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Understanding and Controlling the Metal-Directed Assembly of Terpyridine-Functionalized Coiled-Coil Peptides

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SUPPORTING INFORMATION

Experimental Methods

Synthesis of ligand 1. To 4'-carboxy-2,2':6',2"-terpyridine (279.7 mg, 1.009 mmol), prepared as previously described,[1] was added thionyl chloride (18 mL), and the resulting solution was refluxed for 3 hours. Solvent was removed to dryness by vacuum distillation to afford the acid chloride intermediate, which was taken forward without further purification. To the acid chloride, a solution of excess ethanolamine (7 mL) in dry dichloromethane (7 mL) was added dropwise at 0 °C under nitrogen. The reaction was stirred overnight under nitrogen at room temperature. The reaction was precipitated into water (50 mL) and extracted three times with dichloromethane (50 mL each). The organic layers were combined and concentrated by vacuum distillation. The resulting solid was re-suspended in minimal ethanol and precipitated with water (100 mL). The precipitate was filtered and dried under high vacuum to obtain the product as a white solid (219.6 mg, 68% yield). ^{1}HNMR (500 MHz, DMSO-d_6) δ 9.06 (t, J = 5.4 Hz, 1H), 8.84 (s, 2H), 8.77 (d, J = 4.2 Hz, 2H), 8.65 (d, J = 8 Hz, 2H), 8.04 (dt, J = 7.8, 1.6 Hz, 2H), 7.54 (dd, J = 7.0, 5.4 Hz), 4.80 (t, J = 5.6 Hz, 1H), 3.58 (q, J = 5.9 Hz, 2H), 3.41 (q, J = 5.9 Hz, 2H); ^{13}CNMR (125 MHz, DMSO-d_6) δ 164.8, 155.6, 154.6, 149.4, 144.2, 137.6, 124.7, 120.9, 118.3, 59.5, 42.4; HRMS (ESI) m/z calculated for $C_{18}H_{17}N_4O_2$ $[M+H]^+$: 321.1352; found 321.1351.

Synthesis of peptides 2 and S1. Peptides **2** and **S1** were synthesized by manual microwave-assisted Fmoc solid phase methods using a CEM MARS microwave on NovaPEG Rink Amide resin. Coupling reactions were performed with a 1.5 min ramp to 90 °C followed by a 2 min hold at that temperature, and Fmoc deprotections performed with a 1.5 min ramp to 90 °C followed by a 1 min hold at that temperature.[2] Coupling solutions were composed of Fmoc-protected amino acid (5 equiv. relative to resin), HCTU (4.9 equiv.), and DIEA (7.5 equiv.) in NMP. Fmoc deprotections used 20% v/v 4-methylpiperidine in DMF. The resin was washed three times with DMF between each reaction. Full-length peptide was acetyl capped by treatment with DMF/DIEA/Ac_2O (8/2/1, v/v/v) at room temperature for 20 mins. The terpyridine-modified side chain was introduced by coupling Fmoc-Dab(Alloc)-OH (for peptide **2**) or Fmoc-Lys(Alloc)-OH (for peptide **S1**) at the corresponding site during synthesis of the full-length chain and subsequent on-resin conversion of the Alloc-protected amine as previously published.[3] Peptide was cleaved from the resin by treatment with TFA/H_2O/EDT/TIS (93/3/3/1 by volume) for ~4 h. Peptides were precipitated from the cleavage solution into cold Et_2O, centrifuged and decanted to obtain the crude peptide. Peptides were purified by reverse phase HPLC on gradients between water and acetonitrile with 0.1% TFA. Purity was confirmed by analytical HPLC (Figure S6) and identity was confirmed by MALDI-TOF mass spectrometry on an Applied Biosystems Voyager DE-Pro instrument (Figure S8, S11).

Synthesis of peptides 3 and 4. Peptides **3** and **4** were synthesized by manual microwave-assisted Fmoc solid phase methods using a CEM MARS microwave on NovaPEG Rink Amide resin. Coupling reactions were performed with a 2 min ramp to 70 °C followed by a 4 min hold at that temperature. Coupling reactions were composed of Fmoc-protected amino acid (5 equiv. relative to resin), HCTU (4 equiv.), and DIEA (6 equiv.) in NMP. Fmoc deprotections performed with a 2 min ramp to 90 °C followed by a 2 min hold at that temperature and 5 min cooldown. Fmoc deprotections were performed with 20% v/v 4-methylpiperidine in DMF. The resin was washed three times with DMF between each reaction. Full-length peptide was acetyl capped by treatment with DMF/DIEA/Ac_2O (8/2/1, v/v/v) at room temperature for 20 min. The terpyridine-modified side chain was introduced by coupling Fmoc-Dab(Alloc)-OH at the corresponding site during synthesis of the full-length chain and subsequent on-resin conversion of the Alloc-protected amine, as previously published.[3] Peptides were cleaved from the resin by treatment with TFA/H_2O/EDT/anisole/TIS (90/3/3/3/1 by volume) for ~4 h. Crude peptide was precipitated from the cleavage solution into cold Et_2O, and the resulting mixture centrifuged and decanted. The pellet was dissolved in 30% acetonitrile/water with 1 M guanidinium chloride and purified by reverse phase HPLC on gradients between water and acetonitrile with 0.1% TFA. Purity was confirmed by analytical HPLC (Figure S6) and identity by MALDI-TOF mass spectrometry on a Bruker ultrafleXtreme instrument (Figure S9, S10).

