

Electronic Supplementary Information†

A NIR-Responsive Azobenzene-based Supramolecular Hydrogel Using Upconverting Nanoparticles

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1. Materials and Methods

All reagents were purchased from Sigma Aldrich and used without further purification. ¹H-NMR, FT-IR, UV-Visible, Induced Circular Dichroism, and upconversion emission spectroscopies were performed at Concordia University. MALDI-TOF MS analysis was performed at the Drug Discovery Platform at the McGill University Health Centre. Cryo-ultramicrotoming and TEM analysis was performed at McGill University Facility for Electron Microscopy Research.

All ¹H-NMR and ¹³C-NMR spectra were recorded on a 500 MHz Bruker Scientific NMR spectrometer at 25 °C. All FT-IR spectra were recorded on a Thermo Scientific Nicolet iD5 ATR spectrophotometer. UV-Visible spectroscopy was carried out using a Varian Cary 100 Bio UV-Vis spectrophotometer using a 1 cm path length quartz cuvette. Induced Circular Dichroism spectroscopy was carried out on a JASCO J-815 spectropolarimeter using a 0.2 cm path length quartz cuvette. Zeta Potential was measured on a Zetasizer Nano-S (Malvern Instruments Ltd, Worcestershire, UK). Measurements were performed using a disposable folded capillary cell (Malvern). For each experiment, 10 measurements of 20 runs each were recorded.

2. MALDI-TOF-MS analysis:

The α -Cyano-4-hydroxycinnamic acid (CHCA) matrix solution, used in all experiments, was prepared at saturation in a solvent mixture (water with 0.1% TFA: acetonitrile (ACN), 1:1 v/v). For the sample-matrix crystallization procedure, the aliquot of sample was then mixed with the saturated matrix in a 1:1 ratio (v/v) and 1 μ L of this mixture was directly spotted onto the MALDI target plate. MALDI spectra were acquired on an UltrafleXtreme time-of-flight spectrometer operated in the positive ion, reflectron mode equipped with a 2 KHz repetition rate Smartbeam II laser from Bruker Daltonics. For each spectrum, 1000 shots were accumulated under optimized delayed extraction conditions with a source accelerating voltage of +20kV. A peptide solution standard was used to calibrate the instrument.

3. TEM Analysis of oleate-capped LiYF₄:Tm³⁺/Yb³⁺ UCNPs:

TEM analysis of the oleate-capped LiYF₄:Tm³⁺/Yb³⁺ nanoparticles was performed using a Jeol-JEM-2100F microscope operating at 200 kV equipped with a charge coupled device (CCD) camera (Gatan). Prior to analysis, a 1 wt% sample was dispersed in toluene. A drop of the resulting solution was evaporated on a formvar/carbon film supported on a 3-mm 300-mesh copper grid.

4. Cryo-ultramicrotomy and TEM analysis:

The sample was mounted on an aluminum stub with 2.3 M sucrose in 0.1 M phosphate buffer at 4 °C and frozen in liquid nitrogen. Sectioning was performed with the Leica Microsystems UC7/FC7 cryo-ultramicrotome at -90°C, and the 100-nm ultrathin sections were transferred onto formvar coated 200-mesh Cu TEM grids. Imaging was carried out with a FEI Tecnai G² Spirit BioTwin TEM equipped with a Gatan Ultrascan 4000 CCD camera Model 895 at an accelerating voltage of 120 kV.

5. Emission Spectroscopy:

The upconversion emission spectra of all LiYF₄:Tm³⁺/Yb³⁺ UCNP solutions studied were obtained upon 980 nm excitation, using a Coherent 6-pin 15 fiber-coupled F6 series 980 nm laser diode with a power of 0.460 W, coupled to a 100 μm fiber (core diameter). All studies were carried out in 1 cm path length quartz cuvettes (Thorlabs). UV emission spectra were collected at a right angle with respect to the incident beam using a Spex Minimate 1/4 m monochromator and detected with an Oriel 70680 photomultiplier tube. The PMT signals were processed by a model SR440 Stanford Research Systems preamplifier, and a SR400 Stanford Research Systems gated photon counter was used as an interface between the computer and hardware. The signals were recorded using a Stanford Research Systems SR465 data acquisition/analysis system.

6. Powder X-Ray Diffraction Studies

PXRD was performed using a Bruker D2 Phaser benchtop powder X-ray diffractometer equipped with a LynxEye detector, a copper K-alpha source and a nickel filter.

7. Rheological Studies

Zero-shear viscosity measurements were performed using an Anton-Paar MCR500 rheometer equipped with cone-plate geometry (25 mm diameter) at a gap of 1 mm at 25 °C.

8. Gel-Sol Transition studies under UV Excitation

A UVP LLC. UVGL-58 handheld UV lamp at 365 nm (6W Hg lamp, power density 1.2 mW/cm²) placed approximately 5 cm away from the sample was used to conduct the UV-induced gel-sol transition studies.

9. Gel-Sol Transition studies under NIR Excitation

A 1.3 W 980 nm handheld laser was used with a focusing lens placed at 10 cm distance from the laser output and a chopping blade operated by a Stanford Research Systems Inc. Model SR540 Chopper Controller at a frequency of 4 kHz was placed between the focusing lens and the sample at a distance of 3 inches between each. The measured power of the laser while chopping at 4kHz was 0.7 W.

10. 3-(4-phenylazophenoxy)propanol (AzopropOH) was synthesized according to a procedure described by Liu et al.¹ In summary, 4-phenylazophenol (1.98 g, 10 mmol) was reacted with 3-bromopropanol (1.67 g, 12 mmol) in dry DMF at 75 °C for 6 hours. The crude product was extracted in cold water and chloroform followed by a wash with 1 M HCl and saturated NaCl. The crude product was purified by column chromatography using silica gel and 1:6 v/v EA:DCM mixture as eluent. The product was obtained in 90% yield and analyzed by ¹H-NMR spectroscopy in CDCl₃. **¹H-NMR:** δ= 1.90 (q, 2H), 3.58 (t, 2H), 4.15 (m, 2H), 4.58 (m, 1H), 7.125 (m, 2H), 7.50-7.58 (m, 3H), 7.83-7.89 (m, 4H). Smaller peaks in the aliphatic region may be attributed to different chemical shifts of the propanol group in the *cis*-azopropOH isomer.

