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**Supplementary material**

**Thermodynamic stability of mercury(II) complexes formed with environmentally relevant low-molecular-mass thiols studied by competing ligand exchange and density functional theory**

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**S1. Calculation of stability constants for Hg(MAC)<sub>2</sub> and HgIMAC using I<sup>-</sup> as competing ligand.** Components, reactions and a calculation scheme for determination of the stability constant for the Hg(MAC)<sub>2</sub> and HgIMAC complexes with I<sup>-</sup> as the competing ligand, using WinSGW software. The *pKa* of the RSH and COOH groups for MAC are 10.2 and 3.5, respectively. A similar set of corresponding equations were set-up when calculating constants for Hg(2-MPA)<sub>2</sub>.

$$[I^-]_{\text{total}} = [I^-]_{\text{free}} + [HgI^+] + 2[HgI_2] + 3[HgI_3^-] + 4[HgI_4^{2-}] + [HgIMAC] \quad (\text{Sa})$$

$$[MAC]_{\text{total}} = [MAC]_{\text{free}} + 2[Hg(MAC)_2] + [HgIMAC] + [HgMAC] \quad (\text{Sb})$$

$$[Hg^{2+}]_{\text{total}} = [Hg^{2+}]_{\text{free}} + [HgI^+] + [HgI_2] + [HgI_3^-] + [HgI_4^{2-}] + [HgIMAC] + [Hg(MAC)_2] + [HgMAC] + [Hg(OH)_n](\text{Sc})$$

$$Hg^{2+} + I^- \rightleftharpoons HgI^+ \quad \text{Log } K_1 = 13.42 \quad (\text{S3a})$$

$$Hg^{2+} + 2I^- \rightleftharpoons HgI_2 \quad \text{Log } K_2 = 24.60 \quad (\text{S3b})$$

$$Hg^{2+} + 3I^- \rightleftharpoons HgI_3^- \quad \text{Log } K_3 = 28.32 \quad (\text{S3c})$$

$$Hg^{II} + 4I^- \rightleftharpoons HgI_4^{2-} \quad \text{Log } K_4 = 30.07 \quad (\text{S3d})$$

$$Hg^{2+} + MAC \rightleftharpoons HgMAC \quad \text{Log } K = 32.5 \quad (\text{S4a'})$$

$$Hg^{2+} + 2MAC \rightleftharpoons Hg(MAC)_2 \quad \text{Log } \beta_2 = ? \quad (\text{S1b'})$$

$$Hg^{2+} + I^- + MAC \rightleftharpoons HgIMAC \quad \text{Log } K = ? \quad (\text{S6})$$

Example of calculation

$$[I^-]_{\text{total}} = 20 \mu\text{M}^a$$

$$[MAC]_{\text{total}} = 16 \mu\text{M}^a$$

$$[Hg^{2+}]_{\text{total}} = 4 \mu\text{M}^a$$

$$[Hg(MAC)_2] = 3.2 \mu\text{M}^b$$

$$[HgIMAC] = (\text{Peak Area (HgIMAC)} / \text{Peak Area (Hg(MAC)_2)}) \times [Hg(MAC)_2] = 0.45 \mu\text{M}^b$$

$$[HgI_n^x] = [Hg^{II}]_{\text{total}} - [Hg(MAC)_2] - [HgIMAC] = 0.29 \mu\text{M}^b$$

<sup>a</sup> Concentration of ligand used for complexation

<sup>b</sup> Concentration of complexes determined by LC-ICPMS.

In the experiment, the concentration of I<sup>-</sup> was varied, and in the calculation scheme above it is exemplified for 20  $\mu\text{M}$  I<sup>-</sup>. Constants were recalculated to an ionic strength of 0 M and experimental pH was 2.9. Based on the equations (Sa-Sc); reaction S3(a-d), (S4a'), (S1b'), (S6), and total concentration of Hg<sup>II</sup> and ligands, and measured concentration of Hg(MAC)<sub>2</sub> and HgIMAC complexes, the log *K* of Hg(MAC)<sub>2</sub> and HgIMAC was determined to be 40.9 and 32.2, respectively.

**S2. Calculation of the stability constant for the Hg(Cys)<sub>2</sub> complex using MAC as competing ligand.** Calculation scheme for determination of the stability constant for Hg(Cys)<sub>2</sub> with MAC as the competing ligand using WinSGW software. The *pKa* of the RSH group for MAC and Cys are 10.2 and 8.6, respectively and *pKa* of the COOH group for MAC and Cys are 3.4 and 2.0, respectively. The same corresponding calculation scheme was used for the other Hg(SR)<sub>2</sub> complexes using MAC or 2-MPA as the competing ligand.

$$[\text{MAC}]_{\text{total}} = [\text{MAC}]_{\text{free}} + 2[\text{Hg}(\text{MAC})_2] + [\text{HgMAC}] \quad (\text{Sd})$$

$$[\text{Cys}]_{\text{total}} = [\text{Cys}]_{\text{free}} + 2[\text{Hg}(\text{Cys})_2] + [\text{HgCys}] \quad (\text{Se})$$

$$[\text{Hg}^{2+}]_{\text{total}} = [\text{Hg}^{2+}]_{\text{free}} + [\text{HgMAC}] + [\text{Hg}(\text{MAC})_2] + [\text{HgCys}] + [\text{Hg}(\text{Cys})_2] + [\text{Hg}(\text{OH})_n] \quad (\text{Sf})$$



