

A Dual Encapsulation Strategy to Generate Anion-Responsive Luminescent Lanthanide Hydrogels

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Experimental Details

General Considerations

Reagent grade chemicals, including the anhydrous solvents, were purchased from Sigma Aldrich and Fluorochem. The inhibitors were removed from the monomers before reaction by passing the reagent neat through a basic alumina column. All other reagents were used as received.

Nuclear Magnetic Resonance

NMR spectra were recorded in the stated deuterated solvent on a JEOL ECS-400 or 500 spectrometers (^1H at 400 MHz, ^{13}C at 100 MHz; ^1H at 500 MHz, ^{13}C at 125 MHz, respectively), at 298 K. Chemical shifts are expressed in ppm and are adjusted to the chemical shift of the residual NMR solvent resonances (CDCl_3 : ^1H δ = 7.26 ppm, ^{13}C δ = 77.16 ppm, or D_2O : ^1H δ = 4.79 ppm).

Gel Permeation Chromatography (GPC)

Molecular weight and dispersity analysis were performed using Agilent Technologies 1260 infinity GPC, with a chloroform/triethylamine (98:2 v/v) mobile phase and polystyrene standards. Raw data was accessed using Agilent GPC/SEC software.

Dynamic Light Scattering (DLS)

DLS measurements were performed using a Malvern Zetasizer Nano NS and Zetasizer software, version 7.12. The system uses an avalanche photodiode detector and a diode-pumped solid-state laser operating (wavelength of 532 nm). Light scattered was detected at a 175° angle, and the temperature was stabilised at $25^\circ\text{C} \pm 0.1^\circ\text{C}$. Particle suspensions were diluted 10 μL in 1 mL deionised water and filtered through 400 nm nylon syringe filters.

Luminescence Experiments

Luminescence spectra were recorded on a Camlin Photonics luminescence spectrometer with FluoroSENS version 3.4.7.2024 software. Emission spectra were obtained using a 100 μL or 1000 μL Hellma Analytics quartz cuvette for analysis of particles and hydrogels respectively (see Figure S8). Excitation light was set at the required wavelength (i.e. 330 nm) and emission recorded in the range 400 – 720 nm using an integration time of 0.5 seconds, increment of 1.0 nm, excitation slit of 0.2 nm and emission slit of 0.5 nm.

Anion Binding Titrations

Anion binding titrations were carried out in duplicate in 10 mM HEPES buffer at pH 7.0. Stock solutions of the anion containing Eu(III) complex (5 μM) were made up at 0.9, 9 and 90 mM anion. The appropriate anion stock solution (i.e. bicarbonate) was added incrementally to 100 μL of Eu(III) complex (5 μM) or the hydrogel in a 1 cm x 1 cm quartz cuvette and the emission spectrum was recorded after each addition. The ratio of emission bands 605 – 630 nm/ 585 – 600 nm ($\Delta\lambda = 2 / \Delta\lambda = 1$) was plotted as a function of anion concentration. The data was analysed using a nonlinear least squares curve fitting procedure, based on a 1:1 binding model described by the equation:

$$FB = \frac{\frac{1}{K_a} + [A] + [Eu] - \sqrt{\left(\frac{1}{K_a} + [A] + [Eu]\right)^2 + 4[A][Eu]}}{2[Eu]}$$

where FB is the fraction bound, calculated by $(I - I_0)/(I_1 - I_0)$ where I is the emission intensity at $[A]$, I_0 is the initial emission intensity, and I_1 is the final emission intensity. $[A]$ is the total concentration of anion in solution, $[Eu]$ is the total concentration of Eu(III) complex, K_a is the apparent binding constant.

pH Measurements

pH measurements were carried out using a Jenway 3510 pH/mV meter with a Jenway combination electrode or a Jenway 3020 pH meter with an Aldrich glass combination pH electrode, calibrated using several buffer solutions (pH 4.00 ± 0.01; 7.00 ± 0.01 and 10.00 ± 0.01).

Scanning Electron Microscopy (SEM)

Electron micrographs were recorded at the Loughborough Materials Characterisation Centre (LMCC) using a JEOL 7800F field emission scanning electron microscope (FEG-SEM) at varying accelerating voltages (2 - 10 kV) and magnifications (100 - 150,000 X). Hydrogel samples were freeze-fractures in liquid nitrogen prior to lyophilisation. Subsequent sample coating was performed using a Quorum technology 150 Q TES system equipped with a Gold/Palladium sputter target. A deposition current of 20 mA and tooling factor of 2.3 was used for up to 90 seconds to coat the sample in < 5 nm of Au/Pd. Samples were mounted on aluminium pin stubs (Agar Scientific Ltd.) unless otherwise specified.

Rheology

A 40 mm parallel plate was fitted onto the rheometer and the bottom Peltier plate heated to 80 °C. A standard set-up was created, conducting an oscillation time sweep at 80 °C for 600 seconds and applying an angular frequency of 10.0001 rad/s. The software indicates the minimum volume of sample to run the test which was 0.87 mL and therefore 0.9 mL of hydrogel mixture of the relevant composition were used each time. The experiments were conducted in triplicate for each hydrogel type. The oscillation time sweep was set to run immediately after the sample was placed on the heated Peltier plate to reduce the risk of premature crosslinking before the test begins. Complex viscosity, storage modulus (G') and loss modulus (G'') were plotted against time and the gel point was calculated where the storage and loss moduli intersect. Where the moduli did not quite meet on the curves produced by the rheometer, the storage modulus curve was extrapolated to obtain a representative value of the gel point.

Tensile Properties

Hydrogels were prepared and swollen in deionised water before samples of 4 cm x 0.5 cm were cut. Specimens were loaded onto the Instron tensometer using a 2 cm gauge length and tensile tests using a load of 2 kN were conducted at 1 mm/min crosshead speed at room temperature. The thickness of the specimens was measured with Vernier Callipers. Several measurements were taken across the length of the sample and an average calculated. Five specimens were tensile tested for each hydrogel type, and the hydrogels were fully swollen prior to the tensile testing by submerging them in deionized water for at least 24 hours.

