Supramolecular Hydrogels Prepared From Fluorescent Alkyl Pyridinium Acrylamide Monomers and CB[8]

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Contents

Materials and Methods

Materials. All materials as follows were purchased from commercial suppliers and used without further purification unless otherwise stated: isoquinoline (IsoQ, 96%, Fisher Scientific), 4-phenylpyridine (PhPy, 99%, Fisher Scientific), 3-bromopropylamine hydrobromide (98%, Fisher Scientific), ammonium hexafluorophosphate (99%, Fisher Scientific) , acryloyl chloride (≥ 96.0 %, Merck), Amberlite IRA67 (Sigma-Aldrich), Dowex 1X8-200 Ion-exchange resin (Sigma-Aldrich), acrylamide (AAm, ≥99%, Sigma-Aldrich), 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044, >98.0% TCI Chemicals).

2-(pyridin-4-yl)benzo[d]thiazole (BTPy), 1-methyl-[4,4'-bipyridin]-1-ium (MV), cucurbit[8]uril (CB[8]) and CB[7] were synthesized and purified according to published procedures.[1-3]

All solvents and reagents were purchased from commercial sources (Sigma Aldrich or Fisher Scientific) and used without purification. Milli-Q water (18.2 MΩ·cm) was used in the preparation of all none-deuterated aqueous solutions.

Isothermal Titration Calorimetry (ITC). All ITC experiments were carried out on a Microcal ITC200 at 298.15 K in pure water. In a typical ITC experiment, cucurbit[8]uril was in the sample cell, and the guest molecule was in the injection syringe with a concentration of about ten times the concentration of the CB[8] solution. The concentration of CB[8] was calibrated by titration with a standard solution of 1-adamantanamine. To avoid bias or potentially arbitrary offsets caused by manual adjustment of baseline, all raw data (thermograms) of ITC were integrated by NITPIC (v.1.2.2), fitted in Sedphat (v.15.2b), and visualized through GUSSI (v.1.2.1).

Nuclear Magnetic Resonance (NMR) Spectroscopy ^{1}H NMR were acquired in DMSO-D6, MeOD-D_4 or D_2O at 298K and recorded on a Bruker AVANCE 500 with TCI Cryoprobe system (500 MHz) or a 400 MHz Avance III HD Smart Probe Spectrometer being controlled by TopSpin2.

Fourier-transform infrared spectroscopy (FTIR) FTIR spectra were acquired using a Perkin Elmer Spectrum 100 instrument (Waltham, MA, USA).

High Resolution Mass Spectrometry (HRMS) HRMS spectra were collected on either a Waters Vion IMS Qtof (IsoQPrAm and MVPrAm) or a Waters LCT Premier (PhPyPrAm and BTPyPrAm)

Rheology Rheological measurements were conducted on a TA instruments discovery HR2 Hybrid Rheometer with an 8mm sandblasted geometry to reduce sample slippage. Environmental temperature was recorded using the built-in platinum resistance thermocouple in sandblasted AR-Series Peltier lower plates. Zero gap, rotational mapping (precision bearing mapping; 2 iterations), geometrical inertia and friction calibrations were done prior to each use of the rheometer. Dynamic oscillatory strain amplitude sweeps were conducted at a frequency of 10 rad/s. Dynamic oscillatory frequency sweep measurements were conducted at a 1%

oscillation strain. Each hydrogel type was prepared three times and for each preparation three samples were taken from different areas of the gel to ensure both homogeneity and reproducibility of samples. Data was analysed using TA Instruments TRIOS software and exported into Microsoft Excel for further evaluation For each hydrogel the 9 measurements (3 preparations of each gel, 3 samples per preparation) were averaged and a 95% confidence interval was calculated and plotted as both the negative and positive error bars.

UV/Vis Spectroscopy and Fluorescence Spectroscopy UV/Vis and fluorescence spectra were recorded on a Duetta Fluorescence and Absorbance Spectrometer from Horbia Scientific using an Thorlabs 3 ml quartz fluorescence curvette with a pathlength of 10 mm at 298K

Photography of gels To prepare for the photographs of hydrogels in Figure 5b 500 mg of each hydrogel was cut into small pieces and placed into a 1 mL vial with an additional 200 uL of water in order to swell the hydrogel to the size of the vial.

Synthesis of alkyl pyridinium acrylamides

As exemplified by IsoQPrAm in Figure S **1** (and generalised in Figure 2a) a general synthesis for alkyl pyridinium acrylamides was used.

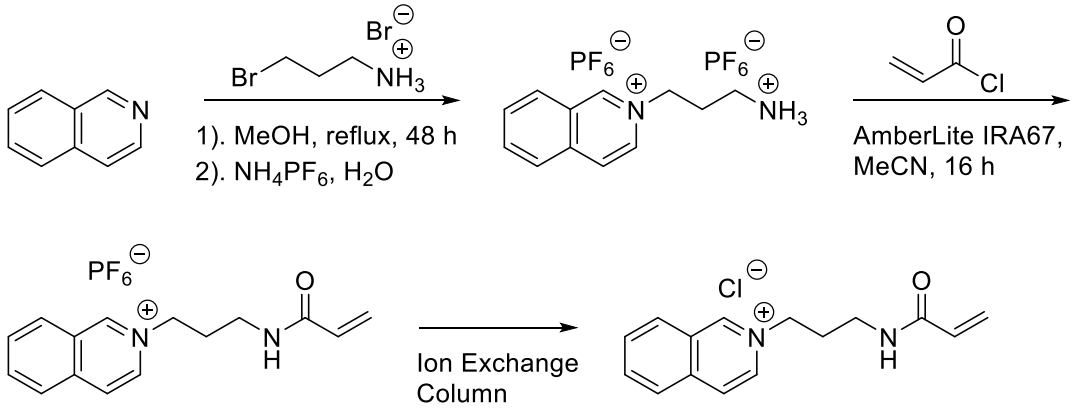

Figure S 1: Synthetic route of 2-(3-acrylamidopropyl)isoquinolin-2-ium chloride (IsoQPrAm)

2-(3-acrylamidopropyl)isoquinolin-2-ium chloride – (IsoQPrAm)

Isoquinoline (2.58 g, 20 mMol) and 3-Bromopropylamine hydrobromide (5.38 g, 20 mMol) were added to methanol (20 mL) and the mixture was heated under vigorous reflux for 48-72 h. Upon cooling the to room temperature the intermediate, 2-(3-ammoniopropyl)isoquinolin-2-ium bromide crystallised into needle like crystals. After washing with DCM these crystals were dissolved into a minimum volume of water. In a separate flask ammonium hexafluorophosphate (16.4 g, 100 mMol, 5x eqv.) was dissolved in a minimum amount of water. Mixing these two solution instantly formed

