

Synthesis, Functionalization, and Biomedical Applications of Multifunctional Magnetic Nanoparticles

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Synthesis of multifunctional magnetic nanoparticles (MFMNPs) is one of the most active research areas in advanced materials. MFMNPs that have magnetic properties and other functionalities have been demonstrated to show great promise as multimodality imaging probes. Their multifunctional surfaces also allow rational conjugations of biological and drug molecules, making it possible to achieve target-specific diagnostics and therapeutics. This review first outlines the synthesis of MNPs of metal oxides and alloys and then focuses on recent developments in the fabrication of MFMNPs of core/shell, dumbbell, and composite hybrid type. It also summarizes the general strategies applied for NP surface functionalization. The review further highlights some exciting examples of these MFMNPs for multimodality imaging and for target-specific drug/gene delivery applications.

1. Introduction

Magnetic nanoparticles (MNPs) have demonstrated great promise for diagnostic and therapeutic applications.^[1–5] At diameters less than 20 nm, these MNPs are often in a superparamagnetic state at room temperature, that is, their magnetization can be saturated under an external magnetic field, but in the absence of this field their net magnetic moments are often randomized to zero by thermal agitation. Owing to their unique magnetic properties and because they are of comparable size to biologically important objects, these MNPs are very useful for biomedical applications. Their responses to external magnetic fields allow biomolecules to be tagged and detected

magnetically, enabling some exciting new approaches to bioseparation, biodetection and targeted drug delivery.^[6–10] In addition, these MNPs can also respond resonantly to an alternating magnetic field and function as a heater, offering a promising therapeutic solution by magnetic fluid hyperthermia.^[11,12]

To perform real-time monitoring and drug treatment with high accuracy, MNPs are often coupled with targeting agents, therapeutic drugs, and other functional probes. However, these multistep conjugations to a single-component NP are usually low yield processes and the presence of different molecules on the same NP surface may interfere with targeting

capabilities and the subsequent uptake of the NP conjugates. Recent advances in NP research allow the synthesis of various composite NPs with core/shell and dumbbell structures that offer a promising solution to this problem encountered with single-component MNPs. For example, Au-Fe₃O₄ dumbbell NPs were applied by conjugating anticancer drug cisplatin (*cis*-diamminedichloroplatinum) on Au and Herceptin (HER) antibodies on Fe₃O₄ with the number of cisplatin molecules and antibody molecules being controlled by the size of the Au and Fe₃O₄ components.^[13] The composite NPs demonstrated the targeted specific delivery of cisplatin to breast tumor cells (SK-BR-3). Alternatively, by proper surface functionalization, FePt NPs were found to release Fe that was highly cytotoxic.^[14] Utilized as a therapeutic agent, these NPs might offer a promising approach to the multistep conjugation problem in NP delivery systems, as one would only need to anchor a targeting agent on the NP surface and the number of targeting molecules could be readily controlled by NP size.

This review summarizes recent progress in the design and fabrication of multifunctional MNPs (MFMNPs) for biomedical applications. It first outlines the general synthesis of composite NPs of iron oxide, alloys, core/shell, dumbbell, and multicomponent adducts. It then discusses the chemistry applied for surface modification to make these MFMNPs biocompatible. Finally, the review highlights the potential applications of these MFMNPs in multimodality imaging and target-specific drug/gene delivery.

2. Chemical Synthesis of MNPs

The general strategy for preparing monodisperse NPs in solution phase is to separate the nucleation and growth of nanocrystals.

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In La Mer theory,^[15] a burst nucleation event first occurs when the monomer concentration quickly increases over critical supersaturation without further formation of nuclei afterwards.^[16] The produced nuclei then grow at the same rate, giving monodisperse particles. Once formed, these NPs have a high surface area and agglomerate easily to minimize their surface energy. A suitable capping agent has to be used to stabilize these NPs to prevent them from agglomerating.

Numerous synthetic methods have been developed to synthesize MNPs,^[17] including coprecipitation,^[18] sol-gel synthesis,^[19] microemulsion synthesis,^[20] sonochemical reaction,^[21] hydrothermal reaction,^[22] thermal decomposition,^[23] electrospray synthesis,^[24] and laser pyrolysis.^[25] Here we briefly review recent advances in the synthesis of monodisperse MNPs.

2.1. Iron Oxide NPs

Co-precipitation of Fe^{3+} and Fe^{2+} salts is a classical method used to prepare iron oxide NPs.^[26] Iron oxide NPs can be obtained on a large scale by aging a stoichiometric mixture of inorganic salts in aqueous media. A series of experimental parameters, such as pH, reaction temperature, and precursor, have been studied to control NP morphology, size, and quantity. The solvothermal process uses a high pressure reaction to obtain well-crystallized MNPs. However, using these two methods it is difficult to produce MNPs with a narrow size distribution.

High temperature decomposition of metal complexes is now routinely applied to prepare MNPs with controlled size and morphology.^[27,28] A typical thermal decomposition method requires the presence of a metal complex and surfactant in an organic solvent with a high boiling point. In the reaction, the precursors are either added via a hot-injection process or directly heated up.^[29] In the hot-injection process, the thermally unstable metal complexes are rapidly injected into a hot solution in the presence of surfactant to create an instant nucleation event, followed by a controlled growth process. In the heating up procedure, all the precursors are mixed and heated, MNPs are made by tailoring reaction temperatures and precursor concentrations. Oleic acid and oleylamine are two common surfactants used for NP stabilization and for the control of NP size and morphology. The metal precursors are usually metal acetylacetonates^[30] and metal oleates.^[31] For example, iron(III) acetylacetonate, $\text{Fe}(\text{acac})_3$, was mixed with 1,2-hexadecanediol, oleic acid, and oleylamine in benzyl ether and the mixture was heated up to 300 °C to make monodisperse Fe_3O_4 NPs with a size range of 4–8 nm.^[30] The same procedure worked for the synthesis of CoFe_2O_4 and MnFe_2O_4 (Figure 1).^[32] Recent developments in this synthesis indicated that an alkyl diol was not necessary in Fe_3O_4 NP synthesis and monodisperse Fe_3O_4 NPs could be made by heating the mixture of $\text{Fe}(\text{acac})_3$ and oleylamine in benzyl ether.^[33] Thermal decomposition of $\text{Fe}(\text{acac})_3$ in a mixture of oleic acid and oleylamine led to the formation of wüstite (FeO) NPs.^[34] The size of the FeO NPs was controlled in the range 14–100 nm and the shapes were either polyhedra or truncated octahedra, depending on the molar ratio of oleic acid to oleylamine. The FeO NPs can be converted to magnetite, maghemite ($\gamma\text{-Fe}_2\text{O}_3$), hematite ($\alpha\text{-Fe}_2\text{O}_3$) NPs and $\text{Fe}/\text{Fe}_3\text{O}_4$, offering a general approach to the synthesis of NPs of various iron oxides.^[34]



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Iron(III) oleate was used to prepare monodisperse iron oxide NPs on a large scale.^[31] The cations of oleate salts, such as hydron, sodium, potassium, and dibutylammonium, were found to have great influence on the shape of NPs, and monodisperse

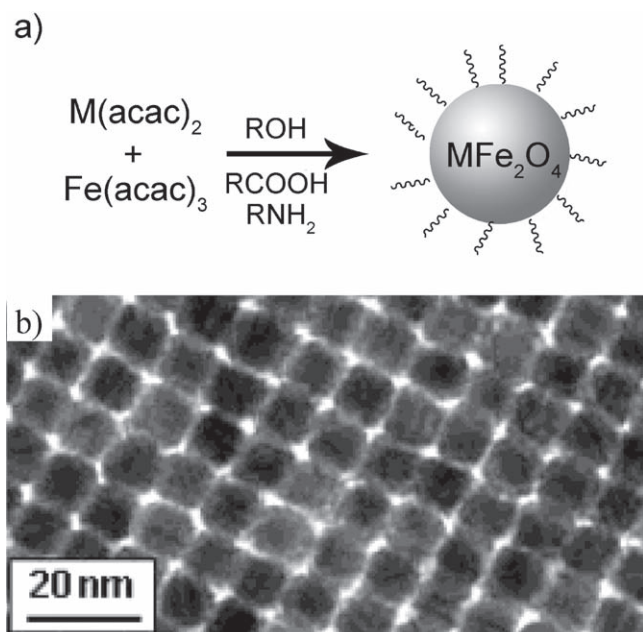


Figure 1. a) Schematic illustration of the synthesis of monodisperse ferrite NPs by employing metal acetylacetonates as the precursor. b) Transmission electron microscopy (TEM) image of Fe₃O₄ NPs. Reproduced with permission.^[32] Copyright 2004 American Chemical Society.