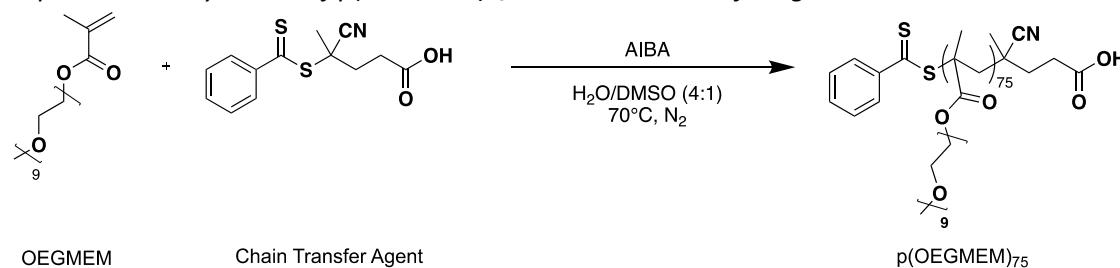
Swelling Studies

Hydrogels were prepared and swollen in deionised water to allow ease of cutting. Square samples of 1 cm x 1 cm were cut and left to dry in a fume hood for at least 24 hours before beginning the experiments at room temperature. Samples were weighed using a 5 decimal place scale before beginning the swelling studies and placed in individual 'holders' and labelled. Three samples were used per experiment. The holder was completely submersed in the required solvent and at each time interval, the samples were gently dabbed dry to remove the surface solvent and weighed. Note: it was important to remove the hydrogel from the holder at the last moment, to reduce the amount of evaporation of the solvent from within the hydrogel. The swelling ratio was calculated using the following equation:

$$\text{Swelling Ratio (\%)} = \frac{W_{\text{swell}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100$$

Polymer Synthesis and Analysis

Representative synthesis of $p(\text{OEGMEM})_{75}$: macro chain transfer agent.



Scheme S1: Synthesis of hydrophilic polymer $p(\text{OEGMEM})_{75}$.

To a stirred 80:20 water/DMSO mixture (8.0 mL) was added OEGMEM₅₀₀ (1.9 mL, 4.0 mmol, 75 equiv.), trioxane (28 mg, 0.31 mmol, 6.2 equiv.), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (14 mg, 0.05 mmol, 1 equiv.) and 2,2'-azobis(2-amidinopropane) dihydrochloride (56 μL of stock solution, 0.01 mmol, 0.2 equiv.). The solution was purged with nitrogen for 5 minutes, before being stirred at 70 $^\circ\text{C}$ for 4 hours. The synthesis of the linear polymer was monitored by ^1H NMR spectroscopy in D_2O (Figure S1). Successful polymerisation was observed by tracking the ratio of the methacrylate monomer signals at 6.0 ppm and 5.6 ppm in relation to the internal standard, trioxane at 5.1 ppm. Plotting of the data ensured that the conversion did not exceed 95%. The product was purified by dialysis (6 \AA , 4 h, 3500 MWCO, 4 L deionised water) removing any monomer present and lyophilized to yield a pink viscous oil.

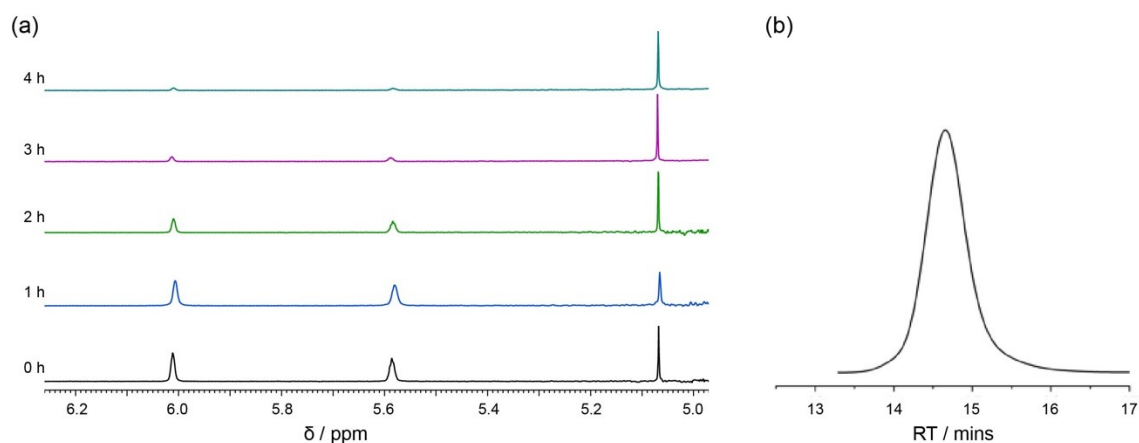
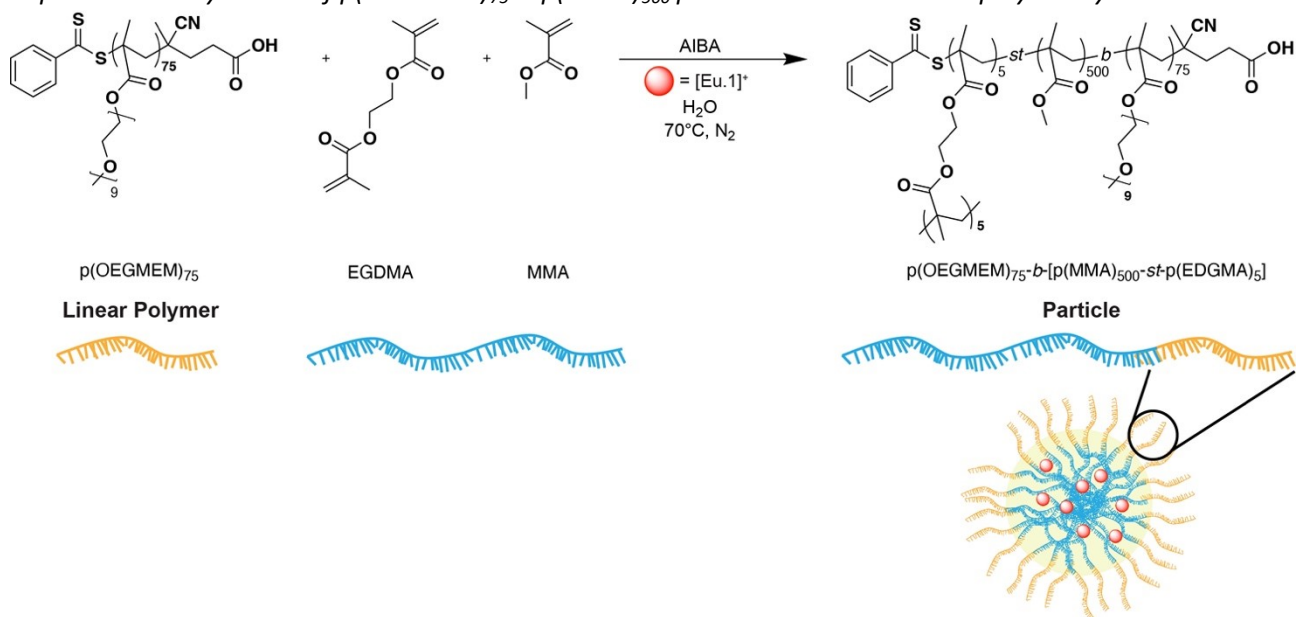


