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Multifunctional Magnetic Nanoparticles: Design, Synthesis, and Biomedical Applications

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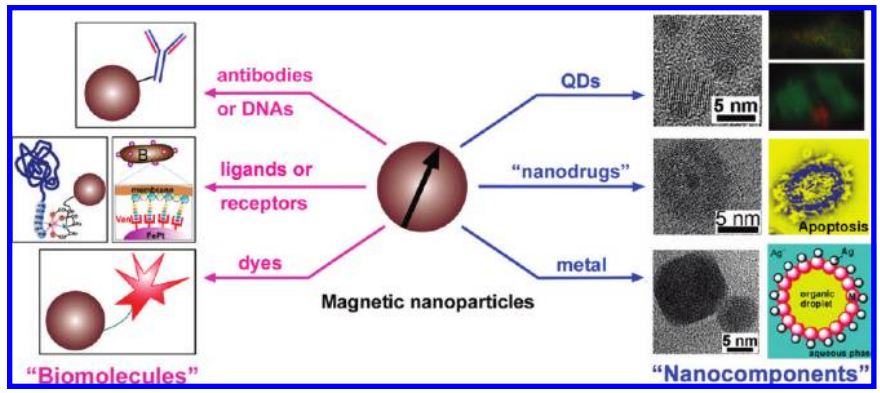

The combination of nanotechnology and molecular biology has developed into an emerging research area: nanobiotechnology. Magnetic nanoparticles are well-established nanomaterials that offer controlled size, ability to be manipulated externally, and enhancement of contrast in magnetic resonance imaging (MRI). As a result, these nanoparticles could have many applications in biology and medicine, including protein purification, drug delivery, and medical imaging.

Because of the potential benefits of multimodal functionality in biomedical applications, researchers would like to design and fabricate multifunctional magnetic nanoparticles. Currently, there are two strategies to fabricate magnetic nanoparticle-based multifunctional nanostructures. The first, molecular functionalization, involves attaching antibodies, proteins, and dyes to the magnetic nanoparticles. The other method integrates the magnetic nanoparticles with other functional nanocomponents, such as quantum dots (QDs) or metallic nanoparticles. Because they can exhibit several features synergistically and deliver more than one function simultaneously, such multifunctional magnetic nanoparticles could have unique advantages in biomedical applications.

In this Account, we review examples of the design and biomedical application of multifunctional magnetic nanoparticles. After their conjugation with proper ligands, antibodies, or proteins, the biofunctional magnetic nanoparticles exhibit highly selective binding. These results indicate that such nanoparticles could be applied to biological medical problems such as protein purification, bacterial detection, and toxin decorporation. The hybrid nanostructures, which combine magnetic nanoparticles with other nanocomponents, exhibit paramagnetism alongside features such as fluorescence or enhanced optical contrast. Such structures could provide a platform for enhanced medical imaging and controlled drug delivery. We expect that the combination of unique structural characteristics and integrated functions of multicomponent magnetic nanoparticles will attract increasing research interest and could lead to new opportunities in nanomedicine.

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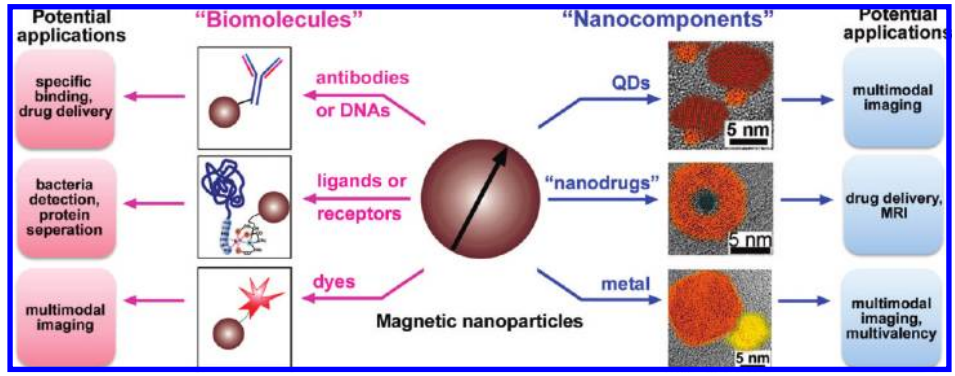

FIGURE 1. The scheme illustrates two strategies to fabricate multifunctional magnetic nanoparticles and their potential applications.

1. Introduction

The integration of nanotechnology with molecular biology and medicine has resulted in active developments of a new emerging research area, nanobiotechnology,[1] which offers exciting opportunities for discovering new materials, processes, and phenomena. Nanoscale magnetic materials have their own advantages that provide many exciting opportunities in biomedical applications. First, they deliver controllable sizes ranging from a few up to tens of nanometers, so their optimization of sizes and properties easily matches with the interest of study. Second, the nanoparticles can be manipulated by an external magnetic force. This "action at a distance" provides tremendous advantages for many applications. Third, magnetic nanoparticles play an important role as MRI contrast enhancement agents because the signal of magnetic moment of a proton around magnetic nanoparticles can be captured by resonant absorption. Recently, techniques and procedures for producing monodispersed and size-controllable magnetic nanoparticles (e.g., FePt, Fe_3O_4, and γ-Fe_2O_3) have advanced considerably,[2,3] which leads to very active explorations of the applications of magnetic nanoparticles, including biomedicine.

In this Account, we describe some potentially useful designs and applications of multifunctional magnetic nanoparticles for biomedicine, which have been attracting increased research effort because of their easily accessible multimodality. Generally, there are two kinds of strategies to fabricate magnetic nanoparticle-based multifunctional nanostructures (Figure 1). The first strategy is molecular functionalization. Biofunctional molecules (e.g., antibodies, ligands, or receptors) coat the magnetic nanoparticles and make them interact with a biological entity with high affinity, thus providing a controllable means of "tagging". After molecular functionalization, the biofunctional magnetic nanoparticles confer high selectivity and sensitivity for many biological applications. The second one is to combine magnetic nanomaterials and other functional nanostructures by sequential growth or coating, which produces a single entity conferring multiple functions in nanoscale. For example, using magnetic nanoparticles as seeds, the growth of semiconducting chalcogenides produces core−shell or heterodimer nanostructures with both magnetic and fluorescent properties, which lead to the demonstration of intracellular manipulation of nanoparticles and a promising candidate for dual-functional molecular imaging (i.e., combining MRI and fluorescence imaging). The integration of magnetic and metallic nanoparticles forms heterodimer structures that offer two distinct surfaces and properties to allow different kinds of functional molecules to attach onto the specific parts of the heterodimers, which may bind to multiple receptors or act as agents for multimodality imaging. The encapsulation of a potential anticancer drug by iron oxide nanoshells results in the yolk−shell nanostructures, which promise novel nanodevices for controlled drug delivery.

