



# Photostable and efficient upconverting nanocrystal-based chemical sensors

Cheryl A. Tajon, Hao Yang, Bining Tian, Yue Tian, Peter Ercius, P. James Schuck<sup>1</sup>, Emory M. Chan, Bruce E. Cohen\*

The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA



## ARTICLE INFO

### Keywords:

Upconverting nanoparticles  
Sensors  
Fluorescence  
Energy transfer  
Photostability

## ABSTRACT

Chemical sensing in living systems demands optical sensors that are bright, stable, and sensitive to the rapid dynamics of chemical signaling. Lanthanide-doped upconverting nanoparticles (UCNPs) efficiently convert near infrared (NIR) light to higher energy emission and allow biological systems to be imaged with no measurable background or photobleaching, and with reduced scatter for subsurface experiments. Despite their advantages as imaging probes, UCNPs have little innate chemical sensing ability and require pairing with organic fluorophores to act as biosensors, although the design of stable UCNP-fluorophore hybrids with efficient upconverted energy transfer (UET) has remained a challenge. Here, we report Yb<sup>3+</sup>- and Er<sup>3+</sup>-doped UCNP-fluorophore conjugates with UET efficiencies up to 88%, and photostabilities 100-fold greater by UET excitation than those of the free fluorophores under direct excitation. Despite adding distance between Er<sup>3+</sup> donors and organic acceptors, thin inert shells significantly enhance overall emission without compromising UET efficiency. This can be explained by the large increase in quantum yield of Er<sup>3+</sup> donors at the core/shell interface and the large number of fluorophore acceptors at the surface. Sensors excited by UET show increases in photostability well beyond those reported for other methods for increasing the longevity of organic fluorophores, and those covalently attached to UCNP surface polymers show greater chemical stability than those directly coordinated to the nanocrystal surface. By conjugating other fluorescent chemosensors to UCNPs, these hybrids may be extended to a series of NIR-responsive biosensors for quantifying the dynamic chemical populations critical for cell signaling.

## 1. Introduction

Our understanding of chemical signaling in living systems has arisen largely from fluorescent chemical sensors able to detect dynamic populations of metal ions, metabolites, secondary messengers, and complex biomolecules within functional cells [1,2]. Starting with probes of Ca<sup>2+</sup> ions [3], an expansive suite of organic fluorophores and genetically encoded proteins has been engineered for chemical sensing of diverse targets with the selectivity, dynamic range, stability, and brightness necessary for imaging complex living systems. The extension of these optical biosensors from simple cell culture systems to intact tissue adds the challenge of excitation and emission through tissue that scatters, absorbs, and fluoresces. Each of these optical processes is dependent on the wavelength of light being used: most biomolecules absorb or emit ultraviolet (UV) or visible light, while scattering decreases significantly at longer wavelengths in the near infrared (NIR) [4,5]. Although the NIR contains tissue-transparent windows that might be exploited for the development of optical biosensors, most useful

chemical sensing probes are excited by UV or visible light [1–3,6].

Lanthanide-doped upconverting nanoparticles (UCNPs) absorb multiple NIR photons and emit at higher energies with efficiencies orders of magnitude higher than those of the best 2-photon fluorophores [7,8]. UCNPs can be imaged in the absence of cellular autofluorescence or measurable photobleaching, even under prolonged single-particle excitation [9,10]. UCNPs make use of energy transfer upconversion between 4F<sup>N</sup> electronic states of neighboring lanthanide (Ln<sup>3+</sup>) ions, in which sensitizer ions sequentially transfer absorbed energy to luminescent emitter ions, both of which are doped into a low-phonon nanocrystal host matrix. For many applications, β-phase NaYF<sub>4</sub> nanocrystals doped with 20% Yb<sup>3+</sup> sensitizer and a low percentage of Er<sup>3+</sup> or Tm<sup>3+</sup> emitter are most efficient and can be excited with modest continuous wave (CW) lasers. Addition of inert epitaxial shells to these UCNPs has been shown to significantly enhance emission by reducing Yb<sup>3+</sup>-mediated energy migration to high-vibrational-frequency modes of surface oleate ligands or solvent [7,11–16].

While UCNPs are inherently sensitive to temperature [17,18] and

\* Corresponding author.

E-mail address: [becohen@lbl.gov](mailto:becohen@lbl.gov) (B.E. Cohen).