the 2-(3-ammoniopropyl)isoquinolin-2-ium hexafluorophosphate salt which after washing with ice cold water and drying in a vacuum oven at 40 °C was recovered in quantitative yield. A portion of the resulting white powdery solid (502 mg, 1.05 mMol) was then dissolved in acetonitrile (10.5 ml) and cooled in an ice bath. Amberlite IRA67 resin (5 g) was then added and the mixture was stirred for 30 minutes. Acryloyl chloride (1 ml, 12 mMol) was added dropwise and the mixture was stirred at room temperature for 16 h. Following filtration to remove the resin, the filtrate was evaporated under reduced pressure resulting in a sticky, brown oil which was then, after suspension in MeOH, precipitated into diethyl ether. This suspension was centrifuged at 10,000 rpm for 10 minutes at 4 °C, after decanting the supernatant the solid was then dissolved in acetone and any undissolved material was removed by further filtration. The hexafluorophosphate salt of the final product was obtained by removing the solvent under reduced pressure. Finally, an ion exchange column (Dowex 1X8-200 Ion-exchange resin, solvent = 50:50 MeCN:Water) was used to afford the chloride salt, which after evaporation of acetonitrile under reduced pressure was lyophilized to give the product 2-(3-acrylamidopropyl)isoquinolin-2-ium chloride as a brown waxy solid 202 mg, 70 % yield.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.07 (s, 1H), 8.81 (dd, J = 6.8, 1.5 Hz, 1H), 8.60 (d, J = 6.8 Hz, 1H), 8.47 (d, J = 8.3 Hz, 1H), 8.35 (d, J = 8.3 Hz, 1H), 8.27 (ddd, J = 8.3, 6.9, 1.2 Hz, 1H), 8.23 (t, J = 6.0 Hz, 1H), 8.09 (ddd, J = 8.2, 6.9, 1.2 Hz, 1H), 6.16 (dd, J = 17.1, 10.0 Hz, 1H), 6.05 (dd, J = 17.1, 2.4 Hz, 1H), 5.58 (dd, J = 10.0, 2.4 Hz, 1H), 4.74 (t, J = 7.2 Hz, 2H), 3.25 (dt J – 6.9, 6.0 Hz 2H), 2.23 (p, J = 6.9 Hz, 2H). 13C NMR (151 MHz, DMSO) δ 165.35, 150.92, 137.40, 137.28, 135.47, 132.16, 131.55, 130.90, 127.70, 127.64, 126.24, 125.44, 59.10, 35.72, 30.85. FTIR (neat) 3468 (N-H Amide), 3425, 3041 (C-H Aromatic), 1646 (C=C), 1641 (C=O Amide), 1542, 1541, 1400, 1238, 983, 838, 775. HRMS: (ESI) m/z [M-Cl]1+ calcd. for $C_{15}H_{17}N_2O^+$: 276.1029, found: 276.1018.

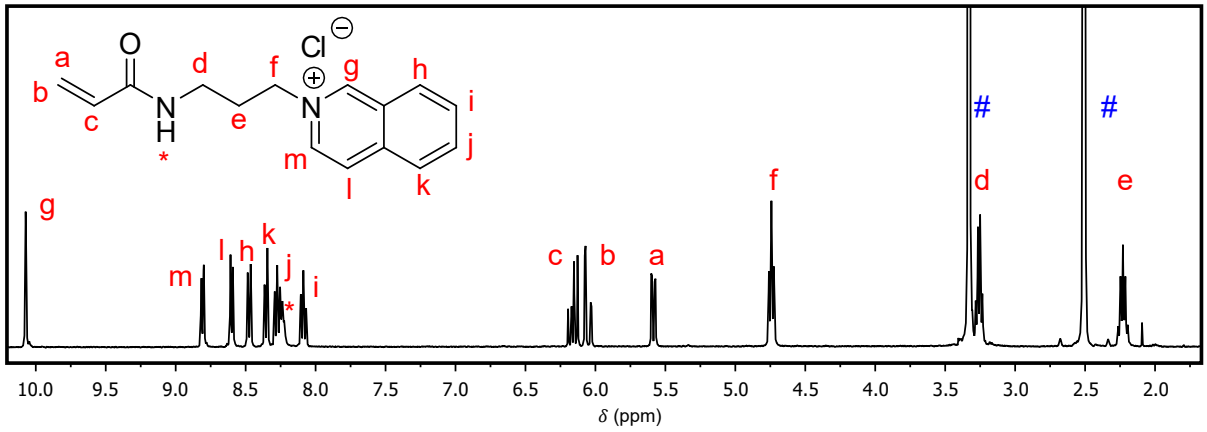

Figure S 2: ^{1}H NMR spectrum of IsoQPrAm. Recorded in DMSO-D_6. (# = DMSO, water)

We note that this synthesis is also possible in higher yield (>90%) using triethylamine (2 eqv.) as a base which can be removed by precipitation of the 2-(3-

acrylamidopropyl)isoquinolin-2-ium PF_6 into water, however a small amount <5% of TEA.HCl always remains after several precipitation cycles.

Products with different pyridine groups were synthesized via the same procedures as IsoQPrAm:

1-(3-acrylamidopropyl)-4-phenylpyridin-1-ium chloride – PhPyPrAm. 250mg (79% yield**)** ^{1}H NMR (400 MHz, Methanol-d4) δ 8.88 (d, J = 7.0 Hz, 2H), 8.32 (d, J = 7.0 Hz, 2H), 7.92 (dd, J = 7.7, 1.9 Hz, 2H), 7.73 – 7.43 (m, 3H), 6.31 – 6.04 (m, 2H), 5.60 (dd, J = 7.2, 4.7 Hz, 1H), 4.57 (t, J = 7.1 Hz, 2H), 3.31 (t, J = 6.5 Hz, 2H), 2.20 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 165.39, 155.09, 145.49, 134.01, 132.53, 132.25, 130.11, 128.59, 125.37, 124.91, 58.23, 35.75, 31.04. FTIR (neat) 3644 (N-H Amide), 3424, 3060 (C-H Aromatic), 1670 (C=C), 1640 (C=O Amide), 1520, 1476, 1178, 996, 819, 774 HRMS (ESI m/z [M-Cl]1+ calcd. for $C_{17}H_{19}N_2O^+$: 267.1497, found: 267.1487

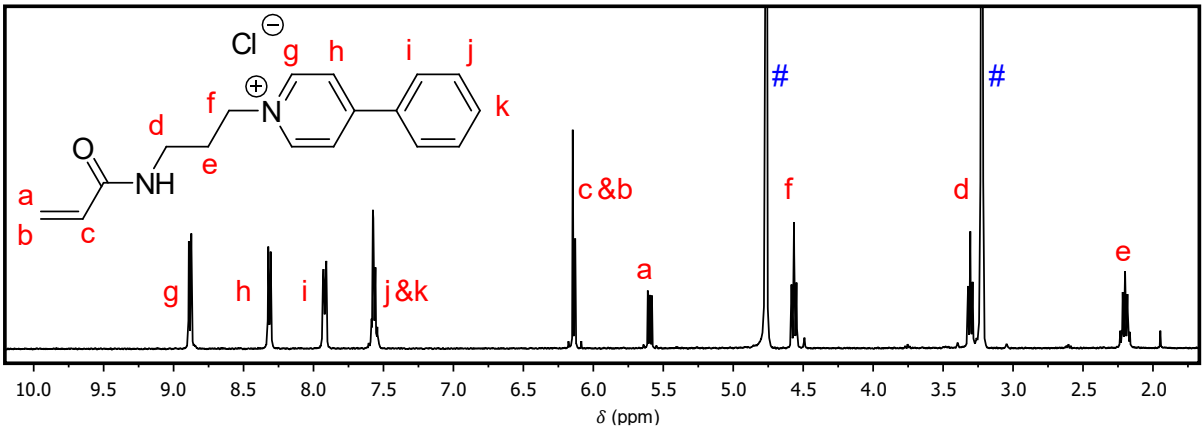

Figure S 3: ^{1}H NMR spectrum of PhPyPrAm. Recorded in MeOD-D_4. (# = MeOH, water)

