Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

Short oligopeptides with three cysteine residues as models of sulphur-rich Cu(I)- and Hg(II)-binding sites in proteins

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Table S1. Analytical HPLC and (+)ESI-MS references of the peptides

Name	t_r (min)	Chemical formula	Molecular weight (g/mol)	m/z $[M+2H]^{2+}$	m/z $[M+H]^{+}$
P^{3C}	10.1	$C_{34}H_{57}N_{13}O_{13}S_3$	951.34	476.8	952.4
1^{C}	10.7	$C_{34}H_{57}N_{13}O_{13}S_3$	951.34	476.8	952.3
1^{L}	10.5	$C_{36}H_{62}N_{14}O_{14}S_3$	1010.37	506.3	1011.4
2^{C}	10.9	$C_{34}H_{57}N_{13}O_{13}S_3$	951.34	476.7	952.3
2^{L}	10.4	$C_{36}H_{62}N_{14}O_{14}S_3$	1010.37	506.7	1011.4
3^{C}	10.8	$C_{37}H_{62}N_{14}O_{14}S_3$	1022.37	512.3	1023.3

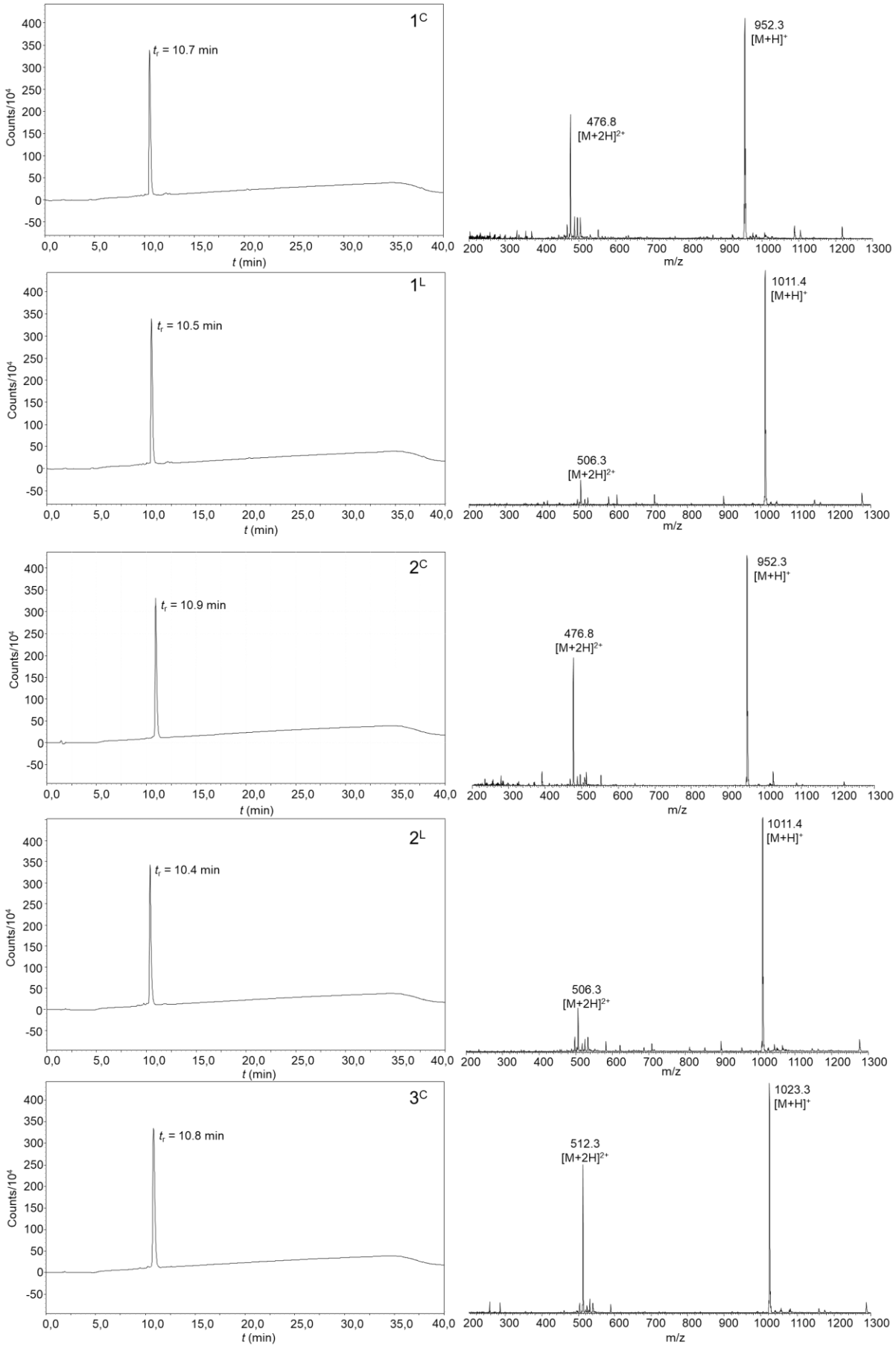


Figure S1. Analytical HPLC chromatogram and (+)ESI-MS spectra of the studied peptides

