

The Annual Final Scientific Report:

**Development of Convergence Nanoparticles (Phase II):
Detection and Therapeutics of Pathogen Targets Using
Multi-Mode Hybrid Nanoparticle Probe**

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Table of Content

1. Abstract
2. Introduction
3. Approach
4. Results and discussions
5. Pay-off
6. Summary
7. References
8. Research outputs
9. Financial reports
- * Appendix: The first page and the page including acknowledgement part of the published papers.

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14. ABSTRACT Various kinds of pathogens and diseases are now threatening human lives, and their precise and accurate detection and imaging are important to prevent the diseases as well as to understand the biological phenomena. Here we reported a multi-mode nanoprobe system which simultaneously provided highly sensitive detection, imaging, and therapeutic functions in treating of pathogens or diseases. The multi-mode probe consisted of magnetic nanoparticle, targeting molecules, fluorescence tag, and radionuclide and each modality complemented each other to not only increase the detection sensitivity but also eventually to enable the false-free detection of pathogens and disease. Concurrently, the multi-mode nanoparticle also provided high therapeutic effect through combination with biological molecules (e.g. siRNA) or by using heat generation effect of magnetic component.					
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1. Abstract

Various kinds of pathogens and diseases are now threatening human lives, and their precise and accurate detection and imaging are important to prevent the diseases as well as to understand the biological phenomena. Here we reported a multi-mode nanoprobe system which simultaneously provided highly sensitive detection, imaging, and therapeutic functions in treating of pathogens or diseases. The multi-mode probe consisted of magnetic nanoparticle, targeting molecules, fluorescence tag, and radionuclide and each modality complemented each other to not only increase the detection sensitivity but also eventually to enable the false-free detection of pathogens and disease. Concurrently, the multi-mode nanoparticle also provided high therapeutic effect through combination with biological molecules (e.g. siRNA) or by using heat generation effect of magnetic component.

2. Introduction

Detection, imaging, and therapeutics of pathogens and diseases have become important not only to understand their biological phenomena but also to elucidate and to prevent diseases.¹ Recently, various pathogens induce many fatal diseases and have been threatening human lives.² Therefore, fast, accurate and false-free detection and imaging of these pathogens and diseases are critical to understand their metabolic phenomena and to develop defense system against them. Furthermore, once found, the effective killing of such biological targets without harming neighboring biological environments is also important.

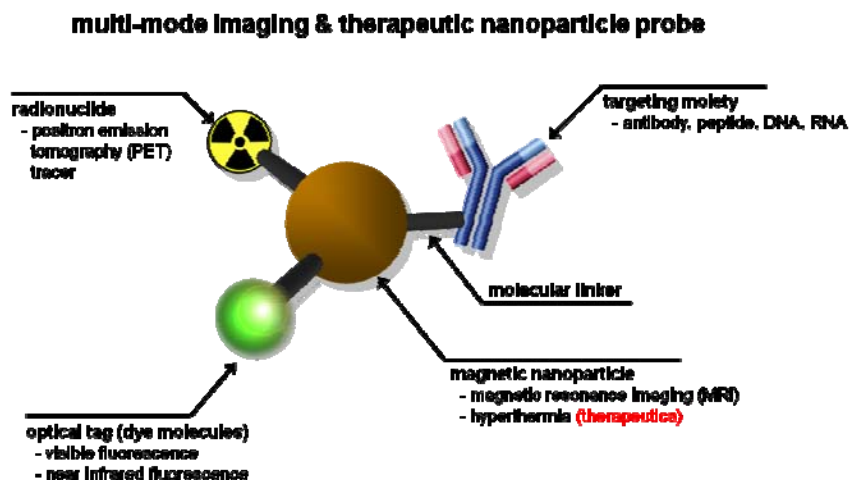


Figure 1. A schematic of multi-mode imaging and therapeutic probe proposed in this research. The magnetic nanoparticle, biomolecules, optical tag, and radionuclide are integrated into one nanoparticulate system which acts as multiplex platform for multi-modal imaging and therapeutics of pathogens.

Typically, imaging techniques including fluorescence, surface plasmon resonance (SPR), and magnetic resonance imaging (MRI)³⁻⁵ have been independently used as a single modality. However, in many cases, single imaging modality is not good enough for accurate imaging and exhibits relatively high false-detection rate. Rather, double or triple imaging modality which complements each modality can provide more accurate information of the pathogens or diseases. (Figure 1) Simultaneously, for the success of multi-mode imaging, the development of nanoparticle based probe is pre-requisite, which can boost up the imaging accuracy and sensitivity significantly. In this project, we developed nanoparticle based multi-mode imaging and therapeutic probe for the highly accurate detection and therapeutics of biological pathogens.

3. Approaches

1) Approach

Our research was focused on the development of multi-mode probes and their

applications on the detection and therapeutic of pathogens and disease. Various imaging modalities (e.g. magnetic core, optical tag, and radionuclide etc.) with target specific biomolecules were integrated into one hybrid nanoparticle via molecular linkers.

In the step 1 (see Figure 2), multi-mode probe was specifically bind to the pathogenic targets through selective biomolecular interactions such as antigen – antibody. In the step 2, such binding events and targeting was detected and imaged by MRI, PET (positron emission tomography), and fluorescence. At this point, MRI and PET provided macroscopic imaging of larger than millimeter sized area and subsequent fluorescence imaging enabled microscopic information of the pathogen targets, where more detailed spatial and chemical/biological information of the pathogens was possible. In the step 3, when these were located, multi-mode nanoparticle could kill the pathogen by using gene (siRNA) or heat generated from magnetic core. As a consequence, multi-mode probes will provide highly sensitive detection/imaging and therapeutic platform of pathogens.

