SUPPORTING INFORMATION

Fluorescence enables high throughput screening of polyelectrolyte – protein binding affinities

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Materials

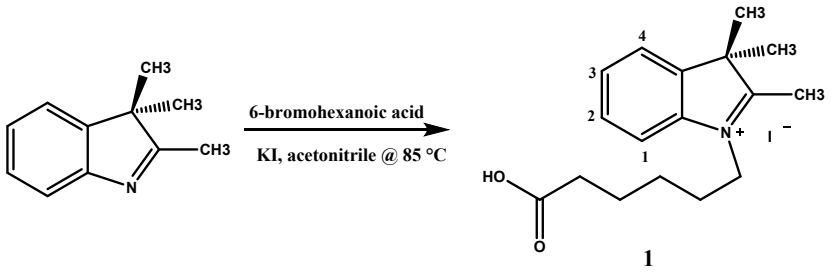
2,3,3-Trimethyl-3*H*-indole, 6-bromohexanoic acid, malonaldehyde bis(phenylimine) monohydrochloride, *N*,*N*′-Diphenylformamidine, and 1,2,3,3-tetramethyl-3*H*-indolium iodide were purchased from Sigma-Aldrich. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) was purchased from Fluka. N-Hydroxysuccinimide (NHS) was purchased from Sigma-Aldrich 4-(Dimethylamino)pyridine was purchased from Merck. *N*-(3-(Dimethylamino)propyl)acrylamide (DMAPAm) was purchased from Tokyo Chemical Industry. Acrylamide, 2-Hydroxyethyl acrylate, methyl acrylate, butyl acrylate, benzyl acrylate, and carboxyethyl acrylate were purchased from Sigma-Aldrich. All liquid monomers were de-inhibited prior to use by passing them over MEHQ inhibitor removal resin. Deuterated solvents were purchased from Cambridge Isotope Laboratories. 2,2′-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) was purchased from Wako and used as received. Deionized water was produced by a milli-Q reverse osmosis system and had a resistivity of 19.6 mΩ cm^{-1}. The RAFT agent 3-(benzylsulfanylthiocarbonyl-sulfanyl)- propionic acid (BSPA) was synthesized according to literature procedures.[1] Glucose oxidase (GOx) from *Aspergillus niger* was purchased from Sigma-Aldrich as lyophilized powder and used as received. All other materials and solvents were purchased from Sigma-Aldrich and used as received, unless otherwise indicated.

Physical and Analytical Methods

^{1}H-NMR spectra were acquired with Bruker 300 MHz or 400 MHz spectrometers and processed using TopSpin 4.1.4.

Gel permeation chromatography (GPC) for the polymers was performed on a Shimadzu modular system equipped with a differential refractive index detector and a three-column set of Agilent PL aquagel-OH 8 µm Guard (7.5 x 50 mm), Agilent PL aquagel-OH 8 µm 30 (7.5 x 300 mm) and Agilent PL aquagel-OH 8 µm MXED-M (7.5 x 300 mm) in series. 40% acetonitrile in water with 0.1% trifluoroacetic acid (TFA) was used as eluent. The apparent molecular weights and dispersities were estimated relative to narrow molecular weight distribution poly(ethylene glycol/oxide) calibration standards (Polymer Laboratories).

Synthesis of 1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indolium iodide (1)



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Synthesis of 1-(5-carboxypentyl)-2,3,3-trimethyl-3*H*-indol-1-ium iodide **1** was adapted from Simmons *et al*.[2] 2,3,3-Trimethyl-3*H*-indole (1.94 g, 12.18 mmol), 6-bromohexanoic acid (3.09 g, 15.84 mmol) and potassium iodide (2.63 g, 15.84 mmol) were heated to 85 ˚C in acetonitrile (30 mL) for 48 hours under reflux. After cooling to room temperature, solid potassium bromide was filtered out and the solution was concentrated *in vacuo*. The product was precipitated in diethyl ether. A yield of 2.91 g, 7.25 mmol (59.52 %) was obtained as a red solid. ^{1}H NMR (400 MHz, CD_3CN): δ = 7.76-7.78 (1H, m, H1), 7.69-7.71 (1H, m, H2), 7.59-7.61 (2H, m, H3 and H4), 4.37 (2H, t, *J* = 7.84 Hz, -CH_2Ar), 2.75 (3H, s, -CH_3), 2.29 (2H, t, *J* = 7.32 Hz -CH_2CO_2H), overlapped with solvent peak (2H, m, -$CH_2CH_2CO_2H$), 1.57-1.65 (2H, m, -CH_2CH_2Ar), 1.53 (6H, s, -$(CH_3)_2$), 1.43-149 (2H, m,- $CH_2CH_2CH_2CO_2H$) ppm.

