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Functionalized Silica Nanoparticles: A Platform for Fluorescence Imaging at the Cell and Small Animal Levels

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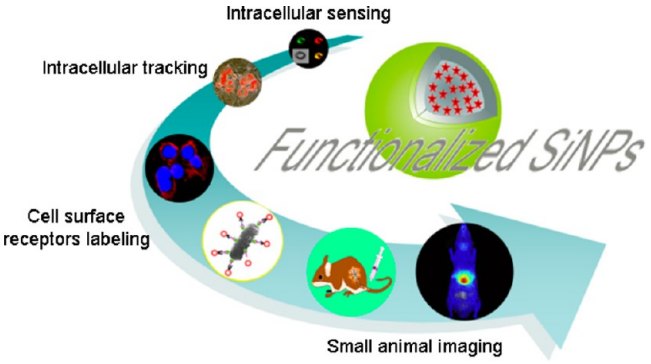

Going *in vivo*, including living cells and the whole body, is very important for gaining a better understanding of the mystery of life and requires specialized imaging techniques. The diversity, composition, and temporal–spatial variation of life activities from cells to the whole body require the analysis techniques to be fast-response, noninvasive, highly sensitive, and stable, *in situ* and in real-time. Functionalized nanoparticle-based fluorescence imaging techniques have the potential to meet such needs through real-time and noninvasive visualization of biological events *in vivo*.

Functionalized silica nanoparticles (SiNPs) doped with fluorescent dyes appear to be an ideal and flexible platform for developing fluorescence imaging techniques used in living cells and the whole body. We can select and incorporate different dyes inside the silica matrix either noncovalently or covalently. These form the functionalized hybrid SiNPs, which support multiplex labeling and ratiometric sensing in living systems. Since the silica matrix protects dyes from outside quenching and degrading factors, this enhances the photostability and biocompatibility of the SiNP-based probes. This makes them ideal for real-time and long-time tracking. One nanoparticle can encapsulate large numbers of dye molecules, which amplifies their optical signal and temporal–spatial resolution response. Integrating fluorescent dye-doped SiNPs with targeting ligands using various surface modification techniques can greatly improve selective recognition. Along with the endocytosis, functionalized SiNPs can be efficiently internalized into cells for noninvasive localization, assessment, and monitoring. These unique characteristics of functionalized SiNPs substantially support their applications in fluorescence imaging *in vivo*.

In this Account, we summarize our efforts to develop functionalized dye-doped SiNPs for fluorescence imaging at the cell and small animal levels. We first discuss how to design and construct various functionalized dye-doped SiNPs. Then we describe their properties and imaging applications in cell surface receptor recognition, intracellular labeling, tracking, sensing, and controlled release. Additionally, we have demonstrated the promising application of dye-doped SiNPs as contrast imaging agents for *in vivo* fluorescence imaging in small animals. We expect these functionalized dye-doped SiNPs to open new opportunities for biological and medical research and applications.

1. Introduction

The *in vivo* approach allows visualization of biology in an intact and native physiological state.[1] Going *in vivo*, including living cells and the whole body, is very important for gaining better understanding of dynamic biochemical processes. Due to the diversity, composition, and temporal–spatial

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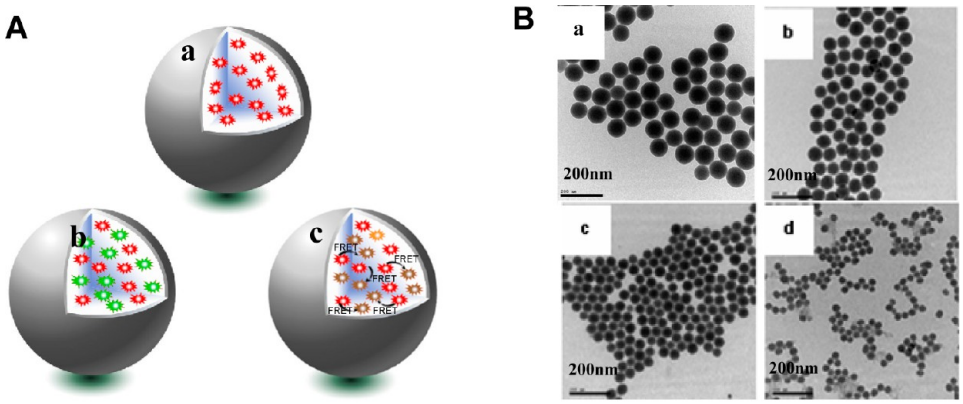

FIGURE 1. (A) Schematic drawing of different kinds of functionalized dye-doped SiNPs. (a) single-dye-doped SiNPs; (b)SiNPs doped with two kind of dyes with ratiometric effects; (c) SiNPs doped with two kind of dyes with FRET effects. (B) TEM imaging of SiNPs with different sizes.

variation of life activities from cells to the whole body, the ability to monitor and visualize biological processes *in vivo* is highly challenging. Therefore, specialized technologies and methods should be developed to meet the following requirements: small enough in spatial scales so as to enter cells and cellular organelles for imaging, high sensitivity to track trace component, fast response to keep up with the rapid changes of biological events, high selectivity to accurately identify the object of analysis in the complex life system, and little disturbance to the living states.[2−4]

