Electronic Supplementary Material (ESI) for Chemical Science

Supporting Information

Functional, metal-based crosslinkers for α-helix induction in short peptides

Sarah J. Smith, Kang Du, Robert J. Radford, and F. Akif Tezcan*
Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Dr., La Jolla, California 92093-0356

Table of Contents

Page

S3 I. General Considerations

S4 II. Synthesis of Unnatural Amino Acids

Scheme S1. Synthetic scheme for the synthesis of Iodoacetamido-8-hydroxyquinolate (IQuin)

Figure S1. ESI-MS of IQuin

Scheme S2. Synthetic scheme for the synthesis of Fmoc-E(Quin)-OH

Figure S2. ESI-MS of Fmoc-E(Quin)-OH

Scheme S3. Synthetic scheme for the synthesis of NH_2MeQuin

Figure S3. ESI-MS of NH_2MeQuin

Scheme S4. Synthetic scheme for the synthesis of Fmoc-E(MeQuin)-OH

Figure S4. ESI-MS of Fmoc-E(MeQuin)-OH

Scheme S5. Synthetic scheme for the synthesis of Re(Cl)(IQuin)$(CO)_3$

Figure S5. ESI-MS of Re(Cl)(IQuin)$(CO)_3$

S10 III. Peptide Synthesis

Figure S6: Peptide Characterization by MALDI-TOF Spectrometry and HPLC

S13 IV. Determination of metal binding affinity by UV-Vis absorbance titration

Figure S7: UV-Vis spectra shows Quin metal binding

Scheme S6: Sample titration fitting scripts in Dynafit

Figure S8: Peptide-metal binding titrations and fits

S17 V. Circular Dichroism Spectroscopy (CD)

Table S1: Percent helicity based on calculation and comparison with TFE

Figure S9: CD Spectra for peptides at 25 ^oC

Figure S10: CD Spectra for peptides at 4 ^oC

S19 VI. Fluorescence Spectroscopy

Figure S11: Absorption spectrum showing the fluorescence excitation band

Figure S12: Reversibility of fluorescence emission

Table S2: Fluorescence quantum yield values

Figure S13: Absorption spectrum of the Re-peptide complex

Figure S14: Shift of fluorescence spectrum upon His coordination after heating

S22 References

I. General Considerations

I a. Supplies

Unless otherwise stated, reagents and solvents were purchased from Fisher Scientific and used without further purification. Peptide supplies (both amino acids and resin) were purchased from Aapptec and used without further purification.

I b. HPLC Purification

Reverse-phase HPLC was performed using an Agilent Technologies 1260 Infinity HPLC equipped with a preparation-scale column (Agilent preHT, 5 μm, 21.1 x 100 mm) using 0.1% trifluoroacetic acid (TFA) in water as solvent A and acetonitrile as solvent B. Peptide absorbance was monitored at 220 nm or 254 nm (the absorbance of Quin), and fractions were manually collected. After initial purification, an analytical column (Agilent Eclipse plus C_{18}, 3.5 μm, 4.6 x 100 mm) using the same gradient to determine purity. All peptides were purified to >90% pure and the masses were verified using MALDI-TOF mass spectrometry. Preparative scale columns were run at 8 ml/min and analytical scale columns were run at 1 mL/min.

I c. Mass Spectrometry

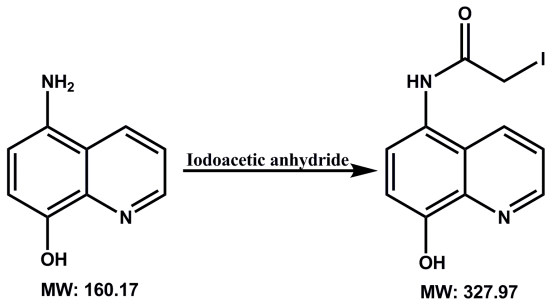
Mass spectrometry analysis was carried out at the Molecular Mass Spectrometry Facility at UCSD. Peptide mass spectrometry was performed on a Bruker Biflex IV MALDI-TOF mass spectrometer. In a typical experiment, 3 μL of a dilute peptide sample (1-10 μM) was combined with 3 μL α-cyano-4-hydroxycinnamic acid (CHCA) (Agilent) as a matrix. 3 μL of this solution was plated on a standard 288 well plate and dried completely before analysis.

Small molecule mass spectrometry was performed using electrospray ionization (ESI) on a Quattro Ultima Triple Quadrupole mass spectrometer. Samples were prepared at a concentration of 0.1-1.0 mg/mL in a 50% MeOH solution in water. Analysis was performed under both the positive and negative ion modes.

II. Synthesis of Unnatural Amino Acids

II a. Synthesis of Iodoacetamido-8-hydroxyquinolate (IQuin)

Iodoacetic anhydride was first synthesized by adding 1.19 g of iodoacetic acid (Sigma, 6.4 mmol) dissolved in 10 mL ethyl acetate to 660 mg of dicyclohexylcarboiimide (DCC) (Sigma, 3.2 mmol) dissolved in an additional 10 mL of ethyl acetate. White precipitate formed immediately and the reaction was allowed to stir for 2 hours in the dark. The white precipitate (dicyclohexylurea) was filtered and the filtrate was evaporated to dryness. Concurrently, 500 mg of 5-amino-8-hydroxyquinolate dihydrochloric acid (2.1 mmol) was added to 10 mL of acetonitrile with 1 mL triethylamine (7 mmol) and refluxed at 80 °C for 2 hours to dissolve, resulting in a clear, dark solution. Iodoacetic anhydride was then dissolved in 5 mL acetonitrile and added to the 5-amino-8-hydroxyquinolate dihydrochloric solution and the mixture was stirred overnight at room temperature in the dark. The product was isolated by filtration and washed with 5% sodium bicarbonate (aq). The resulting solid was dried *in vacuo* to afford the desired product in 80% crude yield (0.55 g, 1.67 mmol). ^{1}H NMR (500 MHz, acetonitrile-d_3): δ(ppm) 8.88 (d, J = 4.6 Hz, 1H), 8.66 (s, 1H), 8.53 (d, J = 8.6 Hz, 1H), 7.72 (m, J = 4.6, 8.6, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 3.96 (s, 2H). ESI-MS (+): *m/z* calculated for ($C_{11}H_9IN_2O$ + H) 328.9 amu $[M+H]^+$, found 329.0 amu. HPLC retention time: 4.9 min, 85% H_2O + 0.1% trifluoroacetic acid, 15% acetonitrile. As synthesized, the product was judged to be approximately 55% pure by HPLC and was used without further purification. The large side product was chloroacetamido-8-hydroxyquinolate which would also react specifically with the cysteine on the target peptides, therefore, it was decided that it was not necessary to remove it. To better structurally characterize the desired product, a small amount was purified by prep-scale HPLC and a fraction was collected that was used for NMR analysis.



Scheme 1. Synthetic scheme for the synthesis of iodoacetamido-8-hydroxyquinolate (IQuin).

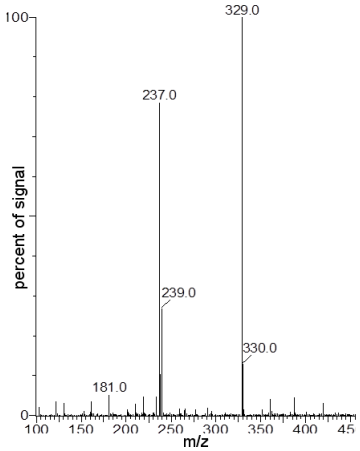
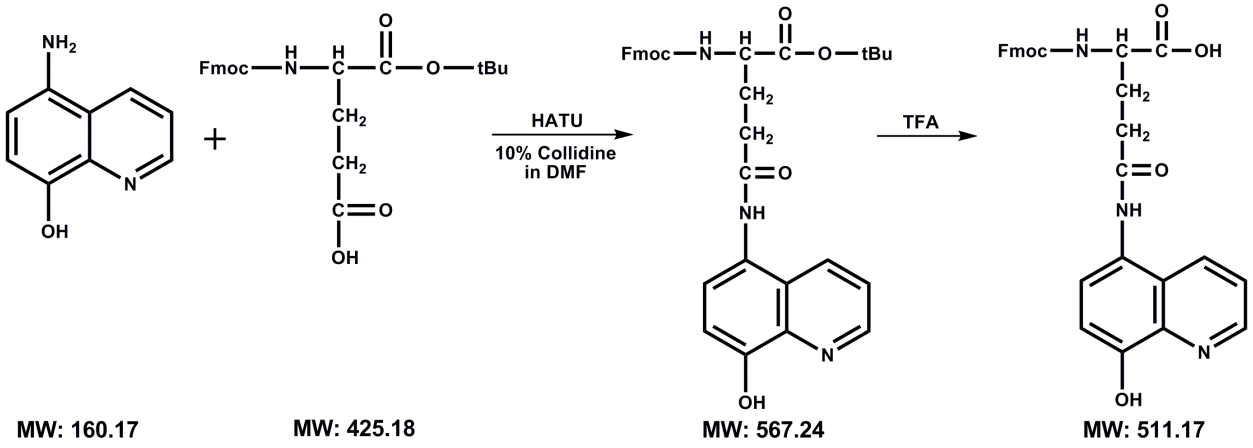


Figure S1. ESI-MS (positive mode) of Iodoacetamido-8-hydroxyquinolate (IQuin). Observed peak at 329.0 amu exp.: 328.9) $[M + H]^+$. The peak at 237.0 amu corresponds to the side product chloroacetamido-8-hydroxyquinolate (exp.: 236.7) $[M + H]^+$.

