Electronic Supplementary Information (ESI)

Near-infrared responsive three-component supramolecular hydrogels of peptide, agarose and upconversion nanoparticles

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Materials

All chemicals used in this study were purchased from commercial suppliers and used without further purification. Chemicals were obtained from Acros Organics (Waltham, Massachusetts, USA), Aldrich (St. Louis, Missouri, USA), Alfa Aesar (Massachusetts, USA), Iris Biotech (Marktredwitz, Germany), Fluorochem (Hadfield, UK), Fluka (St. Louis, Missouri, USA), Merck (Darmstadt, Germany), and TCI (Tokyo, Japan).

UV/Vis Spectroscopy

UV/Vis absorption spectra were recorded using a Jasco V-770 spectrophotometer (Jasco Deutschland GmbH, Pfungstadt, Germany) equipped with High Precision SUPRASIL quartz glass cuvettes (Hellma Analytics GmbH, Müllheim, Germany). Spectra were measured with the Spectra Manager 2 software, version 2.14.06 (Jasco Deutschland GmbH, Pfungstadt, Germany). Samples were dissolved in 20 mM HEPES buffer (pH = 8.01), and the baseline was corrected against the same solvent. Data analysis was performed using OriginPro 2018b (version b9.5.5.409, ORIGINLAB Corporation, Northampton, USA).

Gel preparation

The preparation of the hydrogels followed a standard operation procedure. First, the required amounts of AAP-FGDS (3 wt%), agarose (1.5 wt%), and UCNP (0.1 wt%) were accurately weighed using an analytical balance. These components were transferred to a clean container and dissolved in deionized water with gentle stirring until visibly homogeneous. The pH of the resulting solution was then adjusted to pH = 11 by the dropwise addition of a KOH solution. The pH was monitored continuously using a calibrated pH meter to achieve precise control over the alkalinity of the system.

To ensure complete dissolution of all components and to eliminate any potential aggregates, the solution was subjected to a sonication step. This process was performed using a bath sonicator for 10 min, resulting in a clear and uniform mixture.

Following sonication, the solution was transferred into a heat-resistant container and placed in an oven preheated to 90°C. The solution was heated for 5 min, which facilitated the uniform distribution of the gel matrix components. Upon removal from the oven, the container was left to cool to room temperature under ambient conditions. This cooling step allowed the hydrogel to solidify and stabilize, resulting in a homogeneous gel.

Rheological Measurements

Rheological experiments were conducted using an Anton Paar Modular Compact Rheometer MCR 102 (Anton Paar GmbH, Graz, Austria) with data collection and analysis performed using RheoCompass V1.20.40.496 (Anton Paar GmbH, Graz, Austria) and OriginPro 2018b (version b9.5.5.409, ORIGINLAB Corporation, Northampton, USA).

Hydrogel samples (250 µL each) were prepared individually and allowed to gel overnight in 0.5 mL Eppendorf tubes. For measurements, the material was transferred from an Eppendorf tube to the rheometer. After positioning, the sample was equilibrated for 10 min prior to measurement.

Frequency sweep measurements were performed using a P-PTD200 measuring cell and a CP25-2 spindle (25 mm plate diameter, 2° cone angle). The measuring gap was set to 0.06 mm, with a shear strain of 1%. Measurements were conducted at 20 °C, with the frequency range scanned from 1 Hz to 100 Hz.

