Supplementary Material

Preparation and thermally-induced self-assembly behaviour of elastin-like peptide side-chain polymer-gold nanoparticle (ESP-GNP) hybrids

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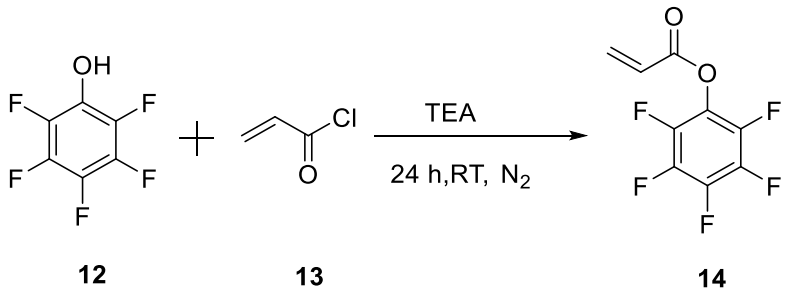
Synthesis of Pentafluorophenyl Acrylate

Pentafluorophenyl acrylate (PFPA) was synthesised as described in the literature (Scheme S1).[2] In a two neck round bottomed flask connected to a N_2 supply, pentafluorophenol (PFP) (10g, 54.3 mmol, 1 eq) and triethylamine (TEA) (6.6g, 65.2 mmol, 1.2 eq) were dissolved in 100 mL of dry diethyl ether and the mixture was cooled in an ice bath. Acryloyl chloride (6.0 g, 66.3 mmol, 1.2 eq) was added drop-wise through a needle under cooling with an ice bath. The clear solution of PFP and TEA turned into a white suspension immediately. Then remove the ice bath and the suspension was stirred overnight at ambient temperature with N_2 protection.[3] The precipitated triethylamine hydrochloride salt was removed by filtration, and the solid was washed with diethyl ether. After evaporation of the solvent, the residue was filtered again to obtain a clear yellow liquid. The reaction mixture containing the unreacted PFP, acryloyl chloride and crude product were analysed by TLC (petroleum ether 40-60^{o}C as eluent). The TLC showed two spots with R_f values of 0.48 and 0.68 which are due to PFP and crude product respectively. The yellow crude product was purified by using an auto column chromatography. The column material was silica gel and the solvent we used was petroleum ether (40-60^{o}C). The collected fraction was putted on the rotary evaporator to remove the petroleum ether. After evaporation, the final product was obtained (6.7 g, 27.5 mmol) and the yield was 52%. Pure PFPA was stored in the refrigerator.

^{1}H-NMR (400 MHz, 298K, $CDCl_3$) δ_H / ppm: 6.65 (1H, dd, Hc), 6.28 (1H, dd, Ha), 6.13 (1H, dd, Hb). ^{13}C-NMR (400 MHz, 298K, $CDCl_3$) δ_C / ppm: 161.7 (1C, s, CO), 141.2 (2C, m, C2), 139.5 (1C, m, C4), 137.9 (2C, m, C3), 135.4 (1C, s, CH_2), 125.3 (1C, s, CH), 125.0 (1C, m, C1).



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Scheme S1: Synthesis of pentafluorophenyl acrylate (PFPA).

^{1}H NMR, ^{13}C-NMR and ^{19}F NMR spectra of purified PFPA are shown in Figures S1-3. In the ^{1}H-NMR spectrum (Figure S1), the signals at 6.70 ppm, 6.35 ppm and 6.16 ppm are respectively correlated to protons c, a and b. In the ^{13}C-NMR spectrum (Figure S2), the signals at 125.34, 135.42 and 161.65 respectively represent C-H, CH_2 and –C=O. In the ^{19}F NMR spectrum (Figure S3), there are three signals at -162 ppm, -158 ppm and -153 ppm which are due to three types of aromatic fluorine environment.

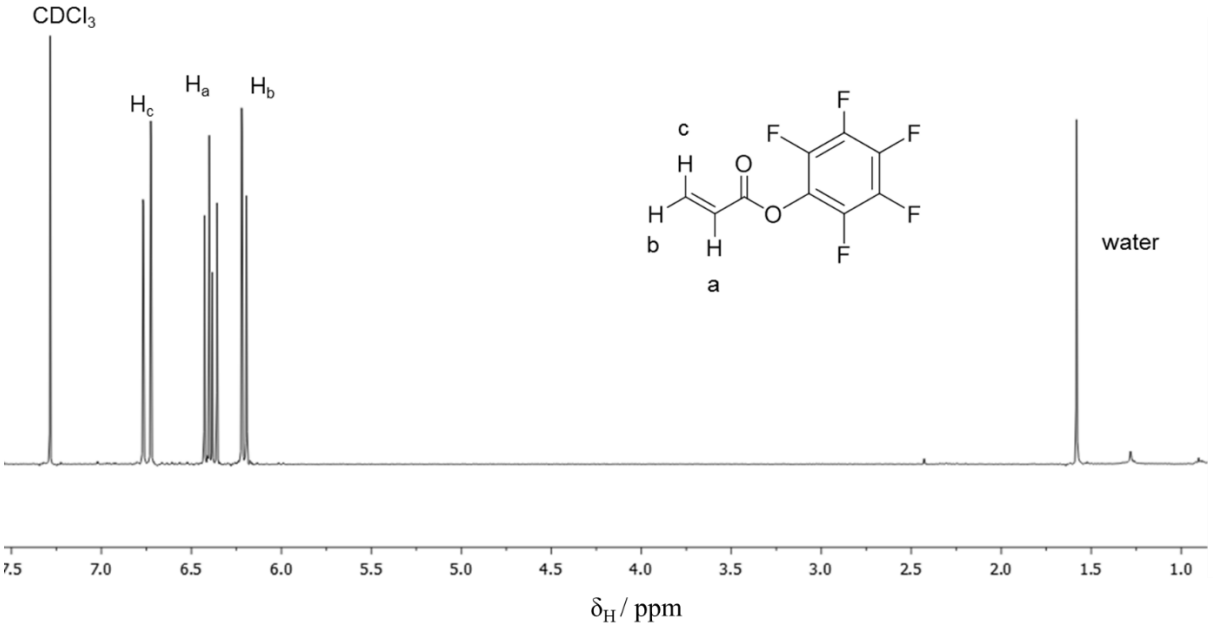


Figure S1: ^{1}H-NMR spectrum of monomer PFPA in $CDCl_3$.

