5th Korea-USA Nanoforum on NanoBiotechnolgy

Synthesis and Biomedical Applications of Uniform-sized Nanoparticles

Taeghwan Hyeon,* Jaeyun Kim, Hyon Bin Na, Kwangjin An,
ekyung Yu, Soon Gu Kwon, Ji Eun Lee, Yong Il Park, & Nohyun Le

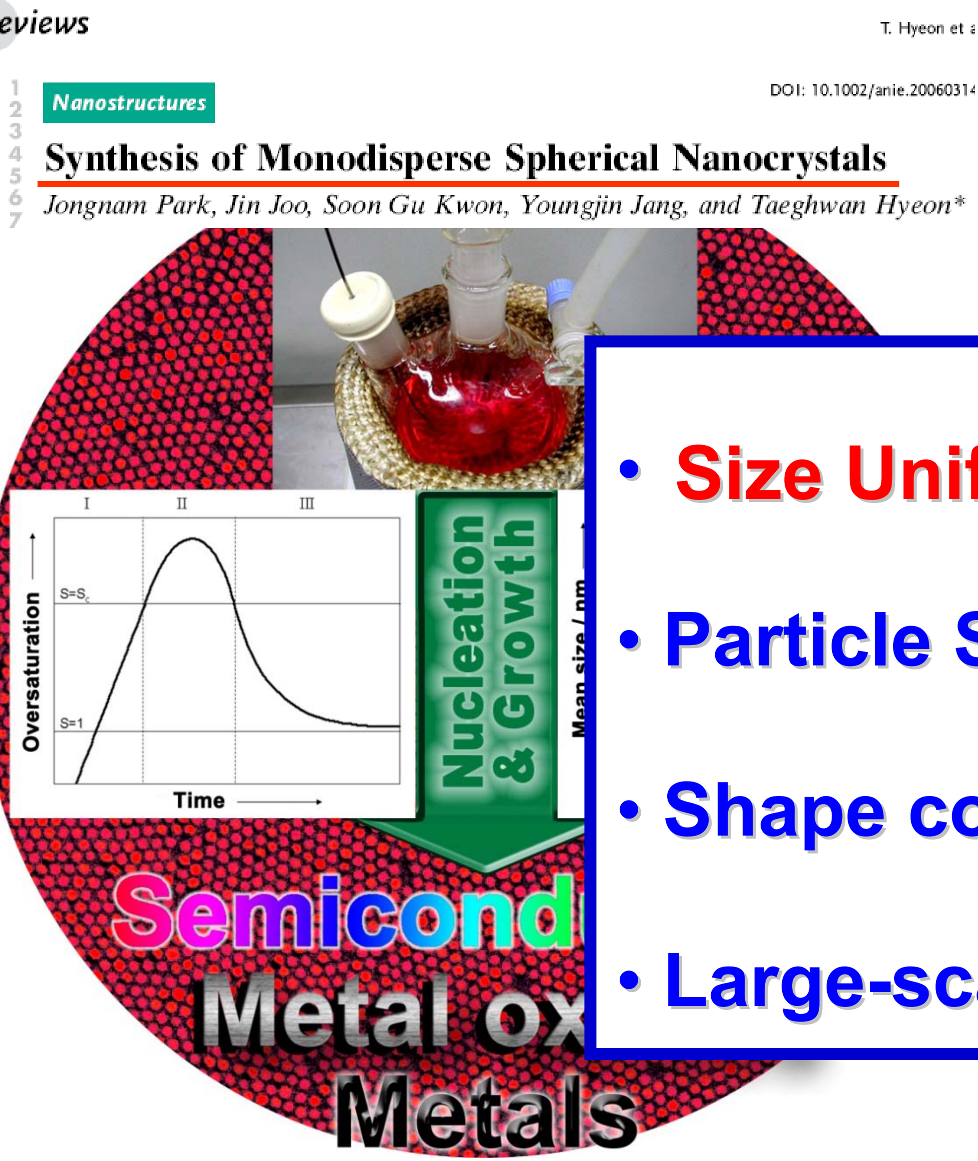
National Creative Research Initiative Center
for Oxide Nanocrystalline Materials
and School of Chemical and Biological Engineering,
Seoul National University, Seoul 151-744, Korea

April 17, 2008 Jeju Shila Hotel, Korea

Part I:

Synthesis and Formation Kinetics of Uniform-sized Iron Oxide Nanocrystals

Key Issues in Nanoparticle Synthesis



- **Size Uniformity**
- **Particle Size Control**
- **Shape control**
- **Large-scale Synthesis**

J. Park, et al. *Angew. Chem. Int. Ed. (Invited Review)* 2007, 46, 4630.

T. Hyeon, *Chem. Comm.* (*Feature Article*), 2003, 927, " Chemical Synthesis of Magnetic Nanoparticles."

Why is Size Uniformity of Nanoparticles Important

Physical properties of nanoparticles are directly dependent on the particle size.

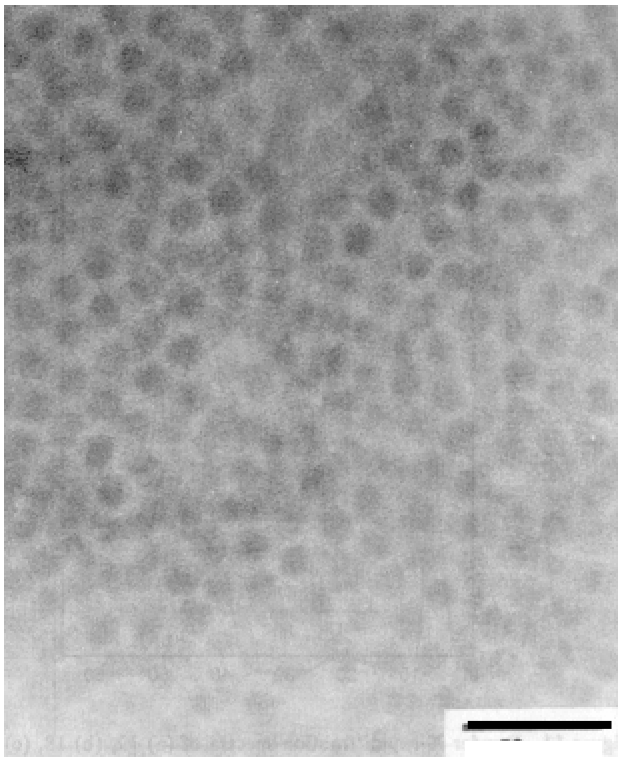
- For Terabit/in^2 magnetic storage media, well-aligned Monodisperse magnetic nanoparticles needed.
- Color sharpness of semiconductor nanoparticle based LED, Lasers, Phosphors depends on the Size Uniformity.
- Nanoparticles for Bio-Medical Applications,

  Uniform size is important for passage of cell membrane

  Important for FDA approval process

ynthesis of Monodisperse Semiconductor Nanosphere
through Burst of Homogeneous Nucleation & Subsequent Aging

5.1 nm CdSe **MIT**

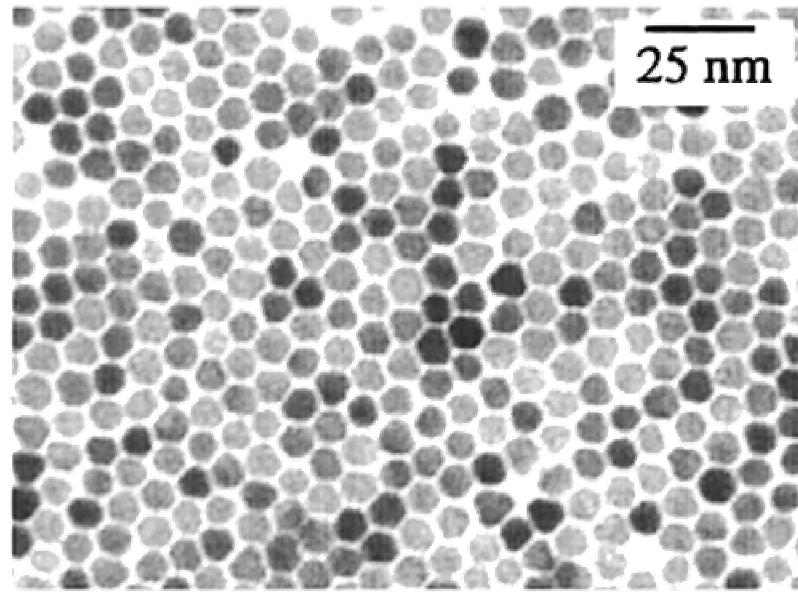


20 nm

urray, Norris, Bawendi, *J. Am. Chem. Soc.* **1993**, 115, 8706

8.5 nm CdSe

UC-Berkeley

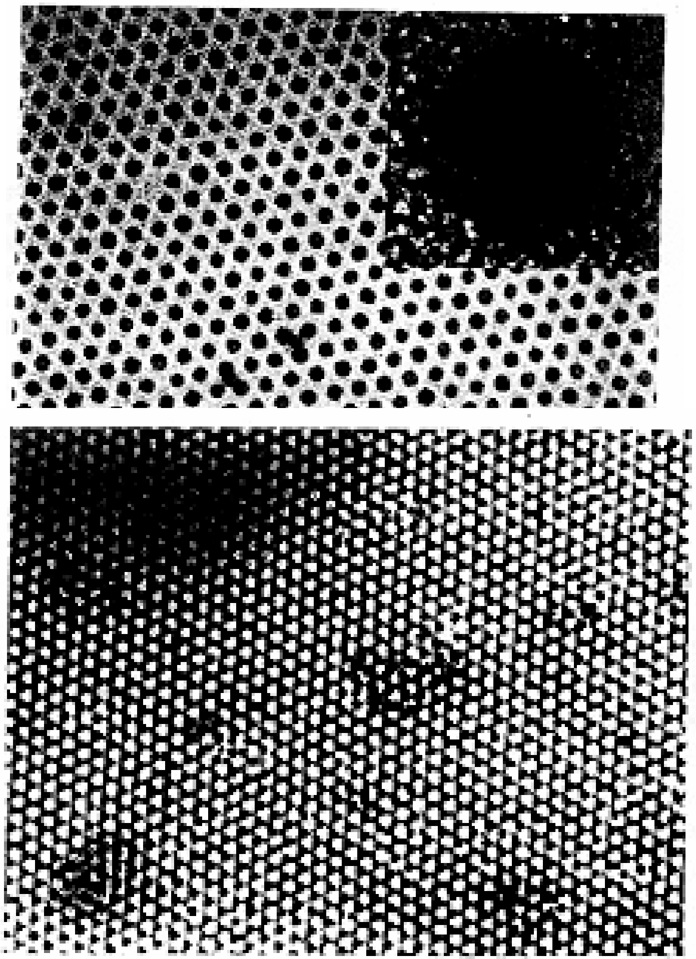


Peng, Alivisatos, *J. Am. Chem. Soc.* **1998**, 120, 5343

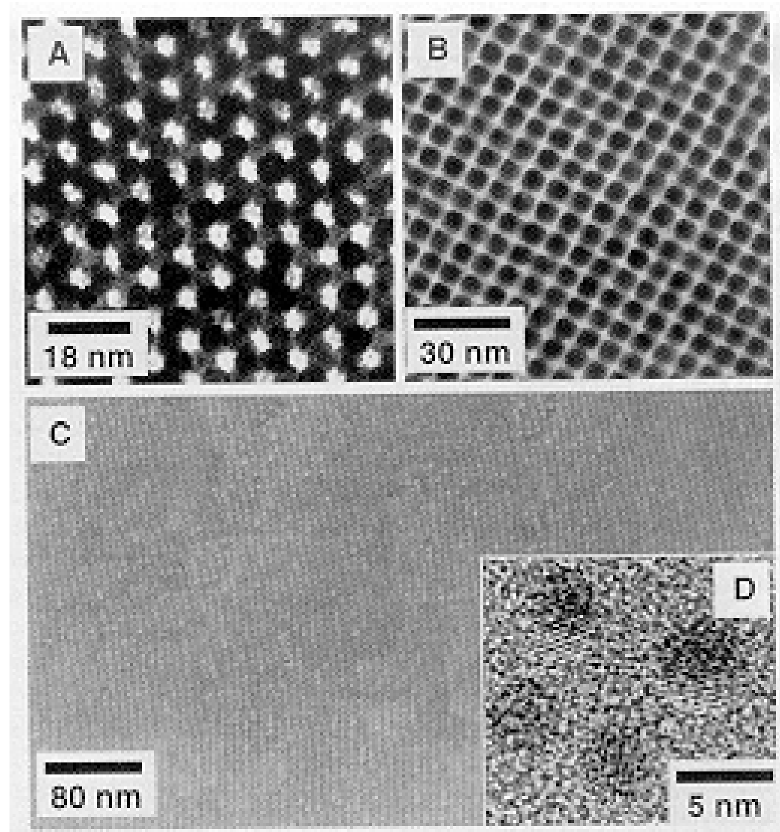
Synthesis of Magnetic Metal Nanoparticles

IBM Watson Research Center

Cobalt

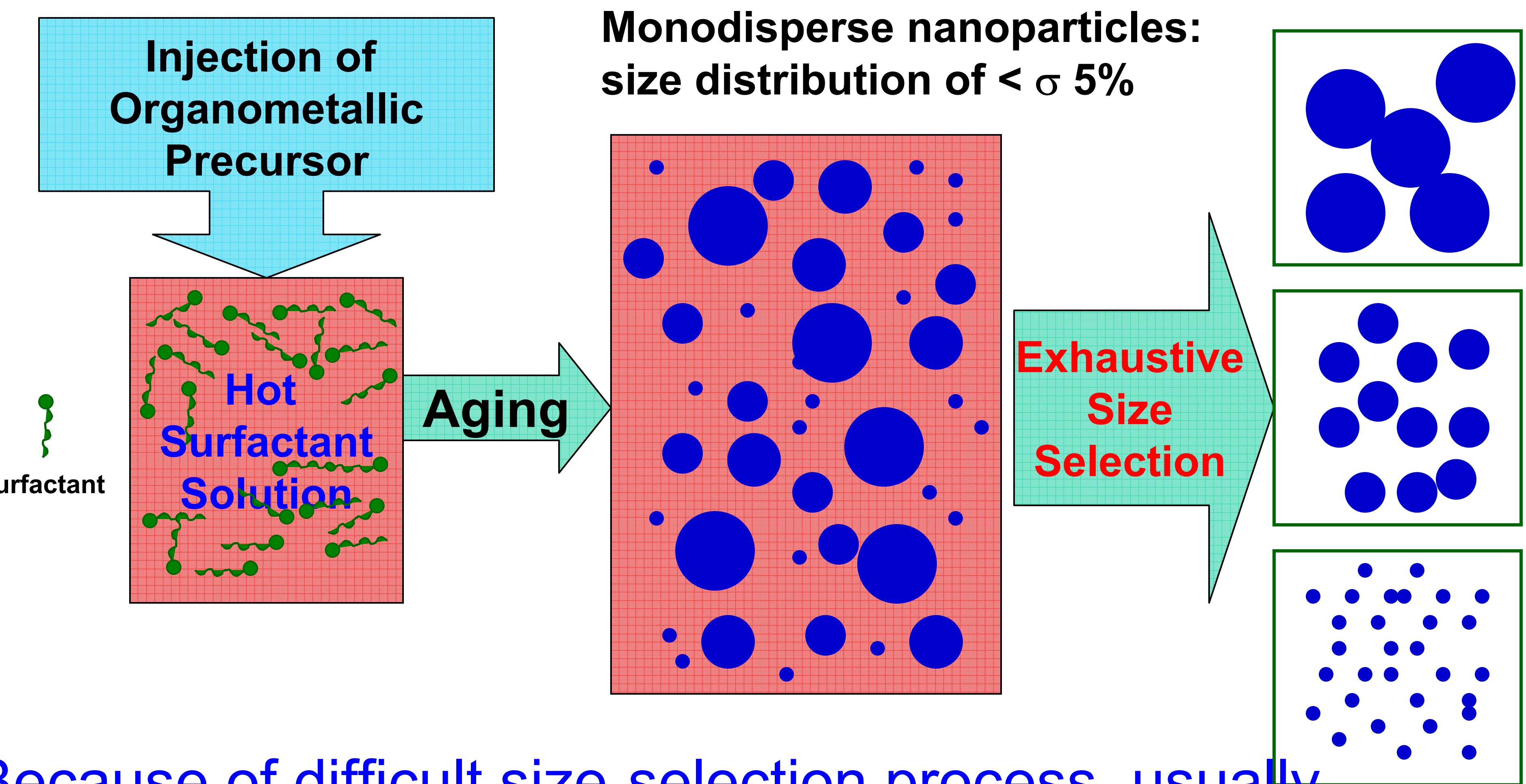


Fe-Pt alloys



Sun and Murray, *J. Appl. Phys*. **1999**, 85, 4325; *Science* **2000**, 287, 1989.

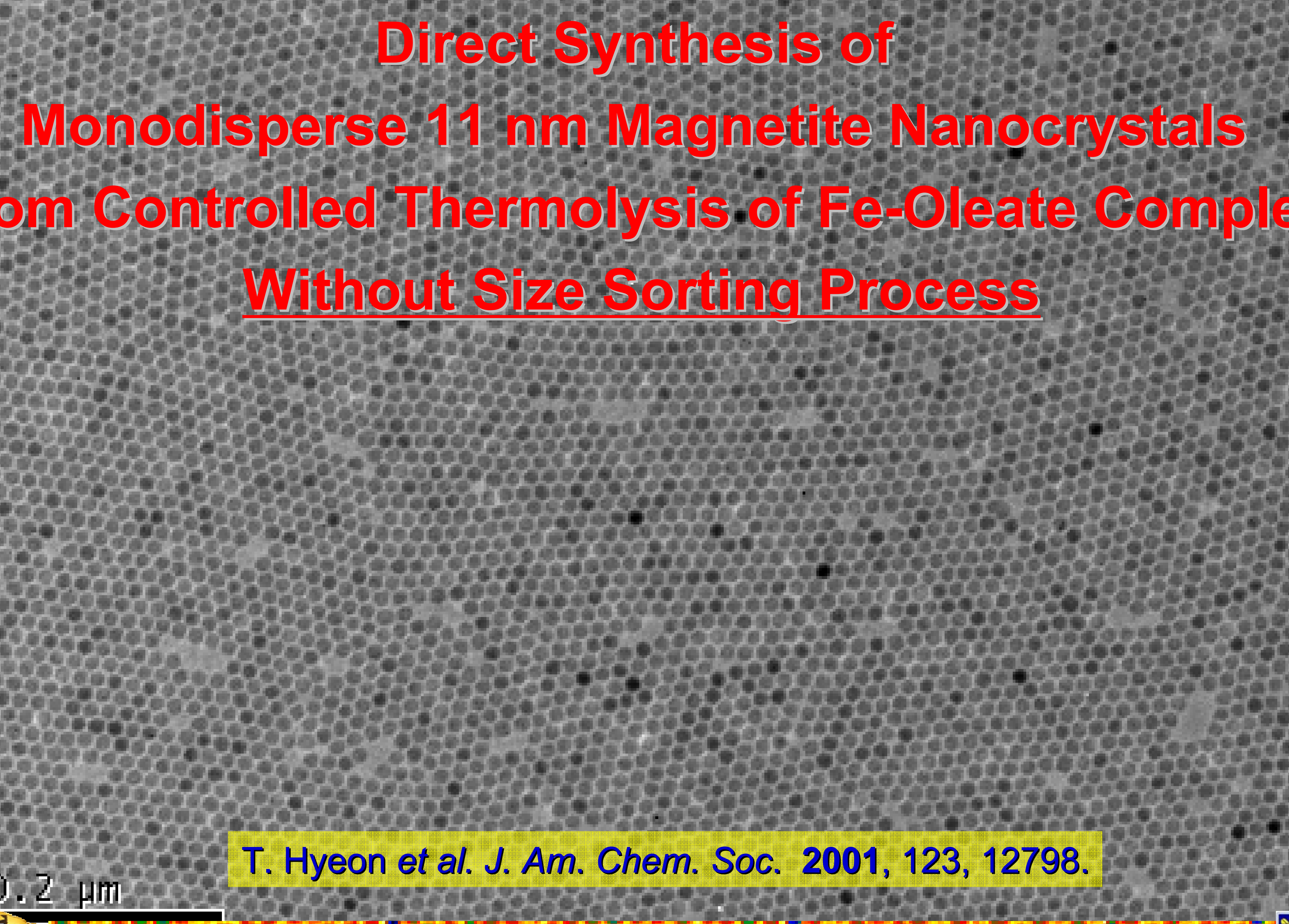
Conventional Synthesis of Monodisperse Nanocrystals
Burst of Nucleation by Hot Injection followed by Aging



Because of difficult size-selection process, usually < *100 mg* of monodisperse nanoparticles is produced.

