Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information: The role of cysteine and sulfide in the interplay between microbial Hg(II) uptake and sulfur metabolism

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Table S1: Composition of the growth medium (MSM) [1]

Media component[a]	Concentration (M)
pH	7.5
KH_2PO_4	5.0×10^{-3}
K_2HPO_4	9.9×10^{-3}
$MgSO_4$	4.1×10^{-4}
NH_4NO_3	1.2×10^{-2}
Isoleucine	7.6×10^{-4}
Leucine	7.6×10^{-4}
Thiamine	3.0×10^{-9}
Glucose	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}

[a] All reagents (> 99% purity) were purchased from Sigma-Aldrich.

HR-XANES reference standard preparation

The Hg references in this study include two forms of Hg(II) bound to inorganic sulfur (α- $HgS_{(s)}$ and β-$HgS_{(s)}$) and three forms of Hg(II) bound to organic sulfur ($Hg(cysteine)_{2(aq)}$ at pH = 3, $Hg(cysteine)_{2(aq)}$ at pH = 11.6, and $Hg(cysteine)_{4(aq)}$ at pH = 11). The α-$HgS_{(s)}$ was obtained from Sigma-Aldrich (CAS Number: 1344-48-5) and the bulk β-$HgS_{(s)}$ was prepared in the lab and is described in detail in our previous publication.[6] The $Hg(cysteine)_{2(aq)}$ standards at pH = 3 and pH = 11.6 were prepared according to the methods reported in Manceau et al.[7] Briefly, H_2Cysteine and $Hg(ClO_4)_2$ were added to 10 mL deionized water (boiled and bubbled with N_2 for ~1 hour while cooling) to achieve a final Hg(II) concentration of 0.5 mM and a final cysteine concentration of 1 mM. The mixture had a pH of 3 at which the amine groups of the cysteine ligands are protonated and thus are not involved in coordinating Hg(II).[7] A replicate solution of H_2Cysteine and $Hg(ClO_4)_2$ was prepared with a pH of 11.6, adjusted with 1 M NaOH, where both amine groups coordinate Hg(II) at ~2.51 Å in addition to the 2 thiols at ~2.35 Å.[7] The $Hg(cysteine)_{4(aq)}$ standard was prepared in a similar manner and had 50 mM total Hg(II), 500 mM total cysteine, and a pH of 10.7.

Table S2: Equilibrium constants used to calculate Hg speciation in this study[a,b]