2. Molecular Functionalization of Magnetic Nanoparticles

Magnetic nanoparticles can take the advantage of specific binding to detect or purify the biological entities after being modified by biomolecules. Because of their unique property, response to a magnetic field, biofunctional magnetic nanoparticles exhibit two features, specificity and magnetism. Most surface modification strategies, either polymer coating or chemical ligand exchange, are based on or derived from self-assembled monolayers (SAMs).[4,5] The following sections discuss the attachment of functional molecules onto magnetic nanoparticles for several applications.

2.1. Specific Binding and Targeting. The interactions of biomolecular pairs with specific high affinity exist prevailingly in nature. If one of the biomolecular entities conjugates with

the magnetic nanoparticles, the resulting biofunctional magnetic nanoparticles can specifically bind to the other biomolecular entity. Then, the most direct consequence is that the external magnetic force can control the location of the biological entity. Based on this concept, several applications using biofunctional magnetic nanoparticles, such as pathogen detection, protein purification, and toxin decorporation, have been demonstrated in research laboratories.

2.1.1. Bacterial Detection. Bacteria at low concentrations are hard to detect and usually require long induction times before further analysis. To detect bacteria at ultralow concentrations without time-consuming procedures is advantageous in clinical diagnosis and environmental monitoring. We developed a simple strategy that uses vancomycin-conjugated FePt nanoparticles (FePt@Van conjugates) to capture and detect pathogens such as vancomycin-resistant enterococci (VRE) and other Gram-positive bacteria at exceptional low concentrations.[6] Figure 2A shows the experimental procedure of bacterial detection using the biofunctional magnetic nanoparticles. The mixing of FePt@Van conjugates with a solution of bacteria results in a sufficient amount of magnetic nanoparticles binding onto the bacteria because of their strong interactions (i.e., polyvalent interactions between Van and D-Ala-D-Ala on the bacterial surface). A small magnet attracts and enriches these bacteria−nanoparticle composites for the analysis. In the control experiment, the use of FePt nanoparticles capped with nonspecific groups (FePt-NH_2) fails to capture the bacteria because of the lack of specific molecular recognition (Figure 2B).

Scanning electron microscope (SEM) can easily distinguish the bacteria from the aggregates due to their micrometer sizes. FePt@Van conjugates capture bacterial strains such as *Staphylococcus aureus*, *S. epidermidis*, and coagulase negative staphylococci (CNS) with ultralow concentrations. Figure 2C shows the SEM image of "magnetized" *S. aureus* aggregate with FePt@Van conjugates. When FePt-NH_2 is used, the SEM image (Figure 2D) shows no *S. aureus*, suggesting that FePt@Van binds to *S. aureus* specifically due to the molecular recognition. Figure 2E shows the SEM image of vancomycin-resistant bacteria (VanA genotype) captured by the FePt@Van nanoparticles. Transmission electron micrograph (TEM, Figure 2F) clearly confirms that the nanoparticles bind on the surface of bacterial cells. The currently achieved detection limit can be as low as 4 cfu/mL. The high-sensitivity bacterial detection using FePt@Van polyvalent nanoparticles may be due to their similar size to antibodies such as IgG. Moreover, FePt@Van nanoparticles can also capture and preconcentrate Gram-negative bacteria such as *Escherichia coli* due to the partial exposure of the proper receptors on the surface of cells.[7,8]

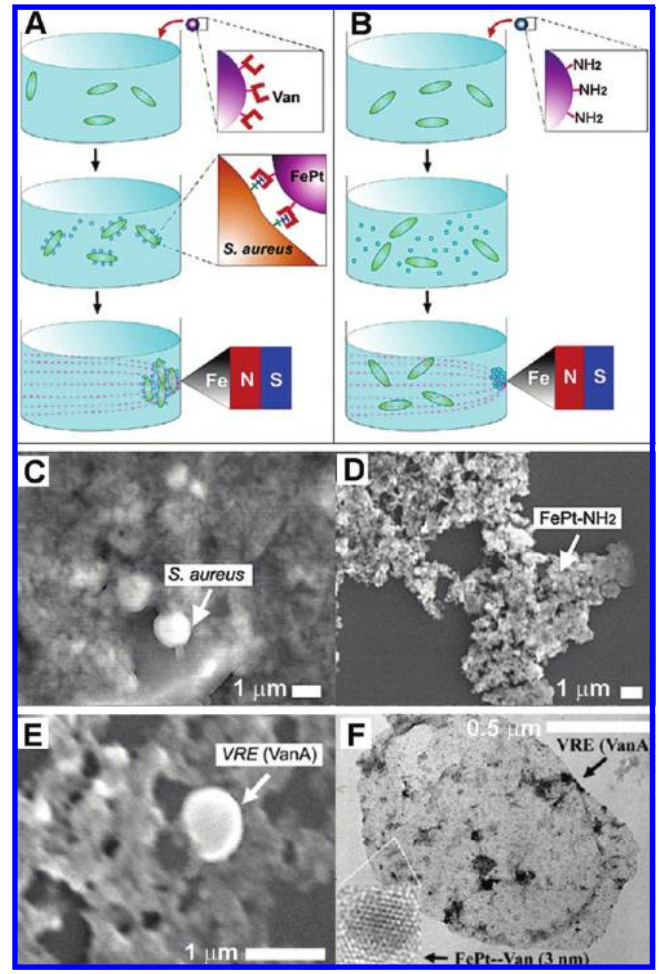

FIGURE 2. (A) Interaction between FePt@Van nanoparticles and bacteria. (B) FePt-NH_2 nanoparticles as the control. SEM images of (C) aggregates of *S. aureus* and FePt@Van nanoparticles and (D) aggregates of FePt-NH_2 nanoparticles. (E) SEM and (F) TEM images of a VanA bacteria captured by FePt@Van nanoparticles (inset, HRTEM of FePt@Van nanoparticles). Reproduced from ref 6. Copyright 2003 American Chemical Society.