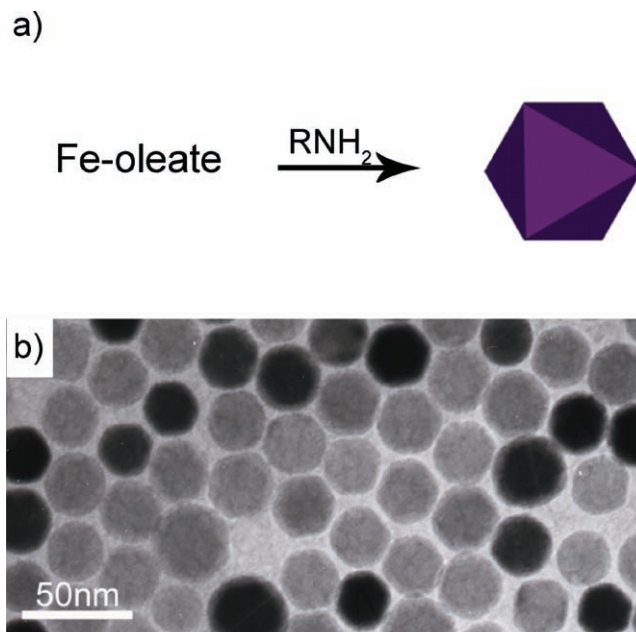


Figure 2. a) A protocol for Fe₃O₄ nanooctahedra synthesis. b) TEM image of octahedral Fe₃O₄ NPs with projection axis {111}. Reproduced with permission.^[36] Copyright 2009 Royal Society of Chemistry.

spherical, cubic, and bipyramidal iron oxide NPs were obtained.^[35] Monodisperse octahedral Fe₃O₄ NPs were obtained by using oleylamine as a stabilizer and reducing agent (**Figure 2**).^[36] The reaction temperature and the molar ratio of precursor to stabilizer are the key for the formation of the octahedral shape.

Porous or hollow iron oxide nanostructures have also been synthesized. Iron oxide nanocapsules were made by a wrap-bake-peel process from akagenite (β-FeOOH) nanorods.^[37] The process involved the coating of akagenite with silica, followed by a heat treatment and silica removal, as illustrated in **Figure 3**. Porous hollow iron oxide NPs were also produced by controlled oxidation of metallic iron NPs in a high temperature (>200 °C) solution phase by the Kirkendall process (**Figure 4**).^[39] Trimethylamine N-oxide was used as an oxidant. The rate of oxygen diffusion towards the core is slower than diffusion of iron outwards from the core, leading to the formation of hollow structures. The Fe₃O₄ shell can be further crystallized at higher reaction temperatures, forming larger crystalline domains and pore structures around the shell. By using technical grade trioctylphosphine oxide (TOPO) as both the solvent and the etchant, hollow MnO or Fe₃O₄ NPs can also be obtained by using relevant nanocrystals as the starting materials. The alkylphosphonic acid impurity in TOPO is believed to be responsible for the etching process and the Kirkendall effect also plays a key role in the formation of hollow NPs.^[38]

2.2. Alloy NPs

FePt and FePd NPs possess high magnetocrystalline anisotropy and good chemical stability. Controlled synthesis of these NPs

and their applications have made considerable progress.^[40–43] FePt NPs are normally synthesized by the thermal decomposition of Fe(CO)₅ and the reduction of Pt(acac)₂.^[40–42] The size and composition of FePt NPs are tuned by tailoring the ratio of precursors and the solvent. Alternatively, FePt alloy NPs can be prepared by reducing FeCl₂ and Pt(acac)₂ by LiBET₃H in the presence of oleic acid and oleylamine.^[41] Recently, one-dimensional FePt nanostructures such as nanowires have been synthesized by a solvothermal route^[44] or a thermal decomposition process in which oleylamine was used both as a solvent and as a surfactant, while dioctyl ether was added to dilute the concentration of oleylamine, nanorods were obtained at 160 °C.^[45] **Figure 5** shows typical TEM images of FePt nanowires/nanorods. The molar ratio of solvent to surfactant is the key to determining the aspect ratio of the FePt nanostructures.

Owing to their high magnetic moment, FeCo alloys are a promising material for various magnetic applications.^[46] FeCo NPs with well-controlled particle size were synthesized by reductive decomposition of Fe(acac)₃ and Co(acac)₂ in the presence of a mixture of oleic acid, oleylamine and 1,2-hexadecanediol under a gas mixture of 93% Ar + 7% H₂ at 300 °C.^[47] Alternatively FeCo NPs were made by sodium borohydride reduction of ferrous and cobalt salts.^[48] Replacing the reducing agent with potassium borohydride resulted in FeCo hollow NPs.

3. Chemical Synthesis of MFMNPs

In addition to the synthesis of MNPs,^[49] great progress has also been made in the synthesis of quantum dots,^[50] gold NPs,^[51] and nanosilicas,^[52] making it possible to integrate different functional NPs into one single nanoentity. Recently, several types of

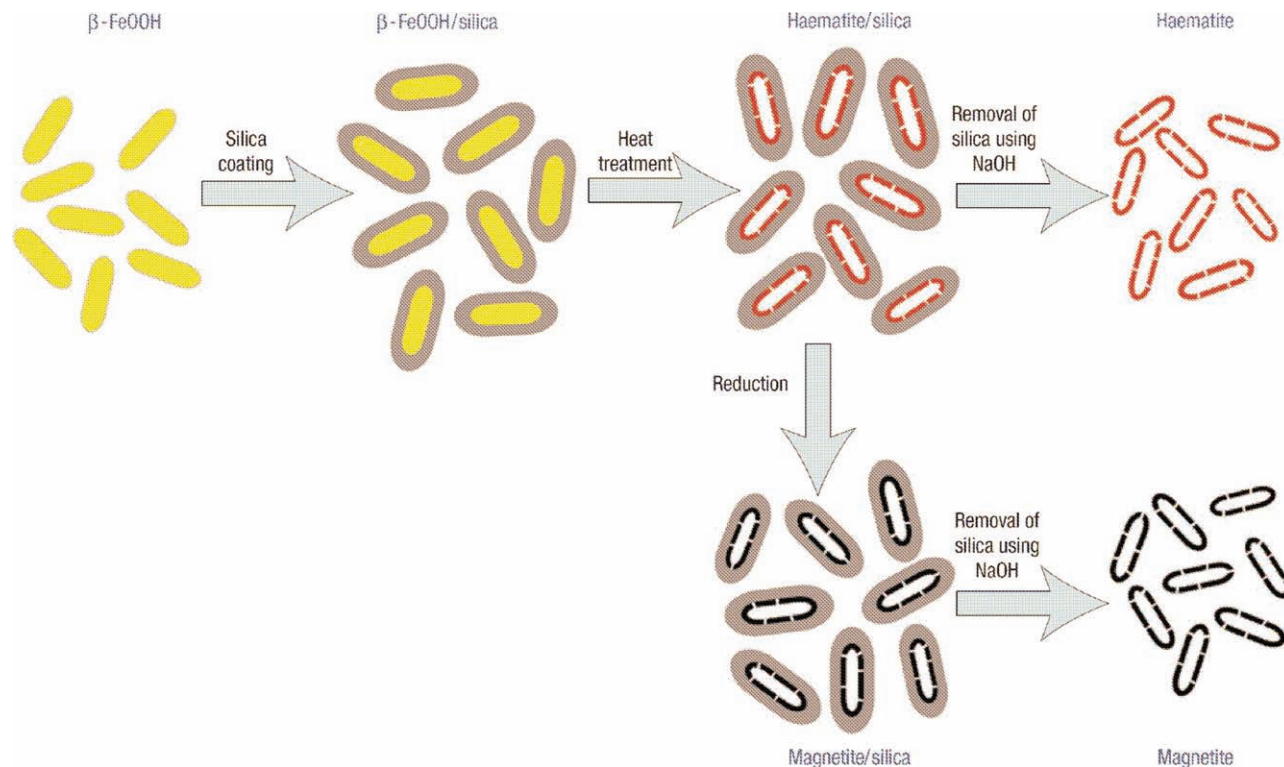


Figure 3. Wrap-bake-peel process to obtain nanocapsules from akagenite. Reproduced with permission.^[37] Copyright 2008 Nature Publishing Group.

