

## Supplementary Information (SI)

### From cysteines to histidines - chemically distinct Cu(II)-binding motifs in the C-terminal region of archaeal CopT regulators

Wojciech Lizak,<sup>1</sup> Klaudia Kłopotowska,<sup>1</sup> Arian Kola,<sup>2</sup> Daniela Valensin,<sup>2,3</sup> Aleksandra Hecel<sup>1\*</sup>

<sup>1</sup> Faculty of Chemistry, University of Wrocław, 50383 Wrocław, Poland.

<sup>2</sup> Department of Biotechnology, Chemistry and Pharmacy, University of Siena, 53100 Siena, Italy.

<sup>3</sup> Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine (CIRMMP), Via L. Sacconi 6, Sesto Fiorentino 50019, Italy

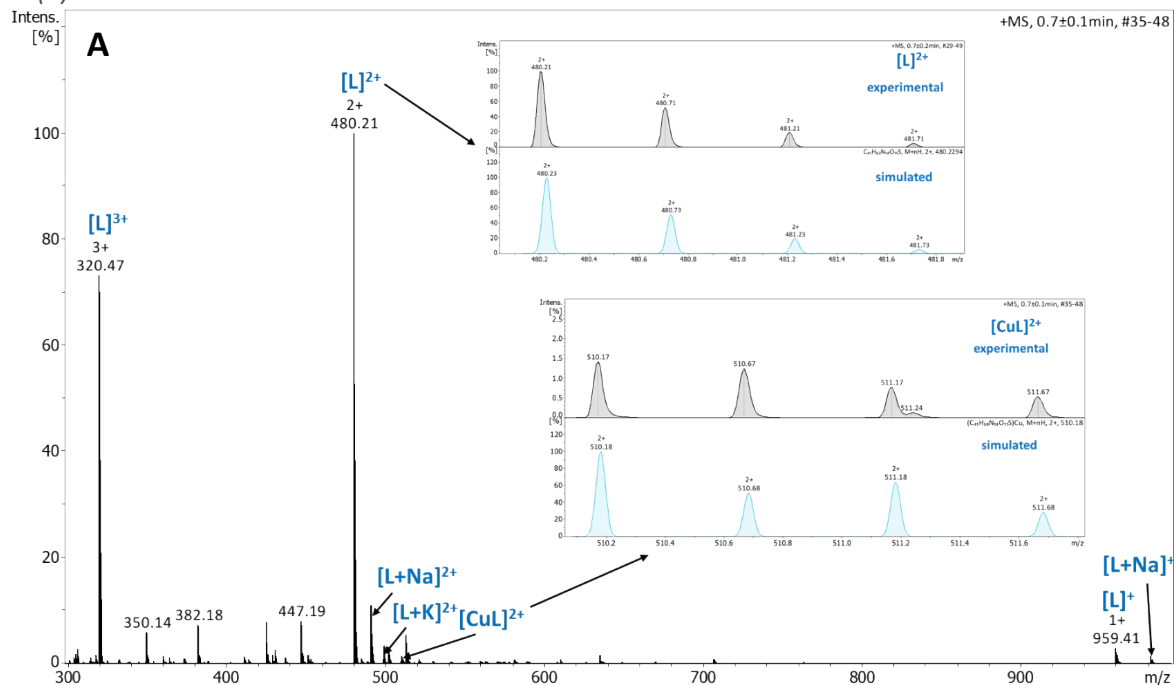
\*[aleksandra.hecel2@uwr.edu.pl](mailto:aleksandra.hecel2@uwr.edu.pl)

Table S1 Stoichiometry, molecular formula and average m/z value for the species present in ESI-MS spectra of Cu(II) complexes with the (A) Ac-GPKGMPHGH-COOH and (B) Ac-GPKGMPGEEGHH-COOH, M:L molar ratio = 0.9:1 in water:methanol 50:50 solution.

