Supporting information for

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

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1. Calculation of stability constants for $Hg(MAC)_2$ and HgIMAC using I^-as competing ligand. Components, reactions and a calculation scheme for determination of the stability constant for the $Hg(MAC)_2$ and HgIMAC complexes with I^- 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 $Hg(2\text{-}MPA)_2$.

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

$[MAC]_{total}= [MAC]_{free} + 2[Hg(MAC)_2] +[HgIMAC] + [HgMAC]$ (b)

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

$Hg^{II} + I^- = HgI^+$ Log K_1= 13.42 (3a)

$Hg^{II} + 2I^- = HgI_2$ Log K_2= 24.60 (3b)

$Hg^{II} + 3I^- = HgI_3^-$ Log K_3=28.32 (3c)

$Hg^{II} + 4I^- = HgI_4^{2-}$ Log K_4= 30.07 (3d)

Hg^{II} + MAC=HgMAC Log K = 32.5 (4a’)

$Hg^{II} + 2MAC = Hg(MAC)_2$ Log β_2= ? (1b’)

$Hg^{II} + I^- +MAC = HgIMAC$ Log K=? (6)

Example of calculation

$[I^-]_{total}$ = 20 μM[a]

$[MAC]_{total}$ = 16 μM[a]

$[Hg^{II}]_{total}$ = 4 μM[a]

$[Hg(MAC)_2]$ = 3.2 μM[b]

[HgIMAC] = (Peak Area (HgIMAC) / Peak Area ($Hg(MAC)_2$)) ×$[Hg(MAC)_2]$ = 0.45 μM[b]

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

[a] Concentration of ligand used for complexation

[b] Concentration of complexes determined by LC-ICPMS.

In the experiment, the concentration of I^- was varied, and in the calculation scheme above it is exemplified for 20 μM I^-. Constants were recalculated to an ionic strength of 0 M and experimental pH was 2.9. Based on the equations (a-c); reaction 3(a-d), (4a’), (1b’), (6), and total concentration of Hg^{II} and ligands, and measured concentration of $Hg(MAC)_2$ and HgIMAC complexes, the log *K* of $Hg(MAC)_2$ and HgIMAC was determined to be 40.9 and 32.2, respectively.

2. Calculation of the stability constant for the $Hg(Cys)_2$ complex using MAC as competing ligand.

Calculation scheme for determination of the stability constant for $Hg(Cys)_2$ 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 $Hg(SR)_2$ complexes using MAC or 2-MPA as the competing ligand.

$[MAC]_{total}= [MAC]_{free} + 2[Hg(MAC)_2] + [HgMAC]$ (d)

$[Cys]_{total}= [Cys]_{free} + 2[Hg(Cys)_2] + [HgCys]$ (e)

$[Hg^{II}]_{total}= [Hg^{II}]_{free} + [HgMAC] + [Hg(MAC)_2] + [HgCys] + [Hg(Cys)_2] +[Hg(OH)_n]$ (f)

$Hg^{II} + MAC = HgMAC$ Log $K = 32.5$ (4’)

$Hg^{II} + Cys = HgCys$ Log $K = 29.0$ (4’’)

$Hg^{II} + 2MAC = Hg(MAC)_2$ Log $\beta_2= 40.9$ (1b’)

$Hg^{II} + 2Cys = Hg(Cys)_2$ Log $\beta_2=?$ (1b’’)

Example of calculation

$[MAC]_{total} = 8$ µM[a]

$[Cys]_{total}= 8$ µM[a]

$[Hg^{II}]_{total}= 4$ µM[a]

$[Hg(MAC)_2] = 2.27$ µM[b]

$[Hg(Cys)_2] = 1.72$ µM[b]

[a] Concentration of ligand used for complexation

[b] 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 (d-f); reactions (4’), (4’’), (1b’), (1b’’) and total concentration of the Hg^{II} and ligands and measured complexes concentration, the log β_2 of $Hg(Cys)_2$ was determined to 37.5.