*II b. Synthesis of 5-{*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-glutamino}-8-hydroxyquinoline (Fmoc-E(Quin)-OH)*

Fmoc-Glu-OtBu (Aapptec) (1.30 g, 3.0 mmol) was mixed with HATU (1.14 g 3.0 mmol) in a round-bottom flask. The mixture was dissolved with 5 mL of 10% 2, 4, 6-collidine in DMF with stirring. In a separate vial, 5-amino-8-hydroxyquinoline (0.70 g, 3.0 mmol) was dissolved in 10% (v/v) 2, 4, 6-collidine in DMF (15 mL), yielding a dark brown solution, which was added to the Fmoc-Glu-OtBu/HATU solution with stirring. The reaction occurred overnight at room temperature. The mixture was then filtered to give a brown solution and the filtrate was evaporated to dryness, resulting in brown oil. The oil was re-dissolved in 100 mL dichloromethane (DCM) and washed with 0.1 M HCl (3 x 30 mL)and brine (2 x 30 mL). The organic phase was dried over $MgSO_4$, and evaporated to dryness, resulting in reddish brown oil. TFA (20 mL) was added to the oil and the mixture was stirred for 2 hours, at which time it was added to cold diethylether (500 mL). The solution was then stored at -80°C for 2 hours to allow for complete precipitation of the amino acid. The light yellow precipitate was collected by filtration, re-dissolved by a minimal amount of acetonitrile and evaporated to dryness, yielding a brown powder. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6): δ(ppm) 9.85 (s, 2H), 8.90 (d, J = 3.9 Hz, 1H), 8.46 (d, J = 8.6 Hz, 1H), 7.90 (d, J = 7.7 Hz, 2H), 7.75 (d, J = 7.7, 2H), 7.73 (d, J = 8.1, 1H),7.65 (q, J = 4.3 Hz, 1H), 7.49 (d, J = 8.2 Hz, 1H), 7.42 (t, J = 7.7 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 7.13 (d, J = 8.1 Hz, 1H), 4.25 (m, 3H), 4.08 (td, J = 8.2, 4.7, 1H), 2.56 (t, J = 7.7, 2H), 2.05 (td, J = 7.3, 8.1, 2H) ESI-MS (+): *m/z* calculated for ($C_{29}H_{25}N_3O_6$ + H) 512.2 amu $[M+H]^+$, found 512.1 amu. HPLC retention time: 10.3 min, 58% H_2O + 0.1% trifluoroacetic acid, 42% acetonitrile.



Scheme 2. Synthetic scheme for the synthesis of Fmoc-E(Quin)-OH.

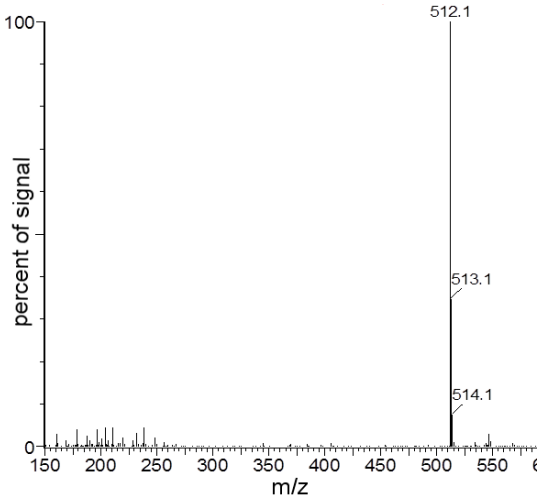
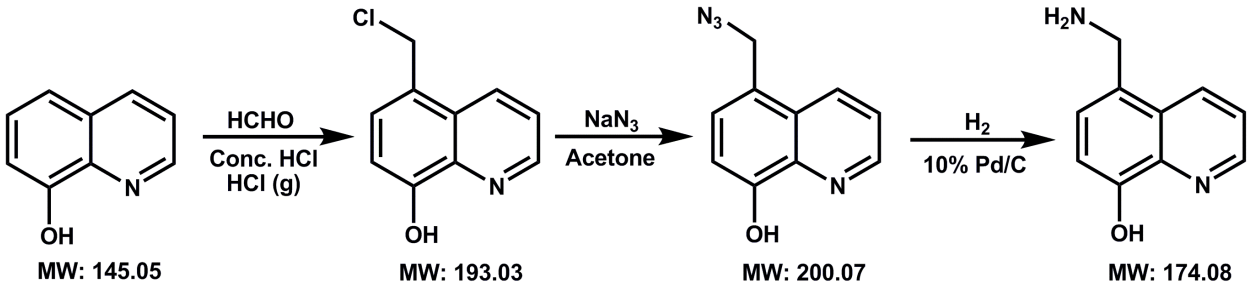


Figure S2. ESI-MS (positive mode) of 5-{N-[(9H-fluoren-9-ylmethoxy(carbonyl)]-L-glutamino}-8-hydroxyquinoline (Fmoc-E(Quin)-OH). Observed peak at 512.1 amu (exp.: 512.2) $[M + H]^+$.

II c. Synthesis of 5-Aminomethyl-8-hydroxyquinoline (NH_2MeQuin)

This synthesis was adapted from a previously published protocol.[1] 8-hydroxyquinoline (11.60 g, 80.0 mmol) was dissolved in concentrated HCl (40 mL). Formaldehyde (30% aqueous solution) (6.4 mL, 80.0 mmol, Fisher) was added to the bright yellow solution. HCl gas was generated by mixing concentrated sulfuric acid and concentrated HCl and the gas was bubbled through the 8-hydroxyquinoline solution. The reaction was held at room temperature for 30 min while a bright yellow precipitate formed. To quench the reaction, dry acetone (100 mL) was added. The precipitate was collected by filtration and washed by dry acetone (200 mL) to yield ClMeQuin in 70% yield (10.81 g, 56.0 mmol.) ClMeQuin (10.30 g, 53.0 mmol) was mixed with sodium azide (10.40 g, 159.0 mmol) in dry acetone (200 mL). The mixture was refluxed at 75 °C overnight. The resulting light green mixture was cooled to room temperature, filtered and the light green filtrate was collected. After evaporating to dryness, the product was re-dissolved in a minimal amount of DMF and then added to 1 L of cold water. A large quantity of pale green precipitate formed and was collected by filtration, to afford N_3MeQuin in 70% yield (7.47 g, 37.4 mmol). N_3MeQuin (2.00 g, 10.0 mmol) was dissolved in a minimal amount of a solution consisting of 50% methanol and 50% dichloromethane (v/v). The solution was hydrogenated under 25 psi of hydrogen gas with a catalytic amount of dry 10% palladium on activated carbon (Fisher). After 1 hour, an additional 200 mL of 50% methanol in dichloromethane was added to ensure complete solvation of the product. The catalyst was discarded by filtration and the light brown filtrate was evaporated to dryness, affording the desired product, a light yellow powder in 69% yield (1.20 g, 6.9 mmol). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6): δ(ppm) 8.84 (d of d, J = 4.4, 1.5 Hz, 1H), 8.38 (d of d, J = 8.4, 1.5 Hz, 1H), 7.55 (q, J = 4.4 Hz, 1H), 7.45 (d, J = 7.7 Hz, 1H), 7.26 (s, 2H), 7.13 (d, J = 7.7 Hz, 1H), 4.66 (s, 2H). ESI-MS (+): *m/z* calculated for ($C_{10}H_{10}N_2O$ + H) 175.1 amu $[M+H]^+$, found 175.0 amu. HPLC retention time: 6.8 min, 76% H_2O + 0.1% trifluoroacetic acid, 24% acetonitrile.



Scheme 3. Synthetic scheme for the synthesis of 5-Aminomethyl-8-hydroxyquinoline. (NH_2MeQuin)

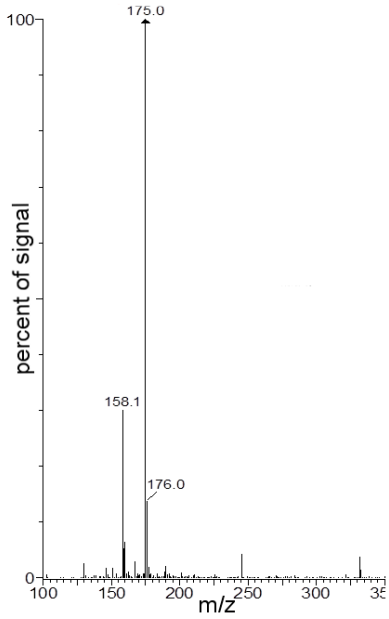
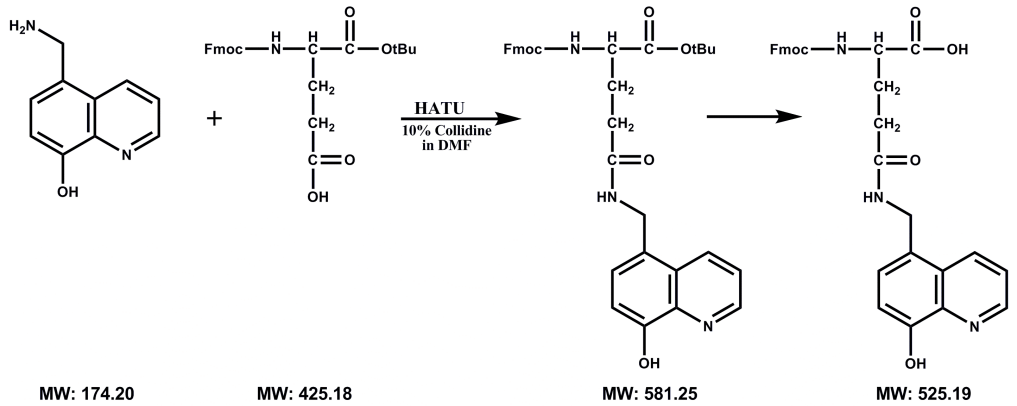


Figure S3. ESI-MS (positive mode) of 5-aminomethyl-8-hydroxyquinoline. (NH_2MeQuin). Observed peak at 175.0 amu (exp.: 175.1) $[M + H]^+$.

