Supporting Information

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Design of a Three-Helix Bundle Capable of Binding Heavy Metals in a Triscysteine Environment**

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Supporting Information

Table S1. Parameters fitted to the ^{199m}Hg PAC data.[a]

α_3DIV (μM)	Hg^{II} (μM)	pH	ν_Q (GHz)	η	$\Delta\omega_0/\omega_0\times100$	$1/\tau_c$ (μs^{-1})	A×100	χ_r^2
200	80	5.8	1.48(2)	0.15(5)	2(2)	18(21)	12(1)	0.63
200	80	7.5	1.48[b]	0.15[b]	2[b]	10(18)	4(1)	0.67
			1.11[b]	0.40[b]	6[b]		11(1)	
200	80	8.6	1.11(2)	0.40(3)	6(2)	0(20)	10(1)	0.62

[a] Numbers in the parenthesis are the standard deviations of the fitted parameters. [b] Fixed in the fit.

Table S2. Parameters fitted to the ^{111m}Cd PAC data.[a]

Peptide ($C_{CdII}/C_{peptide}$)	**ω_0 (rad/ns)**	**η**	**$\Delta\omega_0/\omega_0 \times 100$**	**$1/\tau_c$ (μs^{-1})**	**A $\times 100$**	**χ_r^2**
α_3D**IV** (1/12)	0.350(6)	0.0(1)	14(3)	4.5(6)	5(1)	1.36
	0.268(4)	0.18(7)	1(2)	4.5(6)	0.3(3)	
	0.17(2)	0.5(2)	24(3)	4.5(6)	5.8(8)	

[a] Numbers in the parenthesis are the standard deviations of the fitted parameters.

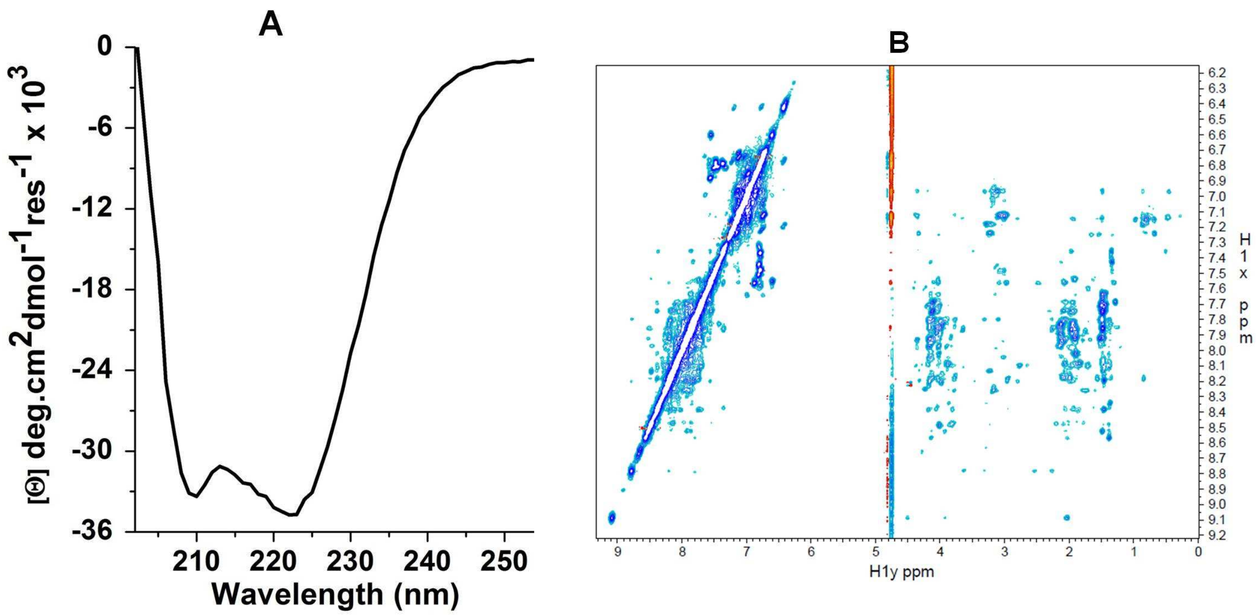

Figure S1. A) CD spectrum of α_3D**IV** at pH 8.0 and 25°C. The double minima at 222 and 208 nm are representative of α helical structure. Molar ellipticity of –34726 deg cm^2 dmol^{-1} res^{-1} at 222 nm corresponds to a 97% folded structure. **B)** ^{1}H-^{1}H NOESY spectrum of α_3D**IV** showing chemical shift dispersion characteristic of a well folded α–helical structure.