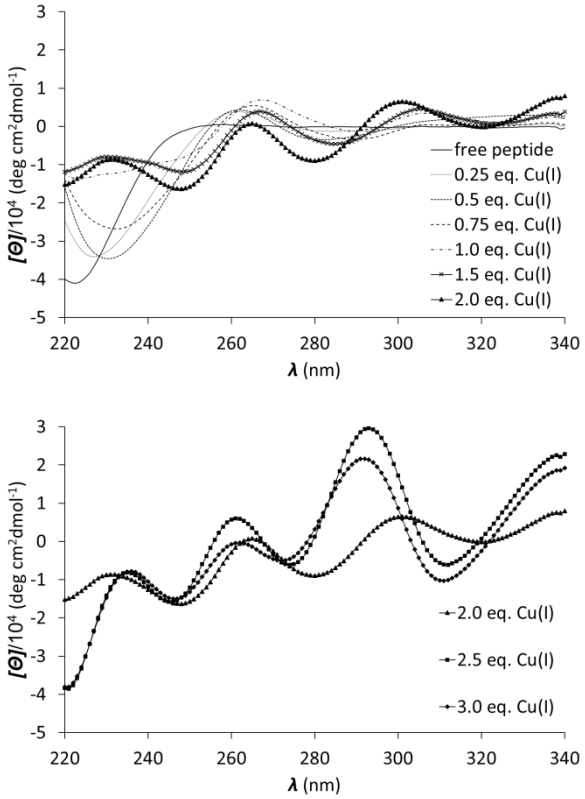


Figure S2. CD titration of $\mathbf{1^C}$ with Cu(I) (c_{peptide} = 30 μM) in phosphate buffer 20 mM, pH = 7.4 + 10 V/V% AcN. The upper panel shows the spectra with 0.0-2.0 equivalents of Cu(I) and the lower with 2.0-3.0 equivalents.

