Supporting Information

Glutathione Complex Formation with Mercury(II) in Aqueous Solution at Physiological pH

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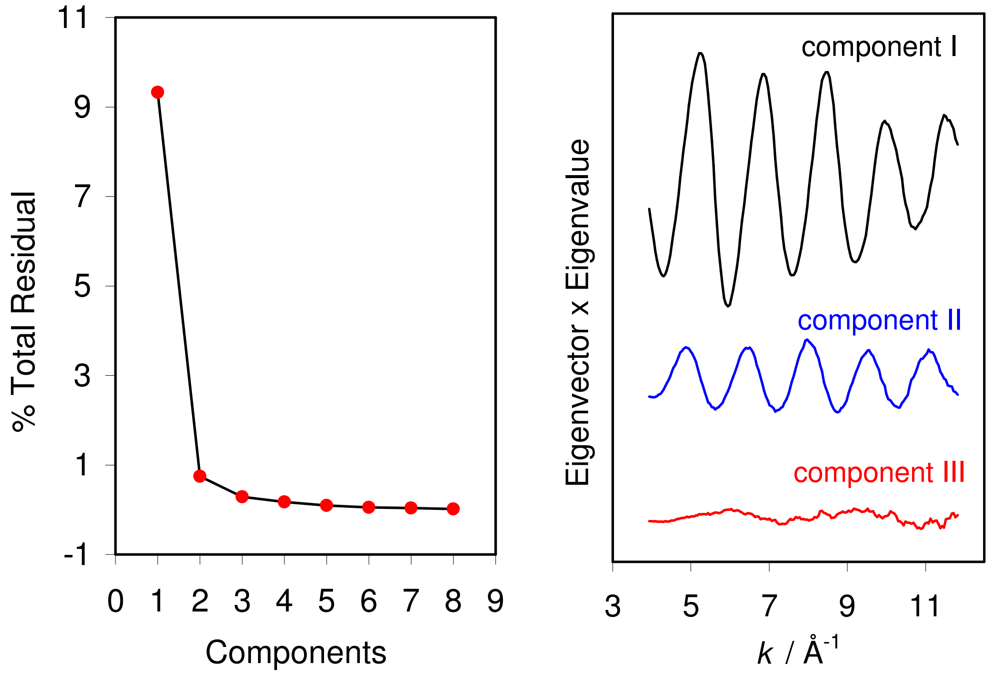


Figure S-1. PCA of k^3-weighted EXAFS oscillations of 9 Hg^{II}-glutathione solutions (pH = 7.0) **C1 - F1** ($C_{Hg(II)}$ ~17 mM) and **B2 - F2** ($C_{Hg(II)}$ ~ 50 mM) over k = 3.9 – 11.9 $Å^{-1}$. (*left*) % Total residual in the reconstructed spectra obtained from consecutive elimination of the components, indicating two major components. (*right*) First three components with eigenvalues 58.6, 18.0 and 4.2, respectively. (*Note*: Solutions **A1**, **B1** and **A2**, where one species is dominating, were not included in PCA).

Table S-1. % Total residual after consecutive elimination of the components in the PCA of solutions **C1** – **F1** and **B2** - **F2** (*see Figure S-1, left*).

Component	*% Total Residual*
8	0.016
7	0.036
6	0.063
5	0.096
4	0.182
3	0.288
2	0.746
1	9.328

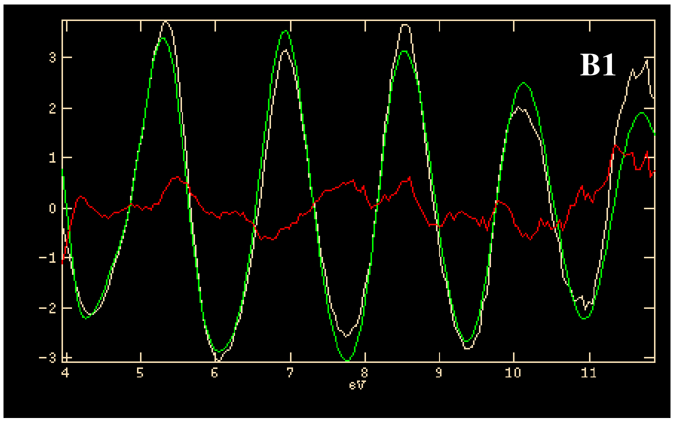
Figure S-2. Linear combination fits for solutions **B1–F1** over the range k = 3.9 - 11.9 $Å^{-1}$, using EXAFS oscillations for models of $[Hg(AH)_2]^{2-}$ (model fit for solution **A1**: Hg-S 2.325 Å, σ^2 = 0.004 $Å^2$) and of $[Hg(AH)_3]^{4-}$ (simulated using 3 Hg-S @ 2.42 Å, σ^2 = 0.006 $Å^2$, ΔE_0 = 9.0, S_0^2 = 1.0).

