Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

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General Information

Standard Fmoc-protected amino acids were purchased from CEM Corporation or Pepceuticals. HCTU and peptide grade DMF were purchased from Pepceuticals. All other reagents were purchased from Sigma Aldrich and used without further purification.

Peptides were synthesised on a Biotage® Initiator+ Alstra™ microwave assisted peptide synthesiser. Peptides were purified on a Dionex reverse-phase high performance liquid chromatography (RP-HPLC) system equipped with Dionex P680 pumps and a Dionex UVD170U UV-vis detector (monitoring at 214 nm and 280 nm), using a Phenomenex, Gemini, C18, 5 μm, 250 x 21.2 mm column. Gradients were run using solvents consisting of A (H_2O + 0.1% TFA) and B (MeCN + 0.1% TFA) and fractions were lyophilised on a Christ Alpha 2-4 LO plus freeze dryer.

Purified peptides were analysed on a Shimadzu RP-HPLC system equipped with Shimadzu LC-20AT pumps, a SIL-20A autosampler and a SPD-20A UV-vis detector (monitoring at 214 nm and 280 nm) using either a Phenomenex, Gemini, C18, 5 μm, 250 x 21.2 mm column or a Phenomenex, Aeris, 5 μm, peptide XB-C18, 150 x 4.6 mm column at a flow rate of 1 mL/min. RP-HPLC gradients were run using a solvent system consisting of solution A (5% MeCN in H_2O + 0.1% TFA) and B (5% H_2O in MeCN + 0.1% TFA). Two gradients were used to characterise each peptide; a gradient from 0% to 100% solution B over 20 min and a 50 min gradient from 0%-100% solution B. Analytical RP-HPLC data is reported as column retention time (t_R) in minutes (min). Low-resolution mass spectrometry (LRMS) was performed on a Thermo Scientific LCQ Fleet quadrupole mass spectrometer using electrospray ionisation in positive mode (ESI^+). High-resolution mass spectrometry (HRMS) was performed on a Bruker microTOF-Q II (ESI^+).

Peptide content was analysed on a Nanodrop 2000c using UV absorption of peptides at 280 nm.

Peptide synthesis – General methods.

Peptides were synthesised on 0.1 mmol scale using Tentagel RAM (Rink amide) resin. Couplings were performed using 4 equivalents (2 equivalents for unnatural amino acids) Fmoc-protected amino acid, 4 equivalents HCTU and 8 equivalents DIPEA. Coupling of standard Fmoc-protected amino acids was carried out for 10 min at 75 °C followed by 4 × 45 s washes with DMF. Arginine was double coupled for 60 min at room temperature followed by 5 min at 75 °C and repeated with fresh reagents followed by washing. Histidine was coupled at room temperature for 5 min followed by 50 °C for 5 min and washed with DMF. *N*-Terminal acetyl capping was achieved using a mixture of DIPEA (2.0 M / NMP, 50 eq.) and acetic anhydride (Ac_2O, 5.0 M / DMF, 50 eq.) at ambient temperature for 10 min. Once capping reagents had been drained, the peptide resin was then washed four times with DMF (4 × 9 mL) for 45 s at ambient temperature. Resin bound peptide was washed with DCM (4 × 9 mL) for 45 s at ambient temperature.

Deprotection was carried out in 20% piperidine in DMF + 5% formic acid for 30 s and then 3 min at 75 °C followed by washing.

Test cleavages were performed using 1 mL of cleavage cocktail containing 95% TFA, 2.5% TIS and 2.5% H_2O for 1 hour at room temperature. Final cleavage was performed using a cleavage cocktail (10 mL) consisting of 95% TFA, 2.5% H_2O and 2.5% TIS. The resin was vortex mixed for 3 hours at room temperature and the cleavage cocktail evaporated using a stream of nitrogen. The peptide was precipitated from solution with ice cold Et_2O, centrifuged at 3200 × g for 5 min and the precipitate washed with ice cold Et_2O a further three times. Finally, the crude peptides were dissolved in H_2O/MeCN and lyophilised.

Ring Closing Metathesis

The resin was swollen in dichloroethane (DCE) (4 mL) for 10 minutes. The resin was reacted with a 5 mM solution of Grubbs 1st generation catalyst (16 mg, 0.02 mmol, 20 mol% relative to resin loading) in DCE (4 mL) and the mixture was left to react for 2 hours at room

temperature, excluding light. The resin was then washed thoroughly with DMF (3 x 4 mL), followed by DCE (3 x 4 mL). The resin was then subjected to a fresh 5 mM solution of Grubbs 1st generation catalyst (16 mg, 0.02 mmol, 20 mol% relative to resin loading) in DCE (4 mL) and left to react for 2 hours, excluding light. The resin was then washed thoroughly with DMF (3 x 4 mL), followed by DCM (3 x 4 mL), then dried and stored in a desiccator.

Compound Name	Sequence	Yield (%)	% Purity	Calculated m/z	Measured m/z	Err [ppm]	T_R 20min (min)	T_R 50min (min)
DM039	*Ac-PGHLKGREIGLWYAKKQGQKNK-NH_2*	13	96	645.1186	645.1161	3.9	11.840	19.287
DM083	*Ac-PGHLKGREIGLWYAS_5KQGS_5KNK-NH_2*	7	97	857.8272	857.8276	-0.5	13.059	24.800
DM102	*Ac-PGHLKGRR_8IGLWYAS_5KQGQKNK-NH_2*	10	98	871.5148	871.5111	4.3	14.105	26.537

DM039

The linear peptide was prepared using microwave assisted SPPS on a 0.1 mmol scale as specified above, leaving the peptide *N*-terminally acetylated. The resin was then washed thoroughly with DMF (3 x 4 mL), followed by DCM (3 x 4 mL), then dried and stored in a desiccator.

DM039 *Ac-PGHLKGREIGLWYAKKQGQKNK-NH_2*: Peptide was cleaved from the resin and acid labile protecting groups removed. The peptide was purified via preparative RP-HPLC as described above. Purified fractions were lyophilised to give the title compound as a white powder (10 mg, yield 13%, 96% purity).

DM083

The linear peptide was prepared using microwave assisted SPPS on a 0.1 mmol scale as specified above, leaving the peptide *N*-terminally acetylated. The resin was then washed thoroughly with DMF (3 x 4 mL), followed by DCM (3 x 4 mL), then dried and stored in a desiccator. The resin was then subjected to the ring closing metathesis methods stated above, to achieve the stapled peptide.