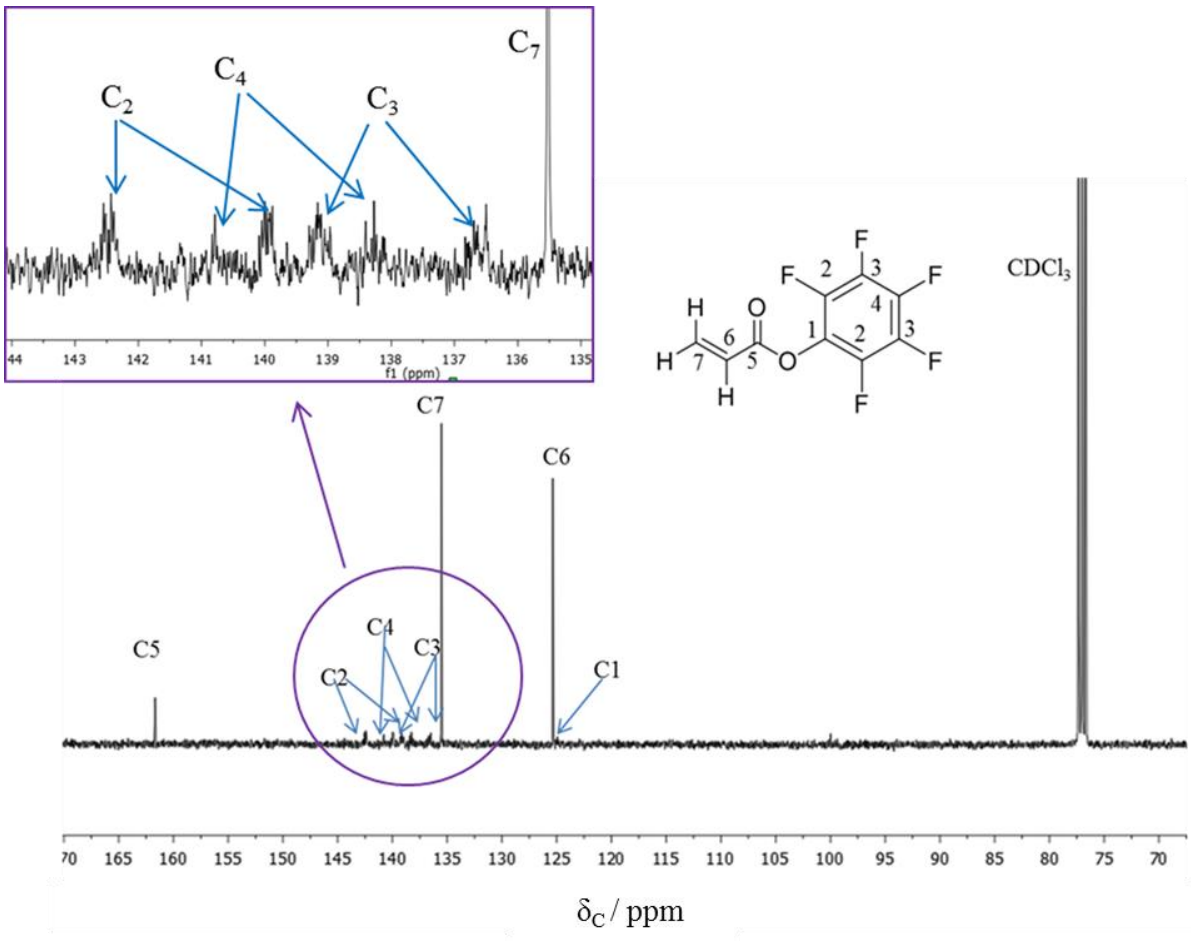


Figure S2: ^{13}C-NMR spectrum of monomer PFPA in $CDCl_3$.

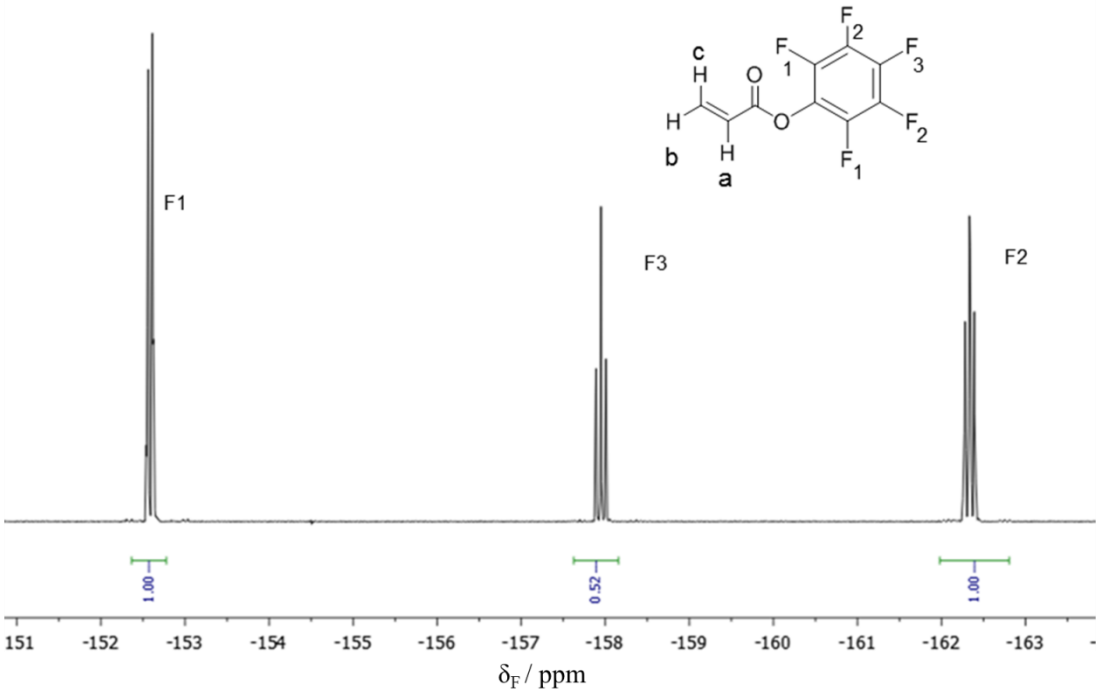
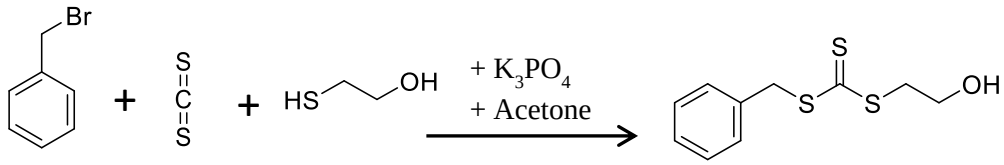


Figure S3: ^{19}F-NMR spectrum of monomer PFPA in $CDCl_3$.

Synthesis of RAFT agent benzyl 2-hydroxyethyl carbonotrithioate

In a one neck round bottom flask, potassium phosphate (27.2g, 128 mmol) was stirred in 350 mL of acetone. After 10 min, mercaptoethanol (10g, 128 mmol, 1 eq) was added into the suspension. The solution was stirred for 10 min and followed by the dropwise addition of carbon disulfide (21.9 g, 128 mmol, 1 eq) in ice bath and the solution was turned to yellow. After another 10 min, benzyl bromide (29.2g, 384.5mmol, 3eq) was added slowly through a funnel and the reaction was continuously stirred at ambient temperature for 30 min. The reaction scheme is presented in Scheme S2.



Scheme S2: Synthesis of RAFT agent benzyl 2-hydroxyethyl carbonotrithioate.

After reaction, the precipitated potassium bromide salt was removed by filtration, and the solid was washed with acetone. After evaporation of the solvent, the residue was filtered again to obtain a clear liquid. The yellow crude product was purified by using an auto column chromatography. The eluent ratio was 3:1 of petroleum ether (40-60 ^{o}C) and ethyl acetate. The final product was putted under high vacuum to remove all solvents. The product was obtained as light yellow oil in a yield of 72% (22.3g). The synthesised RAFT agent was characterised by ^{1}H NMR spectroscopy in solvent $CDCl_3$. Pure benzyl 2-hydroxyethyl carbonotrithioate was stored at -20^{o}C. ^{1}H-NMR ($CDCl_3$): 7.31-7.11 (5H, m, Ph), 4.52 (2H, s, benzyl-CH_2), 3.74 (2H, q, -CH_2-OH), 3.48 (2H, t, -S-CH_2).