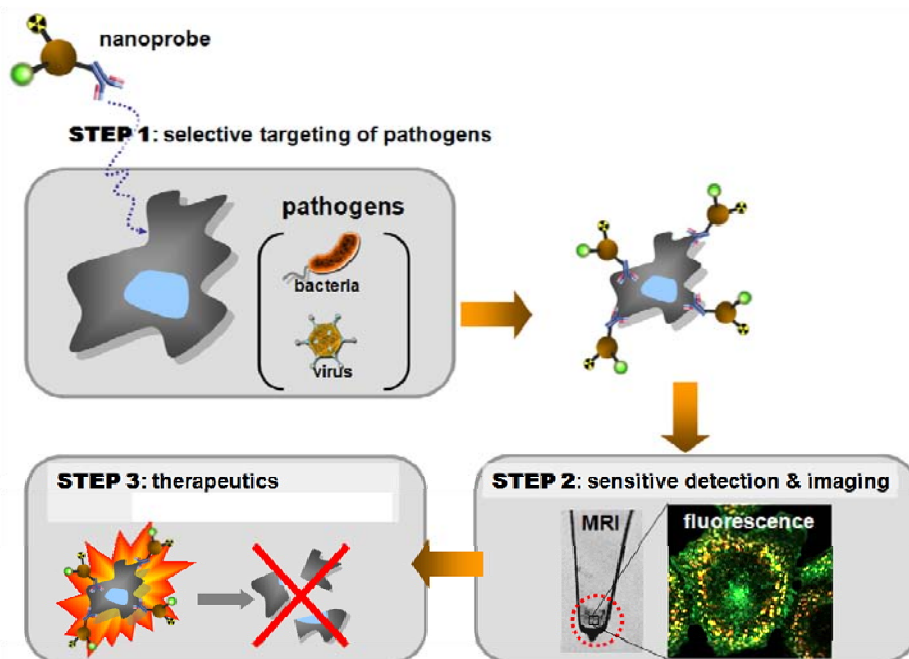


Figure 2. Schematic of multi-modal imaging and therapeutics of pathogens using multi-mode hybrid nanoprobe. The multi-mode hybrid nanoprobe exhibits the specific targeting and provides both macroscale and sub-cellular imaging for attaining biological information of pathogens, which is followed by their death using magnetic hyperthermia.

As a model case study, in the first research year, we performed the feasibility study of dual mode magnetic-radioactive nanoparticle for the dual mode detection. Furthermore, in the following year, the utilization of multi-mode magnetic-bioactive-optical hybrid nanoparticle was demonstrated for accurate detection and therapeutics of

cancer.

2) Uniqueness of the approach

With single modality imaging only, the false-detection rate is high. The major uniqueness of our approach is the accurate detection and simultaneous therapeutics of pathogens by means of multi-modality.

3) Research contents

The 1st year (2008.03~2009.02): Development of multi-mode imaging probe nanoparticles

- Development of magnetic nanoparticles with high magnetism for diagnosis and therapy:
 - a. examination of MR signal enhancement effect of various magnetic nanoparticles
 - b. examination of heat generation ability of various magnetic nanoparticles (therapeutic effect: ~50 %)
- Development of dual-mode convergence nanoparticles: magnetic-radioactive convergence nanoparticles (MRI-PET probe)
- Feasibility test for pathogens or disease detection, imaging and therapy using dual mode nanoprobe
sensitivity: ~ mM of biological targets

The 2nd year (2009.03~2010.02): Convergence nanoparticles for multi-mode diagnosis and therapy

- Development of magnetic nanoparticles with high magnetism for therapy: examination of heat generation ability of various magnetic nanoparticles (therapeutic effect: ~80 %)
- Development of multi-mode convergence nanoparticles: magnetic-optical-radioactive convergences nanoparticles (MRI-PET-fluorescence probe)
- Feasibility test for pathogen detection, imaging and therapy using multi-mode nanoprobe
target pathogens: diseases or bacterial targets
sensitivity: nM~pM of biological targets (single pathogen particle and cellular imaging)

4. Results and discussion

4-1. Development of magnetic nanoparticles with high magnetism for diagnosis and therapy

To enhance the efficacy of disease diagnostic and therapy, the development of magnetic nanoparticles with high magnetism is one of the important issues. Among various magnetic nanoparticles, iron oxide has been mostly utilized in various biological applications because of their low toxicity and chemical stability. In previous project (phase I, FA4869-08-1-4016), to enhance the magnetic property of nanoparticle, we doped Mn ion in iron oxide. In this project, besides Mn, Zn was doped in iron oxide and, as shown in figure 3a, synthesized Zn and Mn doped magnetic nanoparticles had highly monodispersed and possessed single crystallinity. It showed highly enhance magnetic property which was highly enhanced than iron oxide. ($\text{Zn}_{0.4}\text{Mn}_{0.6}\text{Fe}_2\text{O}_4$: 175emu/g, Fe_3O_4 : 110 emu/g) Enhanced magnetic properties of the developed nanoparticle brought the enhanced MR contrast effect. Compared to Feridex, ($\text{Zn}_{0.4}\text{Mn}_{0.6}\text{Fe}_2\text{O}_4$) showed about *ca.* 8 times superior MR signal which make possible ultra-sensitive pathogen detection.

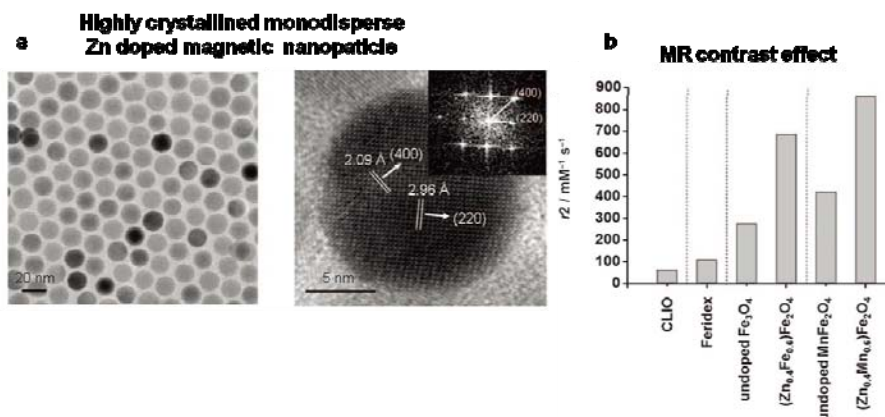


Figure 3. (a) TEM (transmission electron microscope) and HR (high-resolution)-TEM image of Zn doped magnetic nanoparticle. (b) Comparison of MR contrast effect among various magnetic nanoparticles.