Figure S1: (a) ^1H NMR (D_2O , 298 K) spectra for monitoring the $p(\text{OEGMEM})_{75}$ synthesis, showing the consumption of the monomer (6.0 ppm and 5.6 ppm) over 4 hours, 90% conversion. (b) GPC analysis of synthesised polymer after purification, $M_n = 31700 \text{ gmol}^{-1}$. Conditions: chloroform/triethylamine (98:2 v/v) mobile phase and compared to polystyrene standards.

Particle Synthesis and Analysis

Representative synthesis of $p(\text{OEGMEM})_{75}\text{-}b\text{-}p(\text{MMA})_{500}$ particles: extended chain polymer synthesis.



Scheme S2: Synthesis of polymer particles using RAFT polymerisation.

In this exact order, poly(OEGMEM)₇₅ (940 mg, 0.025 mmol, 1 equiv.), trioxane (90 mg, 1.0 mmol, 40 equiv.), 2,2'-azobis(2-amidinopropane) dihydrochloride (28 μL of stock solution, 0.005 mmol, 0.2 equiv.) and the luminescent probe, [Eu.1]⁺ (6 μmol) were added to water (23.7 mL). The solution was adjusted to pH 6.5 – 7 using NaOH (2 M). Whilst stirring, MMA (1.3 mL, 12.5 mmol, 500 equiv.) was added dropwise. The solution was purged with nitrogen for 15 minutes, then heated at 70 °C for 30 minutes, or until the solution turned cloudy, at which point EGDMA (23 μL , 0.12 mmol, 5 equiv.) was added. The solution was heated for a further 1.5 hours (reaction was monitored by ¹H NMR in CDCl₃, Figure S2, tracking the ratio of the methacrylate monomer signals at 6.0 ppm and 5.6 ppm in relation to the internal standard, trioxane at 5.1 ppm). Plotting of the data ensured that the conversion did not exceed 95%. The solution was purified by dialysis against water (6 Å, 4 h, 3500 MWCO, 4 L deionised water) to give the product, a cloudy pink solution.

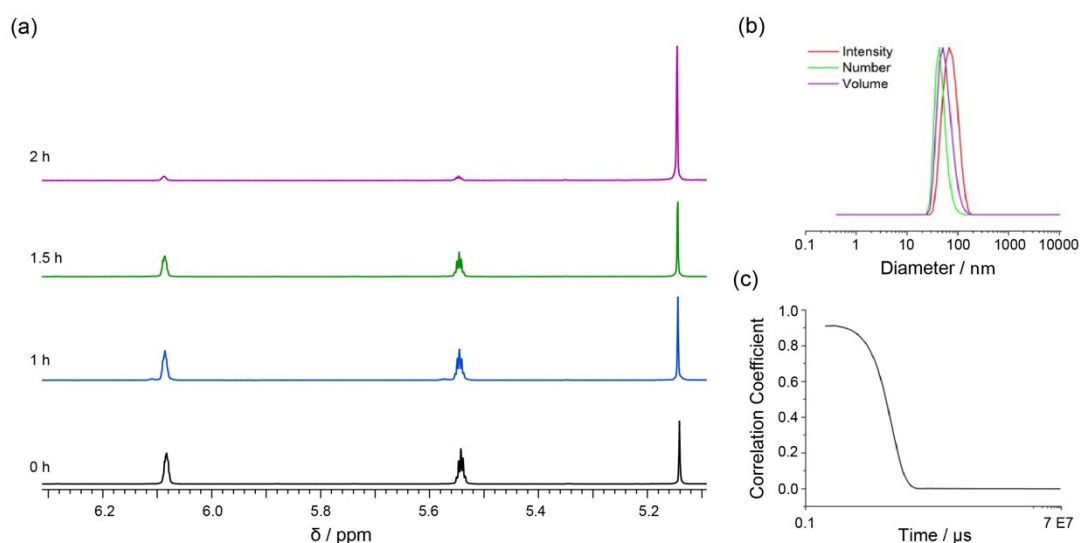


Figure S2: (a) ¹H NMR (CDCl₃, 298 K) spectra for tracking of the $p(\text{OEGMEM})_{75}$ chain extension of the hydrophilic shell, showing the consumption of the monomer (6.1 ppm and 5.5 ppm) over 2 hours. (b) DLS spectrum and (c) DLS correlogram of $p(\text{OEGMEM})_{75}\text{-}b\text{-}p(\text{MMA})_{500}$ particles.

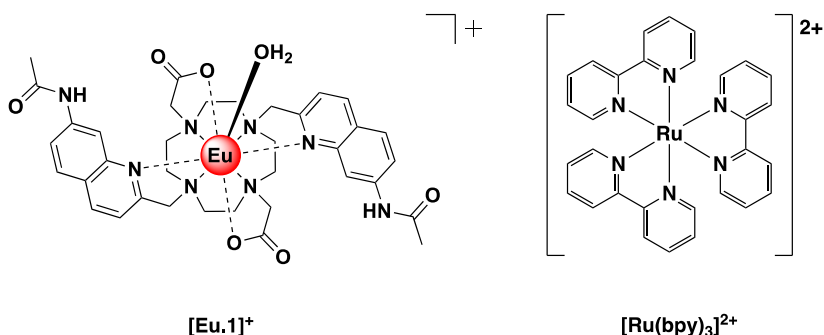


Figure S3: Structures of the emissive metal complexes encapsulated within the polymer matrix. $[Eu.1]^+$ was synthesised using literature procedures¹ and $[Ru(bpy)_3]^{2+}$ was purchased from Sigma Aldrich.