Synthesis of Poly(pentafluorophenyl acrylate) (pPFPA)

Here we give one example of synthesis of poly(pentafluorophenyl acrylate) poly(PFPA)-100 by RAFT polymerisation (Scheme S3). Pentafluorophenyl acrylate (PFPA) (0.5g, 2.1 mmol, 100 eq), benzyl 2-hydroxyethyl trithiocarbonate (5.1mg, $2.1x10^{-5}$ mol, 1 eq), AIBN solution (2.0 mg, $1.05x10^{-5}$ mol, 0.5 eq) and benzene (5.0 mL) were added to a schlenk tube with stirring. The solution was cooled in an ice bath and purged with nitrogen for 30 min. After that, the ice bath was removed and the solution was heated to 70°C for 6 h. Conversion was determined by ^{1}H NMR. After 6 h, the schlenk tube was cooled at ambient temperature with N_2 protection. Then the reaction was quenched by putting the whole tube into a box of dry ice for 10 min. Afterwards the product could be left under atmospheric oxygen. Then solution was partially removed under vacuum and the rest was precipitated in methanol which was cooled to 0°C beforehand. After centrifuging and decanting the methanol, the polymer was dissolved in THF and re-precipitated again. The operation was repeated three times to remove any unreacted RAFT agent and any trace monomers. The polymer was dried under vacuum oven over night to yield a yellow powder. The sample was characterized by THF GPC, ^{1}H-NMR (Figure S4) and ^{19}F NMR (Figure S4) and FT-IR (Figure S5) spectroscopies.

Theoretical M_n was calculated using the following equation:

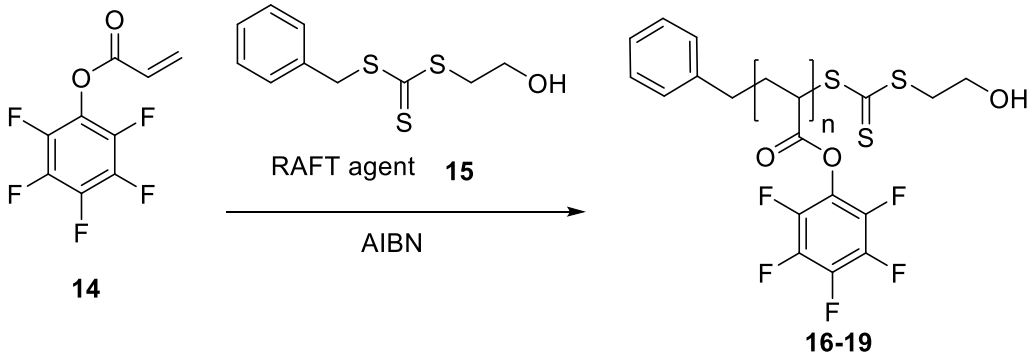
$$M_{n,theor} = [M]/[RAFT] \times MW_{monomer} + MW_{RAFT\ agent}$$

[M]/[RAFT] represents the ratio of monomer and RAFT agent concentration, $MW_{monomer}$ is the molar mass of PFPA (238 g.mol^{-1}) and $MW_{RAFT\ agent}$ is the molar mass of RAFT agent (244 g.mol^{-1}).

Experimental M_n was measured by ^{1}H NMR spectroscopy and calculated by the following equation:

$$M_{n,NMR} = [I^{CH\ at\ 3.0\ ppm} / (I^{phenyl\ group\ at\ 7.2\ ppm}/5)] \times MW_{monomer} + MW_{RAFT\ agent}$$

$I^{CH\ at\ 3.0\ ppm}$ and $I^{phenyl\ group\ at\ 7.2\ ppm}$ are corresponding to the intensity of signals at 3.0 ppm and 7.2 ppm respectively.



Scheme S3: Synthesis of poly(pentafluorophenyl acrylate) (pPFPA).

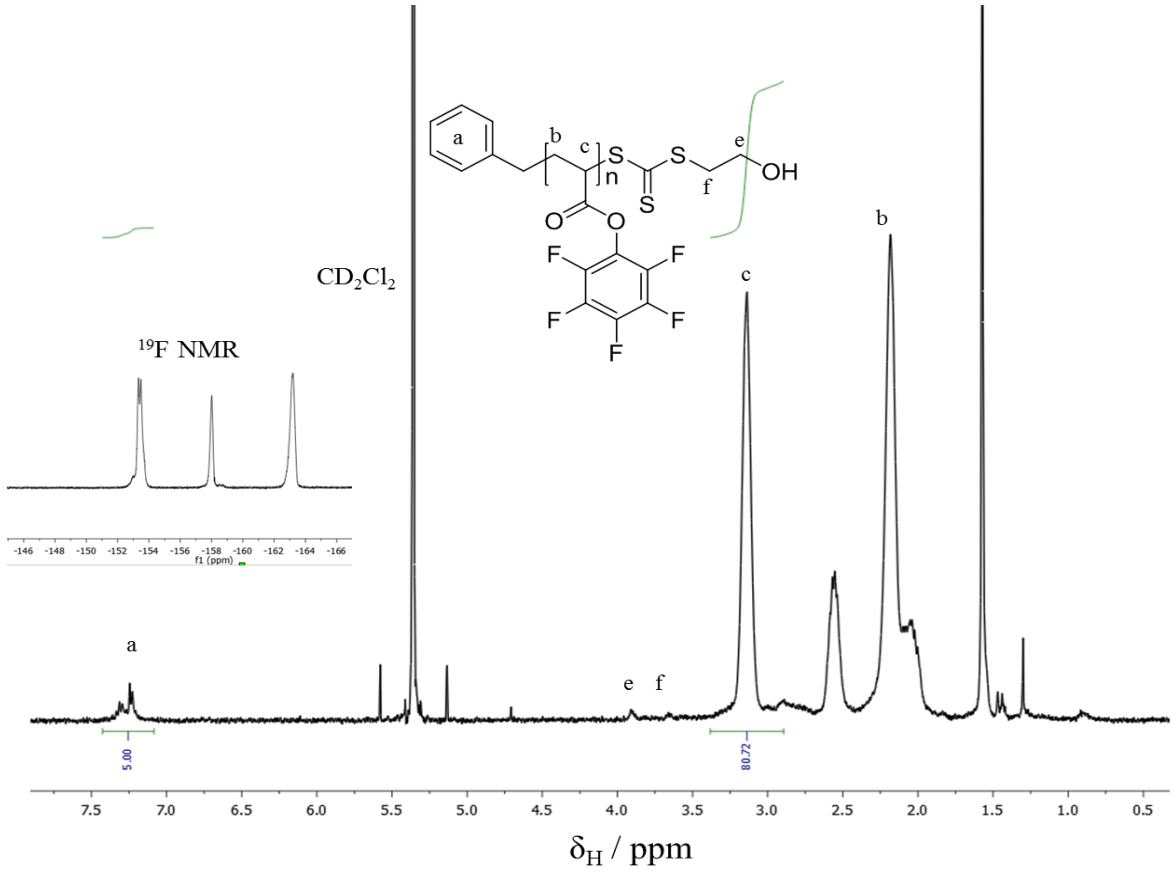


Figure S4: ^{1}H NMR spectrum of purified pPFPA-100 in CD_2Cl_2. Inset shows corresponding ^{19}F NMR spectrum.

