

Supporting Information

Tuning Coordination Chemistry Through the Second Sphere in Designed Metallocoiled Coils – A New Role for Tryptophan

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Materials and Methods, one Supplementary Table and 12 Supplementary Figures.

1. Materials and Methods

Materials: Chemicals were used as received and purchased from; Sigma Aldrich (diethyl ether, gadolinium chloride hexahydrate, terbium chloride hexahydrate, Pepceuticals (Fmoc protected amino acids, HBTU (O-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluorophosphate), synthesis grade DMF (dimethylformamide) and 20% piperidine in DMF premix), AGTC Bioproducts Ltd. (rink amide MBHA resin, NMP (N-methyl-2-pyrrolidone), DIEA (N,N-diisopropylethylamine) and DCM (dichloromethane)), Acros Organics (xylenol orange sodium salt, acetic anhydride, TIPS (triisopropylsilane) and TFA (trifluoroacetic acid)), Rathburn Chemicals Ltd. (>99.9% DMF) and Fisher Scientific Ltd. (HPLC (high pressure liquid chromatography) grade water and acetonitrile, HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), urea, EDTA (ethylenediaminetetraacetic acid) and glacial acetic acid).

Peptide Synthesis, Characterisation and Purification: The peptides used in this work (see Table 1) were synthesized, purified and characterized as reported previously.^{S1}

Models: The cartoon models presented in Figures 1, 2 and S11 were generated in Pymol based on manipulation of PDB 2JGO,^{S2} through amino acid side chain mutagenesis.

UV-visible spectroscopy: UV-visible spectra were recorded on a Shimadzu 1800 UV Spectrophotometer, recorded in single beam mode with a medium scan speed, slit width of 1.0 nm, data interval of 1.0 nm and scan range 420-260 nm (Trp/Tyr), using a 1 cm pathlength, 700 μ L quartz cuvette.

Solution Preparation: All peptide sequences feature a single Trp/Tyr chromophore to allow for accurate concentration determination. The concentration of freshly prepared peptide stock solutions in MilliQ water, were deduced from the absorption of the single Trp residue at 280 nm ($\epsilon_{280} = 5690 \text{ M}^{-1} \text{ cm}^{-1}$) or Tyr residue at 274 nm ($\epsilon_{280} = 1405 \text{ M}^{-1} \text{ cm}^{-1}$) in 7 M aqueous

urea, performed in triplicate for accuracy and left for 10 mins prior to quantitative UV determination.

The concentrations of freshly prepared stock solutions of GdCl_3 and TbCl_3 (~1 mM) in MilliQ water, were determined in triplicate using a xylenol orange indicator and EDTA titration with Ln^{3+} standard solutions, following a procedure previously reported by Fedeli and co-workers.^{S3}

Circular Dichroism: CD spectra were recorded on a Jasco J-715 Spectropolarimeter in a 1 mm pathlength quartz cuvette for 30 μM peptide monomer solutions. The optical chamber was purged with nitrogen and kept under a nitrogen atmosphere throughout the duration of the experiments. 1 mM stock solutions of LnCl_3 (where Ln^{3+} is either Tb^{3+} or Gd^{3+}) were titrated into 30 μM peptide monomer in 10 mM HEPES buffer pH 7.0, as previously reported.^{S4} All solutions were left to equilibrate for 10 minutes before recording spectra, and the observed ellipticity converted into molar ellipticity, with the helical content calculated as the percentage folded, based on the theoretical maximum ellipticity as reported by Scholtz et al.^{S5}

Mass Spectrometry. Solutions of 10 mM ammonium acetate, 10 μM peptide monomer and 10 μM TbCl_3 were made up in a 95:5 water:methanol mixture. The solution was collected and sprayed at 1.5 kV with a gas pressure of 1.5 psi using an Advion Biosciences TriVersa NanoMate electrospray source into a Waters Synapt G2-S mass spectrometer. The backing pressure of the spectrometer was kept around 6 mbar and the trap and transfer collision energies were 4.8 and 4.1 eV, respectively.

Hydration state: Tb^{3+} lifetimes in D_2O and H_2O were determined for the $\text{Tb}(\text{Pep})_3$ complexes. Solutions containing 0.3 equivalents Tb^{3+} (10 μM) and 100 μM peptide monomer, in 10 mM HEPES buffer pH 7.0, were monitored using a μF Flashlamp light source (50 Hz) on the Edinburgh Instruments spectrofluorimeter, collecting over a 10 ms (H_2O) or 20 ms (D_2O) time range, with a lamp trigger delay of 0.1 ms. In the case of MB1-

4(37W) this was performed in the presence of 300 μM peptide so as to ensure complete Tb^{3+} complexation, as the bound Tb^{3+} emission is not sensitized by the Trp in position 37, and is in fact weaker than that for free Tb^{3+} being held at a defined distance of ca. 14-15 \AA from the Trp residue.

Data was fitted to both mono-exponential decay kinetics in the F900 software using the tailfit model, described by the mathematical expression, $R(t)$, where:

$$R(t) = B_1 e^{\left(\frac{-t}{\tau_1}\right)} + B_2 e^{\left(\frac{-t}{\tau_2}\right)}$$

The sample decay model is a function of the variable time, t , and kinetic parameters. B is the pre-exponential factor made up of both instrumental and sample parameters and τ is the characteristic lifetime, measuring the time it takes for the fluorescence to decay to level $1/e$ (ca. 37% its original value). From the observed lifetime the number of coordinated water molecules was determined using the Parker-Beeby equation.^{S6}

NMR Spectroscopy. MRI relaxivity data was collected and processed as previously reported.^{S1}