Functionalized fluorescent nanoparticle based imaging techniques have the potential to meet such needs through real-time and noninvasive visualization of biological events *in vivo*.[5−8] The nanoparticles can specifically interact with their targets in a biological system and transfer the interaction to high temporal−spatial resolution signal readout.[9] With their small size on nanometer scale, the nanoparticles bring minimal physical perturbations to delicate biological systems such as living cells and the whole body.[10] In addition, the nanoparticles can provide a solid support for sensing assays with multiple ligand molecules attached to each nanoparticle, simplifying assay design and increasing the labeling ratio for higher sensitivity in complex biological systems.[9] The miniaturized fluorescent nanoparticles can also be easily taken up by cells through endocytosis and subsequently used for site-specific intracellular measurements and long-term tracking of biomolecules in real time.[11] In addition, nanoparticle-based fluorescent contrast agents can overcome several limitations of conventional fluorescent dyes, such as poor hydrophilicity and photostability, low quantum yield and detection sensitivity, and insufficient stability in whole body systems.[9,12] Especially, taking advantage of the enhanced permeability and retention (EPR) effect of the tumor microvasculature and the poor lymphatic drainage of tumors, fluorescence nanoparticle-based contrast agents tend to preferentially accumulate in tumor tissues for passive targeting imaging.[13] Recently, several examples of fluorescence nanoparticle-based imaging *in vivo* have been reported, including dye-containing nanoparticles, quantum dots (QDs), upconversion nanoparticles, and gold nanoclusters.[12−16]

In the past decade, our group engaged in the preparation of functionalized fluorescent nanoparticles and their imaging applications, including dye-doped silica nanoparticles (SiNPs), QDs, and metal nanoclusters.[17−22] Especially, due to their robust fluorescent brightness, high chemical stability, excellent biocompability, and flexible silica chemistry, dye-doped SiNPs have been systematically studied as a platform for fluorescence imaging at the cell and small animal levels. This Account will describe our recent efforts in the development of functionalized SiNPs, biointeraction analysis of functionalized SiNPs, and their applications in cellular surface reporter recognition and labeling, intracellular sensing and tracking, and fluorescence imaging in small animals.

2. Preparation and Properties of Functionalized SiNPs

Functionalized SiNPs with fluorescent signals consist of the silica matrix and luminescent organic or inorganic dye molecules encapsulated inside (Figure 1A). Generally, thousands of dye molecules can be housed inside one SiNP.[22] Compared with a single dye molecule, the dye-doped SiNPs can produce a highly amplified optical signal, which provides great improvement in imaging sensitivity.[21] In addition, the nontoxic nature, convenient silane-based chemistry, and high stability also make silica an important host material, which can serve as a protective shell or dye isolator, avoiding the effect of the outside environment on the fluorescent dyes doped in the

nanoparticles. Thus, dye leakage from SiNPs can be greatly inhibited and both photobleaching and photodegradation can be obviously reduced.

In our study, dye-doped SiNPs could be prepared through a water-in-oil reverse microemulsion protocol. Three main components make up the reaction mixture: water, surfactant, and oil. The stabilized water nanodroplets formed in the oil solution act as small microreactors, where silane hydrolysis and the formation of nanoparticles with dye trapped inside take place, producing functionalized hybrid SiNPs. We found that electrostatic interaction of dyes with the silica matrix would be dominant whether or not the stable dye-doped SiNPs formed. If some dyes themselves cannot be directly doped, stable dye-doped SiNPs can also be prepared after changing the electric charge polarity by regulating the experimental conditions of relevant materials.[23] Based on the theoretical foundation, ruthenium complexes (RuBpy), methylene blue (MB), Cy5, and other organic dyes can be readily entrapped in the SiNPs, producing single-dye-doped SiNPs. In a like manner, different dyes can be synchronously encapsulated in one SiNP to construct ratiometric fluorescence probes or fluorescence resonance energy transfer (FRET) mediated large Stokes shifting fluorescence probes (Figure 1A).[24,25] Meanwhile, the particle size can also be effectively controlled by adjusting the concentration of reactants and the molar ratio of water to surfactant for the microemulsion process. SiNPs varying in diameter between about 20 and 100 nm can be prepared (Figure 1B).[26]