Determination of molar absorptivity for ligand 1. A stock solution of **1** (905 μM) was prepared in H_2O/DMSO (4:1 v/v). Samples were prepared from this stock solution by dilution with H_2O to final ligand concentrations of 7.5, 15, 30, 60, and 90 μM. UV-vis spectra of each sample were acquired from 200-600 nm, and a linear fit of absorbance at 310 nm as a function of concentration yielded an ε_{310} of 8400 M^{-1} cm^{-1} (Figure S7). This value was used in the determination of the concentration of peptide stock solutions used in CD and EPR experiments.

EPR spectroscopy. Samples used in EPR experiments were 150 μL total volume with 20% v/v glycerol added as a cryoprotectant and were prepared in a 3 mm inner diameter quartz tube. The ligand concentration (small molecule or peptide) was 300 μM in 40 mM N-ethyl-morpholine (NEM) buffer at pH 7.4 with varying concentrations of Cu^{2+} (indicated in figure caption for each relevant experiment). The samples were flash frozen with liquid MAPP within one minute of addition of Cu^{2+}. Experiments were performed at either 80 K or 20 K using an Oxford ITC503 temperature controller and an Oxford CF935 dynamic continuous flow cryostat connected to an Oxford LLT 650 low-loss transfer tube.

Continuous wave (CW) experiments were performed on a Bruker ElexSys E580 CW/FT X-band spectrometer with a Bruker ER4118X-MD5 resonator. The CW spectra were collected consisting of 1024 data points using a center field of 3100 with a 2000 G sweep width. A modulation depth of 4 G, a modulation frequency of 100 kHz, a conversion time of 20.48 ms and a time constant of 10.24 ms were used. All spectra represent the average of 25 scans.

The two dimensional, four-pulse HYSCORE experiments were performed at X-band frequencies on either a Bruker ElexSys E580 CW/FT X-band spectrometer with a Bruker ER4118X-MD5 resonator or a Bruker ElexSys E680 CW/FT X-band spectrometer equipped with a Bruker EN4118X-MD4 resonator. The pulse sequence used was as follows: $\pi/2 - \tau - \pi/2 - \tau_1 - \pi - \tau_2 - \pi/2 -$ echo, where the $\pi/2$ and π pulse lengths were 16 ns and 32 ns respectively. τ_1 and τ_2 were both stepped out by 16 ns. All spectra were collected at the field corresponding to the maximum of the echo-detected field swept spectrum. Data acquisition was 16 hours. Raw data was baseline corrected, zero filled and fast Fourier transformed. The fast Fourier transformation was reported as a contour plot.

The dead time free four-pulse DEER experiment was performed at X-band frequencies on a Bruker ElexSys E680 CW/FT X-band spectrometer equipped with a Bruker EN4118X-MD4 resonator. All DEER experiments were performed at 20 K. The pulse sequence used was as follows: $(\pi/2)\nu_1 - \tau_1 - (\pi)\nu_1 - T - (\pi)\nu_2 - \tau_2 - (\pi)\nu_1 - \tau_2 -$ echo.[4] The observer pulse lengths, $(\pi/2)\nu_1$ and $(\pi)\nu_1$, were 16 ns and 32 ns respectively. The pump pulse length, $(\pi/2)\nu_2$, was 16 ns. The delay, T, was incremented by a step size of 20 ns for 128 points. The pump frequency, ν_2, was positioned at the maximum of the echo detected field swept Cu^{2+} spectrum. The observer frequency, ν_1, was offset 150 MHz downfield from the pump pulse. The raw time domain DEER data were analyzed via DeerAnalysis2013[5] using Tikhonov regularization. Data acquisition time ranged from 6 to 12 hours.

Circular dichroism (CD) spectroscopy. CD measurements were performed in 1 mm quartz cuvettes on an Olis DSM 17 CD spectropolarimeter. Samples contained 100 μM peptide in 10 mM HEPES pH 7. Scans were measured from 200-260 nm with 1 nm increments, 5 second integration and 2 nm bandwidth at 20 °C. Melts were monitored at 222 nm from 2-98 °C with 4 °C intervals equilibrated for 2 minutes at each temperature. Scan data were smoothed by the Savitzky Golay method and melting temperatures determined by fit to a two-state folding model[6] using GraphPad Prism.