2. Figure S1:

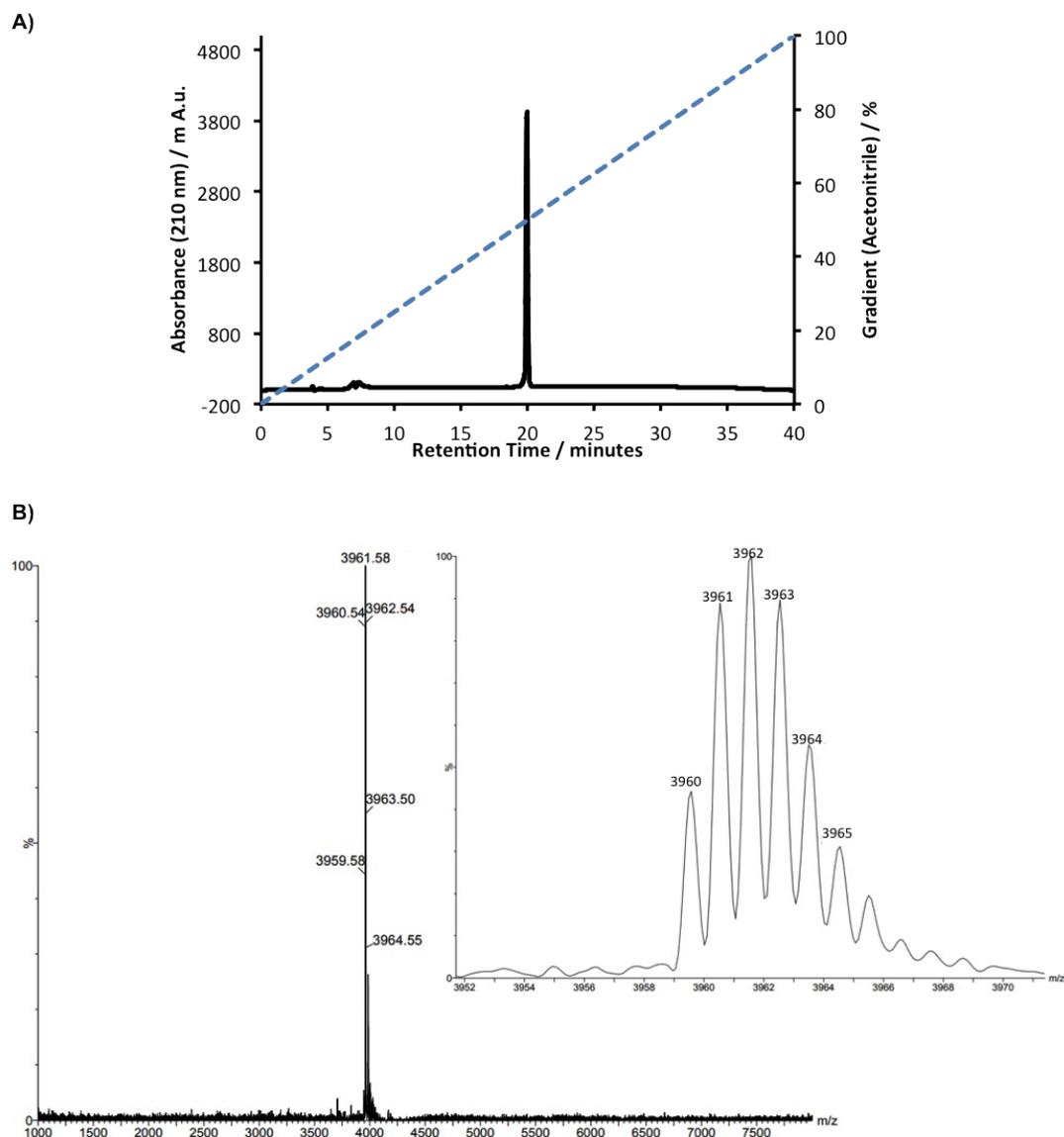
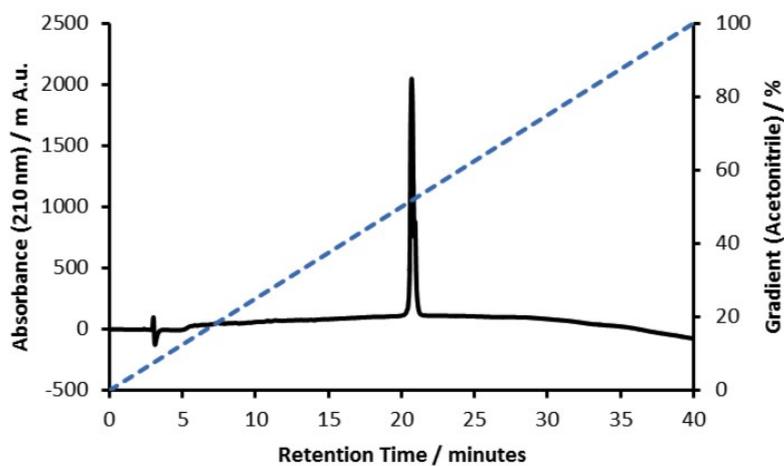


Figure S1. A) C18-analytical HPLC trace of purified MB1-1(2A) using a linear gradient from 0-100% acetonitrile in water with 0.05% TFA (blue dashed line) over 40 minutes. B) MALDI mass spectrum of purified MB1-1(2A) with inset showing the isotope distribution of the +1 charge peak.

3. Figure S2:

A)



B)

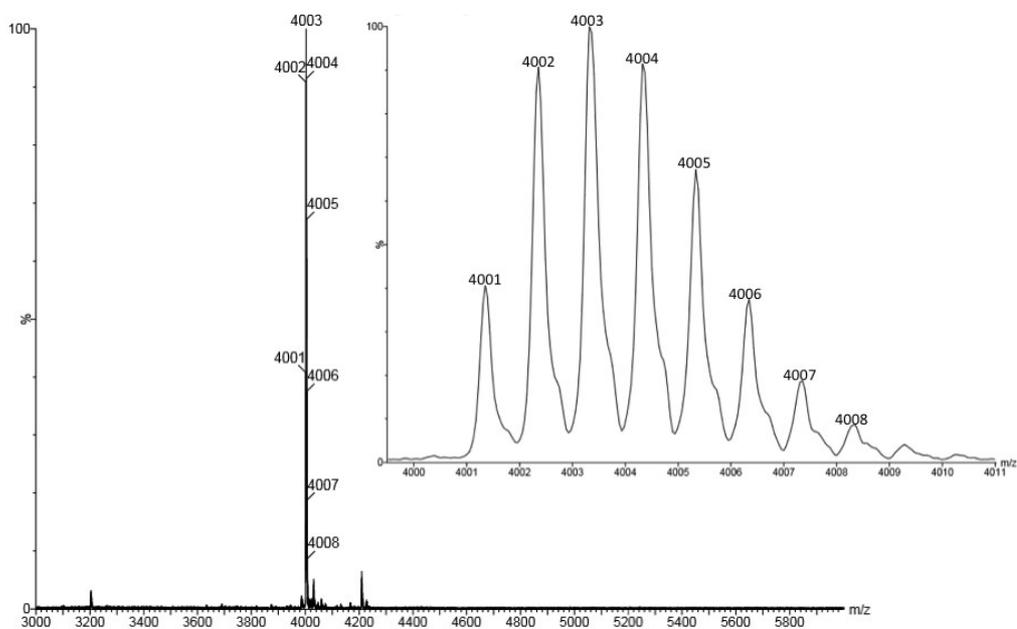
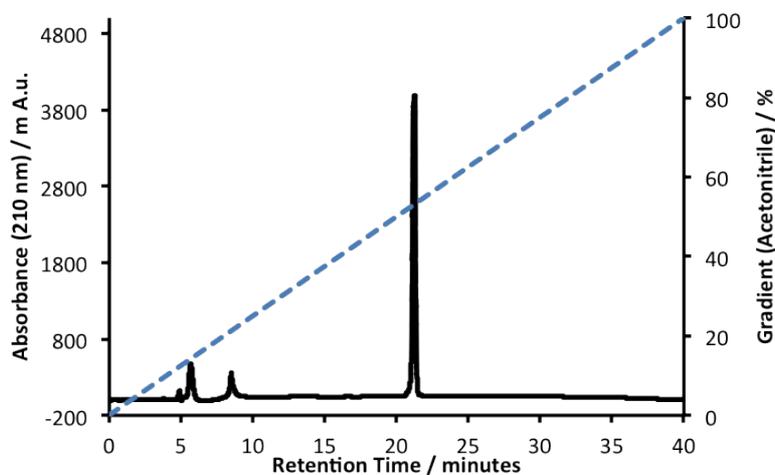