Due to the flexible processing of silica chemistry, the dye-doped SiNPs modified with different functional groups, such as hydroxyl (OH), carboxyl (COOH), phosphonate (CH_3HPO_2), poly(ethylene glycol) (PEG), and amine (NH_2), can be easily prepared through additional coating with different alkoxysilane reagents. The surface functionalization endows the SiNPs with different charges and shows great influences on their colloidal behaviors. For example, we investigated the dielectrophoretic assembly of SiNPs with different chemical groups, which revealed that COOH or CH_3HPO_2 group modification could obviously enhance the dielectrophoretic assembly behavior of SiNPs.[27] Moreover, surface functionalization also influences their interaction with biological molecules.[28] It was found that DNA could be easily enriched onto the positively charged SiNPs and protected from enzymatic cleavage. By using green fluorescence protein (GFP) plasmid DNA as a model DNA, it was demonstrated the plasmid DNA strands are protected from enzymatic cleavage after binding with amino-modified SiNPs to form DNA−SiNP complexes and have the same properties as free DNA strands when released from the SiNPs. More interestingly, it was proven that the released plasmids were still biologically active with GFP synthesized in the cells through the expression of the GFP gene. We believe that the size effects of SiNPs may force the DNA to become bound in such a way that cleavage is either impossible or at least greatly slowed on the NP surface.[28] Utilizing this kind of SiNPs, an anti-ODN (antisense oligonucleotide) carrier for gene therapy has been successfully constructed and demonstrated to greatly improve the inhibition efficiency of anti-ODNs for the proliferation and survivin expression in tumor cells.[29] Besides, based on the electrostatic interaction, SiNPs with different charges have been adopted for selective separation of different proteins. According to the isoelectric points of proteins and SiNPs, the adsorption or desorption between them could be perfectly controlled by adjusting the pH in solution, which is promising for the development of advanced adsorbents with high selectivity and enhanced separation capacity.[30] More importantly, the surface functional groups on the SiNPs can act as a scaffold to graft biological moieties for versatile imaging applications, as we will describe below.

3. Functionalized SiNPs for Fluorescence Imaging at Cellular and Intracellular Levels

3.1. Interaction of Functionalized SiNPs and Cells. Studies on the interaction between functionalized SiNPs and cells are significant issues to guide and assist their applications in cellular and intracellular research. We have investigated the interaction of different functionalized SiNPs with various cell lines, including normal tissue cells, tumor cells, and even stem cells.[31−33] By employing dye-doped SiNPs as a synchronous signal indicator, we assessed the effects of a series of factors on the interaction of SiNPs and cells, such as concentration and chemical modification of SiNPs, incubation time and temperature, and serum and inhibitors in culture medium. It was found that the SiNPs could be taken up by both normal tissue cells and tumor cells, and the internalization of SiNPs was a concentration, chemical modification, time, and energy dependent endocytic process.[32] Moreover, through the uptake inhibition investigation, we found that the internalization of functionalized SiNPs into cells was in part through adsorptive endocytosis and in part through fluid-phase endocytosis.

Another example is stem cells, which are characterized by the ability to renew themselves through mitotic cell division and differentiation into a diverse range of specialized cell types. With the development of stem cell nanotechnology

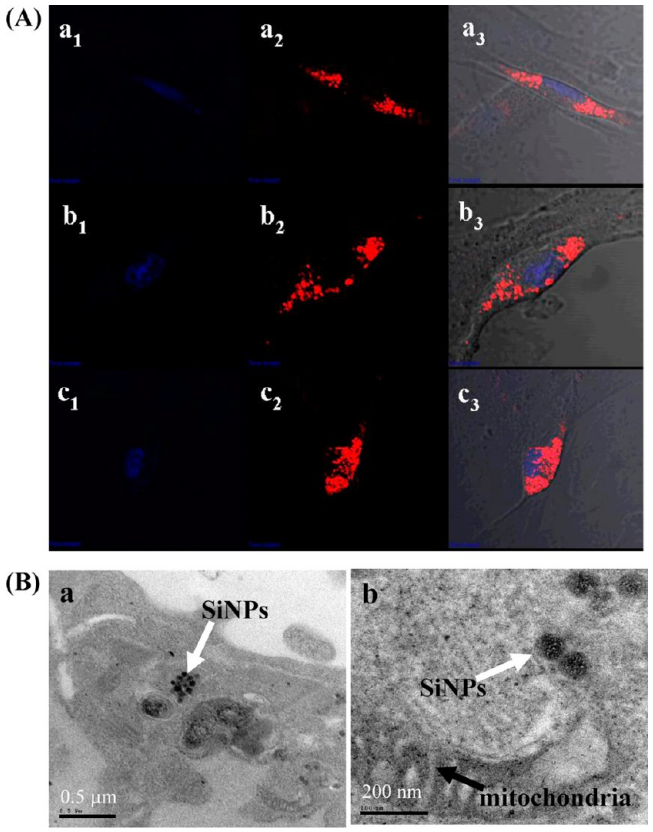

FIGURE 2. (A) Confocal fluorescence images of RMSCs after treatment with SiNPs for (a) 24 h, (b) 48 h, and (c) 72 h. The red fluorescence in RMSCs was derived from the fluorescent SiNP. (B) TEM images of RMSCs after treatment with SiNPs for 72 h. Reproduced from ref 33. Copyright 2010 American Chemical Society.

as a new interdisciplinary field, the biocompatibility of functionalized nanoparticles with stem cells has also emerged to be a fundamental and essential issue to which more and more attention is paid. In our study, a systematic interaction investigation of SiNPs with Sprague–Dawley rat mesenchymal stem cells (RMSCs) was carried out.[33] Results showed that SiNPs at a concentration creating little cytotoxicity on RMSCs could be well taken up by RMSCs and would not affect cellular ultrastructures (Figure 2), adipogenic differentiation, and osteogenic differentiation. It was also demonstrated that insulin could be conjugated onto the SiNPs and retain its biological activity for induction of RMSC adipogenic differentiation in combination with other supplements, which revealed that SiNPs could serve as excellent carriers for differentiation-inducing factors in stem cell research.

