Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information: Hg(II) bacterial biouptake: The role of anthropogenic and biogenic ligands present in solution and spectroscopic evidence of ligand exchange reactions at the cell surface

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Supporting Text

Preparation of Hg standards for XANES measurements

The Hg standards analyzed in this study include $Hg(acetate)_2$ and $Hg(cysteine)_2$ powders as well as aqueous $Hg(cysteine)_3$ and HgEDTA. The $Hg(acetate)_2$ standard was purchased from Sigma Aldrich and finely ground. $Hg(cysteine)_2$ was synthesized according to the method by Jalilehvand et al.[1] 100 mM $Hg(NO_3)_2$ was mixed with 500 mM cysteine in freshly filtered Milli-Q water bubbled with pure N_2 gas. $Hg(cysteine)_2$ formed as a white precipitate. The precipitate was filtered was washed with Milli-Q under a constant stream of N_2 gas, dried under an atmosphere of N_2, and finely ground into a powder for XAS analysis. Powder standards were spread onto the sticky side of a 6" piece of Scotch tape with a razor blade. The tape was then cut into approximately 12 equal pieces and these pieces were stacked (between 2-4 pieces per stack) and sandwiched between 2 pieces of scotch tape. This was done to eliminate "pinholes" and to enable layering for optimal sample thickness at the beamline.

For the aqueous standards, a stock solution of 0.5M $Hg(NO_3)_2$ was prepared in 5% trace metal grade HNO_3. Stock solutions of 1M cysteine and 1M EDTA were also prepared by dissolving the corresponding mass of powdered H_2cysteine in Milli-Q and powdered Na_2H_2EDTA in Milli-Q with 2M NaOH respectively. Both $Hg(cysteine)_3$ and HgEDTA standards were prepared at Hg to ligand ratios of 1:5 (100mM $Hg(NO_3)_2$ and 500mM ligand). Aliquots of 1M HNO_3 or 1M NaOH were added to the solutions to achieve pH=7 for HgEDTA and pH=8 for $Hg(cysteine)_3$. Speciation calculations with ChemEQL indicated 100% of total Hg was as HgEDTA and $Hg(cysteine)_3$ for the respective standards at respective pH. A precipitate initially formed in the $Hg(cysteine)_3$ standard, but it dissolved when pH was increased to 8. Additionally, the $Hg(cysteine)_3$ standard solution was stored in a container with no headspace of air and sealed with Parafilm to minimize the oxidation of excess cysteine.

XANES data collection and analysis

Hg standards were measured in transmission mode, while Hg samples – having lower Hg concentrations – were measured in fluorescence mode. Aqueous Hg standards were contained in ~1cm diameter rubber tubes sealed on both ends with Kapton tape, and the optimal tube length (i.e., absorption length) was calculated with the program Hephaestus (Ravel and Newville 2005). Powdered standards were contained between pieces of Scotch tape, and Hg samples were contained between pieces of Kapton tape. Energy was scanned between 200 eV below to approximately 1000 eV above the Hg L_{III}-edge (12,284 eV) with a Si(111) monochromator. All samples and standards had a pre-edge scanning step size of 0.6 eV, an EXAFS scan increment of 0.06 $Å^{-1}$, a base count time of 1 second, a k weight for the time base of 2, and a final k count time of 10 seconds. Spectra of samples with low Hg concentrations were too noisy for EXAFS analysis; however, energy was still scanned well beyond the edge for normalization purposes. To maintain the energy calibration between samples, a selenium reference foil placed between the transmitted beam detector (I_{T1}) and a reference detector (I_{T2}) was simultaneously scanned for both transmission and fluorescence mode. Incident intensity (I_{T0}), I_{T1}, and I_{T2} were measured with ionization chambers, while fluorescence intensity was measured with a silicon drift detector. Three successive scans of approximately 25 minutes duration per scan were collected for the Hg reference standards. Between 5 and 39 scans of approximately 45 minutes duration per scan were collected for the Hg samples. The beam position was altered for samples that required more scans to prevent beam-induced changes in the sample. XANES data processing was done with the program Athena[2].