Figure S2. A) C18-analytical HPLC trace of purified MB1-1(2I) using a linear gradient from 0-100% acetonitrile in water with 0.05% TFA (blue dashed line) over 40 minutes. B) MALDI mass spectrum of purified MB1-1(2I) with inset showing the isotope distribution of the +1 charge peak.

4. Figure S3:

A)



B)

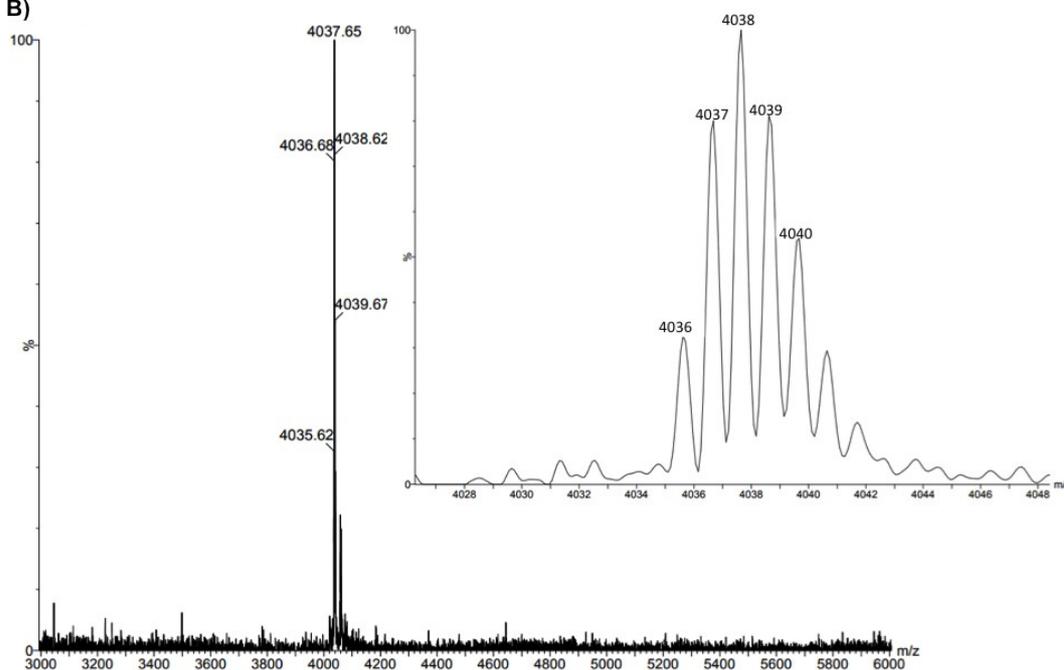
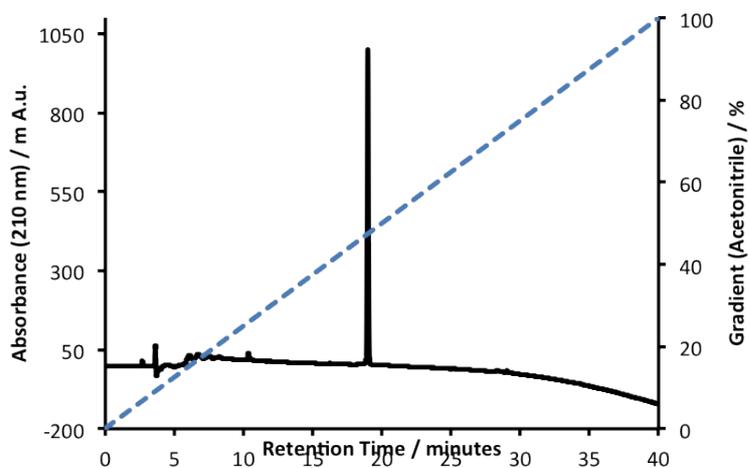


Figure S3. A) C18-analytical HPLC trace of purified MB1-1(2F) using a linear gradient from 0-100% acetonitrile in water with 0.05% TFA (blue dashed line) over 40 minutes. B) MALDI mass spectrum of purified MB1-1(2F) with inset showing the isotope distribution of the +1 charge peak.

