Confinement of folding motifs within central blocks improves single chain polymer nanoparticle folding

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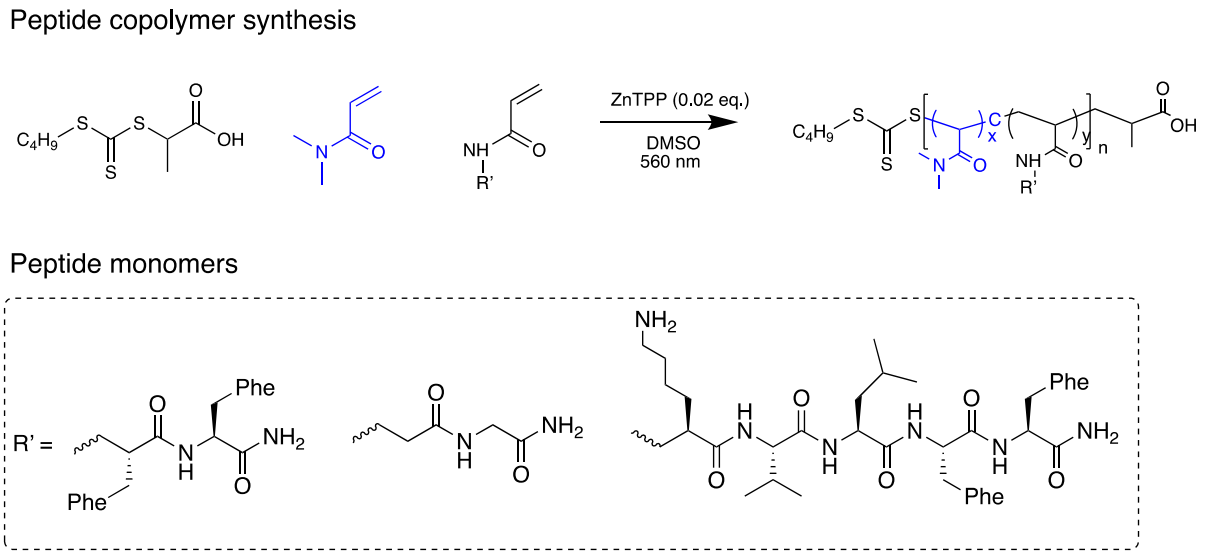
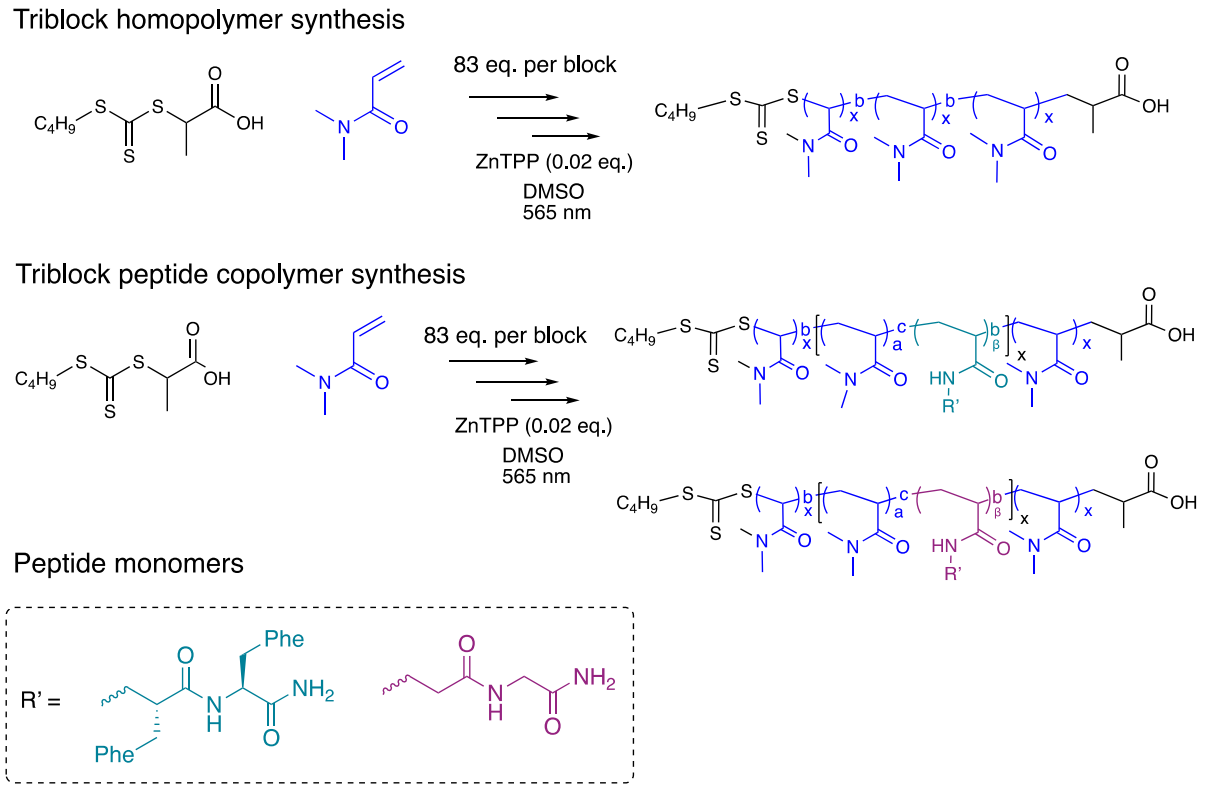
Supporting Information

1. SYNTHETIC SCHEMES



Scheme S1. General PET-RAFT polymerisation of statistical copolymers of DMA, FF, GG^N and GG^O monomers. Mol.% of peptides was varied from 5-15% (y) and of DMA, 85-95% (x) relative to CTA to give a total DP of 250-500 (n).