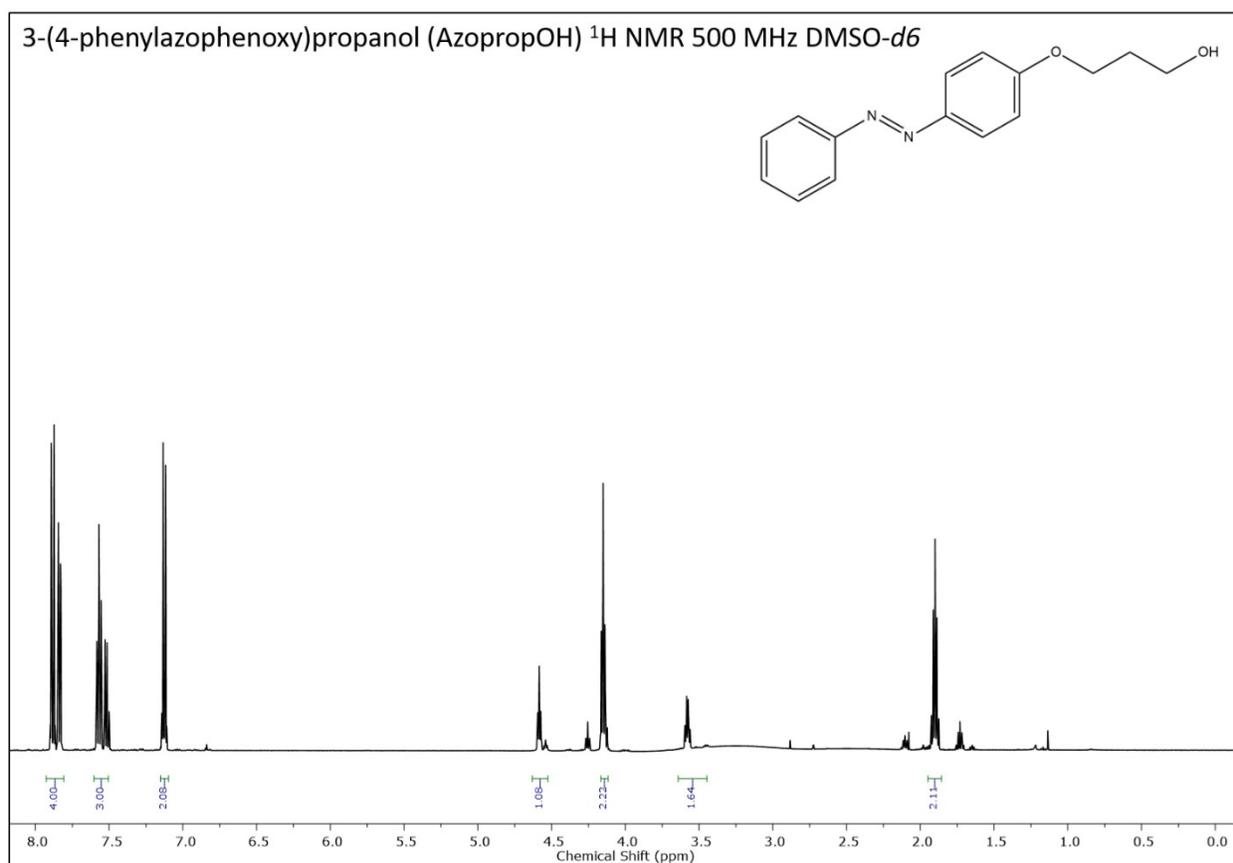


Figure S1. ¹H NMR of AzopropOH in DMSO-*d*₆.

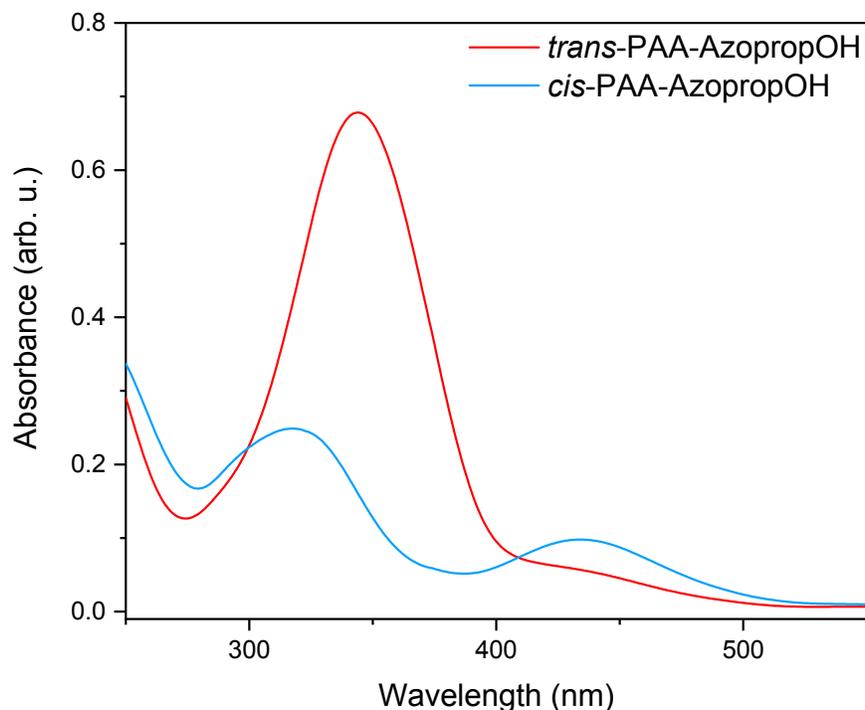


Figure S2. UV-Visible absorption spectra of AzopropOH in 2:1 v/v H₂O:EtOH at 25 °C.

11. PAA-AzopropOH copolymer was synthesized via the Steglich Esterification technique in which Poly(acrylic acid) is reacted with 3-(4-phenylazophenoxy)propanol in the presence of DCC and DMAP to obtain the modified copolymer.² All reactions were performed on a 1 g scale of PAA and performed as follows: 1.00 g of PAA (130,000 M_w) was dissolved in 75 mL anhydrous NMP at 75 °C for 4 hours stirring at 300 rpm, until completely dissolved. The solution was brought to room temperature and 3-(4-phenylazophenoxy)propanol (0.55 g, 2.15 mmol) and DMAP (0.3 g, 2.45 mmol) were added and stirred together for 1 hour. The reaction was then placed on an ice bath and DCC (1.3 g, 6.3 mmol) was added. The reaction was then brought back to room temperature and left to stir for 3 days. Upon completion, the crude azobenzene-modified copolymer was isolated by precipitation in 50/50 v/v EtOAc/Acetone on ice. The precipitate was dried under vacuum for 12 hours and re-dissolved in a minimal quantity of distilled water. Following

dissolution, 50 g of solid NaCl were added to the solution and the mixture was left to stir overnight at 50 °C, after which the pure product precipitates. The precipitate was collected and washed 3x with cold water and then dried under vacuum to obtain the final product in 80% yield. Percentage modification was determined by $^1\text{H-NMR}$ (integration of the methylene protons in the PAA backbone vs. the aromatic protons at 7.13 ppm) and UV-Visible absorption spectroscopy to be 23%, corresponding to an average of 414 out of 1800 repeat units modified with azobenzene. The molecular weight of the polymer was calculated to be 228,000 g/mol based on the 23 % modification. $^1\text{HNMR}$: δ = 1.33-1.85 (m br), 2.06-2.09 (s br), 2.88-3.08(s br), 4.15(s br), 7.13 (m br), 7.50-7.55 (m br), 7.84 (m br). Peaks at 3.33 (s, H₂O), 3.16 (s, MeOH), 2.50 (t, CH₃SO), 2.09 (s, Acetone) are volatile solvent impurities.

