Supporting information

Synthetic macromolecular peptide-mimetics with amino acid substructure residues as protein stabilising excipients

Ruggero Foralosso,[a] Rafał Jerzy Kopiasz,[a,b] Cameron Alexander, [a] Giuseppe Mantovani[a] and Snow Stolnik[a]

[a]University of Nottingham, School of Pharmacy, NG7 2RD, UK
[b] Warsaw University of Technology, Faculty of Chemistry, Noakowskiego 3 St., 00-664, Warsaw, Poland

Materials and methods

Materials

N-hydroxyethylacrylamide, indole 3-acetic acid, phenylacetic acid, methylbutyric acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl, 4-dimethylaminopyridine, bovine pancreatic insulin, hen egg lysozyme, DPBS buffer, *N,N*-diisopropylethylamine (DIPEA), triethylamine (TEA), 4,4'-azobis(4-cyanovaleric acid) (V-501), biotech grade DMF, anhydrous dichloromethane, anhydrous MeOH, piperidine, Fmoc-leu-OH, Fmoc-ile-OH, Fmoc-arg(pbf)-OH, Fmoc-gly-OH, Fmoc-asn-OH, Fmoc-trp(boc)-OH, Fmoc-ser(tbu)-OH, Fmoc-gln(trt)-OH, (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride) (DMTMM), ethylene-dioxy-bis-ethylamine, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) were obtained from Sigma-Aldrich (St. Louis, Missouri, United States). Azobisisobutyronitrile (AIBN) was purchased from FUJIFILM Wako Pure Chemical, Ltd.

Isopropanol, methanol, dichloromethane (DCM), ethyl acetate, diethyl ether, chloroform, tetrahydrofuran (THF), petroleum ether, *N,N*-dimethylformamide (DMF) and acetone were purchased from Fisher Scientific (Bishop Meadow Rd, Loughborough, UK).

Di-*tert*-butyl dicarbonate, fluorescein isothiocyanate and disuccinimidyl carbonate were obtained from Sigma-Aldrich (St. Louis, Missouri, United States). 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) was purchased from Wako Pure Chemical Industries. 3-[Dimethyl-(2-hydroxyethyl)ammonio]-1-propanesulfonate betaine was purchased from Merck Biosciences Ltd, Padge Rd, Beeston, UK.

Peptide IDR 1018 was a kind gift from MedImmune LTD (Cambridge, United Kingdom).

Chemical characterisation methods

^{1}H NMR spectra were recorded at 25 °C on a Bruker Advance III 400 MHz spectrometer. All chemical shifts are reported in ppm (δ) referenced to the chemical shifts of residual solvents resonances of DMSO-d_6 (2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C NMR spectra) and $CDCl_3$ (7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C NMR spectra).

Size exclusion chromatography: a Polymer Laboratories PL-50 instrument equipped with differential refractive index (DRI), viscometry (VS) and dual angle light scatter (LS) was used for SEC analysis. The system was fitted with 2 × PLgel Mixed D columns (300 × 7.5 mm) and a PLgel 5 µm guard column. The eluent used was DMF with 0.1% LiBr. Samples were run at 1 ml min^{-1} at 50 °C.

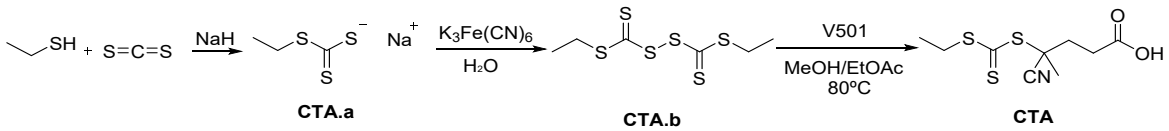
Poly(methyl methacrylate) standards (Agilent EasyVials) were used for calibration between 955 500–550 g mol^{-1}. Analyte samples were filtered through a membrane

with 0.22 µm pore size before injection. Respectively, experimental molar mass ($M_{n,SEC}$) and dispersity (***Đ***) values of synthesized polymers were determined by conventional calibration using Cirrus GPC software.

RP-HPLC was run at a flow rate of 1 mL/min, using a 20-80% acetonitrile gradient in Milli-q water + 0.1% TFA, on a C18 Zorbax Eclipse Plus C18 column (3.5 µm, 95 Å, 4.6 × 12.5 mm). Absorbance was recorded at λ=280 nm.

1) Chain transfer agent (CTA) synthesis

*4-Cyano-4-(((ethylthio)carbonothioyl)thio)pentanoic acid (**CTA**)*



Scheme S1. Synthesis of the chain transfer agent (CTA).

*Sodium ethyl carbonotrithioate (**CTA.a**)***.*** NaH (60 wt % in mineral oil, 2.82 g, 70.4 mmol, 1.06 eq.) was dispersed in diethyl ether (50 mL) and cooled down in an ice bath. Ethanethiol (4.35 g, 70.0 mmol, 1 eq.) was added dropwise under stirring to the suspension and the mixture was stirred for 10 minutes. CS_2 (5.8 mL, 96 mmol, 1.4 eq.) was then added dropwise to the suspension and the reaction was stirred at room temperature for 1 h. The resulting bright yellow solid was filtered and washed with diethyl ether, dried under vacuum (yield: 80 %) and used for the following step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.94 (q, *J*=7.4 Hz, 2H, CH_3CH_2S), 1.13 (t, *J*=7.4 Hz, 3H, CH_3CH_2S); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm): 239.3, 33.8, 14.01.

*Dithiobis-ethyl carbonotrithioate disulfide (**CTA.b**)***.*** **CTA.a** obtained from the previous step was dissolved in 100 mL of an aqueous solution of $K_3Fe(CN)_6$ (16 g, 49 mmol). The mixture was extracted with diethyl ether (4 X 500 mL) and the organic layers were collected and dried over $MgSO_4$. Following filtration, the solvent was removed under reduced pressure, yielding product **CTA.b** as an orange, viscous oil. Yield: 56%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.32 (q, *J*=7.5 Hz, 4H, CH_3CH_2S), 1.36 (t, *J*=7.5 Hz, 6H, CH_3CH_2S); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm): 221.91, 33.10, 12.98.

*4-Cyano-4-(((ethylthio)carbonothioyl)thio)pentanoic acid (**CTA**)*. **CTA.b** (2.80 g, 13.2 mmol, 1 eq.) and V501 (5.38 g, 19.2 mmol, 1.45 eq.) were dissolved in 1:1 v/v ethyl acetate:methanol (80 mL). The solution was degassed for 30 min by argon bubbling, under stirring, then heated to reflux for 17 h. The volatiles were then removed under reduced pressure, and the resulting yellow oily residue was purified by silica gel flash chromatography, using a petroleum ether:Et_2O in gradient, from 9:1 to 7:3 v/v, as the mobile phase. Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.33 (q, *J*=7.4 Hz, kH, CH_3CH_2S), 2.67 (m, 2H, CH_2CH_2COOH), 2.53-2.39 (m, CH_2CH_2COOH), 1.87(s, CH_3), 1.09 (t, *J*=7.4 Hz, 3H, CH_3CH_2S); ^{13}C NMR (101 MHz, DMSO-d6, δ, ppm): 218.19, 172.72, 119.10, 43.08, 31.08, 29.14, 23.74, 12.63; HRMS (ESI): *m/z* $[M-H]^+$

calculated for $C_9H_{14}NO_2S_3$: 264.02 and *m/z* $[M\text{-}Na]^+$ calculated for $C_9H_{13}NNaO_2S_3$: 286.00, found 264.02 and 285.99, respectively; FT-IR: 2932 cm^{-1}, 2235 cm^{-1} ($\upsilon_{C\equiv N}$), 1704 cm^{-1} ($\upsilon_{C=O}$).

2) Peptides synthesis

2.1) Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg-Trp (GILQINSRW).

*Gly-Ile-Leu-Gln(Trt)-Ile-Asn(Trt)-Ser(tBu)-Arg(Pbf)-Trp(tBu)-resin (**1a**)*. In a glass peptide synthesis vessel, 2-chlorotrytilchloride resin beads (1.6 mmol/g, 1.8 g, 2.9 mmol, 1 eq.) were swelled in DMF for 30 min. Afterwards, the resin was washed with fresh DMF, anhydrous methanol, anhydrous DCM and suspended in DMF. The first amino acid, Fmoc-Trp-OH (3.16 g, 6.0 mmol, 2 eq.) was subsequently added to the suspension, followed by DIPEA (2.1 mL, 15 mmol, 5 eq.), and the resulting mixture was stirred for 60 min. After washing with DMF, the resin was treated for 15 min with a mixture of anhydrous DCM/ anhydrous MeOH/ DIPEA (80:15:5 in volume) to cap any remaining reactive trityl group. Finally, the protecting Fmoc group was removed from the attached amino acid treating the resin with a 20% v/v piperidine solution in DMF for 30 min. The remaining 8 amino acids were attached following the same procedure: 2 eq. of Fmoc-protected amino acid were previously dissolved in DMF, mixed with 1.9 eq of HATU and 3 eq. of DIPEA. The solution was added to the resin and the resulting suspension was stirred for 60 min. Fmoc deprotection was performed with the same procedure described above. After each amino acid coupling and Fmoc deprotection step, the excess of reagents was removed from the reaction vessel by filtration, and the resin was washed with DMF, dry methanol, dry DCM, and suspended in DMF, yielding resin-bound protected peptide **1a**.

GILQINSRW. To cleave and deprotect peptide, **1a** was stirred in a mixture of TFA:water:tri-isopropylsilane (95:2.5:2.5 v/v, 10 mL for 200 mg of resin) for 90 min. The supernatant was then collected and dried under vacuum to 1/10 of its volume. The peptide was finally precipitated in diethyl ether and dried overnight under reduced pressure. Yield: 47%; HRMS (ESI): *m/z* $[M\text{-}H]^+$ calculated for $C_{49}H_{80}N_{15}O_{13}$: 1086.6055, found: 1086.6029; RP-HPLC retention time: 12.7 min, purity: 86%.

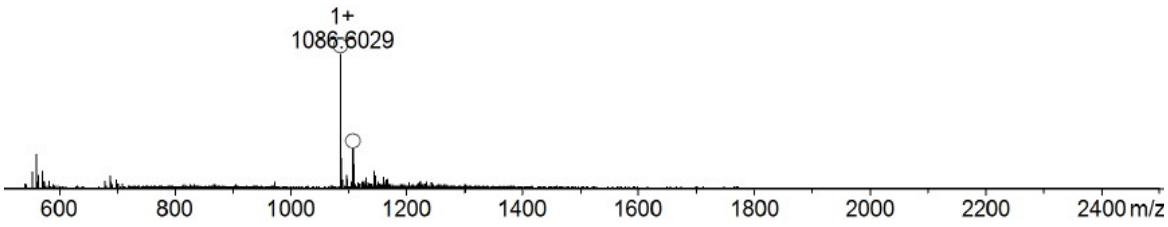


Figure S1. HRMS (ESI) analysis of GILQINSRW.

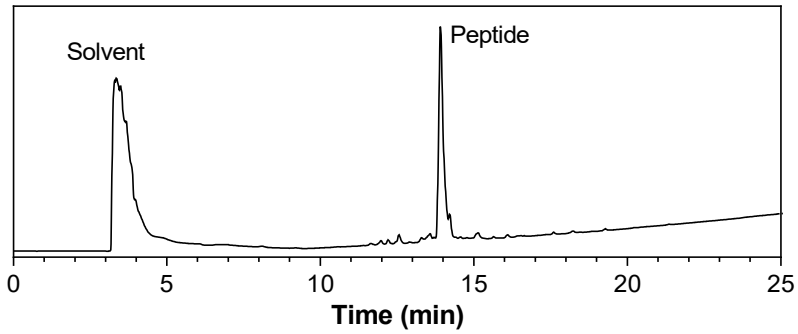


Figure S2. RE-HPLC chromatogram of GILQINSRW, UV detection at λ=280 nm. Purity was estimated by calculating the ratio between the peak area of the peptide and all detected peak areas.

2.2) Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg-Gly.

*Gly-Ile-Leu-Gln(Trt)-Ile-Asn(Trt)-Ser(tBu)-Arg(Pbf)-Gly-resin (**2a**).* Resin-bound peptide **2a** was synthesised in the same manner as **1a**, but at the first stage Fmoc-Gly-OH was used instead of Fmoc-Trp(Boc)-OH.

GILQINSRG. Yield: 41%; HRMS (ESI): *m/z* $[M-H]^+$ calculated for $C_{40}H_{73}N_{14}O_{13}$: 957.5477 and *m/z* $[M+2H]^{2+}$ calculated for $C_{40}H_{74}N_{14}O_{13}$: 479.2775; found: 957.5452 and 479.2805, respectively; RP-HPLC retention time: 8.5 min; purity: 85%.

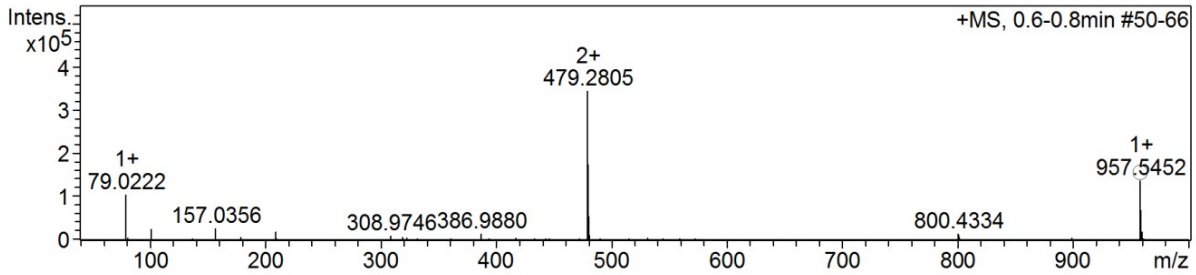


Figure S3. HRMS (ESI) analysis of GILQINSRG.

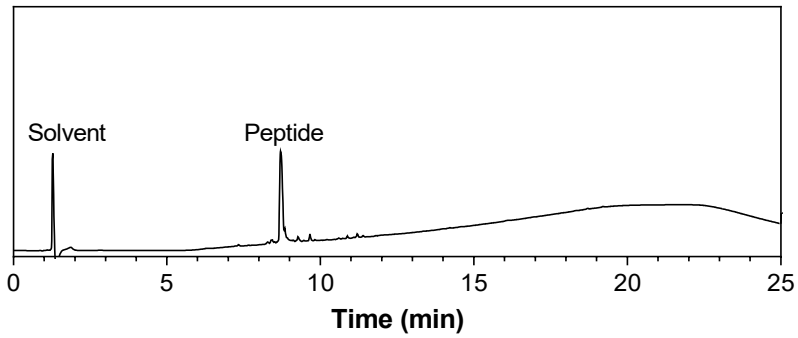


Figure S4. RP-HPLC chromatogram of GILQINSRG, UV detection at λ=280 nm. Purity was estimated by calculating the ratio between the peak area of the peptide and all detected peak areas.

3) Synthesis of HEAn-peptide conjugates.

3.1) HEA_{90}-GILQINSRW

Gly-Ile-Leu-Gln(Trt)-Ile-Asn(Trt)-Ser(tBu)-Arg(Pbf)-Trp(Boc) RAFT agent (**1b**). 400 mg of resin-bound peptide **1a** (containing approximately 246 mg of attached, protected peptide, and 145 mg of starting resin, 1.60 mmol/g, 0.248 mmol, 1 eq.) was swelled for 30 min in DMF. Then, CTA agent (**CTA**) (196 mg, 0.740 mmol, 3 eq.), DIPEA (173 µL, 0.992 mmol, 4 eq.) and HATU (274 mg, 0.720 mmol, 2.9 eq.) were added, and the suspension was left under stirring at room temperature overnight. The resin beads were then repeatedly washed with DCM, and finally treated for 120 min with a DCM:TFE 8:2 v/v solution (10 mL). The suspension was filtered and the filtrate was concentrated under vacuum yielding a protected peptide RAFT agent **1b**. Yield 68%; HRMS (ESI): *m/z* $[M\text{-}2Na]^{2+}$ calculated for $C_{118}H_{150}N_{16}Na_2O_{19}S_4$: 1134.9982, found: 1134.9967.