5. Figure S4:

A)



B)

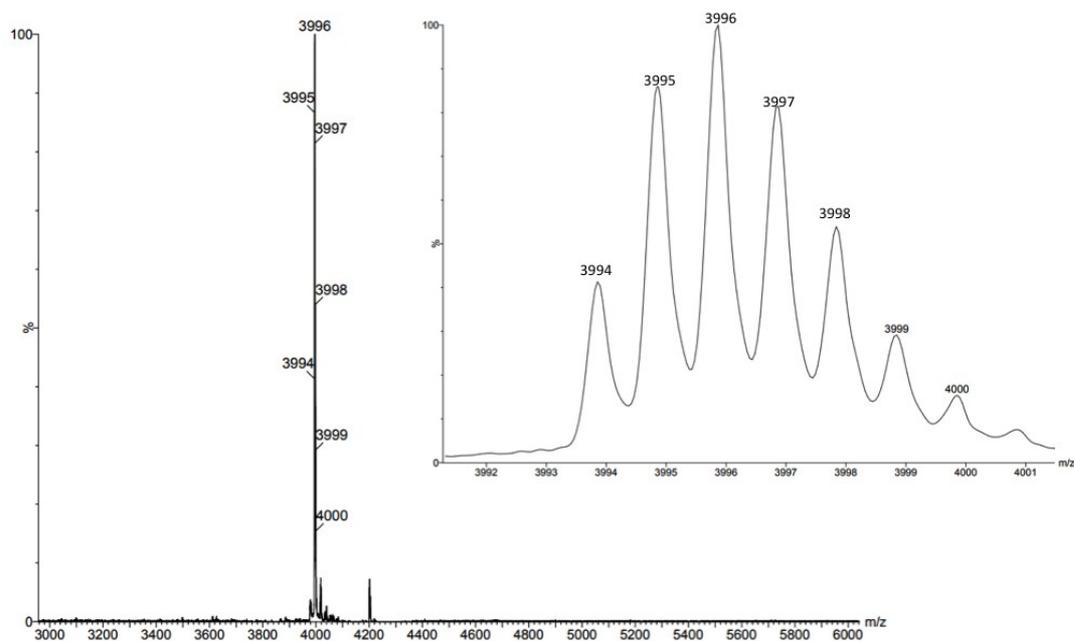
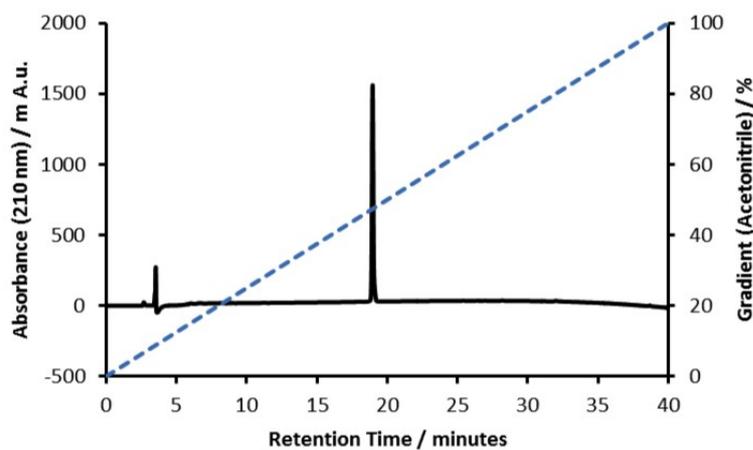


Figure S4. A) C18-analytical HPLC trace of purified MB1-1(2Y) using a linear gradient from 0-100% acetonitrile in water with 0.05% TFA (blue dashed line) over 40 minutes. B) MALDI mass spectrum of purified MB1-1(2Y) with inset showing the isotope distribution of the +1 charge peak.

6. Figure S5:

A)



B)

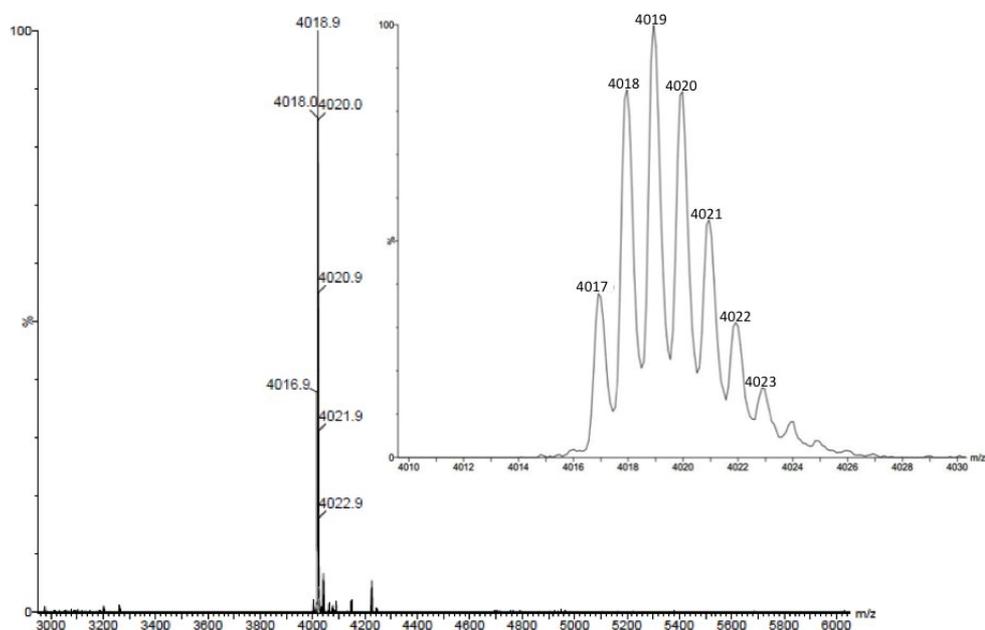
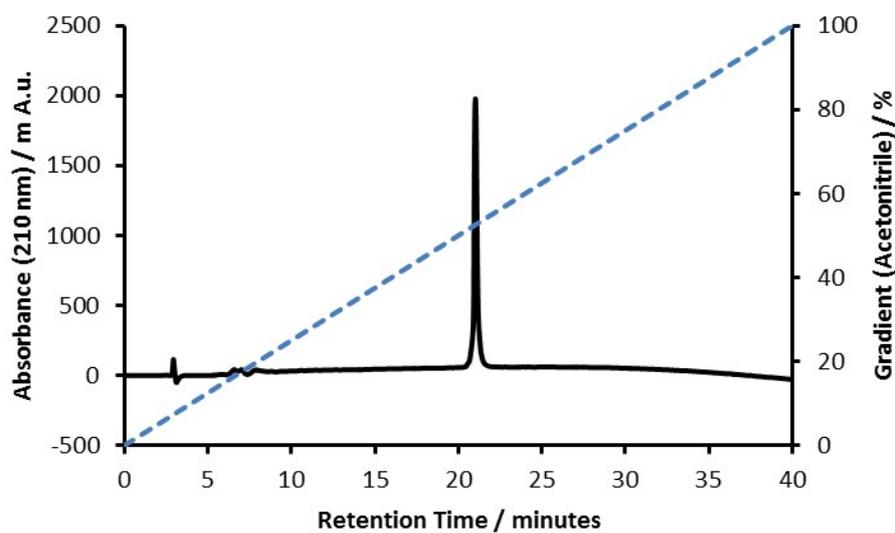


Figure S5. A) C18-analytical HPLC trace of purified MB1-1(2W) using a linear gradient from 0-100% acetonitrile in water with 0.05% TFA (blue dashed line) over 40 minutes. B) MALDI mass spectrum of purified MB1-1(2W) with inset showing the isotope distribution of the +1 charge peak.

