Supporting Information

Self assembling macromolecular chimeras: Preventing fibrillization of a β-sheet forming peptide by polymer conjugation

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Experimental

Materials and Methods

Styrene (99%), divinyl benzene (DVB, 80%) and vinylbenzyl chloride (VBC, 90%) (all Aldrich) were each passed through separate short columns of basic alumina before use. Azobis(*iso*butyronitrile) (AIBN) was purified by recrystallisation from methanol twice and then left to dry under reduced pressure. Trithiocarbonate RAFT agent (2-(butylthiocarbonothioylthio)propanoic acid) was synthesised in accordance with a previously published protocol.[1] Dimethylformamide (DMF) stored over molecular sieves (Labscan, 99%, anhydrous, low amine content) was used as received. Anhydrous dichloromethane (DCM), methanol, anhydrous tetrahydrofuran (THF), tetra-*n*-butylammonium fluoride (TBAF), pyridine, pyridyl disulfide (PDS), copper wire, sodium azide, copper sulfate pentahydrate, calcium sulfate, *n*-methyl morpholine, *N,N,N′,N″,N″*-Pentamethyldiethylenetriamine (PMDETA), tri*iso*propylsilane (TIPS), thioanisole and *N*-Di*iso*propylcarbodiimide were purchased from Sigma Aldrich at the highest purity available and used as received. Copper bromide (Aldrich, 98%) was suspended in glacial acetic acid then filtered and washed with acetic acid (× 5) then ethanol (× 5) before drying under vacuum in a dessicator. Triethylamine (Aldrich), was distilled and stored over potassium hydroxide under nitrogen. Amino acids and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) for peptide synthesis were purchased from Novabiochem and used without purification. Acetic anhydride, *t*-butanol, trifluoroacetic acid, sodium hydroxide and hydrochloric acid were purchased from Ajax fine chemicals and used as received. Purification *via* dialysis was carried out using Spectra Por dialysis tubing with MWCO 2000 Daltons.

Nuclear Magnetic Resonance (NMR)

NMR analyses were carried out on Bruker Ultra Shield Avance 200 or 300 spectrometers. For all NMR analyses, unless stated otherwise, deuterated DMSO (DMSO-d_6) was used as the solvent.

Size exclusion chromatography (SEC).

SEC analyses were carried out at 60 °C using a Shimadzu SEC system equipped with a guard column and two Polymer Laboratories PolarGelM columns attached to a differential refractive index (DRI) detector (Shimadzu, RID-10A) and a UV-Vis detector (Shimadzu, SPD-10A VP). Dimethylformamide (DMF) with lithium bromide (0.25% w/v) was used as the eluent and the flow rate was set at 0.6 ml/min at 60 °C. The system was calibrated using Polymer Laboratories narrow molecular weight distribution polystyrene standards.

Transmission electron microscopy (TEM)

Samples were prepared by placing a drop of sample on Parafilm, onto which a carbon coated copper grid was then placed for one minute. After sample adsorption, the grid was then placed on top of a drop of the staining solution (uranyl acetate). Upon removal from the stain solution, excess solution was carefully blotted off using filter paper and samples were air dried for at least 10 minutes under a tungsten lamp before analysis. TEM images were obtained using a Philips CM120 electron microscope.

Circular Dichroism (CD)

Measurements were performed in triplicate on a Jasco 715 spectropolarimeter using a 1mm quartz cell. Data was collected from 250 nm to 200 nm at 25^{o} C and the spectra reported are an average of four scans. Samples were diluted to give solutions of about 0.1 mg/mL peptide concentration prior to analysis. Mean residue ellipticity ([θ], in deg cm^2 dmol^{-1}) was calculated using the formula reported by Kopecek and co-workers,[2] $[\theta] = [\theta]_{obs}\ MRW/(10\times l\times c)$, where $[\theta]_{obs}$ is the ellipticity measured in millidegrees, MRW is the mean residue molecular weight of the peptide (molecular weight. of the unacetylated peptide, 1 551 Da, divided by the number of amino acid residues), l is the optical path length of the cell in cm (0.1 cm), and c is the peptide concentration in mg /mL.

Fourier transform infra-red (FT-IR)

Solid and pure liquid samples were analysed using a Bruker Optics Alpha-E FT-IR spectrometer equipped with an attenuated total reflectance (ATR) accessory. The number of scans per sample was set at 100.

Liquid samples in DMSO were placed between CaF_2 crystals separated by a Teflon spacer and analysed using a Shimadzu FT-IR 8400S spectrometer. Spectra were averages of 100 scans, recorded with a resolution of 4 cm^{-1} at room temperature and have had the blank solvent spectrum subtracted.

UV-Vis Spectroscopy

UV-Vis measurements were carried out using a Cary 50 Bio UV-visible spectrometer.

Solid phase peptide synthesis (SPPS)

SPPS was undertaken in plastic polypropylene syringes fitted with porous filters (Torviq).

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS)

MALDI-TOF mass spectrometry experiments were undertaken using a Waters (Micromass) TOF SPEC 2E mass spectrometer equipped with a nitrogen laser (λ = 337 nm). The accelerating voltage was 20 kV. Samples were dissolved in methanol at a concentration of 1mg/ml. The spectra were obtained in positive mode and the matrix employed was α-cyano-3-hydroxycinnamic acid. Sample and matrix were mixed and left to dry on a stainless steel plate. Data collection and analysis was carried out using MassLynx software.

Liquid chromatography-mass spectrometry (LC-MS)

LC-MS was conducted using a Thermo separation products spectra system consisting of P400 Pump and a UV6000LP photodiode array detector and a Sunfire C18(2) 5 μm, 2.1 150 mm column at a flow rate of 0.2 mL min^{-1} coupled to a Thermoquest Finnigan LCQ Deca MS detector. The

mobile phase employed was water with 0.1% (*v/v*) formic acid (Solvent A) and acetonitrile with 0.1% (*v/v*) formic acid (Solvent B).

Electrospray ionisation mass spectrometry (ESI-MS)

Mass Spectrometry was conducted using a Thermoquest Finnigan LCQ Deca MS detector with XCalibar Data Processing and Instrument Control Software. Samples of appropriate concentration were made up in methanol before injection into the electrospray ionization unit at 0.2 mL min^{-1}. The electrospray voltage was 5 kV, the sheathing gas was nitrogen at 415 kPa, and the heated capillary was set at 200 °C.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

Analytical reverse-phase RP-HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector and employed a Waters Sunfire C18 column (2.1 x 150 mm column, 5 μm particle size, flow rate of 0.2 mL min^{-1}). Preparative RP-HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with a 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at $\lambda = 230$ employing a Waters Sunfire Prep C18 OBD column (19 x 150 mm, 5 μ m particle size, flow rate 7 mL min^{-1}). The mobile phase consisted of eluents A (0.1% *v/v* TFA in water) and B (0.1% *v/v* TFA in acetonitrile) for all HPLC runs.

pH meter

Measurements were conducted at 27 ± 1 ^{o}C using a calibrated Hach IQ128 miniLab pH meter.

Atmospheric pressure chemical ionization (APCI). APCI was conducted on a Thermo-Finnigan LCQ Ion trap mass spectrometer.

Gas chromatography-mass spectrometer (GC-MS).

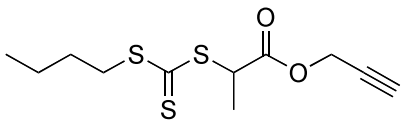
GC-MS was conducted on a Thermo-Finnigan Polaris Q spectrometer operated at 70 eV.

High resolution mass spectrometry (HR-MS)

HR-MS was conducted using a Bruker Daltonics Apex II 7T fourier transform ion cyclotron resonance mass spectrometer.