<sup>1</sup> Present Address: Department of Mechanical Engineering, Columbia University, New York, NY 10027 USA.

mechanical force [19], they have little innate chemical sensing ability and require pairing with external probes to act as biosensors. A series of ions, toxins, and biomolecular interactions have been imaged using UCNPs coupled with organic sensors, with organic small molecules typically quenching one or more  $\text{Er}^{3+}$  luminescence bands, enabling ratiometric imaging [20–25]. UCNP complexes with organic fluorophores [26–30] and fluorescent proteins [31] have also been reported, but upconverted energy transfer (UET) in these systems is less well understood or optimized. Efficiency for UET, defined here as resonant energy transfer from UCNP  $\text{Ln}^{3+}$  donors to energy acceptors, varies widely depending on specifics of UCNP composition and how the UCNP-fluorophore hybrids are constructed [20,26,27,30]. Here, we report chemically stable UCNP-fluorophore complexes with almost 90% UET efficiency and up to 100-fold increase in photostability compared to direct fluorophore excitation. We have characterized UET as a function of nanocrystal composition and structure, fluorophore:UCNP stoichiometry, attachment method, and excitation laser power. We find that 88% UET can be achieved with core/shell UCNPs at confocal laser powers, which can be explained by the large increase in quantum yield of  $\text{Er}^{3+}$  donors at the core/shell interfaces and the large number of fluorophore acceptors at the surface. Thin, inert  $\text{NaYF}_4$  shells improve overall brightness > 1000-fold without decreasing UET efficiency [32], despite adding 2 nm distance between  $\text{Er}^{3+}$  donor and organic acceptor. Sensors excited by UET show increases in photostability well beyond those reported for other methods for increasing the longevity of organic fluorophores, and those covalently attached to UCNP surface polymers show significantly greater chemical stability than those directly coordinated to the nanocrystal surface. These findings can be applied as general design principles for the synthesis of bright, stable UCNP-based fluorescent sensors for imaging a variety of analytes in living systems.

## 2. Experimental section

### 2.1. Synthesis of 8-nm $\beta$ - $\text{NaYF}_4$ : 20% Yb, x% Er, 20% Gd nanocrystals

$\beta$ -Phase UCNPs were synthesized as described [33], with minor modifications, with  $\text{Er}^{3+}$  content from 2 to 60%. To synthesize  $\text{NaYF}_4$ : 20% Yb, 20% Er, 20% Gd nanocrystals:  $\text{YbCl}_3 \cdot \text{H}_2\text{O}$  (0.080 mmol, 32 mg, Strem),  $\text{YCl}_3$  (0.16 mmol, 31 mg),  $\text{ErCl}_3$  (0.08 mmol, 22 mg),  $\text{GdCl}_3$  (0.080 mmol, 21 mg), oleic acid (3.25 g), and 1-octadecene (ODE, 4 mL) were stirred in a flask with an in-reaction thermocouple and were heated at 110 °C under vacuum, and purged with  $\text{N}_2$  every 15 min. After 1 h, the dissolved lanthanides were cooled under  $\text{N}_2$ , and sodium oleate (1.25 mmol, 382 mg),  $\text{NH}_4\text{F}$  (2.0 mmol, 74 mg), and ODE (3 mL) were added to the flask. The reaction mixture was stirred under vacuum at room temperature for 30 min and then heated at 315 °C for 45 min. The reaction flask was cooled with a strong stream of air until the thermocouple read 40 °C. The product was transferred to a 50-mL centrifuge tube, 10 mL of EtOH added, and the tube centrifuged at 3000  $\times$  g for 3 min. The supernatant was decanted and 3 mL of hexane used to wash the reaction flask was added to the pellet, which was sonicated to ensure it was well-dispersed. The tube was centrifuged at 3000  $\times$  g for 3 min and the supernatant transferred to a new tube, leaving behind NaF impurities. To the dispersed UCNPs, 5 mL of EtOH was added and the tube centrifuged again at 3000  $\times$  g for 3 min. The pellet was dispersed in 1 mL of hexane, washed with 5 mL of EtOH two additional times, and the resulting pellet was dispersed in 15 mL of anhydrous hexane.

### 2.2. Synthesis of core/shell UCNPs

$\beta$ - $\text{NaYF}_4$ : 20% Gd shells were grown on  $\beta$ - $\text{NaYF}_4$ : 20% Yb, x% Er, 20% Gd UCNPs with a layer-by-layer protocol [34], using 28 nmol of core UCNPs. For 2-nm shell growth on 8-nm  $\beta$ - $\text{NaYF}_4$ : 20% Yb, 20% Er, 20% Gd UCNPs, a hexane dispersion of core UCNPs was added to a 3-