. Hyeon, *Chem. Comm.* (*Feature Article*), **2003**, 927; J. Park, et al. *Angew. Chem. (Invited Review)* **2007**, 46, 4630.

Direct Synthesis of Monodisperse 11 nm Magnetite Nanocrystals om Controlled Thermolysis of Fe-Oleate Comple <u>Without Size Sorting Process</u>

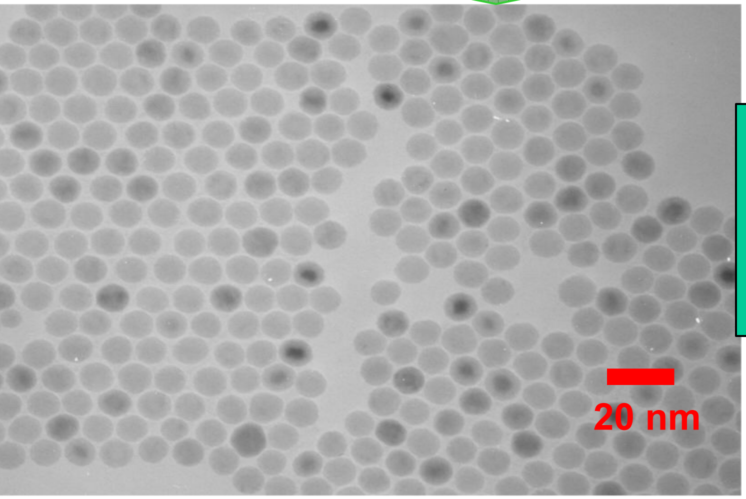


T. Hyeon *et al. J. Am. Chem. Soc.* **2001**, 123, 12798.

Synthesis of Monodisperse Nanocrystals from thermolysis of Metal-surfactant complexe

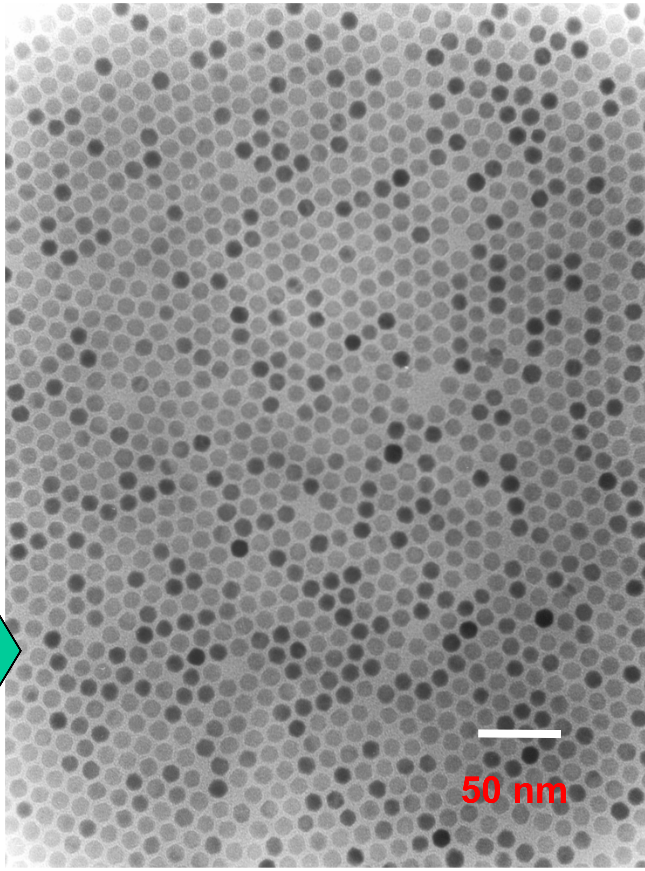
Formation of Fe-oleate complex from $Fe(CO)_5$ + Oleic acid

Aging of the complex By heating at high temp of > 300 °C



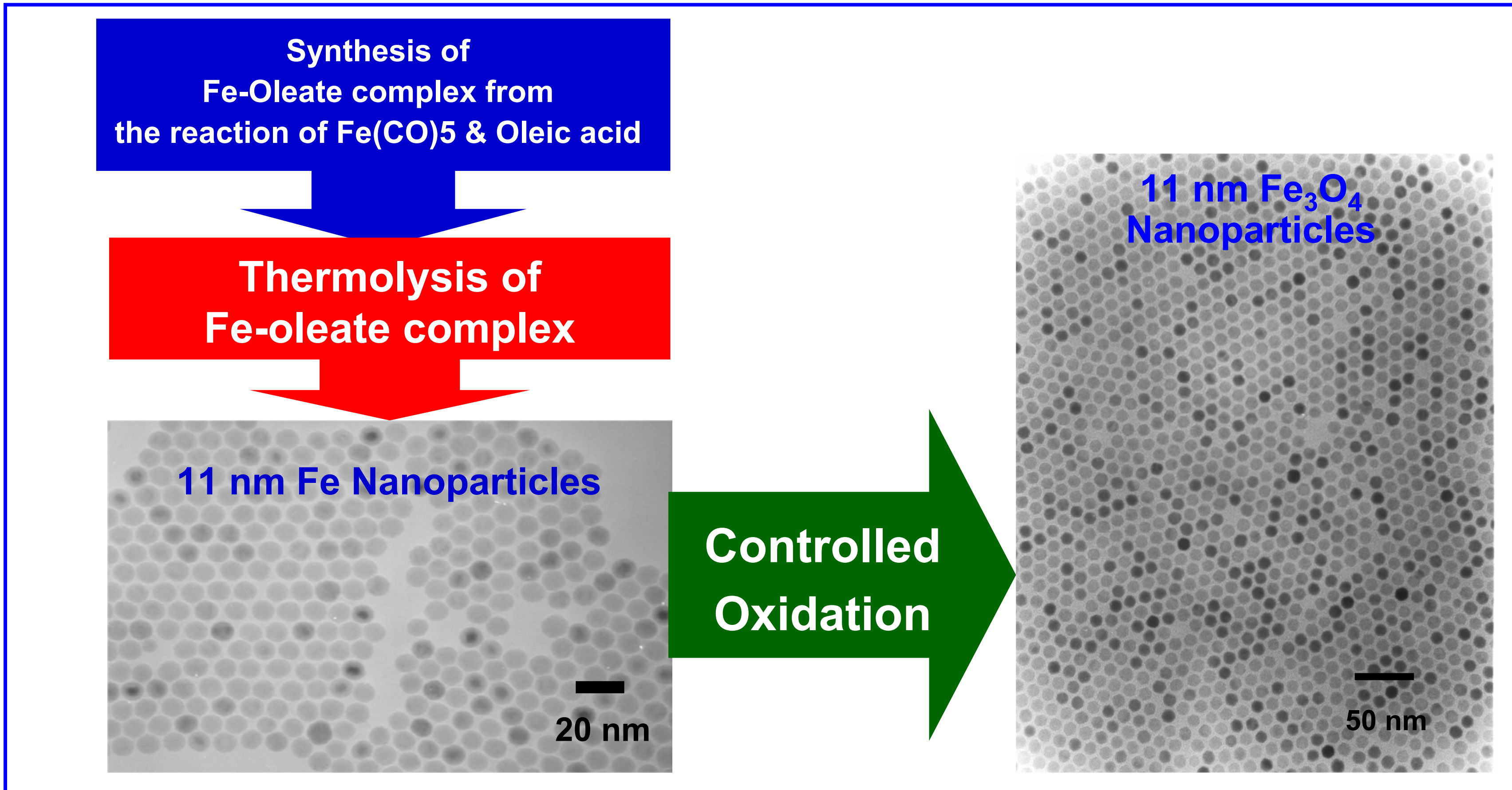
11 nm Fe nanoparticles

Controlled Mild chemical Oxidation



11 nm Iron oxide Nanocrystals

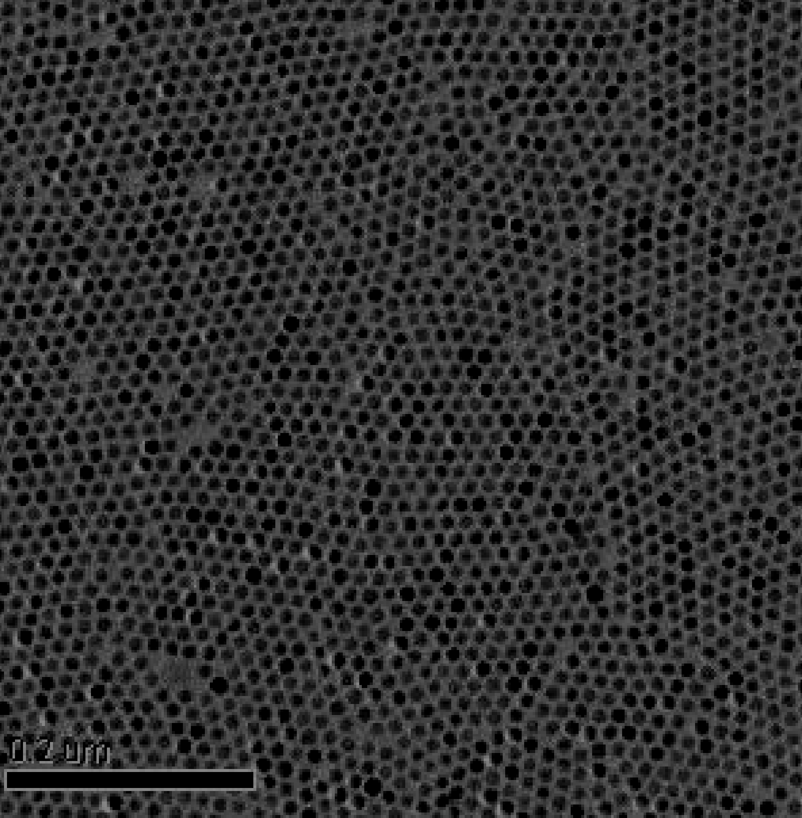
Although we were able to directly synthesize uniform-sized nanoparticles without a size sorting process, We still have to use expensive & toxic chemicals.



Main problem: expensive & toxic precursor $Fe(CO)_5$ (\$ 2000 USD/Kg)

Ultra-large-Scale Synthesis of Uniform-sized Nanoparticles

(*Nature Materials* **2004**, 3, 891.)



40 gram of Monodisperse Magnetite Nanocrystals was produced using $FeCl_36H_2$ without size Sorting Process

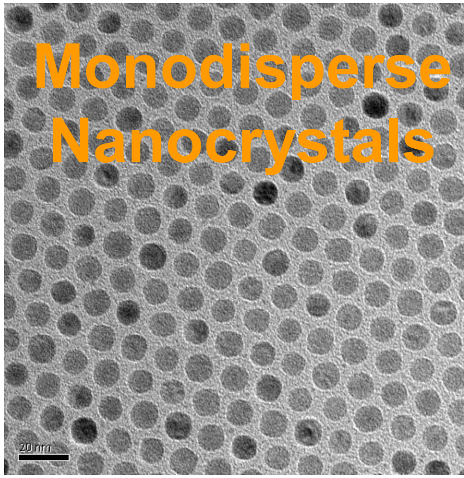
1) Large-scale Synthesis: 40 grams using 1 L reactor

2) Simple and Environmentally-Friendly process

3) Inexpensive using Hydrated Metal chlorides

Metal Chloride + Na-Oleate → **Metal-Oleate Complex** + NaCl

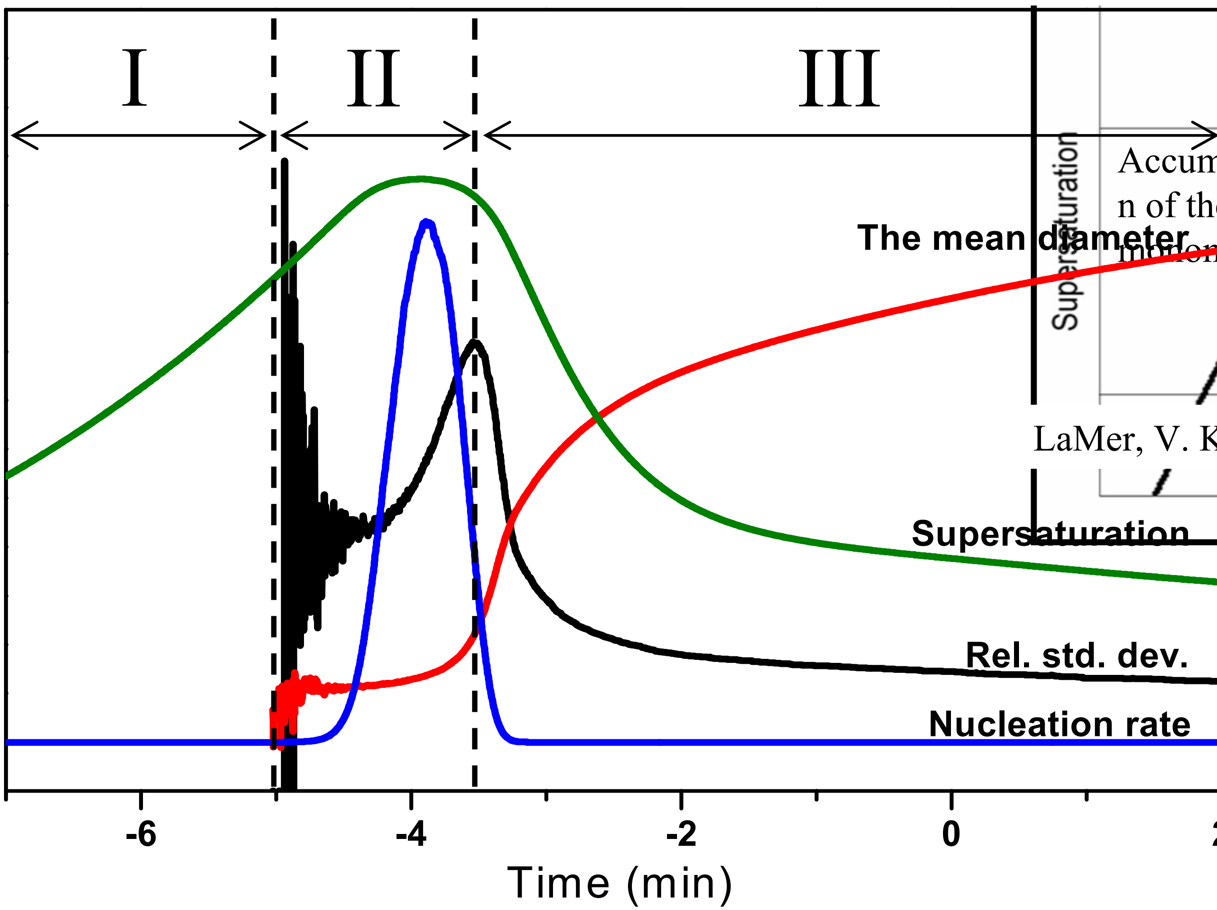
Metal-Oleate Complex $\xrightarrow[\text{in high boiling solvent}]{\text{Thermal decomposition}}$

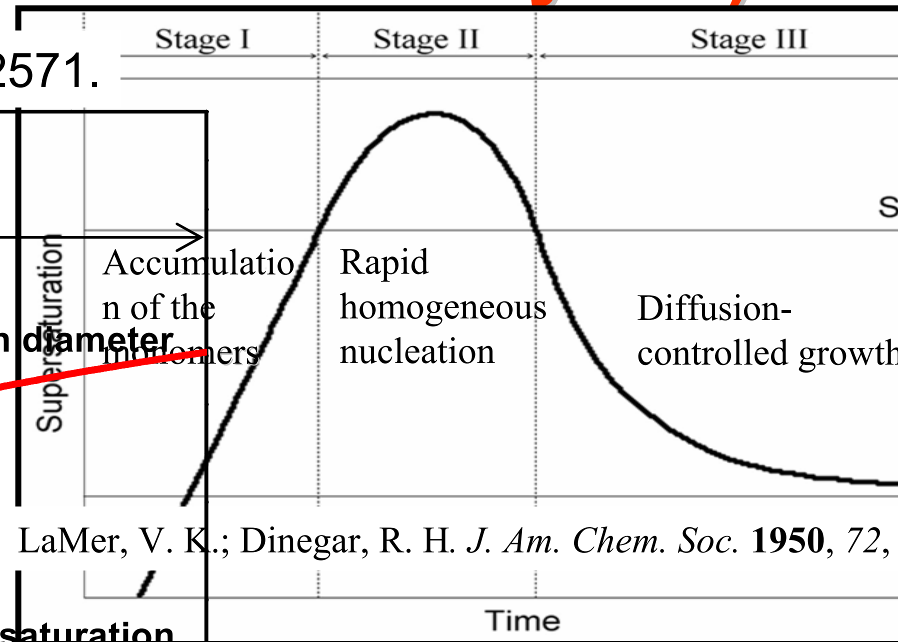


J. Park *et al.*, *Nature Mater*. **2004**, 3, 891.

ow did it happen? (LaMer vs. Kwon&Hyeon)

G. Kwon et al., *J. Am. Chem. Soc.* **2007**, 129, 12571.





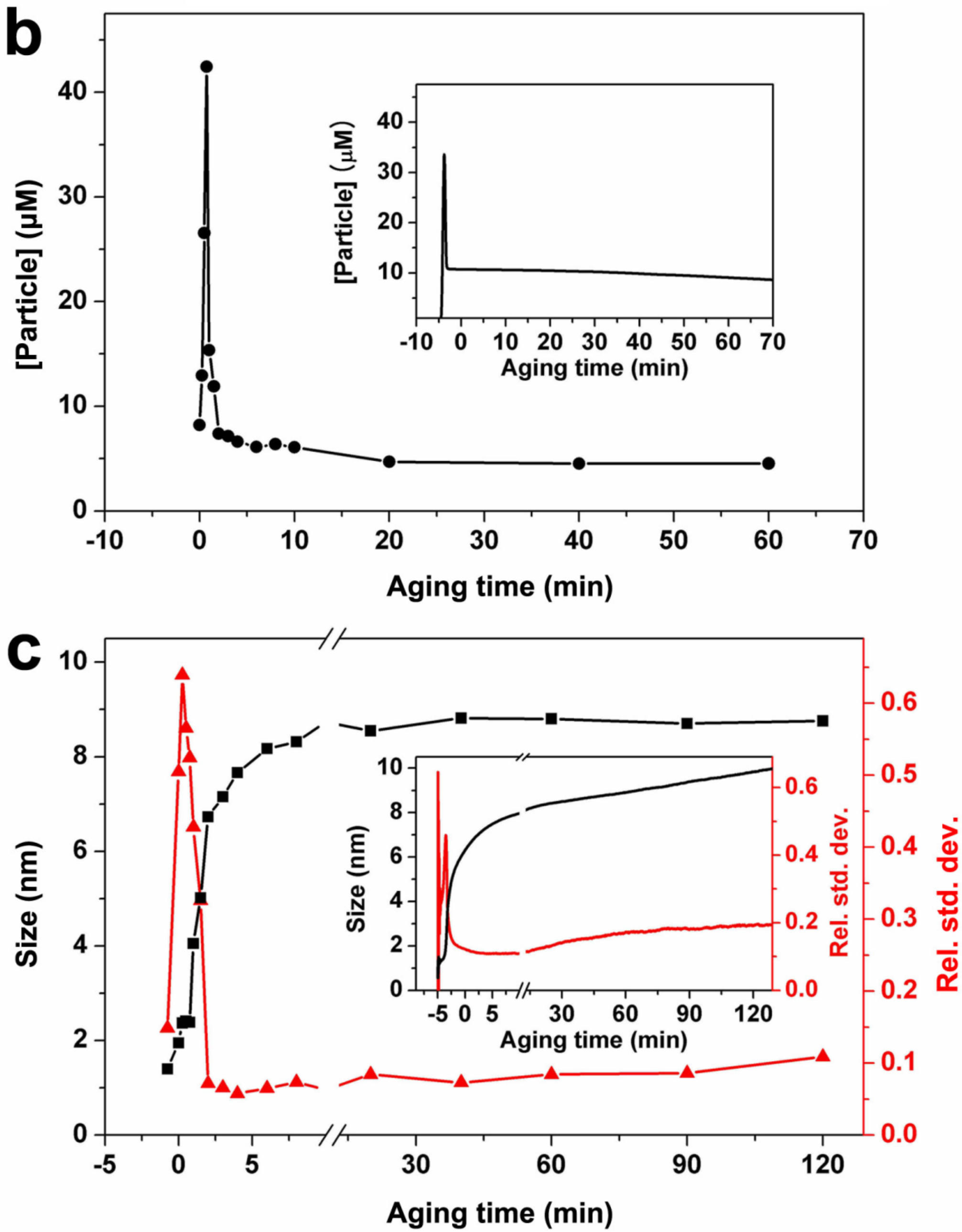
LaMer, V. K.; Dinegar, R. H. *J. Am. Chem. Soc.* **1950**, *72*,

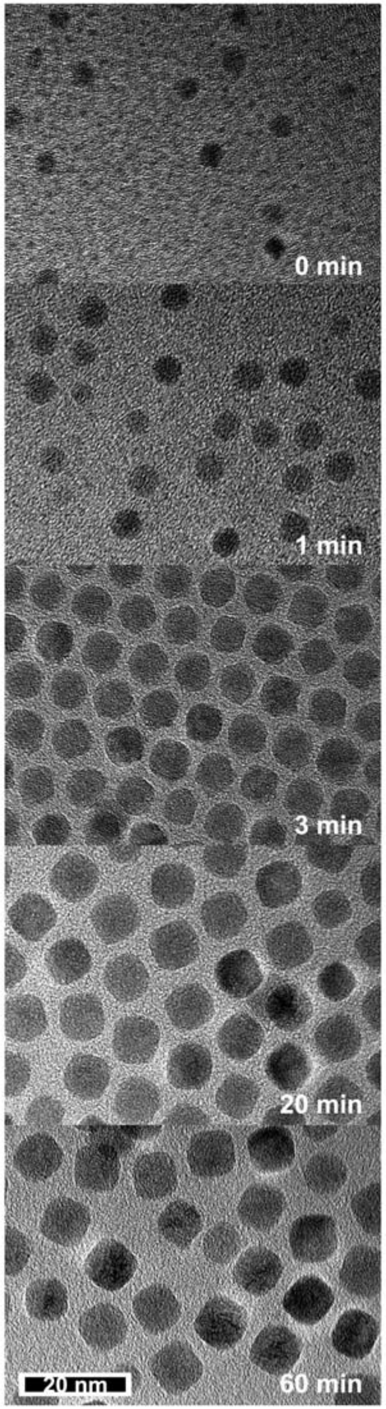
dden increase in nanocrystal concentration (burst of nucleation) is followed by rapid rrowing of size distribution (size focusing), which is well explained by LaMer model.

