

Supporting Information

Two-Colour Fluorescent Imaging in Organisms Using Self-Assembled Nano-Systems of Upconverting Nanoparticles and Molecular Switches

Tuoqi Wu^a, Bob Johnsen^b, Zhaozhao Qin^b, Masakazu Morimoto,^c David Baillie,^{b*} Masahiro Irie^{c*} and Neil R. Branda^{a*}

^a*4D LABS and Department of Chemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6*

^b*Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6*

^c*Department of Chemistry and Research Center for Smart Molecules, Rikkyo University, 3-34-1 Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan*

E-mail: nbranda@sfu.ca, iriem@rikkyo.ac.jp, baillie@sfu.ca

Materials and Methods

General. All solvents and reagents used for synthesis, chromatography, UV-vis spectroscopy measurements and photolysis studies were purchased from Aldrich and used as received, unless otherwise noted. Solvents for NMR analysis were purchased from Cambridge Isotope Laboratories and used as received. The photochromic diarylethene **1o** was synthesized as described in the literature.¹ Poly(propylene glycol)bis(2-aminopropyl ether) (JeffAmine 2070) was received as a gift from Huntsman Inc. Cumene terminated poly(styrene-*co*-maleic anhydride) was purchased from Aldrich. All volumes for absorption, photolysis, and concentration measurements and studies were measured using an autopipette.

Instrumentation

NMR spectroscopy. The ¹H NMR photolysis studies were performed on CD₃CN solutions using a Bruker BioSpin 500 spectrometer by collecting 32 scans per measurement.

Thermo-gravimetric analysis (TGA). TGA was performed using a Shimadzu TGA50 instrument. The sample was prepared by combining multiple dried samples into the weighting pan to make sure data accuracy. The weight loss was analyzed by heating the sample from room temperature to 500°C at the rate of 5°C/min.

Optical Spectroscopy. UV-vis absorption spectroscopy was performed using a Varian Cary 300 Bio spectrophotometer. Fluorescence measurements were performed using a PTI Quantamaster spectrofluorometer. A JDS Uniphase 980 nm laser diode (device type L4-9897510-100M) coupled to a 105 μm (core) fibre was employed as the NIR light excitation source. The output of the diode laser was collimated and directed on the samples using a Newport F-91-C1-T Multimode Fiber

¹ K. Uno, H. Niikura, M. Morimoto, Y. Ishibashi, H. Miyasaka, M. Irie, *J. Am. Chem. Soc.*, 2011, **133**, 13558.

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 FEI Tecnai Osiris Transmission Electron Microscope operating at 200 keV. For the sample preparation, aqueous dispersions of the nanoparticles (5 μL) were placed on thin, carbon formvar-coated copper grids held by anti-capillary tweezers (Ted Pella, Part # 501-4). Water was then slowly removed under reduced pressure in a vacuum desiccator. The shape and size of the core-shell $\text{NaYF}_4\text{:ErYb/NaYF}_4$ nanoparticles were evaluated from the collected TEM images. The size of the nanoparticles was calculated by measuring just over 100 particles located at different areas of the TEM grid.

Dynamic Light Scattering (DLS). DLS measurements were carried out using a Malvern Zetasizer Nano-ZS. The samples were held in a 10 mm path length plastic cuvette (BrandTech, Catalog # 759220). A nanoparticle concentration of $\sim 0.4 \text{ mg/mL}^2$ was diluted 100 times and the dilution was employed for the measurements. All DLS measurements were conducted at 25 $^\circ\text{C}$.

Photoinduced Ring-Closing and Ring-Opening Reactions. All photoreactions of the diarylethene derivative either dispersed in solution ($\mathbf{1o} \rightleftharpoons \mathbf{1c}$) or integrated in the nano-systems ($\mathbf{1o-NP} \rightleftharpoons \mathbf{1c-NP}$) were monitored using UV-vis absorption spectroscopy. The 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^2) to induce the ring-closing reactions ($\mathbf{1o} \rightarrow \mathbf{1c}$ and $\mathbf{1o-NP} \rightarrow \mathbf{1c-NP}$) and a tungsten filament light source with a greater than 434 nm cut-off filter (Elmo Omnigraphic 300AF, 107 mW/cm^2) to induce the ring-opening reactions ($\mathbf{1c} \rightarrow \mathbf{1o}$ and $\mathbf{1c-NP} \rightarrow \mathbf{1o-NP}$). Monochromatic light was generated using a PTI Quantamaster spectrofluorometer. The wavelength was adjusted to be centered at 488 nm, and the slit width of the fluorometer was adjusted to 2 nm.

