Supporting Information

Controlling Self-assembly of a Peptide-Based Material via Metal-Ion Induced Registry Shift

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Contents

Materials. Chemical reagents were purchased from Fisher Scientific, Inc. (Pittsburgh, PA) or Sigma Chemical Co. (St. Louis, MO), unless otherwise noted. Isotopically enriched $^{113}CdCl_2$ (enrichment level: 93.35%) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). Fmoc-amino acid derivatives for solid-phase synthesis were purchased from AnaSpec Inc. (Fremont, CA).

Peptide Synthesis

Peptide synthesis was performed using microwave-assistance on a CEM Liberty solid-phase peptide synthesis instrument. Peptides were synthesized on a 4-(hydroxymethyl)phenoxyacetamido-methyl]-PEG-PS resin from Applied Biosystems, Inc. (Foster City, CA). Standard Fmoc protection chemistry was employed with coupling cycles based on HBTU/DIEA-mediated activation protocols and base-induced deprotection (20% piperidine in DMF with 0.1 M HOBt) of the Fmoc group. The peptides were purified via RP-HPLC on a C18 column with a gradient of water–acetonitrile (0.1% trifluoroacetic acid). The purity was assessed to be above 95% by analytical HPLC. Peptide mass was confirmed using electro-spray ionization mass spectrometry. The peptides were lyophilized, sealed, and stored at -20 °C.

Circular Dichroism Spectropolarimetry. CD spectra were recorded on a Jasco J-810 CD spectropolarimeter in 0.10 mm quartz cells (Hellma Analytics) at a concentration of 100 µM of **TZ1C2** in 10 mM TAPS buffer pH 8.5 with 100 mM NaCl. Spectra were recorded from 190 to 260 nm at a scanning rate of 100 nm/min and a resolution of 0.5 nm. The peptide concentration was determined spectroscopically by measuring the absorbance at 280 nm (A_{280}).[1] For peptides containing Tyr, Trp or Cys residues, the peptide concentration can be calculated from the following equation:

$$MW \times {}^{A_{280}}/_{c} = 1280n_Y + 5690n_W + 120n_C$$

in which c is the concentration of peptide in mg/mL, and n_Y, n_W, and n_C are the numbers of tyrosine, tryptophan and cystine residues, respectively, in the peptide sequence. As **TZ1C2** contains only a single tyrosine residue per molecule, then c = $MW \times A_{280}/1280$. To eliminate error in determination of absorbance that could arise as a result of UV light scattering due to peptide self-assembly, aqueous solutions of peptide were mixed with 6 M guanidinium chloride in 1:9 v/v ratio to completely denature the sample prior to performing the absorbance measurements.

Linear Dichroism Spectroscopy. Linear Dichroism was recorded on JASCO J-810 Circular Dichroism Spectropolarimeter using a microvolume cuvette with a path length of 50 μm^2 at a concentration of 100

μM of **TZ1C2** in 10 mM TAPS buffer pH 8.5 with 100 mM NaCl. The background scattering was obtained by measuring the spectrum at 0 rpm rotation speed. The LD spectrum was measured after 15 minutes rotation at a speed of 3000 rpm to establish Couette flow.

Electron Microscopy. TEM specimens were prepared from aqueous **TZ1C2** solution in 10 mM TAPS buffer with 100 mM NaCl pH 8.5. The samples were deposited onto 200 mesh carbon coated copper grids (Electron Microscopy Sciences). After a 30 seconds incubation period, excess liquid was wicked away and the specimens were stained with 1% methylamine tungstate (Ted Pella, Inc.). Excess stain was wicked away after incubation on the grid for 1 min. The sample grids were dried under vacuum and stored in a desiccator. TEM measurements were acquired on a Hitachi H-7500 transmission electron microscope at an accelerating voltage of 75 kV. The micrographs were recorded at a magnification of 200,000x using a Gatan CCD digital camera.