Peng, X.; Wickham, J.; Alivisatos, A. P. *J. Am. Chem. Soc.* **1998**, *120*, 5343.

apin, D. V.; Rogach, A. L.; Haase, M.; Weller, H. *J. Phys. Chem. B* **2001**, *105*, 12278; *J. Am. Chem. Soc.* **2002**, *124*, 5

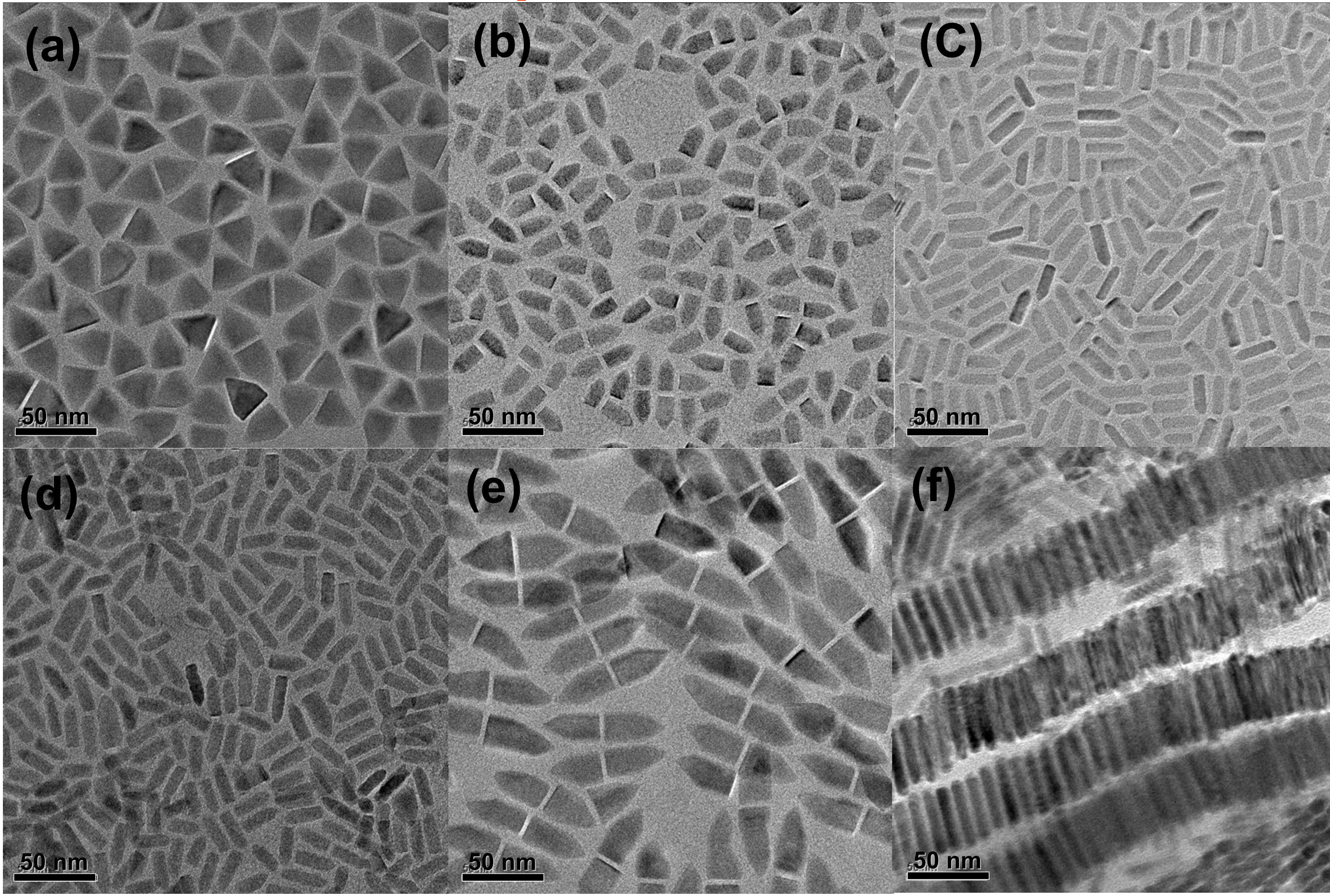
Heating at 320 °C





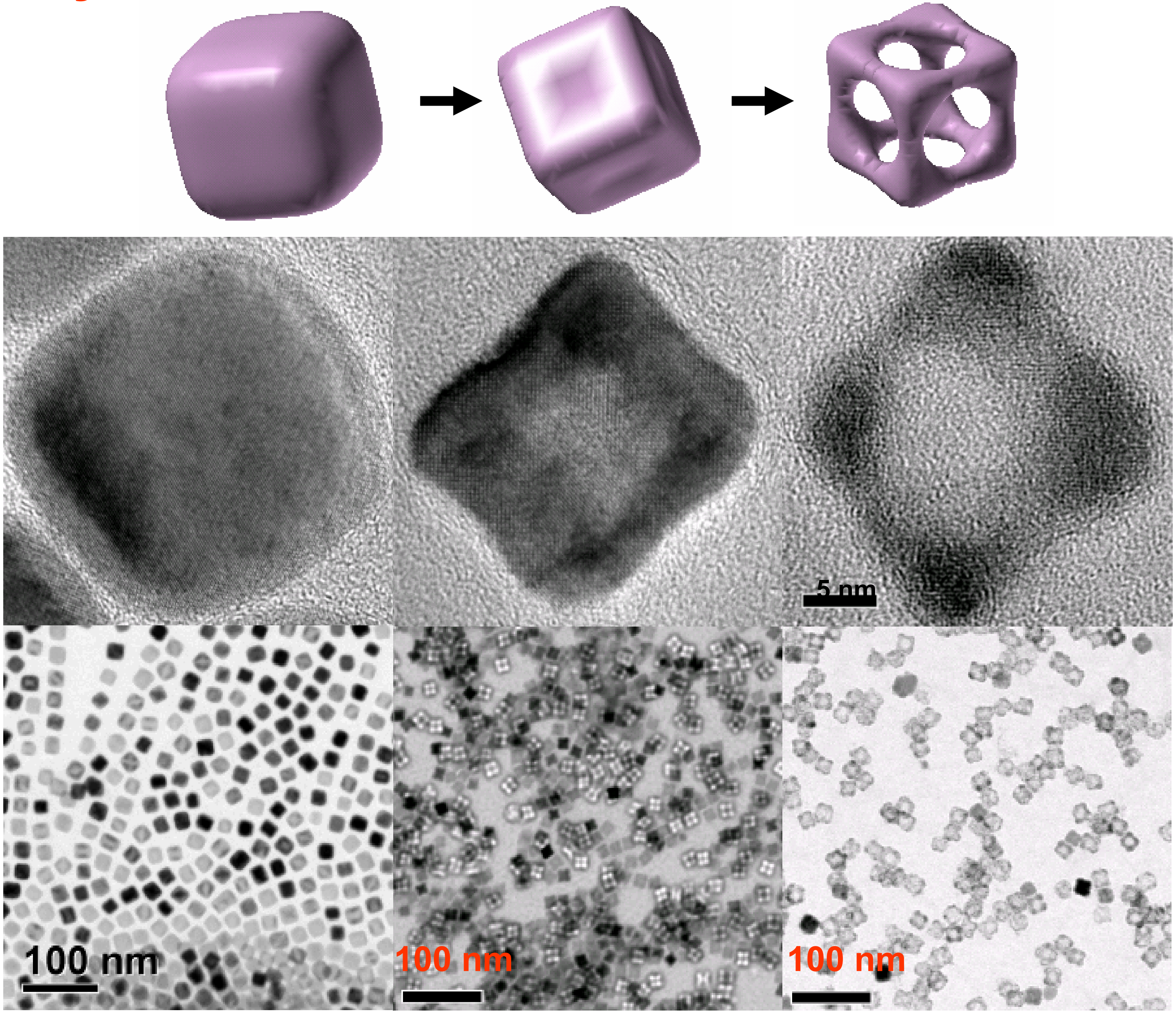
S. G. Kwon & T. Hye
J. Am. Chem. Soc.
2007, 129, 1257.

Pencil-shaped CoO Nanorods



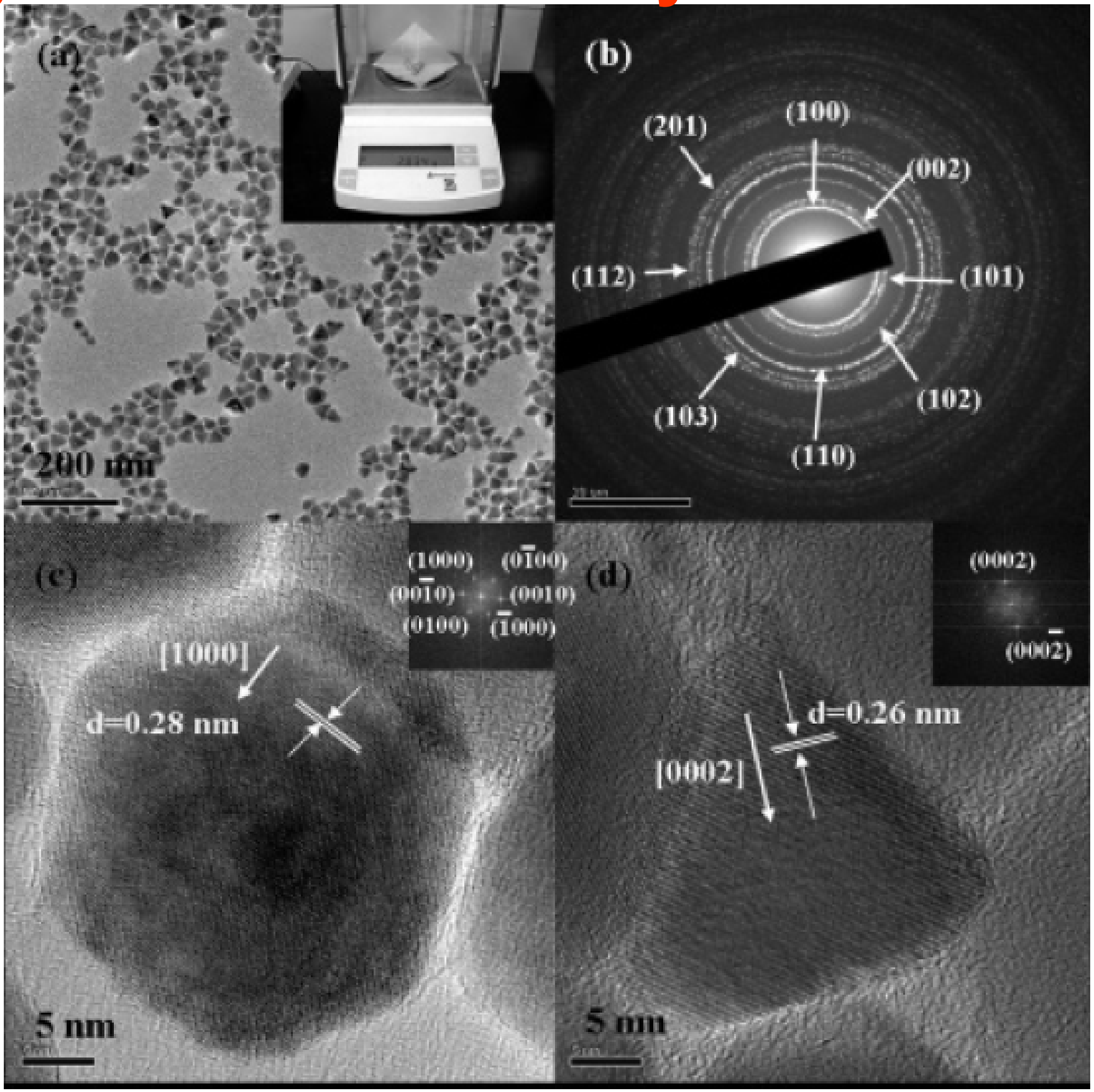
K. An, *et al. J. Am. Chem. Soc.* **2006**, 128, 9753.

Synthesis of Hollow Iron Nanoframes



D. K. Kim *et al. J. Am. Chem. Soc.* **2007**, 129, 5812.

Large-Scale Synthesis of Hexagonal Pyramid-Shaped ZnO Nanocrystals from Thermolysis of Zn-Oleate Compl



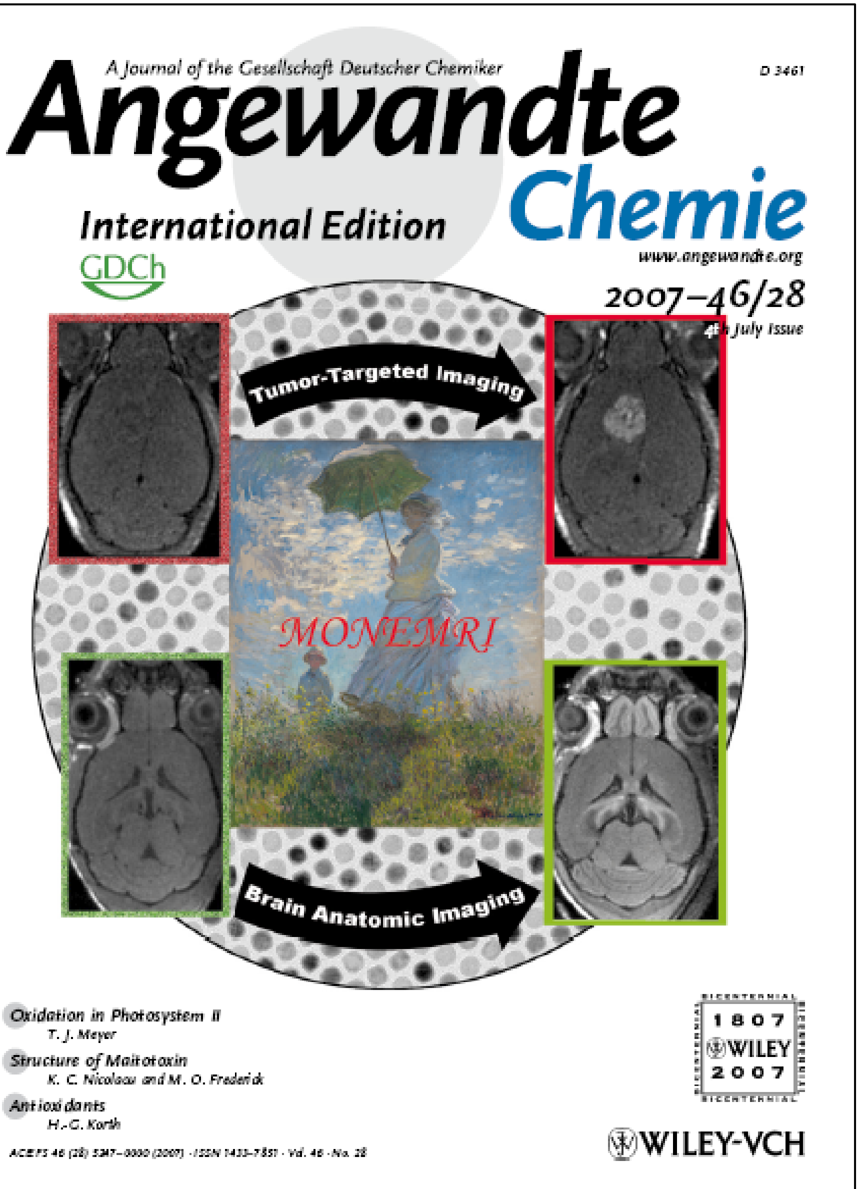
S. Choi *et al. J. Phys. Chem. B* **2005**, 109, 14792.

Part II

Bio-Medical Applications of

Uniform-sized Nanoparticles

Manganese Oxide Nanoparticle contrast Enhanced T1 weighted MRI (MONEMRI)



Hyon Bin Na et al.,

Angew. Chem. Int. Ed. **2007**, 46, 5397.

Cover article in July 4th issue.

Background picture: "Woman with a Parasol" by Claude Monet

Copyright: National Gallery of Art, Washington DC

Magnetic resonance imaging (MRI)

- MRI is one of the most potent imaging techniques for living organisms because MRI provides images with **excellent anatomical details** and functional information in a **non-invasive and real-time monitoring** manner.
- Contrast agents enable more specific and obvious images and enlargements of detectable organs and systems, leading into a wide scope of applications of MRI not only for diagnostic radiology but also for therapeutic medicine.