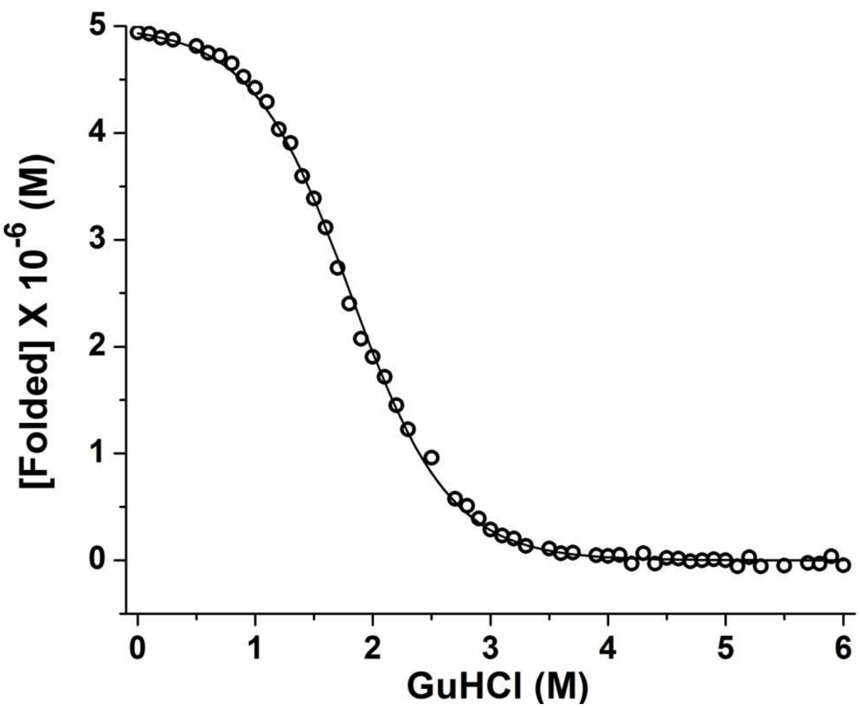

Figure S2. GuHCl denaturation titration curve of α_3D**IV** plotted as a function of concentration of folded protein vs. concentration of GuHCl. The solid line represents fit to the experimental data.

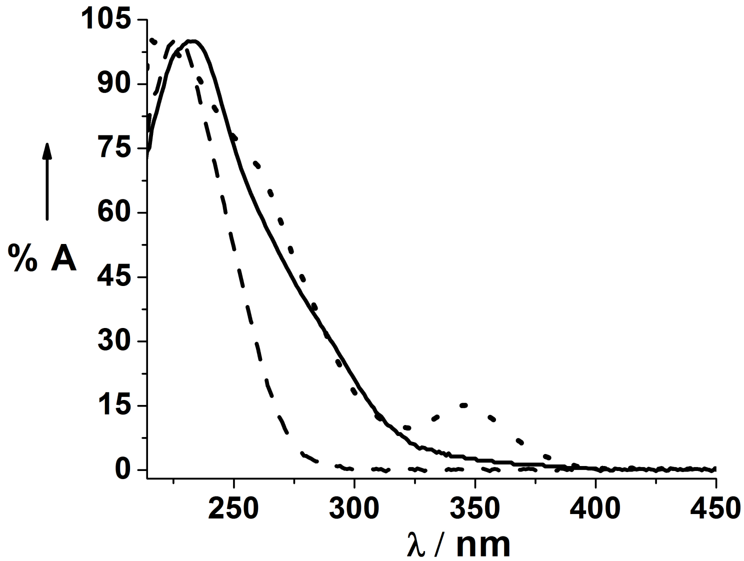

Figure S3. UV/Vis spectra of solutions containing 20 μM α_3D**IV**, 40 μM TCEP, and 1 equivalent of $CdCl_2$ at pH 8.0 (dashed line); 30 μM α_3D**IV**; 60 μM TCEP, and 1 equivalent of $HgCl_2$ at pH 8.6 (solid line), and 20 μM α_3D**IV**, 40 μM TCEP, and 1.0 equivalent of $PbCl_2$ at pH 8 (dotted line). Shown is a plot of normalized absorbance vs. wavelength.