Besides the MR signal enhancing effect, the developed nanoparticle showed superior heat generation effect, which made them possible to be utilized in disease therapy. Magnetic nanoparticles generated heat under AC magnetic field. They could reach 100 Celsius in short time so they could be very effective in killing pathogens. (Figure 4a) The developed nanoparticles showed high heat generation efficacy (specific loss power, SLP) and, as shown in figure 4b, it showed *ca.* 4 times bigger

than Feridex. The high SLP resulted superior cancer therapeutic efficacy than commercial iron oxide, Feridex, when they were treated on cancer cell with same concentration. The percentage of dead cell was 84.4 % in the case of $(\text{Zn}_{0.4}\text{Mn}_{0.6})\text{Fe}_2\text{O}_4$ and 13.5% in the case of the Feridex after treatment of AC magnetic field.

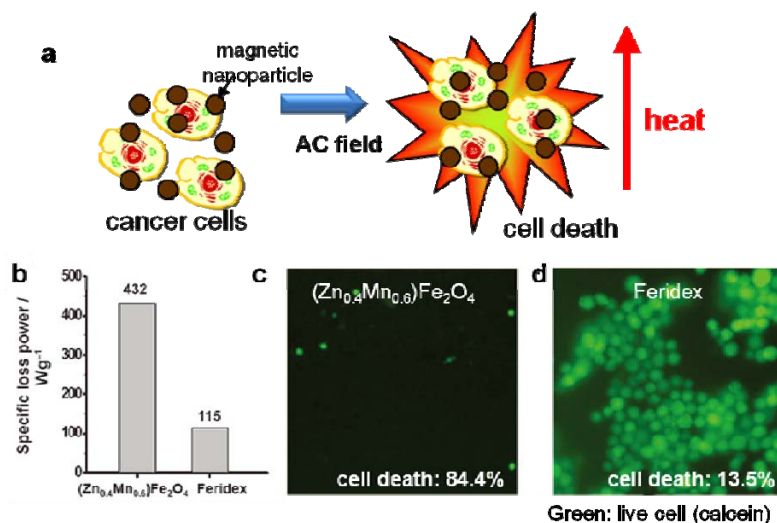


Figure 4. (a) Schematics of magnetic particle induced hyperthermia. The comparison of (b) the SLP value and (c, d) cancer cell killing effect between Feridex and the developed nanoparticle.

Therefore, we could obtain magnetic nanoparticles with high magnetism by doping Zn and Mn ion, which could be utilized for sensitive detection and effective therapy of disease. (*Angew. Chem. Int. Ed.* **2009**, *48*, 1234)

4-2. Development of dual mode magnetic-radioactive convergence nanoparticles (MRI-PET probe)

MR imaging has advantages on the resolution and anatomical information compared to other imaging tools while it has limitation on sensitivity. In this study, as a prototype of multi-mode nanoparticle, magnetic-radioactive dual mode nanoparticles were developed to perform MRI-PET dual mode imaging, which was expected to provide advantages on high sensitivity and resolution. I-124, PET agent, was introduced on MnFe_2O_4 which was developed in phase I. I-124 was selectively bound onto the tyrosine group of albumin which was attached on magnetic core. (Figure 5a) The synthesized dual mode MRI-PET probe showed both MR and PET signals nicely as shown in figure 5b.

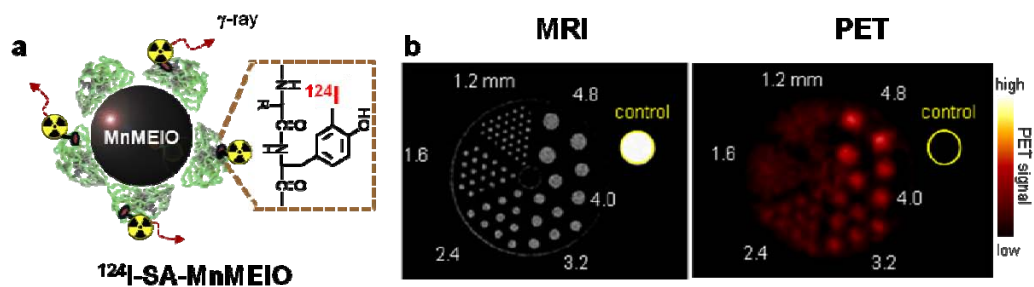


Figure 5. (a) Schematics of the developed MR-PET dual mode probe and (b) MR and PET image of phantom containing the dual mode probe.

The dual mode contrast agent was utilized to detect the sentinel lymph node which was important in the cancer metastasis detection. (Figure 6a) In figure 6b-d, the dual mode contrast agent successfully detected sentinel lymph node and when PET and MR images were overlapped the sentinel lymph node site with high signal intensity in each image perfectly matched. (*Acc. Chem. Res.* **2008**, *41*, 1630.)

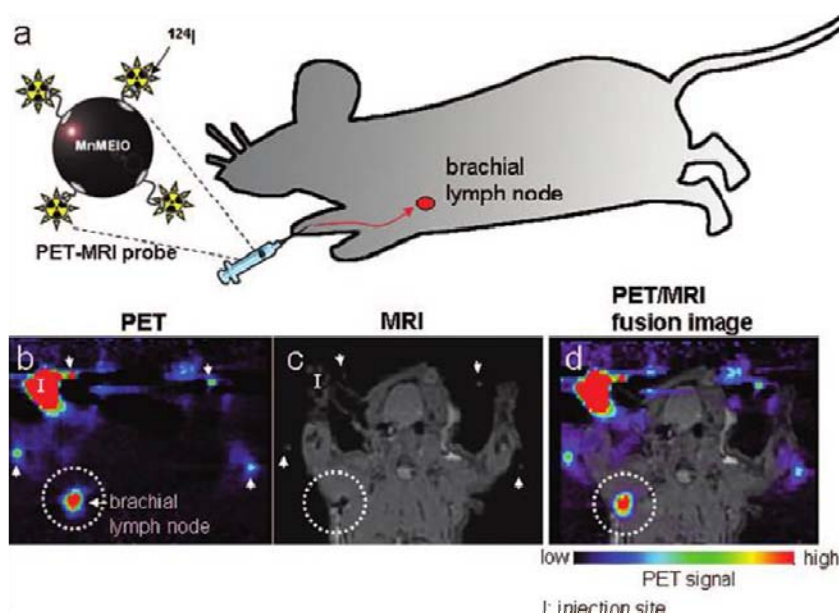


Figure 6. (a) Schematics of the detection of sentinel lymph node using MR-PET dual mode probe and (b)PET, (c) MR, and (d) fusion images of rat which showed high signal in sentinel lymph node in both PET and MRI.