T1 MR Contrast Agents using Gd^{3+} complexes

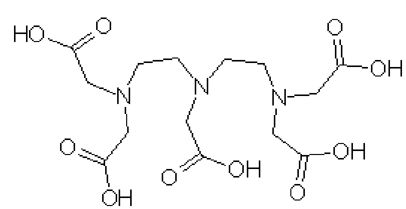
- **Positive Contrast Agents:** **becomes whiter** cause a reduction in the T1 relaxation time (increased signal intensity on T1 weighted images)
- Paramagnetic species have unpaired electrons.
- **Gd3+ and Mn2+ ionic complexes**
- **Most of clinically used MRI contrast is T1 Gd-complex**

1

c N	Ion	3d	4f	Magnetic moment (Bohr magneton)
	Cr 3+	↑ ↑ ↑ _ _		3.8
	Mn 2+	↑ ↑ ↑ ↑ ↑		5.9 (weak field)
	Fe 3+	↑ ↑ ↑ ↑ ↑		5.9 (weak field)
	Cu 2+	↑↓ ↑↓ ↑↓ ↑↓ ↑		1.7-2.2
	Eu 3+		↑↓ ↑ ↑ ↑ ↑ ↑ ↑	(6.9)
	Gd 3+		↑ ↑ ↑ ↑ ↑ ↑ ↑	7.9
	Dy 3+		↑↓ ↑↓ ↑ ↑ ↑ ↑ ↑	(5.9)

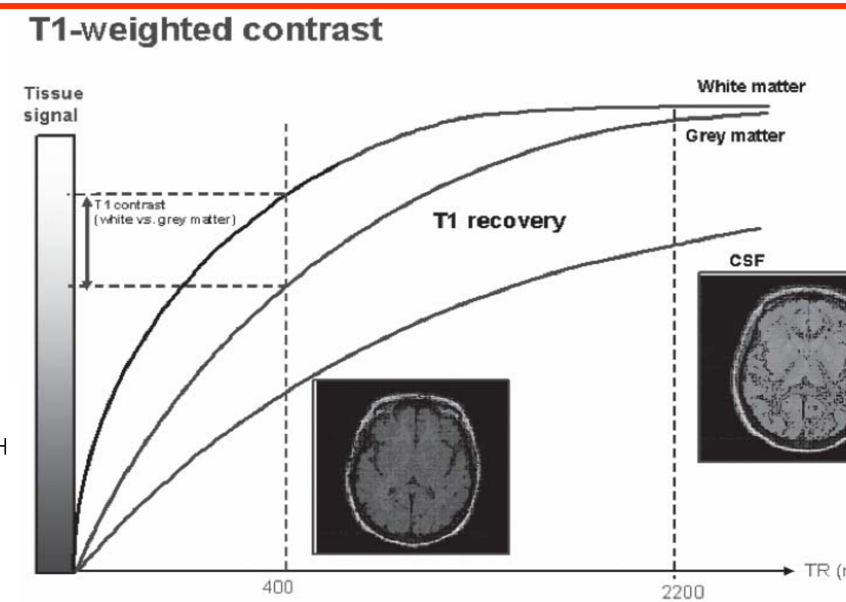
ife in urine = 21.0 min after intravenous injection
in blood = 19.6 min

npound	LD_{50} with intravenous dose in rats (mmol/kg body weight)
DTPA	10
EDTA	0.3
Cl3	0.4
glucamine diatrizoate	18
nmon X-ray contrast agent)	



DTPA (diethylenetriaminepentaacetic acid)

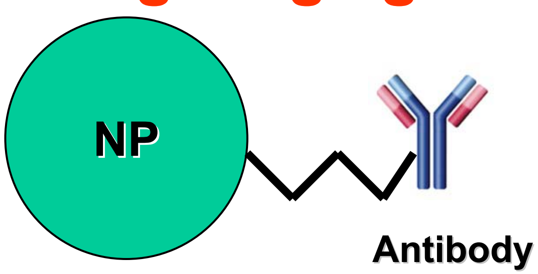
1

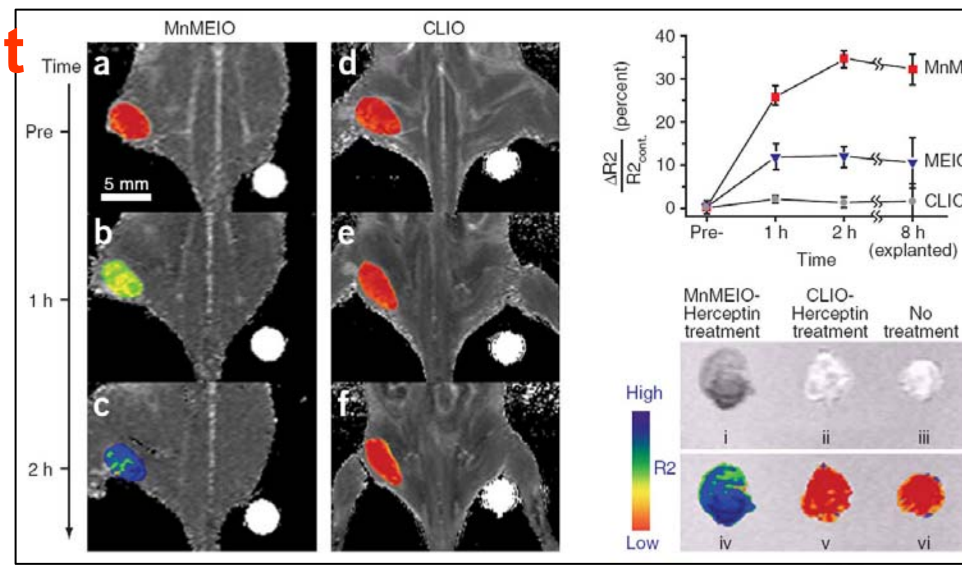


T2 MR Contrast Agents using Ferrite Nanoparticles

- **Negative contrast agents:** **becomes darker**
- Produce spin-spin relaxation effects → Shorter T2 relaxation.
- Superparamagnetic iron oxide (SPIO) nanoparticles
- Problems: 1) Dark signal is confused with pathologic conditions; 2) blooming effect: signal loss or distorted background image, overestimated image

Nonetheless, Magnetic Important for Molecular Imaging because of easy derivatization of targeting agent





Lee et. al., *Nat. Med.* **2007,** *13*, 95.

What is Ideal MRI contrast agent for Molecular & Cellular Imaging?

1) Efficient positive (T1) contrast ability,

2) Nanoparticle shape

for Easy labeling with targeting agents

3) High intracellular uptake and accumulation

for Cellular imaging

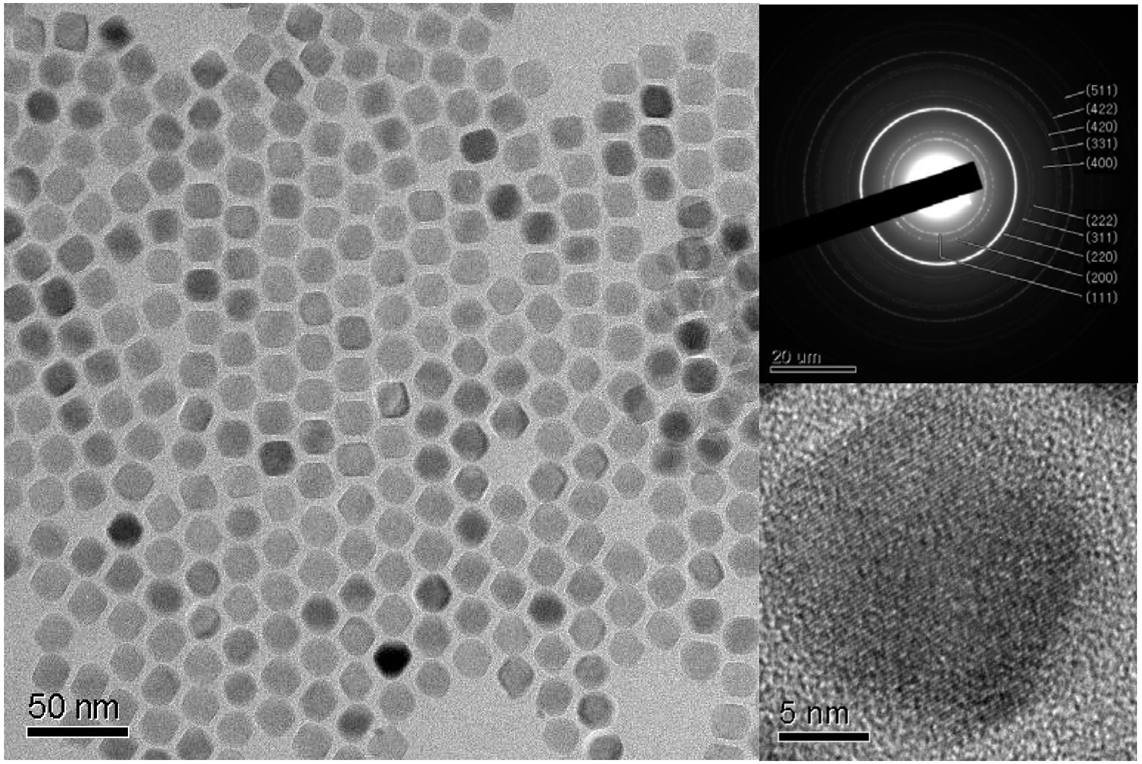
4) Facile delivery, safe clearance,

and minimal side effects.

Manganese Oxide Nanoparticle contrast Enhanced T1 weighted MRI (MONEMRI)

Bulte, J. W. M. & Kraitchman, D. L. *NMR Biomed.* 17, 484-499 (2004).

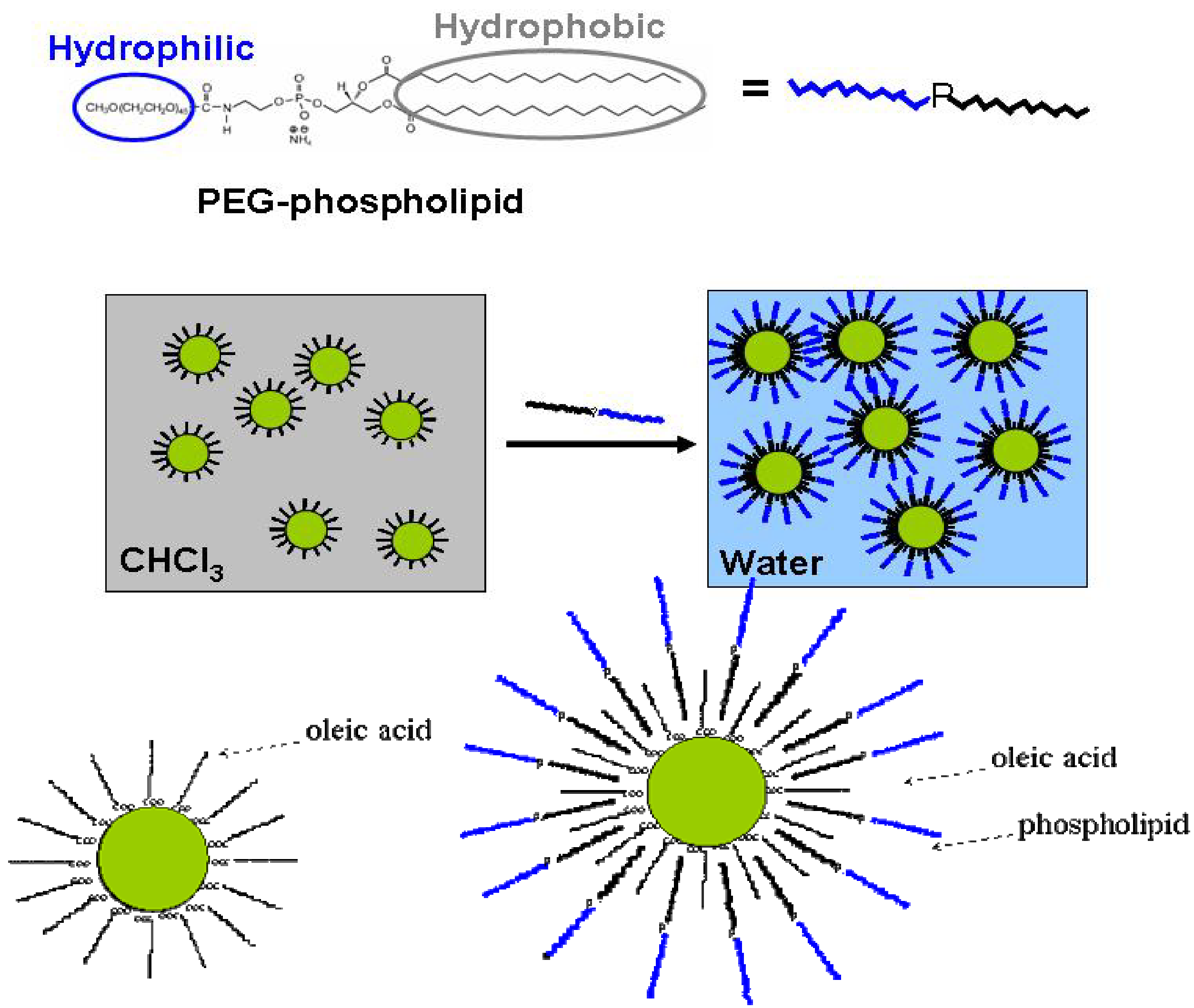
tra-large scale Synthesis of Monodisperse Nanocrysta



Monodisperse MnO Nanocrystals

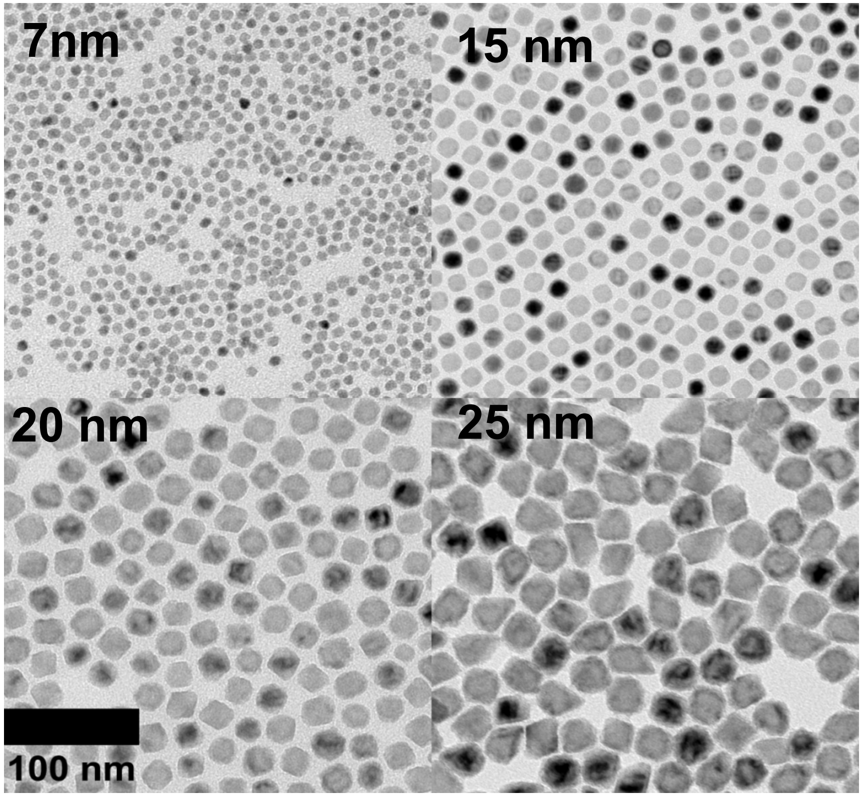
J. Park, et al., *Nature Mater.* **2004**, *3*, 891.

Water-dispersible MnO nanoparticles

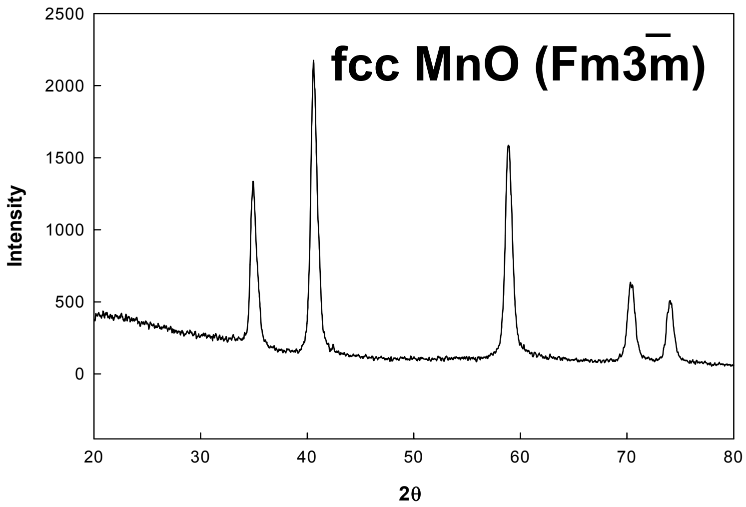


Ligand exchange: B. Dubertret, et al., *Science* **2002**, *298*, 1759.

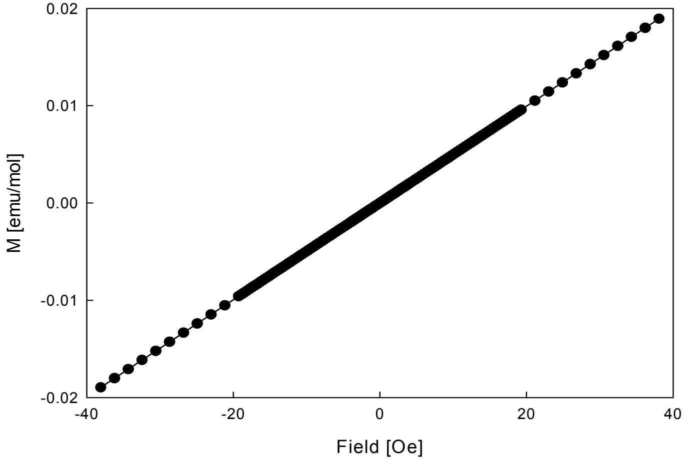
Water-dispersible MnO nanoparticles



XRD of MnO nanoparticles



Magnetic property of MnO nanoparticles at T = 300K



In vivo MRI - mouse

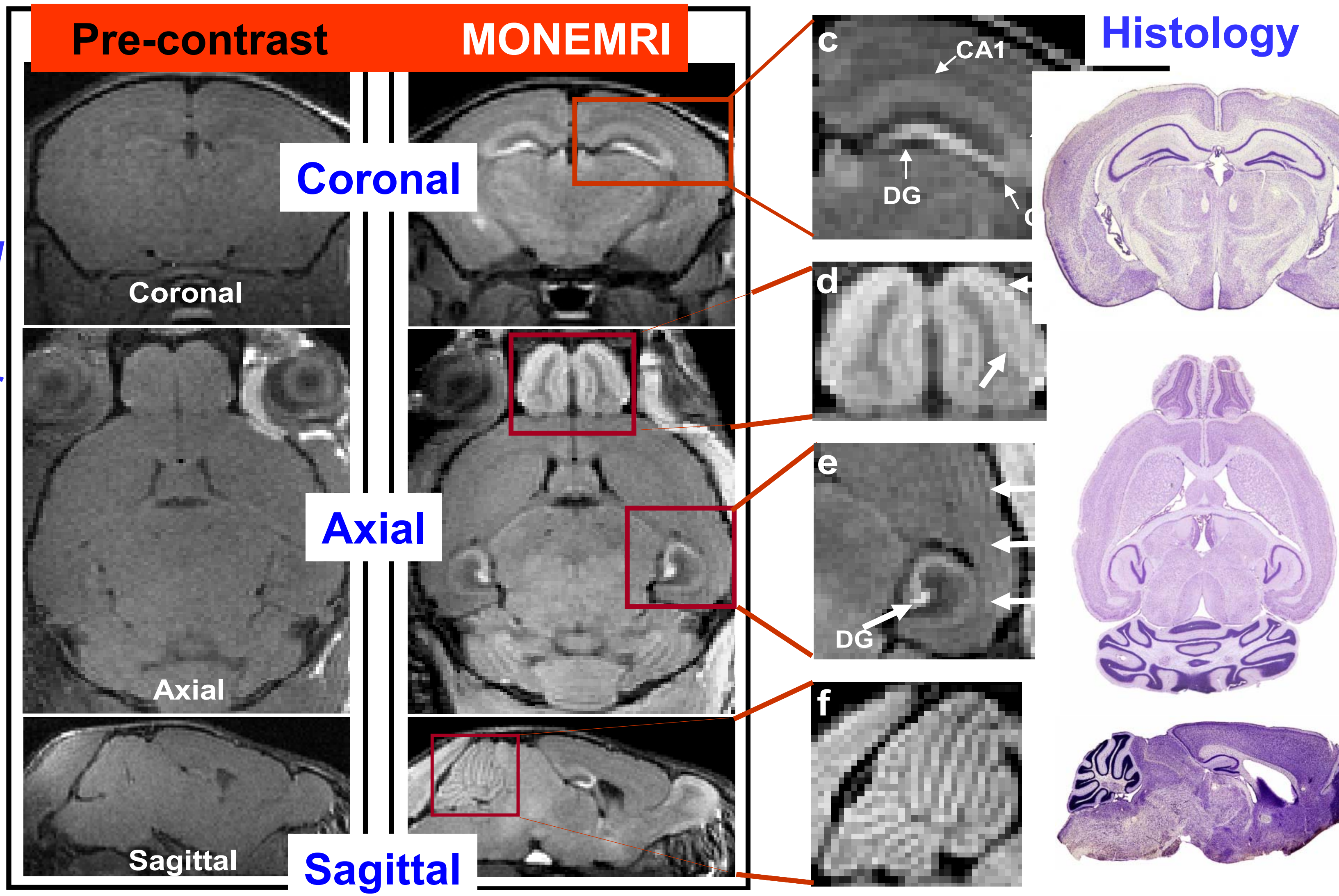
- 8 weeks mouse
- 20~25 g of mouse

Vol (ml)	0.15~0.3
Mn (mg)	0.70
Mouse (g)	20
Dose (mg/kg body)	35.00

WAS BLIND, BUT NOW I SEE*

MONE-MRI of Mouse Brain

nventional
Gd-DTPA
ves similar
mage as
e-contrast



nazing Grace,
lewton, 1779.

