

# Fluorescent Quenching of Lanthanide-Doped Upconverting Nanoparticles by Photoresponsive Polymer Shells

Tuoqi Wu, Danielle Wilson, and Neil R. Branda\*

4D LABS and Department of Chemistry, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6

# Supporting Information

**ABSTRACT:** A photoresponsive amphiphilic polymer was synthesized and used to encapsulate upconverting lanthanidedoped nanoparticles to produce a novel water-dispersible nanoassembly with a high loading of emission quenchers. The nanoassembly exhibits fluorescent emission in the visible region upon irradiation with 980 nm light, which can be reversibly modulated by toggling the isomeric state of photoresponsive chromophores attached to the polymer's backbone using UV and visible light. Photon counting experiments show that the quenching mechanism for this new nanoassembly is a combination of Förster resonance energy transfer (FRET) and emission-reabsorption. Compared to the similar nanoassembly prepared from a reported "plug-and-play" method, this new



nanoassembly has higher overall quenching efficiency due to the increased photoswitch loading (14 times compared to the existing nanoassembly).

Thile nanostructured fluorescent probes based on organic,<sup>1,2</sup> silicon,<sup>3</sup> carbon,<sup>4</sup> and quantum dot<sup>5,6</sup> materials offer many advantages in bioimaging applications, their use would still benefit from improved image resolution. One approach to achieve this goal is to develop methods to toggle a fluorescent probe between "light" emissive and "dark" nonemissive states using an external stimulus.<sup>8-12</sup> Light has been used as the stimulus in imaging technologies such as stimulated emission depletion (STED) microscopy,<sup>13</sup> timegated STED microscopy,14 and stochastic optical reconstruction microscopy (STORM).<sup>15</sup> Light is a particularly appealing stimulus because it is noninvasive and can be easily tuned and focused to provide a high degree of spatial and temporal control. It has been successfully used to reversibly convert photoresponsive chromophores between two isomeric forms, where only one of the isomers acts as an effective Förster resonance energy transfer (FRET) acceptor to quench the fluorescence from nanoparticle donors.<sup>16–18</sup> We<sup>19–21</sup> and others<sup>22,23</sup> have recently focused our attention

We<sup>19–21</sup> and others<sup>22,23</sup> have recently focused our attention on developing variable-emission systems using dithienylethene derivatives to decorate lanthanide-doped upconverting nanoparticles (UCNPs). These hybrid systems combine the beneficial features of the photoresponsive chromophores and the emissive nanoparticles. Dithienylethenes undergo reversible cyclization reactions between colorless ring-open and colored ring-closed isomers when exposed to UV and visible light (Scheme 1)<sup>24–26</sup> usually with a high degree of photofatigue resistance during numerous ring-closing/ring-opening cycles.<sup>27</sup> The members of this family of photoresponsive compounds also tend to exhibit relatively good thermal bistability at ambient temperatures (both isomers are stable in the dark) and can be subjected to a wide range of synthetic conditions to tailor their optoelectronic properties, making them versatile FRET acceptors.<sup>28-37</sup>

The lanthanide-doped UCNPs<sup>38,39</sup> have many advantages over other emissive systems such as quantum dots. While the use of quantum dots  $^{40-45}$  minimizes the photobleaching observed for small molecule probes, it does not overcome the problem of potential toxicity and "blinking" when used in bioimaging applications. The former can be overcome by modifying the surfaces of the quantum dots.<sup>46</sup> But "blinking" is still an unresolved issue.<sup>47,48</sup> The lanthanide-doped UCNPs absorb several photons of near-infrared (NIR) light and emit them in the UV and visible region of the electromagnetic spectrum.<sup>38,39</sup> Because they absorb NIR light, which has deeper tissue penetration and induces few organic photoreactions (and, therefore, produces less damage to the surrounding environment) than high energy UV or visible light, these nanoparticles provide lucrative alternatives as emissive bioimaging probes.<sup>49</sup> They also exhibit high photobleaching resistance.<sup>50</sup> These appealing features explain why we<sup>19-21</sup> and others<sup>22,23,49</sup> have developed several upconverting nanosystems for potential use as fluorescent probes for in vitro and in vivo imaging.

One of the challenges when developing systems that rely on effective photochemical reactions is the fact that many of these

Received: June 12, 2014

Published: June 23, 2014

reactions operate poorly in aqueous conditions due to the poor solubility or interaction between water and the chromophores.<sup>51</sup> This explains why we chose to carry out our initial proof-of-concept studies in organic polymer films<sup>52</sup> and organic solvents.<sup>19,53</sup> Our work in developing water-dispersible systems for live organism imaging required considerable synthetic efforts to modify the chromophores, attach them to the nanoparticles (which had to be subjected to a ligand exchange reaction prior to decorating them with the modified chromophores), and retain the photoactivity of the chromophore.<sup>20</sup> These synthetic steps were avoided when we demonstrated a "plug-and-play" method to prepare water-dispersible nanosystems where both the UCNPs and the photoresponsive diarylethenes were encapsulated by an amphiphilic polymer.<sup>21</sup> A simplified version of this system is illustrated in Figure 1. The fact that both components reside



**Figure 1.** Simplified illustration of the nanosystem containing upconverting nanoparticles wrapped in an amphiphilic polymer shell containing trapped photoresponsive chromophores. Neither the chromophores nor the nanoparticles have to be subjected to synthetic modifications and ligand exchange using this approach.

within the hydrophobic "inner" shell created when the polymer spontaneously wraps around the nanoparticles (1) eliminates the need for ligand exchange of the oleate groups and anchoring the photochromic molecules to the surface (although we did demonstrate that attaching the photoresponsive chromophores to the nanoparticles prior to wrapping them with the polymer has the benefits of higher loading and more effective UV photoswitching),<sup>54</sup> (2) provides a method to conveniently build a library of different systems by swapping the organic chromophores, and (3) most importantly, creates a local hydrophobic environment where photochemistry is not diminished even in water.

