Video Article

A 'Plug and Play' Method to Create Water-dispersible Nanoassemblies Containing an Amphiphilic Polymer, Organic Dyes and Upconverting Nanoparticles

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Abstract

In this protocol, we first describe a procedure to synthesize lanthanide doped upconverting nanoparticles (UCNPs). We then demonstrate how to generate amphiphilic polymers *in situ*, and describe a protocol to encapsulate the prepared UCNPs and different organic dye molecules (porphyrins and diarylethenes) using polymer shells to form stable water-dispersible nanoassemblies. The nanoassembly samples containing both the UCNPs and the diarylethene organic dyes have interesting photochemical and photophysical properties. Upon 365 nm UV irradiation, the diarylethene group undergoes a visual color change. When the samples are irradiated with visible light of another specific wavelength, the color fades and the samples return to the initial colorless state. The samples also emit visible light from the UCNPs upon irradiation with 980 nm near-infrared light. The emission intensity of the samples can be tuned through alternate irradiation with UV and visible light. Modulation of fluorescence can be performed for many cycles without observable degradation of the samples. This versatile encapsulation procedure allows for the transfer of hydrophobic molecules and nanoparticles from an organic solvent to an aqueous medium. The polymer helps to maintain a lipid-like microenvironment for the organic molecules to aid in preservation of their photochemical behavior in water. Thus this method is ideal to prepare water-dispersible photoresponsive systems. The use of near-infrared light to activate upconverting nanoparticles allows for lower energy light to be used to activate photoreactions instead of more harmful ultraviolet light.

Video Link

The video component of this article can be found at https://www.jove.com/video/52987/

Introduction

Today there is still an urgent need to develop new types of bio-imaging agents. Many novel fluorescent probes have been well documented.[1-6] However, substantial improvements in the image resolution remains a challenge.[7] One practical method is to directly modulate the fluorescence probes between a 'light' emissive state and a 'dark' quenched state.[8-12] This particular method has been applied to develop technologies such as stimulated emission depletion (STED) microscopy[13] and stochastic optical reconstruction microscopy (STORM).[14]

Another approach to modulate fluorescence is to couple photoresponsive chromophores together with fluorescent probes.[15,16] Toggling the photoresponsive chromophore between two isomers where only one of the isomers can act as an efficient energy-transfer acceptor, allows control over quenching of the fluorescence from the probe through Förster Resonance Energy Transfer (FRET) and other mechanisms. The result is the creation of an emissive state and a quenched state that can be alternated by exposure of the photoresponsive chromophore to different wavelengths of light.

Photoresponsive diarylethene chromophores can be reversibly toggled between a colorless ring-open isomer and a colored ring-closed isomer upon irradiation with UV and visible light.[17-19] The thermal stability of the two isomers and tunable absorption spectra of the ring-closed isomer make diarylethenes very good candidates as controllable FRET acceptors.[20-23] Lanthanide-doped $NaYF_4$ upconverting nanoparticles are useful for bio-imaging.[24] These nanoparticles absorb near-infrared light and emit light in several regions of the visible spectrum. Examples of fluorescence modulation by combining photoresponsive diarylethene chromophores and nanoparticles have been previously reported by our group.[25-27] However, the systems described in each example required an additional synthetic modification to attach the diarylethenes to the surface of the nanoparticles, which complicates the development of more diverse systems.

Herein we demonstrate a simple 'plug-and-play' method to prepare water-dispersible organic dye molecules and photoresponsive upconverting nanoparticles using a self-assembly strategy. The choice of polymers; poly(styrene-*alt*-maleic anhydride) and polyether amine 2070 provide both a hydrophobic and hydrophilic environment. The hydrophobic sections of the polymer help to hold the normally water insoluble organic molecules and upconverting nanoparticles together, whereas the hydrophilic region of the polymer is critical for maintaining the water-solubility. We will first

demonstrate synthesis of the upconverting nanoparticles by the thermal nucleation method. Then, we will prove how the organic molecules and upconverting nanoparticles are encapsulated within hydrophobic regions of the polymer shell and remain stable in aqueous media by simply co-stirring a solution of the upconverting nanoparticles, polymer and different organic dye molecules, followed by a convenient work-up procedure. We also demonstrate how to modulate fluorescence emission of the assemblies using external light irradiation. We anticipate the scope of using this 'plug-and-play' method to make water-dispersible nanoassemblies will continue to expand.