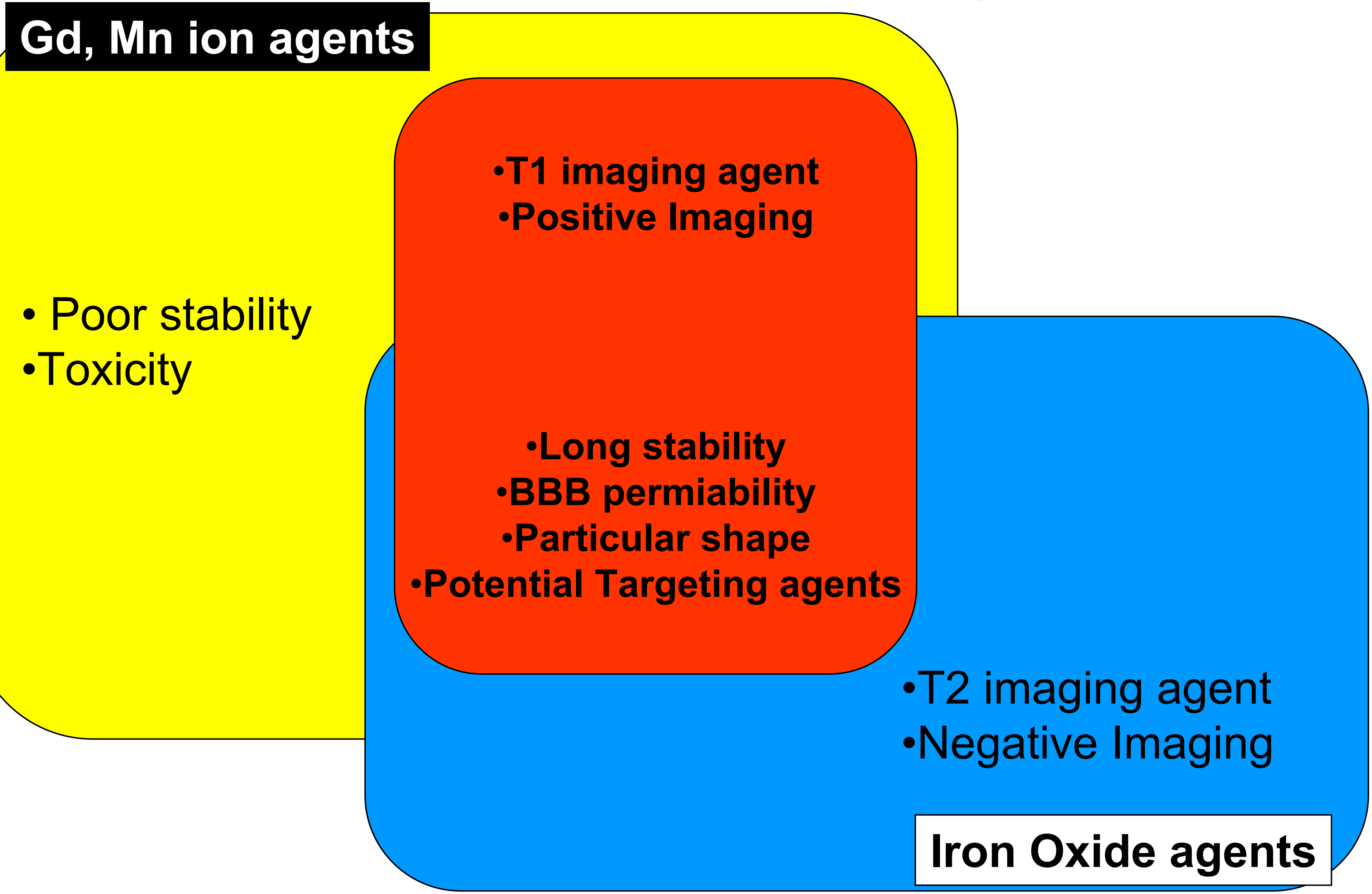
MONEMRI shows clear sub-anatomic structure of brain

Concentration of MnO nanoparticles that inhibited 50% of cell growth (IC50)

Cell line	**Characteristics**	**IC_{50} (Mn mM)**
MRC-5	Human normal lung fibroblast	**4.73**
HEK 293	Human embryonic kidney cell	**1.33**
NCI-H460	Human large cell lung cancer cell	**0.36**
Huh7	Human hepatoma cell	**0.66**
U87-MG	Human glioblastoma cell	**3.57**
MCF-7	Human breast adenocarcinoma cell	**0.44**

- **No appreciable toxicity below 0.82 mM (Mn) was observed in human normal and cancer cell lines**
- **No abnormal behavior of mouse during MRI for > 1 month**

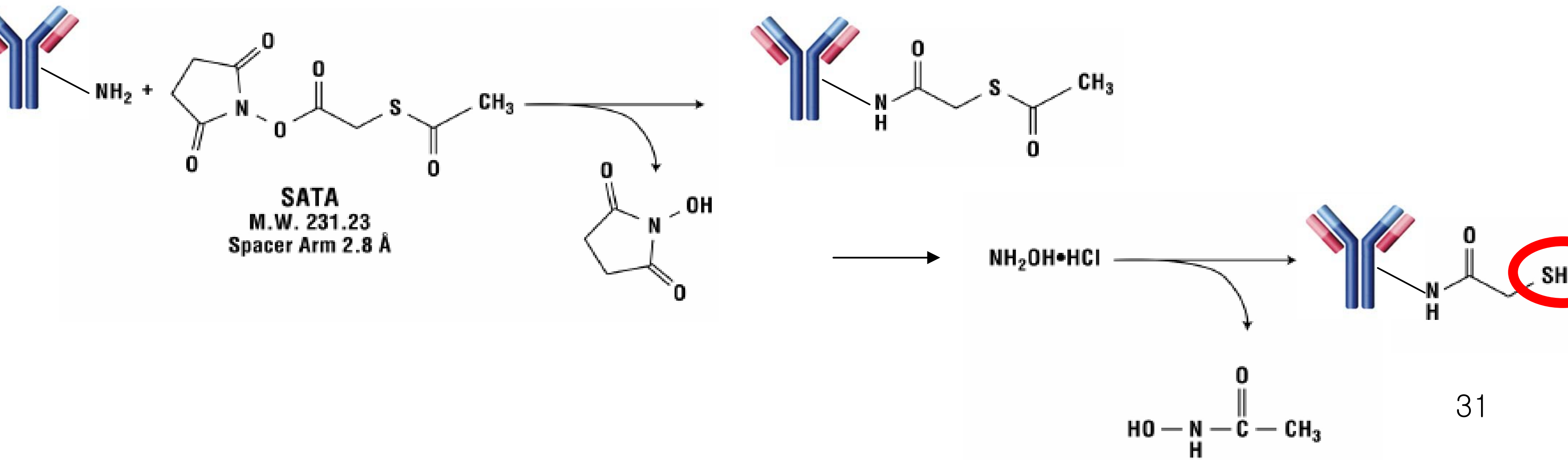
MONEMRI combines T1 positive contrast ability with Nanoparticle Shape for Targeted MRI



Targeting MnO agent – antibody conjugation

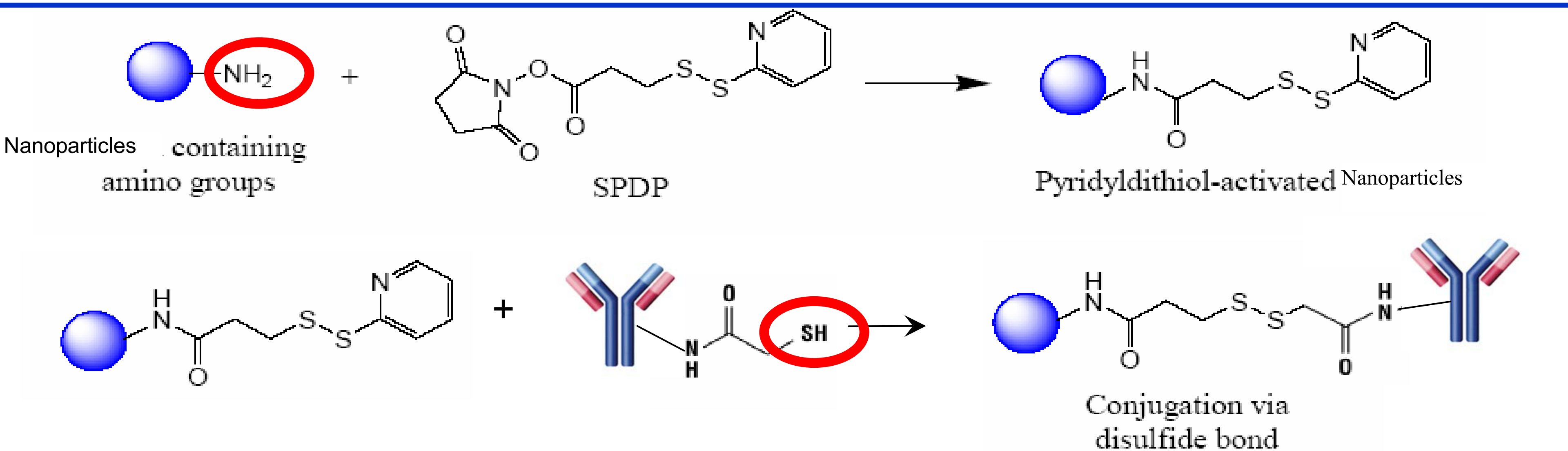
Trastuzumab (Herceptin®) – MW : ~150 kDa
an anti-cancer therapy that acts on the **HER2/neu (erbB2)** receptor (breast cancer). Cells treated with Herceptin undergo arrest during the G1 phase of the cell cycle so there is **reducted proliferation**.

1. Antibody modification – Thiol group

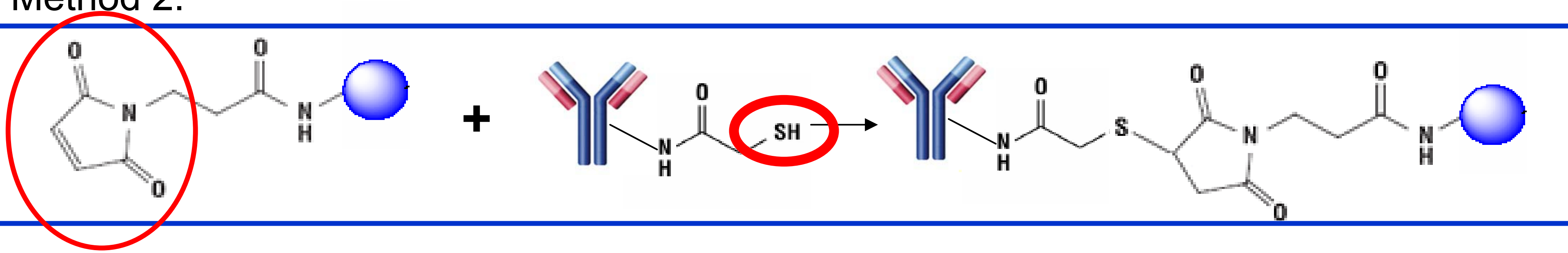


2. NPs modification

Method 1.



Method 2.



Herceptin-MnO Nanoparticles for Targeting to Breast Cancer Brain Metastatic Tumor

III. Collection of MRI images

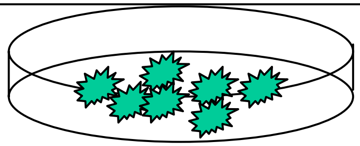
II

Herceptin conjugated MnO nanoparticles

Bolus injected through tail vein

I

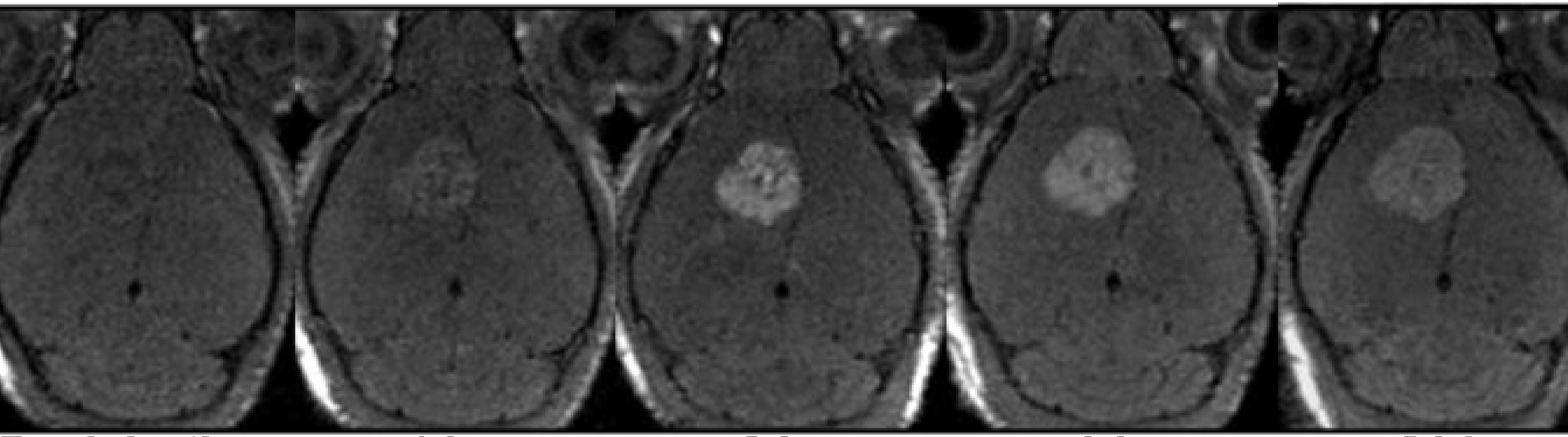
Breast cancer MDA-MB-435 cells



Preparation of
Breast Cancer
Brain Metastatic Tumor Model

The tumor was grown for 12-17 days.

Imaging Selectively the Breast Cancer Cells in the Metastatic Brain Tumor Model Using Herceptin Conjugated MnO Nanoparticles

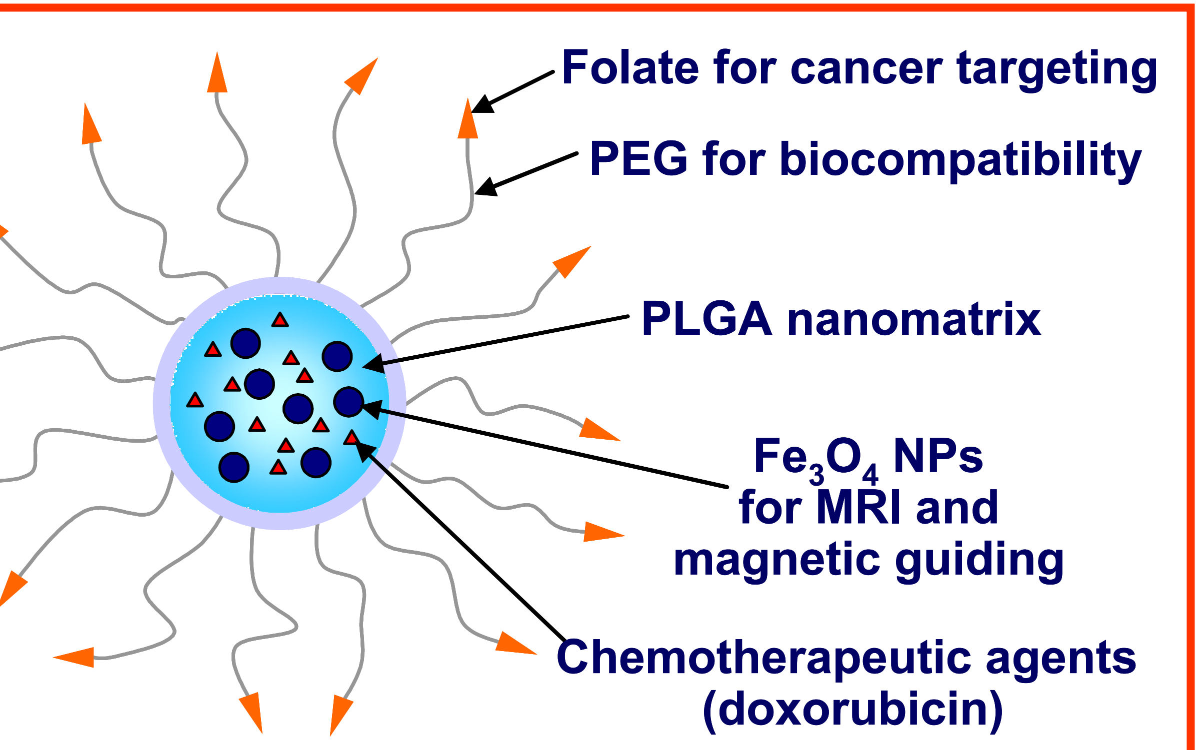


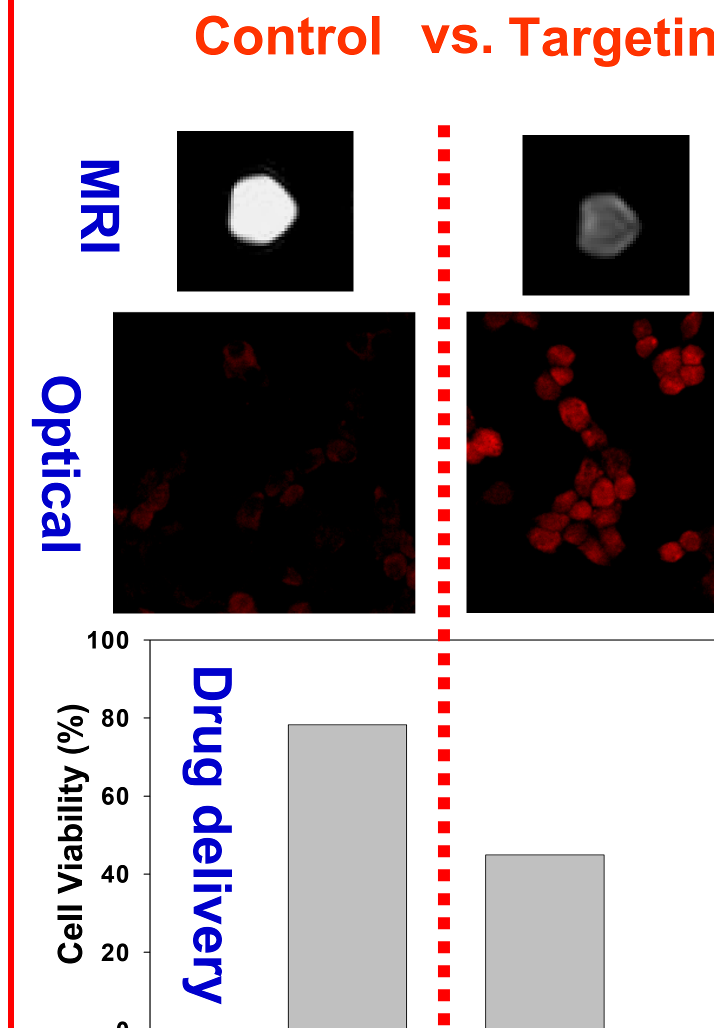
1) Imaging selectively the breast cancer cells in the metastatic brain tumor model

) Boundary between Tumor and Normal Cells are clearly Definec which is very important for brain tumor surgery