Species	Reaction	LogK (I=0, T=298K)
$HCysteine^-$	$H^+ + Cysteine^{2-} = HCysteine^-$	10.87[c]
$H_2Cysteine$	$2H^+ + Cysteine^{2-} = H_2Cysteine$	19.38[c]
$H_3Cysteine^+$	$3H^+ + Cysteine^{2-} = H_3Cysteine^+$	21.67[c]
$HgCysteine$	$Hg^{2+} + Cysteine^{2-} = HgCysteine$	35.73[c]
$HgHCysteine^+$	$Hg^{2+} + H^+ + Cysteine^{2-} = HgHCysteine^+$	43.83[c]
$HgH_2Cysteine^{2+}$	$Hg^{2+} + 2H^+ + Cysteine^{2-} = HgH_2Cysteine^{2+}$	46.12[c]
$Hg(Cysteine)_2^{2-}$	$Hg^{2+} + 2Cysteine^{2-} = Hg(Cysteine)_2^{2-}$	44.55[c]
$HgH(Cysteine)_2^-$	$Hg^{2+} + H^+ + 2Cysteine^{2-} = HgH(Cysteine)_2^-$	53.97[c]
$HgH_2(Cysteine)_2$	$Hg^{2+} + 2H^+ + 2Cysteine^{2-} = HgH_2(Cysteine)_2$	62.04[c]
HS^-	$H^+ + S^{2-} = HS^-$	13.89
H_2S	$2H^+ + S^{2-} = H_2S$	20.91
$HgS(s)$	$HgS(s) + H^+ = Hg^{2+} + HS^-$	–36.8[d]
$Hg(SH)_2^0$	$Hg^{2+} + 2HS^- = Hg(SH)_2^0$	39.1[d]
HgS_2H^-	$Hg^{2+} + 2HS^- = HgS_2H^- + H^+$	32.5[d]
HgS_2^{2-}	$Hg^{2+} + 2HS^- = HgS_2^{2-} + 2H^+$	23.2[d]
$HIsoleucine$	$H^+ + Isoleucine^- = HIsoleucine$	9.76
$H_2Isoleucine^+$	$2H^+ + Isoleucine^- = H_2Isoleucine^+$	12.35
$HgIsoleucine^+$	$Hg^{2+} + Isoleucine^- = HgIsoleucine^+$	12.84
$Hg(Isoleucine)_2$	$Hg^{2+} + 2Isoleucine^- = Hg(Isoleucine)_2$	20.46
$HLeucine$	$H^+ + Leucine^- = HLeucine$	9.74
$H_2Leucine^+$	$2H^+ + Leucine^- = H_2Leucine^+$	11.99
$HgLeucine^+$	$Hg^{2+} + Leucine^- = HgLeucine^+$	12.34
$Hg(Leucine)_2$	$Hg^{2+} + 2Leucine^- = Hg(Leucine)_2$	20.16
$HgSO_4$	$Hg^{2+} + SO_4^{2-} = HgSO_4$	1.34
$Hg(SO_4)_2^{2-}$	$Hg^{2+} + 2SO_4^{2-} = Hg(SO_4)_2^{2-}$	2.40
$HgOH^+$	$Hg^{2+} + H_2O = HgOH^+ + H^+$	-3.4
$Hg(OH)_2$	$Hg^{2+} + 2H_2O = Hg(OH)_2 + 2H^+$	-6.2
$Hg(OH)_3^-$	$Hg^{2+} + 3H_2O = Hg(OH)_3^- + 3H^+$	-21.1
NH_4^+	$H^+ + NH_3 = NH_4^+$	9.24
$HgNH_3^{2+}$	$Hg^{2+} + NH_3 = HgNH_3^{2+}$	8.8
$Hg(NH_3)_2^{2+}$	$Hg^{2+} + 2NH_3 = Hg(NH_3)_2^{2+}$	17.4
$Hg(NH_3)_3^{2+}$	$Hg^{2+} + 3NH_3 = Hg(NH_3)_3^{2+}$	18.4
$Hg(NH_3)_4^{2+}$	$Hg^{2+} + 4NH_3 = Hg(NH_3)_4^{2+}$	19.1
$MgIsoleucine^+$	$Mg^{2+} + Isoleucine^- = MgIsoleucine^+$	1.34[e]
$Mg(Isoleucine)_2$	$Mg^{2+} + 2Isoleucine^- = Mg(Isoleucine)_2$	1.72[e]
$MgHIsoleucine^{2+}$	$Mg^{2+} + H^+ + Isoleucine^- = MgHIsoleucine^{2+}$	10.07[e]
$MgLeucine^+$	$Mg^{2+} + Leucine^- = MgLeucine^+$	1.34[e]
$Mg(Leucine)_2$	$Mg^{2+} + 2Leucine^- = Mg(Leucine)_2$	1.62[e]
$MgHLeucine^{2+}$	$Mg^{2+} + H^+ + Leucine^- = MgHLeucine^{2+}$	10.17[e]
$MgCysteine$	$Mg^{2+} + Cysteine^{2-} = MgCysteine$	4.16
$MgHCysteine^+$	$Mg^{2+} + H^+ + Cysteine^{2-} = MgHCysteine^+$	11.04
$Mg(Cysteine)_2^{2-}$	$Mg^{2+} + 2Cysteine^{2-} = Mg(Cysteine)_2^{2-}$	3.40
$MgOH^+$	$Mg^{2+} + H_2O = MgOH^+ + H^+$	-11.44
$HMOPS$	$H^+ + MOPS^- = HMOPS$	7.20
$H-\beta-glycerophosphate^-$	$H^+ + \beta-glycerophosphate^{2-} = H-\beta-glycerophosphate^-$	6.65
$H_2-\beta-glycerophosphate$	$H^+ + H-\beta-glycerophosphate^- = H_2-\beta-glycerophosphate$	1.33

[a] All complexation constants are from the Joint Expert Speciation System database (http://jess.murdoch.edu.au) or otherwise cited. [b] Only mono- and dithiolate Hg(II)-cysteine complexes are included in our calculations, although new research suggests tri- and tetrathiolate complexes may be stable under our conditions (unfortunately, no thermodynamic constants are available).[3] [c] From Cardiano et al.[4] [d] From Drott et al.[5] [e] These constants were measured at 37 °C.