Protocol

1. Synthesis of the $NaYF_4/Yb^{3+}/Er^{3+}$ Upconverting Nanoparticles (UCNP)

1. Set up the apparatus as followed:
    1. Place a 250 ml heating mantle on a regular stirring plate and plug the mantle onto the thermal couple.
    2. Place a 250 ml round bottom flask equipped with a magnetic stir bar onto the heating mantle with proper clamping.
    3. Attach an air adapter to the left neck of the round bottom flask and connect this air adapter to the Schlenk line with plastic tubing.
    4. Attach a glass adapter to the right neck of the round bottom flask and fix a thermometer adapter onto the glass adapter. Insert the temperature probe into the flask through the thermometer adapter and plug this into the thermocouple.
    5. Attach a distillation head to the middle neck of the round bottom flask. Place a stopper on top of the distillation head. Connect the head to a condenser, followed by a vacuum distillation adapter and a 50 ml round bottom flask. Connect the vacuum distillation adapter to a bubbler through plastic tubing.
2. Weigh 1.17 g (3.9 mmol) of yttrium acetate, 0.439 g of ytterbium acetate and 0.0727 g (0.1 mmol) of erbium acetate and place them in the reaction round bottom flask.
3. Add 30 ml of oleic acid and 75 ml of octadecene to the flask using a graduated cylinder.
4. Rinse the side of the reaction round bottom flask using 5 ml of methanol to make sure that no oleic acid and octadecene is stuck to the sides of the reaction flask.
5. Connect the reaction flask to a double manifold Schlenk line and turn the corresponding valve to keep the reaction flask connected to the nitrogen line.
6. Turn on the thermocouple, set the temperature to 80 °C, and gradually heat the system to this temperature. At 80 °C and after all starting materials are dissolved, remove the heating mantle and allow the reaction to cool down to 30 °C.
7. When temperature reaches 30 °C, take off the distillation head, switch the air adapter from the left neck to the middle neck and close off the left neck with a stopper. Slowly introduce vacuum to the reaction flask by turning the valve on the Schlenk line from the nitrogen line to the vacuum line. All of the low boiling point components will be pulled out from the reaction at this point.
8. When the solution stops bubbling, raise up the temperature to 115 °C in a speed of 5 °C/min.
9. Once the temperature reaches 115 °C, maintain this temperature for 15 min, then remove the heating mantle and cool down the reaction to 50 °C. Afterwards, quickly switch the set up back to the original form by reattaching the distillation head to the middle neck and the air adapter to the left head.
10. Weigh out 0.74 g (12.5 mmol) of NaOH and 0.50 g (20.0 mmol) of NH_4F during the cooling process, and dissolve them in 50 ml of methanol by sonication.
11. After sonication, pour the solution into the reaction round bottom flask and rinse the sides of the flask with 5 ml of MeOH.
12. Leave the solution stirring at 50 °C for 30 min.
13. Increase the temperature to 75 °C to distill the methanol.
14. During the distillation, empty the collection flask when necessary. After the distillation is finished, heat up the reaction to 300 °C under nitrogen protection as fast as possible.
15. Once the temperature reaches 300 °C, maintain this temperature for 1 hr. If needed, cover the setup with aluminum foil to help maintain the temperature. Then remove heat source and allow the reaction to cool down to room temperature.
16. Once it is cooled down to room temperature, split the solution evenly into three centrifugation tubes (50 ml tubes, roughly 35 ml solution per each tube), and top up the tube to the 50 ml scale using anhydrous ethanol. Centrifuge all the tubes at 3,400 x g for 15 min. After centrifugation, the UCNPs should be observed on the side of the tubes as a white precipitate.
17. Discard the supernatant and redisperse the UCNPs pellets in hexanes (7.5 ml of hexanes per each tube), then top up the tube with ethanol to the 50 ml scale. Centrifuge tubes again at 3,400 x g for 15 min.
18. Once the centrifugation is complete, discard the supernatant and redisperse the solid UCNPs in 30 ml of $CHCl_3$ for further use.

2. Assembly of Water-dispersible Nanoassemblies Containing Organic Dye Molecules and Upconverting Nanoparticles

1. Dissolve 25 mg (0.0147 mmol) of poly(styrene-*alt*-maleic anhydride) (PSMA) in 3 ml of $CHCl_3$ into a scintillation vial equipped with a magnetic stir bar. This quantity is an optimized quantity after multiple trials.
2. Add 250 µl (47 mg/ml) of the upconverting nanoparticles chloroform stock solution to the scintillation vial.
3. Cap the vial and place it on the magnetic stirring plate, and stir the solution at room temperature for 2 hr.
4. Weigh 160 mg (0.0773 mmol) of polyether amine 2070, and dissolve it in 1 ml of $CHCl_3$. Then add this solution to the scintillation vial in one portion using a pipette. The solution will turn to pale yellow indicating the reaction of polyether amine 2070 with the anhydride groups on the PSMA.
5. Continue to stir the solution overnight at room temperature.
6. Measure the appropriate quantity of organic dye molecules then dispense it into the scintillation vial in one portion, stir the resulting solution for 1 hr.
    1. For the sample TPP-NP (nanoassembly containing polymer shell, tetraphenyl porphyrin and upconverting nanoparticles), directly add 1 mg of tetraphenyl porphyrin to the scintillation vial. For the sample DAE-UCNP (nanoassembly containing polymer shell, diarylethene

molecules and upconverting nanoparticles), the quantity of each diarylethene molecules is 2×10^{-7} mol. Add the two diarylethene molecules into the reaction solution. The volumes for the two diarylethene molecules are: DAE-1o (1.8 mM), 111 µl and DAE-2o (1.6 mM), 125 µl.