Nematodes Imaging ($\lambda_{\text{ex}} = 488 \text{ nm}$ and 980 nm). Imaging was performed on a Leica confocal, two-photon microscope (model TCS SP5 with DMI 6000 inverted microscope) equipped with a Coherent femtosecond Ti:Sapphire laser (model Chameleon Vision) for 980 nm excitation and an Ar-ion laser for 488 nm excitation. The NIR laser beam was focused onto the sample using a 10 times microscope objective with a numerical aperture of 0.4. The emission detection range was chosen from 500 nm to 700 nm.

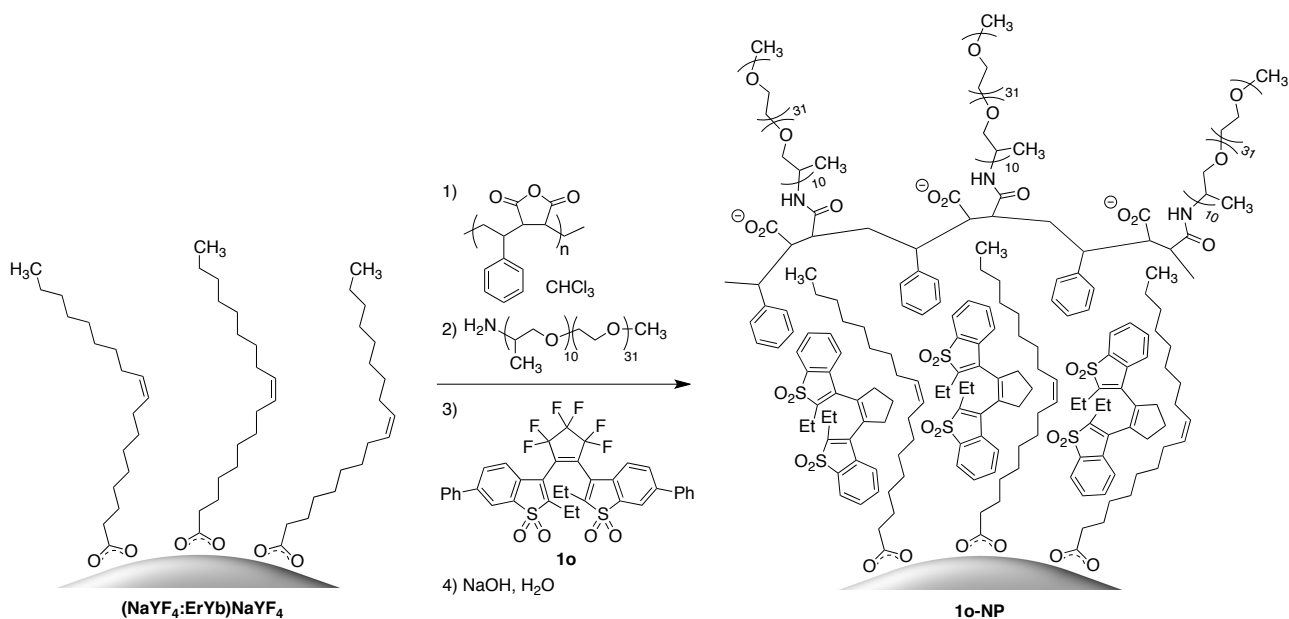
Synthesis of the Nano-Assemblies

Synthesis of $\beta\text{-NaYF}_4$: 2 mol% Er^{3+} , 20 mol% Yb^{3+} core nanoparticles ($\text{NaYF}_4\text{:ErYb}$). In a typical synthesis, $\text{Y}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$ (1170 mg, 3.9 mmol), $\text{Yb}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$ (439 mg, 1.0 mmol) and $\text{Er}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$ (43 mg, 0.10 mmol) were added to a 250 mL three-neck round-bottom flask containing octadecene (75 mL) and oleic acid (30 mL). The solution was stirred magnetically and heated slowly to 120 $^\circ\text{C}$ under vacuum for 30 min to form the lanthanide oleate complexes, and to remove residual water and oxygen. The temperature was then lowered to 50 $^\circ\text{C}$ and the reaction flask placed under a gentle flow of nitrogen gas. During this time, a solution of NH_4F (741 mg, 20 mmol) and NaOH (500 mg, 12.5 mmol) dissolved in methanol (20 mL) was prepared via sonication. Once the reaction reached 50 $^\circ\text{C}$, the methanol solution was added to the