*II d. Synthesis of 5-{*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-glutaminomethyl}-8-hydroxyquinoline (Fmoc-E(MeQuin)-OH)*

Fmoc-Glu-OtBu (0.85 g, 2.0 mmol) and HATU (0.76 g, 2.0 mmol) were mixed and dissolved in 10% (v/v) 2,4,6-collidine in DMF (5 mL) with stirring. In a separate vial, NH_2MeQuin was suspended in 10% (v/v) 2, 4, 6-collidine in DMF (20 mL) with gentle heating to form a slurry and then transferred to the activated amino acid forming an orange, cloudy solution which cleared after 2 hours. The reaction was stirred overnight at room temperature. The resulting mixture was evaporated to dryness under constant heating and vacuum, to yield brown oil. The oil was dissolved in CH_2Cl_2 (100 mL) and then washed by 0.1 M HCl (3 x 100 mL) followed by brine (2 x 50 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness, yielding a light yellow solid. The crude product was purified using flash chromatography, eluting in 5% (v/v) methanol in dichloromethane (R_f ~0.6). The purified product was evaporated to dryness and then re-dissolved in TFA (10 mL). After 2 hours, the mixture was transferred into cold diethylether (500 mL) and stored at -80°C for 2 hours to allow for complete precipitation. The bright yellow precipitate was collected by filtration, re-dissolved in a minimal amount of acetonitrile, and evaporated to dryness, to afford the desired product, a bright yellow solid, in 40% yield. (0.42 g, 0.8 mmol). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6): δ(ppm) 8.92 (d, J = 3.7 Hz, 1H), 8.62 (d, J = 8.4 Hz, 1H), 8.35 (t, J = 5.4 Hz, 1H), 7.88 (d, J = 7.4 Hz, 2H), 7.73 (d, J = 4.4 Hz, 1H), 7.72 (d, J = 7.7 Hz, 2H), 7.69 (dd, J = 8.1, 6.7 Hz, 1H), 7.45 (d, J = 8.1 Hz, 1H), 7.40 (t, J = 7.4 Hz, 2H), 7.31

(t, *J* = 7.4 Hz, 2H), 7.13 (d, *J* = 7.7 Hz, 1H), 4.60 (d, *J* = 5.4, 2H), 4.25 (m, 3H), 3.95 (td, *J* = 8.1, 4.4 Hz, 1H), 2.20 (t, *J* = 7.7 Hz, 2H), 1.80 (td, *J* = 13.1, 7.7 Hz, 2H). ESI-MS (+): *m/z* calculated for ($C_{30}H_{27}N_3O_6$ + H) 526.2 amu $[M+H]^+$, found 526.3 amu. HPLC retention time: 9.4 min, 65% H_2O + 0.1% trifluoroacetic acid, 35% acetonitrile.



Scheme 4. Synthetic scheme for the synthesis of 5-{*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-glutaminomethyl}-8-hydroxyquinoline (Fmoc-E(MeQuin)-OH).

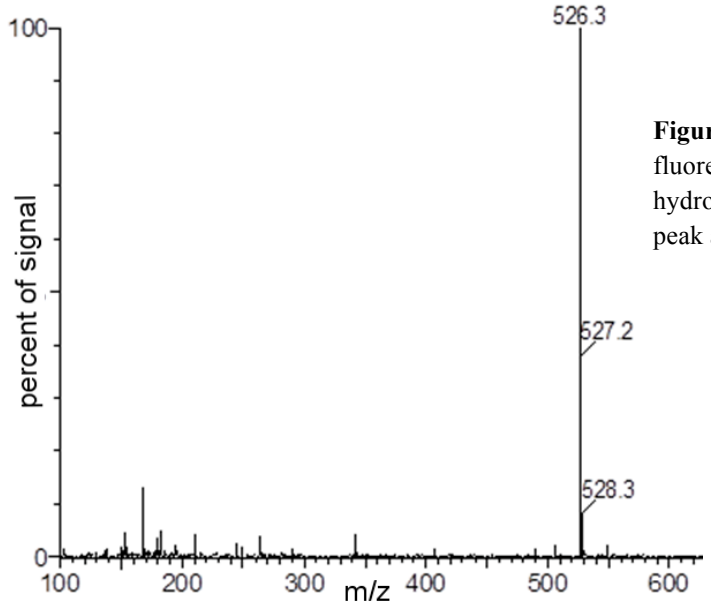
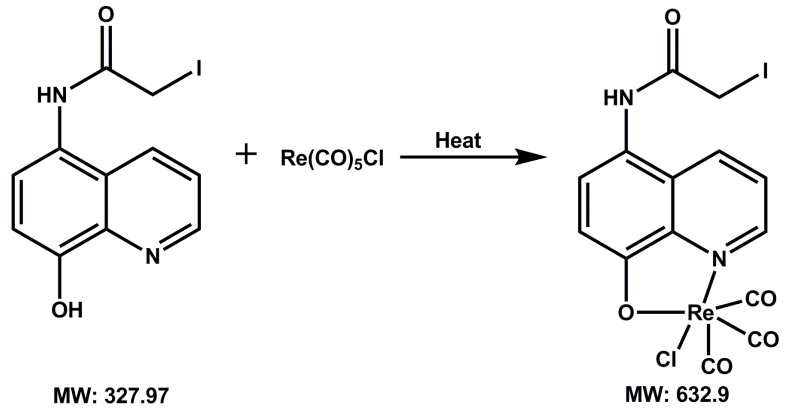


Figure S4. ESI-MS (positive mode) of 5-{N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-glutaminomethyl}-8-hydroxyquinoline (Fmoc-Glu(MeQuin)-OH). Observed peak at *m/z* = 526.3 amu (exp.: 526.2 amu) $[M + H]^+$.

II e. Synthesis of Re(Cl)(IQuin)(CO)$_3$

$Re(Cl)(CO)_5$ (0.03 g, 0.1 mmol) was placed in a round bottom flask. HPLC-purified IQuin (0.03 g, 0.1 mmol) was added to the flask, and the flask was degassed. Degassed, dry acetonitrile (50 mL) was then added, and the reaction was refluxed under argon overnight in the dark. The following day, the reaction was slowly cooled to room temperature and the reaction mixture was then poured into ice cold hexanes. The solvents were removed in vacuo and the product was redissolved in 50% acetonitrile and 50% water + 0.1% trifluoroacetic acid and then HPLC purified on a preparative scale column as discussed in "General Considerations," using a gradient of 5% solvent B to 40% solvent B over 25 min. The product eluted from the column in 35% acetonitrile and was yellow in color. The HPLC fractions

containing the product were dried by lyophilization. Despite presence of an impurity (ESI-MS (-): *m/z* 511.0 amu), peptide labeling was performed (see Section III c.) and the labeled peptide was purified to homogeniety (See Figure S6h). ^{1}H NMR (500 MHz, acetonitrile-d_3): δ(ppm) 8.95 (d, J = 5.2 Hz, 1H), 8.47 (s, 1H), 8.40 (d, J = 8.6 Hz, 1H), 7.56 (m, J = 5.2, 8.6, 1H), 7.38 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 3.95 (s, 2H). ESI-MS (-): *m/z* calculated for ($C_{14}H_8IN_2O_5ReCl$ + H) 632.9 amu $[M]^-$, found 633.0 amu, 629.0 amu $[M - Cl + MeOH]^-$, 597.0 amu $[M - Cl]^-$, 541.1 amu $[M - I + Cl]^-$. IR peaks: 1901, 1917, 2023 cm^{-1}.



MW: 327.97 MW: 632.9

Scheme 5. Synthetic scheme for the synthesis of Re(Cl)(IQuin)(CO)$_3$.

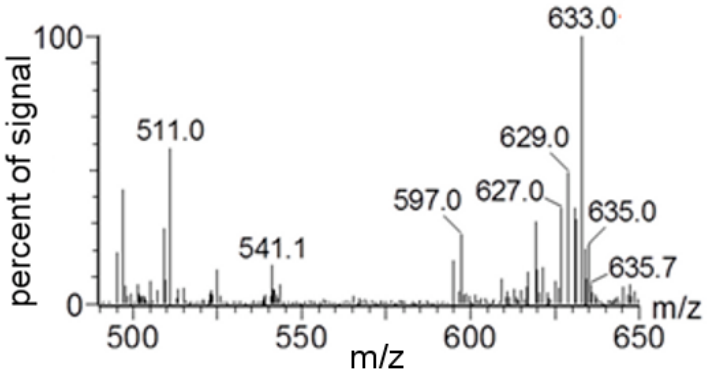


Figure S5. ESI-MS (negative mode) of Re(Cl)(IQuin)(CO)$_3$. Observed peak at *m/z* = 633.0 amu (exp 632.9) $[M]^-$, 629.0 amu (exp 628.9) $[M - Cl + MeOH]^-$, 597.0 amu (exp 596.9) $[M - Cl]^-$, 541.1 amu (exp 540.9) $[M - I + Cl]^-$.

III. Peptide Synthesis

III a. General protocols for Solid Phase Peptide Synthesis

Peptides were synthesized using a MilliPore 9050 or an Aapptec Focus XC peptide synthesizer. All peptides were *N*-terminal acylated and *C*-terminal amidated. Standard FMOC chemistry was used for synthesis, as glutamate-derived non-natural amino acids were incorporated in the same way as natural amino acids.

For each amino acid addition, the FMOC protecting group on the N-terminus of the growing peptide chain was first removed with a solution of 20% (v/v) 4-methylpiperidine in dimethylformamide (DMF). A 4-fold excess of an FMOC-protected amino acid containing a free C-terminus was then added in a solution of 4 M *N,N*-Diisopropylethylamine (DIPEA) in DMF with an equimolar amount of HATU and coupling was allowed to proceed for 40 min with shaking. The resin was then washed with DMF and the cycle was repeated until all amino acids were incorporated. Unnatural amino acids were incorporated during peptide synthesis using the same protocol as for natural amino acids. After the final step of synthesis, the *N*-terminus of each peptide was acylated with a mixture of 0.5 M acetic acid anhydride, 0.5 M N-hydroxybenzaldehyde (HOBt) and 10% (v/v) dichloromethane in DMF.