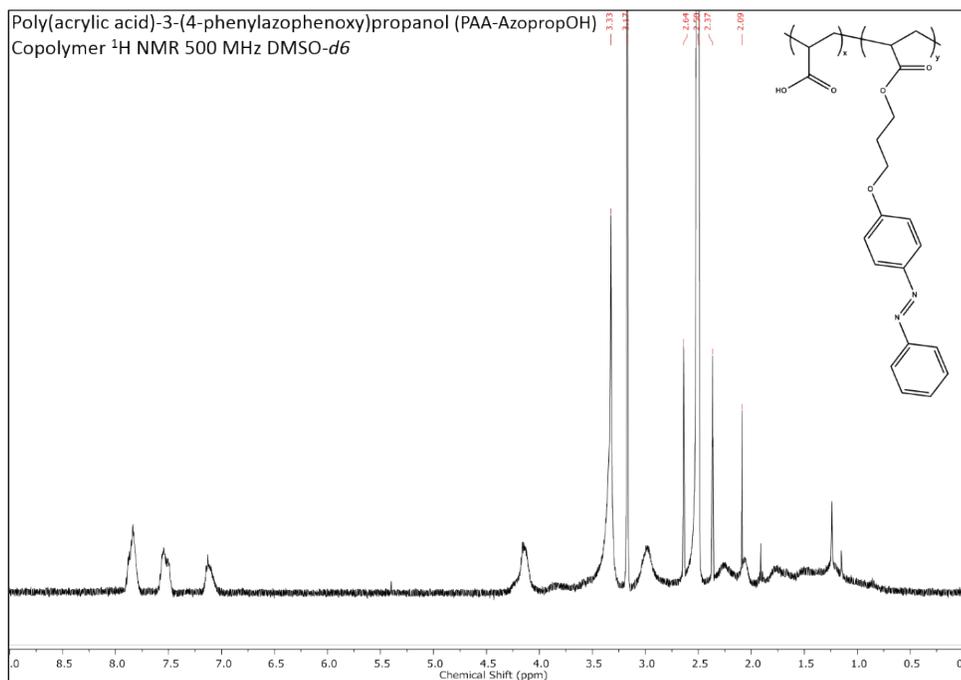


Figure S3. $^1\text{H-NMR}$ of PAA-AzopropOH in DMSO-*d*₆.

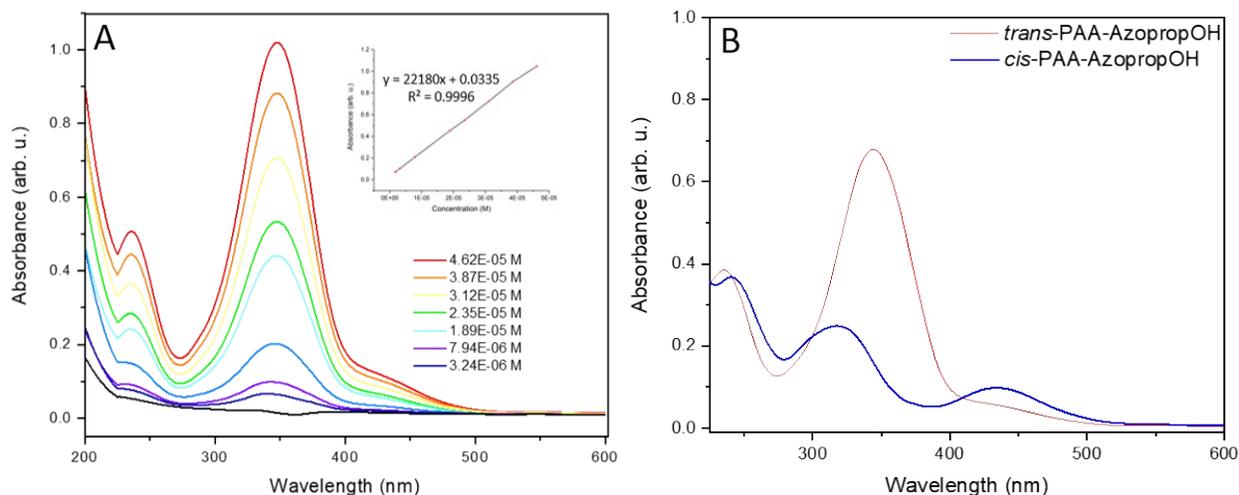


Figure S4. A) Calibration curve of AzopropOH used to determine the % modification of PAA-AzopropOH. B) UV-Vis Absorption spectrum of PAA-AzopropOH used to determine the percent modification (3.8×10^{-5} M by azobenzene units). Calibration curve and PAA-AzopropOH spectra obtained in 2:1 v/v $H_2O:EtOH$ solvent.

12. Synthesis of Oleate-Capped $LiYF_4:Tm^{3+}(0.5 \text{ mol } \%)/Yb^{3+}(25 \text{ mol } \%)$ Upconverting Nanoparticles

The UCNPs are prepared by a two-step thermal decomposition method.³ First, lanthanide trifluoroacetate precursors are prepared by dissolving Tm_2O_3 (0.0024 g, 6.25×10^{-3} mmol), Yb_2O_3 (0.1232 g, 0.313 mmol), and Y_2O_3 (0.2103 g, 0.931 mmol) in a mixture of 1:1 H_2O /trifluoroacetic acid (10 mL) in a 3-neck round bottom flask. The solution is heated at 80 °C under reflux until it becomes clear (approximately 12 hours). The solution is then dried at 60 °C to obtain the final precursor product. Then, in the second step, CF_3COOLi (0.2999 g, 2.5 mmol) is added to the dried lanthanide trifluoroacetate precursors and the mixture was dissolved in a mixture of oleic acid (10 mL, technical grade, 90%) and 1-octadecene (20 mL, technical grade, 90%). The mixture was degassed at 120 °C for 30 minutes and then brought to 315 °C at a rate of 10 °C/min under an argon atmosphere. The reaction was stirred at 315 °C under argon for 1 hour and then cooled to room temperature. To obtain the particles, 99% ethanol was added to the reaction solution to precipitate the nanoparticles, which were then isolated by centrifugation at 3700 rpm for 15 minutes. The

particles were re-dispersed in hexanes and precipitated in ethanol and centrifuged twice more to remove any impurities.

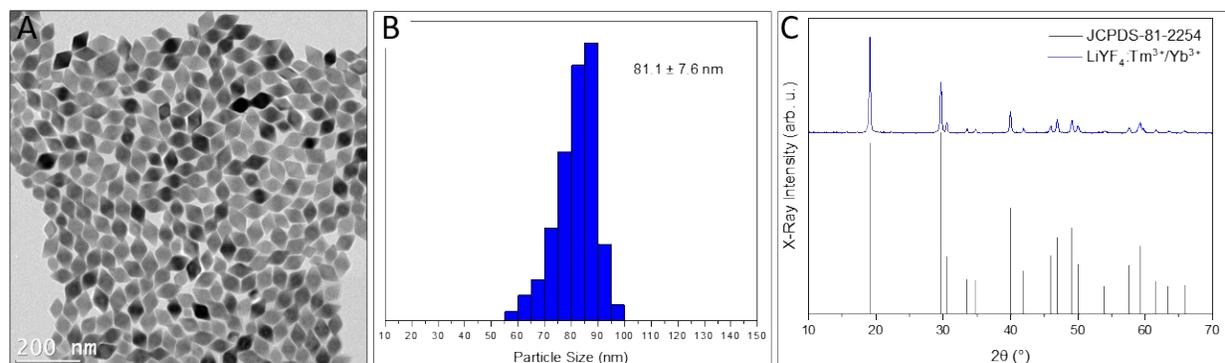


Figure S5. A) TEM image of $\text{LiYF}_4:\text{Tm}^{3+}/\text{Yb}^{3+}$ UCNPs. B) Size distribution of UCNPs based on the measurement of 500 particles. C) PXRD of UCNPs