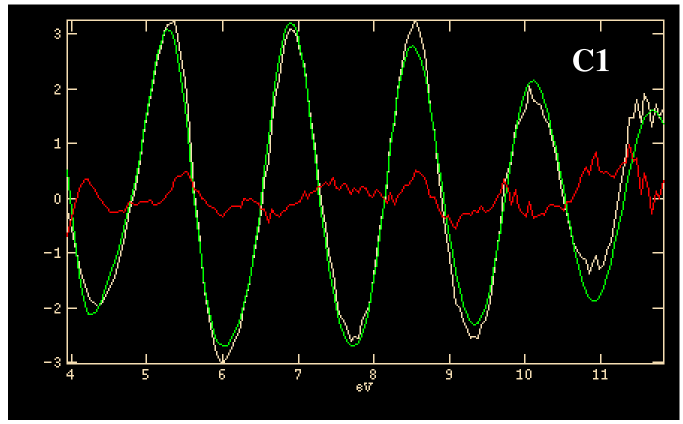
Solution **B1**

$[Hg(AH)_2]^{2-}$	95 %	Residual = 0.177
$[Hg(AH)_3]^{4-}$	5 %	

Solution **C1**

$[Hg(AH)_2]^{2-}$	87 %	Residual = 0.0868
$[Hg(AH)_3]^{4-}$	13 %	



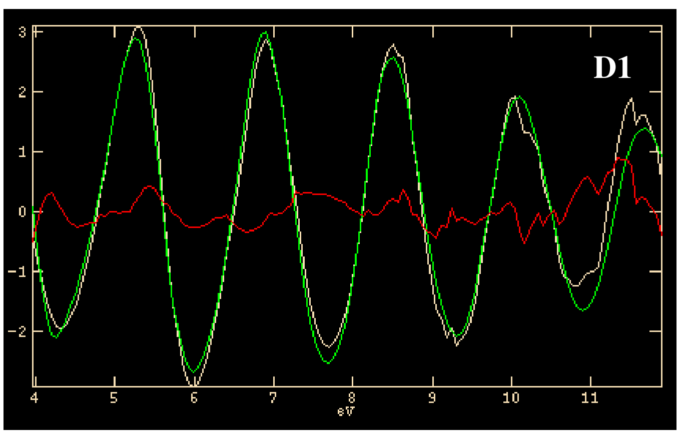


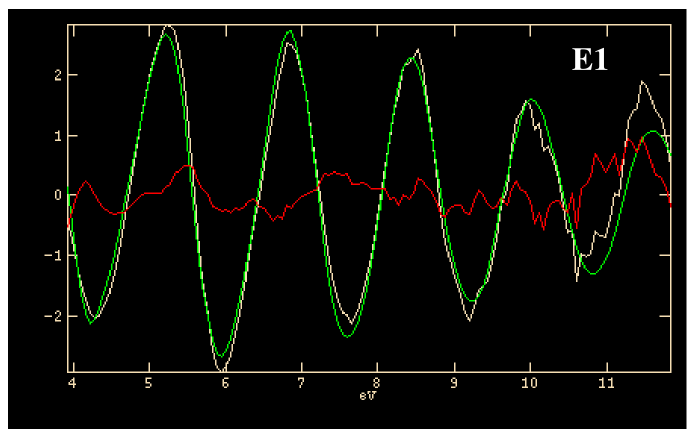
Solution **D1**

$[Hg(AH)_2]^{2-}$	79 %	Residual = 0.0817
$[Hg(AH)_3]^{4-}$	21 %	

Solution **E1**

$[Hg(AH)_2]^{2-}$	65 %	Residual = 0.0998
$[Hg(AH)_3]^{4-}$	35 %	





Solution **F1**

$[Hg(AH)_2]^{2-}$	52 %	Residual = 0.0825
$[Hg(AH)_3]^{4-}$	48 %	

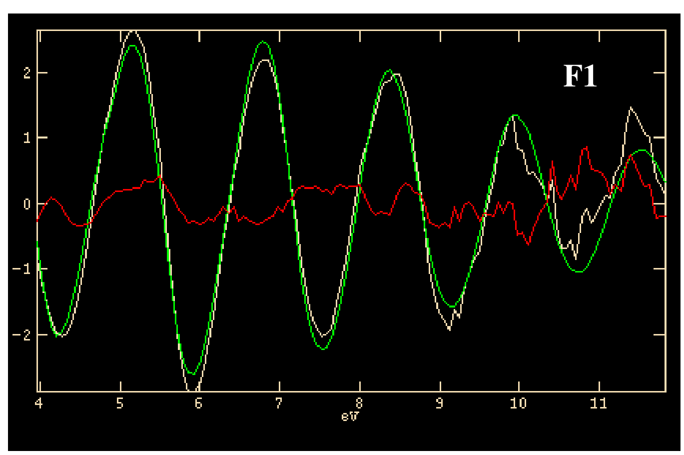


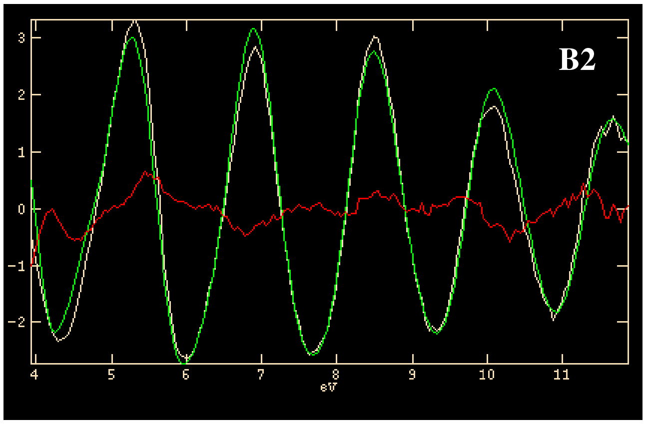
Figure S-3. Linear combination fits for solutions **B2–F2** over k = 3.9 - 11.9 Å^{-1}, using EXAFS models for $[Hg(AH)_2]^{2-}$ (model fit for solution **A2**: Hg-S 2.324 Å, σ^2 = 0.0035 Å^2) and $[Hg(AH)_3]^{4-}$ (simulated using 2 Hg-S @ 2.42 Å, σ^2 = 0.006 Å^2, ΔE_0 = 9.0, S_0^2 = 1.0).