Example of calculation

$$[\text{MAC}]_{\text{total}} = 8 \mu\text{M}^{\text{a}}$$

$$[\text{Cys}]_{\text{total}} = 8 \mu\text{M}^{\text{a}}$$

$$[\text{Hg}^{2+}]_{\text{total}} = 4 \mu\text{M}^{\text{a}}$$

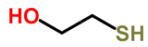
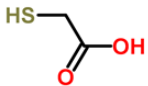
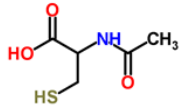
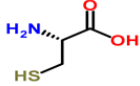
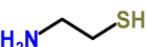
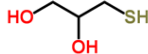
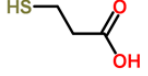
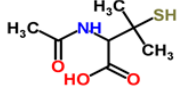
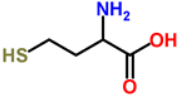
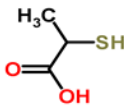
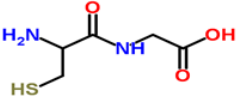
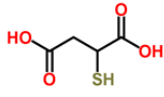
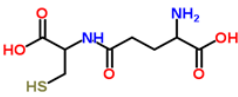
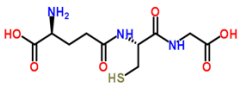

$$[\text{Hg}(\text{MAC})_2] = 2.27 \mu\text{M}^{\text{b}}$$

$$[\text{Hg}(\text{Cys})_2] = 1.72 \mu\text{M}^{\text{b}}$$

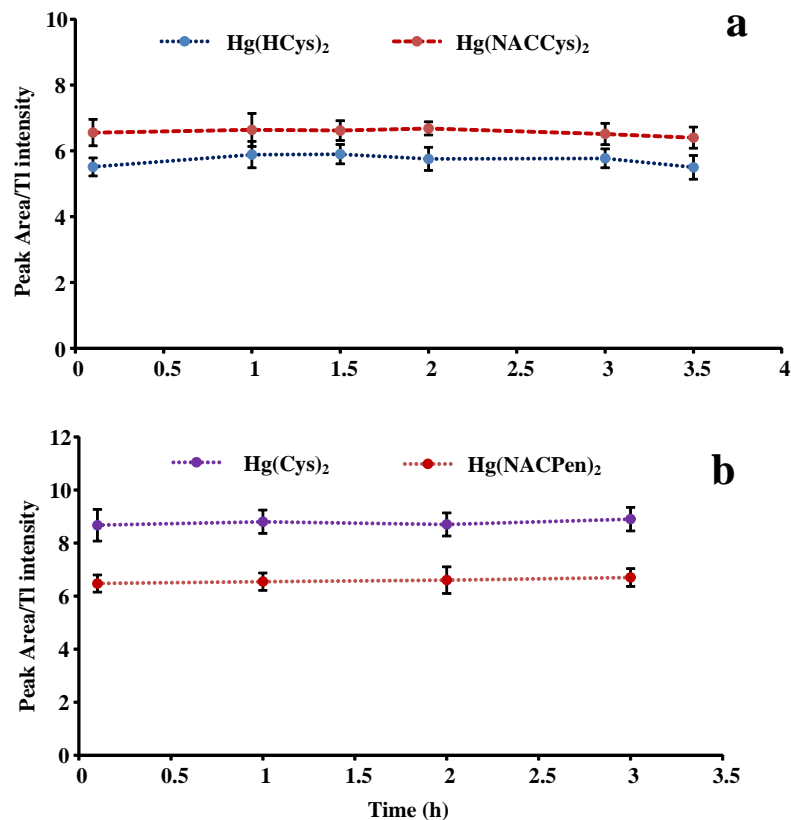
<sup>a</sup> Concentration of ligand used for complexation

<sup>b</sup> Concentration of complexes determined by HPLC-ICPMS

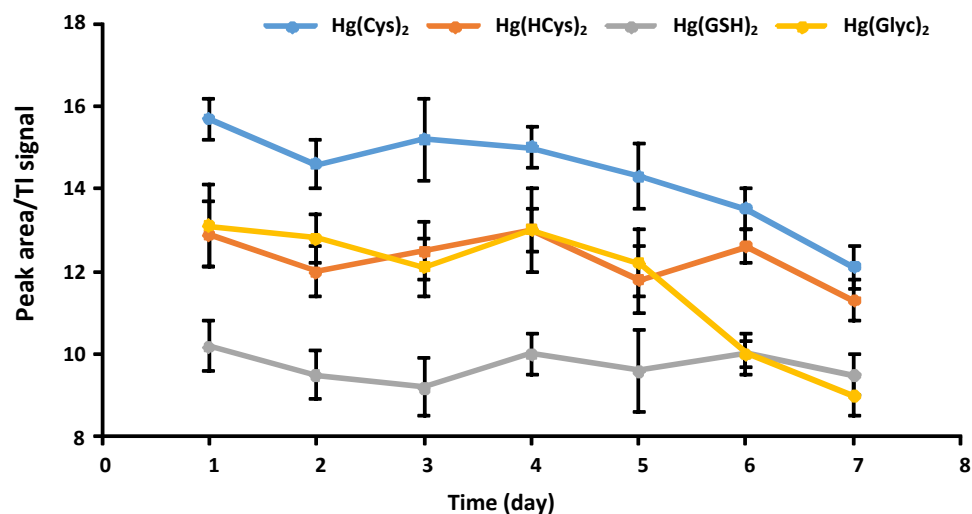
Constants were recalculated to an ionic strength of 0 M and experimental pH was 2.9. Based on the equations (Sd-Sf); reactions (S4'), (S4''), (S1b'), (S1b'') and total concentration of the Hg<sup>II</sup> and ligands and measured complexes concentration, the log  $\beta_2$  of Hg(Cys)<sub>2</sub> was determined to 37.5.