MNP-based multifunctional nanostructures, including core/shell,^[53,54] dumbbell,^[55–57] and multicomponent hybrid^[58] types of nanostructures, have been developed. In these MFMNPs, the MNPs can be combined either with quantum dots (QDs), or with plasmonic metallic NPs.^[59–61] The MFMNPs exhibit unique combinations of magnetic and optical properties and have shown great potential for biomedical applications.^[62,63]

3.1. Core/Shell NPs

Core/shell NPs are the most common type of multicomponent NPs and have been studied extensively. Core/shell structures were first realized in semiconductor NPs,^[64,65] where the heterostructures can lead to carrier confinement or separation, depending on the band alignment of the two semiconductor materials with different gap energies.^[66] Bimagnetic core/shell FePt/Fe₃O₄ NPs were synthesized by reductive decomposition

of Fe(acac)₃ in the presence of monodisperse FePt NPs.^[67,68] Other core/shell NPs with a variety of material combinations have also been realized using similar approaches. Core/shell NPs with a magnetic core and metallic shell, such as Fe₃O₄/Au,

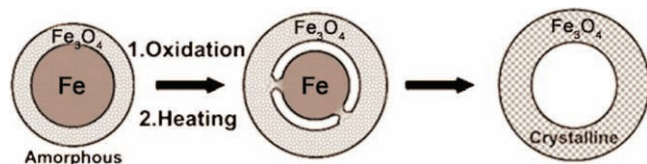


Figure 4. Synthesis of core-shell-void Fe-Fe₃O₄ and hollow Fe₃O₄ NPs from Fe-Fe₃O₄ NP seeds. Reproduced with permission.^[39]

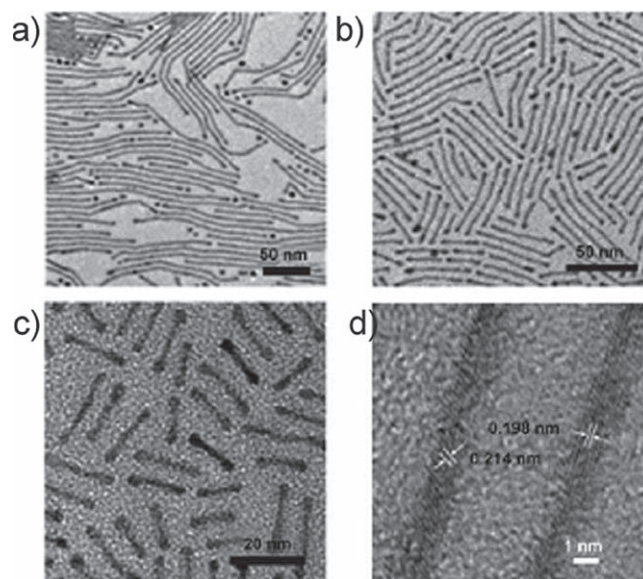


Figure 5. TEM images of FePt nanowires and nanorods. Reproduced with permission.^[45]

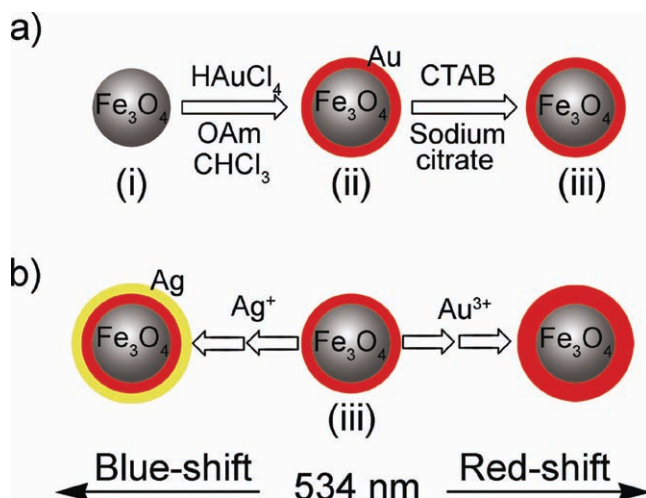


Figure 6. a) Schematic illustration of the surface coating of (i) Fe_3O_4 NPs with Au, to form (ii) hydrophobic $\text{Fe}_3\text{O}_4/\text{Au}$ NPs and (iii) hydrophilic $\text{Fe}_3\text{O}_4/\text{Au}$ NPs. b) Schematic illustration of the formation of $\text{Fe}_3\text{O}_4/\text{Au}$ and $\text{Fe}_3\text{O}_4/\text{Au}/\text{Ag}$. Reproduced with permission.^[69] Copyright 2007 American Chemical Society.

are promising multifunctional nanostructures. The magnetic core provides magnetic functionality and delivery power and the Au shell offers a well-developed surface for biomolecule attachment and plasmonically active components for optical imaging.^[69,70] A schematic illustration of the synthetic strategy for $\text{Fe}_3\text{O}_4/\text{Au}$ core/shell NPs is shown in **Figure 6**. The synthesis starts with low-temperature coating of Au on the surface of Fe_3O_4 NPs by reducing HAuCl_4 in a chloroform solution of oleylamine. Oleylamine is used as a mild reducing agent as well as surfactant. After the synthesis, $\text{Fe}_3\text{O}_4/\text{Au}$ NPs are transferred from the organic environment to water with hexadecyltrimethylammonium bromide (CTAB) and sodium citrate. The water-soluble NPs serve as seeds for the formation of $\text{Fe}_3\text{O}_4/\text{Au}$ NPs with thicker Au coatings achieved by adding more HAuCl_4 under reductive conditions.^[69] Similarly, starting from $\text{Fe}_3\text{O}_4/\text{Au}$, $\text{Fe}_3\text{O}_4/\text{Au}/\text{Ag}$ NPs can be synthesized by adding AgNO_3 to the reaction solution. Recently, FePt/CoS_2 core/shell NPs were also synthesized by the sequential growth of CoS_2 porous nanoshells over FePt NPs.^[63]

3.2. Dumbbell NPs

Dumbbell NPs are generally obtained by sequential growth of a second component on pre-formed NP seeds. This is similar to the synthesis of core/shell NPs with the difference that the nucleation and growth are anisotropically centered on one specific crystal plane around the seed NPs, and not uniformly distributed over the seed NP surface. Therefore, the successful synthesis of dumbbell NPs relies critically on promoting heterogeneous nucleation while suppressing homogeneous nucleation. This can be achieved by tuning the seed-to-precursor ratio and controlling the heating profile so that the concentration of the precursor stays below the homogeneous nucleation threshold throughout the synthesis process.^[16] In the growth

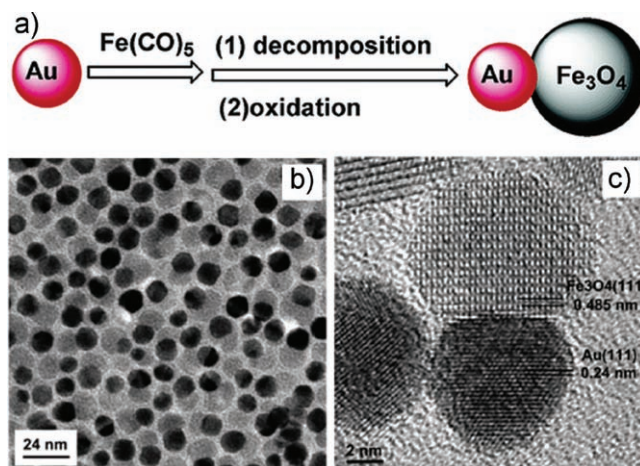


Figure 7. a) Schematic illustration of the growth of $\text{Au}-\text{Fe}_3\text{O}_4$ dumbbell NPs. b) TEM and c) HRTEM images of the 8–14 nm $\text{Au}-\text{Fe}_3\text{O}_4$ dumbbell NPs. Reproduced with permission.^[73] Copyright 2005 American Chemical Society.

process, the lattice spacings of the two components are generally well matched to lower the energy required for epitaxial nucleation of the second component.^[72] However, lattice mismatch can also be applied to make dumbbell NPs by a surface de-wetting process of the core/shell structure.^[59–61] Electron transfer at the interface of two components in the nucleation process may play a key role in controlling the dumbbell morphology, and this transfer process can be modulated by the polarity of the solvent.^[73]