4-3. Development of multi-mode convergence nanoparticle for pathogen detection, imaging and therapy and their feasibility test

Based on the research for proto-type convergence nanoparticle (section 4-2), we finally built multi-functional nanoparticle for simultaneous imaging, detection, and therapy.

- ① Magnetic core was utilized for MR signal enhancing to detect pathogen.
- ② Fluorescence dye was attached for microscopic imaging in cellular level.
- ③ RGD peptide was adopted for pathogen targeting.
- ④ siRNA was introduced for gene therapy and in this study siGFP which deactivated the GFP expression was utilized as a feasibility test.

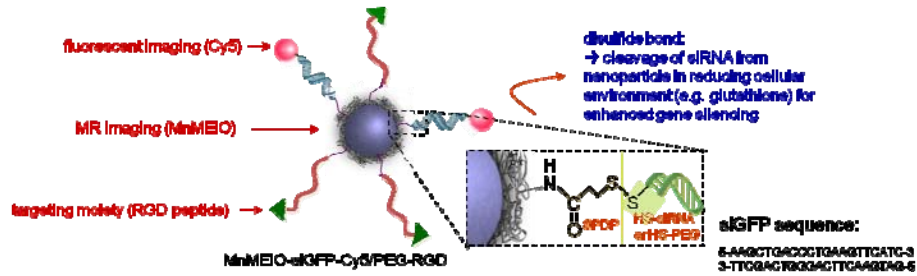


Figure 7. Schematic of multi-modal convergence nanoparticle.

The developed nanoparticle specifically detected cancer cell which have protein $\alpha_v\beta_3$ while no MR signal was observed with cancer cell without the protein. In addition,

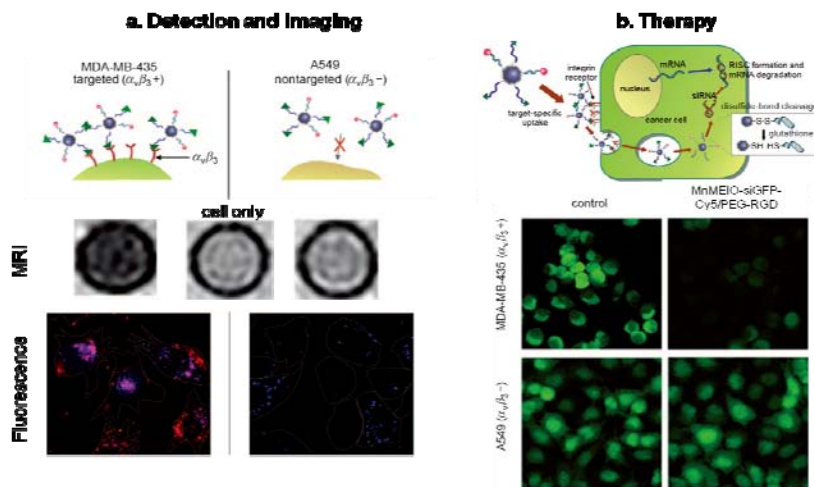


Figure 8. Simultaneous pathogen detection, imaging, and therapy using multi-mode nanoparticle.

red color in fluorescent image revealed the distribution of nanoparticles in cell. Also, the nanoparticle successfully knocked-out the protein expression via gene expression. (*Angew. Chem. Int. Ed.* **2009**, 48, 4174)

5. Pay-off

Based on this project, it will be possible to develop smart and multi-functional nanoparticle probes comprised of sensing, information, and actuation in a single nanosystem and, therefore, can revolutionize current biomedical imaging techniques. The multi-mode nanoparticles with capabilities of target-specificity, ultra-sensitive probing, and therapeutic effects will enable accurate and false-free detection and pinpointing therapy of pathogens or diseases. Furthermore, our multi-mode nanoparticles strategy can serve as a platform concept technology for the next generation biomedical sensing techniques, which will be very useful for anti-pathogenic and anti-bio/chemical warfares as well as normal biomedical applications.

6. Summary

We demonstrated high performance multi-functional nanoparticles. By integrating individual nanoparticle components such as magnetic, bio-active, optical, radioactive, and heat-generating materials into a single nanosystem *via* molecular linkers, it was possible to develop a novel, highly versatile, multi-functional convergence nanoparticle system for their multi-functional biomedical applications. Subsequently, we examined their utility in multi-mode detection/imaging/therapeutic applications to realize simultaneous accurate and false-free diagnosis and therapy of biological targets. The use of the multi-mode nanoparticle probe can bring significant improvements of current diagnosis and therapeutic techniques.

7. References

1. Brandenburg, B.; Zhuang, X. *Nat. Rev. Microbiol.* **2007**, *5*, 197.
2. Lindsay, J. A.; Holden, M. T. *Trends Microbiol.* **2004**, *12*, 378.
3. Herweh, C.; Jayachandra, M. R.; Hartmann, M.; Gass, A.; Sellner, J.; Heiland, S.; Nagel, S.; Hähnel, S.; Meyding-Lamadé U. *J. Neurovirol.* **2007**, *13*, 426.
4. Payne C. K. *Nanomed.* **2007**, *2*, 847.
5. Ro, H. S.; Koh, B. H.; Jung, S. O.; Park, H. K.; Shin, Y. B.; Kim, M. G.; Chung, B. H. *Proteomics* **2006**, *6*, 2108.

8. Research outputs

8-1. Publications

1. Cheon, J. *et al.*
"Synergistically Integrated Nanoparticles as Multimodal Probes for Nanobiotechnology"
Acc. Chem. Res. **2008**, *41*, 1630. [I.F. = 12.176]
2. Cheon, J. *et al.*
"Critical Enhancements of MRI Contrast and Hyperthermic Effects by Dopant-Controlled Magnetic Nanoparticles"
Angew. Chem. Int. Ed. **2009**, *48*, 1234. [I.F. = 10.879]
3. Cheon, J. *et al.*
"All-in-One Target-Cell-Specific Magnetic Nanoparticles for Simultaneous Molecular Imaging and siRNA Deliver"
Angew. Chem. Int. Ed. **2009**, *48*, 4174. [I.F. = 10.879]

8-2. Conferences

1. Cheon, J.
"Dual-modality PET-MRI probe for sentinel lymph node detection"
NanoBio-Seoul 2008, Yonsei University, Seoul, Korea, Oct. 31. 2008.
2. Cheon, J.
"Design of High Performance Nanoparticle Technology for Multi-Modal Biomedical Applications"
NanoBio-Seoul 2008, Yonsei University, Seoul, Korea, Oct. 30. 2008.
3. Cheon, J.
"Synergistically Intergrated Multimodal Nanoparticle Probe for Biomedical Application."
NCI Alliance for Nanotechnology in Cancer Investigators Meeting, Chicago, IL, U S A, Sep. 08. 2008.
4. Cheon, J.
"Design of magnetic nanoparticles for highly sensitive diagnostics and therapeutics."
236th ACS National Meeting & Exposition, Philadelphia, PA, USA, Aug. 18, 2008.