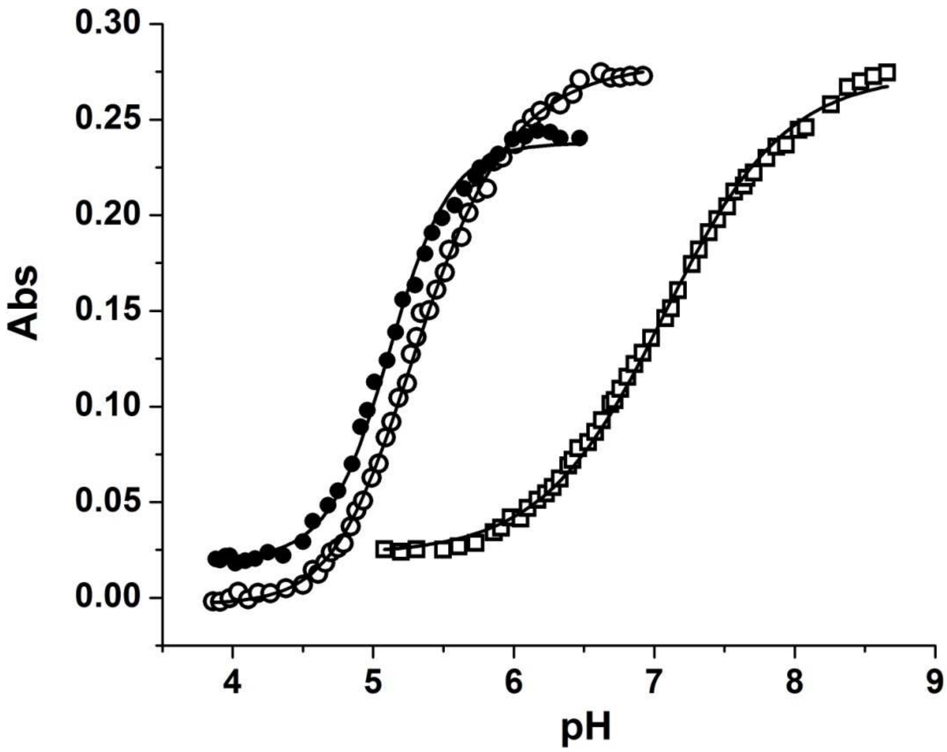

Figure S4. pH dependence of the binding of 1 equivalent of Cd^{II} (open circles), Hg^{II} (squares), and Pb^{II} (filled circles) to 20 μM, 30 μM, and 20 μM α_3D**IV**, respectively, along with the fits of the experimental data. Experiments were followed by monitoring UV/Vis absorbance due to LMCT bands at 232, 247, and 236 nm for Cd^{II}, Hg^{II}, and Pb^{II}, respectively. Cd^{II} and Pb^{II} titration data were fit to the model simultaneous dissociation of two Cys thiol protons; Hg^{II} was fit to a single 1-H step corresponding to the formation of HgS_3 from $HgS_2(SH)$.

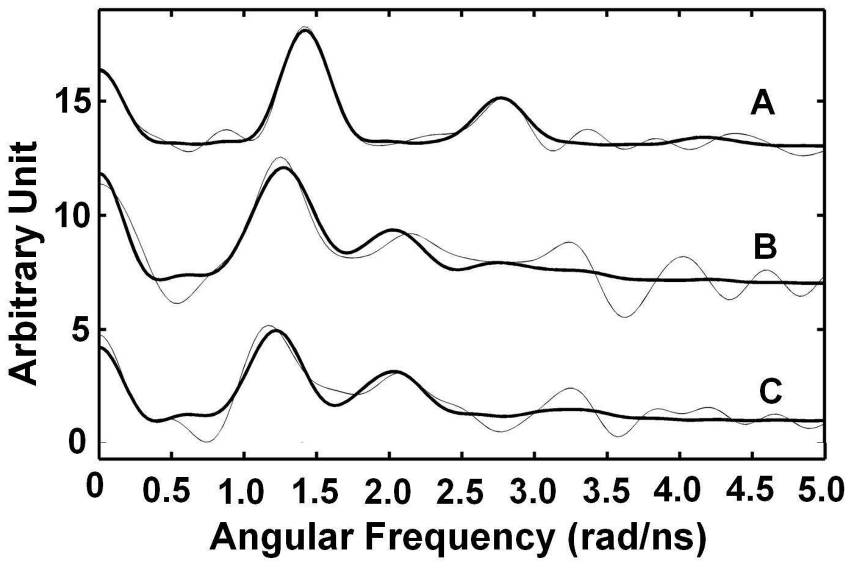

Figure S5. Fourier transformed ^{199m}Hg PAC spectra of α_3D**IV** (faint lines) under different conditions along with the Fourier transformed fits (dark lines). Sample conditions were A) 200 μM α_3D**IV**, 80 μM $HgCl_2$, 100 mM phosphate buffer at pH 5.8, B) 200 μM α_3D**IV**, 80 μM $HgCl_2$, 100 mM phosphate buffer at pH 7.4, and C) 200 μM α_3D**IV**, 80 μM $HgCl_2$, 100 mM CHES buffer at pH 8.6.