1. Hydroxyl	2. Carboxyl	3. Carboxyl/carbonyl + secondary amine	4. Carboxyl/carbonyl + primary amine (w/wo secondary amine)	5. Primary amine
Mercaptoethanol (ETH) 	Mercaptoacetic acid (MAC) 	N-acetyl-cysteine (NACCys) 	Cysteine (Cys) 	Cysteamine (Cyst) 
Monothiolglycerol (Glyc) 	3-Mercaptopropionic acid (3-MPA) 	N-acetyl-penicilamine (NACPen) 	Homocysteine (HCys) 	
	2-Mercaptopropionic acid (2-MPA) 		N-cysteinylglycine (CysGly) 	
	Mercaptosuccinic acid (SUC) 		γ-glutamylcysteine (GluCys) 	
			Glutathione (GSH) 	
			Penicilamine (Pen) 	

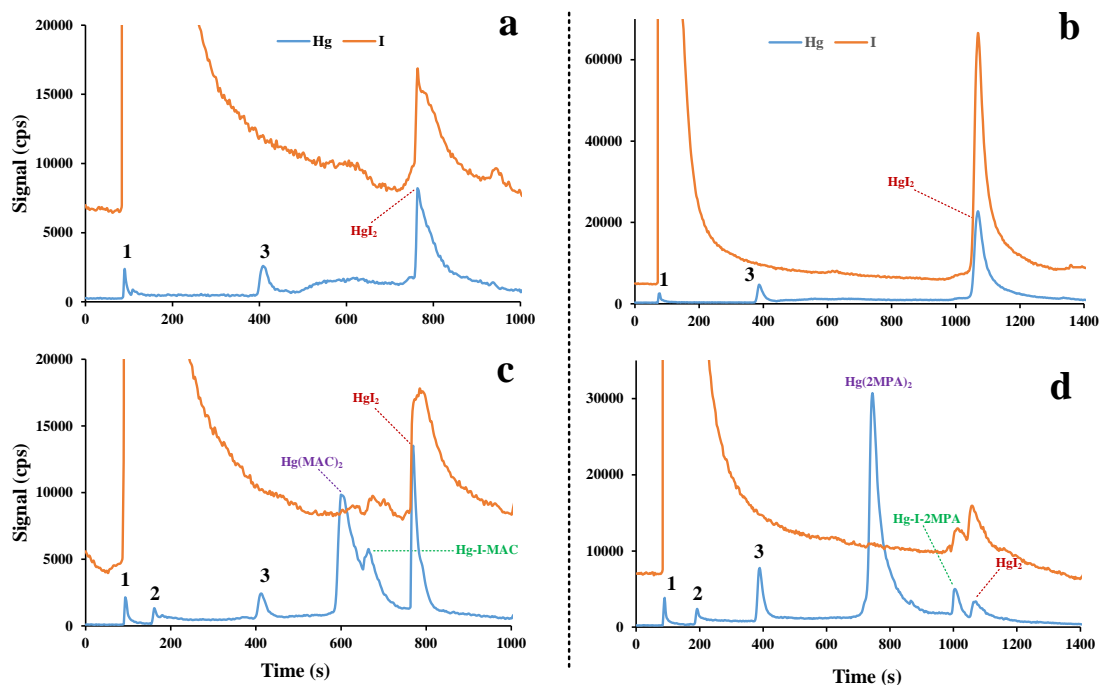
**Fig. S1.** The structure and abbreviation of investigated thiol ligands. The thiols are grouped according to the presence of functional groups in addition to the thiol group.



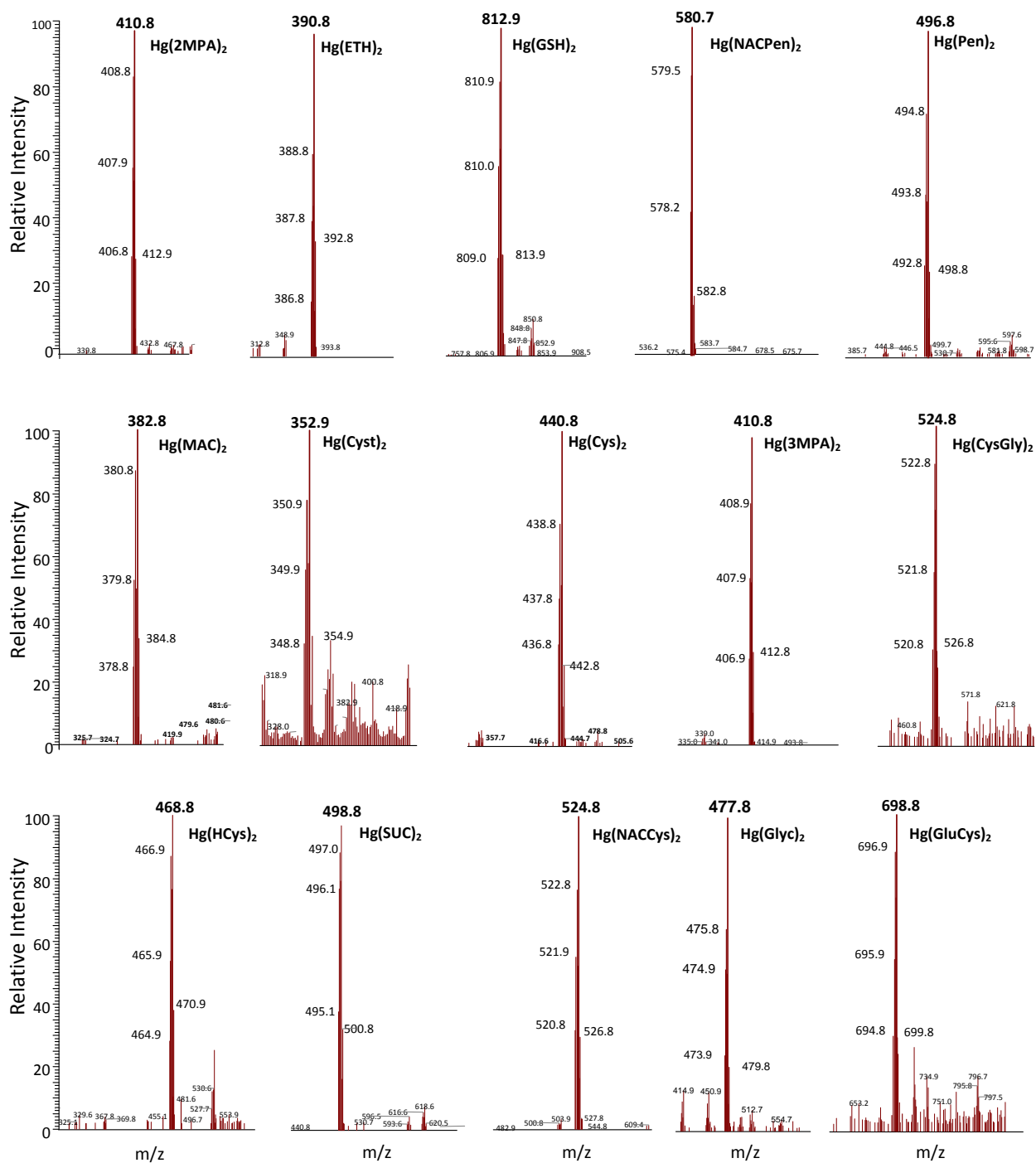
**Fig. S2.** The integrated peak area of Hg(SR)<sub>2</sub> complexes to Thallium (<sup>204</sup>Tl<sup>+</sup>) signal ratios with different reaction time for the Hg(SR)<sub>2</sub> complex synthesis. (a) mixture of 1 μM of Hg<sup>II</sup> and 2 μM of each HCys and NACCys. (b) mixture of 1 μM of Hg<sup>II</sup> and 2 μM of each Cys and NACPen. The experiments were conducted at pH 3.0, and an ion strength of 0 M. A post column flow of a 10 ng ml<sup>-1</sup> Tl solution (flow rate of 100 μl min<sup>-1</sup>) was used to monitor and correct for signal drift of the LC-ICPMS system over time.