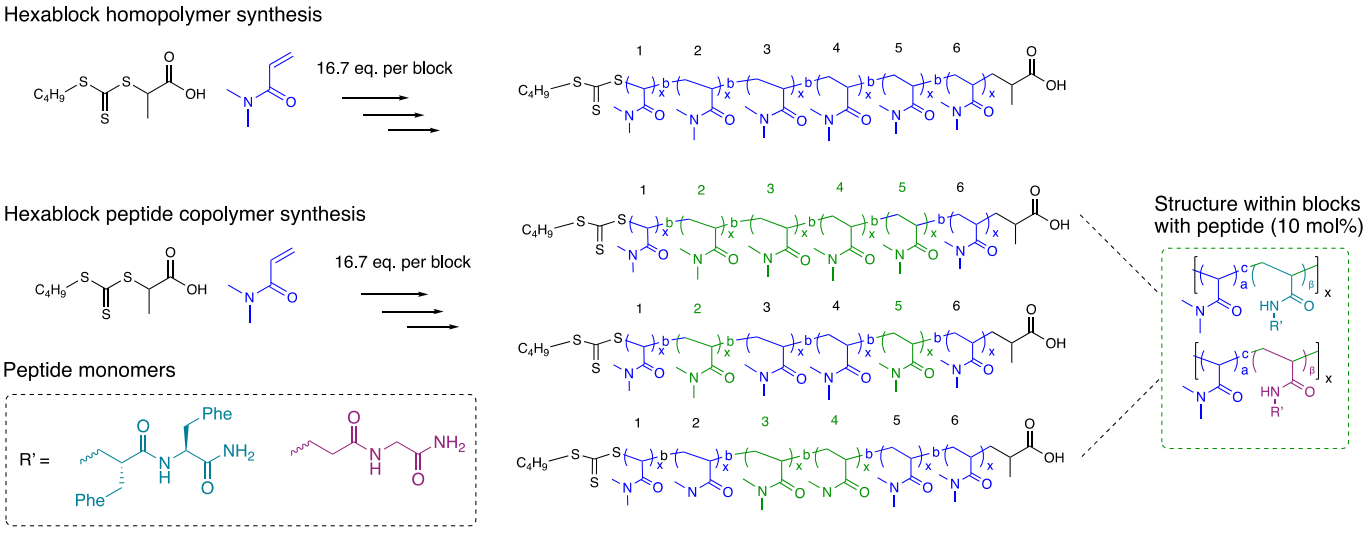


Scheme S2. General PET-RAFT polymerisation of triblock polymers with DMA, FF, GG^O, and GG^N monomers. Mol.% of peptide (β) in the central block was kept constant at 10% and mol.% of DMA (a) kept to 90% relative to CTA. Each block (x) was DP83 to give a total of DP250.

Table S1. Example experimental conditions for the synthesis of triblock polymers of DP250 with either DMA, FF, and GG^O peptide monomers with a sampling volume of 30 uL after each extension, from a macroRAFT agent.

	Block numbers		
	#1	**#2**	**#3**
DP to add to total	83	83	83
[Monomer stock] (M)	2	2	2
Monomer to add (uL)	**90**	**74.7**	**64.6**
[Monomer] (M)	1.00	0.83	0.45
[MacroRAFT] (M)	0.012	0.008	0.006
[ZnTPP] (x 10^{-4} M)	1.2	0.8	0.5
Total volume (uL)	**180**	**224.7**	**259.3**
Sampling volume (uL)	**30**	**30**	**30**
New volume [a]	150	194.7	229.3
ZnTPP: RAFT (%)	**1.00**	**1.00**	**0.83**

Notes: [a] New volume is the volume after sampling and before the next monomer addition



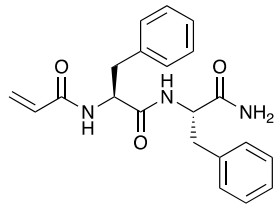
Scheme S3. Structure of the hexablock polymers generated through automation using liquid handling robot with DMA, FF, GG^O, and GG^N monomers. Mol.% of peptide (β) kept constant at 10% and mol.% of DMA (a) kept to 90% relative to CTA. Each block (x) was DP16.7 to give a total of DP100.

Table S2. Example experimental conditions for the automated synthesis of DP100 hexablock polymers with either DMA, FF, and GG^{O} peptide monomers with a sampling volume of 30 uL after each extension, from a macroRAFT agent.

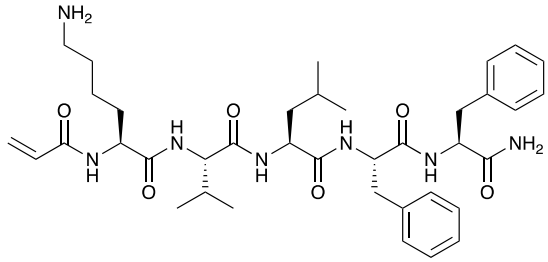
	Block numbers					
	#1	**#2**	**#3**	**#4**	**#5**	**#6**
DP to add total	16.7	16.7	16.7	16.7	16.7	16.7
[Monomer stock] (M)	2	2	2	2	2	2
Monomer to add (uL)	**37.5**	**52.5**	**39.6**	**30.6**	**23.7**	**18.1**
[Monomer] (M)	0.75	0.423	0.300	0.231	0.188	0.158
[MacroRAFT] (M)	0.045	0.026	0.018	0.014	0.011	0.009
[ZnhTPP] (x 10^{-4} M)	4.5	3.4	2.9	2.7	2.5	2.5
Total volume (uL)	**100**	**122.5**	**132.1**	**132.8**	**126.5**	**114.6**
Sampling volume (uL)	**30**	**30**	**30**	**30**	**30**	**30**
New volume [a]	70	92.5	102.1	102.8	96.5	84.6
ZnhTPP: RAFT (%)	1.00	1.32	1.63	1.95	2.27	2.59

Notes: [a] New volume is the volume after sampling and before the next monomer addition

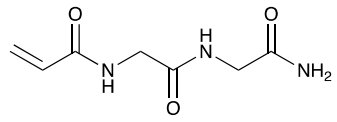
2. CHARACTERISATION DATA



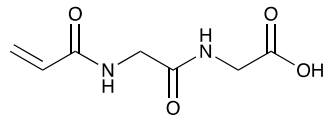
$_L$FF-acrylamide: ^{1}H-NMR (400 MHz, d_6-DMSO): δ 8.30 (d, 1H, NH, *J* = 8.0 Hz), 8.07 (d, 1H, NH, *J* = 8.0 Hz), 7.31(S, 1H, NH2), 7.05 – 7.25 (10H, ArH,), 6.20 (q, 1H, CH_2CHO-, *J* = 8.4 Hz), 6.00 (dd, 1H, CH_2CHO-, *J* = 16 Hz), 5.55 (dd, 1H, CH_2CHO-, *J* = 8 Hz), 4.58 (m, 1H, Cα-H, *J* = 12 Hz), 4.4 (m, 1H, Cα-H, *J* = 8 Hz), 2.8 (m, 2H, CH2, *J* = 16 Hz), 2.7 (m, 2H, CH2, *J* = 12 Hz). ^{13}C-NMR (125 MHz, d_6-DMSO): 40.9, 54.6, 72.9 126.9, 128.5, 129.7, 138.6, 162.2, 165.3, 171.7. ESI-MS: [M + H] calculated: 366.17, found: 366.2.