Synthesis

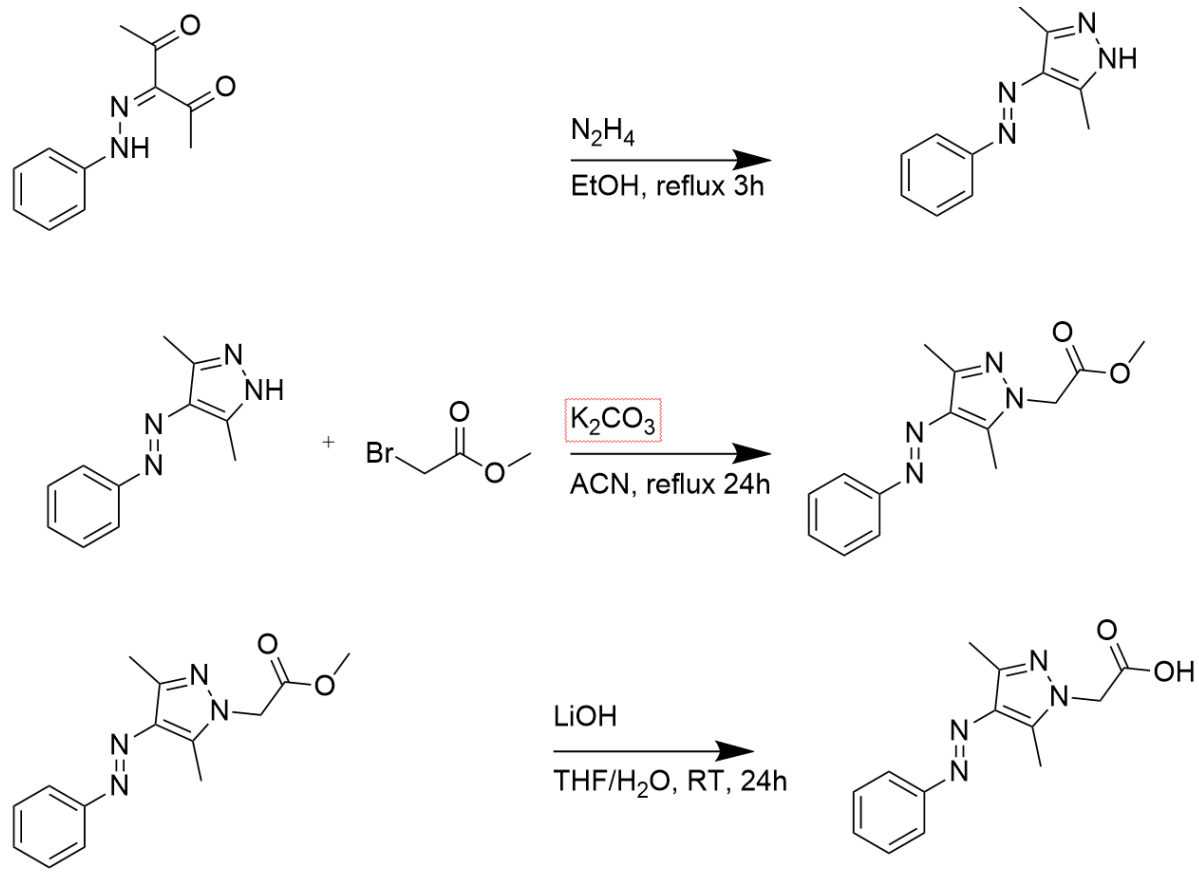
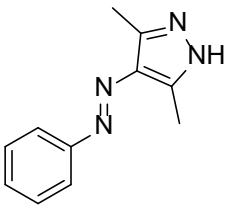


Figure S1: *Schematic representation of the synthesis of AAP-FGDS. 3-(2-phenylhydrazono)pentane-2,4-dione is converted to 3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazole through reaction with hydrazine hydrate under reflux in ethanol. The resulting pyrazole derivative is then alkylated with methyl bromoacetate in acetonitrile under reflux conditions to yield the ester intermediate. Finally, hydrolysis of the ester group using LiOH in a THF/water mixture at room temperature results in the formation of the AAP-FGDS carboxylic acid derivative.The synthesis of AAP-FGDS was performed as described previously.*[1]

Synthesis of 3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazole (AAP)



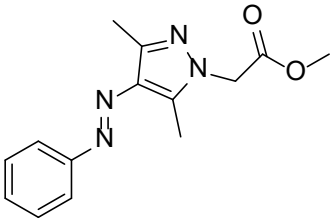
To synthesize 3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazole, 3-(2-phenylhydrazono)pentane-2,4-dione (5.04 g, 24.68 mmol, 1 eq.) was combined with hydrazine hydrate (790 mg, 24.68 mmol, 0,77 mL, 1 eq.) in 200 mL of ethanol (EtOH). The mixture was refluxed for 3 h, resulting

in an orange solution. After the reaction, the solution was concentrated under reduced pressure. The product, 3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazole, was obtained as a yellow solid with no further purification, yielding 4.78 g (23.87 mmol, 97%). The identity of the product was confirmed by 1H-NMR, 13C-NMR, and ESI-MS.

1 H NMR: (400 MHz, $CDCl_3$) δ [ppm]= 10.20 (s, 1H, -NH-), 7.87 – 7.74 (m, 2H, o-Ph H), 7.52 –7.44 (m, 2H, m-Ph H), 7.43 – 7.36 (m, 1H, p-Ph H), 2.64 (s, 6H, $-CH_3$).