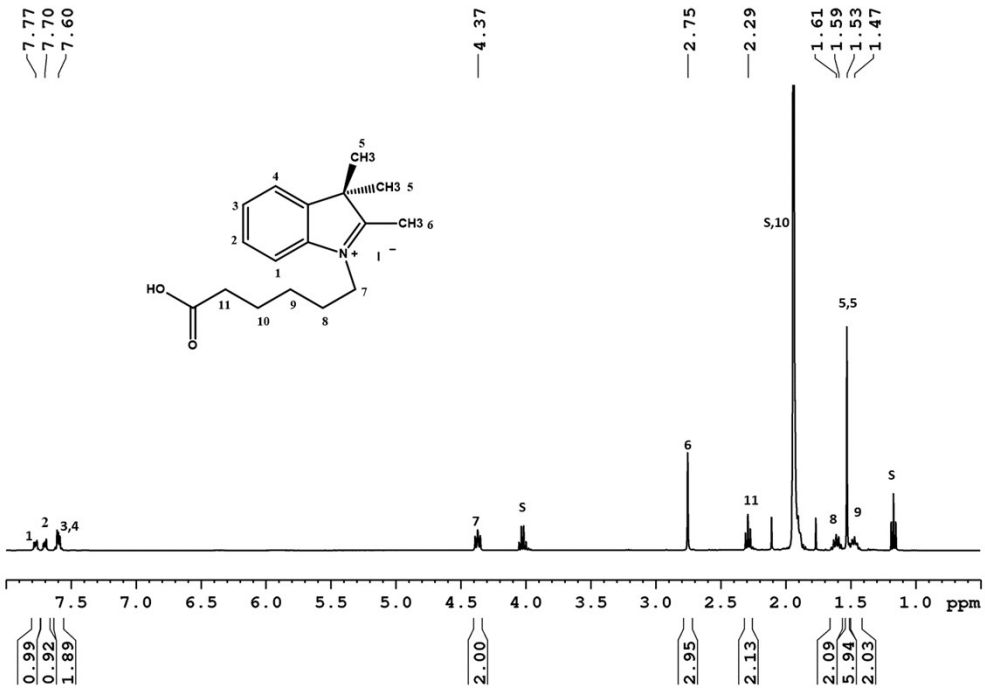
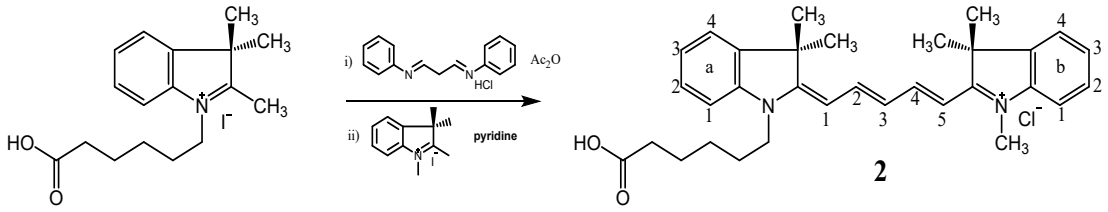


Figure S1 1H NMR (400 MHz, CD3CN) for indole (1), S: Solvent.

Synthesis of Cyanine 5 (2)



Synthesis of Cy5 **2** was adapted from Kvach *et al*.[3] Indole **1** (1.61 g, 4.01 mmol) and malonaldehyde bis(phenylimine) monohydrochloride (1.24 g, 4.81 mmol) were dissolved in acetic anhydride (25 mL) and heated to 120 ˚C for 30 minutes under reflux. After cooling to room temperature 1,2,3,3-tetramethyl-3H-indolium iodide (1.69 g, 5.62 mmol) and pyridine (25 mL) were added, the reaction stirred for 48 hours during which time it turned dark blue. The product was extracted in dichloromethane (100 mL), washed with water (2 x 100 mL) and brine (2 x 100 mL), dried with magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, (9% methanol–dichloromethane initially, grading to 12.5% methanol–dichloromethane) to provide 755.4 mg a blue solid (36.31%). Spectroscopic data were consistent with those previously reported.[3] ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84-7.93 (2H, m, $\underline{H}1_{a/b}$), 7.33-7.40 (4H, m, $\underline{H}2_{a/b}$ and $\underline{H}4_{a/b}$), 7.19-7.24 (2H,

m, H3$_{a/b}$), 7.06-7.11 (2H, m, H2$_v$ and H4$_v$), 7.00 (1H, t, *J* = 12.48 Hz, H3$_v$), 6.62 (1H, d, *J* = 13.52 Hz, H1$_v$), 6.41 (1H, d, *J* = 13.44 Hz, H5$_v$), 4.04 (2H, t, *J* = 7.88 Hz, -CH$_2$Ar), 3.71 (3H, s, ArMe), 2.53 (2H, t, *J* = 6.76 -CH$_2$CO$_2$H), 1.78-1.87 (4H, m, -CH$_2$CH$_2$Ar and -CH$_2$CH$_2$CO$_2$H), 1.70 (6H, s, Ar(CH$_3$)$_2$), 1.70 (6H, s, Ar(CH$_3$)$_2$), 1.54-1.61 (2H, m, -CH$_2$CH$_2$CH$_2$Ar) ppm.

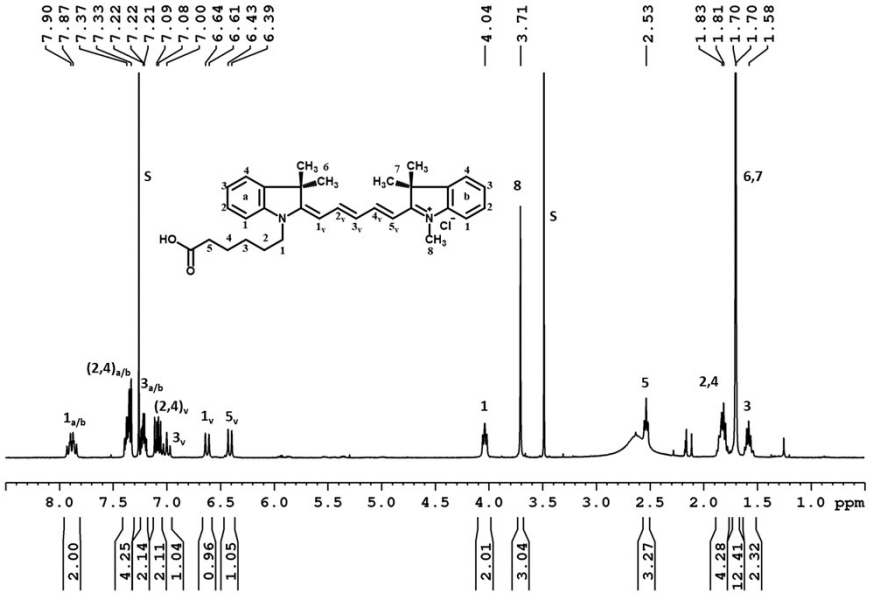
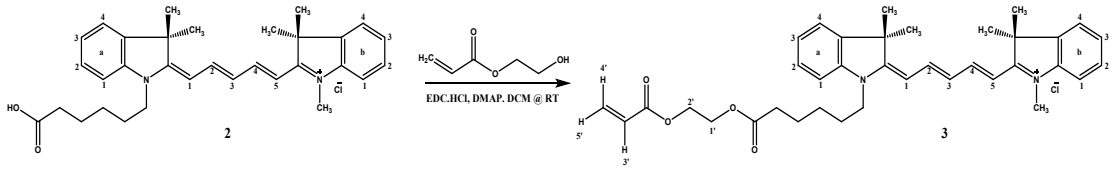