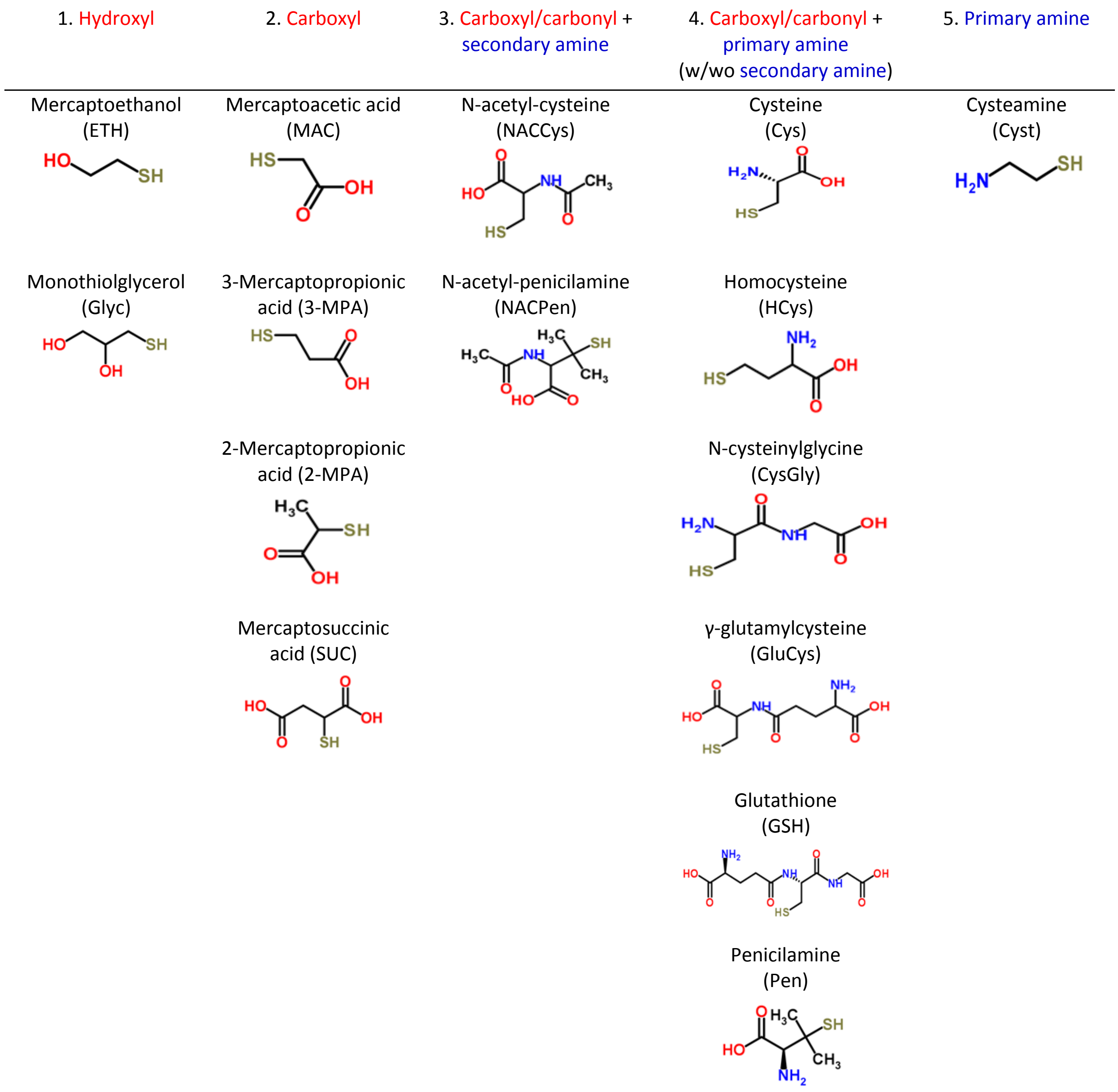

Figure S1. The structure and abbreviation of investigated thiol ligands. The thiols are grouped according to the presence of functional groups in addition to the thiol group.