5. Cheon, J.
 "Dopant Controlled Metal Oxide Nanoparticles for High Performance Magnetic Resonance Imaging and Hyperthermia Effects"
 The University of Kyushu-Yonsei University Joint Symposium, Seoul, Korea, Aug 24, 2009.
6. Cheon, J.
 "Dopant Controlled Magnetism Tuning of Metal Oxide Nanoparticles for High Performance Magnetic Resonance Imaging and Hyperthermic Effects"
 Materials Research Society Meeting, MRS, Boston, MA, USA, Dec. 01, 2009.
7. Cheon, J
 "Four-in-One Targeted Gene Suppression Using Magnetic Nanoparticles for simultaneous Molecular Imaging and siRNA Delivery"
 Materials Research Society Meeting, MRS, Boston, MA, USA, Dec. 01, 2009.

8-3.Press Release

1. "Targeted Gene Suppression in Cancer Cell-Four-in-one"
Nanotechnology Now, May 06, 2009.
2. "Four in one; Targeted Gene Suppression in Cancer Cell"
Wiley InterScience, May 08, 2009.
3. "Combination nanoparticles to fight cancer"
Chemistry World, May 13, 2009.
4. "4-in-1; Targeted gene suppression in cancer cells"
ChemEurope.com & Bionity.com, May 12, 2009
5. "Four-in-one agent for targeted gene suppression in cancer cells"
Nanowerk News May 06, 2009.
6. "Four in one; Targeted Gene Suppression in Cancer Cell"
PhysOrg.com May 06, 2009.
7. "4-in-1"
Bio-Medicine May 07, 2009
8. "Four in one"
e-Science News May 08, 2009
9. "4-in-1"
Association of Cancer Online Resource Published: May 07, 2009
10. "Diagnosis and therapy at a time"
Yonhap News(Korean local news), May 05, 2009.

11. "All-in-One Nano Agent to Combat Tumor"

The Korea Times (Korean local news), May 11, 2009

9. Financial Reports

I. Planned Expenditure (Mar 1, 2008~ Feb. 28, 2010)	II. EXPENDITURES
SALARIES	
A. SENIOR PERSONNEL \$ 52,000	SALARIES
B. OTHER PERSONNEL \$ 20,000	A. SENIOR PERSONNEL: \$ 52,000
C. FRINGE BENEFITS \$ 6,000	Jinwoo Cheon : \$ 2,000 * 24 month = \$ 24,000
	B. OTHER PERSONNEL \$ 20,000
	Jae-Hyun Lee: \$ 1,000 * 20 month = \$ 20,000
	C. FRINGE BENEFITS \$ 6,000
D. EQUIPMENT \$ 0	D. EQUIPMENT \$ 0
E. TRAVEL \$ 9,000	E. TRAVEL \$ 9,000
	Jinwoo Cheon
	San Francisco (Research Discussion)
	Airfare: \$ 2,500 + Travel Cost: \$2,000 = \$ 4,500
	San Diego (Research Discussion)
	Airfare: \$ 2,500 + Travel Cost: \$2,000 = \$ 4,500
F. TUITION REF 2.12.13 \$ 0	F. TUITION REF 2.12.13 \$ 0
G. OTHER DIRECT COSTS \$ 25,500	G. OTHER DIRECT COSTS \$ 25,500
1. SUPPLIES/MATERIALS \$ 21,000	1. SUPPLIES/MATERIALS \$ 21,000
2. PUBLICATION AND REPORTS \$ 2,000	* See the detailed materials sheet.
3. COMPUTER SERVICE \$ 2,500	2. PUBLICATION AND REPORTS \$ 2,000
	Copies \$2,000
	3. COMPUTER SERVICE \$ 2,500
	Computer for analysis 1 set \$ 2,500
J. TOTAL DIRECT EXPENSES AND FACILITIES AND ADMINISTRATION EXPENSES	J. TOTAL DIRECT EXPENSES AND FACILITIES AND ADMINISTRATION EXPENSES
Overhead \$ 37,500	Overhead \$ 37,500
Total: \$150,000	Total: \$150,000

* Detailed materials sheet

Item	Unit price (\$)	Amount	Total (\$)
Iron pentacarbonyl (Strem 26-2800, 250 g)	70	10	700
Dicobalt octacarbonyl (Fluka 60811, 25g)	200	10	2,000
Zn Chloride (Aldrich 45011, 5g)	100	20	2,000
1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (Pierce 25952-53-8, 25g)	500	5	2,500
Oleylamine (Aldrich O7805, 500g)	130	10	1,300
Oleic acid (Sigma O1383, 25 g)	150	10	1,500
Sephadex G-25(Sigma S5772, 25g)	200	5	1,000
TBE buffer (Sigma T4415, 10x, 20 L)	250	5	1,250
1,2-Hexadecanediol, Tech (Aldrich 213748, 50g)	180	10	1,800
Iron(III) acetylacetonate (Aldrich 517003, 50g)	150	10	1,500
Antibody	170	10	1,700
Hexane (Aldrich 156175, 1L)	70	20	1,400
Ethanol (Aldrich 187380, 1L)	70	20	1,400
Acetone (Aldrich 154598, 1L)	70	20	1,400
Total			\$ 21,000

ACCOUNTS of chemical research

Synergistically Integrated Nanoparticles as Multimodal Probes for Nanobiotechnology

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RECEIVED ON FEBRUARY 12, 2008

CON SPECTUS

Multimodal Nanoprobe



Current biomedical imaging techniques including magnetic resonance imaging (MRI), positron emission tomography (PET), and computed X-ray tomography (CT) are vital in the diagnosis of various diseases. Each imaging modality has its own merits and disadvantages, and a single technique does not possess all the required capabilities for comprehensive imaging. Therefore, multimodal imaging methods are quickly becoming important tools for state-of-the-art biomedical research and clinical diagnostics and therapeutics.