Procedures

Synthesis of prop-2-ynyl 2-(butylthiocarbonothioylthio) propanoate (6)



To a dry round bottomed flask was added 2-(butylthiocarbonothioylthio)propanoic acid (2.0 g, 8.4 mmol) and propargyl alcohol (2.4 g, 28.1 mmol). DCM (100 mL) at 0 ^{o}C was added to the flask with swirling to ensure complete dissolution of reactants. The mixture was cooled in an ice bath for 10 minutes. 4-(Dimethylamino)pyridine (1.54 g, 8.4 mmol) and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (2.4 g, 25.2 mmol) dissolved in 40 mL of DCM were then slowly added to the round bottomed flask *via* a pressure equalising dropping funnel. The reaction was stirred for a further 4 h at 0 ^{o}C and at room temperature for a further 16 hours. The reaction mixture was then washed with HCl (0.01 % w/v, 5 × 100 mL), water (10 × 100 mL), brine (3 × 100 mL) then dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification of the crude product by flash chromatography (eluent: 9:1 v/v hexane/ethyl acetate) gave **6** as a yellow oil. (1.6 g, 70 %). ^{1}H NMR (300 MHz, $CDCl_3$, Figure S1): δ (ppm from TMS) 0.93 (t, *J*=7.31 Hz, 3H, -CH_3), 1.42 (m, *J*=7.10 Hz, 2H, -CH_2), 1.61 (d, *J*=7.35 Hz, 3H, -CH_3), 1.7 (m, 2H, -CH_2), 2.49 (t, *J*=2.50 Hz, 1H, ≡CH), 3.36 (t, *J*=7.38 Hz, 2H), 4.73 (d, *J*=2.28 Hz, 2H, -CH_2), 4.85 (q, *J*=7.30 Hz, 1H, -CH). ^{13}C NMR (75 MHz, $CDCl_3$, Figure S2): 14.0 (CH_3), 17.1 (CH_3), 22.5 (CH_2), 30.3 (-CH_2), 37.4 (CH_2-S), 48.0 (CH-S), 53.5 (C-O), 75.8 (≡CH), 77.4 (-C≡), 170.9 (C=O), 222.1 (C=S). APCI (*m/z*): $[M+H]^+$ 277. FT-IR ATR υ (cm^{-1}): 1049 (C=S, str.), 1736 (C=O), 2129 (-C≡C-, str), 3293 (C≡C-H, str.).

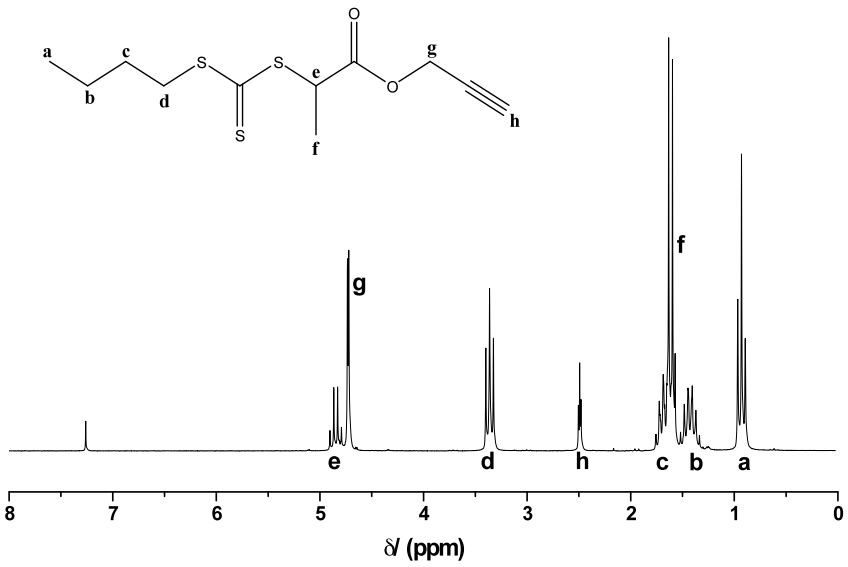


Figure S1: ^{1}H NMR of purified **6.**

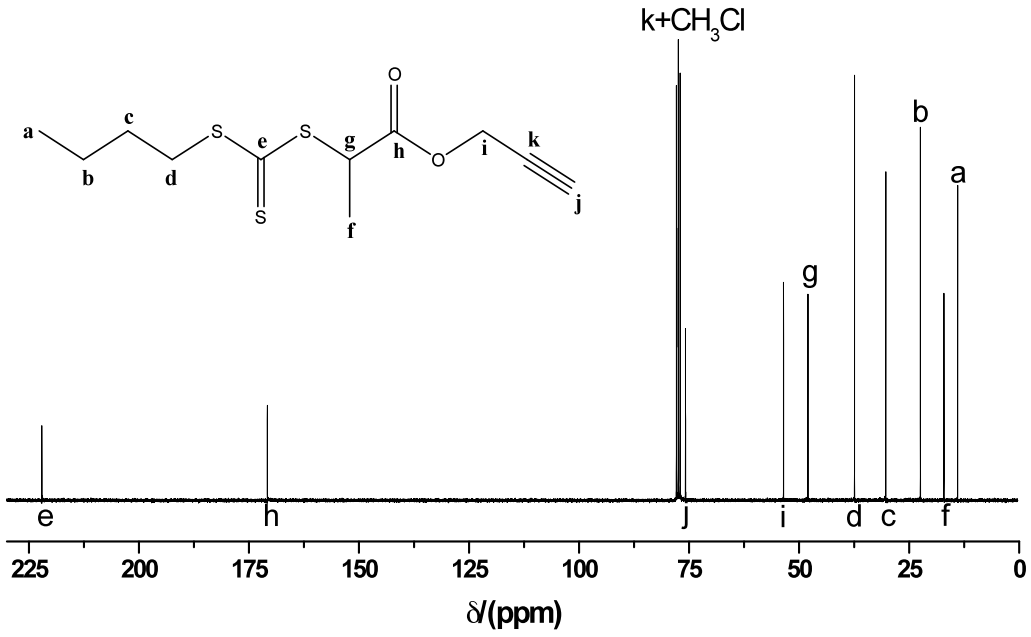


Figure S2: ^{13}C NMR of purified **6**.

Polymerisation of HEA (3, General procedure).

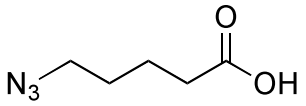
Compound **6** (0.1200 g, 0.43 mmol), HEA (**3**) (2.1g, 18.10 mmol) and AIBN (0.0036 g, 0.02 mmol) were weighed into a glass vial and dissolved in *t*-butanol (5.3 mL). The mixture was thoroughly mixed on a vortex mixer before aliquots (1.2 mL each) were taken and placed in polymerisation test tubes immersed in ice. The polymerisation tubes were sealed using rubber septa and oxygen was removed from the solution by sparging with nitrogen for 15 minutes. After degassing, the vials were transferred to a heated oil bath maintained at 60 ^{o}C and polymerisation was allowed to proceed for the desired period with one polymerisation tube being removed at the end of each period. Upon removal from the oil bath, the reaction was stopped by placing the reaction vessel in an ice bath and opening the reaction to the atmosphere. Conversion was determined by ^{1}H NMR.

P(HEA) polymers **4** and **5** that were used for the 'click' reactions were synthesised using a protocol similar to that described above. Purification of the polymers was achieved *via* repeated precipitation in cold diethyl ether followed by drying under reduced pressure.

Table S1: Characterisation of alkynyl P(HEA) polymers used for 'click' reactions by SEC, ESI and ^{1}H NMR.