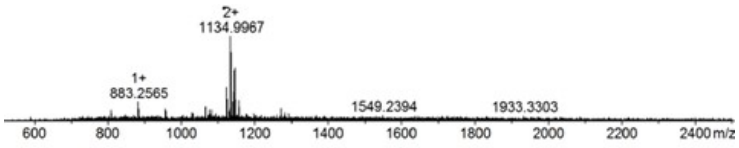


Figure S5. HRMS (ESI) analysis of RAFT agent **1b**.

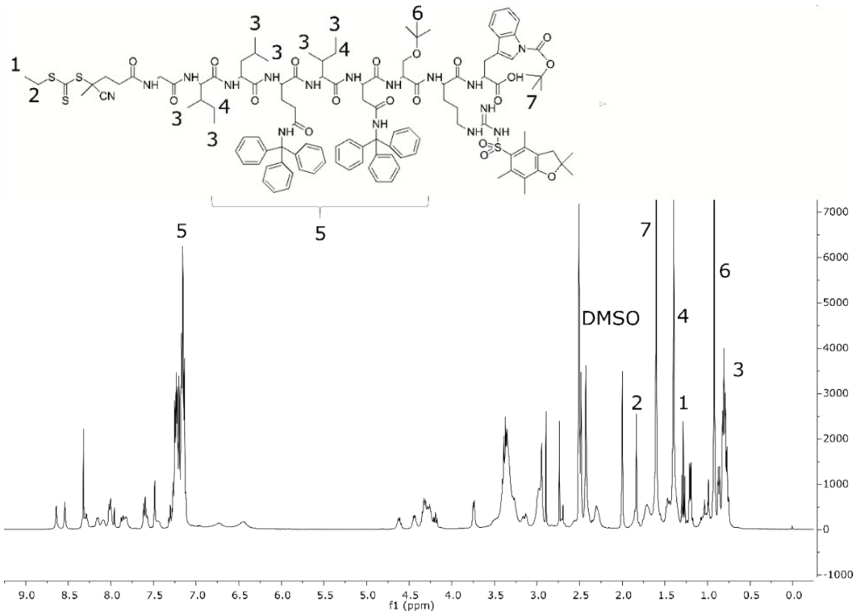


Figure S6. ^1H NMR (DMSO-d_6, 400 MHz) spectrum of RAFT agent **1b**.

*(N-hydroxyethylacrylamide)$_{90}$-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg-Trp (HEA$_{90}$-GILQINSRW; **1**).* RAFT agent (**1b**) (1 eq., 220 mg, 0.096 mmol) was dissolved in DMF (3 mL) and mixed with *N*-

hydroxyethyl acrylamide (100 eq., 1.12 g, 9.60 mmol). The solution was transferred into a small Schlenk tube equipped with a magnetic stirrer, which was placed in ice. Initiator V501 (0.7 mg, 0.002 mmol, 0.1 eq) in DMF (70 µL), was added to the tube. The mixture was degassed for 30 min bubbling argon under stirring at 0°C, and finally the tube was put in a paraffin oil bath at 70 ˚C. The reaction was monitored by ^{1}H NMR, checking the disappearance of acrylamide monomer signals in the 5-6.5 ppm region, until ≈80% conversion was reached. The polymer was then precipitated in THF and dried under high vacuum. Yield: 44%; $M_{n,NMR}$: 12.5 kDa, $M_{n,SEC(DMF)}$: 17.0 kDa, $Đ$ 1.09.

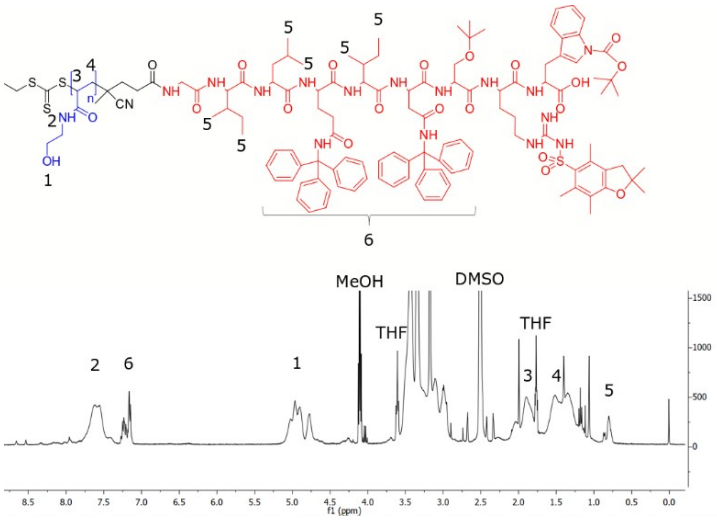


Figure S7. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of protected HEA_{90}-GILQINSRW.

To remove the trithiocarbonate chain-end, peptide-polyHEA (350 mg, 0.028 mmol, 1 eq.) was dissolved in DMF (5 mL) together with AIBN (700 mg, 2.4 mmol, 80 eq.). The mixture was degassed in ice for 30 min by bubbling argon under stirring, and then put in an oil bath at 80°C overnight. The polymer-peptide conjugate was then precipitated in THF. The residue was re-dissolved in MeOH and precipitated again in THF. This precipitation procedure was repeated several times. The precipitate was finally dried under reduced pressure. Successful removal of the trithiocarbonate group was confirmed by UV spectrophotometry, which showed the disappearance of the band for the trithiocarbonate group at λ=300 nm. Yield: 60%; $M_{n,SEC(DMF)}$: 16.8 kDa; $Đ$ 1.08.

To remove the protecting groups, peptide-polyHEA (200 mg) was dissolved in a solution of 0.1 N HCl in hexafluoro-*iso*-propanol (5 mL). The solution was stirred for 6 h at room temperature, then the polymer conjugate was precipitated in diethyl ether, re-dissolved in water, dialysed against ultra-pure water (MWCO 3.5 kDa) for 48 h, and the final product HEA_{90}-GILQINSRW was isolated by freeze-drying. Yield: 62%;. $M_{n,NMR}$: 10.8 kDa, $M_{n,SEC(DMF)}$: 17.0 kDa, $Đ$ 1.07.

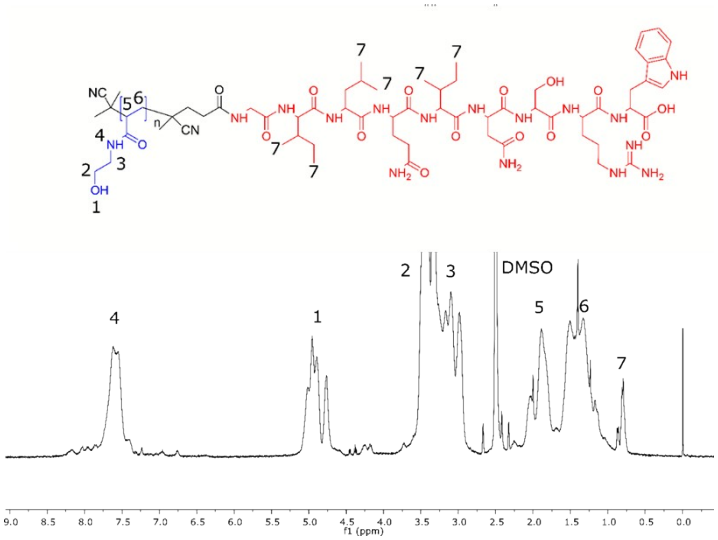


Figure S8. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of deprotected HEA$_{90}$-GILQINSRW.

3.2) HEA$_{95}$-GILQINSRG

Gly-Ile-Leu-Gln(Trt)-Ile-Asn(Trt)-Ser(tBu)-Arg(Pbf)-Gly RAFT agent (**2b**). RAFT agent **2b** was synthesised in the same manner as RAFT agent **1b**. Yield: 66%; HRMS (ESI): *m/z* $[M]^-$ calculated for $C_{104}H_{134}N_{15}O_{17}S_4$: 1993.9004, found 1993.8901.

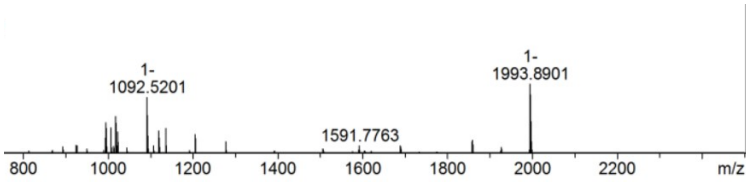


Figure S9. HRMS (ESI) analysis of RAFT agent **2b**.

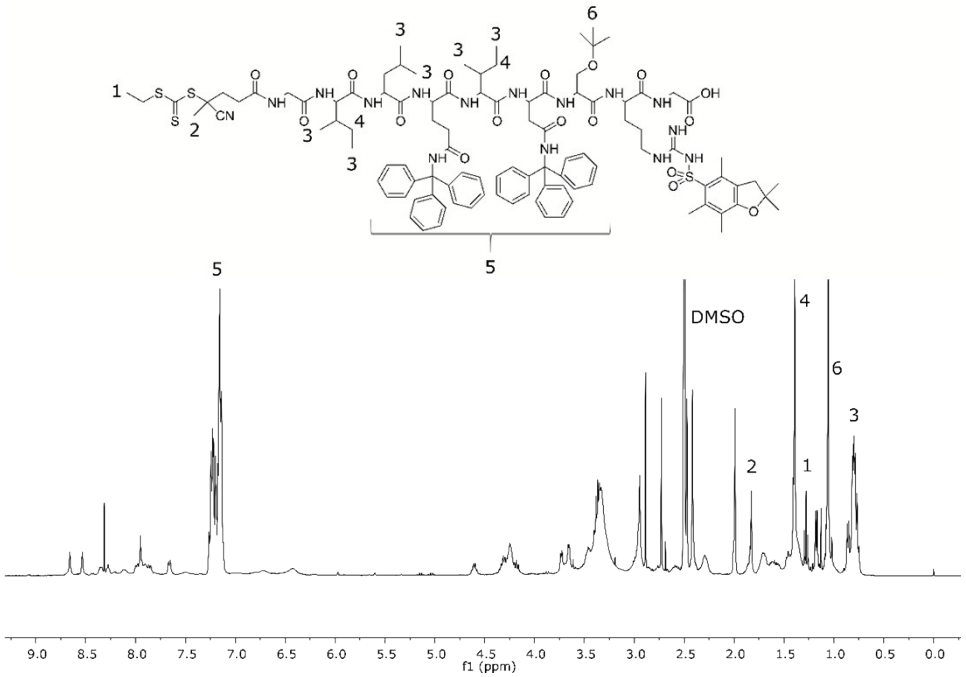


Figure S10. ^{1}H NMR (DMSO-d$_6$, 400 MHz) spectrum of RAFT agent **2b**.

poly(N-hydroxyethylacrylamide)-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg-Gly (HEA$_{90}$-GILQINSRG; ***2****).* Polymer HAE$_{90}$-GILQINSRG **2** was synthesised in the same manner as **1**, using RAFT agent **2b** instead of **1b**.

peptide-polyHEA after RAFT polymerisation. Yield: 47%; $M_{n,NMR}$: 13.1 kDa, $M_{n,SEC(DMF)}$: 19.9 kDa, $Đ$ 1.18.

peptide-polyHEA after trithiocarbonate removal. Yield: 64%; $M_{n,SEC(DMF)}$: 15.0 kDa, $Đ$ 1.13.

Final HEA$_{95}$-*b*-GILQINSRG**.** Yield: 58%; $M_{n,NMR}$: 12.1 kDa, $M_{n,SEC(DMF)}$: 14.8 kDa, $Đ$ 1.13.

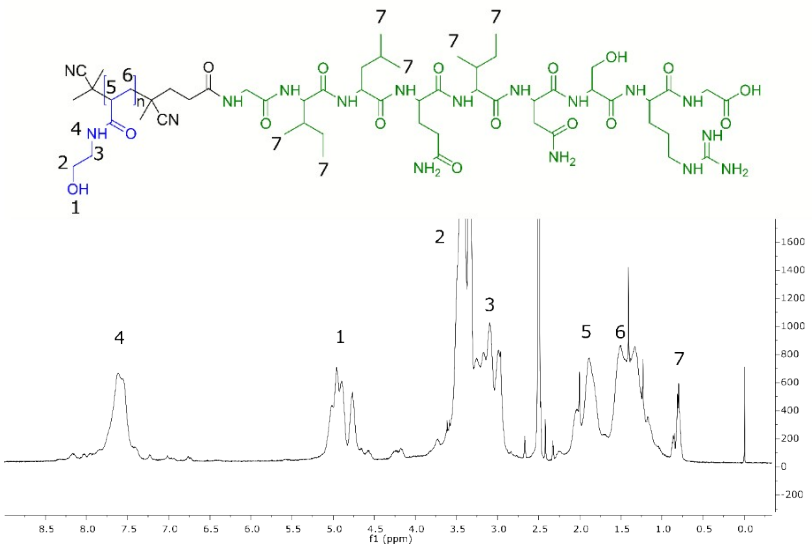
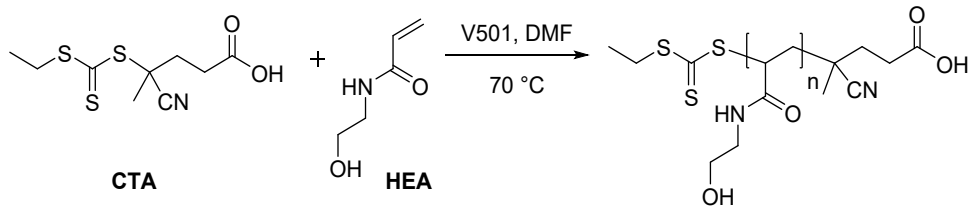


Figure S11. ^{1}H NMR (DMSO-d$_6$, 400 MHz) spectrum of deprotected HEA$_{90}$-GILQINSRG.

4) Synthesis of control homopolymers HEA_{36} and HEA_{100}



Scheme S2. Synthesis of control homopolymers HEA_{36} and HEA_{100}

Poly(N-hydroxyethyl acrylamide) (HEA_{36}). Chain transfer agent **CTA** (150 mg, 0.53 mmol, 1 eq.) and monomer HEA (2.48 g, 21.2 mmol, 40 eq.) were dissolved with in DMF (3 mL). The mixture was transferred into a small Schlenk tube equipped with a magnetic stirrer, which was put in ice. Initiator V 501 (17 mg, 0.053 mmol, 0.1 eq.), previously dissolved in DMF (170 µL), was added to the tube. The mixture was degassed for 30 min bubbling argon under stirring at 0°C, and finally put in a paraffin oil bath at 70 ˚C. The reaction was monitored by ^{1}H NMR, monitoring the disappearance of acrylamide monomer signals in the 5-6.5 ppm region, until ~ 80% conversion was reached. The polymer was then precipitated in THF and dried under high vacuum.