13. Preparation of Oleate-Free $\text{LiYF}_4:\text{Tm}^{3+}/\text{Yb}^{3+}$ UCNPs

Oleate removal was performed by an acid-base reaction.⁴ Briefly, 50 mg of UCNPs were dispersed in 5 mL of toluene and added to a 10 mL 0.1 M HCl aqueous solution. This mixture was allowed to stir at room temperature for 4 hours, or until all nanoparticles migrated to the aqueous layer, as viewed under 980 nm excitation. The aqueous layer was isolated and centrifuged at 14500 rpm for 30 minutes to collect the oleate free nanoparticles. To confirm the removal of the oleate capping ligand from the nanoparticle surface, FT-IR was used. As shown in the FT-IR spectra, some oleate remains on the surface of the nanoparticles, but the majority was successfully removed, in agreement with the literature.⁴

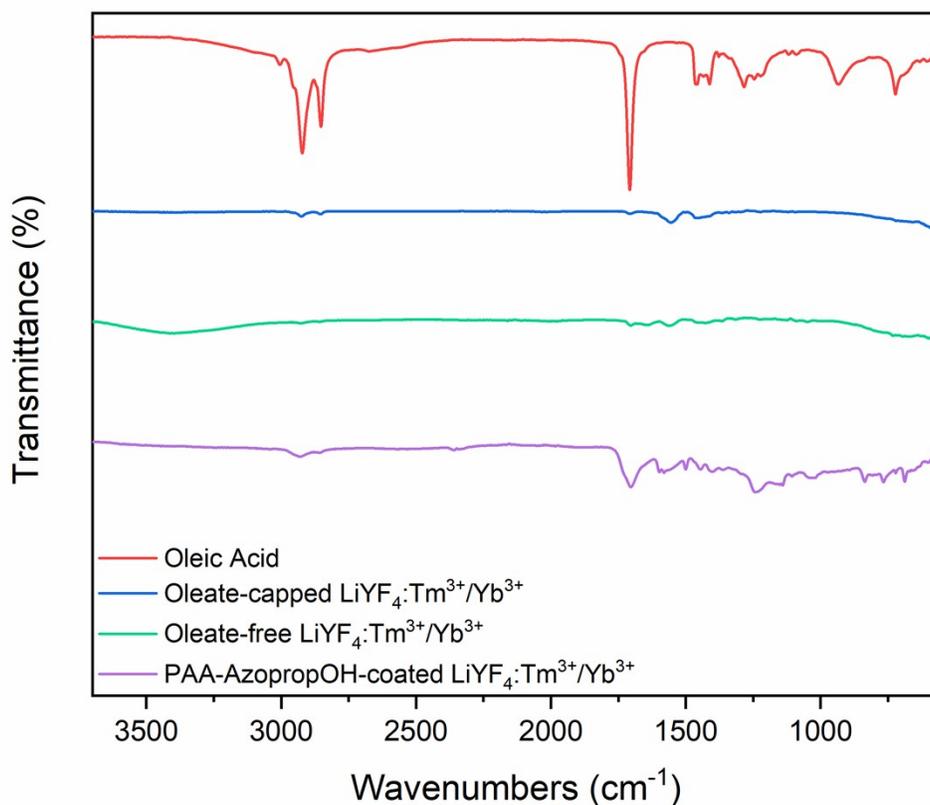


Figure S6. Infrared spectra of (red) oleic acid, (blue) oleate-capped $\text{LiYF}_4:\text{Tm}^{3+}/\text{Yb}^{3+}$ UCNPs, (green) oleate-free UCNPs, (purple) PAA-AzopropOH coated UCNPs

14. Preparation of PAA-AzopropOH copolymer-coated $\text{LiYF}_4:\text{Tm}^{3+}/\text{Yb}^{3+}$ UCNPs and Energy Transfer

Studies

10 mg of oleate-free particles were dispersed in 20 mL of a 20 mg/mL solution of PAA-azopropOH copolymer and stirred at RT overnight. The polymer-coated particles are then collected by centrifugation.

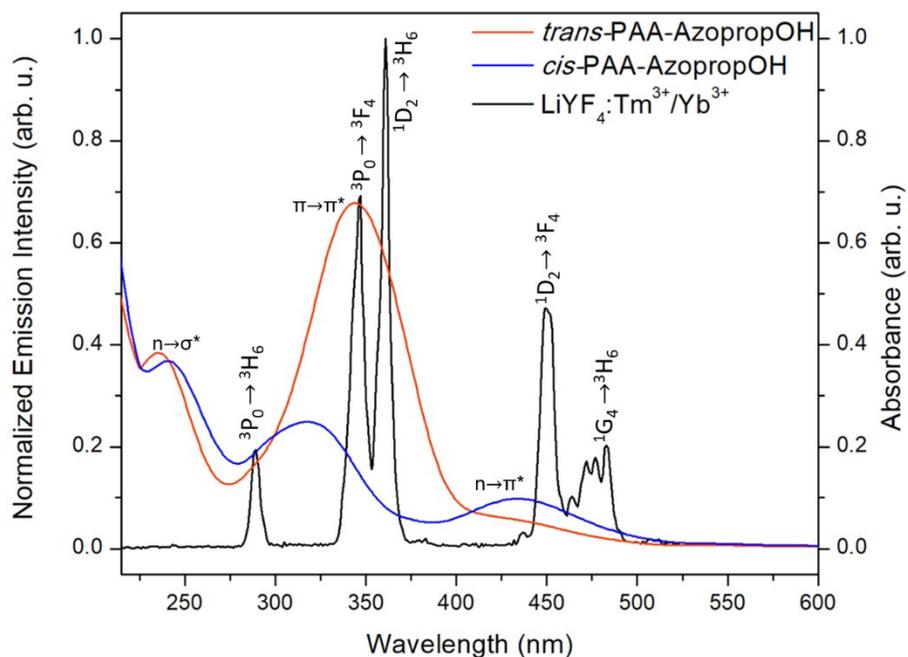


Figure S7. Overlap of absorption spectra of *trans* (orange) and *cis* (blue) PAA-azopropOH (2.90×10^{-5} M by azobenzene units) with the normalized emission spectrum (black) of $\text{LiYF}_4:\text{Tm}^{3+}/\text{Yb}^{3+}$ Upconverting Nanoparticles (1 mg/mL).