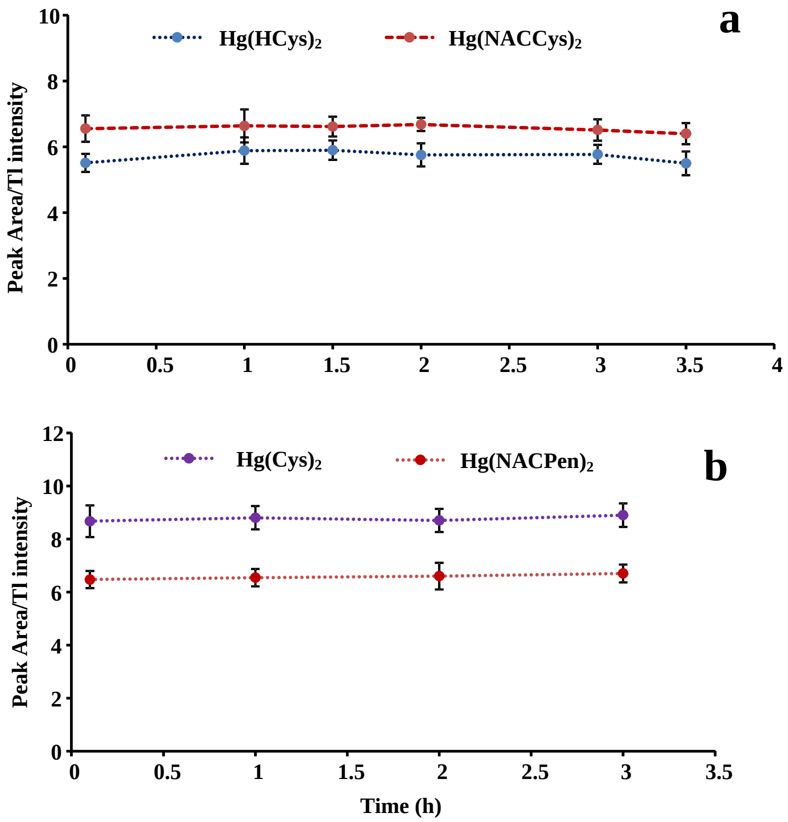

Figure S2. The integrated peak area of Hg(SR)$_2$ complexes to Thalium (^{204}Tl$^+$) signal ratios with different reaction time for the Hg(SR)$_2$ complex synthesis. (a) mixture of 1 µM of Hg^{II} and 2 µM of each HCys and NACCys. (b) mixture of 1 µM of Hg^{II} and 2 µM of each Cys and NACPen. The experiments were conducted at pH 3.0, and an ion strength of 0 M. A post column flow of a 10 ng ml^{-1} Tl solution (flow rate of 100 µl min^{-1}) was used to monitor and correct for signal drift of the LC-ICPMS system over time.

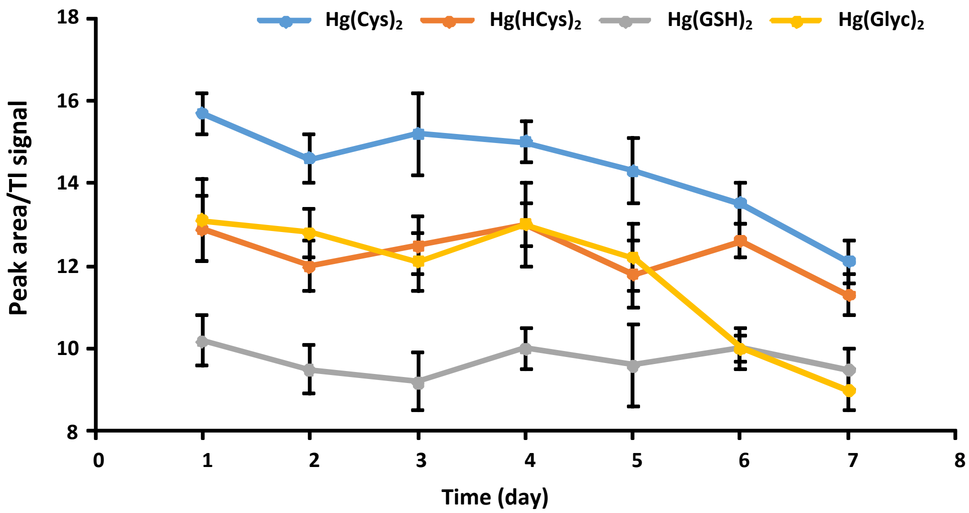

Figure S3. Peak area of $Hg(SR)_2$ complexes to Thalium ($^{204}Tl^+$) signal ratios at different storage time of $Hg(SR)_2$ complex solutions. Samples contain 1 µM of Hg^{II} and 4 µM of individual LMM thiols i.e. Cys, HCys, GSH and Glyc. A post column flow of a 10 ng ml^{-1} Tl solution (flow rate of 100 µl min^{-1}) was used to monitor and correct for signal drift of the LC-ICPMS system over time.