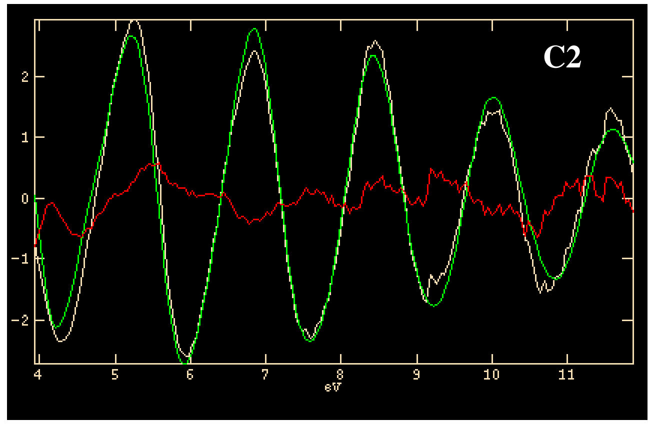
Solution **B2**

$[Hg(AH)_2]^{2-}$	78 %	Residual = 0.074
$[Hg(AH)_3]^{4-}$	22 %	

Solution **C2**

$[Hg(AH)_2]^{2-}$	62%	Residual = 0.082
$[Hg(AH)_3]^{4-}$	38 %	



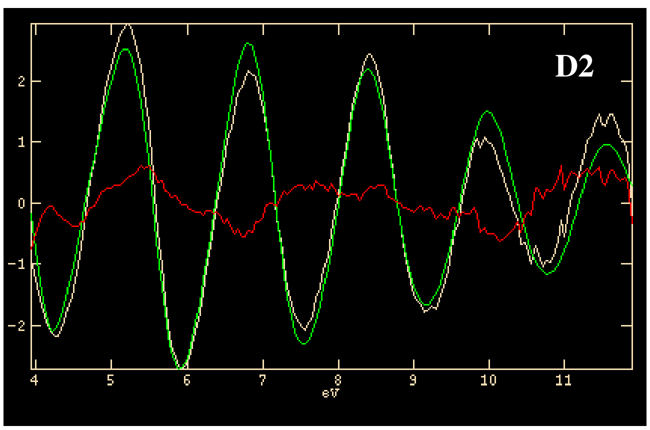


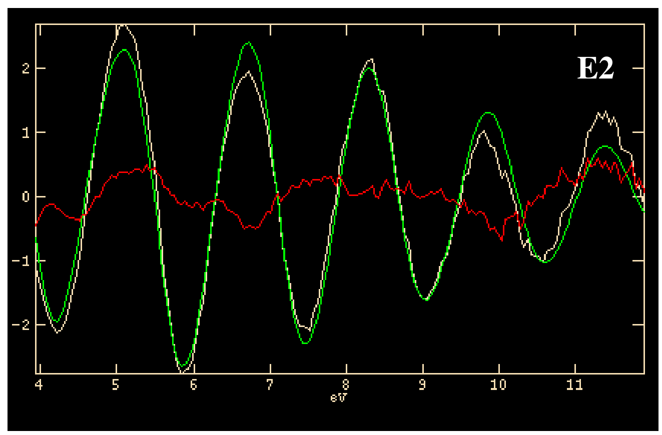
Solution **D2**

$[Hg(AH)_2]^{2-}$	54 %	Residual = 0.102
$[Hg(AH)_3]^{4-}$	46 %	

Solution **E2**

$[Hg(AH)_2]^{2-}$	35 %	Residual = 0.080
$[Hg(AH)_3]^{4-}$	65 %	





Solution **F2**

$[Hg(AH)_2]^{2-}$	30 %	Residual = 0.080
$[Hg(AH)_3]^{4-}$	70 %	

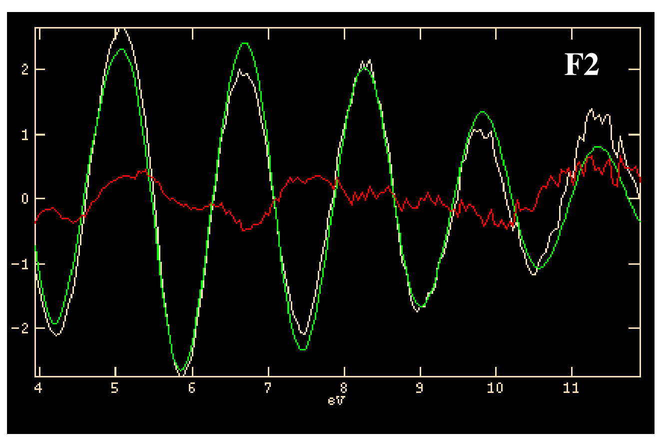
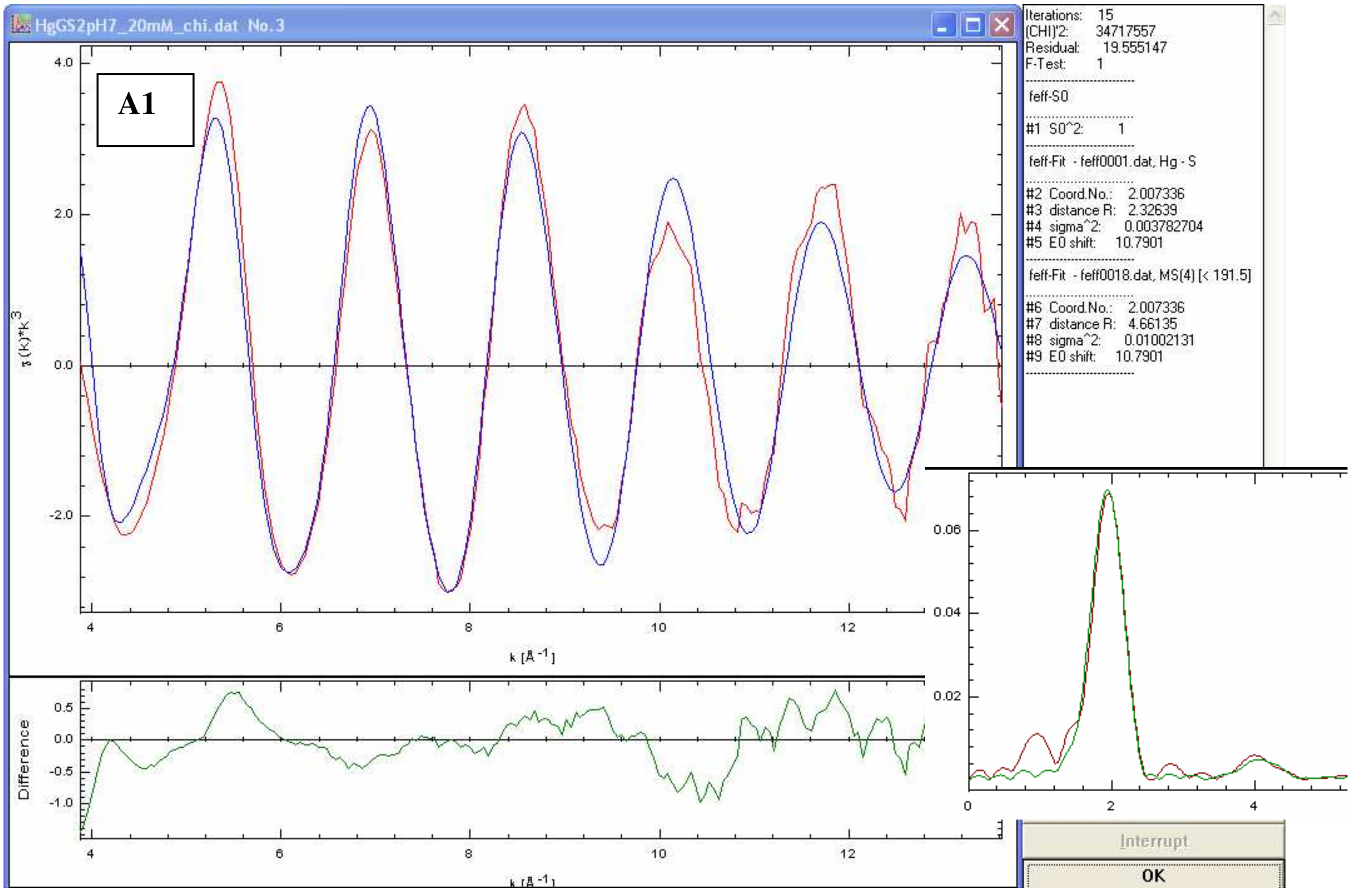


Table S-2. Assignment of Mass Ions Observed in ESI Mass Spectra for Solution **F1** (GSH/Hg(II) = 11.8, pH = 7.0) Measured in the Negative Ion Mode as Shown in Figure 3. Glutathione, GSH ($C_{10}H_{17}N_3O_6S$) *m* = 307.3.

