Supporting Information: Cellular mercury coordination environment, and not cell surface ligands, influence bacterial methylmercury production

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Table S1: Trace metal solution added to growth medium (10 mL per L)

Component	Concentration (g/L)
Nitrilotriacetic acid	1.5
NaOH*	~0.63
$MgSO_4 \cdot 7H_2O$	3.0
$MnSO_4 \cdot H_2O$	0.5
NaCl	1.0
$FeSO_4 \cdot 7H_2O$	0.1
$CoCl_2 \cdot 6H_2O$	0.1
$CaCl_2$	0.1
$ZnSO_4 \cdot 7H_2O$	0.1
$CuSO_4 \cdot 5H_2O$	0.01
$AlK(SO_4)_2 \cdot 12H_2O$	0.01
H_3BO_3	0.01
$Na_2MoO_4 \cdot 2H_2O$	0.01
$NiCl_2 \cdot 6H_2O$	0.01
Na_2SeO_3	0.01

*Added to adjust pH to ~6.5

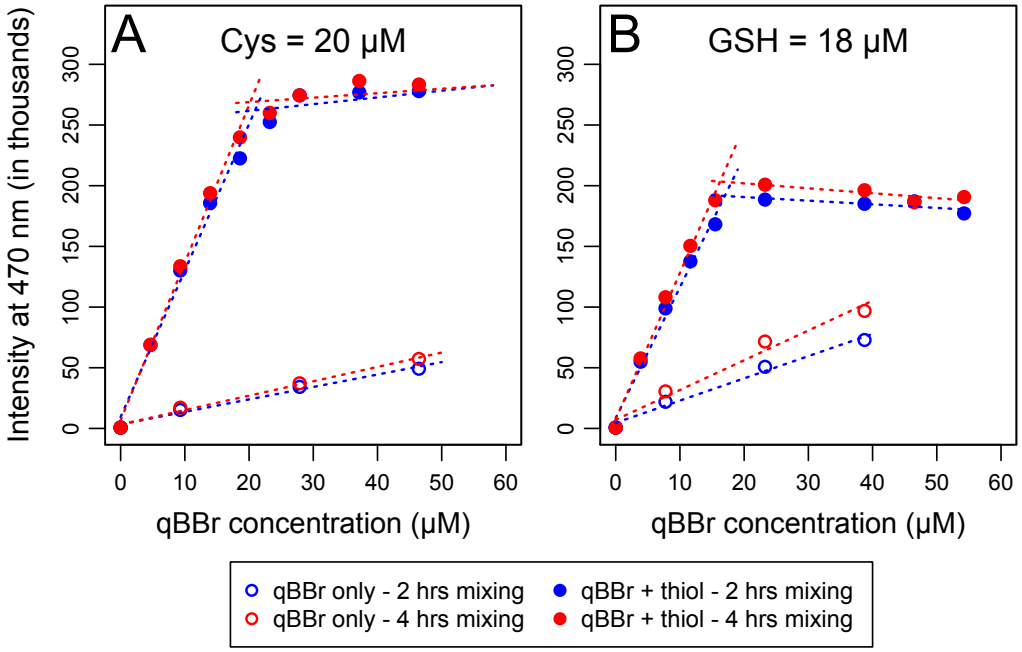

Figure S1: The maximum fluorescence intensity measured at 470 nm (excitation: 380 nm) as a function of added qBBr concentration after mixing with (A) 20 µM cysteine (Cys) and (B) 18 µM glutathione (GSH) in the anaerobic assay medium for 2 or 4 hours. (A) The qBBr titration estimated the Cys concentration as 20.9 µM and 20.2 µM at 2 and 4 hours, respectively. (B) The qBBr titration estimated the GSH concentration as 17.0 µM and 16.2 µM at 2 and 4 hours, respectively.