The detection limit achieved using FePt@Van magnetic nanoparticles is comparable to that of assays based on polymerase chain reaction (PCR), and this protocol is faster than PCR when the bacterium count is low. In addition, using biofunctional magnetic nanoparticles to capture bacteria is particularly useful when PCR is inapplicable. The procedure that combines FePt@Van biofunctional magnetic nanoparticles with fluorescence dyes can achieve quick, sensitive, and low-cost detection of bacteria in blood.[9] FePt nanoparticles provide the platform for introducing Van in order to generate multivalent interactions. The fluorescent vancomycin, conjugate of Van and fluorescein amine (Van-FLA), stains the enriched bacteria for the quick detection using fluorescence microscopy. This

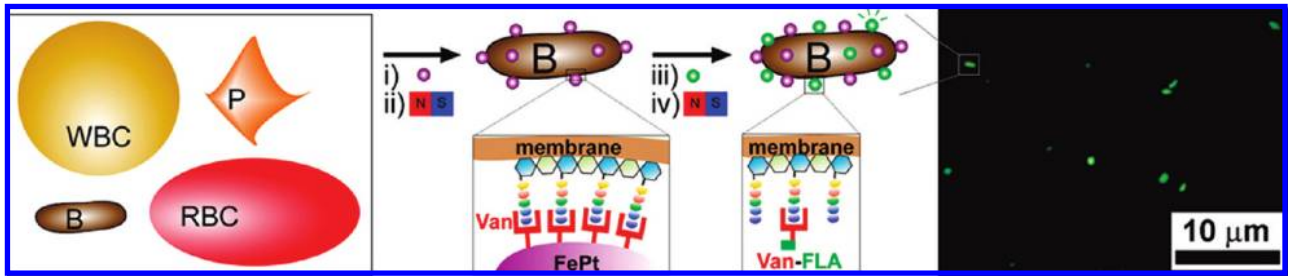

FIGURE 3. Detecting bacteria in blood samples: (i) addition of FePt@Van; (ii) capturing bacteria assisted by a magnet; (iii) addition of Van-FLA for staining the bacteria; and (iv) magnetically separating stained bacteria from Van-FLA solution. Right, a fluorescent image of stained bacteria after the capture. Adapted from ref 9. Copyright 2006 Wiley-VCH.

protocol allows the detection of bacteria from the samples within 2 h and has sensitivity as low as 10 cfu/mL.[9] Figure 3 illustrates the straightforward steps for detecting bacteria in a blood sample: (i) mixing, (ii) separation, (iii) staining, and (iv) washing. Using the fluorescence microscope, one can easily observe the captured bacteria (Figure 3).

Although it is possible to detect the species of bacteria by combining biofunctional magnetic nanoparticles and a specific antibody, it may have limited accuracy and render high cost. In order to quickly and accurately detect the type of strains at low concentration, the integration of magnetic nanoparticles for bacteria accumulation and the PCR method for DNA analysis may confer some advantages. After all, biofunctional magnetic nanoparticles would help to open a new and attractive avenue for pathogen detection and disease diagnosis applications.

2.1.2. Protein Purification. Purification and effective manipulation of proteins is important for their studies and applications in life sciences. Among the existing protocols, magnetic separation and purification is a convenient method for selective and reliable capture of specific proteins, genetic materials, organelles, and cells.[10,11] For example, using dopamine as a robust anchor,[12] the successful synthesis of NTA-terminated magnetic nanoparticles offers a simple and versatile platform for separating six histidine (6xHis)-tagged protein.[13,14] The high surface-to-volume ratio and the good dispersity of the nanoparticles also increase the protein binding capacity. The target proteins cover the surface of the nanoparticles effectively and quickly, thus reducing the overall unoccupied surface area for nonspecific absorption of proteins and achieving higher specificity than microparticles. Moreover, the use of NTA-terminated magnetic nanoparticles eliminates the pretreatment of the cell lysate because of the high specificity of the nanoparticles (Figure 4A,B). It is worth mentioning that the NTA-modified magnetic nanoparticles are reusable without losing efficiency (Figure 4B). Although protein purification based on 6xHis-tag has been widely used, the toxicity of the metal−NTA complexes[15] in this technique requires caution for *in vivo* applications. The specificity of the magnetic nanoparticles exhibited in protein separation suggests that magnetic nanoparticles, as a general and versatile system, should selectively bind with other biological targets at low concentrations if proper anchors and ligands are used.

2.1.3. Toxin Decorporation. Magnetic nanoparticles also play an important role in toxin decorporation,[16] a strategy that reduces and removes toxins from contaminated bodies. For example, biofunctional magnetite nanoparticles decorated by bisphosphonate (BP), which coordinates to a uranyl ion (UO_2^{2+}) with high affinity, can remove UO_2^{2+} efficiently (Figure 4C). The designed magnetic nanoparticles, Fe_3O_4−BP, remove 99% and 69% of UO_2^{2+} from water and blood, respectively (Figure 4D).[17] Besides being the first example of removal of radionuclides from a biological fluid by nanoparticles, this result suggests that functionalized, biocompatible magnetic nanoparticles can act as useful and effective agents of decorporation for selectively and rapidly removing radioactive toxins *in vivo*. Although the successful protocol for removal of UO_2^{2+} using Fe_3O_4−BP nanoparticles provides a potential platform to develop a biocompatible methodology for decorporating radioactive hazards from human bodies, it is important for researchers to demonstrate such a possibility in animal models as the next phase of research.