ESI-MS: [m/z]: found: 201.1146 [M+H] +, calculated: 201.1135

Synthesis of methyl (E)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate



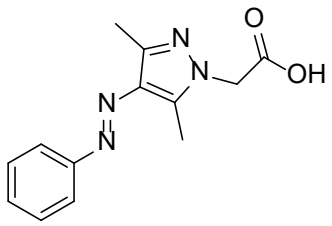
For the synthesis of methyl (E)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate, 4.78 g (23.87 mmol, 1 eq.) of AAP, 9.90 g (71.61 mmol, 3 eq.) of potassium carbonate (K_2CO_3), and 4.75 g (31.03 mmol, 1.3 eq.) of methyl 2-bromoacetate were dissolved in 100 mL of acetonitrile (ACN). The reaction mixture was refluxed for 2 h, then allowed to cool to room temperature and stirred overnight. The resulting orange suspension was concentrated under reduced pressure, and the residue was re-dissolved in a 1:1 mixture of ethyl acetate (EtOAc) and water (300 mL). After phase separation, the aqueous layer was extracted with EtOAc (2 x 70 mL). The combined organic layers were dried over magnesium sulfate ($MgSO_4$), and the solvent was removed under vacuum. The resulting residue was purified by column chromatography using a DCM/MeOH (98:2) eluent, with an Rf value of 0.8. The final product was isolated as an orange oil with a yield of 4.58 g (23.87mmol, 70%).

1 H NMR: (400 MHz, $CDCl_3$) δ [ppm]= 7.83 – 7.76 (m, 2H), 7.46 (t, J = 8.2, 7.7, 1.8 Hz, 2H), 7.41

– 7.35 (m, 1H), 4.85 (s, 2H), 3.79 (s, 3H), 2.57 (s, 3H), 2.51 (s, 3H).

ESI-MS: [m/z]: found: 295.1189 [M+Na] +, calculated: 295.1165

Synthesis of (E)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetic acid (AAP-carboxylic acid)



To synthesize (E)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetic acid, methyl (E)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate (4.78 g, 17.77mmol, 1 eq.) was dissolved in a 4:1 mixture of tetrahydrofuran (THF) and water (250 mL). Lithium hydroxide (LiOH, 630.54 mg, 26.33mmol, 1.5 eq.) was added in one portion. The mixture was stirred overnight at room temperature. After the reaction, the solvent was removed under reduced pressure, and the residue was re-dissolved in water (400 mL). The solution was then acidified to approximately pH 2 using concentrated hydrochloric acid (HCl). Extraction with dichloromethane (DCM, 3 x 150 mL) was performed to isolate the organic layer. The combined organic extracts were dried over magnesium sulfate ($MgSO_4$), and the solvent was evaporated under reduced pressure. The title product was obtained as a yellow solid with a yield of 3.1 g (12 mmol, 68%).

1 H NMR: (400 MHz, DMSO-d6) δ [ppm]= 13.27 (s, 1H), 7.77 – 7.71 (m, 2H), 7.55 – 7.49 (m, 2H), 7.47 – 7.41 (m, 1H), 4.96 (s, 2H), 2.52 (s, 3H), 2.38 (s, 3H).

ESI-MS: [m/z]: found: 257.1045 [M-H]- , calculated: 257.1044

Solid phase peptide synthesis (SPPS)

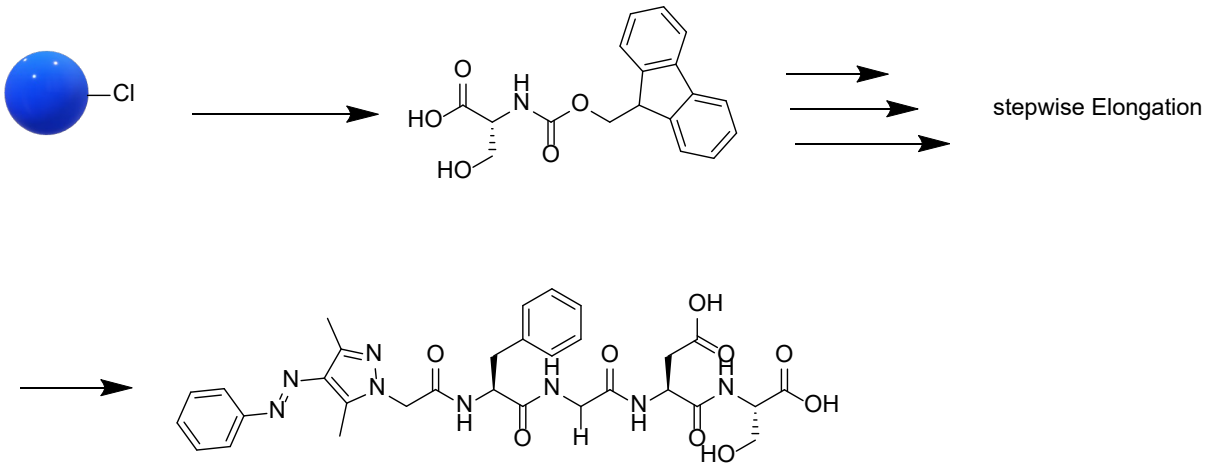


Figure S2: *Schematic representation of the peptide synthesis on 2-chlorotrityl resin. The Fmoc-protected amino acid is coupled to the resin, followed by stepwise elongation of the peptide chain through successive addition of Fmoc-protected amino acids and AAP-carboxylic acid. The final peptide is obtained after cleavage from the resin and purification steps.*[2]