1-(3-acrylamidopropyl)-4-(benzo[d]thiazol-2-yl)pyridin-1-ium chloride – BTPyPrAm. 252 mg (79% yield) ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (d, J = 6.9 Hz, 2H), 8.79 (d, J = 7.1 Hz, 2H), 8.40 (t, J = 5.6 Hz, 1H), 8.38 (d, J = 8.6 Hz, 1H), 8.28 (d, J = 8.2 Hz, 1H), 7.70 (dt, J = 16.5, 7.2 Hz, 2H), 6.24 (dd, J = 17.9, 10.4 Hz, 1H), 6.10 (d, J = 17.9 Hz, 1H), 5.61 (d, J = 10.5 Hz, 1H), 4.72 (t, J = 6.9 Hz, 2H), 3.25 (q, J = 6.3 Hz, 2H), 2.18 (p, J = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 165.39, 161.87, 153.84, 146.75, 146.54, 136.65, 132.15, 128.31, 128.21, 125.59, 125.37, 124.82, 123.69, 59.16, 35.78, 31.21. FTIR (neat) 3424 (N-H Amide), 3387, 3034 (C-H Aromatic), 1661 (C=C), 1640 (C=O Amide), 1524, 1485, 1318, 1259, 982, 861, 809, 776 . HRMS (ESI): m/z [M-Cl]1+ calcd. for $C_{18}H_{18}N_3OS^+$: 324.1171, found: 324.1168

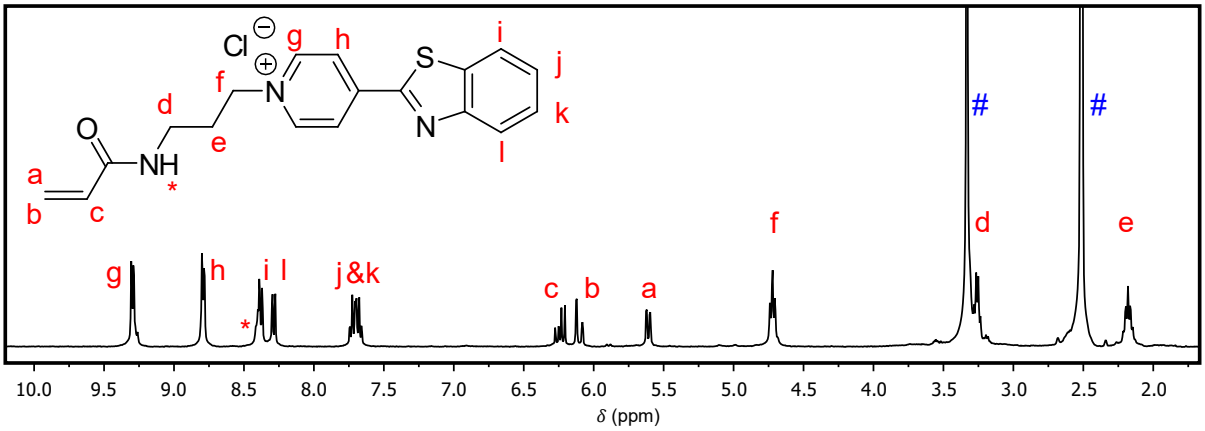

Figure S 4: ^{1}H NMR spectrum of BTPyPrAm. Recorded in DMSO-D_6. (# = DMSO, water)

1-(3-acrylamidopropyl)-1'-methyl-[4,4'-bipyridine]-1,1'-diium chloride - MVPrAm
269 mg (85% yield) ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (d, *J* = 6.8 Hz, 2H), 9.28 (d, *J* = 6.8 Hz, 2H), 8.79 (d, *J* = 6.7 Hz, 2H), 8.75 (d, *J* = 6.7 Hz, 2H), 8.25 (t, *J* = 5.8 Hz, 1H), 6.20 (dd, *J* = 17.1, 9.9 Hz, 1H), 6.10 (dd, *J* = 17.1, 2.3 Hz, 1H), 5.62 (dd, *J* = 9.9, 2.3 Hz, 1H), 4.71 (t, *J* = 7.1 Hz, 2H), 3.25 (q, *J* = 6.0 Hz, 3H), 2.19 (p, *J* = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 165.40, 148.98, 148.51, 147.12, 146.46, 132.12, 126.98, 126.56, 125.63, 59.28, 48.46, 35.78, 31.16. FTIR (neat) 3394 (N-H Amide), 3233, 3046 (C-H Aromatic), 2957, 2874 1646 (C=C), 1639 (C=O Amide), 1542, 1455, 1229, 1182, 879. 830. HRMS: m/z [M-2Cl]1+ calcd. for $C_{17}H_{21}N_3O_2^+$: 283.1685, found: 283.1672

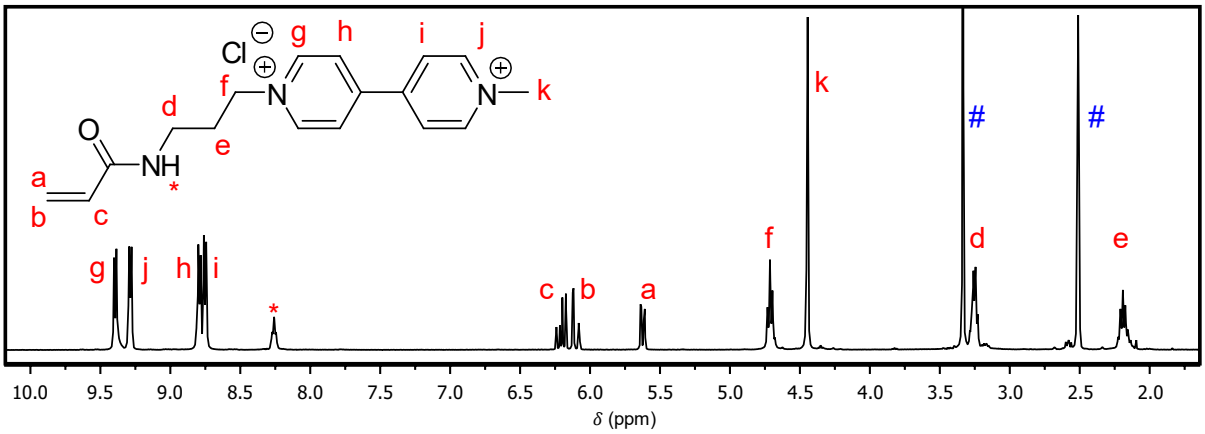

Figure S 5: ^{1}H NMR spectrum of MVPrAm. Recorded in DMSO-D_6. (# = DMSO, water)

Hydrogel synthesis – typical procedure

Hydrogels were prepared at 2 M total monomer concentration of which, 95 mol% comprised acrylamide while 5 mol% was alkyl pyridinium monomer, CB[8] was used in a 1:2 CB[8]:guest ratio in all cases. A typical procedure is detailed as follows: IsoQPrAm (27.7 mg, 0.1 mMol), AAm (135.1 mg, 1.9 mMol), CB[8] (66.5 mg 0.05 mMol) and VA-044 (1.6 mg, 4.8 µM, 0.24% compared to total monomer conc.) were added to 1 ml of water in a 6-dram vial equipped with a stirrer bar. A suba-seal was then forced into the top of a vial and secured with parafilm. The solution was sonicated in ice for 2 mins followed by bubbling with nitrogen in an ice bath for 30 minutes. After degassing the vial was submersed in a 50 °C oil bath with stirring for 16 hours. After the vial was removed from the oil bath it was kept sealed at room temperature for a further 24 h to ensure any evaporated water was reabsorbed. The hydrogel was removed by breaking the vial and ~8 mm samples were cut from it for rheological measurements using an upturned Pasteur pipette.