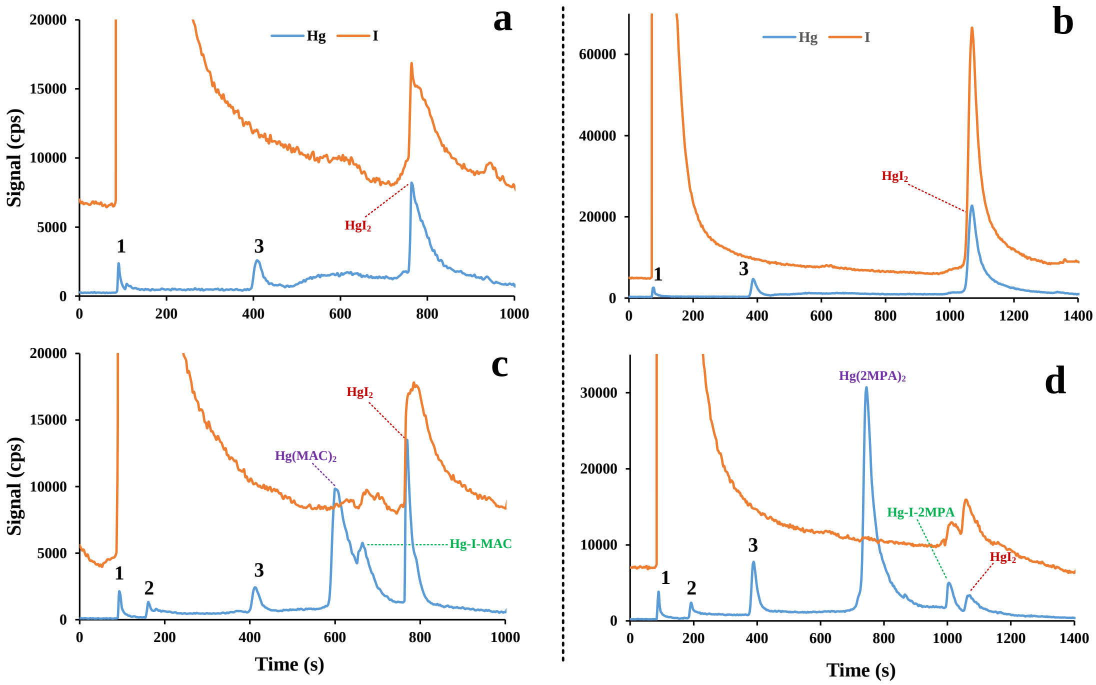

Figure S4. LC-ICPMS chromatograms showing $^{202}Hg^+$ and $^{127}I^+$ signals (counts per second, cps) for **(a)** 4 µM of Hg^{II} and 50 µM of I$^-$. A gradient elution was used with initially 3.5% of 1-propanol during 12 min, then increased to 25% in a step gradient and kept for 4 min and then back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

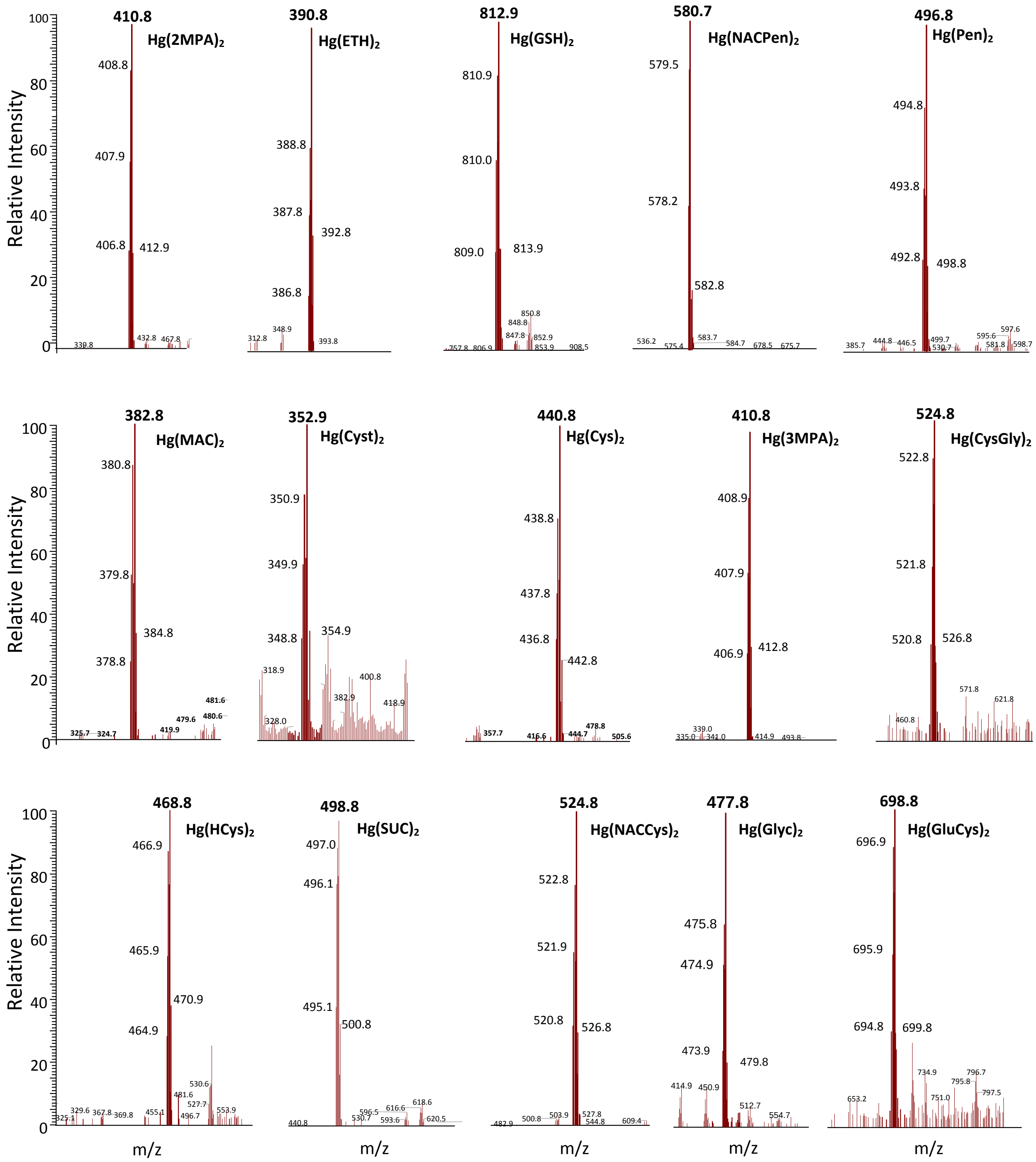

Figure S5. The mass spectra of 15 investigated $Hg(SR)_2$ complexes achieved by direct infusion to ESIMS, showing molecular mass and Hg isotope pattern of the $Hg(SR)_2$ complexes. All $Hg(SR)_2$ complex analyses were conducted in the negative ionization mode with exception of $Hg(Cyst)_2$ which was analysed in positive mode. The concentration of Hg^{II} was 0.1 mM, the molar ratio of RSH to Hg^{II} was 4 and pH was 3.0.