The synthesis of the peptide begins with the loading of the resin. The first Fmoc-protected amino acid, such as Fmoc-Serine(tBu), Fmoc-Ser*-AAP, or Fmoc-Ser*-Ad, is dissolved in 20 mL of dry DCM under an argon atmosphere (1.5 equivalents relative to the resin loading). This solution is added to the 2-chlorotrityl resin (1.6 mmol/g), followed by the addition of DIPEA (2 equivalents). The mixture is agitated for 5 min using an argon stream to facilitate coupling. To ensure complete activation, an additional 3 equivalents of DIPEA are added, and the resin is further agitated for 2 h under argon. Afterward, methanol (1 mL per gram of resin) is added to quench any remaining active resin functionalities, and the suspension is stirred for an additional 15 min. The resin is filtered and sequentially washed with DCM (3 × 20 mL), DMF (3 × 20 mL), DCM (3 × 20 mL), and methanol (3 × 20 mL). Finally, the resin is dried under vacuum, and the loading ratio is determined by measuring the weight increase.

Next, the stepwise elongation of the peptide chain is carried out. The dried resin is pre-swollen by shaking in DMF (20 mL) for 5 min and subsequently washed with DMF (2 × 20 mL). The Fmoc group is removed by treating the resin with 20 mL of 20% piperidine solution in DMF and shaking for 5 min. After removing the solution, a fresh portion of 20% piperidine solution in DMF (20 mL) is added, and the suspension is shaken for 20 min to ensure complete deprotection. The resin is then washed thoroughly with DMF (7 × 20 mL).

For the coupling of the next amino acid, Fmoc-Aspartic acid(OtBu) (3 equivalents relative to resin loading, 0.5 M solution in DMF) is introduced. HOBt (4 equivalents relative to resin loading, 0.4 M solution in DMF) and DIPCDI (4 equivalents relative to resin loading, 0.4 M solution in DMF) are added to activate the coupling reaction. The suspension is gently shaken

for 2.5 h to ensure complete coupling. Afterward, the resin is washed with DMF (3 × 20 mL). This procedure is repeated for subsequent amino acids: Fmoc-Glycine, Fmoc-Phenylalanine, and AAP-carboxylic acid (3 equivalents each, 0.5 M solution in DMF).

Finally, the cleavage of the peptide from the resin is performed. The resin is suspended in a cleavage cocktail consisting of TFA, H_2O, and Triisopropylsilane (95:2.5:2.5, 20 mL). The mixture is stirred for 4 h at room temperature. After cleavage, the solution is filtered, and the resin is washed with TFA (3 × 5 mL). The filtrate is added to a cold Et_2O:pentane solution (3:1 v/v), precipitating the peptide, which is collected via centrifugation. To purify the peptide, it is re-dissolved in a minimal amount of DMSO and re-precipitated by adding MilliQ water. The precipitate is collected via centrifugation, and any remaining water is removed through lyophilization. The resulting peptide is obtained as a yellow powder.

MALDI-TOF: [m/z]: found: 665.29 [M-H]+ , calculated: 665.27

13 C NMR (101 MHz, DMSO): δ 172.16, 171.77, 171.07, 169.04, 166.49, 153.46, 141.44, 141.07, 138.19, 134.97, 130.01, 129.69, 129.67, 128.57, 126.77, 121.87, 120.25, 61.70, 55.28, 54.43, 51.90, 49.61, 42.41, 40.66, 40.45, 38.27, 36.76, 14.34, 9.77.

1 H NMR: (400 MHz, DMSO-d6): δ 8.54 – 8.41 (m, 2H), 8.22 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 7.7 Hz, 2H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.41 (dd, *J* = 15.5, 7.9 Hz, 1H), 7.29 – 7.15 (m, 5H), 4.95 – 4.51 (m, 5H), 4.30 – 4.19 (m, 1H), 3.89 – 3.52 (m, 5H), 3.09 (dd, *J* = 14.1, 4.1 Hz, 1H), 2.74 (m, *J* = 34.0, 15.2, 7.6 Hz, 2H), 2.32 (d, *J* = 18.0 Hz, 6H).

