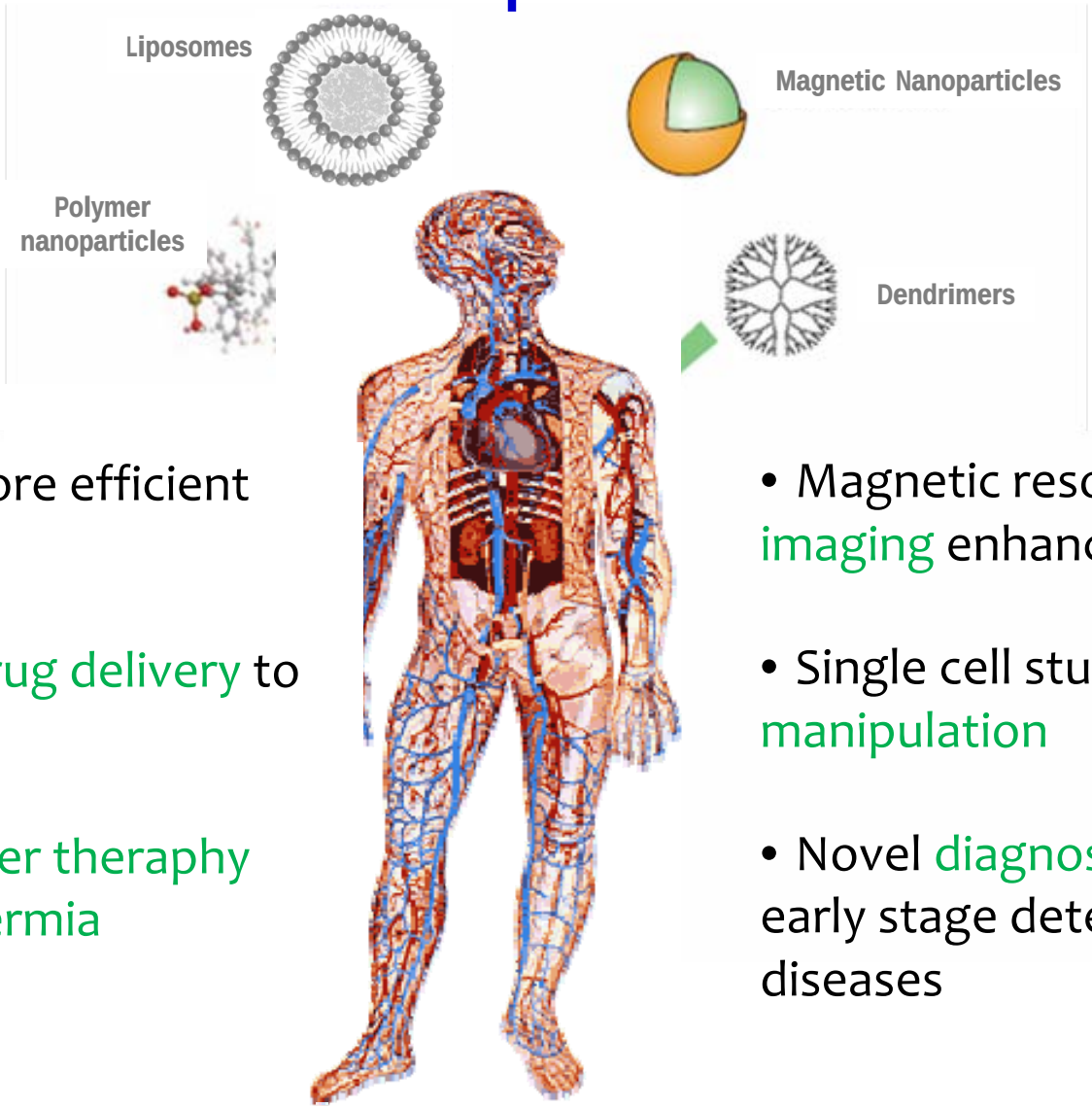
FDA calls it "nanotechnology" only if it involves all of the following:

1.Research and technology development, or products regulated by FDA, that are at the atomic, molecular or macromolecular levels, and where at least one dimension, that affects the functional behavior of the product, is in the length scale range of approximately 1-100 nanometers. (Man-made materials)

2. Creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size.

3. Ability to control or manipulate at the atomic scale.

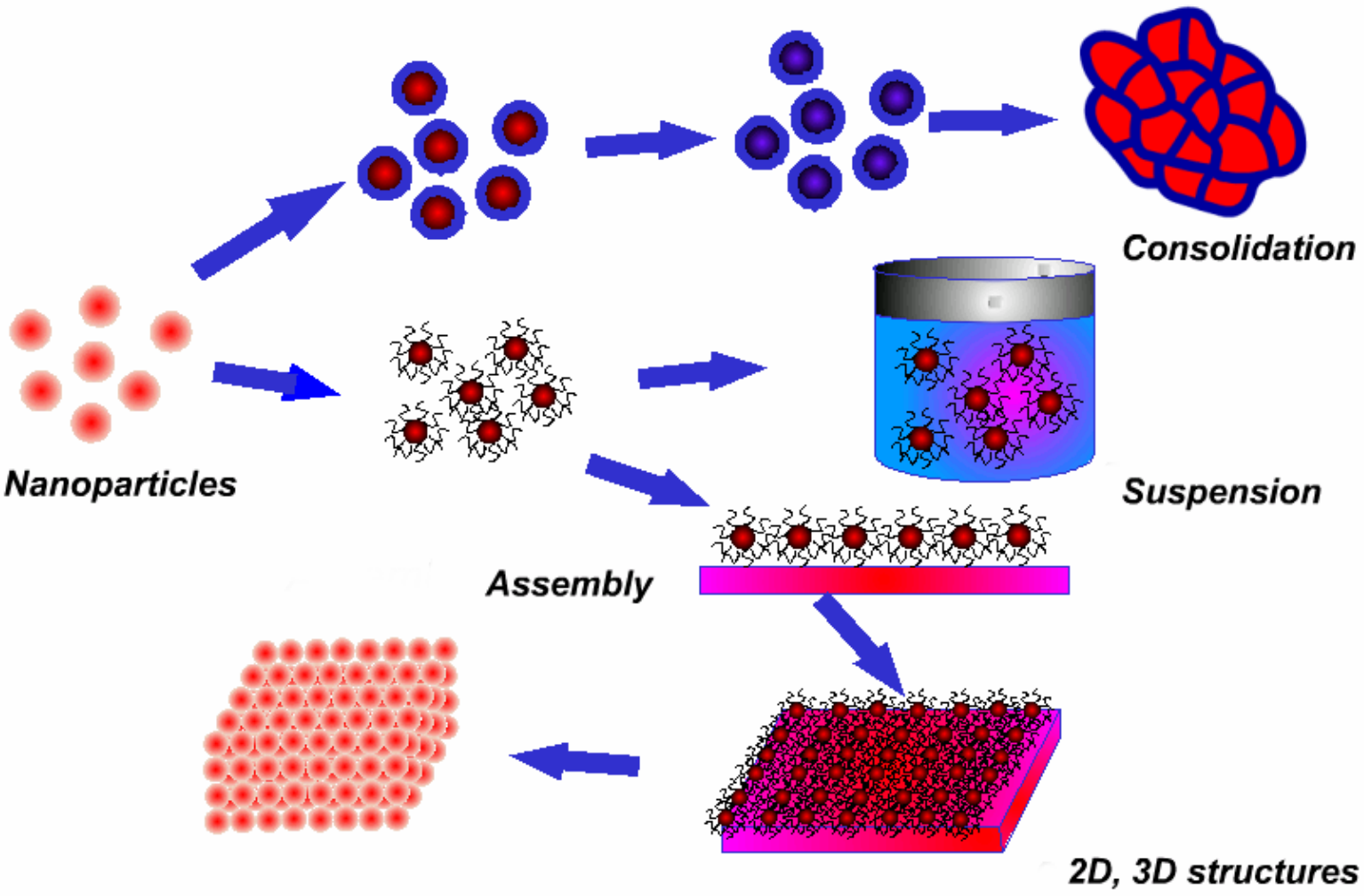
Medical applicaltions of nanoparticles



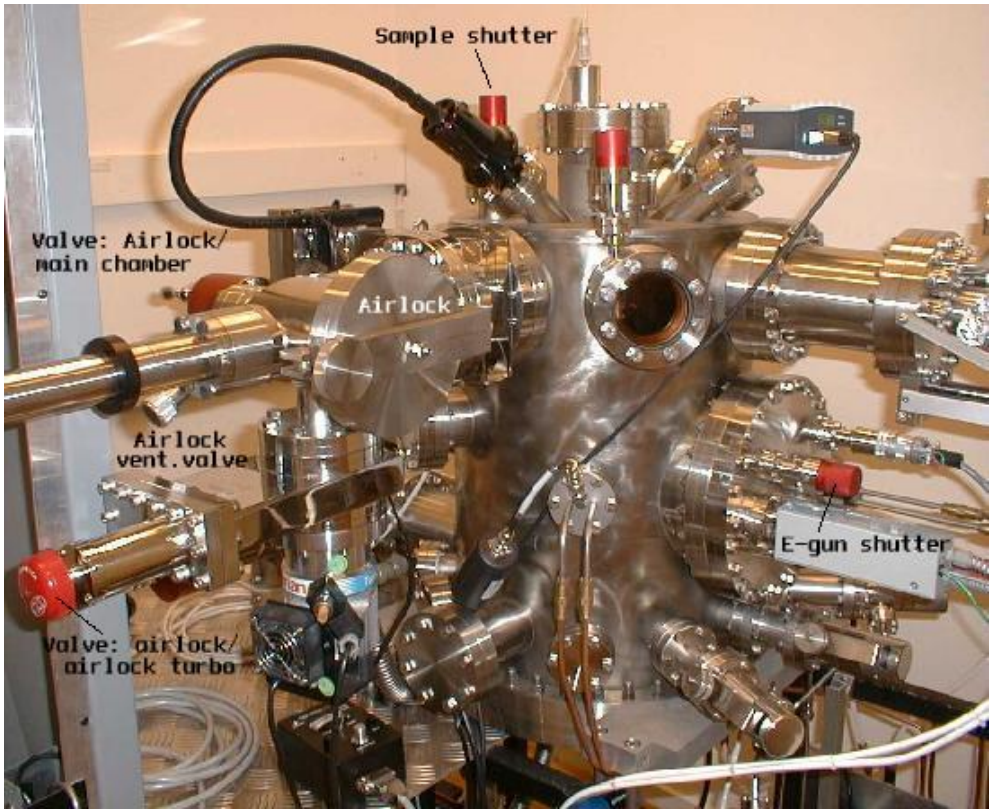
- Fast and more efficient biosensors
- Targeted drug delivery to specific cells
- Novel cancer theraphy and hyperthermia treatments
- Magnetic resonance imaging enhancement
- Single cell study and bio-manipulation
- Novel diagnostic tools for early stage detection of diseases

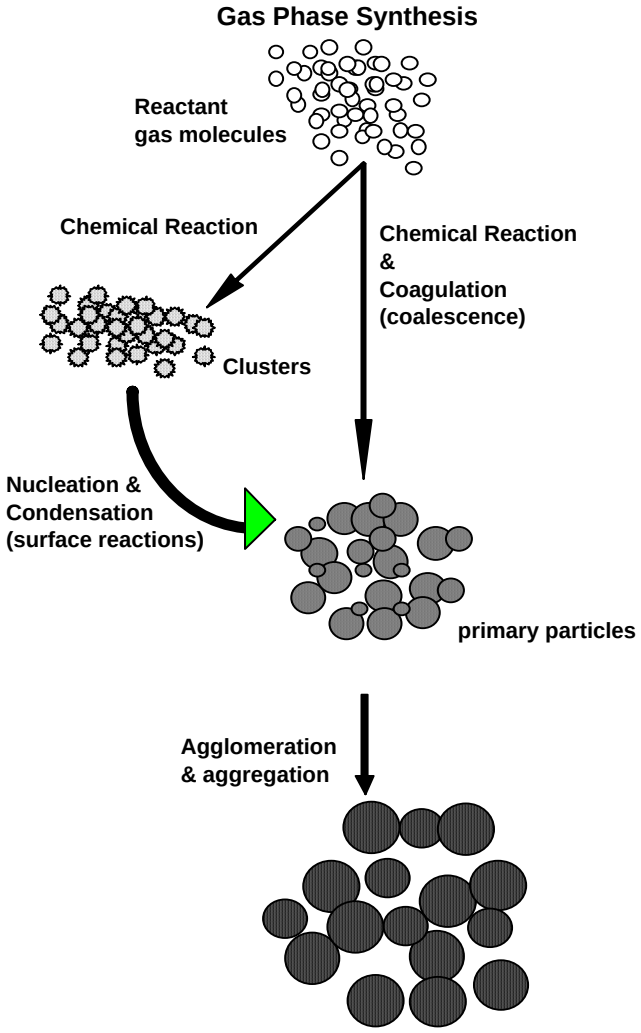
Nature Nanotechnology, **2007**, 2, 469-478

Nanoparticle Engineering



Gas Phase Synthesis





Chemical Solution Methods for Nanoparticles synthesis

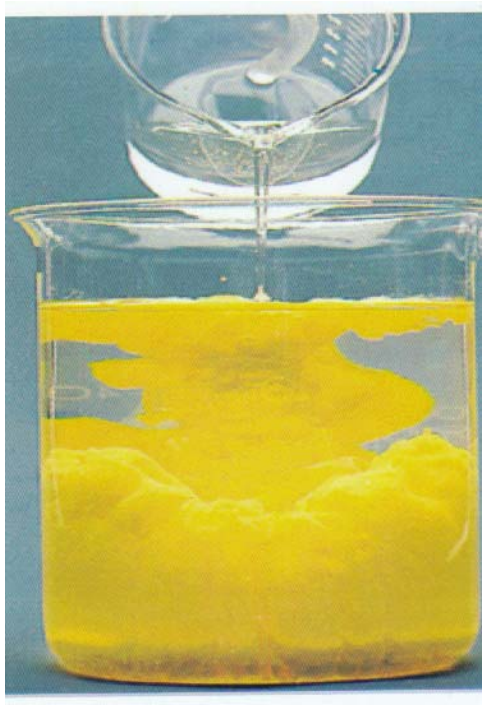
- **Precipitation** Homogenous precipitation
- Co-precipitation
- Hydrolysis
- Oxidative hydrolysis
- Reductive precipitation
- Electrochemical reduction

- **Condensation** Sol-gel technique
- Macro-molecular chemistry

- **Evaporation** Spray-drying
- Spray-pyrolysis
- Freeze-drying
- Aerosol technique

- **Templates** Precipitation in microemulsion
- Precipitation in presence of surfactants

- **Others** Sono-chemical reactions



Design of tailored Magnetic Nanoparticles

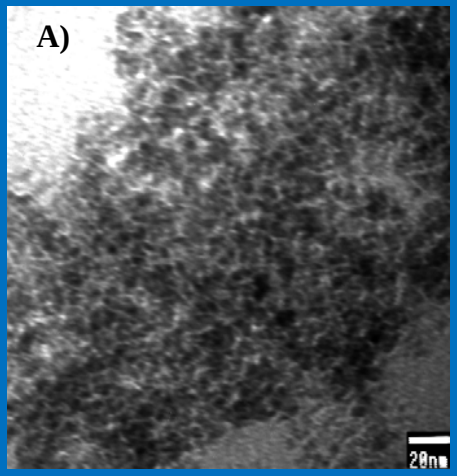
Superparamagnetic and Thermally blocked nanoparticles with strong magnetic response

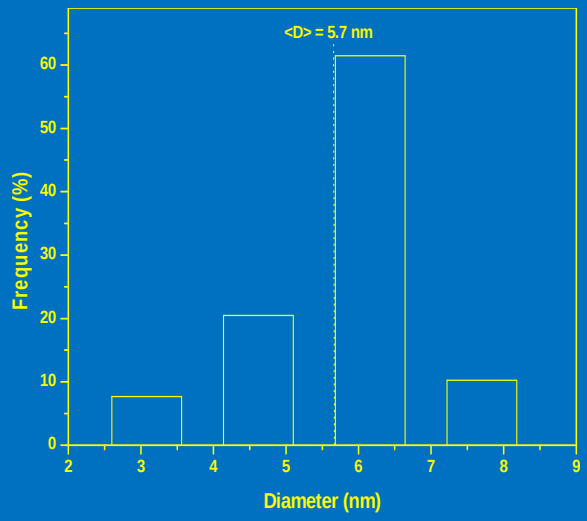
- Magnetite (Fe_3O_4)
- Maghemite (γFe_2O_3)
- Ferrites ($CoFe_2O_4$, $ZnFe_2O_4$, $MnFe_2O_4$, …)
- Iron Platinum (FePt) & CoPt

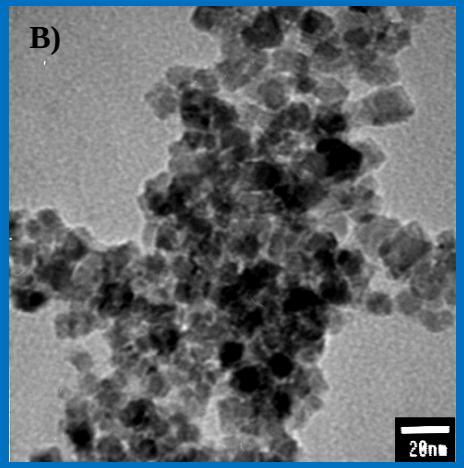
Bio-compatibility and surface functionalisation

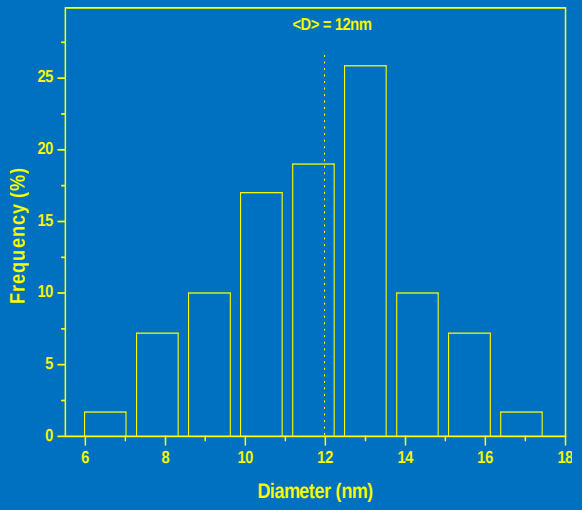
- Inorganic: Gold, Silica, hydroxyappatite, ...
- Organic: Dextran, PVA, PEG, mPEG, …