-*m/z*	Assignment
1229.6	$[5Na + Hg(GSH)_3 - 8H]^-$
1207.6	$[4Na + Hg(GSH)_3 - 7H]^-$
878.7	$[3Na + Hg(GSH)_2 - 6H]^-$
856.7	$[2Na + Hg(GSH)_2 - 5H]^-$
834.7	$[Na + Hg(GSH)_2 - 4H]^-$
812.7	$[Hg(GSH)_2 - 3H]^-$

Figure S-4. Curve-fitting of the Hg L_{III}-edge EXAFS spectra for solutions **A1** (17 mM) and **A2** (50 mM) in long *k*-range (13.5 to 14.5 $Å^{-1}$), showing no Hg-Hg scattering



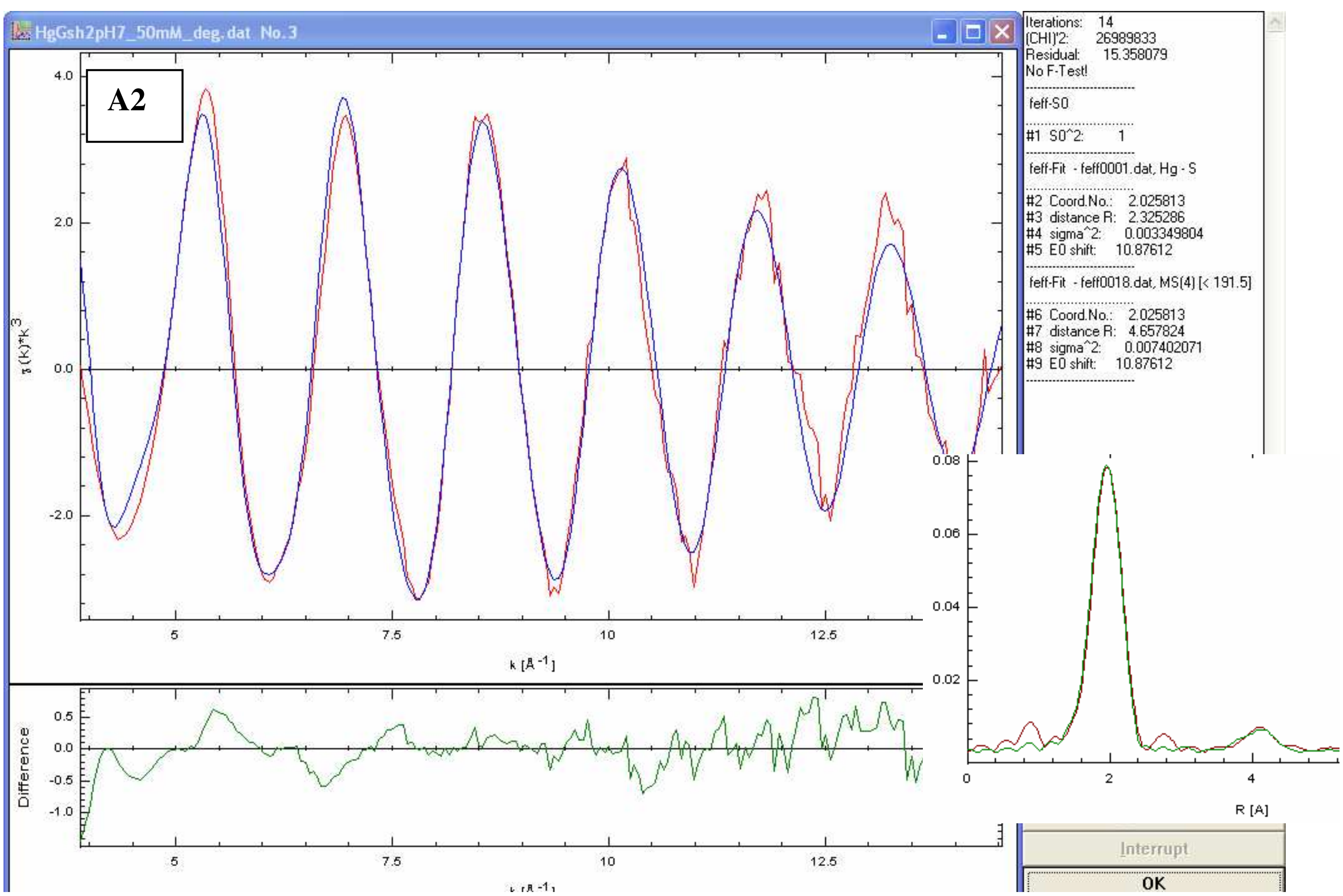
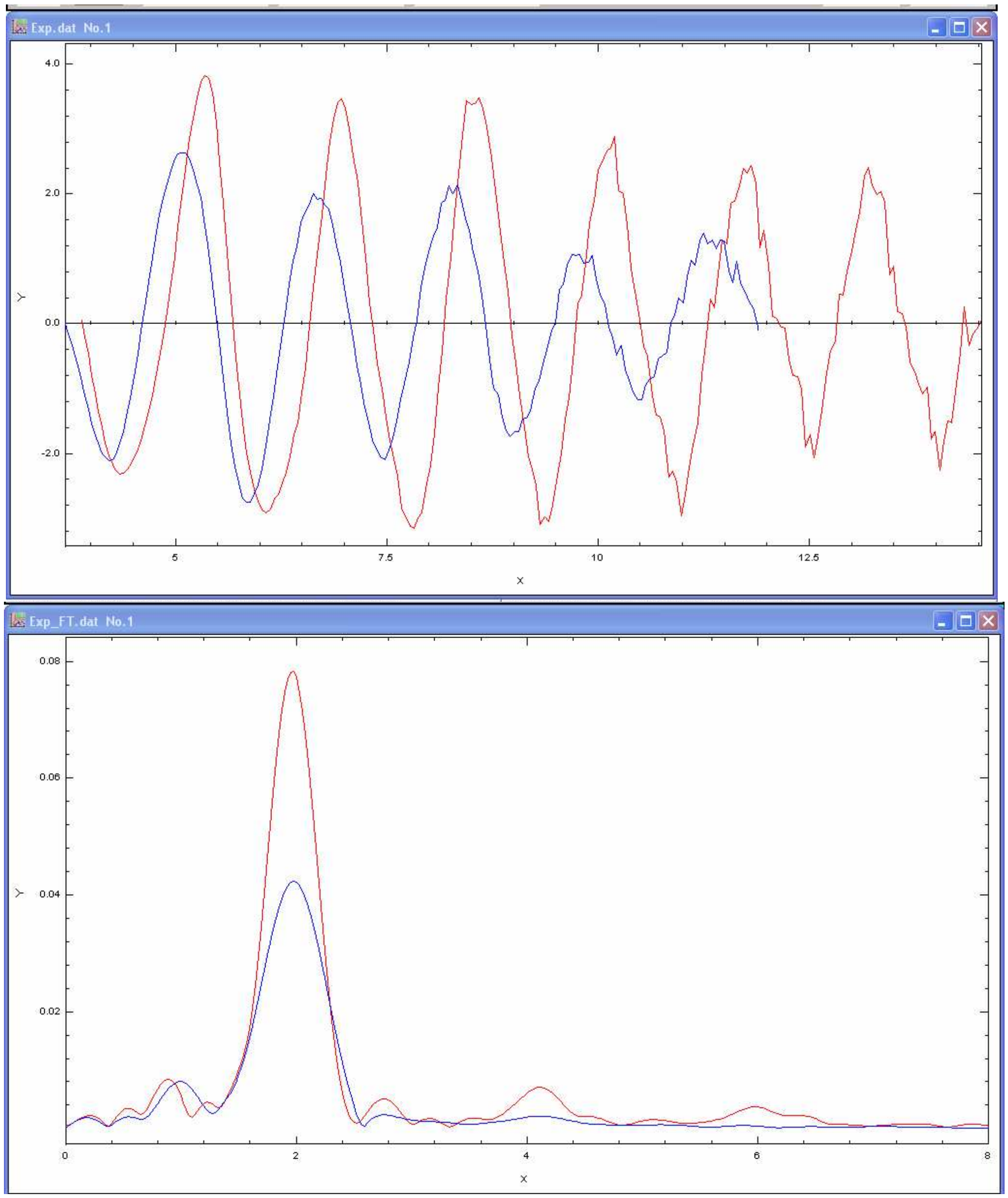