Multifunctional Nanomedical Platforms

Multimodal Imaging
multaneous Diagnosis & Therapy





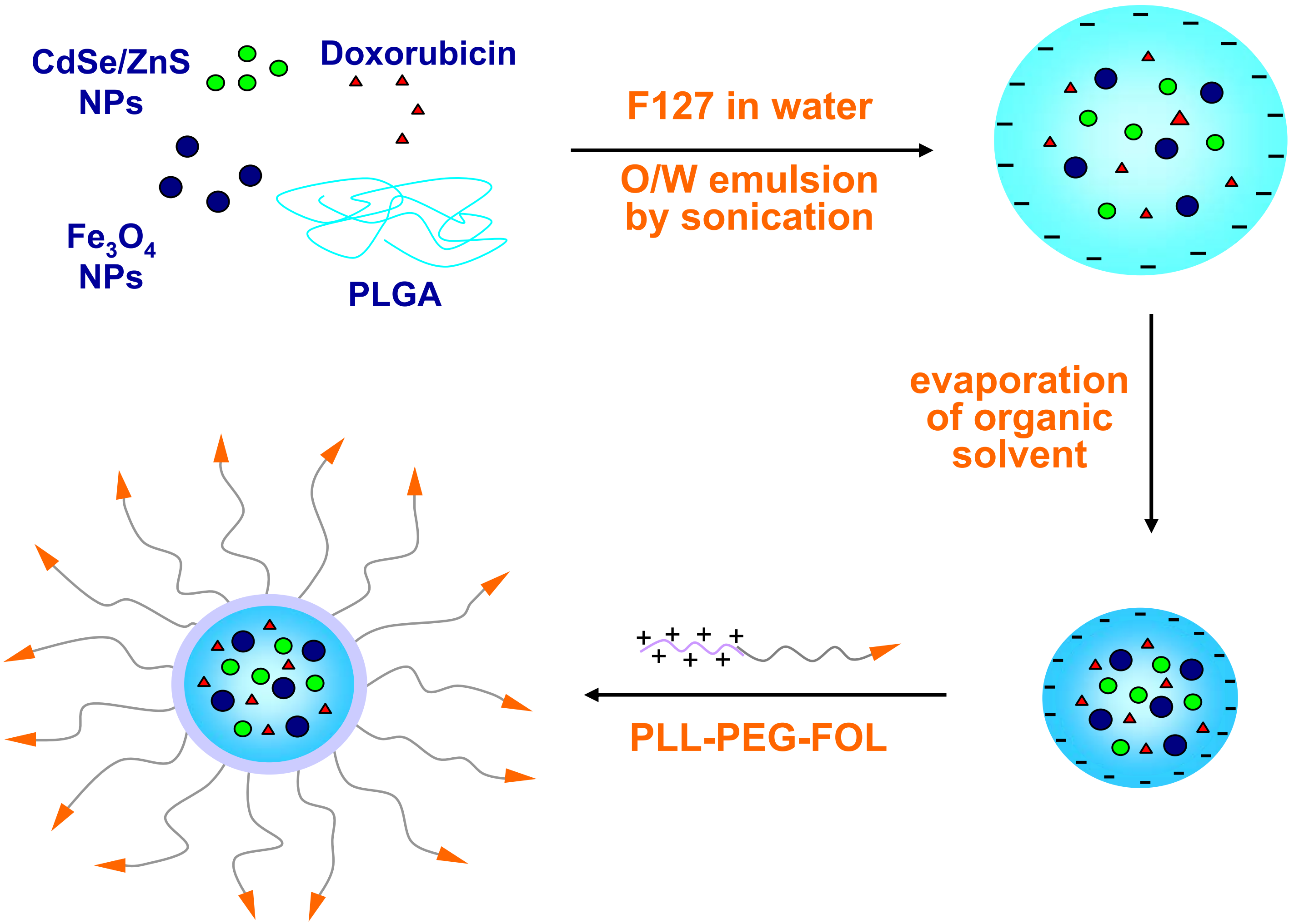
. Kim et al. *Chem. Soc. Rev.* (invited) **2008**. submitted.

Multifunctional Polymer Nanoparticles: Magnetic Guided Diagnostic and Therapeutic Nanomedicine

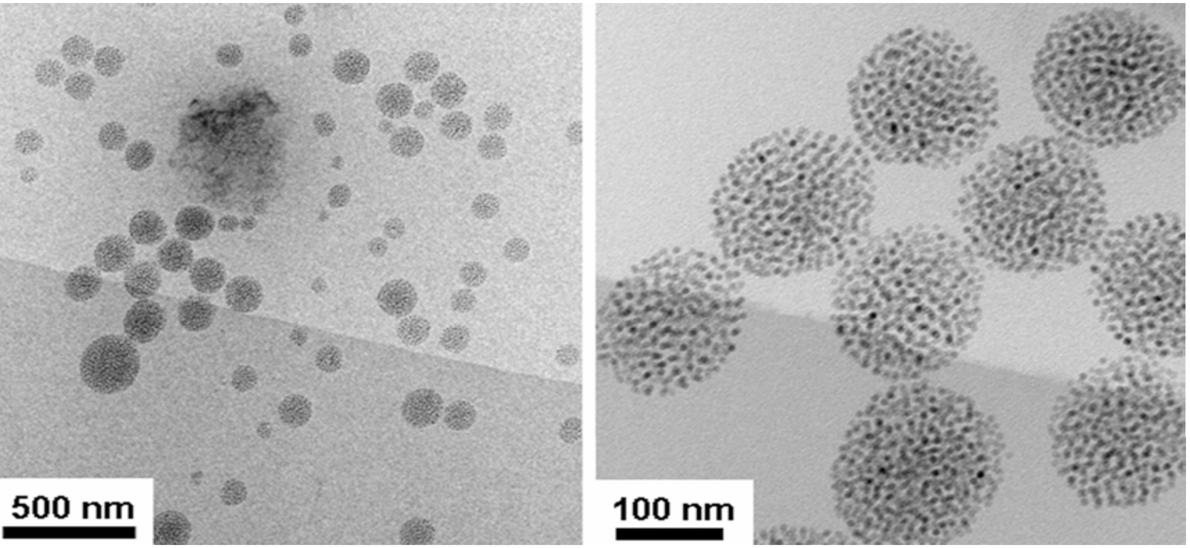
J. Kim et al. *Adv. Mater*. **2008**, *20*, 478.

Scheme

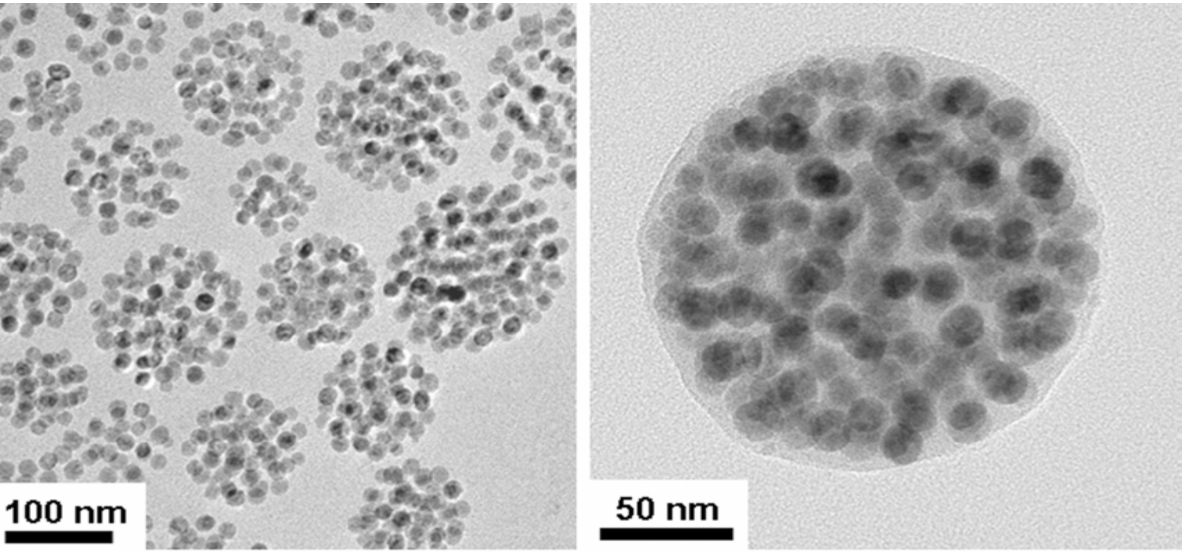
Multifunctional Polymer Nanoparticles for Cancer Imaging and Therapy



PLGA (MNP/DOXO) Nanoparticles

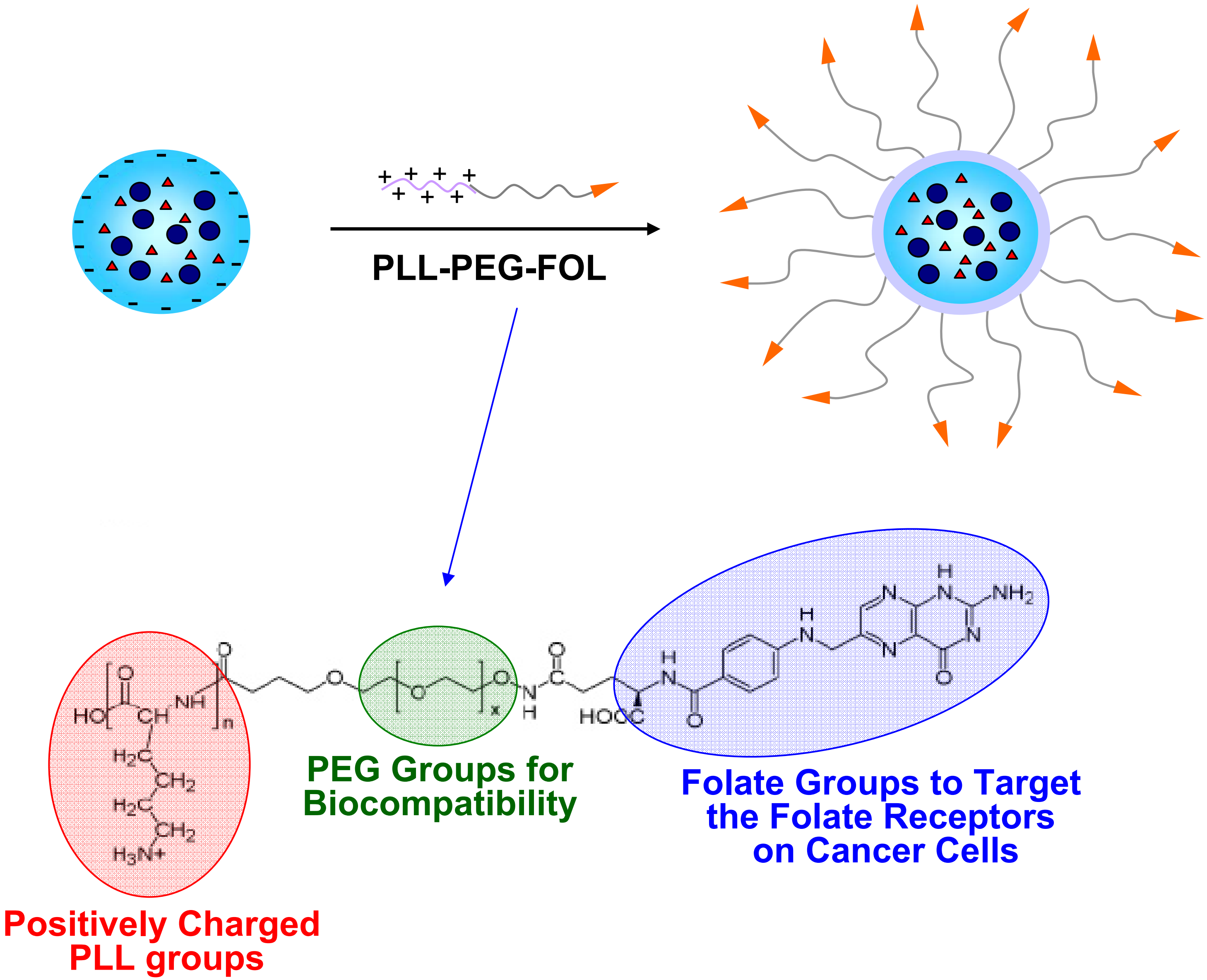


- **7 nm Fe_3O_4 nanoparticles in PLGA nanoparticles**
- **100 ~ 200 nm sized PLGA nanoparticles**



- **15 nm Fe_3O_4 nanoparticles**
- **100 ~ 200 nm sized PLGA nanoparticles**

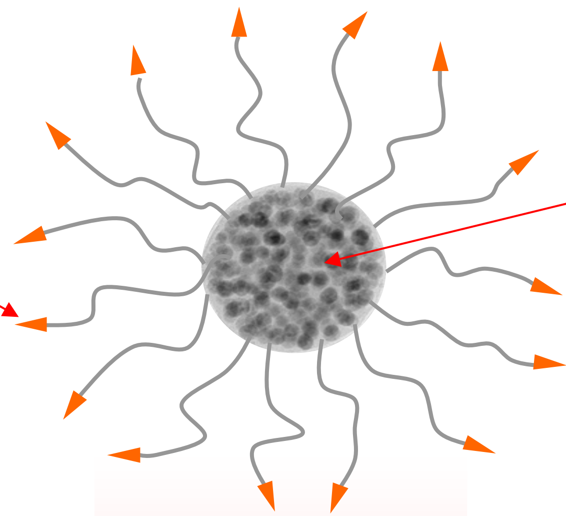
Folate Conjugation



In Vitro Targeted MRI and Drug Delivery

(1) Folate Targeting

(2) Magnetic Attraction
(High Magnetic Moment)



KB cell : a human epidermal carcinoma cell line
folate receptor-overexpressing cell line

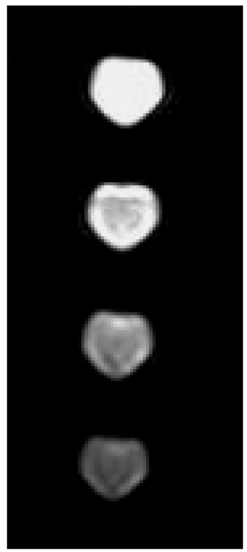
- ► **Imaging of Cancer Cells Using T_2 MRI**
- ► **Cell Growth Inhibition by Doxorubicin**

In Vitro T_2 MRI

KB cells were mixed with 1% agarose solution for MRI scan

T_2-weighted image

Darker



Control

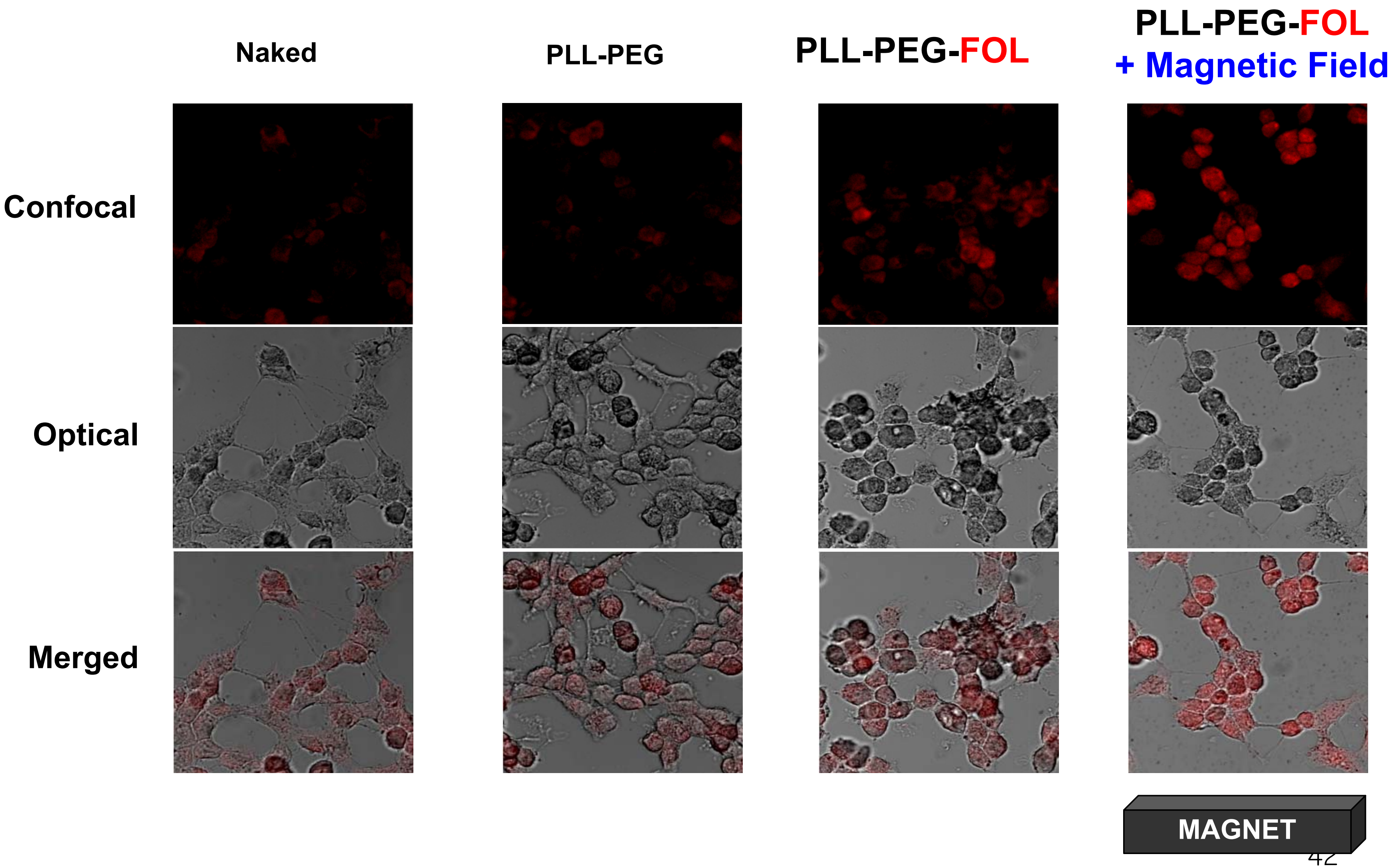
Naked PLGA(MNP/DOXO)

PLGA(MNP/DOXO)-PLL-PEG-FOL

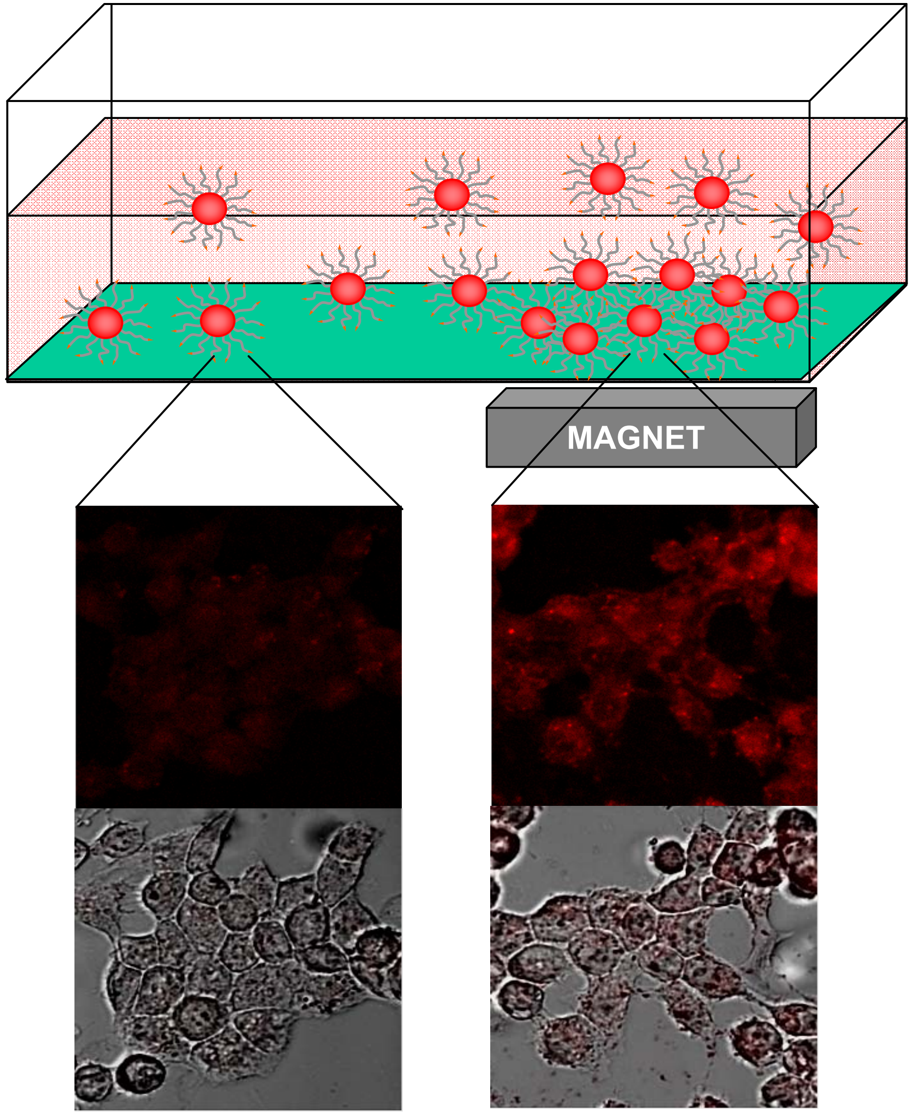
PLGA(MNP/DOXO)-PLL-PEG-FOL + Magnetic Field