Magnetite





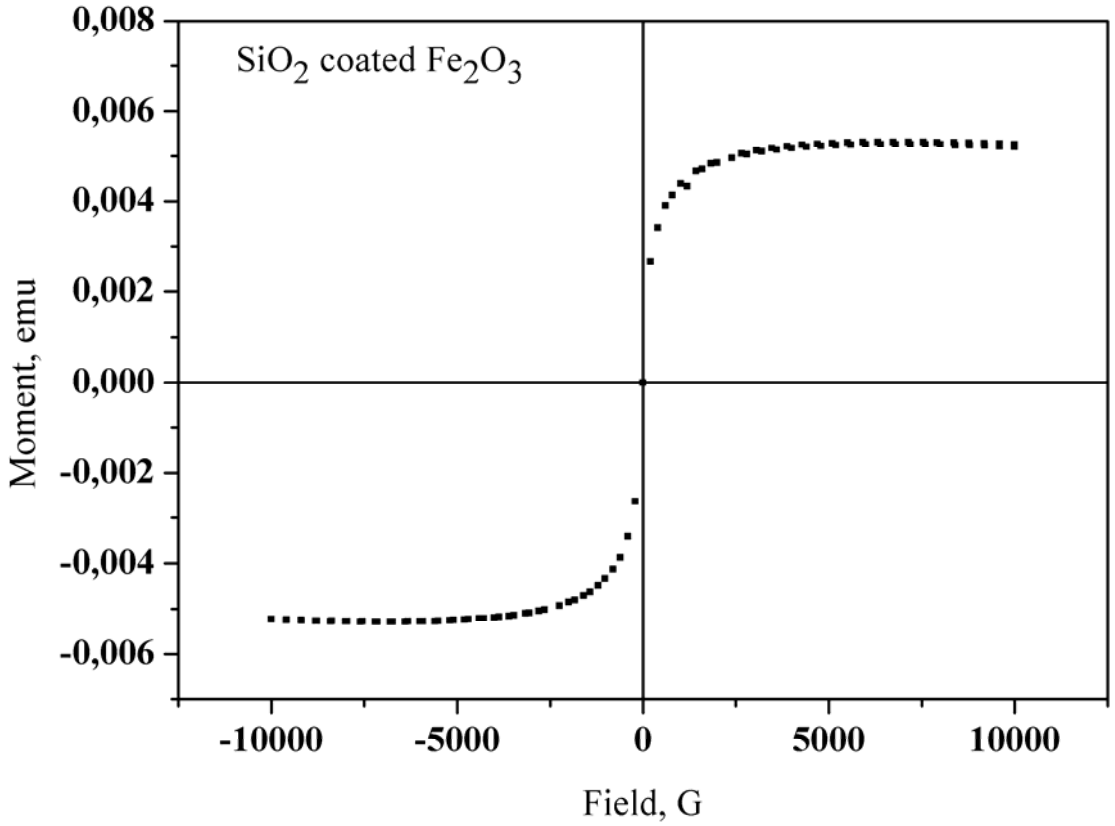




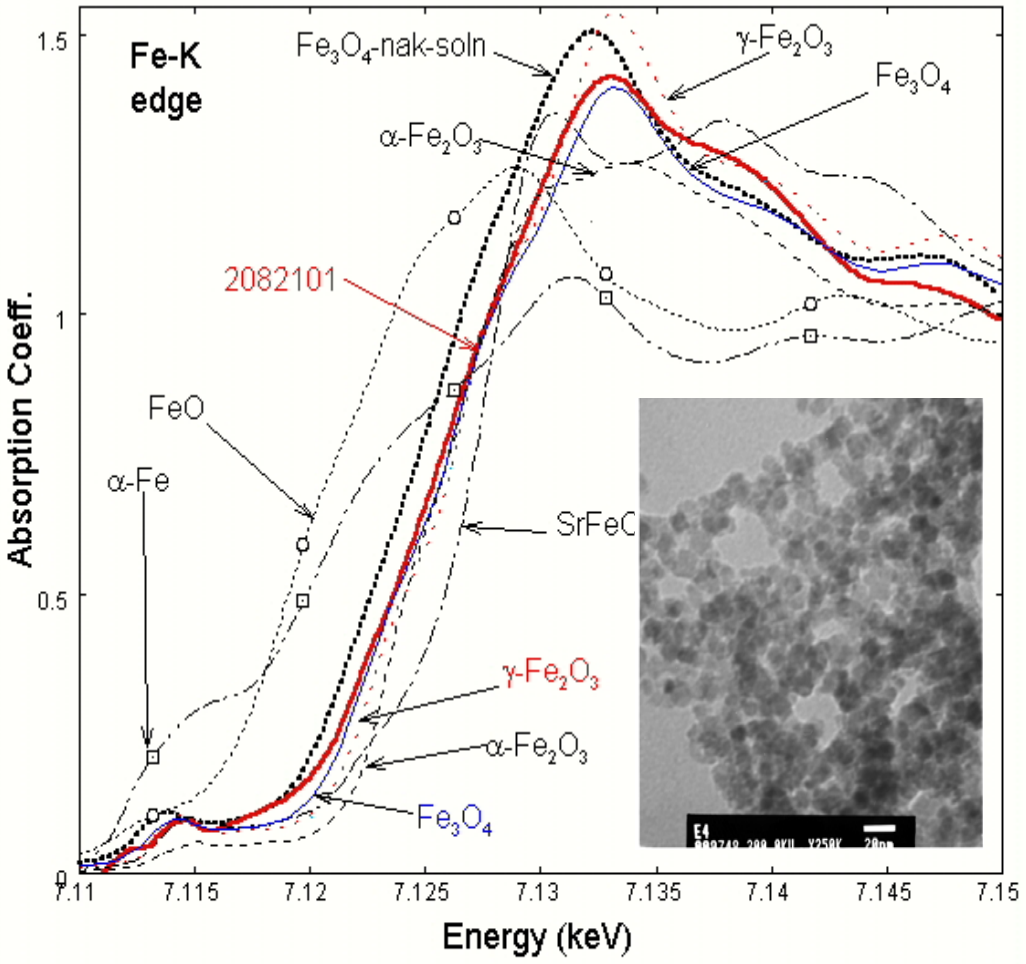
TEM images (left) and the corresponding particle size histograms (right) of magnetite nanoparticles prepared by controlled coprecipitation. (A) without heat treatment and (B) after heat treatment (80°C for 1hrs)

Magnetic characterisation

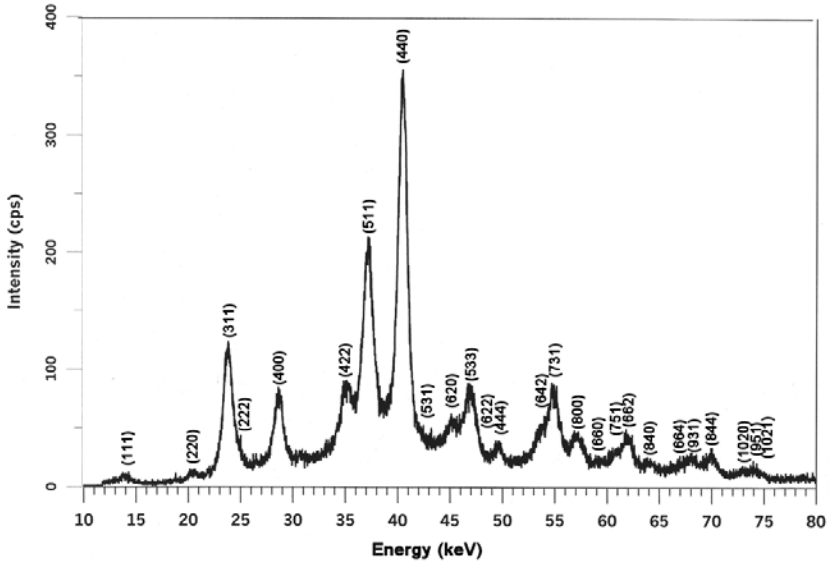
VSM measurement for SiO_2 coated Fe_3O_4 by co-precipitation



Superparamagnetic iron oxide nanoparticles

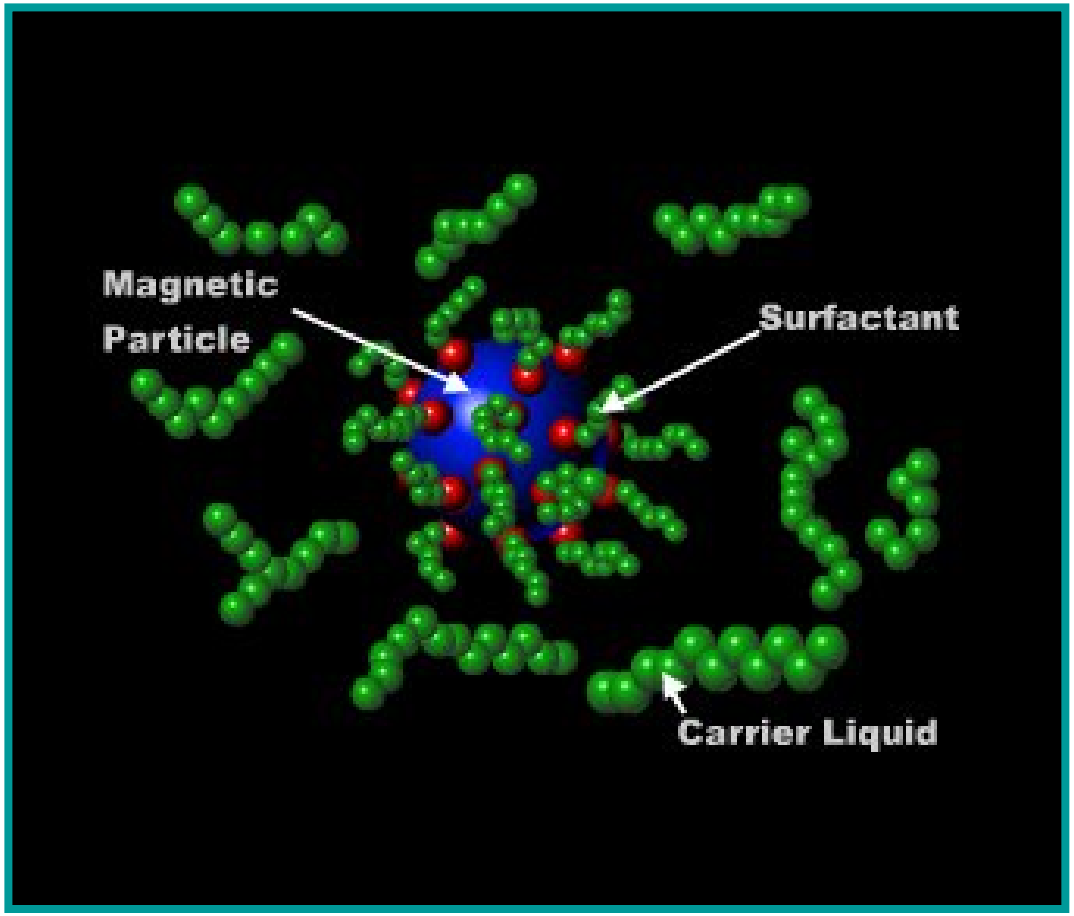


- Average particle size=12 nm
- XAS shows nonstochiometric phase $Fe_3O_{4-\delta}$, the curve shifts to Fe^{2+}.



After one year shelf storage

Surface Functionalization of Magnetic Nanoparticles



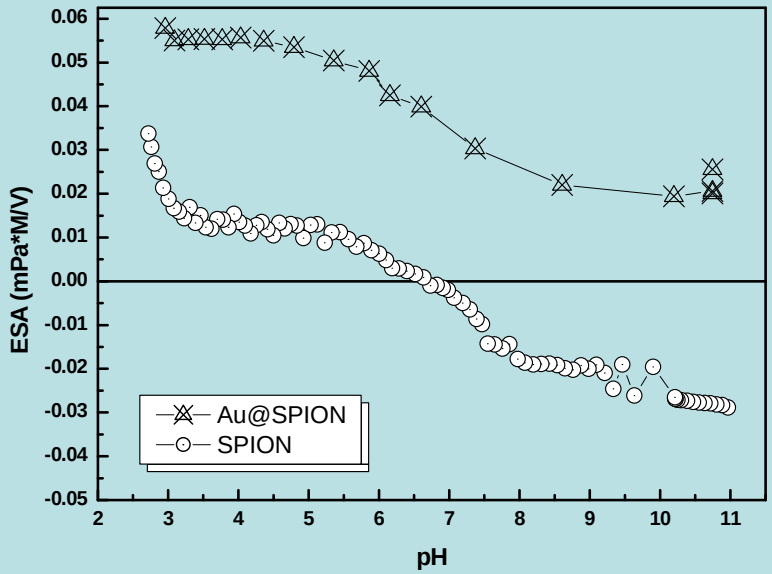
Magnetic Nanoparticles

- **Oxide : magnetite, ferrite**
- **Metal : Fe, Co, PtFe, CoPt**

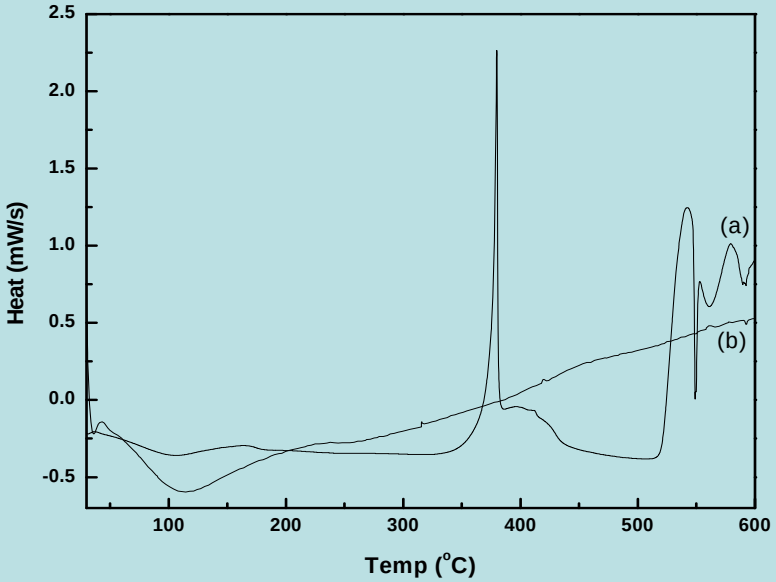
Coating

- Gold
- Silica
- Hydroxyapatite
- Dextran
- Starch
- Albumin
- Sodium Oleate
- Folic acid
- L-aspartic acid
- PVA
- PEG
- mPEG
- PLLA (PDLLA)
- PCL
- PGA

Effect of surface modification Au Coating

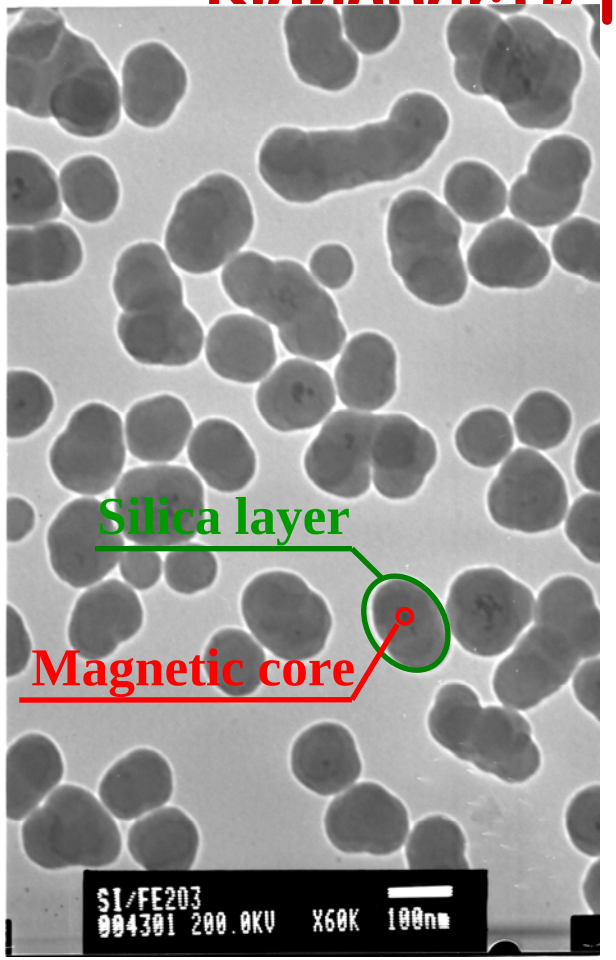


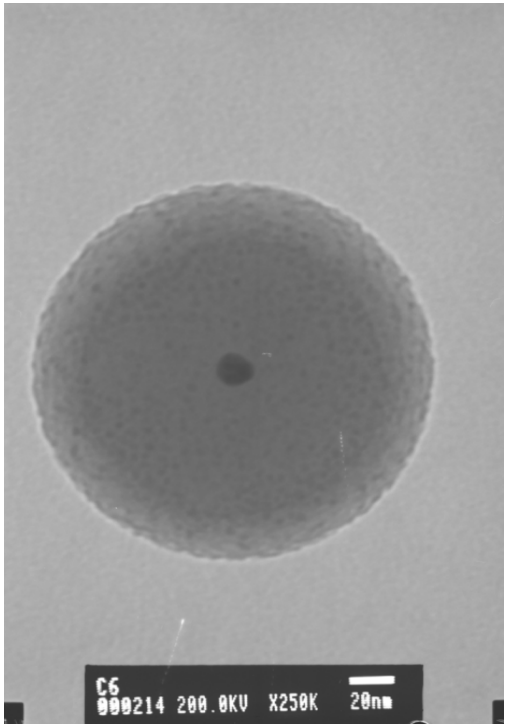
ESA measurement of SPION and Au@SPION prepared by μE system



DSC analysis of bare and coated nanoparticles. (a) Magnetite, and (b) Au coated SPION.

Silica Coated Magnetic Nanoparticles

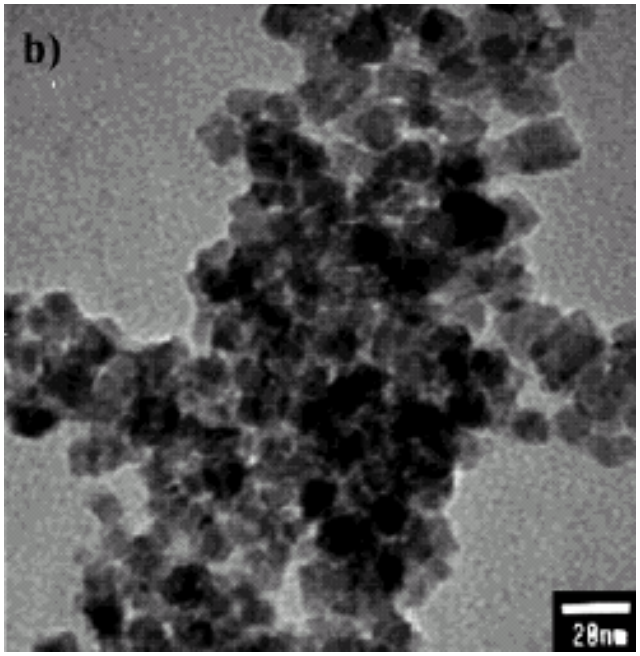




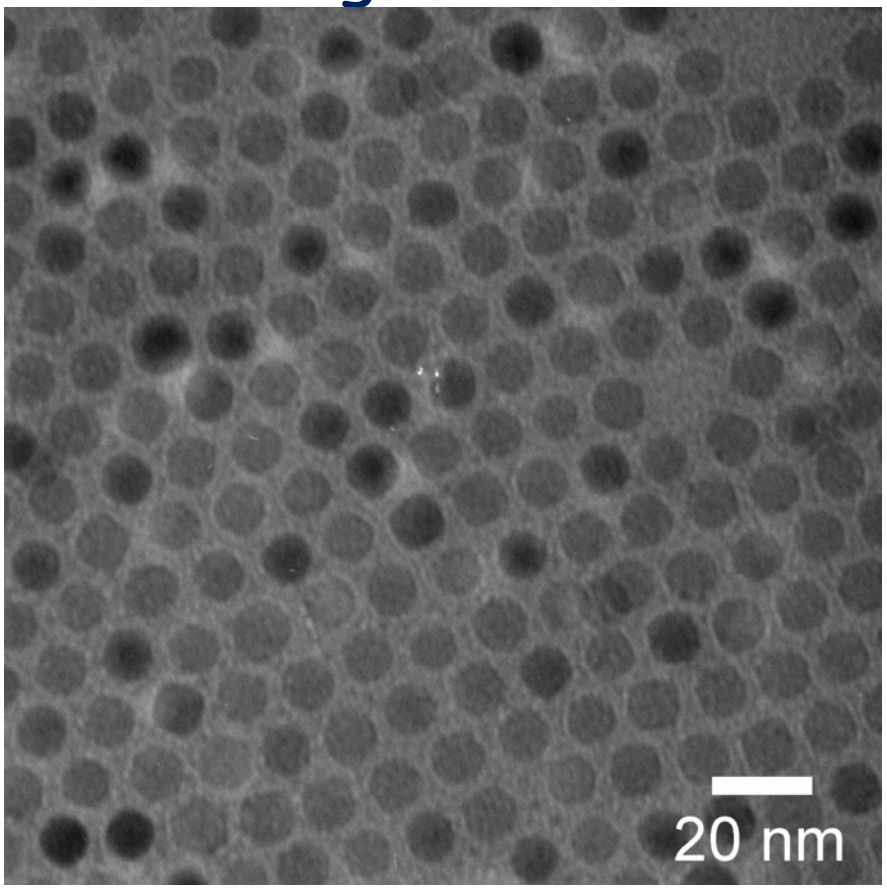
Control of thickness, porosity of coating layer

Visualization

SPION: MRI Contrast Agents



Widely used current commercial T2 contrast agent (Aq. Soln. Synthesis)



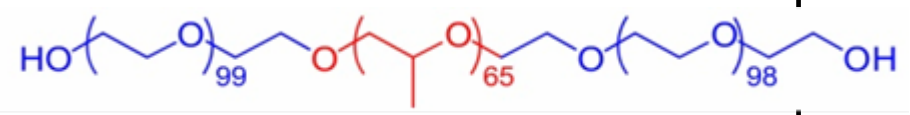
Controlled Synthesis in organic liquids (at 300 C)