Size Exclusion Chromatography. An aliquot (500 μL) of **TZ1C2** (100 μM) in TAPS buffer (10 mM, pH 8.5, 100 mM NaCl) and excess cadmium (200 μM) were injected into a Superdex75 10/300 GL gel filtration column (GE Healthcare) previously equilibrated with TAPS elution buffer (10 mM, pH 8.5, 100 mM NaCl) on an ÄKTA-purifier system (GE Healthcare) at ambient temperature. Elution of the analyte was monitored spectrophotometrically at 280 nm using a flow rate of 0.7 mL min^{-1} for the TAPS elution buffer. The synthetic coiled-coil proteins ccHex and ccTet were employed as molecular mass standards for the analysis.[3]

Analytical Ultracentrifugation. In order to identify the oligomerization state of **TZ1C2** and to characterize mass and shape distributions of the oligomerization products, sedimentation velocity (SV) experiments were performed. SV experiments characterize the solution behavior of macromolecules and can identify dynamic processes such as mass-action driven reversible associations by observing the sedimentation and diffusion behavior of all species in the system simultaneously. The experimental data are analyzed with the van Holde-Weischet analysis,[4] or modeled with solutions of the Lamm equation,[5-6] which are optimized by 2-dimensional spectrum analysis (2DSA).[7] 2DSA solutions were further refined using genetic algorithm (GA) analysis[8] to obtain a parsimoniously regularized solution.[9] The GA solution is then analyzed by Monte Carlo analysis to determine confidence limits for the determined parameters.10 The calculations are computationally intensive and are carried out on high-performance computing platforms.[11] The van Holde-Weischet approach provides diffusion-corrected sedimentation

coefficient distributions from which an assessment of heterogeneity and reversible self-association can be obtained. The 2DSA and GA analyses further provide molecular weight and anisotropy for any species found in the mixture.

All sedimentation experiments were performed with a Beckman Optima XL-I at the Center for Analytical Ultracentrifugation of Macromolecular Assemblies at the University of Texas Health Science Center at San Antonio. Sedimentation velocity data were analyzed with the UltraScan-III software[12] version 2.0.[13] All calculations were performed on the Lonestar and Stampede clusters at the Texas Advanced Computing Center at the University of Texas at Austin, or on the Alamo cluster at the Bioinformatics Core Facility at the University of Texas Health Science Center at San Antonio. **TZ1C2** was measured in the presence of Cd(II) at a loading concentration of 0.24 OD and 0.73 OD at 280 nm, and at 0.22 OD 280 nm in the absence of Cd(II). All measurements were made in a buffer containing 10 mM TAPS and 100 mM NaCl. The experimental data were collected in intensity mode at 20°C, and at 60 krpm, using standard epon 2-channel centerpieces. Hydrodynamic corrections for buffer density, viscosity and partial specific volume were made according to methods outlined in Laue, et al.,[14] and as implemented in UltraScan.[12] The partial specific volume of **TZ1C2** was determined to be 0.743 cm^3/g. All data were first analyzed by 2-dimensional spectrum analysis (2DSA)[7] with simultaneous removal of time- and radial-invariant noise and fitting of the meniscus position, followed by van Holde-Weischet analysis and genetic algorithm (GA) refinement,[9] followed by Monte Carlo analysis (MC).[10] We acknowledge the support of the San Antonio Cancer Institute grant P30 CA054174 for the Center for Analytical Ultracentrifugation of Macromolecular Assemblies at the University of Texas Health Science Center at San Antonio.

^{113}Cd NMR Spectroscopy. Lyophilized **TZ1C2** peptide (20 mg) was dissolved in 400 µL of 15% D_2O/H_2O purged with N_2 in advance to remove dissolved oxygen. The pH was adjusted to 8.5 using 0.1 M NaOH in 15% D_2O/H_2O. The peptide concentration was determined spectrophotometrically by measuring the absorbance at 280 nm (see above for detail). After determination of peptide concentration, two equivalents of isotopically enriched $^{113}CdCl_2$ were added based on peptide concentration. The ^{113}Cd NMR spectrum was collected at room temperature on a Varian Inova 600 spectrometer (113.08 MHz for ^{113}Cd) equipped with a 5 mm broadband probe. A spectral width of 844 ppm (112,360 Hz) was sampled using a 6.0 µs 90° pulse and 0.2 second acquisition time with 0.05 second delay between scans. Chemical shifts were recorded in parts per million relative to 0.1 M $Cd(ClO_4)_2$ in D_2O.