DM083 *Ac-PGHLKGREIGLWYAS_5KQGS_5KNK-NH_2*: Peptide was cleaved from the resin and acid labile protecting groups removed. The peptide was purified via preparative RP-HPLC as described above. Purified fractions were lyophilised to give the title compound as a white powder (1.6 mg, 7% yield, 97% purity).

DM102

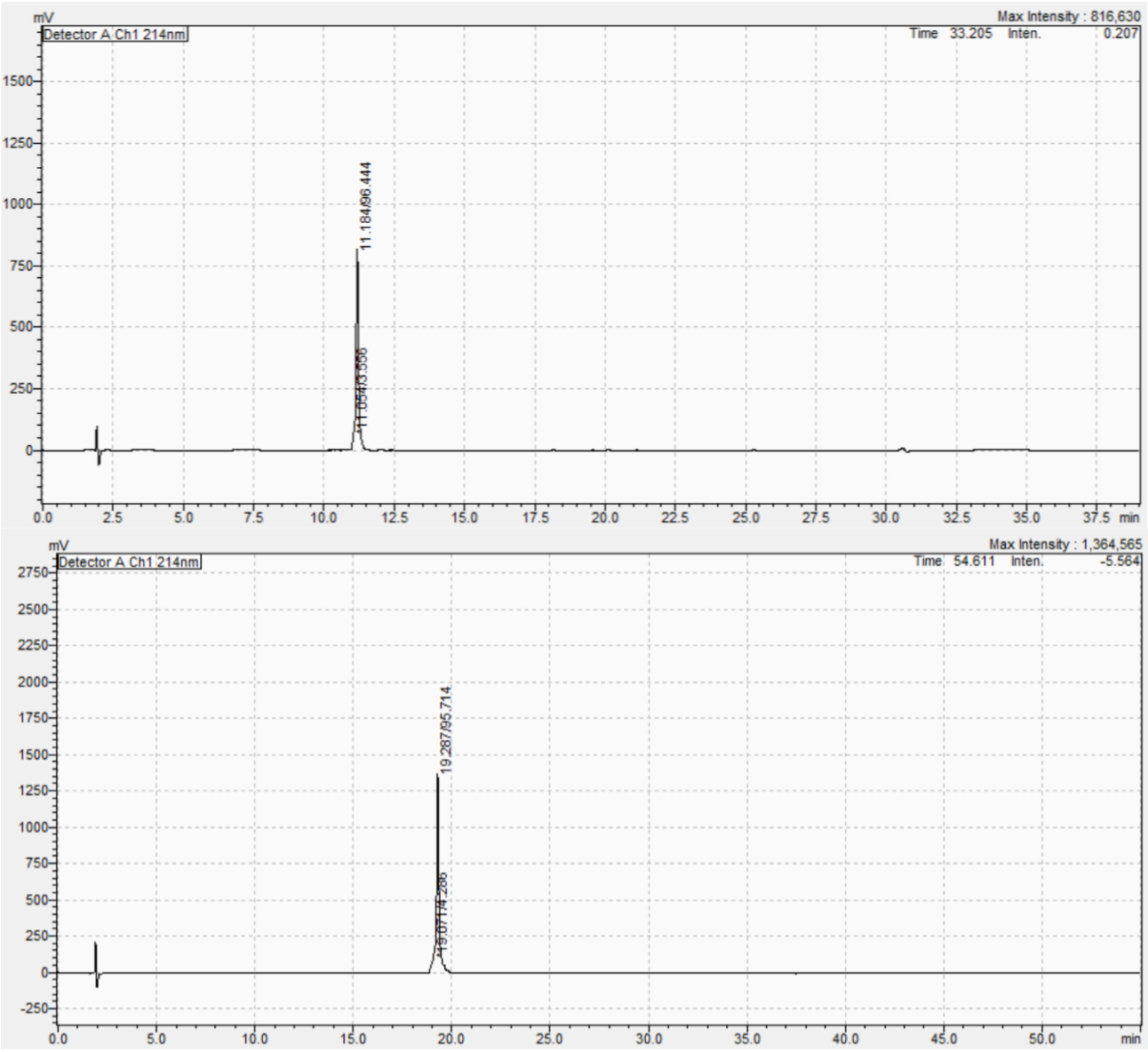
The linear peptide was prepared using microwave assisted SPPS on a 0.1 mmol scale as specified above, leaving the peptide *N*-terminally acetylated. The resin was then washed thoroughly with DMF (3 x 4 mL), followed by DCM (3 x 4 mL), then dried and stored in a

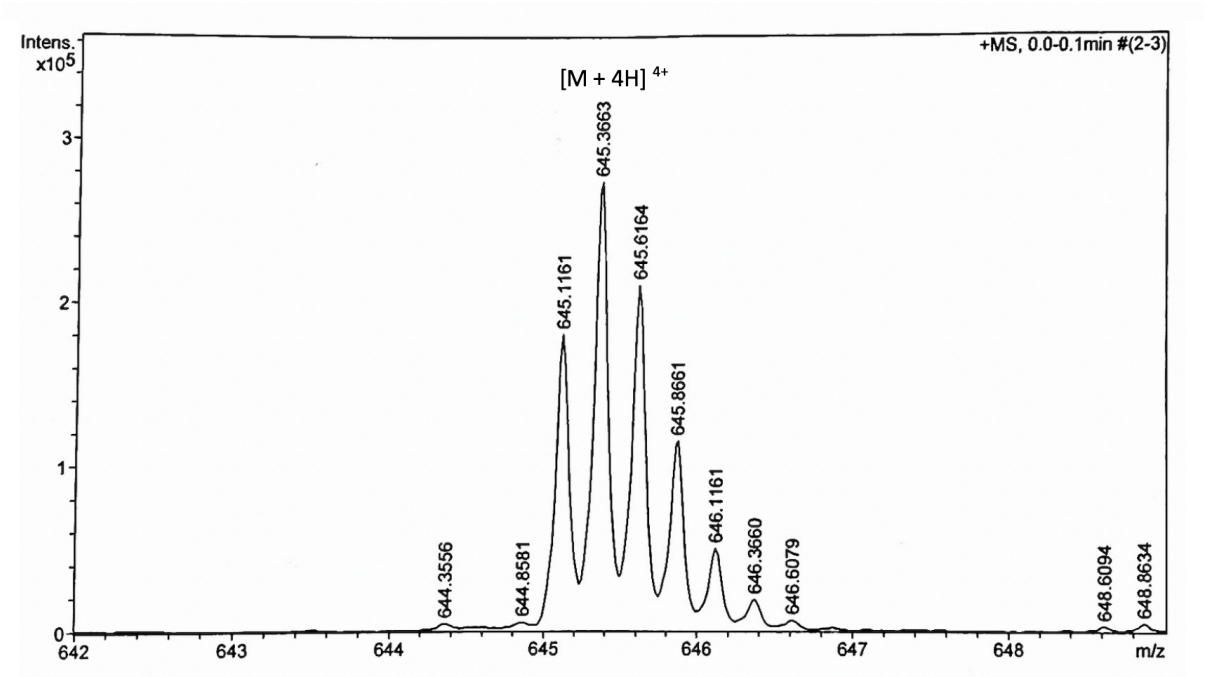
desiccator. The resin was then subjected to the ring closing metathesis methods stated above, to achieve the stapled peptide.