m/z	ion	Molecular formula
<b>(A) Cu(II)-Ac-GPKGMPHGH-COOH</b>		
959.41	[L] <sup>+</sup>	C <sub>41</sub> H <sub>62</sub> N <sub>14</sub> O <sub>11</sub> S
981.39	[L+Na] <sup>+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)Na
997.37	[L+K] <sup>+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)K
480.21	[L] <sup>2+</sup>	C <sub>41</sub> H <sub>62</sub> N <sub>14</sub> O <sub>11</sub> S
491.20	[L+Na] <sup>2+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)Na
499.18	[L+K] <sup>2+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)K
502.19	[L+2Na] <sup>2+</sup>	(C <sub>41</sub> H <sub>60</sub> N <sub>14</sub> O <sub>11</sub> S)Na <sub>2</sub>
510.17	[CuL] <sup>2+</sup>	(C <sub>41</sub> H <sub>59</sub> N <sub>14</sub> O <sub>11</sub> S)Cu
320.47	[L] <sup>3+</sup>	C <sub>41</sub> H <sub>62</sub> N <sub>14</sub> O <sub>11</sub> S
<b>(B) Cu(II)-Ac-GPKGMPGEEGHH-COOH</b>		
1274.50	[L] <sup>+</sup>	C <sub>53</sub> H <sub>79</sub> N <sub>17</sub> O <sub>18</sub> S
1296.48	[L+Na] <sup>+</sup>	(C <sub>53</sub> H <sub>78</sub> N <sub>17</sub> O <sub>18</sub> S)Na
1318.46	[L+2Na] <sup>+</sup>	(C <sub>53</sub> H <sub>77</sub> N <sub>17</sub> O <sub>18</sub> S)Na <sub>2</sub>
1335.41	[CuL] <sup>+</sup>	(C <sub>53</sub> H <sub>77</sub> N <sub>17</sub> O <sub>18</sub> S)Cu
1357.39	[CuL+Na] <sup>+</sup>	(C <sub>53</sub> H <sub>76</sub> N <sub>17</sub> O <sub>18</sub> S)CuNa
637.75	[L] <sup>2+</sup>	C <sub>53</sub> H <sub>79</sub> N <sub>17</sub> O <sub>18</sub> S
648.74	[L+Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>78</sub> N <sub>17</sub> O <sub>18</sub> S)Na
659.73	[L+2Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>77</sub> N <sub>17</sub> O <sub>18</sub> S)Na <sub>2</sub>
668.21	[CuL] <sup>2+</sup>	(C <sub>53</sub> H <sub>77</sub> N <sub>17</sub> O <sub>18</sub> S)Cu
679.20	[CuL+Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>76</sub> N <sub>17</sub> O <sub>18</sub> S)CuNa
690.19	[CuL+2Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>75</sub> N <sub>17</sub> O <sub>18</sub> S)CuNa <sub>2</sub>
701.18	[CuL+3Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>74</sub> N <sub>17</sub> O <sub>18</sub> S)CuNa <sub>3</sub>
432.83	[L+Na] <sup>3+</sup>	(C <sub>53</sub> H <sub>78</sub> N <sub>17</sub> O <sub>18</sub> S)Na
440.16	[L+2Na] <sup>3+</sup>	(C <sub>53</sub> H <sub>77</sub> N <sub>17</sub> O <sub>18</sub> S)Na <sub>2</sub>
445.80	[CuL] <sup>3+</sup>	(C <sub>53</sub> H <sub>77</sub> N <sub>17</sub> O <sub>18</sub> S)Cu

447.48	$[L+3Na]^{3+}$	$(C_{53}H_{76}N_{17}O_{18}S)Na_3$
453.13	$[CuL+Na]^{3+}$	$(C_{53}H_{76}N_{17}O_{18}S)CuNa$
460.45	$[CuL+2Na]^{3+}$	$(C_{53}H_{75}N_{17}O_{18}S)CuNa_2$