7. Remove the $CHCl_3$ solvent under reduced pressure using a rotary evaporator, then add 3 ml of 0.001 M aqueous NaOH (pH ≈ 11) to the scintillation vial, then sonicate the vial until a milky suspension is formed.
8. Place the vial back on the rotary evaporator, and carefully remove the remaining $CHCl_3$ until the suspension has turned to a clear solution.
9. Transfer the solution from the scintillation vial to two 1.5 ml conical centrifugation tubes, then centrifuge the solution at 20,600 x g for 25 min.
10. Discard the supernatant, then add a total of 3 ml of deionized H_2O into the two tubes (1.5 ml per tube), sonicate the tubes to redisperse the pellets in the deionized H_2O.
11. Centrifuge the two tubes again at 20,600 x g for 25 min.
12. Discard the supernatant, then add a total of 3 ml of deionized H_2O into the two tubes (1.5 ml per tube). Sonicate the tubes to redisperse the pellets in the deionized H_2O.
13. Filter the aqueous nanoparticles dispersion sample through a 0.2 µm syringe filter to obtain the final sample for further testing.

Representative Results

Absorption spectra and photoluminescence spectra were collected for sample DAE-UCNP. The absorption spectra are used for comparing the spectral overlap between the closed diarylethene chromophores and the upconverting nanoparticles. Photographs of the samples (both TPP-UCNP and DAE-UCNP) were also included to demonstrate successful encapsulation of organic dye molecules and upconverting nanoparticles, which are located within the amphiphilic polymer shells in the aqueous phase. The modulation of photochemistry and fluorescence was also shown by illumination of the samples with different light sources.

The chemical theory 'like dissolves like' explains why when an aliquot of the porphyrin or UCNPs in chloroform is added to water even after vigorously shaking, both remain in the organic layer (**Figure 2, a, b, d** and **e**). However, when using the 'plug-and-play' encapsulation method (**Figure 1**), a water-dispersible nanoassembly (TPP-UCNP) containing both porphyrin and UCNPs is produced. The reason why we chose tetraphenyl porphyrin as a model compound to study is because it is a non-water soluble organic compound and it has interesting applications in photodynamic therapy. When an aqueous solution containing the nanoassemblies is added to chloroform, even after vigorously shaking, the nanoassemblies remained in the water layer (**Figure 2, c** and **f**). The use of the amphiphilic polymer shell has two advantages: (1) it creates an internal hydrophobic environment that traps both the porphyrin and the UCNPs, and (2) It creates an external hydrophilic environment that interacts with surrounding water molecules to maintain water-dispersibility of the entire assembly. The red color of the sample (**Figure 3**) is attributed to the porphyrin molecules trapped within the assembly, and the presence of the porphyrin molecules was demonstrated using UV-vis absorption spectroscopy. Upon irradiation with a near-infrared 980 nm laser, green emission is produced from the sample (**Figure 2, c** and **f**, **Figure 3**), which is assigned to the emission from the Er^{3+}-doped $NaYF_4$ upconverting nanoparticles. The encapsulation protocol does not require any specific modifications to be made to the encapsulated molecules nor ligand exchange of the UCNPs, we therefore propose that this 'plug-and-play' protocol can be applied as a general strategy to transfer a variety of different organic molecules from an organic solvent to an aqueous medium.

To demonstrate the versatility of our procedure, we simultaneously transferred two hydrophobic diarylethenes (**DAE-1o** and **DAE-2o**) from organic solvent to water (**Figure 4**) to generate a mixed nanoassembly (DAE-UCNP). Diarylethenes are photoresponsive molecules that undergo conversion between a ring-open isomer and a ring-closed isomer.[28] Upon irradiation with UV light, the colorless ring-open isomer is converted to the colored ring-closed isomer, and exposure to visible light triggers the reverse process. These reactions are illustrated in **Figure 4**. Interconversion between the ring-open and ring-closed isomers can be repeated many times without significant degradation of the chromophores. These photoreactions are typically conducted in organic solvents, not only for solubility reasons but also because the cyclization process is frequently hindered in water. The poor performance of photoreactions in water is primarily due to: (1) suppressed reactivity of the excited diarylethene molecules in polar solvents due to intramolecular charge transfer interactions, and (2) the possibility of collision between excited organic molecules and water molecules that lead to quenching of the excited states and shutting down the photocyclization reaction. However, these issues can be overcome through encapsulation of the diarylethene within an amphiphilic polymer shell to form water-dispersible nanoassemblies.