The peptide resin was removed from the synthesizer and dried *in vacuo*. The peptide was then cleaved from the resin using a cleavage cocktail containing 5% (v/v) thioanisole, 3% (v/v) ethane dithiol and 2% (v/v) anisole in trifluoroacetic acid (TFA) over a period of two hours. After cleavage was complete, the peptide solution was filtered to remove the resin and the filtrate was added to a solution of cold ether and incubated at -80 °C overnight to precipitate the peptide from solution. The solution was then filtered, the peptide was collected as a precipitate, and dried *in vacuo*. The crude solid was redissolved in water, and purified via HPLC.

III b. Functionalization of cysteine-containing peptides (H-CQuin, H-CQuin-A, A-CQuin)

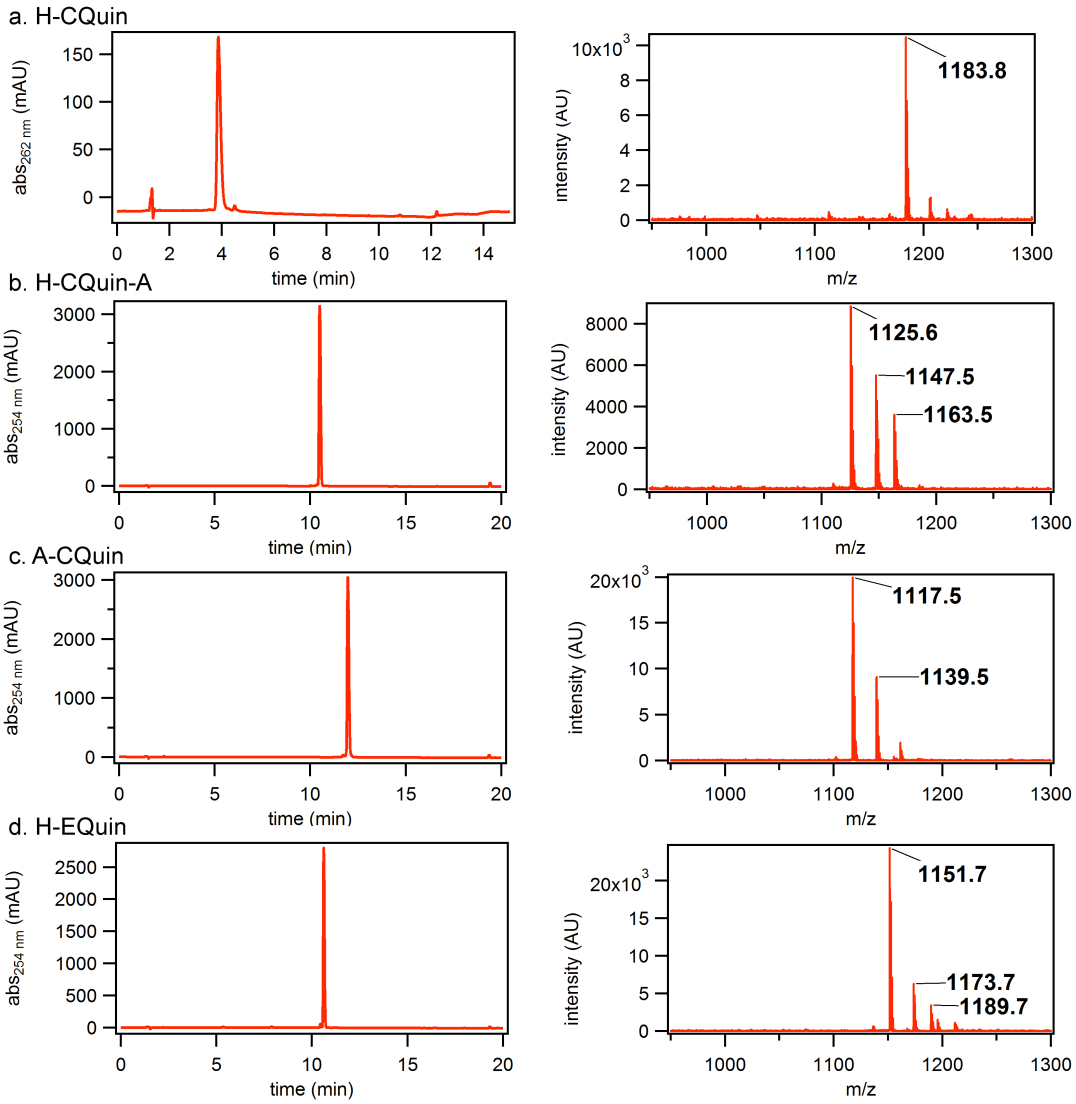
Under an argon atmosphere, 20 mg of each peptide [HCys, HCys-A, or ACys] was dissolved in 3 mL of degassed 10 mM HEPES buffer (pH 7.5) with constant stirring. A 3- to 5-fold excess of iodoacetamido-8-hydroxyquinolate (Iquin) was dissolved in 1 mL degassed DMF and added dropwise to the peptide solution. The mixture was stirred in the dark for 4 hours. The resulting dark brown solution was centrifuged and the supernatant was collected. The solution was then run on a PD MidiTrap G-10 desalting column (GE Healthcare) to remove excess IQuin and DMF. The resulting crude peptide solution was purified by HPLC, and the mass of the labeled peptide was verified by MALDI-TOF mass spectrometry.

III c. Incorporation of Re(Cl)(IQuin)(CO)$_3$

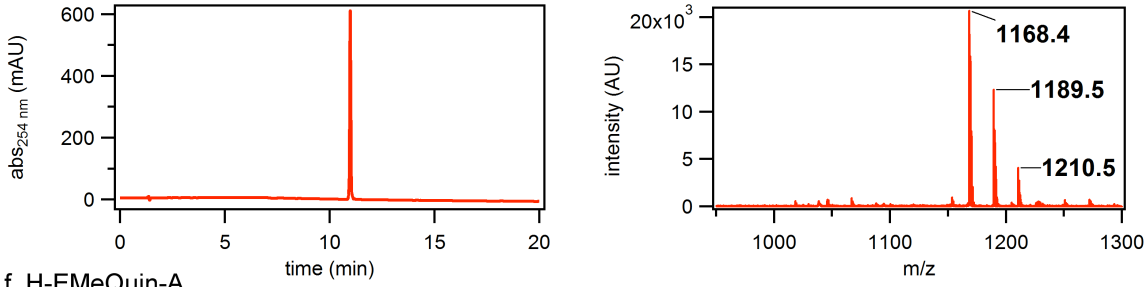
Under an argon atmosphere, 5 mg of the HC peptide was dissolved in 0.1M HEPES at pH 7.4. Re(Cl)(IQuin)(CO)$_3$ was dissolved in acetonitrile and added to the peptide. The reaction mixture stirred at room temperature in the dark for 4 hours and the resulting product was purified twice by HPLC, and the correct product was verified by mass spectrometry. The observed mass corresponded to the product without a chloride coordinated to the Re center. It was not determined whether this was due to replacement of the chloride ion by histidine, due to the mass spectrometry conditions themselves, or due to replacement of the chloride ion by a solvent species. However, there were obvious spectral changes (fluorescence, CD, UV-vis) observed when the peptides were later heated which corresponded to His coordination, indicating that His had not coordinated to the Re center at that point. Finally, the peptide

was heated to 65 °C for 30 min, and the coordination of the His residue was observed by CD, UV-vis, and fluorescence spectroscopy. Histidine coordination also occurred by leaving the peptide at room temperature for approximately one to two hours.

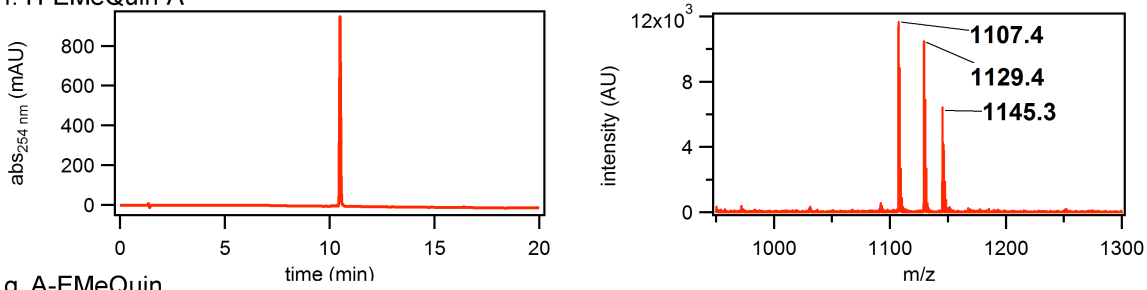
Figure S6. Analytical HPLC traces and MALDI-TOF spectra of various peptide constructs. HPLC gradient used: 1. 0 to 5 min, 95% solvent A, constant. 2. 5 to 20 min, 95% to 60% solvent A, gradient. except for H-CQuin and H-CQuin(Re): 1. 0 to 2 min, 90% solvent A, constant. 2. 2 to 12 min, 90% to 55% solvent A, gradient. **(a)** H-CQuin: Expected: 1185 amu. Observed: 1183.8 $[M + H]^+$. **(b)** H-CQuin-A: Expected 1128 amu. Observed: 1125.6 $[M + H]^+$, 1147.5 $[M + Na]^+$, 1163.5 $[M + K]^+$. **(c)** A-CQuin: Expected 1119 amu. Observed: 1117.5 $[M + H]^+$, 1139.5 $[M + Na]^+$. **(d)** H-EQuin: Expected 1153 amu. Observed: 1153.5 $[M + H]^+$, 1176.6 $[M + Na]^+$, 1191.9 $[M + K]^+$. **(e)** H-EMeQuin: Expected 1167 amu. Observed: 1166.7 $[M + H]^+$, 1187.7 $[M + Na]^+$, 1203.7 $[M + K]^+$. **(f)** H-EMeQuin-A: Expected 1110 amu. Observed: 1107.4 $[M + H]^+$, 1129.4 $[M + Na]^+$, 1145.4 $[M + K]^+$. **(g)** A-EMeQuin: Expected 1099 amu. Observed: 1099.7 $[M + H]^+$, 1121.6 $[M + Na]^+$, 1137.6 $[M + K]^+$. **(h)** H-CQuin(Re)CO_3: Expected 1455 amu. Observed: 1452.7 $[M + H]^+$. **(i)** Bax-HCM: Expected 3302 amu. Observed: 3310.2 amu $[M + H]^+$.