Sample ID	***Conversion %***	***M_n ESI g/mol***	***M_n ^{1}H NMR g/mol, (DP)***	***M_n SEC g/mol***	***PDI (SEC)***
$P(HEA)_{27}$	63	3490	3132 (27)	4100	1.1
$P(HEA)_{16}$	41	1832	1856 (16)	1650	1.1

Synthesis of 5-azido pentanoic acid (2)



The method followed is a combination of protocols described by Srinivasan *et al*.[3] and Khoukhi *et al*.[4] Bromovaleric acid (7.24 g, 40 mmol) was dissolved in methanol under an inert atmosphere and the solution was cooled to 0 ^{o}C. To this solution was added thionyl chloride (120 mmol), dropwise, via a droping funnel and the resulting solution was stirred at 0 ^{o}C for 30 minutes then for 16 hours at room temperature. After the reaction, MeOH was removed *in vacuo* and the resulting residue was suspended in ethyl acetate (50 mL) followed by extraction with $NaHCO_3$ (3 × 40 mL), water (3 × 40 mL) and brine (1 × 30 mL). The organic layer

was then dried over sodium sulfate and methyl-5-bromopentanoate was obtained after solvent removal. The methyl-5-bromopentanoate was used without purification for the azidation step.

Methyl 5-bromopentanoate was dissolved in DMSO (30 mL) and sodium azide (5 g, 77 mmol) was added to the solution with rapid stirring. The suspension was stirred at 50 ^{o}C for 24 hours before it was cooled and taken up in water. The resulting mixture was extracted with ether (4 × 50 mL) and the combined ether extracts were then washed with brine followed by drying over sodium sulfate. Removal of the solvent was done on the rotary evaporator to yield methyl 5-azidopentanoate as a brown oil (5.7 g, 91%) which was then dissolved in 30 mL of THF/water solution (3:1 *v/v*) followed by addition of aqueous LiOH (73 mmol in 20 mL water). The mixture was left to stir for 4 hours after which the solvent was removed *in vacuo*. The resulting residue was taken up in ethyl acetate (50 mL) then washed with 1N HCl (3 × 50mL), water (5 × 50 mL) and brine (2 × 50mL). After washing, the organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo* to yield **2** as a brown oil (4.8 g, 93%).
^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): (2H, -CH_2), δ 11.73 ppm (1H, s, -COOH), 3.27 (t, *J*= 6.45 Hz, 2H, -CH_2), 2.36 (t, *J*= 6.95 Hz, 2H, -CH_2), 1.75 (m, 4H); FT-IR (N_3 str, 2019 cm^{-1}, COOH 1670 cm^{-1}, 3000 cm^{-1}). These data are in agreement with that previously reported by Srinivasan *et al.*[3]

Synthesis of peptides.

Loading of the Fmoc protected Rink resin

Rink amide resin (1 g, 0.74 mmol/g) was swollen in DCM (~ 12 mL) for one hour before use then washed with DMF (× 5). Deprotection of the Fmoc protected resin was achieved by treating the resin with piperidine in DMF (20% v/v) (2 × 3 mins). The resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5) before addition of the first amino acid. Fmoc-Gln(Trt)-OH (5 eq.) and HOBt (5 eq) were dissolved in DMF. Di*iso*propylcarbodiimide (DIPCDI) (5 eq.) in DMF was then added to the amino acid solution and the resulting solution was left to stand for 10 minutes prior to addition to the resin. The reaction mixture was left on a shaker for 6 hours. After coupling, the resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5) before capping with acetic anhydride/pyridine solution 1:9 v/v. Finally, the resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5). Resin loading was found to be 0.56 mmol/g as determined by deprotecting with 20%

piperidine in DMF (2 × 3 min) and measuring the absorbance of piperidine-fulvene adduct at λ = 301 nm.

Iterative peptide assembly (Fmoc-strategy)

Deprotection: The resin was treated with 20% piperidine/DMF (2 × 3 min) and washed with DMF (× 5), DCM (× 5) and DMF (× 5). *Amino acid coupling:* A pre-activated solution of protected amino acid (4 eq.), PyBOP (4 eq.) and NMM (8 eq.) in DMF (final concentration 0.1 M) was added to the resin. After shaking for 2 h, the resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5). *Capping of unreacted chains*: Unreacted chains were capped using a solution of acetic anhydride/pyridine (0.3 M/0.03 M) in DMF. The solution was taken up into the syringe and the mixture left on the shaker for 10 minutes. After capping the resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5).

Azidopentanoic acid coupling: After adding the last amino acid residue of P_{11} (Gln), the Fmoc deprotection step was carried out, then a pre-activated solution of **2** (4 eq.), PyBOP (4 eq.) and NMM (8 eq.) in DMF (final concentration 0.1 M) was added to the resin. After shaking for 4 h, the resin was washed with DMF (× 5) and DCM (× 10) and the capping step omitted.

P_{11}-2: After adding the last amino acid in the P_{11} sequence, Fmoc deprotection was performed followed by *N*-acetylation of the peptide with acetic anhydride/pyridine solution (0.3 M/0.03M) in DMF for 10 minutes. The resin was washed with DMF (× 5) and DCM (× 10) before cleaving. *Cleavage*: A mixture of TFA, thioanisole, tri*iso*propylsilane (TIPS) and water (85:5:5:5 *v/v*) was added to the resin. After shaking for 4 h, the resin was washed with TFA (3 × 2 mL) *Work-up*: The combined cleavage solution and TFA washings were concentrated *in vacuo* and the product was precipitated in cold diethyl ether. The mixture was centrifuged to collect product as a white precipitate which was then washed with ether (× 5). The traces of solvent were removed on a rotary evaporator to obtain dry product which was dissolved in DMSO and purified by preparative HPLC using the at column dilution method[5] (Gradient 5 to 35% B over 40 min).

Characterization: (i) **P_{11}-2**, MALDI-TOF (*m/z*): $[M+H]^+$ 1594. This result is in agreement with that previously published by Aggeli *et al*.[6] (ii) **N_3-P_{11}**, HPLC (Figure S3, gradient 5 to 35% B over 40 min, R_t = 20.3 mins), ESI (*m/z*): $[M+H]^+$ 1677; $[M+2H]^{2+}$ 839. FT-IR ATR ν_{max}/cm^{-1} (2109 N_3, str.). HR-MS (*m/z*): Calculated for $C_{75}H_{106}N_{25}O_{20}$: $[M+H]^+$, 1676.7993. Found $[M+H]^+$, 1676.7993.