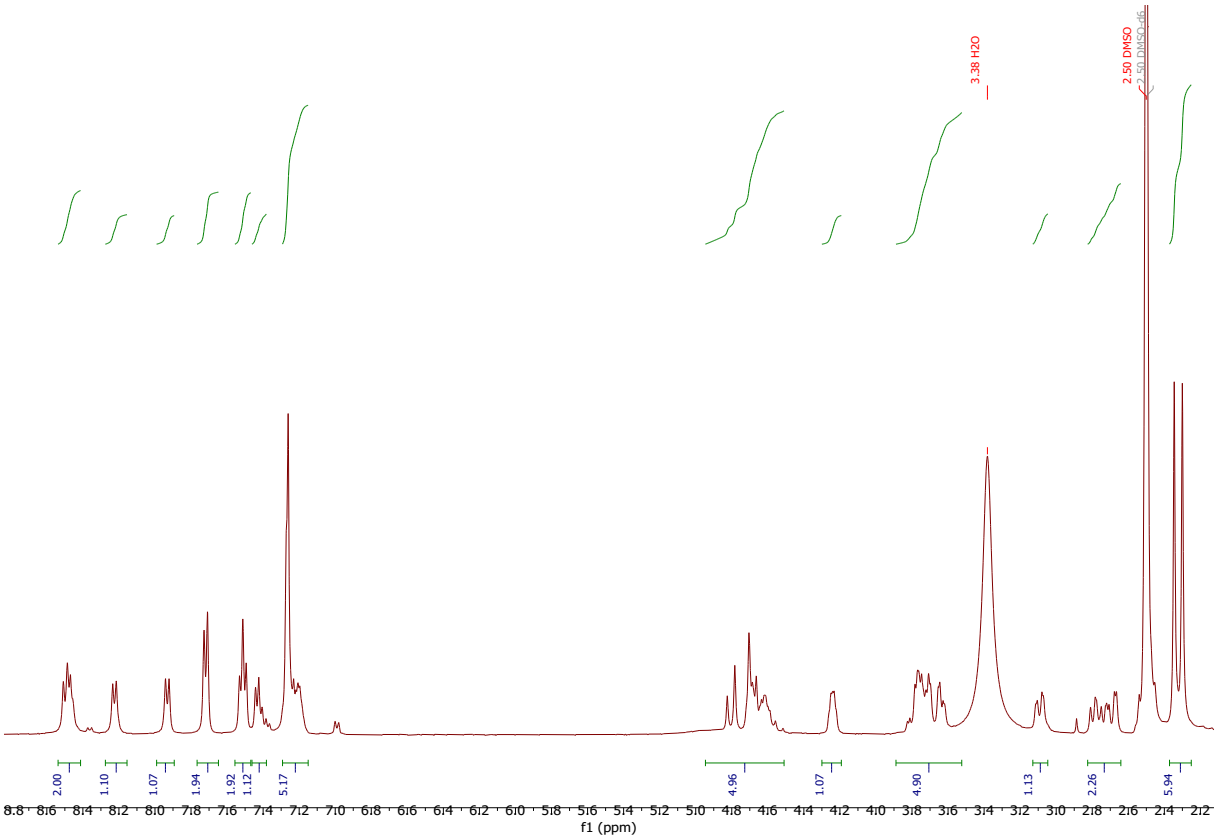


Figure S3: *1 H NMR: (400 MHz, DMSO-d6) of AAP-FGDS.*

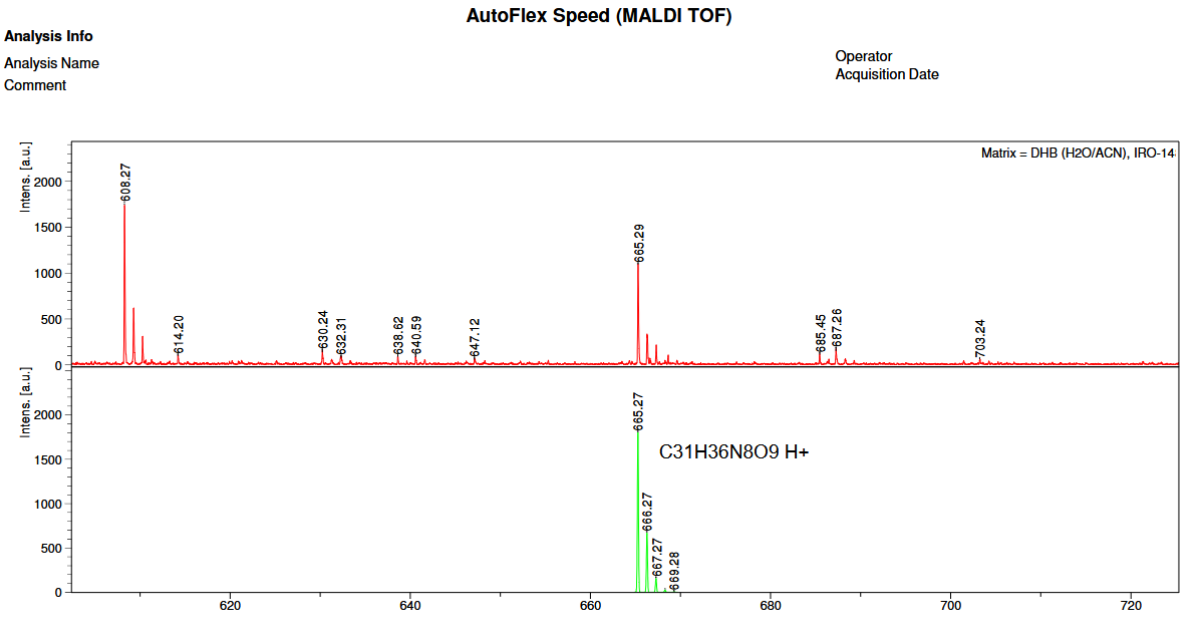


Figure S4: *MALDI TOF spectrum of AAP-FGDS.*

Preparation of upconversion nanoparticles (UCNP)