DM102 *Ac-PGHLKGRR$_8$IGLWYAS$_5$KQGQKNK-NH$_2$*: Peptide was cleaved from the resin and acid labile protecting groups removed. The peptide was purified via preparative RP-HPLC as described above. Purified fractions were lyophilised to give the title compound as a white powder (0.8 mg, 10% yield, 98% purity).

Peptide characterisation

DM039

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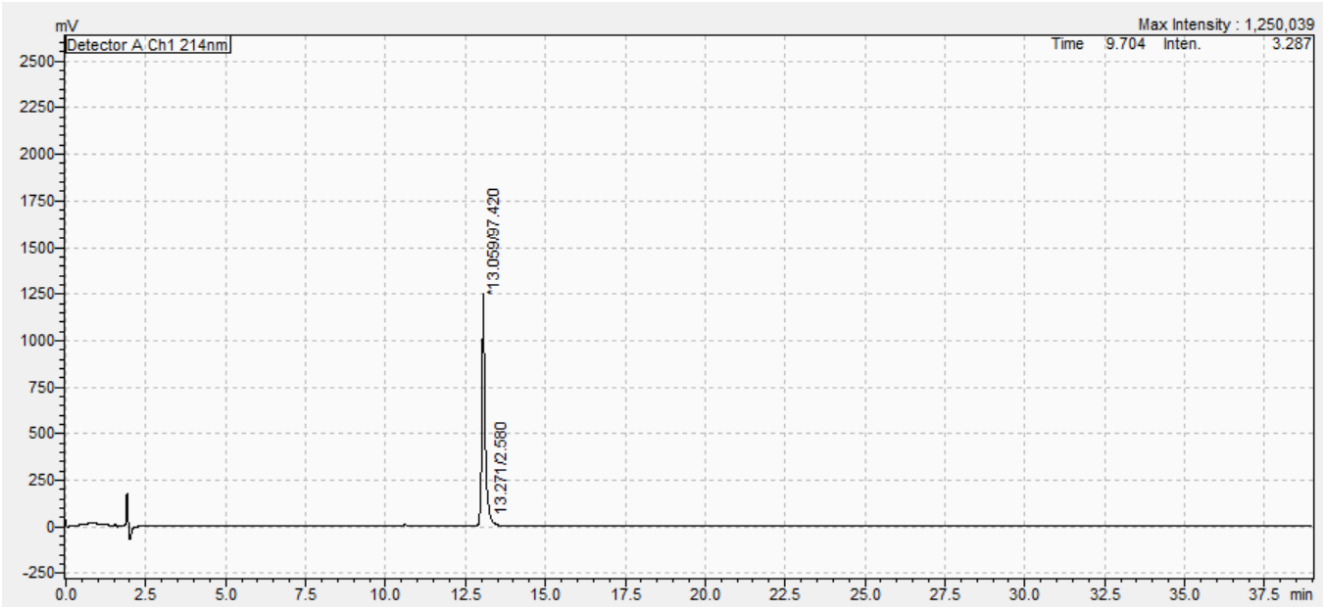
m/z