7. Figure S6:

A)



B)

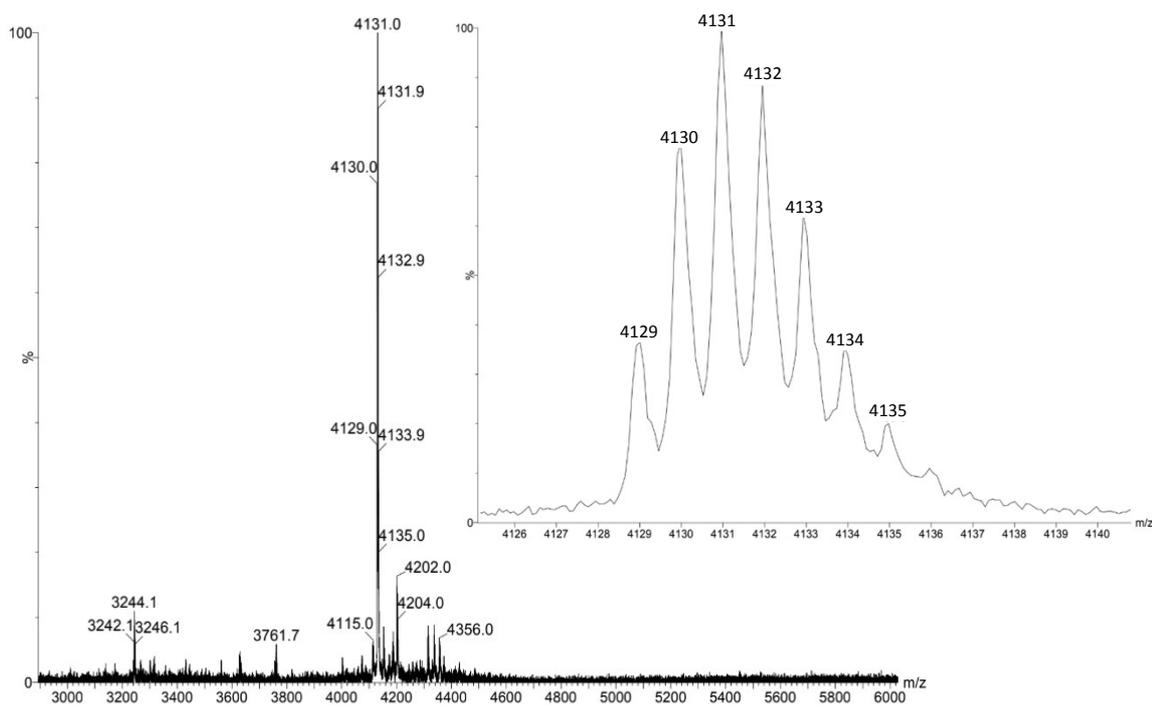


Figure S6. A) C18-analytical HPLC trace of purified MB1-4(37W) using a linear gradient from 0-100% acetonitrile in water with 0.05% TFA (blue dashed line) over 40 minutes. B) MALDI mass spectrum of purified MB1-4(37W) with inset showing the isotope distribution of the +1 charge peak.