All other hydrogels were prepared in a similar manner with the following quantities of reagents:

Table S 1: Mass and number of moles of each reactant for each hydrogel synthesis.

Sample name	*Alkyl pyridinium quantity (mg, mmol)*	*Aam Mass quantity (mg, mmol)*	*CB[8] mass quantity (mg, mmol)*	*VA-044 quantity (mg, mmol)*	*Milli-Q water volume (ml)*
IsoQPrAm	27.7, 0.1	135.1, 1.9	66.5, 0.05	1.6, 0.0048	1
PhPyPrAm	30.2, 0.1	135.1, 1.9	66.5, 0.05	1.6, 0.0048	1
BTPyPrAm	40.0, 0.1	135.1, 1.9	66.5, 0.05	1.6, 0.0048	1
MVPrAm	35.4, 0.1	135.1, 1.9	66.5, 0.05	1.6, 0.0048	1
AAm	0, 0	142.2, 2.0	0, 0	1.6, 0.0048	1

Thermodynamic parameters from ITC

Table S 2: Thermodynamic data for alkyl pyridinium acrylamide complexes with CB[8] obtained by ITC (H_2O, 298K)

Guest	K_1 /M^{-1}	ΔH_1 /cal mol^{-1}	ΔS_1 /cal mol^{-1} K^{-1}
PhPyPrAm	$1.05 \pm 0.20 \times 10^7$	−8097 ± 165	4.96
IsoQPrAm	$3.63 \pm 0.41 \times 10^7$	−11210 ± 37	−3.00
BTPyPrAm	$8.65 \pm 0.16 \times 10^6$	−9837 ± 73	−1.26
MVPrAm	$1.20 \pm 0.18 \times 10^7$	−6072 ± 33	12
Guest	K_2 /M^{-1}	ΔH_2 /cal mol^{-1}	ΔS_2 /cal mol^{-1} K^{-1}
PhPyPrAm	$3.05 \pm 0.27 \times 10^5$	−4158 ± 113	11.1
IsoQPrAm	$3.62 \pm 0.18 \times 10^5$	−7080 ± 66	1.68
BTPyPrAm	$4.85 \pm 0.57 \times 10^5$	−4332 ± 466	11.5
MVPrAm	-	-	-

^{1}H NMR Titrations

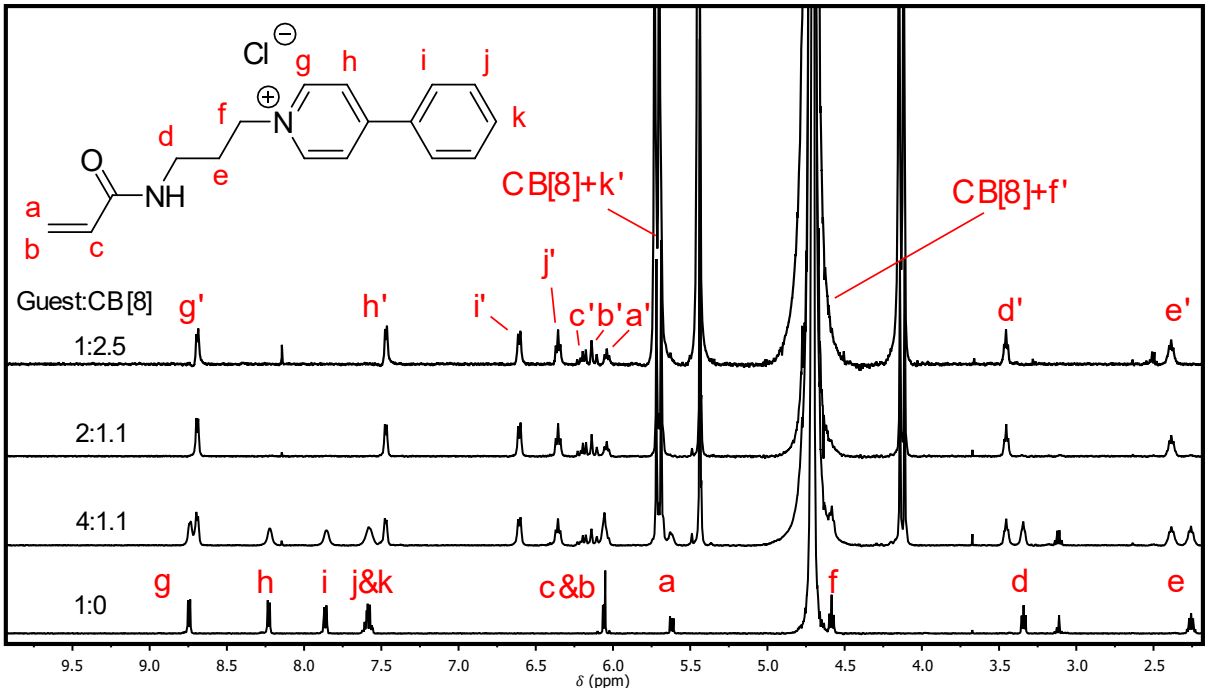

Figure S 6: Titration of PhPyPrAm into CB[8] monitored using ^{1}H NMR spectroscopy and showing upfield shifts for H_g-H_k and downfield shifts for H_a-H_f.

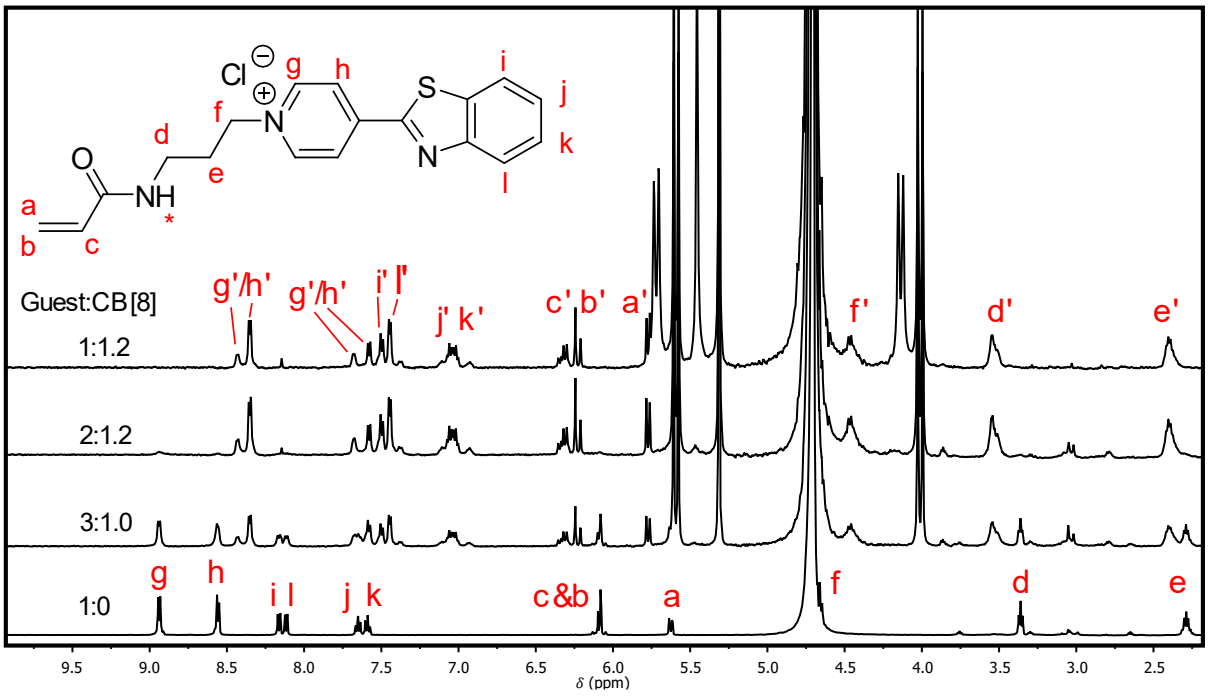

Figure S 7: Titration of BTPyPrAm into CB[8] monitored using ^{1}H NMR spectroscopy and showing upfield shifts for H_g-H_l and downfield shifts for H_a-H_f.

