

Supplemental information for:

“The metal selectivity of a short peptide maquette imitating the high-affinity metal-binding site of *E. coli* HypB”

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Experimental Procedures

Peptide synthesis and purification. The B7 peptide was prepared via solid-phase peptide synthesis using Fmoc-protection based chemistry and an Applied Biosystems 431A peptide synthesizer. The peptide sequence consists of the first seven residues of the HypB protein and a C-terminal tryptophan added for detection purposes, yielding a final sequence of CTTCGCGW. NovaPEG Rink Amide resin (NovaBioChem) was used as the solid phase, and the synthesis was performed at the 0.1 mmol scale. Fmoc-L-cysteine-(Trt)-OH, Fmoc-L-threonine-(tBu)-OH, Fmoc-L-glycine-OH, and Fmoc-L-tryptophan-(BOC)-OH (Anaspec) were used for synthesis. Coupling used four equivalents of O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), six equivalents of N,N-diisopropylethylamine (DIPEA), and four equivalents of amino acids. Acetic anhydride was used to cap the N-terminus of uncoupled amino acids.

After synthesis, the peptide was cleaved from the resin by using a solution of 87.5% trifluoroacetic acid, 5% MilliQ water, 5% thioanisole, and 2.5% 1,2-ethanedithiol.¹ For a typical cleavage, 16 mL of solution was mixed with 0.5 g of peptide-loaded resin and incubated at room temperature. After two hours, the resin was filtered from the solution by using a Biospin disposable chromatography column (Bio-Rad). The TFA was evaporated by using a stream of nitrogen gas, and then 45 mL of diethyl ether was added in order to precipitate the peptide. The solution was centrifuged, the supernatant removed, and then the precipitant was washed with ether two more times. The washed pellet was dried with nitrogen gas, and then dissolved in 10 mL of MilliQ water.

The crude peptide was purified via two methods. Small scale preparations were purified using a Waters Sep-Pak C18 solid phase extraction cartridge. Briefly, the solid phase was washed with 3 mL of

acetonitrile and 3 mL of MilliQ water. One milliliter of crude peptide was slowly loaded onto the column, and then eluted with a gradient of 0 – 30% acetonitrile with 0.1% TFA as an ion-pairing reagent². This procedure was performed in an anaerobic glovebox. The collected fractions were screened via electronic absorption spectroscopy, and peptide-containing fractions were confirmed by using matrix assisted laser desorption ionization mass spectrometry (MALDI-MS). The singly-charged sodiated peptide was observed at 851.29 m/z, which corresponds to the predicted mass to charge of the $[C_{32}H_{48}N_{10}O_{10}S_3Na]^+$ ion of 851.27 m/z. Appropriate fractions were pooled and lyophilized to dryness for storage.

Large scale peptide preparations were purified by using reversed-phase HPLC and a prep-scale monomeric C₁₈ column (Grace Vydac) (Figure S9). Five milliliters of crude peptide was injected, then eluted with a gradient of 0 – 85% acetonitrile (0.1% TFA) with a flow rate of 4 mL/minute over sixty minutes. Collected fractions were screened and dried as described above.

Metal titration and competition experiments. Lyophilized B7 peptide was resuspended in buffer A (25 mM HEPES, 100 mM KCl, pH 7.5) and quantified in triplicate by using electronic absorption spectroscopy ($\epsilon_{280nm} = 5550 \text{ M}^{-1}\text{cm}^{-1}$, calculated with ExPASy Proteomics Server³). For metal titrations, increasing amounts of metal salt (NiSO₄, ZnSO₄, CdSO₄, CoSO₄, Cu(CH₃CN)₄PF₆, purity >99.99%) dissolved in deionized water were added to a constant concentration of peptide. These samples were equilibrated in an anaerobic glove box at room temperature. Direct metal titrations were incubated for at least an hour, while competition experiments were allowed at least twelve hours to equilibrate. The electronic absorption spectra (190 – 700 nm) of the samples were then measured by using an Agilent 8452 spectrophotometer. For nickel affinity experiments, 5.0 or 7.5 mM glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA, Sigma Aldrich) was included in the sample. The concentrations of free nickel were calculated as described previously⁴, and the data fit to the equation $r = [Ni]/(K_d + [Ni])$, where r is the fraction of peptide bound to nickel, $[Ni]$ is the free nickel concentration, and K_d is the free nickel concentration required for 50% saturation. Copper saturation experiments were performed in an anaerobic fluorescence cuvette and monitored with a Fluorolog (Jobin-Yvon) fluorimeter. Samples were

excited at 295 nm, and the sulfur-copper(I) luminescence signal was observed at 600 nm.⁵ The data were analyzed as described previously,⁶ by using extinction coefficients calculated from direct titrations.