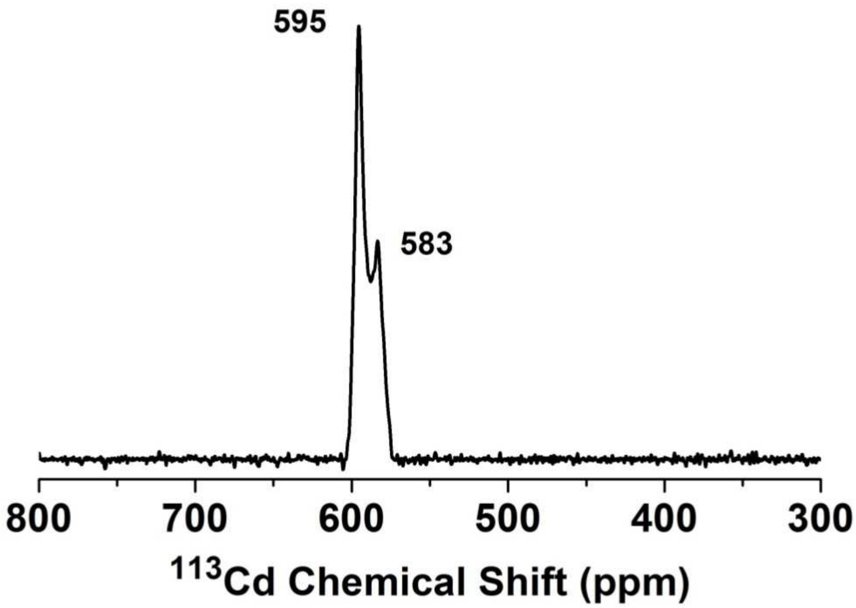

Figure S6. ^{113}Cd NMR spectrum of 3 mM α_3D**IV** with 0.8 equivalents of $^{113}Cd(NO_3)_2$ recorded at pH 8.

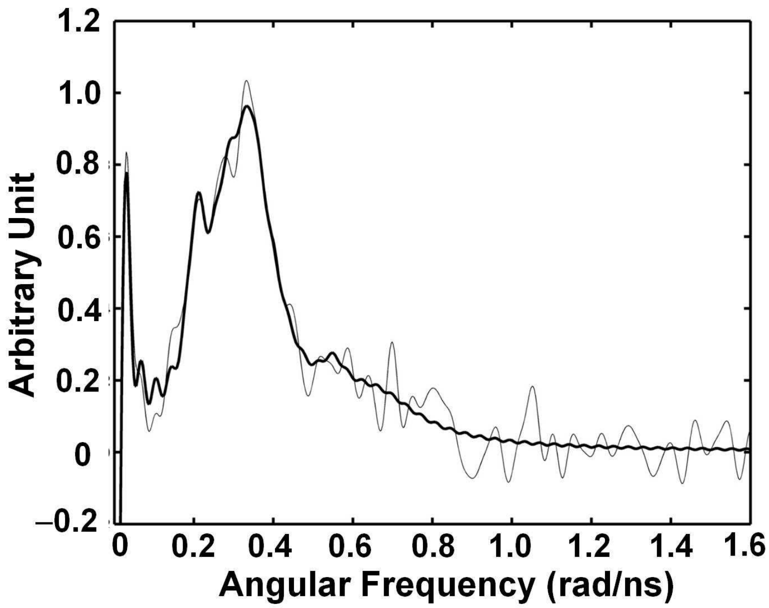

Figure S7. Fourier transformed ^{111m}Cd PAC spectrum of 300 μM α_3D**IV** loaded with 1/12 equivalents of Cd^{II} (faint line) along with the Fourier transformed fits (dark line). Sample was prepared in 20 mM TRIS buffer at pH 8.1. The data analysis of this spectrum relied on identification of typical signals from CdS_3O species reported in the literature.[37]