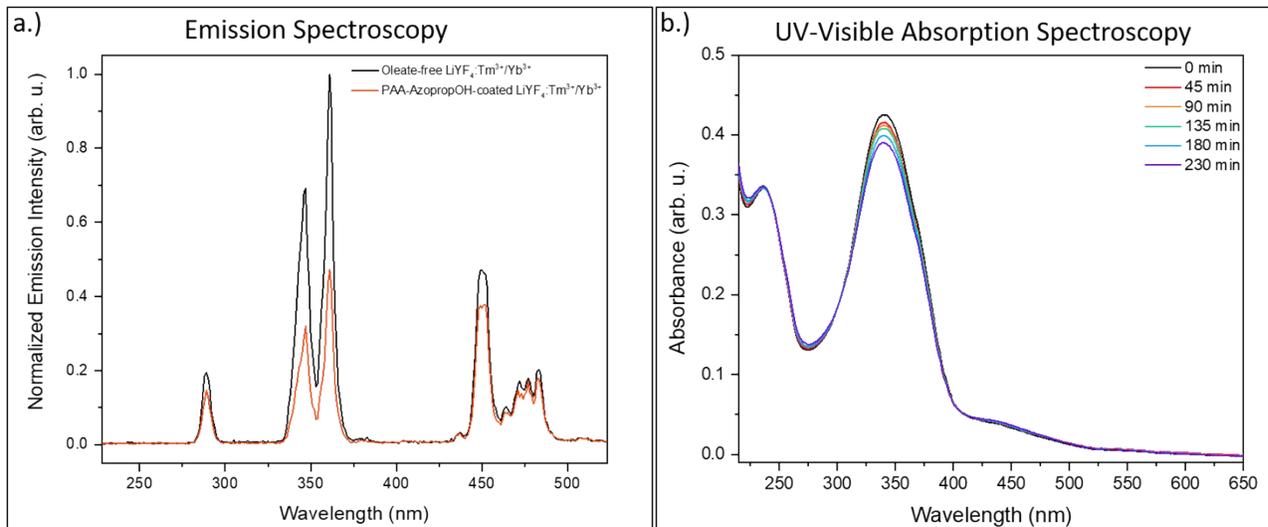


Figure S8. **a.)** Energy Transfer studies carried out with LiYF₄:Tm³⁺/Yb³⁺ (1 mg/mL pH 9 H₂O) with and without the PAA-azopropOH copolymer. **b.)** UV-Vis absorption spectra of LiYF₄:Tm³⁺/Yb³⁺ coated with the PAA-azopropOH copolymer (1mg/mL pH 9 H₂O)

15. Synthesis of Mono-(6-O-(p-tolylsulfonyl))-β-Cyclodextrin (BCDTos) was performed according to the procedure published by Yasen *et al.* with some modifications.⁵ β-Cyclodextrin (2 g, 1.76 mmol) was dissolved in 100 mL aqueous 0.4 M NaOH solution at 0 °C. *p*-toluenesulfonyl chloride (0.34 g, 1.76 mmol) is added slowly over the course of 1 hour keeping the solution temperature below 5 °C and stirring at 700 rpm. After the addition is completed, the reaction is left to stir on ice for 30 minutes and then vacuum filtered to remove unreacted TsCl. The filtrate is then brought to pH 8.5 with 1 M HCl solution and stirred at room temperature for another hour. The resulting precipitate is then vacuum filtered and washed 3x with cold distilled water and dried under vacuum for 12 hours. 0.39 g of product was obtained in a 17.5% yield. Mono-substitution of β-Cyclodextrin was confirmed by MALDI-MS, LC-MS analysis and ¹H-NMR spectroscopy. **¹H-NMR BCDTos:** 2.42 (s, 3H), 3.15-3.73 (m, 46H), 4.14-3.33 (m, 8H), 4.75-4.83 (m, 7H), 5.70 (s, 13H), 7.43

(d, 2H), 7.75 (m, 2H) $^{13}\text{C-NMR}$: 21.65, 60.36, 70.16, 72.32, 73.51, 81.95, 102.38, 128.02, 130.33, 133.12, 145.25 **MALDI-MS (m/z)**: 1165 (β -CD), 1311 (M+Na), 1327 (M+K), 1465 (ditosylated- β -CD)

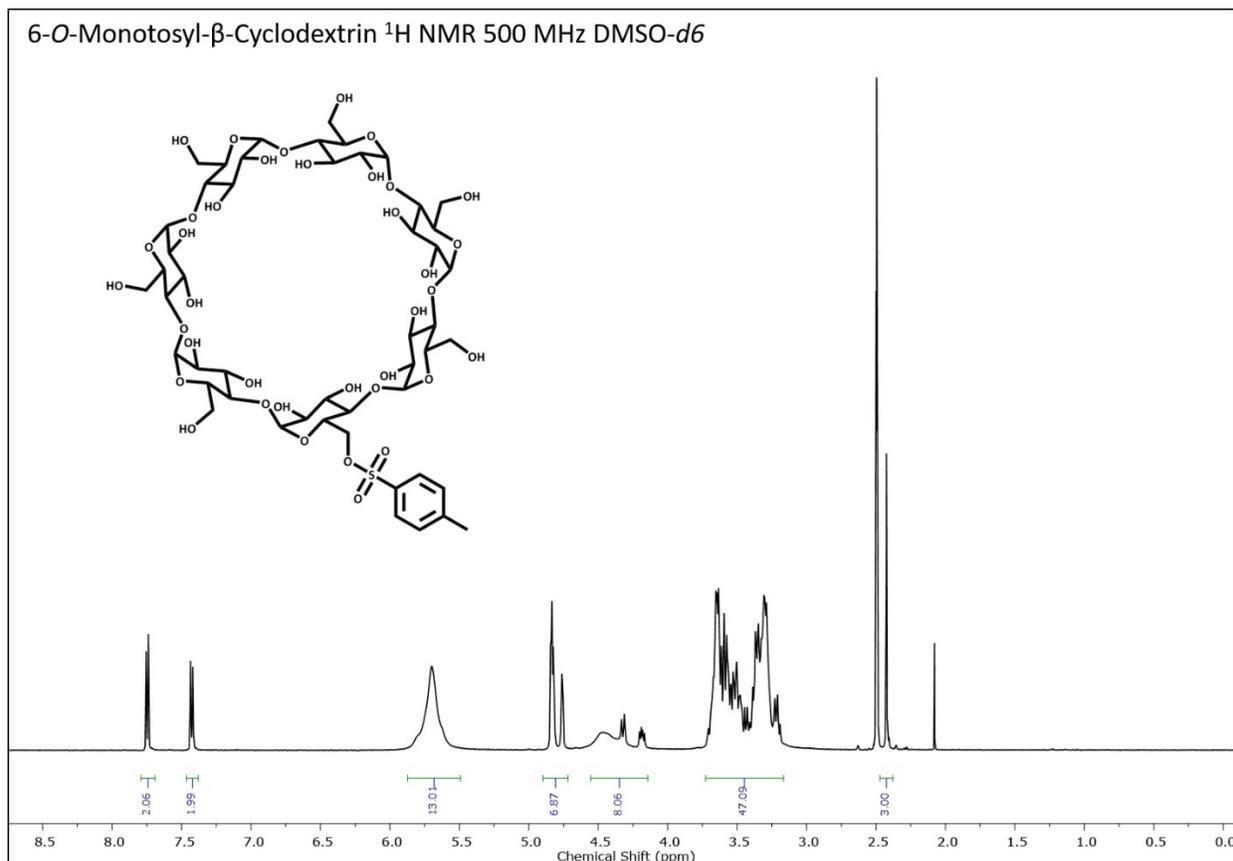


Figure S9. $^1\text{H-NMR}$ of BCDTos in DMSO- d_6 .