DM083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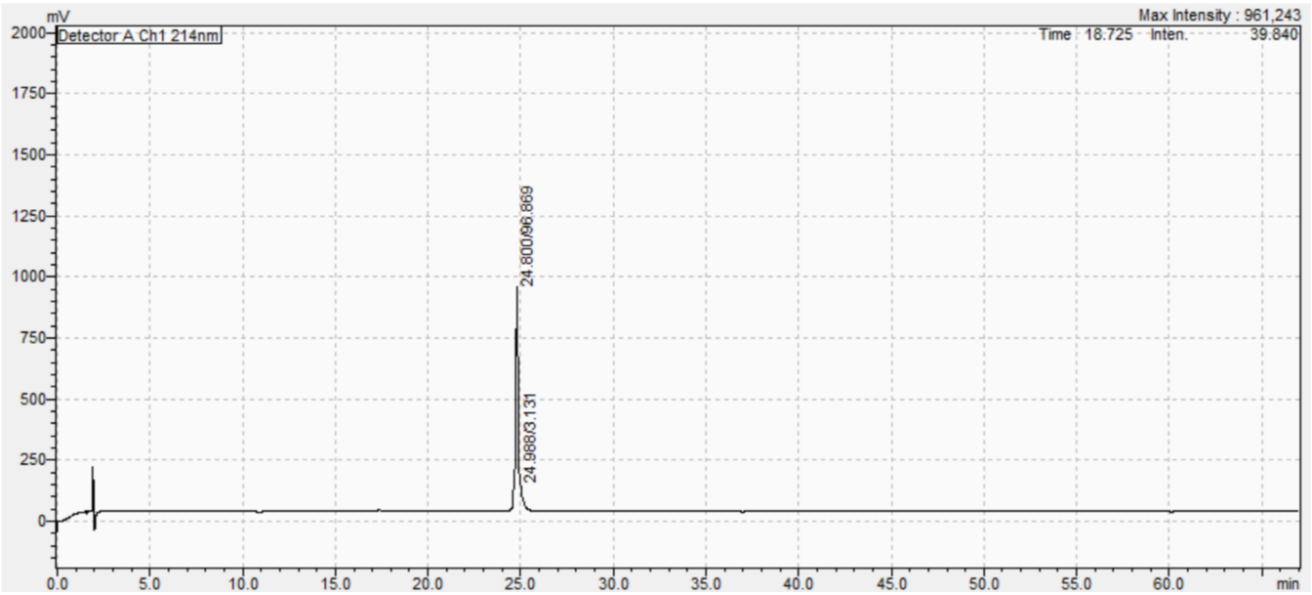
13.271/2.580
min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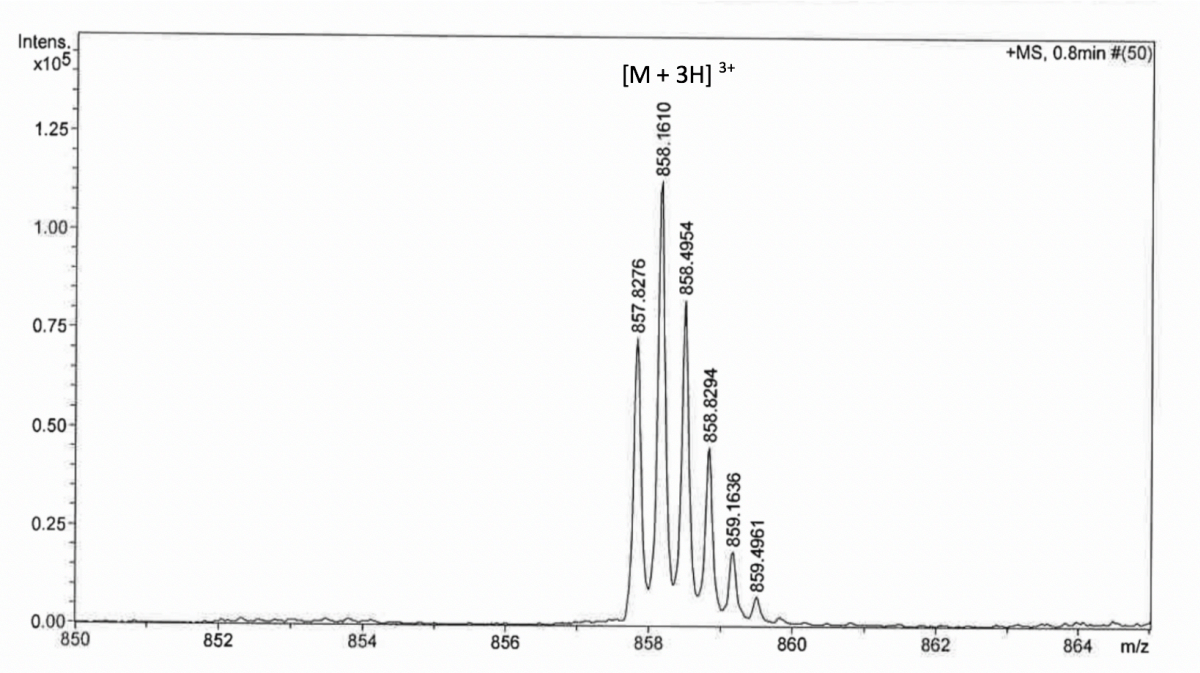
min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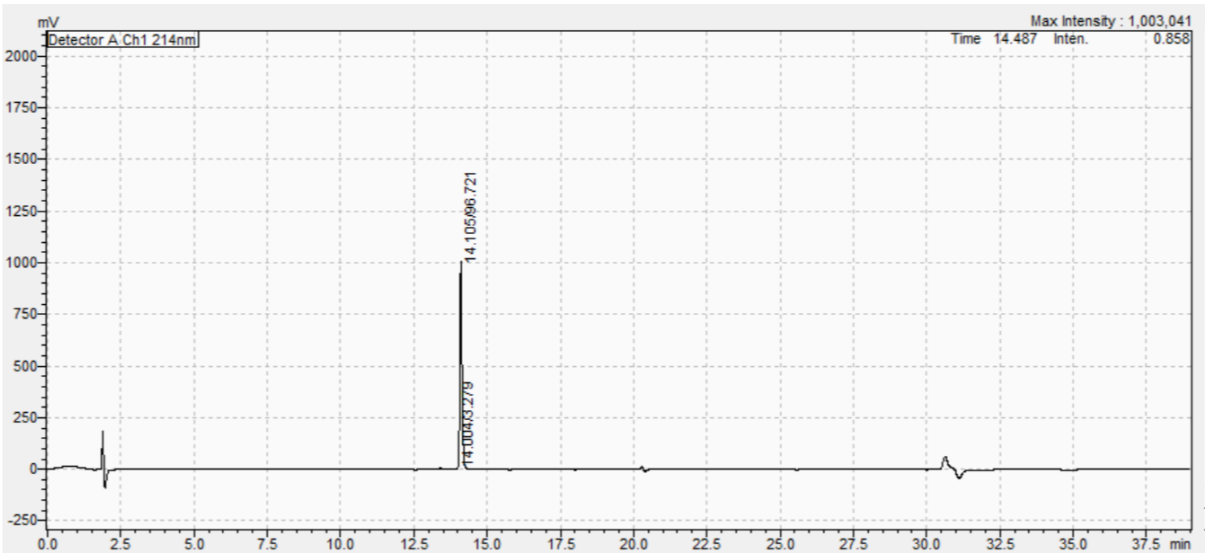
m/z