neck, 50-mL flask and the hexane evaporated under  $\text{N}_2$ . Oleic acid (4 mL) and ODE (6 mL) were added and the flask stirred at 70 °C for 1 h under vacuum. In separate flasks, Ln oleates were prepared by heating  $\text{YCl}_3$  (0.40 mmol, 78 mg),  $\text{GdCl}_3$  (0.10 mmol, 26 mg), oleic acid (2 mL), and 1-octadecene (3 mL) at 110 °C for 1 h under vacuum; and sodium trifluoroacetate (1.20 mmol, 16 mg) was dissolved in oleic acid (3 mL) and stirred at room temperature for 1 h under vacuum. The UCNPs flask was purged with  $\text{N}_2$  and heated at 280 °C for 10 min, allowing the temperature to stabilize. Shell precursors were injected as in Table S2, with sequential injections of lanthanide and Na/F precursors performed every 15 min. After four rounds of injections, the reaction was allowed to stir for an additional 30 min at 280 °C, and a strong stream of air to the flask was used for cooling. Core/shell UCNPs were isolated and stored using the same protocol for core UCNPs.

### 2.3. Synthesis of POA amphiphilic copolymer

To a round-bottom flask was added 800 mg of polyacrylic acid ( $M_w \sim 2000$ , Sigma Aldrich, 0.40 mmol), 1.40 g of *N*-hydroxysuccinimide (12 mmol), 60 mL of 200 mM HEPES buffer (pH 7.5), and 20 mL of EtOH. In separate 50 mL tubes, 1.90 mL of *tert*-butyl *N*-(2-aminoethyl)carbamate (12.0 mmol) and 992  $\mu\text{L}$  of 1-octylamine (6.0 mmol) were each dissolved in 20 mL of EtOH. Both amine solutions of amine were added to the reaction and the pH adjusted to pH 7.5 using 5 M NaOH. EDC (22.0 mmol, 3.40 g) was added in 3 parts over 18 h, with the pH readjusted to 7.5 before the final addition. The resulting product was distributed equally among four 50 mL tubes and each concentrated to 5 mL under  $\text{N}_2$ . The tubes were centrifuged at 5000  $\times$  g for 10 min at 4 °C, the supernatant decanted, and 20 mL of  $\text{H}_2\text{O}$  was added to each tube. The tubes were sonicated for 15 min and then centrifuged at 5000  $\times$  g for 10 min at 4 °C. Supernatants were decanted and each pellet was dissolved in 5 mL of trifluoroacetic acid, which was then evaporated overnight under a stream of  $\text{N}_2$ . The resulting oil was dissolved in 20 mL of 30% EtOH and the pH raised to 5 with concentrated  $\text{NH}_4\text{OH}$ . The solution was distributed equally among two dialysis cassettes (3500 Da MWCO, Thermo Fisher) and dialyzed against 3  $\times$  4 L of  $\text{H}_2\text{O}$ , causing the polymer to precipitate. The polymer was removed from the cassettes with EtOH and concentrated under  $\text{N}_2$  to remove all EtOH. The product, poly(*n*-octylacrylamide)-co-poly(2-aminoethylacrylamide) random amphiphilic copolymer (POA) with a 2:1 amine: $\text{C}_8$  ratio, was lyophilized to a white powder 1.30 g ( $\sim 90\%$  yield).

### 2.4. UCNP polymer encapsulation

Following similar encapsulation procedures for quantum dots with amphiphilic polymers [35], the UCNP-polymer stoichiometries were 1:2900 for core UCNPs and 1:5800 for core/shell UCNPs. POA copolymer (10 mg, 2.90  $\mu\text{mol}$ ) was stirred in 500  $\mu\text{L}$  of MeOH, and 15 mL of  $\text{CHCl}_3$  was added. UCNPs (0.5 nmol) in 50  $\mu\text{L}$  of hexane were added, solvents evaporated under a gentle stream of  $\text{N}_2$ , and 10 mL of 10 mM MES (pH 6.0) buffer was added. The vial was sonicated for 30 min, heated at 80 °C for 45 min, cooled slowly, and sonicated again for 15 min. The UCNP dispersion was added to a 15-mL 100 kDa MWCO spin filter (Millipore), concentrated, washed 3 times with 10 mM MES (pH 6) buffer, and concentrated to a final volume of 250  $\mu\text{L}$  (2  $\mu\text{M}$ ). Concentrations were determined using a standard emission versus concentration curve measured for the parent hydrophobic core/shell UCNPs.

### 2.5. UCNP-fluorophore conjugation

For functionalization of POA amines, the UCNP-fluorophore stoichiometry was varied by co-addition of MeO-PEG<sub>8</sub>-succinimidyl ester (SE; Thermo Fisher) with the fluorophore SE, using ratios from 4:1 to 19:1 PEG:fluorophore. To fluorophore and PEG SEs (250 nmol total) in

Download English Version:

<https://daneshyari.com/en/article/7906402>

Download Persian Version:

<https://daneshyari.com/article/7906402>

[Daneshyari.com](https://daneshyari.com)