² This value was determined by TGA measurements.

reaction flask and the resulting cloudy mixture was stirred for 30 min at 50 °C. The reaction temperature was then increased to 70 °C and the methanol evaporated from the reaction mixture. Subsequently, the reaction temperature was increased to 300 °C as quickly as possible and maintained at this temperature for 90 min under the nitrogen gas flow. During this time the reaction mixture became progressively clearer until a completely clear, slightly yellowish solution was obtained. The mixture was allowed to cool to room temperature. The nanoparticles were precipitated by the addition of EtOH and isolated via centrifugation at 4500 rpm corresponding to a relative centrifugal field (RCF) of approximately 1000. The resulting pellet was dispersed in a minimal amount of hexanes and precipitated with excess EtOH. The nanoparticles were isolated via centrifugation at 4500 rpm and then dispersed in CHCl₃ (10–15 mL) for the subsequent shell growth procedure.

Synthesis of β -NaYF₄: 2 mol% Er³⁺, 20 mol% Yb³⁺ / β -NaYF₄ core-shell nanoparticles. Y(CH₃CO₂)₃•xH₂O (1125 mg, 3.75 mmol) was added to a 250 mL three-neck round-bottom flask containing octadecene (56 mL) and oleic acid (22 mL). The solution was heated slowly to 120 °C under vacuum with magnetic stirring for 30 min. The temperature was lowered to 80 °C, the reaction flask was placed under a gentle flow of nitrogen and the dispersion of the core nanoparticles (**NaYF₄:ErYb**) in CHCl₃ was added. The resulting solution was slowly heated to 110 °C and maintained at this temperature until all the CHCl₃ was removed. The reaction mixture was cooled to 50 °C and a solution of NH₄F (556 mg, 15 mmol) and NaOH (375 mg, 9.375 mmol) dissolved in MeOH (20 mL) was added. The resulting cloudy mixture was stirred for 30 min at 50 °C at which time the reaction temperature was increased and the MeOH evaporated. After the evaporation of the MeOH, the reaction temperature was increased to 300 °C as quickly as possible and maintained at this temperature for 90 minutes under the nitrogen gas flow. The mixture was allowed to cool to room temperature and the nanoparticles were isolated using the same procedure of precipitate and isolation described for the core nanoparticles. The isolated core-shell (**NaYF₄:ErYb**)NaYF₄ nanoparticles were dispersed in CHCl₃ (~15 mL) for subsequent experiments.



Scheme S1. Synthesis of nano-assembly **1o-NP**.

Synthesis of hybrid nano-assembly 1o-NP. A stirring solution of cumene terminated poly(styrene-co-maleic anhydride) (25 mg, 14.6 μmol , $M_n = 1700$) in CHCl_3 (3 mL) was treated with a solution of the oleate-coated core-shell UCNP (**(NaYF₄:ErYb)NaYF₄**) (200 μL , 15 mg/mL) in CHCl_3 .³ After stirring at room temperature for 2 h, the reaction was treated with a solution of Jeffamine 2070 (160 mg, 77 μmol , $M_n = 2070$) in CHCl_3 (1.0 mL). The reaction was then stirred at room temperature for 16 h, at which time it was treated with a CHCl_3 stock solution of the photoresponsive compound **1o** (1.2 mM, 84 μL , 0.1 μmol) and stirred for 1 h. The reaction 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_3 were carefully removed using a rotary evaporator to afford a clear aqueous solution. This solution was transferred using a pipette into two 1.5 mL conical centrifugation tubes and centrifuged at 15000 RPM for 25 min (in IEC micromax). The supernatant was removed from the pellets of nanoparticles using a pipette, and the pellets were re-dispersed in deionized water (1.5 mL for each tube) with the help of sonication. The tubes were centrifuged for 25 min at 15000 RPM (in IEC micromax) and the supernatant was removed from the pellets of nanoparticles using a pipette. The nanoparticles were re-dispersed in deionized water (1.5 mL for each tube) with the help of sonication, the two samples were combined and passed through a 0.2 μm filter (Acrodisc[®] Syringe Filter) to obtain the final stock solution of encapsulated nanoparticles for further use.