The synthesis of UCNP consists of two parts and was performed according to the experimental procedure described by Capobianco et al.[4] In a second step, the UCNP were water-solubilized with a silica coating for application. The concentrations of the individual lanthanides were chosen to maximize the yield of Tulium emission in the wavelength range required for the isomerization.

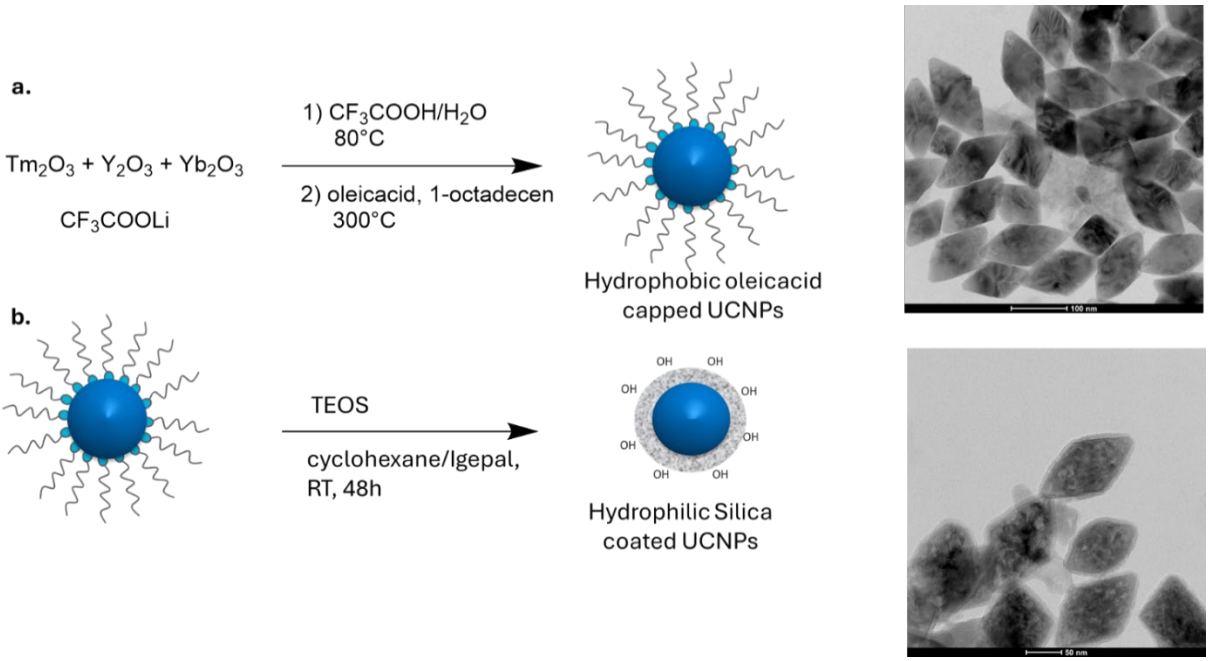


Figure S5: *Schematic illustration and TEM images of UCNP synthesis and surface modification. (a) The synthesis of hydrophobic oleic acid-capped UCNPs begins with the reaction of Tm_2O_3, Y_2O_3, and Yb_2O_3 with trifluoroacetic acid at 80 °C, followed by decomposition in oleic acid and 1-octadecene at 300 °C. The resulting UCNPs exhibit a hydrophobic oleic acid surface, as confirmed by the TEM image (top right). (b) The hydrophobic UCNPs are further modified to hydrophilic silica-coated UCNPs by reacting with TEOS in the presence of Igepal and cyclohexane at room temperature for 48 h. The TEM image (bottom right) shows the resulting silica-coated UCNPs with a uniform surface modification.*[3,4]

Step 1

All chemicals used in the synthesis of the nanocrystals were purchased from Aldrich. The lanthanide trifluoroacetate precursors were prepared from the respective lanthanide and yttrium oxides and trifluoroacetic acid (99%). For the $NaYF_4$:Tm^{3+} (0.5 mol%), Yb^{3+} (25 mol%) codoped sample, 19.29 mg (0.05 mmol) of Tm_2O_3, 98.52 mg (0.25 mmol) of Yb_2O_3, and 225.81 mg (1.0 mmol) of Y_2O_3 were dissolved in 10 mL of 50% aqueous trifluoroacetic acid at 80 °C. The water and excess acid were evaporated to dryness at 50 °C.