3.2. Labeling and Tracking of Cell Surface Receptors. *In situ* labeling and tracking cell surface receptors with high sensitivity and selectivity holds great potential for the diagnosis of early stage diseases and the monitoring of some life processes. By utilizing the significant properties of dye-doped SiNPs, we developed an immunofluorescence labeling and tracking technique platform for cell surface receptor-related imaging. Based on the affinity and specificity associated with the antigen–antibody or ligand–receptor recognition process, dye-doped SiNPs were covalently conjugated with different antibodies or ligands and then applied to label antigens or receptors in several cell lines selectively and efficiently, such as $SmIgG^{+}B$ lymphocyte for the immune-diagnosis of systemic erythema lupus, HepG liver cancer cells, MDA-MB-231, and MCF-7 breast cancer cells.[21,34–36]

A representative example is the identification of live liver cancer cells in a mixed cell system using lactobionic acid (LA)-conjugated RuBpy-doped SiNPs.[37] The LA–SiNP bioconjugates were specific enough not only to effectively identify liver cancer cells from mixed heterogeneous cells (Figure 3A) but also to precisely label a few liver cancer cells in blood. Besides, multilabeling of target cells could also be carried out by conjugating different antibodies or ligands with different dye-doped SiNPs. Relying on the antigen–antibody recognition of CEA and CK19 in MGC-803 cells with anti-CEA antibody and anti-CK19 antibody on fluorescein isothiocyanate (FITC) doped SiNPs and RuBpy doped SiNPs, respectively, both *in vitro* cultured MGC-803 cells in blood and the *ex vivo* primary MGC-803 cells that came from the tumor tissues of mice bearing MGC-803 gastric cancer tumor xenografts were verified to be double-labeled and distinguished (Figure 3B).[38]

Similarly, some life processes have also been monitored by labeling and tracking cell membrane surface receptors using biofunctionalized SiNPs.[39] The externalization of phosphatidylserine (PS) from the inner to the outer membrane is an early and major event in the apoptotic process, which has been used to detect early stage apoptosis. A novel bioprobe based on Rhodamine B isothiocyanate-doped SiNPs modified with annexin V has been explored, which could not only specifically label early stage apoptotic cells but also track the physiological change process of PS. With the extension of culture time with paclitaxel, the number of the apoptotic cells labeled by the bioprobe increased ever more. Meanwhile, red fluorescence of the SiNPs on the outer membrane of the recognized apoptotic cells changed from weak to strong, from partially to completely surrounding the cell membrane (Figure 3C), which revealed a gradual translocation process of PS in the early stage apoptotic cell membrane.

In addition, by combination of flow cytometry, ligand–magnetic nanoparticles enrichment strategy or the microfluidic

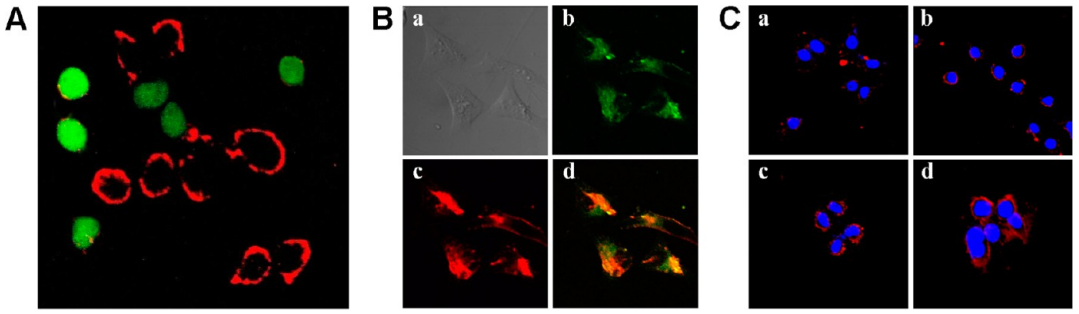

FIGURE 3. (A) Recognition of liver cancer cells (red) from mixed heterogeneous cells with LA-conjugated RuBpy-doped SiNPs. Reproduced from ref 37. Copyright 2007 Elsevier. (B) Biofunctionalized dye-doped SiNPs mediated double immunofluorescence imaging of MGC-803 gastric cancer cells. Reproduced from ref 38. Copyright 2008 Elsevier. (C) Images of early stage apoptotic breast cancer cells treated with paclitaxel for different time periods, and then stained with RBITC-doped SiNPs conjugated to annexin V (red). Reproduced from ref 39. Copyright 2007 Elsevier.