X-Ray crystallography. Peptides **3** and **4** were crystallized by hanging drop vapor diffusion in 24-well plates. The crystallization drop was prepared by mixing stock solutions of 10 mg/mL peptide in water, crystallization buffer (details below), and 15 mM $CuCl_2$ in a 1:1:1 ratio. For peptide **3**, the well buffer consisted of 0.1 M Bis-Tris pH 6.5, 25% w/v PEG 3350; for peptide **4**, the well buffer consisted of 0.1 M Tris pH 8.5, 25% w/v PEG 3350. The drop prepared as described above was equilibrated over well buffer at room temperature. Crystals were harvested and flash frozen in liquid nitrogen. X-ray diffraction data were collected using Cu/Kα radiation on a Rigaku diffractometer (FR-E generator, VariMax optics) with a

Saturn 944 CCD detector. Data were processed using d*TREK and structures were solved by molecular replacement using Phaser. The search models employed were derived from the previously reported *de novo* designed trimeric coiled coil on which the designs of **3** and **4** were based (PDB 4DZL).[7] The search model was a trimer for peptide **3** and a single chain for peptide **4**. Refinement was performed using Phenix[8] and Coot.[9] Geometric restraints for the Cu^{2+}-bound terpyridine residue were based on a crystal structure of bis(2,2':6',2"-Terpyridyl)-copper(II) hexafluorophosphate (CSD entry 1108163),[10] and restraints for the Tpy-Cu^{2+}-carboxylate coordination were based on a crystal structure of μ_2-succinato-bis[aqua(2,2':6',2"-terpyridine)copper(II)] dinitrate dihydrate (CSD entry 769916).[11] Data collection and refinement statistics for all crystal forms are in Table S1. Coordinates and structure factors are deposited in the PDB under accession codes 6OLN (**3**) and 6OLO (**4**).

Table S1. X-ray data collection and refinement statistics for **3** and **4**.

Data Collection	Peptide **3**	Peptide **4**
Unit cell dimensions (Å, °)	$a = 48.8$, $b = 51.0$, $c = 79.3$ $\alpha = \beta = \gamma = 90$	$a = b = 39.7$, $c = 42.0$ $\alpha = \beta = 90$, $\gamma = 120$
Space group	$P22_12_1$	H3
Resolution (Å)	41.54–2.50 (2.59–2.50)	26.61–2.30 (2.38–2.30)
Total observations	26,729	5,748
Unique observations	7,260	1,094
Redundancy	3.7 (3.4)	5.3 (3.8)
Completeness (%)	99.9 (98.4)	100 (100)
I/σ	18.0 (11.6)	16.3 (2.4)
R_{merge} (%)	5.7 (8.1)	7.0 (36.9)
Refinement		
Resolution (Å)	41.54–2.50	19.87–2.30
R (%)	27.1	25.9
R_{free} (%)	29.5	27.3
Avg. B factor (Å^2)	30.2	46.9
RMSD		
Bonds (Å)	0.004	0.004
Angles (°)	0.88	0.97