PLGA(MNP/DOXO) nanoparticles can be used as a T_2 contrast agent in targeted MRI for the detection of cancer cells

Cellular Uptake (CLSM)



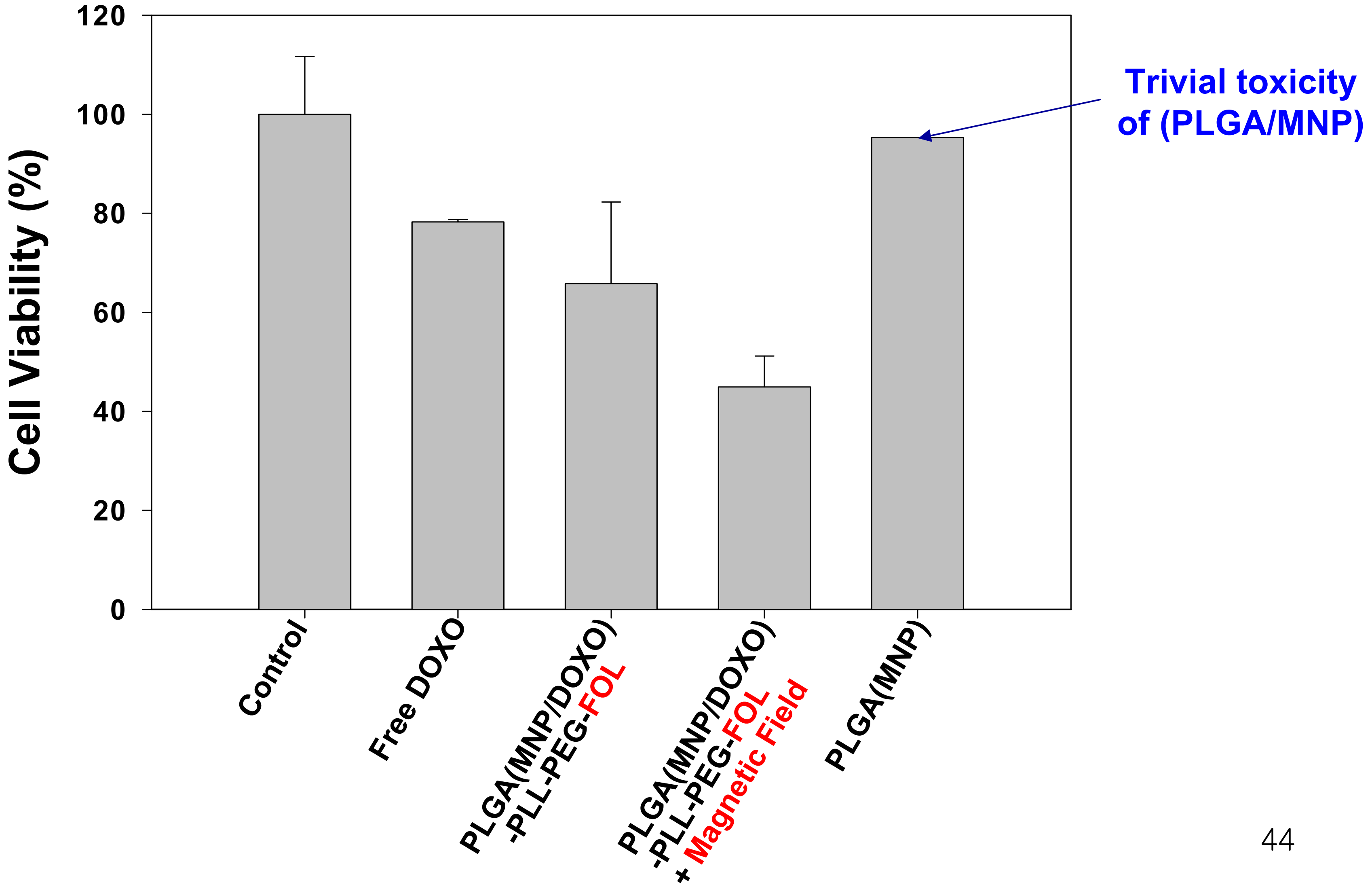
Magnetic Guiding Effect



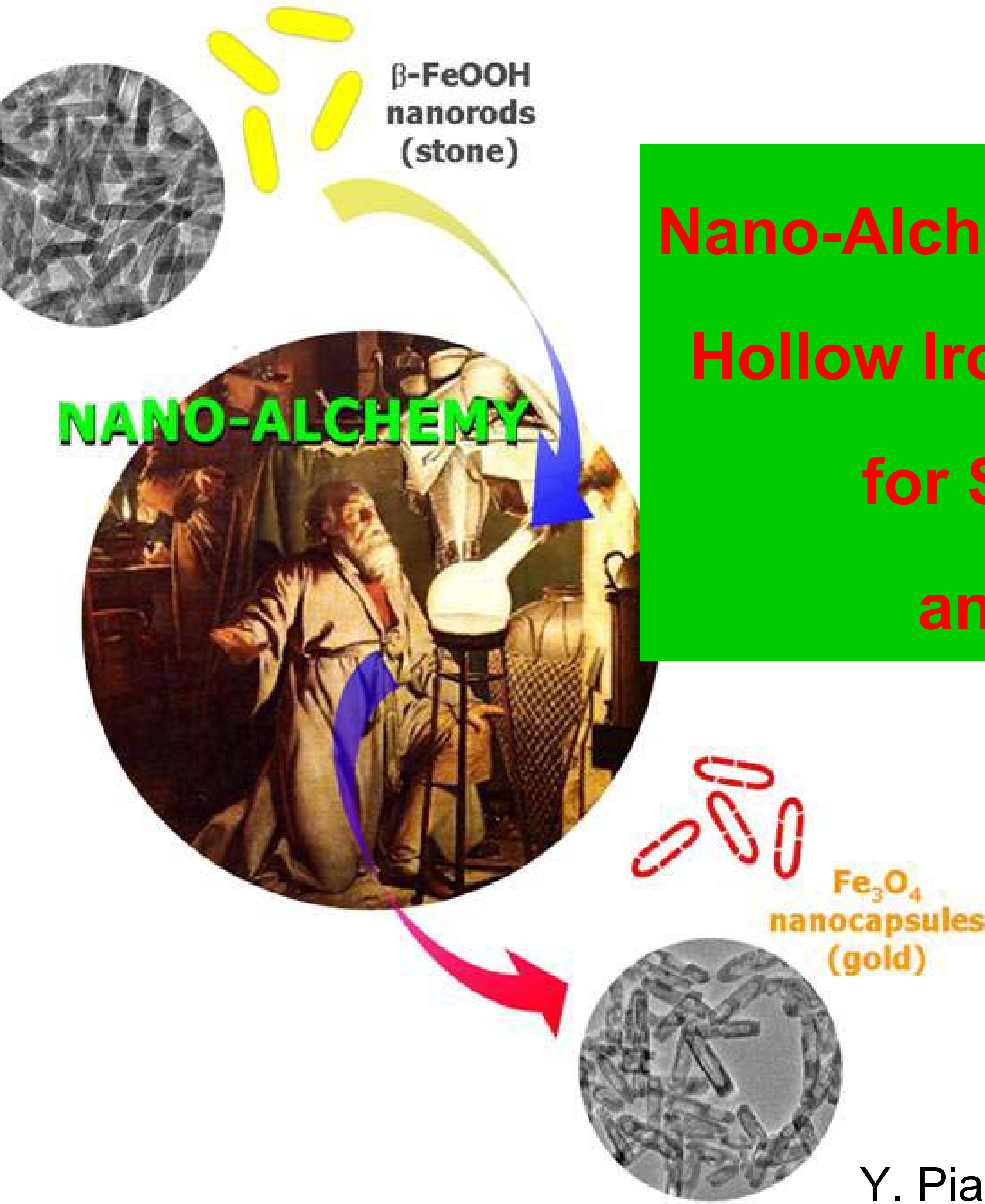
The Magnetic Field Enhanced the Uptake of PLGA(MNP/DOXO) Nanoparticles

Cytotoxicity Assay

0.4 μM DOXO, 4h incubation at 37 °C,
After further incubation for 5 days, CCK-8 assay

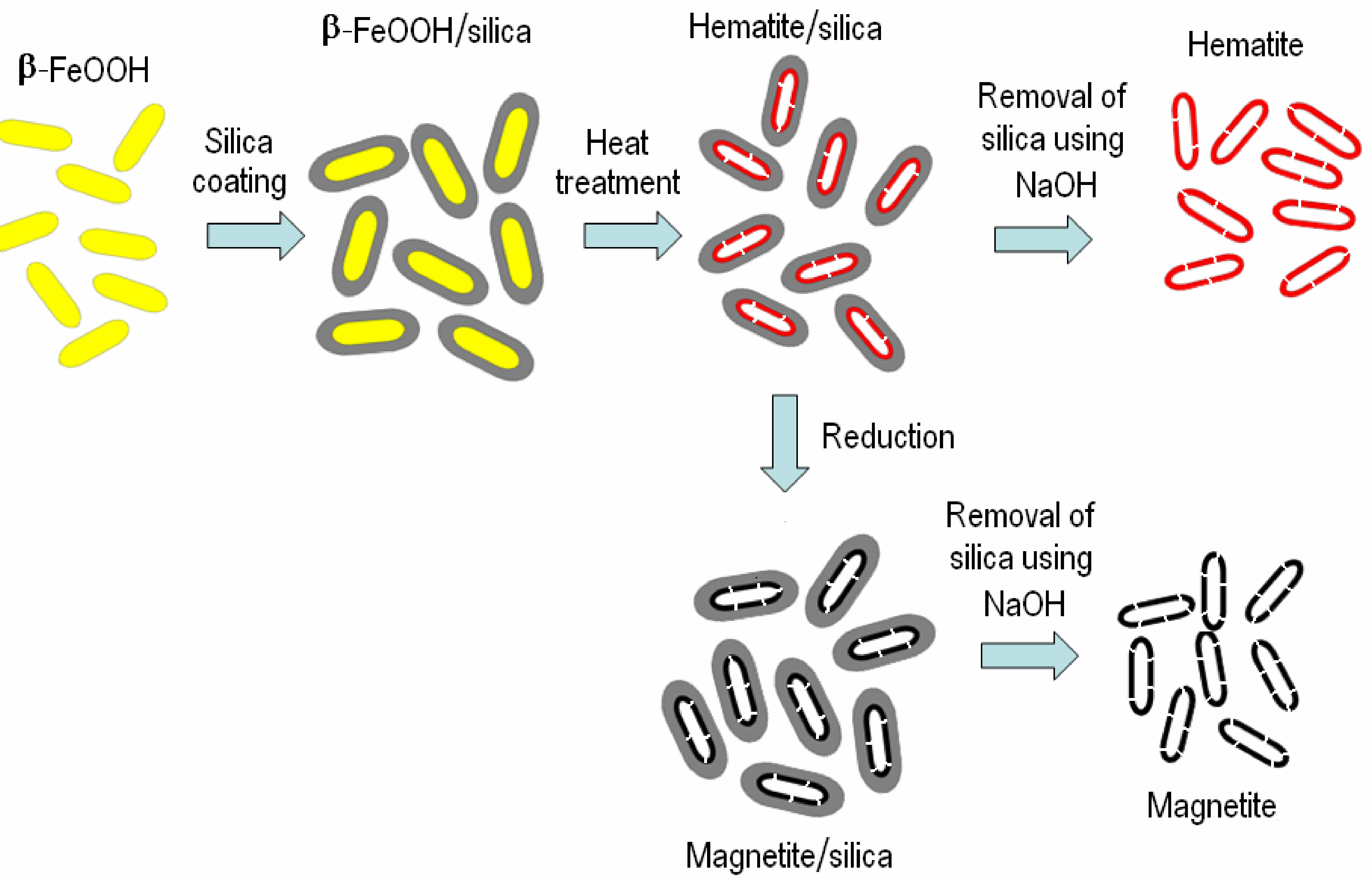


Nano-Alchemical (?) Fabrication of Hollow Iron Oxide Nanocapsules for Simultaneous MRI and Drug Delivery

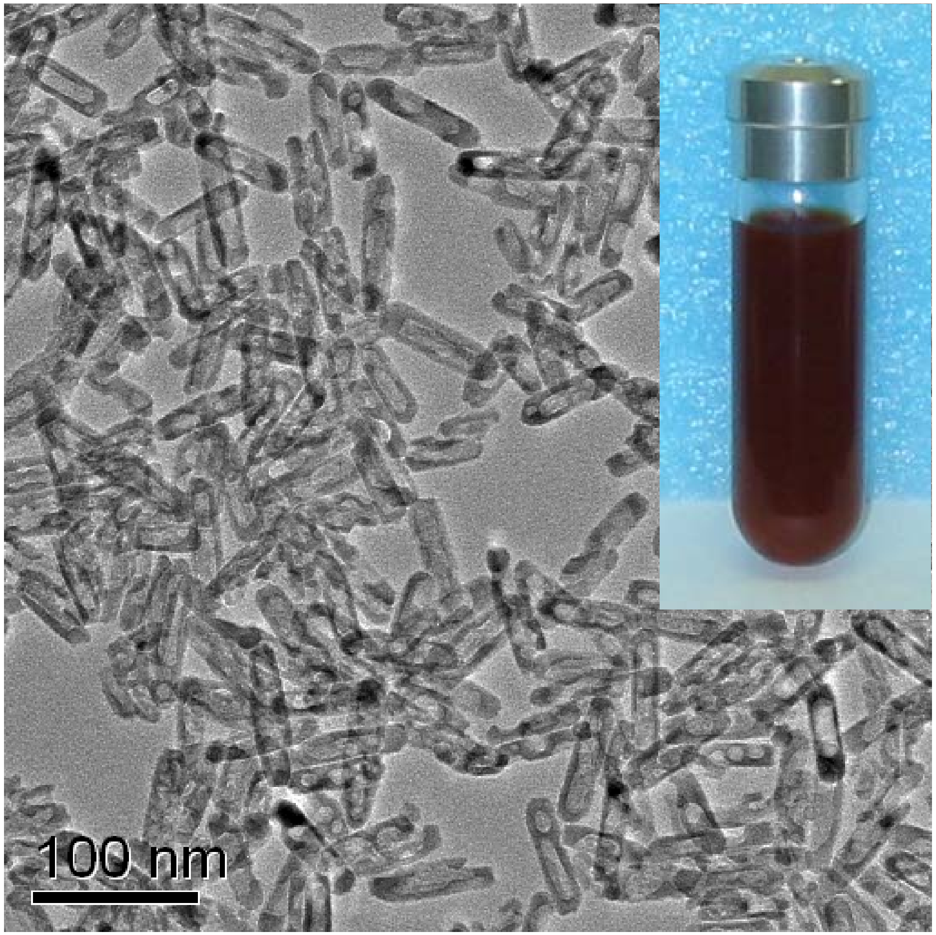


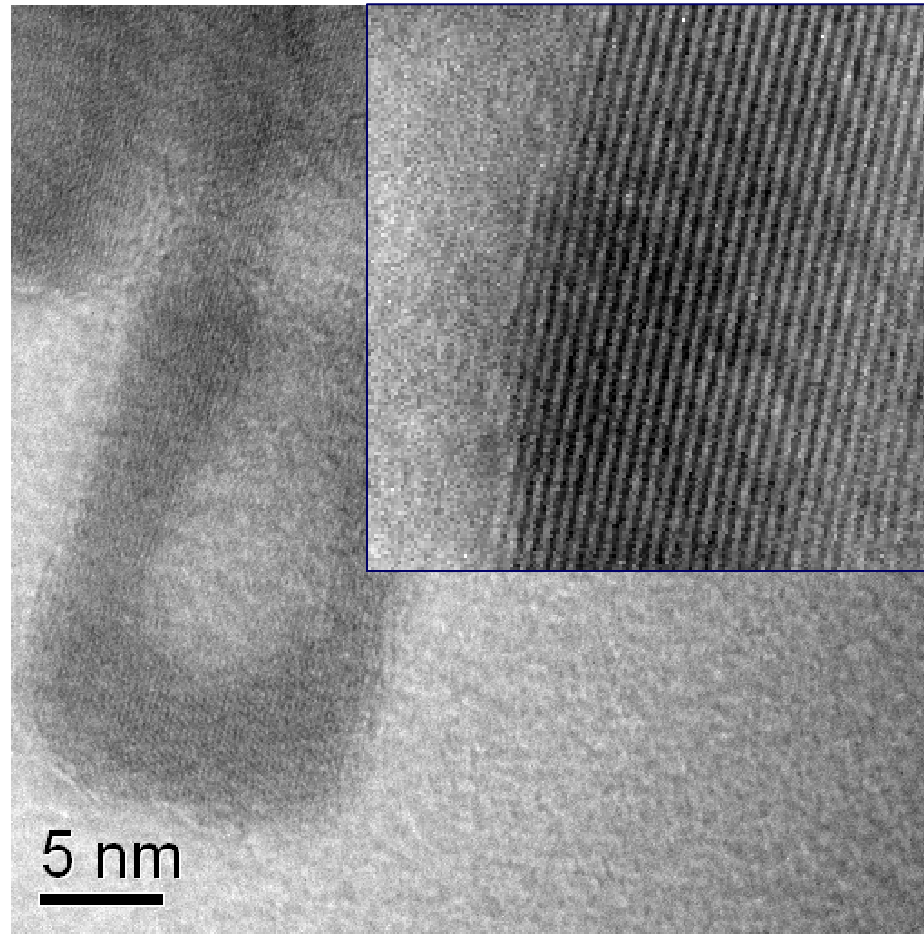
Y. Piao et al., *Nature Mater*. **2008**, 7, 24

Wrap/Bake/Peel Process for Nanostructural Transformation
om FeOOH Nanorods to Biocompatible Iron Oxide Nanocapsule