Qin, J. et al., Adv. Mat. 2007

Phase transfer through Surface Coating

ABA type triblock copoly Pluronic F127

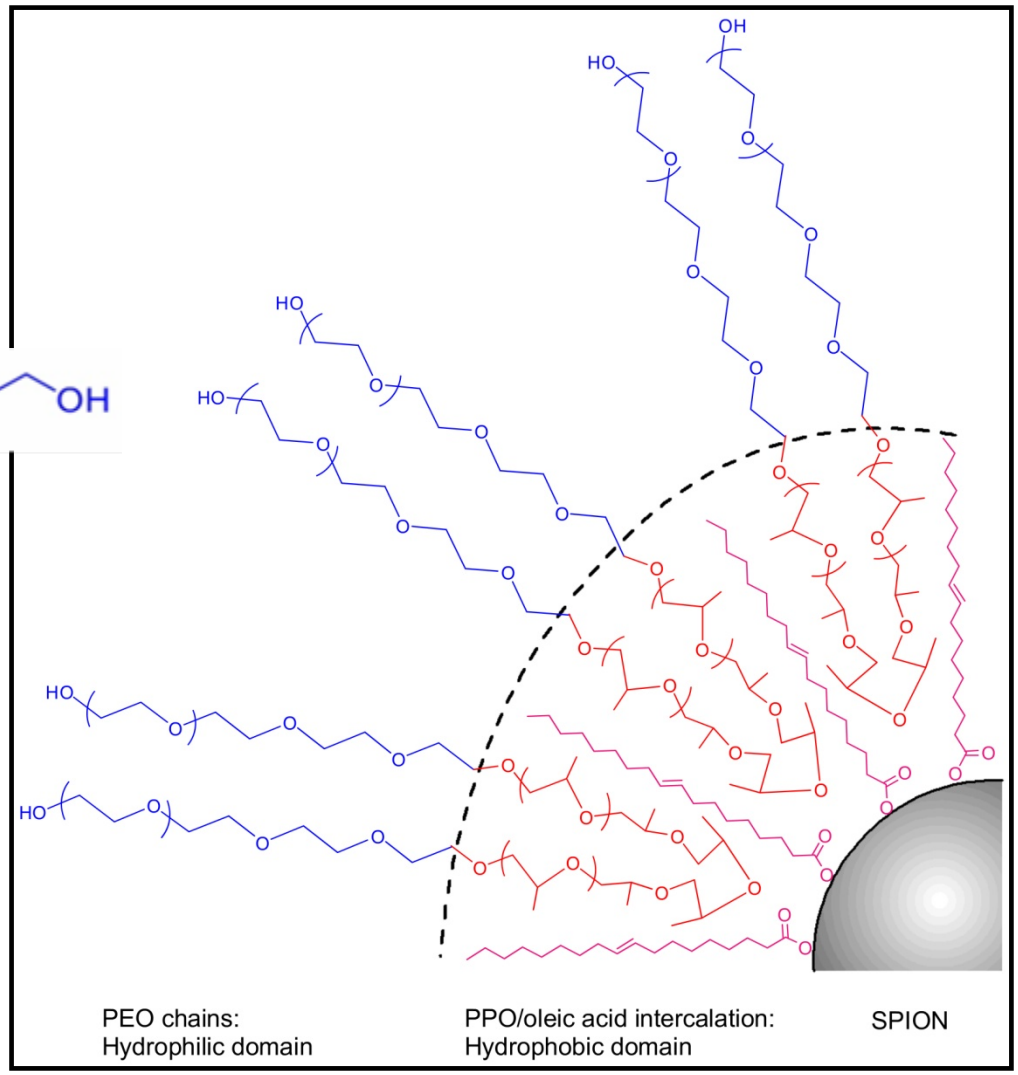
Hydrophilic poly(ethylene oxide)

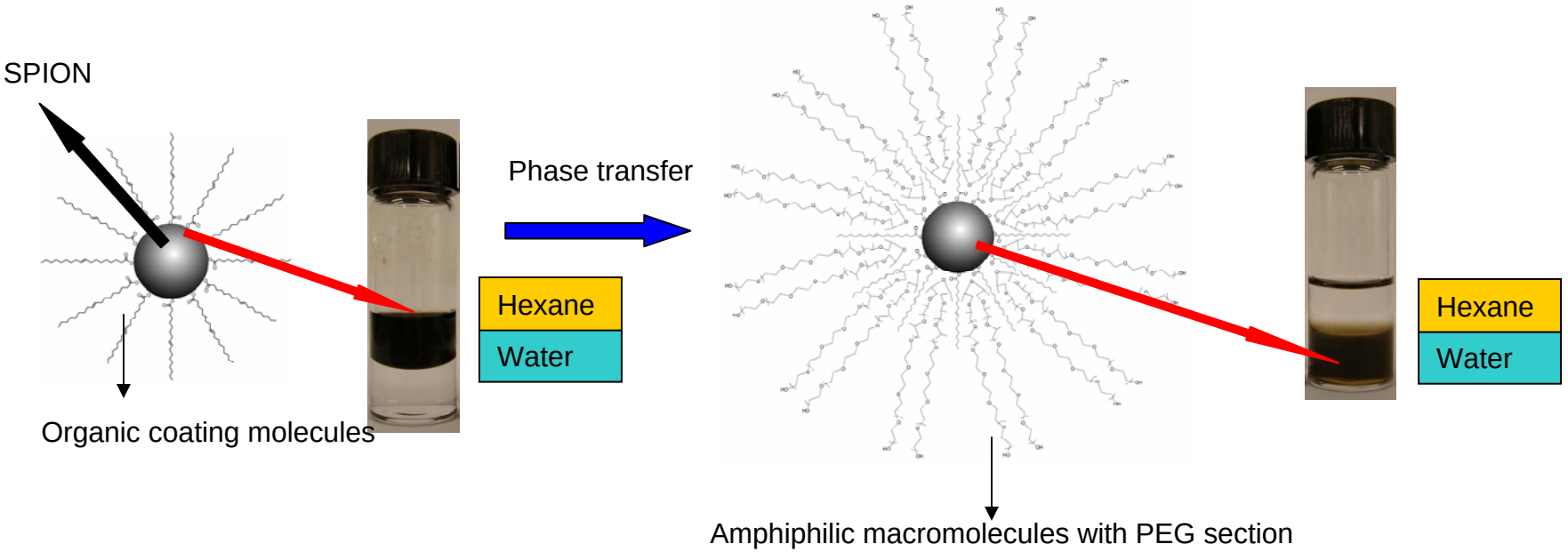


Hydrophobic poly(propylene oxide)

Amphiphilic coating layer

PF127/Oleic acid (POA)





J. Qin et al, Adv Mat (2007)

SPION: Suiperparamagnetic iron oxide nanoparticles
PEG: Poly(ethylene glycol)

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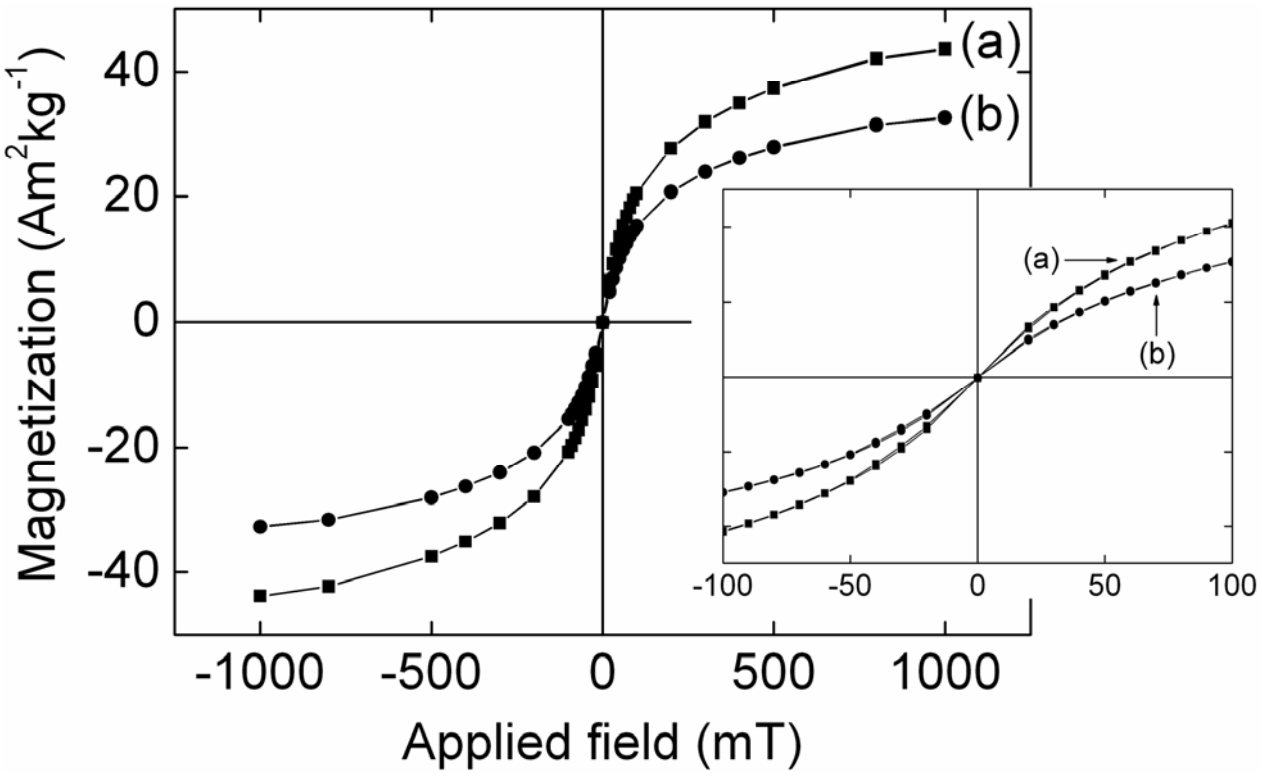
Superparamagnetism Retained



Magnetization curve of (a) as-synthesized SPION and (b) POA@SPION

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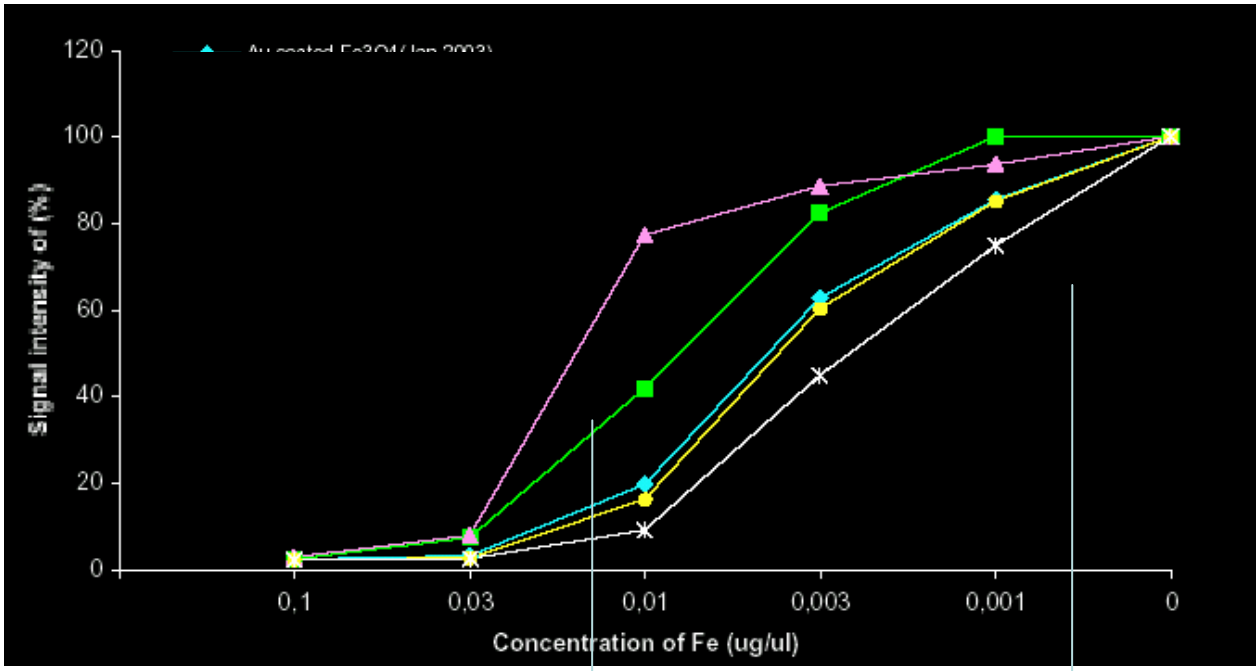
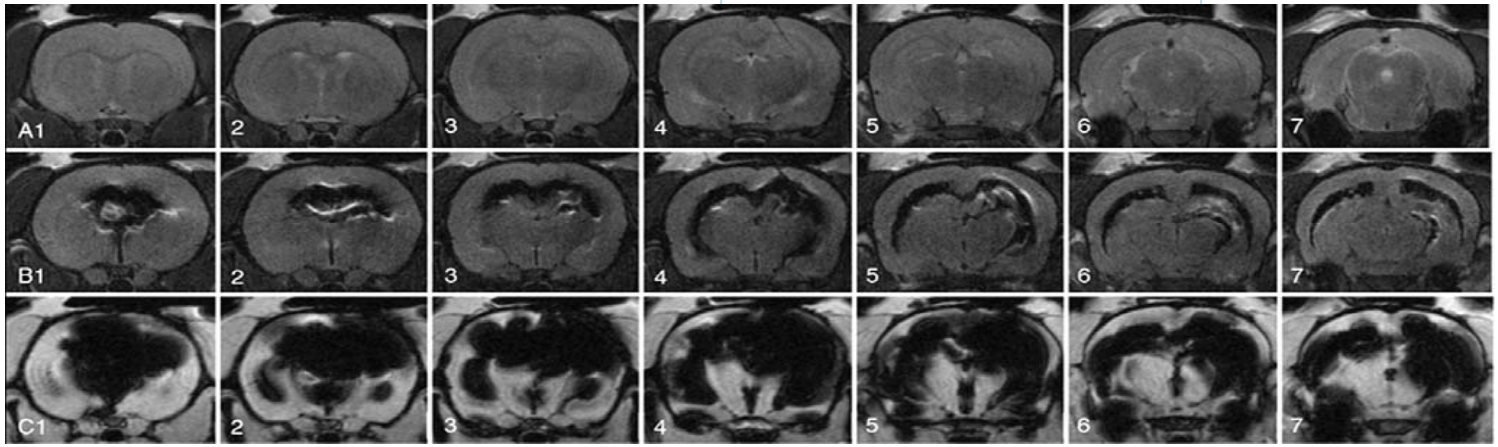
Compare with Conventional Iron Oxide Nanoparticle Based Contrast Agents

Particles name	Surface polymer	r_2/r_1 ratio (0.47 T, 310 K)	Mean hydrodynamic diameter (nm)
POA@SPION	Pluronic F127 + Oleic acid	41.5	116
AMI-25 (Feridex; Advanced Magnetic s, Cambridge, Mass)	Dextran	4.0	72
AMI-227 (Combidex; Advanced Mag netics, Cambridge, Mass)	Dextran	2.2	19
MION-37 (R. Weissleder, Massachus etts General Hospital, Boston, Mass)	Dextran T10	2.2	16-28
MION-37 (R. Weissleder, Massachus etts General Hospital, Boston, Mass)	Dextran T10	2.2	18-24
NC100150 (Clariscan, Nycomed, A mersham, Oslo, Norway)	Oxidized Starch	1.6	11.9
SH U 555 A (Schering AG, Berlin, Ge rmany)	Carboxydextran	7.1	65
USPIO S (Schering AG, Berlin, Germa ny)	Carboxydextran	2.3	21

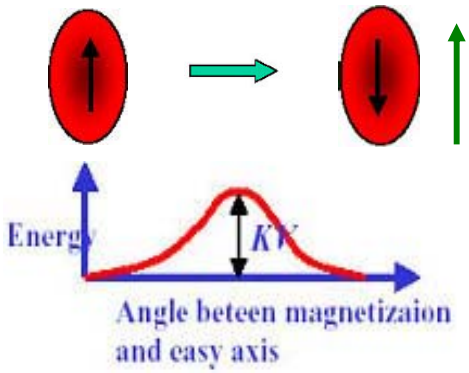
Dose response of Fe_3O_4 nanoparticles in MRI





Thermally Blocked Nanoparticles
Magnetic Relaxation for Bio-Diagnostics

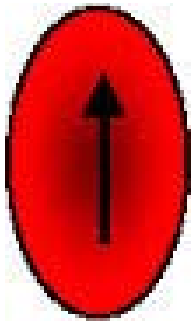
Néel relaxation



$$\tau_N = \tau_0 e^{\frac{KV}{kT}}$$

τ_N = Nèel rel. time
τ_0 = characteristic rel. time
k = Boltzmann constant
T = temperature
K = magnetic anisotropy
V = single domain volume

Brownian relaxation



$$\tau_B = \frac{3V_H\eta}{kT}$$

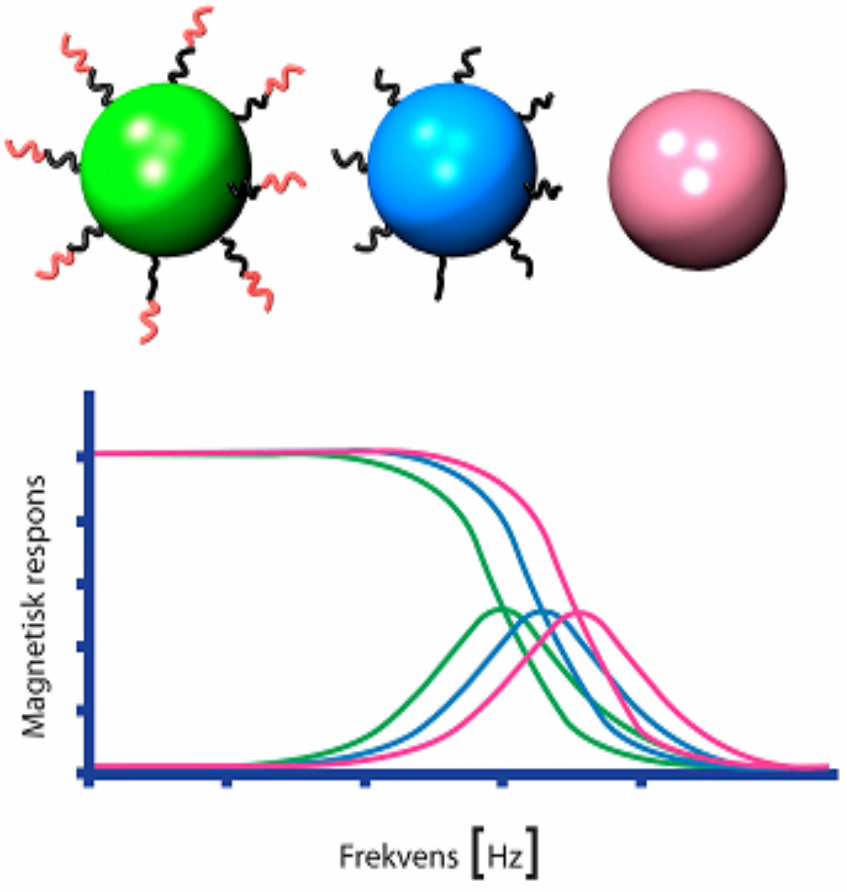
τ_B = Brownian rel. time
V_H = Hydrodynamic particle volume
η = viscosity

KTH Materials Science and Engineering

Biosensor Based on Magnetic Relaxation

Detect specific biomolecules by measuring changes in Brownian relaxation of thermally blocked magnetic nanoparticles.

shift in the maximum of the imaginary magnetic susceptibility.