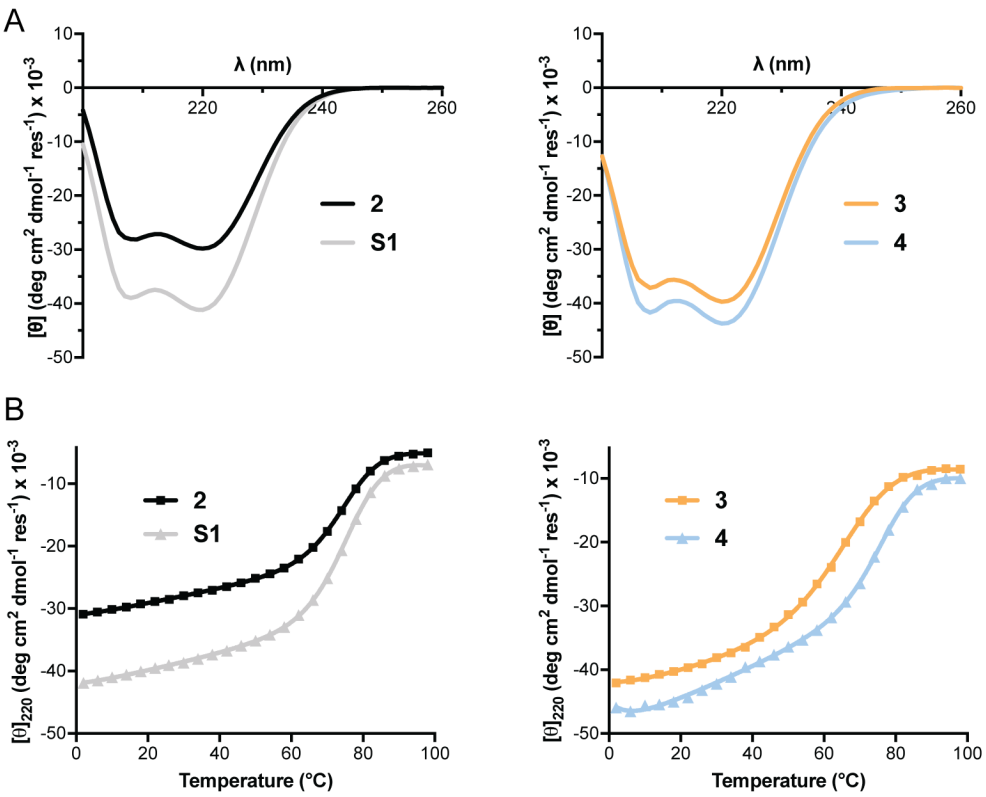

Figure S1 CD scans (A) and thermal melts (B) for peptides **2**-**4** and **S1**.

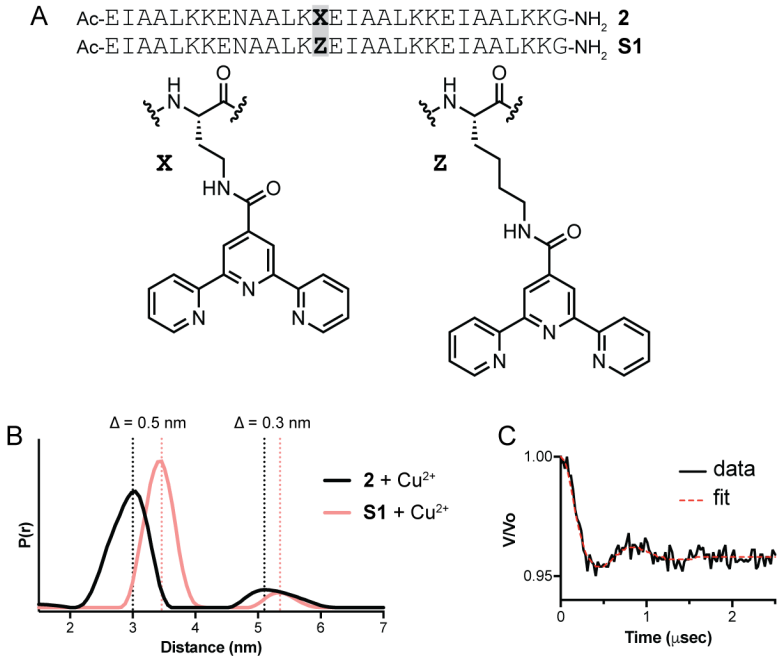

Figure S2 (A) Sequence of trimeric coiled-coil peptides **2** and **S1**, which differ only in the length of the Tpy-functionalized side chain (residue **X** in **2** vs. residue **Z** in **S1**). (B) Comparison of DEER distance distributions observed for **2** and **S1** in the presence of 0.5 equiv. Cu^{2+}. (C) Baseline corrected DEER signal and fit for peptide **S1**.

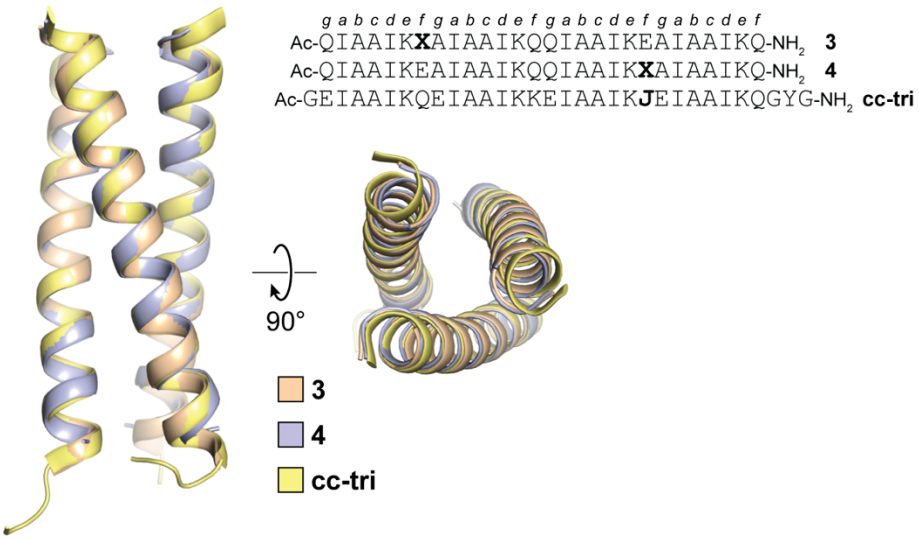

Figure S3 Sequence and structural alignment of trimeric coiled coil peptides **3** and **4** with the *de novo* designed sequence **cc-tri** (PDB 4DZL)[7] that served as the blueprint for their design. **X** is the Tpy-functionalized residue described in the main text; **J** is 4-iodo-Phe.