*Cu(II)-Ac-GPKGMPHGH-COOH*



*Cu(II)-Ac-GPKGMPGEEGH-COOH*

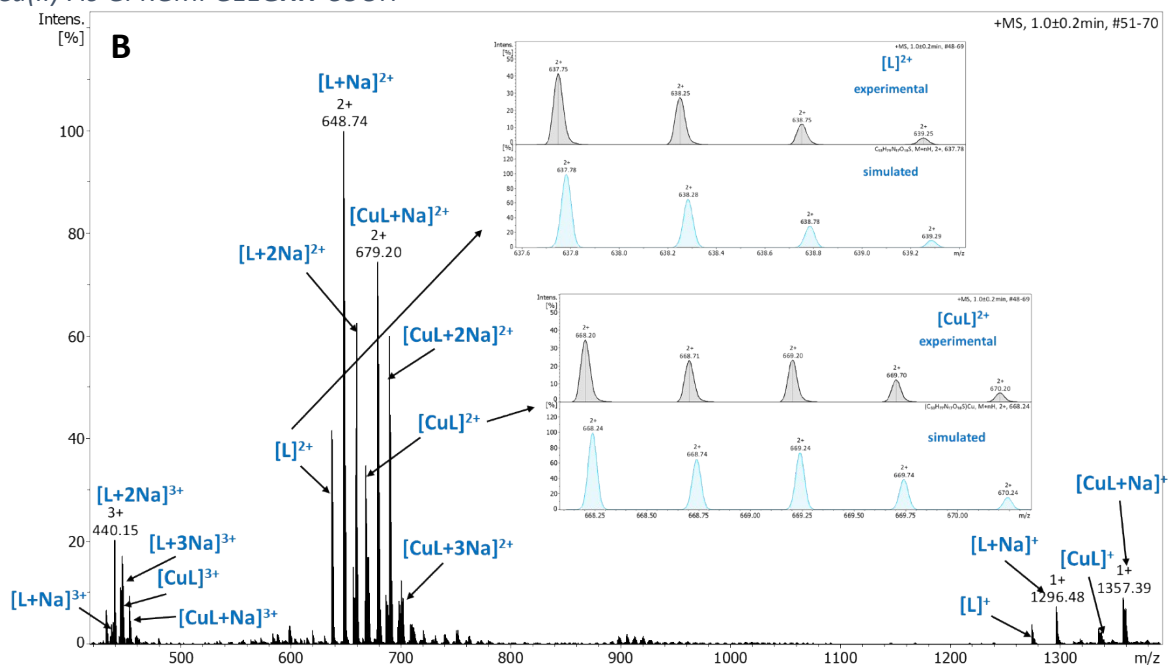
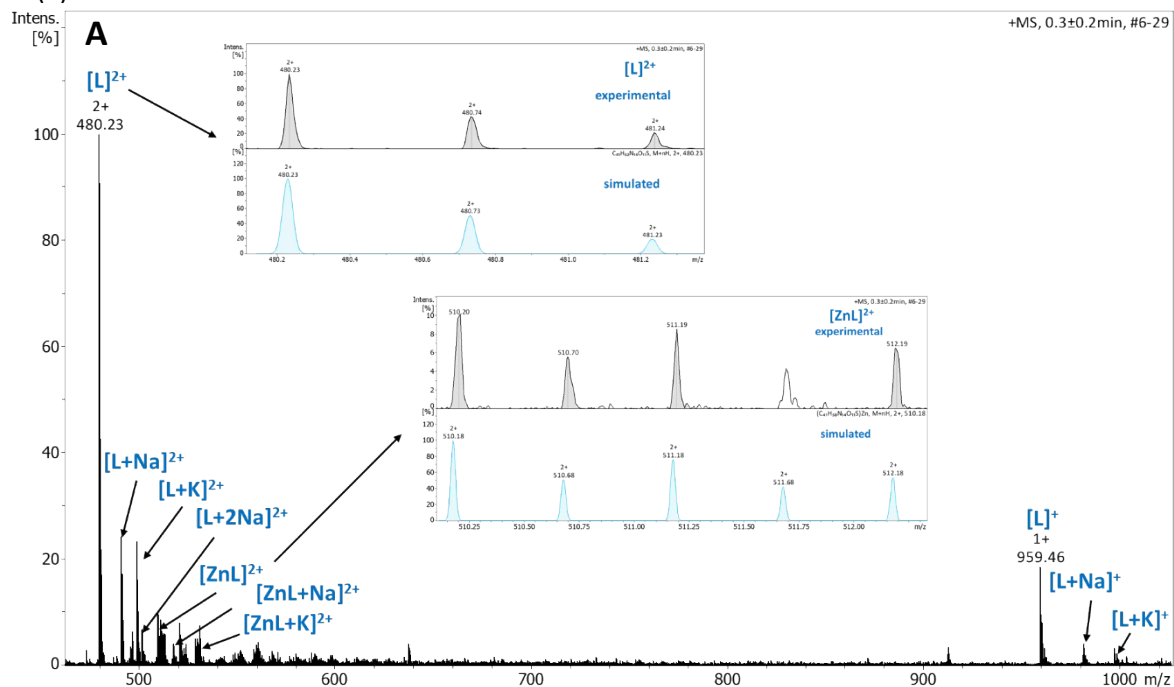


Figure S1 High-resolution ESI-MS spectrum of Cu(II) complexes with the (A) Ac- Ac-GPKGMPHGH-COOH and (B) Ac-GPKGMPGEEGH-COOH. M:L molar ratio = 0.9:1 in water:methanol 50:50 solution. Inside the spectra: comparison of experimental and simulated isotopic patterns for selected ligand and complex species.