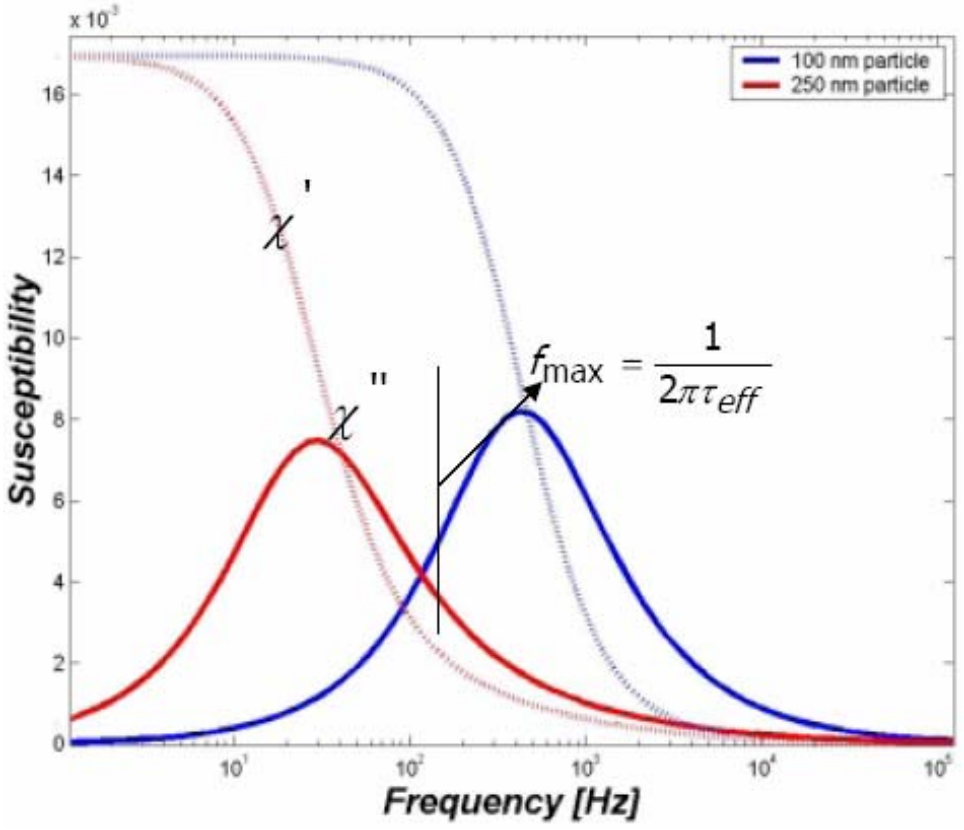
Fornara, A. et al, NanoLetters (2008)

Bio-diagnostics based on Magnetic Relaxation

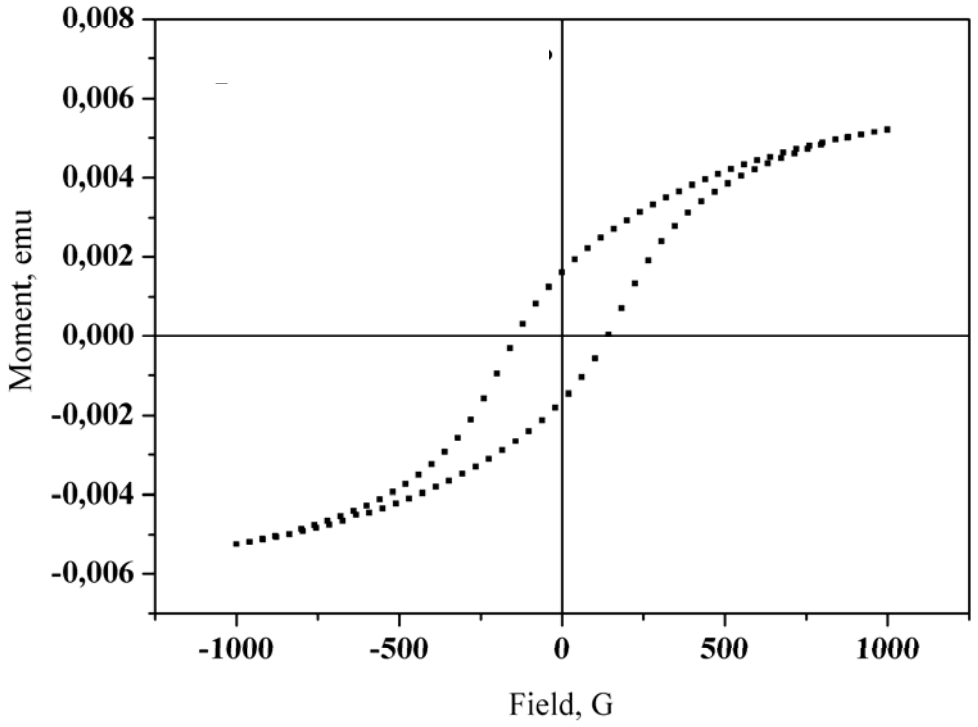
Brownian relaxation process can be detected in the frequency domain

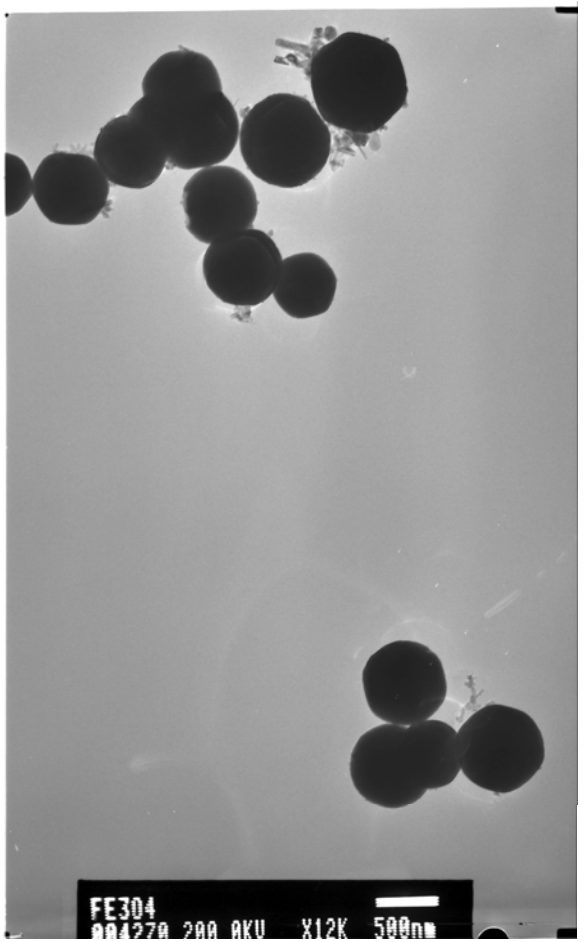
$$M = \chi H = (\chi' - i\chi'')H$$

M = magnetisation
H = alternating external magnetic field
χ = complex magnetic susceptibility

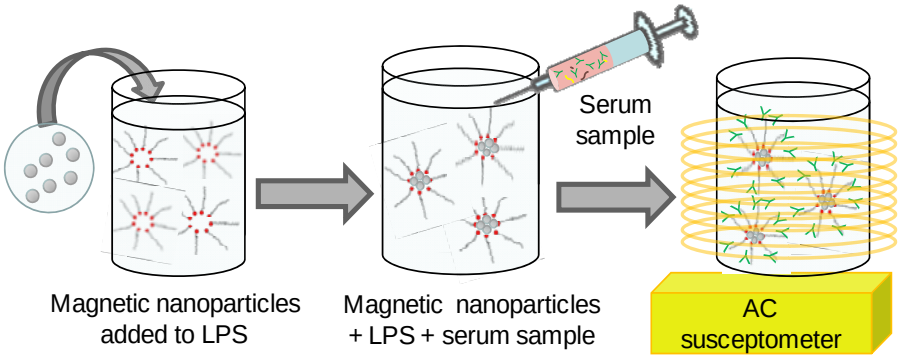


Synthesis of Thermally blocked Magnetic nanoparticles





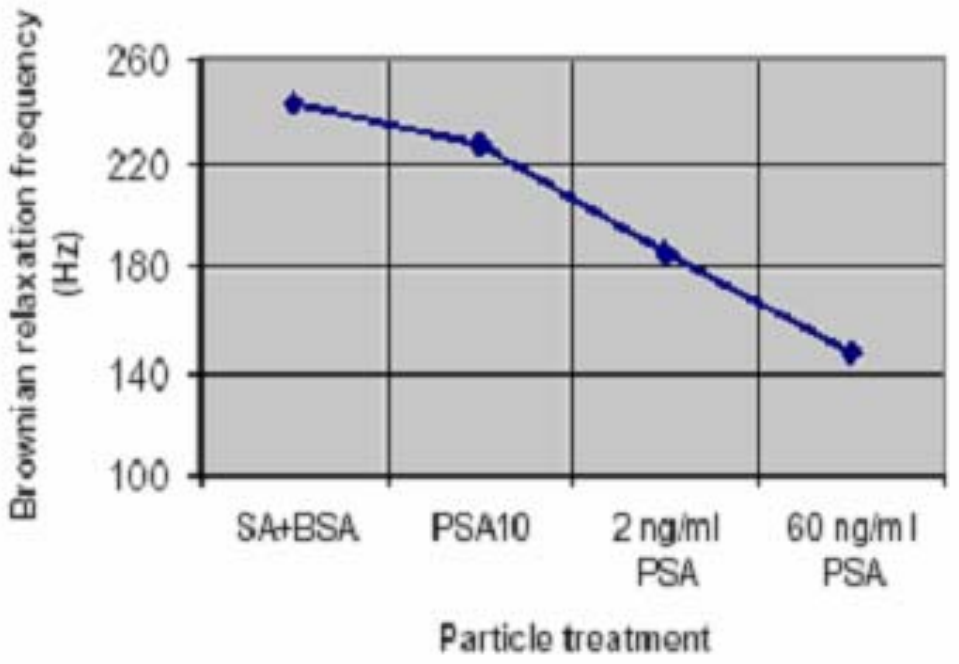
Quantitative detection of PSA by Brownian relaxation frequency measurements

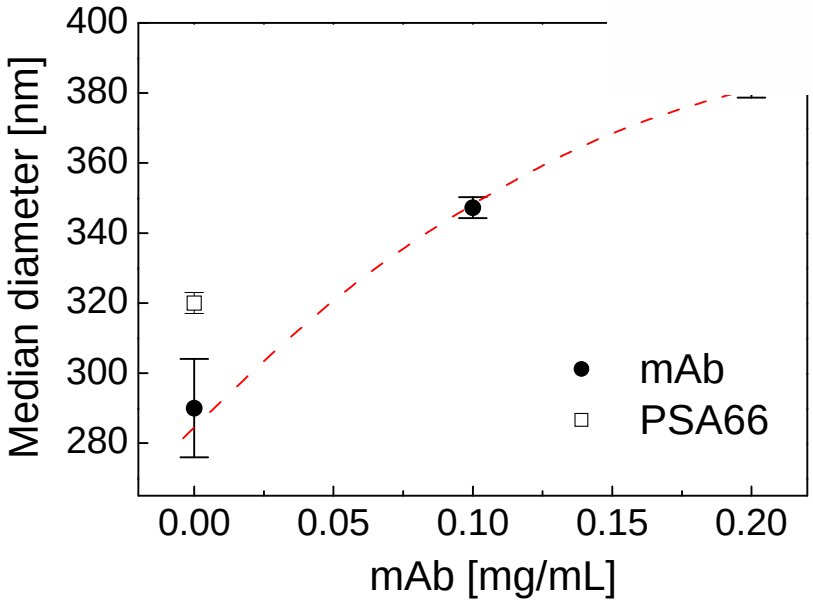


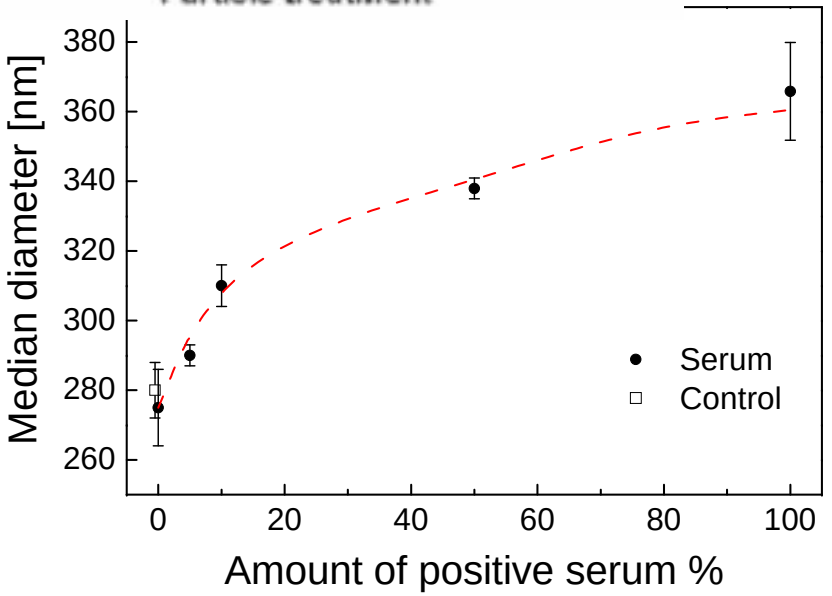
- No pretreatment
- Simple mixing of fluids
- Fast
- Multiple bio molecules detection
- Practical for point of use

100 1000

Detection of Brucella Antibodies in Serum



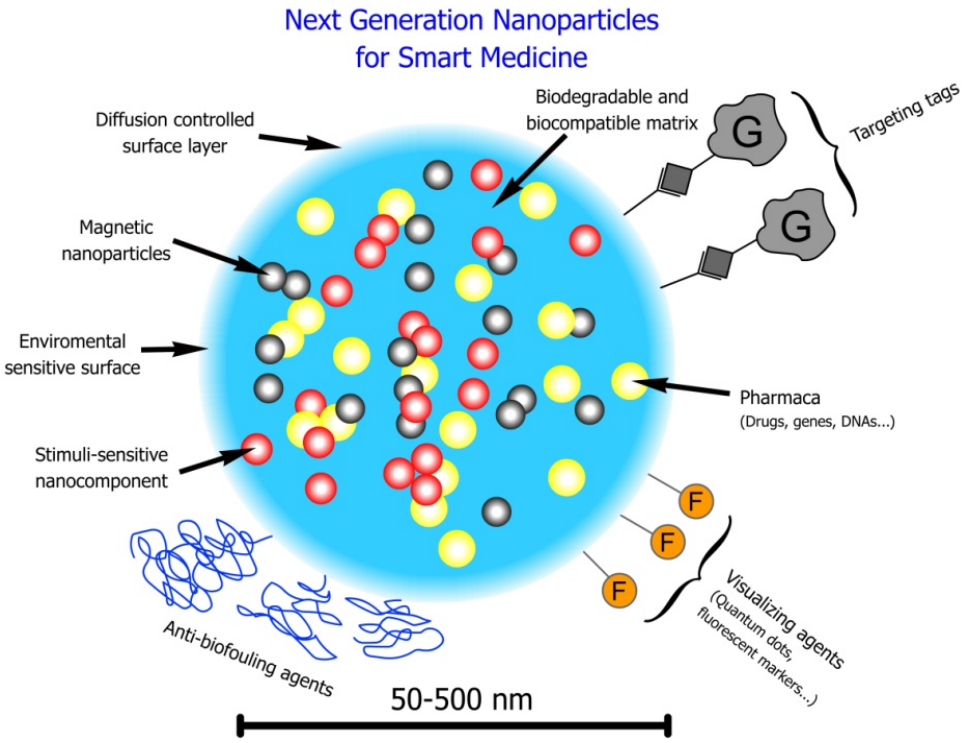




Detection limit: 0.05 µg/ml

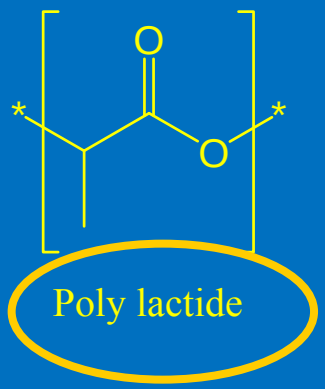
Fornara, A. et al, NanoLetters (2008)

Nanomaterials in Biology and Medicine

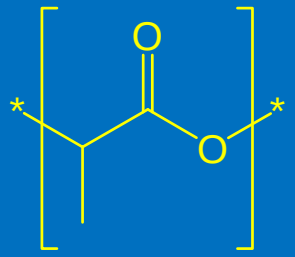


<u>Targeted drug delivery</u> – Targeted drug delivery using a multicomponent nanoparticle containing therapeutic as well as biological surface modifying agents

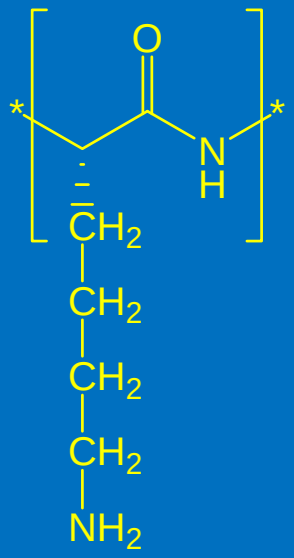
Biocompatible Polymers



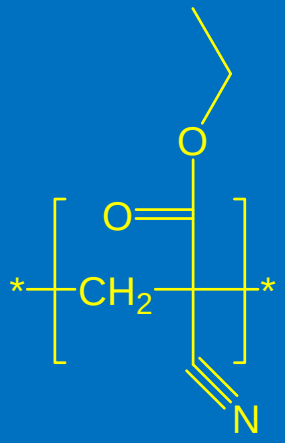
Poly lactide



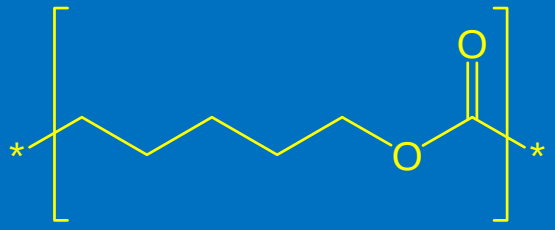
Poly glycolide



Poly L-lysine



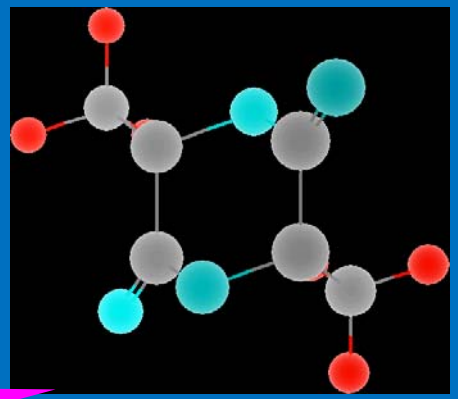
Poly(ethyl-2-cyanoacrylate)



Poly ¥å-caprolactone

Amphiphilic copolymer for biodegradable nanosphere fabrication

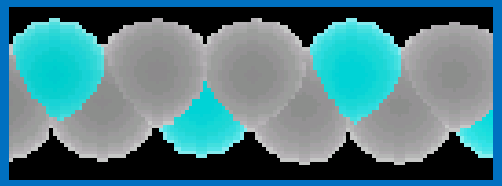
Lactide (3,6-dimethyl-1,4-dioxane-2,5-dione)



- Biocompatible
- Degradable under physiological condition
- Applicable as a hydrophobic segment in amphiphilic copolymer

copolymerization

poly ethylene glycol (PEG)



- Biocompatible
- Applicable as a hydrophilic segment in amphiphilic copolymer

Strategies for Drug Delivery Systems

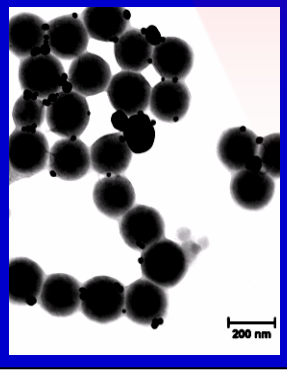
Hydrophobic drug
i.e. steroids

Emulsion/evaporation
(o/w)

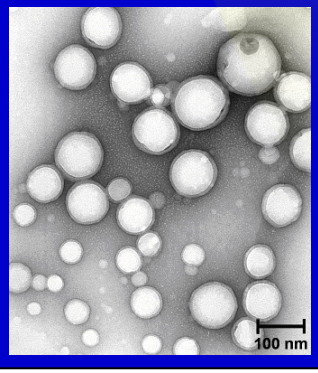
Hydrophilic drug
i.e. proteins

Modified-double-emulsion
(w/o/w)