Figure S2 1H NMR (400 MHz, CDCl3) for Cy5 (2), S: Solvent.

Synthesis of Cy5 monomer (3)



Cy5 was coupled with 2-Hydroxyethyl acrylate via EDC coupling. Cy5 (0.32 g, 0.61 mmol), EDC.HCl (0.47 g, 2.46 mmol) and 4-Dimethylaminopyridine, DMAP, (0.08 g, 0.61 mmol) were dissolved in 3 mL DCM in a 10 mL round bottom flask. 2-Hydroxyethyl acrylate (0.14 g, 1.3 mmol) was added to the mixture and the reaction was stirred for 48 hours at room temperature. The residue was washed with water (2 x 25 mL) and brine (2 x 25 mL), dried with magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography, (9% methanol–dichloromethane) to provide 0.22 g a blue solid (36.31%). Spectroscopic data were consistent with those previously reported.[3] ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.25-8.38 (2H, m, H1$_{a/b}$), 7.33-7.38 (4H, m, H2$_{a/b}$ and H4$_{a/b}$), 7.19-7.23 (2H, m, H3$_{a/b}$), 7.10 (1H, d, *J* = 7.88 Hz, H2$_v$), 7.04 (1H, d, *J* = 8.36 Hz, H4$_v$), 6.79-6.86 (1H, m, H3$_v$), 6.32-6.48 (3H, m, H1$_v$, H5$_v$ & H5'), 6.10-6.17 (1H, m, H3'), 5.86 (1H, dd, *J* = 10.44, 1.36 Hz, H4'),

4.30-4.37 (4H m, H1' and H2') 4.05 (2H, t, *J* = 7.60 Hz, -CH_2Ar), 3.73 (3H, s, ArMe), 2.32-2.38 (2H, m, CH_2CO_2), 1.82-1.86 (2H, m, -CH_2CH_2Ar), 1.78 (6H, s, Ar(CH_3)$_2$), 1.74 (6H, s, Ar(CH_3)$_2$), 1.66-1.74 (2H, m, -$CH_2CH_2CO_2$H) 1.46-1.55 (2H, m, -$CH_2CH_2CH_2$Ar) ppm.

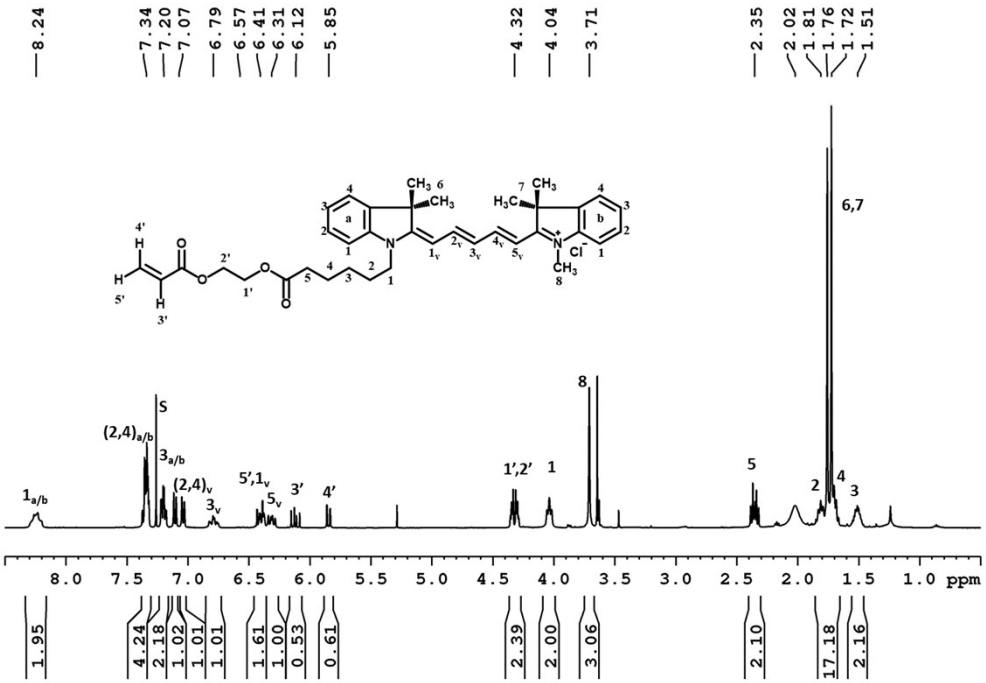


Figure S3 1H NMR (400 MHz, CDCl3) for Cy5 monomer (3), S: Solvent.