While these systems represent effective examples to demonstrate the "plug-and-play" concept and light-induced reversible quenching of the emission from the nanoparticles, their performance would have been substantially more impactful if the loading of the chromophores was higher to achieve a greater extent of fluorescence quenching. Our assumption is based on the fact that, using the coencapsulation method, only a small number of photochromic components could be loaded into the hybrid system. Attaching the chromophores directly into the polymer used for wrapping should increase the loading and emission quenching. This is the focus of the studies reported in this paper.

# EXPERIMENTAL SECTION

General Details. All solvents and reagents used for synthesis, chromatography, UV-vis spectroscopy measurements, and photolysis studies were purchased from Aldrich, Caledon, and Anachemia and used as received, unless otherwise noted. Solvents for NMR analysis were purchased from Cambridge Isotope Laboratories and used as received. Column chromatography was performed using silica gel 60 (230-400 mesh) from Silicycle Inc. The NaYF<sub>4</sub> nanoparticles codoped with 2% Er<sup>3+</sup> and 20% Yb<sup>3+</sup> (NaYF<sub>4</sub>:ErYb) were synthesized using a modification of a recently reported procedure.<sup>20</sup> The photochromic amine (10) was synthesized as described in the literature<sup>55</sup> Poly(propylene glycol)bis(2-aminopropyl ether) (Jeff-Amine 2000) was received as a gift from Huntsman Inc. Cumene terminated poly(styrene-co-maleic anhydride) was purchased from Aldrich. Amino functionalized PEG 2000 was purchased from Rapp Polymere GmbH Inc. All volumes for absorption, photolysis, and concentration measurements/studies were measured using an autopipette.

**Instrumentation.** <sup>1</sup>H NMR and <sup>13</sup>C NMR characterizations of synthetic precursors were performed on a Bruker BioSpin 400 spectrometer and were found to match the reported data. The characterizations of the final compounds were performed on QNP 600 cryoprobe (proton sensitive, inverse probe) working at 600.33 MHz for <sup>1</sup>H and 150.97 MHz for <sup>13</sup>C. The <sup>1</sup>H NMR photolysis studies were performed on CD<sub>3</sub>CN solutions using a Bruker BioSpin 500 spectrometer.

**Optical Spectroscopy.** UV–vis absorption spectroscopy was performed using a Varian Cary 300 Bio spectrophotometer. Fluorescence measurements were performed using a PTI Quanta-master spectrofluorometer. A JDS Uniphase 980 nm laser diode (device type L4-9897510-100M) coupled to a 105  $\mu$ m (core) fiber was employed as the excitation source. The output of the diode laser was collimated and directed on the samples using a Newport F-91-C1-T Multimode Fiber Coupler. The visible emissions were collected from the samples at  $\pi/2$  from the incident beam in the plane of the spectrometer. All of the colloidal samples were held in a square quartz cuvette with an interior width 4 mm and length 10 mm (Starna Cells, Part # 9F/Q/10). All spectra were corrected for instrument sensitivity.

**Transmission Electron Microscopy (TEM).** TEM images were obtained using a Hitachi 8100 scanning transmission electron microscope operating at 200 keV. For the nanoparticles dispersed in hexanes, a small amount of this dispersion was drop-cast on a carbon Formvar-coated copper grid (400 mesh, Ted Pella, Part no. 01754-F) and air-dried before imaging. The shape and size of the NaYF<sub>4</sub>:ErYb nanoparticles were evaluated from the collected TEM images. The size of the nanoparticles was calculated from over 50–70 particles located at different areas of the TEM grid. For aqueous samples, dilute colloids of the nanoparticles dispersed in water (5  $\mu$ L) were placed on thin, carbon Formvar-coated copper grids held by anticapillary tweezers (Ted Pella, part no. 501-4). Water was then slowly removed under reduced pressure in a vacuum desiccator.

**Dynamic Light Scattering (DLS).** DLS measurements were carried out using a Malvern Zetasizer Nano-ZS. The colloidal samples were held in a 10 mm path length plastic cuvette (BrandTech, Catalog no. 759220). A nanoparticle concentration of  $\sim$ 0.4 mg/mL was employed for the measurements. All DLS measurements were conducted at 25 °C.

Photoinduced Ring-Closing and Ring-Opening Reactions. All photoreactions of the dithienylethene derivatives as small molecules in solution ( $3o \rightleftharpoons 3c$ ) or integrated in the nanoparticle systems (2o-NP  $\rightleftharpoons 2c$ -NP and  $3o(NP) \rightleftharpoons 3c(NP)$ ) were monitored using UV–vis absorption spectroscopy. All photoreactions were carried out using the light source from a lamp used for visualizing TLC plates at 365 nm (Spectroline E-series, 1.3 mW/cm<sup>2</sup>) to induce the ring-closing reactions ( $3o \rightarrow 3c$ , 2o-NP  $\rightarrow 2c$ -NP, and  $3o(NP) \rightarrow$  3c(NP)) and a tungsten filament light source with a greater than 490 nm cutoff filter (Elmo Omnigraphic 300AF, 107 mW/cm<sup>2</sup>) to induce the ring-opening reactions (3c  $\rightarrow$  3o, 2c-NP  $\rightarrow$  2o-NP, and 3c(NP)  $\rightarrow$  3o(NP)). Monochromatic light was generated using a PTI Quantamaster spectrofluorometer. The wavelength was adjusted to be centered at 550 and 650 nm, and the slit width of the fluorometer was adjusted to 1 nm in width.