Competition experiments between metals were fit in DynaFit 3⁷ using a custom script. Data and concentration of components varied between experiments. The body of the script was as follows:

```
[task]
data = equilibria
task = fit

[mechanism]
; Where N = Ni(II)
;       Z = Competitor metal
;       B = Peptide
N.B <=> N + B : Kd1  dissociation
Z.B <=> Z + B : Kd2  dissociation
```

Electrospray measurements. Peptide samples analyzed via electrospray mass spectrometry (ESI-MS) were resuspended in buffer B (10 mM ammonium acetate, pH = 7.5). Samples were prepared in an anaerobic glove box, and then capped with a septum to prevent oxidation prior to analysis. Mass spectra were acquired on an AB/Sciex QStarXL mass spectrometer equipped with an ion spray source in the positive mode. Ions were scanned in the 400 – 2000 m/z range with accumulation times of one second per spectrum, with no interscan time delay. The instrument parameters are as follows: ionSpray voltage 5200 V; declustering potential 60 V; focusing potential 60 V; ion source gas 45 psi; curtain gas 45 psi.

NEM modification procedure. To verify the oxidation state of the peptide, the cysteine residues were modified with N-ethyl maleimide (Sigma Aldrich) and the peptide then observed by MALDI-MS. Briefly, a 50 μ M solution of peptide in buffer B (10 mM HEPES, 100 mM KCl, pH 6.9) was incubated anaerobically with 750 μ M NEM for approximately two hours, at room temperature. Upon completion, the sample was mixed with α -cyano-4-hydroxycinnamic acid and analyzed via MALDI-MS. Fully reduced samples were thrice modified by NEM, and a +375 m/z peak was observed. Partially oxidized samples were less readily reacted with NEM, and single modifications (+125 m/z) were observed. Only fully oxidized peptide was used for experiments.

X-ray absorption spectroscopy. Ni(II)-B7 peptide samples were dissolved in buffer A to a concentration of 1.5 mM, and 0.8 equivalents of nickel sulfate were added. Nickel binding was confirmed via electronic absorption spectroscopy. The sample was then transferred to a Lucite sample cuvette (2 x 10 x 10 mm) and frozen in liquid nitrogen for data acquisition.

Measurements were carried out at the Stanford Synchrotron Radiation Laboratory (SSRL) with the SPEAR 3 storage ring containing between 85 and 100 mA at 3.0 GeV. Nickel K-edge data were collected on structural biology beamline 7-3 with a wiggler field of 2T and employed a Si(220) double-crystal monochromator. Harmonic rejection was achieved by setting the cut-off angle of the mirrors to 13 keV. Samples were maintained at 10 K using an Oxford instruments liquid helium flow cryostat. Three to six scans were accumulated for each sample, and the energy was calibrated with respect to nickel foil measured concurrently in each scan. A lowest energy inflection point of 8331.6 was assumed for Ni(II).

EXAFS data were quantitatively analyzed via curve fitting using the EXAFSPAK package⁸, using *ab initio* theoretical phase and amplitude functions calculated using FEFF, version 7.2⁹.

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Model	A-Bs	Bond Length (Å)	σ^2 (Å²)	ΔE_0 (eV)	F-factor
4S	Ni-S	2.2003 ± 0.0017	0.00711 ± 0.00022	-9.602	0.3650
3S-1N	Ni-S	2.1781 ± 0.0030	0.00520 ± 0.00055	-13.533	0.2358
	Ni-N	2.0533 ± 0.0197	0.00946 ± 0.00958		
2S-2N	Ni-S	2.1999 ± 0.0041	0.00367 ± 0.00032	-9.602	0.2294
	Ni-N	2.0140 ± 0.0089	0.00541 ± 0.00115		
1S-3N	Ni-S	2.2258 ± 0.0036	0.00019 ± 0.00028	-6.442	0.2374
	Ni-N	2.0129 ± 0.0054	0.00360 ± 0.00043		

Table S1 Parameters from fitting of EXAFS data. A-Bs denotes absorber and backscatterer interactions. The bond length represents the interatomic distances, σ^2 are the Debye-Waller factors (mean-square deviations in interatomic distance); the threshold energy shifts, ΔE_0 , are given in eV. The F-factor or fit-error function is defined as $(\sum k^6(\chi(k)_{\text{calcd}} - \chi(k)_{\text{exptl}})^2 / \sum k^6 \chi(k)_{\text{exptl}}^2)^{1/2}$. Based on both the weighted F-factor, and the threshold energy shifts (ΔE_0), the curve of best fit was determined to correspond to a coordination sphere of three sulfur and one nitrogen (shown in figure S10). While the 2S-2N model resulted in a lower F-factor, the threshold energy shift of the 3S-1N model is much more characteristic of a square planar complex, which we expect based on the edge features.