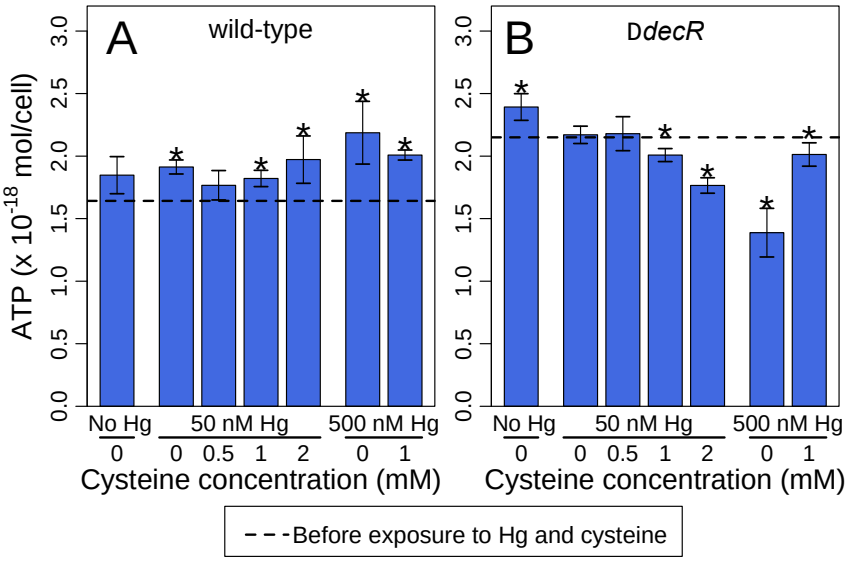

Figure S1: The amount of ATP per cell measured after exposing (A) wild-type and (B) *decR* mutant strains to Hg (50 nM and 500 nM) and cysteine (0, 0.5, 1, and 2 mM) for 3 hours. The dotted black line represents the ATP concentration in an aliquot from the same sample of cells directly before Hg and cysteine addition. The effect of cysteine without Hg was also tested and the results were not statistically different (data not shown). The bars are averages of duplicates from 2 independent experiments, the error bars are ± 1 S.D, and the asterisks mark samples with ATP concentrations that are statistically different than the concentrations measured before exposure to Hg and cysteine ($p < 0.05$).

Table S3: Total concentrations of cysteine, cystine, and sulfide as well as dissolved and total recoverable Hg in the exposure medium after 0, 1, 2, and 3 hours of exposure of wild-type and *decR* mutant strains to Hg and cysteine.

