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Imaging Agents

Biomimetic Surface Engineering of Lanthanide-Doped Upconversion Nanoparticles as Versatile Bioprobes**

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The past decade has witnessed an explosion of interest in the development of luminescent nanoparticles because of their excellent potentials in biomedical application.[1] Most recently, lanthanide ion (Ln^{3+}) doped upconversion nanoparticles (UCNPs), typically $NaMF_4$:Yb^{3+}/Ln^{3+} (M = yttrium or gadolinium, Ln = erbium or thulium), have become an exciting new class of nanophosphors that convert near-infrared (NIR) excitation light (typically ca. 980 nm) into shorter-wavelength luminescence.[2,3] The major appeal of bioimaging using UCNPs is that deep tissue penetration as well as high-contrast optical imaging can be achieved owing to the ability to suppress autofluorescence and minimize photodamage to living cells.[3–5] Furthermore, UCNPs can afford tunable multicolor emission with exceptional photostability. These properties coupled with low toxicity to cells make UCNPs an ideal choice for long-term imaging in vitro and in vivo.[3–5]

Despite these promises, a major challenge in the field of UCNPs is the lack of a general methodology to make water-dispersible, bio-compatible, and functionalizable UCNPs, because they are normally prepared in organic solvents and capped with hydrophobic ligands that lack any functional groups for surface modification; meeting this challenge is a prerequisite for many biomedical applications of this class of materials.[3–5] Toward this goal, silica coating has been developed to render the UCNPs dispersible in water, but further surface modification steps are still required to attach functional groups on the silica shell for bioconjugation. Oxidation of the capped oleic acid ligands to azelaic acid could make UCNPs both water-dispersible and functionalizable,[4c] but the oxidation process produces adventitious MnO_2 that can quench the luminescence.[4d] Despite the recent advances in the direct synthesis of water-dispersible UCNPs[4b] and in the postsynthesis ligand oxidation with ozone[4q] or in the ligand-free treatments[4r] to render them water-dispersible and functionalizable, there is a need for a simple and general method for producing biocompatible UCNPs with versatile chemical surface properties that allow coupling of various biomolecules; such bionanomaterials can be used in diverse applications, such as selective sensing or imaging and targeted therapy.[3a,f] Herein, we report such an approach to engineer the UCNP surface coating with a monolayer of functional phospholipids (Figure 1). These phospholipids afford biocompatibility by mimicking the composition and functionality of the cell's external membrane.

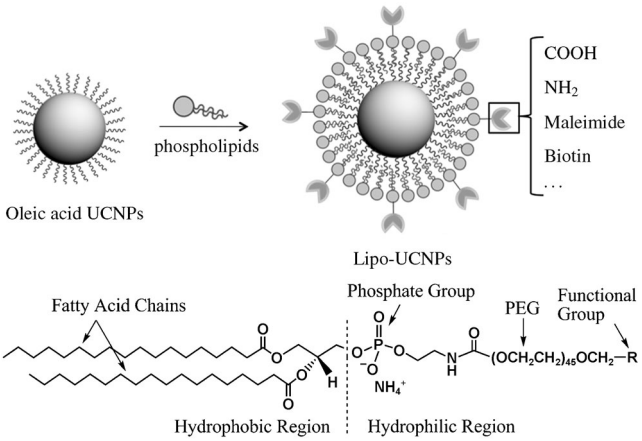

Figure 1. Illustration of the assembly of the water-dispersible and functionalizable UCNPs by adding a monolayer of phospholipids, which mimick the composition of the external cell membrane, to the oleic acid capped UCNPs. The molecular structure of the designed phospholipid is shown at the bottom.