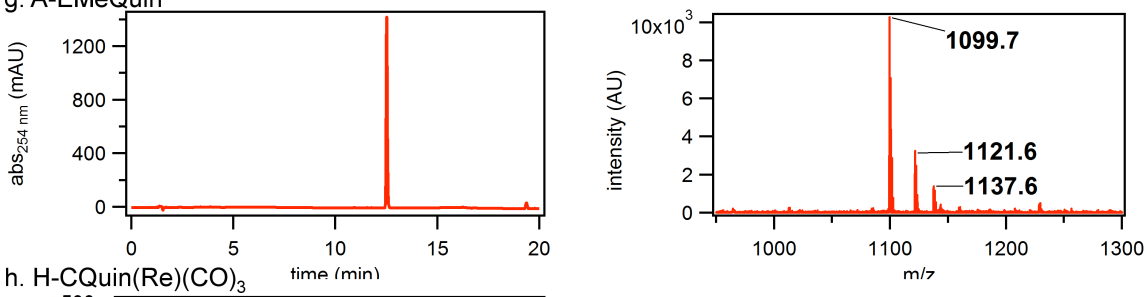
e. H-EMeQuin



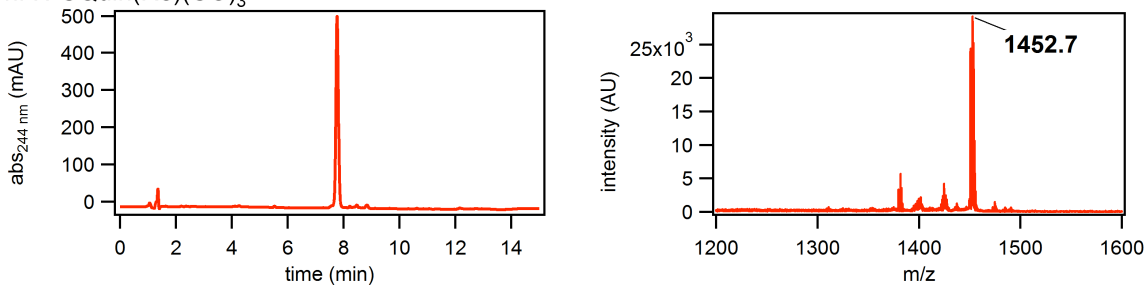
f. H-EMeQuin-A



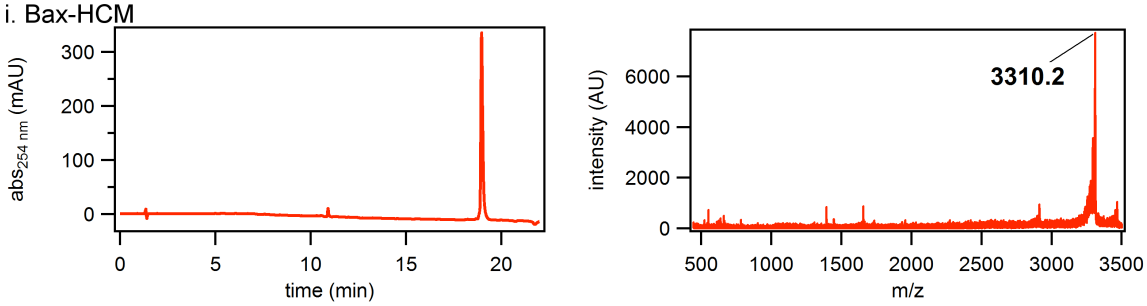
g. A-EMeQuin



h. H-CQuin(Re)(CO)$_3$



i. Bax-HCM



IV. Determination of metal binding affinity

Metal binding titrations were performed by monitoring the Quin π–π* absorption band, which shifts approximately 20 nm upon metal binding (Figure S6a). 1-mL samples were prepared containing 10-20 μM peptide; peptide concentrations were determined using the extinction coefficient ε_{244} = 22,000 $M^{-1}cm^{-1}$. Each sample was prepared in 5 mM MOPS buffer at pH 7.1 pretreated with Chelex resin (BioRad) with a 3-fold excess of chelator (30-60 μM). All pipet tips were rinsed 3 times with analytical grade 10% HNO_3 (Fluka) before use. For the Ni^{II} and Cu^{II} samples, ethylene glycol tetraacetic acid (EGTA) was used as a competing chelator, and for the Zn^{II} and Co^{II} samples, N-(2-acetamido)iminodiacetic acid (ADA) was used. 2 mM metal stocks were prepared and added stepwise to the peptide solution so that the total amount never exceeded 50 μL (5% of the total volume); each sample was equilibrated for 3 min with stirring after metal addition before the absorbance was measured. UV-vis measurements were performed on a Hewlett Packard 8452A diode array spectrophotometer. The largest changes in absorbance were measured at 262 nm upon the addition of metal, and these values were plotted as a function of M^{II} concentration (Figure S6b). All spectra were corrected for dilution. M^{II} dissociation constants for either EGTA or ADA were calculated using MaxChelator (http://maxchelator.stanford.edu) and fixed during data fitting. The titration data were separately fit to two models using non-linear regression through Dynafit 3 (Biokin), where one model assumes a 1:1 peptide:metal stoichometry while the other model takes account of the possibility of metal-induced peptide dimerization (1:1 and 2:1 peptide:metal stoichometry) (Scheme S5, Figure S7).

Scheme S6. Dynafit scripts for describing metal-peptide binding equilibria. Two models for binding were used: **(a)** A 1:1 peptide:metal competitive binding model, and: **(b)** A 1:1 and 2:1 peptide:metal competitive binding model, taking into account the possibility for metal-induced peptide dimerization at limiting metal concentrations. Metal-chelator dissociation constants were obtained using MaxChelator and held fixed. The variables used include: peptide (p), metal (m), peptide-metal complex (pm), metal-induced peptide dimer (d), chelator (e), and metal-chelator complex (em). Parameteres that were allowed to float during the fitting process are followed by "?" and all other parameters were held fixed.

a.

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

[mechanism]
p + m <==> pm     :     kd1 dissoc
e + m <==> em     :     kd2 dissoc

[constants]
kd1  = 5e-8 ?
kd2 = 2.803e-9

[concentrations]
p = 13.8e-6
e = 45e-6

[responses]
p = 0
Pm = 15000
```

b.

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

[mechanism]
p + m <==> pm     :     kd1 dissoc
e + m <==> em     :     kd2 dissoc
pm + p  <==> d    :     kd3 dissoc

[constants]
kd1 = 1e-10 ?
kd2 = 2.803e-9
kd3 = 1e-5 ?

[concentrations]
p = 13.8e-6
e = 45e-6

[responses]
p = 0
pm = 15000
d = 30000 ?
```

Figure S7. UV-vis spectral changes upon Quin-M^{II} coordination. A solution containing 16 µM H-EMeQuin and 45 µM EGTA was titrated with increasing amounts of M^{II}. **(a)** An approximately 20 nm red-shift is observed upon Ni^{II} binding, shifting the maximum absorbance of the π–π* transition from approximately 244 nm to 262 nm. **(b)** The difference spectrum between the first, completely metal-free spectrum and the last, completely Ni^{II}-bound spectrum. **(c)** The spectral shift as a result of Co^{II} binding. **(d)** The spectral shift as a result of Cu^{II} binding. **(e)** The spectral shift as a result of Zn^{II} binding.