8. Figure S7:

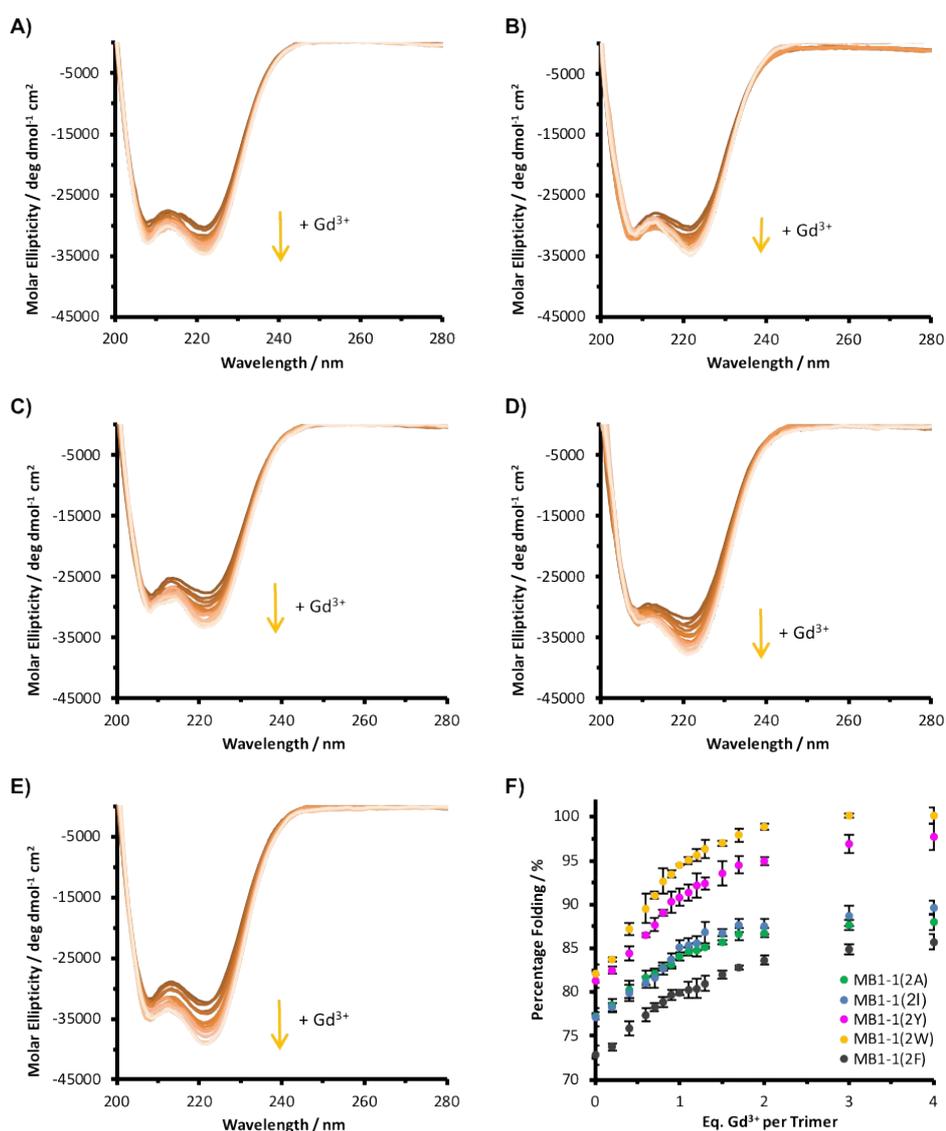


Figure S7. Representative Gd³⁺ binding titrations monitored by CD of 30 μM peptide monomer solutions of A) MB1-1(2A), B) MB1-1, C) MB1-1(2F), D) MB1-1(2Y) and E) MB1-1(2W) in 10 mM HEPES buffer pH 7.0. F) Plot of percentage folded, based on molar ellipticity at 222 nm as a function of Gd³⁺ equivalents per trimer, of 30 μM peptide monomer solutions of MB1-1(2A) (green), MB1-1(2I) (blue), MB1-1(2F) (black), MB1-1(2Y) (pink) and MB1-1(2W) (yellow) in 10 mM HEPES buffer pH 7.0. Data is a result of the average of three repeats where the error bars represent the standard deviation.

9. Figure S8:

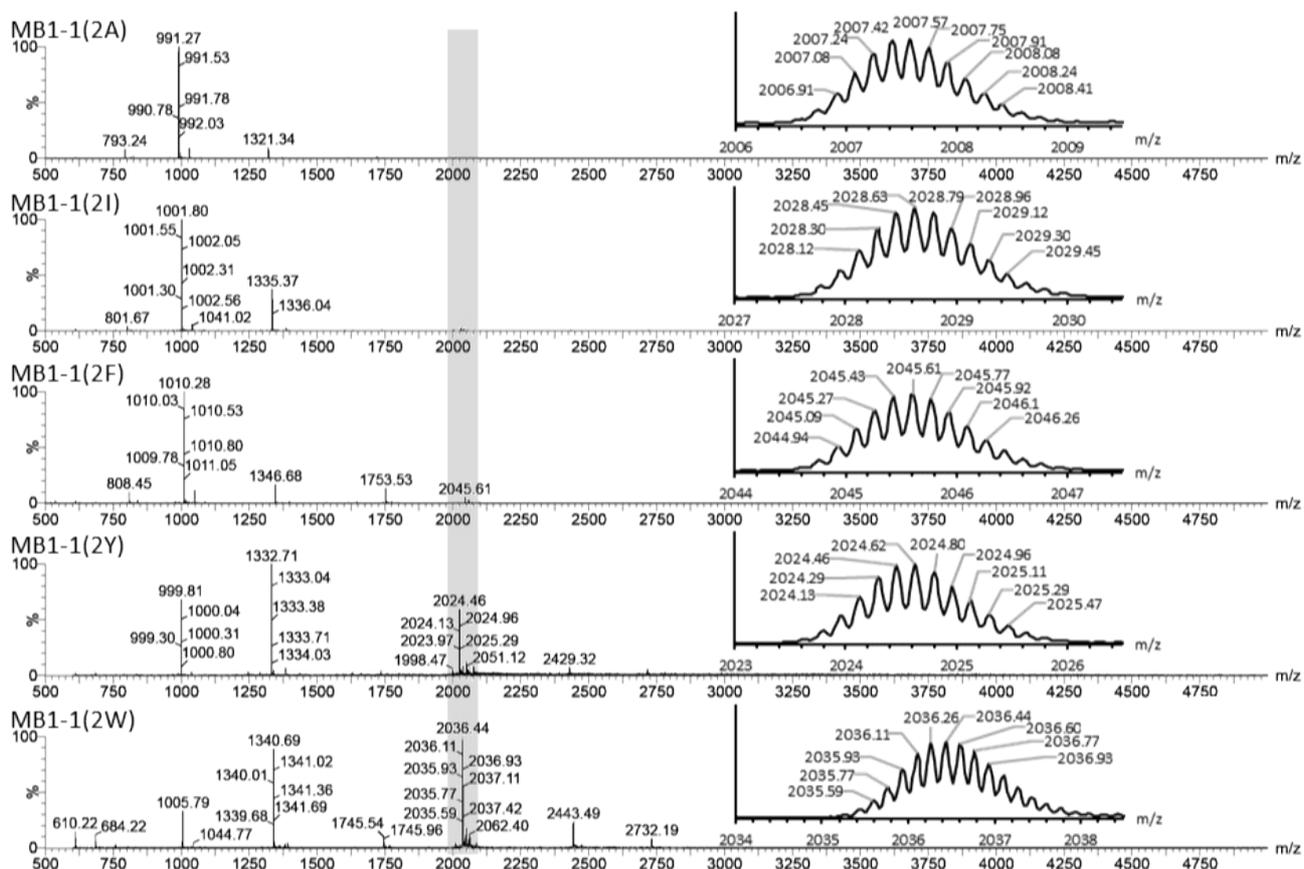


Figure S8. Electrospray ionisation mass spectra of 10 µM peptide monomer in the presence of 10 µM TbCl₃, in 10 mM ammonium acetate buffer made up in a 95:5 water:methanol mixture, where peptide is A) MB1-1(2A), B) MB1-1(2I), C) MB1-1(2F), D) MB1-1(2Y) and E) MB1-1(2W). Though the main species detected corresponds to the non metallated peptide monomer, the intact Tb(Pep)₃ trimer species could be detected. A zoom-in of the +6 peak is shown and highlighted in grey.