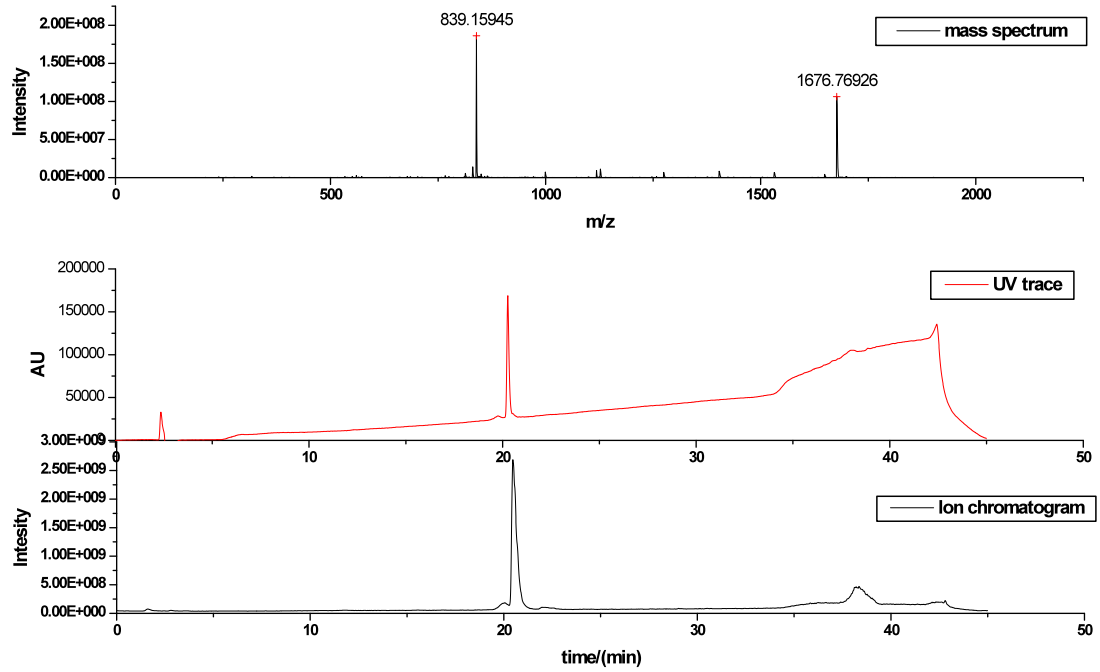
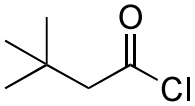


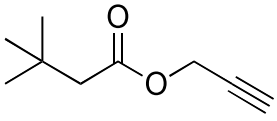
Figure S3: LC-MS spectrum of N_3-P_{11}. (Gradient 5 to 35% B over 40 min).

Synthesis of 3,3-dimethylbutanoyl chloride



To 3,3-dimethylbutynoic acid (3.4 g, 29.3 mmol) in dried DCM (30 mL) in an ice bath at 0 ^{o}C was added dropwise an excess of thionyl chloride (5.0 g , 42.4 mmol) and the reaction was stirred at room temperature for 16 hours. The remaining thionyl chloride and solvent were then removed *in vacuo* to afford 3,3-dimethylbutanoyl chloride as a yellow oil (4.1 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$), δ 1.11 (9H, s, -CH_3), 2.87 (2H, s, -CH_2). These data are in agreement with that previously reported by Dubois *et al.*[8]

Synthesis of prop-2-ynyl 3,3-dimethylbutanoate (7)



A solution of 3,3-dimethylbutanoyl chloride (4.0 g, 26 mmol) in anhydrous DCM (10 mL) was added dropwise to an excess of propargyl alcohol (7.3 g, 130 mmol) and triethylamine (4.4 mL, 31 mmol) in anhydrous DCM (20 mL) under nitrogen at 0 ^{o}C . The reaction was stirred for 30 minutes at 0 ^{o}C then at room temperature for 4 hours before it was diluted to 50 mL with DCM and extracted with $NaHCO_3$ (3 × 50 mL), 0.1 M HCl (3 ×50 mL), water (10 × 100 mL) and brine (1 × 50 mL) . The organic layer was dried over

anhydrous Na_2SO_4 and **7** was obtained as a yellow oil (3.3 g, 82%) after removal of solvent *in vacuo*. ^{1}H NMR (300 MHz, $CDCl_3$), δ 1.00 (9H, s, CH_3), 2.30 (2H, s, CH_2CO), 2.54 (1H, m, C), 4.69 (2H, s, CH_2-O). ^{13}C (75 MHz, $CDCl_3$): δ 30.0 (CH_3), 31.4 (-C-(CH_3)), 48.0 (-CH_2), 51.7 (-CH_2-O), 75.1 (≡CH), 78.3 (-C≡) , 171.9 (C=O) GC-MS (*m/z*): 154 $[M]^+$. FT-IR ATR ν_{max} /cm^{-1}: 1735 (C=O), 2129 (-C≡C-, str), 3298 (C≡C-H, str.)

'Click' reaction between N_3-P_{11} (1) and 7 to produce PDB-P_{11} (8)

To a vial equipped with a stirrer bar, **1** (0.0150 g, 8.9 μmol), and CuBr (0.0065 g, 45.5 μmol) were added and the vial was sealed with a suba seal then carefully evacuated and backfilled with nitrogen three times. In a separate vial, **7** (1 mL of stock solution, 0.00210g, 13.6 μmol) and PMDETA (1 mL of stock solution, 7.8 mg, 45 μmol) from 10 mL stock solutions (in DMSO) were added and the vial was sealed with a suba seal then purged with nitrogen for 20 minutes. The **7** / PMDETA solution was then cannulated into the azide/copper containing vial under nitrogen. The resulting solution was thoroughly mixed on a vortex mixer and the vial was placed in a pre-heated oil bath at 40 ^{o}C. Monitoring of the reaction progress was accomplished by LC-MS and the reaction was judged to have gone to completion after 24 hours. The reaction mixture was also analysed by FT-IR which showed the characteristic azide absorbance peak at 2100 cm^{-1} had completely disappeared, indicating that the reaction conversion was 100%. Purification of the reaction mixture was achieved by HPLC (Figure S4, gradient 5 to 50% B over 80 min, R_t = 42 mins). HR-MS (*m/z*): Calculated for $C_{84}H_{120}N_{25}O_{22}$: $[M+H]^+$, 1830.9034. Found $[M+H]^+$, 1830.9015.

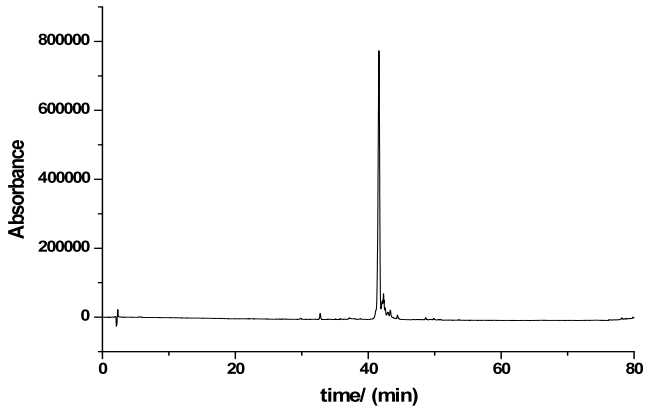


Figure S4: Characterisation of purified **8** by HPLC.

Synthesis of azide functionalised solid supports

The method followed is similar to that described by Monteiro and co-workers.[7] To a glass vial equipped with a magnetic stirrer was added VBC (8 mL, 0.056 mol), styrene (6.4 mL, 0.028 mol), DVB (0.240 mL, 8.42×10^{-4} mol) and AIBN (14 mg, 8.4×10^{-5} mol). The vial was sealed with a rubber septum then degassed for 15 minutes by passing nitrogen through the system whilst the vial was submerged in an ice bath. The vial was then transferred to a temperature controlled oil bath set at 50 ^{o}C for 24 hours then at 100 ^{o}C for 6 hours. The resulting cross-linked polymer was ground with mortar and pestle then washed by stirring in 50 mL of DMF at 50 ^{o}C for 1 h which was followed by the filtration of the mixture whilst hot. Washing of the product was repeated four more times after which the product was then washed with acetone followed by drying under vacuum overnight. After drying, the cross-linked polymer (4 g), sodium azide (5.68 g, 0.087 mol) and DMF (40 mL) were added to a 50 mL round bottom flask equipped with a magnetic stirrer and the mixture was placed in an oil bath at 50 ^{o}C for 48 h. The reaction mixture was filtered hot and washed with copious quantities of water to remove sodium azide then washed with acetone. The azide functionalised cross-linked polymer was then added to a round bottom flask containing 50 mL of DMF and left to stir at 90 ^{o}C for 30 min after which the mixture was filtered hot. The washing procedure was repeated twice after which the polymer was washed several times with DMF and then acetone. The resulting azide functionalised cross-linked polymer was then dried under vacuum overnight. FT-IR ATR ν_{max} /cm^{-1}: 2109 N_3 str.(Figure S5).