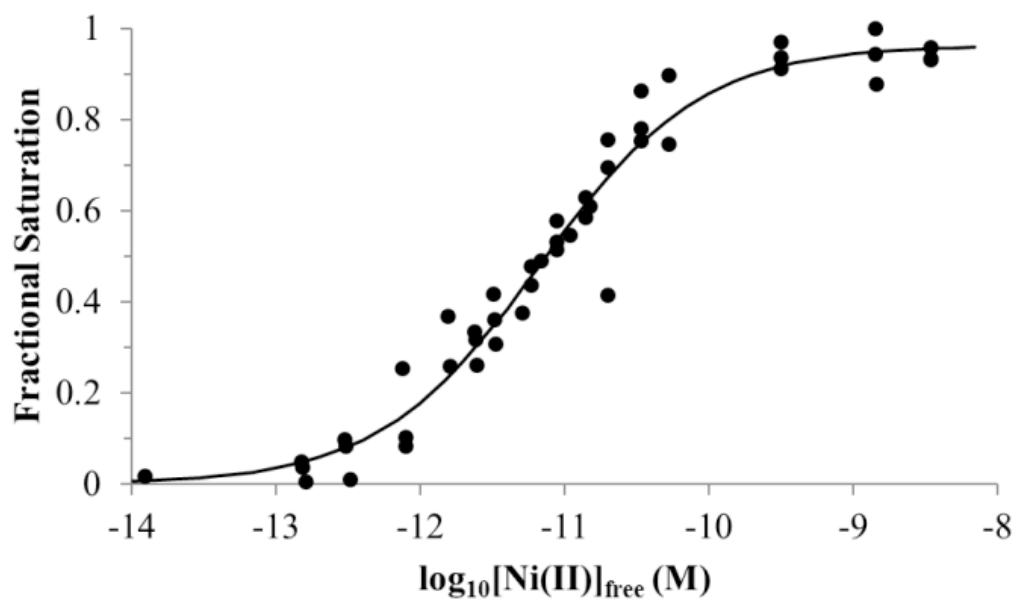


Figure S1. Addition of Ni(II) sulfate to a mixture of 15 μ M B7 peptide and 5 mM EGTA. The free nickel (nickel which is bound to neither EGTA or peptide) is plotted along the x-axis, and was calculated based on published EGTA-Ni stability constants.⁶

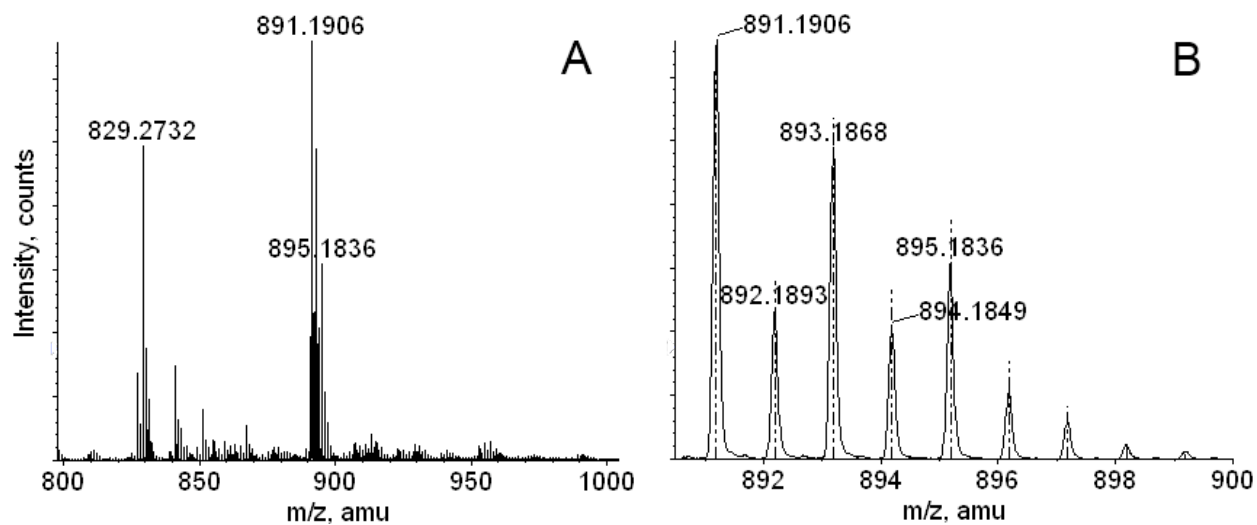


Figure S2. (A) ESI-MS of 5 μM B7 peptide loaded with one equivalent of Zn(II). Peaks of note include the reduced peptide (at 829.27 m/z) and the zinc-peptide complex (at 891.19 m/z). Unlike the nickel-peptide complex, some of the zinc-peptide complex does not remain intact when it is observed by ESI-MS, contrary to solution experiments that indicate that the peptide is saturated at equimolar concentrations of zinc and peptide. (B) Expected isotope distribution of the B7-Zn(II) complex, [C₃₂H₄₇N₁₀O₁₀S₃Zn]⁺ (dotted lines) and the observed signal (solid line).