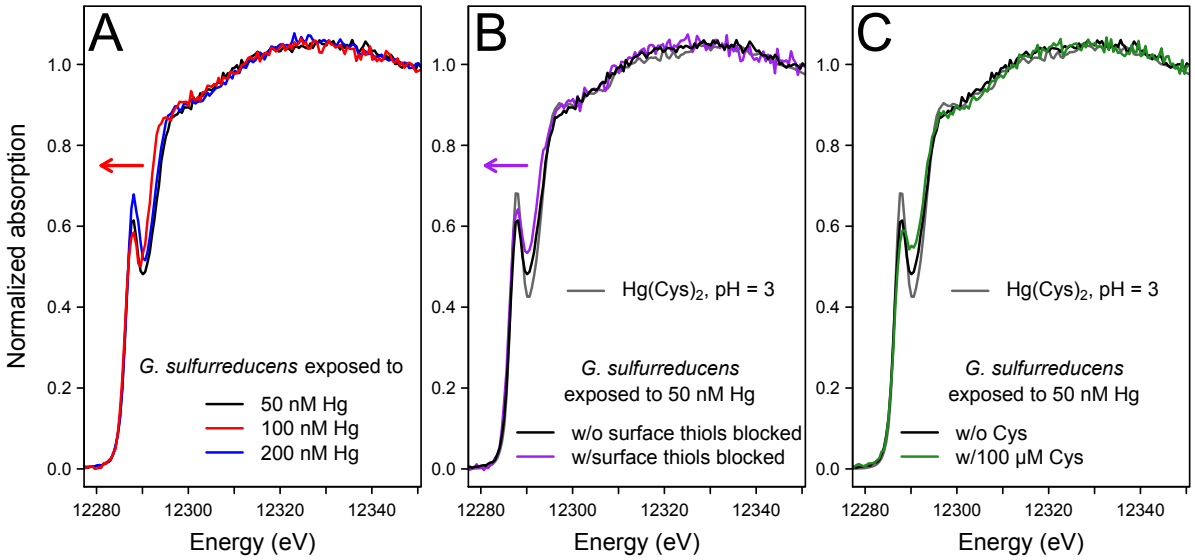

Figure S2: Hg L_3-edge HR-XANES spectra of cell pellets of *G. sulfurreducens* that were exposed to Hg as $Hg(NO_3)_2$ for 2 hours as a cell suspension prior to collection. The exposure conditions include (A) 50 nM, 100 nM, and 200 nM total Hg, (B) 50 nM total Hg with and without the cell surface thiols quantitatively blocked by qBBr, and (C) 50 nM Hg with and without the addition of 100 µM Cys (pre-equilibrated with Hg for 1 hour). A spectrum of the $Hg(Cys)_2$ reference prepared at pH = 3 is included in subplots B and C for comparison with sample spectra. The red and purple spectra from subplots A and B, respectively, have left-shifted edge energies above the near-edge peak indicating mixed Hg-S/N coordination.[1] Information on the bacterial density and mercury concentration for each sample measured by HR-XANES is provided in Table S2.

Table S2: Cell densities and Hg concentrations for the samples analyzed by HR-XANES

No.	Sample name	Bacterial density (g per L; wet weight)	Total added Hg		Total recovered Hg[a]		Total measured MeHg (nM)
			(nM)	(µg per L)	(nM)	(µg per L)	
1	50 nM Hg	1.1	50	10	23.1 ± 0.1	4.6 ± 0.0	0.8 ± 0.6
2	100 nM Hg	1.0	100	20	70.0 ± 2.3	14.0 ± 0.5	2.0 ± 1.8
3	200 nM Hg	1.0	200	40	125.4 ± 3.8	25.1 ± 0.8	2.3 ± 1.5
4	50 nM Hg + surface thiols blocked	1.1	50	10	40.2 ± 0.3	8.0 ± 0.1	1.1 ± 0.9
5	50 nM Hg + 100 µM Cys	1.1	50	10	22.2 ± 1.9	4.4 ± 0.4	7.9 ± 2.1

[a] The total Hg concentration in the cell suspension after the 2 hour mixing period.