Perturbed Angular Correlation Spectroscopy. Methods for sample preparation and data acquisition followed the general procedures described in Matzapetakis, et al.[15] Perturbed angular correlation (PAC) experiments were performed with a setup using six detectors[16] at a temperature of 1 ± 2 °C that was controlled using a Peltier element. The radioactive cadmium was produced on the day of the experiment at the University Hospital cyclotron in Copenhagen. The ^{111m}Cd solution (10−40 μL) was mixed with nonradioactive cadmium acetate and TRIS buffer. The **TZ1C2** peptide was then added (dissolved in 10 mM TAPS buffer, pH 8.5, 100 mM NaCl), and the sample was left to equilibrate for 10 min to allow for metal ion binding. All buffers were purged with Ar and treated so as to lower metal contamination. Finally, sucrose was added to produce in order to further reduce the Brownian tumbling of the molecules. Final sample conditions were 300 μM peptide, 1.9/3 eq. Cd(II), 10 mM TAPS, 100 mM NaCl, (final pH value of 9.1 at 1 °C), and 55 % w/w sucrose. The experiments were carried out at 1 °C. The time resolution was 0.860 ns, and time per channel was 0.562 ns. All fits were carried out with 300 data points, disregarding the first 5 points due to systematic errors. The PAC spectroscopic results are presented in Table S1.

Non-denaturing Nano-electrospray Ionization Mass Spectrometry. A solution (500 μL) of peptide **TZ1C2** (100 μM) was incubated with tris(2-carboxyethyl)phosphine (TCEP, 500 μM) for 1 h. The sample was dialyzed for two days against 1200 mL of 500 mM ammonium acetate, pH 8.5. The sample was removed from dialysis and incubated with a one to one molar ratio of $^{113}CdCl_2$ for 12 h at 4 °C. Mass spectrometry experiments were performed on a Thermo LTQ-FTMS with a 7 T magnet. The ionization was performed using a nanospray source with PicoTip Emitters (New Objective). The voltage on the PicoTip emitter was maintained at 1.5 kV. The capillary temperature was approximately 40 °C. The capillary voltage was 10 V and the tube lens was 100 V. The automatic gain control (AGC) was set to 1.00×10^6. The maximum inject time was 5000 μs. The spectrum was taken at a resolution of 500,000, and the mass range was set to 1800 to 2000 m/z. The spectrum was signal averaged for ten minutes (54 scans).

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Table S1. Parameters fitted to PAC-data. The numbers in parenthesis are the standard deviations of the fitted parameters.

Peptide	t	pH	ω_0	η	$\Delta\omega_0/\omega_0$	$1/\tau_c$	A	χ_r^2
	°C	(@ 1 °C)	(rad/ns)		×100	μs^{-1}	×100	
TZ1C2	1	9.1	0.3993(5)	0.614(2)	1.5(2)	4.7(8)	7.4(3)	1.35