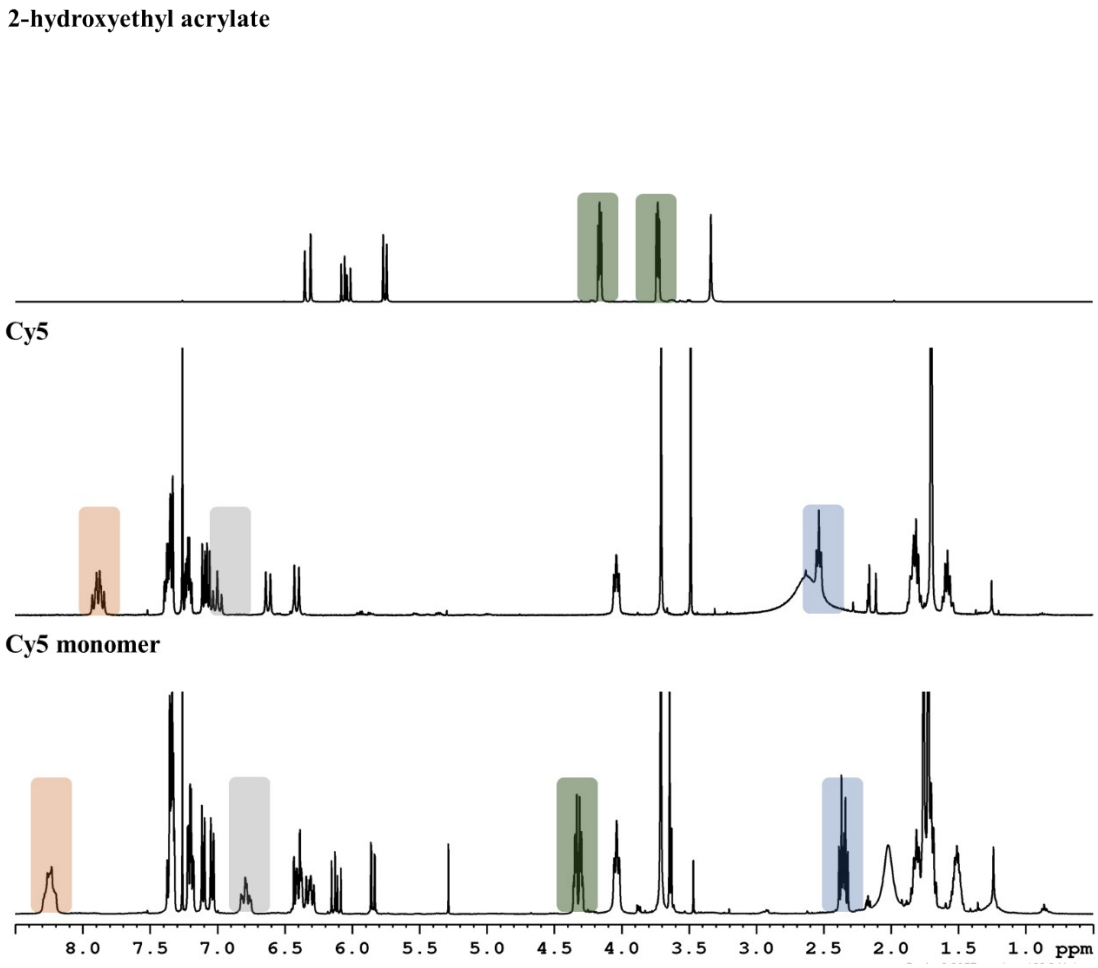


Figure S4 1H NMR (400 MHz, CDCl3) for Cy5 monomer, Cy5, and HEA monomer

Enz-RAFT polymerization using BSPA RAFT agent

Enzyme assisted RAFT polymerization[4,5] was utilised to synthesise the polymers library. Stock solutions of BSPA RAFT agent (0.26 M) and Cy5-moomer (0.02 M) were prepared in DMSO. Monomer (2M), VA-044 initiator (0.05 M), glucose (0.63 M), sodium pyruvate (1.00 M) and GOx (0.02 mM) in PBS buffer, hydrophobic monomers where prepared in DMSO. For a typical polymerization, monomer stock (125 µL, 0.25 mmol) was added to the RAFT agent (9.8 µL, 2.5 µmol) and initiator stocks (9.2 µL, 0.5 µmol) in an 8-strips PCR tubes. To this was added glucose (20 µL, 12.5 µmol), sodium pyruvate (12.5 µL, 12.5 µmol) and Cy5 monomer (25 µL, 0.5 µmol). After all components were mixed, GOx stock (25 µL, 0.5 nmol) was the last to be added and the final volume was adjusted to 250 µL with PBS. The tubes were capped and incubated at 44 ˚C with agitation at 100 rpm for 8 hours. At the end of the polymerization, 10 µL of the solution was diluted in D_2O for measurement of monomer conversion by ^1H NMR spectroscopy, 10 µL was diluted in 40% acetonitrile in water for measurement of the molecular

weight distribution. The polymers were used without further purification for spectroscopic measurements.