S1. Protocol for Spectral Deconvolution

Spectral deconvolutions of the Hg L_3-edge HR-XANES were performed to obtain the average Hg coordination number to sulfur. Using the minimize function in Larch,[2] we performed deconvolutions on the normalized HR-XANES into 4 Gaussian peaks and one error function. The error function was fixed for all deconvolutions with an amplitude of 0.477, a width of 0.159 eV, a center at 12302.2 eV, and an offset of 0.473. The amplitude and width (2σ) of all 4 Gaussians were allowed to float, while the center of each peak was constrained to (Peak 1) 12287.5 – 12288.2 eV, (Peak 2) 12292 – 12296 eV, (Peak 3) 12294 – 12301 eV, and (Peak 4) 12323 – 12335 eV. A fifth Gaussian peak with a center between the values of 12310 – 12315 eV was included in the fits to α-HgS. The Gaussian peak at lowest energy (12287.7 – 12288.2 eV), referred to as Peak 1, was used for further analysis.

To determine how to extract coordination information from the properties of Peak 1, we assessed the correlations between the height, area, and width (2σ) of Peak 1 with the known Hg-S coordination number of reference spectra. Specifically, we tested weighted sum spectra of $Hg(cysteine)_2$ (pH = 3) and β-$HgS_{(s)}$ with known average Hg-S coordination numbers of 2, 2.4, 2.8, 3.2, 3.6 and 4 (Figures S3 and S4A). The ratio of the height to the width of Peak 1 correlates well (R^2 = 0.995) with the known Hg coordination number to S (Figure S4B). Linear Hg-S_2 complexes generally have a sharp and intense near-edge peak.[3] The addition of more S atoms into the coordination sphere causes distortions from linearity and leads to spectra with a weak (Hg-S_3) or absent (Hg-S_4) near-edge peak.[1] Therefore, it is reasonable that there is a strong correlation between the height/width of the Gaussian peak that fits the near-edge region of the spectrum and the Hg coordination number to S. We thus chose the ratio of the height to the width of Peak 1 to estimate the Hg-S coordination number in our bacterial samples. Subsequently, we created a standard curve from a variety of known Hg standards with Hg-S_2, Hg-S_3, and Hg-S_4 coordination, which is described further and presented in Figure 3B of the main text. We provide the results and parameters of the spectral deconvolutions of standards from this study as well as Manceau et al.[4, 5] and Bourdineaud et al.[6] in Tables S3 and S4 and Figures S5 and S6.