Figure S-5. *Top)* Comparison between k^3-weighted Hg L_{III}-edge EXAFS spectra of Hg(II)-GSH solutions **A2** (red) and **F2** (blue) at pH = 7.0, containing $C_{Hg(II)}$ = 50 and C_{GSH} of 0.2 and 0.5 M, respectively; *below*) corresponding Fourier-transforms.



Appendix I : Stability Constants For Glutathione and Hg(II)-GSH Complexes

Previously reported stability constants for Hg(II)-GSH complexes obtained from Ref. 8 and 19:

Eqn. 15 and Table VI, Ref. 8:	$Hg^{2+} + 2A^{3-} \leftrightarrow HgA_2^{4-}$	$\log \beta_1 = 41.58$
Ref. 19:	$Hg^{2+} + 3A^{3-} \leftrightarrow HgA_3^{7-}$	$\log \beta_2 =$ **44.76**
	$A^{3-} + H^+ \leftrightarrow HA^{2-}$	$\log \beta_3 = 9.56$
	$A^{3-} + 2H^+ \leftrightarrow H_2A$	$\log \beta_4 = 18.16$
	$Hg^{2+} + 2A^{3-} + 2H^+ \leftrightarrow HgA_2H_2^{2-}$	$\log \beta_5 = 60.24$
	$Hg^{2+} + 2A^{3-} + H^+ \leftrightarrow HgA_2H^{3-}$	$\log \beta_6 = 51.21$
	$Hg^{2+} + 3A^{3-} + 3H^+ \leftrightarrow HgA_3H_3^{4-}$	$\log \beta_7 =$ **72.75**
	$Hg^{2+} + 3A^{3-} + 2H^+ \leftrightarrow HgA_3H_2^{5-}$	$\log \beta_8 =$ **63.90**
	$Hg^{2+} + 3A^{3-} + H^+ \leftrightarrow HgA_3H^{6-}$	$\log \beta_9 =$ **54.57**

"Adjusted" stability constants for Hg(II)-GSH complexes used in the current study:

$Hg^{2+} + 3A^{3-} \leftrightarrow HgA_3^{7-}$	$\log \beta_2 =$ **44.23** (= **44.76** - 0.53)
$Hg^{2+} + 3A^{3-} + 3H^+ \leftrightarrow HgA_3H_3^{4-}$	$\log \beta_7 =$ **72.22** (= **72.75** - 0.53)
$Hg^{2+} + 3A^{3-} + 2H^+ \leftrightarrow HgA_3H_2^{5-}$	$\log \beta_8 =$ **63.37** (= **63.90** - 0.53)
$Hg^{2+} + 3A^{3-} + H^+ \leftrightarrow HgA_3H^{6-}$	$\log \beta_9 =$ **54.04** (= **54.57** - 0.53)

The stability constant values for the HgS_3 species have been adjusted by -0.53 logarithmic units in this work to account for the relative ratio of $[Hg(AH)_2]^{2-}$ / $[Hg(AH)_3]^{4-}$ complexes obtained from the EXAFS linear combination fittings of HgS_2 and HgS_3 models (see Figure S-6a).

In our previous work (*20*), we obtained distributions of deprotonated HgA_n^{2-3n} species with n = 2, 3 and 4 at pH 10.5 from linear combination fitting of EXAFS models. The formation constant $\log \beta_{10} \approx 44.8$ for the formation of $[HgA_4]^{10-}$ complex ($Hg^{2+} + 4A^{3-} \leftrightarrow HgA_4^{10-}$) provides a fraction diagram that approximately accounts for the EXAFS distribution of the HgS_n species (see Figure S-6b).

Figure S-6a. The fraction diagram *vs.* total glutathione concentration, C_{GSH} or $[A^{3-}]_{TOT}$, shows the $[Hg(AH)_2]^{2-}$ and $[Hg(AH)_3]^{4-}$ complexes to be the major Hg(II)-GSH species formed at pH 7.0 in solutions **A2** - **F2**, containing $[Hg^{2+}]_{total}$ = 50 mM. The calculations were made with the MEDUSA program (see http://www.kemi.kth.se/medusa/), with the stability constant" log β_7 adjusted -0.53 logarithmic units to 72.22 for the $[Hg(AH)_3]^{4-}$ complex ($HgA_3H_3^{4-}$ in the figure; see Appendix I). The adjustment shifts the C_{GSH} value for equal amounts of $[Hg(AH)_2]^{2-}$ and $[Hg(AH)_3]^{4-}$ from 0.17 M to 0.27 M. Dots (•) show the proportion of the HgS_3 complex obtained from EXAFS linear combination fitting (Table 4).