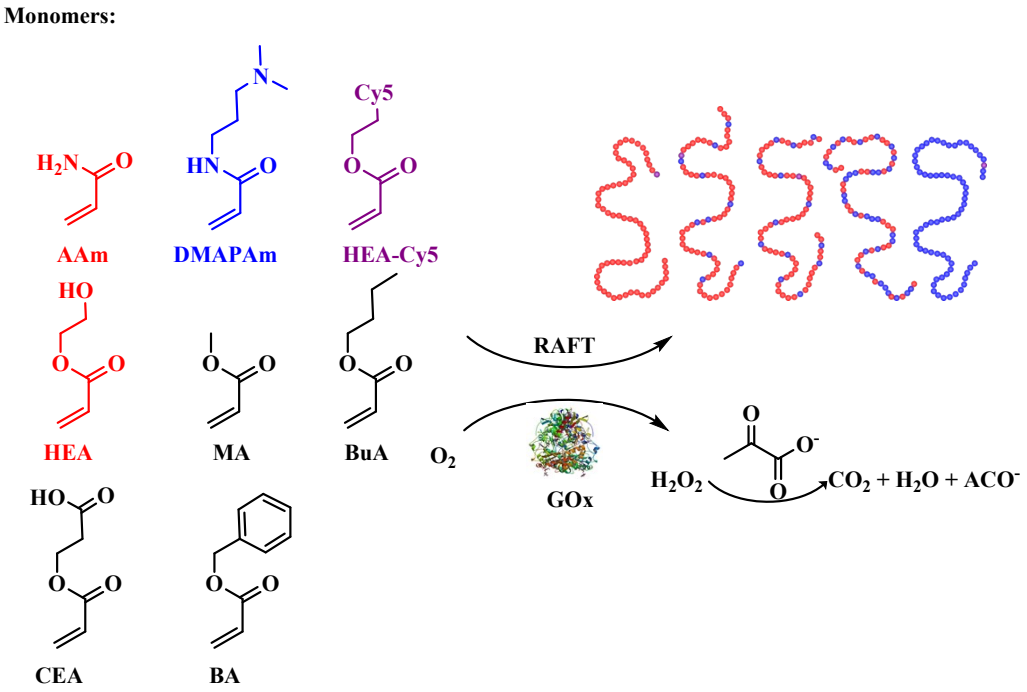


Figure S5 The structures of the monomers explored to synthesize fluorescent polymers through Enz-RAFT polymerization.

Table S1 Characterization data (1H NMR and GPC) for the synthesized fluorescent polymers. The polymerizations were performed in 8-strip PCR tubes, 250 µL/vial. The polymerizations were carried out for 7 hours in 14 % DMSO at 44 °C. [RAFT]/[initiator] = 5, [GOx] = 2 µM.

Polymer	Mw Theo (g mol^{-1}) [b]	(Đ) [c]
Poly(A_{75}-co-D_{20}-co-MA_5)	9200	1.32
Poly(H_{75}-co-D_{20}-co-MA_5)	12500	1.41
Poly(A_{75}-co-D_{20}-co-Bu_5)	9400	1.16
Poly(H_{75}-co-D_{20}-co-Bu_5)	12700	1.23
Poly(A_{75}-co-D_{20}-co-Bn_5)	9500	1.20
Poly(H_{75}-co-D_{20}-co-Bn_5)	12900	1.25
Poly(A_{70}-co-D_{20}-co-MA_{10})	9200	1.16
Poly(H_{70}-co-D_{20}-co-MA_{10})	12400	1.42
Poly(A_{70}-co-D_{20}-co-Bu_{10})	9700	1.17
Poly(H_{70}-co-D_{20}-co-Bu_{10})	12800	1.25
Poly(A_{70}-co-D_{20}-co-Bn_{10})	10000	1.23
Poly(H_{70}-co-D_{20}-co-Bn_{10})	13100	1.39

[a] Theoretical molecular weight calculated from monomer conversion. [b] Dispersity (= M_w/M_n) calculated from GPC.

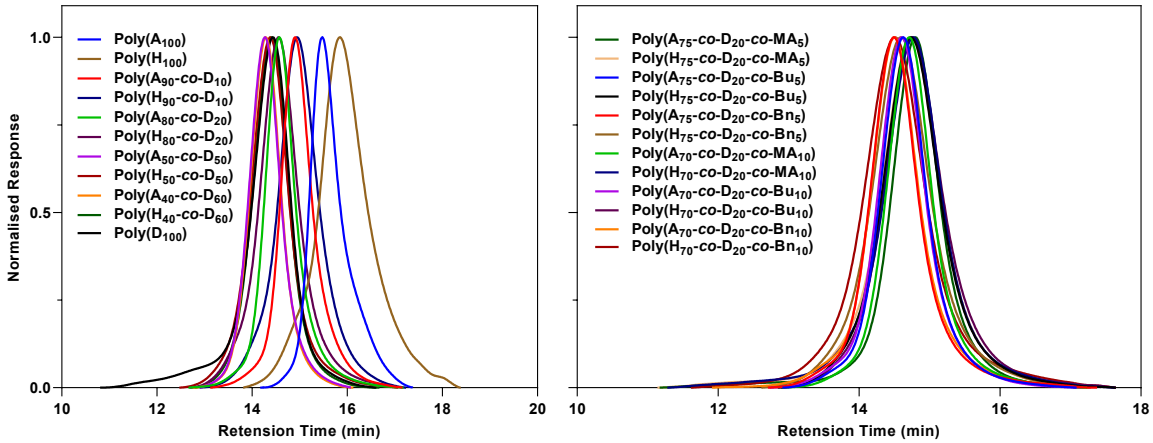


Figure S6 The GPC traces of the synthesized fluorescent polymers.