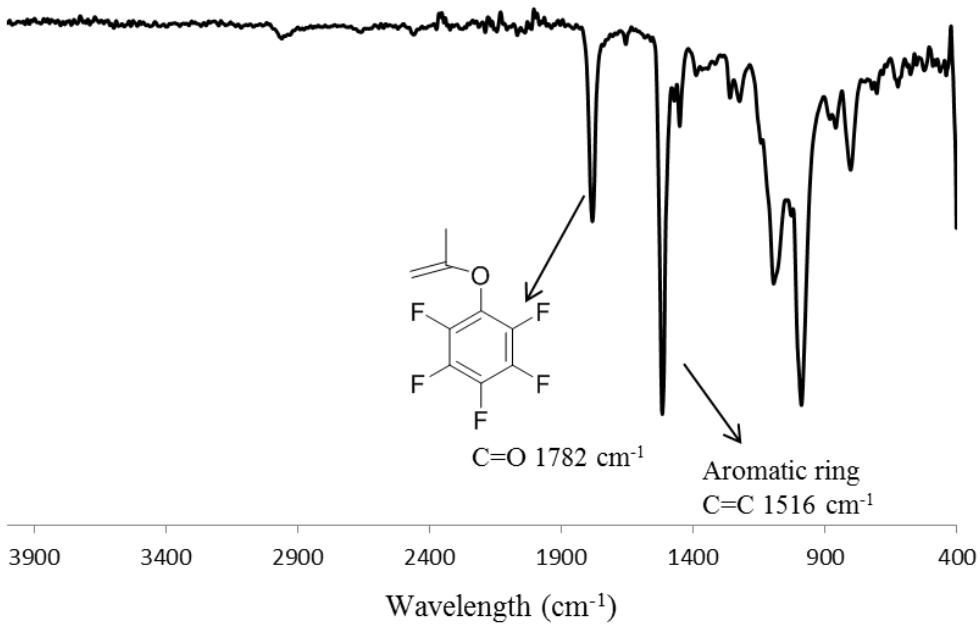


Figure S5: ATR-FTIR spectrum of pPFPA-100.

Peptide Synthesis and Purification

Peptides 1-3 were synthesised according to our previously described protocol. As an example, the procedure for Peptide 1 is included here. A suspension of Rink Amide resin (0.25 g, 0.19 mmol, 1 eq) was pre-swollen in DCM for 30 min. and washed by DMF three times. Then, 20% v/v piperidine in DMF was added for 5 minutes to remove the Fmoc group and this was repeated three times. After each time the resin was washed with DMF (3 x 5 mL). Fmoc-Gly-OH (0.11 g, 0.37 mmol, 2eq) was pre-activated with PyBOP (0.19 g, 0.36 mmol, 2 eq) and NMM (40µL, 0.37 mmol, 2eq) in DMF for 10 min and then was added into the pre-swollen resin, the reaction mixture was constantly agitated for 2 h. This procedure was then repeated with the following four amino acids: Fmoc-Val-OH (0.13g, 0.37 mmol, 2 eq), Fmoc-Gly-OH (0.11 g, 0.37mmol, 2 eq), Fmoc-Pro-OH (0.13 g, 0.37 mmol, 2eq), and Fmoc-Val-OH (0.13 g, 0.37 mmol, 2 eq). Piperidine (20% *v/v*) in DMF was added to the resin to remove the Fmoc group. The resin was washed repeatedly with DMF, DCM and diethyl ether**.** Peptide 1 was then cleaved from the resin using 3 mL of 95% TFA containing 2.5% of water and 2.5% of TIPS. After cleavage for 3 hours, the resin was removed by filtration and the obtained peptide was precipitated in diethyl ether. The product was then dissolved in water and freeze-dried. For purification process, 10 mg of crude peptide was dissolved in H_2O (containing 0.1% TFA) and purified by semi-preparative reverse phase HPLC. Purified Peptide 1 was obtained in 56% yield (Figure S6). A small sample of purified Peptide 1 was dissolved in water and analyzed by LC-MS (Figure S7). The peak at 1.5 min is the main product. The mass is 427.2 which represents $[M+H]^+$. The small peaks that appear at 0.5 min and 2.8 min are solvent peaks which also appear in a blank sample. Analytical HPLC analysis of Peptide 1 was carried out and the trace is shown in Figure S8.

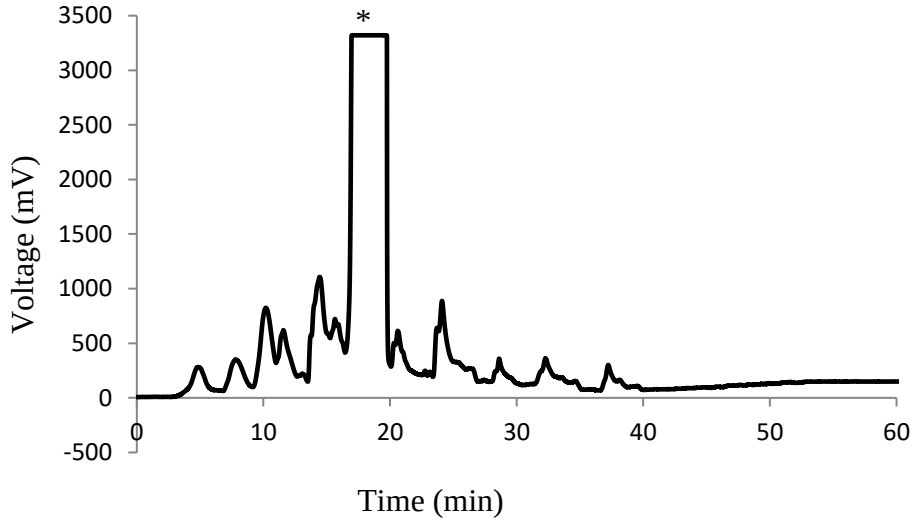


Figure S6: Semi-preparative HPLC chromatogram for Peptide 1 (VPGVG-NH_2). 45 min from 0-100% B, retention time range from 16.5-20.2 min.