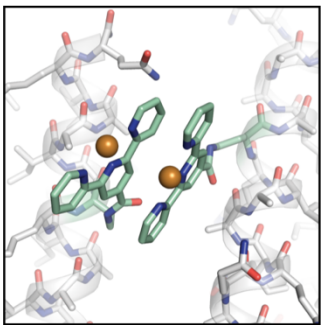

Figure S4 Representative π-stacking of **X**-residue Tpy moieties at an interface between adjacent coiled coils in the crystal structure of peptide **3**.

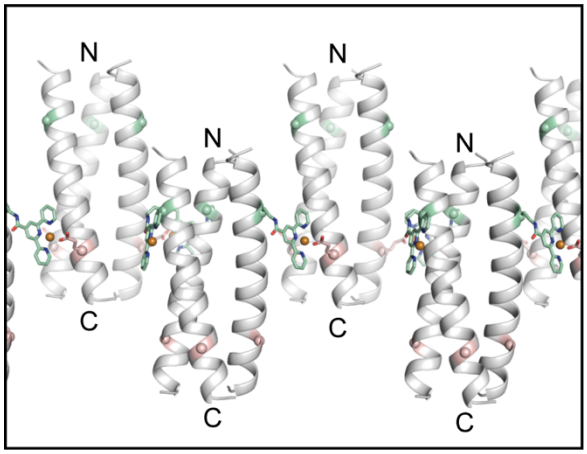

Figure S5 Parallel relative orientation of trimeric coiled coils in a single layer from the crystal structure of peptide **4** (*ab* plane, viewed from side).

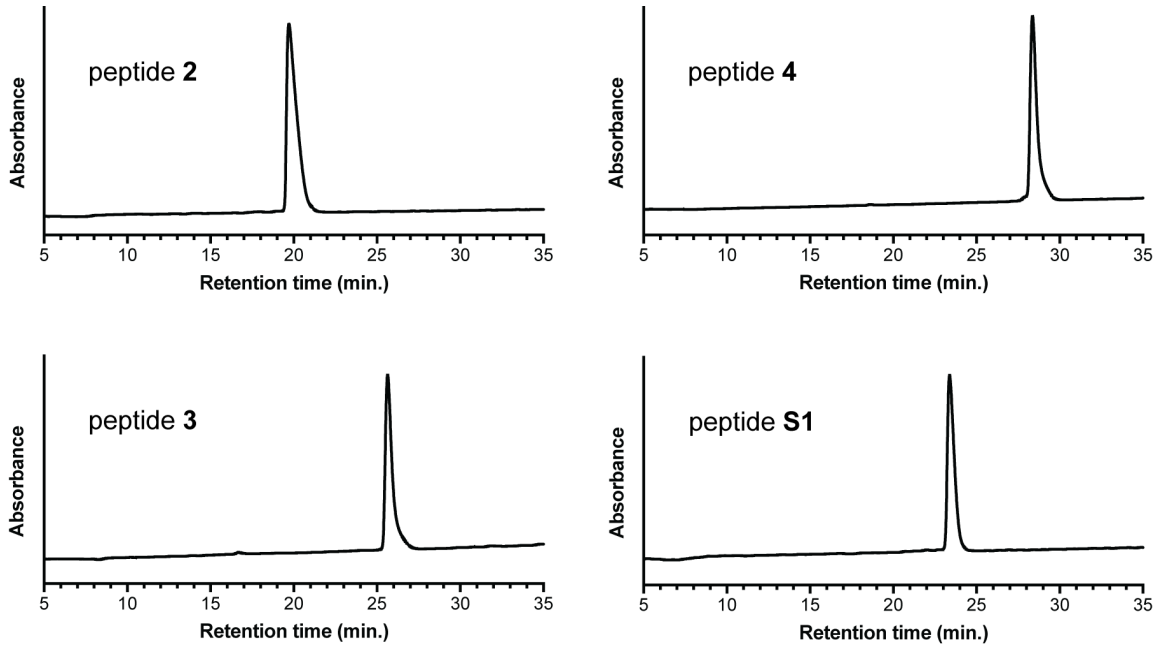

Figure S6 Analytical HPLC chromatograms for purified peptides **2**-**4** and **S1**.