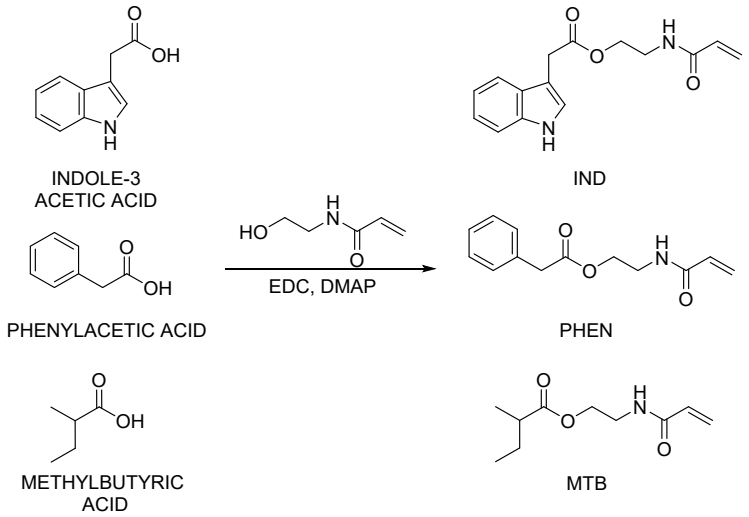
Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.80-7.40 (m, 36H, NH), 5.50-4.30 (m, 36H, -CH_2O*H*), 3.43 (m, 72H, C*H*$_2$OH), 3.26-3.00 (m, 72H, -C*H*$_2$NH-), 2.10-1.70 (m, 36H, *CH*CH_2) 1.60-1.20 (m, 72H, CH*CH*$_2$); $M_{n,THEO}$: 4.5 kDa; $M_{n,SEC(DMF)}$: 8.5 kDa; *Đ*=1.04.

Poly(N-hydroxyethyl acrylamide) (HEA_{100}). Chain transfer agent **CTA** (0.15 g, 0.53 mmol, 1 eq.) and *N*-hydroxyethyl acrylamide (5.0 g, 42 mmol, 80 eq.) were dissolved in DMF (8 mL). The mixture was transferred into a glass tube equipped with a magnetic stirrer bar, which was put in ice. Initiator V501 (16 mg, 0.050 mmol, 0.1 eq.), in DMF (160 µL), was added to the tube. The mixture was degassed for 30 min bubbling argon under stirring at 0°C, and finally put on a paraffin oil bath at 70 ˚C. The reaction was monitored by ^{1}H NMR, checking the disappearance of acrylamide monomer peaks into the 5-6.5 ppm region, until ~80% conversion was reached. The polymer was then precipitated in THF and dried under high vacuum.

Yield: 72%; ^{1}H NMR (400 MHz, d_6-DMSO, δ, ppm): 7.80-7.40 (m, 100H, NH), 5.10-4.70 (m, 100H, OH), 3.43 (m, 200H, C*H*$_2$OH), 3.26-3.00 (m, 200H, -C*H*$_2$NH-), 2.10-1.70 (m, 100H, C*H*CH_2), 1.60-1.20 (m, 200H, CHC*H*$_2$); $M_{n,THEO}$: 12.9 kDa; $M_{n,SEC(DMF)}$ 12.1 kDa; *Đ* 1.14.

5) Synthesis of block co-polymeric peptide mimetics

5.1) Synthesis of IND, PHEN and MTB monomers



Scheme S3. Synthesis of IND, PHEN and MTB monomers.

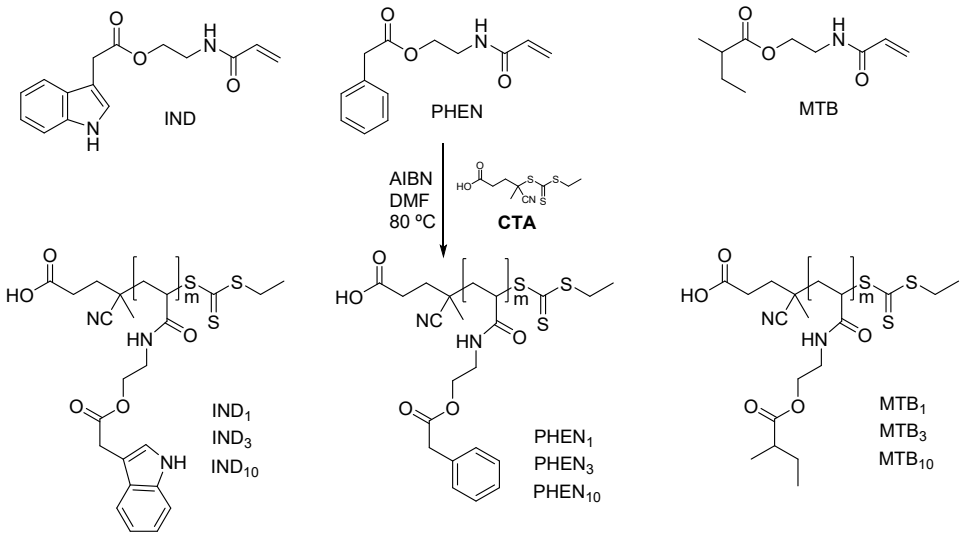
The synthesis of the acrylamide monomers IND, PHEN and MTB was carried out by reaction of 2-hydroxyethyl acrylamide (HEA) with 3-indole-acetic acid, phenyl-acetic acid and methylbutyric acid, respectively. In a typical reaction, a solution of EDC-HCl (1.2 g, 6.3 mmol, 1.1 eq.) in anhydrous DCM (20 mL) was added dropwise over 1 h to a solution of 3-indole-acetic acid, phenyl-acetic acid, or methylbutyric acid (5.7 mmol, 1 eq.), DMAP (0.07 g, 0.6 mmol, 0.1 eq.) and HEA (0.89 mL, 8.6 mmol, 1.5 eq) in DCM (50 mL), at 0°C. The reaction was then stirred for 16 hours at room temperature. The solution was washed twice with brine and twice with 2M HCl. The organic phase was then dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The resulting monomers were used for the polymerisation experiments without further purification.

IND. Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), 8.68 (s, 1H, indole NH), 7.6 (d, *J*=6.5 Hz, 1H, aromatic), 7.34 (d, *J*=8.1 Hz, 1H, aromatic), 7.20 (t, *J*=8.2 Hz, 1H, aromatic), 7.13 (m, 1H, aromatic), 7.07 (d, *J*=2.4 Hz, 1H, =C*H*-NH-C), 6.13 (dd, *J*=17.0, 1.4 Hz, 1H, *CH*=CH_2), 5.80 (m, 1H, -CH_2N*H*-C(O)), 5.74 (dd, *J*=17.0, 10.3 Hz, 1H, CH=C*H*H), 5.53 (dd, *J*=10.4, 1.4 Hz, 1H, CH=CH*H*), 4.16 (t, *J*=5.3 Hz, 2H, O*CH*$_2$$CH_2$NH), 3.77 (s, 2H, CH_2COO) 3.49 (q, *J*=5.5 Hz, 2H, OCH$_2$*CH*$_2$NH); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 172.31, 166.05, 136.24, 130.38, 126.61, 123.66, 121.95, 119.48, 118.42, 111.65, 107.50, 63.11, 38.41, 31.31; FT-IR: 3346 cm^{-1} (υ_{N-H}), 1721 cm^{-1} ($\upsilon_{C=O\ ester}$), 1660.4 cm^{-1} ($\upsilon_{C=Oamide}$), 1545.5 cm^{-1}($\upsilon_{C-Namide}$); MS (ESI): *m/z* $[M-H]^+$ calculated for $C_{16}H_{17}N_2O_3$: 273.12 and *m/z* $[M-Na]^+$ calculated for $C_{16}H_{16}N_2O_3Na$: 295.10, found 273.12 and 295.10, respectively.

PHEN. Yield: 67%; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), 7.4-7.2 (m, 5H, aromatic), 6.23 (dd, *J*=17.0, 1.3 Hz, 1H, *CH*=CH_2), 5.99 (dd, *J*=17.0, 10.3 Hz, 1H, CH=C*H*H), 5.70 (m, 1H, -CH_2N*H*-C(O)), 5.64 (dd, *J*=10.3, 1.3 Hz, 1H, CH=CH*H*), 4.22 (t, *J*=5.2 Hz, 2H, O*CH*$_2$$CH_2$NH), 3.65 (s, 2H, C*H*$_2$C(O)O) 3.58 (q, *J*=5.6 Hz, 2H, OCH$_2$*CH*$_2$NH); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 171.69, 165.74, 133.84, 130.56, 129.22, 128.7, 127.28, 127.27, 126.62, 63.41, 41.29, 38.67; FT-IR: 1731 cm^{-1} ($\upsilon_{C=Oester}$), 1654.5 cm^{-1} ($\upsilon_{C=Oamide}$), 1560.3 cm^{-1}($\upsilon_{C\text{-}N\ amide}$); MS (ESI): *m/z* $[M\text{-}H]^+$ calculated for $C_{13}H_{16}NO_3$: 234.11 and *m/z* $[M\text{-}Na]^+$ calculated for $C_{13}H_{16}NO_3Na$: 256.10, found 234.11 and 256.10, respectively.

MTB. Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), 6.28 (dd, *J*=17.0, 1.3 Hz, 1H, *CH*=CH_2), 6.09 (dd, *J*=17.0, 10.3 Hz, 1H, CH=C*H*H), 5.89 (m, 1H, -CH_2N*H*-C(O)), 5.66 (dd, *J*=10.3, 1.3 Hz, 1H, CH=CH*H*), 4.23 (t, *J*=5.4Hz, 2H, O*CH*$_2$$CH_2$NH), 3.62 (q, *J*=5.6 Hz, 2H, OCH$_2$*CH*$_2$NH), 2.4 (h, *J*=7 Hz 1H, *CH*CH_3), 1.15 (d, *J*=7Hz, 3H, CH*CH*$_3$), 0.90 (t, *J*=7.4Hz, 3H, CH_2*CH*$_3$); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 177.08, 165.87, 130.70, 126.75, 62.76, 41, 39.05, 26.78, 16.62, 11.64; FT-IR: 1732.6 cm^{-1} ($\upsilon_{C=Oester}$), 1655.8 cm^{-1} ($\upsilon_{C=Oamide}$), 1541.8 cm^{-1}($\upsilon_{C\text{-}Namide}$); MS (ESI): *m/z* $[M\text{-}H]^+$ calculated for $C_{10}H_{18}NO_3$: 200.13 and *m/z* $[M\text{-}Na]^+$ calculated for $C_{10}H_{17}NO_3Na$: 222.11, found 200.13 and 222.11, respectively.

5.2) Synthesis of oligomeric chain transfer agents IND_m, $PHEN_m$, and MTB_m



Scheme S4. Synthesis of oligomeric chain transfer agents IND_1, IND_3, IND_{10}, $PHEN_1$, $PHEN_3$, $PHEN_{10}$, MTB_1, MTB_3 and MTB_{10}.

The synthesis of the oligomeric IND_m, $PHEN_m$, and MTB_m CTAs was carried out by RAFT polymerization, using different [monomer]:[**CTA**] molar ratios in combination with IND, PHEN and MTB. In a typical reaction, IND, PHEN, or MTB (3.68 mmol) and **CTA** (0.78 g, 2.94 mmol for IND_1, $PHEN_1$ and MTB_1; 0.24 g, 0.92 mmol for IND_3, $PHEN_3$ and MTB_3; 0.08g, 0.3 mmol for IND_{10}, $PHEN_{10}$ and MTB_{10}) were mixed together in 1 mL of DMF. The solutions were

transferred into a small Schlenk tube equipped with a magnetic stirrer, which was put on ice. Radical initiator AIBN (0.08 g, 0.29 mmol for IND_1, $PHEN_1$ and MTB_1; 0.026 g, 0.09 mmol for IND_3, $PHEN_3$ and MTB_3; 0.008 g, 0.03 mmol for IND_{10}, $PHEN_{10}$ and MTB_{10}), previously dissolved in DMF (80 µL for IND_1, 26 µL for $PHEN_1$ and 8 µL for MTB_1) was added to the tubes. The mixtures were degassed for 30 min bubbling argon under stirring at 0°C and finally put in a paraffin oil bath at 80 ˚C. The reactions were monitored by ^{1}H NMR, monitoring the disappearance of acrylamide monomer signals in the 5-6.5 ppm region, until ~80% conversion was reached. The reaction solutions were then transferred into a 25 mL round bottom flask, and DMF was removed under reduced pressure to give a residue that was purified as follows:

IND_1, *$PHEN_1$* and *MTB_1* were purified by flash chromatography using petroleum ether:EtOAc 2:8 v/v as the eluent.

IND_3, *$PHEN_3$* and *MTB_3* were purified by reversed phase chromatography on an Agilent 971-FP automated flash purification system using a BIOTAGE KP-C18-HS 12 g column. Elution was performed using a linear gradient from 20% to 80% MeCN in water over 250 min at a flow rate of 8 mL min^{-1}.

IND_{10}, *$PHEN_{10}$* and *MTB_{10}* were re-dissolved in acetone and purified by multiple precipitations in Et_2O. The final precipitates were dried under vacuum and used as macro CTAs without any further purification.

IND_1. Yield: 32%; ^{1}H NMR (400 MHz, d6-DMSO, δ, ppm), 10.93 (s, 1H, indole NH), 8.84 (s, 1H, acrylamide NH), 7.48 (d, *J*= 7.8Hz, 1H, CH aromatic), 7.34 (d, *J*= 8.1Hz, 1H, CH aromatic), 7.24 (d, *J*= 2.0Hz, 1H, CH aromatic), 7.07 (m, 1H, CH aromatic), 6.98 (t, *J*= 7.3Hz, 1H, CH aromatic), 4.85 (m, 1H, CH), 4.06 (m, 2H, CH_2), 3.73 (d, *J*= 2.3Hz, 2H, CH_2), 1.26 (m, 3H, CH_3); ^{13}C NMR (101 MHz, d_6-DMSO, δ, ppm): 222.64, 176.20, 172.03, 169.28, 136.14, 127.17, 123.32, 127.17, 122.31, 119.78, 118.69, 111.56, 108.11, 62.83, 49.03, 39.16, 36.9, 30.95, 24.34, 23.41, 20.85, 18.44, 12.83; HRMS (ESI): *m/z* $[M]^-$ calculated for $C_{24}H_{28}N_3O_5S_3$: 534.12, found 534.12.

$PHEN_1$. Yield: 38%; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), 7.23-7.27 (m, 5H, aromatic), 6.60-6.55 (m, 1H, acrylamide NH), 4.85 (dd, *J*=8.1, 4.8Hz, 1H, CH), 4.09 (d, *J*= 5.3Hz, 2H, CH_2CH_2NH), 3.57 (m, 2H, CH_2), 3.42 (m, 2H, CH_2CH_2NH), 3.32 (q, *J*= 7.3Hz, 2H, CH_2CH_3); ^{13}C NMR (101 MHz, $CDCl_3$, δ, ppm): 223.01, 176.55, 171.58, 169.28, 133.68, 129.33, 128.7, 127.28, 122.26, 63.08, 48.69, 41.18, 39.09, 34.42, 33.55, 32.23, 29.47, 24.48, 23.68, 12.84; HRMS (ESI): *m/z* $[M]^-$ calculated for $C_{22}H_{27}N_2O_5S_3$: 495.11, found 495.10.

MTB_1 Yield: 41%. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), 6.83-6.79 (m, 1H, acrylamide NH), 4.93 (dd, *J*= 8.0, 4.9Hz, 1H, CH_2CH), 4.12 (t, *J*= 5.2Hz, 2H, CH_2CH_2NH), 3.50 (m, 2H, CH_2CH_2NH), 3.37 (q, *J*= 7.4Hz, 2H, CH_2CH_3), 2.53 (m, 2H, CH_2CH_2) 2.35 (h, *J* =6.9 Hz, 1H, $CHCH_3$), 2.15-1.8 (m, 2H, CH_2CH_2),1.7-1.4 (m, 2H, CH_2CH_3), 1.33 (m, 3H, CH_2CH_3), 1.13 (m 3H, $CHCH_3$), 0.88 (td, *J*= 7.4, 2.5Hz, 3H, CH_2CH_3); ^{13}C NMR (101 MHz, d_3- $CDCl_3$, δ, ppm): 223.25, 177.17, 169.88, 122.65, 62.27, 49.21, 41.3, 39.59, 36.29, 34.86, 30.00, 24.02, 19.66, 16.86, 13.21, 12.02; HRMS (ESI): *m/z* $[M]^-$ calculated for $C_{19}H_{29}N_2O_5S_3$: 461.12, found 461.13.