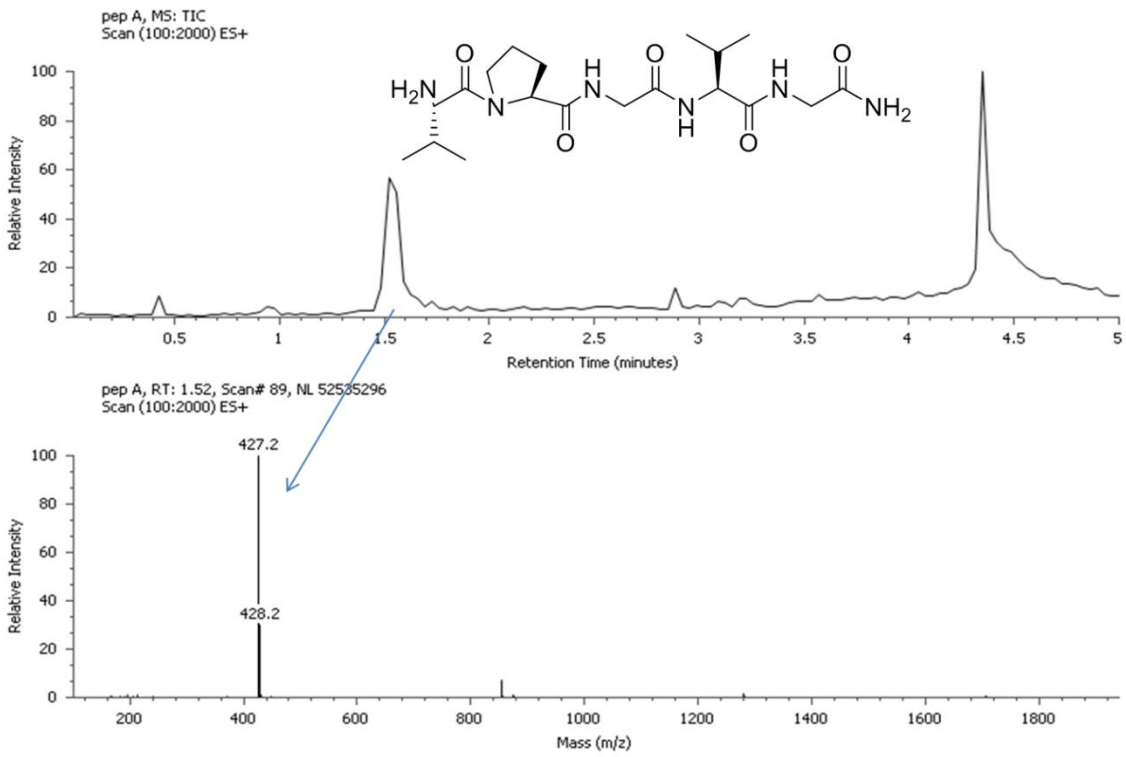


Figure S7: LC-MS spectrum of purified Peptide 1 (VPGVG-NH_2).

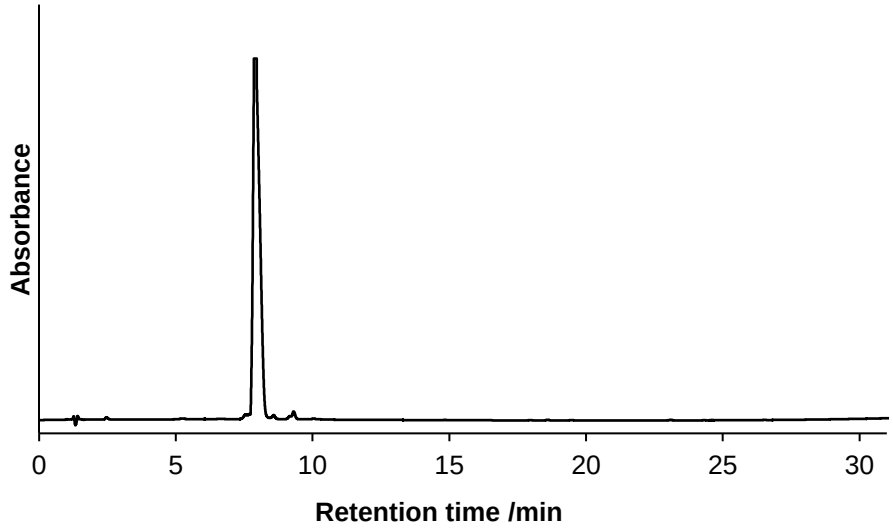


Figure S8: Analytical HPLC trace of purified Peptide 1 (VPGVG-NH_2). HPLC was carried out on an analytical column (C18, 4.6 x 100 mm, 3.5 m particle size). Peptides were eluted in H_2O/MeCN + 0.05 % trifluoroacetic acid. Retention time = 7.8 min.

The same method was applied to Peptides 2 and 3. Crude products were purified by semi-preparative HPLC using the same conditions as described above. After purification, 53% of pure Peptide 2 (GVPGVG-NH_2) was obtained. A small sample of Peptide 2 was dissolved in water and analyzed by LC-MS (Figure S9). The peak eluting at 2 min is the main product. The mass is 484.0 which represents $[M+H]^+$. The other peaks that appear

at 3.0 min and 4.5 min are solvent peaks which also appear in the blank sample. Analysis by analytical HPLC was carried out to confirm the purity of Peptide 2 (Figure S10).

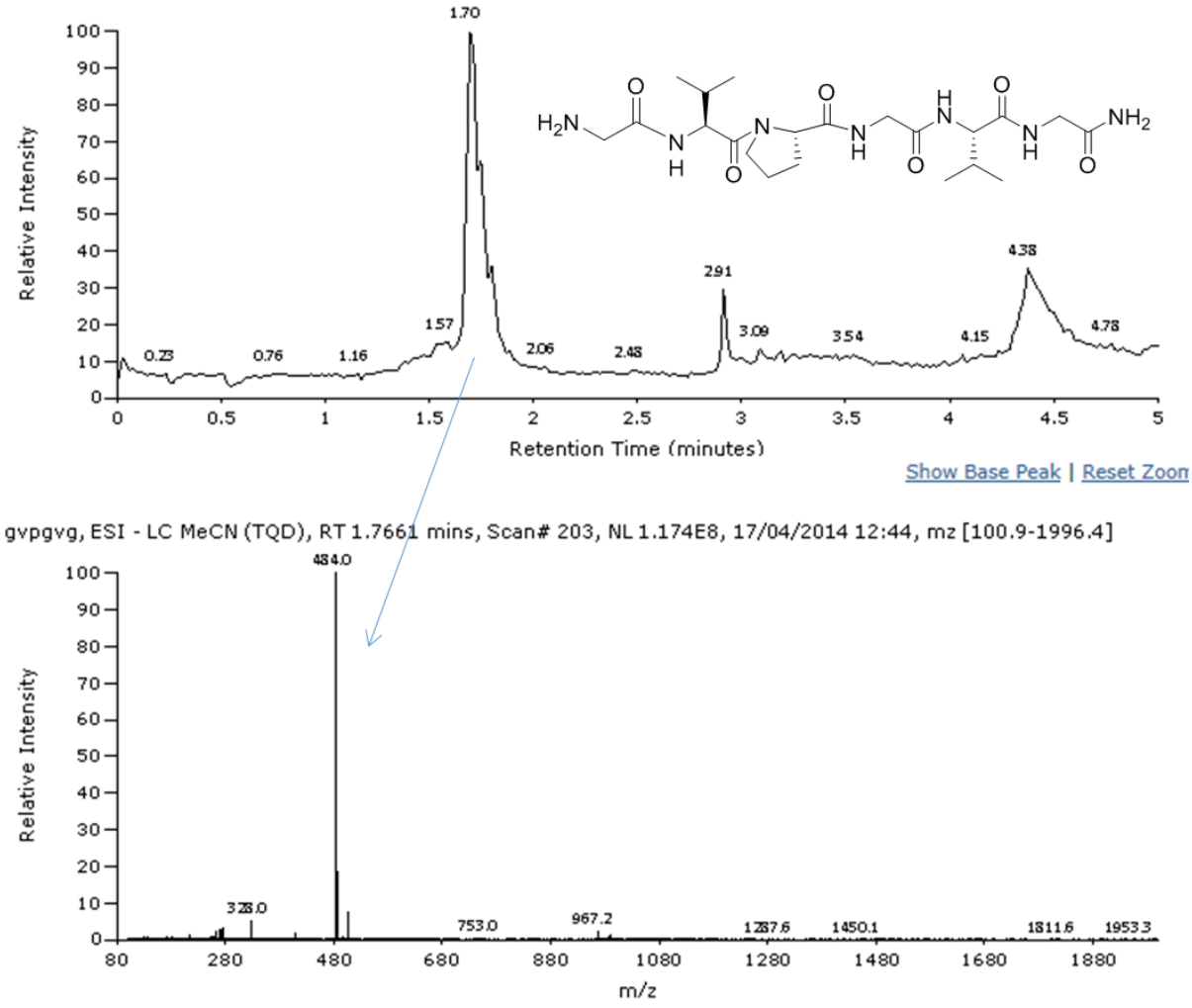


Figure S9: LC-MS spectrum of purified Peptide 2 (GVPGVG-NH_2).