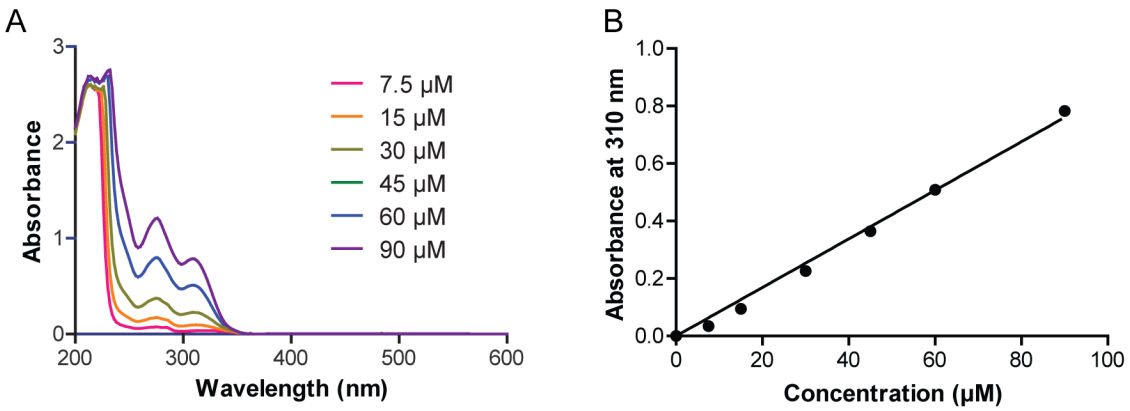

Figure S7 Determination of molar absorptivity of ligand **1**. (A) UV-vis spectra at varying concentrations; (B) linear regression of A_{310} as a function of concentration.

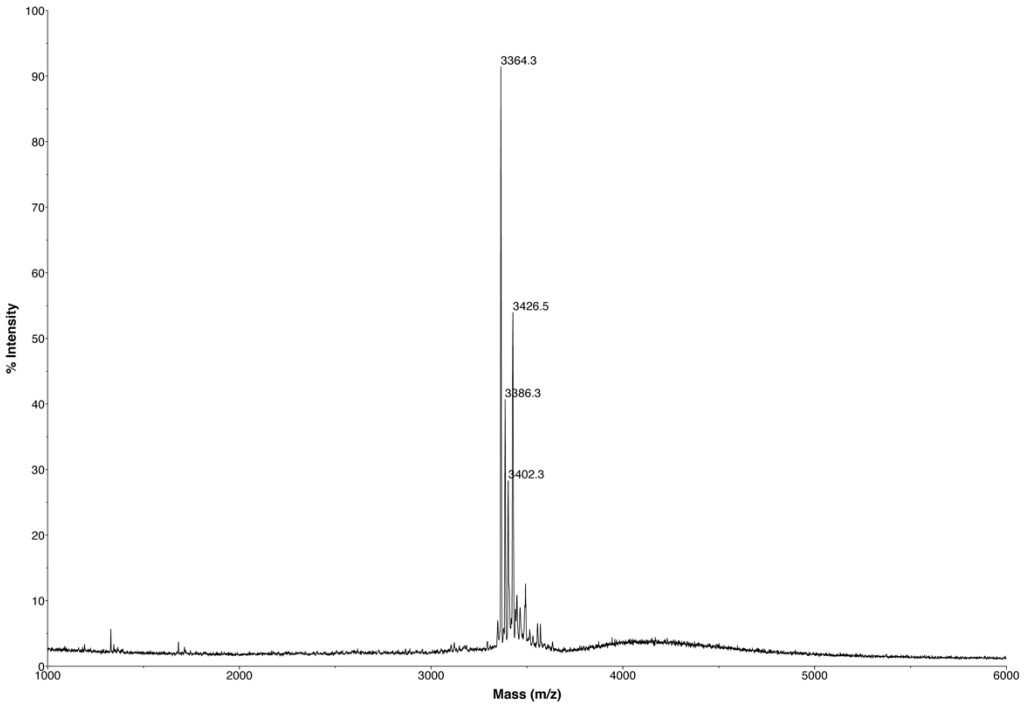

Figure S8 MALDI-MS spectrum of peptide **2**; $[M+H]^+$ (*m*/*z*) calc = 3365.0 (avg).