16. Mono(6-triethylenetetramino-6-deoxy)- β -Cyclodextrin (BCDTrien) was prepared according to the procedure outlined by Ren *et al.* with a 74 % yield of product.⁶ $^1\text{H-NMR}$: 1.02 (s, 1.22H), 2.36-2.76 (m, 12 H), 3.11-3.77 (m, 48H), 4.82 (m, 6H) **MALDI-MS (m/z)**: 1263 (M+H), 1285 (M+Na), 1301 (M+K)

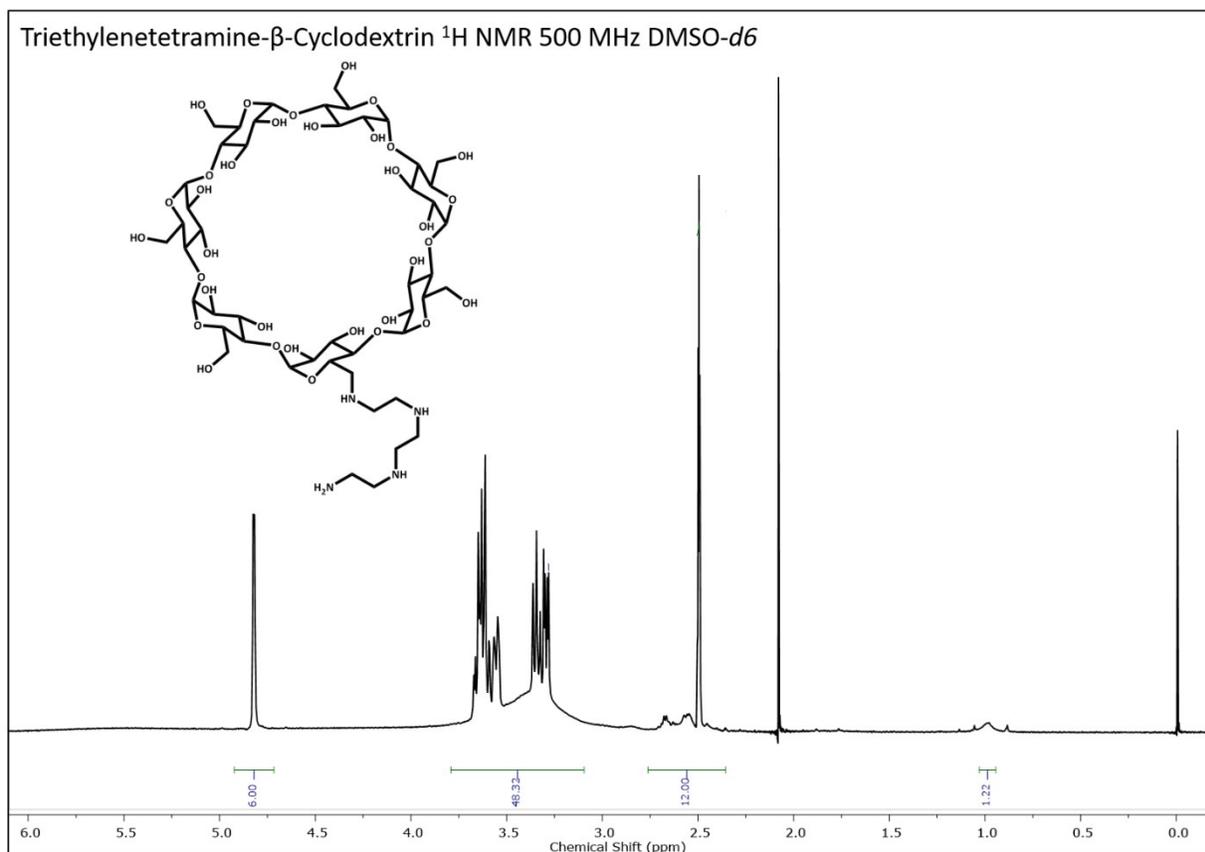


Figure S10. ^1H -NMR of BCDtrien in DMSO- d_6 .

17. Deoxycholate- β -Cyclodextrin (deoxyBCD) was prepared via EDC/NHS coupling of mono(6-triethylenetetramino-6-deoxy)- β -Cyclodextrin with deoxycholic acid. Deoxycholic acid (66 mg, 0.1675 mmol), EDC·HCl (96 mg, 0.51 mmol) and NHS (116 mg, 1.01 mmol) were dissolved in 5 mL anhydrous DMF and left to stir at room temperature (700 rpm, 25 °C) for 4 hours. Separately, mono(6-triethylenetetramino-6-deoxy)- β -Cyclodextrin (211 mg, 0.17 mmol) was dissolved in 2 mL anhydrous DMF. The deoxycholic acid-containing mixture was placed on ice and once the solution reached a temperature below 5 °C, the mono(6-triethylenetetramino-6-deoxy)- β -cyclodextrin solution was added dropwise to the reaction mixture. The resulting solution was left to stir overnight at room temperature. The product was crashed out in acetone (50 mL) and centrifuged

(4000 rpm, 15 minutes) to obtain the crude product. The product was isolated by washing 3x with acetone, vacuum drying between each wash step. The final product was obtained in a 63.7% yield (175 mg). **¹H-NMR deoxyBCD:** 0.84-2.22 (m, 35H) 2.54-2.89 (m, 12H), 3.24-3.75 (m, 44H) 4.83 (m, 7H), 5.57-5.84 (m, 12H) **MALDI-MS (m/z):** 1285 (β -CDtrien), 1638 (M+H), 1735 (impurity)

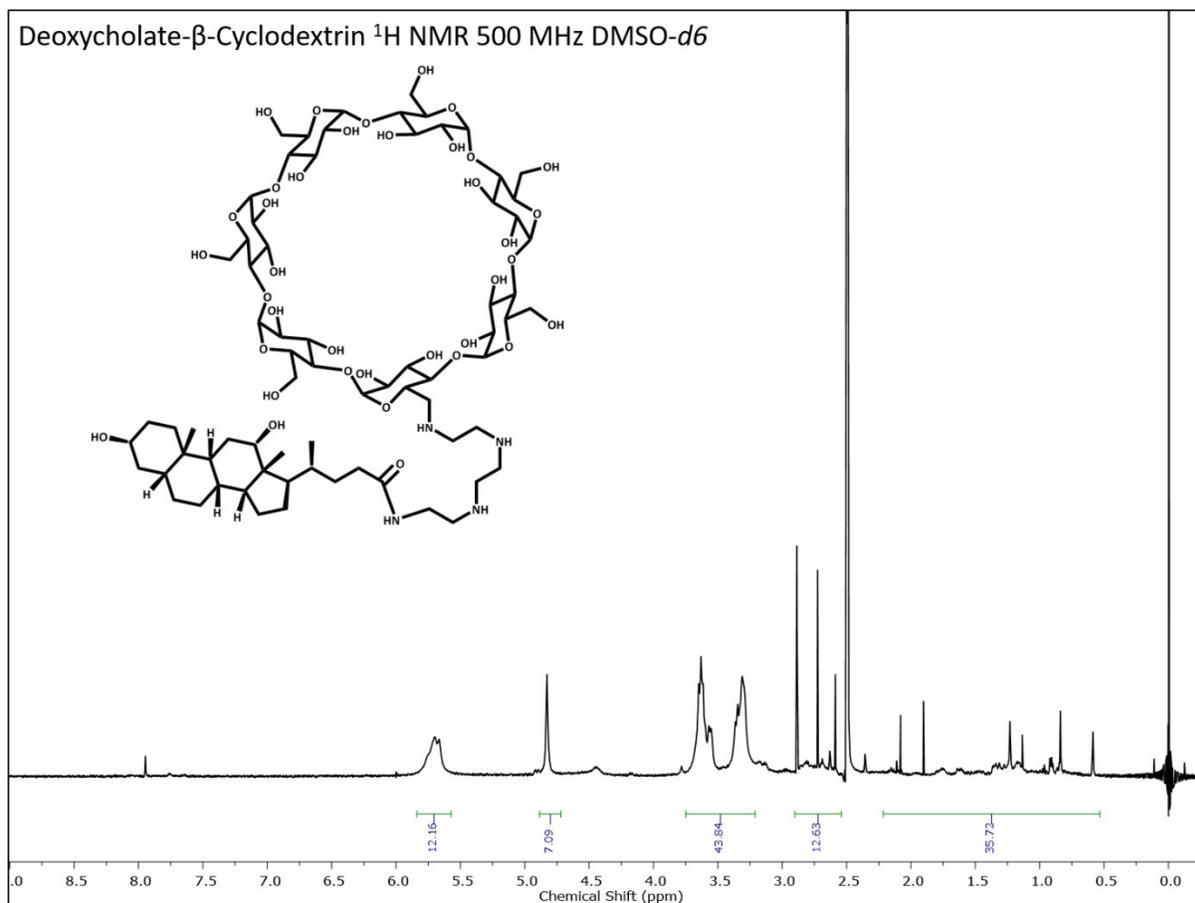


Figure S11. ¹H-NMR of deoxyBCD in DMSO-*d*6.