2.2. Dyes or Drugs. It is also attractive to conjugate magnetic nanoparticles with other functional molecules. Because organic dyes (e.g., Cy5.5, FITC) have widely served as probes to visualize biological processes and magnetic nanoparticles can act as MRI contrast agents, the conjugation of magnetic nanoparticles and dyes will offer the multifunctional nanoprobes combining MRI and optical imaging.[18] For example, the conjugation of Fe_3O_4 nanoparticles and porphyrin[19] may lead to a bimodal imaging nanoprobe. Moreover, the well-studied pharmacokinetics and low systemic toxicity of porphyrin derivatives have already led to some clinical trials and usage of porphyrin derivatives in photodynamic therapy (PDT),

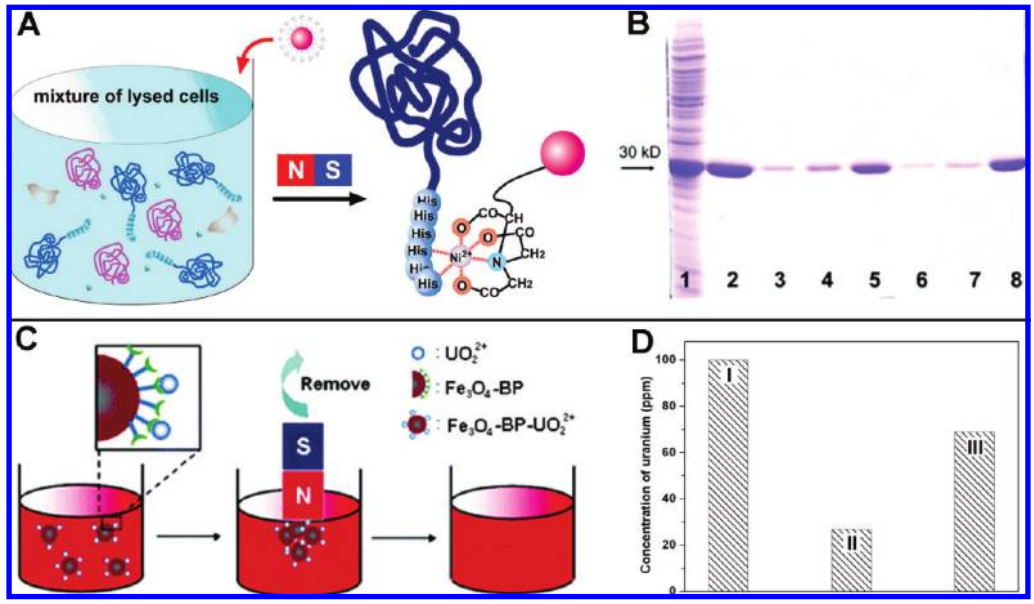

FIGURE 4. Protein purification and toxin decorporation using biofunctional magnetic nanoparticles: (A) NTA-terminated magnetic nanoparticles selectively binding to histidine-tagged proteins; (B) SDS/PAGE analysis of the fraction of proteins washed off the magnetic nanoparticles by imidazole solution at 10 (lane 3), 80 (lane 4), and 500 mM (lane 5) and the fractions washed off the reused nanoparticles using imidazole solution at 10 (lane 6), 20 (lane 7), and 500 mM (lane 8); (C) Fe_3O_4−BP nanoparticles remove UO_2^{2+} from blood; (D) the amount of UO_2^{2+} in blood (I) before and (II) after the removal, and (III) the amount of UO_2^{2+} on the magnetic nanoparticles.

so the porphyrin-modified Fe_3O_4 nanoparticles can act as a multifunctional nanomedicine that combines PDT anticancer treatment and noninvasive MR imaging. Although fluorescence imaging may be the fastest growing area for *in vivo* analysis, limited tissue penetration of the fluorescence restricts its application in small animal imaging, which requires organic fluorophores emitting at above 700 nm in the near-infrared (NIR). However, there are very limited options for available NIR-emitting organic probes, and the potential cytotoxicity and stability of organic fluorophores for *in vivo* experiments should be of concern.

3. Combination of Magnetic Nanomaterials and Other Nanocomponents

Nanostructures provide an excellent platform to integrate different functional nanocomponents into one single nanoentity to exhibit multifunctional properties. To assemble different nanoparticles into a single entity as the novel building block itself is exciting and holds great potential. As briefly discussed in the following sections, based on the magnetic nanoparticles, one can combine QDs to exhibit magnetic and fluorescent properties,[20−22] sequentially grow metallic nanocomponents[23] or form exotic nanostructures, such as yolk−shell nanoparticles for the exploration of nanomedicines.[24,25]

3.1. Quantum Dots. Several useful applications in the study of subcellular processes of fundamental importance in biology have highlighted the potential of QDs in nanobiotechnology.[26] As now well-recognized, QDs have much greater temporal stability and resistance to photobleaching than fluorescent dyes do. Moreover, there are many alternatives in QDs with NIR emission for *in vivo* imaging compared with organic fluorophores. Such unique and attractive properties of the QDs have inspired the fabrication of hybrid nanostructures that exhibit both fluorescence and magnetism, such as Co@CdSe core−shell nanocomposites[27] and FePt−ZnS nanosponges.[28]

Based on the FePt nanoparticles and semiconducting chalcogenide nanocomponents, systematic studies show that the reaction conditions control the formation of different hybrid nanostructures. In one-pot reaction, the sequential growth of CdX (X = S or Se) onto FePt nanoparticles under the lower reaction temperature results in the formation of FePt@CdX core−shell nanoparticles (Figure 5A,B).[21] However, the use of a higher boiling point solvent gives FePt−CdX heterodimer nanoparticles (Figure 5C,D).[20,21] The formation of heterodimers at higher temperature is probably due to the difference in phase transition temperatures between the FePt and CdX components. The CdX components may melt at the higher temperature and induce dewetting from the FePt cores, resulting in the formation of heterodimeric nanostructures. The synthesis of these core−shell and heterodimer nanoparticles is highly reproducible and general. Although the optical properties of these nanostructures still need to be improved for achieving higher quantum yields, this rather simple approach

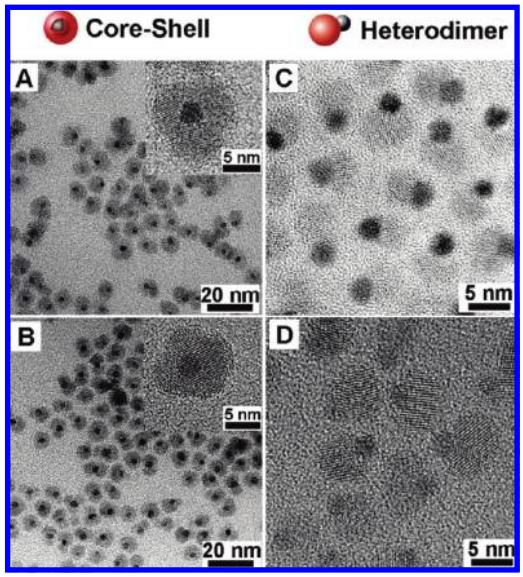

FIGURE 5. Formation of core−shell or heterodimer nanostructures by FePt and CdX (X = S or Se). The core−shell nanostructures of (A) FePt@CdS and (B) FePt@CdSe nanoparticles (insets, HRTEM images). The heterodimeric nanostructures of (C) FePt−CdS and (D) FePt−CdSe.[20,21] Reproduced in part from ref 21. Copyright 2007 American Chemical Society.

opens an avenue for designing and synthesizing sophisticated and multifunctional nanostructures.