Wild-type		50 nM Hg				500 nM Hg	
Exposure time		0 mM Cys	0.5 mM Cys	1 mM Cys	2 mM Cys	0 mM Cys	1 mM Cys
0 hr	[cysteine][a]	0 µM	333.7 µM	781.9 µM	1660.7 µM	0 µM	781.9 µM
	[cystine][a]	0 µM	84.9 µM	89.7 µM	123.3 µM	0 µM	89.7 µM
	[sulfide][a,b]	0 µM	0 µM	0 µM	0.03 µM	0 µM	0 µM
	[$Hg_{dissolved}$][b]	24.9 nM	44.3 nM	48.9 nM	51.1 nM	286.6 nM	479.9 nM
	[$Hg_{total,rec.}$][c]	38.0 nM	51.1 nM	51.5 nM	48.7 nM	478.4 nM	540.8 nM
1 hr	[cysteine]	0 µM	255.7 µM	697.9 µM	1632.1 µM	0 µM	697.9 µM
	[cystine]	0 µM	106.9 µM	109.0 µM	135.5 µM	0 µM	109.0 µM
	[sulfide]	0 µM	22.2 µM	25.6 µM	21.3 µM	0 µM	25.6 µM
	[$Hg_{dissolved}$]	0.5 nM	4.0 nM	18.5 nM	39.9 nM	29.8 nM	27.0 nM
	[$Hg_{total,rec.}$]	28.0	26.6 nM	33.6 nM	44.9 nM	333.4 nM	314.2 nM
2 hr	[cysteine]	0 µM	195.2 µM	697.4 µM	1597.9 µM	0 µM	697.4 µM
	[cystine]	0 µM	103.0 µM	81.5 µM	95.9 µM	0 µM	81.5 µM
	[sulfide]	0 µM	39.4 µM	36.2 µM	40.0 µM	0 µM	36.2 µM
	[$Hg_{dissolved}$]	0.0 nM	1.4 nM	4.3 nM	29.5 nM	0.3 nM	4.3 nM
	[$Hg_{total,rec.}$]	19.3	27.4 nM	26.0 nM	43.1 nM	287.8 nM	264.1 nM
3 hr	[cysteine]	0 µM	183.2 µM	664.2 µM	1561.9 µM	0 µM	664.2 µM
	[cystine]	0 µM	92.4 µM	81.8 µM	125.6 µM	0 µM	81.8 µM
	[sulfide]	0 µM	27.4 µM	19.1 µM	23.8 µM	0 µM	19.1 µM
	[$Hg_{dissolved}$]	0.0 nM	8.0 nM	13.1 nM	34.9 nM	27.8 nM	17.9 nM
	[$Hg_{total,rec.}$]	17.8	31.2 nM	34.9 nM	45.3 nM	233.1 nM	284.7 nM
Δ*decR*		50 nM Hg				500 nM Hg	
Exposure time		0 mM Cys	0.5 mM Cys	1 mM Cys	2 mM Cys	0 mM Cys	1 mM Cys
0 hr	[cysteine]	0 µM	389.8 µM	801.0 µM	1728.1 µM	0 µM	801.0 µM
	[cystine]	0 µM	66.4 µM	87.9 µM	111.8 µM	0 µM	87.9 µM
	[sulfide]	0 µM	0 µM	0 µM	1.0 µM	0 µM	0 µM
	[$Hg_{dissolved}$]	21.1 nM	52.0 nM	51.4 nM	48.4 nM	402.2 nM	464.6 nM
	[$Hg_{total,rec.}$]	35.3 nM	49.0 nM	47.9 nM	50.1 nM	438.8 nM	470.5 nM
1 hr	[cysteine]	0 µM	346.2 µM	774.2 µM	1685.1 µM	0 µM	774.2 µM
	[cystine]	0 µM	71.6 µM	76.9 µM	93.6 µM	0 µM	76.9 µM
	[sulfide]	0 µM	5.7 µM	8.1 µM	9.8 µM	0 µM	8.1 µM
	[$Hg_{dissolved}$]	0.1 nM	35.5 nM	40.3 nM	45.3 nM	38.5 nM	29.7 nM
	[$Hg_{total,rec.}$]	25.1 nM	38.5 nM	41.7 nM	46.3 nM	348.8 nM	360.9 nM
2 hr	[cysteine]	0 µM	329.5 µM	744.8 µM	1610.4 µM	0 µM	744.8 µM
	[cystine]	0 µM	76.2 µM	85.6 µM	118.0 µM	0 µM	85.6 µM
	[sulfide]	0 µM	9.2 µM	10.5 µM	11.0 µM	0 µM	10.5 µM
	[$Hg_{dissolved}$]	0 nM	1.6 nM	34.3 nM	43.4 nM	0 nM	10.8 nM
	[$Hg_{total,rec.}$]	20.9 nM	37.1 nM	40.4 nM	45.9 nM	348.7 nM	313.4 nM
3 hr	[cysteine]	0 µM	315.0 µM	724.0 µM	1586.8 µM	0 µM	724.0 µM
	[cystine]	0 µM	75.6 µM	85.5 µM	102.1 µM	0 µM	85.5 µM
	[sulfide]	0 µM	4.3 µM	5.6 µM	6.7 µM	0 µM	5.6 µM
	[$Hg_{dissolved}$]	0 nM	5.0 nM	35.6 nM	44.1 nM	0 nM	0.5 nM
	[$Hg_{total,rec.}$]	20.6 nM	35.7 nM	41.4 nM	46.5 nM	363.1 nM	364.3 nM

[a] Measurements for total sulfide and cysteine/cystine were performed in absence of Hg, which was previously shown to have no effect on the concentrations.[2] [b] Slightly negative values, as determined from calibration curves, for total sulfide and Hg are written as zero in this table. [c] The total recoverable Hg (cell-bound + dissolved).