Phospholipids are a major component of all cell membranes that possess special amphiphilic structure and excellent biocompatible properties. Phospholipid-based nanostructures (e.g. liposomes) have been widely used for encapsulating drugs and delivering drugs to numerous cell lines and have been approved by the US Food and Drug Administration for disease treatment in the clinic.[6] Because of their intriguing properties, phospholipids have been successively used to provide biofunctionality to various inorganic nanoparticles, such as quantum dots (QDs),[7] iron nanoparticles,[8] and

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mesoporous silica nanoparticles[9] for different biomedical applications. Herein we propose to engineer UCNPs with phospholipids to overcome one of the biggest challenges in UCNP applications mentioned above. As shown in Figure 1, the designed phospholipids contain four segments: two fatty acid chains, a phosphate group, a poly(ethylene glycol) (PEG) segment, and a functional group to realize the biomimetic surface engineering of UCNPs. The UCNPs would become water-dispersible driven by the hydrophobic van der Waals interactions between the hydrophobic tail of the phospholipids and the primary oleate ligands on the UCNP surface. The fatty acid chains of the phospholipids are embedded in the hydrophobic surface of the UCNPs, while the hydrophilic part points out toward the aqueous environment. In such a way, the surfaces of UCNPs can be modified with PEG, an inert, nontoxic, and nonimmunogenic hydrophilic polymer. Furthermore, various functional groups (e.g. carboxylic acid, amine, maleimide, biotin) at the end of the PEG segment of the Lipo-UCNPs can be easily conjugated with various biomolecules for specific biomedical applications.

Highly efficient upconverting $NaYF_4$:18% Yb^{3+}/2% Er^{3+} nanoparticles were synthesized according to the literature methods using oleic acid as the stabilizing agent.[10] As shown in Figure 2a, these nanocrystals display uniform hexagonal plate-like morphology with mean sizes of approximately 60 nm and high crystallinity. To demonstrate the feasibility of

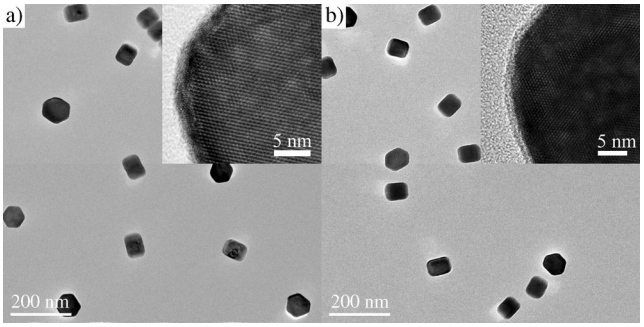

Figure 2. TEM images of the a) as-prepared UCNPs and b) UCNPs coated with phospholipids DSPE-PEG-COOH. Inset shows the high-resolution TEM image of the respective sample.

the method described in Figure 1, a PEGylated phospholipid, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[carboxy(polyethylene glycol)-2000] (DSPE-PEG-COOH), was used to coat the above UCNPs. Representative TEM images (Figure 2b, Figure S1 in the Supporting Information) of the resulting Lipo-UCNPs show that they remain monodisperse in size without obvious change in shape and without aggregation. High-resolution TEM investigation (Figure 2b, inset) confirms the core/shell crystallized nanocrystals with a uniform, approximately 2 nm thick, hydrophobic oleic acid/lipid layer around the surface. Dynamic light scattering (DLS) measurements indicated that the Lipo-UCNPs were well-dispersed in water with a mean hydrodynamic diameter of approximately 88 nm (Figure S2 in the Supporting Information). In comparison with oleic UCNPs dispersed in cyclohexane (ca. 66 nm), this increase of approximately 22 nm in diameter is in agreement with a monolayer of the PEGylated phospholipids.