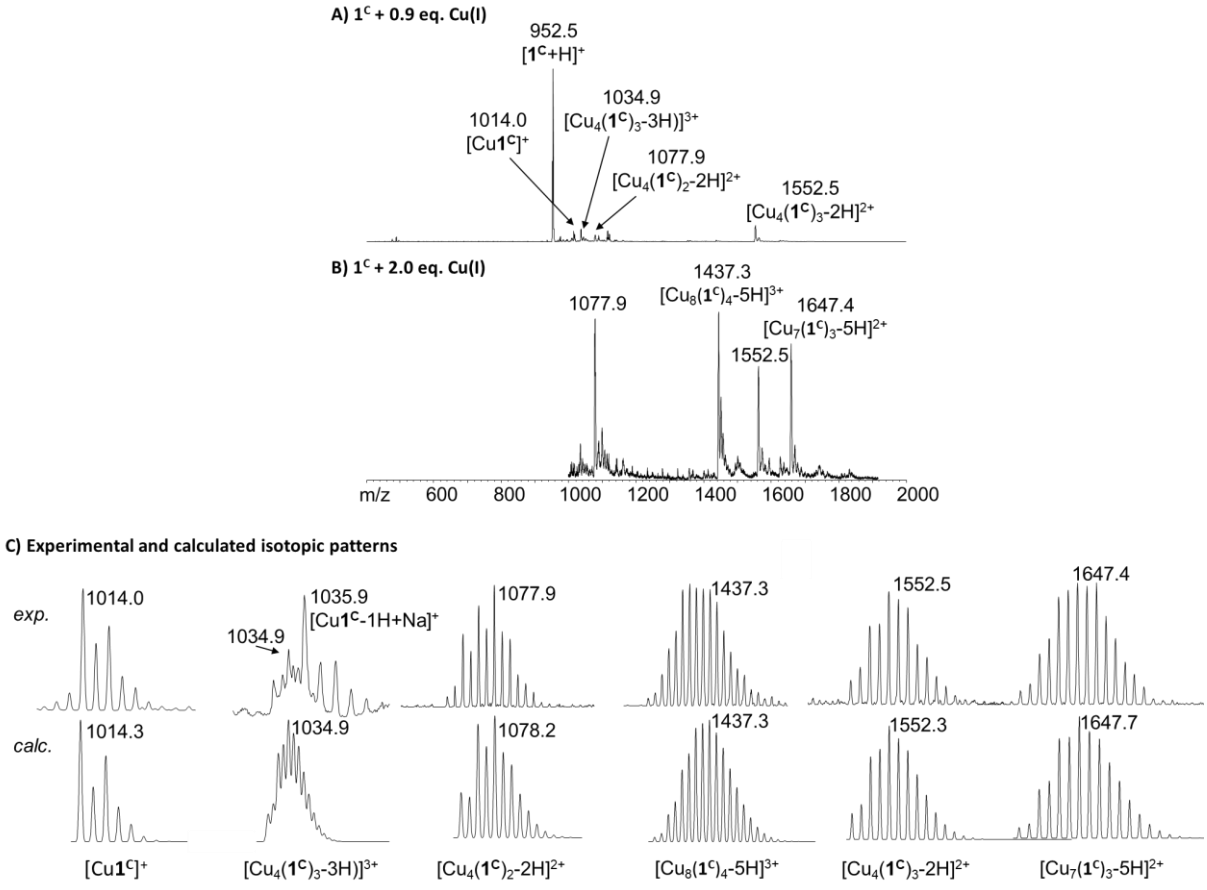


Figure S3. (+) ESI-MS spectra recorded for **1^{C}** with Cu(I). $c_{peptide}$ = 100 μM in NH_4AcO buffer 20 mM, pH = 7.0 + 10 V/V% AcN. **A)** 0.9 Cu(I) equiv. **B)** 2.0 Cu(I) equiv. **C)** Experimental and calculated isotopic patterns of the main cluster species. The notation **1^{C}** refers here to the neutral free peptide.

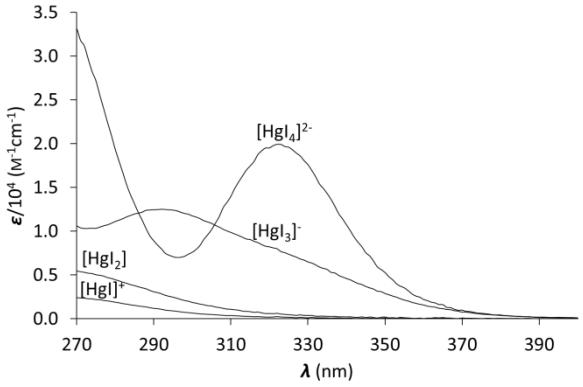


Figure S4. Molar spectra of the Hg(II)-I¯ complexes at pH = 2.0 obtained by SPECFIT.

Calculation of the formation constants of the HgHL and HgL complexes

Thermodynamic formation constants for the mono-protonated and parent Hg(II)-complexes were estimated from the apparent stabilities of the HgP mononuclear complexes determined at pH = 2.0. These calculations involve the stepwise proton dissociation constants (K_a^{HL}, $K_a^{H_2L}$, $K_a^{H_3L}$) of the ligands, expressed in a form of the overall formation (association) constant, β_{H_3L}, of the fully protonated peptides:

$$\frac{[H_3L]}{[L][H]^3} = \beta_{H_3L} = \frac{1}{K_a^{HL} \times K_a^{H_2L} \times K_a^{H_3L}} \quad (1)$$

Such data had been determined only for one of the peptides, $\mathbf{1^L}$, nevertheless, the same protonation/deprotonation constants were extrapolated for all other studied ligands. Consequently, the calculations detailed below can be considered as rather precise estimates for the complexes of $\mathbf{1^L}$ but less reliable predictions for the other five peptides. The deduction leading to the final formulae are as follows:

The apparent stability of the mononuclear complexes at pH = 2.0 is defined as:

$$\beta_{HgP}^{pH2.0} = \frac{[HgP]}{[Hg][P]} \quad (2)$$

Considering that the spectrophotometrically determined pK_a values, attributed to the release of one equivalent proton from the Hg(II)-bound peptides, span the range of 4.3 – 5.1, a plausible assumption is that the peptides are bound to Hg(II) as mono-protonated ligands (HL) at pH = 2.0 and the equilibrium concentration of the sum of complexed ligand forms, [HgP], can be approximated with the concentration of the HgHL complex, i.e. [HgP] = [HgHL]. Additionally, at pH = 2.0 the concentration of the free peptide, [P], can be substituted with that of the fully protonated ligand, [H_3L]. Above equation is then transformed to:

$$\beta_{HgP}^{pH2.0} = \frac{[HgHL]}{[Hg][H_3L]} \quad (3)$$

[H_3L] in the above equation can be substituted by

$$[H_3L] = \beta_{H_3L} \times [L] \times [H]^3 \quad (4)$$

and rearranged to

$$\beta_{HgP}^{pH2.0} \times \beta_{H_3L} \times [H]^2 = \frac{[HgHL]}{[Hg][L][H]} \quad (5)$$

Latter equation can be easily combined with the expression of the formation constant of the HgHL complex (6).

$$\beta_{\mathrm{HgHL}} = \frac{[\mathrm{HgHL}]}{[\mathrm{Hg}][\mathrm{L}][\mathrm{H}]} \quad (6)$$

The combination of (5) and (6) leads to an expression allowing the calculation of β_{HgHL} from the experimentally measured stability data:

$$\beta_{\mathrm{HgHL}} = \beta_{\mathrm{HgP}}^{\mathrm{pH2.0}} \times \beta_{\mathrm{H_3L}} \times [\mathrm{H}]^2 \quad (7)$$

$$\log\beta_{\mathrm{HgHL}} = \log\beta_{\mathrm{HgP}}^{\mathrm{pH2.0}} + \log\beta_{\mathrm{H_3L}} - 2 \times \mathrm{pH} \quad (8)$$

Formation constants for the parent HgL complexes can be obtained by using the spectrophotometrically determined deprotonation constants ($\mathrm{p}K_{\mathrm{a}}^{\mathrm{HgHL}}$) for the HgHL ⇌ HgL+ H process:

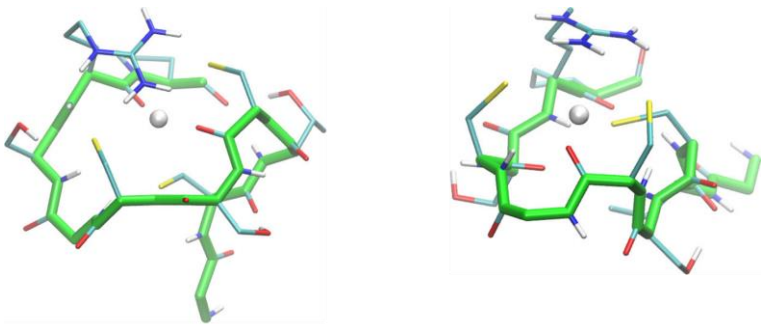
$$\log\beta_{\mathrm{HgL}} = \log\beta_{\mathrm{HgHL}} - \mathrm{p}K_{\mathrm{a}}^{\mathrm{HgHL}} \quad (9)$$

From the above thermodynamic stability constants, apparent stabilities of the HgP mono-complexes may be re-calculated for any desired pH values allowing a direct comparison of the Cu(I)- and Hg(II)-binding affinities of the studied peptides.

Table S2. Average energies (kcal/mol) of the peptides in their apo or Hg(II)-bound forms measured during the last 40 ns (of 85 ns or more) MD simulations. Internal energy is sum of Bonds + Angles + Dihedrals + Impropers – (Standard deviations in parentheses). The energy differences (holo – apo) correlated to the stability constant β_{HgP} are also given.

Peptide	$\mathbf{P^{3C}}$	$\mathbf{1^{C}}$	$\mathbf{2^{C}}$	$\mathbf{3^{C}}$ §	$\mathbf{1^{L}}$	$\mathbf{2^{L}}$
E(HgP)	−186.0 (7.0)	−184.8 (6.9)	−182.3 (6.7)	−202.3 (7.0)	−187.1 (7.1)	−181.3 (7.1)
E(P)	−173.2 (7.2)	−173.4 (7.5)	−170.3 (7.0)	−191.3 (7.1)	−174.0 (6.9)	−171.8 (7.2)
Δ*E*(HgP-P)	−12.8	−11.4	−12.0	−11.0	−13.1	−9.5

§The higher total energies calculated for the $\mathbf{3^{C}}$ peptide is a consequence of the larger number of amino acids (11 against 10 for the other peptides).



$\mathbf{1^{L}}$ $\mathbf{2^{L}}$

Figure S5. Energy minimized structures of the 2 linear peptides in their Hg-bound form. (oriented with respect to the position of backbone atom coordinates of residues 1 to 10)