DM102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14.004/3.279
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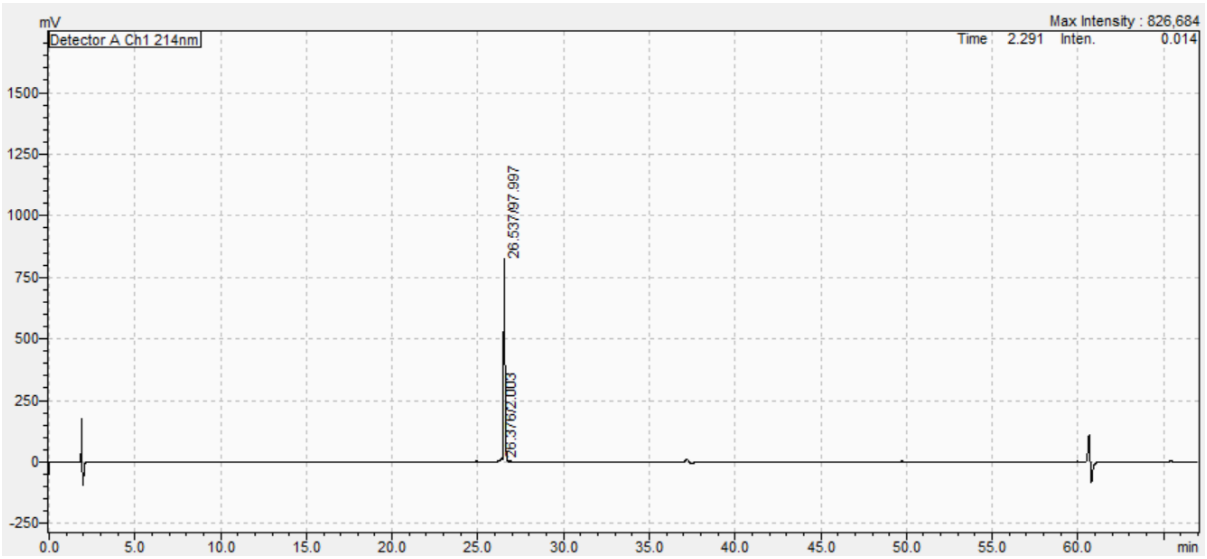
1750
1500
1250
1000
750
500
250
0
-250
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 32.5 35.0 37.5 min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26.376/2.003
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-250
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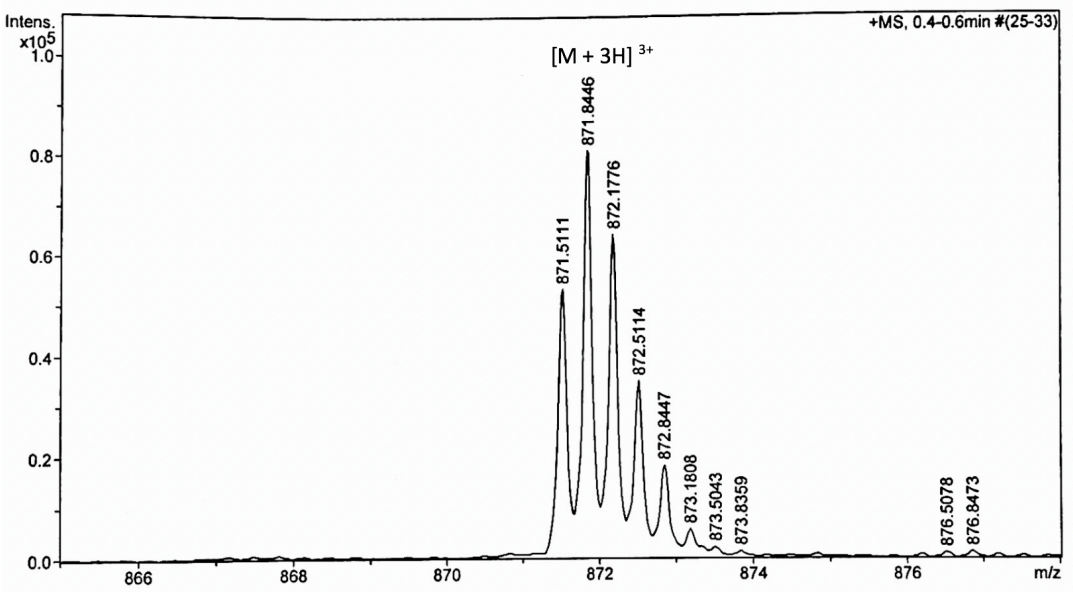


FP experimental

Annellation of the oligonucleotides

The 5'-FAM-labelled oligonucleotide sequences were purchased from Eurogentec and provided as a dry powder. As suggested from the supplier, the oligonucleotides were resuspended in water to obtain a stock concentration of 100 µM. The samples were further diluted in Inner cell buffer (25 mM Hepes pH 7.6, 110 mM KCl, 10.5 mM NaCl, 1 mM $MgCl_2$) down to a concentration of 500 nM. For the dsDNA, an equal amount of ssDNA-FAM and its complementary strand were mixed together. The samples were then warmed to 95°C for 10 min and left to cool slowly overnight at room temperature. The samples were left in a dark environment as the fluorophore labelling made them light sensitive.

Oligomer	Sequence
HRAS-FAM	TCG-GGT-TGC-GGG-CGC-AGG-GCA-CGG-GCG
Complementary c-MYC-mutant ssDNA	CGC-CCA-CGC-ACT-ATG-CC

c-MYC-mutant ssDNA-FAM	GGC-ATA-GTG-CGT-GGG-CG
c-kit2-FAM	TGG-GCG-GGC-GCG-AGG-GAG-GGG
c-MYC-FAM	TGA-GGG-TGG-GTA-GGG-TGG-GTA-A
h-Telo-FAM	TAG-GGT-TAG-GGT-TAG-GGT-TAG-GGT
c-kit1-FAM	TGG-GAG-GGC-GCT-GGG-AGG-AGG-G
BCL2-FAM	TGG-GCG-CGG-GAC-GAG-GGG-GGC-GGG

Fluorescence polarisation measurements

All Fluorescence Polarisation (FP) measurements were performed on a multifunctional microplate reader (EnVision, Perkin Elmer) in black 384-well microplates (Corning, Cat No 3575) with 15 µL of sample solution per well. The EnVision software required optimisation for a specific oligonucleotide concentration prior to the experiment. Optimisation was performed by following the standard protocol highlighted in the software guidelines. The inputted minimum and maximum concentrations of oligonucleotide were respectively 0 nM (Inner cell buffer) and 20 nM. 492-nm excitation and 518-nm emission filters were used for the FP measurements.

Fluorescence polarisation binding assay[1]

A 5 µM stock concentration of each peptide was prepared in Inner cell buffer. Individual samples containing a fixed concentration of fluorescently 5'-FAM-labelled oligonucleotide (20 nM) and increasing concentrations of peptide (0, 5, 10, 20, 50, 100, 200, 300, 500, 700, 1000, 1500, 2000, 2500, 3000, 4000 and 5000 nM) were dissolved in Inner cell buffer. The samples were left to incubate at room temperature for 30 min to allow the interaction between the peptide and the oligonucleotide. 15 µL of each sample were then loaded on a 384-well black plate and tested for FP. Each measurement was repeated three times for statistical significance.

Data Analysis

Raw data were normalized using baseline correction, where the baseline was defined as the fluorescence polarisation measured in the absence of peptide. Normalized data were then plotted in Prism7.0 and fitted by using the quadratic ligand depletion model (Equation 1).

$$Y = \frac{K_d + R_t + X - \sqrt{(K_d{}^2 + (R_t - X)^2 + 2\,K_d\,(R_t + X))}}{2} \qquad \text{Equation 1}$$

Equation 1. Y is the fluorescent polarisation signal measured at each datapoint. R_t is the total concentration of receptor (FAM-labelled DNA). X is the total concentration of ligand (peptide) and K_d is the dissociation constant (the inflection point of the curve on a logarithmic scale).