Labelling of glucose oxidase with cyanine dye, Cy3

GOx was labelled with Cy3-CO2H using EDC.HCl and NHS coupling reagents. Stock solutions of Cy3-CO_2H (1.23 mg/mL), EDC.HCl (3.59 mg/mL) and NHS (5.76 mg/mL) were prepared in MES buffer (100 mM, pH 5.5). To 200 µL MES buffer in 4.0 mL vial was added 100 µL of Cy3-CO2H (0.25 µmol), 100 µL EDC.HCl (1.87 µmol) and 100 µL NHS (5.00 µmol) stock solutions. The mixture was stirred for 15 minutes at room temperature. To that was added (10 mg, 0.06 µmol) GOx in 1.0 mL carbonate buffer (50 mM, pH 9.6). The reaction continued for 2 hours. The labelled GOx was separated using sephadex G-15 column. Aliquots of 2.0 mg/mL GOx-Cy3 were stored at −20 °C until use.

FRET measurements using fluorometer

FRET measurements were performed on a Cary Eclipse fluorescence spectrophotometer (Agilent) using a quartz cuvette of 1.0 cm path length at 25 °C. Stock solution of GOx-Cy3 and Cy5 labelled polymer solutions were prepared in PBS buffer. Polyion complexes were prepared by titrating stock solution of Cy5 labelled polymers with GOx-Cy3 solutions in the cuvette to yield polymer/GOx molar ratio ranging from 1.0 to 6.0. Mixture made from Poly(A_{100}), Poly(A_{50}-*co*-D_{50}) and Poly(D_{100}), were excited at 500 nm and fluorescence intensity (FI) was measured from 510-800 nm.

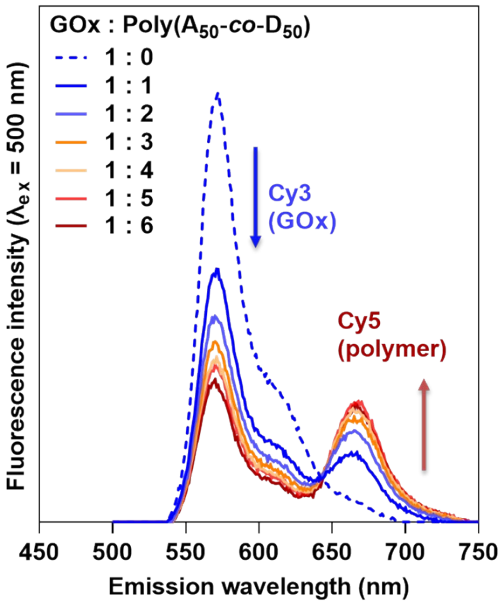


Figure S7 Fluorescence spectra of Poly(A50-co-D50) polymer mixed with GOx in increasing molar equivalents in PBS.

FRET measurements using fluorescence microplate reader

Experiments were performed on Molecular Devices microplate reader equipped with SoftMax® Pro 4.3 software. PIC particles were prepared by mixing 30 µL polymer and 30 µL GOx-Cy3 (0.5 mg/mL) to give polymer/protein molar ratio ranging from 1.0 to 16.0 in 96-well plates at 25 °C in PBS buffer. Samples were excited at 485 nm. FI for Cy3 and Cy5 were measured at 570 nm and 670 nm, respectively.

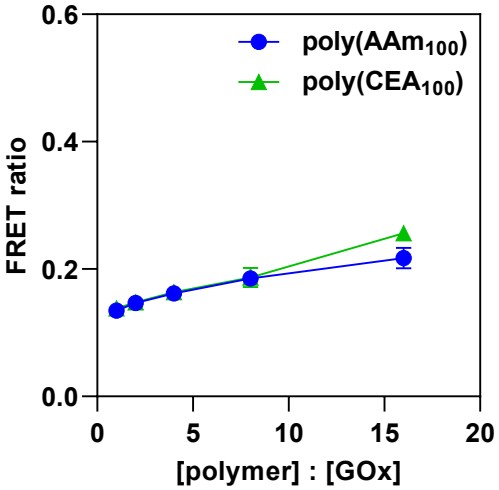


Figure S8 FRET ratio of polyacrylamide and poly(carboxyethyl acrylate) polymers after mixing with Cy3-GOx at increasing molar equivalents in PBS.

Small-Angle X-ray Scattering

SAXS experiments were carried at the Australian Synchrotron on the small-/wide-angle X-ray scattering beamline using X-rays with a wavelength, λ, of 1.127 Å. Isotropic scattering patterns were collected on a Pilatus 1 M detector with an active area of 981 × 1043 pixels of 170 m^2 each with a 2.7 m sample to detector distance. The magnitude of scattering vector (q) is defined by $q = 4\pi/\lambda \sin(\theta/2)$, where θ is the scattering angle. The samples were placed in a 96-well plate solution autoloader from where samples were taken automatically, and the SAXS was measured in a consistent position in a quartz capillary. Scatterbrain (ANSTO) was used to radially average the raw pixels in the SAXS images and subtract the background patterns, which were collected for each individual solvent/LiCl mixture. The data were then fit using the NIST NCR scattering software package in IGOR Pro.32. Where the errors in the data at low Q were very large, the data points indicated in the figures were excluded from the fit.

Table S2 Sphere model fitting parameters for small angle X-ray scattering (SAXS) fits shown in Figure 2. Data obtained` upon assembly of the polymers with GOx (poly/GOx = 6) in PBS.