In this Account, we will discuss synergistically integrated nanoparticle probes, which will be an essential tool in multimodal imaging technology. When inorganic nanoparticles are introduced into biological systems, their extremely small size and their exceptional physical and chemical properties make them useful probes for biological diagnostics. Nanoparticle probes can endow imaging techniques with enhanced signal sensitivity, better spatial resolution, and the ability to relay information about biological systems at the molecular and cellular levels.

Simple magnetic nanoparticles function as MRI contrast enhancement probes. These magnetic nanoparticles can then serve as a core platform for the addition of other functional moieties including fluorescence tags, radionuclides, and other biomolecules for multimodal imaging, gene delivery, and cellular trafficking. For example, MRI–optical dual-modal probes composed of a fluorescent dye-doped silica (DySiO_2) core surrounded by magnetic nanoparticles can macroscopically detect neuroblastoma cancer cells via MRI along with subcellular information via fluorescence imaging.

Magnetic nanoparticles can also be coupled to radionuclides (^{124}I) to construct MRI–PET dual-modal probes. Such probes can accurately detect lymph nodes (LNs), which are critical for assessing cancer metastasis. *In vivo* MRI/PET images can clearly identify small (~ 3 mm) LNs along with precise anatomical information. Systems using multicomponent nanoparticles modified with biomolecules can also monitor gene expression and other markers in cell therapeutics studies. We have used hybrid stem cell–magnetic nanoparticle probes with MRI to monitor *in vivo* stem cell trafficking. MRI with hybrid probes of magnetic nanoparticles and adenovirus can detect target cells and can monitor gene delivery and the expression of green fluorescent proteins optically. Each component of such multimodal probes complements the other modalities, and their synergistic materials properties ultimately provide more accurate information in *in vitro* and *in vivo* biological systems.

1. Introduction

Inorganic nanoparticles possess unique nanoscale size-dependent physical and chemical properties

that can be controlled in a manner that is not allowed in bulk size materials.¹ When these tiny materials are introduced into biological systems, their extremely small size and their exceptional

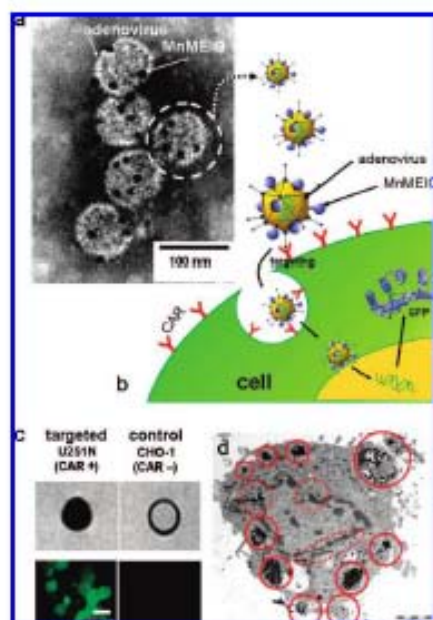


FIGURE 11. Adenovirus-MnMEIO hybrid nanoparticle probes for targeted MR imaging and gene delivery: (a) TEM image of the adenovirus-MnMEIO hybrid nanoparticles; (b) a schematic of the targeting and gene delivery processes using the adenovirus-MnMEIO hybrid nanoparticle probes, which are internalized through CAR-mediated endocytosis processes; (c) such events are imaged only in the CAR-positive cells by a dark contrast in MRI and the vivid GFP (green fluorescent protein) gene expression; (d) TEM image of adenovirus-MnMEIO hybrid nanoprobe treated U251N cells. Solid circles indicate nanoprobe either under endocytosis or trapped inside endosomes. Dashed circles indicate some nanoprobe found near the nuclear membrane. Reproduced with permission from ref 30. Copyright 2007 Wiley-VCH.

ment as a heat generator for hyperthermia or as a guiding vector to the targeted area.³⁴ Although still in its early stages with only a handful of successfully demonstrated cases, the continued development of such multimodal probes is increasingly important for advancing this exciting and rapidly changing research field.

We acknowledge Dr. Young-wook Jun for his helpful discussion. This research is supported by the National Research Laboratory (Grant M10600000255), Nano-Bio Science & Technology Program (Grant M1050300218-05M0300-21810), AOAD-AFOSR, NCI Center for Cancer Nanotechnology Exce-

llence (CCNE), NBIT (Grant K20716000001-07A0400-00110), and 2nd stage BK21 for Chemistry.

BIOGRAPHICAL INFORMATION

Jinwoo Cheon is a chemistry professor of Yonsei University, the director of Convergence Nanomaterials National Research Laboratory, and the head of the Nanomaterials Division of the Nano-Medical National Core Research Center of Korea. He graduated from Yonsei University with B.S. and received his Ph.D. from University of Illinois, Urbana-Champaign, in 1993. After postdoctoral training at U.C. Berkeley and also at UCLA, he joined KAIST as an assistant professor. In 2002, he moved to Yonsei University. His current research interest includes the development of functional inorganic nanostructures and their applications for biomedical and energy related sciences.

Jae-Hyun Lee, born in Seoul, Korea, graduated from Yonsei University in 2003 with his B.S. He is a graduate student pursuing his Ph.D. in chemistry under the supervision of Professor Jinwoo Cheon. His current research interests are the fabrication of bioactivatable hybrid magnetic nanoparticles for molecular imaging and therapeutics. He is a recipient of the Korea Research Foundation Fellowship (2004), Seoul Science Fellowship (2005), and Yonsei Graduate Student Research Award (2007).