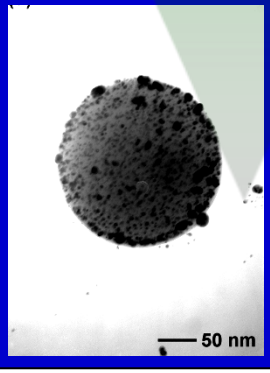
Surface
modification
s and
activation



drug



drug



biomolecules

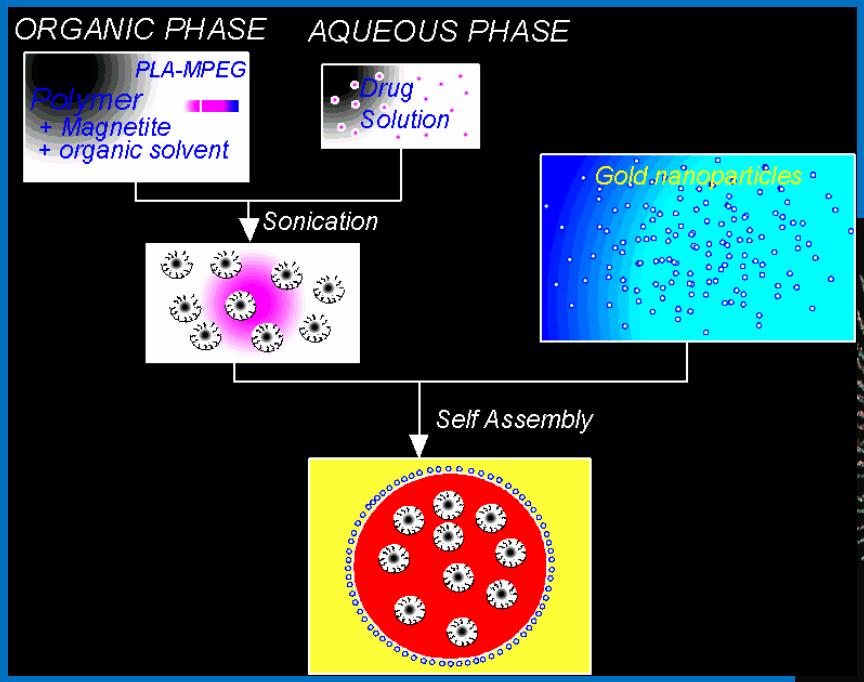
drug

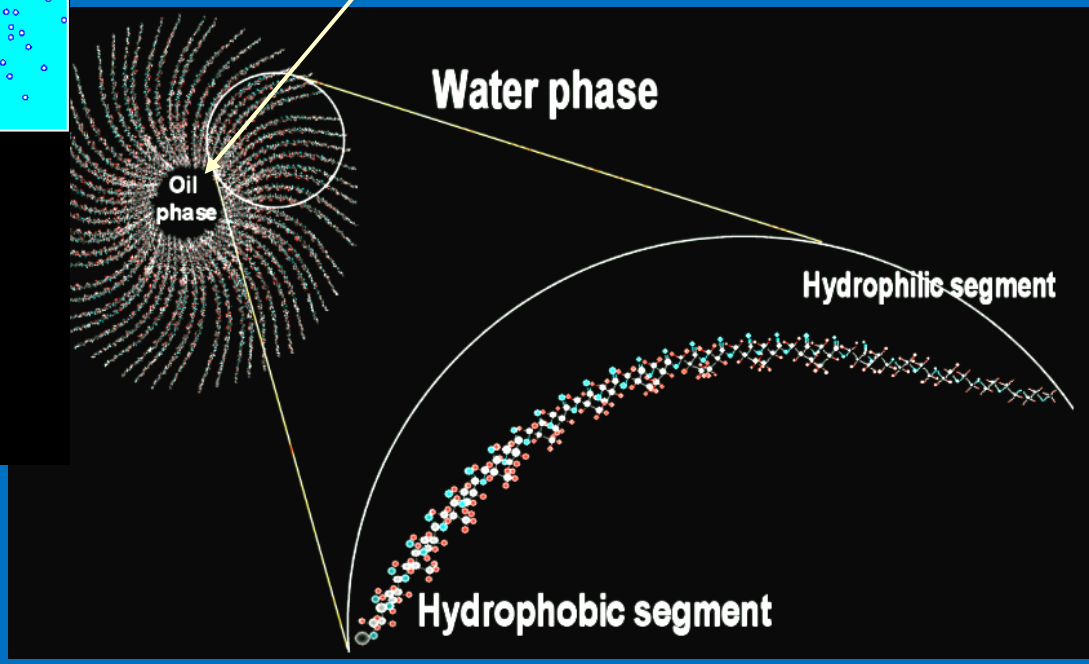
biomolecules

biomolecules

Procedures for preparation of drug-loaded Nanospheres

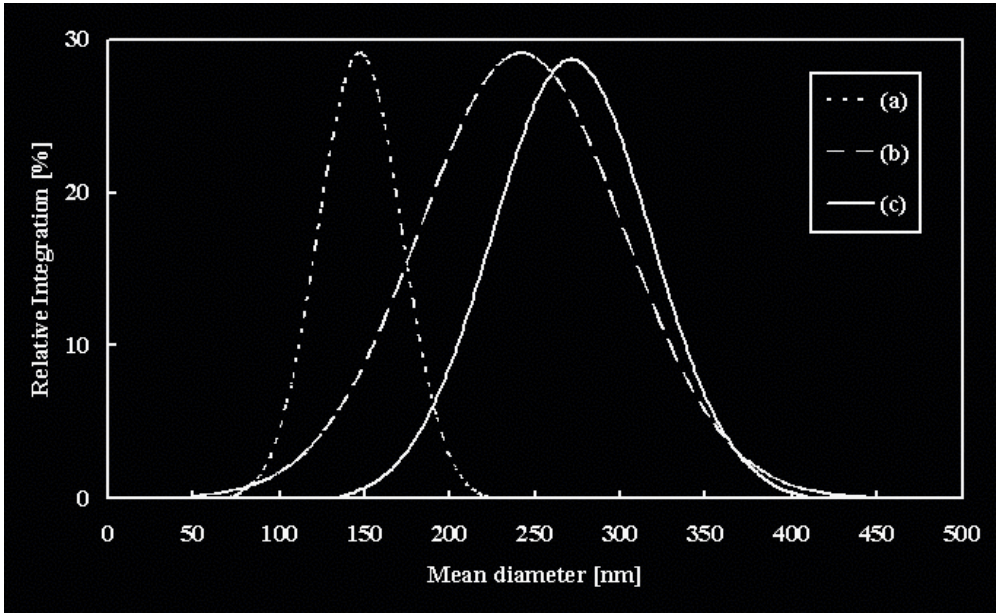
Drug , Fe_3O_4 , & Quantum dots loaded in the cavity





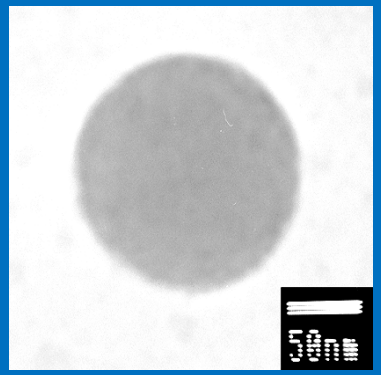
Compositions of PLA-mPEG diblock copolymers

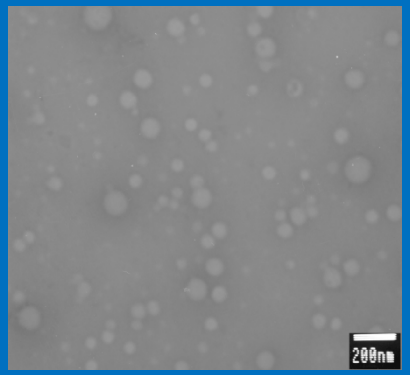
Code	PLA		mPEG
	Enantiomers	Mw[kDa]	Mw [kDa]
M90	PDLLA(P-*meso*-LA)	90	5
M45	PDLLA(P-*meso*-LA)	45	5
M25	PDLLA(P-*meso*-LA)	25	5
M5	PDLLA(P-*meso*-LA)	5	5
L45	PLLA	45	5
D45	PDLA	45	5
R45	PLLA+PDLA(P-*rac*-LA)	45	5



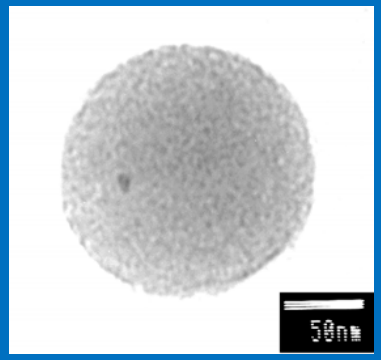
TEM images of BSA-loaded nanospheres

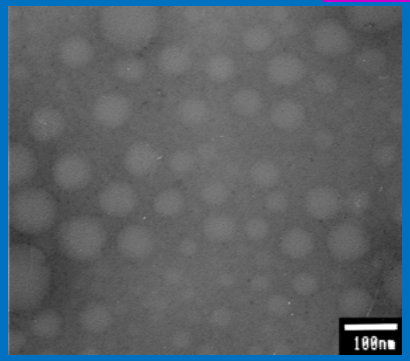
BSA-loaded PLA-mPEG nanospheres

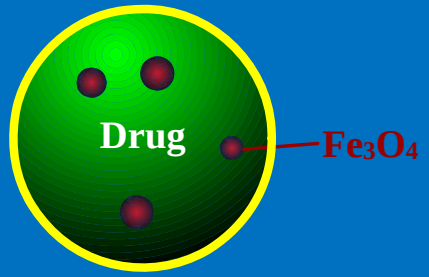




BSA and Fe_3O_4-loaded PLA-mPEG nanospheres



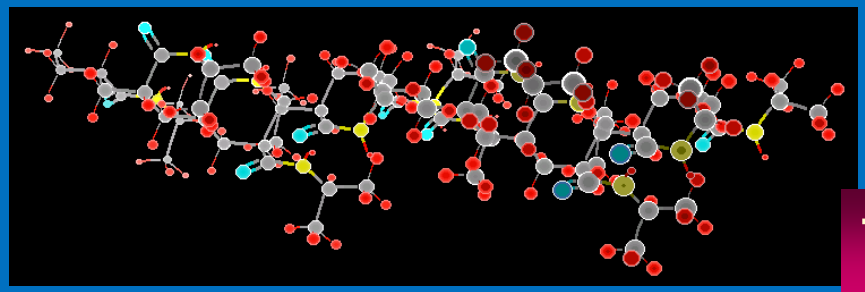




Thermo- sensetive Drug delivery system

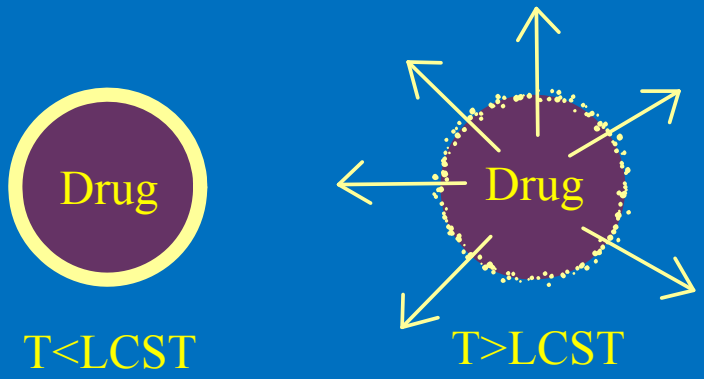
- Stable suspension at temperatures **below** body temperature
- Unstable at temperatures **above** body temp.
  - Use body increase of temp to trigger release
  - Use external heating source -hyperthermia

‘Smart’ polymeric Nanoparticles Systems



Poly(*N*-isoprorylacrylamide)

Thermosensitive polymer



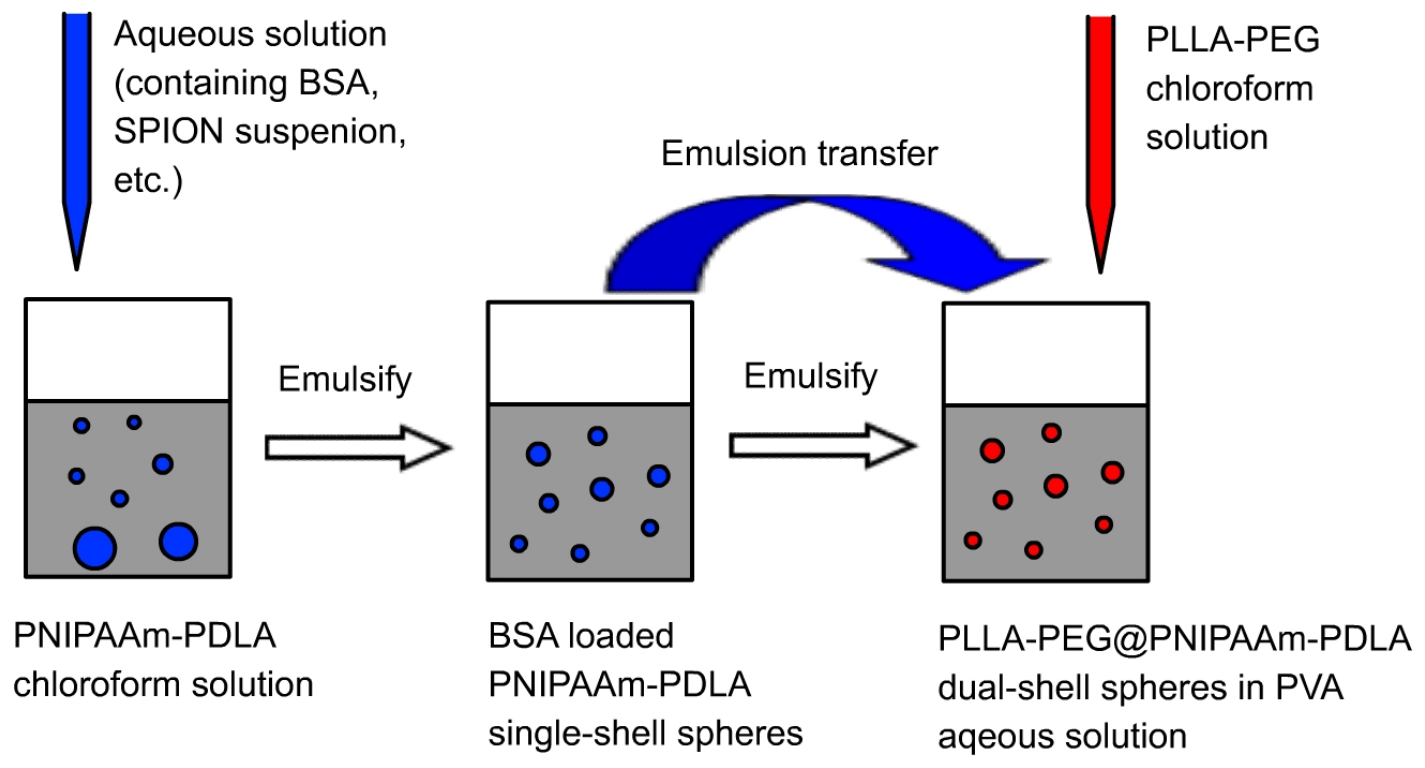
LCST : Lower critical solution temperature

Under the condition that temperature
exceeds the LCST, amphiphlic micelles are collapsed so as to start to release
the entrapped drug.

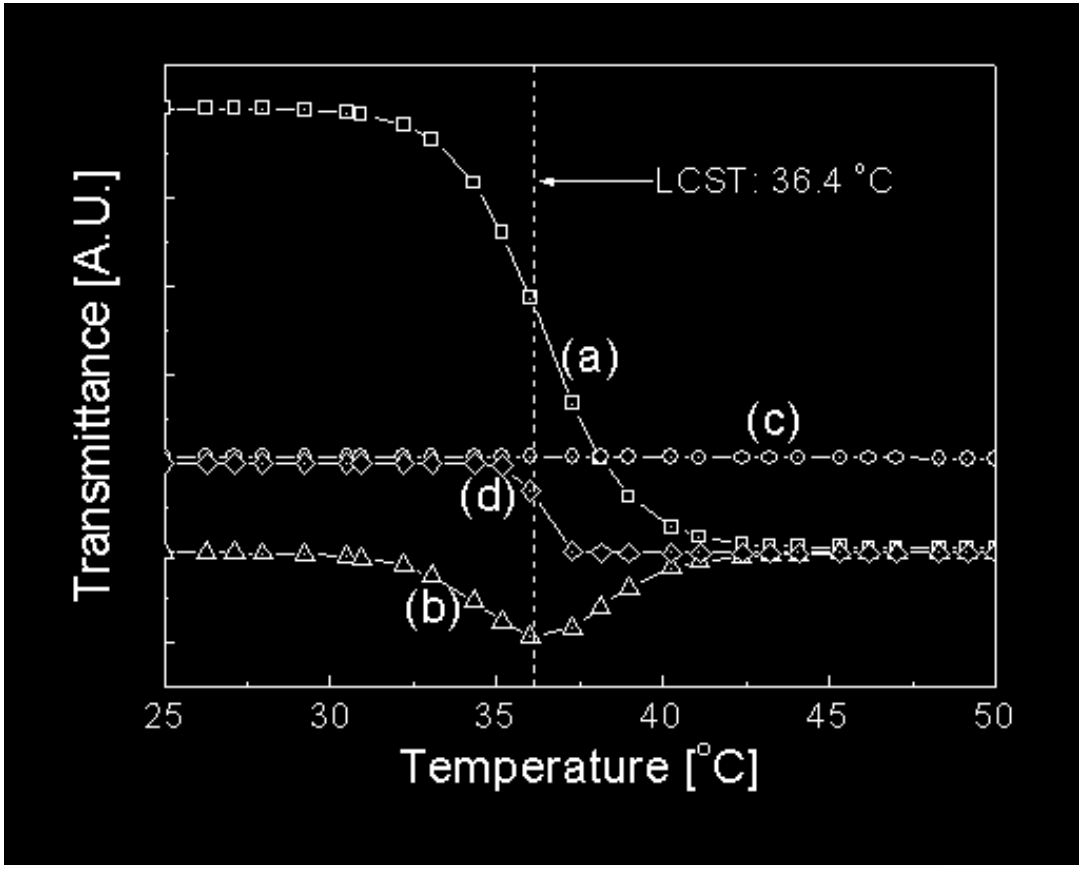
*Jo, Y. S., et al., Macromolecular Rapid Communications **2003**, 24, 957.*

Qin et al (2006)

Schematic View of Modified-Double-Emulsion-Method (MDEM)

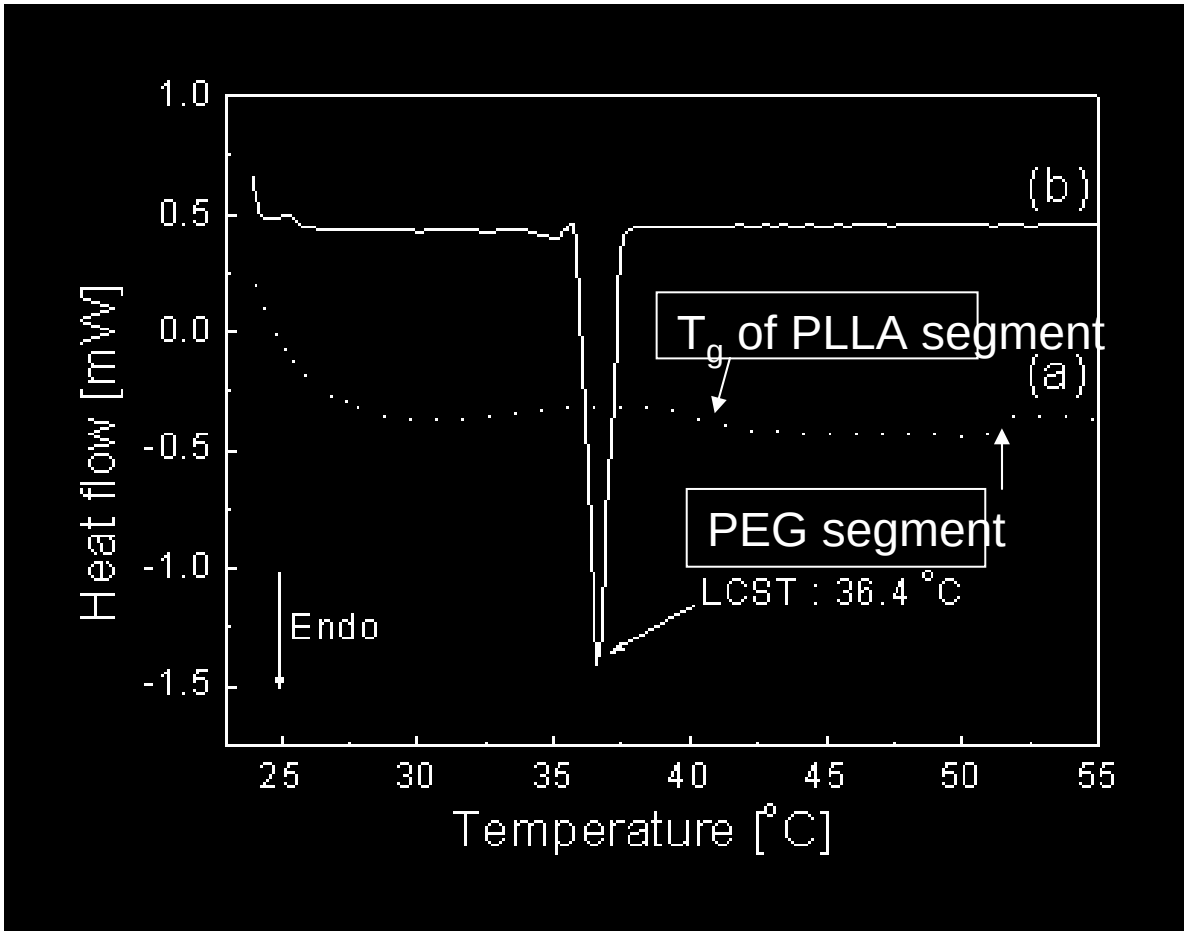


BSA: Bovine serum albumin
PEG: Poly(ethylene glycol)
PDLA: Poly(D, D-lactide)
PLLA: Poly(L, L-lactide)
PNIPAAm: Poly(*N*-isopropylacrylamide)
PVA: Poly (vinyl alcohol)
SPION: Superparamagnetic iron oxide nanoparticles