Subsequently, 0.34 g (2.5 mmol) of sodium trifluoroacetate (98%) was added to the reaction vessel along with 20 mL of octadecene (90%) and 20 mL of oleic acid (90%). The mixture was heated to 100 °C under vacuum with magnetic stirring for 30 min to remove residual water and oxygen. The flask was periodically purged with dry argon gas. The resulting clear, slightly yellow solution was then heated to 300 °C at a rate of 10 °C/min under argon and held at this temperature for 1 h. Gas bubble formation at ~250 °C indicated the decomposition of the metal trifluoroacetates, and a burst of nucleation was observed between 280 and 300 °C, causing the solution to turn turbid.

After cooling to room temperature, the solution became clear, yielding a yellow colloidal solution. The nanocrystals were precipitated by adding hexane/acetone (1:4 v/v) and isolated via centrifugation at 3000 rpm (~1000 RCF). The pellet was washed with ethanol and further purified by dispersing in a small amount of chloroform, followed by precipitation with excess ethanol. The final nanocrystals were dried under vacuum for at least 24 h. These nanocrystals could be dispersed in nonpolar solvents such as hexane, toluene, or dichloromethane by sonicating the suspension for 10–20 min. To aid dispersion, a drop of oleic acid was occasionally added. To avoid this step, it was preferable to leave the nanocrystals in a semi-dried ‘muddy’ state rather than as a fully dried powder.[24]

Step 2

To coat the nanocrystals with a silica layer, 50 mg of the synthesized nanocrystals were dispersed in 24 mL of cyclohexane. Initially, 0.2 mL of Igepal CO-520 was added to the suspension, which was stirred for 20 min. After 10 min of stirring, an additional 0.8 mL of Igepal CO-520 and 0.15 mL of ammonia solution (25%) were introduced to the mixture, followed by sonication for 20 min to ensure uniform dispersion.

Subsequently, 0.04 mL of tetraethyl orthosilicate (TEOS) was added dropwise, and the reaction mixture was stirred at room temperature for 48 h to allow for the formation of a uniform silica shell around the nanocrystals. The resulting silica-coated nanocrystals were isolated by centrifugation, washed with ethanol, and dried under vacuum. These silica-coated nanocrystals are well-dispersed and suitable for further integration into the hydrogel matrix.

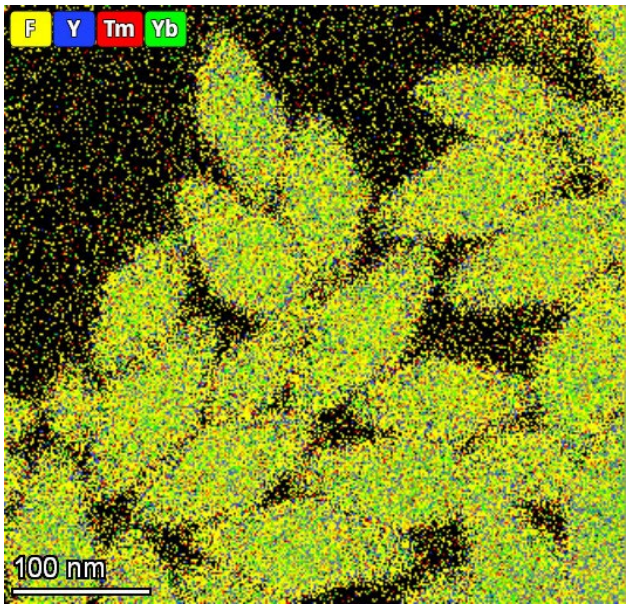


Figure S6: *High-angle annular dark field (HAADF) TEM image of upconversion nanoparticles (UCNP), displaying elemental mapping for Fluorine (F, yellow), Yttrium (Y, blue), Thulium (Tm, red), and Ytterbium (Yb, green). The distribution confirms the uniform incorporation of dopant ions into the nanostructures. Scale bar: 100 nm.*