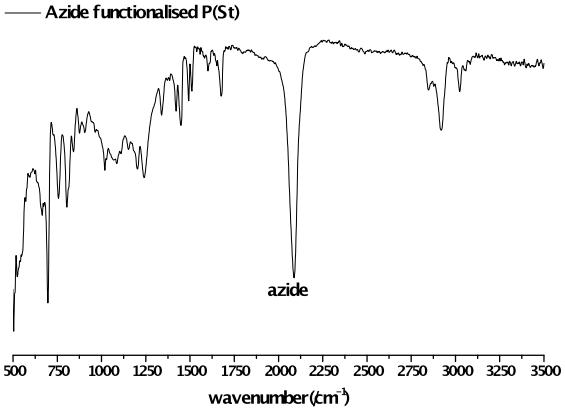


Figure S5: FT-IR spectrum of azide functionalised solid supports

Reactivity of azide functionalised polymer and estimation of azide loading

3-(Ethynyloxy)-4,5-dihydroxybenzoic acid (20 mg, 96 μmol) and crosslinked azide functionalised polymer (20 mg, 50 μmol) were weighed into a glass vial equipped with a magnetic stir bar. The vial was sealed using a rubber septum and then degassed by evacuation and backfilling with nitrogen five times. Copper bromide (26 mg, 181 μmol) was weighed into a separate glass vial which was also sealed with a rubber septum and degassed by evacuation and backfilling with nitrogen. Previously degassed DMF containing PMDETA (40 μL, 197 μmol) was then added to the copper bromide containing glass vial under nitrogen and the resulting solution was then cannulated into the vial containing the crosslinked polymer. The reaction mixture was placed in an oil bath at 40 ^{o}C and left to stir for 72 hrs (to achieve maximum possible conversion). The crude reaction mixture was then analysed by HPLC and the loading was estimated based on the peak areas of the alkyne compound in the crude reaction mixture and of a solution of known concentration of the alkyne. Azide loading based on HPLC results was 2.5 mmol/g (theoretical loading = 3.8 mmol/g, assuming all VBC was incorporated into polymer and converted into azide).

Typical procedure for 'Click' reactions between 1 and alkynyl P(HEA) polymers (4 and 5)

$P(HEA)_{16}$ (**4**) (67 mg, 36.1 μmol) and N_3-P_{11} (40 mg, 23.9 μmol) were added to a dry vial which was subsequently sealed then evacuated and backfilled with nitrogen five times. Previously degassed DMSO (2.5 mL) was then added to solubilise the polymer and peptide. Copper bromide (0.0172 g, 119.9 μmol) was weighed into a vial containing a stirrer bar and the vial was also sealed then degassed by evacuation and nitrogen backfilling. In another vial, a solution of PMDETA (25 μL, 120.0 μmol) in DMSO (1.5 mL) was made by adding previously degassed DMSO into a thoroughly degassed vial containing PMDETA and the resulting solution was degassed using nitrogen gas for a further 10 minutes. Using a cannula, the PMDETA solution was then added to the copper bromide containing vial followed by the transfer of the polymer-peptide solution to the same vial. The resulting solution was mixed thoroughly then placed in a pre-heated oil bath at 40 ^{o}C for 3 days. Analysis of the reaction mixture by FT-IR at the end of the reaction period showed the disappearance of the azide peak (2109 cm^{-1}) indicating the reaction had gone to completion. The reaction mixture was analysed by HPLC (10% to 70% B over 80 min) which showed the absence of the peak expected from the peptide.

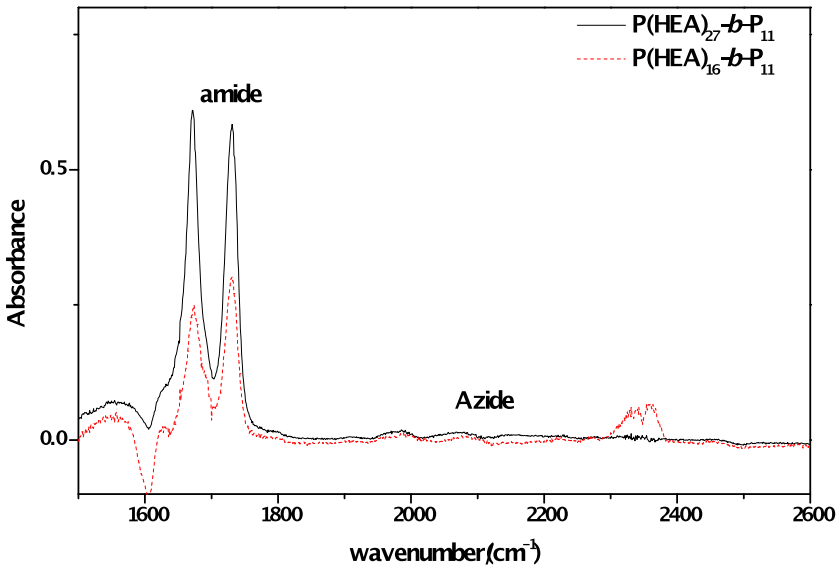


Figure S6: FT-IR spectra of crude 'click' reaction mixtures after 72 hours of reaction between alkynyl P(HEA) polymers and N_3-P_{11}.

Purification of conjugates 9 and 10

To remove excess polymer from solution, azide functionalised polystyrene supports (1.5 g, 3.75 mmol) were swollen in DMF (3 mL) and the 'click' reaction mixture obtained above was then added. Copper wire (0.5 g) and PMDETA (20 μL) were also added to the vial and the reaction was left to stir 48 hours at 50 ^{o}C. The mixture was then centrifuged and the solution was carefully drawn off using a pipette. To the remaining resin was added DMSO/DMF (1:1) solution followed by stirring for 15 minutes at 50 ^{o}C. The mixture was then centrifuged and the resultant solution decanted. This washing step was performed three times. The decanted solutions were then combined and filtered before dialysis against deionised water for 4 days (membrane MWCO = 2000 g/mol). The conjugates were analysed by HPLC (10% to 70% B over 80 min): (i) $P(HEA)_{27}$-*b*-P_{11} **(10)**, R_t = 32 min, (ii) $P(HEA)_{27}$-*b*-P_{11} **(9)**, R_t = 38 mins; and FTIR (Figure S7).

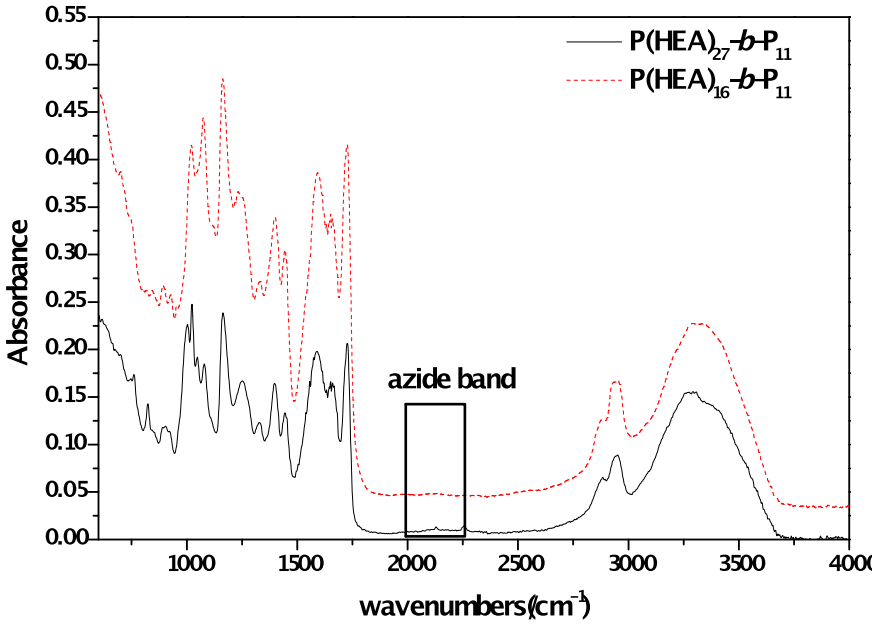


Figure S7: FT-IR spectra of freeze dried chimeras after purification.