Dumbbell $\text{Au}-\text{Fe}_3\text{O}_4$ NPs are now well-known and have been extensively studied. They are prepared by the decomposition of iron pentacarbonyl, $\text{Fe}(\text{CO})_5$, over the surface of the pre-made Au NPs, followed by oxidation in air, as illustrated in **Figure 7a**.^[73] The size of the Au NPs is controlled either by tailoring the ratio of HAuCl_4 to oleylamine, or by controlling the temperature at which the HAuCl_4 solution is injected. The size of the Fe_3O_4 NPs is tuned by adjusting the $\text{Fe}(\text{CO})_5/\text{Au}$ ratio, as shown in **Figure 8**. **Figure 7b,c** are typical TEM and HRTEM images of a dumbbell NP with an Fe_3O_4 component of 14 nm and Au component of 8 nm. In the structure, an Fe_3O_4 (111) plane grows onto a Au (111) plane, giving the dumbbell-like structure. Similarly, dumbbell NPs of $\text{Pt}-\text{Fe}_3\text{O}_4$ can be produced by controlled nucleation and growth of Fe on Pt NPs, followed by oxidation in air.^[71]

$\text{FePt}-\text{Au}$ NPs were prepared by the growth of Au over FePt NPs.^[74] Other dumbbell NPs composed of various combinations of metals (Au, Ag, Pt, or Ni) and oxides (Fe_3O_4 or MnO) were synthesized from thermal decomposition of mixtures of metal-oleate and metal-oleylamine complexes.^[75] In this case, noble metal NP seeds were formed first by employing oleylamine as a reductant. Then, MnO or Fe_3O_4 components grew on the noble metal NP seeds by a seed-mediated growth process at 300 °C through the thermal decomposition of the relevant oleate complex, resulting in heterostructured metal-oxide NPs. Heterostructures based on FePt NPs and semiconducting chalcogenide NPs have been systematically studied.^[76] The sequential growth of CdX ($\text{X} = \text{S}$ or Se) onto FePt NPs at a lower

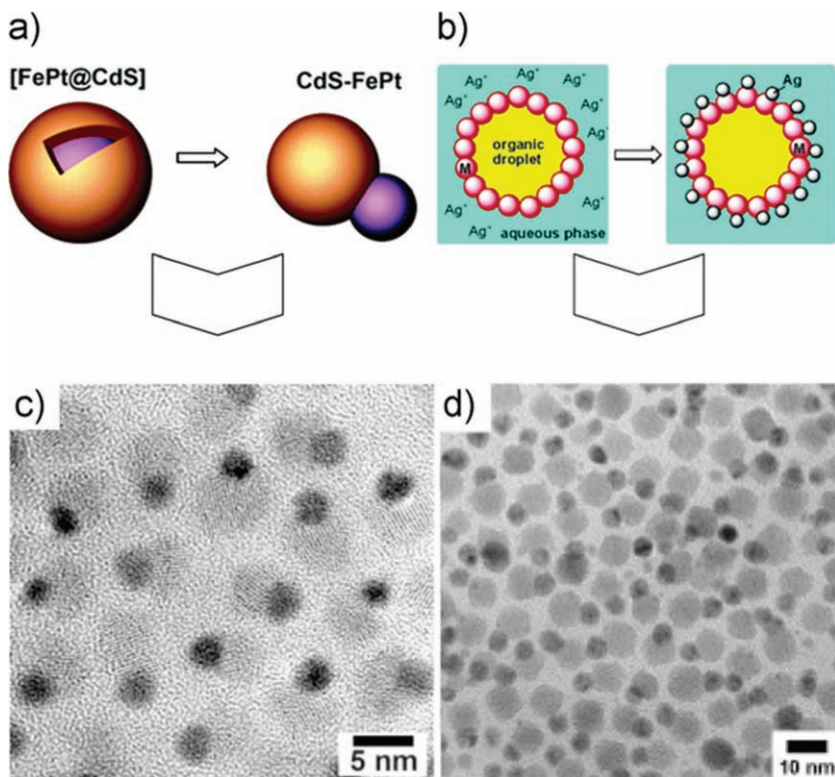


Figure 8. Schematic illustration of the synthesis of CdS-FePt heterodumbbells (a,c), and the micellar synthesis of Ag-Fe₃O₄ heterodumbbells (b,d). Reproduced with permission.^[76,77] Copyright 2004, 2005 American Chemical Society.

reaction temperature results in the formation of FePt/CdX core/shell NPs.^[77] However, due to the dewetting of CdX from the FePt surface at higher reaction temperature, the core/shell FePt/CdX NPs can be converted to FePt-CdX dumbbell NPs, as shown in Figures 8a,c. This synthesis has been extended to γ -Fe₂O₃-MS (M = Zn, Cd, Hg) NPs.^[72] Note that Fe₃O₄-CdSe NPs show an emission wavelength peak at 610 nm with quantum yield of about 38%. The resulting fluorescent MNPs have two attractive features—superparamagnetism and fluorescence—which allow them to be controlled using magnetic force and to be imaged using a fluorescence microscope.^[77] Ag-Fe₃O₄ dumbbell NPs were made in a micellar structure by ultrasonication of a heterogeneous solution of as-prepared Fe₃O₄ NPs in an organic solution and AgNO₃ in water, as exhibited in Figures 8b,d. The sonication provides the energy required to form a microemulsion, which is stabilized by the NPs that self-assemble at the liquid/liquid interface. Fe(II) in the NPs acts as a catalytic center for the reduction of Ag⁺ to Ag NPs. The partial exposure of the NPs to the aqueous phase and the self-catalyzed reduction of Ag⁺ and nucleation of Ag are proposed as two factors that lead to the heterodimer morphology. The synthesis can be readily extended to make Ag-FePt NPs as well.^[77]

Using combinations of the chemistry described above, more exotic dumbbell NPs that contain a noble metal, MNPs, and QDs can be made. For example, PbS-Au-Fe₃O₄ NPs were obtained by mixing Au-Fe₃O₄ NPs with a Pb-oleate complex and elemental S. The competition between the adsorption of S onto the Au surface and reaction with the Pb-oleate to form PbS NPs led to heterogeneous nucleation of PbS on the Au surface.^[78]

3.3. Multicomponent Hybrid NPs

Self-assembly processes provide an approach to fabricating multicomponent hybrid NPs with integrated multifunctionality. By employing intermolecular forces, it is easy to prepare assembled nanostructures from various individual NPs, as illustrated in Figure 9.^[79] Hydrophobic Fe₃O₄, CdSe/ZnS NPs, doxorubicin (DOX), and poly(D,L-lactic-co-glycolic acid) (PLGA) were incorporated into the hydrophobic part of Pluronic F127 ((EO)₉₇(PO)₆₉(EO)₉₇) micelles upon sonication. A mixture dispersion of hydrophobic magnetite (or CdSe/ZnS) NPs, DOX, and

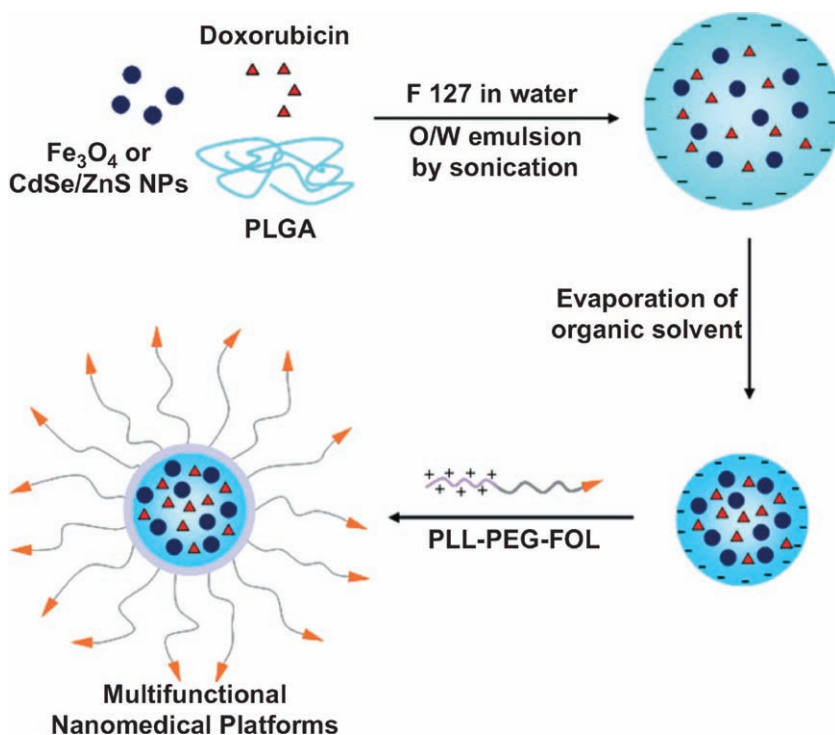


Figure 9. Scheme for preparation of nanoassemblies as multifunctional nanoplateforms. Reproduced with permission.^[79]