SPR experimental

SPR measurements were performed on a four channel BIAcore S200 (GE Life Sciences) by using a streptavidin coated sensor chip (Series S sensor chip SA). A biotinylated G-quadruplex sequence for Myc (biotin- [TGA GGG TGG GTA GGG TGG GTA A] and a biotinylated mutant form of Myc (biotin- [TGA GTG TGT GTA GTG TGT GTA A] were used. The biotin-labelled oligonucleotide sequences were purchased from Eurogentec and provided as a dry powder. As suggested from the supplier, the oligonucleotides were resuspended in water to obtain a stock concentration of 100 µM. The DNA oligonucleotides were folded in filtered and degassed running buffer (50 mM Tris•HCl pH 7.4, 100 mM KCl + 0.005% TWEEN), diluted to 50 nM from the stock solution, kept at 95 °C for 10 minutes and then cooled to room temperature overnight. After conditioning the surface with three 1-minute injections of 1M NaCl, 50 mM NaOH; a solution of biotin-Myc-mutant and biotin-Myc was injected at 2 µL min^{-1} until the attainment of final response of 250 RU and 235 RU respectively. All experiments were performed at 25˚C in running buffer. Binding experiments were performed by injecting the ligand (DM039) at increasing concentrations from 0.1 nM to 10 µM, in both channels at a flow rate of 30 µL min^{-1} with a contact time of 80 seconds and dissociation of 200 seconds with running buffer to regenerate the chip. Ligand solutions were freshly prepared with

running buffer by serial dilutions from a stock solution. The final graphs were obtained by subtracting the myc-mutant sensorgrams from the myc sensorgrams. The response at equilibrium (R_{eq}) was plotted against the concentration of the ligand [DM039] to generate a hyperbolic binding curve. A binding constant was determined by fitting the binding curve using a Hill 1 equation. The Hill equation was fitted using Origin software (Microcal Inc. USA) (*Equation 2*).

$$Fraction\ of\ bound\ ligand = \frac{[L]^n}{(K_d + [L]^n)} \qquad \textit{Equation 2}$$

The Hill equation; where K_d is the equilibrium dissociation constant, [L] is the free (unbound) ligand concentration and n is the Hill coefficient.[2]

CD experimental

CD spectra were recorded on a JASCO J-810 circular dichroism spectrophotometer using a 1 mm path length quartz cuvette. CD measurements were performed at 298 K over a range of 200-320 nm using a response time of 2 s, 1 nm pitch and 0.5 nm bandwidth. The recorded spectra represent a smoothed average of three scans, zero-corrected at 320 nm and normalized (Molar ellipticity ϑ is quoted in 10^5 deg cm^2 dmol^{-1}). The absorbance of the buffer was subtracted from the recorded spectra. CD titrations of unlabeled, annealed DNA were performed in 10 mM Tris + 100 mM KCl (pH 7.4) buffer. DNA (c-MYC) was annealed at 10 μM (heated at 95 °C for 10 minutes and slowly cooled to room temperature overnight) and spectra were recorded at a final concentration of 5 μM. Spectra were overlaid to analyse the observed trend.

The raw circular dichroism data was processed by subtraction of solvent and then converted into MRE (mean residue ellipticity)[3]

$$MRE = \frac{\theta_{obs}}{(10 \times c \times l)(R-1)}$$

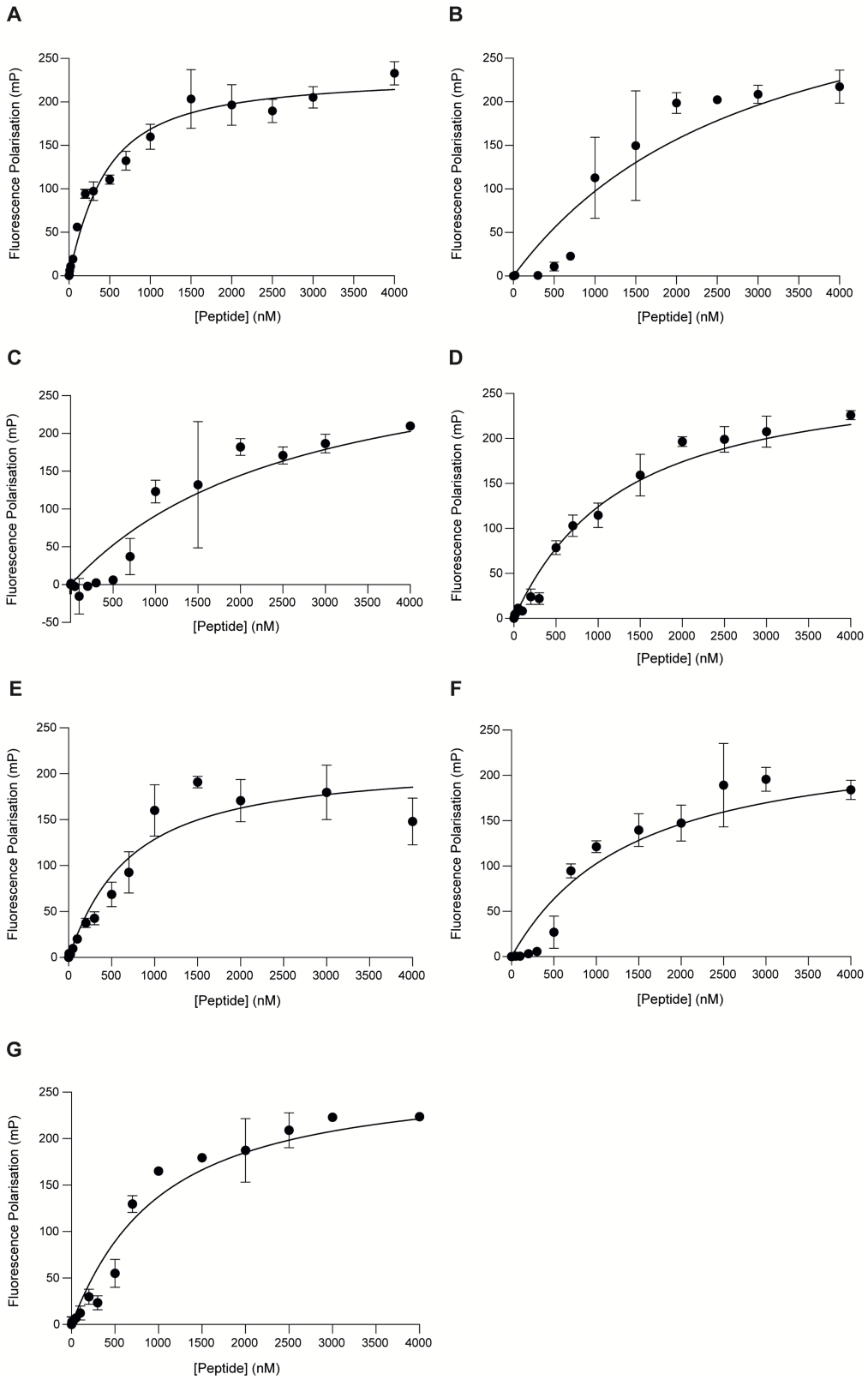
Where $[\theta]_{MRE}$ = molar ellipticity per residue, θ = circular dichroism at a given wavelength (222 nm), c = molar concentration, l = path length in cm, R = number of residues in the peptide sequence.