The DSPE-PEG-COOH-coated Lipo-UCNPs possess excellent water solubility by virtue of their cloaks of hydrated PEG molecules (Figure 3a). Upon continuous excitation at 980 nm, the luminescence of the lipid-coated $NaYF_4$:18% Yb^{3+}/2% Er^{3+} nanoparticles in water appears

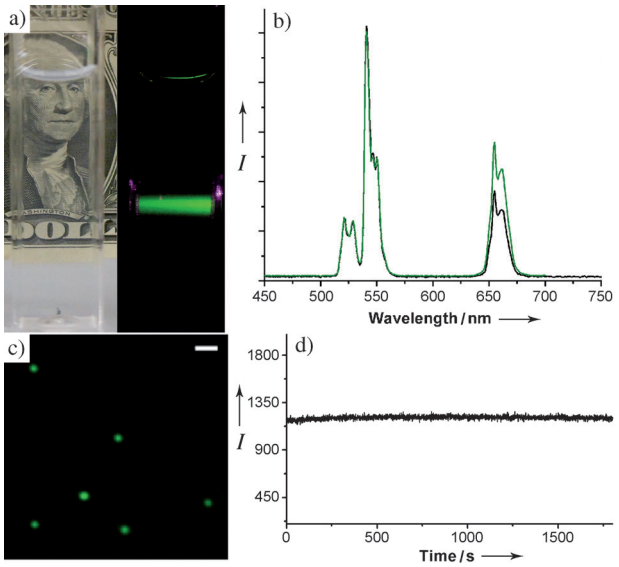

Figure 3. a) Photographs of the transparent water solution of Lipo-UCNPs without laser illumination (left) and the upconverted visible luminescence under continuous-wave 980 nm laser illumination (right). b) Upconversion luminescence spectra measured at room temperature of as-prepared UCNPs in cyclohexane (black trace) and Lipo-UCNPs in water (green trace) under excitation at 980 nm. c) Upconversion luminescence image of individual Lipo-UCNPs attached to a coverslip under 980 nm excitation. Scale bar = 1 μm. d) Super photostability of a single Lipo-UCNP. Emission collected by using a 510–560 nm channel. Exposure time for imaging data collection = 0.5 s.

predominantly green (Figure 3a). The corresponding upconversion luminescence spectrum of Lipo-UCNPs in water is similar to that of the as-prepared samples in cyclohexane with a slight decrease in the relative integrated green/red emission ratio owing to the surface quenching effect of water molecules (Figure 3b).[5b] These results strongly indicate that the characteristic upconversion property of the nanoparticles was unaffected by the phospholipid coating. The NIR-to-visible upconverting property of the Lipo-UCNPs was further investigated at the single-molecule level. Immobilized individual Lipo-UCNPs were imaged by excitation at 980 nm, which resulted in bright individual green spots on the coverslip surface (Figure 3c). These Lipo-UCNPs spots exhibited neither blinking nor photobleaching over 30 min continuous laser excitation (Figure 3d). Figure S3 in the Supporting Information shows the point-spread functions (PSF) of a few single Lipo-UCNP luminescent spots excited at 980 nm, 50 kW cm^{-2}. At 30 ms exposure time, we detected approximately 6000 photons from one single spot, allowing us

to localize the center of the spot to 1.6 nm accuracy. These properties make the Lipo-UCNP an excellent probe for single-molecule imaging.[11] Furthermore, the Lipo-UCNPs showed long-term stability in water and resistance to aggregation over an extended period of several months (Figure S4 in the Supporting Information).