³ This value was determined by weighing dried aliquot amount of the stock solution of the core-shell UCNP.

Sizes of the Nano-Assemblies Using TEM and DLS.

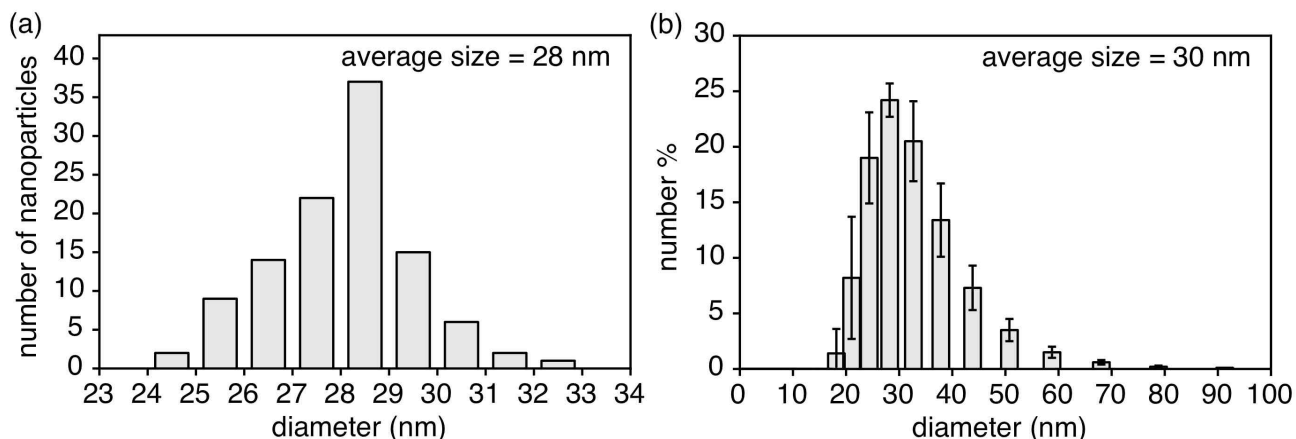


Figure S1. Size distribution as measured by (a) TEM and (b) DLS of the nano-assemblies **1o-NP**. For the DLS measurements, the original aqueous dispersion (0.4 mg/mL as determined by TGA; 0.8×10^{-6} M **1o**) was diluted 100 times with water and this diluted sample was employed for measurements. The sample was incubated at 25 °C for 2 min before the DLS measurements were acquired. The DLS plot is the averaged result of the measurements from 5 runs. Average size values were obtained directly from the DLS instrument using Malvern General Purpose & Multiple Narrow Mode NNLS (non-negative least squares) algorithm to treat the autocorrelation equation of the intensity trace describing sample intensity fluctuation recorded during the experiment.

Thermogravimetric Analysis.

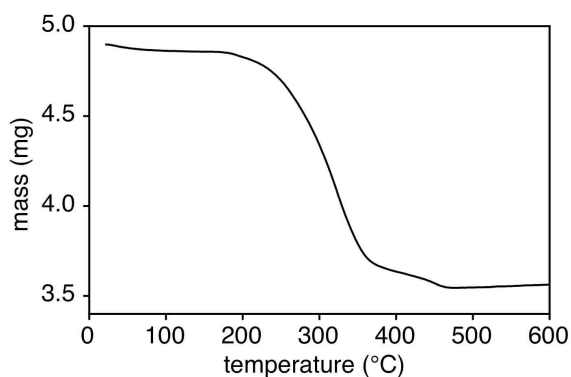


Figure S2. TGA graph of a sample of nano-assemblies **1o-NP**. The weight loss was analyzed by heating the sample from room temperature to 600 °C at the rate of 5°C/min.