IND$_3$. Yield: 8% as a mixture of IND_2 and IND_3; ^{1}H NMR (400 MHz, d6-DMSO, δ, ppm), 7.49 (m 2.52H, CH), 7.35 (d, *J*= 8.2Hz, 2.52H, CH, CH aromatic), 7.23 (m, 2.52H, CH, CH aromatic), 7.07 (m, 2.52H, CH, CH aromatic), 6.97 (m, 2.52H, CH, CH aromatic), 4.03 (m, 5.04H, CH_2), 3.72 (m, 5.04H, CH_2); HRMS (ESI): *m/z* $[M]^-$ calculated for $C_{54}H_{60}N_7O_{11}S_3$: 1078.3518 (n = 3) and *m/z* $[M]^-$ calculated for $C_{39}H_{44}N_5O_8S_3$: 806.2357 (n = 2), found 1078.3528 (52% of area) and 806.2365 (48% of area).

PHEN$_3$. Yield: 12%; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), 7.5-7.2 (m, 15H, aromatic), 7.2-6.5 (m, 3H, acrylamide NH), 4.9-4.5 (m, 3H, CH), 4.18 (m, 6H, CH_2CH_2NH), 3.63 (m, 6H, CH_2CO), 3.55-3.25 (m, 6H, CH_2CH_2NH, and m, 2H, CH_2CH_3); HRMS (ESI): *m/z* $[M]^-$ calculated for $C_{48}H_{57}N_4O_{11}S_3$: 961.3191, found 961.3205.

MTB$_3$. Yield: 10%; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm), 7.2-6.8 (m, 3H, acrylamide NH), 4.4-4.0 (m, 6H, CH_2CH_2NH), 3.55-3.25 (m, 6H, CH_2CH_2NH, and m, 2H, CH_2CH_3), 1.14 (m, 9H, $CHCH_3$), 0.9 (m, 9H, CH_2CH_3); HRMS (ESI): *m/z* $[M]^-$ calculated for $C_{39}H_{63}N_4O_{11}S_3$: 859.3661, found 859.3679.

IND$_{10}$. Yield: 58%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm), 10.90 (s, 10H, indole NH), 7.46 (m, 10H, CH, CH aromatic), 7.34 (m, 10H, CH, CH aromatic), 7.21 (m, 10H, CH, CH aromatic), 7.05 (m, 10H, CH, CH aromatic), 6.95 (m, 10H, CH, CH aromatic), 4.04 (m, 20H, CH_2), 3.7 (m, 20H, CH_2), 2.38-1.88 (m, 10H CH_2CH), 1.8-1 (m, 20H CH_2CH); $M_{n,THEO}$: 3.00 kDa; $M_{n,SEC(THF)}$: 1.56 kDa, *Đ*=1.12.

PHEN$_{10}$. Yield: 64%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm), 8.4-7.4 (m, 10H, acrylamide NH), 7.35-7.15 (m, 50H, aromatic), 7.2-6.5 (m, 10H, acrylamide NH), 4.03 (m, 20H, CH_2CH_2NH), 3.83 (m, 20H, CH_2CO), 2.4-1.85 (m, 10H CH_2CH), 1.8-1 (m, 20H CH_2CH). $M_{n,THEO}$: 2.6 kDa; $M_{n,SEC(THF)}$: 1.6 kDa, *Đ*=1.09.

MTB$_{10}$. Yield: 57%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm), 8.4-7.1 (m, 10H, acrylamide NH), 4.01 (m, 20H, CH_2CH_2NH), 2.33 (m, 1H, $CHCH_3$), 1.06 (m, 30H, $CHCH_3$), 0.83 (m, 30H, CH_2CH_3); $M_{n,THEO}$: 2.3 kDa. $M_{n,SEC(THF)}$: 1.47 kDa, *Đ*=1.10.

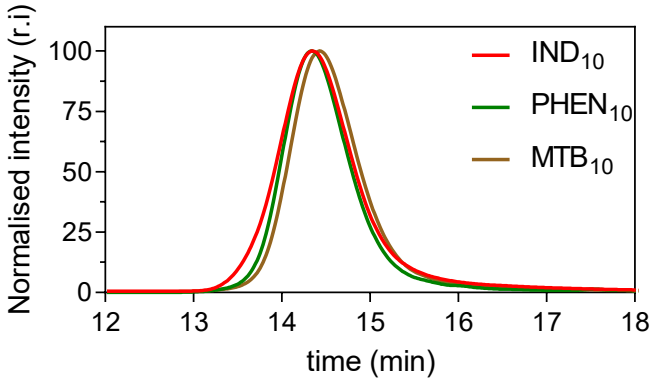
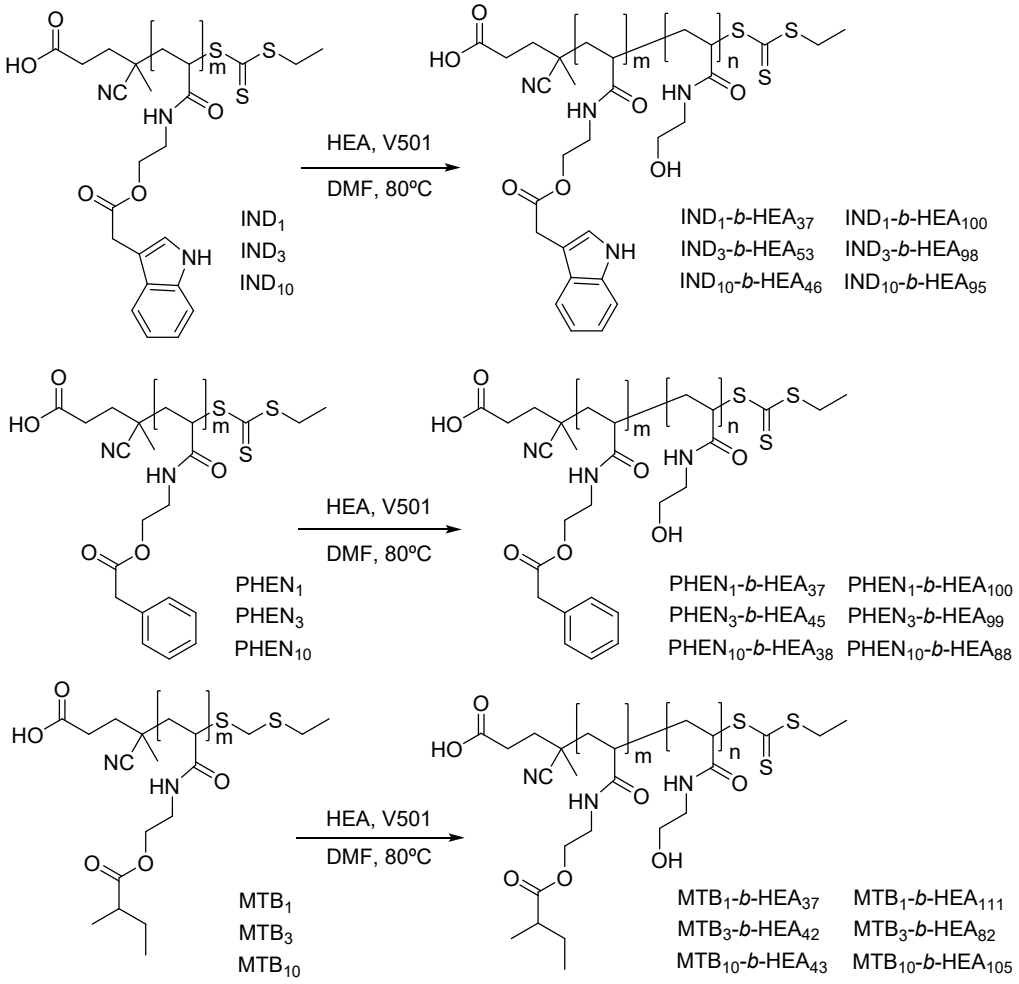


Figure S12. Normalised SEC traces of IND_{10}, $PHEN_{10}$ and MTB_{10}. SEC analyses were performed using THF as the mobile phase (PMMA standards).

5.3) Synthesis of IND_m-HEA_n, $PHEN_m$-HEA_n, and MTB_m-HEA_n



Scheme S5. Synthesis of IND_1-, IND_3-, IND_{10}-, $PHEN_1$-, $PHEN_3$-, $PHEN_{10}$-, MTB_1-, MTB_3- and MTB_{10}-*b*-HEA_n block copolymers.

IND_m, $PHEN_m$, and MTB_m were utilised as macro-chain transfer agents to mediate the RAFT polymerisation of *N*-hydroxy-ethylacrylamide (Scheme S5). Two different chain lengths, with DP 40 and 100, were targeted, to investigate the influence of the size of the polyHEA block on protein-copolymer interaction. The combination of nine oligomeric RAFT agents and two different p(HEA) chains length resulted in the synthesis of a library of eighteen amphiphilic IND_m-*b*-HEA_n, $PHEN_m$-*b*-HEA_n, and MTB_m-*b*-HEA_n block copolymers.

In a typical reaction, the chosen CTA (0.10 mmol for IND_1, $PHEN_1$, MTB_1; 0.050 mmol for IND_3, $PHEN_3$, MTB_3; 0.050 mmol for IND_{10}, $PHEN_{10}$, MTB_{10}) was dissolved in DMF along with HEA (50 eq. for the target DP=40, 100 eq. for the target DP=100). The initial monomer concentration in the reaction solution was 3.0 M. The mixtures were transferred into small schlenk tubes equipped with a magnetic stirrer, which were cooled to 0°C. Initiator V501 (0.1 eq.),

previously dissolved in DMF, was added to each tube. The mixtures were degassed for 30 min bubbling argon under stirring at 0°C, and finally placed in a paraffin oil bath at 70 ˚C. The reactions were monitored by ^{1}H NMR, monitoring the disappearance of acrylamide monomer signals in the 5-6.5 ppm region until ~ 80% conversion was reached. The polymers were then precipitated in a 8:2 v/v THF:Et_2O. The residue was re-dissolved in MeOH and precipitated again in THF. This precipitation procedure was repeated three times, then the polymers were dried under reduced pressure.

IND_1-b-HEA_{37}. Yield: 75%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm), 7.80-7.40 (m, 37H, polyacrylamide NH), 7.34 (d, 1H, CH, CH aromatic), 7.24 (m, 1H, CH, CH aromatic), 7.08 (t, 1H, CH, CH aromatic), 6.99 (t, 1H, CH, CH aromatic), 5.50-4.30 (m, 37H, polyacrylamide OH), 4.04 (m, 2H, CH_2), 3.78 (m, 2H, CH_2), 2.10-1.70 (m, 37H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 74H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 5.0 kDa; $M_{n,SEC(DMF)}$: 8.7 kDa; *Đ*=1.07.

$PHEN_1$-b-HEA_{37}. Yield: 78%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.80-7.40 (m, 37H, polyacrylamide NH), 7.35-7.22 (m, 5H, aromatic), 5.50-4.30 (m, 37H, polyacrylamide OH), 4.04 (m, 2H, CH_2), 2.10-1.70 (m, 37H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 74H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 4.8 kDa; $M_{n,SEC(DMF)}$: 9.0 kDa, *Đ*=1.07.

MTB_1-b-HEA_{37}. Yield: 82%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.80-7.40 (m, 37H, polyacrylamide NH), 5.50-4.30 (m, 38H, polyacrylamide OH), 4.02 (m, 2H, CH_2), 2.10-1.70 (m, 38H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 76H, polyacrylamide CH*CH_2*), 1.07 (d, 3H, CH_3), 0.84 (t, 3H, CH_3); $M_{n,NMR}$: 4.8 kDa; $M_{n,SEC(DMF)}$: 10.4 kDa, *Đ*=1.05.

IND_3-b-HEA_{53}. Yield: 46%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm), 7.80-7.40 (m, 53H, polyacrylamide NH), 7.34 (d, 2.52H, CH, CH aromatic), 7.24 (m, 2.52H, CH, CH aromatic), 7.08 (t, 2.52H, CH, CH aromatic), 6.99 (t, 2.52H, CH, CH aromatic), 5.50-4.30 (m, 53H, polyacrylamide OH), 4.03 (m, 5.04H, CH_2), 3.74 (m, 5.04H, CH_2), 2.10-1.70 (m, 53H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 106H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 7.2 kDa; $M_{n,SEC(DMF)}$: 11.7 kDa; *Đ*=1.09.

$PHEN_3$-b-HEA_{45}. Yield: 70%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.80-7.40 (m, 45H, polyacrylamide NH), 7.35-7.22 (m, 15H, aromatic), 5.50-4.30 (m, 45H, polyacrylamide OH), 4.05 (m, 6H, CH_2), 2.10-1.70 (m, 45H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 90H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 6.2 kDa; $M_{n,SEC(DMF)}$: 9.6 kDa, *Đ*=1.07.

MTB_3-b-HEA_{42}. Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.80-7.40 (m, 42H, polyacrylamide NH), 5.50-4.30 (m, 42H, polyacrylamide OH), 4.02 (m, 6H, CH_2), 2.10-1.70 (m, 42H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 84H, polyacrylamide CH*CH_2*), 1.07 (d, 9H, CH_3), 0.84 (t, 9H, CH_3); $M_{n,NMR}$: 5.8 kDa; $M_{n,SEC(DMF)}$: 10.9 kDa; *Đ*=1.05.

IND_{10}-b-HEA_{40}. Yield: 46%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm), 7.80-7.40 (m, 40H, polyacrylamide NH), 7.34 (d, 10H, CH, CH aromatic), 7.24 (m, 10H, CH, CH aromatic), 7.08 (t, 10H, CH, CH aromatic), 6.99 (t, 10H, CH, CH aromatic), 5.50-4.30 (m, 40H, polyacrylamide OH), 4.03 (m, 20H, CH_2), 3.74 (m, 20H, CH_2), 2.10-1.70 (m, 40H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 80H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 7.7 kDa; $M_{n,SEC(DMF)}$: 8.9 kDa; *Đ*=1.07.

PHEN$_{10}$-b-HEA$_{38}$. Yield: 70%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.80-7.40 (m, 38H, polyacrylamide NH), 7.35-7.22 (m, 50H, aromatic), 5.50-4.30 (m, 38H, polyacrylamide OH), 4.03 (m, 20H, CH_2), 2.10-1.70 (m, 38H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 76H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 7 kDa; $M_{n,SEC(DMF)}$ 10.7 kDa; *Đ*=1.08.

MTB$_{10}$-b-HEA$_{43}$. Yield: 68%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.80-7.40 (m, 43H, polyacrylamide NH), 5.50-4.30 (m, 43H, polyacrylamide OH), 4.02 (m, 20H, CH_2), 2.10-1.70 (m, 43H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 86H, polyacrylamide CH*CH_2*), 1.07 (d, 30H, CH_3), 0.84 (t, 30H, CH_3); $M_{n,NMR}$: 7.7 kDa; $M_{n,SEC(DMF)}$ 10 kDa; *Đ*=1.07.

IND$_1$-b-HEA$_{100}$. Yield: 67%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm), 7.80-7.40 (m, 100H, polyacrylamide NH), 7.34 (d, 1H, CH, CH aromatic), 7.24 (m, 1H, CH, CH aromatic), 7.08 (t, 1H, CH, CH aromatic), 6.99 (t, 1H, CH, CH aromatic), 5.50-4.30 (m, 100H, polyacrylamide OH), 4.04 (m, 2H, CH_2), 3.78 (m, 2H, CH_2), 2.10-1.70 (m, 100H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 200H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 8.7 kDa; $M_{n,SEC(DMF)}$: 13.8 kDa; *Đ*=1.07.

PHEN$_1$-b-HEA$_{100}$. Yield: 81%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.80-7.40 (m, 100H, polyacrylamide NH), 7.35-7.22 (m, 5H, aromatic), 5.50-4.30 (m, 100H, polyacrylamide OH), 4.04 (m, 2H, CH_2), 2.10-1.70 (m, 100H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 200H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 12.2 kDa; $M_{n,SEC(DMF)}$: 18.6 kDa; *Đ*=1.08.