18. Synthesis of the PAA-AzopropOH-Deoxycholate- β -Cyclodextrin Hydrogel

The gel was prepared and described according to the protocol outlined by Zhao *et al.*⁷ A typical synthesis is as follows: Upon weighing a sample of the PAA-azopropOH copolymer, the number of azobenzene molecules present in a given mass of polymer was determined assuming the 23% modification that as

measured by $^1\text{H-NMR}$ and UV-Vis spectroscopy. The mass of deoxycholate- β -Cyclodextrin was then calculated to give a 1:1.5 ratio of azobenzene molecules to deoxyBCD molecules. The PAA-azopropOH copolymer was dissolved in 500 μL deionized water adjusted to pH 8, and let to stir at 70°C until fully dissolved. Separately, deoxyBCD was dissolved in 100 μL deionized water at pH 8. The deoxyBCD solution was then added dropwise to the copolymer solution and stirred for a few seconds to allow for complete incorporation. The solution thickens immediately upon addition of deoxyBCD, and is then allowed to sit without stirring for 1 hour at 70°C. During this time, the mixture is concentrated to approximately $3/4^{\text{th}}$ of the original volume. The resulting mixture is then placed in the refrigerator for an additional hour, allowing for the gel to set. Upon setting, the gel is stable at room temperature, and the excess water is removed by gently patting the gel with a filter paper. This works in various scales, from 5mg to 30 mg of copolymer, but has not been tested at scales larger than 30 mg. To incorporate the Ln-UCNPs into the hydrogel matrix, a pre-formed hydrogel is freeze-dried and re-hydrated with a 3 mg/mL aqueous solution of PAA-azopropOH coated $\text{LiYF}_4:\text{Tm}^{3+}/\text{Yb}^{3+}$ UCNPs. The gel is re-hydrated to give a gel matrix concentration of 0.4 mg/mL.

19. Binding Constant Determination – Benesi Hildebrand Plot

The binding constant experiment was performed under the following conditions: the AzopropOH copolymer was analyzed at a constant concentration (2.0×10^{-4} M accounted for by azobenzene units) and varying amounts of β -Cyclodextrin ($0 - 3.5 \times 10^{-4}$ M) in pH 9 phosphate-buffered aqueous solution at 25°C

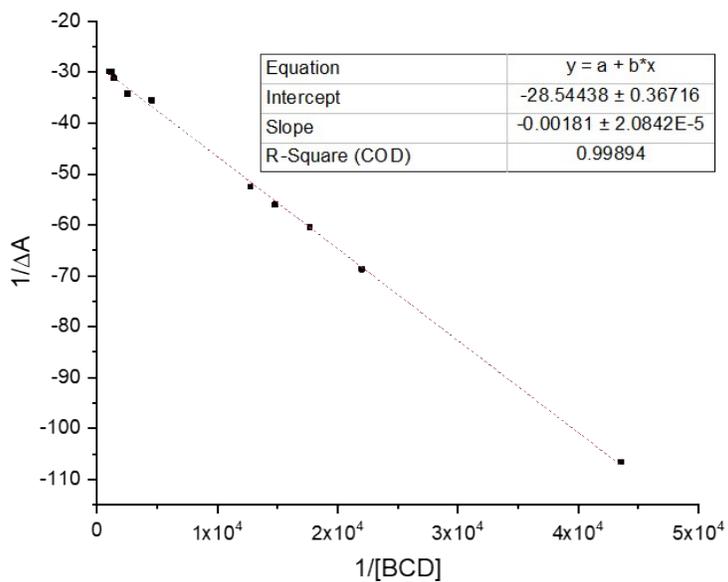


Figure S12. Benesi-Hildebrand plot of $1/[\text{BCD}]$ vs $1/\Delta A$.

20. Gel-Sol Transition Under Direct UV Excitation at 365 nm

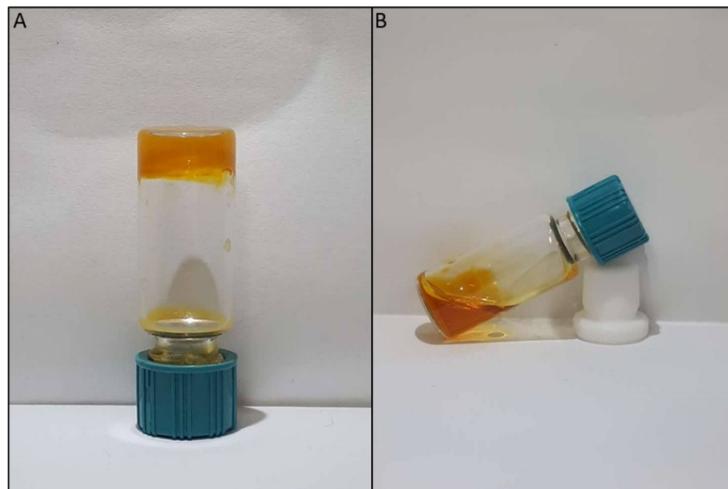


Figure S13. A) A sample of the PAA-azopropOH hydrogel in gel form. B) The hydrogel in sol form after 365 nm irradiation for 25 minutes.

21. Rheometry of PAA-AzopropOH-Deoxy- β -Cyclodextrin Hydrogel with UCNPs Embedded in

Matrix

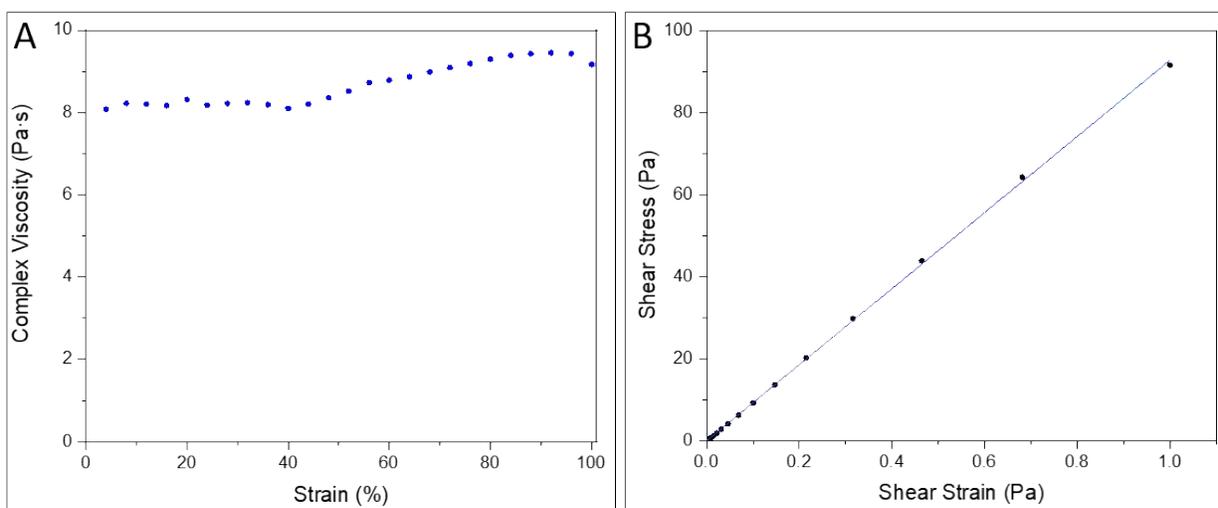


Figure S14. A) Viscosity vs. Strain % graph. B) Shear strain vs. Shear Stress graph.