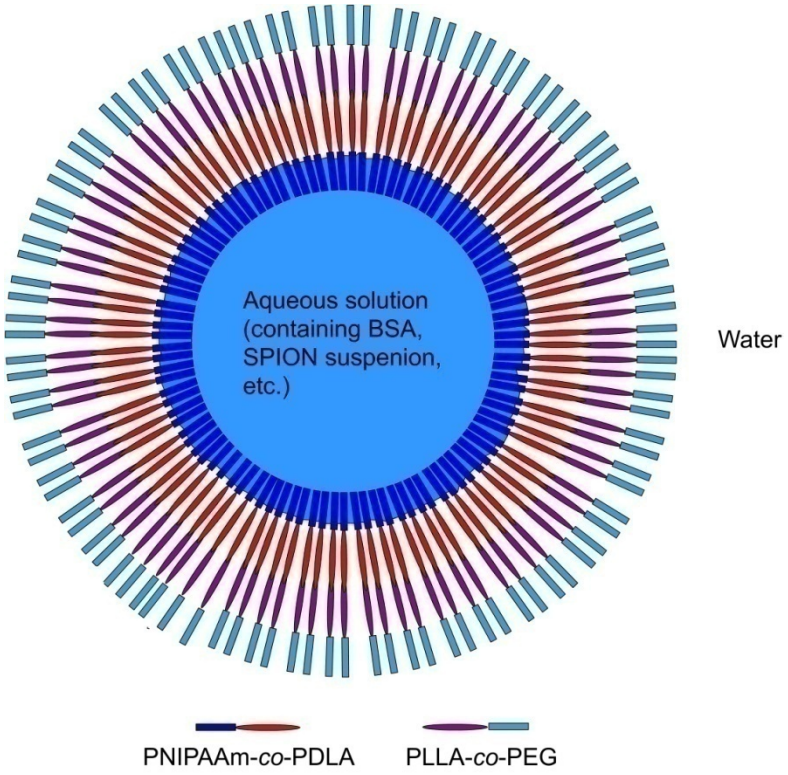
(a) transparency change of the PNIPAAm-PDLA solution
(b) differentiated curve of (a)
(c) w/o/w' emulsion

Thermogram of Polymeric Structures



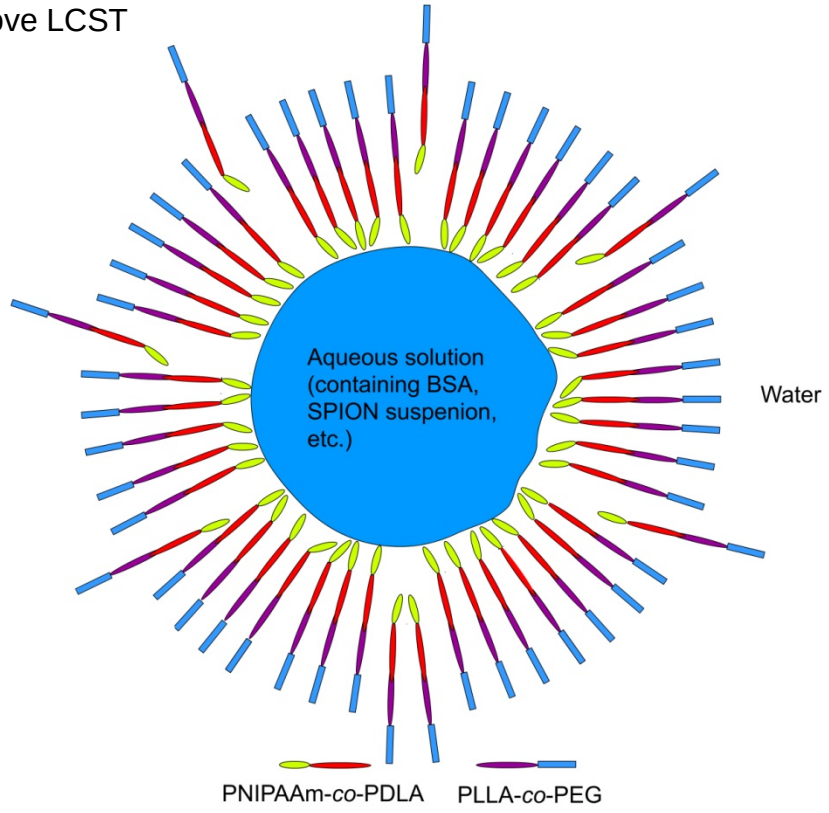
Schematic View of the “Shell-in-Shell” Structure

Below LCST



PNIPAAm: hydrophilic, stable

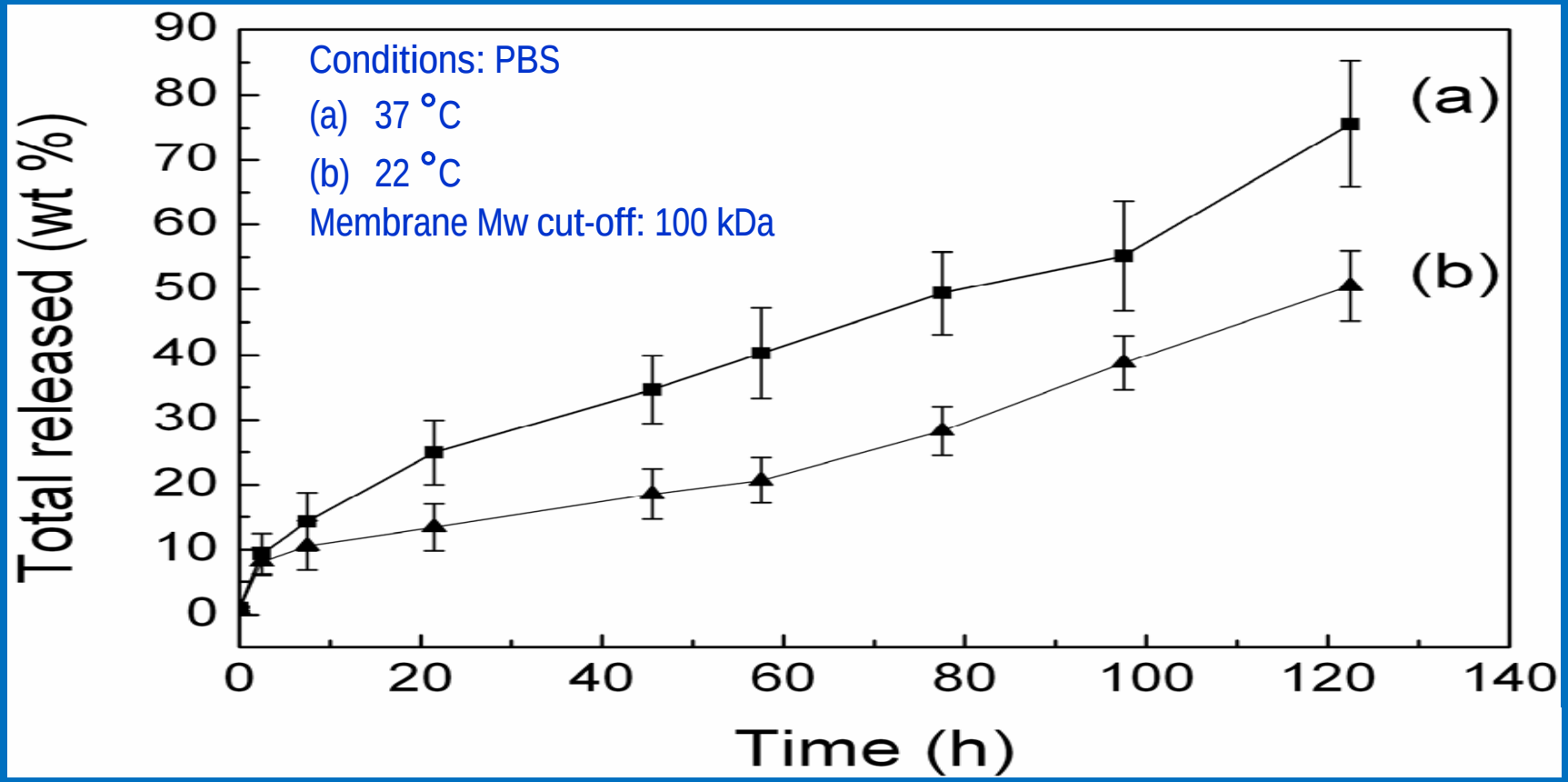
Above LCST



PNIPAAm: hydrophobic, unstable → Drug release

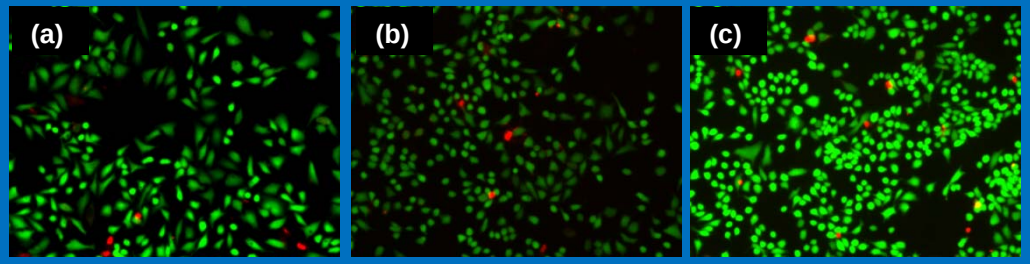
LCST: Lower critical solubility temperature

Temperature-dependent Bovine Serum Albumin (BSA) Release



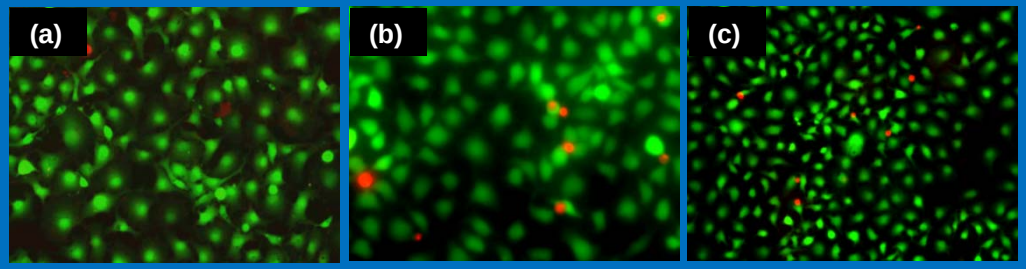
Suk, J et al (adv. Funct mat 2005)

Toxicity Assay
Au@PLLA-PEG@PNIPAAm-PDLA and PLLA-PEG@PNIPAAm-PDLA



1000 μg/mL Au | 1000 μg/mL uncoated | control

TCCV assay



1000 μg/mL Au | 1000 μg/mL uncoated | control

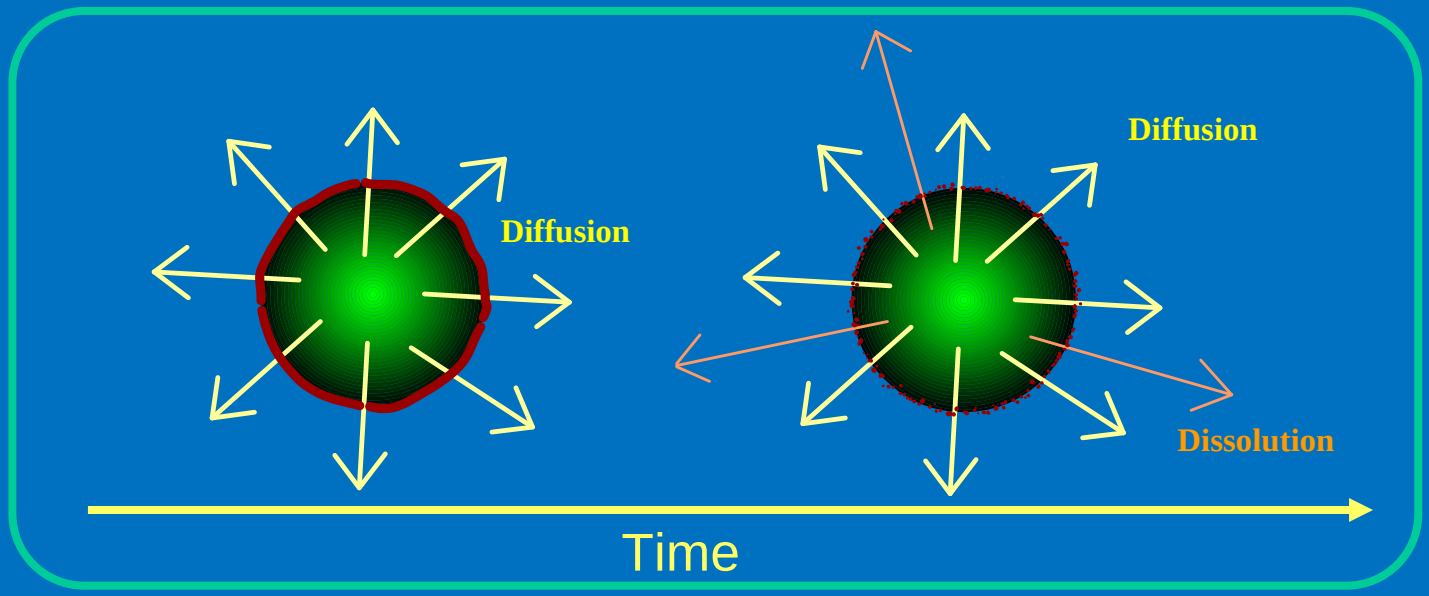
Green spots = living cells
Red spots = dead cells

MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium

TCCV: Two-color cell fluorenscence viability

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r}\right)$$

Mathematical modeling of controlled release rate



- Diffusion model

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r}\right)$$

$$\frac{c_1}{c_{1\infty}} = 1 - \sum_{n-1}^{\infty} \frac{6(\alpha+1)\alpha}{(9+3\alpha+q_n^2\alpha^2)} e^{-\frac{q_n^2}{R^2}Dt}$$

- Dissolution model

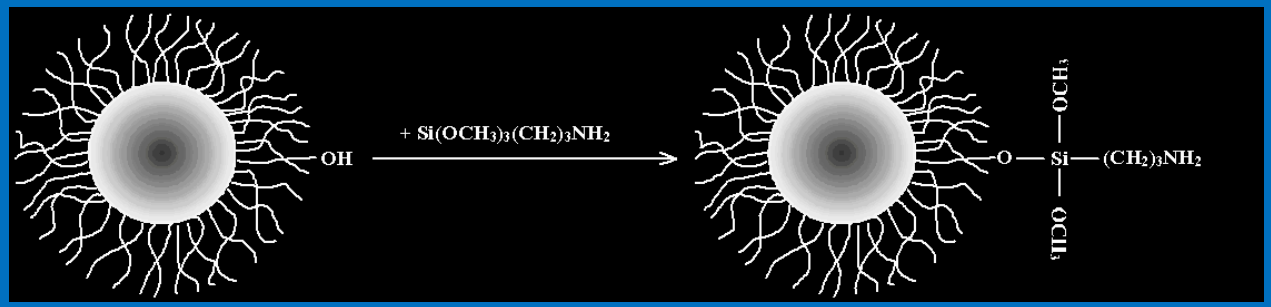
$$r_d = -\frac{dc}{dt} = k(c - K_p c_1)$$

$$c_1 = \frac{c_0}{K_p(\alpha+1)}\left[1 - \exp\left(-\frac{\alpha+1}{\alpha}kt\right)\right]$$

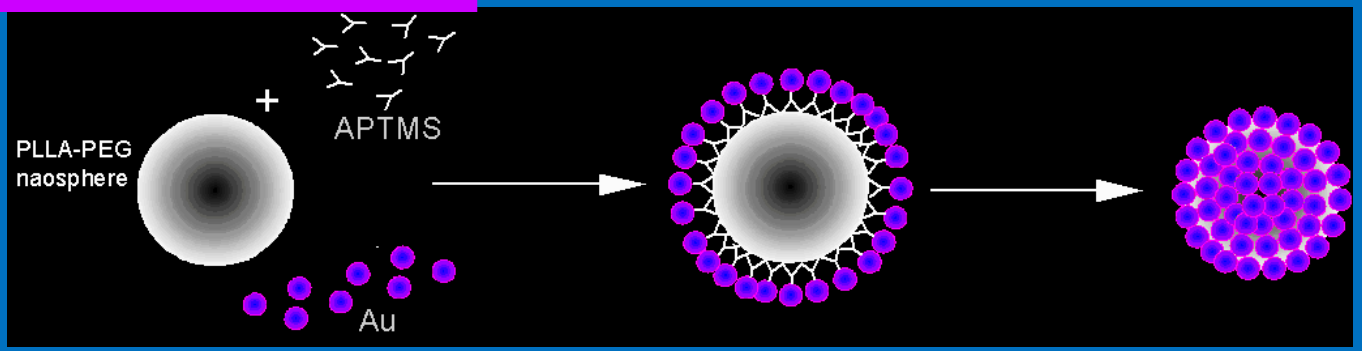
Jo. Y. S,et al, *Nanotechnology* **2004**, 15 (9),1186-1194.

Self-assembly of gold nanoparticles on the surface of PLA-mPEG nanospheres

Silanization

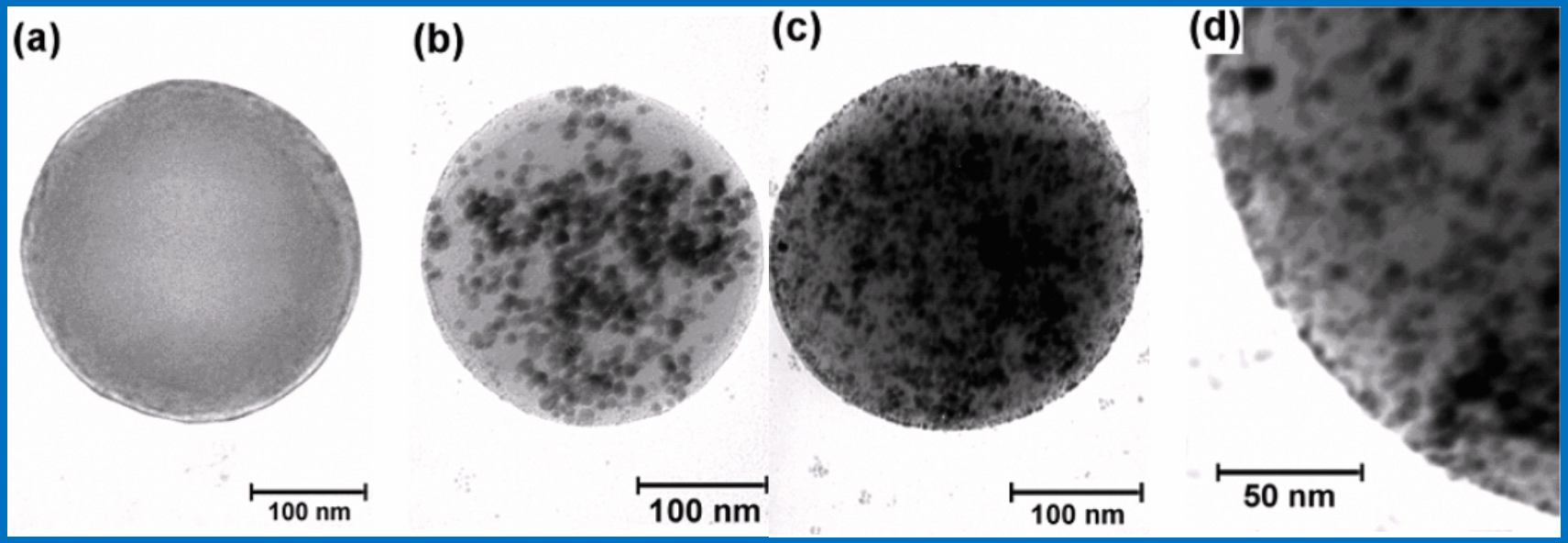


Gold nanoparticle self-assembly



Y. S. Jo, M. Muhammed, et al., *J. Materials Science: Materials in Medicine*, **2005**

TEM images of ‘shell-in-shell’ structure nanoparticles

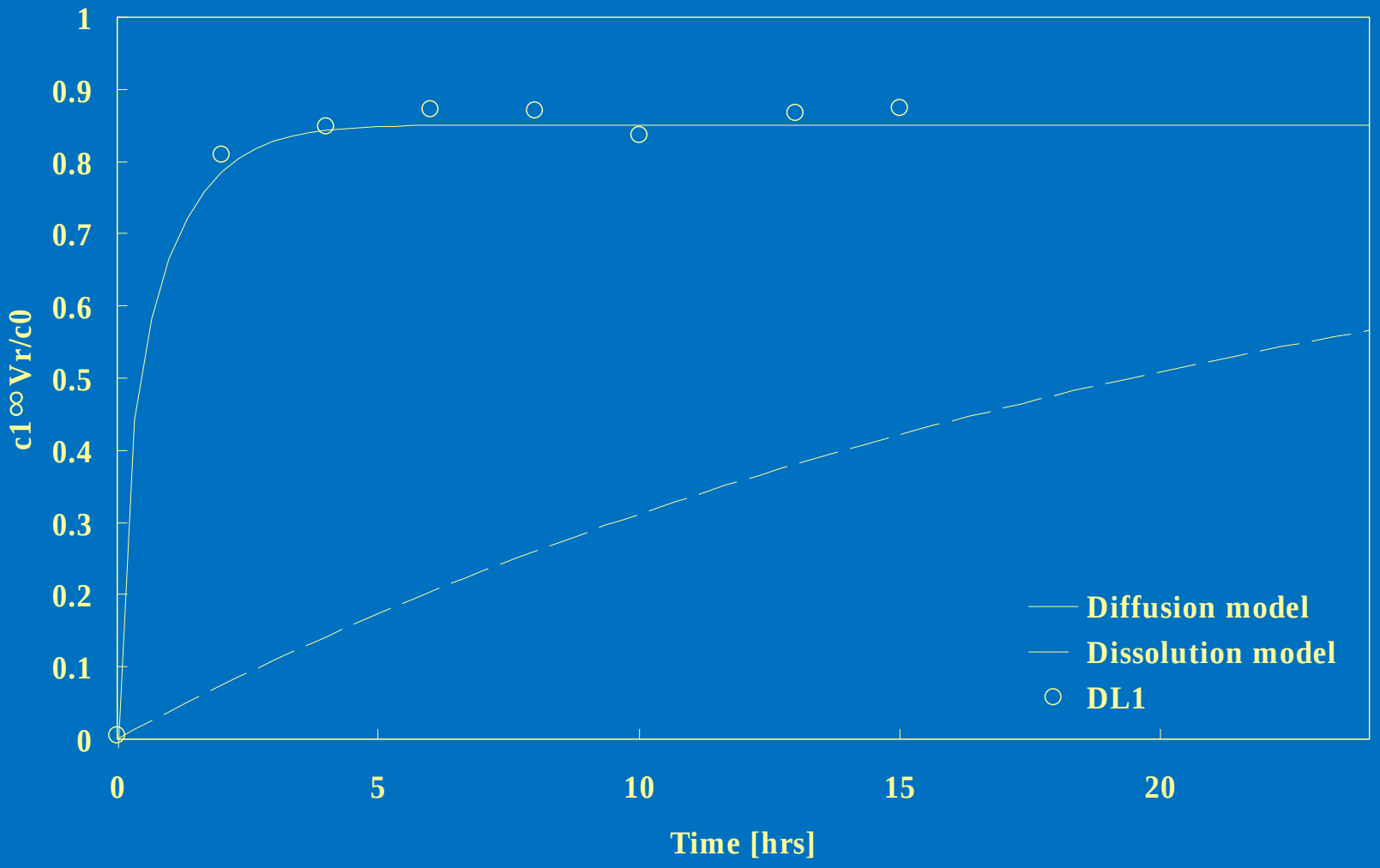


a) PLLA-PEG micelle
b) ‘shell-in-shell’ structures covered with Au nanoparticles in part
c) - d) ‘shell-in-shell’ structures fully covered with Au nanoparticles