The combination of superparamagnetism and fluorescence at nanometer scale should help the biological applications of multifunctional nanomaterials. Although the fluorescence of QDs is partially quenched by metallic nanoparticles in FePt−CdX hybrid nanostructures, the replacement of metallic nanoparticles with metal oxide nanoparticles results in a good quantum yield. For example, the Fe_3O_4−CdSe heterodimer nanoparticles show the emission wavelength peak at 610 nm with quantum yield of about 38%. The resulting fluorescent magnetic nanoparticles bear two attractive features with high quality, superparamagnetism and fluorescence, which allow their intracellular movements to be controlled using magnetic force and to be monitored using a fluorescent microscope.[22] As shown in Figure 6, the confocal microscopic imaging of cells reveals the movement of Fe_3O_4−CdSe nanoparticles inside the cells upon applying a magnetic force. After incubation, HEK293T cells can take up the GSH-modified Fe_3O_4−CdSe nanoparticles together with pEGFP-N1 vector. Without the application of magnetic force, Fe_3O_4−CdSe@GSH nanoparticles distribute nonspecifically inside the cells because of their simple surface modification (Figure 6E). The homogeneous fluorescent spots suggest that the nanoparticles mainly distribute in the cytosol. After being attracted by a small magnet, the Fe_3O_4−CdSe@GSH nanoparticles aggregate on the side of the cell nearest the magnet (Figure 6F). Because of their significant magnetic moment, the magnetic nanoparticles drift to

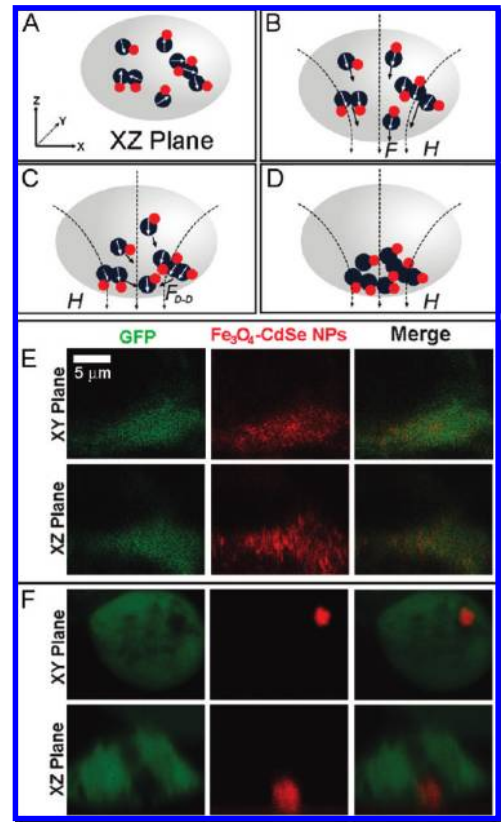

FIGURE 6. Intracellular manipulation of Fe_3O_4−CdSe: (A) distribution of the nanoparticles in a cell without a magnetic field; (B) movement of the nanoparticles with the magnetic field; (C) aggregation due to strong magnetic dipolar−dipolar interactions (F_{D-D}); (D) the aggregates of the nanoparticles inside the cells. The confocal images of HEK293T cells after being incubated with the Fe_3O_4−CdSe@GSH nanoparticles and pEGFP-N1 vector (E) without and (F) with a magnetic field. Reproduced from ref 22. Copyright 2008 American Chemical Society.

the magnet due to the magnetic field gradient (*H*). However, the movement of each nanoparticle is partially hindered inside the cells due to the high viscosity of the cytosol, resulting in a rather slow response. When the nanoparticles approach each other, the magnetic dipolar−dipolar interactions (F_{D-D}) become the dominant forces and drive the nanoparticles much closer to each other (Figure 6C). After several hours, the interplay of these two forces leads to the result of round aggregates of nanoparticles inside the cells.

The successful demonstration of intracellular manipulation of magnetic nanoparticles will be useful for biological applications because this strategy may offer a useful tool to investigate the difference between the apical and basolateral domains in polarized cells by the asymmetric localization of magnetic nanoparticles that carry specific ligands. To realize these promises, the fluorescent magnetic nanoparticles should have fast response to the magnetic force, which has yet to be improved.