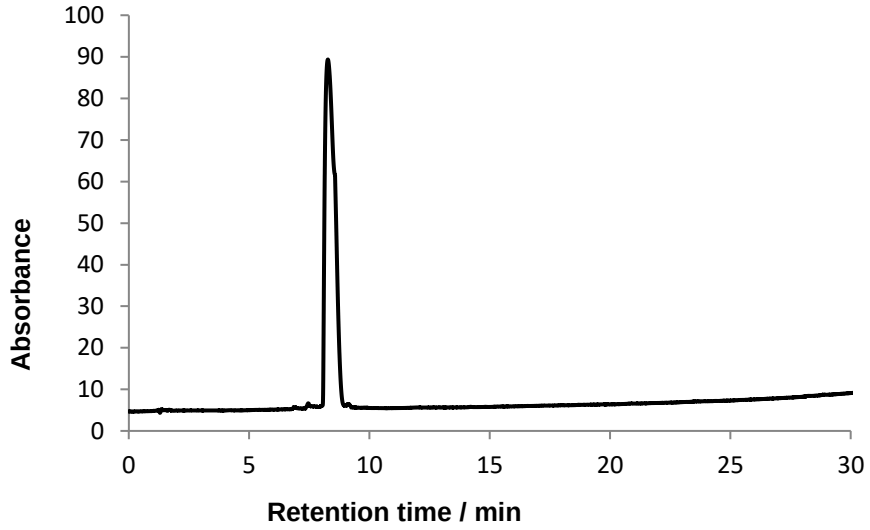


Figure S10: Analytical HPLC trace of Peptide 2 (GVPGVG-NH_2). HPLC was carried out as for Peptide 1. Retention time = 8.3 min.

Similarly, purification yielded 55% of Peptide 3. The LC-MS spectrum of Peptide 3 is shown in Figure S11. The peak that elutes at 1.7 min is the main product. The mass is 511.9, which represents $[M+H]^+$. The other peaks that appear at 2.7 min and 4.5 min are solvent peaks which also appear in the blank sample. Analytical HPLC of Peptide 3 was carried out and the trace is shown in Figure S12.

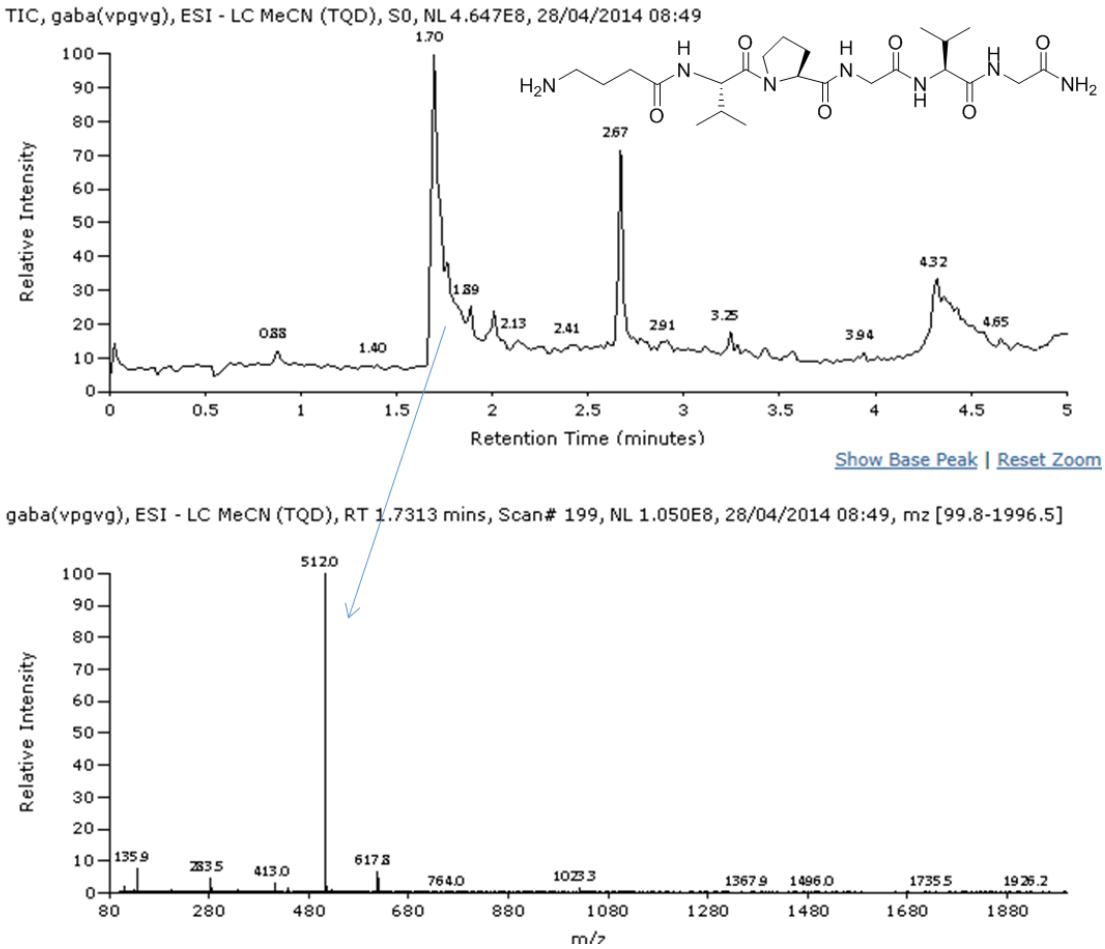


Figure S11: LC-MS spectrum of purified Peptide 3.

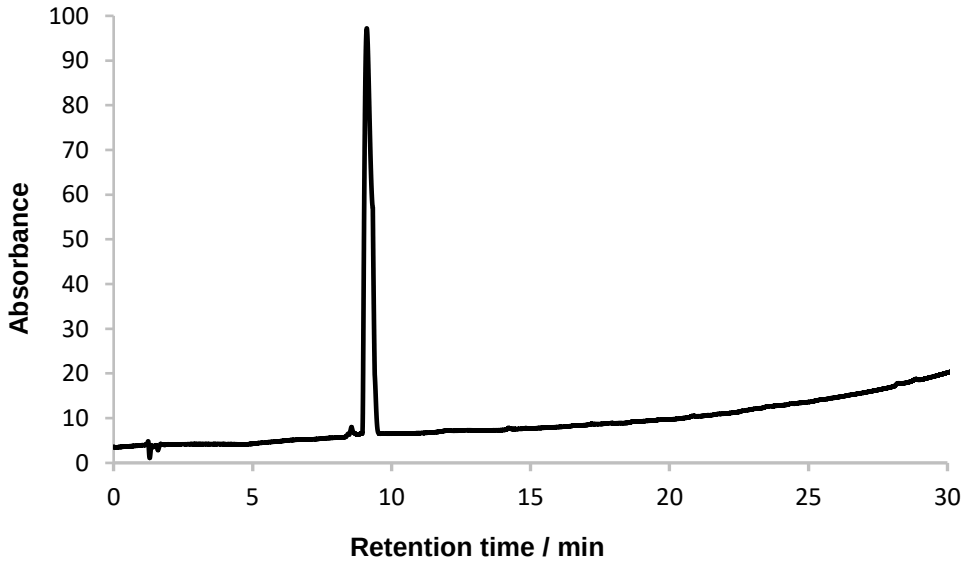


Figure S12: Analytical HPLC trace of purified Peptide 3. HPLC was carried out as for Peptide 1. Retention time = 9.1 min.