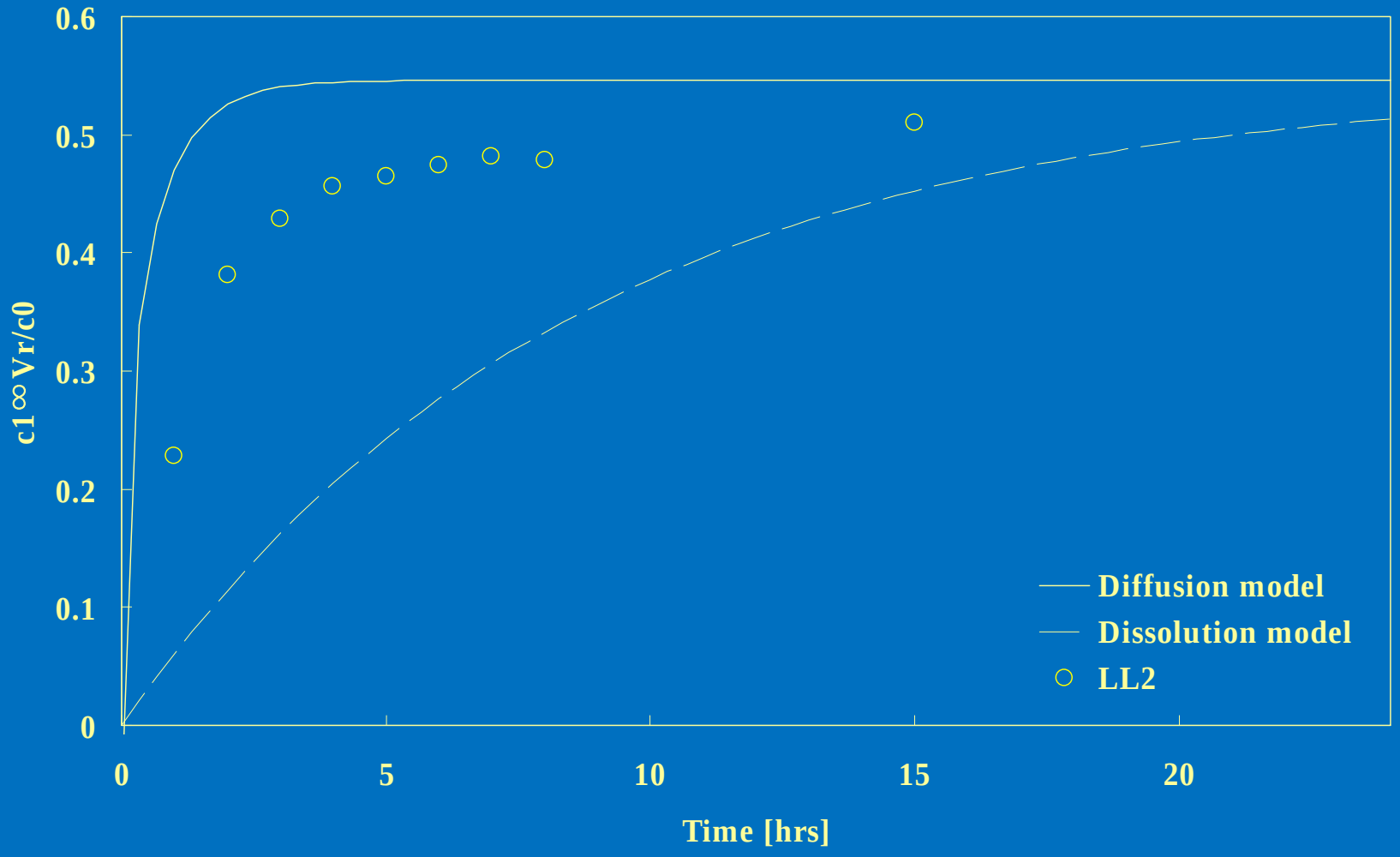
Y. S. Jo, *J Mat Sci.; Mat in Medicine* (2004)

Drug Release Profile

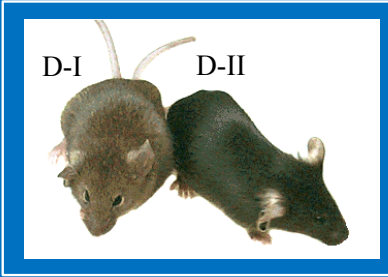
Dissolution regime



Drug Release Profile
Dissolution- Diffusion Regimes

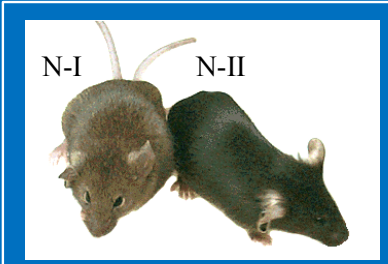


in vivo test: estrogen release by PLA-PEO DDS



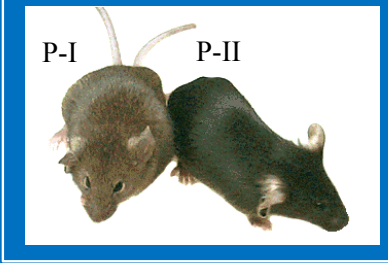
Group D

PLA-PEO DDS (loading 17¥âestradiol) injected
Dosage;
(39.6 ¥g / 39.6 ¥g / 550 ¥L = 17¥âestradiol / PLA-PEO DDS / PBS buffer solution)



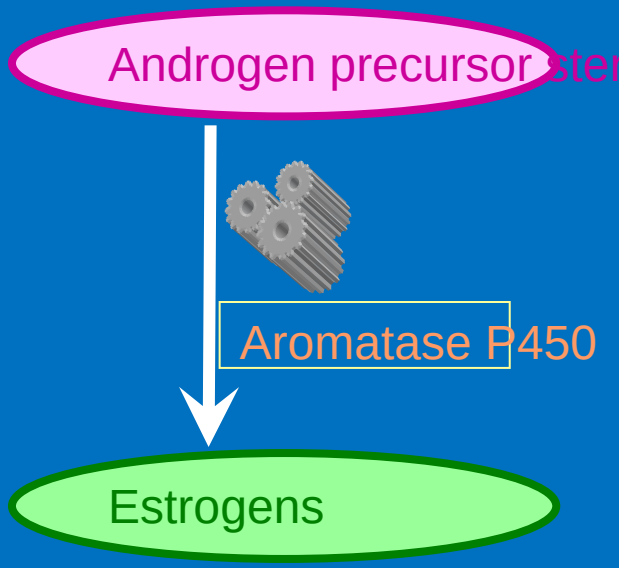
Group N

550 ¥L of PBS buffer solution injected



Group P

P-I; 550 ¥L of positive control solution (17¥âestradiol dissolved in olive oil) injected
P-II; 17¥âestradiol tablet implanted



Aromatase P450 (P450arom) Knocked-Out (ArKO) mice used in this work

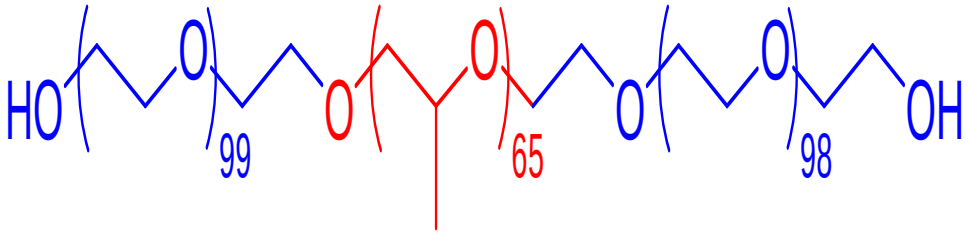
Injectable Drug Delivery Systems

Hydrogels - Ferrogels

Injectable Drug Delivery Systems

- Biocompatible
- Liquid at Room temperature
- Solid at Body temperature
- Can be loaded with drugs
- Controlled drug release

Pluronic F127

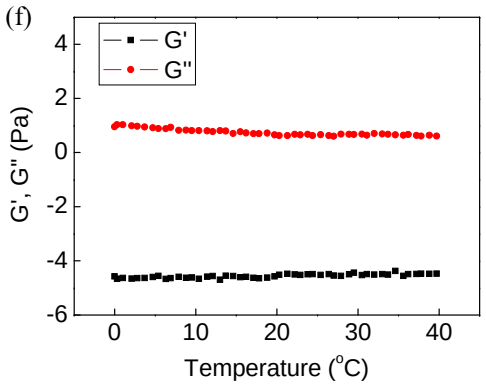


Pluronic F127 copolymer undergoes reversible sol-gel transition at different T as a function of concentration

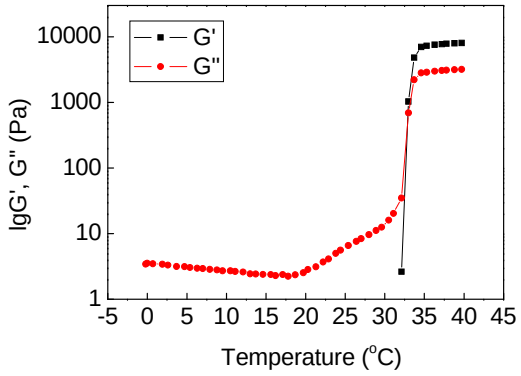
Effect of Concentration on Gelation Temperature

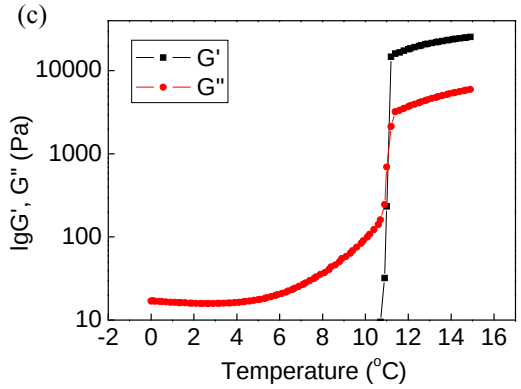
8.75%

(e)