PLGA in methylene chloride was poured into an aqueous solution containing Pluronic F127 and the solution was subjected to ultrasonication using a probe-type sonicator. After that the organic solvent was evaporated at room temperature by mechanical stirring and subsequently washed with deionized water for several times, PLGA NPs incorporating inorganic NPs and DOX were obtained. The positively charged, poly(L-lysine) domain of poly(L-lysine)-poly(ethylene glycol)-folate (PLL-PEG-FOL) was adsorbed on the NP surface through electrostatic interactions with the negatively charged surface of the PLGA NPs derived from the terminal carboxylate groups of the PLGA chain. This platform is composed of four components. First, biodegradable PLGA NPs are used as a matrix for loading and subsequent controlled release of hydrophobic therapeutic agents into cells. Second, two kinds of inorganic NPs (Fe_3O_4 and CdSe/ZnS) are incorporated into the PLGA matrix: MNPs can be employed for both magnetically guided delivery and as a T2 magnetic resonance imaging (MRI) contrast agent, and semiconductor NPs are incorporated into the polymer particles for optical imaging. Third, DOX is a therapeutic agent for cancers. Finally, cancer-targeting folates conjugated to the PLGA NPs by PEG groups can be used to target KB cancer cells that have over-expressed folate receptors on their cell surfaces. Similarly, fluorescent magnetic nanohybrids (FMNHs) conjugated with Cetuximab were also made.^[80]

Self-assembly processes can be further enhanced by direct chemical linkage to form hybrid NPs. An example is the

self-assembly of dye-doped silica mixed with MNPs, as shown in **Figure 10**. The integrated structures can be used for simultaneous optical and magnetic imaging.^[81]

4. Surface Functionalization of NPs

Monodisperse NPs with controlled shape and sizes are usually coated with a long-chain hydrocarbon, leading to a hydrophobic surface. To make these NPs biocompatible for biological applications, their surfaces are often functionalized by means of surfactant addition or surfactant exchange, as illustrated in **Figure 11**. Surfactant addition is achieved through the adsorption of amphiphilic molecules that contain both a hydrophobic segment and a hydrophilic component. The hydrophobic segment forms a double layer structure with the original hydrocarbon chain, while hydrophilic groups are exposed to the outside of the NPs, rendering them water soluble. Surfactant exchange is the direct replacement of the original surfactant with a new bifunctional surfactant. This bifunctional surfactant has one functional group capable of binding to the NP surface tightly via a strong chemical bond and the second functional group at the other end has a polar character so that the NPs can be dispersed in water or be further functionalized.

There are various kinds of materials that can be chosen for coating NPs.^[82] Polymer coating materials of lipids, proteins, dendrimers, gelatin, dextran, chitosan, pullulan, PEG, poly(ethylene-co-vinyl acetate), poly(vinylpyrrolidone) (PVP), PLGA, or poly(vinyl alcohol) (PVA) are often chosen for this purpose.^[82–87] Other special molecules, such as bifunctional 2,3-dimercaptosuccinic acids (DMSA),^[88] dopamine,^[89–91] and silanes,^[92] were also investigated for use in NP functionalization. For example, dopamine was found to be a stable anchor on the Fe_3O_4 NP surface.^[90,91] Silanes were employed to exchange the hydrophobic ligands on ferrite magnetic nanostructures.^[92] The end group of silanes, including isocyanine, acrylate, thiol, amino, and carboxylic groups, offer extensive chemistry for the modification of nanostructures. Dopamine and saline as stabilizers often need to be combined with PEG or other polymers to offer long-term NP stabilization in biological solutions.^[91,93] Besides PEG, chitosan,^[94] alginate,^[95] dextran,^[25,96] and poly(acrylic acid) (PAA)^[97] can also be used to stabilize nanostructures and offer long-term stability and biocompatibility. As an amphiphilic surfactant, phospholipid-PEG can readily coat FeCo NPs having graphitic carbon shells by means of hydrophobic interactions (**Figure 12**).^[98] The carbon-coated FeCo NPs, which are superparamagnetic with a high magnetic moment, showed not only ultrahigh R1 and R2 relaxivities but also high aqueous solubility and biocompatibility.

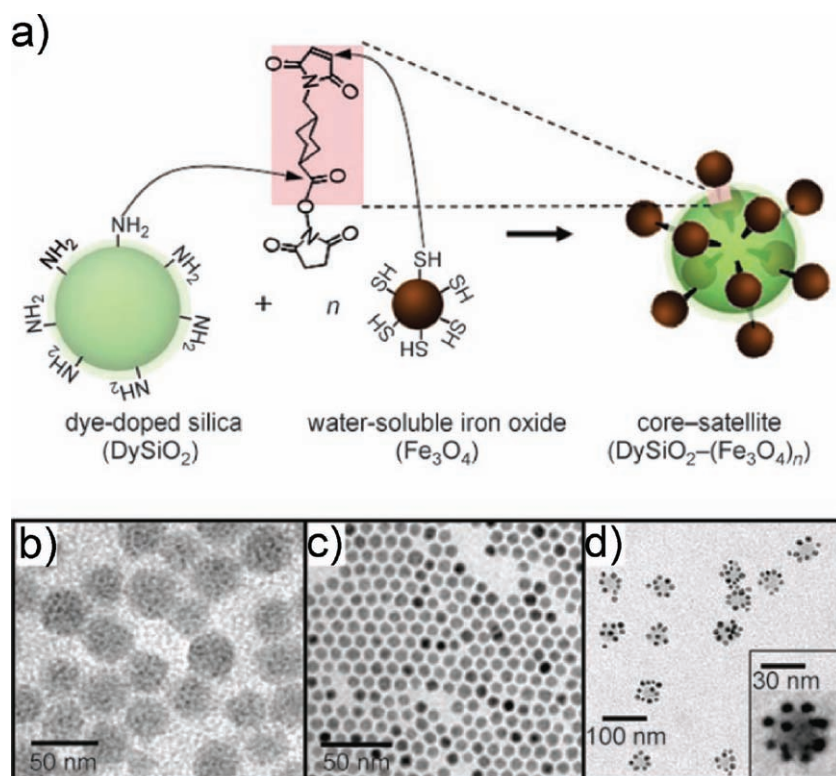


Figure 10. a) Schematic diagram of the synthesis of core-satellite $\text{DySiO}_2-(\text{Fe}_3\text{O}_4)_n$ NPs. b–d) TEM images of b) rhodamine-doped silica (DySiO_2), c) iron oxide (Fe_3O_4), and d) core-satellite $\text{DySiO}_2-(\text{Fe}_3\text{O}_4)_n$ NPs. Reproduced with permission.^[81]

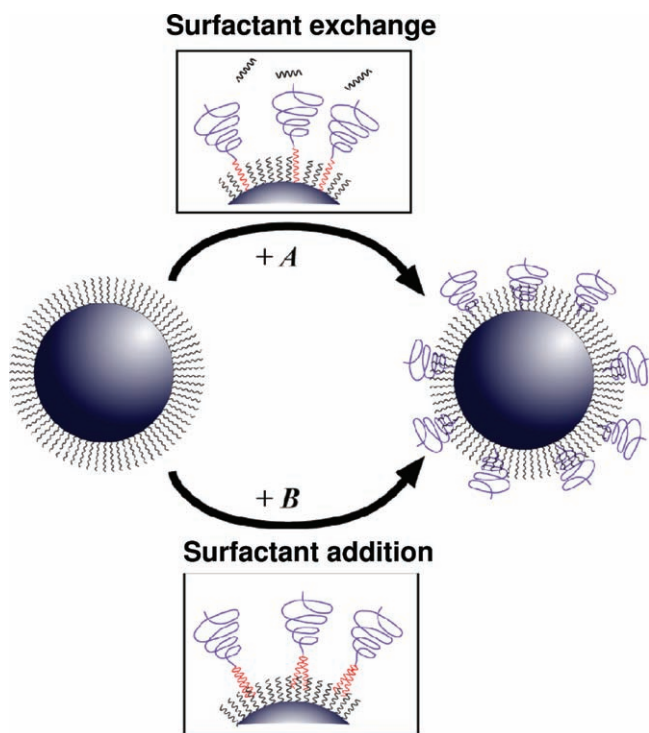


Figure 11. Strategies for NP surface modification.