Using the same 'plug-and-play' protocol described for the porphyrins, diarylethenes and upconverting nanoparticles were encapsulated within polymer shells to form water-dispersible nanoassemblies (**Figure 2** and **Figure 5**). The UV-vis absorption spectra of the two isomers that undergo light induced cyclization and cycloreversion reactions within the nanoassemblies dispersed in water are shown in **Figure 6**. As is typical for diarylethenes, none of the ring-opened isomers (**DAE-1o** or **DAE-2o**) absorb in the visible region of the electromagnetic spectrum (**Figure 6a**). Irradiation of the ring-opened isomers with 365 nm light produces their ring-closed counterparts (**DAE-1c** and **DAE-2c**). This is also why the colorless sample (**Figure 5a**) changed to an orange-colored sample (**Figure 5b**) and showed a strong visible band in the UV-vis spectrum (**Figure 6a**). Irradiation of the colored sample with visible light of a wavelength greater than 434 nm fades the sample to its original colorless state containing the ring-opened isomers (**DAE-1o** and **DAE-2o**). All spectral changes were completed within 3 minutes. Selective photochromism was observed (**Figure 6c**) because the two chromophores encapsulated within the polymer shell of **DAE-UCNP** have well separated absorption bands. When the sample **DAE-UCNP** was irradiated with visible light of a wavelength greater than 650 nm, only the ring-closed isomer **DAE-2c** is responsive to this particular wavelength of light and was converted to the ring-opened isomer **DAE-2o**. This results in a decrease in the visible absorption band at 647 nm and yields a solution with a deeper orange color (**Figure 5c**) from the selective fading of blue ring-closed isomers. Under these conditions, the band corresponding to **DAE-1c** is almost unchanged (orange solid line in **Figure 6c**). This data supports the conclusion that the amphiphilic polymer shell helps to retain the efficiency of photoreactions in water.

When the aqueous dispersion of nanoassembly **DAE-UCNP** is excited with 980 nm light, the two bands centered at 537 nm and 650 nm can be detected with a fluorometer, which are typical for erbium-doped nanoparticles. The band centered at 537 nm (denoted as green emission) can be attributed to $[^2H_{11/2}, {}^4S_{3/2}]^4I_{15/2}$ transitions while the band centered at 650 nm (denoted as red emission) is the result of $[^4F_{9/2}, {}^4S_{3/2}]^4I_{15/2}$ transitions (**Figure 6b**). The ring-opened isomers (**DAE-1o** and **DAE-2o**) do not absorb any visible light, and as a result the fluorescence emission of the sample **DAE-UCNP** is not quenched by either of the ring-opened isomers. However, irradiation of the sample with 365 nm

light converts the ring-opened isomers to their ring-closed counterparts (**DAE-1c** and **DAE-2c**) and both of them strongly absorb visible light. Since the emission bands from the UCNPs overlap with the absorption bands of the ring-closed isomers, the quenching of the UCNPs emission is achieved through an energy transfer process (**Figure 6b**). This process is a combination of both FRET and emission-reabsorption mechanisms.[26] The original emission can be regenerated by irradiation of the sample with visible light of a wavelength greater than 434 nm light, which converts the ring-closed isomers back to the corresponding ring-opened isomers. As discussed before, the green and red emission bands can be selectively quenched because of the selective photochromism of the sample and the capability of quenching the emission bands by the ring-closed isomers. When the sample is irradiated with visible light of a wavelength greater than 650 nm, only the ring-closed isomer **DAE-2c** is returned to the ring-opened isomer **DAE-2o** and the red emission is regenerated while the green emission is still quenched to some extent (**Figure 6d**).

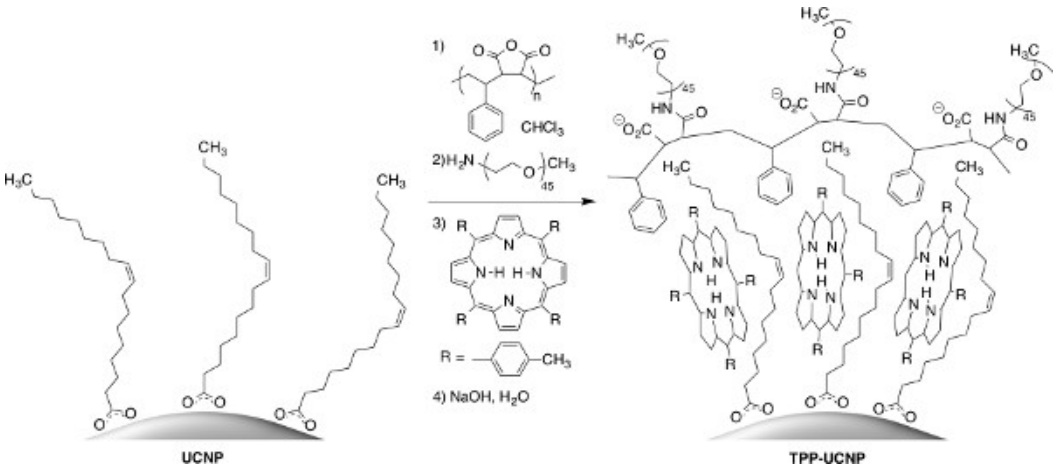

Figure 1. Synthesis of the nanoassemblies (**TPP-UCNP**) containing the polymer encapsulated both the upconverting nanoparticles and the tetraphenyl porphyrin.