$_L$KVLFF-acrylamide: ^{1}H-NMR (400 MHz, *d6* - DMSO): δ 8.30 (d, 1H, NH, *J* = 8 Hz), 8.04 (d, 1H, NH, *J* = 8 Hz), 7.97 (d, 1H, NH, *J* = 8 Hz), 7.87 (d, 1H, NH, *J* = 4.8 Hz), 7.82 (d, 1H, NH2, *J* = 8 Hz), 7.69 (d, 1H, NH, *J* = 8 Hz), 7.1 – 7.3 (m, 10H, ArH), 6.34 (q, 1H, CH_2CHO-, *J* = 6.8 Hz), 6.1 (dd, 1H, CH_2CHO-, *J* = 16 Hz), 5.61 (dd, 1H, CH_2CHO-, *J* = 8.8 Hz), 4 - 4.5 (m, 5H, Cα-H), 2.6 – 3.1 (m, 12, CH_2 (Phe), CH_2 (Lys)), 1.9 (m, 2H, CH_2 (Val), 1.5 (m, 6H, CH_3 (Leu)), 1.3 (m, 6H, CH_3 (Val). ESI-MS: [M+H] calculated: 706.89, found: 706.35.



GG-acrylamide: ^{1}H-NMR (400 MHz, d_6-DMSO): δ 8.41 (d, 1H, NH, J = 8.8 Hz), 8.13 (d, 1H, NH, J = 8.8 Hz), 7.24 (s, 1H, NH_2), 6.38 (dd, 1H, CH_2CHO-, *J* = 9.6 Hz), 6.10 (dd, 1H, CH_2CHO, *J* = 16.4 Hz-), 5.62 (dd, 1H, CH_2CHO-, *J* = 9.6 Hz), 3.81 (d, 2H, Cα-H, *J* = 2.4 Hz), 3.62 (d, 2H, Cα-H, *J* = 6 Hz). ^{13}C-NMR (125 MHz, d_6-DMSO): δ 42.3, 42.7, 125.8, 132.4, 166.4, 169.4, 171.9.



GG-carboxylic acid: Gly-Gly-OH (2 g, 15.2 mmol) was dissolved in methanol (15 mL), with potassium hydroxide (1.7g, 30.3 mmol), sodium carbonate (0.48 g, 4.5 mmol) and magnesium sulphate (3.82 g, 31.8 mmol), with and stirred. The solution was cooled to 0 ºC in an ice bath, and acryloyl chloride (1.35 mL, 1.51 g, 16.7 mmol) was added dropwise. The mixture was left to react for 3h while warming to room temperature, after which it was heated to 50°C and filtered to remove the salts. The filtrate was concentrated to ~20mL and triturated from isopropyl alcohol to yield product as a white solid (2.16 g, 77%). ^{1}H-NMR (400 MHz, d_6-DMSO): δ 13.03 (s, 1H, OH), 8.41 (d, 1H, NH, *J* = 8 Hz), 8.14 (d, 1H, NH, *J* = 8 Hz), 6.48 (q, 1H, CH2CHO-), 6.09 (dd, 1H, CH2CHO-), 5.74 (dd, 1H, CH2CHO), 3.81 (d, 2H, Cα -H), 3.49 (d, 2H, Cα -H). ^{13}C-NMR (125 MHz, d_6-DMSO): δ 42.6, 43.7, 126.8, 132.1, 165.8, 169.3, 174.0.

Table S3. Summary of the reaction conditions, conversion and molecular weight of homopolymers synthesised with DMA, KVLFF, FF, GG^N, and GG^O peptide monomers on DMF GPC with 2.5 g/L of LiBr and 0.1 g/L of BHT. [a] Description of the homopolymers with the grafting density of peptide and target DP. [b] Conversion (%) calculated using ^{1}H-NMR. [c] M_w calculated relative to linear PMMA standards without correction.

#	Name [a]	[CTA]/[Monomer]/[ZnTPP]	Time (h)	X % [b]	$M_{n\ Theo}$	$M_{n\ DMF\ GPC}$ [c]	$Đ_{DMF\ GPC}$	$M_{n\ Aq.\ GPC}$ [d]	$Đ_{Aq.\ GPC}$
P1	$(D)_{500}$	1:500: 0.02	1.5	98	48812	31685	1.22	41624	1.59
P2	$(G^N)_{5\%,\ 500}$	1:500: 0.02	2.5	95	52955	24312	1.2	48822	1.16
P3	$(G^o)_{5\%,\ 500}$	1:500: 0.02	2.5	93	43980	-	-	-	-
P4	$(G^N)_{10\%,\ 500}$	1:500: 0.02	2.5	95	51814	43933	1.27	60763	1.17
P5	$(G^o)_{10\%,\ 500}$	1:500: 0.02	2.5	92	45785	-	-	-	-
P6	$(G^N)_{15\%,\ 500}$	1:500: 0.02	2.5	94	49100	14518	1.08	16443	1.31
P7	$(G^o)_{15\%,\ 500}$	1:500: 0.02	2.5	95	49620	-	-	-	-
P8	$(F)_{5\%,\ 500}$	1:500: 0.02	2.5	95	54600	23698	1.11	42402	1.15
P9	$(F)_{10\%,\ 500}$	1:500: 0.02	2.5	93	59647	29965	1.12	63013	1.19
P10	$(F)_{15\%,\ 500}$	1:500: 0.02	2.5	93	61161	18661	1.18	9082	1.41
P11	$(K)_{5\%,\ 500}$	1:500: 0.02	2.5	92	60714	32634	1.17	-	-
P12	$(K)_{10\%,\ 500}$	1:500: 0.02	3	90	73052	27913	1.13	-	-
P13	$(K)_{15\%,\ 500}$	1:500: 0.02	3	91	83088	14472	1.25	-	-
P14	$(D)_{250}$	1:250: 0.02	3	95	20929	23854	1.24	16546	1.15
P15	$(G^N)_{5\%,\ 250}$	1:250: 0.02	3	95	22581	18145	1.27	60922	1.27
P16	$(F)_{5\%,\ 250}$	1:250: 0.02	3	93	20959	20668	1.2	46083	1.16
P18	$(G^N)_{10\%,\ 250}$	1:250: 0.02	3	92	27452	-	-	-	-
P19	$(F)_{10\%,\ 250}$	1:250: 0.02	3	97	28623	41031	1.38	35544	1.3
P20	$(K)_{10\%,\ 250}$	1:250: 0.02	3	92	35368	39382	1.28	29769	1.21
P21	$(G^N)_{15\%,\ 250}$	1:250: 0.02	3	93	42118	33116	1.29	-	-
P22	$(F)_{15\%,\ 250}$	1:250: 0.02	3	94	31027	14968	1.03	20047	1.26
P23	$(K)_{15\%,\ 250}$	1:250: 0.02	3	91	41663	14833	1.02	15937	1.35

Table S4. Summary of triblock polymers synthesised with DMA, FF, and GG^{O} peptide monomers on DMF GPC with 2.5 g/L of LiBr and 0.1 g/L of BHT. [a] Description of the multiblock polymers with the position of peptide monomer and the target DP. [b] Mw and dispersity calculated relative to linear PMMA standards without correction.