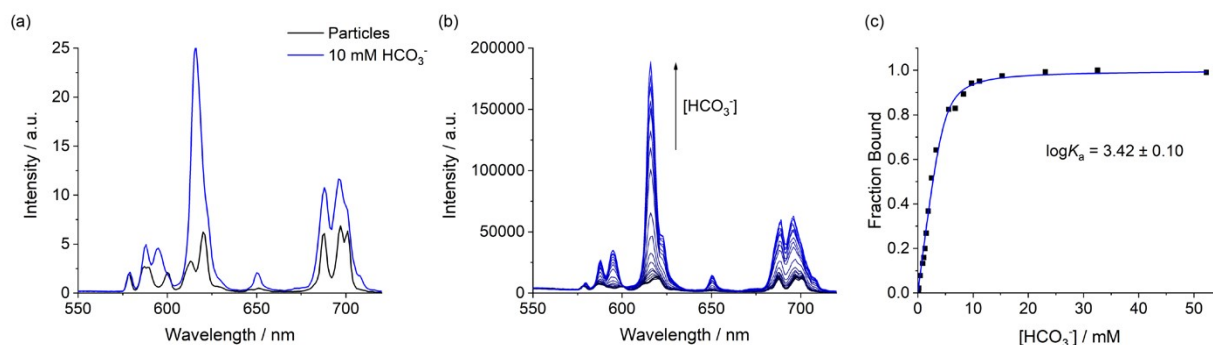


Figure S4: (a) Steady-state emission of $[Eu.1]^+$ loaded particles with and without bicarbonate (10 mM); (b) Variation in emission spectra of $[Eu.1]^+$ upon incremental addition of bicarbonate; (c) Plot of fraction bound (determined from $\Delta I = 2 / \Delta I = 1$ intensity ratio) versus bicarbonate concentration, showing the fit to a 1:1 binding isotherm. Measured in 10 mM HEPES at pH 7.0 and 295 K, $\lambda_{ex} = 330$ nm.

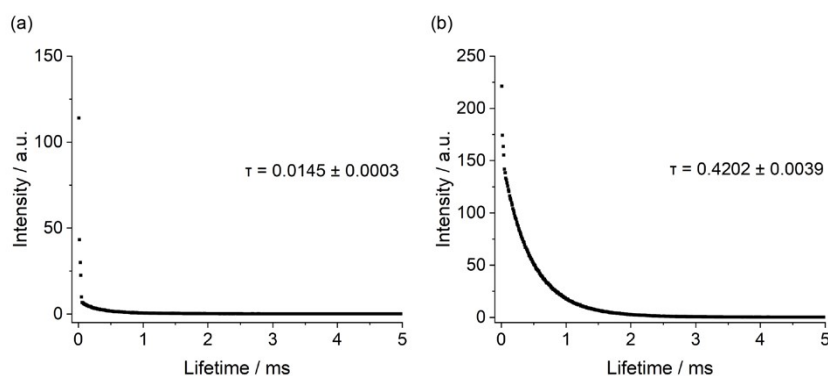


Figure S5: Emission lifetimes of the Eu-loaded particles (a) with no anion present; (b) with 30 mM bicarbonate present. Measured in 10 mM HEPES at pH 7.0 and 295 K, $\lambda_{ex} = 330$ nm.

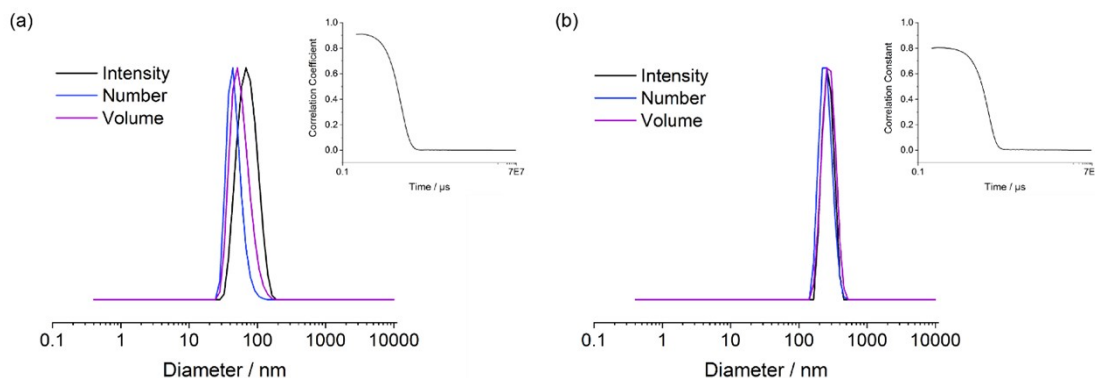
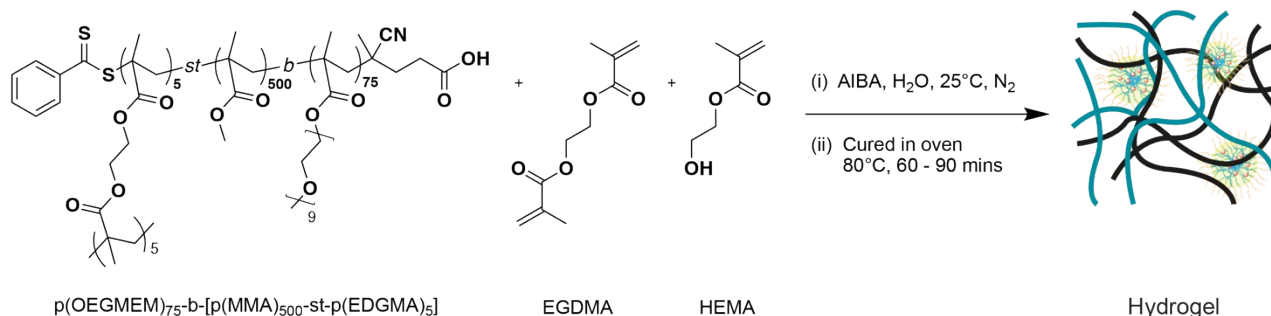


Figure S6: DLS spectrum and DLS correlogram (inset) of probe-loaded particles (a) Z = 65 nm, (b) Z = 270 nm.