Calculation of percentage helicity was performed using the following equation:

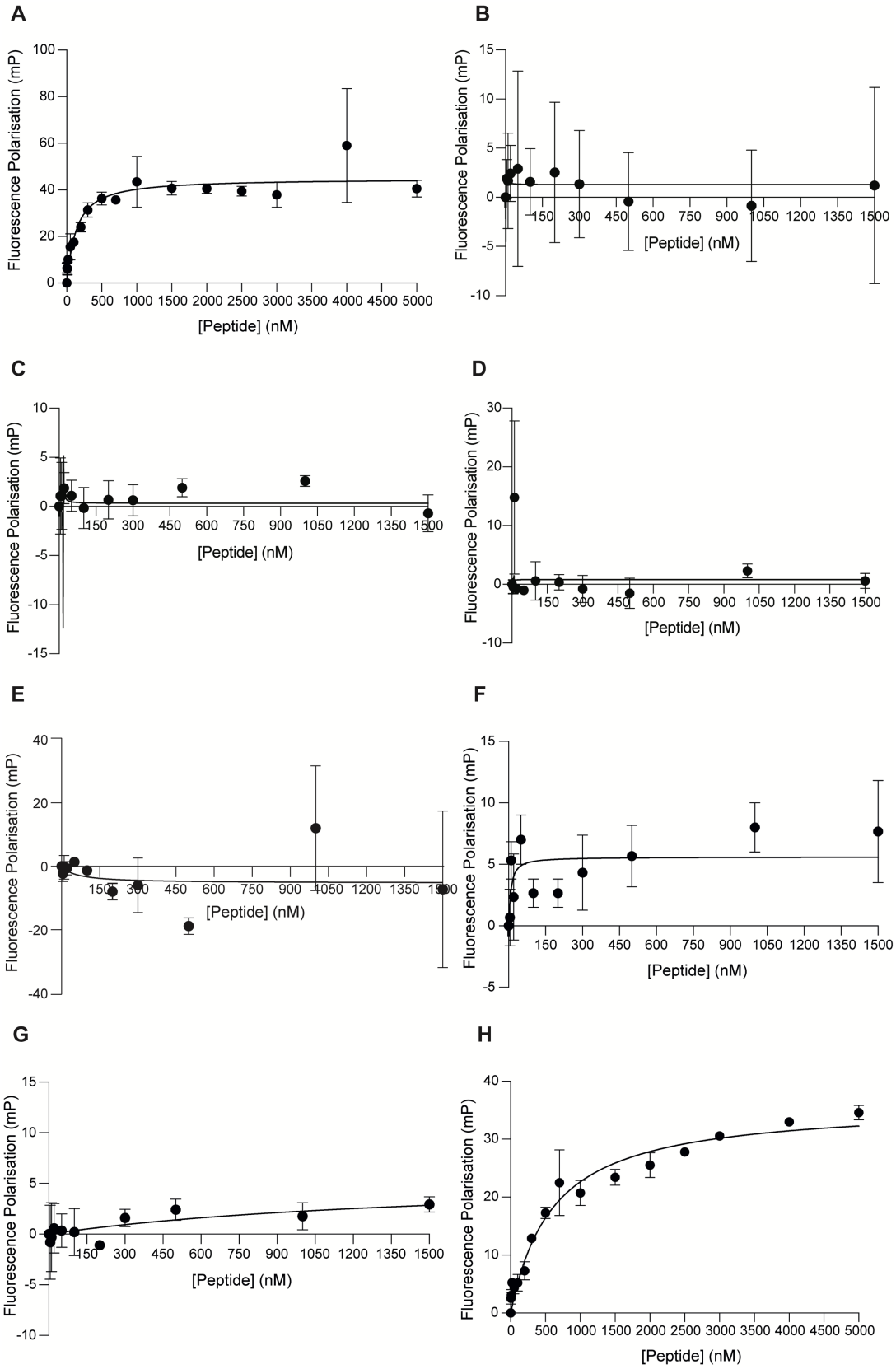
$$fH = \frac{\theta_O - \theta_C}{\theta_H - \theta_C}$$

Where: θ_O = MRE at 222nm θ_C = the signal for 100% coil structure = 2200-53xT, T=Temperature, θ_H = Theoretical MRE for 100% helicity at 222 nm = (-44000 (Theoretical MRE for 100% helicity at 222 nm at 0 °C) +250 (temperature dependence of infinite helix) x T) x (1-3/number of amino acid residues).