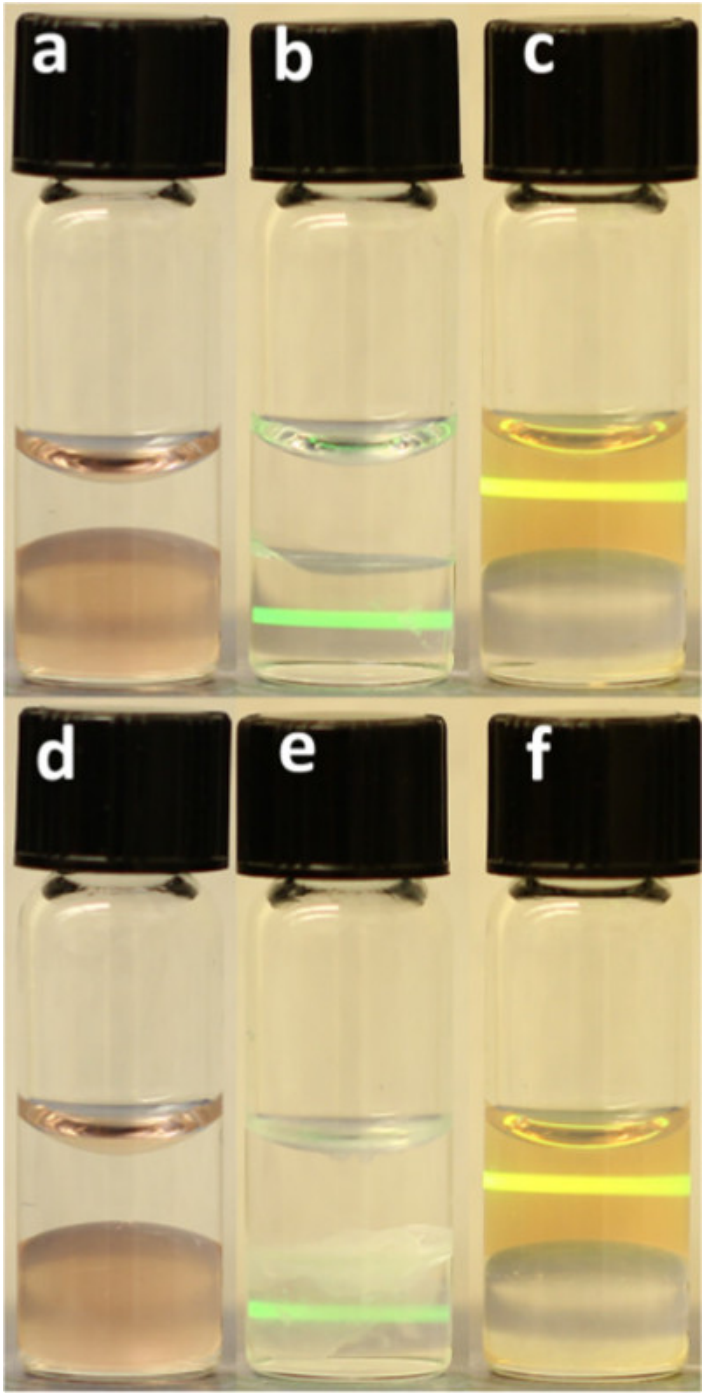

Figure 2. Photographs showing water gently layered on top of $CHCl_3$ containing (**a**) TPP in the $CHCl_3$ phase, (**b**) the UCNPs in the $CHCl_3$ phase, (**c**) the nanoassemblies (**TPP-UCNP**) in the water phase. Images (**d**), (**e**) and (**f**) are of the identical vials after they have been vigorously shaken and showing no transfer of the components to the other liquid phases. The green and yellow light observed in images (**b**), (**c**), (**e**) and (**f**) are due to the irradiation with a 980 nm laser to show the location of upconverting nanoparticles.