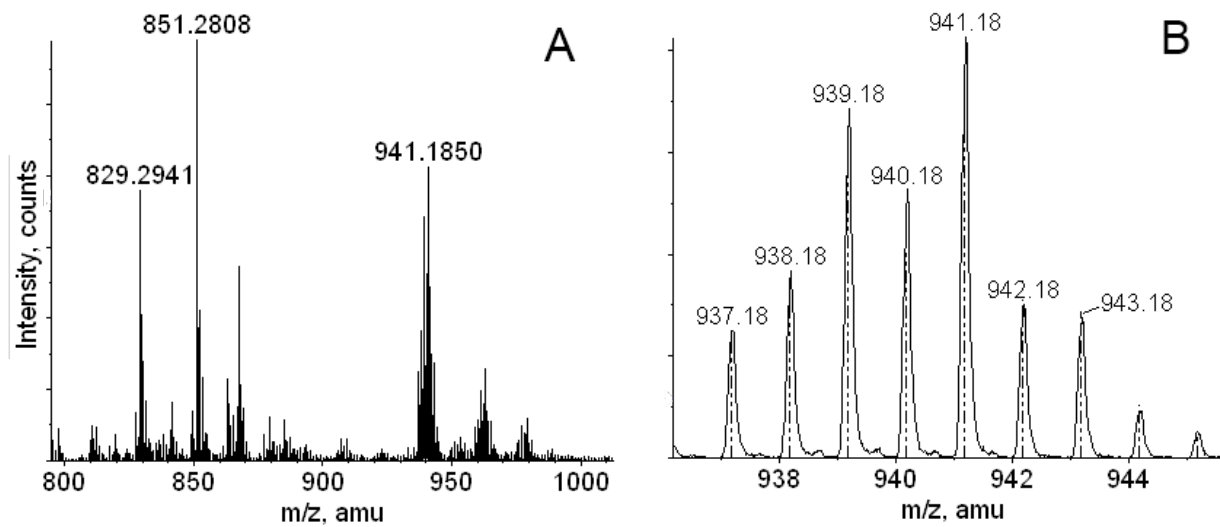


Figure S3. (A) ESI-MS of 5 μM B7 peptide loaded with one equivalent of Cd(II). Peaks of note include the reduced peptide (at 829.27 m/z), the sodiated reduced peptide (at 851.28 m/z) and the cadmium-peptide complex (at 941.18 m/z). Similar to the zinc-peptide complex, the peptide does not appear saturated upon the addition of one equivalent of cadmium (B) Isotopic fitting of the 941 m/z cluster to $[\text{C}_{32}\text{H}_{47}\text{N}_{10}\text{O}_{10}\text{S}_3\text{Cd}]^+$, which corresponds to a 1:1 peptide-cadmium complex.