FOOTNOTES

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REFERENCES

- (a) Alkaskas, A. P. Semiconductor Dots, Nanocrystals, and Quantum Dots. *Science* 1996, 271, 933-937. (b) Murray, C. B.; Norris, D. J.; Bawendi, M. G. Synthesis and Characterization of Nearly Monodisperse CdE (E = sulfur, selenium, tellurium) Semiconductor Nanocrystallites. *J. Am. Chem. Soc.* 1993, 115, 8706-8715. (c) Link, S.; El-Sayed, M. A. Spectral Properties and Relaxation Dynamics of Surface Plasmon Electronic Oscillations in Gold and Silver Nanoparticles and Nanorods. *J. Phys. Chem. B* 1999, 103, 8410-8426.
- (a) Cao, Y. W.; Jin, R.; Minko, C. A. Nanoparticles with Raman Spectroscopic Fingerprints for DNA and RNA Detection. *Science* 2002, 297, 1536-1540. (b) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Lathrop, R. L.; Minko, C. A. Selective Colorimetric Detection of Polynucleotides Based on the Distance-Dependent Optical Properties of Gold Nanoparticles. *Science* 1997, 277, 1078-1081. (c) Qian, X.; Peng, X.-H.; Ansari, D. O.; Yin-Guo, G.; Chen, G. Z.; Shin, D. M.; Yang, L.; Young, A. M.; Wang, M. D.; Nie, S. In Vivo Tumor Targeting and Spectroscopic Detection with Surface-Enhanced Raman Nanoparticle Tags. *Nat. Biotechnol.* 2007, 25, 83-90.
- (a) Michael, K.; Prasad, F. F.; Baritola, L. A.; Tsay, J. M.; Doss, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. Quantum Dots for Live Cells, In Vivo Imaging, and Diagnostics. *Science* 2005, 307, 539-544. (b) Mochly, I. L.; Uyeda, H. T.; Goldman, E. R.; Mochly, H. Quantum Dot Bioconjugates for Imaging, Labeling and Sensing. *Nat. Mater.* 2006, 4, 435-446. (c) Kim, S.; Lim, Y. T.; Soltesz, E. G.; De Gracia, A. M.; Lee, J.; Nakagawa, A.; Parker, J. A.; Mihaljovic, T.; Laurence, R. G.; Dor, D. M.; Cohn, L. H.; Seward, M. G.; Frangioni, J. V. Near-Infrared Fluorescent Type II Quantum Dots for Sentinel Lymph Node Mapping. *Nat. Biotechnol.* 2004, 22, 93-97.
- (a) Laurent, S.; Forge, D.; Port, M.; Roch, A.; Roble, C.; Elst, L. V.; Muller, R. N. Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications. *Chem. Rev.* 2008, 108, 2064-2110. (b) Jun, Y.-w.; Bao, J.-w.; Cheon, J. Nanosizing Laws of Magnetic Nanoparticles and Their Applications in Biomedical Sciences. *Acc. Chem. Res.* 2008, 41, 179-189. (c) Goodwin, S.; Peterson, C.; Hoh, C.; Blitzer, C. Targeting and Retention of Magnetic Targeted Carriers (MTCs) Enhancing Intratumoral Chemotherapy. *J. Magn. Magn. Mater.* 1999, 194, 132-139. (d) Yew, C. T.; Mayo, J. T.; Yu, W. W.; Prasad, A.; Fekken, J. C.; Yoon, S.; Gong, L.; Shipley, H. J.; Kan, A.; Tomson, M.; Nabelek, D.; Colvin, V. L. Low-Field Magnetic Separation of Monodisperse Fe₃O₄ Nanocrystals. *Science* 2006, 314, 964-967. (e)

Critical Enhancements of MRI Contrast and Hyperthermic Effects by Dopant-Controlled Magnetic Nanoparticles**

Jung-tak Jang, Hyunsoo Nah, Jae-Hyun Lee, Seung Ho Moon, Min Gyu Kim, and Jinwoo Cheon*

Magnetic characteristics are crucial for the successful performances of magnetic nanoparticles in biomedical applications such as magnetic resonance imaging (MRI), drug delivery, cellular signaling, and hyperthermia.^[1–4] Therefore, the development of new types of nanoparticles is particularly important. In this regard, a metal dopant substitution strategy of metal ferrite nanoparticles has been pursued to achieve high and tunable nanomagnetism.^[5] In the case of Zn^{2+} doping, however, the use of nonequilibrium reactions has typically resulted in nonstoichiometric or metastable states in which Zn^{2+} ions are disordered between T_d and O_A sites.^[6–8] A recent report of successful Zn^{2+} doping includes the use of diethyl zinc (Et_2Zn) as a new Zn^{2+} ion source;^[9] however, because of the highly unstable and pyrophoric nature of the precursor, such a synthetic protocol for nanoparticles is still far from ideal for the achievement of large-scale reproducibility and precise dopant controls. In this study, we have overcome a number of previous challenges; not only is size monodispersity with a large-scale (ca. 10 g) synthesis achieved, the proper positioning of Zn^{2+} dopants in T_d sites in metal ferrite nanoparticles is also demonstrated, which ultimately leads to successful magnetism tuning. Our obtained nanoparticles exhibit an extremely high magnetization value (175 emu g^{-1}) and provide the largest MRI contrast effects ($r_2 = 860 \text{ mM}^{-1} \text{ s}^{-1}$) among the contrast agents reported to date. They have an eight- to fourteenfold increase in MRI contrast and a fourfold enhancement in hyperthermic effects compared to conventional iron oxide nanoparticles.

For decades, iron oxide (Fe_3O_4) nanoparticles have served as the model material in the biomedical research field associated with magnetism.^[10] However, considering that the effects of magnetic nanoparticles for biomedical applications

are strongly dependent on their magnetic characteristics, it is important to devise nanoparticles with high and tunable magnetism, especially saturation magnetization (M_s) values, while maintaining high size monodispersity. For example, nanoparticles with tunable magnetism, such as manganese-doped metal ferrite and FeCo nanoparticles, have enhanced MRI contrast effects that are significantly superior to that of conventional iron oxide nanoparticles.^[10,11] This enhancement is significant for clinical purposes as the nanoparticle probe dosage level can be progressively lowered when using probes that have improved contrast enhancement effects. In the first part of this study, we present a large-scale, simple, and reliable synthetic protocol to achieve Zn^{2+} doping controlled metal ferrite nanoparticles. A one-pot thermal decomposition method was used, which involved a metal chloride (MCl_2 , $\text{M} = \text{Zn}^{2+}$, Mn^{2+} , and Fe^{2+}) and iron tris-2,4-pentadionate ($[\text{Fe}(\text{acac})_3]$) in the presence of oleic acid, oleylamine, and octyl ether.^[12] The Zn^{2+} doping level, a key parameter, was carefully controlled by varying the initial molar ratio of the metal chloride precursors. As shown in Figure 1 a–c, a series of 15 nm sized Zn^{2+} doped nanoparticles of $(\text{Zn}_x\text{Mn}_{1-x})\text{Fe}_2\text{O}_4$ and $(\text{Zn}_x\text{Fe}_{1-x})\text{Fe}_2\text{O}_4$ ($x = 0, 0.1, 0.2, 0.3, 0.4$, and 0.8) with single crystallinity and size monodispersity ($\sigma < 5\%$) were