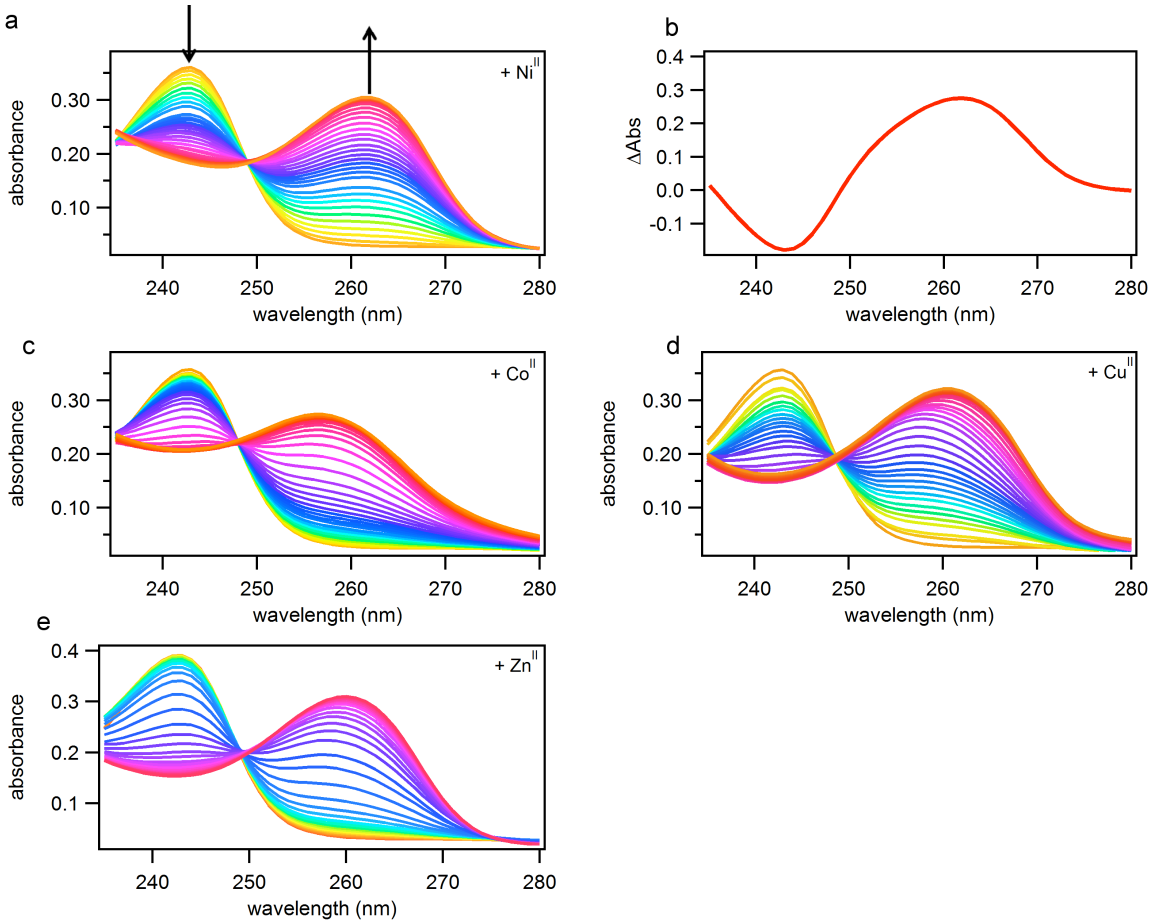
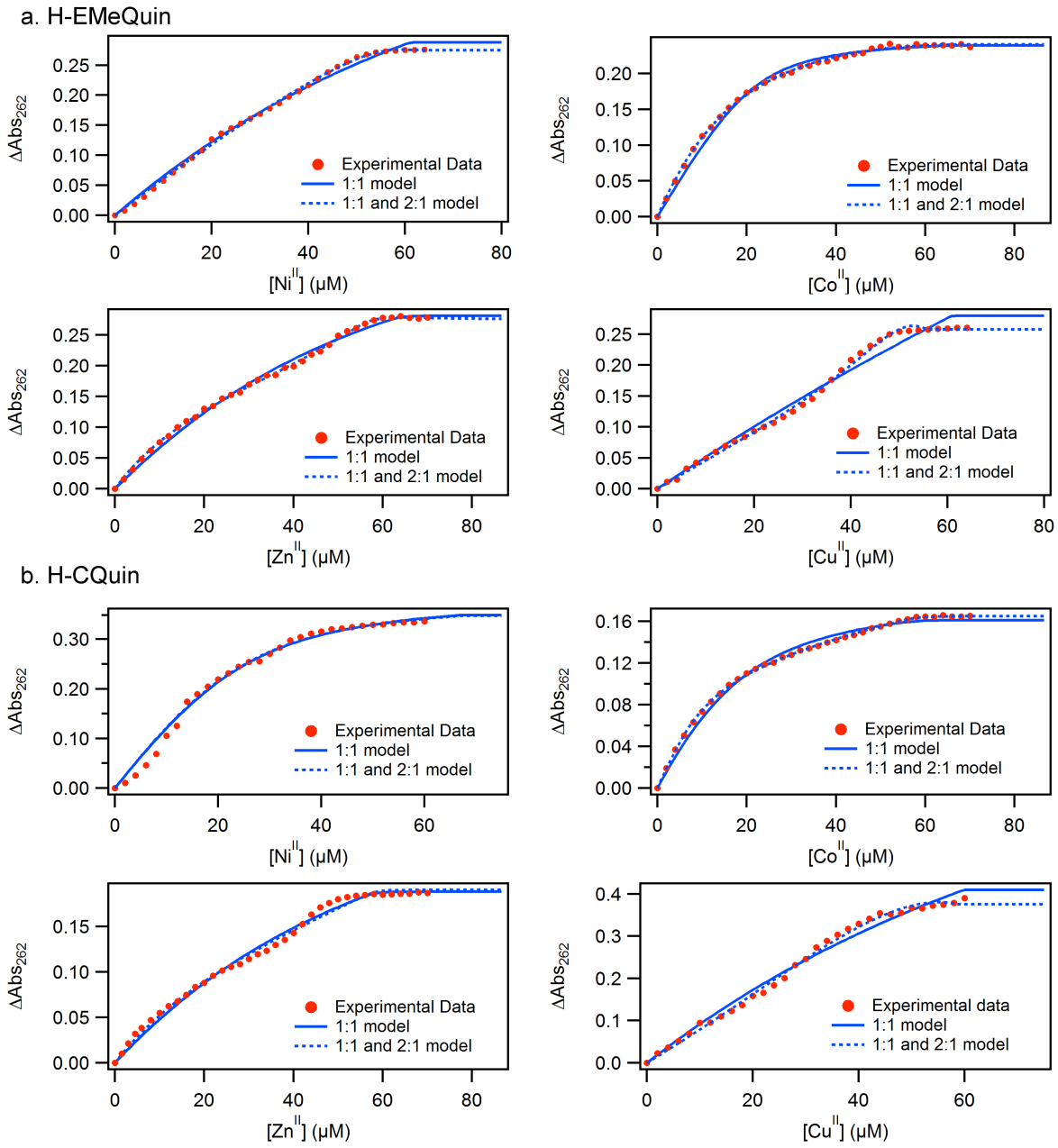
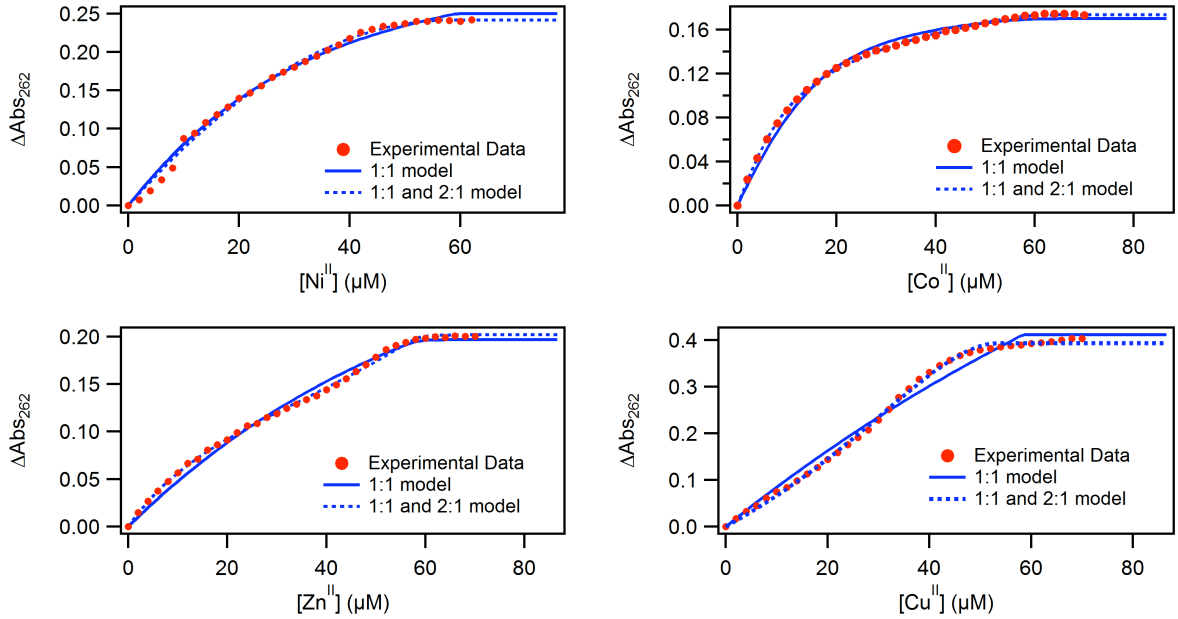


Figure S8. Peptide-metal binding titrations and fits. Titrations of **(a)** H-EMeQuin, **(b)** H-CQuin, and **(c)** H-EQuin with late first row transition metals as monitored by UV-vis spectroscopy. Two different models were used for fitting, one assuming a 1:1 binding stoichometry between the peptide and metal (solid blue line), and the second accounting for the possibility of metal-induced dimerization (dotted blue line). The 1:1 and 2:1 model fit the data better, indicating that a peptide-metal dimer was likely forming at low metal concentration. In all cases, the values for metal-binding obtained through the 1:1 and 2:1 model were reported.



c. H-EQuin



V. Circular Dichroism Spectroscopy (CD)

Samples for circular dichroism spectroscopy (CD) analyses contained 10 μM peptide in 5 mM sodium borate buffer at pH 7.5. The buffer had previously been treated using Chelex resin (BioRad) and all pipet tips were washed 3x with 10% analytical grade nitric acid (Fluka) before use. Peptide concentration was calculated by measuring the absorbance on a UV-vis spectrometer at 244 nm and calculating the corresponding concentration using an extinction coefficient of ε_{244} = 22,000 cm^{-1} M^{-1} for the metal-free peptide. A 10 μM peptide stock was first prepared, and then split into five 750 μL samples. To each sample, a 3-fold excess of either EDTA or M^{II} was added to ensure the formation of a completely metal-free or metal-bound peptide, respectively. 5 mM metal stock solutions had previously been made using $NiCl_2$, $CuSO_4$, $ZnCl_2$, or $CoCl_2$. CD spectra were measured using an Aviv 215 spectrometer. The CD spectrum of each sample was measured from 190-260 nm, using a slit width of 1 nm, scanning at 1 nm intervals with a 1 s integration time. Measurements were taken at both 25 °C and 4 °C with constant stirring. Each measurement was repeated five times, averaged and smoothed with a binomial function, and corrected for any background signal from the buffer solution. For samples containing trifluoroethanol (TFE), peptides were prepared both with a 3-fold excess of EDTA and with a 3-fold excess of Cu^{II} in 60% TFE, but no notable difference was observed whether the peptide was metal-free or metal-bound.