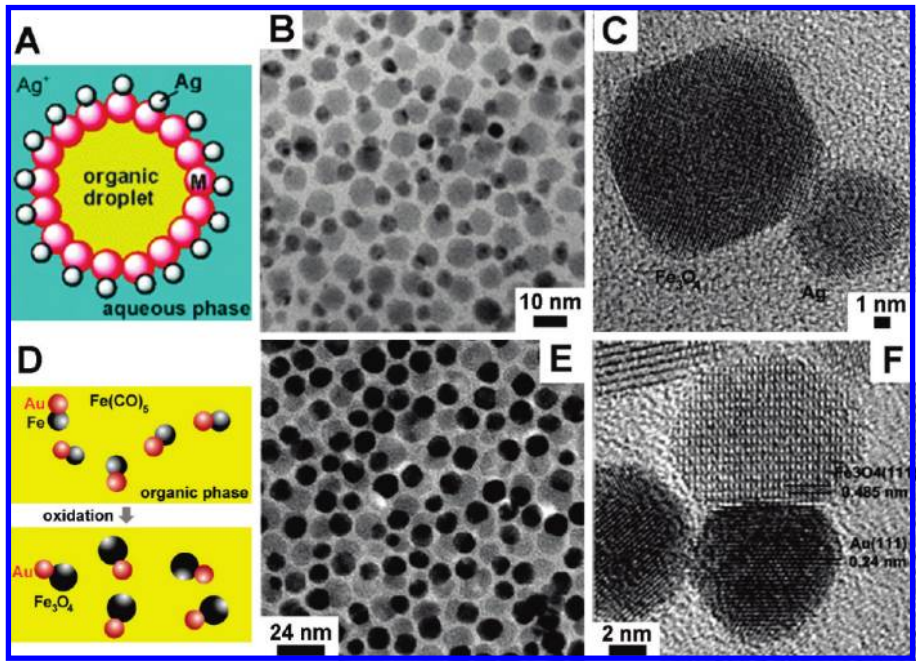

FIGURE 7. Two approaches for synthesis of Fe_3O_4−M (M = Ag or Au) heterodimers: (A) Fe_3O_4−Ag heterodimers form at a liquid−liquid interface; (B) TEM and (C) HRTEM images of Fe_3O_4−Ag heterodimers;[23] (D) the formation of Fe_3O_4−Au heterodimers by the decomposition of $Fe(CO)_5$ on the surface of Au nanoparticles; (E) TEM and (F) HRTEM images of Fe_3O_4−Au heterodimers.[30]

3.2. Metallic Nanoparticles. Metallic nanoparticles (e.g., Ag, Au, and Pt) have fascinated scientists for several centuries, partly because of the colorful colloidal solutions of metallic nanoparticles. Because of their excellent optical properties, metallic nanoparticles are especially useful for biomedical applications, such as optical contrast agents, multimodal sensors combining optical imaging and scattering imaging, and photothermal therapy.[29] The combination of metallic nanoparticles and magnetic nanoparticles likely will lead to new applications in biomedicine.

One of the simplest and most efficient methods is the sequential growth of metallic components (e.g., Ag or Au) onto a "colloidosome" (i.e., the self-assembly of nanoparticles at a liquid−liquid interface) of magnetic nanoparticles.[23] The metallic nucleation takes place on the exposed surface of the magnetic nanoparticles and produces the heterodimers of two distinct nanospheres (Figure 7A). TEM and HRTEM images (Figure 7B,C) show Fe_3O_4−Ag heterodimers with relative uniform size and structure, indicating the method of liquid−liquid interface heterogeneous growth is a general way to make heterodimer nanoparticles. Recently, Sun et al. reported another way to fabricate Fe_3O_4−Au heterodimers in a homogeneous organic solvent,[30] where the thermal decomposition of $Fe(CO)_5$ onto the surface of the Au nanoparticles and the following oxidation of intermediate result in uniform Fe_3O_4−Au heterodimers (Figure 7D−F).

The heterodimer structure offers particles with two distinct surfaces. Different kinds of functional molecules can covalently bind to the specific parts of the heterodimers. For instance, Fe_3O_4 can support a specific biomolecule for targeting using dopamine as a robust anchor.[12,13] For the Ag or Au component, one can use the well-developed gold−thiol chemistry for the attachment of thiol-terminated biomolecules.[5,31] Together with their own distinct functionalities, these multifunctional heterodimers can respond to magnetic forces, show enhanced resonance absorption and scattering, and bind with specific receptors. Using epidermal growth factor receptor isoform A (EGFRA)-conjugated Fe_3O_4−Au heterodimer nanoparticles, Sun et al. demonstrated their dual-functional imaging property for cell tracking.[32] This type of heterodimer nanoparticle may have great potential in multimodal biomedical applications,[33] especially for molecular imaging, but a substantive amount of work need to be done to achieve these promising applications.

3.3. Yolk−Shell Nanostructures. Using magnetic nanostructures as drug delivery carriers is an active and promising research subject on biomedical applications.[34] The conventional and straightforward strategy for drug delivery using magnetic nanoparticles is the use of polymers to coat magnetic nanoparticles and to encapsulate drugs to form nanocapsules or micelles,[35] which may require complicated processes and result in modest efficiency. Based on the nanoscale Kirkendall effect reported by Alivisatos's group,[36] we