To take advantage of the wide availability of the phospholipids with various headgroups, we then investigated the versatility of our method in preparing Lipo-UCNPs with different functional groups, which then allow attachment of a wide range of molecules on the surface. As shown in Figure S5 in the Supporting Information, Lipo-UCNPs functionalized with an organic dye could be synthesized through this one-step assembly by using a dye-labeled phospholipid. Förster resonance energy transfer (FRET) between the dye and the UCNPs was observed; such a system may represent a versatile hybrid probe to study FRET in fundamental and biological processes. Furthermore, to explore the functionalization of Lipo-UCNPs with biomolecules, we performed bioconjugation of DNA to the $NaYF_4$:18 % Yb^{3+}/2 % Er^{3+} UCNPs: First we replaced the DSPE-PEG-COOH phospholipid that coats the UCNP with a mixture of DSPE-PEG and DSPE-PEG-maleimide (1:1) to introduce a maleimide functionality to the outer surface of the Lipo-UCNPs. Thiol-modified DNA was then covalently attached to the Lipo-UCNPs through the sulfhydryl–maleimide coupling reaction. Such a reaction was confirmed by the observation of typical absorbance of DNA at 260 nm after the conjugation (Figure S6 in the Supporting Information). To test the functionality of the DNA on the Lipo-UCNPs, these nanoparticles were then incubated with 5 nm gold nanoparticles (AuNPs) that were modified with thiolated complementary DNA (cDNA). As shown in Figure 4 and Figure S7 in the Supporting Information, Lipo-UCNPs were surrounded by a number of AuNPs, forming a satellite nanostructure. As a control, the assembly of the nanoparticles was not observed when AuNPs were functionalized with noncomplementary DNA (Figure S7 in the Supporting Information), thus indicating that DNA molecules were not only attached to Lipo-UCNPs in a large number but also retained their specific hybridization properties to the complementary DNA targets. The UV/Vis spectrum of the DNA-directed Lipo-UCNP–AuNPs nanoassemblies shows a broad absorption band around 520 nm (Figure S8 in the Supporting Information), consistent with the plasmon resonance frequencies observed in similar AuNPs. Interestingly, minimal gold-induced luminescence quenching was observed (Figure S9 in the Supporting Information), thus suggesting that the phospholipid/DNA layer prevented the contact between the UCNP and the AuNPs.[12] Owing to its high sequence specificity and addressability, DNA has recently attracted great attention as a promising building block for biosensing, nanomedicine, and assembly of nanoparticles.[13] The versatile conjugation of DNA on UCNPs reported herein will therefore be beneficial to expanding these applications.

Having demonstrated the general and versatile method to prepare functionalizable Lipo-UCNPs, we then explored the usage of the Lipo-UCNPs for targeted imaging of cancer cells. Because folate can bind to overexpressed folate receptors on

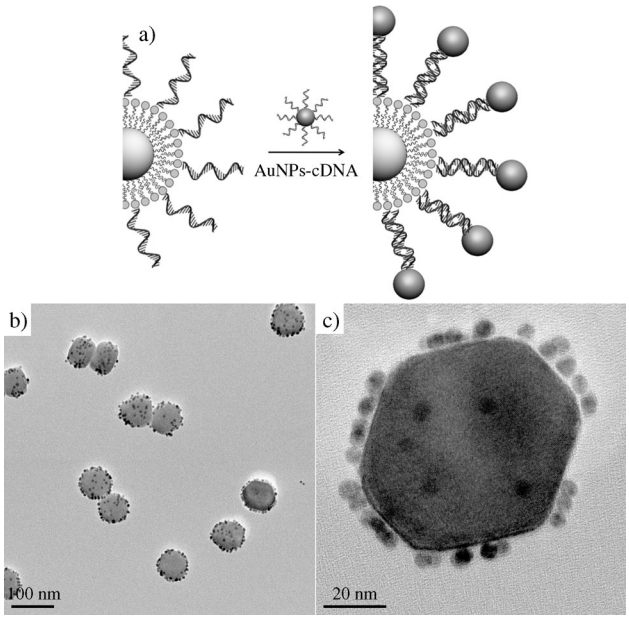

Figure 4. a) Illustration and b, c) TEM images of bioinspired Lipo-UCNP–AuNPs nanoassemblies directed by DNA.

several cancer cell lines, DSPE-PEG-folate was mixed with DSPE-PEG (1:9) and used for the coating of the $NaYF_4$:18 % Yb^{3+}/2 % Er^{3+} UCNPs to introduce the targeting folate ligand on the surface of Lipo-UCNPs to give Lipo-UCNPs-FA (Figure 5 a). The HeLa cells treated with the resulting Lipo-UCNPs-FA were then imaged with widefield or multipoint matrix scan excitation by an NIR laser.[11c] The HeLa cells exposed to Lipo-UCNPs-FA showed many luminescent spots within the cell cytoplasm, thus indicating excellent binding and internalization of nanoparticles into the cancer cells (Figure 5 b). The cellular uptake of the nanoparticles was further confirmed by the 3D reconstruction movie from the data of 3D scan imaging, showing that the Lipo-UCNPs were distributed mainly inside the cells (see Figure S10 and Movie S1 in the Supporting Information). These results are consistent with the projected receptor-mediated endocytosis mechanism. In contrast, when the Lipo-UCNPs without the exterior folate ligands were incubated with HeLa cells under the same physiological conditions minimum uptake of the nanoparticles in the live cells was observed (Figure 5 c), thereby confirming the role of the folate ligand in cell targeting. The availability of various cancer-cell targeting ligands and the ease of conjugating them with the Lipo-UCNPs, as well as their unique optical properties, make the Lipo-UCNPs promising nanoprobes for targeted imaging of cancer cells.