Table S1. Percent helicities for various metal-peptide complexes calculated (top) by comparison with a sample containing 60% TFE, and (bottom) using the ratio $[\theta]_{222}/[\theta]_{max}$. Here $[\theta]_{222}$ is the molar ellipticity measured at 222 nm, and $[\theta]_{max} = (-44000 + 250T)(1/k/n)]$, where k is a constant and equal to 4, and n is the number of amide bonds and equal to 10.[2]

Peptide	Cu^{II} (4 °C)	Zn^{II}(4 °C)	EDTA(4 °C)	Cu^{II}(25 °C)	Zn^{II}(25 °C)
Percent Helicity, vs. TFE					
H-CQuin	69.9	42.3	18.8	53.4	32.7
H-CQuin-A	83.1	69.0	8.9	65.6	52.2
H-EMeQuin	102.2	54.7	23.3	88.3	40.9
H-EMeQuin-A	87.1	66.4	25.8	70.1	52.4
Percent Helicity, calculated					
H-CQuin	34.0	20.6	9.1	26.2	15.9
H-CQuin-A	41.2	34.2	4.4	32.5	25.9
H-EMeQuin	65.5	35.0	14.9	51.8	24.0
H-EMeQuin-A	53.4	40.7	15.8	43.0	32.1

Figure S9. CD spectra of various peptides at 25 °C. Each sample was prepared with 10 µM peptide and 30 µM of either M^{II} or EDTA. For each graph, EDTA = black, Ni^{II} = green, Co^{II} = red, Cu^{II} = blue, Zn^{II} = pink, and TFE = cyan. TFE samples were prepared with metal-free peptide in 40% TFE. No further induction of helicity was observed upon the addition of metal to the TFE sample.

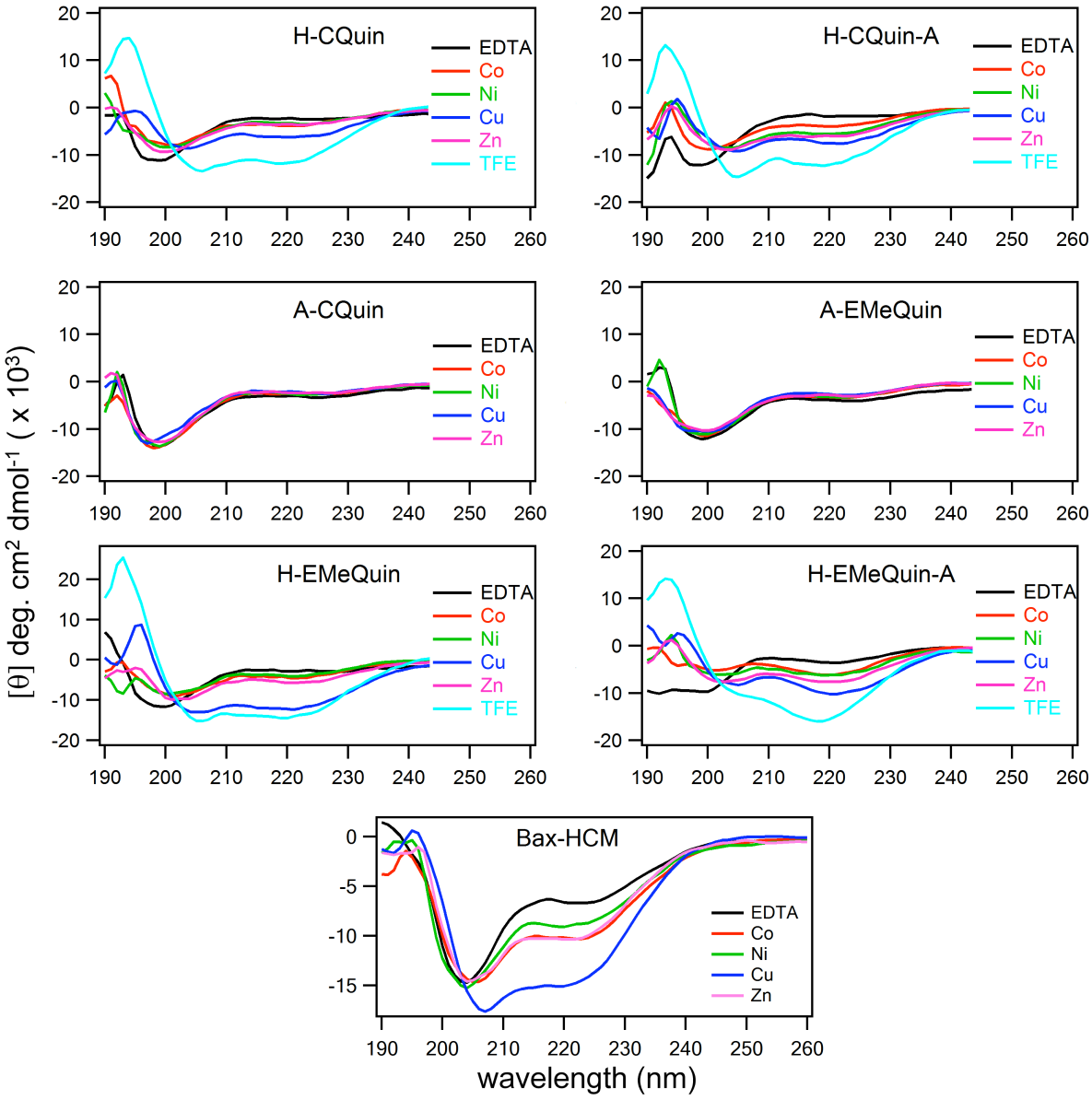
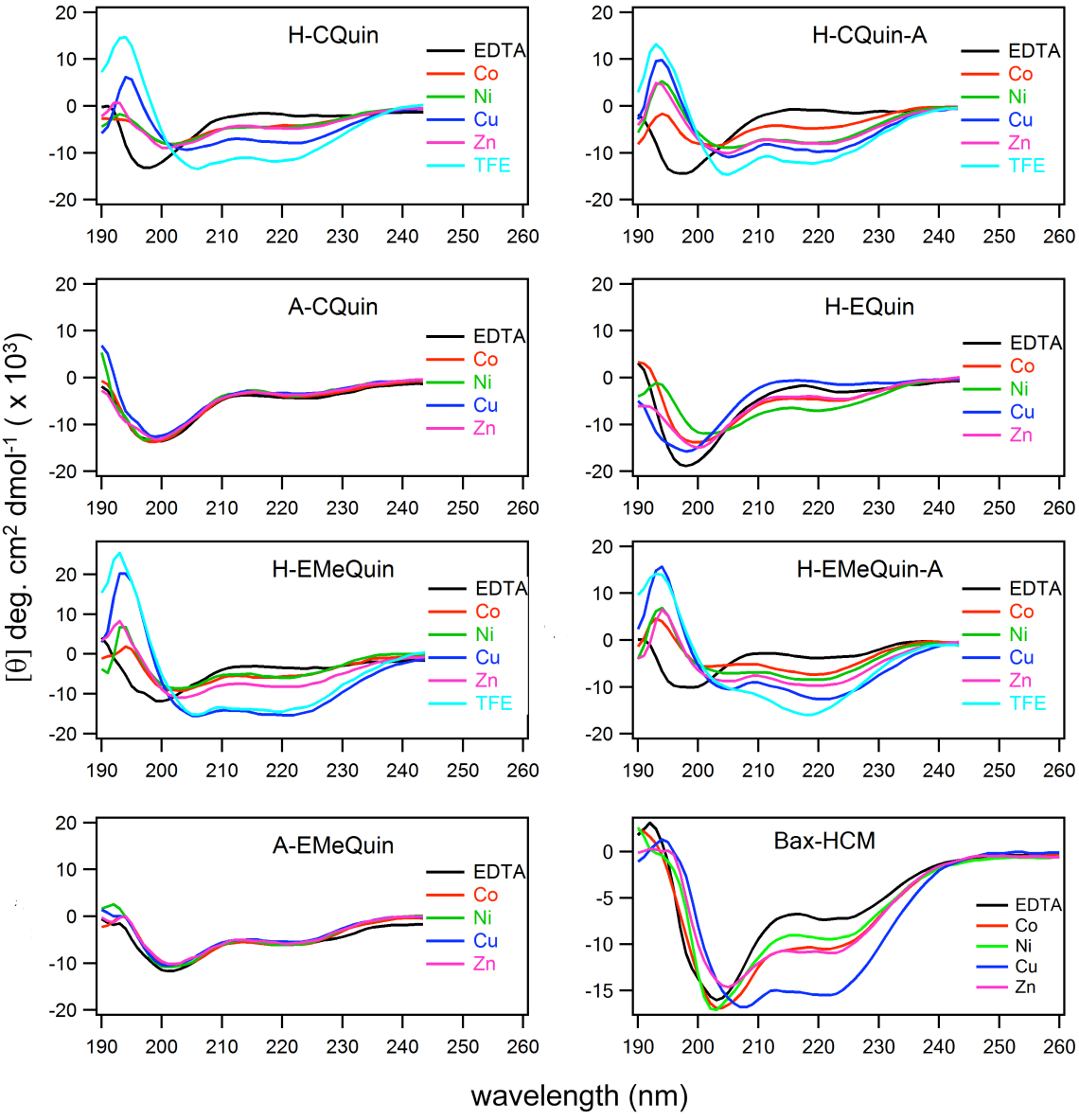


Figure S10. CD spectra of various peptides at 4 °C. Each sample was prepared with 10 µM peptide and 30 µM of either M^{II} or EDTA. For each graph, EDTA = black, Ni^{II} = green, Co^{II} = red, Cu^{II} = blue, Zn^{II} = pink, and TFE = cyan. TFE samples were prepared with metal-free peptide in 40% TFE. No further induction of helicity was observed upon the addition of metal to the TFE sample.