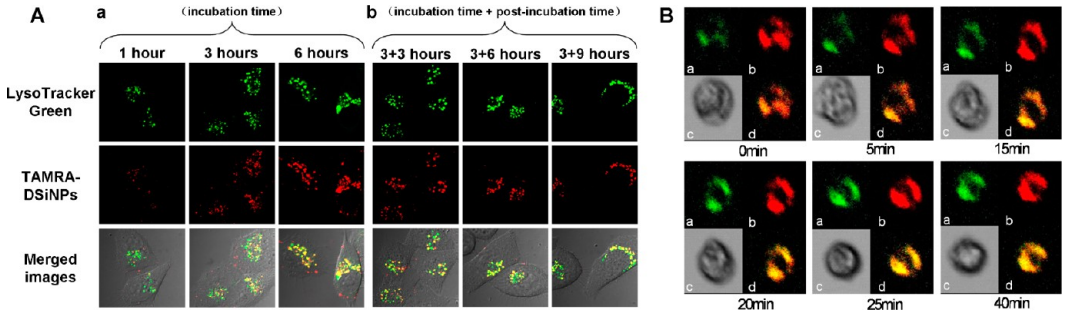

FIGURE 4. (A) Feasibility investigation of TAMRA-doped SiNPs as the lysosome marker. Reproduced from ref 44. Copyright 2010 American Chemical Society. (B) Change in lysosomal pH, as monitored by two-fluorophore doped SiNPs, in murine macrophages after treatment with chloroquine. Reproduced from ref 24. Copyright 2007 Springer.

system, this SiNP-based immunofluorescence labeling was also introduced into rapid and ultrasensitive detection of pathogens, such as tuberculosis, *Escherichia coli*, and *Salmonella*.[40−43]

3.3. Intracellular Labeling, Tracking, Sensing and Controlled Release. Noninvasive intracellular labeling, tracking, sensing, and controlled release in living cells is an important and challenging issue in cell-related research. By utilizing the property that SiNPs can be transported into living cells through endocytosis, we developed a novel biocompatible and long-lived lysosome labeling and tracking method based on dye-doped SiNPs.[44] It was demonstrated that tetramethylrhodamine (TAMRA)-doped SiNPs could selectively accumulate in lysosomes of HeLa cells (Figure 4A), and the photostability of TAMRA-doped SiNPs associated with lysosomes was detectable, at least 30 times longer than that of LysoTracker Green. Compared with LysoTracker Green and Alexa 488-dextran, the SiNPs' fluorescence could be detected over a 5-day postrecultivation period and was demonstrated to be adoptable for lysosome-related studies in living cells, fixed cells, and permeabilized cells. With this method, we achieved long-time lysosome labeling and tracking in chloroquine-treated HeLa cells, which indicates its potential application as a biocompatible, long-life, and highly photostable lysosome marker for lysosome-related studies.

Dye-doped SiNPs could also be used for real-time and *in situ* sensing of intracellular physiological parameters change.[24] We constructed a ratiometric pH nanosensor based on two-fluorophore-doped SiNPs that contained a pH-sensitive indicator (FITC) and a reference dye (RuBpy) for noninvasive monitoring of intracellular pH changes (Figure 4B). The pH nanosensor with an average diameter of 42 nm could easily be taken up by cells and exhibited excellent pH sensitivity, reversibility, and a dynamic range of pH 4−7 for biological studies. We used this novel pH nanosensor for monitoring pH changes in living cells by drug stimulation. Both lysosomal pH changes in murine macrophages stimulated by chloroquine and the intracellular acidification in apoptotic cancer cells were monitored in real time and with high pH sensitivity. Furthermore, the relationship of intracellular acidification and apoptosis in HeLa cells induced by vincristine sulfate was studied with this ratiometric pH nanosensors, which revealed that apoptosis was preceded by intracellular acidification.[45] These results would provide theoretical foundation for the therapy

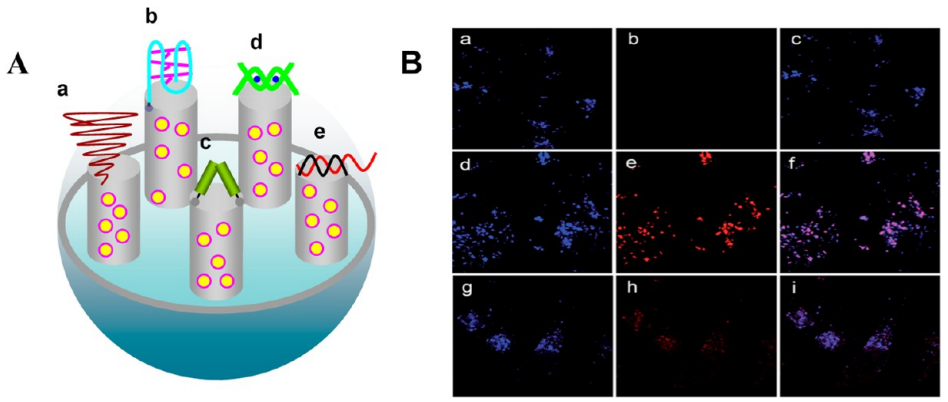

FIGURE 5. (A) Schematic illustration of the stimuli-responsive controlled release system based on various modifications (a, polymers; b, i-motif; c, thymine dimer; d, e, DNA duplex). (B) Fluorescence imaging of the cellular uptake and controlled-release behavior of dsDNA−RuBpy-doped mesoporous SiNPs (red) after incubation with HeLa cells for 0 h (a−c), 3 h (d−f), and 8 h (g−i). Reproduced from ref 47. Copyright 2012 Royal Society of Chemistry.

of cancer through the interference of cells' pH by use of vincristine sulfate or other anticancer drugs.