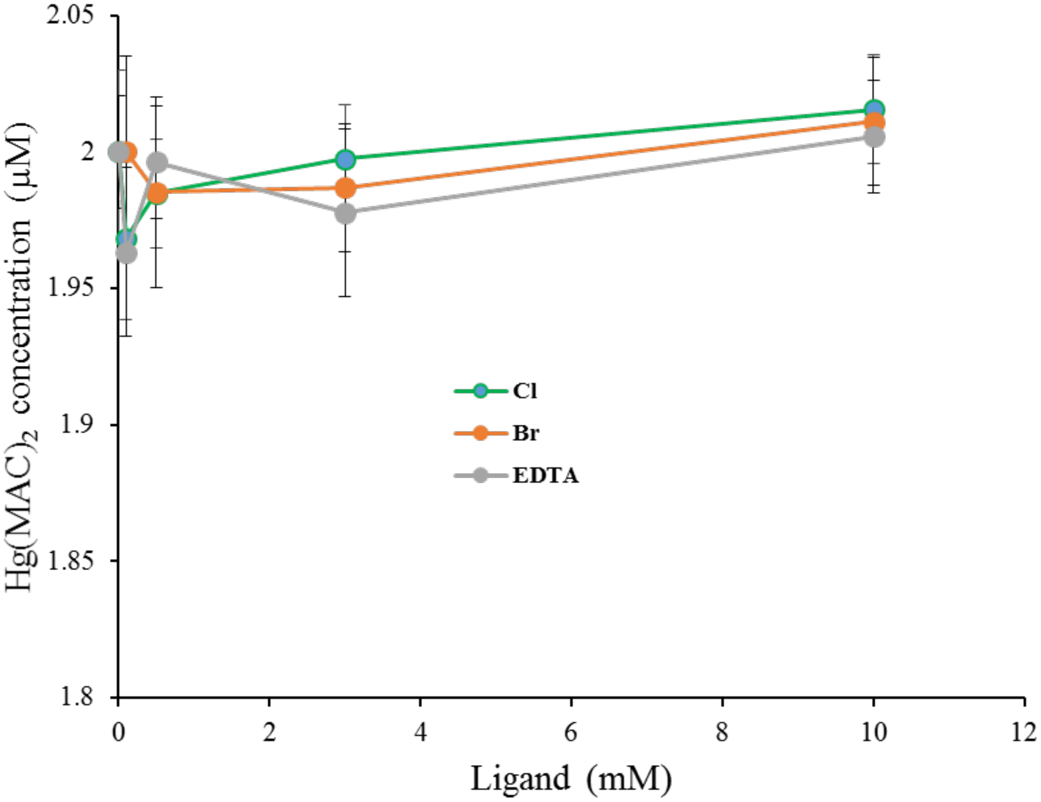

Figure S6. Measured concentration of the $Hg(MAC)_2$ complex (2 µM of Hg^{II} and 8 µM of MAC) at different added concentrations of the competing ligands EDTA, Cl^-, and Br^-. The experiments were carried out at constant ionic strength of 0.5 M ($NaClO_4$) and pH of 3.0.

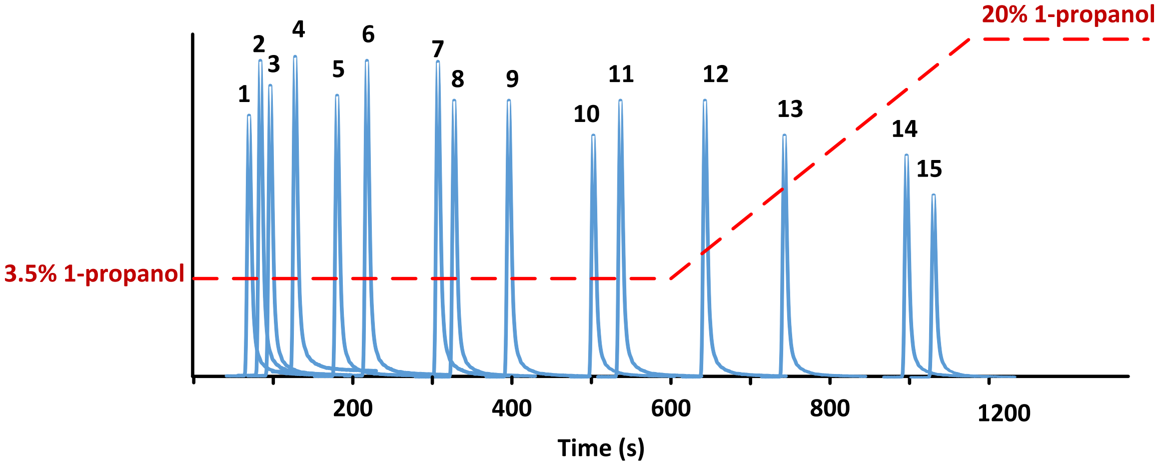

Figure S7. LC-ICPMS chromatograms of $^{202}Hg^{+}$ signals Illustrating of retention time of the 15 investigated $Hg(SR)_2$ complexes on the Kinetic Biphenyl LC column used in the LC-ICPMS measurements. The retention time increased as, 1. $Hg(Cyst)_2$, 2. $Hg(CysGly)_2$, 3. $Hg(Cys)_2$, 4. $Hg(HCys)_2$,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