References

1. F. Jalilehvand, B. O. Leung, M. Izadifard, E. Damian, *Inorg Chem* 2006, **45**. 66-73.
2. B. Ravel, M. Newville, *J Synchrotron Radiat* 2005, **12**. 537-541

Supporting Tables

Table S1: Composition of MSM and MCM

Media component	MSM (M)	MCM (M)
KH_2PO_4	5.0×10^{-3}	
K_2HPO_4	9.9×10^{-3}	
3-(N-Morpholino)propane-sulfonic acid (MOPS buffer)		2.0×10^{-2}
Na-β-glycerophosphate		1.0×10^{-3}
$MgSO_4$	4.1×10^{-4}	4.1×10^{-4}
NH_4NO_3	1.2×10^{-2}	1.2×10^{-2}
Isoleucine	7.6×10^{-4}	7.6×10^{-4}
Leucine	7.6×10^{-4}	7.6×10^{-4}
Thiamine	3.0×10^{-9}	3.0×10^{-9}
Glucose	1.0×10^{-2}	1.0×10^{-2}
MgO	2.5×10^{-5}	
$CaCO_3$	2.0×10^{-6}	
$Fe(NO_3)_3$	2.0×10^{-6}	
$ZnSO_4$	5.0×10^{-7}	
$CuSO_4$	1.0×10^{-7}	
$CoSO_4$	1.0×10^{-8}	
H_3BO_3	1.0×10^{-6}	
Na_2MoO_4	2.0×10^{-7}	
HNO_3	8.0×10^{-5}	
NaOH		9.1×10^{-3}

Table S2: Hg(II)-organic ligand complexation constants

Species	Reaction	LogK
EDTA		
$HgEDTA^{2-}$	$Hg^{2+} + EDTA^{4-} = HgEDTA^{2-}$	23.50 [a]
$HgOHEDTA^{3-}$	$H_2O + Hg^{2+} + EDTA^{4-} = H^+ + HgOHEDTA^{3-}$	13.7
$HgHEDTA^{-}$	$Hg^{2+} + H^+ + EDTA^{4-} = HgHEDTA^{-}$	27.0
EDDS		
$HgEDDS^{2-}$	$Hg^{2+} + EDDS^{4-} = HgEDDS^{2-}$	17.50
$HgOHEDDS^{3-}$	$H_2O + Hg^{2+} + EDDS^{4-} = H^+ + HgOHEDDS^{3-}$	10.69
$HgHEDDS^{-}$	$Hg^{2+} + H^+ + EDDS^{4-} = HgHEDDS^{-}$	22.32
DTPA		
$HgDTPA^{3-}$	$Hg^{2+} + DTPA^{5-} = HgDTPA^{3-}$	26.3
$HgHDTPA^{2-}$	$Hg^{2+} + H^+ + DTPA^{5-} = HgHDTPA^{2-}$	30.4
NTA		
$HgNTA^{-}$	$Hg^{2+} + NTA^{3-} = HgNTA^{-}$	15.9
Cysteine		