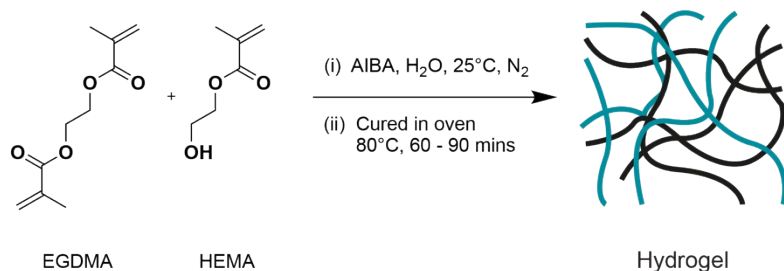
Hydrogel Synthesis and Analysis

Representative synthesis of particle loaded hydrogels.

(a) Hydrogel Synthesis with Particles



(b) Hydrogel Synthesis with No Particles (Control)



Scheme S3: Synthetic scheme displaying the preparation of the hydrogels, (a) incorporating particles within the hydrogel matrix and (b) no particles.

To a clean dry round-bottom flask, was added $p(\text{OEGMEM})_{75}\text{-}p(\text{MMA})_{500}$ particle solution (132.8 μL) and HEMA (900 μL , 7.40 mmol). EGDMA (100 μL , 0.53 mmol) then 2,2'-azobis(2-amidinopropane) dihydrochloride (67.2 μL from stock solution, 7.4 mmol) were then added and stirred for 5 minutes. The reaction was then purged with nitrogen for 5 minutes (no longer, to avoid evaporation). The resulting solution was transferred to a silicon mould then placed in an oven for 60 – 90 minutes at 80 °C. After which, the mould was removed from the oven and while still warm, the hydrogel was pried away from the glass slides. The hydrogel was allowed to cool, transferred to an appropriate length dialysis tubing, and dialysed for 24 hours.

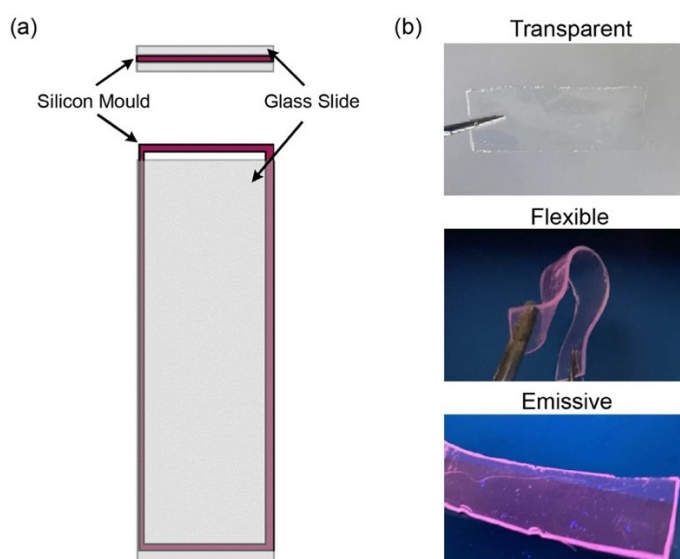


Figure S7: (a) Silicon mould diagram used for hydrogel synthesis. (NB: One slide is offset slightly to allow for hydrogel solution to be syringed in between the glass slides). (b) Photos of the hydrogels synthesised, showing they are transparent, flexible and emissive when they contain a luminescent probe, e.g. [Eu.1]⁺.

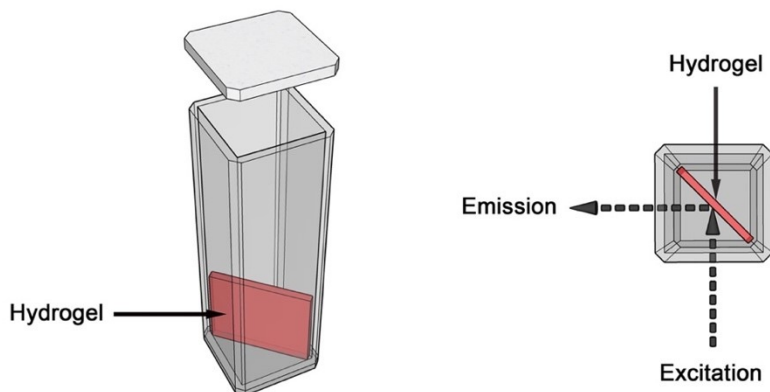


Figure S8: Schematic representation of the hydrogel analysis *via* fluorimetry, by cutting the hydrogel and placing the material diagonally across the bottom of the quartz cuvette.

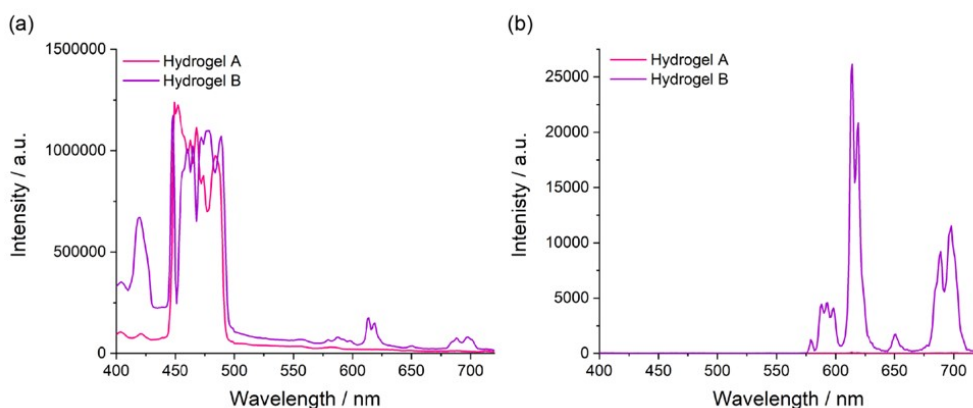


Figure S9: (a) Steady-state emission spectra of the hydrogels showing light scattering between 400 – 500 nm and (b) time-resolved emission (60 – 400 μ s) of hydrogels containing no particles (hydrogel A, baseline plot) and [Eu.1]⁺ loaded particles (hydrogel B) showing complete removal of light scattering. Measured in deionised water at 295 K, λ_{ex} = 330 nm.