**Fig. S3.** Peak area of Hg(SR)<sub>2</sub> complexes to Thallium (<sup>204</sup>Tl<sup>+</sup>) signal ratios at different storage time of Hg(SR)<sub>2</sub> complex solutions. Samples contain 1 μM of Hg<sup>II</sup> and 4 μM of individual LMM thiols i.e. Cys, HCys, GSH and Glyc. A post column flow of a 10 ng ml<sup>-1</sup> Tl solution (flow rate of 100 μl min<sup>-1</sup>) was used to monitor and correct for signal drift of the LC-ICPMS system over time.

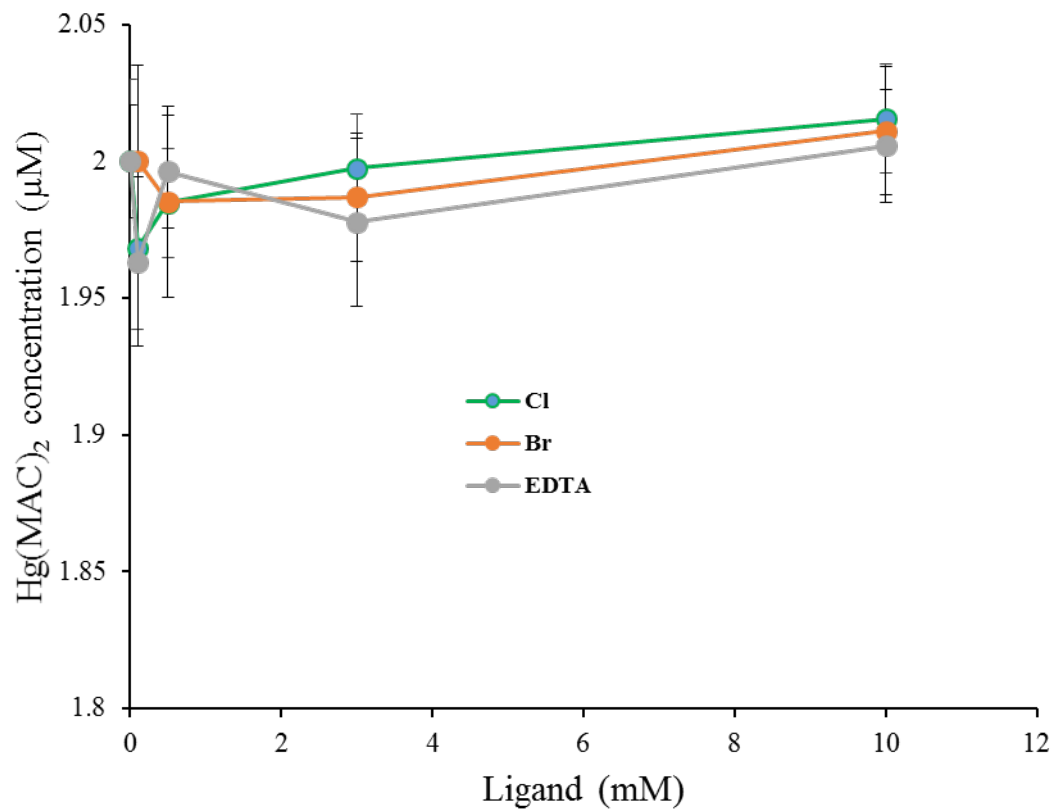


**Fig. S4.** LC-ICPMS chromatograms showing  $^{202}\text{Hg}^+$  and  $^{127}\text{I}^+$  signals (counts per second, cps) for (a)  $4\ \mu\text{M}$  of  $\text{Hg}^{\text{II}}$  and  $50\ \mu\text{M}$  of  $\text{I}^-$ . A gradient elution was used with initially 3.5% of 1-propanol during 12 min, then increased to 25% in a step gradient and kept for 4 min and then back to the initial 3.5% concentration. (b)  $4\ \mu\text{M}$  of  $\text{Hg}^{\text{II}}$  and  $50\ \mu\text{M}$  of  $\text{I}^-$ . A gradient elution was used with initially 8.5% 1-propanol during 14 min, then increased to 25% in a step gradient and kept for 4 min and then back to the initial 8.5% concentration. (c)  $4\ \mu\text{M}$  of  $\text{Hg}^{\text{II}}$ ,  $16\ \mu\text{M}$  of MAC and  $100\ \mu\text{M}$  of  $\text{I}^-$ . The elution gradient was the same as in (a). (d)  $4\ \mu\text{M}$  of  $\text{Hg}^{\text{II}}$ ,  $16\ \mu\text{M}$  of 2MPA and  $100\ \mu\text{M}$  of  $\text{I}^-$ . The elution gradient was the same as in (b). The complexes of  $\text{Hg}(\text{MAC})_2$ ,  $\text{HgI}(\text{MAC})$ ,  $\text{Hg}(\text{2MPA})_2$ , and  $\text{HgI}(\text{2-MPA})$  eluted at 600 s, 670 s, 780 s, and 1010 s, respectively. The  $\text{HgI}_2$  complex eluted at 790 s with the gradient of (a) and at 1070 s with the gradient of (b). The peaks 1, 2 correspond to the complexes of  $\text{HgI}_n^{n-2}$  ( $n=1, 3, 4$ ) and the peak 3 to a system background signal.

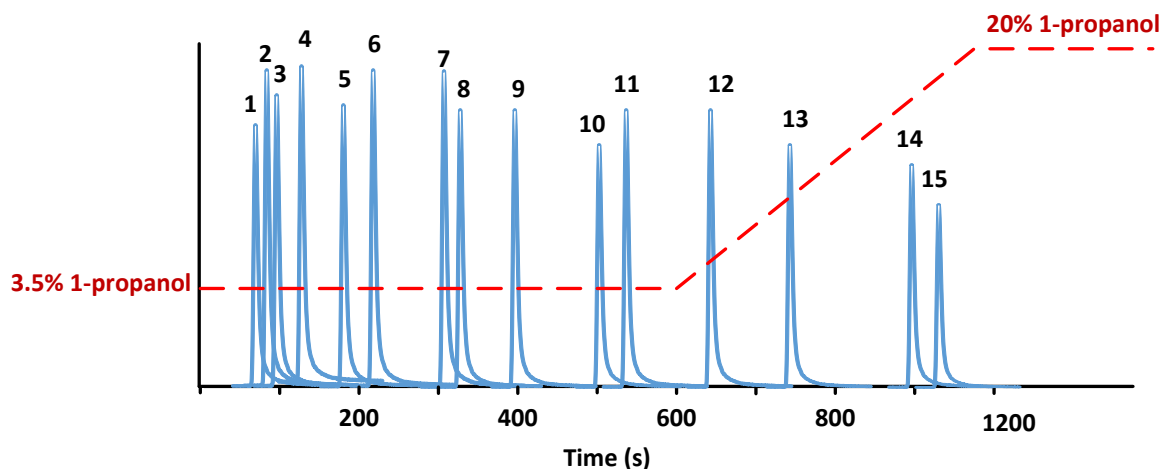


**Fig. S5.** The mass spectra of 15 investigated Hg(SR)<sub>2</sub> complexes achieved by direct infusion to ESIMS, showing molecular mass and Hg isotope pattern of the Hg(SR)<sub>2</sub> complexes. All Hg(SR)<sub>2</sub> complex analyses were conducted in the negative ionization mode with the exception of Hg(Cyst)<sub>2</sub>, which was analysed in positive mode. The concentration of Hg<sup>II</sup> was 0.1 mM, the molar ratio of RSH to Hg<sup>II</sup> was 4 and pH was 3.0.