10. Figure S9:

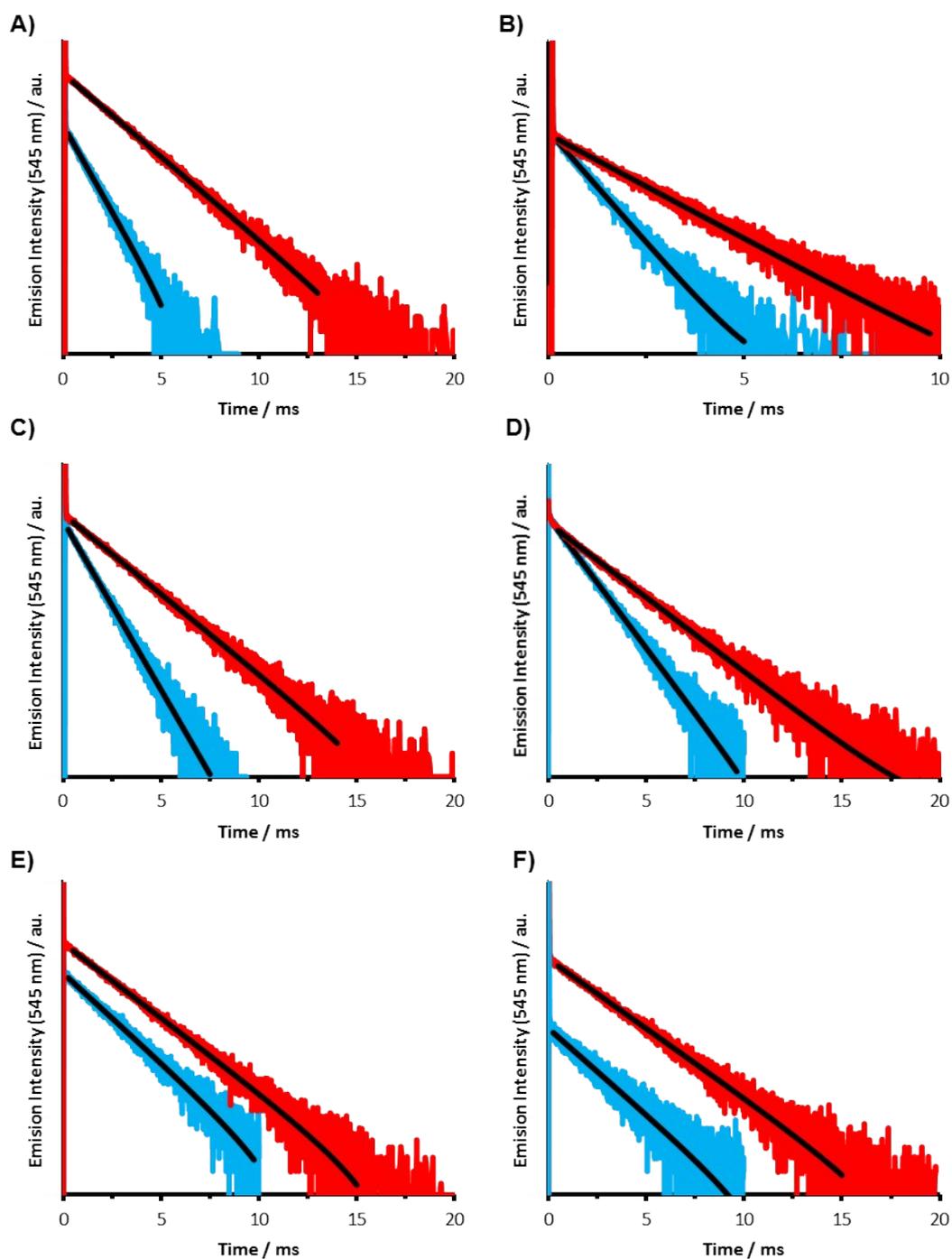


Figure S9. Representative decay profiles for Tb^{3+} emission at 545 nm, recorded in H_2O (blue) and D_2O (red), in the presence of 0.3 equivalents Tb^{3+} per peptide trimer for 100 μM (or 300 μM in the case of MB1-4(37W)) peptide monomer solutions of A) MB1-1(2A), B) MB1-1(2I), C) MB1-1(2F), D) MB1-1(2Y), E) MB1-1(2W) and F) MB1-4(37W) in 10 mM HEPES buffer pH 7.0. Lifetimes are fitted using mono-exponential decays (black line).

11. Table S1 The average number of water molecules coordinated to 0.3 equivalents Tb^{3+} per peptide trimer, for 100 μ M peptide monomer solutions in 10 mM HEPES buffer pH 7.0. where the error represents the standard deviation, based on three repeats.

Repeat	Peptides					
	MB1-1(2A)	MB1-1(2I)*	MB1-1(2F)	MB1-1(2Y)	MB1-1(2W)	MB1-4(37W)
1	3.3	3.3	2.3	1.3	0.1	-0.1
2	2.8	3.1	2.1	1.3	0.2	0.2
3	2.8	2.8	1.9	1.4	0.2	0.2
Average	3.0 ± 0.3	3.1 ± 0.2	2.1 ± 0.2	1.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.2

* Data from reference S1

12. Figure S10:

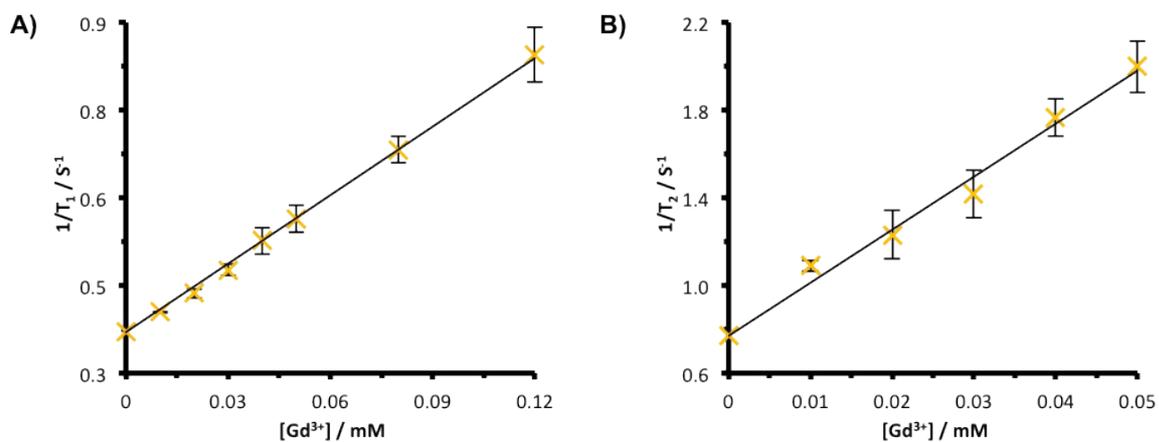


Figure S10. A) T_1 and B) T_2 relaxivity plots, showing reciprocal of relaxation time as a function of Gd^{3+} concentration for relaxation rates of MB1-1(2W) in the presence of 0.5 equivalents Gd^{3+} per peptide trimer. Samples recorded at 293 K in the presence of 10 mM HEPES buffer pH 7.0. Data represents the average of three repeats with error bars are the standard deviation.