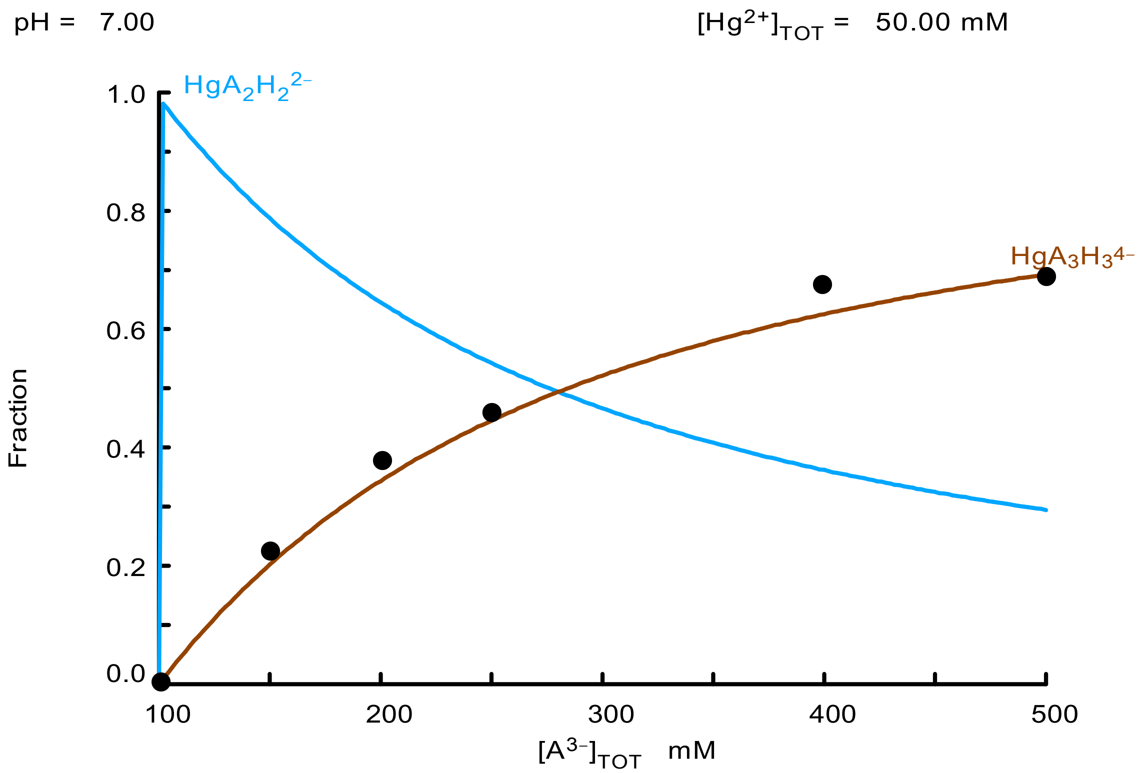
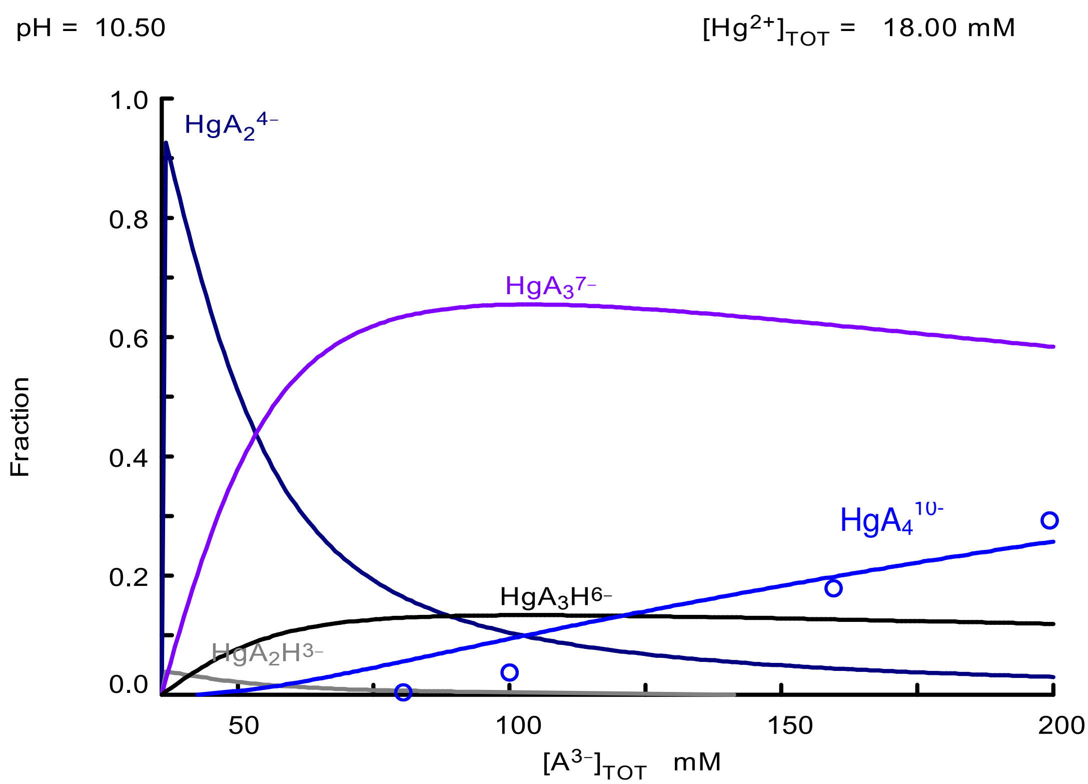


Figure S-6b. Fraction diagram *vs.* total glutathione concentration, C_{GSH} or $[A^{3-}]_{TOT}$, showing the distribution of Hg(II)-GSH species formed at pH 10.5 in solutions containing $C_{Hg(II)}$ or $[Hg^{2+}]_{TOT}$ = 18 mM (*20*). The calculations were made with the MEDUSA program (see http://www.kemi.kth.se/medusa/), using the adjusted stability constants for HgS_3 complexes above (see Appendix I), and log $\beta_{10} \approx 44.8$ for the formation of the $[HgA_4]^{10-}$ complex: ($Hg^{2+} + 4A^{3-} \leftrightarrow HgA_4^{10-}$). Circles (**o**) represent the proportion of the HgS_4 species obtained from EXAFS linear combination fitting (Table 3, Ref. 20).