22. TEM Images of the hydrogel sliced by Cryo-Ultramicrotomy

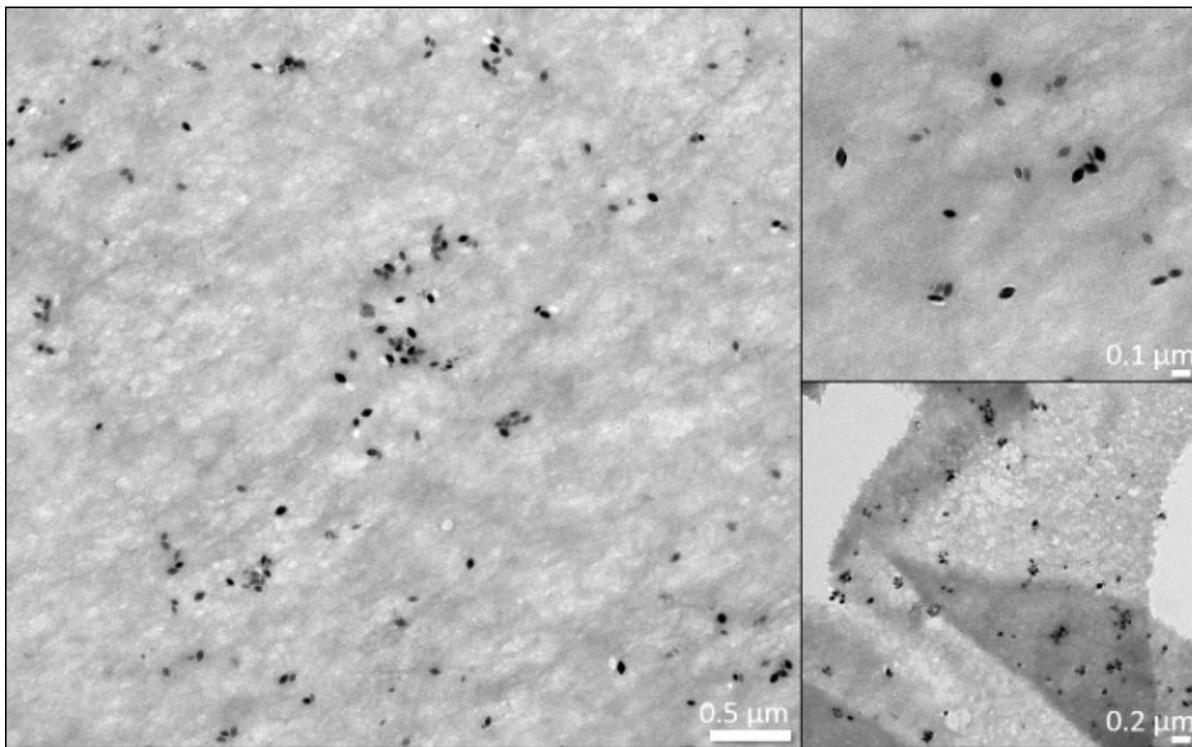


Figure S15. TEM images of cryo-ultramicrotomed slices (100 nm thick) of the hydrogel matrix. The $\text{LiYF}_4:\text{Tm}^{3+}/\text{Yb}^{3+}$ upconverting nanoparticles are visible throughout the hydrogel matrix and do not display significant aggregation.

23. Before and After 980 nm Irradiation of the PAA-AzopropOH-Deoxy- β -Cyclodextrin Hydrogel

with no Ln-UCNPs

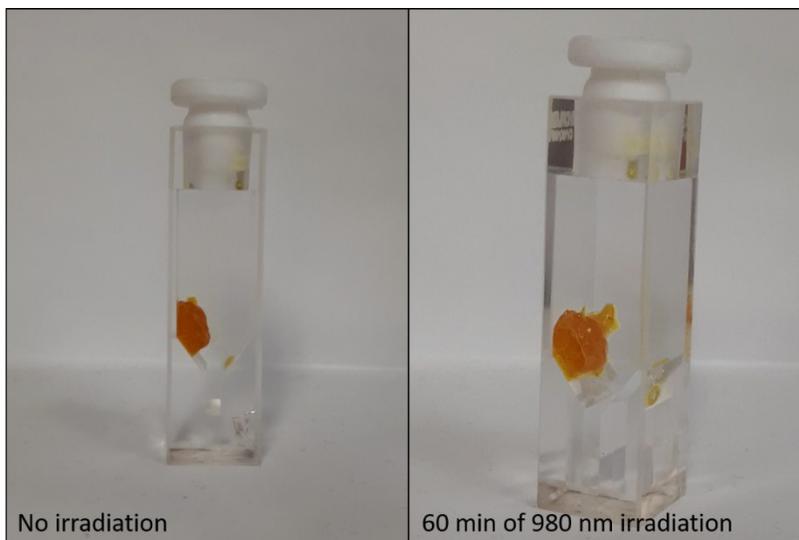


Figure S16. Before (left) and after (right) a gel sample containing no Ln-UCNPs was irradiated with 980 nm light at a power of 0.7 W for 60 minutes while chopping the laser at 4 kHz. This experiment was performed to confirm that the NIR irradiation was not responsible for the gel-sol transition. To ensure the NIR light was passing through the sample, a detector card was placed before and after the sample to align the gel with the laser beam.

References

1. Y. Liu, C. Yu, H. Jin, B. Jiang, X. Zhu, Y. Zhou, Z. Lu, D. Yan, *J. Am. Chem. Soc.*, 2013, **135**, 4765-4770.
2. B. Neises, W. Steglich, *Angew. Chem. Int. Ed.*, 1978, **17**, 522-524.
3. V. Mahalingam, F. Vetrone, R. Naccache, A. Speghini, J. A. Capobianco, *Adv. Mater.*, 2009, **21**, 4025-4028.
4. N. Bogdan, F. Vetrone, G. A. Ozin, J. A. Capobianco, *Nano Lett.*, 2011, **11**, 835-840.
5. W. Yasen, R. Dong, L. Zhou, J. Wu, C. Cao, A. Aini, X. Zhu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 9006-9014.
6. Y. Ren, B. Yang, X. Liao, *RSC Advances*, 2016, **6**, 22034-22042.
7. Y. L. Zhao, J. F. Stoddart, *Langmuir*, 2009, **25**, 8442-8446.