5. Biomedical Applications of MFMNPs

5.1. Molecular Imaging

Molecular imaging refers to the characterization and measurement of biological processes at the cellular and/or molecular level,^[99,100] its modalities include optical bioluminescence, optical fluorescence, ultrasound, MRI, magnetic resonance spectroscopy (MRS), single-photon-emission computed tomography (SPECT), and positron emission tomography (PET). Since no single imaging modality can provide complete information about a subject's structure and function, using multiple imaging modalities that combine two or more imaging techniques has been an attractive goal.^[101–103] The MFMNPs summarized in this

review can serve as multifunctional probes for sensitive imaging applications.^[104] These MFMNPs are also effective carriers for a larger payload of drug molecules for improving therapeutic efficiency.^[105,106]

5.1.1. MRI Probes

MRI is an extremely useful diagnostic tool for medical science.^[104,107] According to different relaxation pathways, MRI images can be classified as T1 (longitudinal relaxation time)- or T2 (transverse relaxation time)-weighted images. MRI contrast agents can help to clarify images and to allow better interpretation.^[108] Commonly, paramagnetic complexes, such as Gd-DTPA (gadolinium diethylene triamine pentaacetic acid), are used as T1 contrast agents and MNPs are selected as T2 contrast agents. The acting mechanism of T2 contrast agents is as follows.^[109] Under an applied magnetic field B_0 , a magnetic moment is induced in superparamagnetic NPs, which perturbs magnetic relaxation processes of the water protons around them, shortening the spin-spin relaxation time T2. Such changes result in darkening of the corresponding area in T2-weighted MR images. The degree of the T2 contrast effect is typically represented by the spin-spin R2 relaxivity ($R_2 = 1/T_2$), where higher values of R2 result in a greater contrast effect. The relaxivity coefficient r_2 , which is obtained as the gradient of the plot of R2 versus the molarity of magnetic atoms, is a standardized contrast enhancement indicator.

Metal oxide MNPs can provide a strong contrast effect in T2-weighted images.^[104] Metal alloy MNPs, such as FeCo and FePt, also serve as MRI contrast agents.^[109] A significant challenge associated with the application of these MNPs is their behavior in vivo. All of these MNPs are almost nonspecifically taken up by the reticuloendothelial system (RES),^[110] including the liver, spleen, and lymph nodes,^[111] making their detection not very effective. The next generation of NP-based MRI contrast agents should incorporate novel nanocrystalline cores, coating materials, and functional ligands to improve their detection and specific delivery/targeting.^[112]

It is important that nanostructures accumulate around the target tissue in biomedical applications. This is achieved by functionalizing the surface of nanostructures with targeting agents. The special interactions between targeting agents and receptors allow accumulation of the nanostructures near specific tissue. MNPs have been modified with many different kinds of targeting molecules, for example, EGF (epidermal growth factor),^[113] Herceptin,^[114] and CEA (carcinoembryonic antigen),^[115] for active detection of tumors. In a recent test, $MnFe_2O_4$ NPs were coupled with Herceptin, an antibody specifically binding to the over-expressed HER2/neu marker on the surface of breast and ovarian cancer cells.^[116] Figure 13 shows the color-coded MR image of a mouse implanted with the cancer cell line NIH3T6.7 and treated with 50 mg of NP–Herceptin. It can be seen that the tumor treated with the $MnFe_2O_4$ –Herceptin NPs shows color changes from red to blue in the color-coded MR images (Figs. 13a–c). In contrast, those treated with the Fe_3O_4 –Herceptin NPs at the same dosage

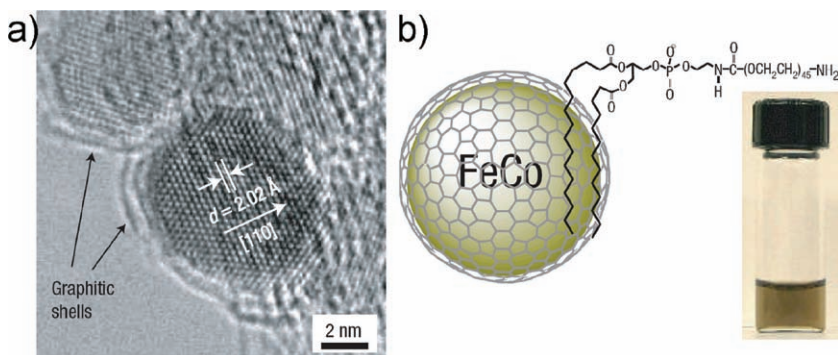


Figure 12. FeCo NPs with graphitic carbon shells for biomedical applications. Reproduced with permission.^[98] Copyright 2006 Nature Publishing Group.

those treated with the Fe_3O_4 –Herceptin NPs at the same dosage

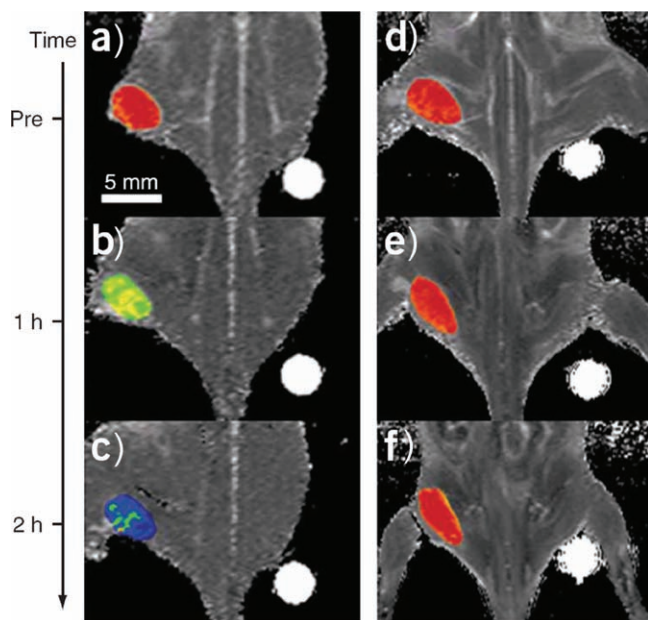


Figure 13. Color maps of T2-weighted MR images of a mouse implanted with the cancer cell line NIH3T6.7 at different time points after injection of MnFe_2O_4 -Herceptin (a–c) and Fe_3O_4 -Herceptin (d–f) conjugates. Adapted from,^[116] with permission from Nature Publishing Group.

have no apparent change in the color-coded MR images (Figs. 13d–f), indicating the high MR sensitivity of MnFe_2O_4 -Herceptin conjugates. An even higher MRI sensitivity can be achieved by using high moment superparamagnetic NPs.

5.1.2. MRI/Optical Imaging Probes

Optical imaging has been a popular imaging modality.^[117,118] Organic dyes are normally employed to offer fluorescent features. Fluorescein isothiocyanate (FITC),^[119] DiI (a hydrophobic and lipophilic cyanine dye),^[120] and cy5.5 (another cyanine dye)^[121] are commonly conjugated with magnetic nanostructures for combining both optical and MRI modalities into one nanostructure.^[122,123] In fabricating dual probes, MNPs coated with dextran can be further linked with HIV-Tat peptide (a membrane translocation signal peptide) and FITC.^[124] MNPs were also modified with PEG-modified phospholipids with Texas Red and Tat peptide attached to the PEG chains.^[125] Iron oxide MNPs coated with bifunctional PEGs were conjugated with Cy5.5 and chlorotoxin, a MMP2 (matrix metalloproteinase 2)-targeting peptide was also used.^[126]

Because of its chemical stability and biocompatibility, silica has also been widely used in biomedicine.^[127–132] Mesoporous silica is a kind of alternative material for drug delivery because porous structures allow the diffusion of drugs into and out of the structure.^[130] By the addition of functional organic molecules, such as dyes,^[127] nanoscale silica can be made into multifunctional nanostructures. For example, optically active iron oxide/silica MFMNPs were made by the copolymerization of an Eu^{3+} complex, free (3-aminopropyl) triethoxysilane (APS) and tetraethyl orthosilicate (TEOS) in the presence of PVP-stabilized Fe_3O_4 NPs. Similarly, FITC was incorporated into the iron oxide/silica system.^[127] Most recently, another kind of optical/MRI probe was fabricated by coating with PAA and by grafting a near infrared (NIR) dye in the PAA matrix (Figure 14).^[133] Such IR and MRI