HgCysteine	$Hg^{2+} + Cysteine^{2-} = HgCysteine$	15.30
$Hg(Cysteine)_2^{2-}$	$Hg^{2+} + 2Cysteine^{2-} = Hg(Cysteine)_2^{2-}$	41.8[d]
$HgH(Cysteine)_2^{-}$	$Hg^{2+} + H^+ + 2Cysteine^{2-} = HgH(Cysteine)_2^{-}$	50.74[d]
$HgH_2(Cysteine)_2$	$Hg^{2+} + 2H^+ + 2Cysteine^{2-} = HgH_2(Cysteine)_2$	58.11[d]
$Hg(Cysteine)_3^{4-}$	$Hg^{2+} + 3Cysteine^{2-} = Hg(Cysteine)_3^{4-}$	45.39 [b]
$HgH(Cysteine)_3^{3-}$	$Hg^{2+} + H^+ + 3Cysteine^{2-} = HgH(Cysteine)_3^{3-}$	55.85[b]
$HgH_2(Cysteine)_3^{2-}$	$Hg^{2+} + 2H^+ + 3Cysteine^{2-} = HgH_2(Cysteine)_3^{2-}$	64.55[b]
Penicillamine		
HgPEN	$Hg^{2+} + PEN^{2-} = HgPEN$	16.15
$HgH(PEN)_2^{-}$	$Hg^{2+} + H^+ + 2PEN^{2-} = HgH(PEN)_2^{-}$	52.03[c]
$HgH_2(PEN)_2$	$Hg^{2+} + 2H^+ + 2PEN^{2-} = HgH_2(PEN)_2$	59.0 [c]
$HgH_3(PEN)_2^{+}$	$Hg^{2+} + 3H^+ + 2PEN^{2-} = HgH_3(PEN)_2^{+}$	61.02 [c]
$HgH_3(PEN)_3^{-}$	$Hg^{2+} + 3H^+ + 3PEN^{2-} = HgH_3(PEN)_3^{-}$	72.43 [c]
Glutathione		
$HgGSH^{-}$	$Hg^{2+} + GSH^{3-} = HgGSH^{-}$	26.0
$Hg(GSH)_2^{4-}$	$Hg^{2+} + 2GSH^{3-} = Hg(GSH)_2^{4-}$	41.58
$HgH(GSH)_2^{3-}$	$Hg^{2+} + H^+ + 2GSH^{3-} = HgH(GSH)_2^{3-}$	51.21[e]
$HgH_2(GSH)_2^{2-}$	$Hg^{2+} + 2H^+ + 2GSH^{3-} = HgH_2(GSH)_2^{2-}$	60.24[e]
$Hg(GSH)_3^{7-}$	$Hg^{2+} + 3GSH^{3-} = Hg(GSH)_3^{7-}$	44.76[e]
$HgH(GSH)_3^{6-}$	$Hg^{2+} + H^+ + 3GSH^{3-} = HgH(GSH)_3^{6-}$	54.70[e]
$HgH_2(GSH)_3^{5-}$	$Hg^{2+} + 2H^+ + 3GSH^{3-} = HgH_2(GSH)_3^{5-}$	63.90[e]
$HgH_3(GSH)_3^{4-}$	$Hg^{2+} + 3H^+ + 3GSH^{3-} = HgH_3(GSH)_3^{4-}$	72.75[e]