Estimate of the Loading of the Photoresponsive Compound (**1o**) within Nano-System **1o**-NP

The total number of UCNPs was determined by the TGA analysis and the TEM images. The total number of chromophores integrated within the nanoparticle system was estimated using the UV-vis absorption data of the chromophore by comparing their absorptivity within the nanoparticle system **1o**-NP to that of the free chromophores (**1o/c**) in solution as shown below.⁴

calculated mass of single nanoparticle (NaYF ₄ :ErYb)NaYF ₄			
the shape of the particle is approximated to be a sphere and the density of (NaYF ₄ :ErYb)NaYF ₄ is taken to be the same as for NaYF ₄			
average radius of one particle (cm)	= 1.40 × 10 ⁻⁶		
volume of one particle (cm ³) using v = 4/3πr ³	= 4/3 × 3.14 × (1.400 × 10 ⁻⁶) ³		= 1.15 × 10 ⁻¹⁷
density of NaYF ₄ (g/cm ³)	= 4.23		
mass of one nanoparticle (g)	= 4.23 × 1.15 × 10 ⁻¹⁷		= 4.86 × 10 ⁻¹⁷
calculated loading of chromophores within the hybrid system 1o-NP			
for the solution of the free chromophore (1o/c)			
concentration (M) of 1o in CH ₃ CN	= 1.10 × 10 ⁻⁵		
A _{1c} (456 nm) in the photostationary state	= 0.200		
% ring-closed 1c at the photostationary state ⁵	= 100		
concentration (M) of ring-closed 1c at the photostationary state	= (1.10 × 10 ⁻⁵) × 100%		= 1.10 × 10 ⁻⁵
following Beer's law, ε (M ⁻¹ •cm ⁻¹)	= 45454		
for the solution of the chromophore within 1o-NP:			
A _{1c-NP} (456 nm) in 1o/c-NP ⁶	= 0.0147		
concentration (M) of ring-closed isomer in 1o/c-NP ⁷	= 8.09 × 10 ⁻⁷		
V (L) of sample	= 3.00 × 10 ⁻³		
moles of ring-closed isomer	= (8.09 × 10 ⁻⁷) × (3.00 × 10 ⁻³)		= 2.43 × 10 ⁻⁹
molecular equivalents of ring-closed isomer ⁸	= (2.43 × 10 ⁻⁹) × (6.023 × 10 ²³)		= 1.46 × 10 ¹⁵
total mass (g) of sample ⁹	= 1.53 × 10 ⁻³		
mass (g) of particles in sample ¹⁰	= 1.53 × 10 ⁻³ × 72%		= 1.10 × 10 ⁻³
mass (g) of one particle	= 4.86 × 10 ⁻¹⁷		
number of particles	= 1.10 × 10 ⁻³ / 4.86 × 10 ⁻¹⁷		= 2.26 × 10 ¹³
loading of photoswitch component 1o per particle	= (1.46 × 10 ¹⁵) / (2.26 × 10 ¹³)		= 65

⁴ All calculations are based on the assumption that the photostationary state and molar absorptivity coefficient (ϵ) of the photoswitches **3o/c** free in solution and integrated in the nanoparticle systems are the same.

⁵ This value was determined by ¹H NMR spectroscopy.

⁶ This value was obtained from the spectrum of the photostationary state (100% **1c**) at the wavelength corresponding to **1c**. The spectrum of the photostationary state was corrected by subtracting the absorbance of the sample (due to the scattering of nanoparticles) at 456 nm before irradiation with 365 nm from the absorbance of the sample at 456 nm after irradiation with 365 nm to the photostationary state.

⁷ Following Beer's law.

⁸ Because at the photostationary state, there are only the ring-closed isomers in the sample, the total number of ring-closed isomers represents the total number of photoswitches.

⁹ The total mass was determined by weighing the mass of dried sample (dried from a 3 mL aqueous sample).

¹⁰ The wt-% of the nanoparticle was determined by TGA.