Fluorescence Lifetime Measurements. An aqueous solution of 20-NP  $(1.17 \times 10^{-4} \text{ M})$  or 3o(NP)  $(1.85 \times 10^{-5} \text{ M})$  in a quartz cuvette (Starna Cells, Part # 9F/Q/10) was excited with picosecond laser pulses at 980 nm with an energy of 500  $\mu$ J/pulse. The two concentrations reported here are total concentration of photoswitches encapsulated in the nanoassemblies obtained using the concentration of the ring-closed isomers of each photoswitch at its photostationary state (calculated by UV-vis spectroscopy after baseline scattering subtraction) divided by percentage of ring-closed isomers in this state (measured by <sup>1</sup>H NMR spectroscopy). The laser pulses were generated by a picosecond optical parametric generator (OPG) from EKSPLA, Model PG401. This OPG was pumped by an EKSPLA PL2241 ps laser at 10 Hz repetition rate and 30 ps pulse duration. The laser beam was focused to a 2 mm light column passing through the sample cuvette along the 10 mm interior direction. The emission spectral detection and lifetime measurements were performed by a combination of spectrograph from Princeton Instruments, model SP2300i, and streak camera system from Hamamatsu, model C7700. The spectrograph used a 150 lines/mm grating providing 362 nm spectral coverage centered at 532 nm, and the streak camera was operated in photon counting mode with a temporal range of 500  $\mu$ s. The decay times were calculated from these curves by fitting them with a single exponential model using Origin software (version 8.5) after the in-growth portion of the curve.

Synthesis of Photoresponsive Polymer (20). In a glass vial equipped with a magnetic stir bar, a solution of diarylethene 10 (15 mg, 0.028 mmol) in  $CHCl_3$  (3 mL) was treated with vigorous stirring with cumene terminated poly(styrene-co-maleic anhydride) (PSMA, 47.6 mg, 0.028 mmol,  $M_{\rm n}$  = 1700). The vial was covered with aluminum foil and the solution was stirred at room temperature for 20 h. The reaction mixture was cooled down to 0 °C and treated with mPEG-NH<sub>2</sub> (168 mg, 0.084 mmol,  $M_n = 2000$ ), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC, 16 mg, 0.084 mmol), and triethylamine (17 mg, 0.168 mmol) all in one portion. The reaction mixture was stirred at 0 °C for 1 h, allowed to warm to room temperature, and stirred for an additional 20 h. The solvent was removed using a rotary evaporator, and the oily residue was treated with deionized water (3 mL). After sonication, the aqueous solution containing the crude polymer product was dialyzed against deionized water for 96 h in a dialysis bag with a molecular weight cutoff of 14 kDa (Spectrum Laboratories, Inc.). The purified polymer was isolated by lyophilizing the resulting aqueous solution to dryness to afford 155 mg (34%) of polymer 20 as a green power. (The slight green color is due to the small amount of ring-closing to generate some colored 2c.) <sup>1</sup>H NMR (600 MHz,  $CD_2Cl_2$ )  $\delta$  7.17 (br, s, 65 H, phenyl protons of PSMA), 3.63 (s, 701 H, OCH<sub>2</sub>CH<sub>2</sub> of mPEG-NH<sub>2</sub>), 3.37 (s, 13H, OCH<sub>3</sub> of mPEG-NH<sub>2</sub>).

Synthesis of Hybrid Nanosystem 20-NP. In a typical synthesis, a stirring solution of photoresponsive polymer 20 in CHCl<sub>3</sub> (3 mL) was treated with a solution of the oleate-coated UCNPs (NaYF<sub>4</sub>:ErYb) in CHCl<sub>3</sub> (375  $\mu$ L, 43 mg/mL). This value is a calculated value of the nanoparticle concentration based on the volume and mass of air-dried aliquots sample of the original UCNPs in CHCl<sub>3</sub> solution. After stirring at room temperature for 20 h, the reaction mixture was evaporated to dryness using a rotary evaporator. The oily residue was treated with aqueous NaOH (3 mL, 0.001 M, pH 11), sonicated for 5 min, and any trace amounts of CHCl<sub>3</sub> were carefully removed using a rotary evaporator to afford a clear aqueous solution. This solution was transferred using a pipet into two 1.5 mL conical centrifugation tubes and centrifuged at 20600 RCF for 25 min. The supernatant was removed from the pellets of nanoparticles using a pipet, and the pellets were redispersed in deionized water (3 mL for each sample) with the help of sonication. The tubes were centrifuged

for 25 min at 20600 RCF, and the supernatant was removed from the pellets of nanoparticles using a pipet. The nanoparticles were redispersed in deionized water (3 mL for each sample) with the help of sonication, and the two samples were combined and passed through a 0.2  $\mu$ m filter (Acrodisc Syringe Filter) to obtain the final stock solution of encapsulated nanoparticles for further use.

Synthesis of Diarylethene Amide 3o. A stirred solution of amino-diarylethene 10 (57 mg, 0.1 mmol) in Et<sub>2</sub>O (5 mL) was treated with acetic anhydride (20  $\mu L,$  22 mg, 0.22 mmol). The reaction mixture was stirred at room temperature for 16 h, at which time it was quenched by pouring it into 50 mL of saturated NaHCO3. The mixture was extracted with  $Et_2O$  (2 × 50 mL), and the organic layers were combined, dried over MgSO<sub>4</sub>, filtered, and evaporated to dryness using a rotary evaporator. Purification using column chromatography (silica, hexanes: EtOAc 1:2,  $r_f = 0.6$ ) afforded 27 mg (47% yield) of amide 30 as yellow crystals. Mp 208-210 °C. <sup>1</sup>H NMR (600 MHz,  $CDCl_3$ )  $\delta$  7.55–7.52 (m, 4H), 7.49 (d, J = 8.4 Hz, 2H), 7.38 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.2 Hz, 1H), 7.28 (s, 1H), 7.21 (s, 1H), 2.20 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$ 168.4, 142.4, 141.9, 141.4, 141.1, 137.7, 136.2 (m, 2 carbons), 133.5, 129.6, 129.2, 128.1, 126.4, 126.0, 125.8, 122.5, 122.1, 120.3, 116.3 (tt, J = 254, 24 Hz, 2 carbons), 111.0 (tq, J = 269, 24 Hz, 1 carbon), 24.8, 14.70, 14.6. (The splitting is due to coupling with the fluorine atoms on the cyclopentene ring.) Anal. Calcd for C<sub>29</sub>H<sub>21</sub>F<sub>6</sub>NOS<sub>2</sub>: C, 60.30; H, 3.66; N, 2.42. Found: C, 60.59; H, 3.48; N, 2.43. HRMS (M + H<sup>+</sup>) expected: 578.1047; found: 578.1040.