Since functionalized SiNPs could enter into cells and locate in some certain organelles, they could also be utilized by nanoparticle-based controlled release systems for intracellular drug delivery and therapy studies. In order to increase the drug load amount, mesoporous SiNPs were selected to fabricate stimuli-responsive intracellular controlled release systems. By modifying different kinds of capping agents on mesoporous SiNPs, including organic small molecules, polymer, and nucleic acid strands, etc., we recently constructed several controlled release systems that could be triggered by a range of stimuli, such as light, pH, and biomolecules (Figure 5A).[46−49] For example, by grafting the duplex DNA with C−Ag^{+}−C structure as the smart molecule-gated switch, we have designed a mesoporous SiNPs-based thiol-responsive system and investigated its intracellular release behavior.[47] It was demonstrated that the carriers could internalized remarkably into the cells by endocytosis within 3 h and distributed mainly into the lysosomes (Figure 5B). Subsequently, the entrapped cargo molecules could be released into the cytoplasm matrix or cell nuclei from the pores, triggered by intracellular thiol-containing molecules, such as glutathione and cystine. Currently, intensive research on the use of these responsive controlled release systems for *in vivo* imaging and therapy is being implemented and will be reported in due course.

4. Functionalized SiNPs for Fluorescence Imaging in Small Animals

4.1. Biodistribution and Fate of Functionalized SiNPs. With exploration of the functionalized nanoparticles for *in vivo* applications, their bioeffects have received much attention. Most studies on bioeffects of SiNPs were focused on the pulmonary and skin toxicity, which were carried out mainly using *in vitro*/*ex vivo* histopathology, body weight analysis, and biochemical evaluation.[50] We used an *in vivo* optical imaging technique to study the interaction of intravenously injected SiNPs with *in vivo* system by using RuBpy-doped SiNPs as an adoptable method to track SiNPs.[51] SiNPs with a size of ~45 nm, which were modified with three types of functional groups including OH, COOH, and PEG, were investigated. *In vivo* imaging results indicated that these SiNPs could all be cleared from systemic blood circulation, but both the clearance time and subsequent biological organ deposition were dependent on the surface modification of the SiNPs. By comparison, PEG-SiNPs exhibited relatively longer blood circulation time and lower uptake by the liver than OH-SiNPs and COOH-SiNPs (Figure 6A). The *ex vivo* organ optical imaging results also demonstrated that intensive fluorescence could be detected in the whole body and all of the organs removed from those mice examined at 4.5 h after PEG-SiNP administration, even though the signal intensity of the liver was much lower than that of either OH-SiNPs or COOH-SiNPs (Figure 6B). More interestingly, it was revealed that SiNPs were partly excreted through the renal route. TEM and energy-dispersed X-ray spectrum analysis of urine samples further confirmed the presence of intact SiNPs. By using the same *in vivo* imaging technique, the biodistribution and urinary excretion of SiNPs with different sizes of 118.4 ± 6.0, 72.1 ± 5.3, 46.7 ± 4.2, and 26.1 ± 5.4 nm were also investigated *in situ* and in real time.[26] Results showed that the smaller the size of SiNPs, the longer the blood circulation, and the more obvious in whole body distribution. It was also found that except for SiNPs with size of 118.4 ± 6.0 nm,