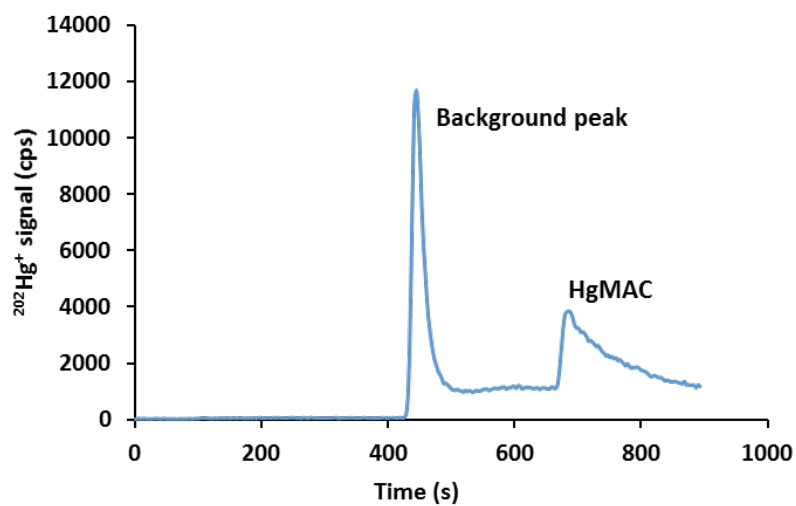




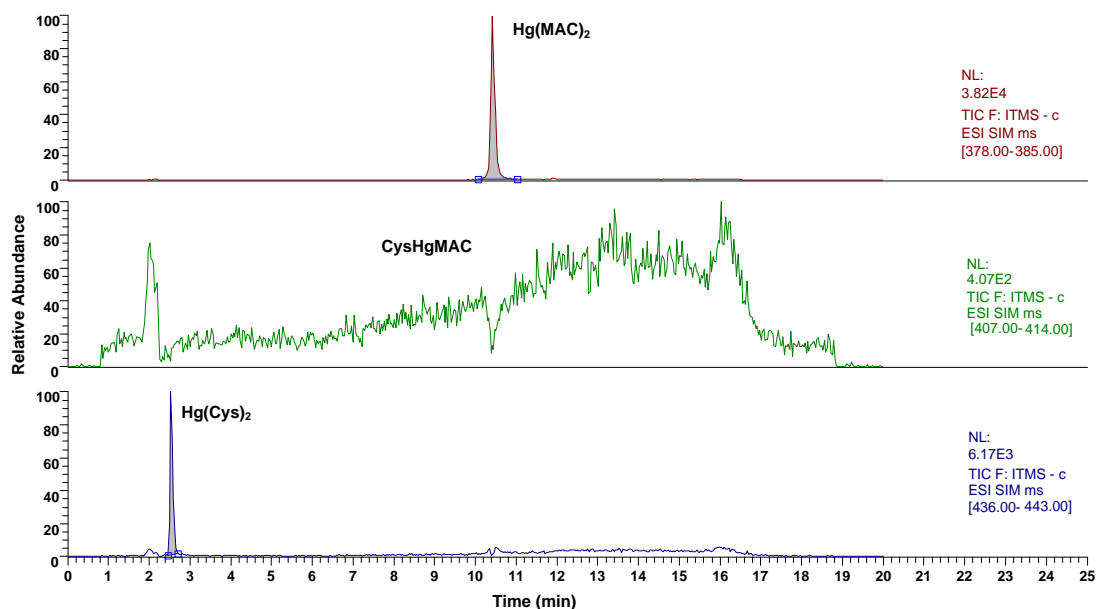
**Fig. S6.** Measured concentration of the  $\text{Hg}(\text{MAC})_2$  complex ( $2 \mu\text{M}$  of  $\text{Hg}^{\text{II}}$  and  $8 \mu\text{M}$  of MAC) at different added concentrations of the competing ligands EDTA,  $\text{Cl}^-$ , and  $\text{Br}^-$ . The experiments were carried out at constant ionic strength of  $0.5 \text{ M}$  ( $\text{NaClO}_4$ ) and pH of  $3.0$ .



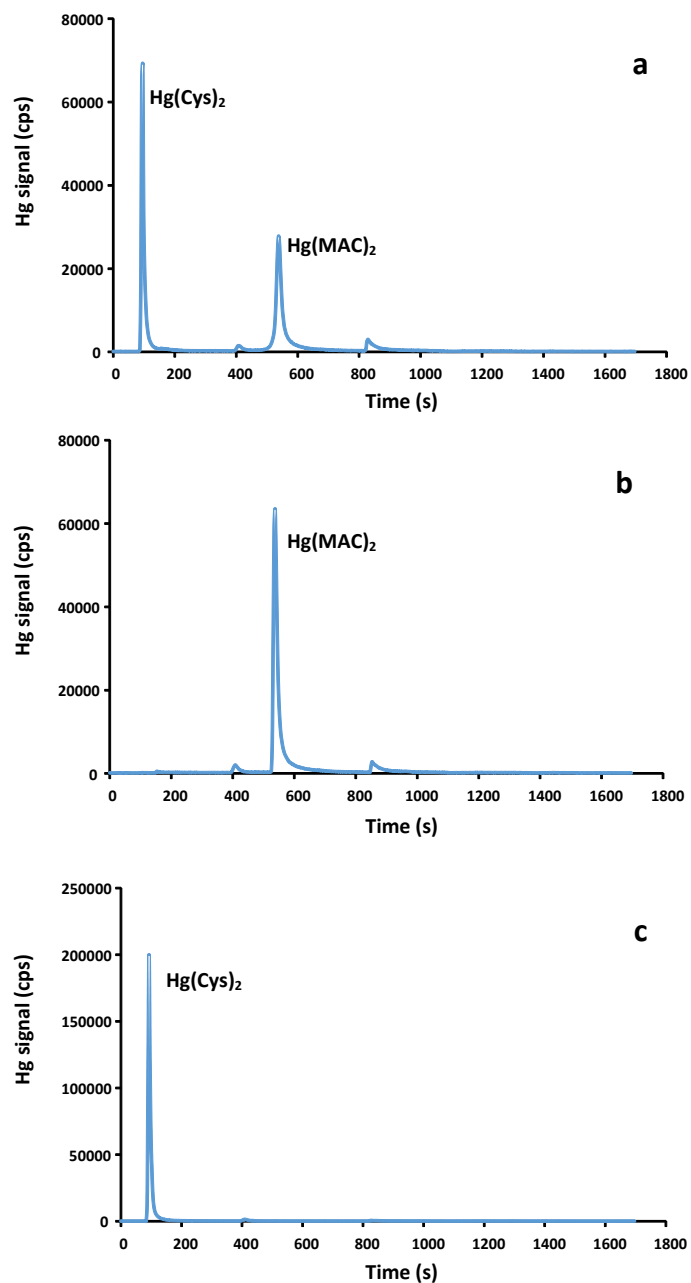
**Fig. S7.** LC-ICPMS chromatograms of  $^{202}\text{Hg}^+$  signals illustrating of retention time of the 15 investigated  $\text{Hg}(\text{SR})_2$  complexes on the Kinetic Biphenyl LC column used in the LC-ICPMS measurements. The retention time increased as, 1.  $\text{Hg}(\text{Cyst})_2$ , 2.  $\text{Hg}(\text{CysGly})_2$ , 3.  $\text{Hg}(\text{Cys})_2$ , 4.  $\text{Hg}(\text{HCys})_2$ , 5.  $\text{Hg}(\text{GSH})_2$ , 6.  $\text{Hg}(\text{GluCys})_2$ , 7.  $\text{Hg}(\text{Pen})_2$ , 8.  $\text{Hg}(\text{Glyc})_2$ , 9.  $\text{Hg}(\text{NACCys})_2$ , 10.  $\text{Hg}(\text{ETH})_2$ , 11.  $\text{Hg}(\text{MAC})_2$ , 12.  $\text{Hg}(\text{SUC})_2$ , 13.  $\text{Hg}(\text{3MPA})_2$ , 14.  $\text{Hg}(\text{NACPen})_2$ , 15.  $\text{Hg}(\text{2MPA})_2$ .