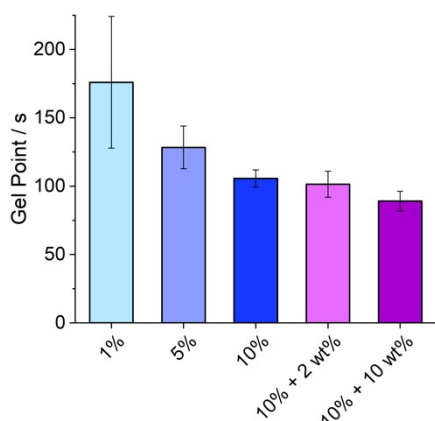


Figure S10: Comparison of the rheological properties (gel point) of the hydrogels composed of (i) 1% crosslinker, (ii) 5% crosslinker and 10% crosslinker with (iii) no particles, (iv) 2 wt% particles and (v) 10 wt% particles. Data is the average of three independent experiments.

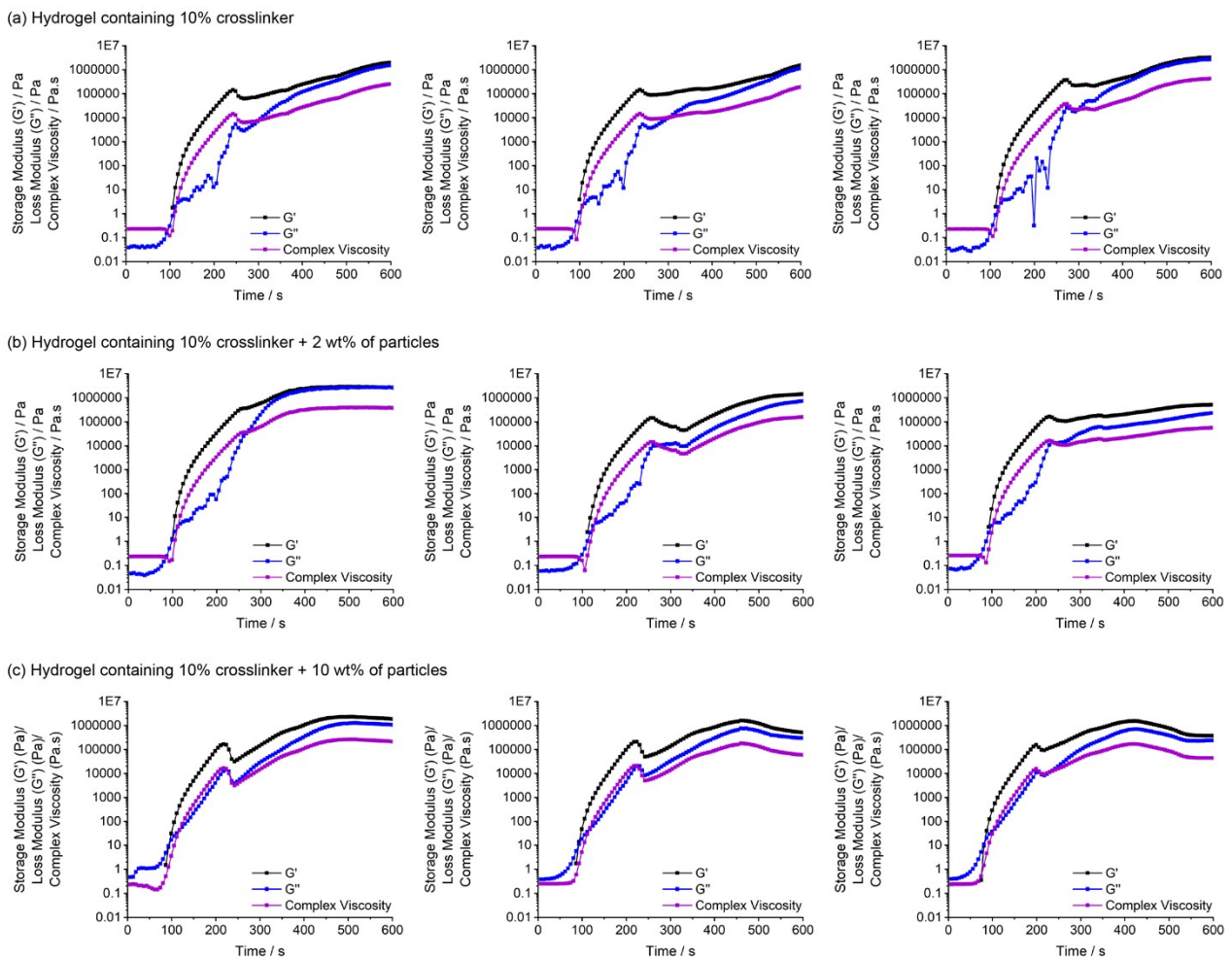


Figure S11: Representative raw data (storage moduli (G' , black) and loss moduli (G'' , blue) and complex viscosity (purple)) used to calculate the gel point (Figure S9 above) of the hydrogels containing (a) 10% crosslinker, (b) 10% crosslinker and 2 wt% particles and (c) 10% crosslinker and 10 wt% particles.

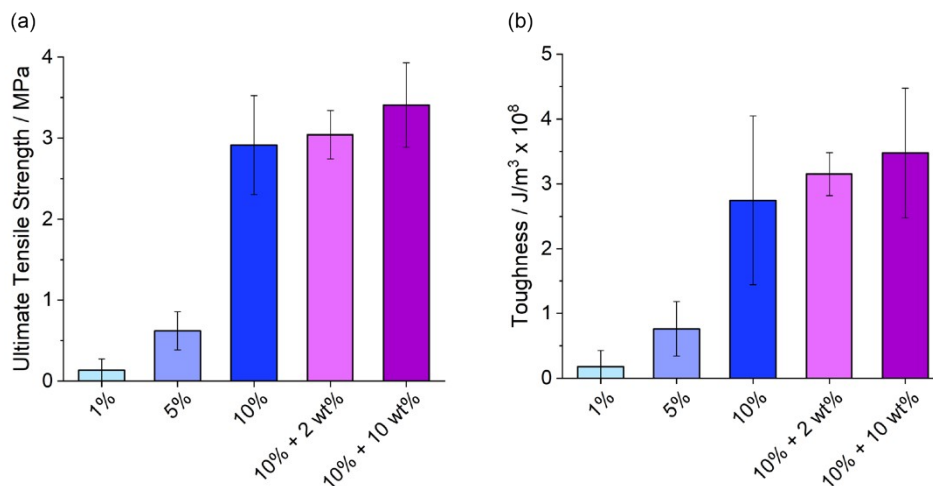


Figure S12: Comparison of the mechanical properties (a) ultimate tensile strength and (b) toughness of the hydrogels composed of (i) 1% crosslinker, (ii) 5% crosslinker and 10% crosslinker with (iii) no particles, (iv) 2 wt% particles and (v) 10 wt% particles. Data is the average of five independent experiments.