[a] All complexation constants are obtained from the Joint Expert Speciation System database (http://jess.murdoch.edu.au/jess_home.htm) or otherwise cited.

[b] Complexation constant obtained from Cheesman, Arnold, and Rabenstein (1).

[c] Complexation constants obtained from Koszegi-Szalai and Paal (2).

[d] Complexation constants obtained from Berthon (3).

[e] Complexation constants obtained from Shoukry, Cheesman, and Rabenstein (4).

References

1. B. V. Cheesman, A. P. Arnold, D. L. Rabenstein, *J Am Chem Soc* 1988, **110**. 6359-6364.

2. H. Koszegi-Szalai, T. L. Paal, *Talanta* 1999, **48**. 393-402.

3. G. Berthon, *Pure Appl Chem* 1995, **67**. 1117-1240.

4. M. M. Shoukry, B. V. Cheesman, D. L. Rabenstein, *Can J Chem* 1988, **66**. 3184-3189.

Table S3: Speciation of 300 nM Hg(II) (as % THg) in presence of varying concentrations of organic ligand in Milli-Q water.

[Organic ligand]		1μM	10μM	100μM	1mM	10mM
EDTA	pH	5.5	5.9	7.0	7.7	7.6
	$HgEDTA^{2-}$	98.91	99.58	99.80	99.26	99.37
	$HgOHEDTA^{3-}$	–	0.01	0.17	0.74	0.63
	$HgHEDTA^{-}$	1.09	0.41	0.03	–	–
NTA	pH	5.4	5.4	5.5	5.7	6.4
	$HgNTA^{-}$	62.21	95.16	99.51	99.92	99.96
	$Hg(OH)_2$	36.82	4.70	0.49	0.08	0.04
	$HgCl_2$	0.97	0.14	–	–	–
EDDS	pH	5.6	6.4	8.4	9.8	9.8
	$HgEDDS^{2-}$	73.04	69.61	2.39	0.11	0.10
	$HgOHEDDS^{3-}$	4.21	27.72	97.52	99.89	99.90
	$HgHEDDS^{-}$	12.98	1.79	–	–	–
	$Hg(OH)_2$	9.61	0.88	0.09	–	–
	$HgCl_2$	0.16	–	–	–	–
DTPA	pH	5.5	5.5	5.7	5.8	5.6
	$HgDTPA^{3-}$	96.17	96.17	97.54	98.05	96.80
	$HgHDTPA^{2-}$	3.83	3.83	2.46	1.95	3.20
Cysteine (CYS)	pH	5.4	5.4	5.4	5.3	5.3
	$HgH_2(CYS)_2$	98.96	99.01	98.89	99.14	99.17
	$HgH(CYS)_2^{-}$	1.04	0.99	1.11	0.86	0.83
Penicillamine (PEN)	pH	5.4	5.4	5.3	5.2	5.2
	$HgH_2(PEN)_2$	99.96	99.95	99.93	99.82	98.78
	$HgH_3(PEN)_2^{+}$	0.04	0.05	0.05	0.06	0.06
	$HgH_3(PEN)_3^{-}$	–	–	0.02	0.12	1.16
Glutathione (GSH)	pH	5.3	4.6	4.1	3.4	2.9
	$HgH(GSH)_2^{3-}$	0.02	–	–	–	–
	$HgH_2(GSH)_2^{2-}$	99.98	100	100	100	100

Supporting Figures and Figure Legends

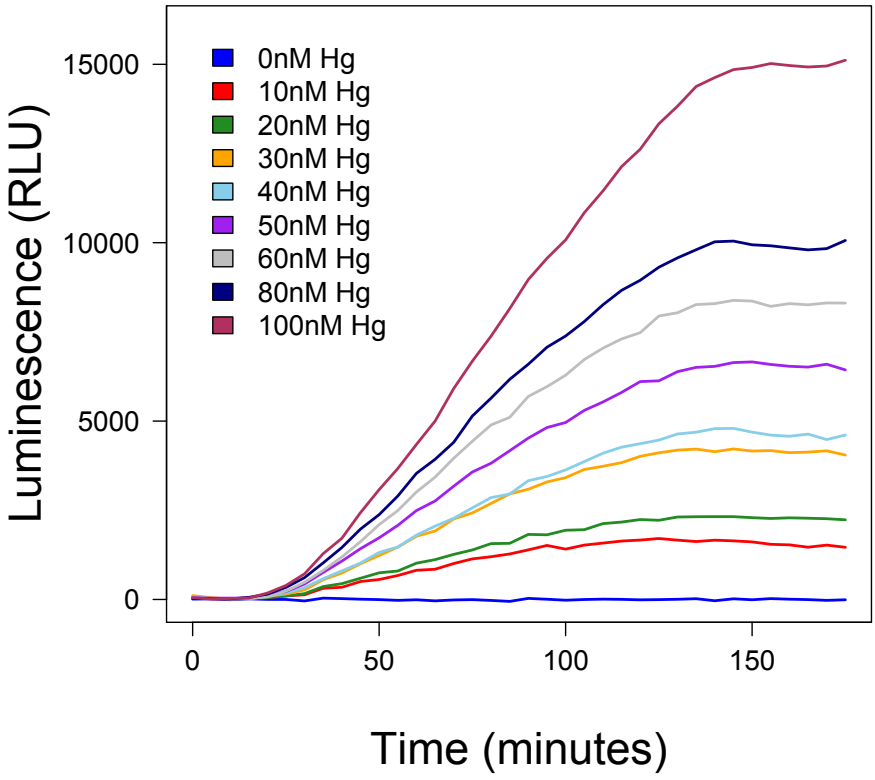


Fig. S1: The luminescence output of *E. coli* ARL1 in the presence of 0-100 nM THg recorded every 5 minutes for 3 hours in MCM. The dominant Hg(II) species for all Hg concentrations are $Hg(isoleucine)_2$ and $Hg(NH_3)_2^{2+}$. Data points are the average of 3 replicates.