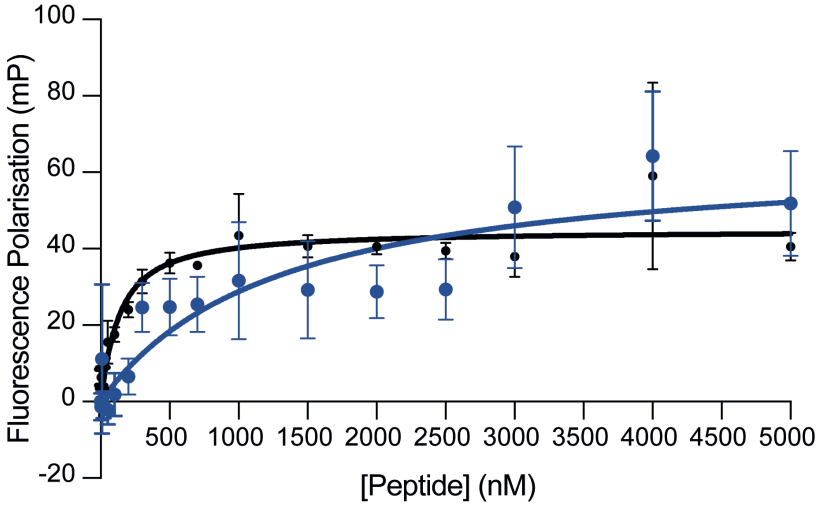
Supplementary Figures



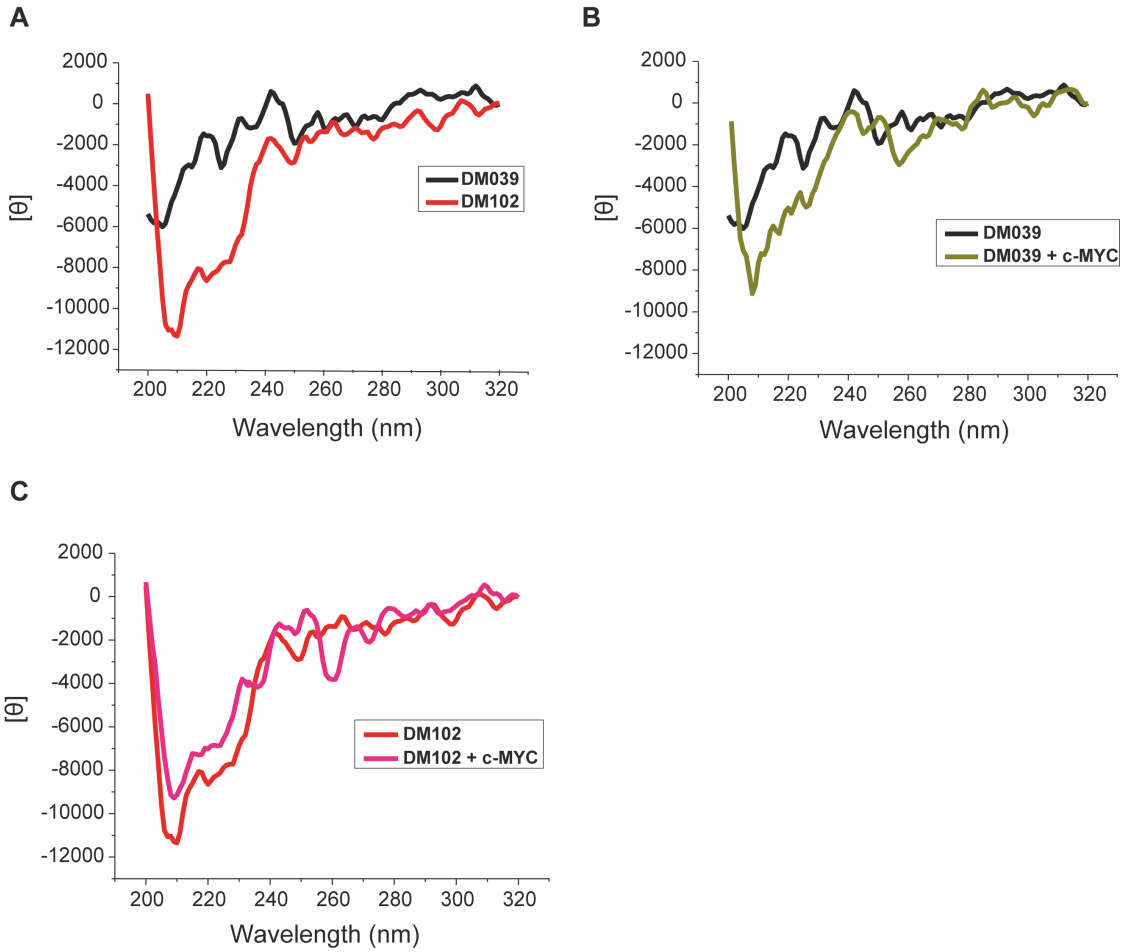
Supplementary Figure 1: FP binding curves obtained with DM102 in the presence of (A) c-MYC, (B) single-stranded DNA, (C) double-stranded DNA, (D) h-Telo, (E) c-KIT1, (F) HRAS and (G) BCL-2. All curves were fitted using the quadratic ligand depletion model (Equation 1).



Supplementary Figure 2: FP binding curves obtained with DM039 in the presence of (A) c-MYC, (B) single-stranded DNA, (C) double-stranded DNA, (D) h-Telo, (E) c-KIT1, (F) c-KIT2, (G) HRAS and (H) BCL-2. All curves were fitted using the quadratic ligand depletion model (Equation 1).

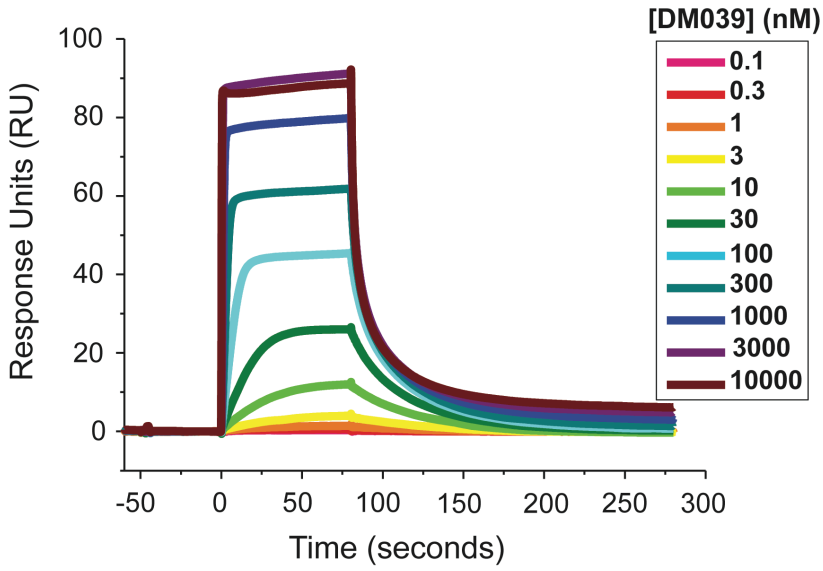


Supplementary Figure 3: Results of the FP-assay performed with DM039 on the MYC G4 sequence, in Li^+ (blue) and K^+ (black) buffers. Both the curves were fitted using the quadratic ligand depletion model (Equation 1).



Supplementary Figure 4: CD spectra of **A**) DM039 and DM102 and **B,C)** DM039 and DM102 after incubation in the presence of c-MYC. Subtraction of MYC G4 signal was performed to allow detection of peptides conformational changes upon G4-binding. Experiments were performed in 10 mM Tris (pH

= 7.4) supplemented with 100 mM KCl at 5 µM DM039, DM102 and c-MYC. Spectra are plotted as the average of 3 independent repeats.



Supplementary Figure 5: SPR sensorgrams related to the interaction of DM039 with the MYC G4, where the ligand concentration was varied from 0.1 nM to 10 µM.

Supplementary References

1 N. J. Moerke, *Curr. Protoc. Chem. Biol.*, 2009, **1**, 1–15.

2 H. Prinz, *J. Chem. Biol.*, 2010, **3**, 37–44.

3 C. M. Grison, G. M. Burslem, J. A. Miles, L. K. A. Pilsl, D. J. Yeo, Z. Imani, S. L. Warriner, M. E. Webb and A. J. Wilson, *Chem. Sci.*, , DOI:10.1039/c7sc01342f.