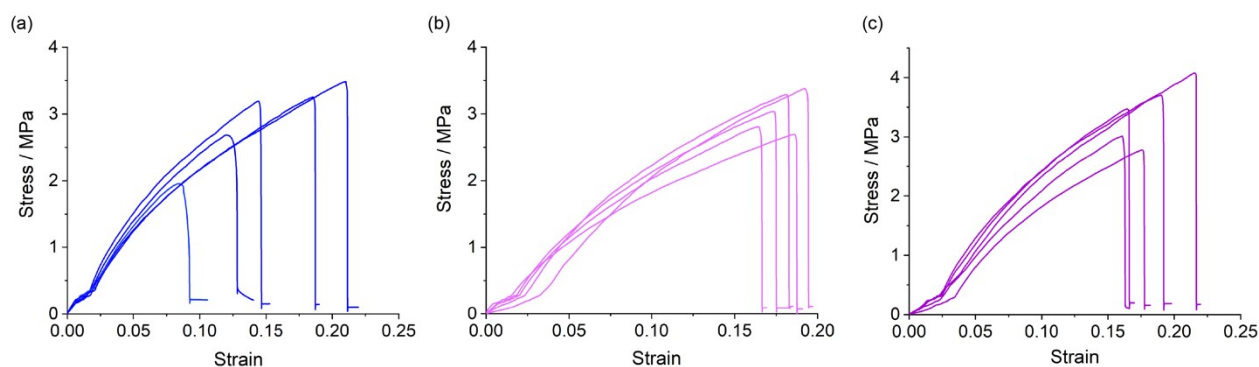


Figure S13: Representative raw data used to calculate the stress-strain of the hydrogel (Figure S11 above) containing 10% crosslinker with (a) no particles, (b) 2 wt% particles and (c) 10 wt% particles.

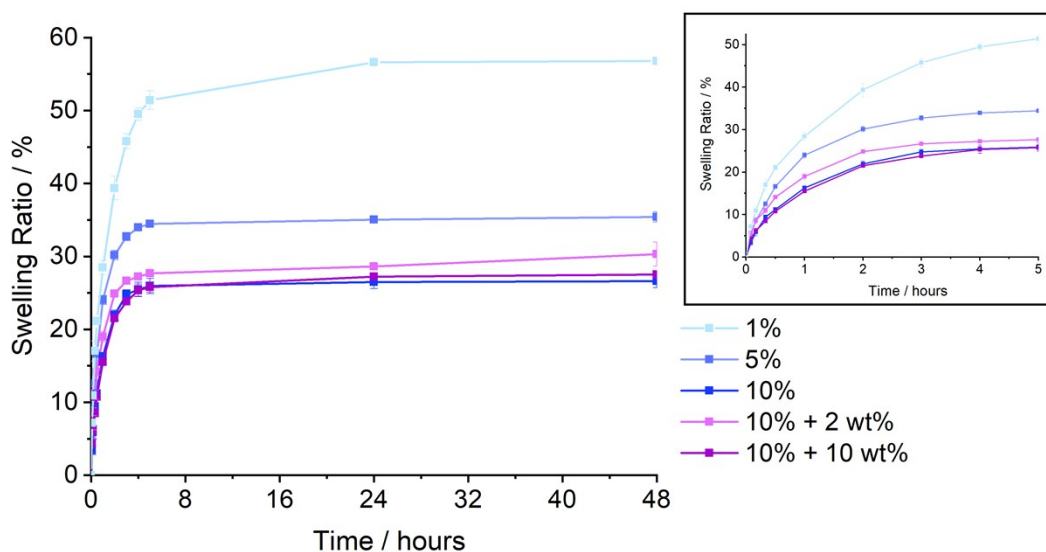


Figure S14: Comparison of the swelling ratio over 48 hours of the hydrogels composed of (i) 1% crosslinker, (ii) 5% crosslinker and 10% crosslinker with (iii) no particles, (iv) 2 wt% particles and (v) 10 wt% particles. Inset: swelling of the hydrogels over the first 5 hours. Measured in deionised water at 295 K.

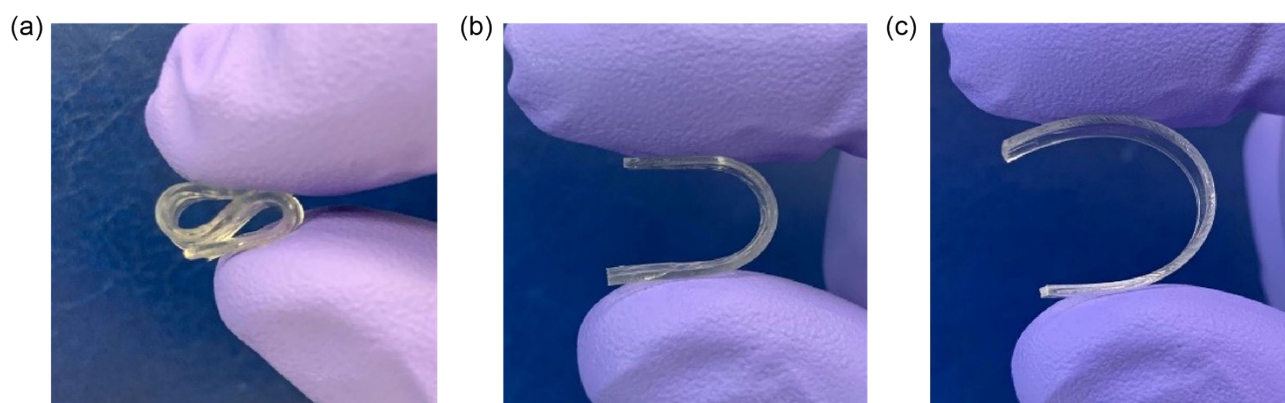


Figure S15: Photos of swollen hydrogels of composition of (a) 1% crosslinker, (b) 5% crosslinker and (c) 10% crosslinker.