Optical Spectroscopy of Photoswitches in Solution and Encapsulated in the UCNP

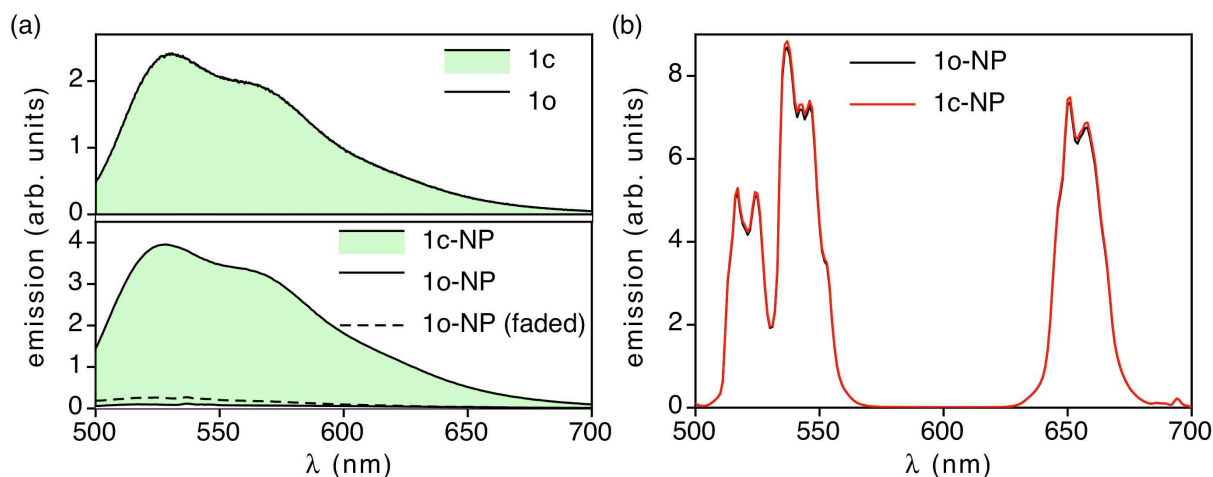


Figure S3. Fluorescent emission spectra of (a) a CH₃CN solution of chromophore **1o** (1.10×10^{-5} M, top spectrum) upon excitation of 488 nm light before irradiation with 365 nm light (black line) and after irradiation with 365 nm light until the photostationary state is reached (black line and green shaded area), and a water dispersion of **1o-NP** (8.09×10^{-7} M,¹¹ bottom spectrum) upon excitation of 488 nm light before irradiation with 365 nm light (black line) and after irradiation with 365 nm light until the photostationary state is reached (black line and green shaded area). The residual emission after exposure to greater than 434 nm light is due to the anti-stokes lines of light shorter than 460 nm, which can induce the cyclization reaction. (b) Fluorescent emission spectra of a water dispersion of **1o-NP** (8.09×10^{-7} M)¹¹ upon excitation of 980 nm light before irradiation with 365 nm light (black line) and after irradiation with 365 nm light until the photostationary state is reached (red line).

Photochromism and Fluorescent Emission of **1o**, UCNP and **1o-NP**

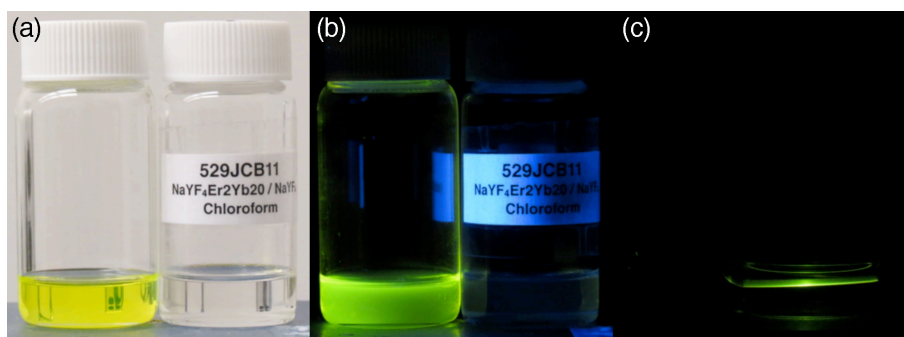


Figure S4. Photographs of vials containing (a) **1c** in CH₃CN (1.10×10^{-5} M, left vial) after irradiating **1o** with 365 nm light until the photostationary state is reached, and oleic acid coated core-shell (NaYF₄:ErYb)NaYF₄ nanoparticles in CHCl₃ (15 mg/mL¹², right vial). (b) Photographs of the same vials upon irradiation with 365 nm light. (c) Photographs of the same vials upon irradiation with 980 nm laser light.

¹¹ This concentrations is total concentration of chromophores encapsulated in the nano-assemblies obtained using the concentration of the ring-closed isomers (**1c** and **1c-NP**) at their photostationary states (calculated by UV-vis spectroscopy after correcting for scattering) divided by percentage of ring-closed isomers in this state (as measured by ¹H NMR spectroscopy).

¹² This value is determined by weighing a dried aliquot amount of the core-shell UCNP.