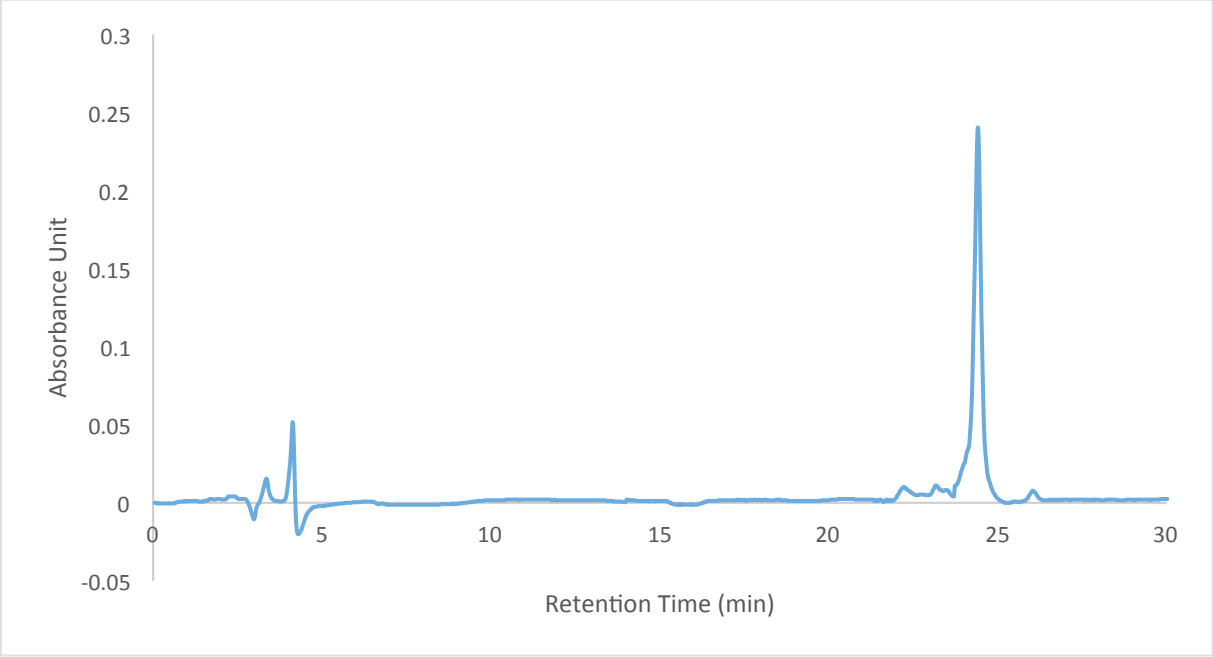

Figure S1. Analytical HPLC trace of peptide **TZ1C2**.

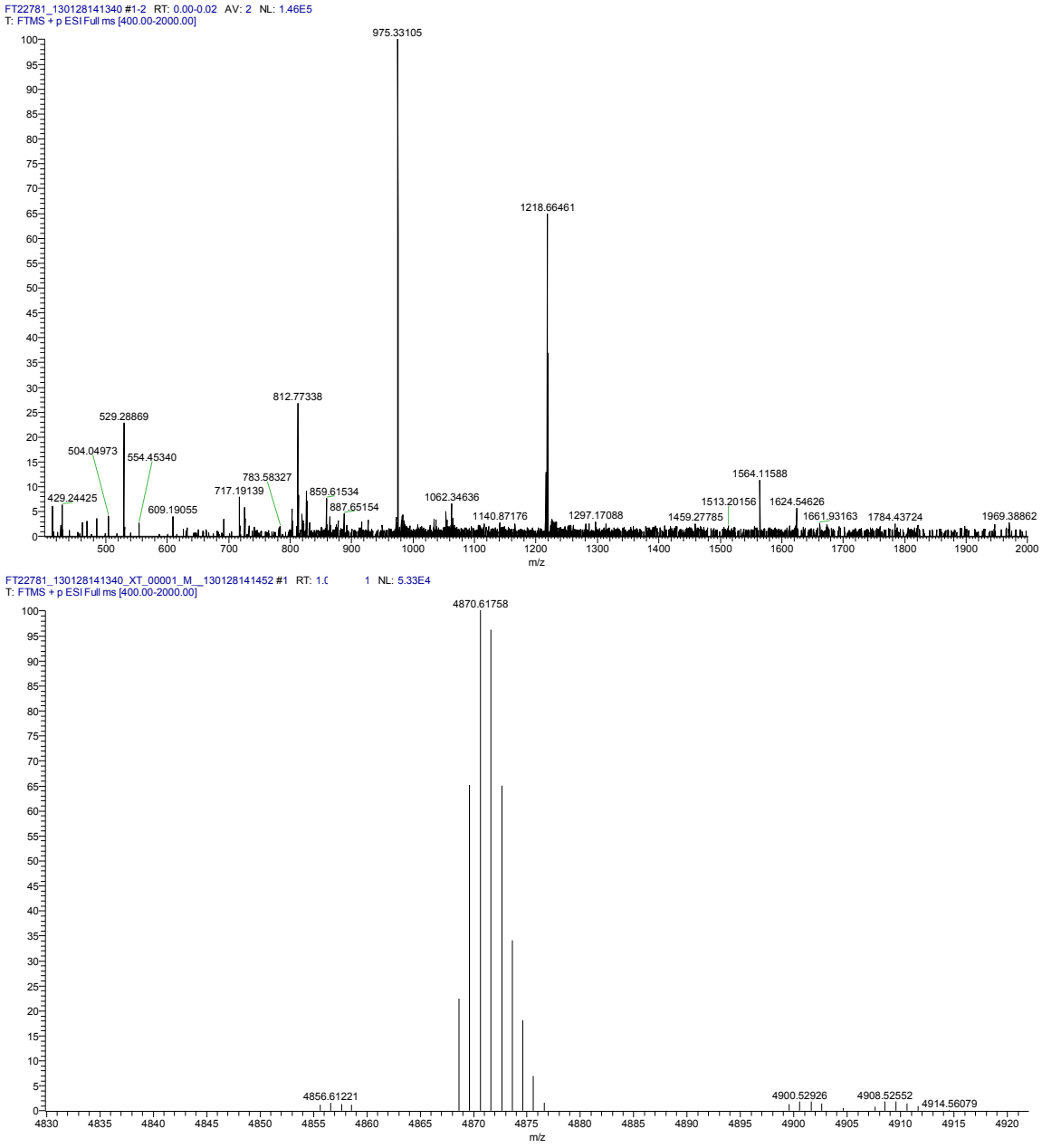

Figure S2. ESI-mass spectrum (upper) and the corresponding spectral deconvolution for purified peptide **TZ1C2**.