Polymer	*GOx*	*Poly(A_{100})*	*Poly(A_{90}-co-D_{10})*	*Poly(A_{80}-co-D_{20})*	*Poly(A_{50}-co-D_{50})*	*Poly(A_{40}-co-D_{60})*	*Poly(D_{100})*
Volume Fraction (scale)	3.41E-04	6.92E-04	7.08E-04	7.20E-04	7.13E-04	7.24E-04	7.25E-04
Radius (nm)	3.18	3.19	3.18	3.19	3.34	3.35	3.43
SLD core ($Å^{-2}$)	1.00E-06	1.00E-06	1.00E-06	1.00E-06	1.00E-06	1.00E-06	1.00E-06
SLD solvent ($Å^{-2}$)	3.00E-06	3.00E-06	3.00E-06	3.00E-06	3.00E-06	3.00E-06	3.00E-06

The core polydispersity is fixed as 0.28

Dynamic light scattering

DLS was performed using a Malvern Zetasizer Nano ZS instrument equipped with a 4 mV He-Ne laser operating at λ = 632 nm and noninvasive backscatter detection at 173°. Stock solution of GOx (1.0 mg/mL) was prepared in PBS. The nanoparticles were prepared by mixing stock solutions of the desired polymers with GOx to have 6.0 molar equivalents of polymer relative to GOx in 0.5 mL solution.

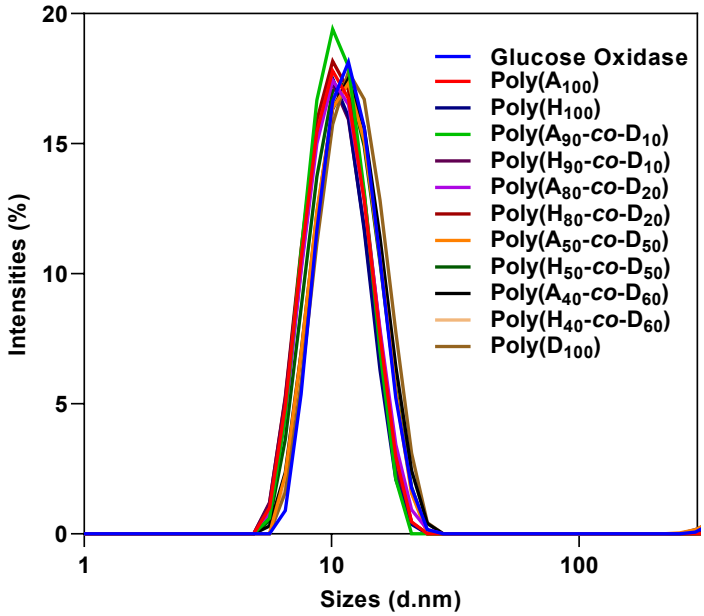


Figure S9 DLS results upon assembly of the polymers with GOx (poly/GOx = 8) in PBS.

Isothermal Titration Calorimetry

ITC measurements were conducted on a GE Healthcare iTC200 isothermal calorimeter. The polymers and GOx stock solutions were dissolved in the same PBS buffer to ensure that all the compositions have similar pH value and salt contents. In the microcalorimeter, two cells were used: the reaction cell containing 200 µL of 0.1 mM GOx while the reference cell containing 200 µL distilled water. Stock solutions of polymers (2.5 mM) were injected into GOx solution with 2 µL injection volume at 150 seconds intervals. This time is required by the reference cell to compensate for the heat difference generated during the binding and return the two cells to equal temperature. After 20 injections, the change in heat signal was insignificant. The experiment was run at 25 °C with constant stirring at 750 rpm. Control experiments were conducted by injected the polymers solution into PBS. The collected data were then fitted using MicroCal LLC ITC program in Origin after deducting the heat from the control measurements.

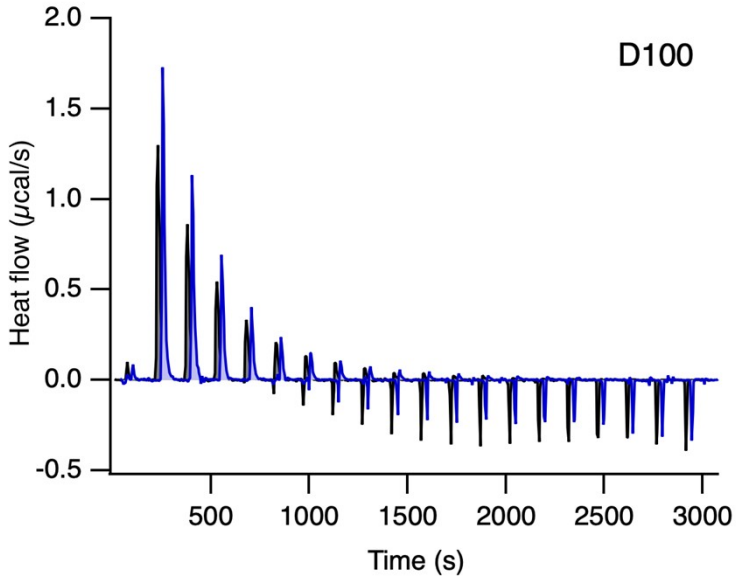


Figure S10: Raw ITC data for duplicate titrations of 2.5 mM of P(D_{100}) into GOx (0.1 mM) in PBS.