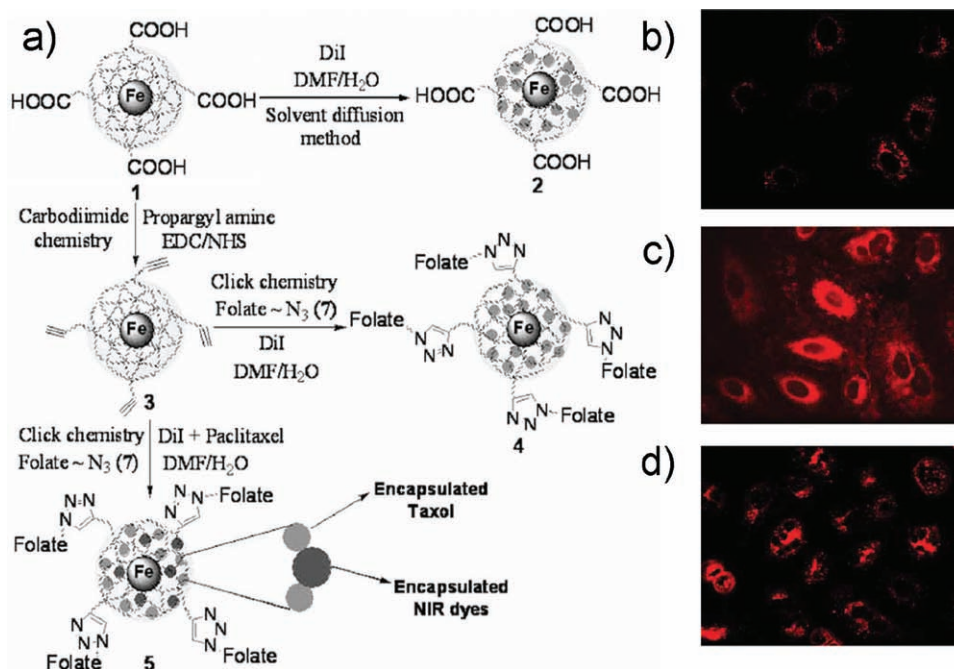


Figure 14. Multimodality imaging probe. a) Schematic representation of the synthesis of theranostics and multifunctional iron oxide NPs. b–d) Assessment of iron oxide NP cellular uptake via confocal laser-scanning microscopy using lung carcinoma A549 cells. b) No internalization was observed in cells treated with carboxylated iron oxide NPs, as no DiI fluorescence was observed in the cytoplasm. c) Enhanced internalization was observed upon incubation with the folate-immobilized iron oxide NPs. d) Cells incubated with paclitaxel (Taxol) and DiI co-encapsulating folate-functionalized iron oxide NPs induced cell death. Reproduced with permission.^[133]

dual modality probes offer much-needed deep tissue imaging at great sensitivity.

5.1.3. MRI/PET Probes

Positron emission tomography (PET) is a useful imaging modality that uses the signals emitted by positron-emitting radiotracers to construct images.^[134] A series of positron-emitting elements, including ^{11}C , ^{18}F , ^{64}Cu , ^{68}Ga , and ^{124}I , are commonly used as tracers in PET imaging. PET, as an extraordinarily sensitive imaging modality, has comparatively low resolution, while MRI gives high anatomical spatial resolution. By combining both of them, it is possible to get both highly sensitive and high-resolution images.

To combine PET with MRI in one probe, a ^{64}Cu complex can be grafted onto dextran in the dextran coating of MNPs via a chelating bifunctional ligand (p-SCNBz-DOTA).^[135] PET/MRI probes with targeting capabilities were also fabricated by coupling ^{64}Cu and cyclic arginine-glycine-aspartic acid (RGD) peptides to poly(aspartic acid) (PASP) in PASP-coated MNPs.^[136] The PET/MRI dual probe has been demonstrated as an *in vivo* dual-modality imaging agent in a sentinel lymph node (SLN) model biological subject.^[137] In the synthesis, serum albumin (SA)-coated MnFe_2O_4 was applied as the MRI probe and ^{124}I , a PET radionuclide, was conjugated to the tyrosine residue in SA. In the *in vivo* experiment, two different types of LNs were clearly identified and accurately localized in a PET/MR fusion image (Figure 15).

5.1.4. MRI/CT Probes

Noble-metal NPs such as gold and silver NPs have attracted significant attention owing to their potential applications in

computed tomography (CT) imaging as a result of their biocompatibility and their high electron density.^[49,138,139] The density of gold (19.32 g cm^{-3}) is much higher than that of commercial CT probe iodine (4.9 g cm^{-3}). Therefore, nanoscale gold can serve as an excellent contrast probe for CT imaging.^[140,141] In this regard, $\text{MnFe}_2\text{O}_4/\text{silica}/\text{Au}$ should be a powerful bimodal contrast agent for both MRI and CT imaging.^[142,143]

5.2. MFMNPs for Drug Delivery

With the rapid development of nanotechnology, NP-based drug carriers have been emerging as effective tools for drug delivery in cancer therapy. There have been many different kinds of macromolecular structures designed for drug delivery systems, such as micelles,^[144–146] liposomes,^[146] NPs,^[147,148] dendrimers^[149] and polymers.^[150] In these systems, the drug is entrapped, attached, adsorbed, or encapsulated into or onto nano-matrices.^[151] A number of studies have demonstrated the advantages of using MNPs for drug delivery. MNPs conjugated with methotrexate (MTX), a therapeutic drug, can target cancer cells that overexpress folate receptors.^[152,153] Such NPs show higher internalization by cancer cells than healthy cells do. MTX can be released from the NPs by lower environmental pH and intracellular enzymes to induce apoptosis of cancer cells.

Recently, various novel MFMNPs have been designed for simultaneous drug delivery and molecular imaging. For example, dumbbell-like $\text{Au-Fe}_3\text{O}_4$ NPs were made to bind covalently with cisplatin complexes,^[13,154] a kind of therapeutic drug that binds strongly to DNA and interrupts DNA transcription,^[155,156] as shown in Figure 16. These dumbbell-like $\text{Au-Fe}_3\text{O}_4$ NPs can

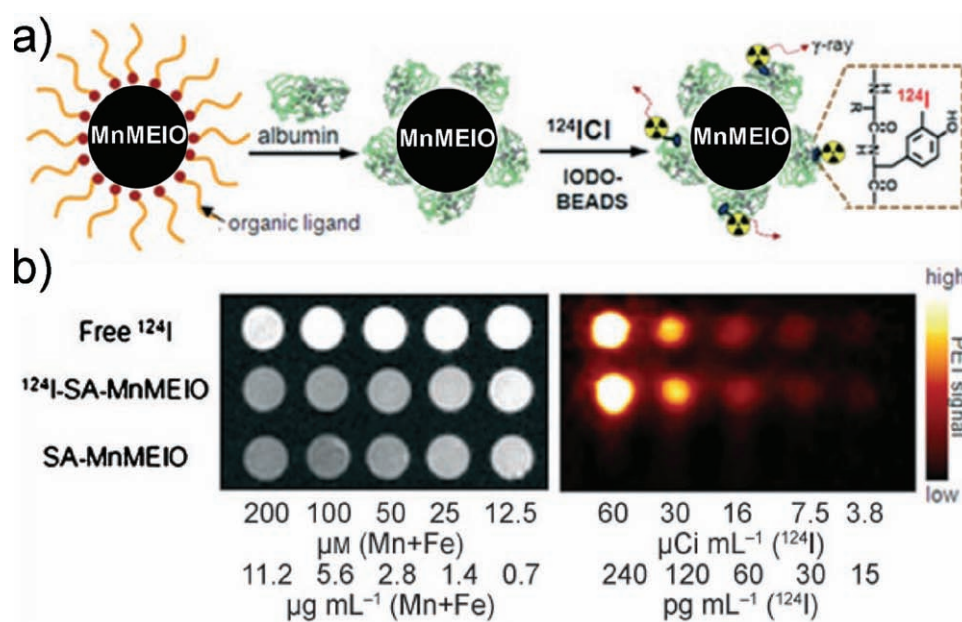


Figure 15. a) Preparation of ^{124}I -SA-MnMEIO (where MnMEIO is Mn-doped magnetism-engineered iron oxide). b) MR (left) and PET images (right) of free ^{124}I , ^{124}I -SA-MnMEIO, and SA-MnMEIO at various concentrations. ^{124}I -SA-MnMEIO clearly exhibits the dual characteristics of PET and MRI. Reproduced with permission.^[137]