MTB$_1$-b-HEA$_{111}$. Yield: 72%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.80-7.40 (m, 111H, polyacrylamide NH), 5.50-4.30 (m, 111H, polyacrylamide OH), 4.02 (m, 2H, CH_2), 2.10-1.70 (m, 111H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 222H, polyacrylamide CH*CH_2*), 1.07 (d, 3H, CH_3), 0.84 (t, 3H, CH_3); $M_{n,NMR}$: 13.2 kDa; $M_{n,SEC(DMF)}$: 19.8 kDa, *Đ*=1.09.

IND$_3$-b-HEA$_{98.}$ Yield: 57%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm), 7.80-7.40 (m, 98H, polyacrylamide NH), 7.34 (d, 2.6H, CH, CH aromatic), 7.24 (m, 2.52H, CH, CH aromatic), 7.08 (t, 2.52H, CH, CH aromatic), 6.99 (t, 2.52H, CH, CH aromatic), 5.50-4.30 (m, 98H, polyacrylamide OH), 4.05 (m, 5.04H, CH_2), 3.74 (m, 5.04H, CH_2), 2.10-1.70 (m, 98H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 196H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 12.0 kDa; $M_{n,SEC(DMF)}$: 17.0 kDa, *Đ*=1.11.

PHEN$_3$-b-HEA$_{99}$. Yield: 66%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.80-7.40 (m, 99H, polyacrylamide NH), 7.35-7.22 (m, 15H, aromatic), 5.50-4.30 (m, 99H, polyacrylamide OH), 4.04 (m, 6H, CH_2), 2.10-1.70 (m, 99H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 198H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 12.5 kDa; $M_{n,SEC(DMF)}$: 17.25 kDa; *Đ*=1.06.

MTB$_3$-b-HEA$_{82}$. Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.80-7.40 (m, 82H, polyacrylamide NH), 5.50-4.30 (m, 82H, polyacrylamide OH), 4.01 (m, 6H, CH_2), 2.10-1.70 (m, 82H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 164H, polyacrylamide CH*CH_2*), 1.07 (d, 9H, CH_3), 0.84 (t, 9H, CH_3); $M_{n,NMR}$: 10.5 kDa; $M_{n,SEC(DMF)}$ 16.8 kDa; *Đ*=1.07.

IND$_{10}$-b-HEA$_{95}$. Yield: 57%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm), 7.80-7.40 (m, 95H, polyacrylamide NH), 7.34 (d, 10H, CH, CH aromatic), 7.24 (m, 10H, CH, CH aromatic), 7.08 (t, 10H, CH, CH aromatic), 6.99 (t, 10H, CH, CH aromatic), 5.50-4.30 (m, 95H, polyacrylamide OH), 4.03 (m, 20H, CH_2), 3.74 (m, 2H, CH_2), 2.10-1.70 (m, 95H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 190H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 12 kDa; $M_{n,SEC(DMF)}$: 18.4 kDa, *Đ*=1.11.

$PHEN_{10}$-b-HEA_{88}. Yield: 60%; ^{1}H NMR (400 MHz, DMSO, δ, ppm): 7.80-7.40 (m, 88H, polyacrylamide NH), 7.35-7.22 (m, 50H, aromatic), 5.50-4.30 (m, 88H, polyacrylamide OH), 4.02 (m, 20H, CH_2), 2.10-1.70 (m, 88H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 176H, polyacrylamide CH*CH_2*); $M_{n,NMR}$: 12.9 kDa; $M_{n,SEC(DMF)}$: 17.4 kDa; *Đ*=1.07.

MTB_{10}-b-HEA_{105}. Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.80-7.40 (m, 105H, polyacrylamide NH), 5.50-4.30 (m, 105H, polyacrylamide OH), 4.02 (m, 20H, CH_2), 2.10-1.70 (m, 105H, polyacrylamide *CH*CH_2) 1.60-1.20 (m, 210H, polyacrylamide CH*CH_2*), 1.07 (d, 30H, CH_3), 0.84 (t, 30H, CH_3); $M_{n,NMR}$: 12.5 kDa; $M_{n,SEC(DMF)}$: 18.7 kDa; *Đ*=1.07.

5.3.1) SEC traces of IND_m-HEA_n, $PHEN_m$-HEA_n, and MTB_m-HEA_n

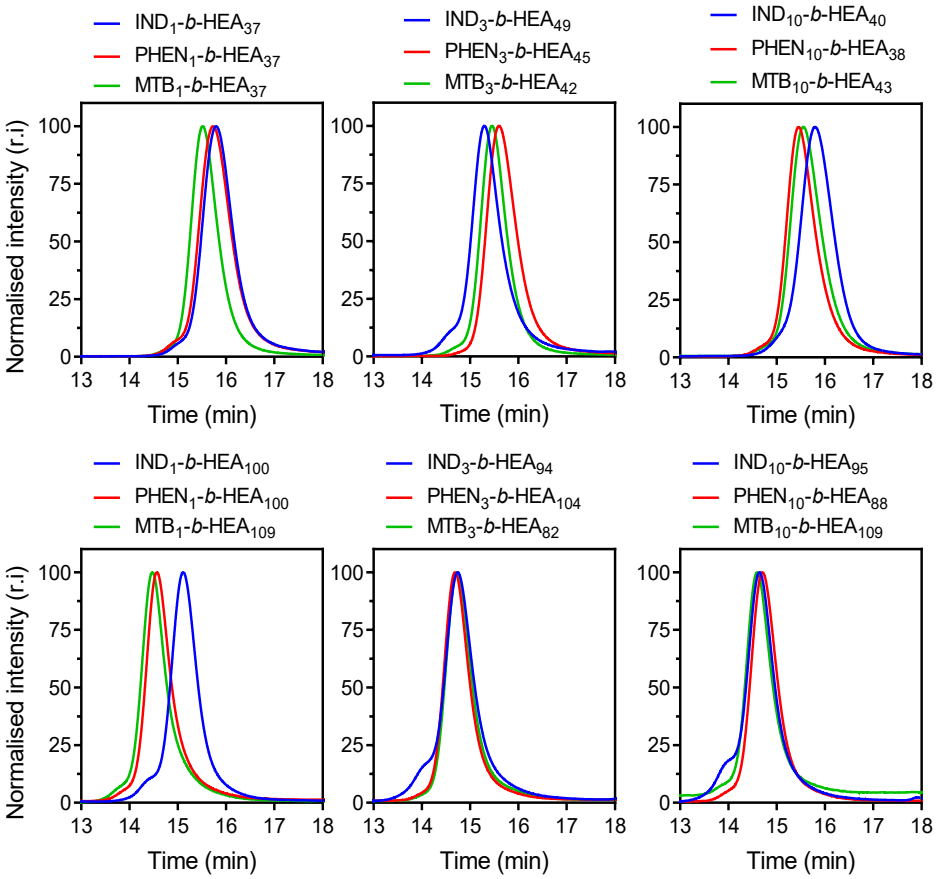


Figure S13. Normalised SEC traces of (top row) HEA_{40} and (bottom row) HEA_{100} $PHEN_m$-, MTB_m-, and IND_m- block co-polymers. SEC analyses were performed using DMF + 0.1 % LiBr as the mobile phase (PMMA standards).

5.3.2) ^{1}H NMR spectra of IND$_m$-HEA$_n$, PHEN$_m$-HEA$_n$, and MTB$_m$-HEA$_n$

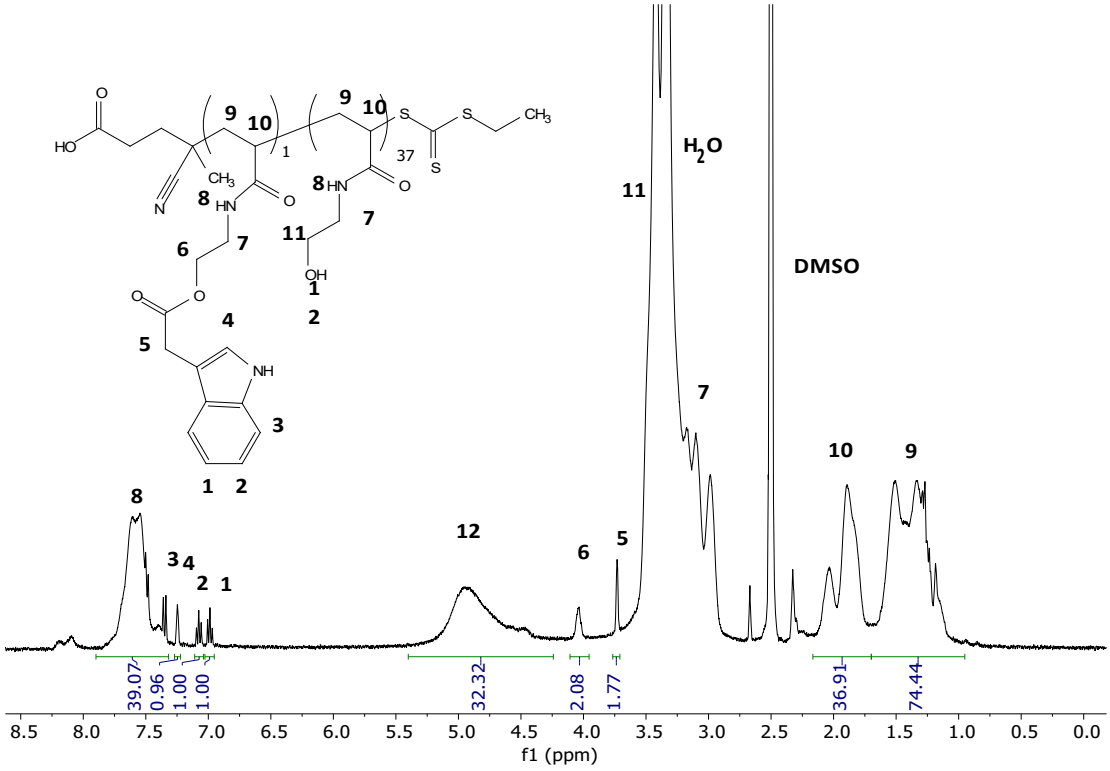


Figure S14. ^{1}H NMR (400 MHz, DMSO-d$_6$) of copolymer IND$_1$-*b*-HEA$_{37}$.

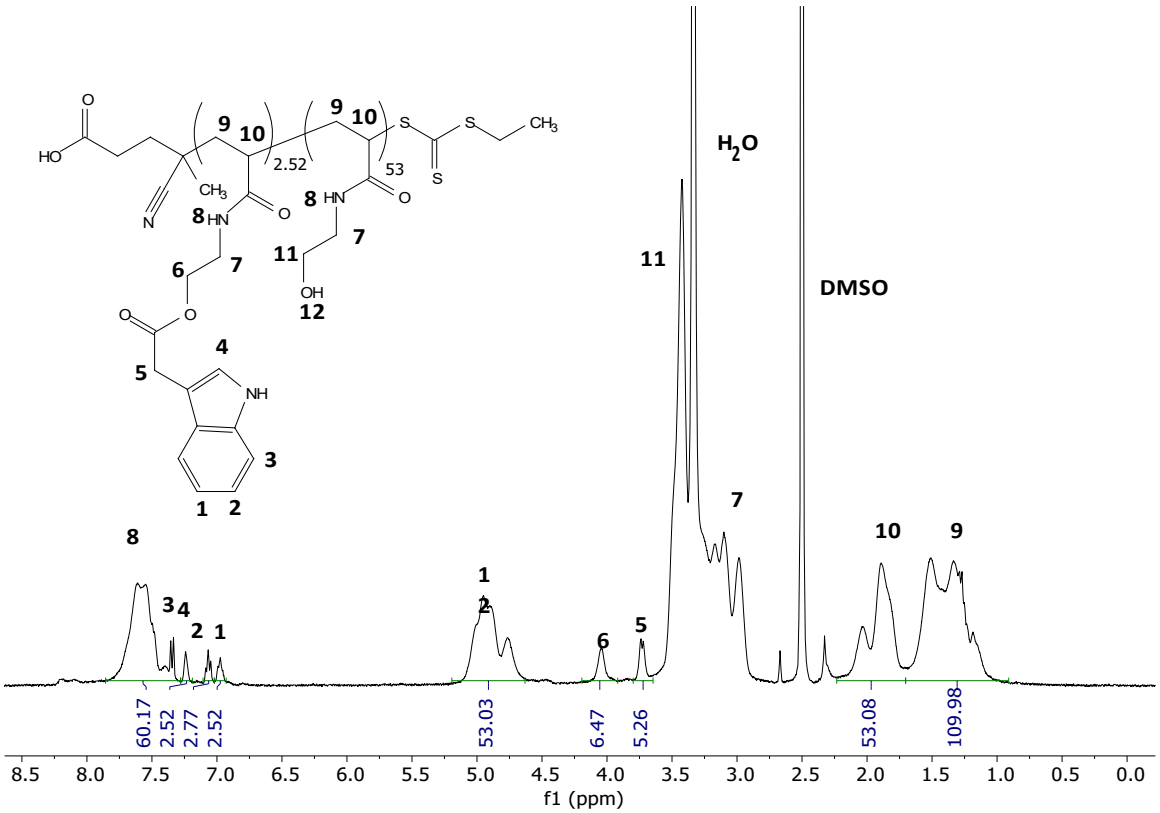


Figure S15. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer IND_3-*b*-HEA_{53}.

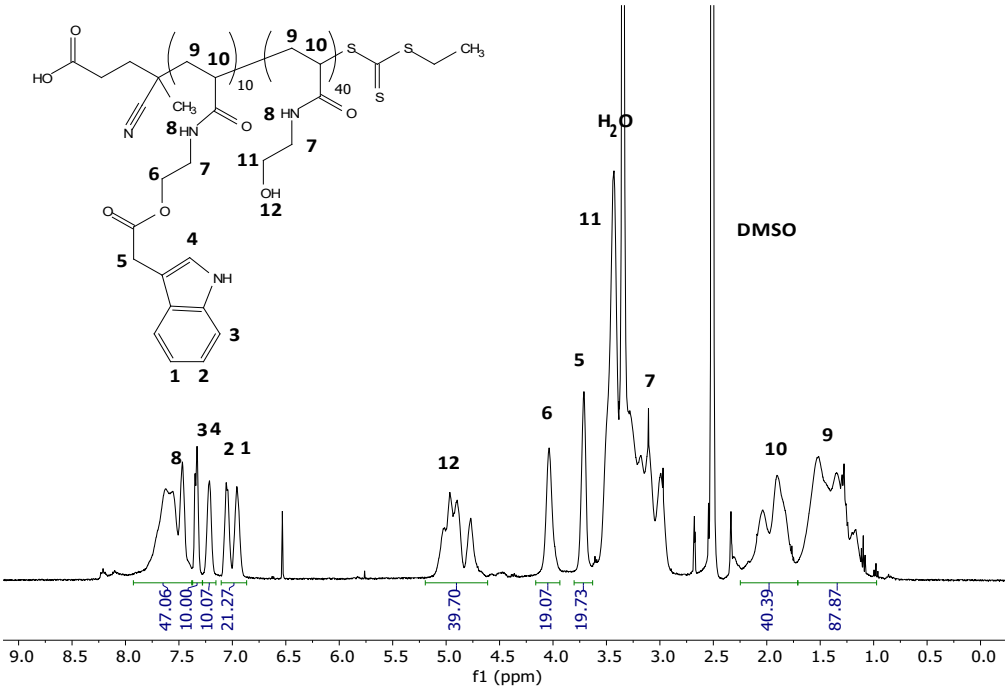


Figure S16. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer IND_{10}-b-HEA_{40}.