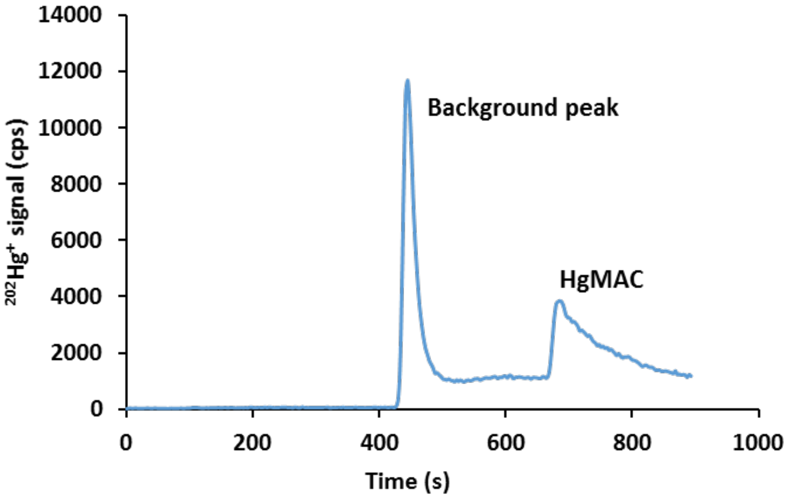

Figure S8. LC-ICPMS chromatogram of $^{202}Hg^{+}$ signals for a mixture of 4 µM of Hg^{II} and 4 µM of MAC with isocratic elution using 3.5% 1-propanol indicating the presence of the one-coordinated HgMAC complex with a retention time of 700 s.

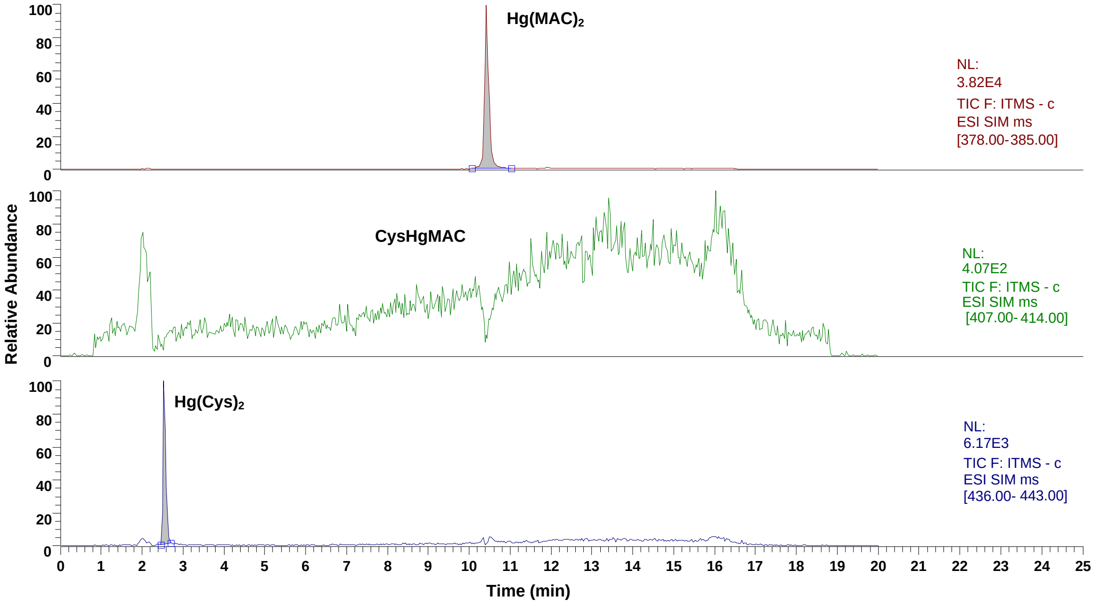

Figure S9. LC-ESIMS chromatograms with selected ion monitoring (SIM) mode targeting m/z of $Hg(MAC)_2$, $Hg(Cys)_2$, and CysHgMAC and their corresponding Hg isotope pattern 378-385 m/z, 436-443 m/z and 407-414 m/z, respectively. An elution gradient was used with initially 8% of MeOH, 0.1%FA and 92% H_2O, 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.