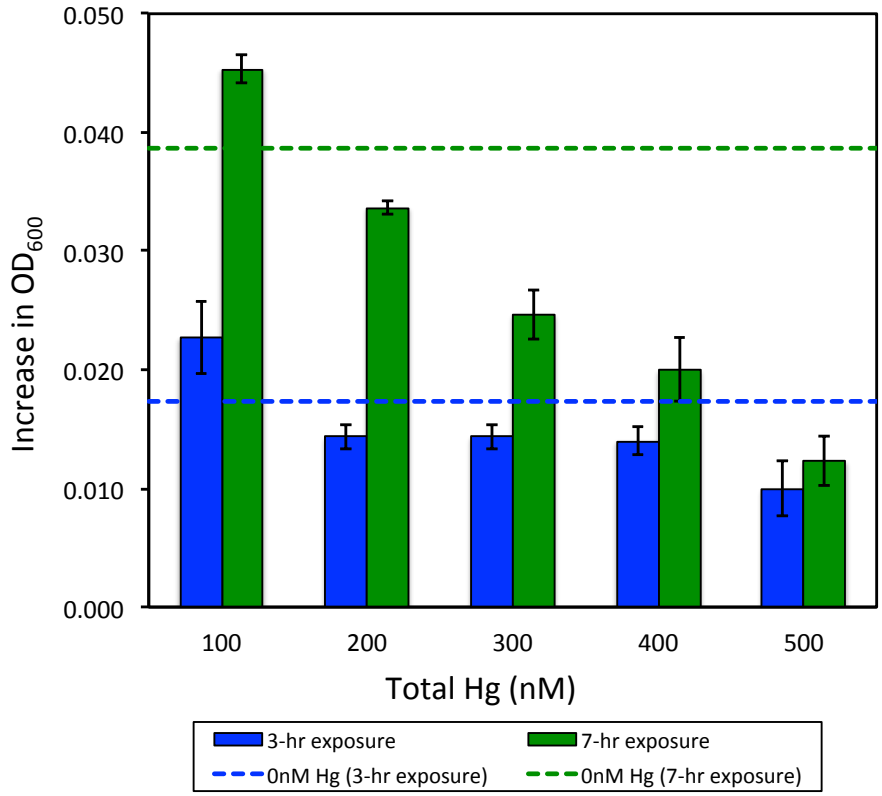


Fig. S2: The growth of *E. coli* ARL1 reported as increase in OD_{600} in the presence of 0-500 nM THg in the bioreporter exposure medium (MCM) with no organic ligands recorded during a 3-hour exposure period (blue) and a 7-hour exposure period (green). The dashed lines represent the growth of *E. coli* ARL1 in the absence of Hg. MCM contains a limited amount of nutrients to support the growth of *E. coli*, thus growth for all conditions is minimal. The initial OD_{600} of *E. coli* ARL1 for each exposure condition was approximately 0.18. The points represent the average of 3 replicates, and error bars are ± 1 SD.