Table S2 Stoichiometry, molecular formula and average m/z value for the species present in ESI-MS spectra of Zn(II) complexes with the (A) Ac-GPKGMPHGH-COOH and (B) Ac-GPKGMPGEEGHH-COOH, M:L molar ratio = 0.9:1 in water:methanol 50:50 solution.

m/z	ion	Molecular formula
<b>(A) Zn(II)-Ac-GPKGMPHGH-COOH</b>		
959.46	[L] <sup>+</sup>	C <sub>41</sub> H <sub>62</sub> N <sub>14</sub> O <sub>11</sub> S
981.45	[L+Na] <sup>+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)Na
997.41	[L+K] <sup>+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)K
480.23	[L] <sup>2+</sup>	C <sub>41</sub> H <sub>62</sub> N <sub>14</sub> O <sub>11</sub> S
491.23	[L+Na] <sup>2+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)Na
499.21	[L+K] <sup>2+</sup>	(C <sub>41</sub> H <sub>61</sub> N <sub>14</sub> O <sub>11</sub> S)K
502.22	[L+2Na] <sup>2+</sup>	(C <sub>41</sub> H <sub>60</sub> N <sub>14</sub> O <sub>11</sub> S)Na <sub>2</sub>
510.20	[ZnL] <sup>2+</sup>	(C <sub>41</sub> H <sub>58</sub> N <sub>14</sub> O <sub>11</sub> S)Zn
521.20	[ZnL+Na] <sup>2+</sup>	(C <sub>41</sub> H <sub>57</sub> N <sub>14</sub> O <sub>11</sub> S)ZnNa
529.19	[ZnL+K] <sup>2+</sup>	(C <sub>41</sub> H <sub>57</sub> N <sub>14</sub> O <sub>11</sub> S)ZnK
<b>(B) Zn(II)-Ac-GPKGMPGEEGHH-COOH</b>		
637.79	[L] <sup>2+</sup>	C <sub>53</sub> H <sub>79</sub> N <sub>17</sub> O <sub>18</sub> S
648.78	[L+Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>78</sub> N <sub>17</sub> O <sub>18</sub> S)Na
656.77	[L+K] <sup>2+</sup>	(C <sub>53</sub> H <sub>78</sub> N <sub>17</sub> O <sub>18</sub> S)K
659.77	[L+2Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>77</sub> N <sub>17</sub> O <sub>18</sub> S)Na <sub>2</sub>
668.75	[ZnL] <sup>2+</sup>	(C <sub>53</sub> H <sub>75</sub> N <sub>17</sub> O <sub>18</sub> S)Zn
679.74	[ZnL+Na] <sup>2+</sup>	(C <sub>53</sub> H <sub>74</sub> N <sub>17</sub> O <sub>18</sub> S)ZnNa