#	Block	Time (h)	X (%) [a]	$M_{n\ (Theo)}$	$M_{n\ (GPC)}$ [b]	Đ
P24	#1	24	89	7469	5132	1.09
P25		24	91	7469	6871	1.1
P26		24	98	7469	7341	1.11
P24	#2	15	90	14700	10176	1.15
P25		15	92	17176	12483	1.09
P26		15	93	21682	16083	1.26
P24	#3	15	91	20929	25398	1.29
P25		15	90	24407	17706	1.18
P26		15	95	28623	27832	1.2

Table S5. Summary of hexablock polymers synthesised with DMA, FF and GG^{O} peptide monomers on DMF GPC with 2.5 g/L of LiBr and 0.1 g/L of BHT. [a] Mw and dispersity calculated relative to linear PMMA standards without correction.

#	Name	$M_{n\ (Theo)}$	$Mn_{\ (GPC)}$ [a]	Đ
P27	$(D\text{-}D\text{-}D\text{-}D\text{-}D\text{-}D)_{100}$	8968	6245	1.14
P28	$(D\text{-}G^{O}\text{-}D\text{-}D\text{-}G^{O}\text{-}D)_{100}$	9645	4986	1.07
P29	$(D\text{-}D\text{-}G^{O}\text{-}G^{O}\text{-}D\text{-}D)_{100}$	9645	5271	1.10
P30	$(D\text{-}G^{O}\text{-}G^{O}\text{-}G^{O}\text{-}G^{O}\text{-}D)_{100}$	9645	5843	1.14
P31	$(D\text{-}F\text{-}D\text{-}D\text{-}F\text{-}D)_{100}$	11358	5372	1.07
P32	$(D\text{-}D\text{-}F\text{-}F\text{-}D\text{-}D)_{100}$	11358	4516	1.05
P33	$(D\text{-}F\text{-}F\text{-}F\text{-}F\text{-}D)_{100}$	11358	5360	1.08

3. SUPPLEMENTARY FIGURES

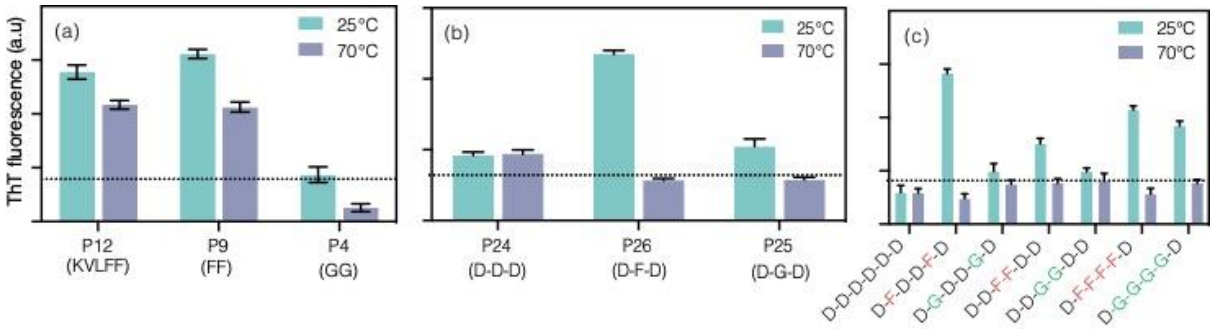


Figure S1. a) Comparison of fluorescence intensity of ThT in homoblock copolymers of either FF, KVLFF, and GGN peptide monomers (DP = 500) in water at 1 mg/mL at 25°C and when heated to 70°C. b) Comparison of fluorescence intensity of ThT in triblock polymers of either DMA, FF, and GGO peptide monomers (DP = 250) in water at 1 mg/mL at 25°C and when heated to 70°C. c) Comparision of fluorescence intensity of ThT in hexablock polymers of either DMA, FF, and GGO peptide monomers (DP = 100) in water at 1 mg/mL at 25°C and when heated to 70°C.

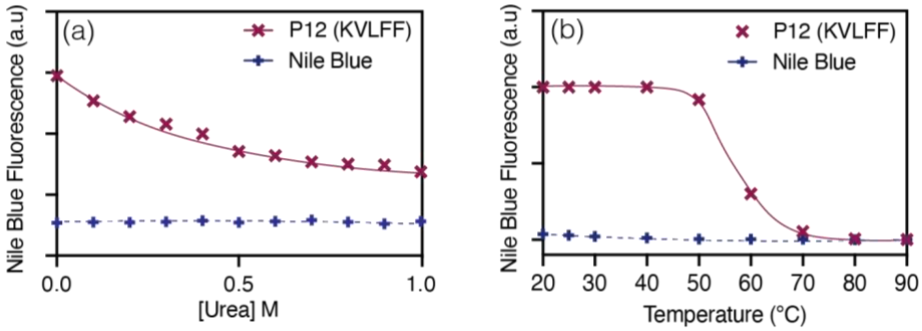


Figure S2. Nile blue fluorescence showing the disassembly of KVLFF copolymer at 10% peptide loading (**P12**) with a) increasing urea concentration and b) with increasing temperature. The intensity of the nile blue blank (0.5 μM) is indicated by blue line in each case.