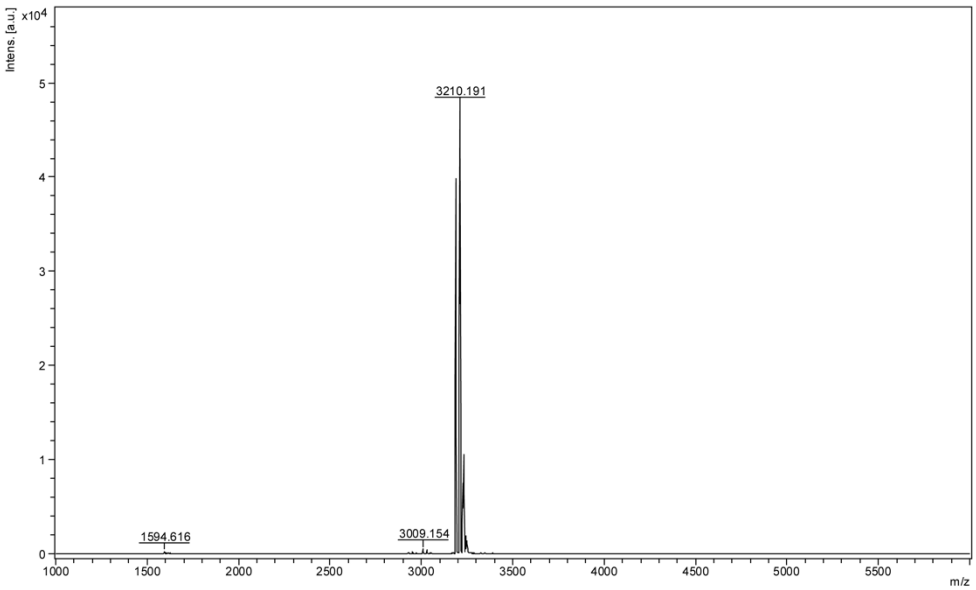

Figure S9 MALDI-MS spectrum of peptide **3**; $[M+Na]^+$ (*m*/*z*) calc = 3209.9 (monoisotopic).

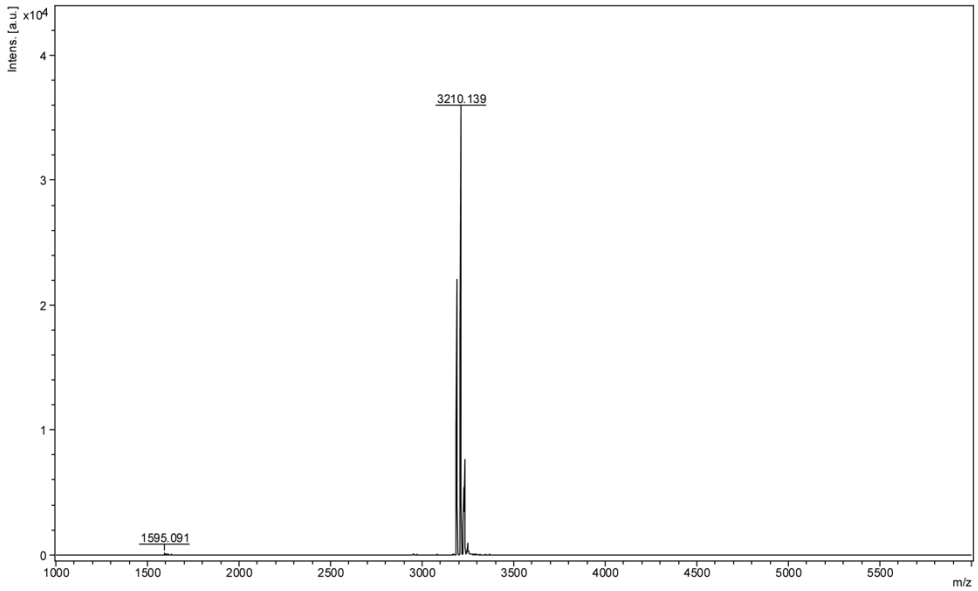

Figure S10 MALDI-MS spectrum of peptide **3**; $[M+Na]^+$ (*m*/*z*) calc = 3209.9 (monoisotopic).