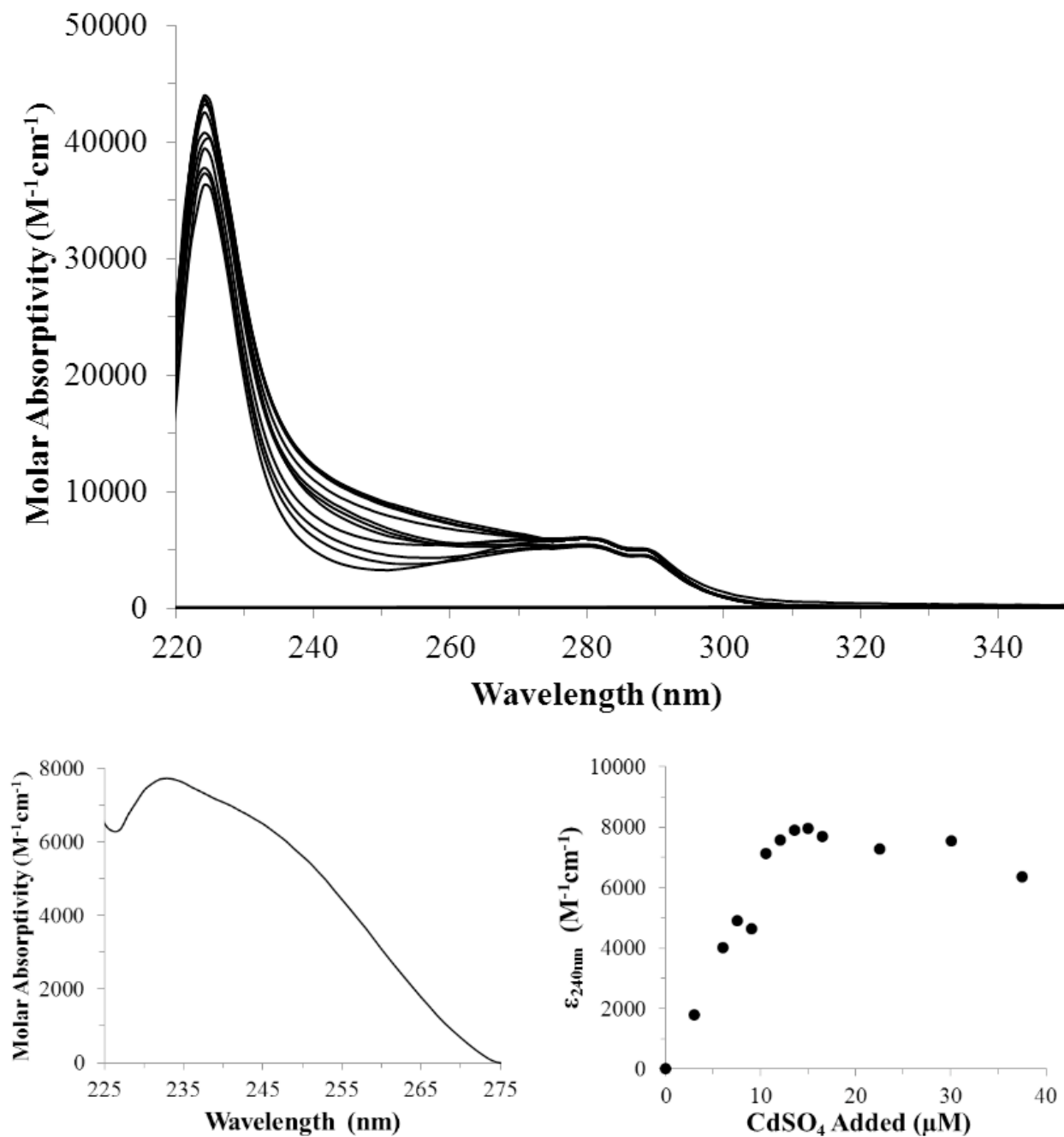


Figure S4. A cadmium titration of 15 μM B7 peptide. **Top**, the electronic absorption spectra of 15 μM B7 with increasing amounts of cadmium sulfate added. **Bottom left**, the difference spectrum of the apo peptide and the peptide with one equivalent of cadmium added. A broad absorption band at 240 nm was observed upon the addition of cadmium, which is characteristic of cadmium-thiolate LMCT. **Bottom right**, the intensity of the band at 240 nm increases linearly with Cd(II) concentration. The band saturates when there was one equivalent of cadmium present, suggesting the formation of a 1:1 complex.

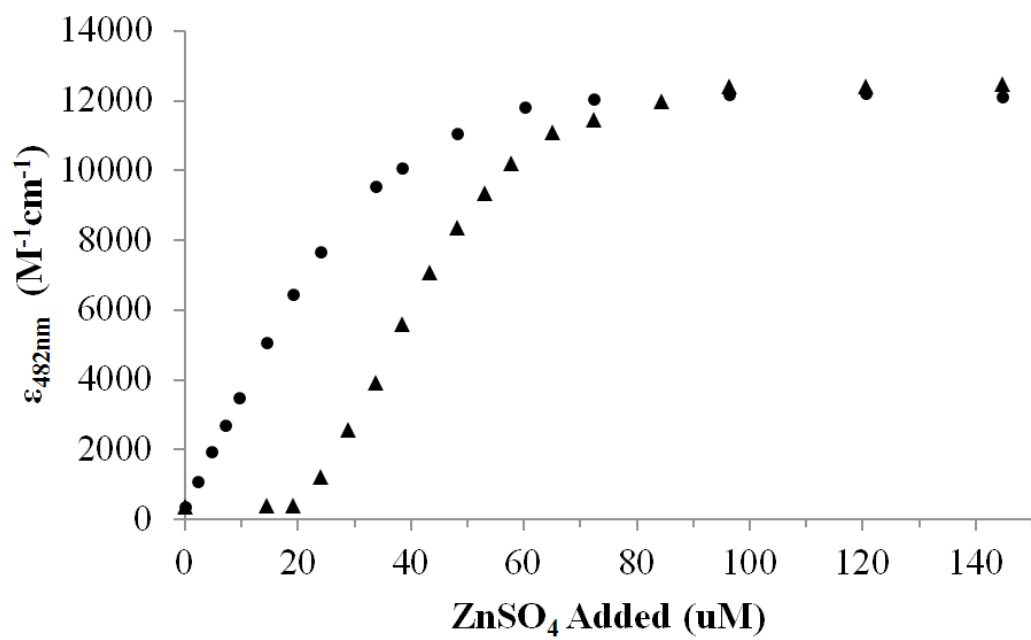


Figure S5. A titration of 50 μM zincon without (circles), or in the presence of 20 μM B7 (triangles). The addition of B7 caused the curve to shift on the x-axis by 20 μM, suggesting a 1:1 complex between the peptide and Zn(II).