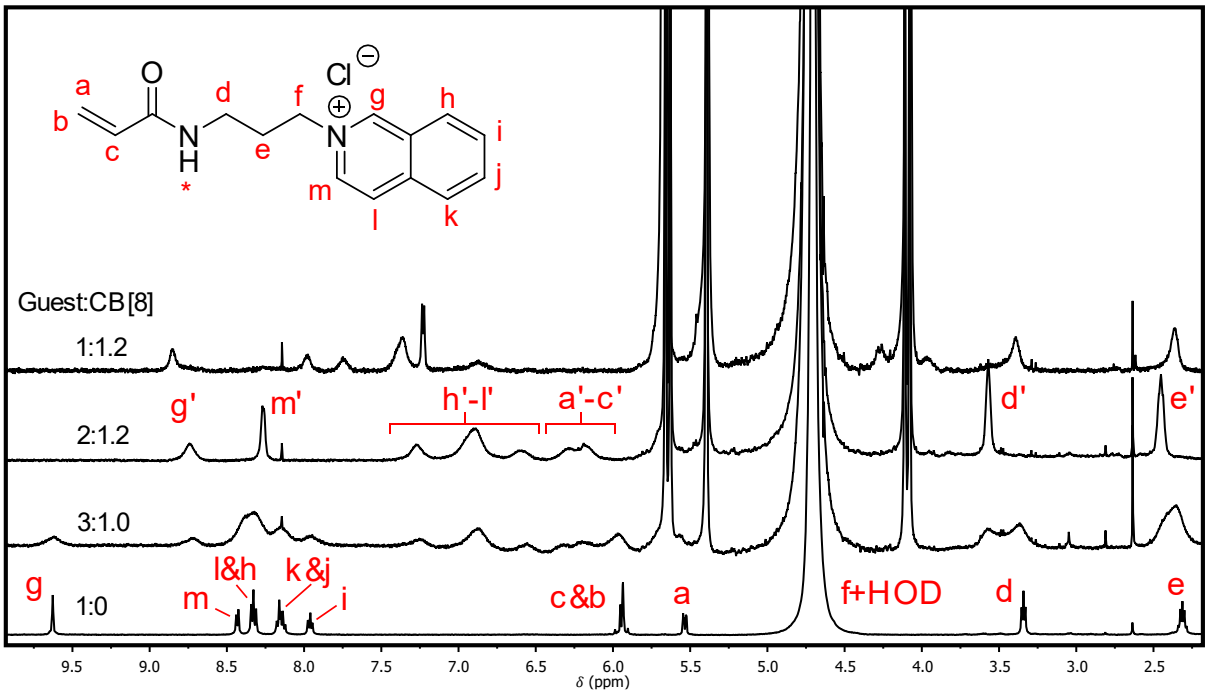

Figure S 8: Titration of IsoQPrAm into CB[8] monitored using ^{1}H NMR spectroscopy and showing upfield shifts for H_g-H_m and downfield shifts for H_a-H_f. The broad signals indicate fast exchange dynamics in the complexes on an NMR timescale.

Rheology

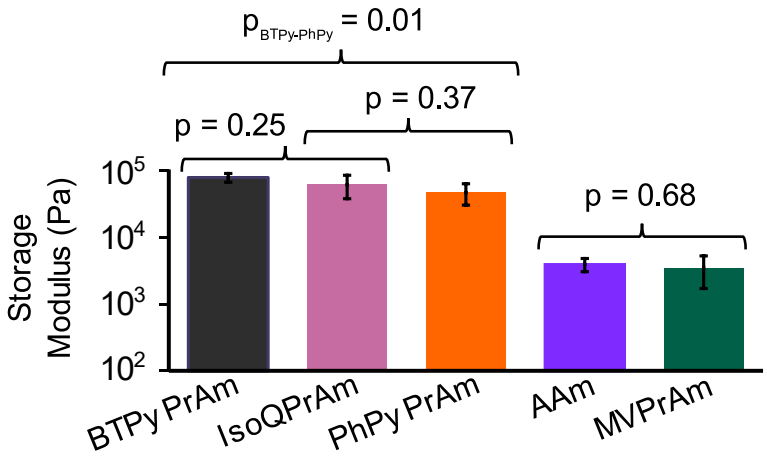

Figure S 9: Storage modulus (G') taken from the linear viscoelastic region of the amplitude sweep (Fig. 4a) error bars plotted as 95% confidence intervals, p-values are calculated from a two-sample unequal variance t-test.

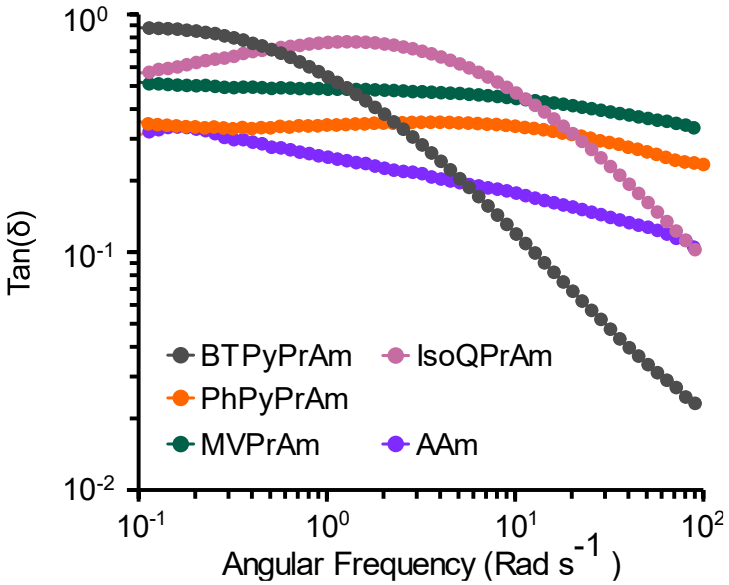

Figure S 10: Tan δ (G' /G'') vs. frequency calculated from the frequency sweep (Fig. 4c) performed at 1% strain**.**

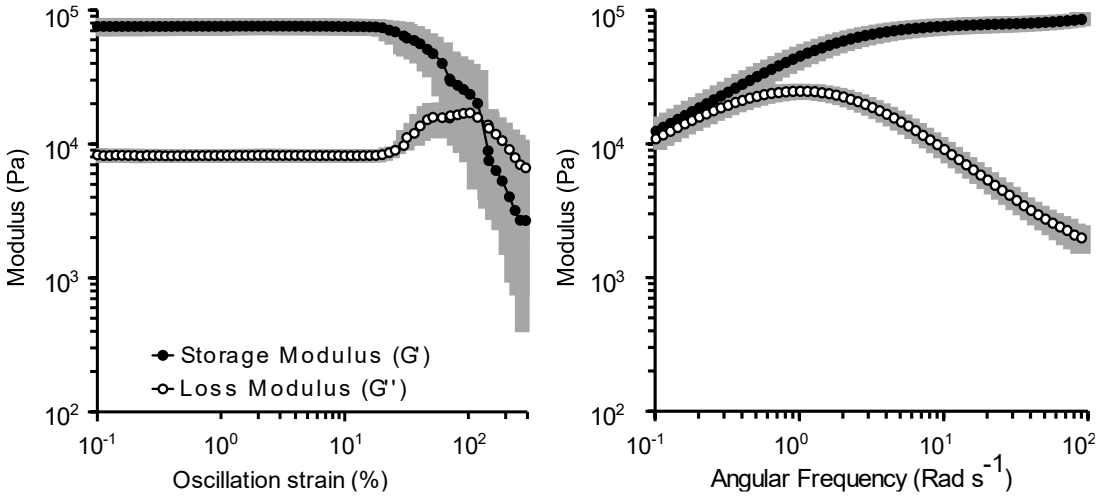

Figure S 11: Rheological characterisation of hydrogels prepared from BTPyPrAm. Left: amplitude sweep performed at 10 rad/s, right: frequency sweep performed at 1% strain. 95% confidence intervals plotted as shaded error bars.

intervals.