### Zn(II)-Ac-GPKGMPHGH-COOH



Zn(II)-Ac-GPKGMPGEEGHH-COOH

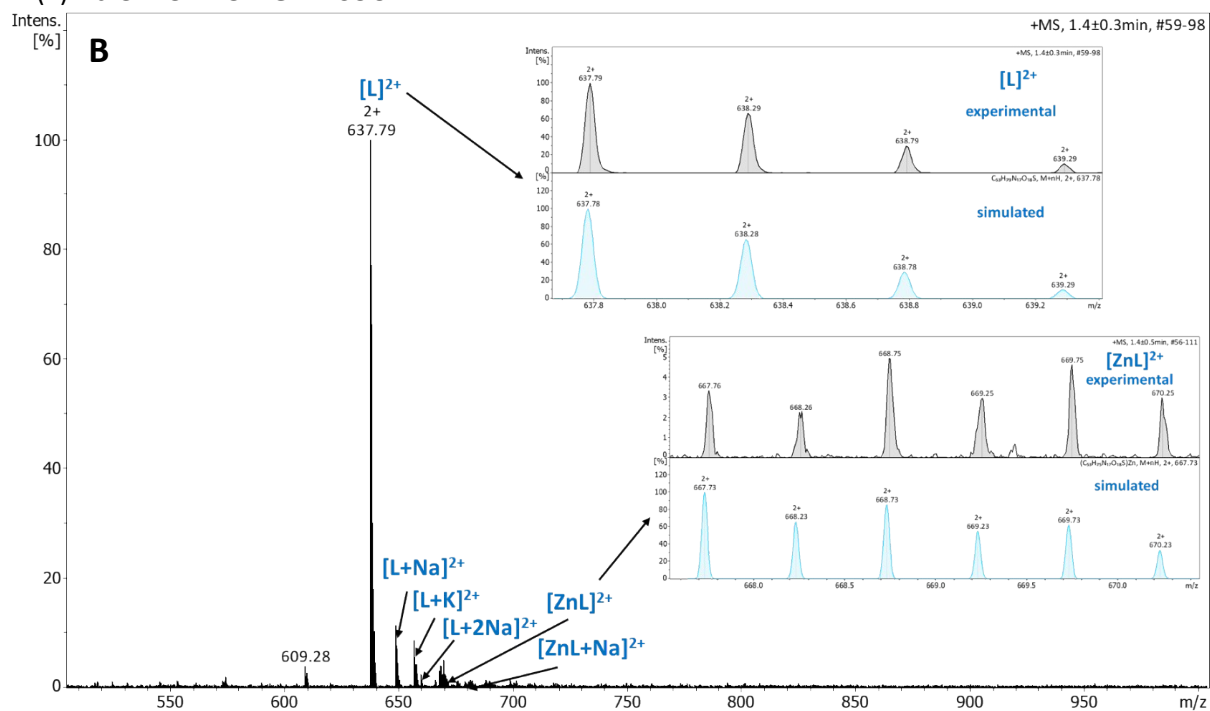


Figure S2 High-resolution ESI-MS spectrum of Zn(II) complexes with the (A) Ac- Ac-GPKGMPHGH-COOH and (B) Ac-GPKGMPGEEGHH-COOH. M:L molar ratio = 0.9:1 in water:methanol 50:50 solution. Inside the spectra: comparison of experimental and simulated isotopic patterns for selected ligand and complex species.

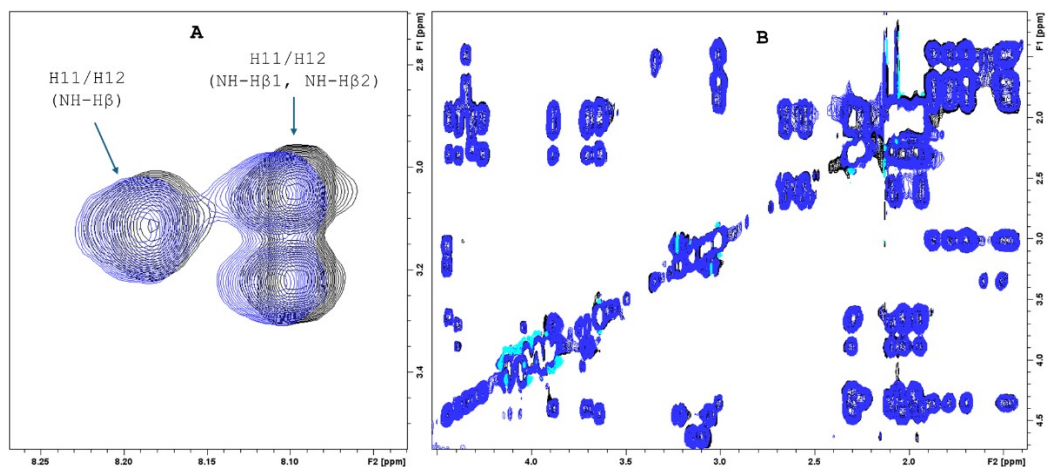


Figure S3 Superimposition of the amide (A) and aliphatic (B) regions of 2D  $^1\text{H}$ - $^1\text{H}$  TOCSY spectra of Ac-GPKGMPGEEGHH-COOH recorded in absence (black) and in presence of 0.9 Zn(II) eqs. (blue).  $T=288\text{ K}$ ,  $C_M=0.5\text{ mM}$ , phosphate buffer=20 mM, pH 7.4.