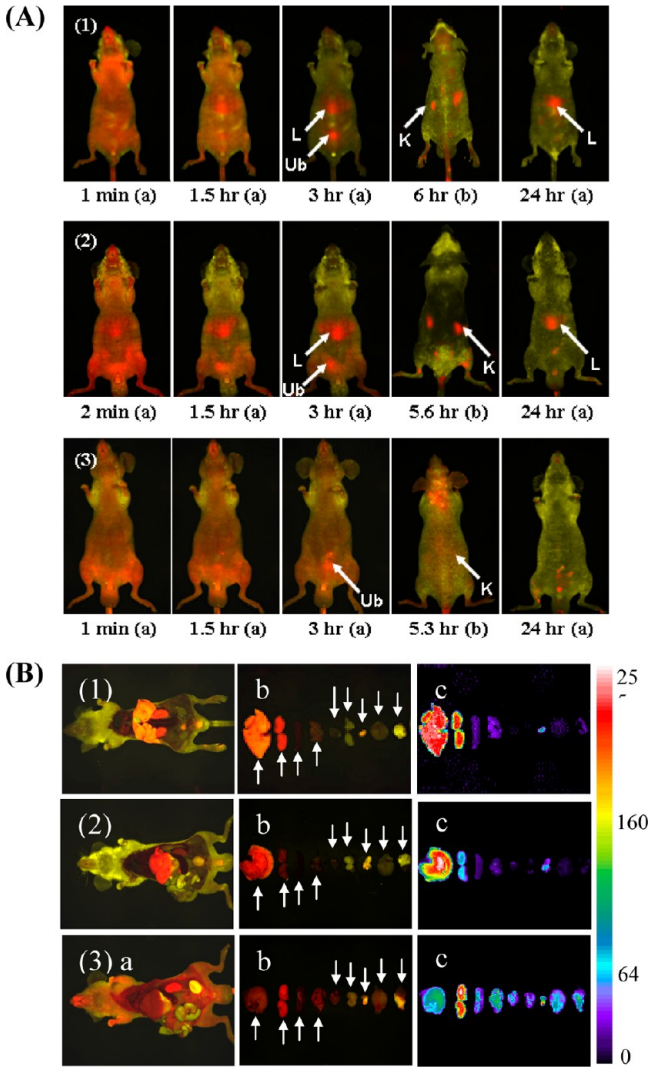

FIGURE 6. (A) *In vivo* imaging biodistribution of different intravenous injected surface-modified silica NPs at different postinjection time points. (B) *Ex vivo* optical imaging of anatomized mice with injection of surface-modified SiNPs and some resected organs during necropsy at 4.5 h pi. Reproduced from ref 51. Copyright 2008 American Chemical Society.

the other three dye-doped SiNPs could be partly excreted from mice via the renal route following intravenously adminstration. This work demonstrated that the *in vivo* optical imaging method is helpful for biodistribution and fate investigation of functionalized SiNPs in small animals.

4.2. Near-Infrared Fluorescence Imaging. The above study presents an effective demonstration that dye-doped SiNPs hold great potential as contrast agents for *in vivo* fluorescence imaging. However, RuBpy is not the ideal indicator due to its inability to produce near-infrared (NIR) fluorescence, which could result in low absorption and autofluorescence from biological tissues.[52] In order to further improve *in vivo* imaging quality, methylene blue (MB), the most inexpensive of the commercially available NIR fluorescent dyes, was selected to be doped in the phosphonate-terminated SiNPs for the development of a novel NIR fluorescence imaging probe.[53] It was found that the fluorescence of MB-doped SiNPs was very stable in water, PBS, and serum, and dye leakage was little. It was also demonstrated that sites subcutaneously injected with different concentrations of MB-doped SiNPs emitted detectable NIR fluorescence (Figure 7A). *In vivo* imaging of mice intravenously injected with MB-doped SiNPs indicated that the whole mouse body emitted strong NIR fluorescence after administration. Subsequently, most of the MB-doped SiNPs gradually accumulated in the reticuloendothelial system such as liver and spleen (Figure 7B). It was also observed that some MB-doped SiNPs were excreted through the renal routes. These results effectively proved that MB-doped SiNPs could serve as a promising probe for *in vivo* NIR fluorescence imaging. In addition, because MB is a phenothiazinium photosensitizer with promising applications in photodynamic therapy (PDT) for its high quantum yield of singlet oxygen generation, we then applied MB-doped SiNPs to perform tumor PDT. Results showed that MB-doped SiNPs could effectively protect MB against reduction by diaphorase, and the PDT effect toward the xenograft tumor *in vivo* was exciting after imaging the MB-doped SiNP injected tumor.

4.3. FRET-Mediated Large Stokes Shift NIR Fluorescence Imaging. The single NIR dye-doped SiNPs has indeed improved the *in vivo* imaging quality to a certain extent, but it is still limited by background and cross-talking between the excitation light and the emitting signals due to the small Stokes shift of the traditional NIR dyes. To address this issue, we present novel large Stokes shift NIR fluorescent SiNPs (LSS-NFSiNPs) based on the principle of FRET.[25] By using two highly water-soluble dyes, RuBpy and MB, as the model donor–acceptor pair, we prepared LSS-NFSiNPs by synchronously doping these two dyes in SiNPs. After optimization of their doping molar ratio, FRET from RuBpy to MB occurred in the silica matrix, resulting in a NIR fluorescent SiNPs with strong signals and large Stokes shift (>200 nm). It was demonstrated that the LSS-NFSiNPs could effectively help to increase the discrimination of the fluorescence signal of interest over other background signals like the autofluorescence from mouse body and the interfering signal from the alimentary canal (Figure 8I). With a combination of excellent stability, large Stokes shift, and NIR spectral properties, this novel LSS-NFSiNPs provides real-time (Figure 8II), deep-tissue fluorescent imaging of live animals. More importantly, the LSS-NFSiNPs can also be gradually cleared from the body through the urinary clearance system. We anticipate this design concept can lay a foundation for further development

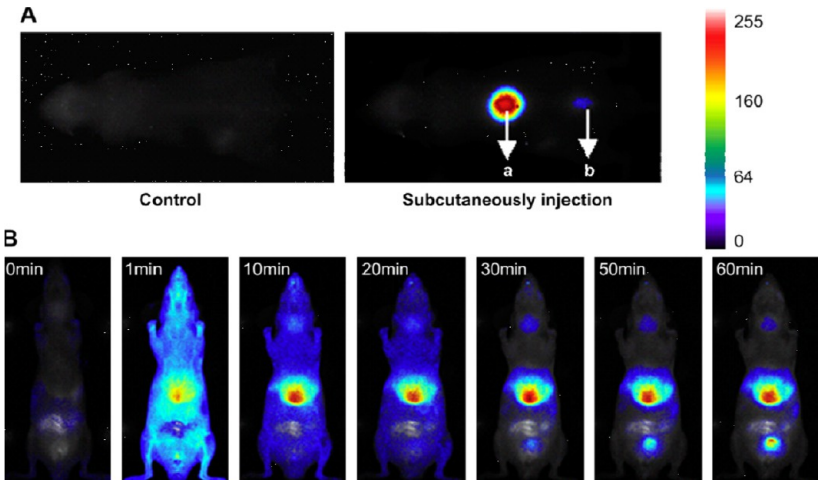

FIGURE 7. *In vivo* NIR imaging of mice with the (A) subcutaneous and (B) intravenous injection of MB-doped phosphonate-terminated SiNPs. Reproduced from ref 53. Copyright 2009 Elsevier.