Synthesis of Hybrid Nanosystem 30(NP). In a typical synthesis, a stirring solution of cumene terminated poly(styrene-co-maleic anhydride) (PSMA, 25 mg, 14.6  $\mu$ mol, Mn = 1700) in CHCl<sub>3</sub> (3 mL) was treated with a solution of the oleate-coated UCNPs (NaYF<sub>4</sub>:ErYb) (250  $\mu$ L, 43 mg/mL) in CHCl<sub>3</sub>. After stirring at room temperature for 2 h, the reaction was treated with a solution of JeffAmine 2070 (150 mg, 80  $\mu$ mol, Mn = 2070) in CHCl<sub>3</sub> (1.0 mL) and a stock solution (1.2 mM) of the photoresponsive compound 30  $(334 \,\mu\text{L}, 0.4 \,\mu\text{mol})$  in CHCl<sub>3</sub> using a pipet. The reaction was stirred at room temperature for 16 h, at which time it was evaporated to dryness using a rotary evaporator. The oily residue was treated with aqueous NaOH (3 mL, 0.001 M, pH 11) and sonicated for 5 min, and any trace amounts of CHCl<sub>3</sub> were carefully removed using a rotary evaporator to afford a clear aqueous solution. This solution was transferred using a pipet into two 1.5 mL conical centrifugation tubes and centrifuged at 20600 RCF for 25 min. The supernatant was removed from the pellets of nanoparticles using a pipet, and the pellets were redispersed in deionized water (1.5 mL for each sample) with the help of sonication. The tubes were centrifuged for 25 min at 20600 RCF, and the supernatant was removed from the pellets of nanoparticles using a pipet. The nanoparticles were redispersed in deionized water (1.5 mL for each sample) with the help of sonication, and the two samples were combined and passed through a 0.2  $\mu$ m filter (Acrodisc Syringe Filter) to obtain the final stock solution of encapsulated nanoparticles for further use.

#### RESULTS AND DISCUSSION

In all reported examples, the amphiphilic polymer was prepared by ring-opening the anhydride functional groups in copolymers of maleic anhydride and hydrophobic alkenes with amines as schematically shown in the top of Scheme 1. The previously reported dithienylethene derivative bearing a single amino functional group  $(10)^{55}$  was chosen to prepare the polymers. The dithienylethene amide **30** (Scheme 1) was prepared for comparison by acetylating **10**.

The photoresponsive polymer **20** was prepared using a modified procedure from our recent work<sup>54</sup> and work published by others.<sup>56–58</sup> In a typical synthesis (Scheme 2),<sup>59</sup> poly(styrene-*co*-maleic anhydride) (PSMA) was reacted with **10** in CHCl<sub>3</sub>. The resulting carboxylate was coupled to mPEG-NH<sub>2</sub> using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC). The final polymer was isolated by removing the organic

Scheme 1. (top) Ring-Opening of Copolymers of Maleic Anhydride with Amines to Produce Functionalized Amphiphilic Polymers for Wrapping Upconverting Nanoparticles<sup>a</sup> and (bottom) Photoresponsive Chromophores Used in These Studies Showing How They Undergo Reversible Photochemical Reactions When Exposed to UV and Visible Light



<sup>*a*</sup>The black circle represents a hydrophobic group such as phenyl rings or alkyl chains. The white circle is typically water dispersible chains such as polyethylene glycols.

solvent, followed by dialysis against deionized water and lyophilization. We estimate the ratio of the photochromic component to the mPEG-2000 chain to be 1:4 according to the <sup>1</sup>H NMR spectrum of 20.<sup>60</sup>

Polymer **20** was used to encapsulate oleate-coated NaYF<sub>4</sub> upconverting nanoparticles doped with 2% Er and 20% Yb (NaYF<sub>4</sub>:ErYb) as shown in Scheme 2 by first mixing the two components in CHCl<sub>3</sub> followed by treatment with water to induce encapsulation. <sup>S9</sup> The final assembly (**20-NP**) was isolated by centrifugation. Another nanosystem where both

organic (30) and inorganic components (NaYF<sub>4</sub>:ErYb) were coencapsulated with a nonphotoactive amphiphilic polymer was prepared using the original coencapsulation method<sup>21</sup> as shown in Scheme 3. This system was prepared to compare the loading of the chromophores in the two systems as well as the effects of loading on the fluorescence quenching.

Dynamic light scattering (DLS) analyses of the nanoassemblies show an average hydrodynamic size of 34.2 nm for 20-NP and 32.2 nm for 30(NP).59 The similarity in the diameters was supported by TEM imaging (Figure 2 and Supporting Information Figure S1), which show average diameter of 24 nm for 20-NP and 23 nm for 30(NP). The smaller size of the nanoparticles measured using TEM compared to DLS is expected as the former highlights the inorganic core while the latter measures the hydrodynamic size of the entire assembly including the polymer shell. What is also revealed in the TEM images is a faint outline of the polymer shell around the nanoparticles. The shell was estimated to be approximately 3 nm thick, which correlates well with the increased diameters of the nanoassemblies measured using DLS techniques (Supporting Information Figure S2). An idealized representation of the polymer shell with the mPEG chains fully extended puts the maximum thickness of the coating at 10-11 nm, which is much greater than what was measured using either TEM or DLS analysis. It is reasonable to assume the polymer exists in a more compressed form, and it is unlikely that each UCNP is encapsulated by more than one polymer layer.