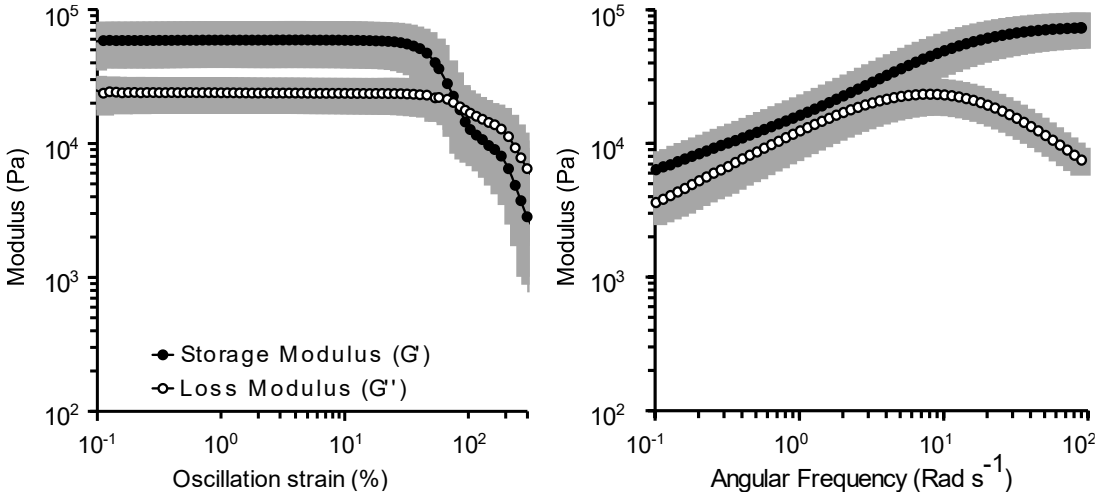

Figure S 12: Rheological characterisation of hydrogels prepared from IsoQPrAm. Left: amplitude sweep performed at 10 rad/s, right: frequency sweep performed at 1% strain. 95% confidence intervals plotted as shaded error bars.

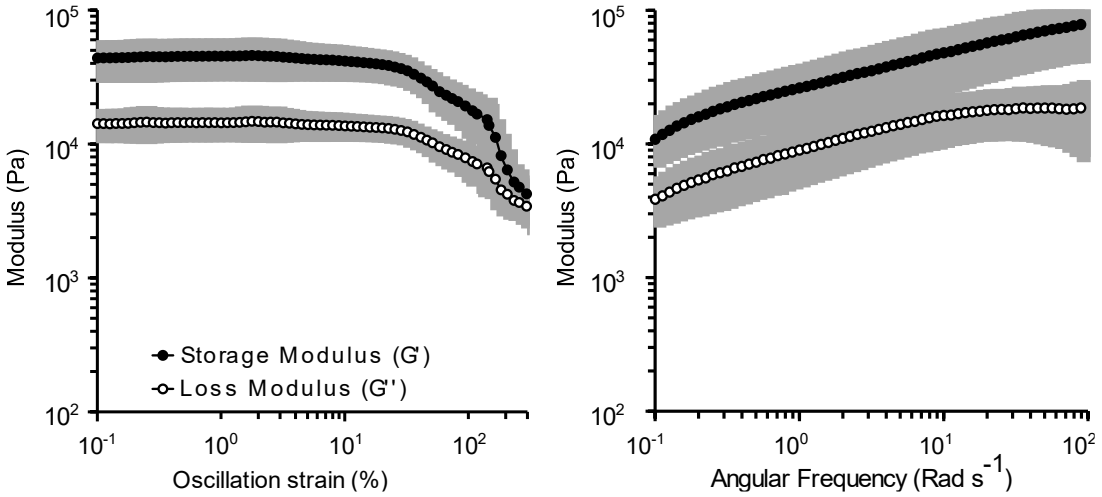

Figure S 13: Rheological characterisation of hydrogels prepared from PhPyPrAm. Left: amplitude sweep performed at 10 rad/s, right: frequency sweep performed at 1% strain. 95% confidence intervals plotted as shaded error bars.

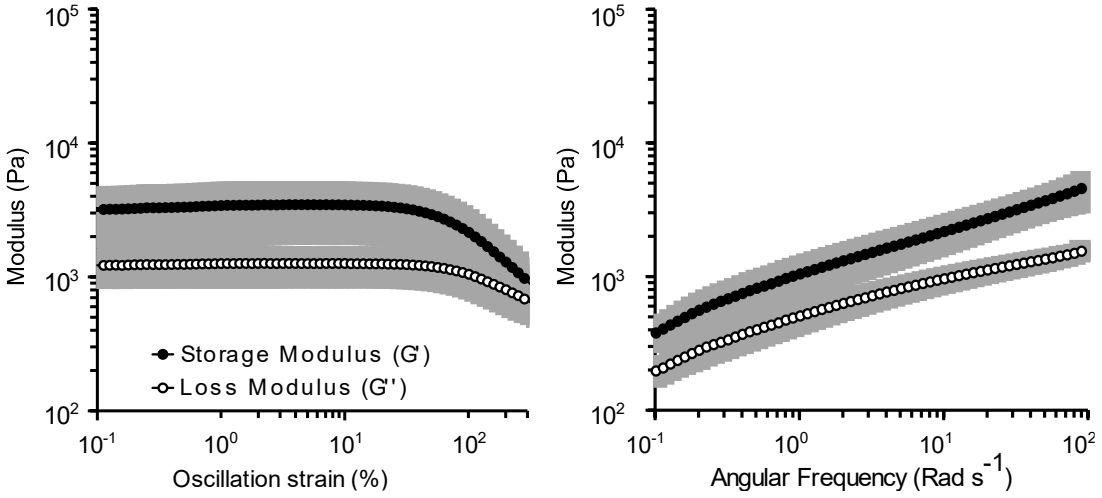

Figure S 14: Rheological characterisation of hydrogels prepared from MVPrAm. Left: amplitude sweep performed at 10 rad/s, right: frequency sweep performed at 1% strain. 95% confidence intervals plotted as shaded error bars.