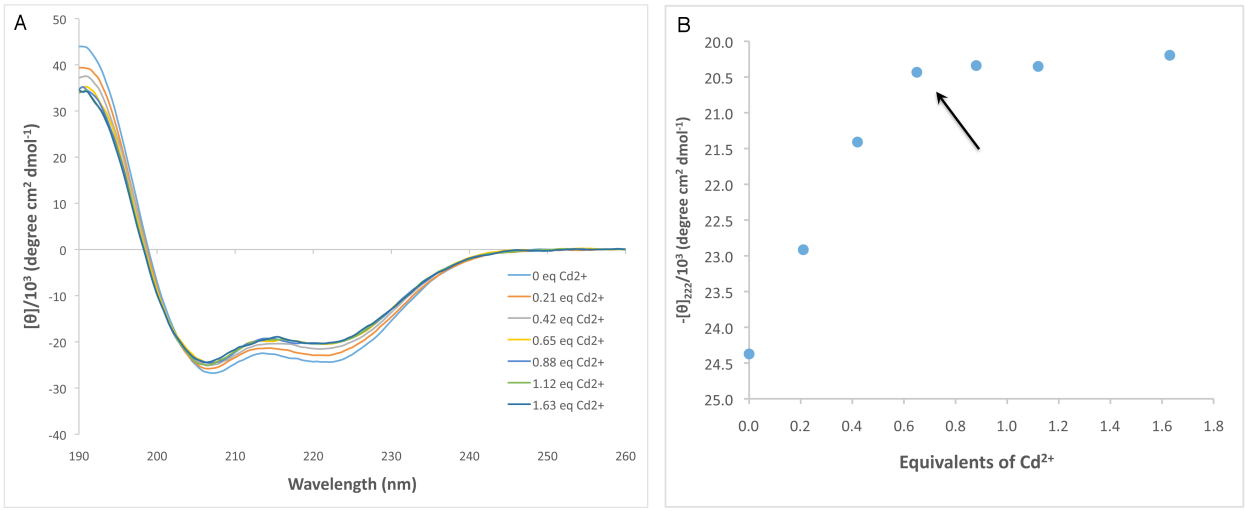

Figure S3. **A**. CD spectropolarimetric titration of **TZ1C2** (75 μM) in TAPS buffer (10 mM, pH 8.5, 100 mM NaCl) with increasing concentrations of Cd(II)Cl_2. **B**. Change in CD spectropolarimetric signal at 222 nm ($[\theta]_{222}$) as a function of the number of equivalents of Cd(II) ion. The arrow indicates that the binding sites saturate at circa 0.65 equivalents of Cd(II), which closely corresponds to the expected value of 0.67 equivalents Cd(II) per peptide for binding of two cadmium ions to a coiled-coil trimer.