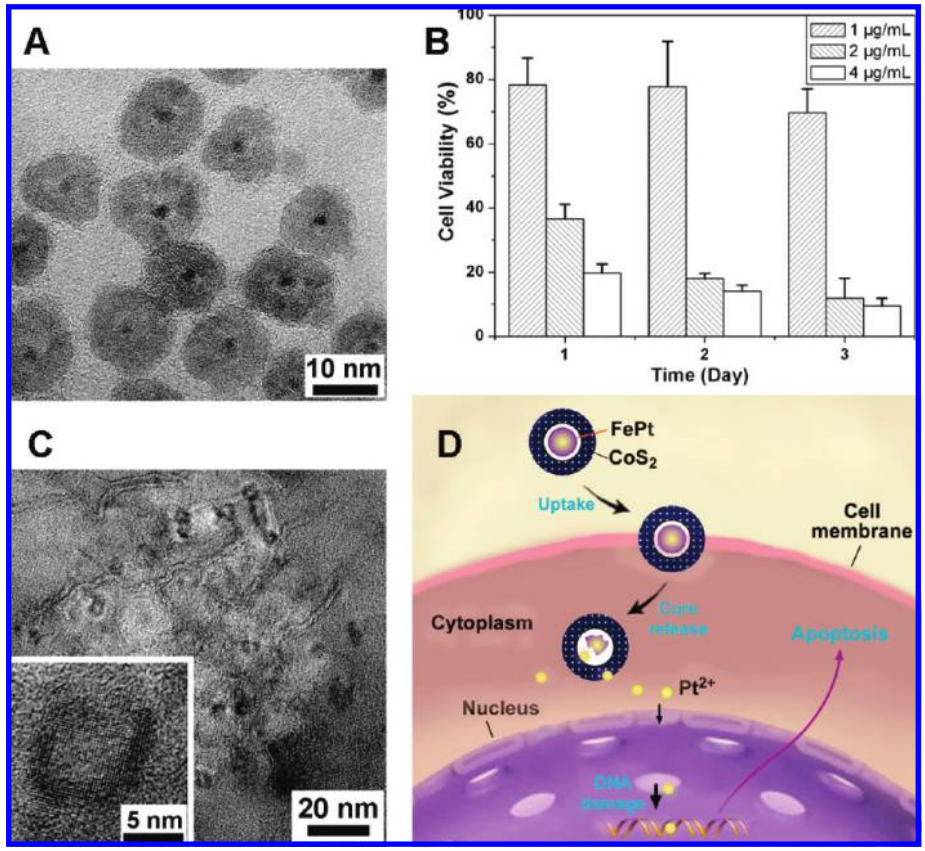

FIGURE 8. The ultrahigh cytotoxicity of FePt@CoS_2 yolk−shell nanoparticles to HeLa cells: (A) TEM image of FePt@CoS_2 yolk−shell nanoparticles; (B) MTT assay results of FePt@CoS_2 nanoparticles on HeLa cells; (C) representative TEM image of FePt@CoS_2 nanoparticles in mitochondria of HeLa cells; (D) the possible mechanism accounts for FePt@CoS_2 nanoparticles killing HeLa cells. Reproduced from ref 24. Copyright 2007 American Chemical Society.

developed FePt@CoS_2 yolk−shell nanoparticles as a potential nanodevice for controlled drug release.[24] The unique structure and properties of FePt@CoS_2 yolk−shell nanoparticles give an unconventional example of a novel drug delivery system that uses the magnetic nanomaterials to directly encapsulate the potential anticancer drug. The premise is that platinum-containing nanoparticles (e.g., FePt) without any surface coating may act as potential anticancer drugs, like the well-known anticancer drug, cisplatin. The sequential growth of CoS_2 porous nanoshells by Kirkendall effect[36,37] provides the feasibility to produce the "naked" FePt nanoyolks and the special interface between outside and inside of the shells (Figure 8A).

According to MTT assay results (Figure 8B), FePt@CoS_2 nanoparticles show an IC_{50} value lower than that of cisplatin. TEM images show that there are many nanoparticles in organelles such as mitochondria, indicating the cellular uptake of nanoparticles. More interestingly, there are no more FePt@CoS_2 nanoparticles except some hollow nanospheres in mitochondria bodies (Figure 8C), indicating that the FePt@CoS_2 nanoparticles enter into the organelles and the FePt yolks disintegrate after the cellular uptake. Figure 8D shows the possible mechanism of cytotoxicity of FePt@CoS_2 nanoparticles against the HeLa cells: After cellular uptake through the endocytosis pathway, under the acidic environment inside the secondary lysosomes, FePt yolks are oxidized (probably by O_2 in the presence of oxidase inside cells) and destroyed to produce platinum(II) species because the reactivity of unprotected iron promotes the disintegration of FePt. The permeability of CoS_2 shells allows these Pt(II) species to diffuse out of shells, enter into the nucleus and mitochondria, damage the DNA double helix chains, and lead to the apoptosis of the HeLa cells, which is similar to the interaction between cisplatin and DNA. Although this mechanism (involving DNA damage) requires more concrete evidence and needs further studies, the cytotoxicity of FePt@CoS_2 nanoparticles clearly originates from the slow, intracellular dissolution of the FePt yolk, which has been further confirmed by the toxicity assays of CoS_2 hollow nanoparticles[24] and FePt@Fe_2O_3 yolk−shell nanoparticles.[25] The mechanism implies that intracellular drug release from a magnetic nanostructure can be a new and powerful