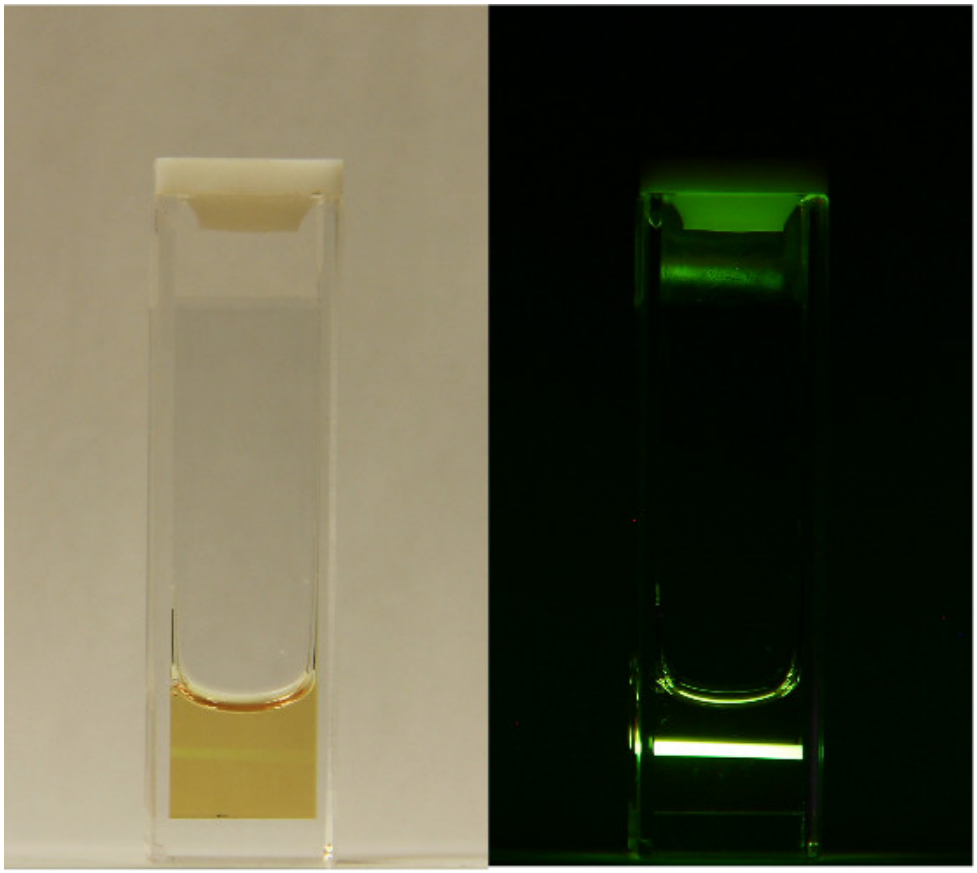

Figure 3. Photographs of an aqueous solution of the nanoassemblies (**TPP-UCNP**) upon irradiation with a 980 nm laser in ambient light (left) and in the dark (right).

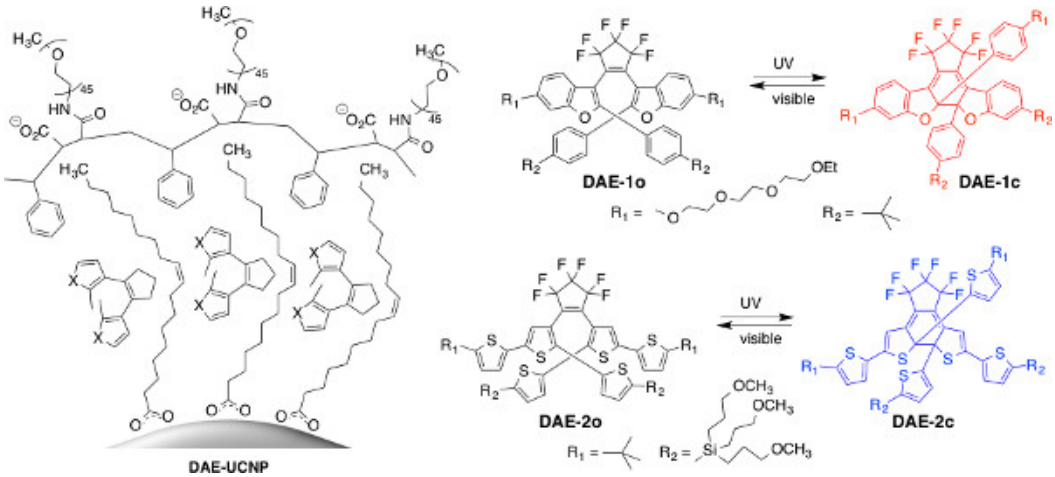

Figure 4. A mixed nanoassembly (**DAE-UCNP**) containing the polymer encapsulated upconverting nanoparticles and two different diarylethenes. The photoinduced ring-closing and ring-opening reactions of the diarylethenes are shown on the right.