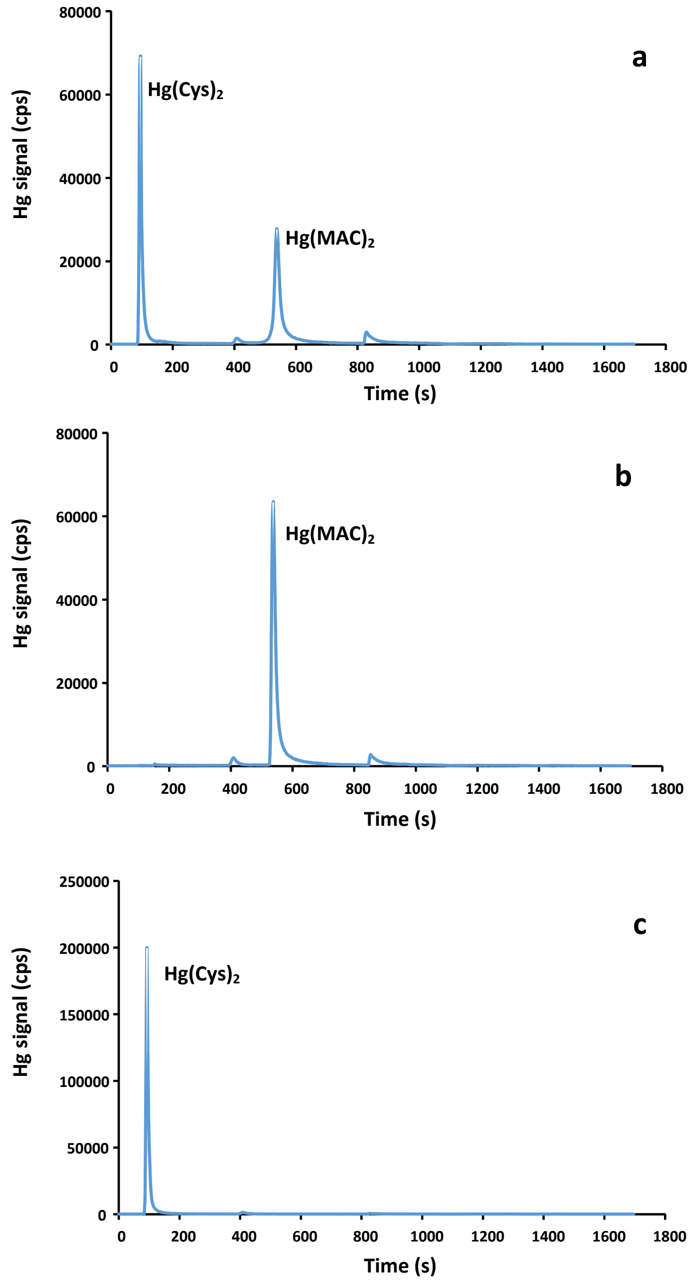

Figure S10. LC-ICPMS chromatograms showing $^{202}Hg^{+}$ signals of (a) a mixture containing 4 µM of Hg^{II} and 8 µM of each Cys and MAC, (b) a mixture containing 4 µM of Hg^{II} and 16 µM of MAC, (c) a mixture containing 4 µM of Hg^{II} and 16 µM of Cys. The retention time of $Hg(Cys)_2$ and $Hg(MAC)_2$ 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.