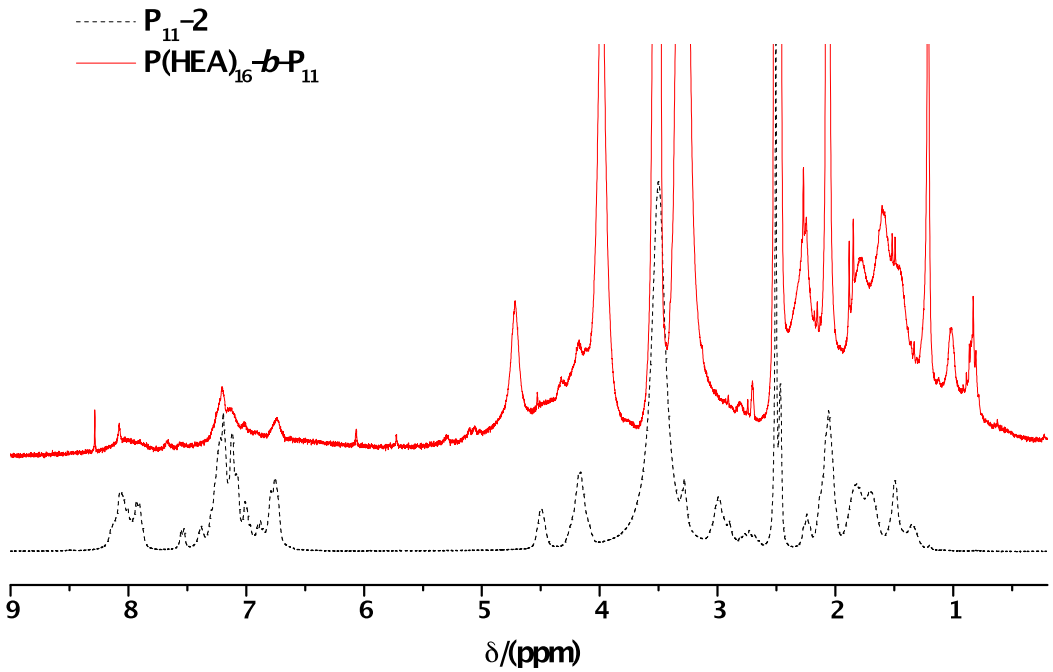


Figure S8: ^{1}H NMR spectra of **9** and P_{11}-2. The spectrum of **10** is similar to that observed for **9**.

Self assembly experiments

HCl (5 mL, 0.1 M) and NaOH (5 mL, 0.1 M) were mixed and then diluted to 100 mL. The pH of this solution was measured and then adjusted to pH 7 by addition of acid or base to give an aqueous solution that was used for self assembly studies of conjugates or peptides. Pre-weighed peptides or conjugates were dissolved in the pH 7 aqueous solution and the pH was measured using a mini pH meter. The pH was then adjusted to the desired value using NaOH (1 M) or HCl (1 M). Samples were then left in the dark for up to a maximum of 10 days before analysis by CD and TEM.

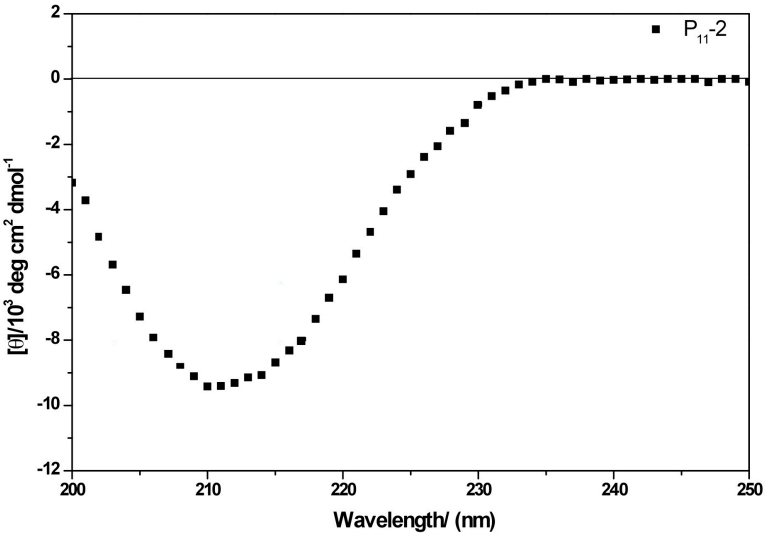


Figure S9: Far UV-CD spectrum of P_{11}-2 at pH 2 (6mM) after 3 days incubation.

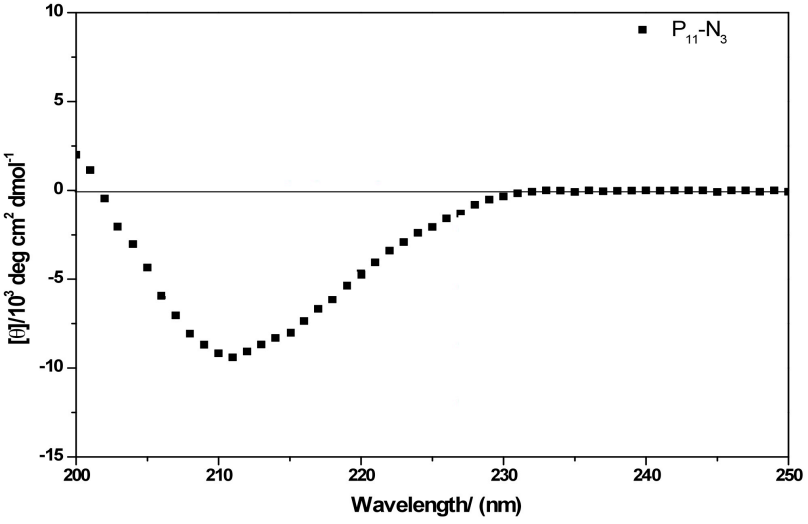


Figure S10: Far UV-CD spectrum of $\mathbf{N_3}$-$\mathbf{P_{11}}$ at pH 2 (6mM) after 3 days incubation.