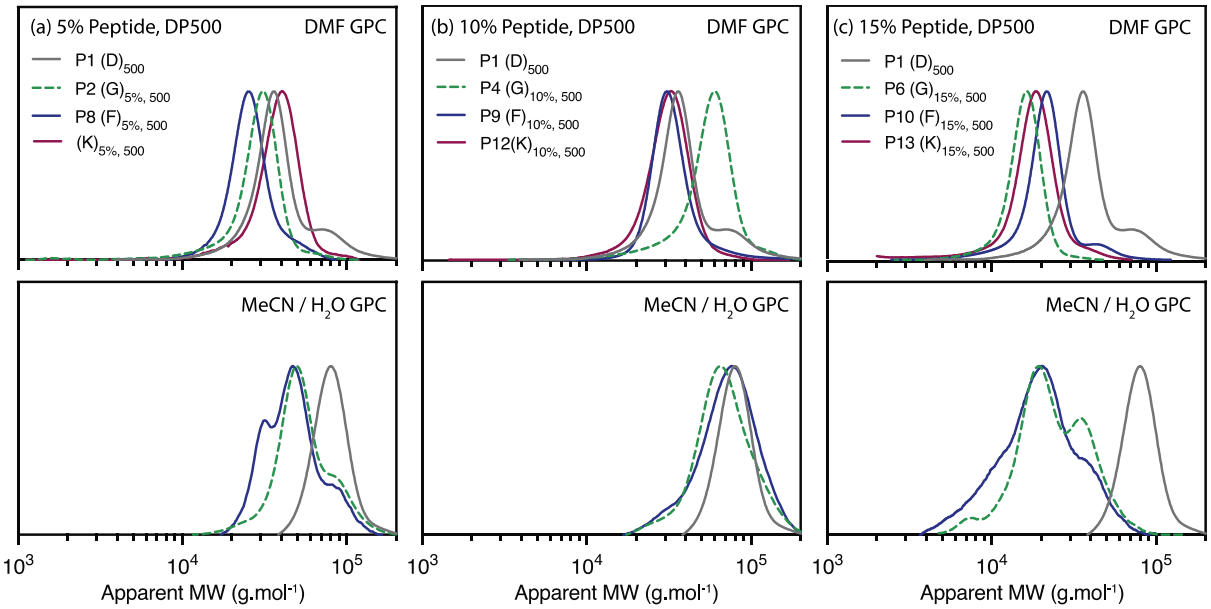


Figure S3. DMF and 40% (vol) MeCN / H_2O GPC traces of KVLFF, FF, and GG copolymers with DMA at DP500 with a peptide density of a) 5%, b) 10%, and c) 15%. Molecular weights are calculated relative to PMMA standards (DMF GPC) and PEO standards (for the MeCN / H_2O GPC) without correction.

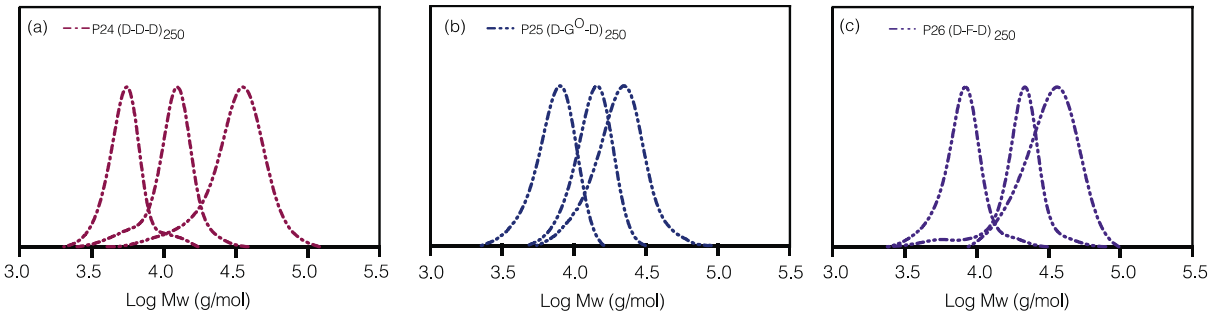


Figure S4. DMF traces of the block extensions of triblock polymers. (a) Block extensions of triblock polymers with DMA at DP250 (b) Block extensions of triblock polymers with GG^{O} monomer positioned in middle block with 10 mol.% peptide at DP250 (c) Block extensions of triblock polymers with FF monomer positioned in middle block with 10 mol.% peptide at DP250.

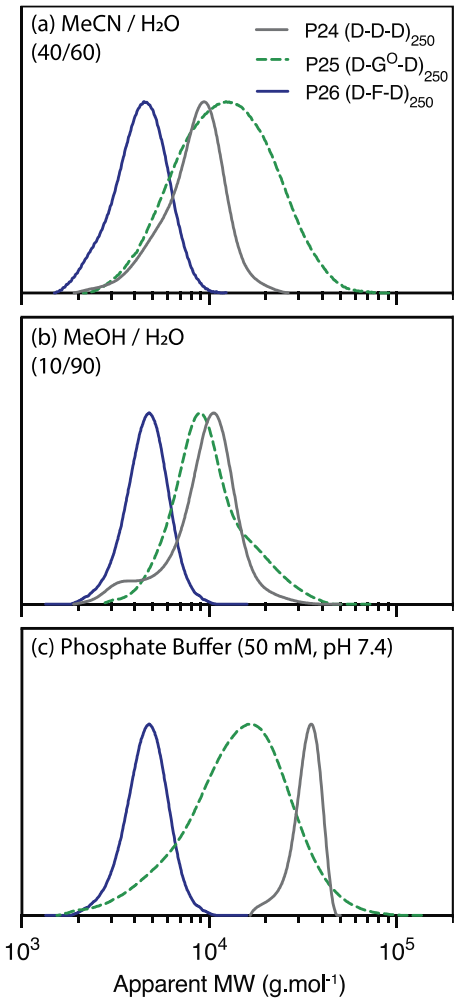


Figure S5. GPC traces of the DMA, FF, and GG^{O} triblock polymers at DP250 using an eluent of a) 40% MeCN / H_2O, b) 10% MeOH / H2O or c) phosphate buffer. Molecular weights are calculated relative to PEO standards without correction in all cases.

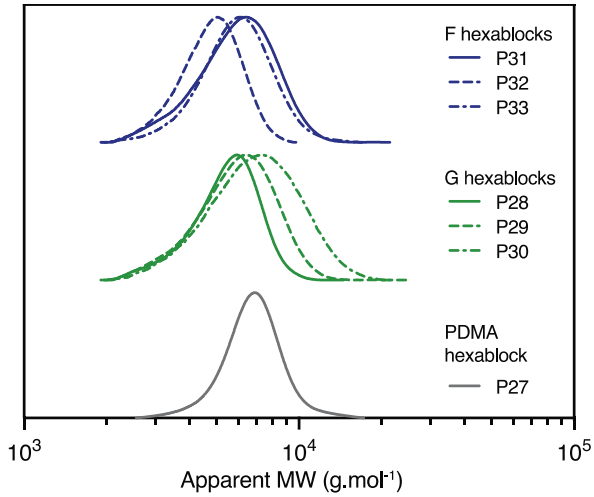


Figure S6. DMF GPC traces of the DMA, FF, and GG^{O} hexablock polymers at a total DP of 100 (16.7 per block). Molecular weights are calculated relative to PEO standards without correction in all cases.

Table S6. Diffusion constants (D) and associated errors, and corresponding hydrodynamic radii (r_h) and associated errors calculated from the DOSY-NMR experiments in 10% MeOD/D_2O (v/v) for peptide copolymers. [a] Description of the multiblock polymer structure with the position of peptide monomer and target DP.