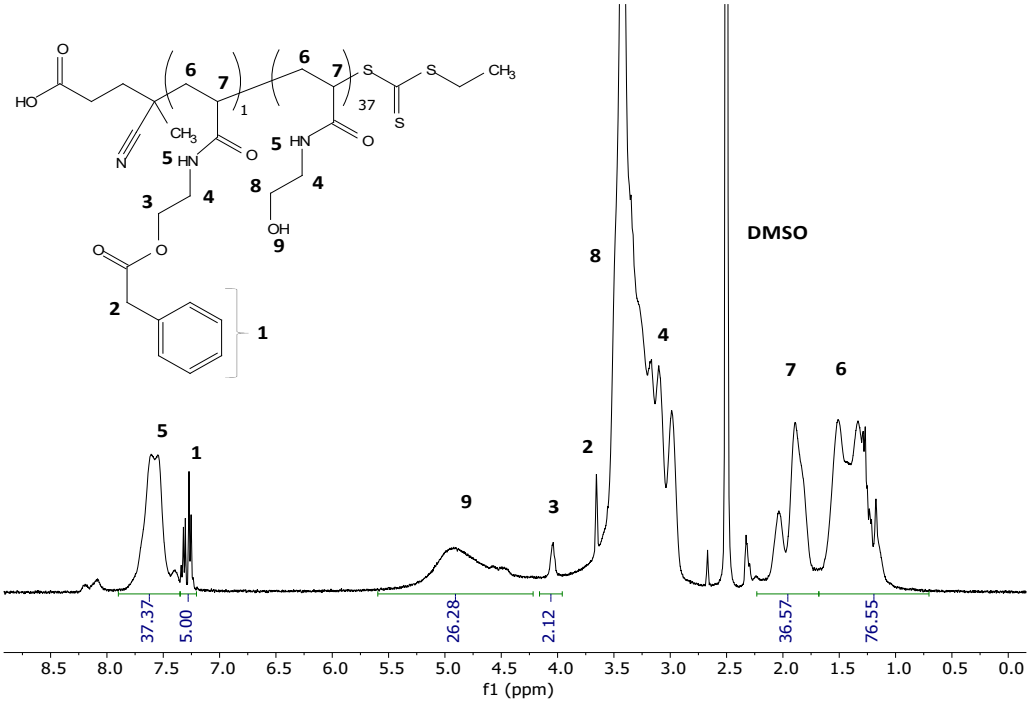


Figure S17. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer PHEN$_1$-*b*-HEA$_{37}$.

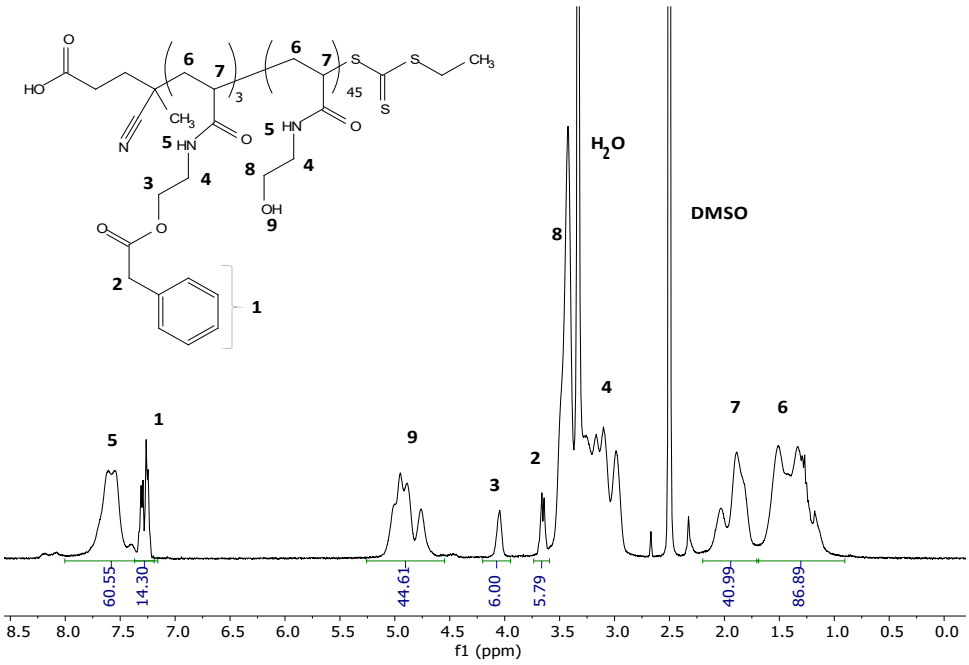


Figure S18. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer PHEN$_3$-*b*-HEA$_{45}$.

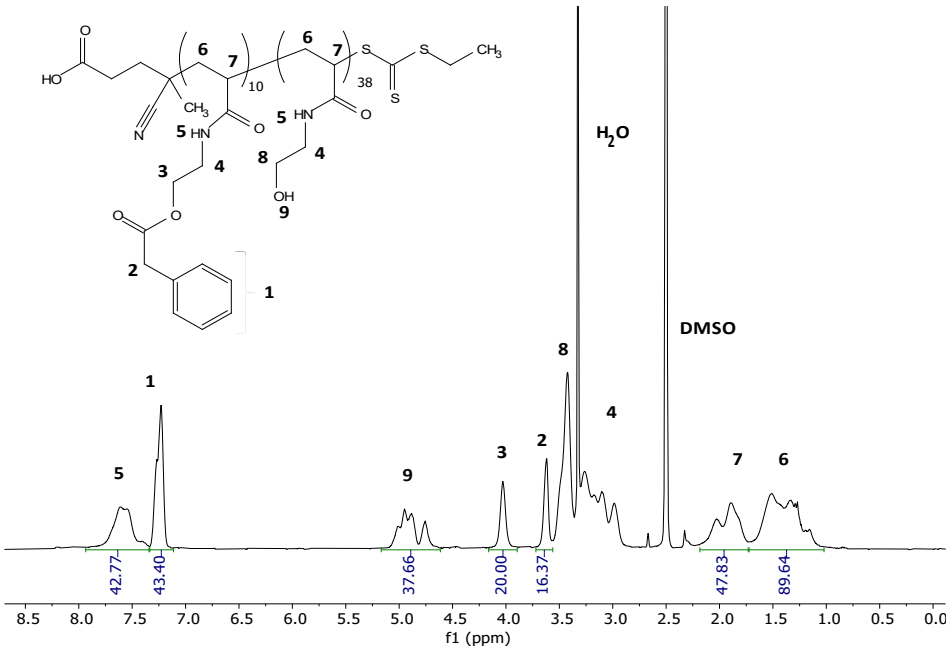


Figure S19. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer PHEN$_{10}$-*b*-HEA$_{38}$.

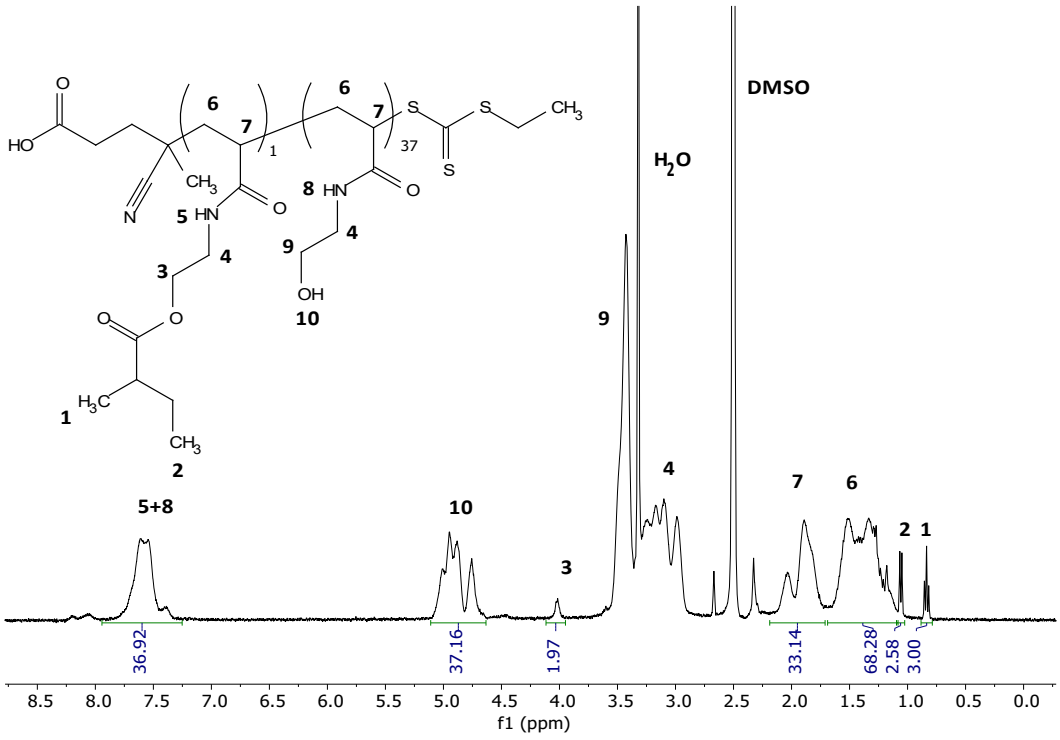


Figure S20. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer MTB$_1$-*b*-HEA$_{37}$.

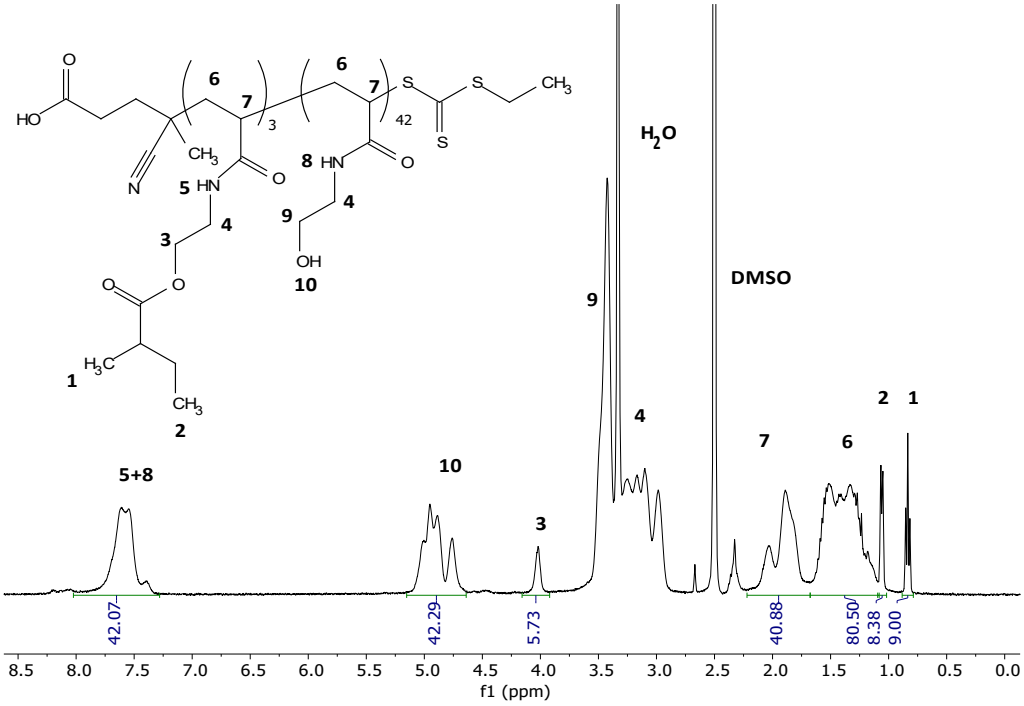


Figure S21. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer MTB_3-*b*-HEA_{42}.

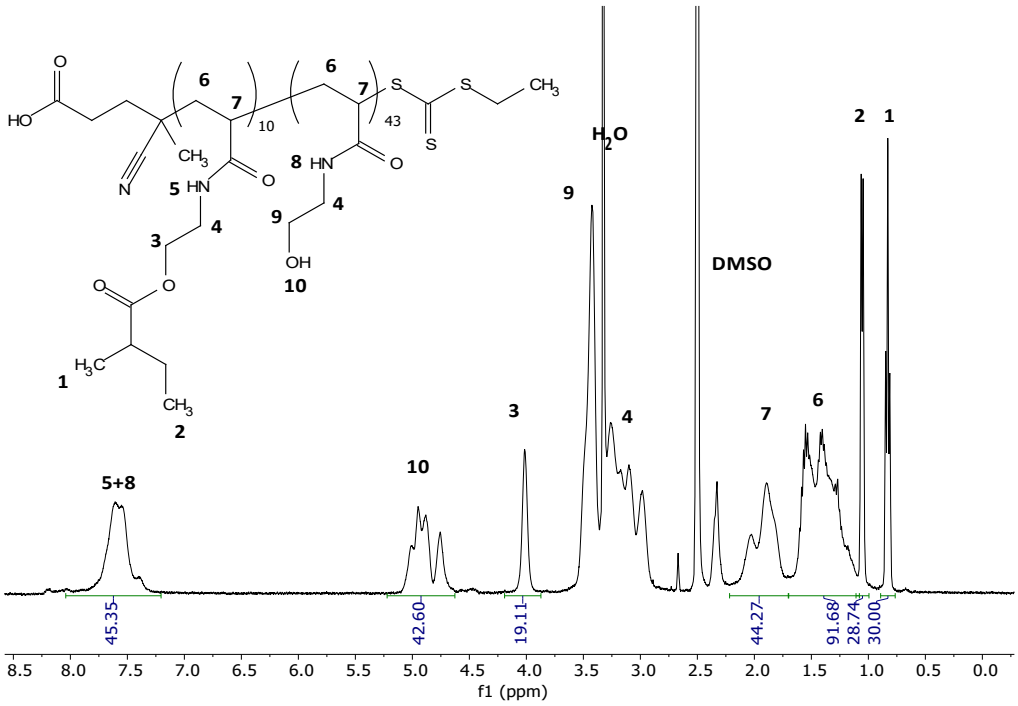


Figure S22. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer MTB_{10}-*b*-HEA_{43}.

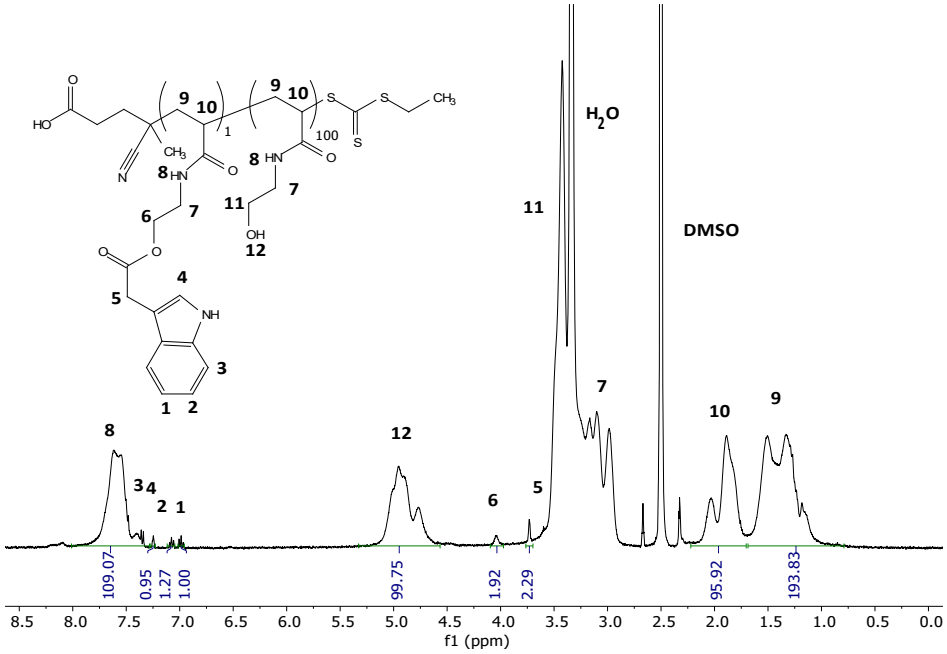


Figure S23. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer IND_1-*b*-HEA_{100}.