Preparation of Elastin-based Side Chain Polymers

^{1}H and ^{19}F NMR spectra of ESPs are shown in Figures S13-15.

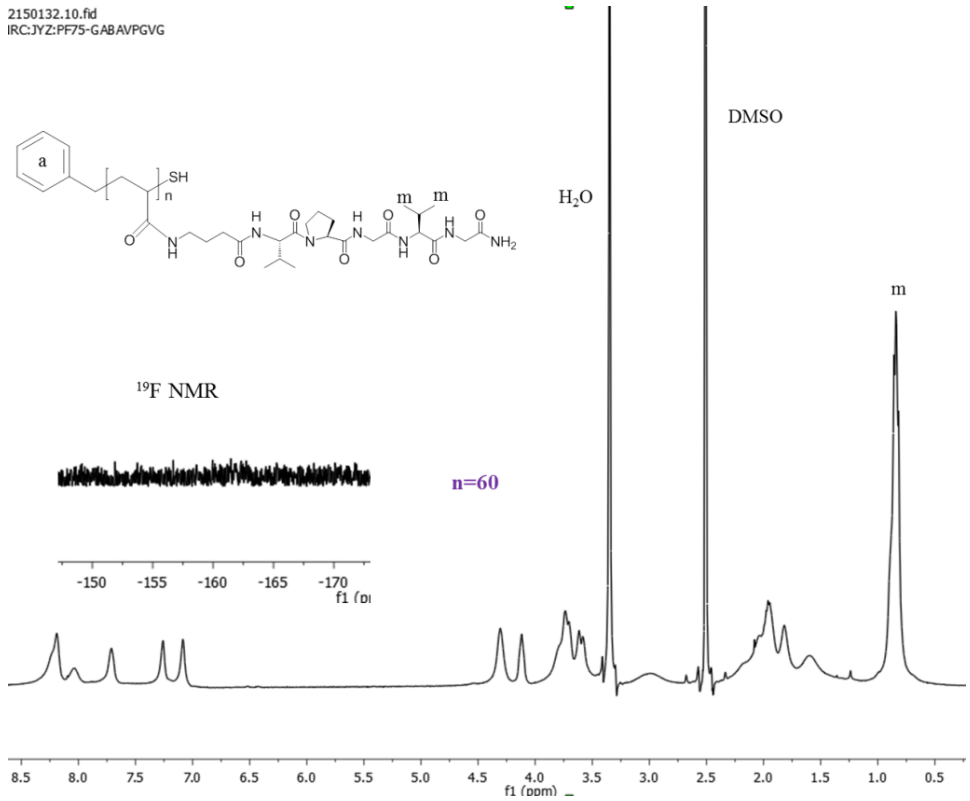


Figure S13: ^{1}H NMR and ^{19}F NMR spectra of PF75-GABA(VPGVG-NH_2) in d_6-DMSO.

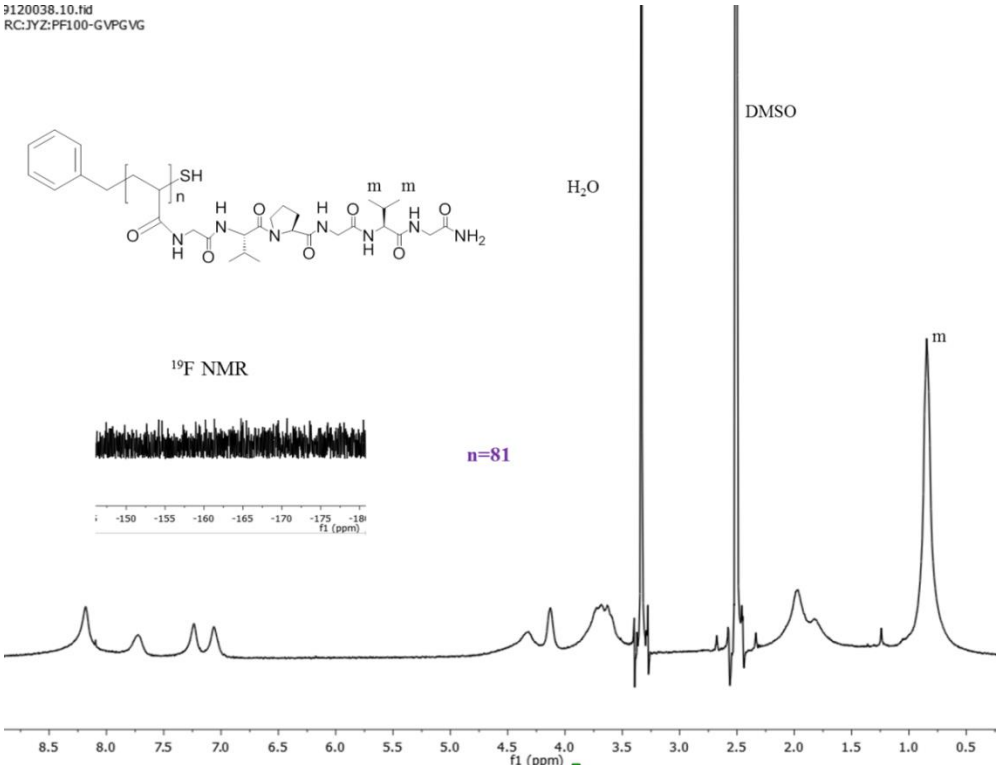


Figure S14: ^{1}H NMR and ^{19}F NMR spectra of PF100-GVPGVG-NH_2 in d_6-DMSO.