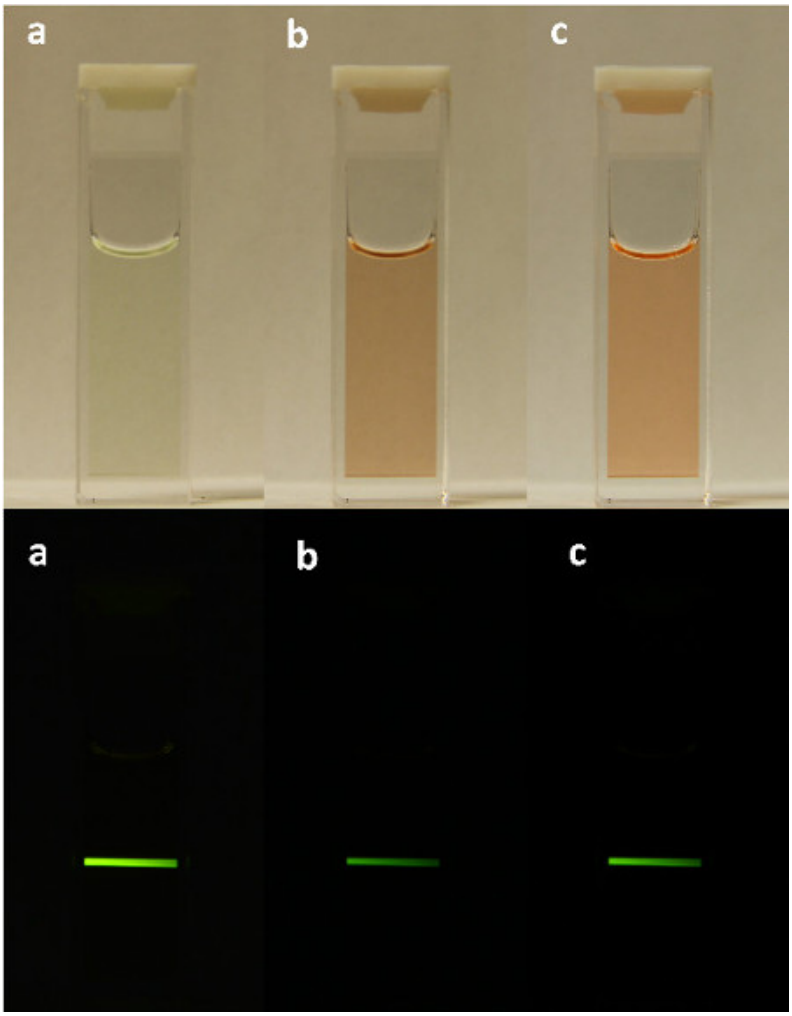

Figure 5. Photographs of aqueous solutions of the mixed nanoassemblies (**DAE-UCNP**) containing the diarylethenes (**a**) in their ring-open states (**DAE-1o** and **DAE-2o**), (**b**) at their photostationary states containing **DAE-1c** and **DAE-2c**, and (**c**) with **DAE-1o** at its photostationary state and **DAE-2o** in its ring-open form. The photostationary states were generated by irradiation of the sample with 365 nm light for 2 min. The mixed state in (**c**) was generated by selectively ring opening **DAE-2c** with light of a wavelengths greater than 490 nm. The bottom photographs show the same samples when they are irradiated with a 980 nm laser in the dark.