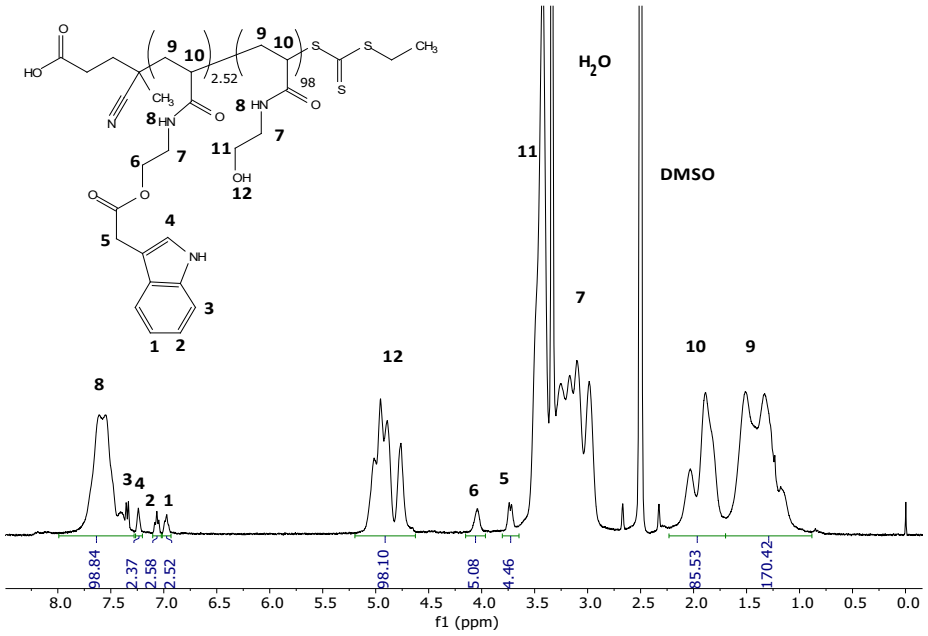


Figure S24. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer IND_3-*b*-HEA_{98}.

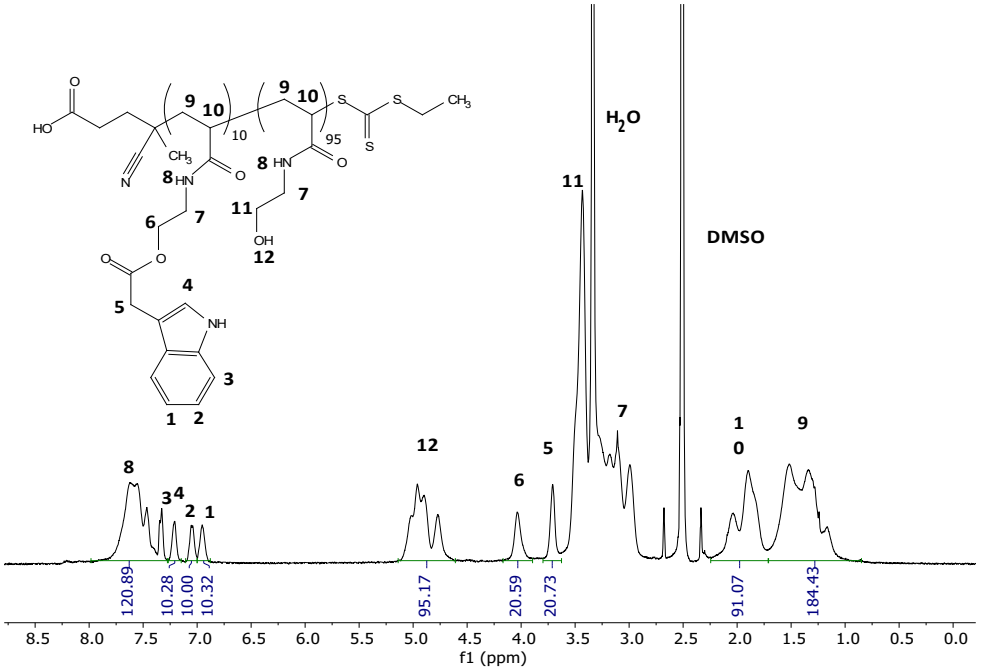


Figure S25. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer IND_{10}-*b*-HEA_{95}.

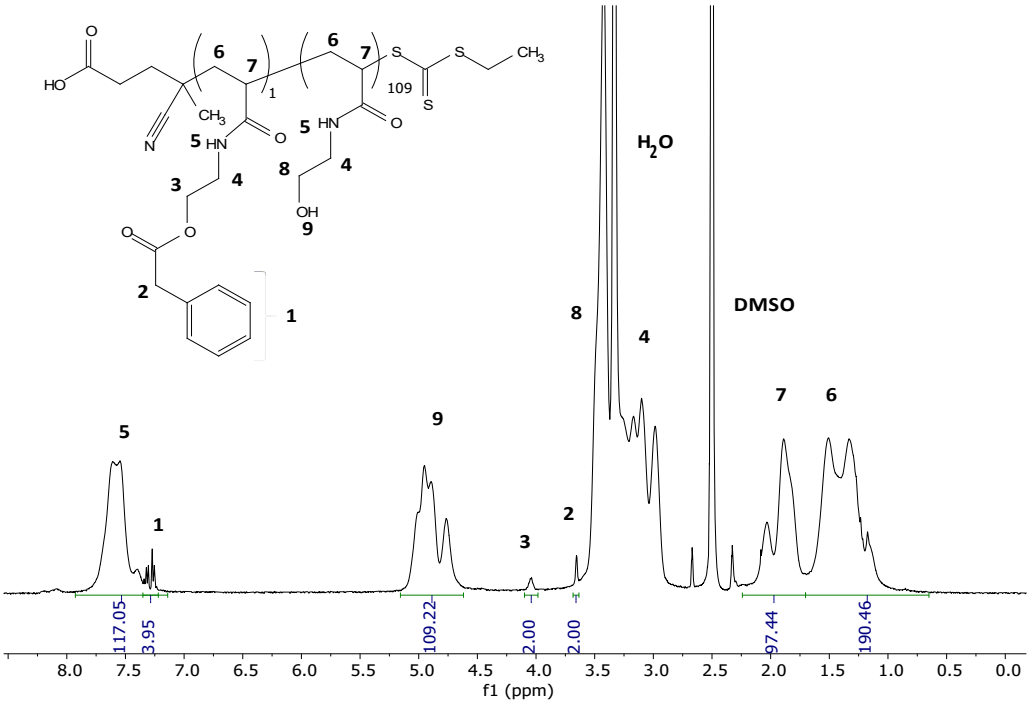


Figure S26. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer $PHEN_1$-*b*-HEA_{109}.

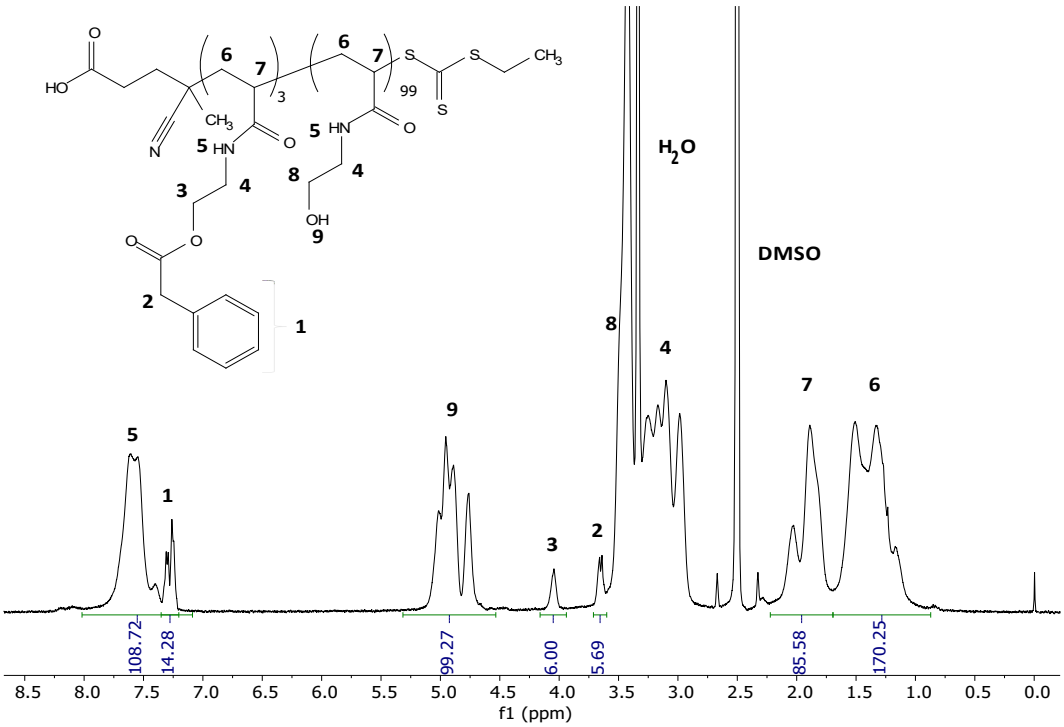


Figure S27. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer PHEN$_3$-*b*-HEA$_{99}$.

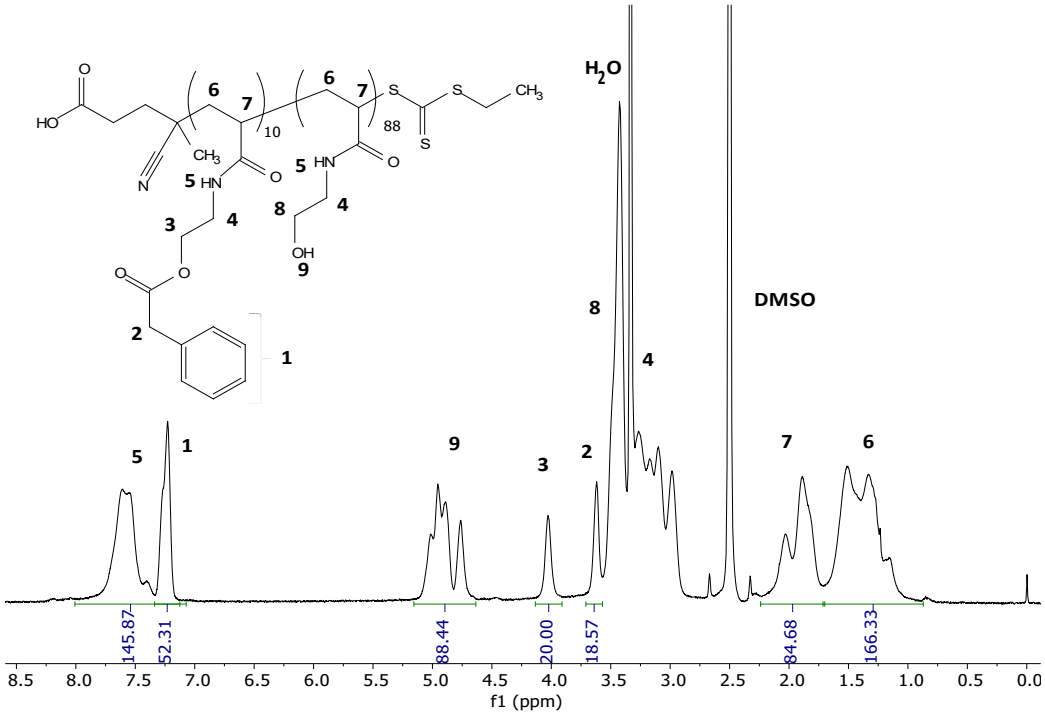


Figure S28. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer PHEN$_{10}$-*b*-HEA$_{88}$.

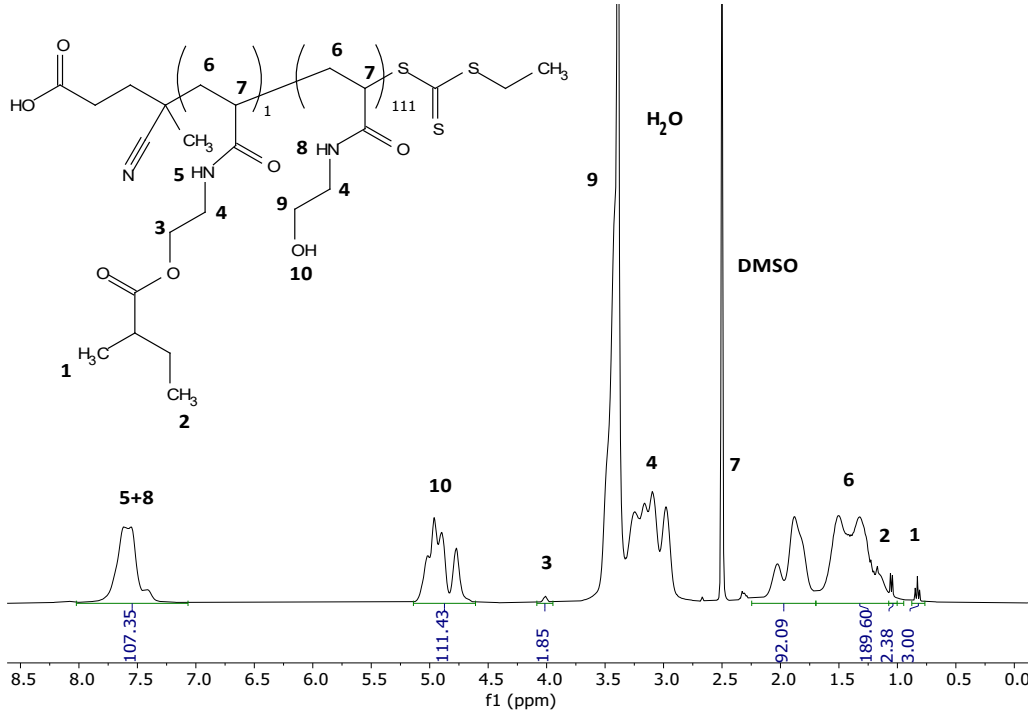


Figure S29. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer MTB_1-*b*-HEA_{111}.

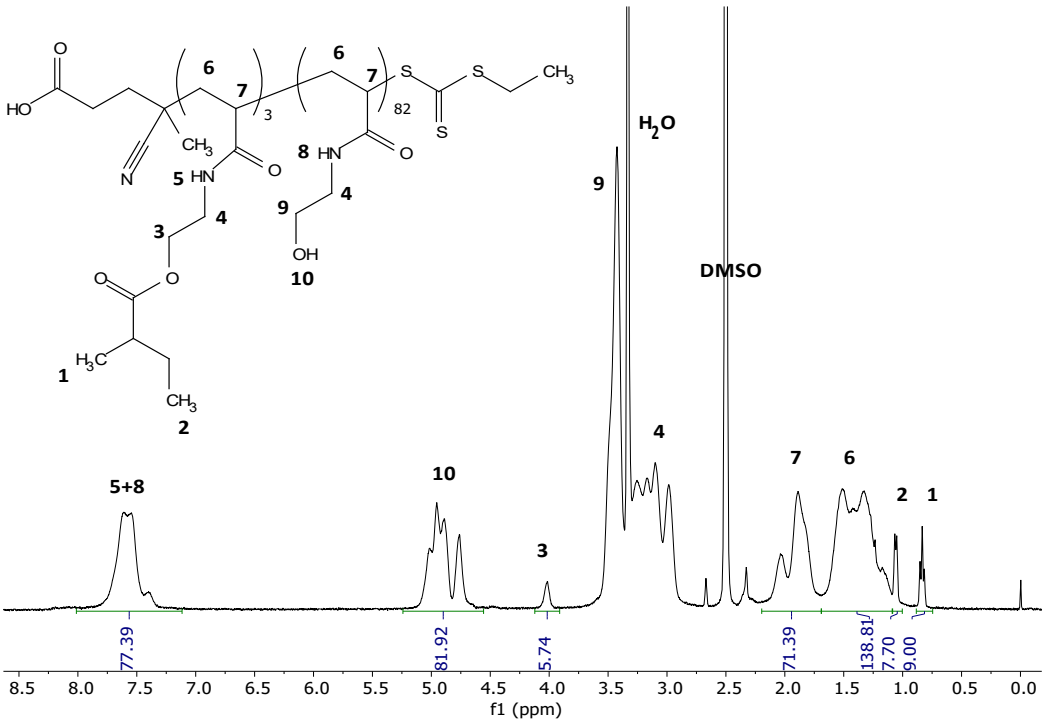


Figure S30. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer MTB_3-*b*-HEA_{82}.