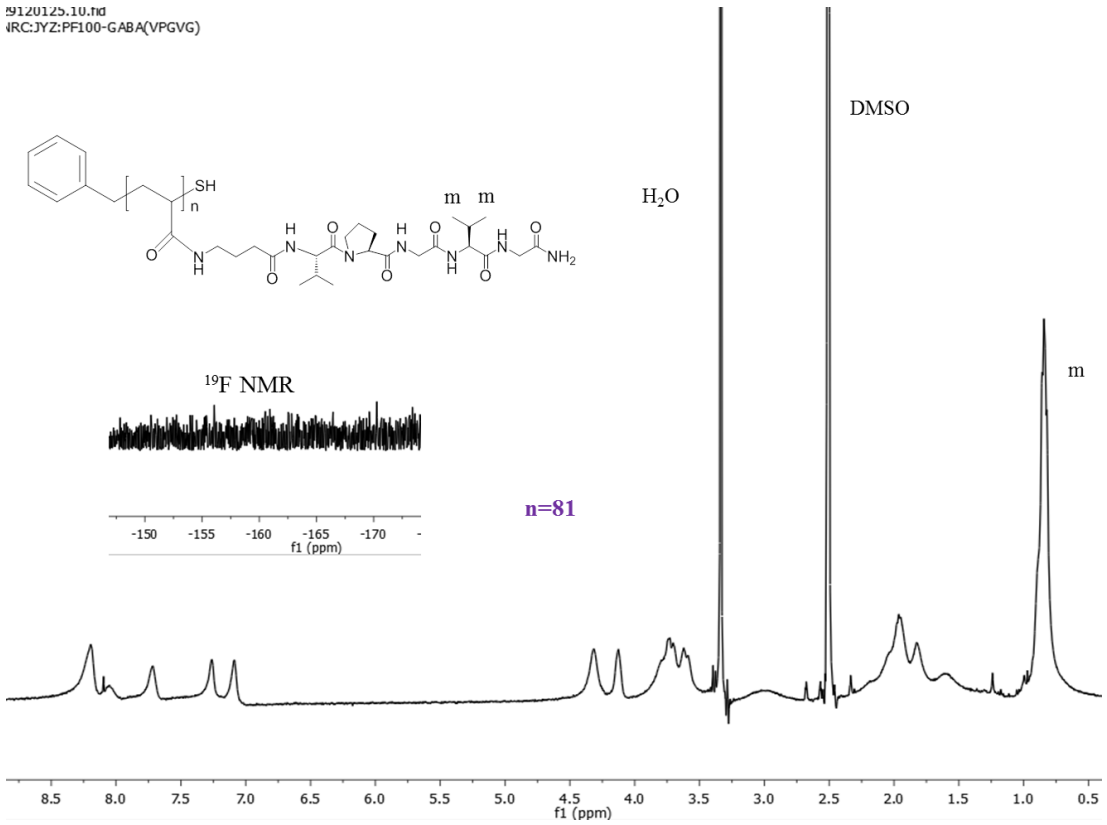


Figure S15: ^{1}H NMR and ^{19}F NMR spectra of PF100-GABA(VPGVG) in d_6-DMSO.

ATR FTIR spectra of ESPs are shown in Figure S16.

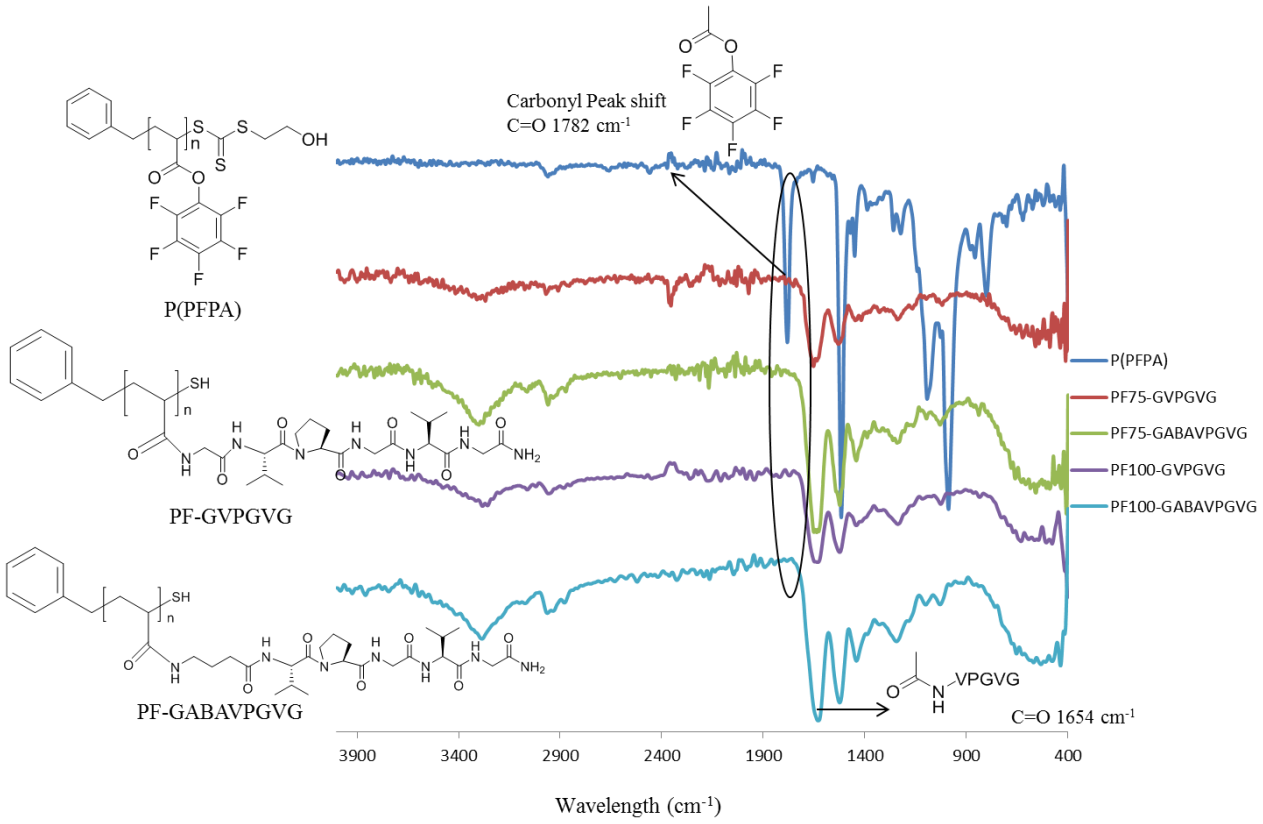


Figure S16: ATR-FTIR spectra of pPFPA, PF(75,100)-GVPGVG-NH_2 and PF(75,100)-GABA(VPGVG-NH_2).

Cloud Point Behaviour of ESPs

Cloud point curves of ESPs from turbidity measurements are shown in Figure S17.

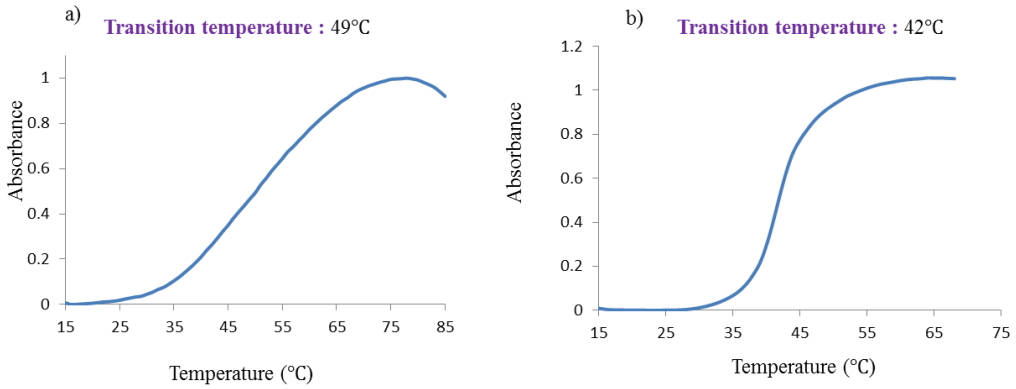


Figure S17: Turbidity measurements of polymer solutions (1 mg mL^{-1}) as observed by UV-Vis absorbance at 300 nm: (a) PF75-VPGVG-NH_2 and (b) PF100-VPGVG-NH_2.

Synthesis of ESP-GNPs

Gold nanoparticles were synthesized using the Turkevich-Frens method.[31, 32] All glassware used for the synthesis of colloidal gold was cleaned using aqua regia (3:1 HCl:HNO_3), rinsed thoroughly with water, and dried in the oven at 60 °C. A $HAuCl_4 \bullet 3H_2O$ solution (0.01 wt%, 0.3mM, 50 mL) was brought to boiling point with rapid stirring in a 100 mL round-bottom flask fitted with a reflux condenser. Once the solution began boiling, trisodium citrate solution (1 wt%, 1.0 mL) was added quickly with continued stirring, after which the solution started to turn dark-blue and finally red-purple. The reaction mixture was refluxed for 5 minutes and stirred for a further 30 minutes. The colloidal gold suspension was cooled to room temperature and filtered through a 0.2 μm filter. The diameter of the colloids was determined by transmission electron microscopy (TEM) and dynamic light scattering (DLS).