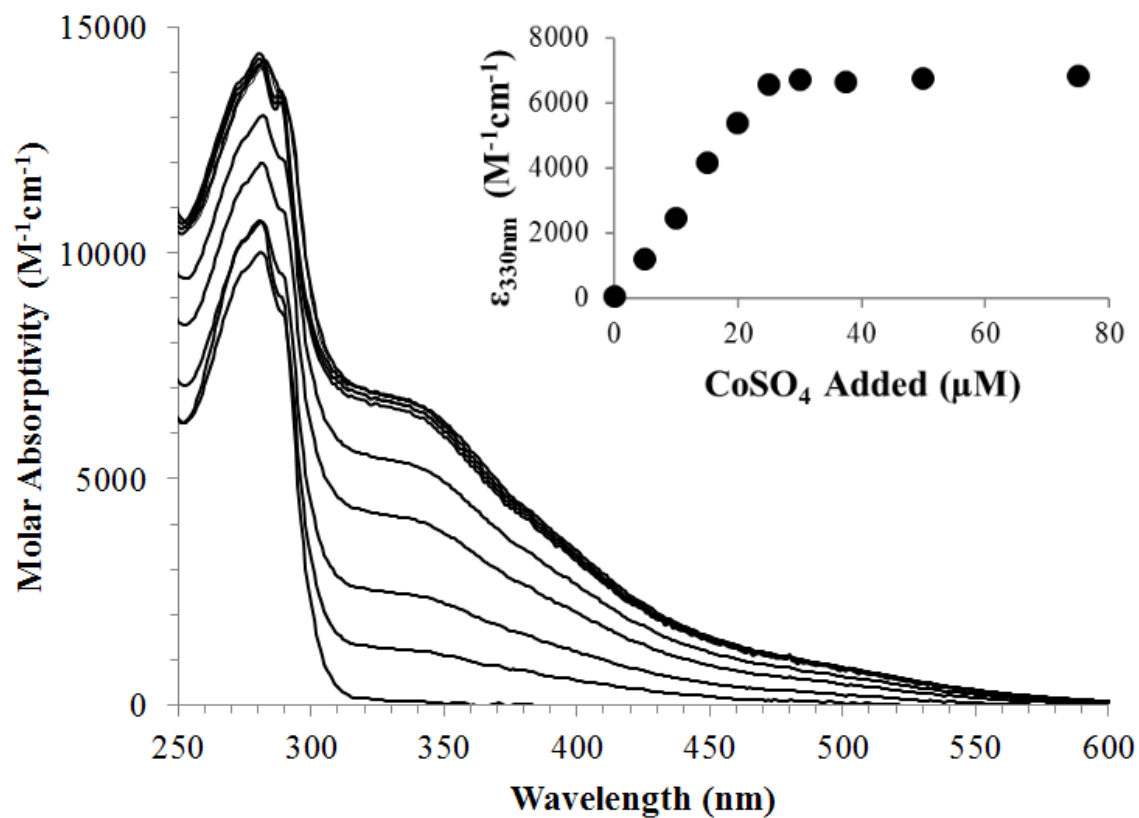


Figure S6. A Co(II) titration of 50 μM B7 peptide. An absorption band at 340 nm was observed upon the addition of cobalt sulfate. The band increased linearly with cobalt concentration, and saturated when there was a half equivalent of cobalt present, suggesting the formation of a 1:2 complex (inset).