#	Description [a]	D ($cm^2.s^{-1}$)	D_{err}	r_h (nm)[b]	r_{err}
P8	$(F)_{5\%,\ 500}$	3.7 x 10^{-7}	3.1 x 10^{-9}	5.30	0.04
P9	$(F)_{10\%,\ 500}$	2.7 x 10^{-7}	1.5 x 10^{-8}	7.30	0.40
P10	$(F)_{15\%,\ 500}$	4.2 x 10^{-7}	7.0 x 10^{-9}	4.60	0.07
P2	$(G^N)_{5\%,\ 500}$	3.5 x 10^{-7}	2.6 x 10^{-8}	5.60	0.41
P4	$(G^N)_{10\%,\ 500}$	3.1 x 10^{-7}	5.2 x 10^{-10}	6.40	0.01
P6	$(G^N)_{15\%,\ 500}$	3.9 x 10^{-7}	3.4 x 10^{-9}	5.00	0.04
P14	$(D)_{250}$	4.1 x 10^{-7}	5.6 x 10^{-9}	5.00	0.07
P16	$(F)_{5\%,\ 250}$	4.1 x 10^{-7}	6.5 x 10^{-9}	4.80	0.07
P19	$(F)_{10\%,\ 250}$	5.4 x 10^{-7}	4.9 x 10^{-9}	3.60	0.03
P22	$(F)_{15\%,\ 250}$	3.4 x 10^{-7}	2.4 x 10^{-8}	3.80	0.38
P15	$(G^N)_{5\%,\ 250}$	3.3 x 10^{-7}	3.2 x 10^{-9}	4.10	0.04
P18	$(G^N)_{10\%,\ 250}$	2.6 x 10^{-7}	1.1 x 10^{-9}	4.50	0.02
P21	$(G^N)_{15\%,\ 250}$	5.7 x 10^{-7}	4.4 x 10^{-8}	3.60	0.27

Table S7. Diffusion constants (D) and associated errors, and corresponding hydrodynamic radii (r_h) and associated errors calculated from the DOSY-NMR experiments in 10% MeOD/D_2O (v/v) for multiblock polymers. [a] Description of the multiblock polymer structure with the position of peptide monomer and target DP.

Polymer	Description [a]	D ($cm^2.s^{-1}$)	D_{err}	r_h (nm)	r_{err}
P24	$(D\text{-}D\text{-}D)_{250}$	6.8 x 10^{-7}	5.9 x 10^{-9}	3.00	0.03
P26	$(D\text{-}F\text{-}D)_{250}$	1.7 x 10^{-6}	1.2 x 10^{-8}	1.20	0.01
P25	$(D\text{-}G^O\text{-}D)_{250}$	5.7 x 10^{-7}	3.5 x 10^{-9}	3.50	0.02
P27	$(D\text{-}D\text{-}D\text{-}D\text{-}D\text{-}D)_{100}$	1.0 x 10^{-6}	1.0 x 10^{-8}	2.00	0.02
P31	$(D\text{-}F\text{-}D\text{-}D\text{-}F\text{-}D)_{100}$	1.9 x 10^{-6}	6.5 x 10^{-8}	1.00	0.03
P28	$(D\text{-}G\text{-}D\text{-}D\text{-}G\text{-}D)_{100}$	8.7 x 10^{-7}	7.2 x 10^{-9}	2.30	0.01
P32	$(D\text{-}D\text{-}F\text{-}F\text{-}D\text{-}D)_{100}$	1.1 x 10^{-6}	1.9 x 10^{-8}	1.90	0.03
P29	$(D\text{-}D\text{-}G\text{-}G\text{-}D\text{-}D)_{100}$	8.6 x 10^{-7}	5.0 x 10^{-9}	2.40	0.01
P33	$(D\text{-}F\text{-}F\text{-}F\text{-}F\text{-}D)_{100}$	1.0 x 10^{-6}	5.8 x 10^{-9}	2.00	0.01
P30	$(D\text{-}G\text{-}G\text{-}G\text{-}G\text{-}D)_{100}$	8.3 x 10^{-7}	4.9 x 10^{-9}	2.50	0.01