VI. Fluorescence Spectroscopy

Samples containing approximately 10 μM of peptide were prepared in a solution of 5 mM sodium borate (pH 7.5). Each sample also contained a 3-fold excess of either Zn^{II} or EDTA. In order to determine the quantum yield of each peptide construct when bound to Zn^{II}, quinine sulfate in 0.1 M H_2SO_4 was used as a standard. The absorbance of both the quinine sulfate standard and each peptide were measured at 375 nm, with initial absorption values ranging from 0.3 to 0.5. Each solution was then diluted 10-fold for fluorescence measurements. For each sample, the excitation wavelength used was 375 nm with a slit width of 2 nm, and emission was measured between 290 and 725 nm with a slit width of 2 nm and an integration time of 0.2 s; each measurement was repeated in triplicate. The following equation was used to calculate the quantum yield for each measurement, where Φ_F is the quantum yield, A is the absorption at the excitation wavelength, F is the area under the emission curve, and n is the refractive index of the solvents (in this case, the same solvent was used for each sample, so that term is equal to 1).[3]

$$\Phi_{F(X)} = (A_S/A_X)(F_X/F_S)(n_X/n_S)^2\Phi_{F(S)}$$

In order to determine the x-fold fluorescence turn-on for each peptide, fluorescence emission spectra were measured for both metal-free and Zn^{II} bound peptides, and the emission value at 550 nm for the Zn^{II} bound peptides were divided by the emission value at 550 nm for the metal-free samples. H-EMeQuin samples were also prepared containing a 3-fold excess of either Co^{II}, Ni^{II}, or Cu^{II}, and it was observed that no fluorescence turn-on was observed with these other metals.

Figure S11. UV-vis absorption spectrum of Zn-HCQuin. Inset: The absorption band at 380 nm used for fluorescence excitation.

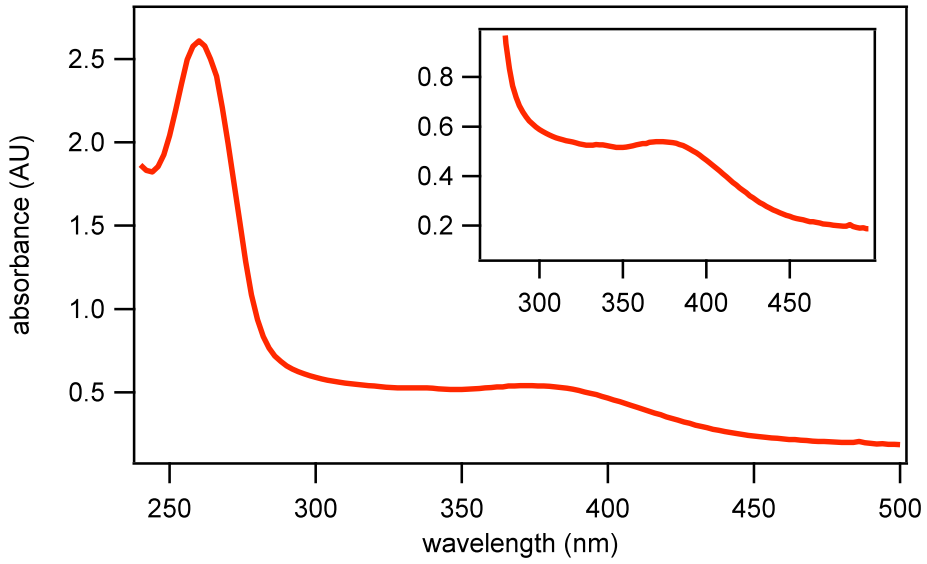


Figure S12. Reversibility of Zn^{II}-induced H-EMeQuin fluorescence .

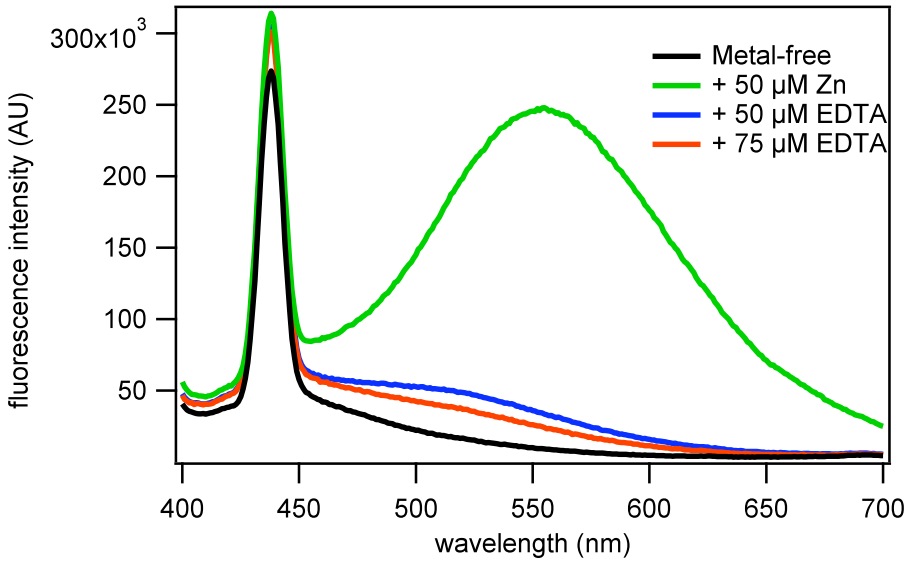


Table S2. Fluorescence quantum yields for the Zn-adducts of various peptides.

Peptide	Quantum Yield
H-CQuin	2.7(1) x 10^{-3}
H-CQuin-A	1.9(2) x 10^{-3}
A-CQuin	1.6(2) x 10^{-3}
H-EQuin	3.1(1) x 10^{-3}
H-EMeQuin	6.4(2) x 10^{-3}
H-EMeQuin-A	8.4(1) x 10^{-3}
A-EMeQuin	3.8(1) x 10^{-3}

The H-CQuin(Re)$(CO)_3$(His) peptide was prepared as described previously. In all cases, the peptide was diluted to approximately 25 µM in water in a quartz cuvette. The peptide was then heated to 65 ^{o}C, and CD, UV-vis and fluorescence spectra were taken at 30-min intervals. Any shifts observed in the measured spectra were complete by 1 hr of heating. Fluorescence spectra were measured using an excitation wavelength of 410 nm. The absorption band is shown in Figure S13. Samples were degassed and measured in an inert environment. Figure S14 shows the enhancement of emission in an oxygen-free sample.

Figure S13. UV-vis spectrum of H-CQuin(Re)(CO)$_3$(His). The inset shows a close-up of the band at 410 nm. This band was used as the excitation wavelength for luminescence.

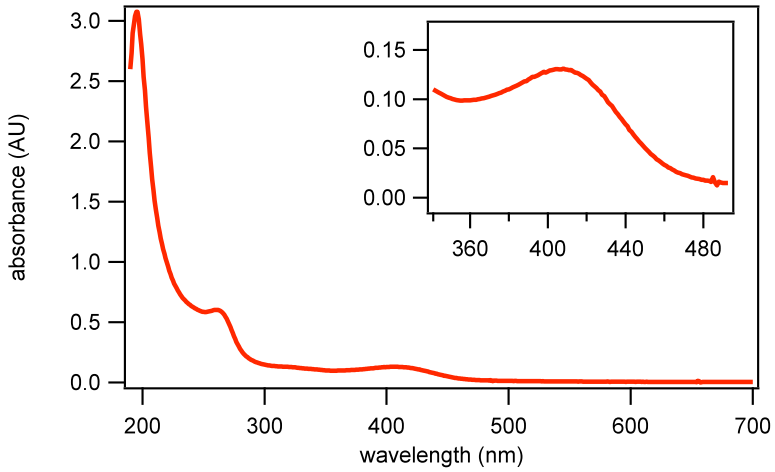
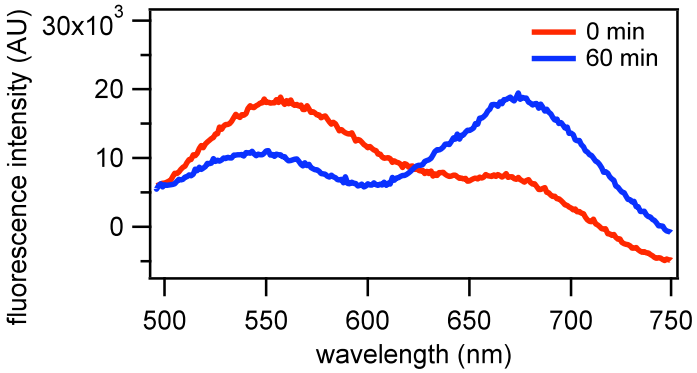


Figure S14. Changes in the H-CQuin(Re)(CO)$_3$(His) luminescence spectrum upon His coordination after heating. 25 µM peptide in 2.5 mL water. The sample was excited at 410 nm.



References

1. L. Li and B. Xu, *Tetrahedron*, 2008, **64**, 10986.
2. D. Wang, K. Chen, J. L. Kulp and P. S. Arora, *J. Am. Chem. Soc.*, 2006, **128**, 9248.
3. S. Fery-Forgues and D. Lavabre, *J. Chem. Ed.*, 1999, **76**, 1260.