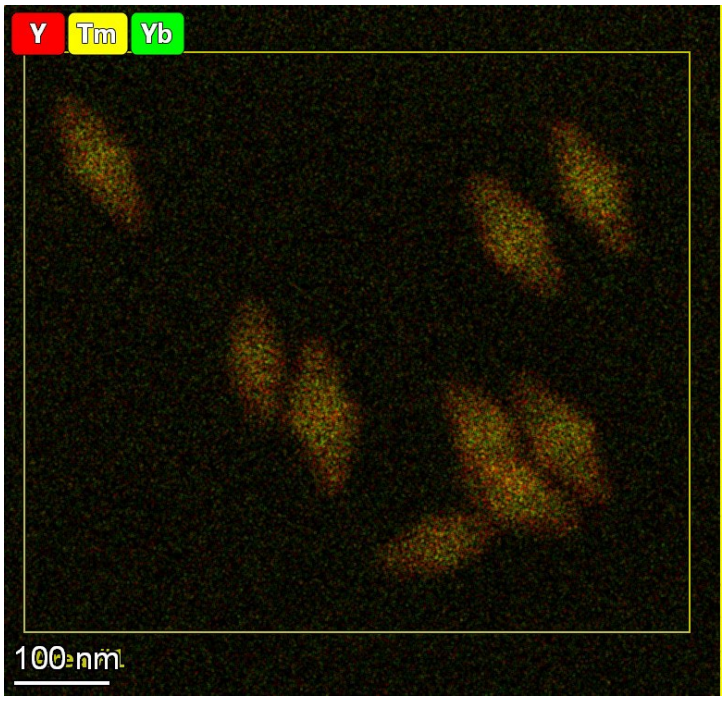


Figure S7: *TEM-EDX mapping of the three-component hydrogel sample containing UCNPs, showing the spatial distribution of Y (red), Tm (yellow), and Yb (green). The scale bar represents 100 nm.*

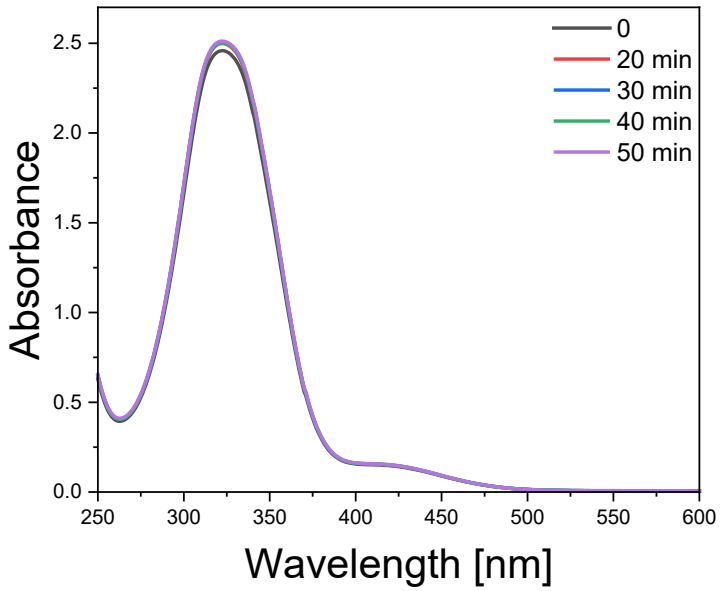


Figure S8: *UV-Vis absorption spectra of a negative control sample consisting of a hydrogel of AAP-FGDS and agarose without UCNP, recorded at different time points (0 min, 20 min, 30 min, 40 min, and 50 min).*

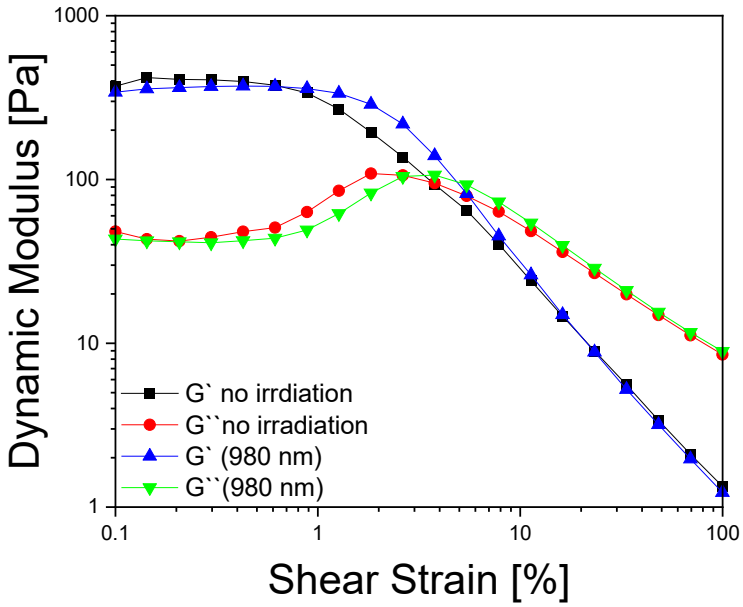


Figure S9: *Amplitude sweep of a negative control sample consisting of a hydrogel of AAP-FGDS and agarose without UCNP. The graph illustrates the dynamic modulus (G' and G'') as a function of shear strain (%) under conditions with and without 980 nm irradiation.*

References

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