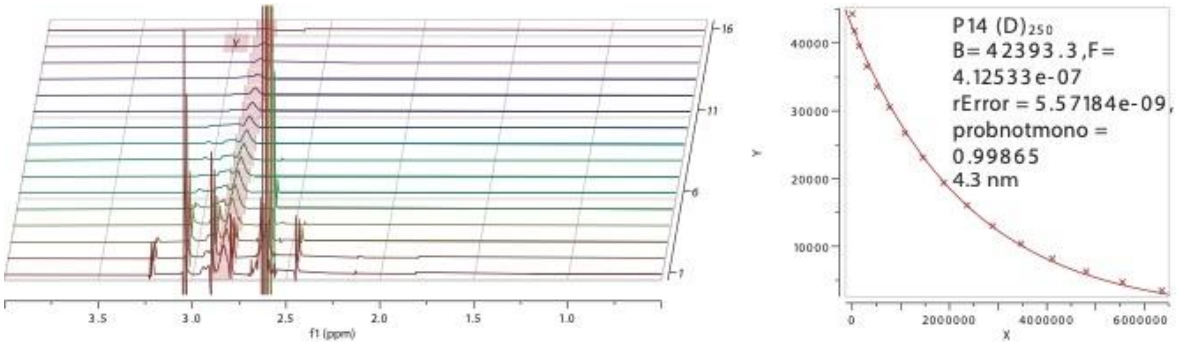


Figure S7. DOSY-NMR data for P14

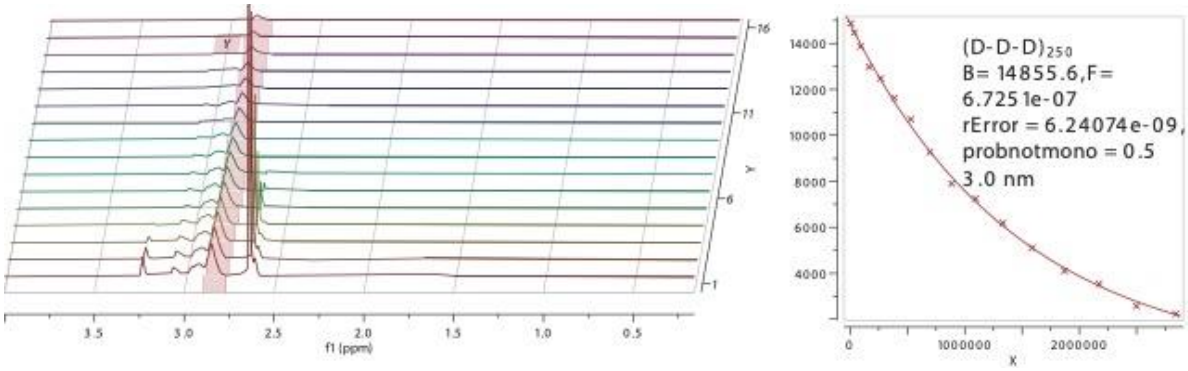


Figure S8. DOSY-NMR data for P24

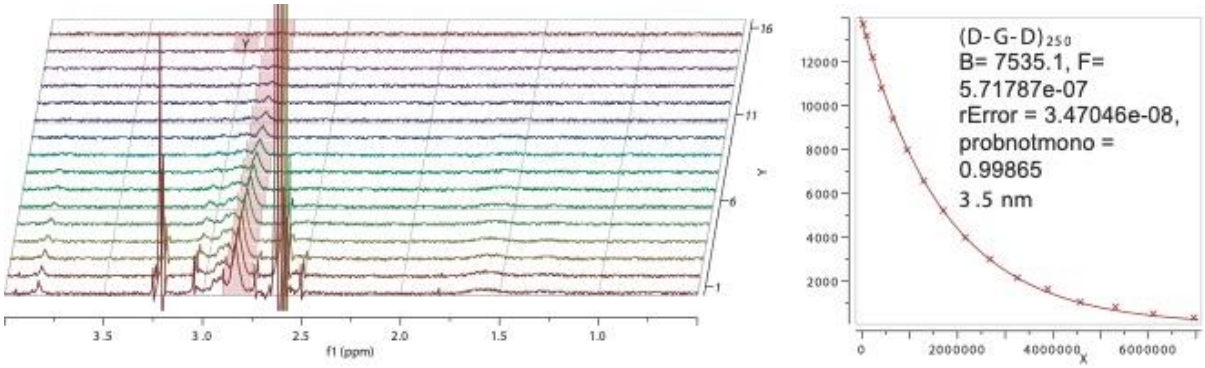


Figure S9. DOSY-NMR data for P25

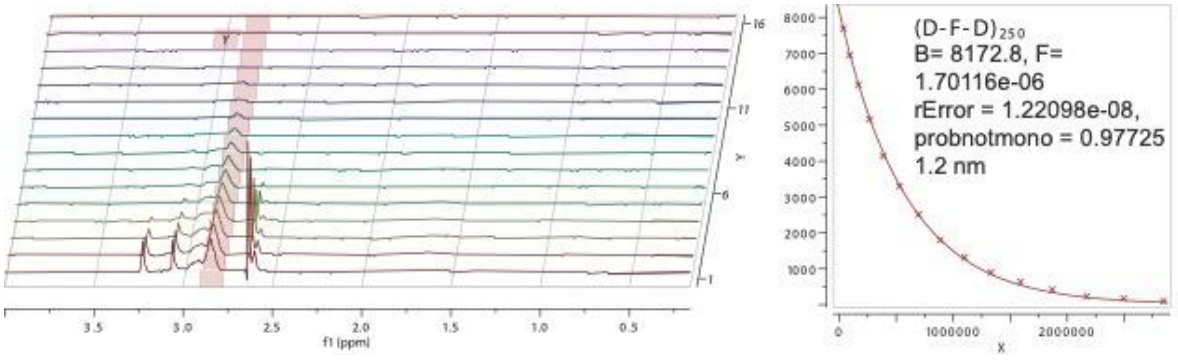


Figure S10. DOSY-NMR data for P26

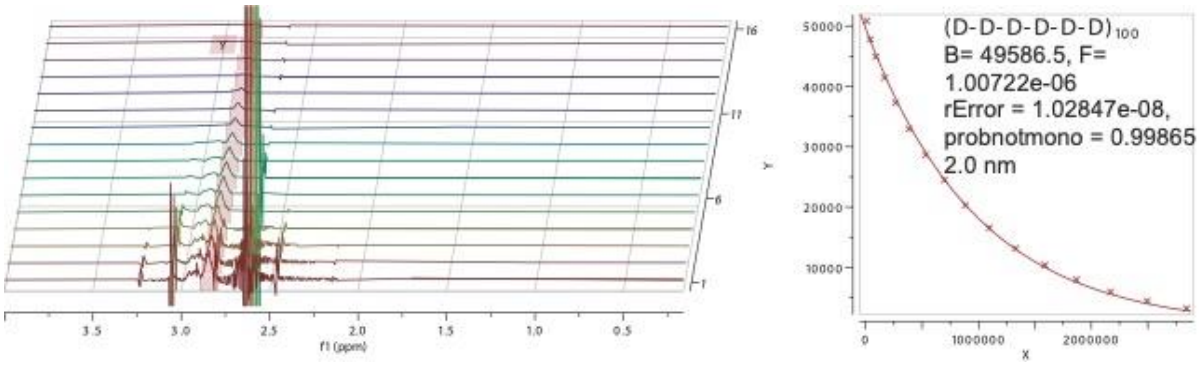


Figure S11. DOSY-NMR data for P27

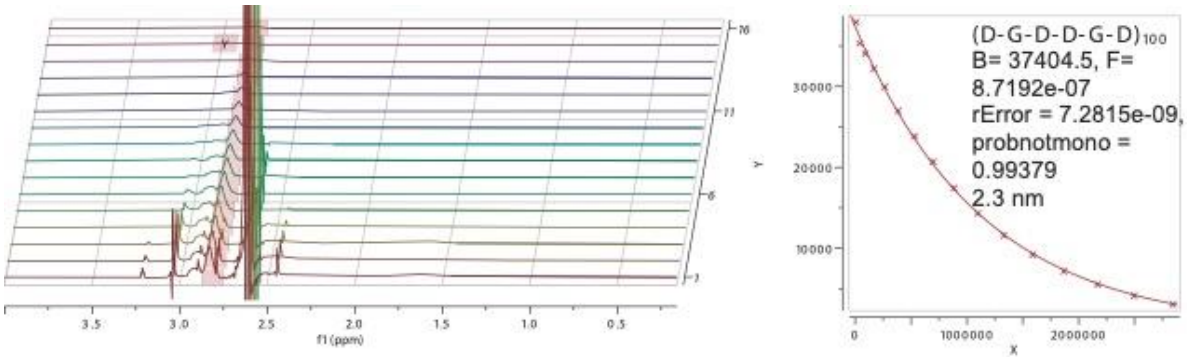