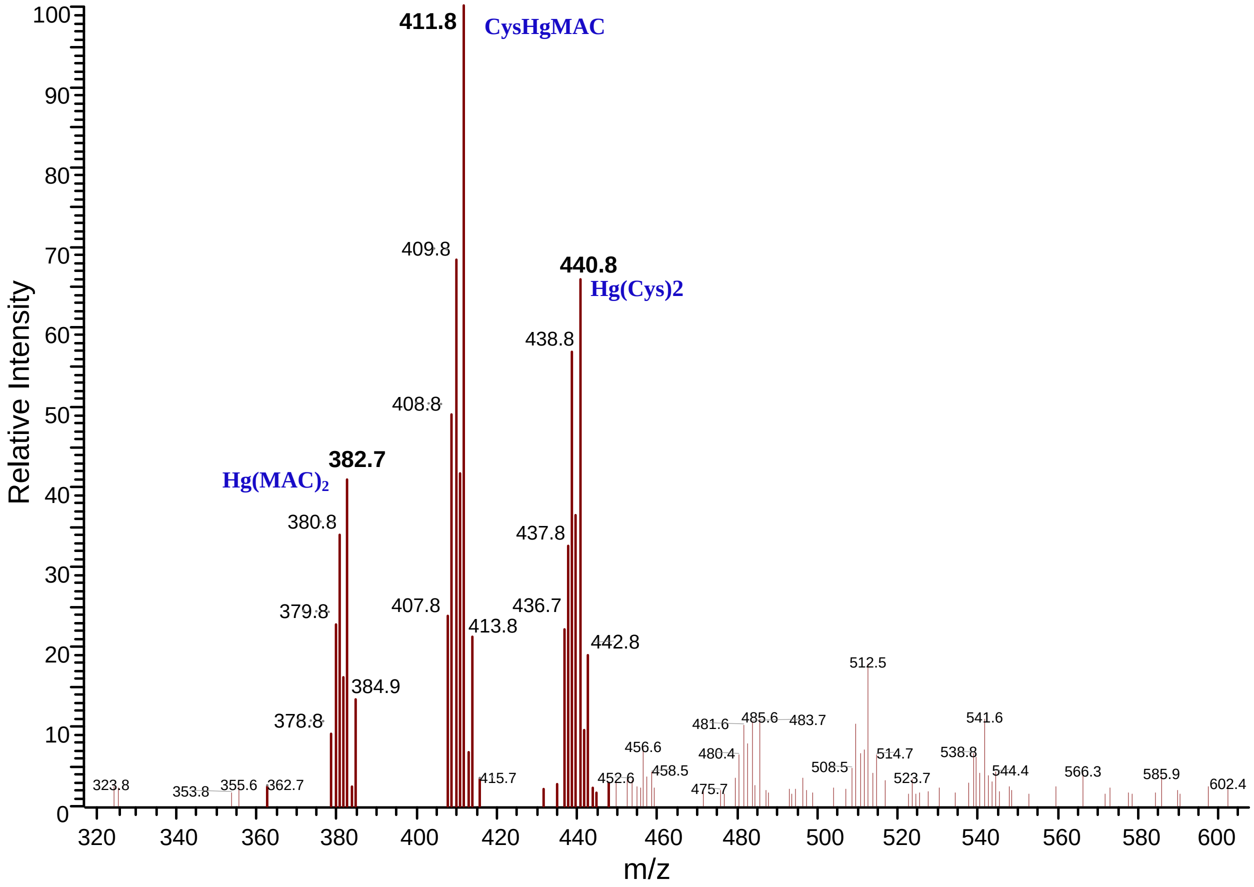

Figure S11. Full scan mass spectrum of a solution containing 0.1 mM of Hg^{II} and 0.2 mM of each Cys and MAC by direct infusion to ESI-MS with a flow rate of 50 µl min^{-1}. Observed signals indicate the presence of $Hg(MAC)_2$, $Hg(Cys)_2$ and CysHgMAC complexes, matching their molecular mass and Hg isotope pattern.

Table S1. Thermodynamic stability constants for 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.[1]

Reaction	Stability constant (log *K*)			
	I=0.5*	I=0	I=0.1	I=1
$Hg^{2+} + I^- = HgI^+$	12.87	13.42	13.02	12.90
$Hg^{2+} + 2I^- = HgI_2$	23.82	24.60	24.00	23.92
$Hg^{2+} + 3I^- = HgI_3^-$	27.6	28.32	27.74	27.74
$Hg^{2+} + 4I^- = HgI_4^{2-}$	29.83	30.07	29.73	30.15

* Reference Martell et al. (2004)

Table S2. Computed complex formation free energies, bond lengths and angles of the $Hg(SR)_2$ complexes determined at the B3LYP level of theory. All geometries were optimized at the gas phase with a mixed basis set containing Def2TZVPP for Hg and 6-31++G(d,p) for all other atoms, respectively, and an initial geometry with all ligands fully extended.

	ΔG_2[a]	Bond (Å)		Angle (°)		
Complexes	(kcal/mol)	Hg-S[b]	Hg-O[c]	S-Hg-S	Hg-S-C[b]	O-Hg-O[c]
$Hg(2\text{-}MPA)_2$	53.1	2.381	3.042	179.7	101.4	158.4
$Hg(NACPen)_2$	56.4	2.364		175.6	105.0	
$Hg(NACCys)_2$	51.1	2.383	3.087	175.1	99.3	65.4
$Hg(SUC)_2$	54.0	2.376	3.146	176.7	102.8	96.4
$Hg(3\text{-}MPA)_2$	58.0	2.361		177.6	103.0	
$Hg(MAC)_2$	56.6	2.373	3.176	177.6	102.9	173.4
$Hg(Glyc)_2$	55.2	2.361		177.7	103.0	
$Hg(GluCys)_2$	77.4	2.365		177.0	103.5	
$Hg(ETH)_2$	56.9	2.360		177.7	102.7	
$Hg(Pen)_2$	86.6	2.383	2.876	176.8	108.4	
$Hg(GSH)_2$	54.7	2.380	3.062	170.7	104.6	63.6
$Hg(Cys)_2$	92.1	2.373		177.4	105.7	
$Hg(CysGly)_2$	91.7	2.375		176.6	106.0	
$Hg(HCys)_2$	87.3	2.372		176.7	106.1	
$Hg(Cyst)_2$	90.7	2.374		175.6	106.1	

[a] The ΔG values are for the reaction 1a, i.e., $Hg^{2+} + 2RSH = Hg(SR)_2 + 2H^+$, in the gas phase at 298.15 K.

[b] The Hg-S distances and Hg-S-C angles are the averages over the two Hg-S distances and Hg-S-C angles, respectively.

[c] In the geometry optimizations, 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 $Hg(Pen)_2$, 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. Martell, A.; Smith, R.; Motekaitis, R., National Institute of Standard and Technology, NIST. *Critically selected stability constants of metal complexes. PC-based Database, Gaithersburg, MD* **2004,** *20899*.