The ring-closing and ring-opening reactions of all photochromic systems (**2o** in **2o-NP**, **3o** in **3o**(**NP**)) were monitored using UV–vis absorption spectroscopy, where characteristic bands in the visible region of the spectrum appear as is typical for the ring-closing reactions of dithienylethene derivatives when they are exposed to UV light (Figure 3, Supporting Information Figure S3, and Table 1). In all cases, the highenergy bands in the UV region (~300 nm) decrease in intensity as the colorless isomers (**2o** and **3o**) are converted into their colored ring-closed counterparts (**2o** and **3c**) when solutions of them (CH<sub>3</sub>CN for **3o** and H<sub>2</sub>O for **2o-NP** and **3o**(**NP**)) are exposed to 365 nm light. The bands are centered between 590 and 600, which explains why the solutions become blue.<sup>61</sup> After

Scheme 2. Synthesis of Photoresponsive Polymer and Encapsulation of Upconverting Nanoparticles



# Scheme 3. Synthesis of Nanosystems Containing Both Upconverting Nanoparticles and Dithienylethene 30 Simultaneously Coencapsulated by an Amphiphilic, Non-Photoactive Polymer





Figure 2. Representative TEM images of the nanoassemblies used in these studies. (a) Encapsulated system using the photoresponsive polymer 20-NP and (b) the system with coencapsulated components (3o(NP)).

about 30-90 s of exposure to this light, the cyclization reactions have reached their photostationary states (20/c-NP, 3o/c, and 3o/c(NP)). In the case of the free chromophore (3o/c), the amount of ring-closed isomer in the photostationary state was measured to be 75% by <sup>1</sup>H NMR spectroscopy. While the amounts of ring-closed isomers in the nanoassemblies are impossible to measure directly by <sup>1</sup>H NMR spectroscopy, we estimated them to be similar to 30/c based on the fact that there are almost no observed differences between the all absorbance spectra for systems when they are normalized at the  $\lambda_{max}$  (300 nm) for the ring-open isomers (Supporting Information Figure S3d). Based on this assumption, we calculated the average loading of the chromophores per nanoparticle in the assemblies to be 1212 for 2c-NP and 87 for 3c(NP).<sup>62</sup> As anticipated, the number of photoresponsive components significantly increased when they were directly conjugated into the polymer used to wrap the nanoparticles, and this will have a consequence on the ability of the systems to quench the emission from the UCNPs.

When any of the colored solutions containing the photostationary states are exposed to visible light of wavelengths greater than 490 nm, they all revert to their colorless states as the ringopen isomers are regenerated. The original spectra are also reproduced. These ring-closing/ring-opening cycles can be



**Figure 3.** UV–vis absorption spectra of H<sub>2</sub>O solutions of (a) **2o-NP** (1.17 × 10<sup>-4</sup> M) and (b) **3o(NP)** (1.85 × 10<sup>-5</sup> M),<sup>63</sup> before (solid) and after (broken line) irradiation with 365 nm light. The green and red shaded areas in each absorption spectrum correspond to the region where the UCNPs emit when irradiated with 980 nm light to show the spectral overlap. (c) Normalized emission spectra ( $\lambda_{ex}$  = 980 nm) of **2o-NP** before irradiation with 365 nm light (white shaded) and at photostationary state (dark green and red shaded) and **3o(NP**) before irradiation with 365 nm light (white shaded) and at photostationary state (light green and red shaded). (d) Relative emission of **2o-NP** before irradiation with 365 nm light (white shaded) and at photostationary state (dark green and red shaded) and at photostationary state (dark green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded) and at photostationary state (light green and red shaded).

repeated numerous times with minimal observable changes in the spectra.

Figure 2 also illustrates how the chromophores in the nanoassemblies can reversibly modulate the emission from the

Table 1. Absorption Characteristics, Photochem	istry, and Loading of the	Chromophores in Nanoassembl	ies 20-NP and 30(N	NP)
			· · · · · · · · · · · · · · · · · · ·	

	optical properties					
	ring-o	open isomer	ring-closed isomer			
photoresponsive system	$\lambda$ (nm)	$\varepsilon ~(\mathrm{cm}^{-1}~\mathrm{M}^{-1})$	$\lambda$ (nm)	$\varepsilon ~(\mathrm{cm}^{-1}~\mathrm{M}^{-1})$	photostationary state $^{c}$ (%)	number of chromophores per nanoparticle
$30/c^a$	300	61882	591	26175	75	-
$20/c-NP^b$	304	_	595	_	$\_^d$	1212
$3o/c(NP)^b$	303	_	600	_	$\_^d$	87

<sup>*a*</sup>In CH<sub>3</sub>CN. <sup>*b*</sup>In H<sub>2</sub>O, after correcting for baseline scattering. <sup>*c*</sup>As measured using <sup>1</sup>H NMR spectroscopy. <sup>*d*</sup>These values are assumed to be the same as for the free chromophore (3o/c) in solution.

Table 2. Emission	Characteristics	and Fluorescence	Lifetimes f	for 20-NP	and 3o(	NP)
-------------------	-----------------	------------------	-------------	-----------	---------	-----

	relative emission <sup>a</sup>		fluorescent lifetime $(\mu s)^b$		FRET quenching (%)	
system	505-570 nm	625–690 nm	ring-open isomer	ring-closed isomer	total	per chromophore
20/c-NP	$0.35 \pm 0.02$	$0.15 \pm 0.01$	$50.4 \pm 0.7$	$37.1 \pm 0.9$	26 ± 1	$0.021 \pm 0.001$
3o/c(NP)	$0.85 \pm 0.03$	$0.76 \pm 0.02$	$51.9 \pm 0.5$	$49.9 \pm 0.5$	3.9 ± 1	$0.045 \pm 0.01$

<sup>*a*</sup>Ratio of the area under the curve in the emission spectrum of the nanosystems after irradiation with 365 nm light until the photostationary states were reached to the area under the curve in the emission spectrum of the nanosystems prior to irradiation (2o-NP and 3o(NP)). The mean values and standard deviations are based on six ring-closing/ring-opening cycles. <sup>*b*</sup>Obtained by using an exponential fit of the photon-counting data. The errors are from the fitting uncertainty. The data were collected only from 505 to 570 nm due to the weak signal between 625 and 690 nm under optimized conditions.