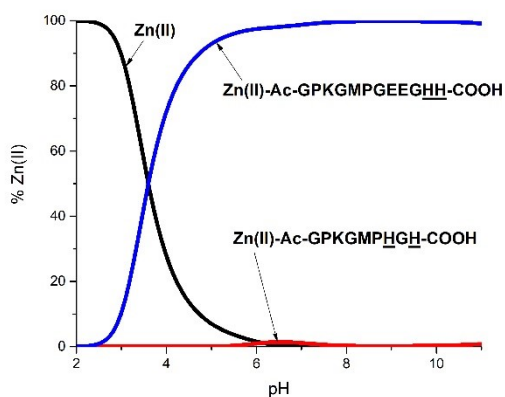


Figure S4 Competition plots for a simulated solution containing equimolar concentrations of Zn(II), Ac-GPKGMPHGH-COOH and Ac-GPKGMPGEEGHH-COOH. The diagram is calculated from the overall stability constants of the binary complexes and simulate a situation in which equimolar concentrations of all of the chosen ligands and metal are present in a solution.

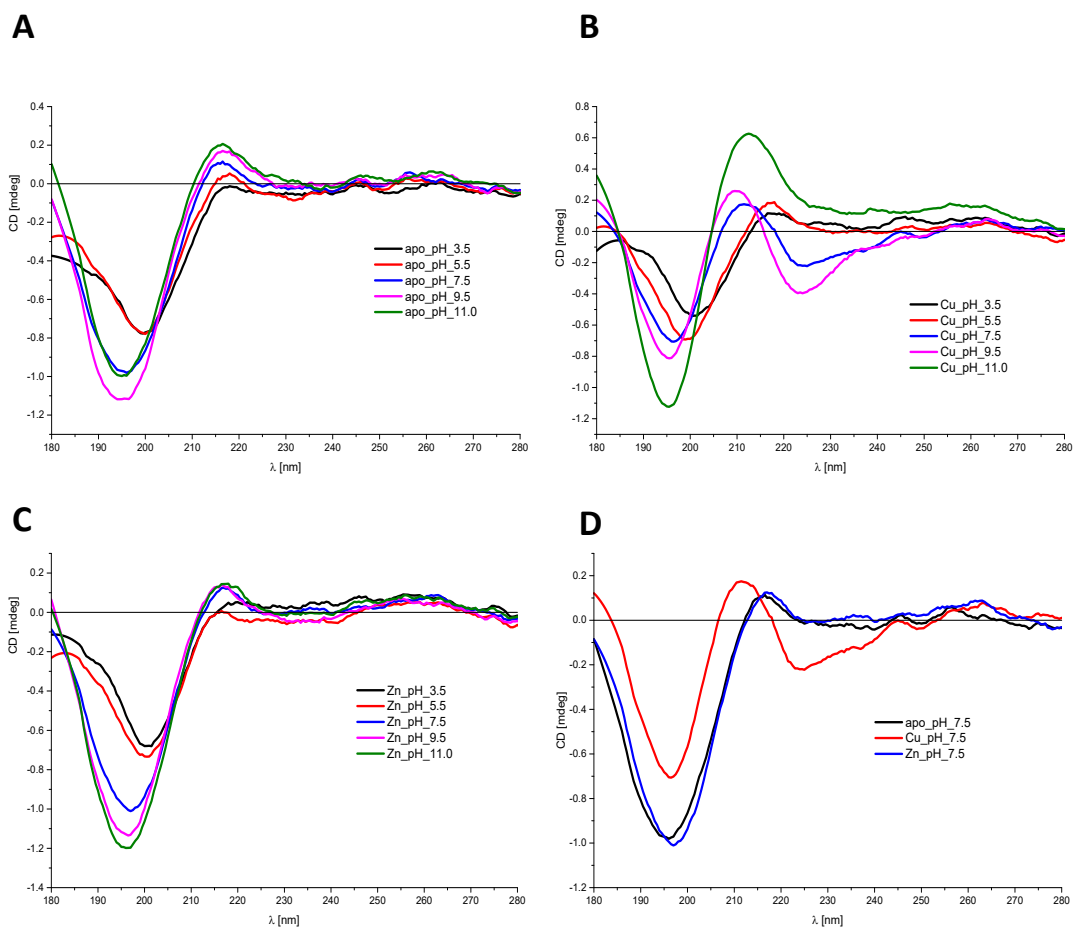


Figure S5 CD spectra for (A) Ac-GPKGMPHGH-COOH; (B) Cu(II)-Ac-GPKGMPHGH-COOH, (C) Zn(II)-Ac-GPKGMPHGH-COOH recorded over the entire pH range (pH 3.5–11.0) in aqueous solution of 4 mM HClO<sub>4</sub> with I = 0.1 M NaClO<sub>4</sub>, T = 298 K, M:L molar ratio = 0.9:1, C<sub>l</sub> = 0.1 mM. (D) Comparison of CD spectra of apo, Cu(II), and Zn(II) species at pH 7.5.