In conclusion, a universal and generalized method has been developed for the synthesis of UCNPs with flexible chemical surface properties for a wide range of applications. The approach has several significant advantages. First, the biomimetic surface offers water-soluble and biocompatible UCNPs, in which the PEGylated surface could afford the nanoparticles with a prolonged circulation half-life, reduced nonspecific binding, and prevention of the reticuloendothelial

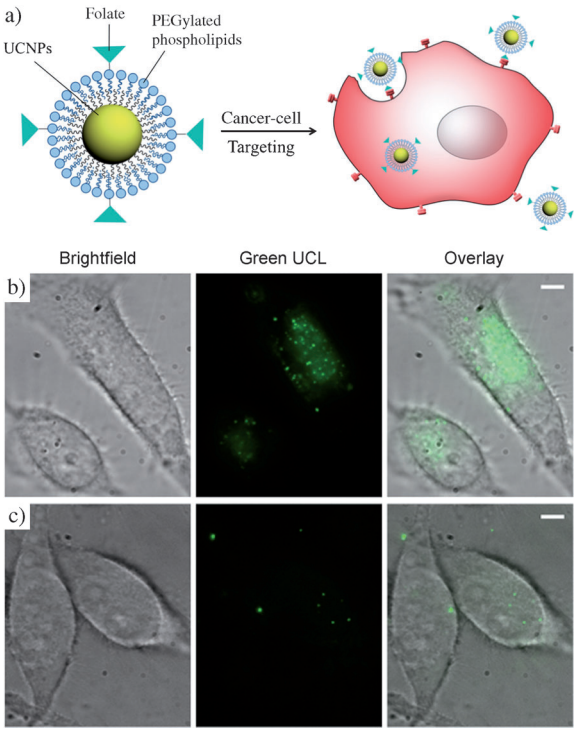

Figure 5. a) Illustration of the targeted imaging of cancer cells with Lipo-UCNPs-FA. b, c) Transmission and luminescence microscopy images of HeLa cells treated with b) Lipo-UCNPs-FA and c) Lipo-UCNPs without folate ligand. For UCNP images, $\lambda_{ex} = 980$ nm, and emission was collected in the range $\lambda = 510$–560 nm. Scale bar = 5 μm. UCL = upconversion luminescence.

system.[14] Second, commercially available lipids with various headgroups (e.g. COOH, NH_2, SH, maleimide, biotin, cyanur chloride) at the end allow for easy functionalization of UCNPs and thus wide applications of bioconjugated UCNPs. Third, the method provides a way to fine-tune the physical surface properties of UCNPs to be positive, negative, or zwitterionic by simply using lipids with different charged headgroups. Forth, the method can be used for construction of multifunctional materials. For example, dye-labeled lipids enable the design of FRET systems on UCNPs; lipids modified with paramagnetic gadolinium(III) complexes will allow for the synthesis of UCNP-based nanoprobes for both upconversion luminescence imaging and magnetic resonance imaging (MRI). Finally, this lipid-coating procedure is also generally applicable to other UCNPs with different sizes. Hydrophobic UCNPs with sizes of approximately 6 and 130 nm have also been functionalized with phospholipid monolayers (Figure S11 and S12 in the Supporting Information). Therefore, the approach demonstrated herein will enable much wider applications for UCNPs.

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