Figure S12. DOSY-NMR data for P28

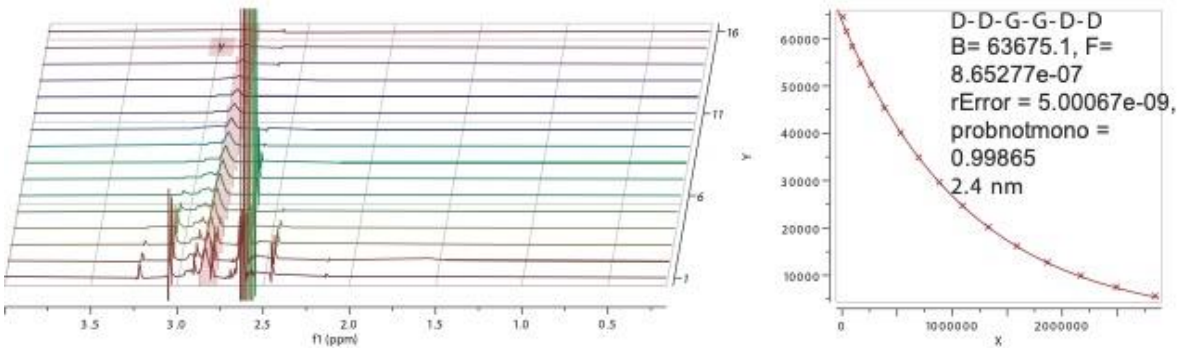


Figure S13. DOSY-NMR data for P29

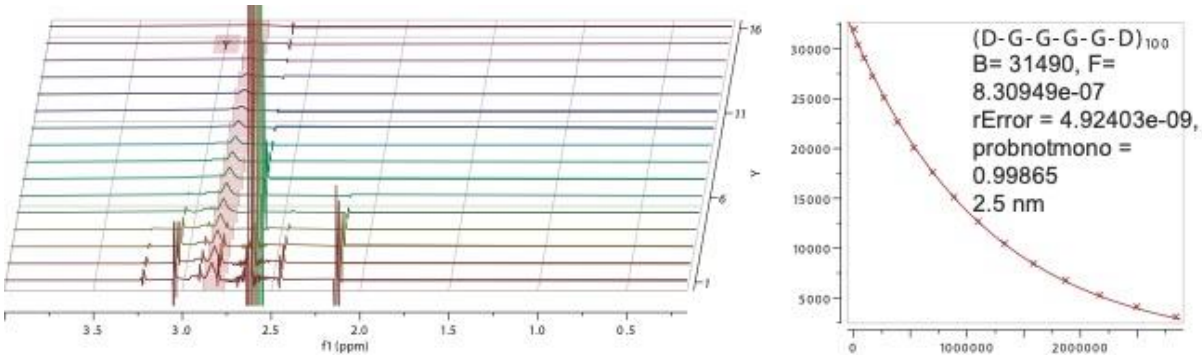


Figure S14. DOSY-NMR data for P30

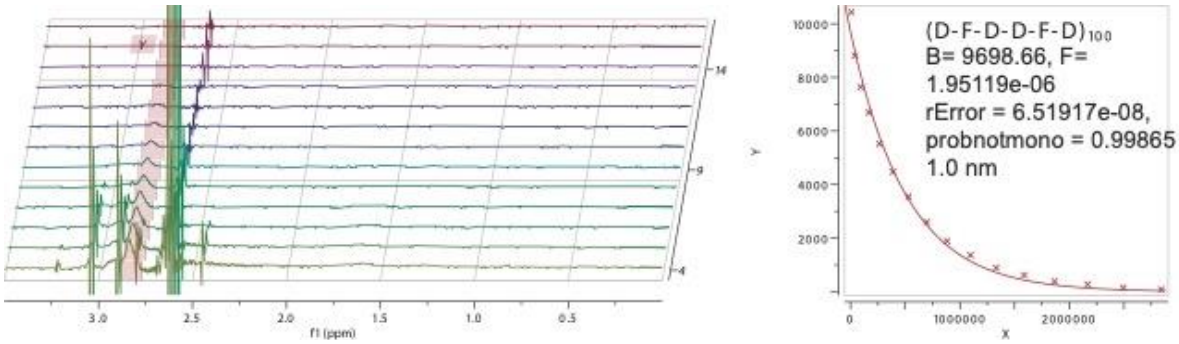


Figure S15. DOSY-NMR data for P31

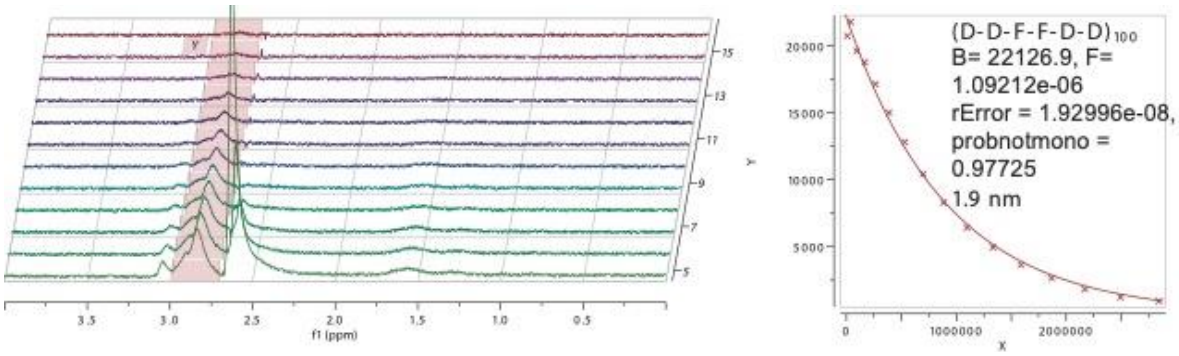


Figure S16. DOSY-NMR data for P32

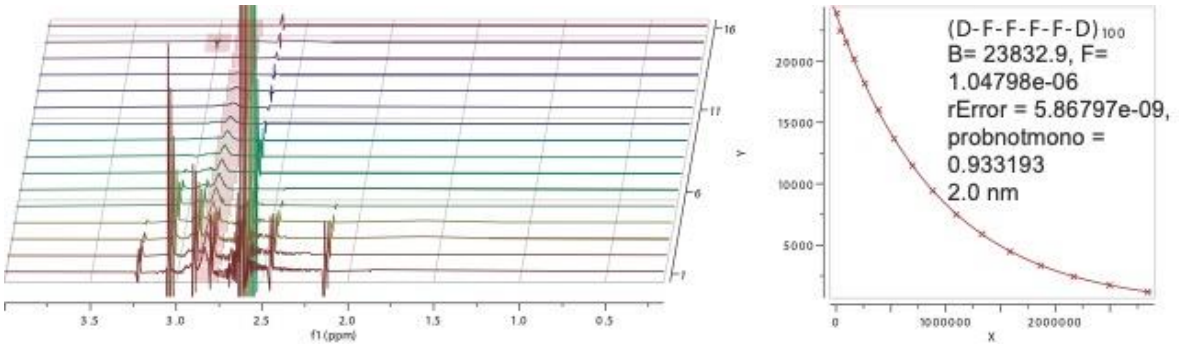


Figure S17. DOSY-NMR data for P33