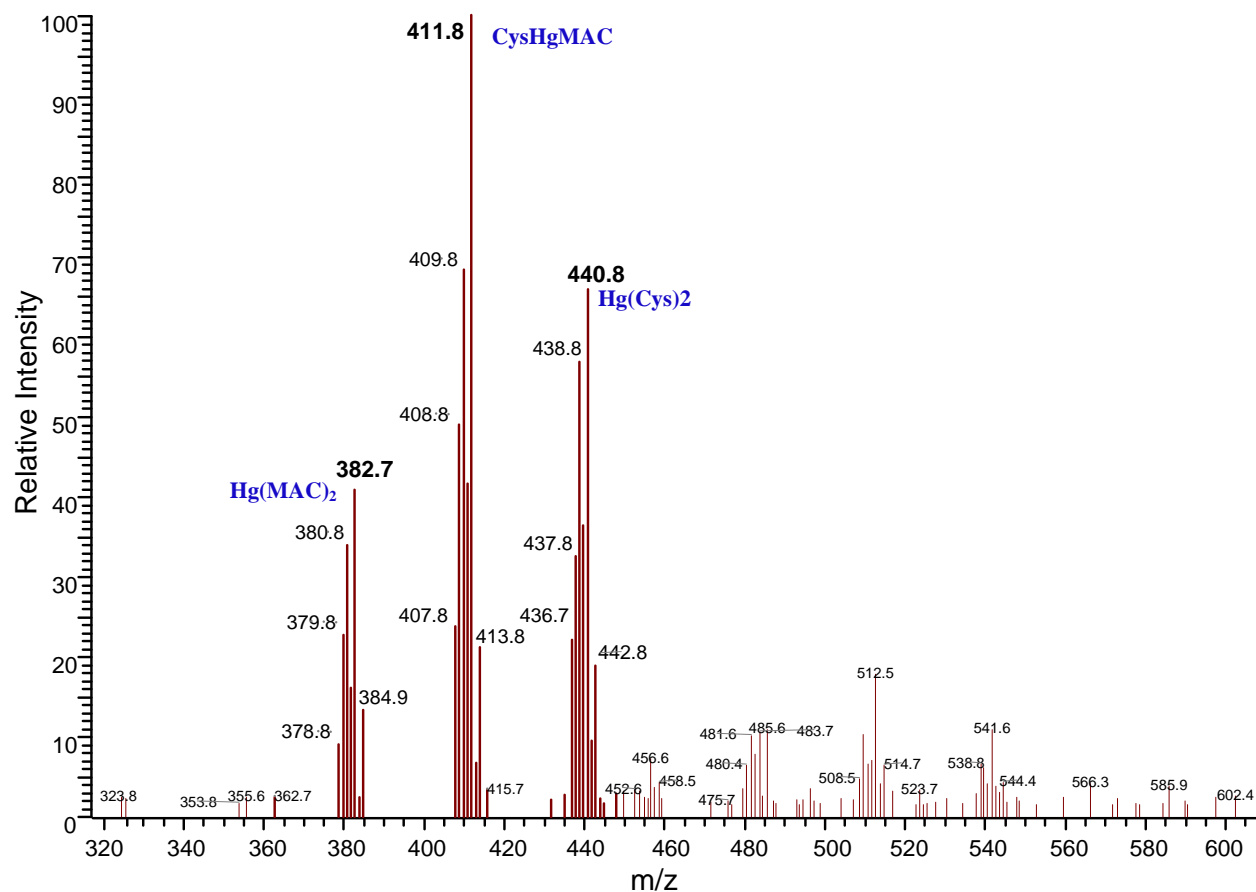
**Fig. S8.** LC-ICPMS chromatogram of  $^{202}\text{Hg}^+$  signals for a mixture of 4  $\mu\text{M}$  of  $\text{Hg}^{\text{II}}$  and 4  $\mu\text{M}$  of MAC with isocratic elution using 3.5% 1-propanol indicating the presence of the one-coordinated HgMAC complex with a retention time of 700 s.



**Fig. S9.** LC-ESIMS chromatograms with selected ion monitoring (SIM) mode targeting  $m/z$  of  $\text{Hg(MAC)}_2$ ,  $\text{Hg(Cys)}_2$ , and CysHgMAC and their corresponding Hg isotope pattern 378-385  $m/z$ , 436-443  $m/z$  and 407-414  $m/z$ , respectively. An elution gradient was used with initially 8% of MeOH, 0.1%FA and 92%  $\text{H}_2\text{O}$ , 0.1%FA from 3 to 10 min followed by a linear gradient to 90% of MeOH, 0.1%FA which was kept for 3 min. From 13 to 16 min the concentration of MeOH, 0.1%FA was reduced from 90% to 8% in a linear gradient and was kept for 9 min. The increased background after 6 min is caused by the increase of MeOH in the mobile phase.