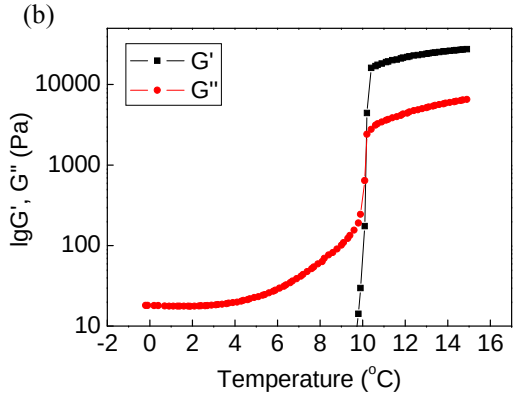


17.5%

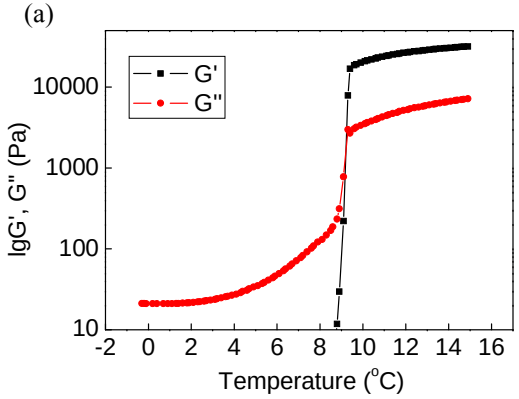




33%

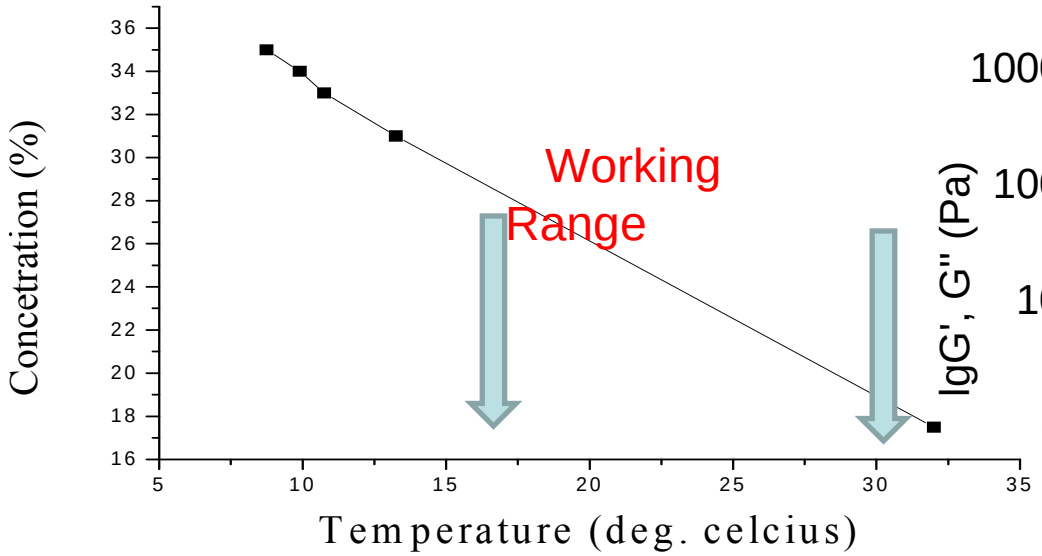


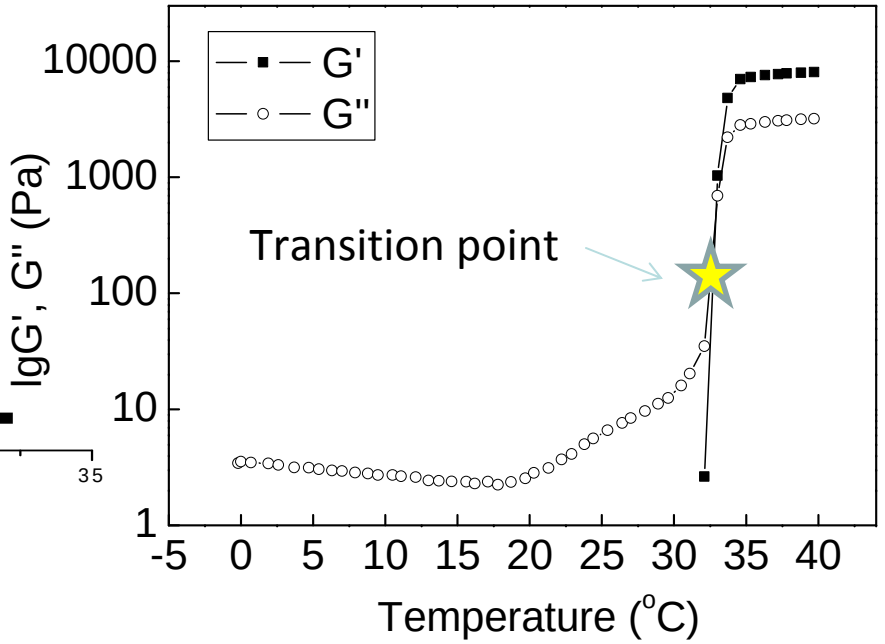
34%



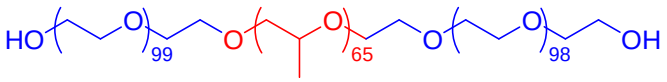
35%

Injectable Drug Delivery Systems
Magnetic-Sensitive Ferrogel

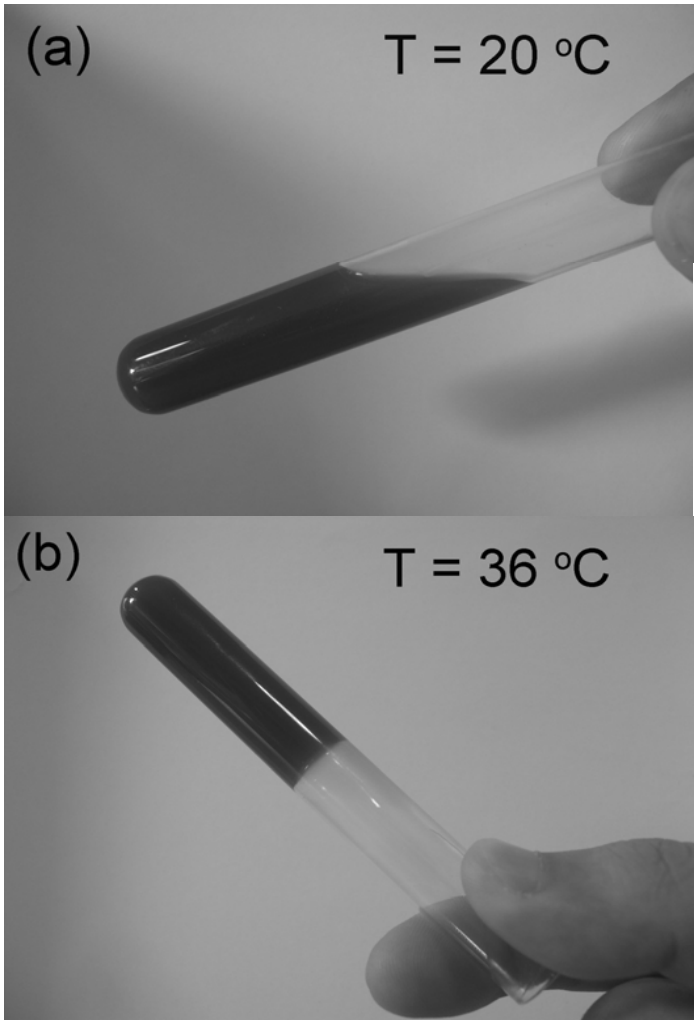


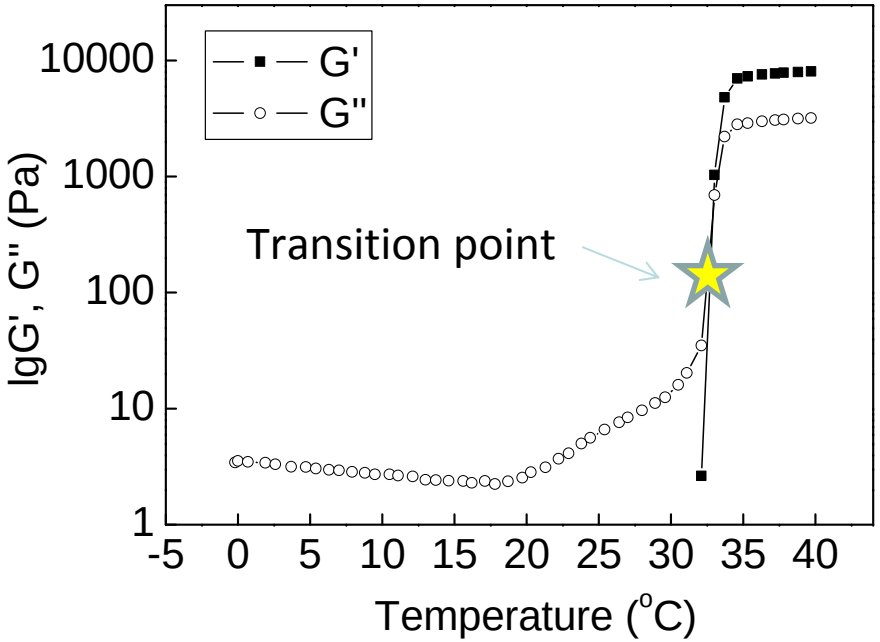


Pluronic F127



Reversible sol-gel transition

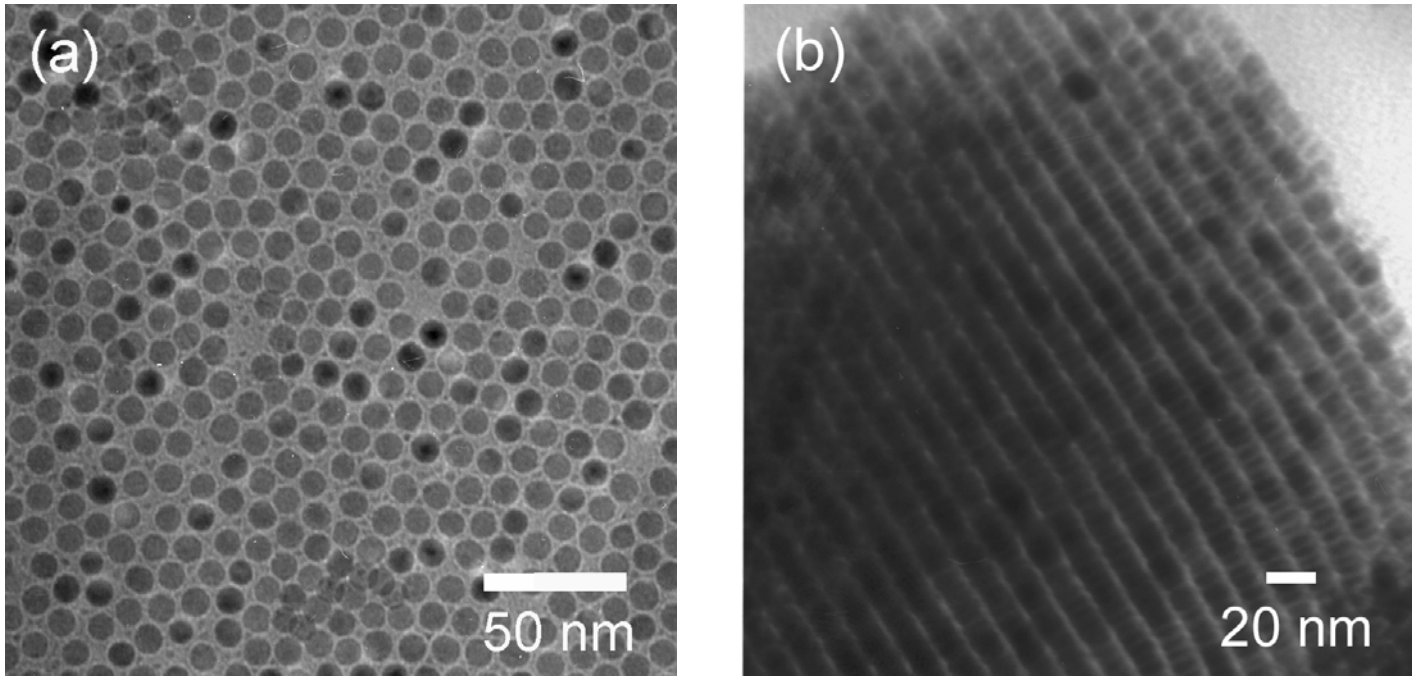




17.5%

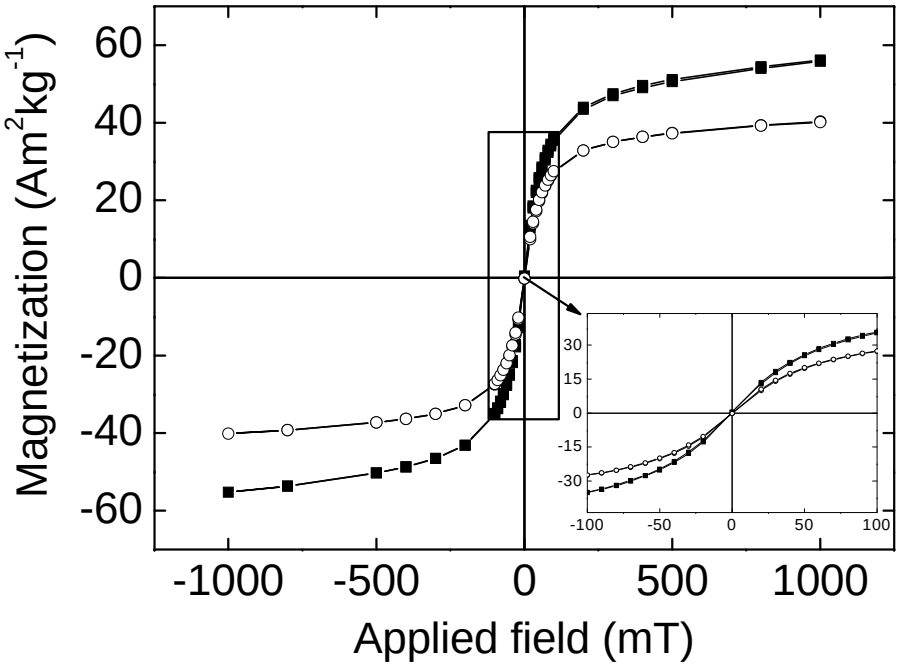
Qin. J., et al Adv. Func . Mat. 2008

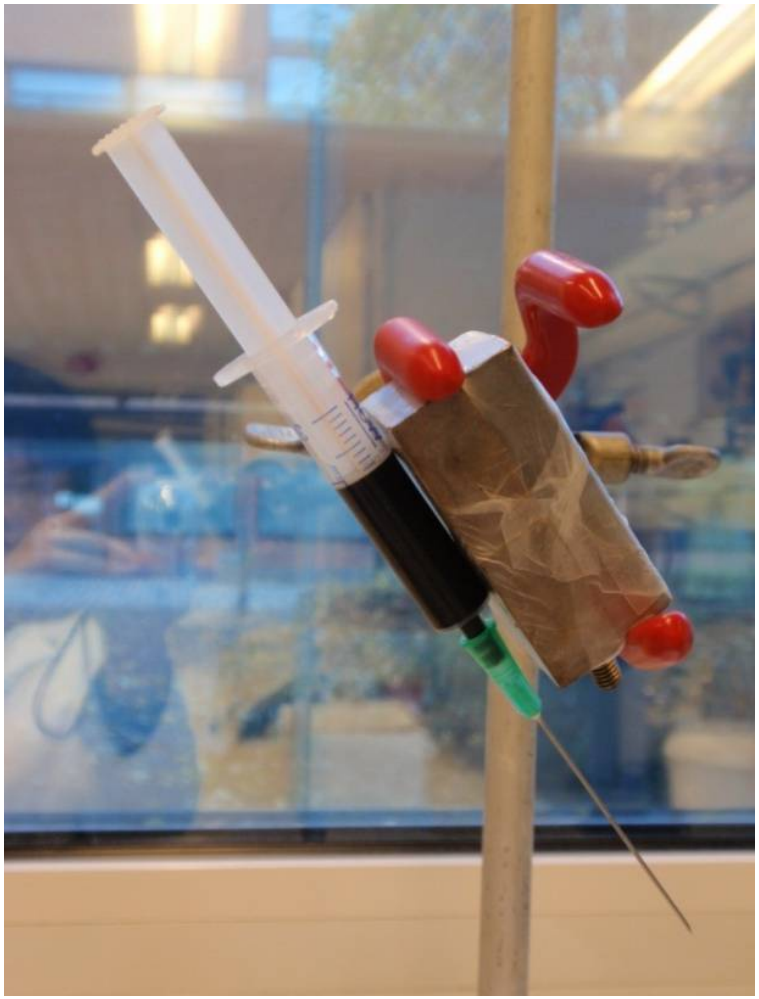
Magnetic-Sensitive Ferrogel



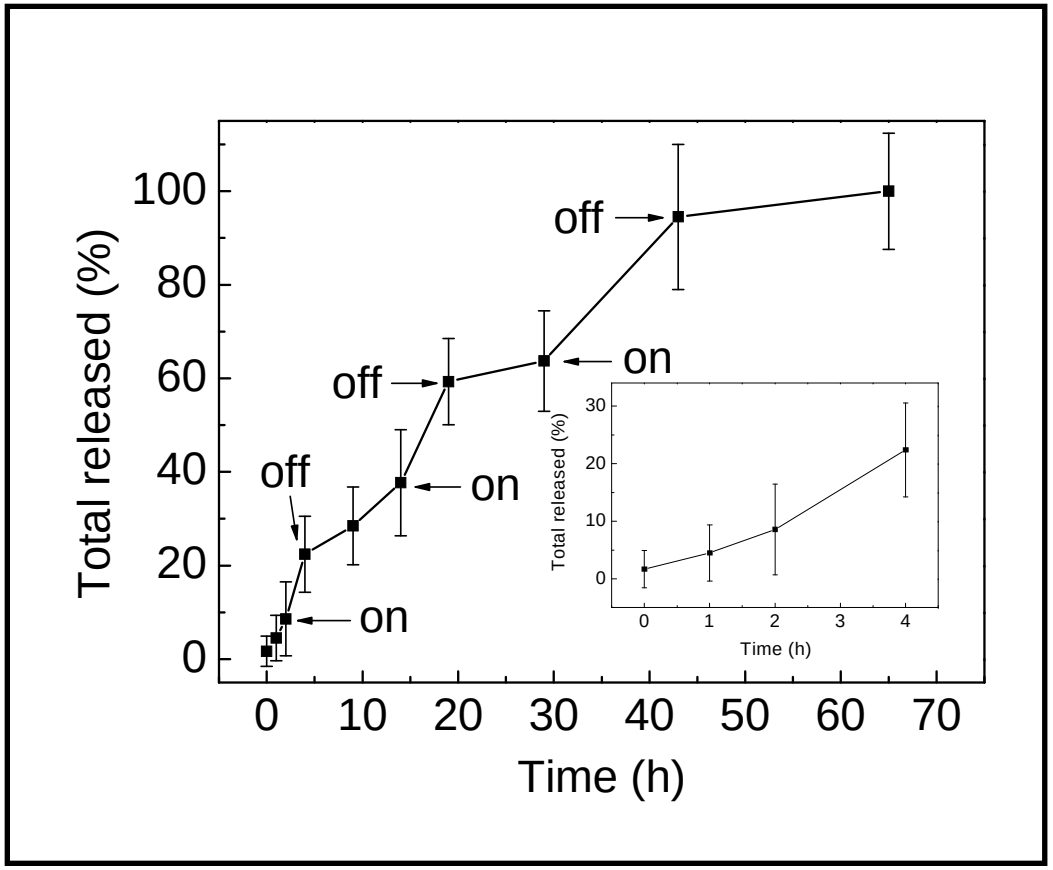
SPION in organic solvent and
(b) embedded in the ferrogel

Sensitive to the External Magnetic Field





Magnetic Sensetive Release rate



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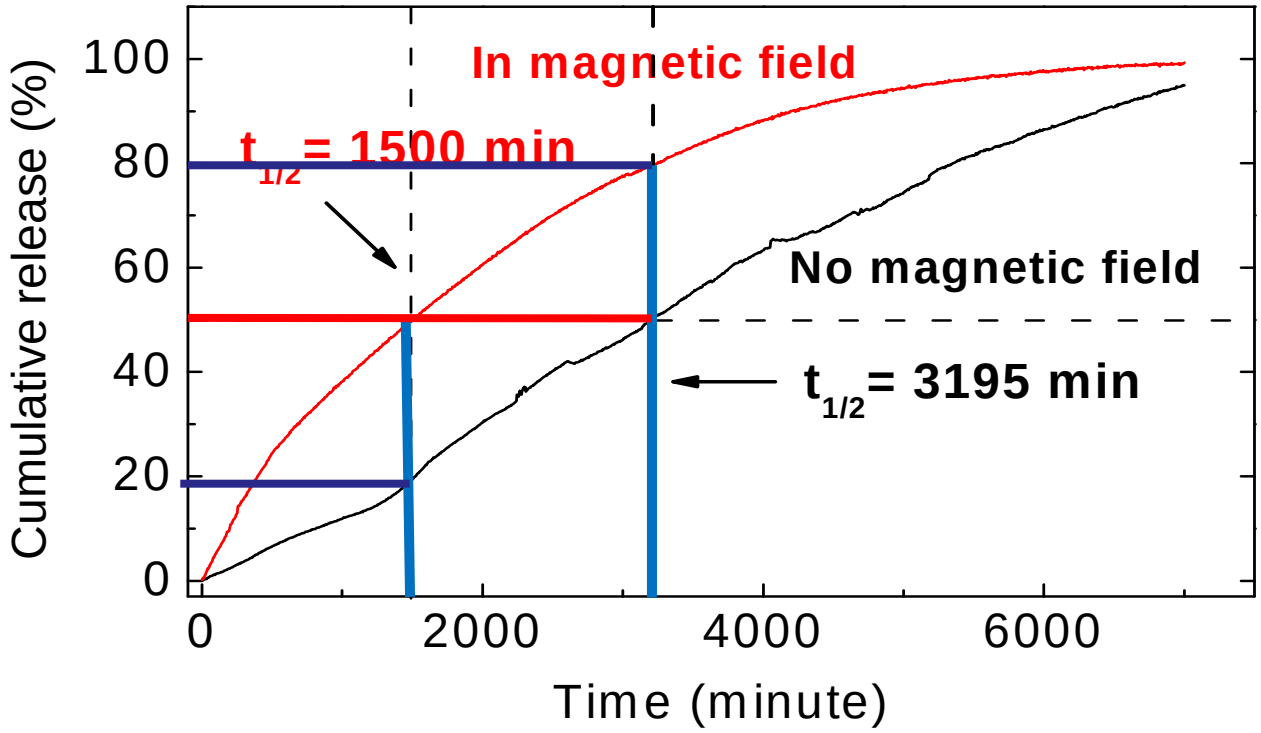
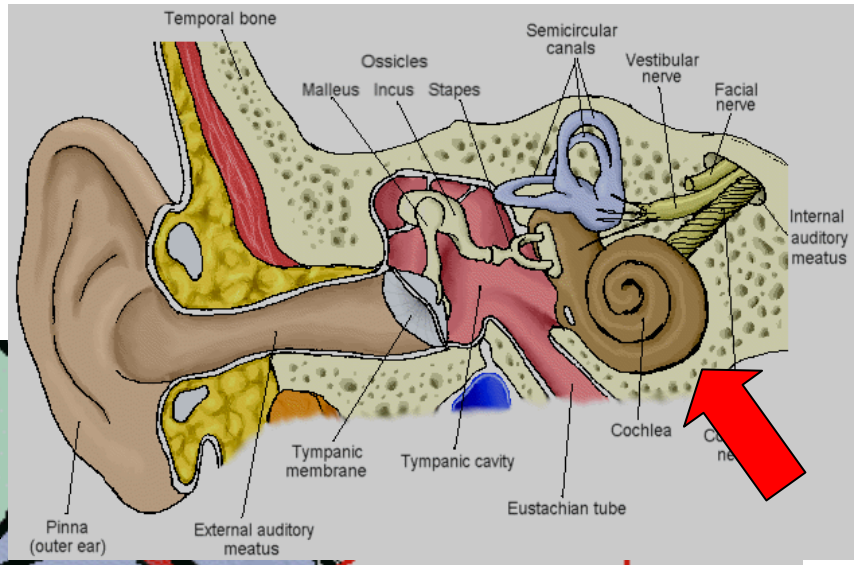
Magnetic Enhanced Drug delivery

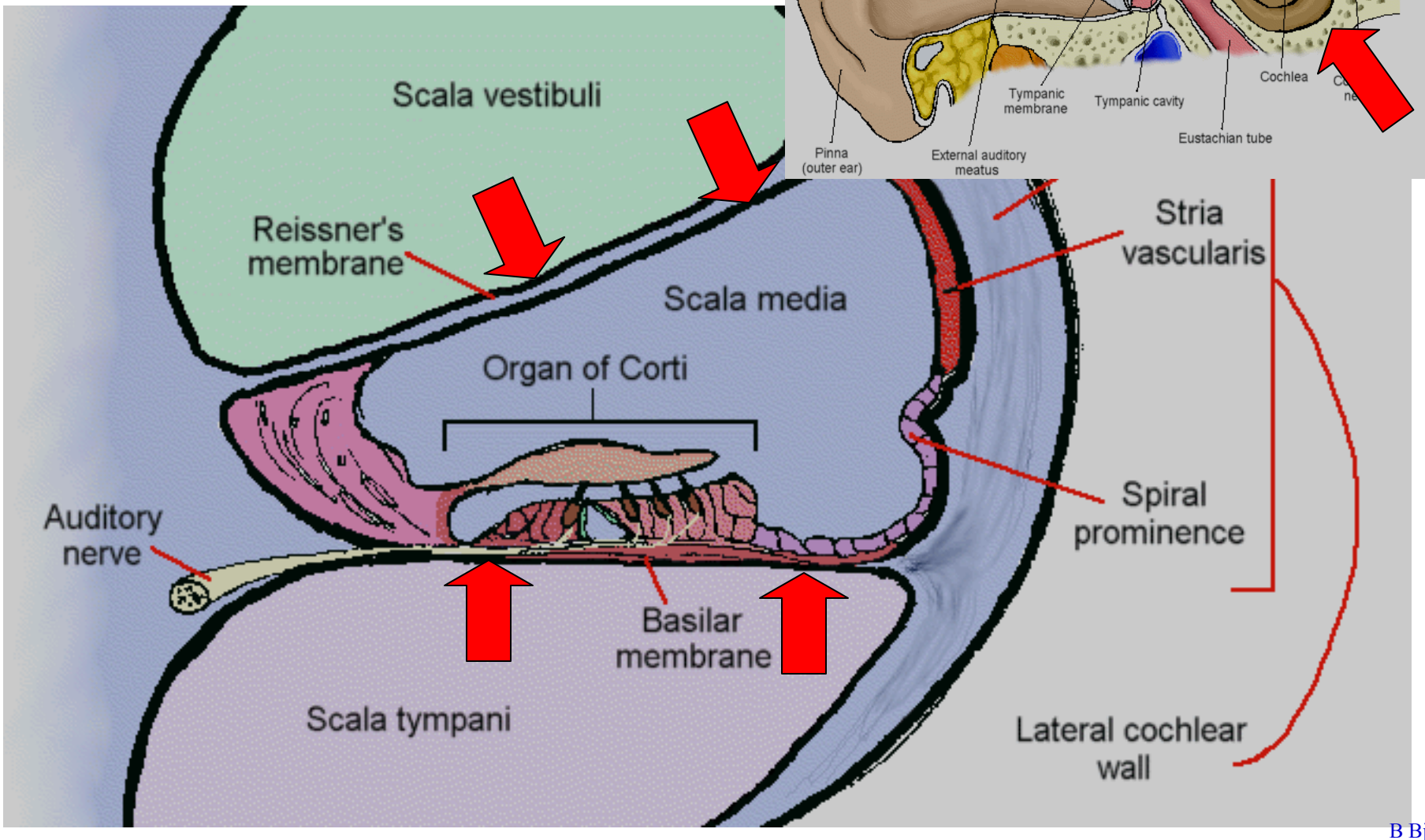
Drug release profiles



Qin. J., et al Adv. Func . Mat. 2008

Hearing Research
NanO ear





Summary

- Nanomaterials and Multifunctional Nanoparticles have tremendous potentials for developing smart and novel medical treatments
- MFNP
  - ✓can carry different Payloads; pharmca, genes, DNA, etc
  - ✓can be designed to be environmentally resposnive
    - Magnetic ,
    - Temperature ,
    - Concentration of biomolecules (sugar, pH,..)
  - ✓ Targeting devices can be attached for delivery at specific sites
- Injectable hydrogels offers new ways for intoducing DDS without surgical operation

Division of Functional Materials (FNM)

School of Information and Communication Technology (ICT)

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