13. Figure S11:

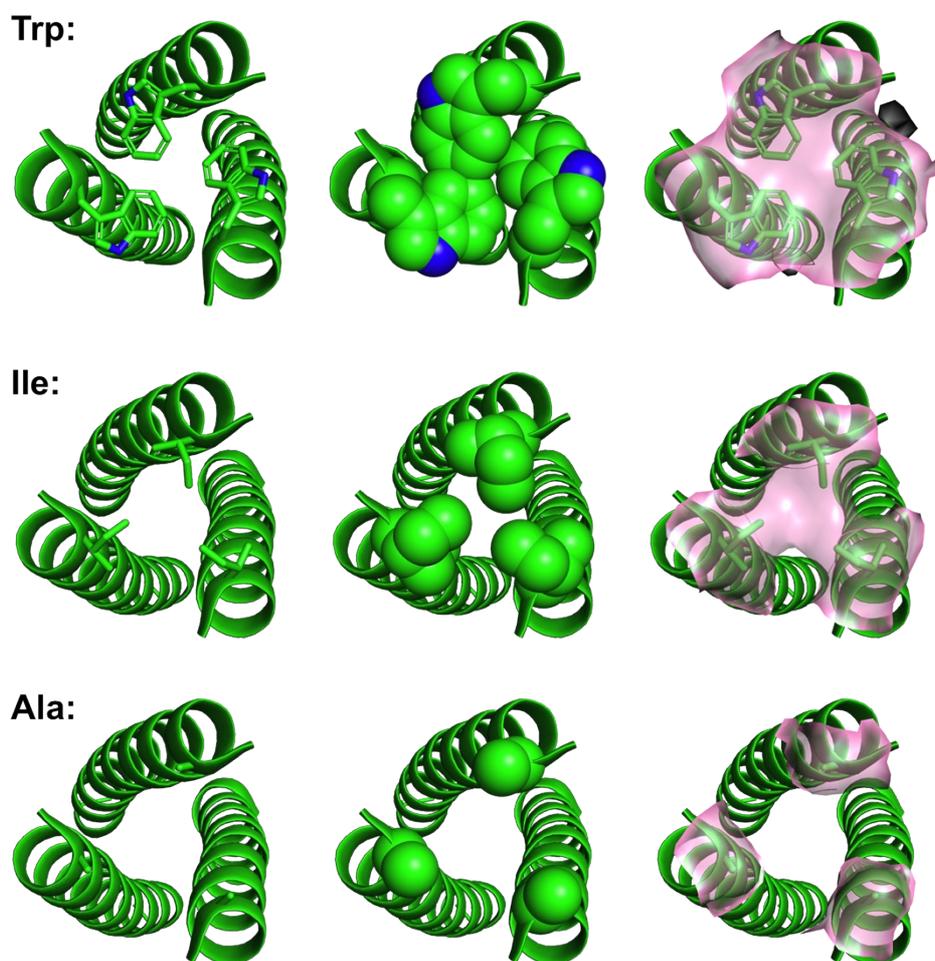


Figure S11. Models of MB1-1(2X) where X = Trp, Ile and Ala, generated based on PDB 2JGO,^{S2} a crystal structure of a three stranded coiled coil that features a Trp in position 2 (*a* site of the first heptad). Pymol representation showing the main chain atoms as green ribbons, and the side chains in position 2 (Trp, Ile or Ala) in either stick form (left), as spheres (center), or with the surface shown in pink (right). In the crystal structure one Trp is present in two conformations (equal occupancy), but in the model above only one of these is shown. The models presented are consistent with a well-packed Trp layer, which appears to block the full face of the N-terminus. In contrast, the Ala layer, and to a lesser extent the Ile layer, appear to generate a less efficiently packed and therefore more solvent penetrable layer.

14. Figure S12:

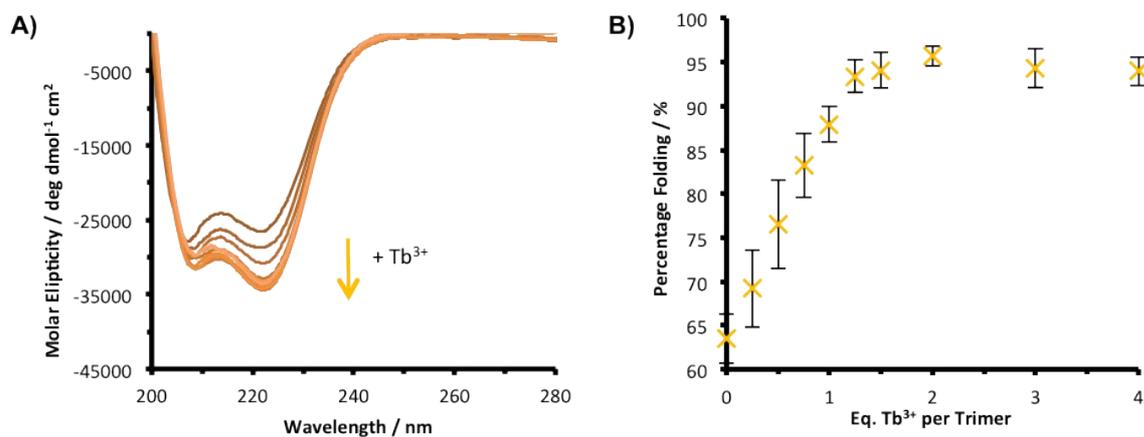


Figure S12. A) Representative Tb³⁺ binding titration monitored by CD of 30 μ M Mb1-4(37W) peptide monomer in 10 mM HEPES buffer pH 7.0. B) Plot of percentage folded, based on molar ellipticity at 222 nm as a function of Tb³⁺ equivalents per trimer. Data is a result of the average of three repeats where the error bars represent the standard deviation.

15. Supporting Information References

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