**Fig. S10.** LC-ICPMS chromatograms showing  $^{202}\text{Hg}^+$  signals of (a) a mixture containing 4  $\mu\text{M}$  of  $\text{Hg}^{\text{II}}$  and 8  $\mu\text{M}$  of each Cys and MAC, (b) a mixture containing 4  $\mu\text{M}$  of  $\text{Hg}^{\text{II}}$  and 16  $\mu\text{M}$  of MAC, (c) a mixture containing 4  $\mu\text{M}$  of  $\text{Hg}^{\text{II}}$  and 16  $\mu\text{M}$  of Cys. The retention time of  $\text{Hg}(\text{Cys})_2$  and  $\text{Hg}(\text{MAC})_2$  is 85 s and 540 s, respectively with a mobile phase gradient of initially 3.5% of 1-propanol for 12 min then a step gradient increase to 25% of 1-propanol. The appearance of a small peak at 850 s is caused by the changed concentration of 1-propanol in the mobile phase.



**Fig. S11.** Full scan mass spectrum of a solution containing 0.1 mM of Hg<sup>II</sup> and 0.2 mM of each Cys and MAC by direct infusion to ESI-MS with a flow rate of 50  $\mu\text{l min}^{-1}$ . Observed signals indicate the presence of Hg(MAC)<sub>2</sub>, Hg(Cys)<sub>2</sub> and CysHgMAC complexes, matching their molecular mass and Hg isotope pattern.

**Table S1. Thermodynamic stability constants for  $\text{HgI}_n^{2-n}$  complexes at different ionic strengths**

The stability constants were corrected to different ion strength using free software Ionic Strength Corrections for Stability Constants from IUPAC with Specific Interaction Theory (SIT) method<sup>[1]</sup>

Reaction	Stability constant ( $\log K_1$ , $\log \beta_2$ – $\beta_4$ )			
	I=0.5 <sup>*</sup>	I=0	I=0.1	I=1
$\text{Hg}^{2+} + \text{I}^- = \text{HgI}^+$	12.87	13.42	13.02	12.90
$\text{Hg}^{2+} + 2\text{I}^- = \text{HgI}_2$	23.82	24.60	24.00	23.92
$\text{Hg}^{2+} + 3\text{I}^- = \text{HgI}_3^-$	27.6	28.32	27.74	27.74
$\text{Hg}^{2+} + 4\text{I}^- = \text{HgI}_4^{2-}$	29.83	30.07	29.73	30.15

<sup>\*</sup> Reference Martell et al. (2004)<sup>[2]</sup>

**Table S2. Computed complex formation free energies, bond lengths and angles of the Hg(SR)<sub>2</sub> complexes determined at the B3LYP level of theory**

All geometries were optimized at the gas phase with a mixed basis set containing Def2-TZVPP for Hg and 6-31++G(d,p) for all other atoms, respectively, and an initial geometry with all ligands fully extended

Complexes	$\Delta G_2^a$ (kcal/mol)	Bond (Å)		Angle (°)		
		Hg-S <sup>b</sup>	Hg-O <sup>c</sup>	S-Hg-S	Hg-S-C <sup>b</sup>	O-Hg-O <sup>c</sup>
Hg(2-MPA) <sub>2</sub>	53.1	2.381	3.042	179.7	101.4	158.4
Hg(NACPen) <sub>2</sub>	56.4	2.364		175.6	105.0	
Hg(NACCys) <sub>2</sub>	51.1	2.383	3.087	175.1	99.3	65.4
Hg(SUC) <sub>2</sub>	54.0	2.376	3.146	176.7	102.8	96.4
Hg(3-MPA) <sub>2</sub>	58.0	2.361		177.6	103.0	
Hg(MAC) <sub>2</sub>	56.6	2.373	3.176	177.6	102.9	173.4
Hg(Glyc) <sub>2</sub>	55.2	2.361		177.7	103.0	
Hg(GluCys) <sub>2</sub>	77.4	2.365		177.0	103.5	
Hg(ETH) <sub>2</sub>	56.9	2.360		177.7	102.7	
Hg(Pen) <sub>2</sub>	86.6	2.383	2.876	176.8	108.4	
Hg(GSH) <sub>2</sub>	54.7	2.380	3.062	170.7	104.6	63.6
Hg(Cys) <sub>2</sub>	92.1	2.373		177.4	105.7	
Hg(CysGly) <sub>2</sub>	91.7	2.375		176.6	106.0	
Hg(HCys) <sub>2</sub>	87.3	2.372		176.7	106.1	
Hg(Cyst) <sub>2</sub>	90.7	2.374		175.6	106.1	

<sup>a</sup> The  $\Delta G$  values are for the reaction 1a, i.e.,  $\text{Hg}^{2+} + 2\text{RSH} = \text{Hg}(\text{SR})_2 + 2\text{H}^+$ , in the gas phase at 298.15 K.

<sup>b</sup> The Hg-S distances and Hg-S-C angles are the averages over the two Hg-S distances and Hg-S-C angles, respectively.

<sup>c</sup> In the geometry optimisations, several complexes form an additional coordination between Hg and the ligand's carbonyl or carboxyl oxygen (bidentate coordination). The Hg-O distances and O-Hg-O angles are the averages over the two Hg-O distances and O-Hg-O angles, respectively, except Hg(Pen)<sub>2</sub>, which only forms a single Hg-O interaction.

## References

- [1] IUPAC, Ionic Strength Corrections for Stability Constants using Specific Interaction Theory (SIT), version 1. **2004**.
- [2] A. Martell, R. Smith, R. Motekaitis, National Institute of Standard and Technology, NIST. *Critically selected stability constants of metal complexes* **2004** (PC-based Database: Gaithersburg, MD).