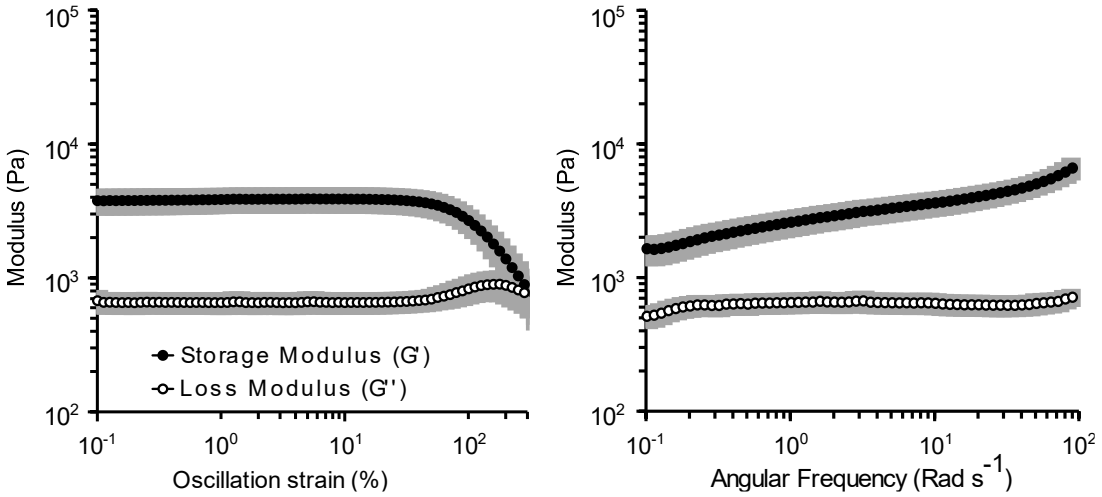

Figure S 15: Rheological characterisation of hydrogels prepared from pure acrylamide (AAm). Left: amplitude sweep performed at 10 rad/s, right: frequency sweep performed at 1% strain. 95% confidence intervals plotted as shaded error bars.

Fluorescence

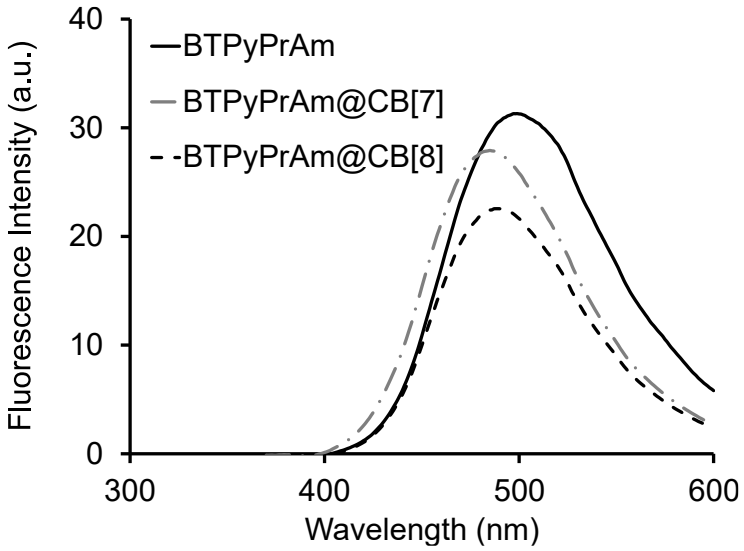

Figure S 16: Fluorescence emission spectrum for BTPyPrAm; unbound, with CB[7] and with CB[8].

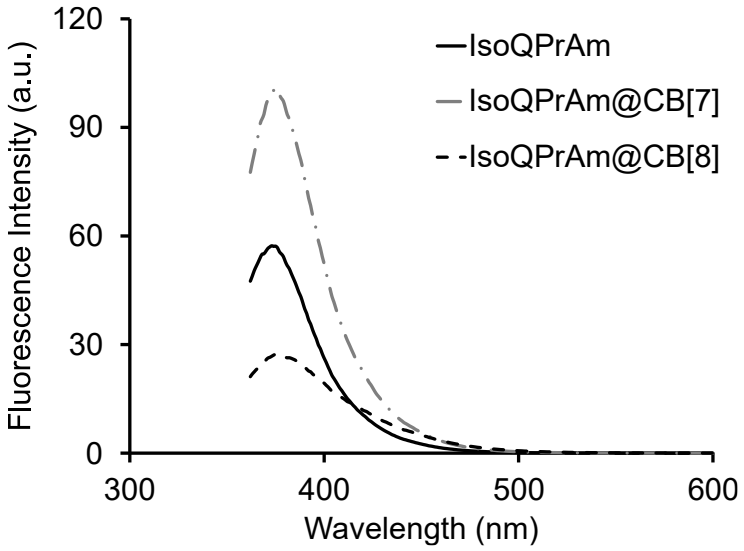

Figure S 17: Fluorescence emission spectrum for IsoQPrAm; unbound, with CB[7] and with CB[8].

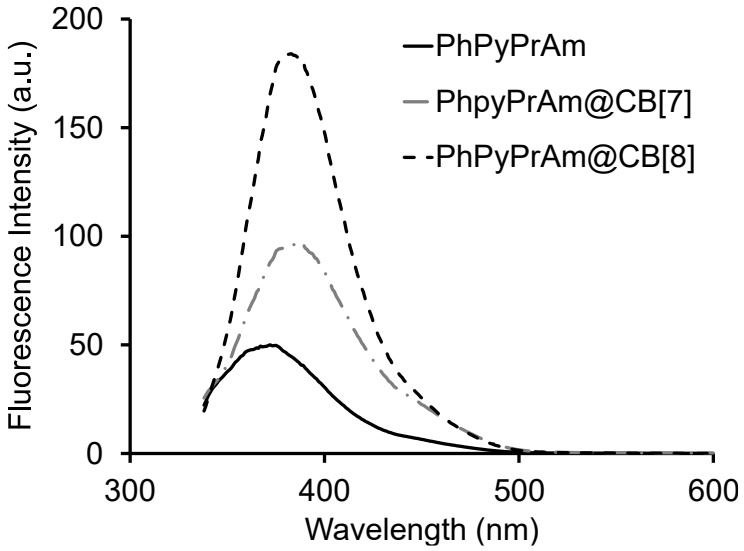

Figure S 18: Fluorescence emission spectrum for PhPyPrAm; unbound, with CB[7] and with CB[8].

Crystallographic data of PhPyPrAm

Suitable crystals for x-ray diffraction were prepared by recrystallisation of PhPyPrAm•PF_6 *via.* A vapour diffusion methodology in which diethyl ether was slowly diffused into a methanolic solution of PhPyPrAm•PF_6. The resulting crystals were colourless needles. A suitable crystal was selected, and data was collected on a Bruker D8-QUEST PHOTON-100 diffractometer. The crystal was kept at 180(2) K during data collection. The structure was solved with SHELXT[4] and refined with SHELXL[5].