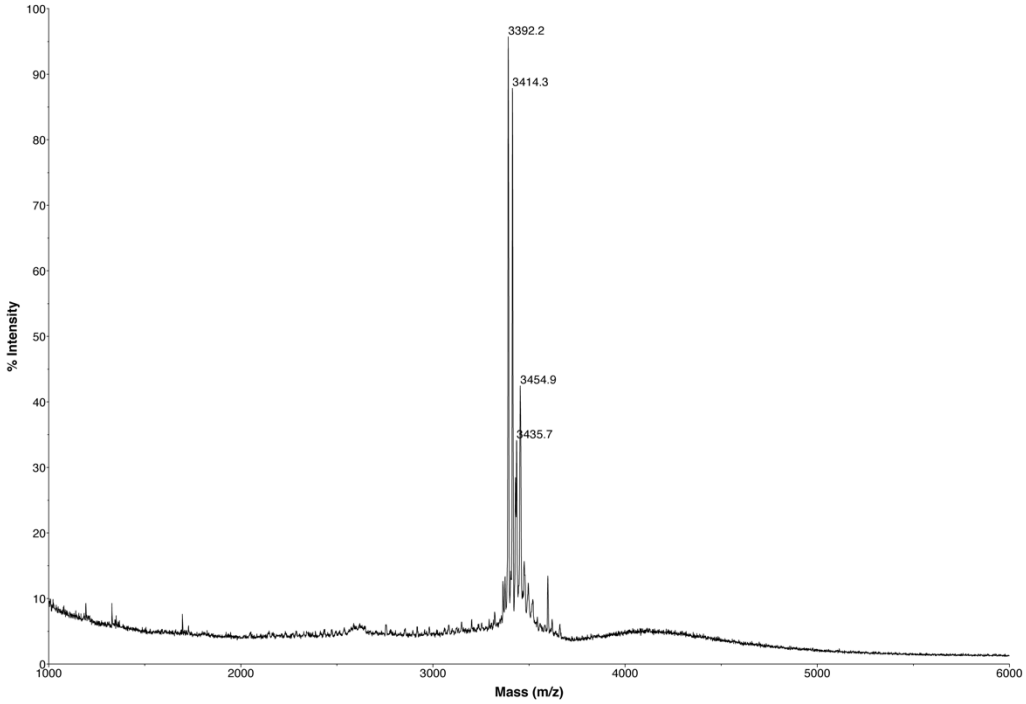

Figure S11 MALDI-MS spectrum of peptide **S1**; $[M+H]^+$ (*m*/*z*) calc = 3393.1 (avg).

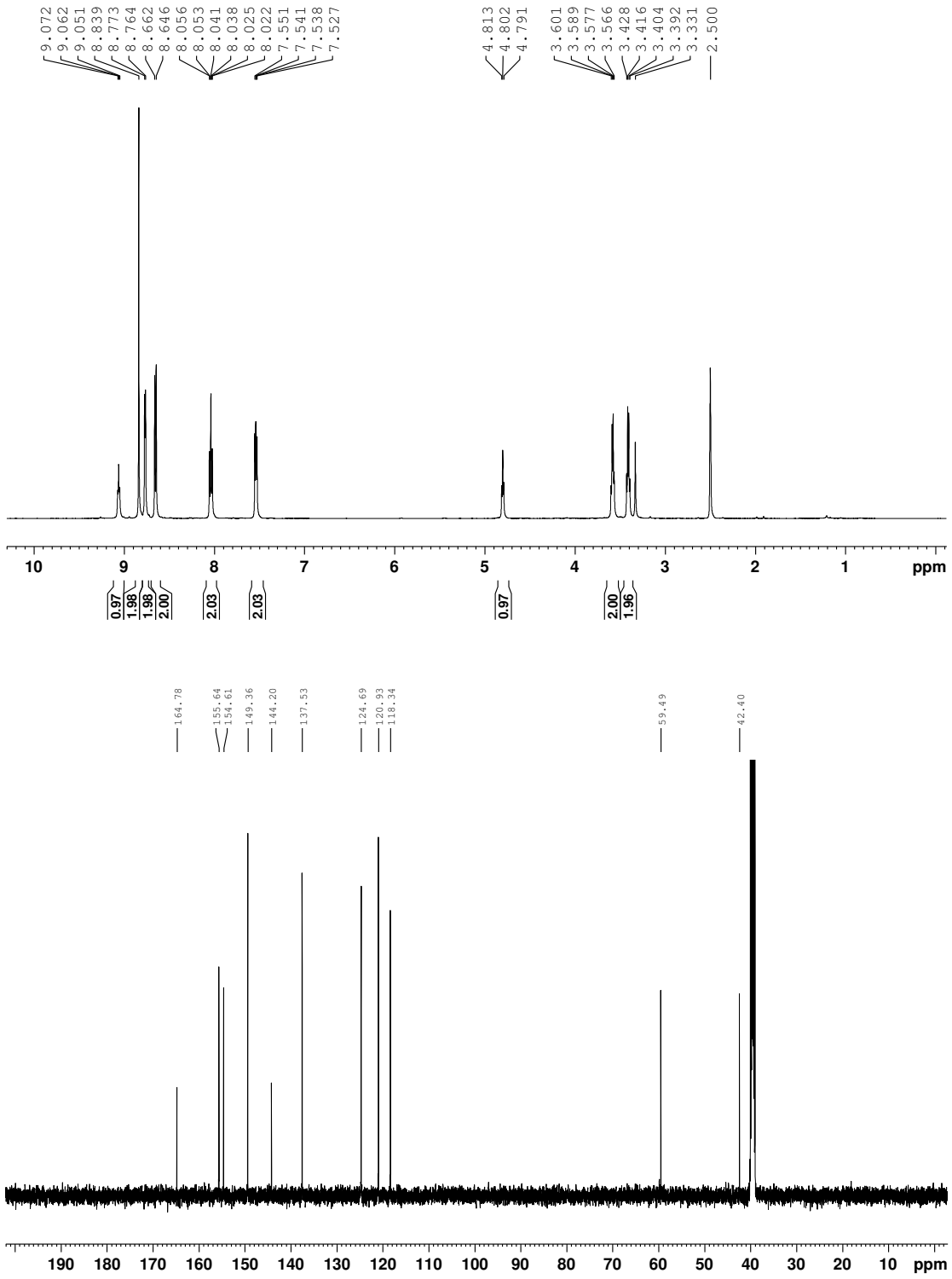

Figure S12 ^{1}H and ^{13}CNMR spectra of ligand **1** in DMSO-d_6.

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