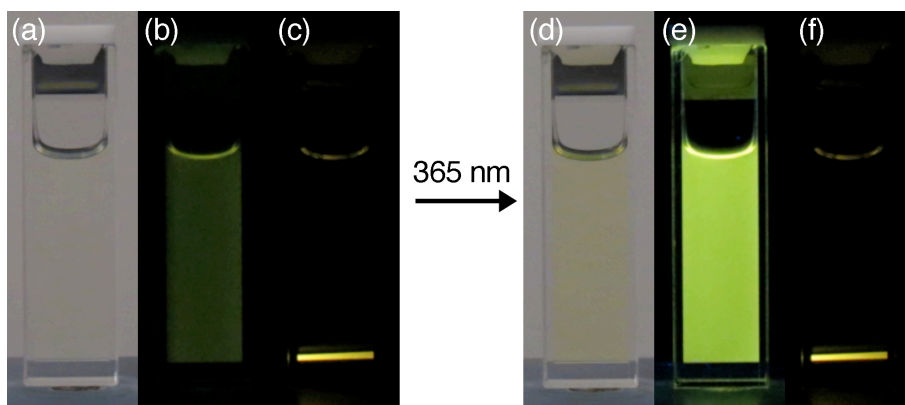


Figure S5. Pictures of cuvettes containing a water dispersion of **1o-NP** before exposure to 365 nm light (a) in white light, (b) in the dark while being exposed briefly to 365 nm light and (c) in the dark while being exposed to 980 nm laser light. Pictures of cuvettes containing a water dispersion of **1o-NP** after long exposure to 365 nm light to trigger the ring-closing reaction of the chromophore (**1o-NP** \rightarrow **1c-NP**) until photostationary state is reached (d) in white light (e) in the dark while being exposed briefly to 365 nm light and (f) in the dark while being exposed to 980 nm laser light.

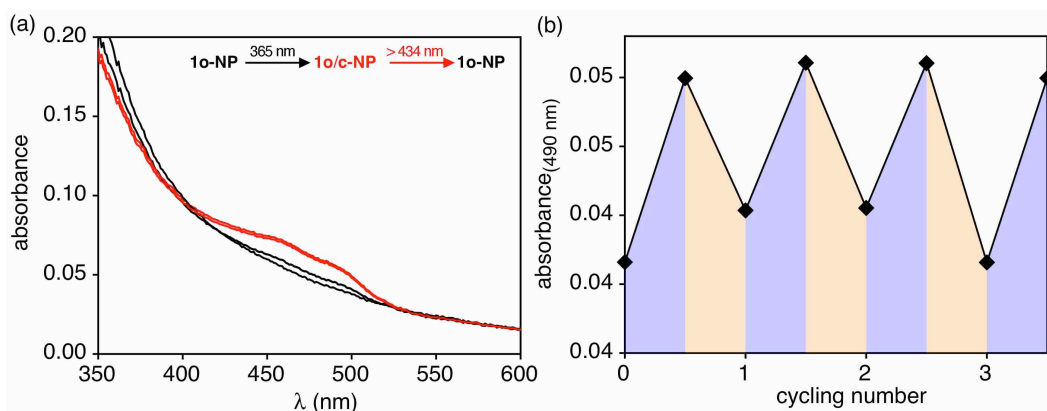


Figure S6. (a) UV-vis absorption spectra of an aqueous dispersion (0.8×10^{-6} M) of the nano-assemblies in their ring-open **1o-NP** (black lines) and ring-closed (red lines) states over four ring-closing and ring-opening cycles using 365 nm light for the former and > 434 nm light for the latter. (b) Changes in the absorption intensities at 490 nm during the four ring-closing and ring-opening cycles.

Biological Experiments

The worm handling methods described by Brenner were used.¹³ *Caenorhabditis elegans* N2 hermaphrodites were incubated on OP50-seeded plates at 20 °C. After 4 days, the nematodes were lightly washed off the plates with 0.05 M NaCl buffer and aliquoted into 1.5 mL eppendorf tubes containing 0.25 mg of hybrid nanoparticles (**1o[NaYF₄:ErYb]**) in H₂O. After a 3 h incubation period, the nematodes were washed in 500 μ L of 0.05 M NaCl buffer and then centrifuged at 500 rpm for 20 seconds. This wash was repeated twice before the nematodes were immobilized using 5 μ L 300mM NaN₃ (in 0.05 M NaCl buffer) per 15 μ L~20 μ L nematodes solution. The immobilized nematodes were eventually mounted on 2% agarose gel and imaged using the two-photon upconversion imaging microscope. Representative images are shown in Figure S6.