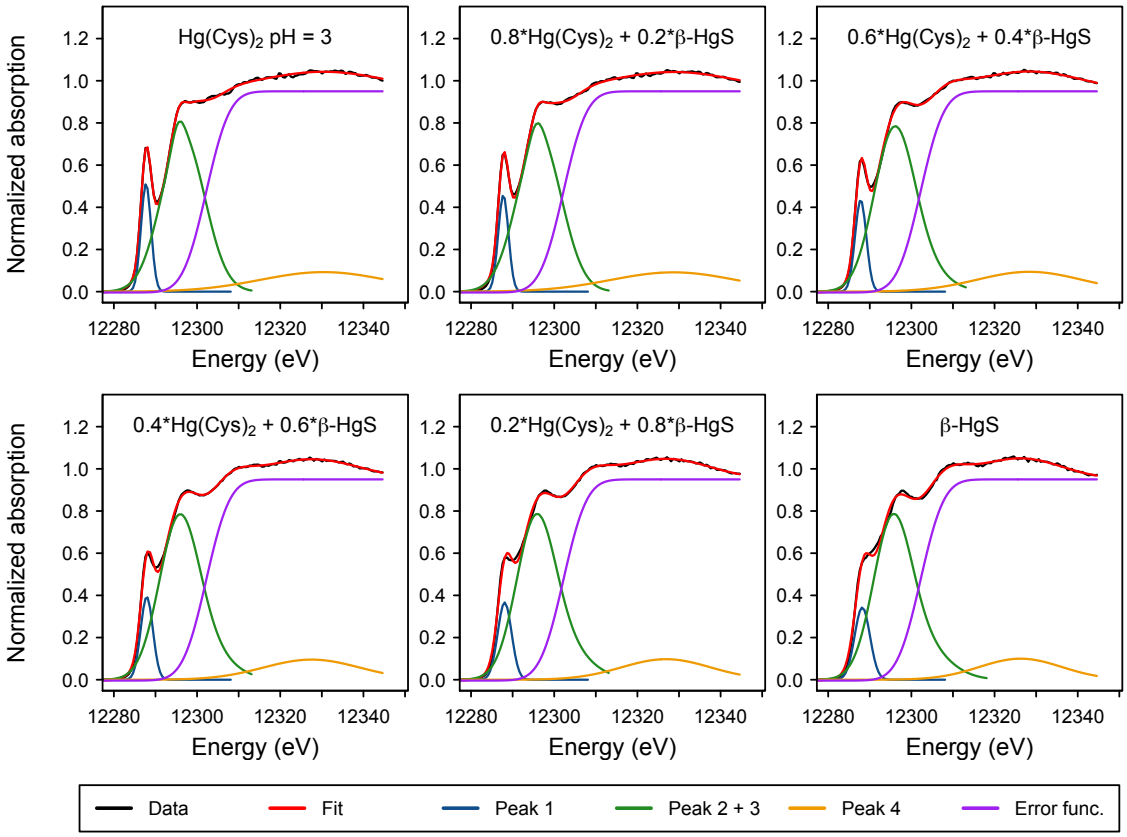

Figure S3: Spectral deconvolutions of weighted sum Hg L_3-edge HR-XANES spectra of Hg(cysteine)$_2$ (pH = 3) and β-HgS into 4 Gaussian peaks and one error function.

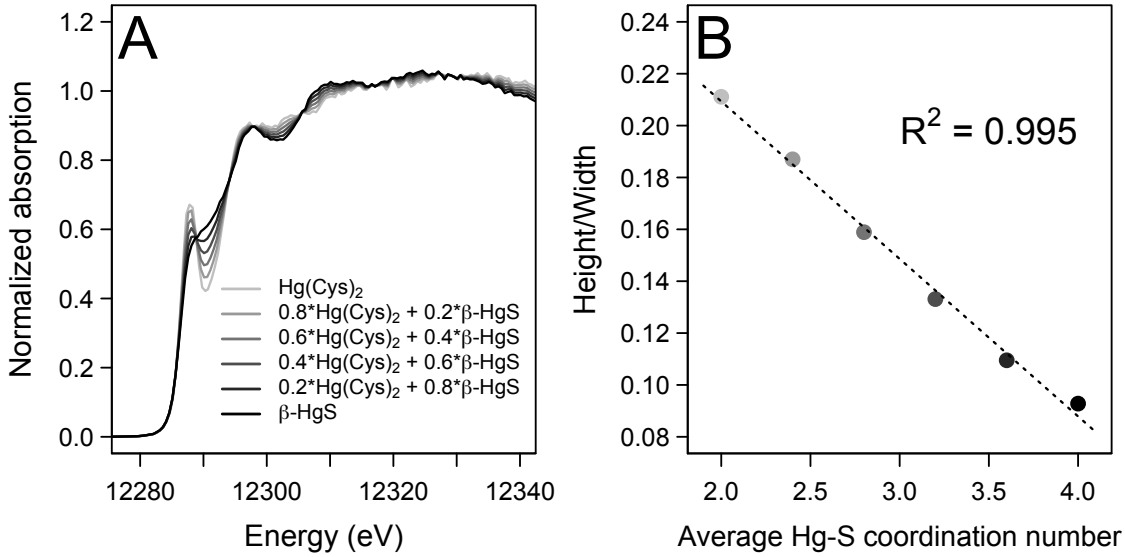

Figure S4: (A) Weighted sum Hg L_3-edge HR-XANES spectra of Hg(cysteine)$_2$ (pH = 3) and β-HgS standards. (B) After spectral deconvolution into 4 Gaussian peaks and one error function (as shown in Figure S4 below), the ratio of the height to the width of Peak 1 (see Figure S3) is well-correlated (R^2 = 0.995) with the average Hg-S coordination number.