The full input file to the MEDUSA computer program (see http://www.kemi.kth.se/medusa/) after the above additions and adjustments is given below. The following log β values are used for the equilibrium formation constants:

```
3,  15,  1,  0   /MEDUSA, t= 25 C, p= 1
H+
Hg 2+
A 3-
Hg(OH)2                 , -6.097   -2  1  0
Hg(OH)3-                , -21.1    -3  1  0
Hg2OH 3+                , -3.33    -1  2  0
Hg3(OH)3 3+             , -6.42    -3  3  0
HgA2 4-                 ,  41.58    0  1  2
HgA2H 3-                ,  51.21    1  1  2
HgA2H2 2-               ,  60.24    2  1  2
HgA3 7-                 ,  44.23    0  1  3 (adjusted)
HgA3H 6-                ,  54.04    1  1  3 (adjusted)
HgA3H2 5-               ,  63.37    2  1  3 (adjusted)
HgA3H3 4-               ,  72.22    3  1  3 (adjusted)
HgA4 10-                ,  44.8     0  1  4 (this work)
H2A-                    ,  18.16    2  0  1
HA 2-                   ,  9.56     1  0  1
OH-                     , -14.0    -1  0  0
Hg(OH)2(c)              , -2.601   -2  1  0
A 3-,  H+,
LAV, -5.0   -12.0
T,  0.05
T,  0.5
```

Figure S-7a. Fraction diagram showing the distribution of Hg(II) complexes *vs*. pH for $C_{\mathrm{Hg(II)}}$ = 0.017 M, (*top*) C_{GSH} = 0.06 M as in solution **B1**, and (*below*) $C_{\mathrm{Hg(II)}}$ = 0.017 M and C_{GSH} = 0.2 M as in **F1**, calculated with the adjusted stability constants (Appendix I).

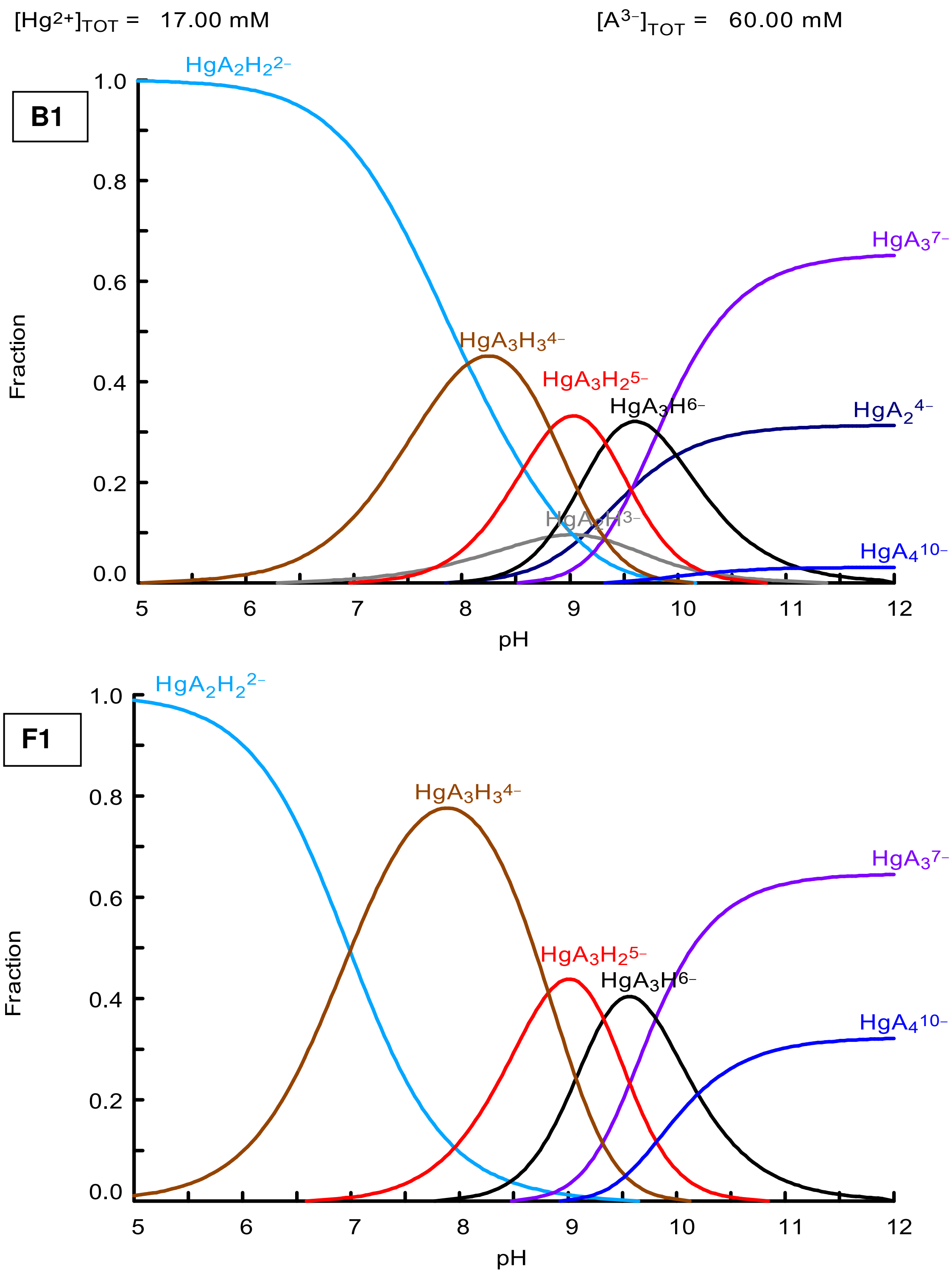


Figure S-7b. Fraction diagram showing the distribution of Hg(II) complexes *vs*. pH for $C_{Hg(II)} = 0.050$ M and $C_{GSH} = 0.15$ M as in solution **B2**, calculated according to the adjusted formation constants (Appendix I).