upconverting nanoparticles. When 980 nm light is used as the excitation source, both nanosystems (20-NP and 30(NP)) show similar emission characteristics. This is expected considering there is minimal spectral overlap between the emission bands of the nanoparticles and the absorption bands of the ring-open isomers (20 and 30) so there should be minimal effect from either chromophore no matter what the level of loading. As is typical for NaYF<sub>4</sub>:ErYb nanoparticles,<sup>3</sup> two sets of relatively sharp bands are observed in the region between 500 and 700 nm. The higher energy "green" emissions (504–568 nm) correspond to the  $[{}^{4}H_{11/2}, {}^{4}S_{3/2}] \rightarrow {}^{4}I_{15/2}$ transitions, while the lower energy "red" emissions (627-684 nm) are a result of the  ${}^{4}F_{9/2} \rightarrow {}^{4}I_{15/2}$  transitions. Both transitions overlap with the absorption band of the ring-closed isomer in the photostationary states (2c-NP and 3c(NP)), which is shown by the reduced amount of emission from either nanosystem when it is irradiated with 365 nm light (Figure 3c). Because the ring-closed isomers integrated in both nanoassemblies have similar absorption profiles, both "green" and "red" nanoparticle emissions are reduced, albeit to different extents (Figure 3c,d and Table 2).<sup>64</sup> As anticipated, visible light (>490 nm) restores the original fluorescence intensity since it triggers the ring-opening reactions and regenerates the isomers that do not effectively quench the emission for the nanoparticles. As in the case of the absorption changes, the emission quenching can be subjected to several ring-closing/ringopening cycles without observable degradation.

The nanoassembly that has the photochromic component directly conjugated into the wrapping polymer (2o-NP) is over four times more effective at quenching the "green" emission as the assembly that has the photochromic component coencapsulated (3o(NP)). It is three times as effective for the "red" emission. Both observations can be attributed to the increased loading of the fluorescence quencher in the nanoassembly (2o-NP has almost 14 times the number of photochromic components as 3o(NP) as shown in Table 1). However, if the relative "green" quenching is considered per chromophore in the two nanoassemblies, it is clear that the chromophores in 3o(NP) are more effective quenchers (0.05% quenching per chromophore in 2o-NP vs 0.17% quenching per chromophore in 3o(NP)). The "red" quenching shows similar results (0.07% and 0.28%, respectively). These differences are somewhat unexpected given their similar absorption spectra and can be attributed to the chromophores in 3o(NP) residing closer to the nanoparticles. This conclusion is supported by lifetime measurements as discussed in the next section of this report.

The two major mechanisms responsible for the quenching of emission from the nanoparticles are Förster resonance energy transfer (FRET) and "emission-reabsorption".65 The former involves discrete energy donors and acceptors that transfer the excited state energy through collisions.<sup>66</sup> The latter is often ignored. In this case, quenching is a result of the light emitted from the nanoparticle being absorbed by chromophores in close proximity but not necessarily anchored to the nanoparticle that is emitting the light. Although the net observable effect is the same, the FRET mechanism is important to enhance when quenching is required in many environments of use. One good example is bioimaging applications, where the concentration of the emissive probe at any one time and at any one place is too low to rely on the contribution of "emissionreabsorption". Because FRET is independent of concentration in a hybrid system containing both "donor" and "acceptor" intimately linked it will still operate. In our previous examples of photoresponsive upconverting nanoparticles we provided evidence that the major contribution to the quenching was "emission-reabsorption", and FRET was only responsible for 7-26% of the quenching.<sup>21</sup> This was the incentive to prepare and study the photoresponsive polymer systems reported in this paper (20-NP).

Fluorescence lifetime measurements for both nanoassemblies show a more significant increase in the "green" emission decay kinetics<sup>67</sup> for **2o-NP**  $\rightarrow$  **2o/c-NP** (from 50 to 37  $\mu$ s) when compared to **3o(NP)**  $\rightarrow$  **3o/c(NP)** (from 52 to 50  $\mu$ s). These data are listed in Table 2. These values correspond to FRET efficiencies of 26% for **2o/c-NP** and 4% for **3o/c(NP)**. Again, the argument for a greater extent of FRET quenching due to higher chromophore loading in **2o/c-NP** can be used. However, if the relative amounts of FRET quenching per chromophore are compared (0.021% quenching per chromophore in 20-NP vs 0.045% quenching per chromophore in 3o(NP)), it is once again clear that the chromophores in the latter system are more effective quenchers. Because the FRET mechanism is highly dependent on the relative orientation and proximity of the acceptor to the donor, the freedom the chromophores exhibit in 3o(NP) results in their finding more suitable positions. In the case of 20-NP the chromophores are anchored to the polymer backbone, which limits their mobility.

The focus of this paper is the enhanced (but still reversible) fluorescence quenching of nanoassemblies that have the photoresponsive chromophore directly integrated into the polymer used to wrap upconverting nanoparticles. While attaching the chromophores as pendant groups onto the polymer backbone somewhat reduces the modular "plug-andplay" approach to make libraries of these systems, the increase in loading makes them attractive. We believe these systems will help in the design of the next generation of organic–inorganic hybrid nanoassemblies for use in bioimaging applications. The fluorescent probe we developed has the potential to enhance the spectral resolution of fluorescent microscopy. Future research will focus on increasing loading while allowing mobility of the chromophores to enhance FRET quenching in UCNP–diarylethene hybrid system.

# ASSOCIATED CONTENT

# **S** Supporting Information

Details on the materials for synthesis, instrumentation for analysis, and microscopy and spectral characterizations. This material is available free of charge via the Internet at http:// pubs.acs.org.

# AUTHOR INFORMATION

#### **Corresponding Author**

\*E-mail: nbranda@sfu.ca (N.R.B).

#### **Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

#### Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canada Research Chairs Program, and Simon Fraser University. This work made use of 4D LABORATORIES shared facilities supported by the Canada Foundation for Innovation (CFI), British Columbia Knowledge Development Fund (BCKDF), and Simon Fraser University. We thank Dr. Saeid Kamal for performing photon counting experiments and providing useful suggestions.