¹³ S. Brenner, *Genetics*, 1974, **77**, 71.

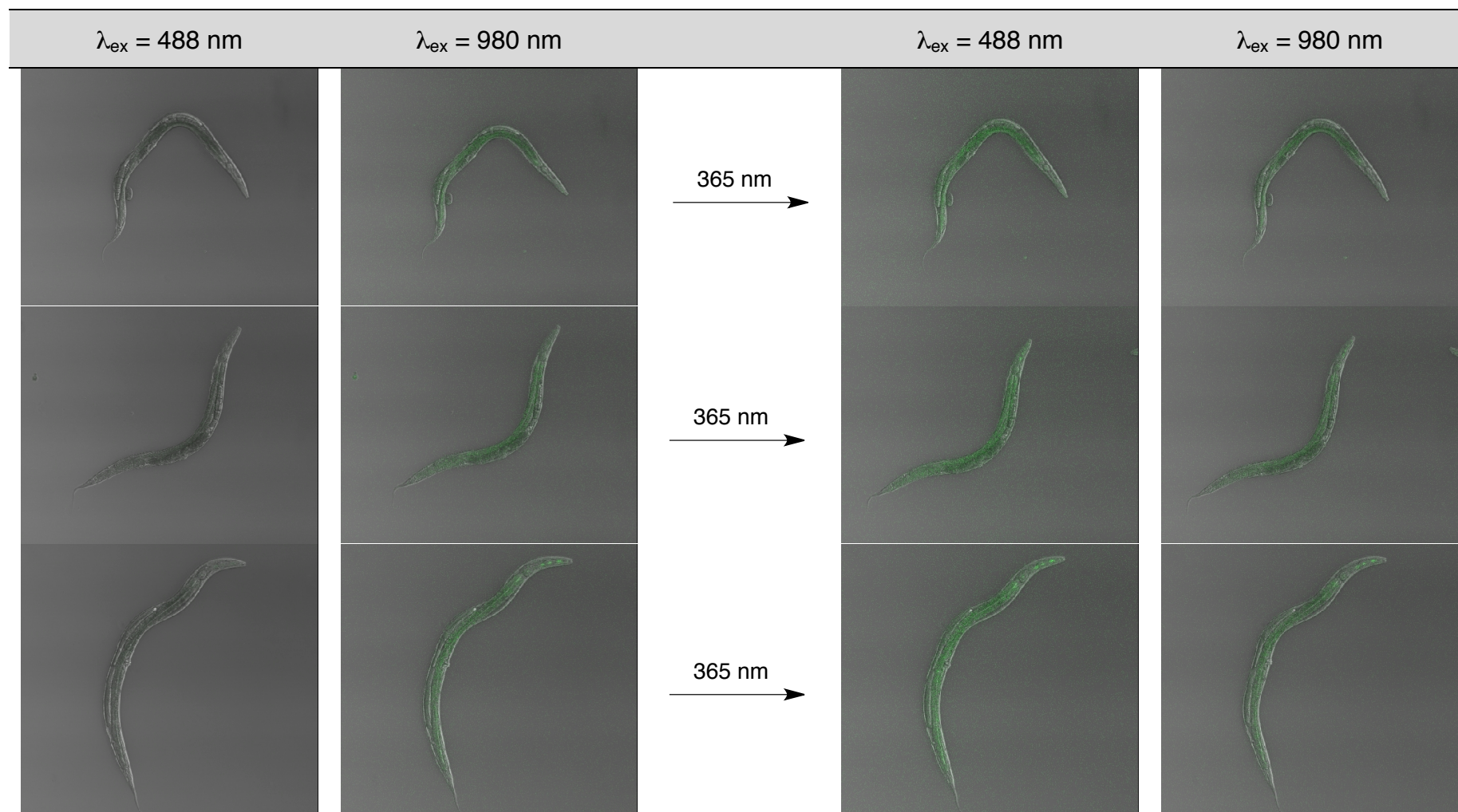


Figure S7. Two-photon upconversion fluorescence microscopy images of wild-type *N2 C. elegans* incubated with **1o-NP** showing the changes in fluorescence due to the photoswitching of the chromophore within the bodies of the worms, while the UCNPs emit in both states.