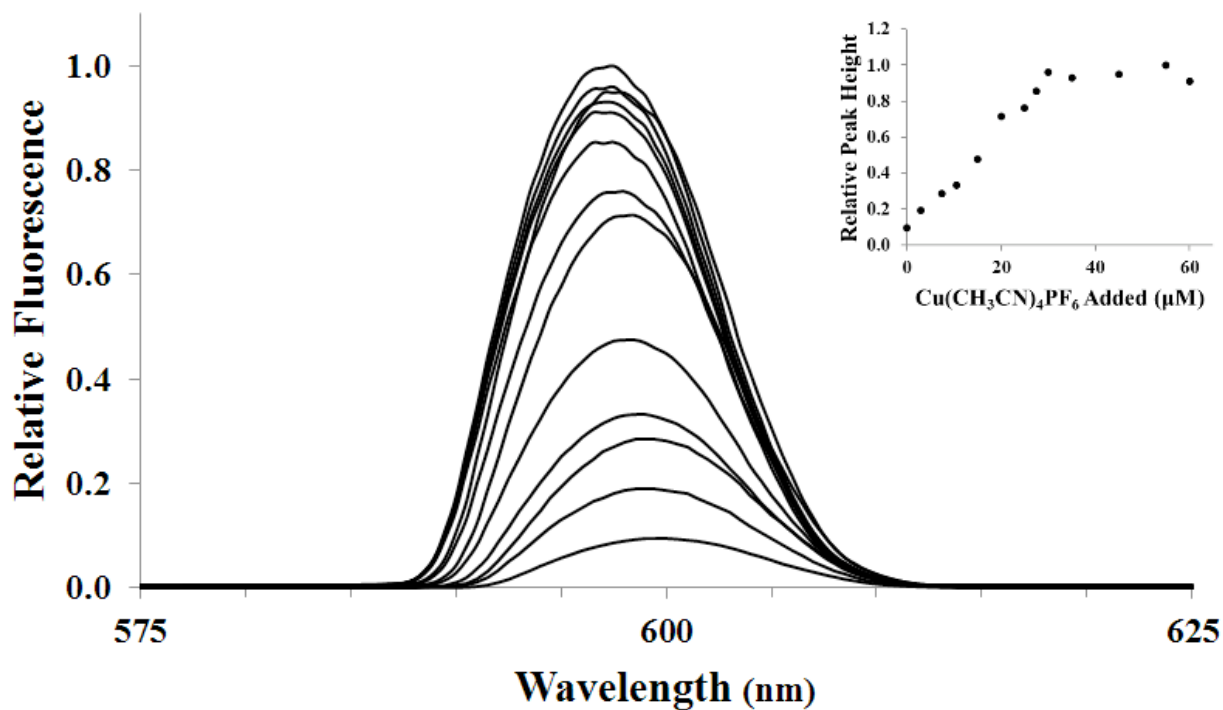


Figure S7 Luminescence measurements upon titration of Cu(I) into a 20 μM solution of B7 peptide. When the sample was excited at 295 nm, a band at 600 nm appeared that increased linearly with the amount of Cu(I) added. The signal saturated at 30 μM of Cu(I) added (inset), suggesting the formation of a 2:3 complex between peptide and copper.

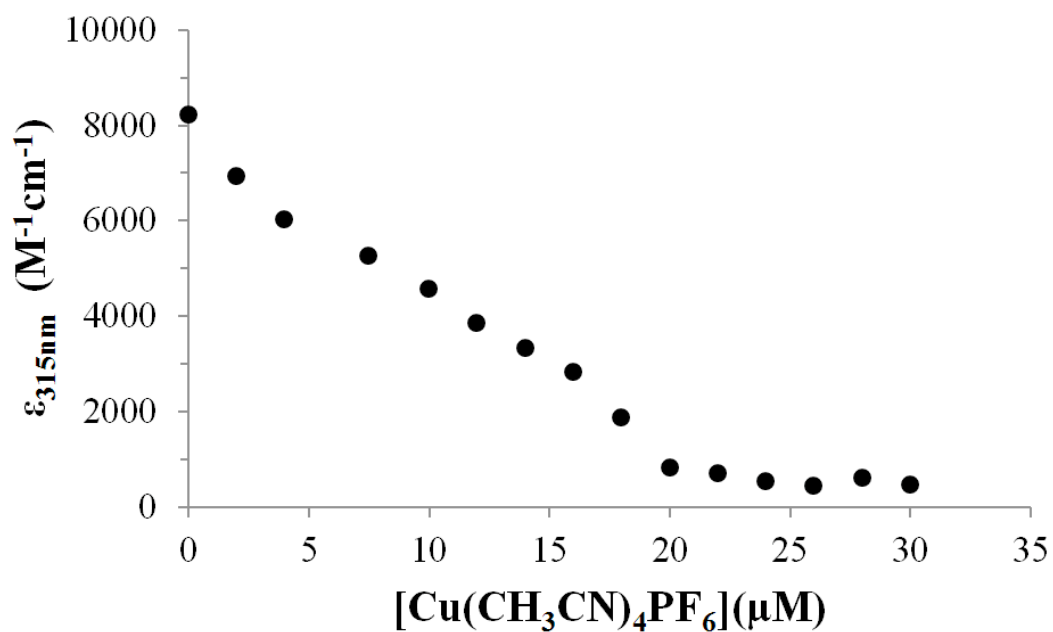


Figure S8. Titration of 20 μM B7 and 20 μM nickel sulfate with a Cu(I) salt. The signal at 315 nm was monitored, which correlates with the amount of nickel bound to B7. As increasing amounts of copper were added the nickel was displaced from B7, suggesting that Cu(I) binds more tightly than Ni(II). Similar results were obtained when the peptide was supplemented with 20 mM nickel sulfate.