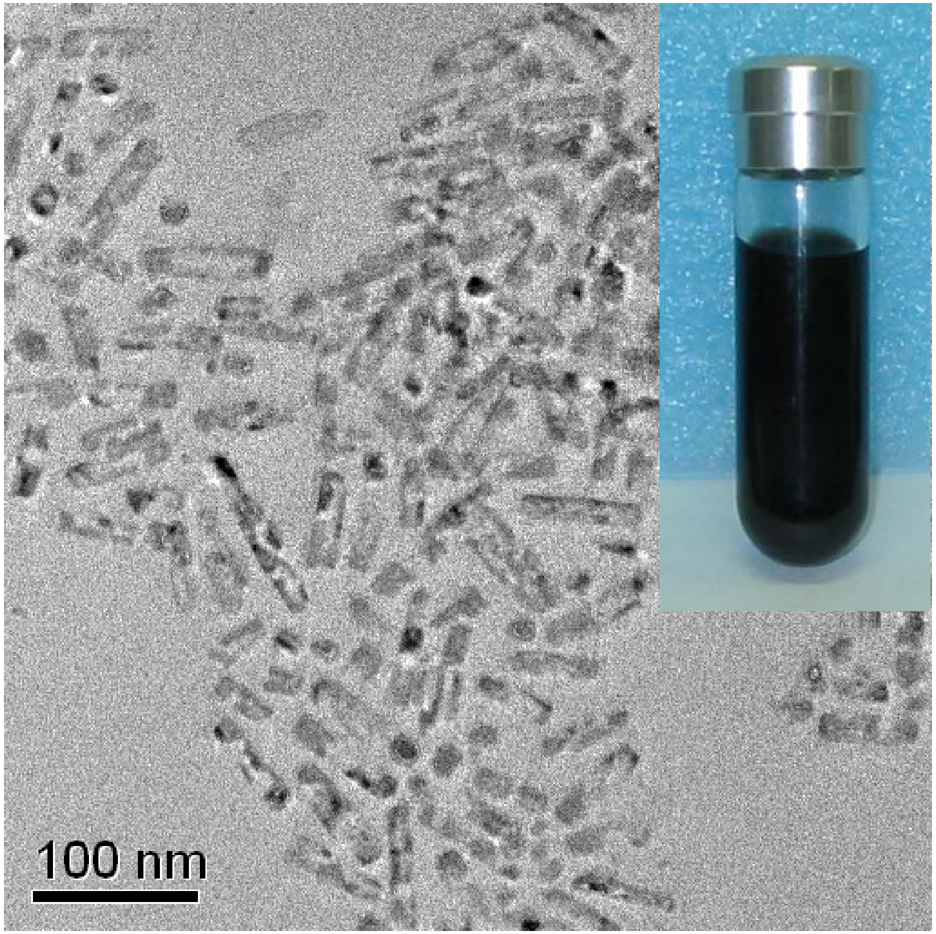


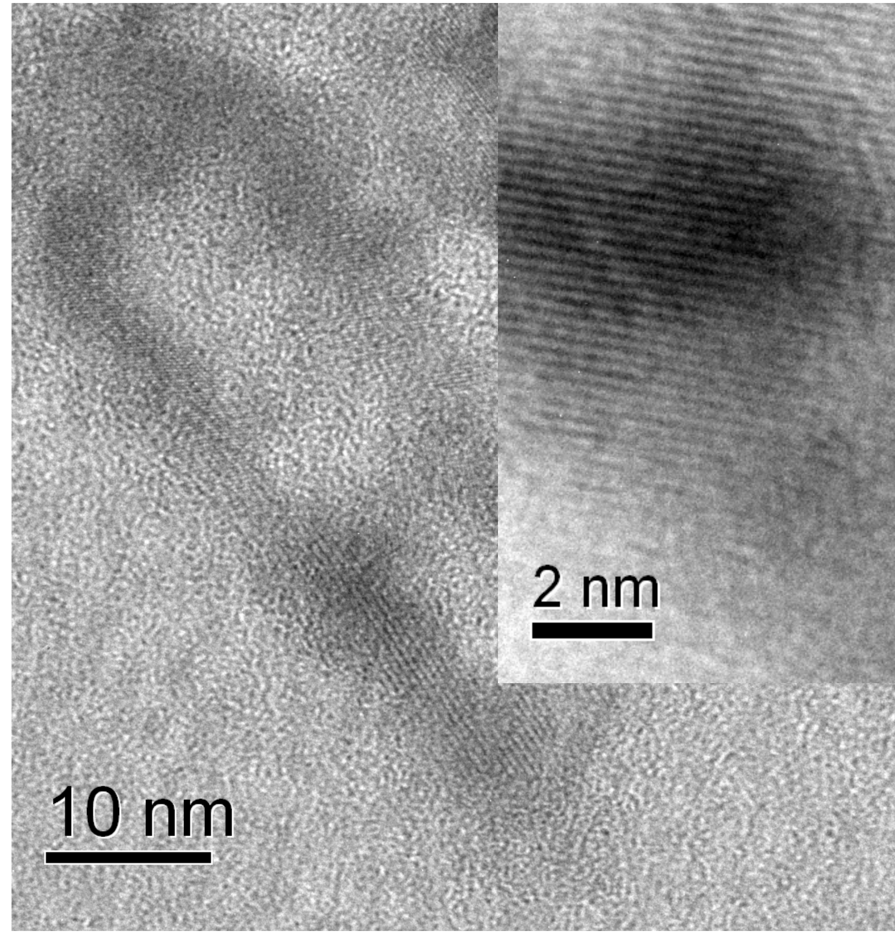
Hollow Hematite (α-Fe_2O_3) Nanocapsules



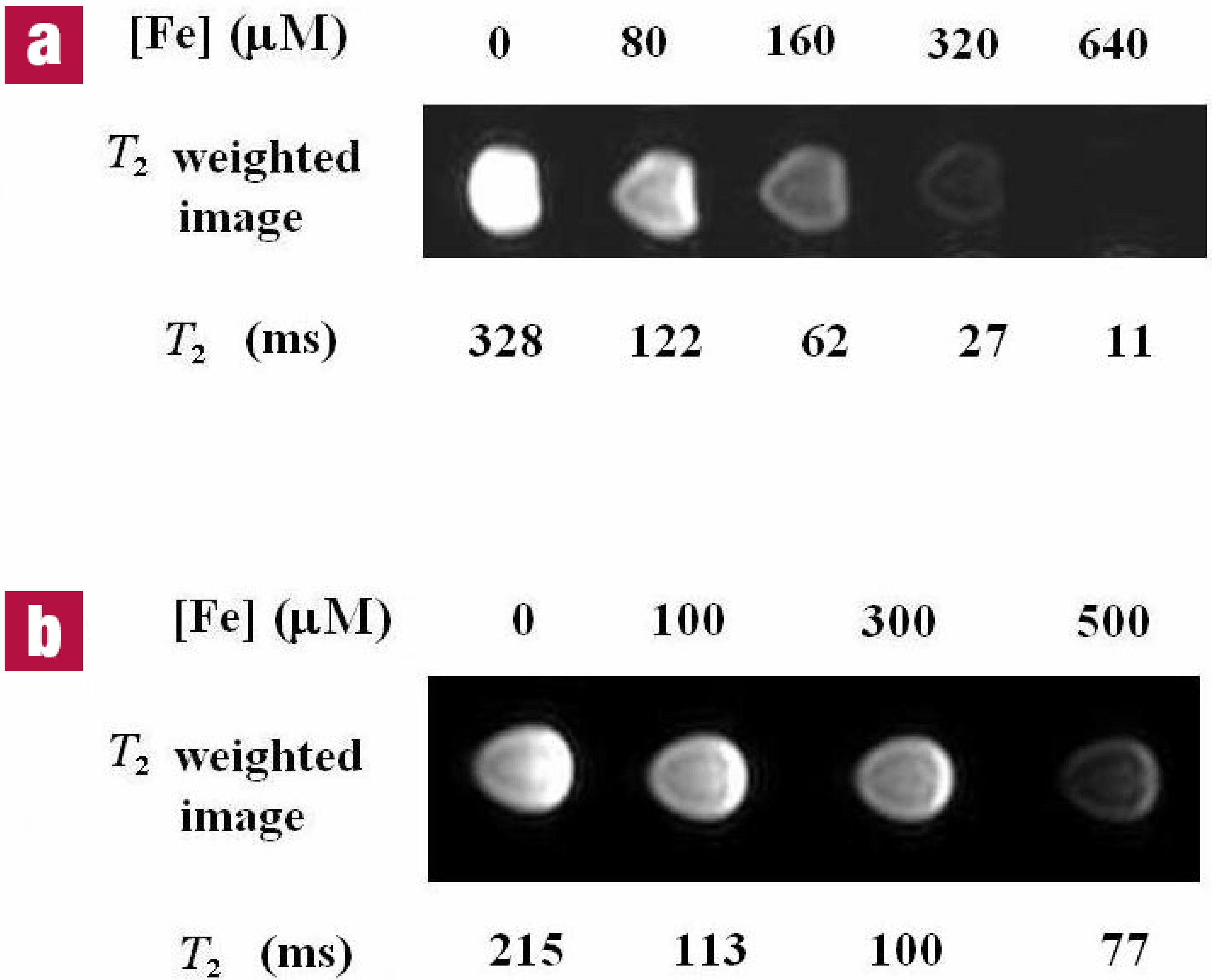


Hollow Magnetite (Fe_3O_4) Nanocapsules

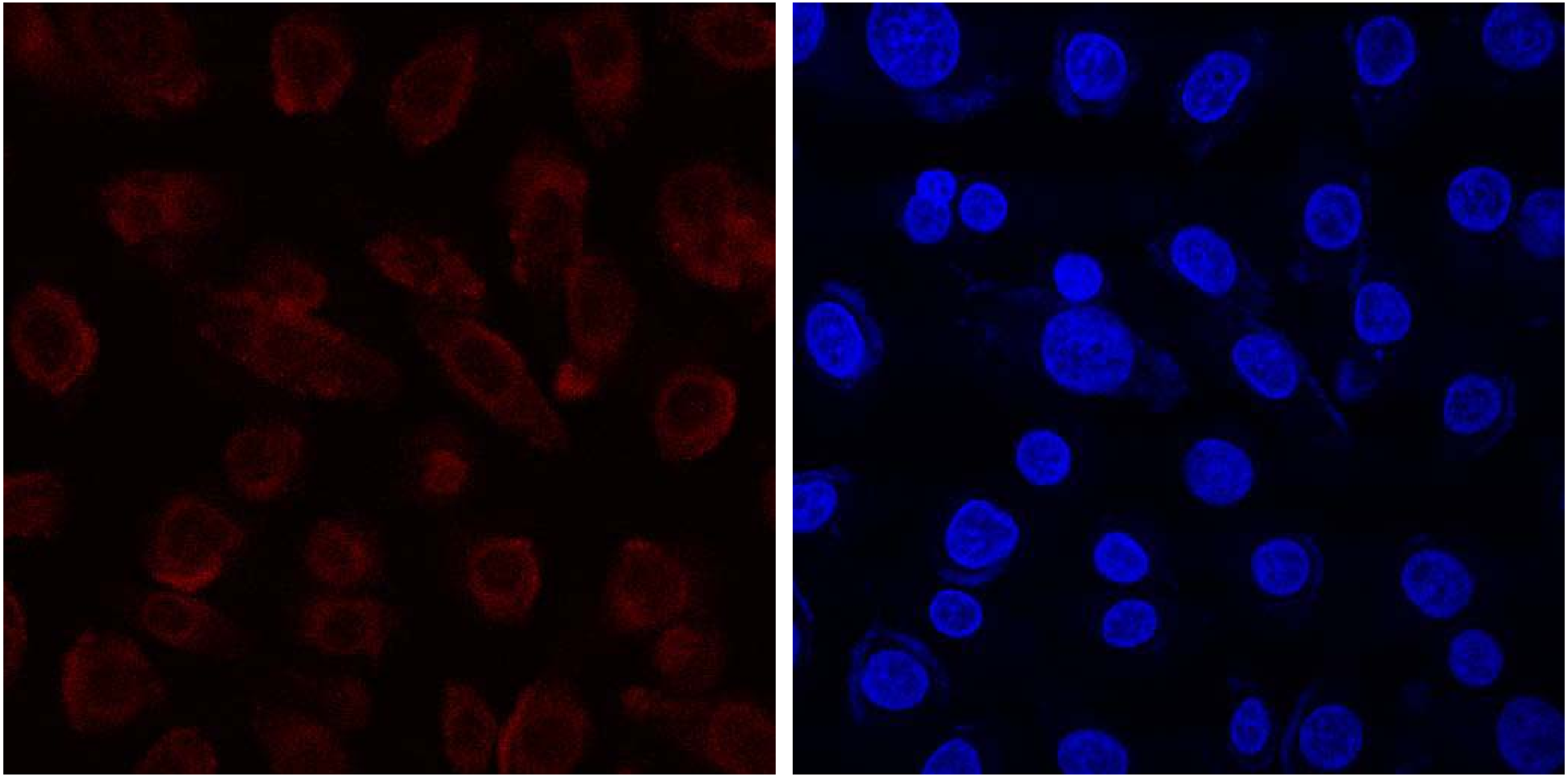




*T*2-weighted MR images of the magnetite nanocapsule



Uptake of free DOX and PEG-MNC-DOX in cancer cells



PEG-MNC-DOX was accumulated mostly in the cytoplasm, whereas free DOX was mostly found in the cell nuclei

SUMMARY

We developed a new T1 MRI contrast agent using MnO anoparticles. We demonstrated MnO nanoparticle contrast-nhanced MRI (MONEMRI) with clear imaging of various mouse rain structures as well as selective imaging of metastatic cance ells in the brain.

Multifunctional drug delivery vehicle based on monodisperse anoparticles embedded in uniform pore-sized mesoporous silic pheres and PLGA particles were fabricated.

We fabricated water-dispersible and biocompatible hollow iron xide nanocapsules via wrap/bake/peel process. The synthesize ater-dispersible magnetite nanocapsules were successfully mployed not only as a drug delivery vehicle, but also as a T2 MR ontrast agent.

We fabricated magnetic gold nanoshells consisting of gold anoshells that are embedded with Fe_3O_4 nanoparticles, and onjugated them with cancer targeting agent for simultaneous M naging and NIR cancer therapy.

I thank God for giving me the opportunity
to work with these excellent and wonderful students.

anocrystal Synthesis: Dr. Jongnam Park, Dr. Joo Jin, Dr. Won Chul Piao, aekyung Yu, Sang-Hyun Choi, Kwangjin Ahn, Jungho Yu, Youngjin Jang, Sun-Ku won, Sung Chul Kim, Jae Sung Son, Dokyun Kim, Mi Hyun Park, Jin Kyung Park

iomedical Applications: Dr. In Su Lee, Hyon Bin Na, Jaeyun Kim, Yong Il Park, Eun Lee, No Hyun Lee, Eung-Gyu Kim, Byunghyo Kim, and Hwan Kim

ast Members: Prof. Sang-Wook Kim (Ajou U.), Dr. Jinwoo Lee (Cornell U.) r. Sangjin Han (U. Minnesota), Kwonnam Sohn; Minsuk Kim; Eunae Kang; oungkwang Yun; In Kyu Park Su (LG Chem); Dr. Seong Lee (IBN, Singapore), rof. Seung Uk Son (SKKU), Sang-Jae Park (Penn. S. U.), Eunwoong Lee (Purdue i Youl Yoon; Bonil Koo (U. Texas), Youjin Lee (MIT), Seon Keun Lee, unhee Chung (Patent Lawyer), Youngjin Yoon, Jin Ahn Lee (Honam Pet.)

$$ Funding $$

- **National Creative Research Initiative Program,**

Korean Ministry of Science & Technology

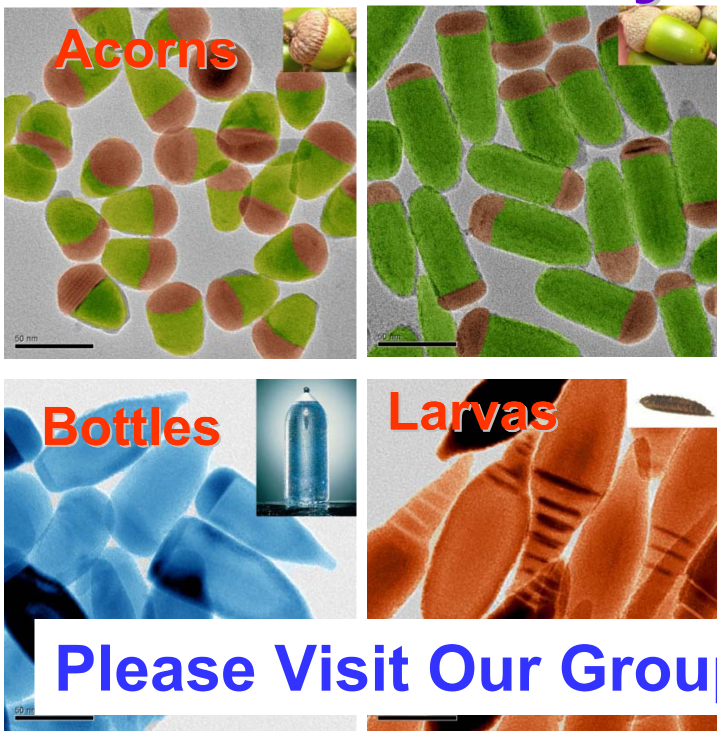
- **Korean Presidential Young Scientist Award,**

Korea Academy of Science and Technology

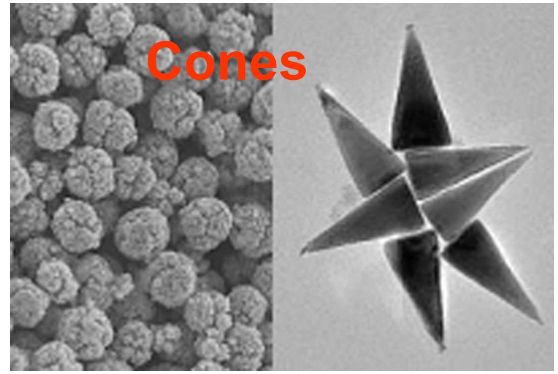
- BK21 Program, Ministry of Education

Thank you very much!

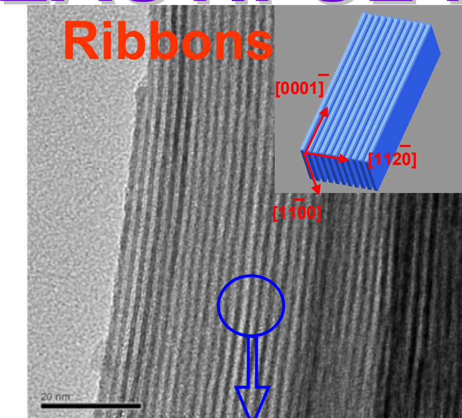
NANO is not only Useful But also BEAUTIFUL !!



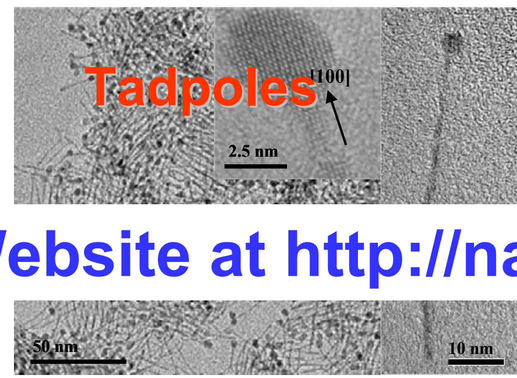
. H. Choi, et al., *J. Am. Chem. Soc* **2006**, 128, 2520.

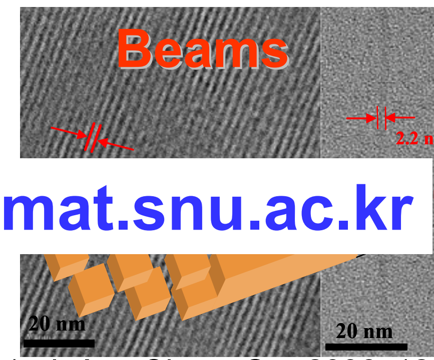


J. Joo, *et al. Adv. Mater.* **2005**, 17, 1873.



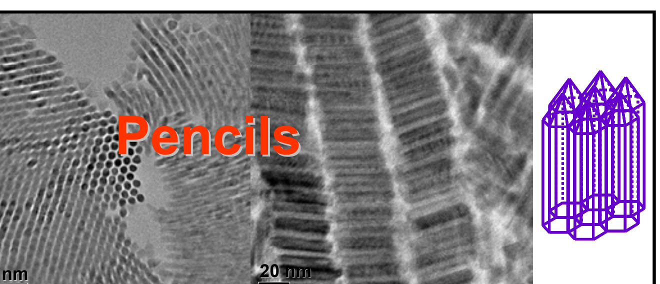
J. Joo et al., *JACS* **2006**, ASAP.



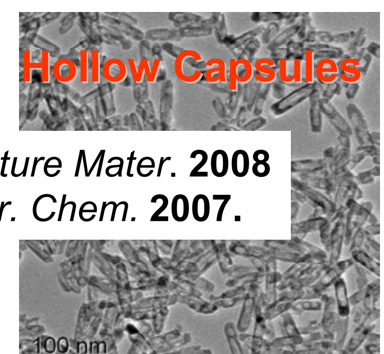


Please Visit Our Group Website at http://nanomat.snu.ac.kr

T. Yoo et al., *Angew. Chem. Int. Ed.* **2005**, 44, 7411; *J. Am. Chem. Soc* **2006**, 128, 1



K. J. An, et al., *J. Am. Chem. Soc* **2006**, 128.



Y. Piao, *Nature Mater.* **2008**
& *J. mater. Chem.* **2007.**