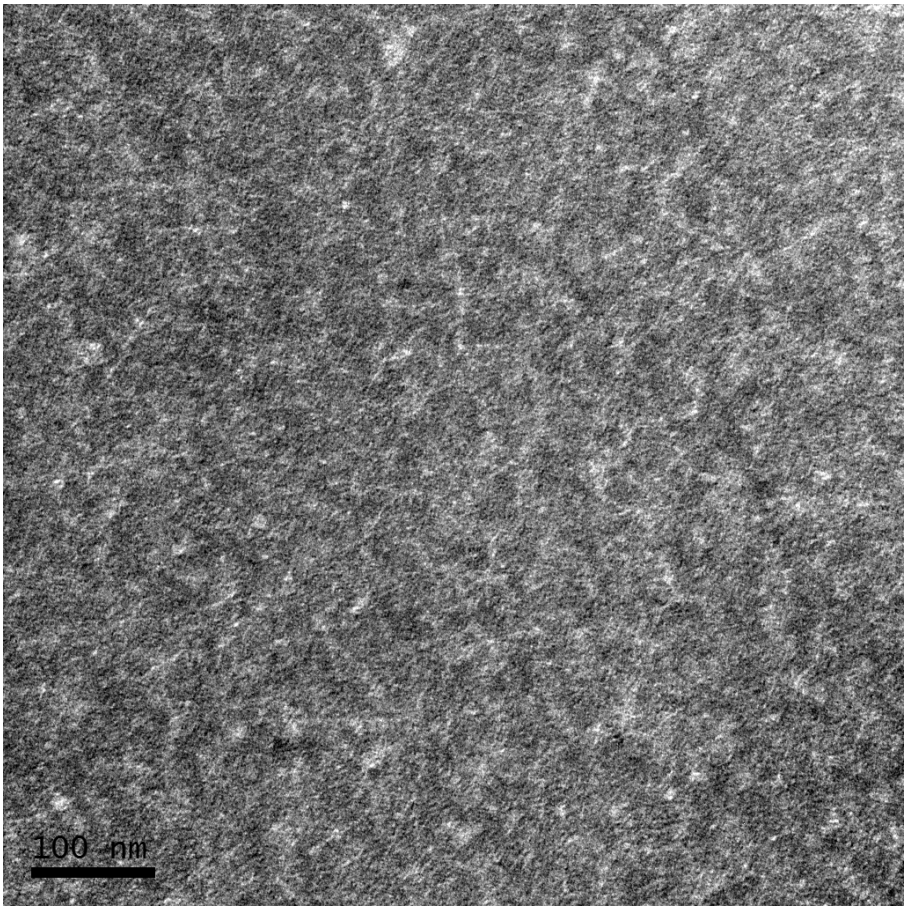

Figure S4. TEM image of peptide **TZ1C2** (500 μM) in TAPS buffer (10 mM, pH 8.5, 100 mM NaCl) in the presence of excess cadmium(II) nitrate (400 μM). TEM indicates the absence of high aspect-ratio fibrils.

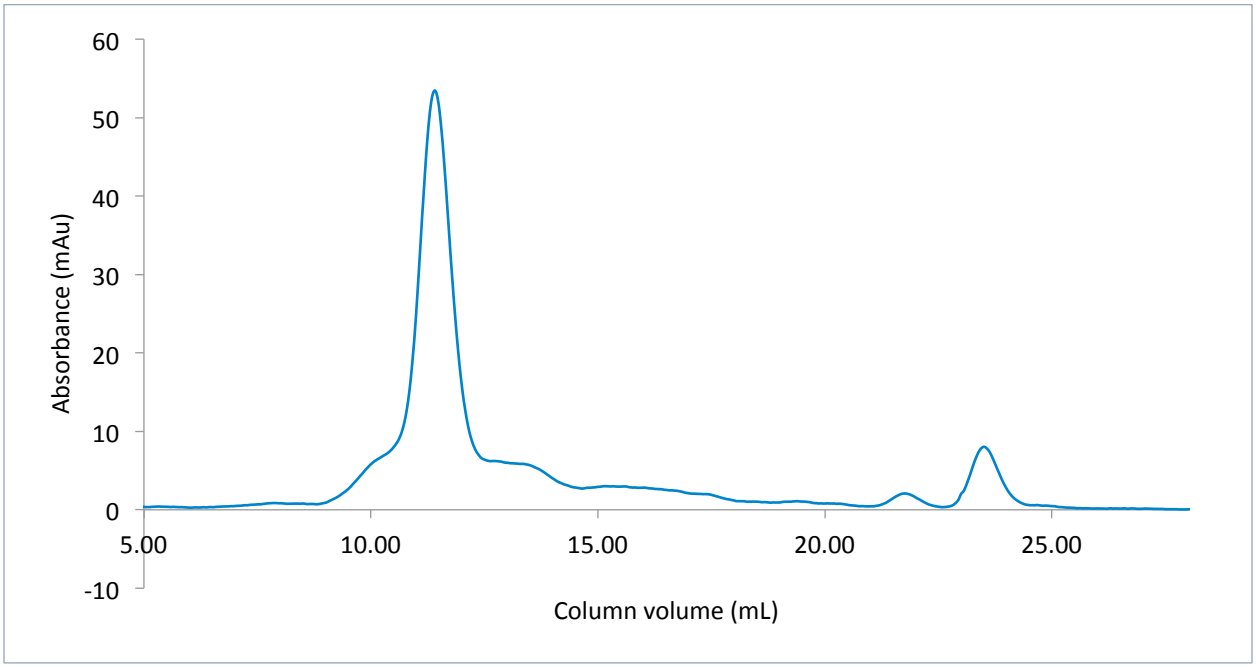

Figure S5. Size-exclusion chromatography of peptide **TZ1C2** (100 μM) in TAPS buffer (10 mM, pH 8.5, 100 mM NaCl) in the presence of excess $Cd(NO_3)_2$.