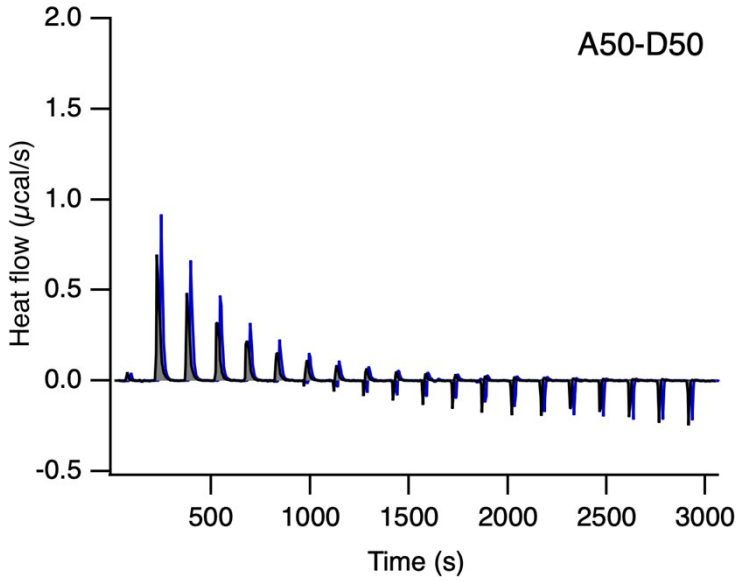


Figure S11: Raw ITC data for duplicate titrations of 2.5 mM of P($A_{50}D_{50}$) into GOx (0.1 mM) in PBS.

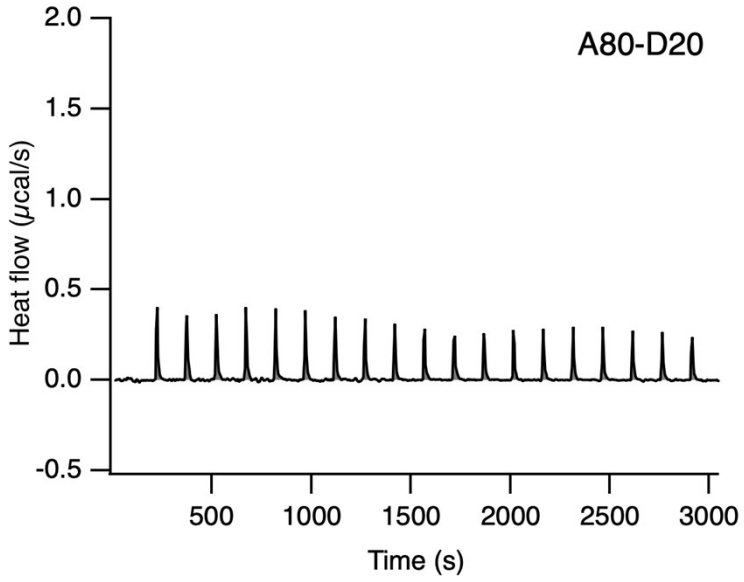


Figure S12: Raw ITC data for a titration of 2.5 mM of $P(A_{80}D_{20})$ into GOx (0.1 mM) in PBS.

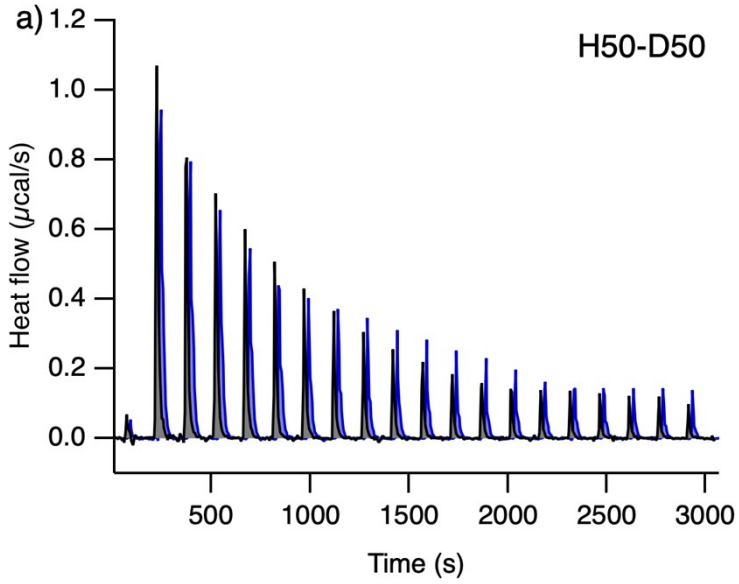


Figure S13: Raw ITC data for duplicate titrations of 2.5 mM of $P(H_{50}D_{50})$ into GOx (0.1 mM) in PBS.

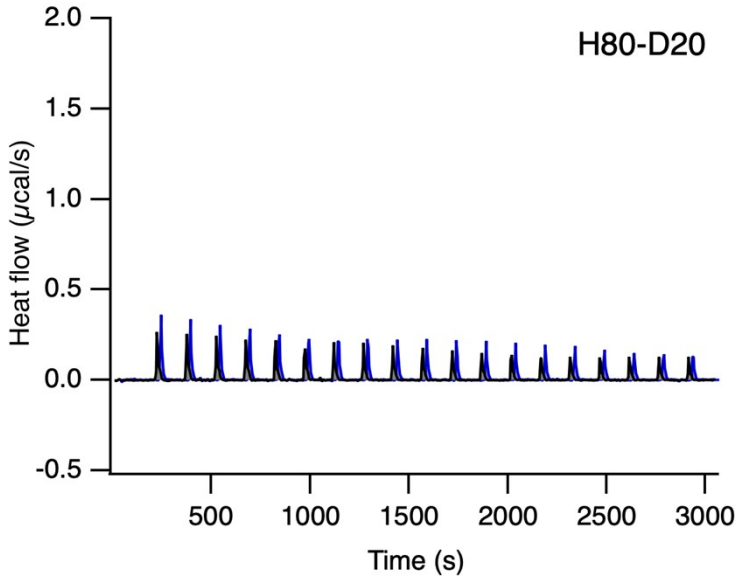


Figure S14: Raw ITC data for a titration of 2.5 mM of $P(H_{80}D_{20})$ into GOx (0.1 mM) in PBS.

References

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