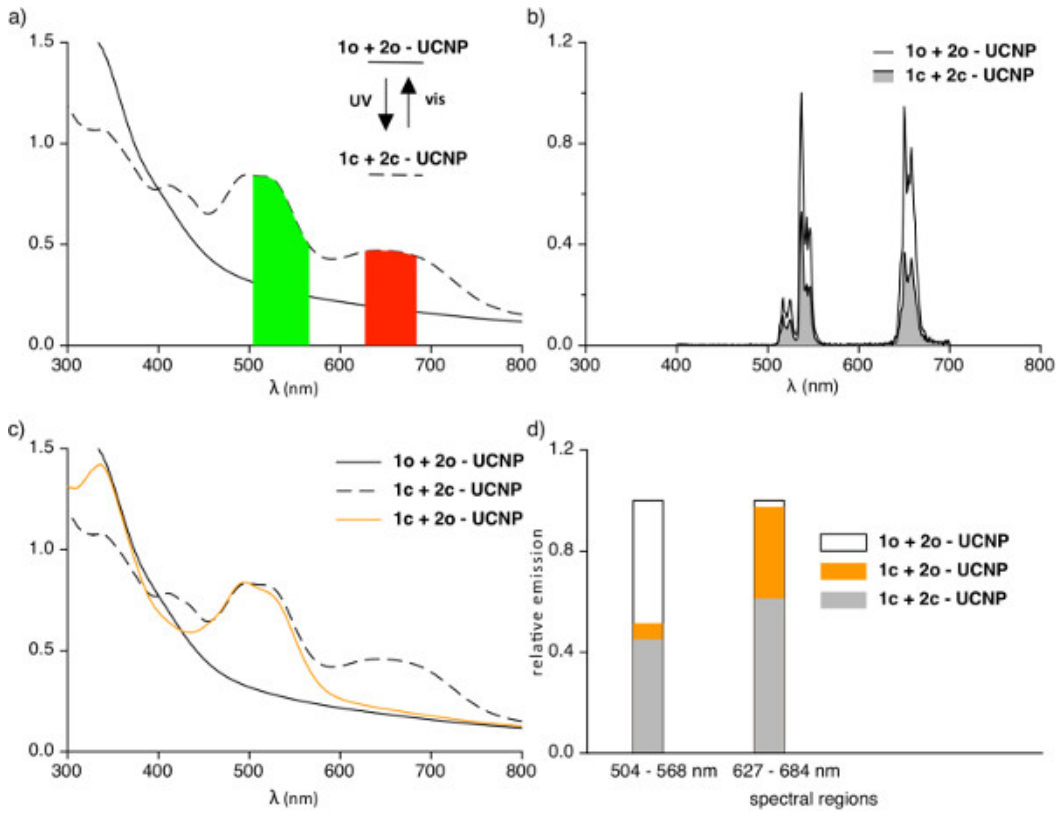

Figure 6. (**a**) UV-vis absorption spectra of the water-dispersed nano-system **DAE-UCNP** containing diarylethenes **1o** and **2o** before (solid line) and after 365 nm light irradiation (dashed line). The green and red bars represent the emission bands of UCNPs when excited with 980 nm light to show the spectral overlap between the emission of the UCNPs and the absorption of diarylethenes at the photostationary state. (**b**) Fluorescent emission spectra of the same sample (λ_{ex} = 980 nm) before (black line) and after (black line with grey shaded area) irradiation with 365 nm light. (**c**) UV-vis absorption spectra of **DAE-UCNP** at the photostationary state (dashed line), after irradiation with >490 nm light from photostationary state (black line), and after irradiation with >650 nm light from photostationary state (orange line). (**d**) Relative emission of **DAE-UCNP** measured when the sample was at the photostationary state (grey bar), after irradiation with >650 nm light from the photostationary state (orange bar), and after irradiation with >490 nm light from the photostationary state (white bar).

Discussion

The nanoparticles synthesized according to this protocol have a size distribution from 20 to 25 nm centered at around 22.5 nm.[26,27] They can be classified as spherical particles with a α-$NaYF_4$ host lattice structure. There are two critical steps in this protocol. In the UCNP synthesis, it is crucial to maintain the heating temperature and time as precise as possible to assure a narrow distribution of particle size. Simultaneous addition of NaOH and NH_4F along with the addition of lanthanides ions at the beginning of the reaction did not yield nanoparticles of a well-distributed size and good morphology. After the addition of NaOH and NH_4F, ensure that the temperature is kept at 75 °C for a long enough period of time to completely distill off all of the methanol from the high boiling point solvent mixture and then raise the temperature to 300 °C as fast as possible after distillation to control the size of nanoparticles.[24]

When making water-dispersible nanoassemblies, it can sometimes be challenging to determine the quantity of UCNPs (Step 2.2) and organic molecules (Step 2.6). One suggestion is to start with a small volume of the UCNPs (*i.e.* 50 µl) and then gradually increase this quantity until a threshold is reached. Based on our trials, a combination of 10 mg of particles and 2 × 10^{-7} mol organic molecules is the optimal quantity for this type of encapsulation. However, although this method can successfully transfer water-insoluble organic compounds and nanoparticles to aqueous medium and hold the two components together in close proximity, this protocol still has limitations. This encapsulation process is not applicable to water soluble molecules or nanoparticles synthesized in an aqueous environment (*i.e.* gold nanoparticles) because the major interaction holding the nanoassembly together is the hydrophobic effect. If a water soluble molecule or nanoparticle is used, it will likely leach out of the hydrophobic polymer layer even if the polymers initially form micelles.

In conclusion, using a 'plug-and-play' protocol, we demonstrate how to conveniently encapsulate hydrophobic organic chromophores and inorganic upconverting nanoparticles within an amphiphilic polymer shell to generate photoresponsive water-dispersible hybrid organic-inorganic nanoassemblies. The polymer shell helps to maintain the hydrophobic environment that is beneficial for organic photoreactions, which makes this 'plug-and-play' protocol ideal for preparation of complex photoresponsive systems for applications in aqueous environments. The existing methods for fabricating water-dispersible nanosystems often requires complicated chemical modification, however, this protocol is capable of transferring non water-soluble components into water conveniently without the for specific modification to those components. The use of near-infrared light to activate upconverting nanoparticles opens the opportunity for low energy light activated photoreactions which is an advantageous feature for biological applications since it causes less damage to cells and tissues in living organisms. A possible drawback to this technique is the upconverted UV light emitted from the nanoparticles, and used to trigger the higher energy photoreactions (*i.e.* photo-isomerization of diarylethene molecules), could potentially cause damage to cells or live organisms. To overcome this issue, an UV protection layer can be coated

on the nanoparticles to prevent the upconverted UV photons from coming out. The nanosystem with tunable fluorescence we demonstrated in this article has the potential to be developed as a novel bioimaging reagent for super-resolution imaging. We anticipate the scope of using this 'plug-and-play' method to make water-dispersible nanoassemblies will continue to expand.

Disclosures

The authors have nothing to disclose.

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