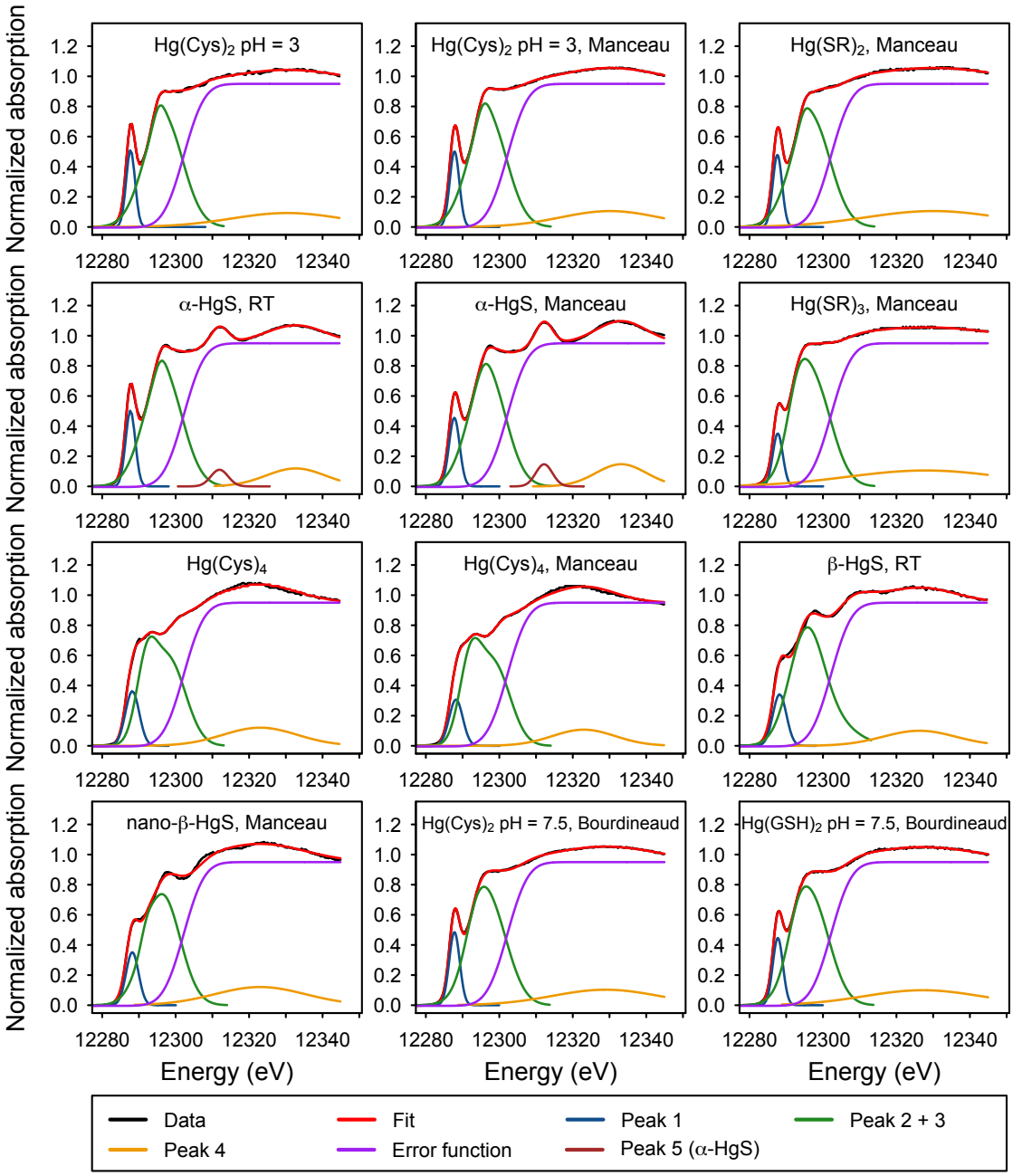

Figure S5: Spectral deconvolutions of Hg L_3-edge HR-XANES spectra of standards with Hg-S coordination into 4/5 Gaussian peaks and one error function from this study, Manceau et al.[4, 5] and Bourdineaud et al.[6] The parameters of Peak 1 (position, width, and height) are presented in Table S3 below.