#### ABBREVIATIONS

NP, nanoparticle; Mp, melting point; HRMS, high resolution mass spectroscopy

# REFERENCES

- (1) Fery-Forgues, S. Nanoscale 2013, 5, 8428.
- (2) Vollrath, A.; Schubert, S.; Schubert, U. S. J. Mater. Chem. B 2013, 1, 1994.
- (3) Cheng, X.; Lowe, S. B.; Reecec, P. J.; Gooding, J. J. Chem. Soc. Rev. 2014, 43, 2680.

- (5) Wang, Y.; Hu, R.; Lin, G.; Roy, I.; Yong, K.-T. ACS Appl. Mater. Interfaces. 2013, 5, 2786.
- (6) Kairdolf, B. A.; Smith, A. M.; Stokes, T. H.; Wang, M. D.; Young, A. N.; Nie, S. Annu. Rev. Anal. Chem. 2013, 6, 143.
- (7) Huang, B.; Bates, M.; Zhuang, X. Annu. Rev. Biochem. 2009, 78, 993.

(8) Fölling, J.; Belov, V.; Kunetsky, R.; Medda, R.; Schönle, A.; Egner, A.; Eggeling, C.; Bossi, M.; Hell, S. W. Angew. Chem., Int. Ed. 2007, 46, 6266.

(9) Fölling, J.; Belov, V.; Riedel, D.; Schönle, A.; Egner, A.; Eggeling, C.; Bossi, M.; Hell, S. W. *ChemPhysChem* **2008**, *9*, 321.

(10) Bossi, M.; Fölling, J.; Belov, V. N.; Boyarskiy, V. P.; Medda, R.; Egner, A.; Eggeling, C.; Schönle, A.; Hell, S. W. *Nano Lett.* **2008**, *8*, 2463.

(11) Berns, M. W.; Krasieva, T.; Sun, C.-H.; Dvornikov, A.; Rentzepis, P. M. J. Photochem. Photobiol., B 2004, 75, 51.

(12) Zou, Y.; Yi, T.; Xiao, S.; Li, F.; Li, C.; Gao, X.; Wu, J.; Yu, M.; Huang, C. J. Am. Chem. Soc. **2008**, 130, 15750.

(13) Westphal, V.; Rizzoli, S. O.; Lauterbach, M. A.; Kamin, D.; Jahn, R.; Hell, S. W. Science **2008**, 320, 246.

(14) Moffitt, J. R.; Osseforth, C.; Michaelis, J. Opt. Express 2011, 19, 4242.

(15) Zhuang, X. Nat. Photonics 2009, 3, 365.

(16) Yildiz, I.; Deniz, E.; Raymo, F. M. Chem. Soc. Rev. 2009, 38, 1859.

(17) Klajn, R.; Stoddart, J. F.; Grzybowski, B. A. Chem. Soc. Rev. 2010, 39, 2203.

(18) The focus of this report is on the use of photoresponsive systems to modulate the emissive properties of nanoparticles. The numerous examples in which photochromic compounds and light have been used to control the fluorescence and phosphorescence of small molecules have not been included in this paper. We encourage the reader to refer to several illustrative reviews on this subject. (a) Cusido, J.; Deniz, E.; Raymo, F. M. *Eur. J. Org. Chem.* **2009**, 2031. (b) Raymo, F. M.; Tomasulo, M. *Chem. Soc. Rev.* **2005**, 34, 327.

(19) Carling, C.-J.; Boyer, J.-C.; Branda, N. R. Org. Biomol. Chem. 2012, 10, 6159.

(20) Boyer, J. C.; Carling, C. J.; Chua, S. Y.; Wilson, D.; Johnsen, B.; Baillie, D.; Branda, N. R. *Chem.—Eur. J.* **2012**, *18*, 3122.

(21) Wu, T.; Boyer, J.-C.; Barker, M.; Wilson, D.; Branda, N. R. Chem. Mater. 2013, 25, 2495.

(22) Zhou, Z.; Hu, H.; Yang, H.; Yi, T.; Huang, K.; Yu, M.; Li, F.; Huang, C. Chem. Commun. 2008, 4786.

(23) Zhang, C.; Zhou, H.; Liao, L.; Feng, W.; Sun, W.; Li, Z.; Xu, C.; Fang, C.; Sun, L.; Zhang, Y.; Yan, C. *Adv. Mater.* **2010**, *22*, 633.

(24) Feringa, B. L. Molecular Switches; Wiley-VCH: Weinheim, 2010.

(25) Tian, H.; Yang, S. Chem. Soc. Rev. 2004, 33, 85.

(26) Ubaghs, L.; Sud, D.; Branda, N. R. *Handbook in Thiophene-Based Materials: Applications in Organic Electronics and Photonics*; Perepichka, I. D., Perepichka, D., Eds.; John Wiley & Sons: Chichester, 2009; Vol. 2.

(27) Other classes of photochromic compounds tend to show less stability than dithienylethenes when integrated into emissive nanoparticle systems. Yildiz, I.; Impellizzeri, S.; Deniz, E.; McCaughan, B.; Callan, J. F.; Raymo, F. M. J. Am. Chem. Soc. **2011**, 133, 871. Although even some dithienylethenes undergo degradation. Erno, Z.; Yildiz, I.; Gorodetsky, B.; Raymo, F. M.; Branda, N. R. Photochem. Photobiol. Sci. **2010**, *9*, 249.

- (28) Norsten, T. B.; Branda, N. R. J. Am. Chem. Soc. 2001, 123, 1784.
  (29) Giordano, L.; Jovin, T. M.; Irie, M.; Jares-Erijman, E. A. J. Am. Chem. Soc. 2002, 124, 7481.
- (30) Fölling, J.; Polyakova, S.; Belov, V.; Blaaderen, A. V.; Bossi, M. L.; Hell, S. W. *Small* **2008**, *4*, 134.
- (31) Jeong, J.; Yun, E.; Choi, Y.; Jung, H. Y.; Chung, S. J.; Song, N. W.; Chung, B. H. Chem. Commun. **2011**, *47*, 10668.