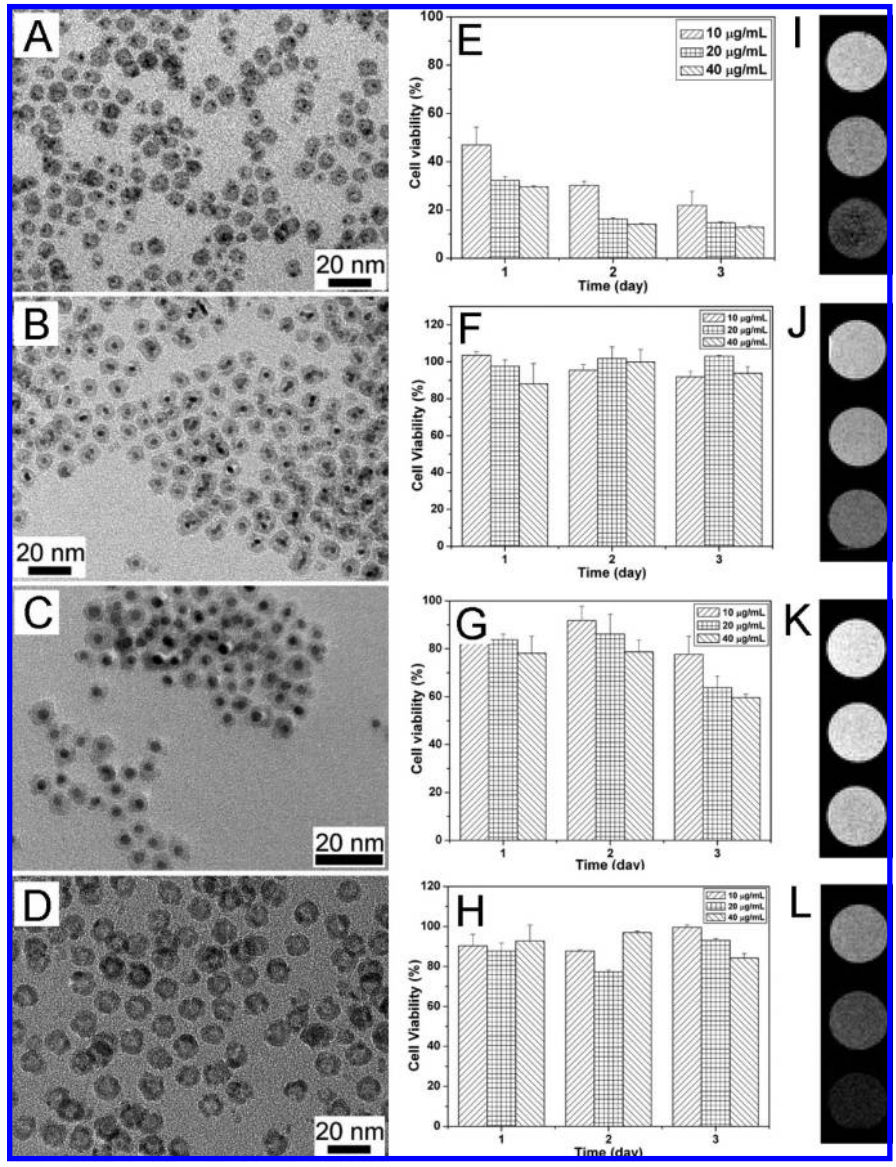

FIGURE 9. TEM images and MTT assay results for HeLa cells of FePt@Fe_2O_3 yolk−shell nanoparticles (A, E), Pt@Fe_2O_3 yolk−shell nanoparticles (B, F), FePt@Fe_3O_4 core−shell nanoparticles (C, G), and γ-Fe_2O_3 hollow nanoparticles (D, H). T_2^*-weighted MR images of (I) FePt@Fe_2O_3 yolk−shell nanoparticles, (J) Pt@Fe_2O_3 yolk−shell nanoparticles, (K) FePt@Fe_3O_4 core−shell nanoparticles, and (L) γ-Fe_2O_3 hollow nanoparticles at 25, 50, and 100 μg/mL, respectively. Reproduced from ref 25. Copyright 2008 American Chemical Society.

strategy for delivering therapeutics, which deserves further exploration and development.

Following the encouraging result of the FePt@CoS_2 yolk−shell nanoparticles, we further reported the bifunctional FePt@Fe_2O_3 yolk−shell nanoparticles combining high cytotoxicity and strong MR contrast enhancement.[25] Recent development in the synthesis of iron oxide hollow nanoparticles[38] facilitates the production of FePt@Fe_2O_3 yolk−shell nanoparticles. Among four types of nanoparticles (Figure 9A−D), FePt@Fe_2O_3 yolk−shell nanoparticles have the highest cytotoxicity (Figure 9E−H). Likely due to the same possible mechanism of cytotoxicity as FePt@CoS_2 yolk−shell nanoparticle, FePt@Fe_2O_3 yolk−shell nanoparticles show an ultralow IC_{50} value in terms of Pt. The Pt nanocrystals in Pt@Fe_2O_3 yolk−shell nanoparticles are stable because of the relative inertness of Pt noble metal. The FePt nanoparticles in FePt@Fe_3O_4 core−shell nanoparticles are very stable because the biocompatible, compact Fe_3O_4 shells prevent the oxidative species from reaching the FePt cores.[24,25] These results suggest two essential requirements of this kind of nanostructure for serving as an effective anticancer drug: (i) the relatively reactive platinum-containing yolks to form platinum(II) species; (ii) the good permeability of shells to allow the metal ions (or complexes) to release from the shells. Obviously, FePt@CoS_2 yolk−shell nanoparticles and FePt@Fe_2O_3 yolk−shell nanoparticles both satisfy these two requirements

and may have effective anticancer activity as potential nanomedicine. Although the *in vivo* experiments have yet to be completed, these results suggest that yolk−shell nanoparticles may evolve to become useful and effective nanodevices for controllable drug release in cancer treatment.

T_2^*-weighted MR image results (Figure 9I−L) indicated γ-Fe_2O_3 hollow nanoparticles exhibit the strongest MR signal attenuation effect among those four types of particles. FePt@Fe_2O_3 yolk−shell nanoparticles and Pt@Fe_2O_3 yolk−shell nanoparticles also show strong MR relaxation enhancement. It is noteworthy that the hollow magnetic nanoparticles exhibit the advantages in MR relaxation enhancement due to their unique geometry and may attract increased research interest for their MRI applications. The interface between the iron oxide hollow nanoparticles and the water phase is much larger than that of solid iron oxide nanoparticles under the same concentrations, which may contribute to the stronger MR contrast enhancement per unit weight.[25]

FePt@Fe_2O_3 yolk−shell nanoparticles offer several distinct advantages as a nanodevice model for controlled drug delivery. First, the biocompatibility of Fe_2O_3 nanoshells ensures the origin of cytotoxicity from the "naked" FePt yolk. Second, the Fe_2O_3 nanoshells allow cancer-targeting moieties to be anchored on the shell surface, which would significantly reduce the side effects. Finally, MR relaxation enhancement effects of Fe_2O_3 nanoshells may provide a direct means for measuring the prognosis during the cancer treatments. Given the capability of surface functionalization of these yolk−shell nanoparticles using disease-specific molecules, one could develop yolk−shell multifunctional nanoparticles that target a specific tissue for delivering the therapeutic agents and monitoring the transformation of the tumor by MRI. Despite the lack of *in vivo* testing results, it is likely that the initial verification of these novel magnetic nanoparticles as multifunctional nanomedicines will drive their further development for multimodal biomedical applications.

4. Conclusion and Perspectives

In this Account, we have summarized some recent design and synthesis of two classes of multifunctional magnetic nanoparticles: molecular-functionalized and nanocomponent-hybridized. These two approaches would be complementary to other methods that integrate the different individual nanoparticles via polymer coating[39] or silica encapsulation.[40] Biofunctional magnetic nanoparticles with high selectivity and high sensitivity not only promise biological applications in bacterial detection and protein purification, but also offer advantages in tumor targeting and multimodal imaging. The hybrid magnetic nanoparticles open up a new avenue to multimode and multipurpose biomedical applications because of their integrated functions. For example, based on the magnetic nanoparticles as MRI contrast agents, one of the most exciting applications is the multimodal imaging that integrates optical imaging (e.g., dyes, QDs, and Au nanoparticles) or positron emission tomography (PET) imaging (e.g., isotopes) with MRI.[41−44]

Although there are many exciting potential biomedical applications of multifunctional magnetic nanoparticles, considerable challenges and issues remain to be resolved. For example, heterogeneity of nanomaterials remains a major problem, and it is hard to precisely control the number of functional molecules on the surface of nanoparticles. Researchers need to develop better strategies for producing nanoparticles that have precise composition, uniform surface modification, and reproducible functionalization. For *in vivo* biomedical applications, the purity, dispersity, and stability of the multifunctional magnetic nanoparticles in a physiological environment are highly important.[45] Therefore, it is necessary to further study and explore multifunctional magnetic nanoparticles for creating successful nanobiotechnology of biomedical applications.

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FOOTNOTES

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