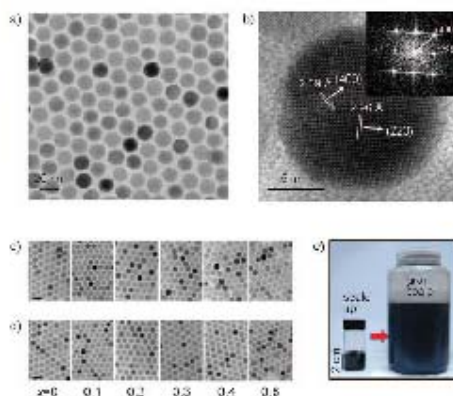


Figure 1. a) TEM image of 15 nm $(\text{Zn}_{0.8}\text{Fe}_{0.2})\text{Fe}_2\text{O}_4$ nanoparticles. b) High-resolution TEM image of 15 nm $(\text{Zn}_{0.8}\text{Fe}_{0.2})\text{Fe}_2\text{O}_4$ nanoparticles. The inset shows the FFT pattern. c, d) TEM images of 15 nm $(\text{Zn}_{0.8}\text{Mn}_{0.2})\text{Fe}_2\text{O}_4$ (c) and $(\text{Zn}_{0.8}\text{Fe}_{0.2})\text{Fe}_2\text{O}_4$ (d) nanoparticles (scale bar: 20 nm). e) Photograph showing that the synthesis of 15 nm $(\text{Zn}_{0.8}\text{Fe}_{0.2})\text{Fe}_2\text{O}_4$ nanoparticles can be scaled up to ca. 10 g.

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All-in-One Target-Cell-Specific Magnetic Nanoparticles for Simultaneous Molecular Imaging and siRNA Delivery**

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There has been considerable interest in the development of a variety of functional inorganic nanoparticles that can be applied in biomedical technologies, including nanotherapeutics, diagnostics, biosensors, and medical imaging.^[1,2] Of particular significance is the use of magnetic nanoparticles for magnetic resonance imaging (MRI), cell and protein separation, heat generation, and magnetism-induced cellular mechanotransduction.^[3,4] The ease of the synthesis of magnetic nanoparticles and subsequent surface modifications to introduce additional therapeutic and diagnostic functionalities has enabled these systems to be employed as smart nanomedicines that incorporate combinations of different components.^[5] Various cell-specific targeting, imaging, and therapeutic functions can be incorporated into a single magnetic nanoparticle which is designed for simultaneous diagnostic and therapeutic use, without losing the individual properties of each component.

It is known that synthetic small interfering RNA (siRNA) can inhibit specific protein expression by suppressing a target gene selectively at the posttranscriptional mRNA level by a mechanism called RNA interference (RNAi).^[6] siRNAs have been studied extensively to treat various genetic diseases, including cardiovascular diseases and various cancers.^[7] The development of appropriate carrier systems is crucial for practical applications of siRNAs as therapeutics. However, many siRNA-carrier systems, including cationic polymers and lipids, possess inherent deficiencies associated with target-cell-specific gene inhibition and biocompatibility.^[8,9]

A number of inorganic nanocrystalline materials have been emerging as potential siRNA carriers in systems devised for simultaneous imaging and therapeutic purposes.^[10,11] Magnetic nanoparticles are highly attractive platform materials for siRNA delivery owing to their unique properties which include uniform size, biocompatibility, superior imaging characteristics, and facile surface modification. Cell-specific targeting strategies for high-performance magnetic nanoparticles with dual (diagnostic and therapeutic) functions also include the immobilization of various target-specific moieties, such as antibodies, small peptides that can be recognized by cells, and aptamers.^[12] In particular, Arg-Gly-Asp (RGD) conjugated nanoparticles bind strongly to $\alpha_v\beta_3$ integrin, which is overexpressed in both endothelial and specific cancer cells, and internalize into the cells by receptor-mediated endocytosis, whereas RGD itself does not promote the endosomal escape of nanoparticles into the cytoplasm.^[13]

In this study, we developed "all-in-one" nanoparticle probes for simultaneous delivery and multimodal imaging. The magnetic nanoparticle probes were conjugated with siRNAs, cancer-cell-specific targeting moieties, and fluorescent dyes. Our purpose in designing the multifunctional nanoprobe was to enable highly accurate imaging to be carried out simultaneously with the delivery of therapeutics. This approach is clinically important, as it can minimize invasiveness and deleterious side effects. As a result of the ability of the probes to target specific cells, siRNAs will be unloaded only inside targeted cells with certain receptors; the disulfide linkages between siRNAs and the nanoparticles should be cleaved inside the cells. In principle, it is possible to monitor where the probes with therapeutics are located in the targeted areas. More specifically, magnetic nanoparticles are useful for visualizing the location and trafficking of siRNA for in vivo applications. However, the innate limit of spatial resolution in MRI (ca. 100 μm) makes it impossible to monitor the intracellular transfection of siRNA by MRI. For this reason, the fluorescent dye is required for sensitive subcellular visualization.

For the fabrication of multifunctional magnetic nanoparticles, manganese-doped magnetism-engineered iron oxide (MnMEIO) nanoparticles of 15 nm in size coated with bovine serum albumin (BSA) were used as the core material (Figure 1a) owing to their high degree of size monodispersity, the ease with which their surface can be modified, and their higher magnetic moment than that of other conventional iron oxide based magnetic nanoparticles.^[22] The last property translates into enhanced MRI signals. For bioconjugation and nanoparticle delivery into the cytoplasm of cells, cationized BSA with a pI (isoelectric point) value of 6.1 was used.^[13,14] The primary amine groups of

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