Table S3: Peak 1 parameters of the spectral deconvolution of standards with Hg-S coordination

		Hg-S_2 standards			
No.	Standard	Position	Width	Height	Height/Width
1	$Hg(Cys)_{2,\ pH\ =\ 3}$	12287.7	2.41	0.509	0.211
2	bulk-α-HgS[a]	12287.7	2.63	0.503	0.191
3	$Hg(SR)_2$[b,c]	12287.6	2.52	0.477	0.189
4	$Hg(Cys)_{2,\ pH\ =\ 3}$[c]	12287.8	2.61	0.500	0.192
5	bulk-α-HgS[c]	12287.8	2.89	0.466	0.161
		Hg-[S_2 + O/N] standards			
		Position	Width	Height	Height/Width
6	$Hg(Cys)_{2,\ pH\ =\ 7.5}$[e]	12287.8	2.86	0.485	0.170
7	$Hg(GSH)_{2,\ pH\ =\ 7.5}$[e]	12287.8	2.76	0.446	0.162
		Hg-S_3 standards			
		Position	Width	Height	Height/Width
8	$Hg(SR)_3$[b,c]	12287.7	2.73	0.351	0.129
		Hg-S_4 standards			
		Position	Width	Height	Height/Width
9	$Hg(cysteine)_4$	12288.2	3.766	0.363	0.096
10	bulk-β-HgS[a]	12288.2	3.688	0.342	0.093
11	$Hg(cysteine)_4$[d]	12288.2	3.718	0.306	0.082
12	nano-β-HgS[d]	12288.2	3.429	0.351	0.102

[a] Measured at room temperature, at which the collected spectrum of the bulk materials resembles their nanoparticulate counterparts at 10 K.[3] [b] SR is cyclohexanethiolate. [c] From Manceau et al.[5] [d] From Manceau et al.[4] [e] From Bourdineaud et al.[6]