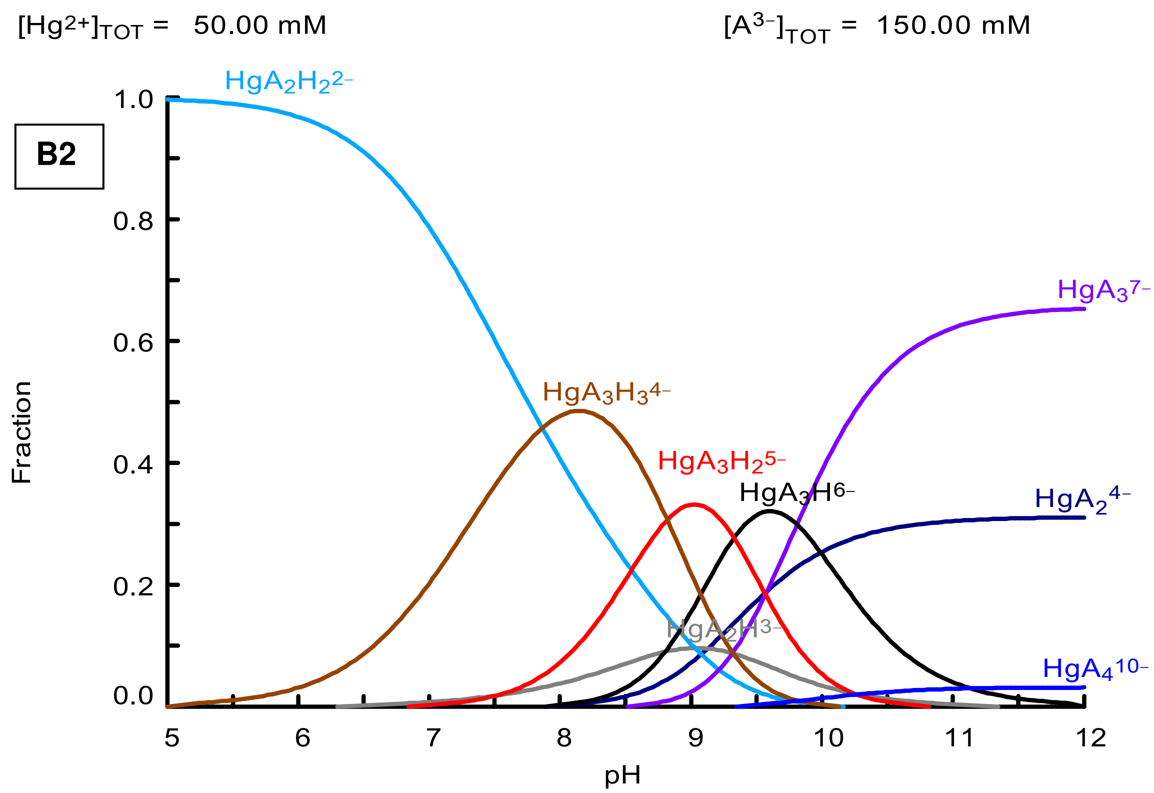


Figure S-7c. Diagram showing the fraction of glutathione (AH_3) species *vs*. pH in an aqueous solution containing $C_{Hg(II)}$ = 0.050 M and C_{GSH} = 0.5 M (as in solution **F2**), calculated based on the adjusted formation constants (Appendix I).

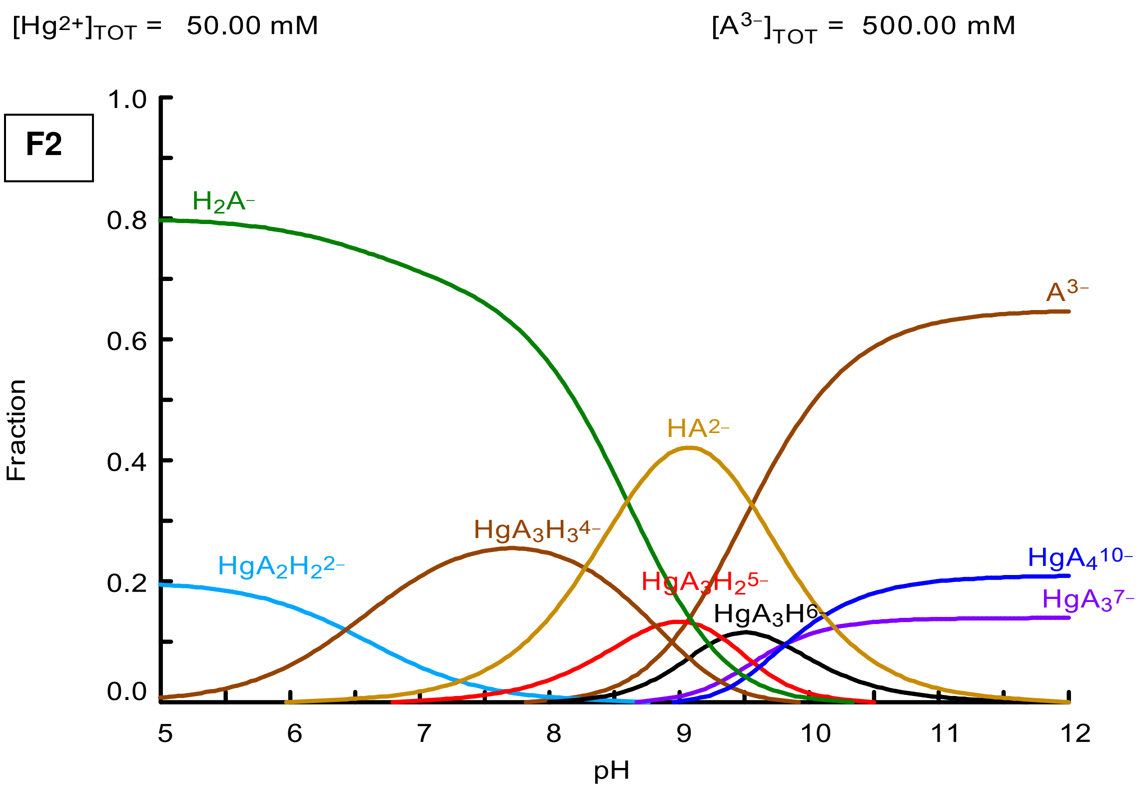


Figure S-8. The diagrams below are calculated for $C_{Hg(II)}$ from 0 to 1 mM in glutathione GSH solution under physiological conditions (pH 7.4, C_{GSH} = 2.2 mM) with the adjusted stability constants in Appendix I. *(top)* The concentrations (logarithmic scale) shows that the dominating Hg(II) complex is $[Hg(AH)_2]^{2-}$ with about 2% present as the first deprotonated HgS_2 species, $[Hg(AH)(A)]^{3-}$ (grey line). *(below)* The fraction diagram shows that the proportions of the two major complexes, $[Hg(AH)_2]^{2-}$ and $[Hg(AH)_3]^{4-}$ are about 95% and 3% of the total Hg(II) amount in dilute Hg(II) solutions (up to 0.1 mM); the percentage goes down at higher Hg(II)-concentration because of the lower free GSH concentration.