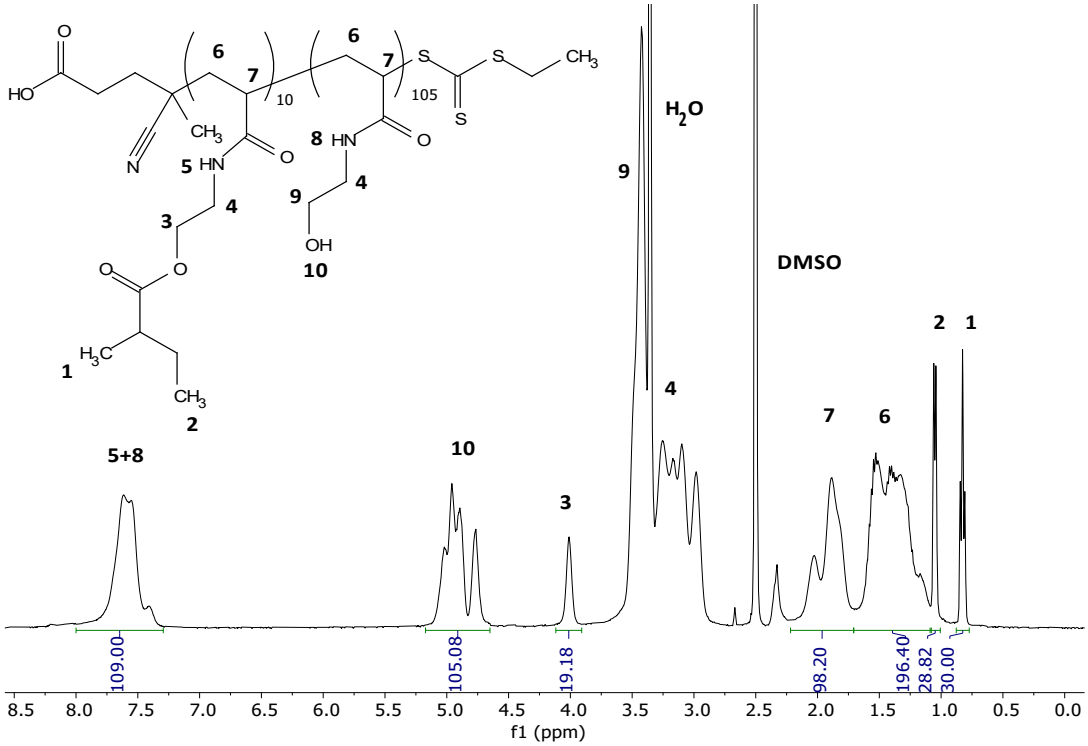


Figure S30. ^{1}H NMR (400 MHz, DMSO-d_6) of copolymer MTB_{10}-*b*-HEA_{105}.

6) Methods

Analysis of polymers self-assembly by dynamic light scattering

The polymers were dissolved in Milli-Q water at a concentration of 1.0 mg/mL. The solutions were left mixing for 2 h, and then analysed using a Malvern Zetasizer Nano. 3 repetitions were made for each sample.

Surface tension measurements of IND_3-*b*-HEA_{98} and IND_{10}-*b*-HEA_{95} solutions

IND_3-*b*-HEA_{98} and IND_{10}-*b*-HEA_{95} were dissolved in Milli-Q water at concentrations ranging from 0.05 to 15 mg/mL. The surface tension of each copolymer dilution was measured on a Kruss DSA 100 using the "pendant drop" method. Two droplets were analysed per sample, taking multiple measurement from each droplet.

Lysozyme aggregation assay

A lysozyme stock solution was prepared by dissolving the protein in water at a concentration of 100 mg/mL. 200 µL of this solution were added to 1800 µL of 100 mM phosphate buffer pH 12.3 in which the polymers had been previously dissolved, for a final lysozyme concentration of 10 mg/mL and a polymer: protein molar ratio of 1:1. From each polymer-protein mixture, 5 aliquots of 300 µL were transferred into a 96-well plate. The plate was loaded into a TECAN Spark 10M Multi-function Platereader. Absorbance at λ=500 nm was measured every 30 min for 24 h at 30°C to monitor the increase of turbidity resulting from protein aggregation.

Insulin aggregation experiment.

An insulin stock solution was prepared by dissolving the protein in 10 mM phosphate buffer pH 2 at a concentration of 4 mg/mL. Polymers stock solutions were prepared by dissolving various amounts of polymers in the same buffer as polyacrylate pendant units are known to be significantly more stable under mild acidic conditions than they are under basic pH.[1] Different amounts of insulin and polymers stock solutions were mixed to have a final protein concentration of 2 mg/mL and polymer:protein molar ratio of 5:1 and 10:1 in a final volume of 485 µL. After mixing, polymer/protein solutions were let under gentle stirring for 2 h. The pH was then raised to 5.3 by adding 15 µL of NaOH 0.5 M solution to each of the polymers:protein mixtures, which were left overnight at ambient temperature. The mixture was then centrifuged, the supernatant discarded, and the precipitate re-dissolved in 10 mM pH2 phosphate buffer to be analysed by RP-HPLC, using a Zorbax Eclipse Plus C18 column (3.5 µm, 95 Å, 4.6 × 12.5 mm), with a linear gradient of 20-90% acetonitrile in Milli-q water with 0.1% TFA.

Insulin aggregation study: Circular Dichroism analysis

Circular Dichroism (CD) spectra were recorded on an Applied Photo-physics Chirascan circular dichroism spectropolarimeter using a 1 mm path length quartz cuvette. CD measurements were performed at 25°C over a 180-280 nm wavelength range, using a response time of 1 s, 1 nm pitch and 0.5 nm bandwidth. The recorded spectra represent a smoothed of the original

scan. An insulin stock solution was prepared by dissolving the protein in 10 mM pH 2 phosphate buffer at a concentration of 4 mg/mL. IND_{10}-*b*-HEA_{95} stock solution was prepared by dissolving the polymer in the same buffer at 98.4 mg/mL. Different amounts of insulin and polymers stock solutions were mixed to have a final protein concentration of 2 mg/mL and polymer:protein molar ratio of 5:1 and 10:1 in a final volume of 990 µL. After mixing, polymer/protein solutions were let under gentle stirring for 2 h. The pH was then raised to 5.3 by adding 10 µL of NaOH 1 M solution to each of the polymers/protein mixtures. The mixtures were then purified by semi-preparative HPLC, using a Phenomenex Jupiter C18 column (10 µm, 300 Å, 10 × 250 mm), with a linear gradient of 30-60% acetonitrile. Purified protein samples were lyophilised and re-dissolved in 10 mM pH 2 phosphate buffer at a concentration of 0.2 mg/mL. pH was raised again to 7.4 before the analysis.

IDR1018 peptide aggregation study

A 1.0 mM peptide stock solution was prepared in water. 0.11 mM polymers stock solutions were prepared by dissolving the polymers in 100 mM phosphate buffer, pH 7.2. 50 µL of peptide were diluted in 450 µL of each polymer solution, for a final volume of 500 µL and a concentration of 0.10 mM for both peptide and the polymers. The solutions were let under stirring for 5 h and finally centrifuged. The supernatant was analysed by RP-HPLC to detect the amount of peptide left in solution, using a Zorbax Eclipse Plus C18 column (3.5 µm, 95 Å, 4.6 × 12.5 mm), with a linear gradient of 25-90% acetonitrile in Milli-q water with 0.1% TFA. Absorbance was recorded at λ= 280 nm.

7) Other supporting data

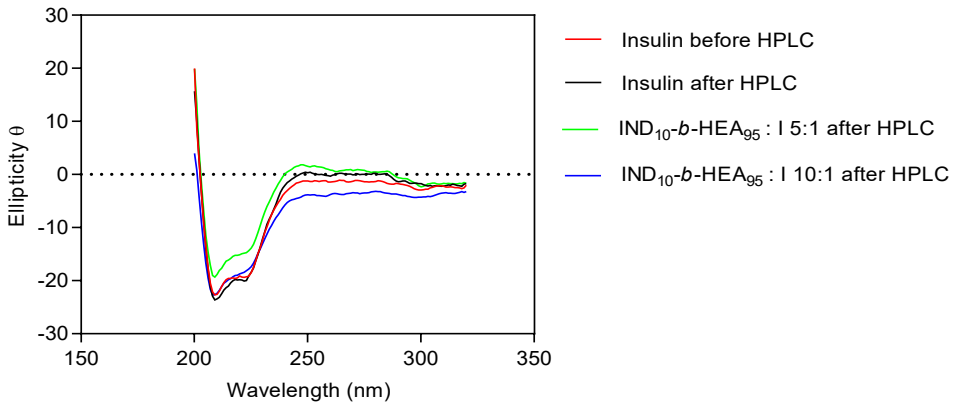


Figure S31. Effect of IND_{10}-*b*-HEA_{95} on insulin secondary structure: circular dichroism (CD) analysis. Profiles are showing native insulin, insulin purified by RP-HPLC, and IND_{10}-*b*-HEA_{95}: insulin mixtures at 5:1 and 10:1 molar ratio purified by RP-HPLC. RP-HPLC.

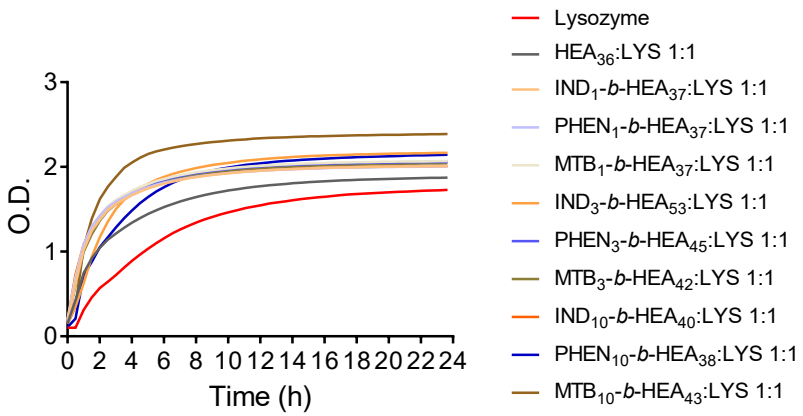


Figure S32. Aggregation assay of lysozyme and a mixture between 40 HEA-DP copolymers and lysozyme at 1:1 copolymers:lysozyme molar ratio. [Lysozyme]= 10 mg/mL in 100 mM pH 12.3 phosphate buffer. Lysozyme aggregation was assessed by turbidimetry, recording Abs λ=500 nm every 30 min for 24.

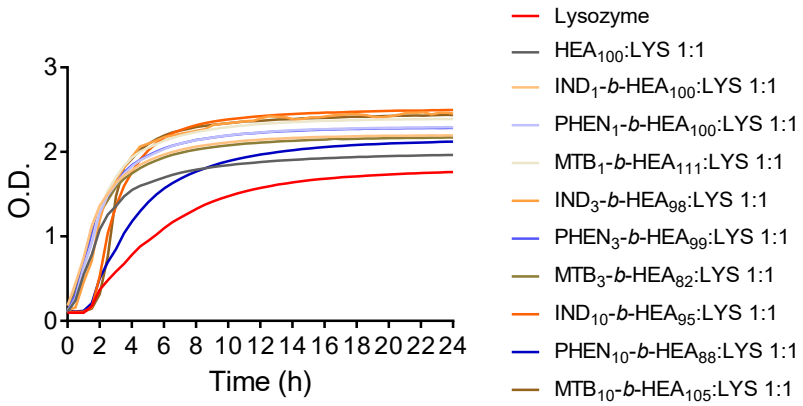
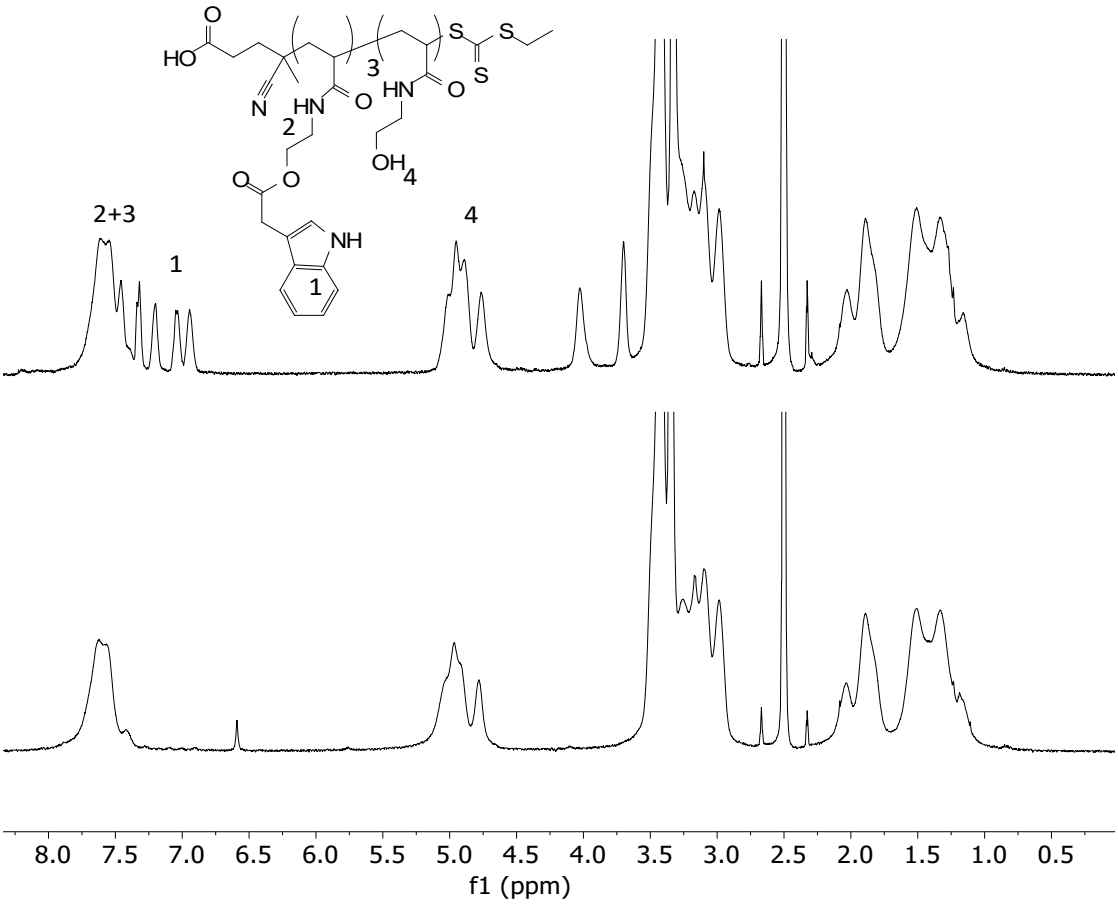


Figure S33. Aggregation assay of Lysozyme and a mixture between 100 HEA-DP copolymers and lysozyme at 1:1 copolymers:lysozyme molar ratio. [Lysozyme]= 10 mg/mL in 100 mM pH 12.3 phosphate buffer. Lysozyme aggregation was assessed by turbidimetry, recording Abs at 500 nm every 30 min for 24.



Spectrum S34. ^{1}H NMR (400 MHz, DMSO-d_6) of IND_{10}-*b*-HEA_{95} (a) before and (b) after 24 h treatment in 100 mM pH 12 phosphate buffer and subsequent precipitation in THF.

References

1 S. Fujisawa and Y. Kadoma, *Int J Mol Sci*, 2012, **13**, 5789–5800.