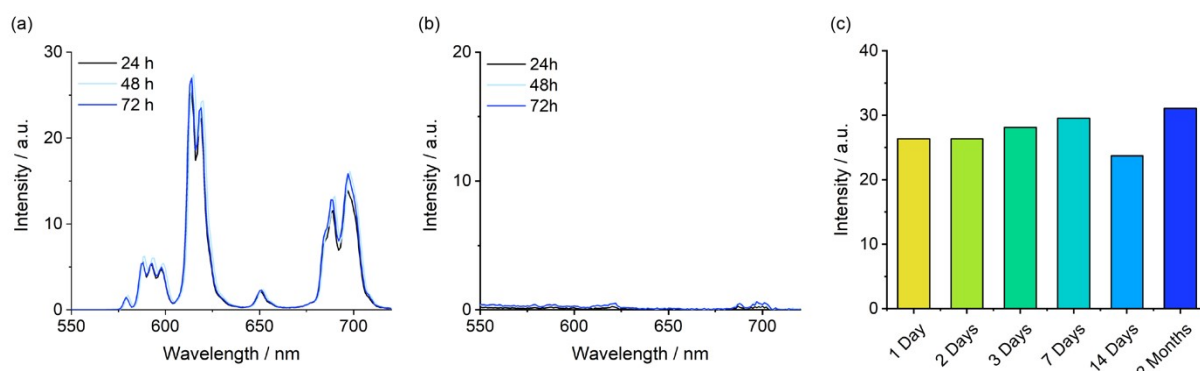


Figure S16: Leaching experiment showing (a) the stable emission of the hydrogel containing [Eu.1]⁺ loaded particles, (b) minimal emission of the hydrogel incubation solution, (c) stable emission intensity ($\Delta\lambda = 2$, 605 – 630 nm) of the hydrogel containing [Eu.1]⁺ loaded particles over a 2 month period. Measured in 10 mM HEPES at pH 7.0 and 295 K, $\lambda_{\text{ex}} = 330$ nm.

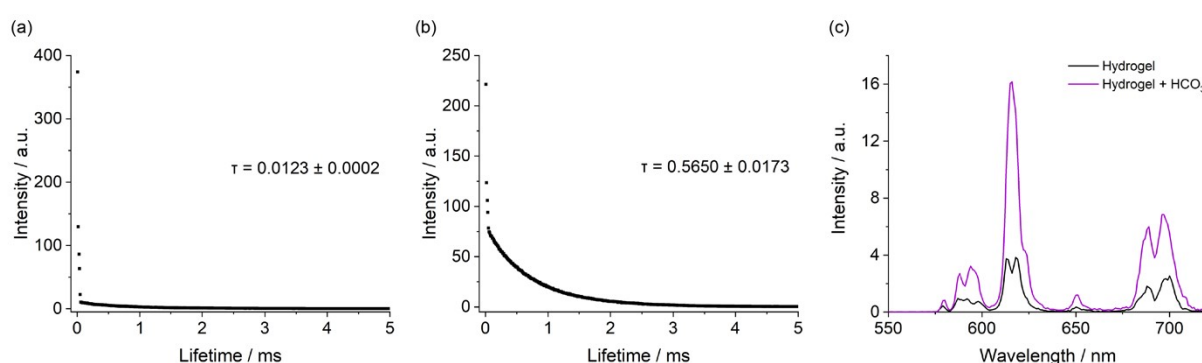


Figure S17: Emission lifetimes of the Eu-loaded hydrogel (a) with no anion present; (b) with 30 mM bicarbonate present; and (c) time-resolved emission in the absence and presence of 30 mM bicarbonate. Measured in 10 mM HEPES at pH 7.0 and 295 K, $\lambda_{\text{ex}} = 330$ nm, time delay 60 – 400 μs to acquire spectra in (c).

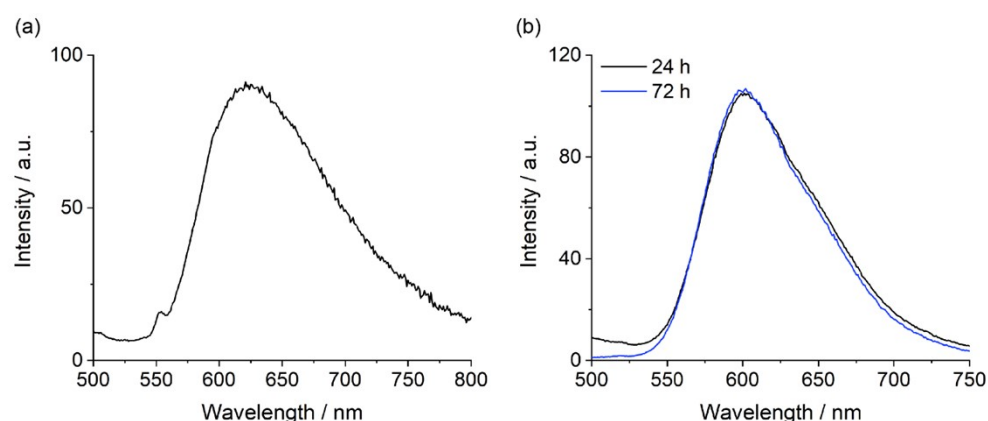


Figure S18: Emission of (a) [Ru(bpy)₃]²⁺ loaded particles and (b) hydrogel containing [Ru(bpy)₃]²⁺ loaded particles showing stability of the emission over 72 hours. Measured in deionised water at 295 K, $\lambda_{\text{ex}} = 450$ nm.

Short video highlighting the ion sensing technology presented herein –

<https://www.youtube.com/watch?v=2AaGlsiAd3U>

References

- 1 S. J. Butler, *Chem. Commun.*, 2015, **51**, 10879–10882.