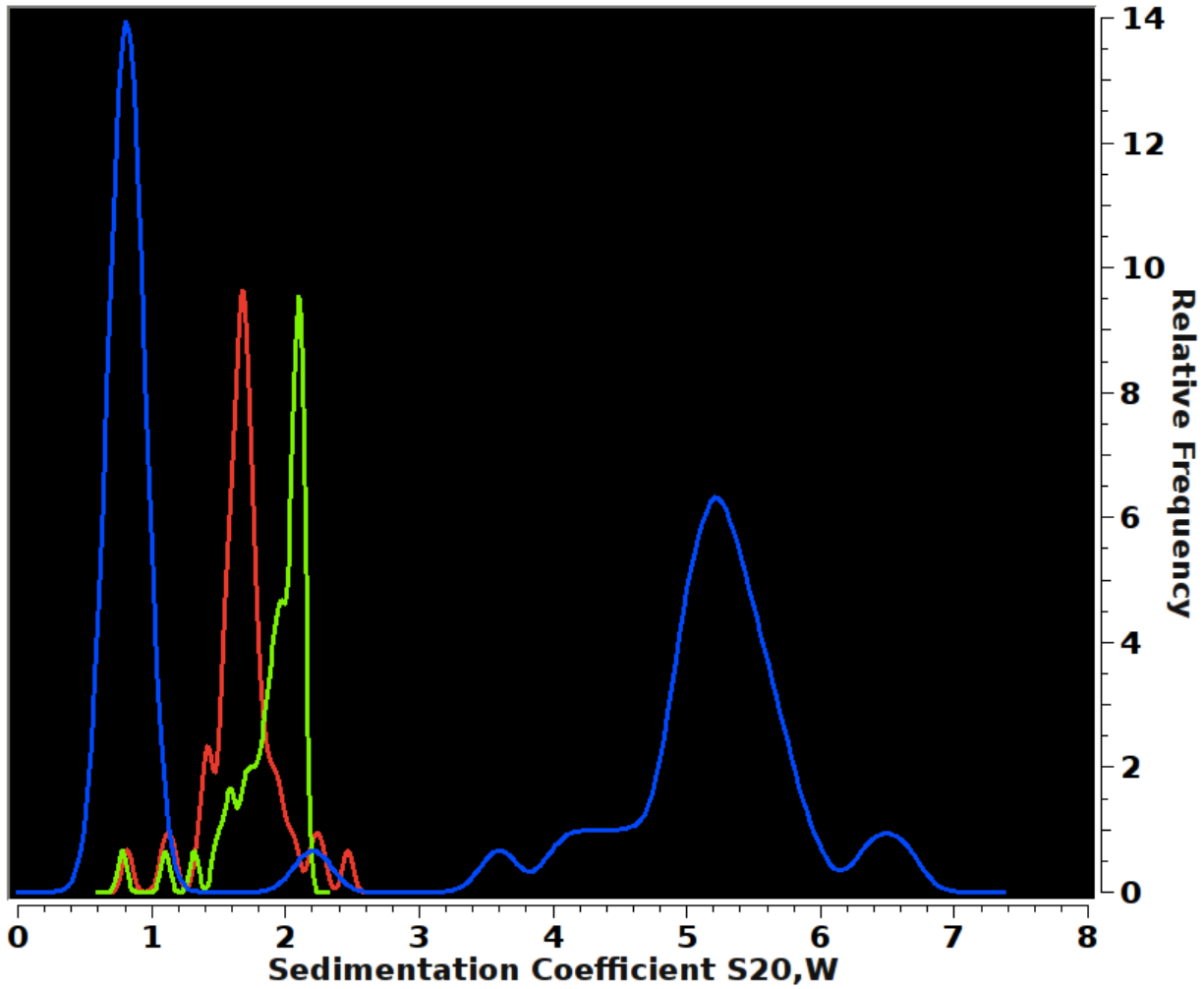

Figure S6. The g(s) distributions for **TZ1C2** in the presence of excess Cd(II) at 160 μM (red) and 480 μM (green) and in the absence of Cd(II) at 140 μM (blue) loading concentration.