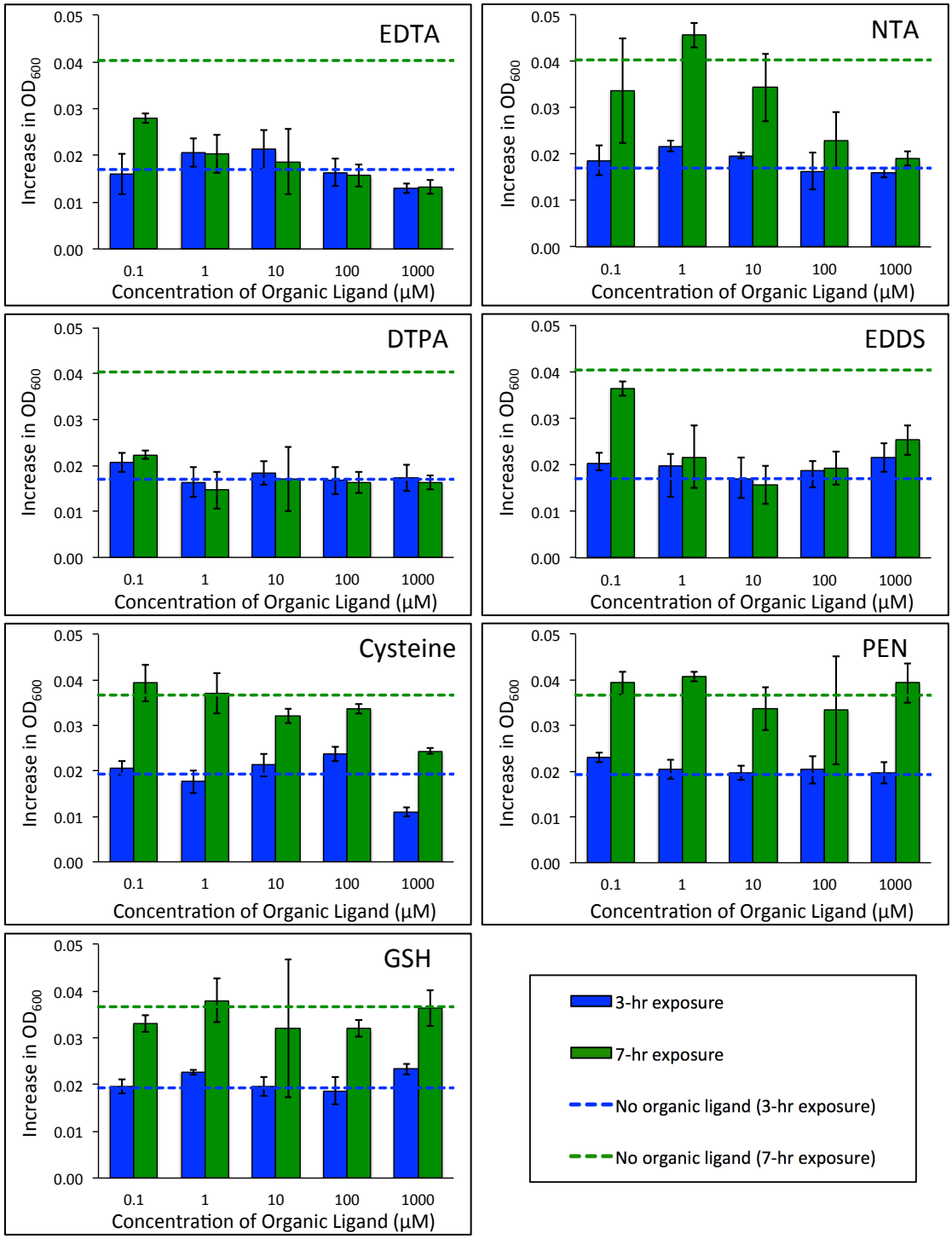


Fig. S3: The growth of *E. coli* ARL1 reported as increase in OD_{600} in the presence of 30 nM THg with 0-1000 μM organic ligand in the bioreporter exposure medium (MCM) for a 3-hour (blue) and a 7-hour (green) exposure period. The dashed lines represent the increase in OD_{600} of *E. coli* ARL1 in the presence of 30 nM THg in the absence of organic ligand for a 3-hour exposure

period (blue) and a 7-hour exposure period (green). MCM contains a limited amount of nutrients to support the growth of *E. coli*, thus growth for all conditions is minimal. The initial OD_{600} of *E. coli* ARL1 was approximately 0.18 for all samples. The values presented are the average of three replicates, and error bars are ± 1 SD.