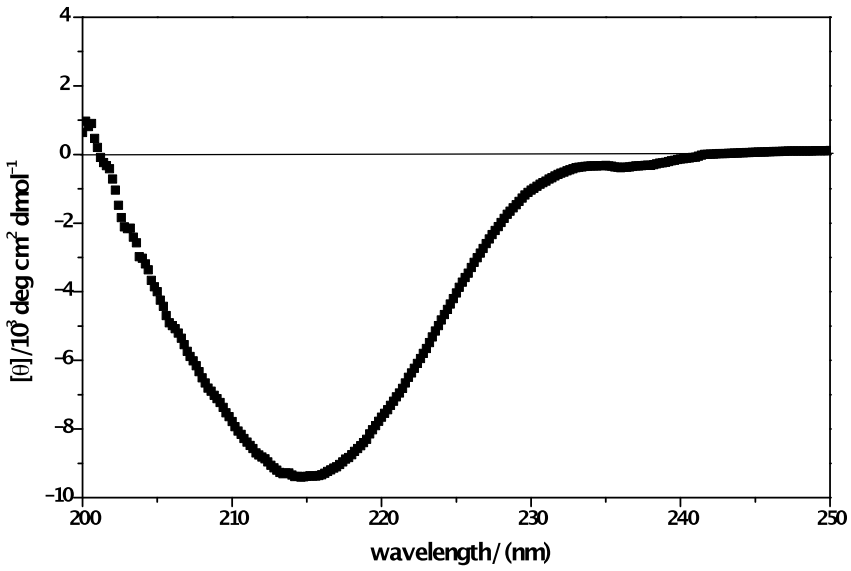


Figure S11: Far UV-CD spectrum of PDB-P_{11} **(8)** at pH 2 (6mN) after 10 days of incubation.

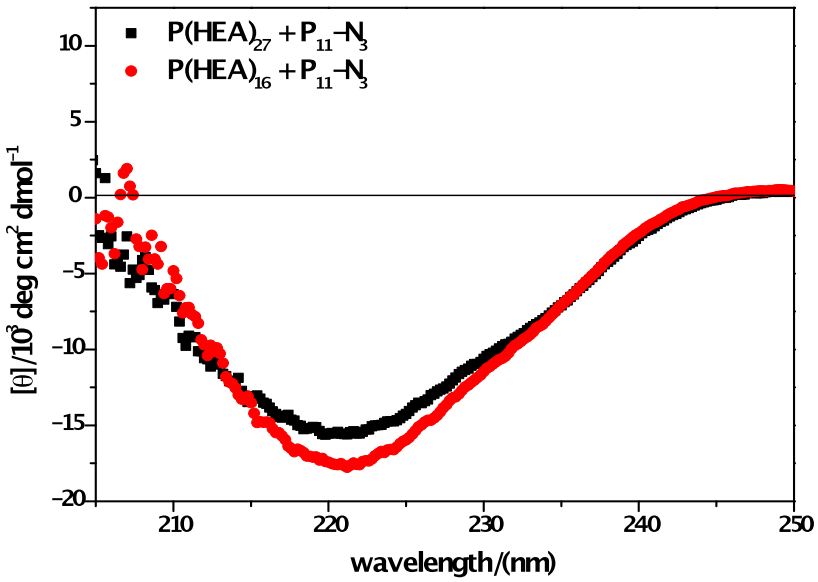


Figure S12: Typical far UV-CD spectrum of $P(HEA)_n$ mixed with $\mathbf{N_3\text{-}P_{11}}$ (6mM) at pH 2 after 10 days of incubation. In this figure, the ratio of P(HEA): $\mathbf{N_3\text{-}P_{11}}$ is 1:1 (mol/mol).

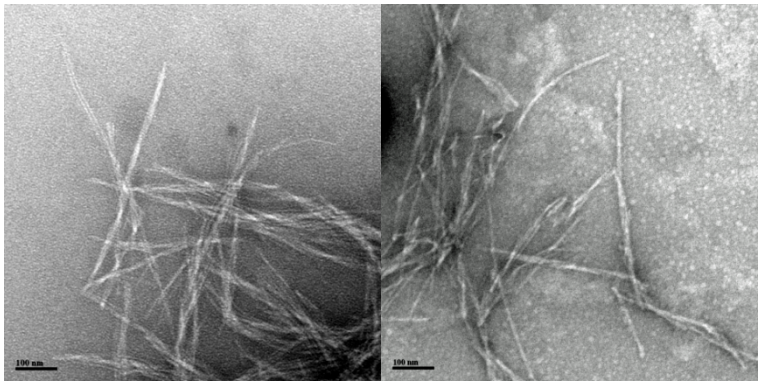


Figure S13: TEM images of **N_3-P_{11}** mixed with P(HEA) polymers [$P(HEA)_{16}$ left and $P(HEA)_{27}$ right] at pH 2 after 10 days of incubation. Scale bar = 100 nm.

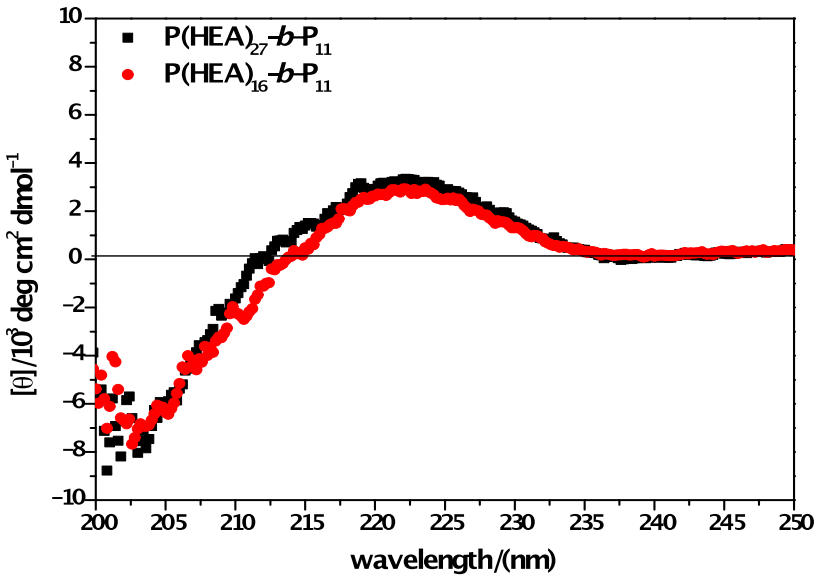


Figure S14: Far UV-CD spectrum of $P(HEA)_n$-*b*-P_{11} conjugates **9** and **10** at pH 11 (6mM) after 10 days of incubation.

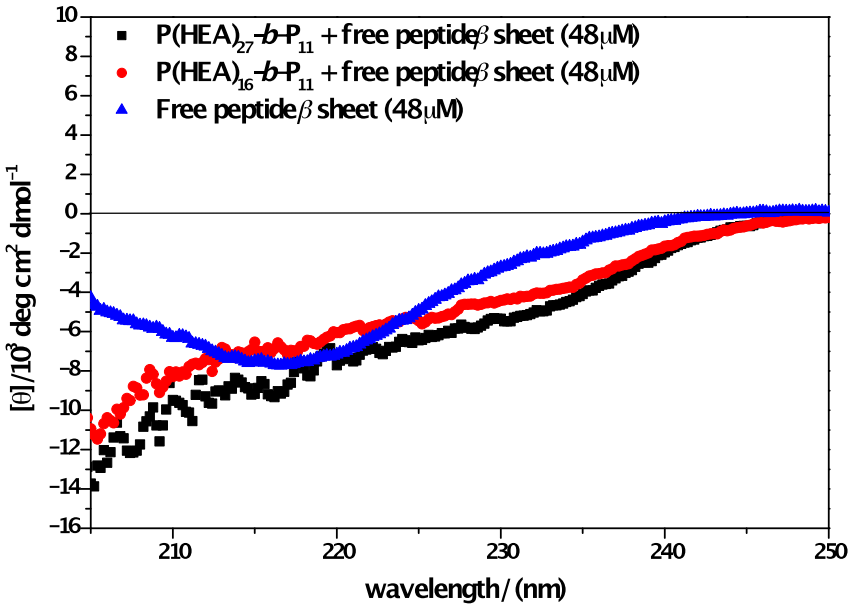


Figure S15: Far UV-CD spectrum of pre-self assembled **N_3-P_{11}** and pre self assembled **N_3-P_{11}** / $P(HEA)_n$-*b*-P_{11} solutions at pH 2 after 10 days of incubation. The concentration of free peptide β-sheets was kept constant in all samples. Pre-self assembly of **N_3-P_{11}** was done at concentration of 4 mM (above C_{agg}) before seeding.