(32) Fukaminato, T.; Doi, T.; Tamaoki, N.; Okuno, K.; Ishibashi, Y.; Miyasaka, H.; Irie, M. J. Am. Chem. Soc. **2011**, 133, 4984. (33) Osakada, Y.; Hanson, L.; Cui, B. Chem. Commun. 2012, 48, 3285.

- (34) Berberich, M.; Natali, M.; Spenst, P.; Chiorboli, C.; Scandola, F.; Würthner, F. *Chem.*—*Eur. J.* **2012**, *18*, 13651.
- (35) Berberich, M.; Würthner, F. Chem. Sci. 2012, 3, 2771.
- (36) Pu, S.; Liu, G.; Wang, R.; Chen, B. Dyes Pigm. 2013, 98, 238.
- (37) Ding, H.; Liu, G.; Pu, S.; Zheng, C. Dyes Pigm. 2014, 103, 82.
- (38) Nyk, M.; Kumar, R.; Ohulchanskyy, T. Y.; Bergey, E. J.; Prasad, P. N. *Nano Lett.* **2008**, *8*, 3834.
- (39) Haase, M.; Schäfer, H. Angew. Chem., Int. Ed. 2011, 50, 5808.
- (40) Medintz, I.; Trammell, S.; Mattoussi, H.; Mauro, J. J. Am. Chem. Soc. 2004, 126, 30.
- (41) Medintz, I.; Clapp, A.; Trammell, S.; Mattoussi, H. Proc. SPIE-Int. Soc. Opt. Eng. 2004, 5593, 300.
- (42) Zhu, L.; Zhu, M.; Hurst, J.; Li, A.-D.-Q. J. Am. Chem. Soc. 2005, 127, 8968.
- (43) Jares-Erijman, E. A.; Giordano, L.; Spagnuolo, C.; Lidke, K.; Jovin, T. M. Mol. Cryst. Liq. Cryst. 2005, 430, 257.
- (44) Tomasulo, M.; Yildiz, I.; Raymo, F. Aust. J. Chem. 2006, 59, 175.
  (45) Binder, W.; Sachsenhofer, R.; Straif, C.; Zirbs, R. J. Mater. Chem.
  2007, 17, 2125.
- (46) Winnik, F. M.; Maysinger, D. Acc. Chem. Res. 2013, 46, 672.
- (47) Chan, W. C. W.; Nie, S. Science 1998, 281, 2016.
- (48) Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. *Science* **2005**, *307*, 538.
- (49) For a review of this area, see: Gai, S.; Li, C.; Yang, P.; Lin, J. *Chem. Rev.* **2014**, *114*, 2343.
- (50) Park, Y.; Kim, J. H.; Lee, K. T.; Jeon, K.-S.; Na, H. B.; Yu, J. H.; Kim, H. M.; Lee, N.; Choi, S. H.; Baik, S.; Kim, H.; Park, S. P.; Park, B.-J.; Kim, Y. W.; Lee, S. H.; Yoon, S.-Y.; Song, I. C.; Moon, W. K.; Suh, Y. D.; Hyeon, T. *Adv. Mater.* **2009**, *21*, 4467.
- (51) Examples of photolabile compounds released in water: Klań, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. *Chem. Rev.* **2013**, *113*, 119. Examples of photochromic spiropyran photochemistry in water: Tian, W.; Tian, J. Dyes Pigm. **2014**, *105*, 66.
- (52) Carling, C.-J.; Boyer, J.-C.; Branda, N. R. J. Am. Chem. Soc. 2009, 131, 10838.
- (53) Boyer, J.-C.; Carling, C.-J.; Gates, B. D.; Branda, N. R. J. Am. Chem. Soc. 2010, 132, 15766.
- (54) Wu, T.; Barker, M.; Carling, C.-J.; Arafeh, K. M.; Boyer, J.-C.; Branda, N. R. *Angew. Chem., Int. Ed.* **2013**, *52*, 11106.
- (55) Lim, S.-J.; Carling, C.-J.; Warford, C. C.; Hsiao, D.; Gates, B. D.; Branda, N. R. *Dyes Pigm.* **2011**, *89*, 230–235.
- (56) Díaz, S. A.; Giordano, L.; Azcárate, J. C.; Jovin, T. M.; Jares-Erijman, E. A. J. Am. Chem. Soc. **2013**, 135, 3208.
- (57) Díaz, S. A.; Giordano, L.; Jovin, T. M.; Jares-Erijman, E. A. *Nano Lett.* **2012**, *12*, 3537.
- (58) Díaz, S. A.; Menéndez, G. O.; Etchehon, M. H.; Giordano, L.; Jovin, T. M.; Jares-Erijman, E. A. ACS Nano 2011, 5, 2795.
- (59) See Supporting Information for details.
- (60) From the <sup>1</sup>H NMR spectrum of the polymer sample, signals corresponded to styrene protons, mPEG-NH<sub>2</sub>, and partial diarylethene protons were integrated. Most of the diarylethene protons were hidden underneath the broad styrene peak. See Supporting Information for details.
- (61) See Supporting Information for more details and images.
- (62) See Supporting Information for the detailed calculations.
- (63) All concentrations are total concentration of photoswitches encapsulated in the nanoassemblies obtained using the concentration of the ring-closed isomers of each photoswitch at its photostationary state (measured by UV–vis spectroscopy) divided by percentage of ring-closed isomers in this state (measured by <sup>1</sup>H NMR spectroscopy).
- (64) For photographs of both nanoparticle systems in their emissive and reduced emissive state; see Figure S4 in the Supporting Information.
- (65) Niebling, T.; Zhang, F.; Ali, Z.; Parak, W. J.; Heimbrodt, W. J. Appl. Phys. 2009, 106, 104701.

- (66) Theodor, F. Ann. Phys. 1948, 437, 55.
- (67) The data were collected only from 505 to 570 nm due to the weak signal between 625 and 690 nm under optimized conditions.