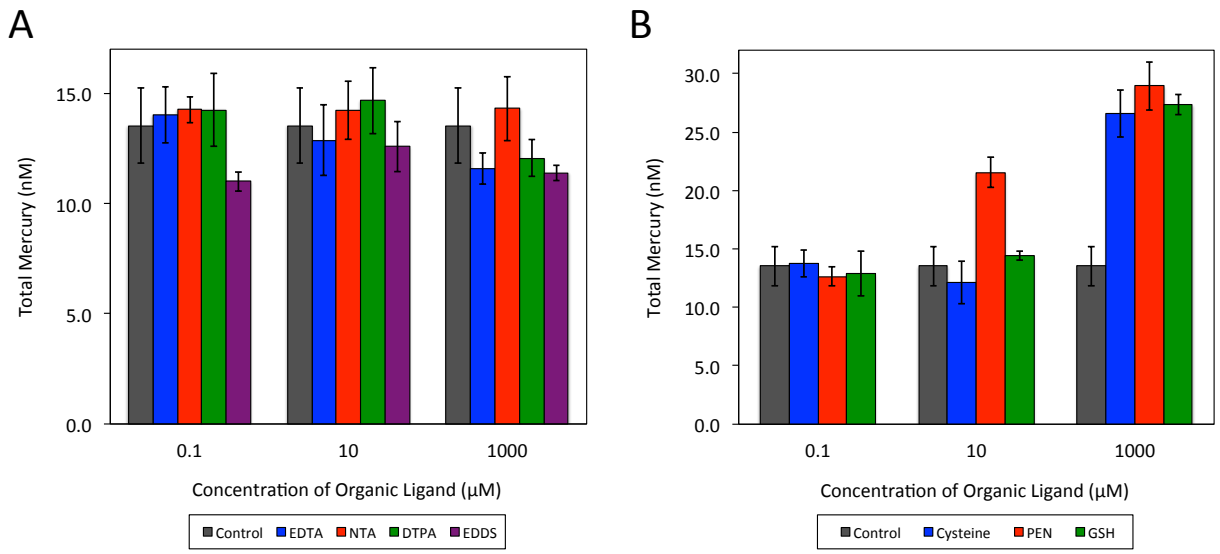
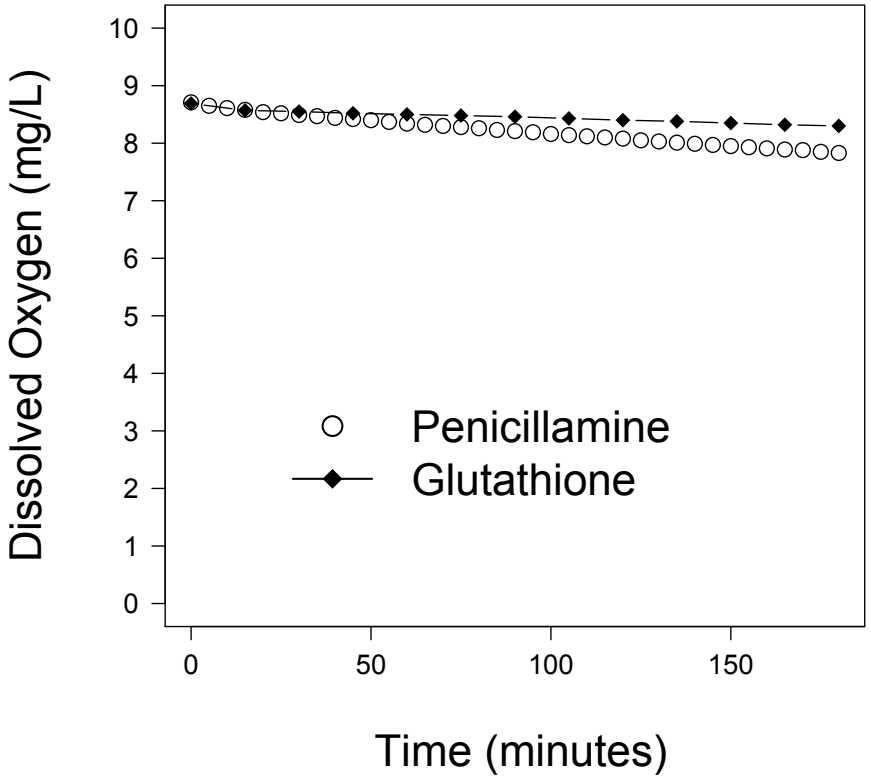


Figure S4: The concentration of THg recovered in the wells of a 96-well plate after exposure of *E. coli* ARL1 to 30 nM THg in the presence of 0.1, 10, and 1000 μM (A) aminopolycarboxylate ligand and (B) thiol-containing ligand in MCM for a 3-hour exposure period. The values presented are the average of three replicates, and error bars are ± 1 SD.



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162 Fig. S5: The concentration of dissolved oxygen measured in a solution of 1 mM penicillamine or
163 glutathione dissolved in the bioreporter exposure medium (MCM) over a period of 3 hours. The
164 solution was prepared in a BOD bottle and sealed from the atmosphere for the entire exposure
165 period.