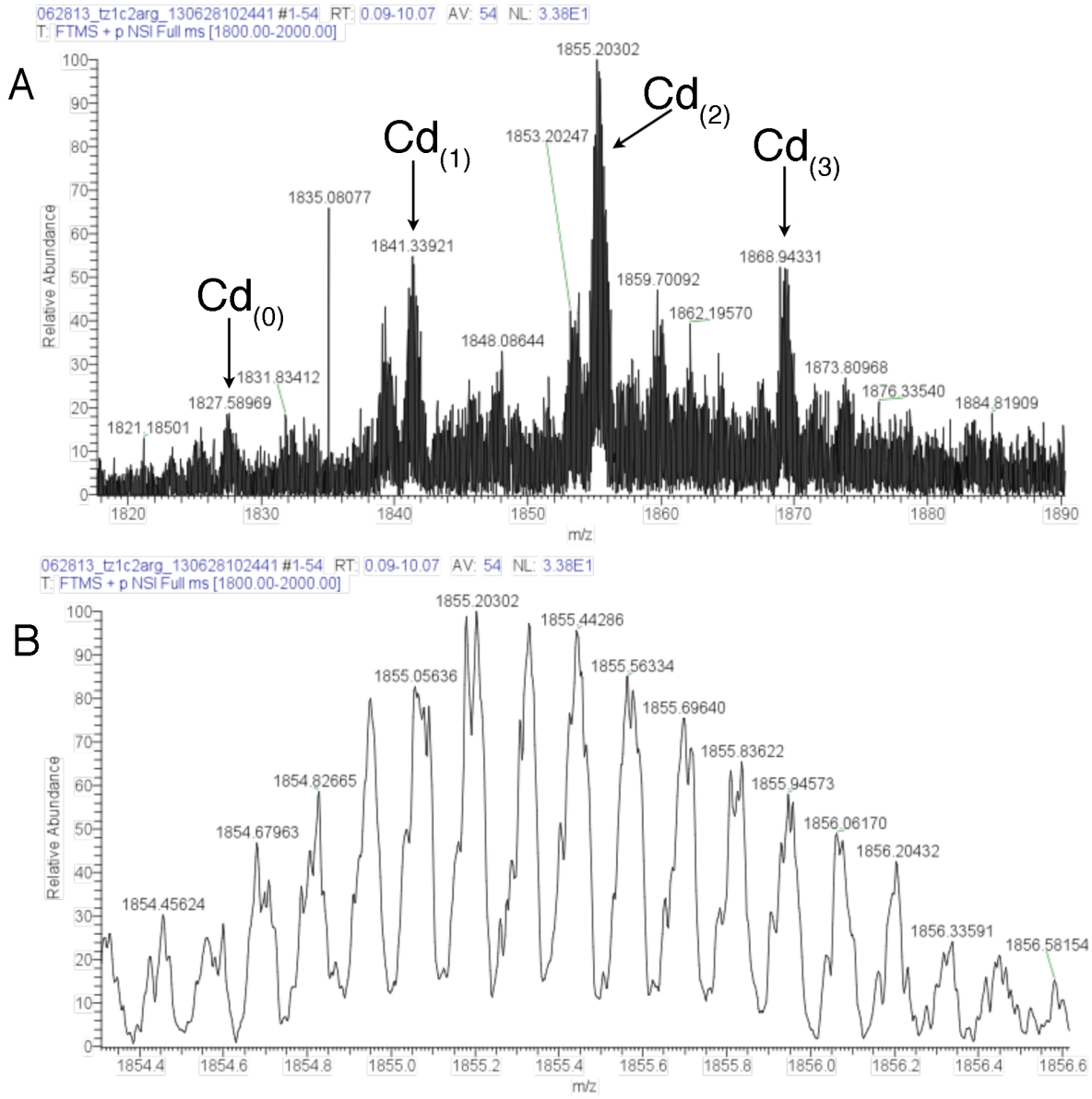

Figure S7. Non-denaturing nano-electrospray ionization mass spectrometry of the **TZ1C2** complex with Cd(II). **A**. Mass spectrum of the **TZ1C2** trimer in the +8 charge state. Arrows indicate the positions of adducts of the **TZ1C2** trimer with 0, 1, 2, or 3 ^{113}Cd(II) ions. The largest population of species corresponds to the adduct with two Cd(II) ions per trimer. **B**. Expansion of the region of the mass spectrum corresponding to the di-cadmium adduct of the **TZ1C2** trimer in the +8 charge state.