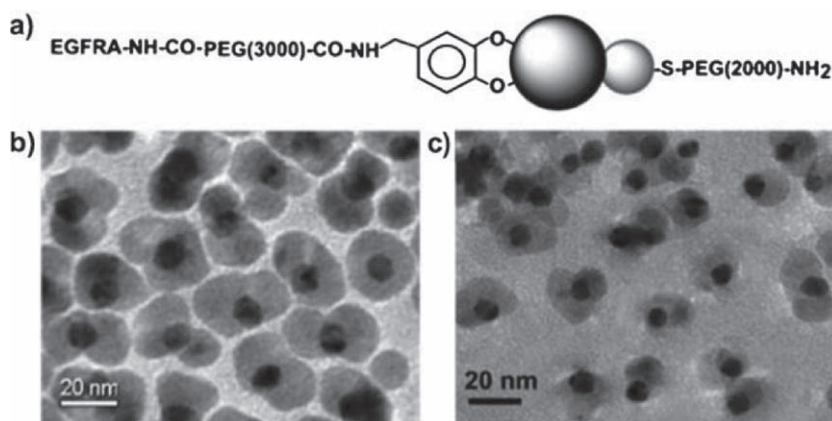


Figure 16. a) Schematic illustration of surface functionalization of the Au-Fe₃O₄ nanoparticles. b, c) TEM images of the 8–20-nm Au-Fe₃O₄ particles before (b) and after (c) surface modification. Reproduced with permission.^[13]

serve as a multifunctional platform for target-specific cisplatin delivery. The release of the therapeutic cisplatin under low-pH conditions renders the NP conjugates more toxic to the targeted tumor cells than free cisplatin. In this structure, a cisplatin complex was conjugated to the Au side by reacting Au-S-CH₂CH₂N(CH₂COOH)₂ with cisplatin. Herceptin (HER2-specific antibody) was coated onto Fe₃O₄ through PEG3000-CONH-Herceptin for targeting abilities. The specificity and efficacy of the cisplatin-Au-Fe₃O₄-Herceptin NPs was examined with Sk-Br3 cells (HER2-positive breast cancer cells) and MCF-7 cells (HER2-negative breast cancer cells), as shown in Figure 17a. The cisplatin-Au-Fe₃O₄-Herceptin NPs target and kill HER2-positive cancer cells (Sk-Br3 cells) more effectively (Figs. 17c,d).

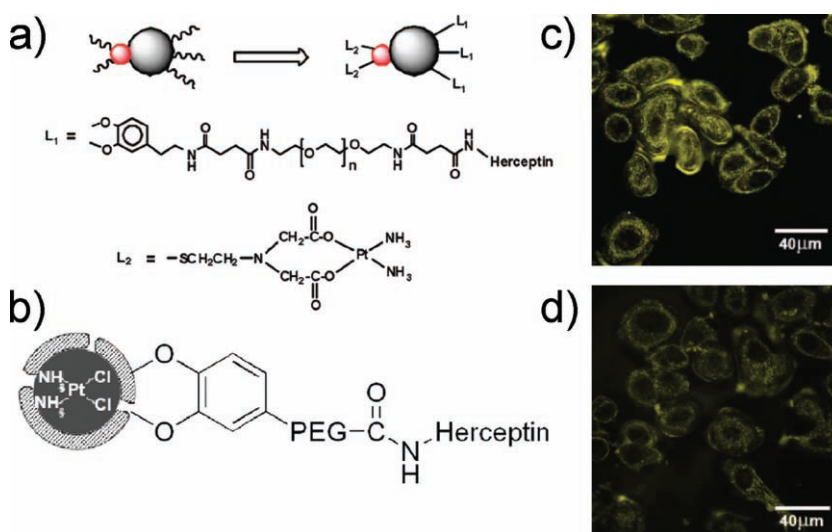


Figure 17. a) Schematic illustration of dumbbell-like Au-Fe₃O₄ NPs coupled with Herceptin and a cisplatin complex for target-specific cisplatin delivery. b) Schematic illustration of simultaneous surfactant exchange and cisplatin loading into a porous hollow NP (PHNP) and functionalization of this PHNP with Herceptin. c,d) Reflection images of Sk-Br3 (c) and MCF-7 cells (d) after incubation with the same concentration of cisplatin-Au-Fe₃O₄-Herceptin NPs. Reproduced with permission.^[154,157]

Fe₃O₄ porous hollow NPs (PHNPs) were also demonstrated to be an efficient carrier for targeted delivery and controlled release of cisplatin (Figure 17b).^[157] Cisplatin was loaded into the core of the NPs by diffusion. After coupling with Herceptin, the cisplatin-loaded hollow NPs provided efficient delivery of cisplatin to HER2-positive breast cancer cells (SK-BR-3).

Different drugs can be delivered using various kinds of strategies.^[158,159] Fluorescent magnetic nanohybrids (FMNHs),^[80] which were stabilized by pyrene-labeled poly(ϵ -caprolactone)-*b*-poly(methacrylic acid) copolymer (Py-PCL-*b*-PMAA), were explored for simultaneous cancer-targeted MRI or optical imaging and magnetically-guided drug delivery. This platform is composed of Fe₃O₄ or CdSe/ZnS with DOX embedded in a biodegradable PLGA polymer matrix. PEG-folate

was coated on the surface of the polymer NPs for active targeting of cancer cells. Most recently, a lipophilic near infrared (NIR) dye and an anticancer drug (Taxol) were incorporated into the polymeric matrix of PAA-coated MNPs for combined optical imaging/MRI detection and targeted cancer therapy.^[133]

5.3. MFMNPs for Gene Delivery

With the development of DNA technology, especially recombination technology, gene therapy, which transfers DNA or RNA into target cells, has become a novel approach for disease therapy.^[160] However, finding effective transfection methods is a major objective in gene therapy research due to rapid degradation of DNA and RNA by enzymes and their poor diffusion across cell membranes.^[161,162]

There are numerous gene delivery methods, such as microinjection, electroporation, micro-particle bombardment, calcium phosphate co-precipitation, and liposome technology.^[163] NP-based gene delivery systems are potentially more effective and active. For example, MNPs coated with adeno-associated virus (AAV) can encode green fluorescent protein (GFP) by using a cleavable heparin sulfate linker.^[164] In addition, MNPs coated with polyamidoamine (PAMAM) dendrimers and the cationic polymer polyethyleneimine (PEI) have been tested as a gene delivery system.^[165,166] However, PEI is highly toxic to cells as it easily disrupts cell membrane function.^[167,168] Its toxicity can be reduced by grafting PEG to PEI.^[169] The MFMNPs are made of MNPs and a copolymer of PEI and PEG, allowing efficient loading and the protection of nucleic acids. All these studies show that this NP method can potentially be used to advanced gene delivery and therapy.

6. Concluding Remarks and Perspective

Multifunctional magnetic nanoparticles (MFMNPs) are important emerging platforms for multimodality imaging probes. The multifunctional surfaces of MFMNPs allow rational conjugations of biological and drug molecules of interest. These recent rapid developments in the synthesis and surface modification of MFMNPs have enabled the use of these NPs for more effective diagnosis and therapy. Next-generation molecular probes fusing multiple fluorescent dyes, drugs, and multiple MNPs into a single nanoprobe should provide superior fluorescence, enhanced MRI contrast, and targeted drug delivery capabilities.

Before these MFMNPs can be used practically as probes for diagnostic and therapeutic applications, numerous challenges need to be met. They should be tested thoroughly for their biocompatibility and biodistribution. Their targeting capabilities need to be enhanced. Once their diagnostic and therapeutic purposes are achieved, these NPs should be eliminated by biological systems without any other detrimental effects. The long-term effects of these NPs and their conjugates on biological systems should also be studied. Despite these numerous challenges, MFMNPs are considered the most promising tools to achieve the desired sensitivity and efficacy required for future medical diagnostics and therapeutics. Extensive research efforts are needed and any one specific solution to biocompatibility, biodistribution, and bioelimination with enhanced diagnostic/therapy abilities may lead to an exciting breakthrough in nanomedicine.

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