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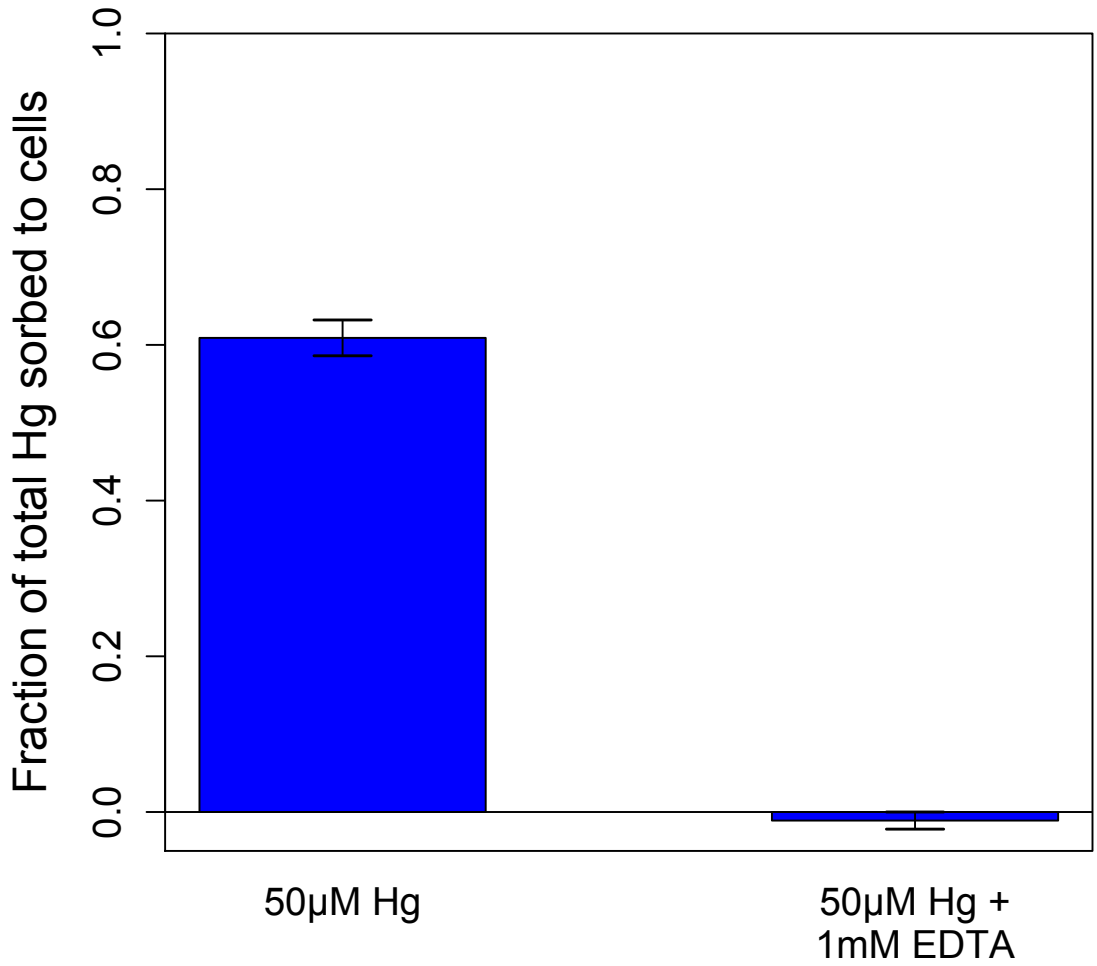

Figure S6: The fraction of total Hg associated with *E. coli* ARL1 after a 3-hour exposure period to 50 μM Hg in the absence of organic ligand and in the presence of 1 mM EDTA. The exposure medium was MCM without glucose and cell density was approximately 3×10^8 cells/mL. The fraction of sorbed Hg was calculated as the concentration of dissolved Hg (passed through 0.22μm filter) subtracted from THg then divided by THg. The bars represent averages from at least 3 independent experiments, and error bars are ±1 SD.