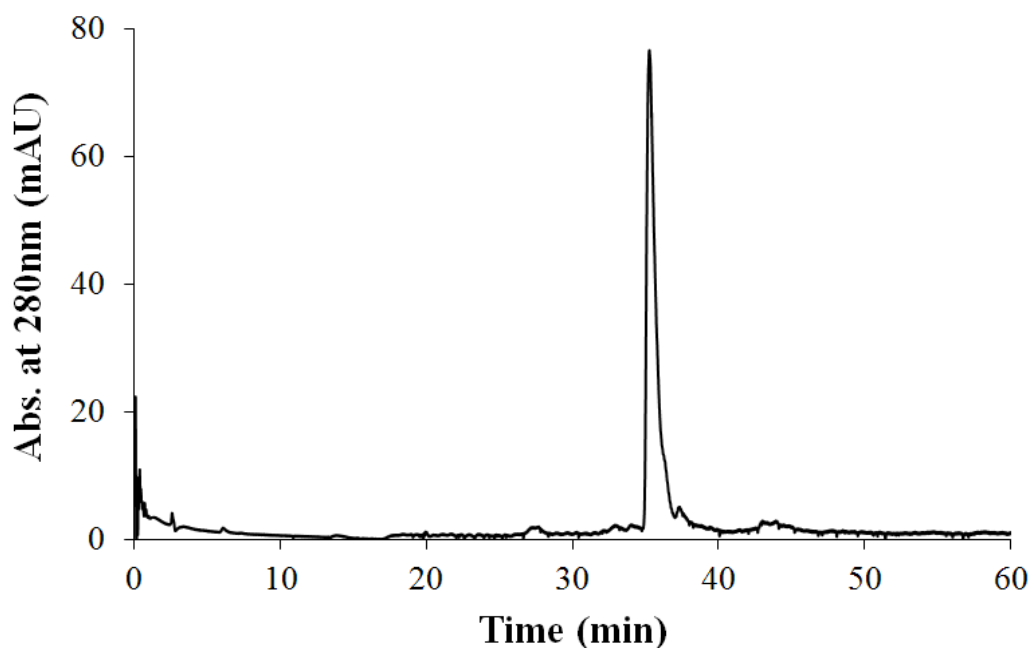


Figure S9 Representative HPLC trace of purified B7 peptide. After loading, the column was washed with water (with 0.1% trifluoroacetic acid) for ten minutes. The peptide was eluted using a gradient of acetonitrile, increasing 1.2 %/min over 40 minutes. The B7 peptide elutes at 35 minutes, at approximately 20% acetonitrile. During the final 10 minutes of the run, the column was washed with 100% acetonitrile. The flow rate during the run was 1 mL/min.

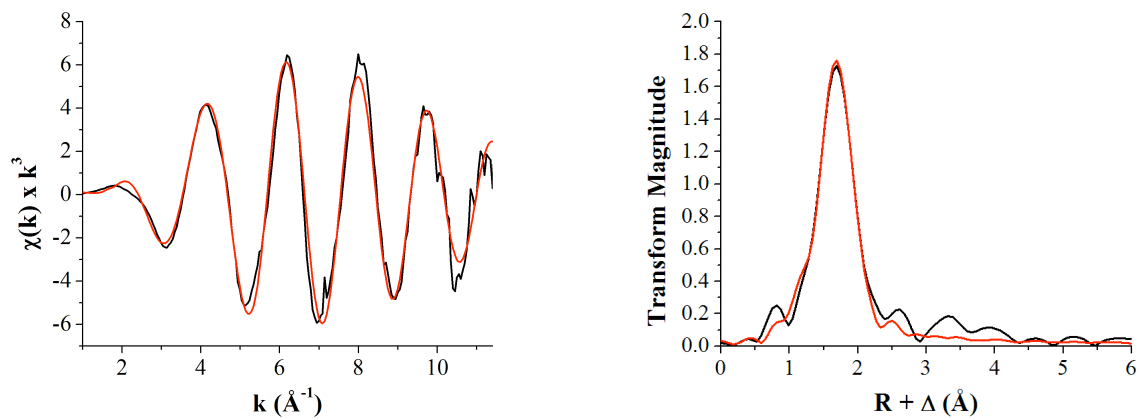


Figure S10 k^3 -Weighted Ni(II) EXAFS data (left) and Fourier transform data (right) of B7 peptide loaded with 0.8 equivalents of nickel. Black line shows the raw data, while the red line shows the best fit, according to table 1 (see above). The data were fit to a k of 11.4\AA^{-1} .