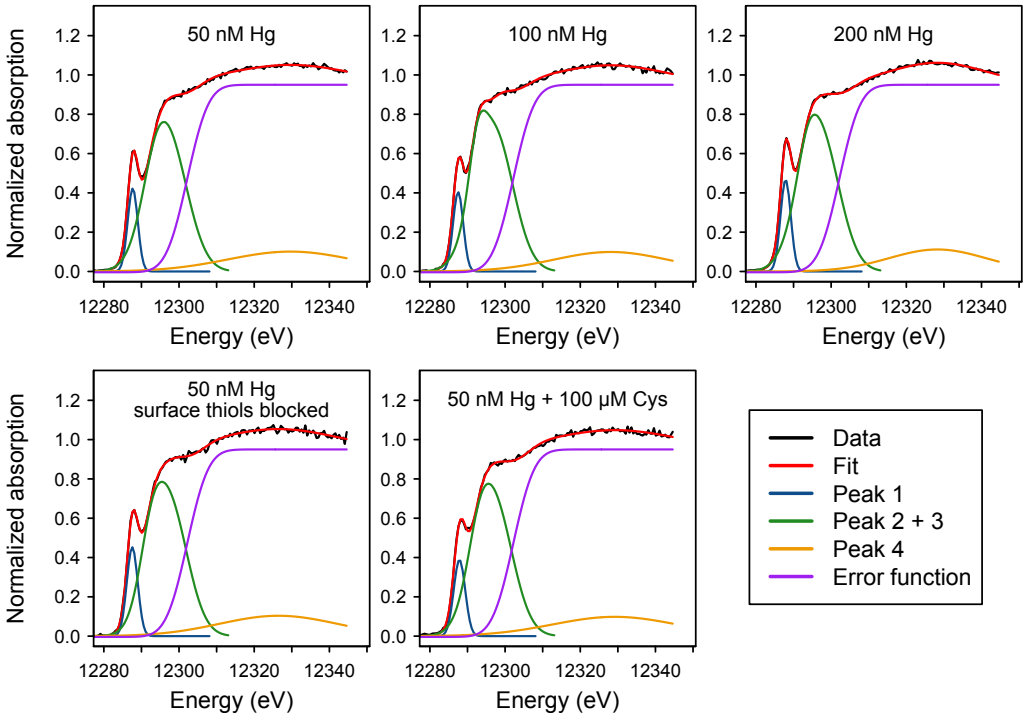

Figure S6: Spectral deconvolutions of Hg L_3-edge HR-XANES spectra of samples of *G. sulfurreducens* containing Hg into 4 Gaussian peaks and one error function. *G. sulfurreducens* was exposed to 50 nM Hg, 100 nM Hg, 200 nM Hg, and 50 nM Hg with 100 µM Cys for 2 hours. The sample with cell surface thiols blocked was exposed to qBBr at a qBBr:cell surface thiol mole ratio of 4:3 for 2 hours prior to Hg exposure. The parameters of Peak 1 (position, width, and height) are presented in Table S4 below.

Table S4: Peak 1 parameters of the spectral deconvolution of *G. sulfurreducens* samples containing Hg

No.	Sample	Position	Width	Height	Height/Width
1	50 nM Hg	12287.7	2.682	0.422	0.157
2	100 nM Hg	12287.5	2.714	0.403	0.148
3	200 nM Hg	12287.8	2.739	0.462	0.169
4	50 nM Hg + surface thiols blocked	12287.6	2.908	0.452	0.155
5	50 nM Hg + 100 µM Cys	12287.9	3.060	0.384	0.125

References

1. Manceau, A.; Enescu, M.; Simionovici, A.; Lanson, M.; Gonzalez-Rey, M.; Rovezzi, M.; Tucoulou, R.; Glatzel, P.; Nagy, K. L.; Bourdineaud, J. P., Chemical forms of mercury in human hair reveal sources of exposure. *Environmental Science & Technology* **2016,** *50*, (19), 10721-10729.

2. Newville, M., Larch: An analysis package for XAFS and related spectroscopies. *J Phys Conf Ser* **2013,** *430*.

3. Manceau, A.; Lemouchi, C.; Enescu, M.; Gaillot, A. C.; Lanson, M.; Magnin, V.; Glatzel, P.; Poulin, B. A.; Ryan, J. N.; Aiken, G. R.; Gautier-Luneau, I.; Nagy, K. L., Formation of mercury sulfide from Hg(II)-thiolate complexes in natural organic matter. *Environmental Science & Technology* **2015,** *49*, (16), 9787-9796.

4. Manceau, A.; Bustamante, P.; Haouz, A.; Bourdineaud, J. P.; Gonzalez-Rey, M.; Lemouchi, C.; Gautier-Luneau, I.; Geertsen, V.; Barruet, E.; Rovezzi, M.; Glatzel, P.; Pin, S., Frontispiece: Mercury(II) binding to metallothionein in *Mytilus edulis* revealed by high energy-resolution XANES spectroscopy. *Chemistry – A European Journal* **2019,** *25*, (4), 997-1009.

5. Manceau, A.; Lemouchi, C.; Rovezzi, M.; Lanson, M.; Gatzel, P.; Nagy, K. L.; Gautier-Luneau, I.; Joly, Y.; Enescu, M., Structure, bonding, and stability of mercury complexes with thiolate and thioether ligands from high-resolution XANES spectroscopy and first-principles calculations. *Inorganic Chemistry* **2015,** *54*, (24), 11776-11791.

6. Bourdineaud, J.-P.; Gonzalez-Rey, M.; Rovezzi, M.; Glatzel, P.; Nagy, K. L.; Manceau, A., Divalent mercury in dissolved organic matter is bioavailable to fish and accumulates as dithiolate and tetrathiolate complexes. *Environmental Science & Technology* **2019,** *53*, (9), 4880-4891.