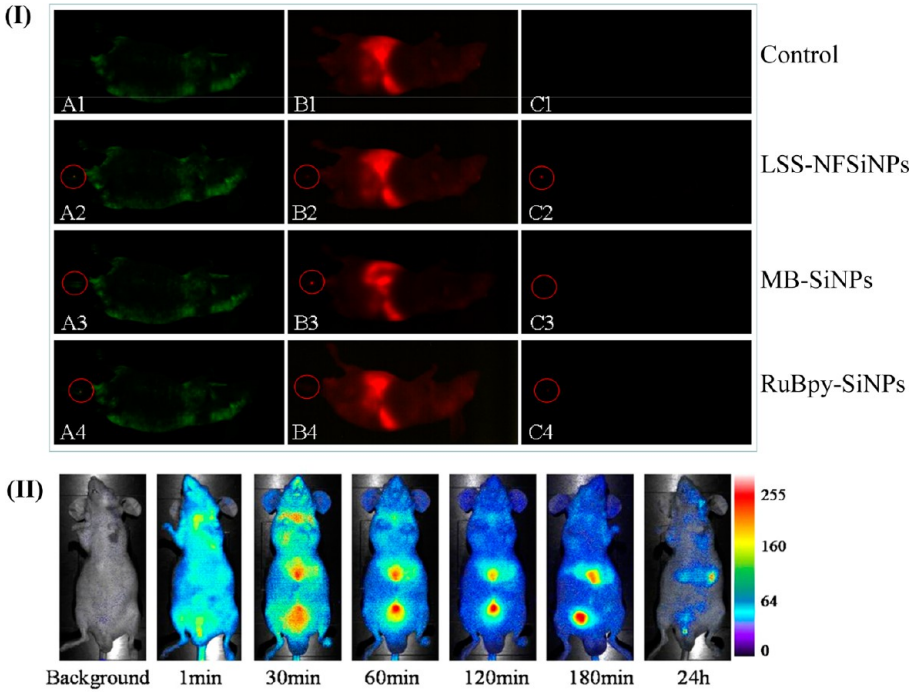

FIGURE 8. (I) *In vivo* imaging of nude mice after dipping with different nanoparticles on the tails. (A) Excited at 465−495 nm and recorded at 515−600 nm. (B) Excited at 615−665 nm and recorded at 680−800 nm. (C) Excited at 465−495 nm and recorded at 680−800 nm. The red circle in every image locates the site of the dipping nanoparticles. (II) Real-time *in vivo* abdomen FRET imaging of nude mice intravenously injected with the LSS-NFSiNPs. Reproduced from ref 25. Copyright 2012 American Chemical Society.

of *in vivo* optical nanoparticulate contrast toward clinical applications.

5. Perspective

The unique characteristics, such as hydrophilic nature, nanometric size, high payload capacity, and suitability for a variety of conjugation methods, have enabled SiNPs to be useful for fluorescence imaging at the cell and small animal levels. Ongoing and future efforts will focus on the clinical translation of SiNPs for molecular imaging, disease diagnostics, and therapy. A great number of challenges, or opportunities, are still present in this area. First, the highly reproducible synthesis of functionalized SiNPs with controllable size and shape, good dispersibility, and multifunction for clinical applications needs to be standardized. Second, to endow SiNPs with more versatile and efficacious functions, more targeting

ligands against a wider range of cellular proteins or tumor cells should also be developed, such as aptamers, which have recently attracted much attention as a new and promising type of targeting molecules and have been successfully introduced into primary attempts for fluorescence imaging at the cell and small animal levels in our laboratory.[54−59] Third, attempts at surface engineering of SiNPs have to be made to reduce nonspecific SiNP uptake by the reticuloendothelial system. Moreover, further understanding of the interaction between SiNPs and large animals, such as biocompatibility, toxicity, and stability, should be investigated because fluorescence imaging experiments in cultured cells and in rodents may not be readily reproduced in larger animals or humans. Finally, the development of multifunctional SiNPs combining diagnostic and therapeutic capabilities is worthy of special attention, which would have great advantages in providing a "see and treat" strategy for tumors or other disease. We anticipate that such challenges or opportunities could potentially be raveled out through the interdisciplinary conversation of materials science, chemistry, biology, imaging, and preclinical and clinical medicine specialists.

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BIOGRAPHICAL INFORMATION

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FOOTNOTES

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The authors declare no competing financial interest.

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