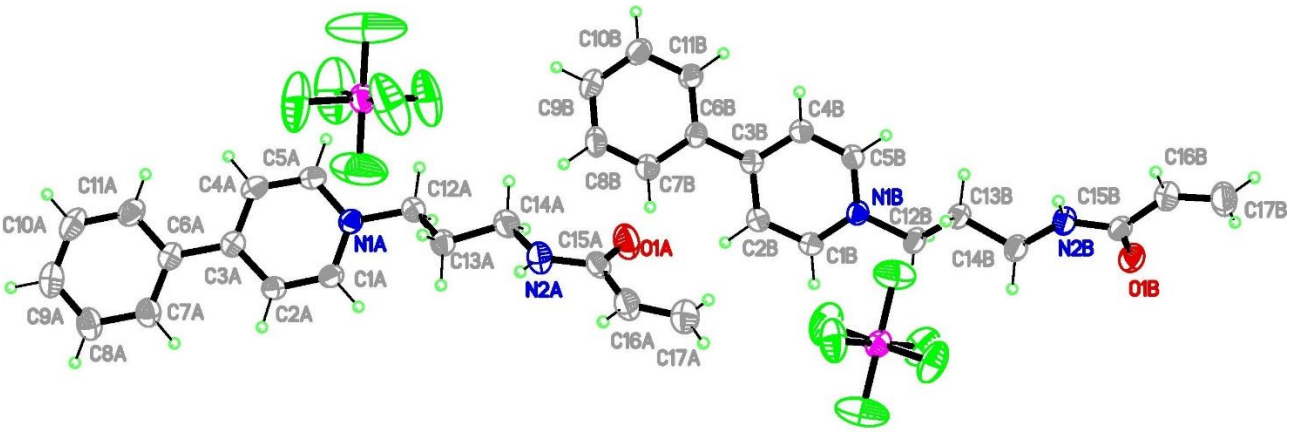

Figure S 19: Displacement ellipsoid plot of the asymmetric unit of PhPyPrAm.PF_6.

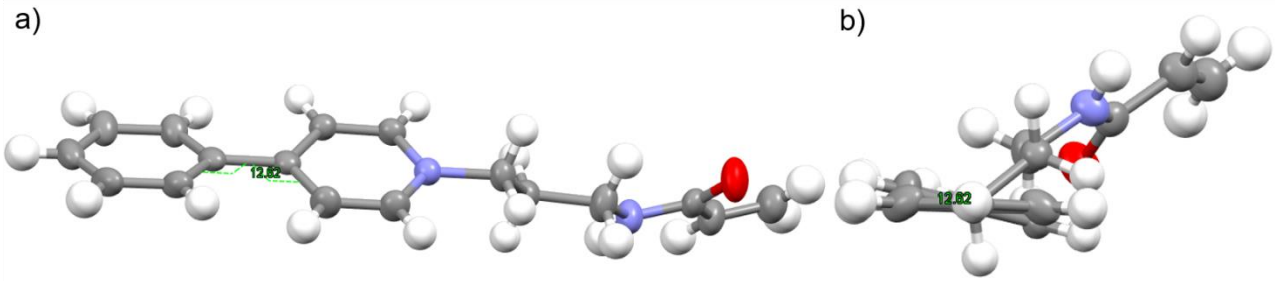

Figure S 20: Crystal structure of PhPyPrAm.PF_6 showing torsion angle of 12.62° between the phenyl and pyridyl rings. a) normal view. b) view along phenyl-pyridyl c-c bond.

Table S 3: Crystal data and refinement results for PhPyPrAm.PF_6.

Chemical formula	$C_{17}H_{19}F_6N_2OP$
M_r	412.31
Crystal system, space group	Monoclinic, $P2_1/c$
T (K)	180(2)
a, b, c (Å)	25.6008(9), 7.0486(2), 21.7397(7)
α, β, γ (°)	90, 114.9510(10), 90
V (Å^3)	3556.8(2)

Z	8
μ (mm^{-1})	2.038
Crystal shape, crystal size (mm)	Colourless block, 0.180 x 0.150 x 0.060
Index ranges	$-29 \leq h \leq 30$, $-8 \leq k \leq 8$, $-25 \leq l \leq 25$
Reflections collected	50740
Independent reflections	6314 [R_{int} = 0.0437, R_{sigma} = 0.0228]
T_{min}, T_{max}	
Data/ restraints/ parameters	6314/0/495
Goodness-of-fit on F^2	1.032
Final R indices [I>2 (I)]	R_1 = 0.0570, wR_2 = 0.1477
R indices (all data)	R_1 = 0.0667, wR_2 = 0.1554
Largest diff. peak and hole (e $Å^{-3}$)	1.03/-0.73

Determination of Critical Gel Concentration

Inverted vial tests were performed to determine whether the presence of alkyl pyridinium guests and CB[8] affected the critical gel concentration. The PhPyPrAm containing hydrogel was selected as a representative material to compare with a purely acrylamide gel. When using 100 mol% acrylamide a freestanding gel is formed at 2 M concentration while at 1 M the polymer network begins to slowly flow after inversion, at concentrations of 0.5 M and below the solution is free flowing (Figure S21). When using 95 mol% acrylamide with 5 mol% of PhPyPrAm a freestanding gel is formed from 2-0.5 M, slow flowing is observed from 0.4-0.3 M and a free-flowing solution is observed from 0.2 M and below (Figure S22) which is in good agreement with our previously reported hydrogels prepared from in-situ polymerisation.[6] Together these results demonstrate that the presence dynamic cross-linking greatly lowered the critical gel concentration providing further evidence of the alkyl pyridinium monomers ability to enhance the hydrogels mechanical properties.

Figure S 21: Photographs of hydrogels comprising 100 mol% acrylamide at different concentration. White text shows the overall polymer concentration in Mol dm^{-3}.

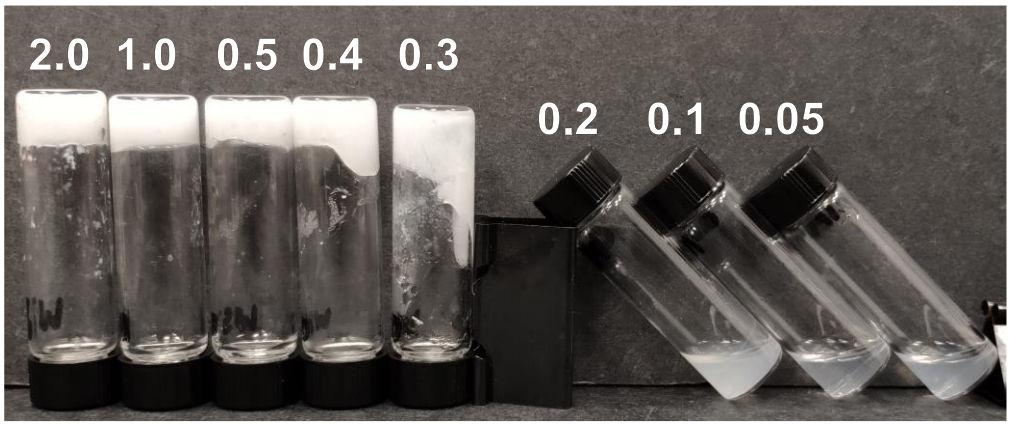

Figure S 22: Photographs of hydrogels comprising 95 mol% acrylamide and 5 mol% PhPyPrAm at different concentration. White text shows the overall polymer concentration in Mol dm^{-3}.

References

1. Hi, X., Guo, J., Liu, J., Ye, M. and Xu, Q., *Chem. Eur. J.,* **2015,** 21: 9988-9993.
2. Park Y. S., Lee E. J., Chun Y. S., Yoon Y. D., and, and Yoon K. B. *J. Am. Chem. Soc.* **2002** *124* (24), 7123-7135
3. Day, A.; Arnold, A. P.; Blanch, R. J.; Snushall, B. *J. Org. Chem*. **2001**, 66, 8094–8100.
4. Sheldrick, G. M. *SHELXT* -- Integrated space-group and crystal structure determination *Acta Crystallographica Section A,* **2015***, 71*, 3-8
5. Sheldrick, G. M.Crystal structure refinement with *SHELXL Acta Crystallographica Section C,* **2015***, 71*, 3-8
6. J. Liu, C. S. Y. Tan, Z. Yu, N. Li, C. Abell and O. A. Scherman, Adv. Mater., **2017**, 29, 1605325.