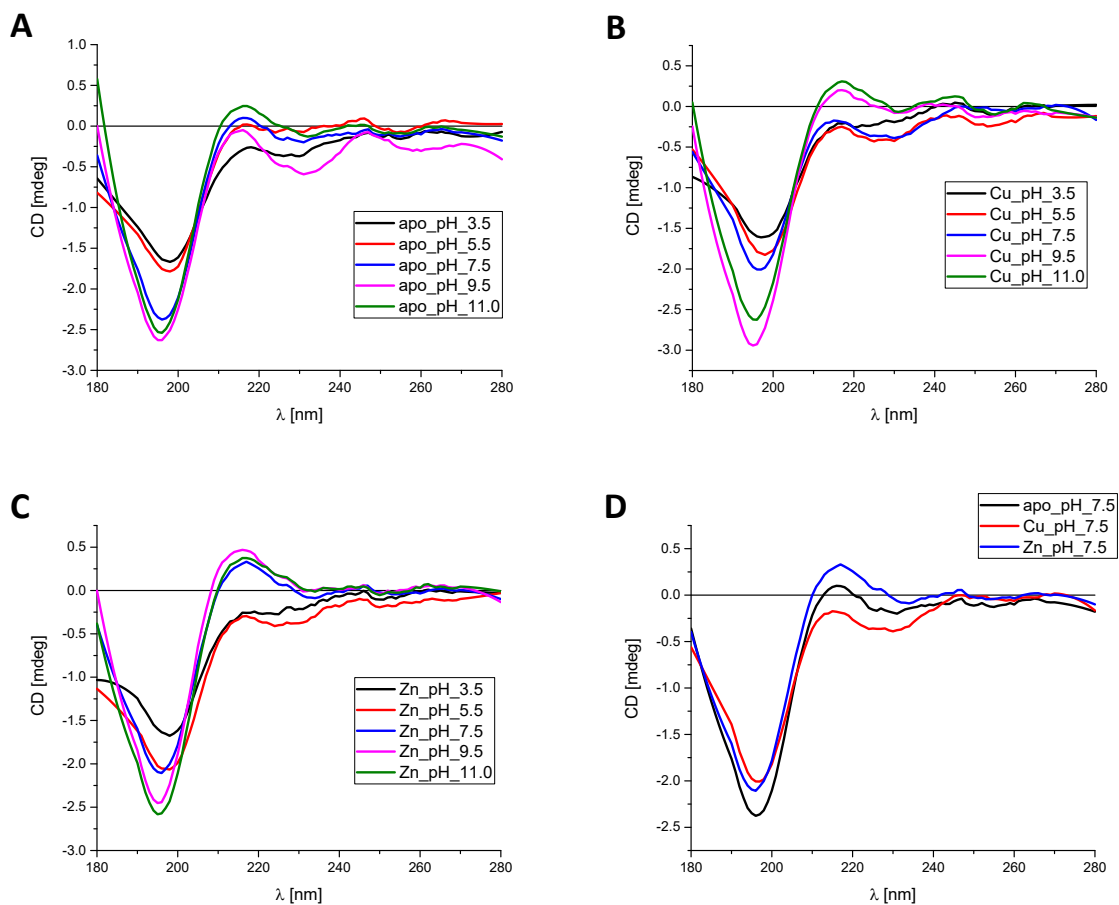


Figure S6 CD spectra for (A) Ac-GPKGMPGEEGHH-COOH; (B) Cu(II)-Ac-GPKGMPGEEGHH-COOH, (C) Zn(II)-Ac-GPKGMPGEEGHH-COOH recorded over the entire pH range (pH 3.5–11.0) in aqueous solution of 4 mM HClO<sub>4</sub> with  $\text{I} = 0.1$  M NaClO<sub>4</sub>,  $T = 298$  K, M:L molar ratio = 0.9:1,  $C_L = 0.1$  mM. (D) Comparison of CD spectra of apo, Cu(II), and Zn(II) species at pH 7.5.