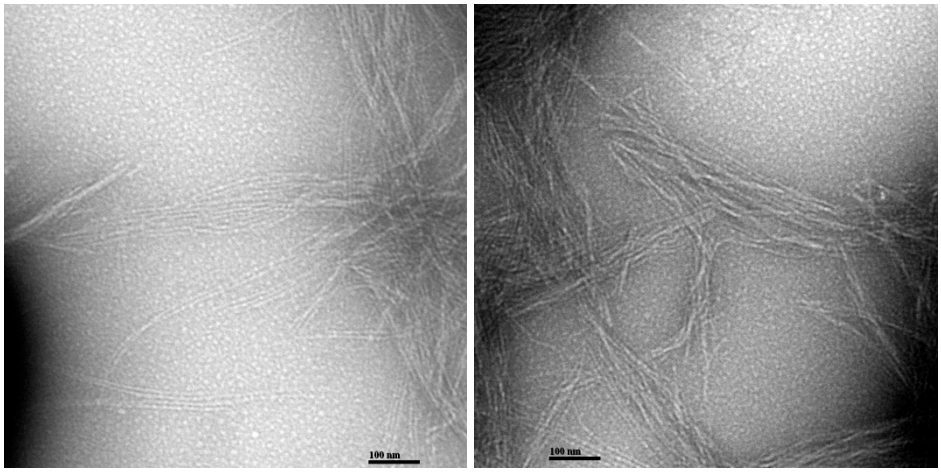


Figure S16: TEM images of $P(HEA)_n$-*b*-P_{11} solutions seeded with pre-self assembled **N_3-P_{11}** after 10 days of incubation at pH 2. Left $P(HEA)_{16}$-*b*-P_{11} and right $P(HEA)_{27}$-*b*-P_{11}. Concentration of pre-self assembled peptide β-sheet = 48 μM.

Table S2: Summary of seeding experiments in which preformed *β*-sheets obtained from **N_3-P_{11}** self assembled at pH 2 were added to chimera solutions and incubated at pH 2 for 10 days before TEM and CD analyses

Chimera	[Preformed ***β***-sheet Peptide] **μM**	[Chimera] **μM**	CD results (conjugate formed ***β***-sheets)	TEM results (***β***-sheet nanostructures retained)
P(HEA)$_{16}$-*b*-P$_{11}$	48	1144	No	Yes
P(HEA)$_{16}$-*b*-P$_{11}$	96	1144	No	Yes
P(HEA)$_{16}$-*b*-P$_{11}$	192	1144	No	Yes
P(HEA)$_{27}$-*b*-P$_{11}$	48	1132	No	Yes
P(HEA)$_{27}$-*b*-P$_{11}$	96	1132	No	Yes
P(HEA)$_{27}$-*b*-P$_{11}$	192	1132	No	Yes

Table S3: Summary of seeding experiments in which free peptide (**N_3-P_{11}**) was added to chimera solutions and incubated at pH 2 for 10 days before TEM and CD analyses. Total concentration of peptide, [T] = [Peptide]+[Chimera]. [T] > C_{agg} (~ 77 μM)

Chimera/peptide	[Peptide] **μM**	[Chimera] **μM**	Ratio peptide: chimera	CD results (conjugate formed ***β***-sheets)	TEM results (***β***-sheet)
P$_{11}$-N$_3$	81	0	100:0	Yes	Yes
P(HEA)$_{16}$-*b*-P$_{11}$	60	21	74:26	Yes	Yes
P(HEA)$_{16}$-*b*-P$_{11}$	60	60	50:50	No	No
P(HEA)$_{27}$-*b*-P$_{11}$	60	21	74:26	Yes	Yes
P(HEA)$_{27}$-*b*-P$_{11}$	60	60	50:50	No	No

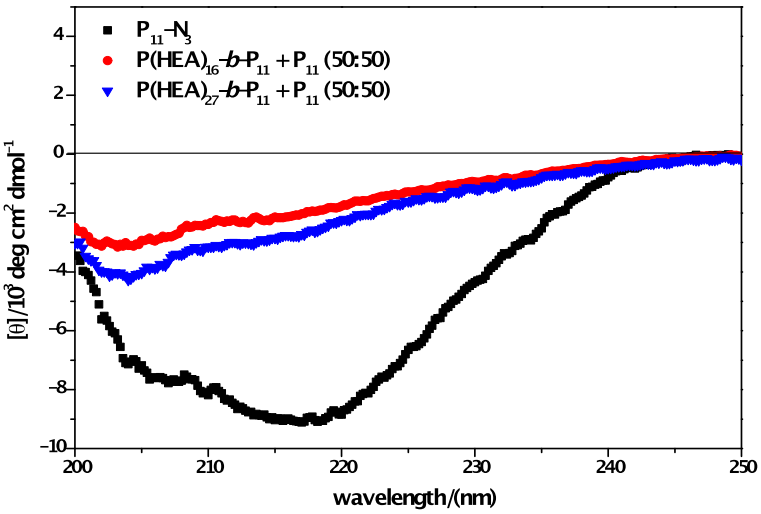


Figure S17: Far UV-CD spectra of **N_3-P_{11}** and **N_3-P_{11}** / P(HEA)$_n$-*b*-P_{11} solutions at pH 2 after 10 days of incubation. Ratio of P(HEA)$_n$-*b*-P_{11} to **N_3-P_{11}** (50:50).

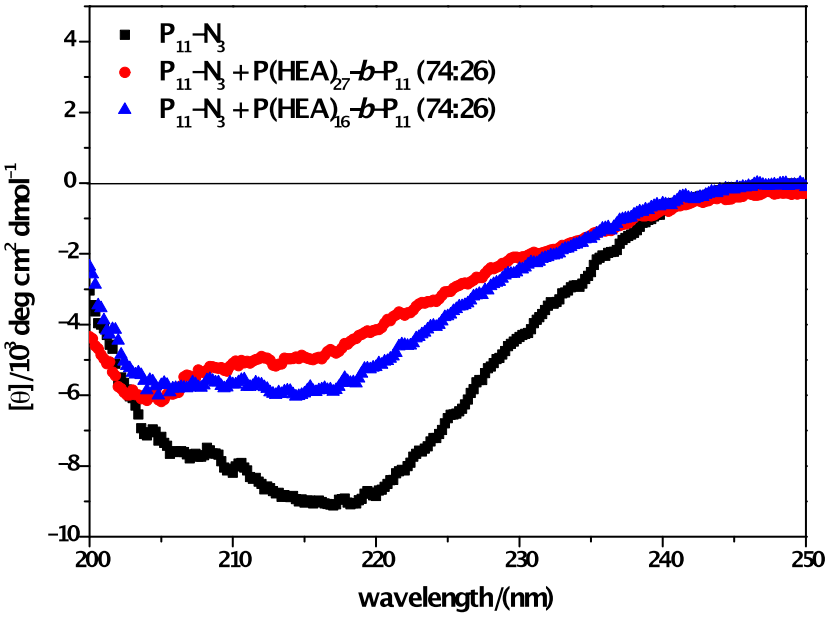


Figure S18: Far UV-CD spectra of **N_3-P_{11}** and **N_3-P_{11}** / P(HEA)$_n$-*b*-P_{11} solutions at pH 2 after 10 days of incubation. Ratio of P(HEA)$_n$-*b*-P_{11} to **N_3-P_{11}** (74:26).

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