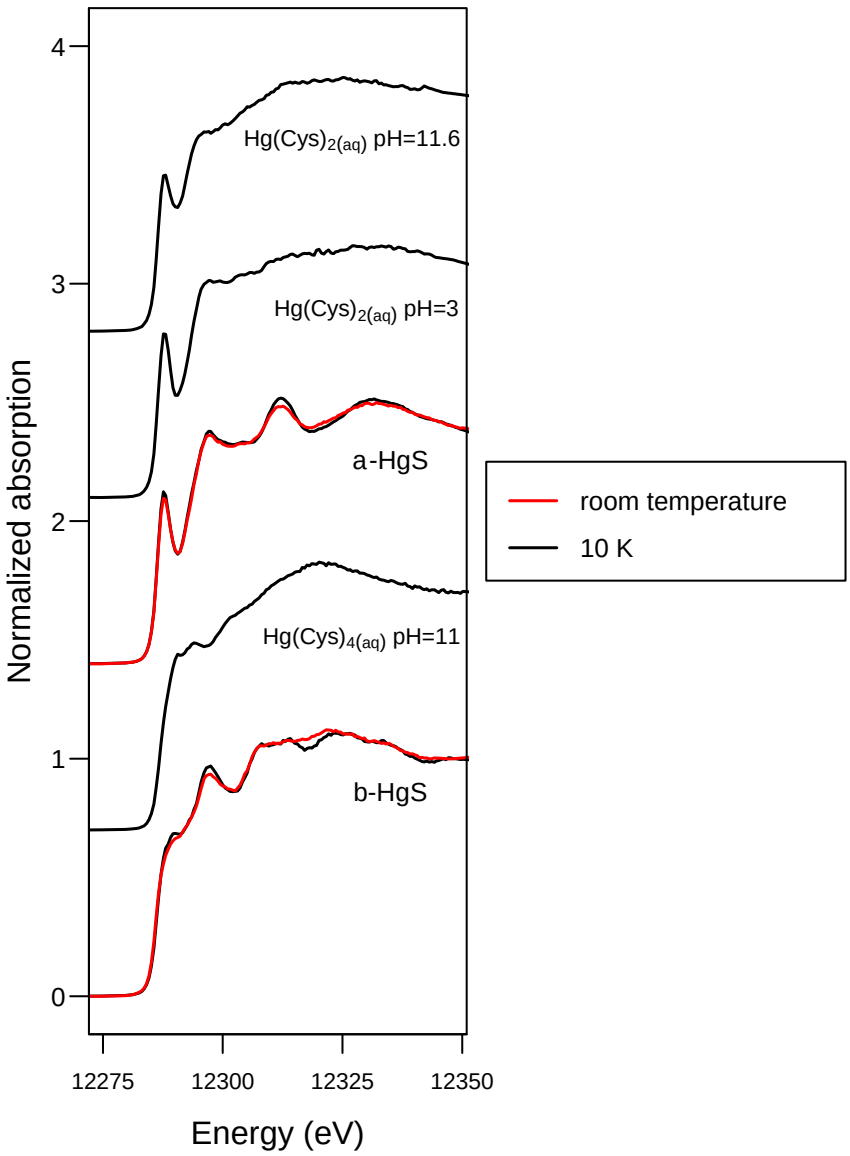

Figure S2: The Hg L_{III}-edge HR-XANES of reference compounds in this study.

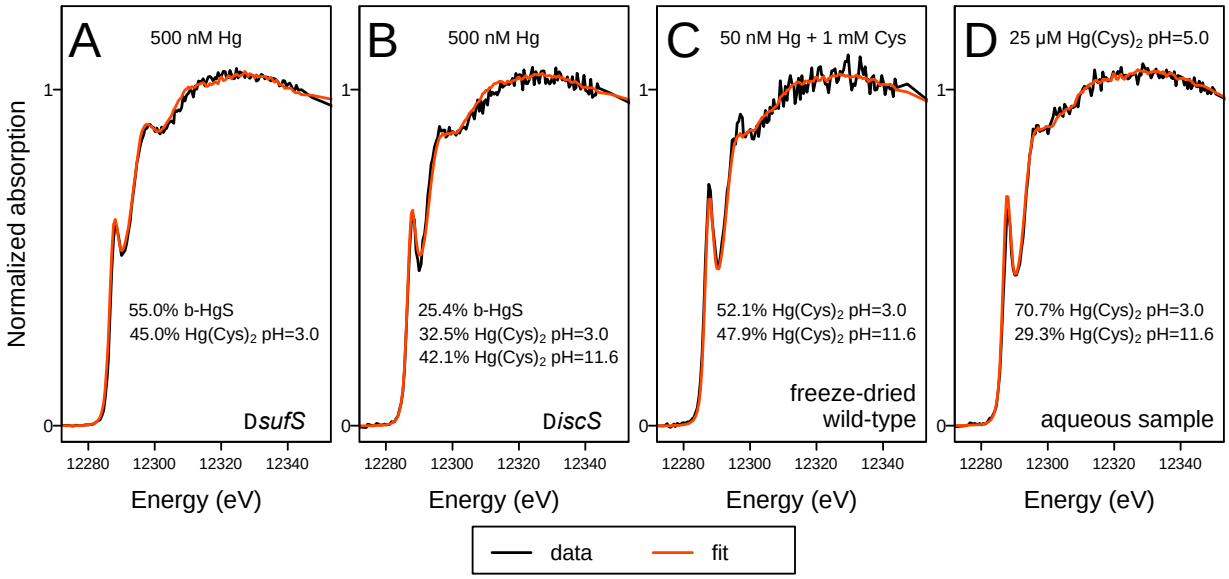

Figure S3: The Hg L_{III}-edge HR-XANES of (A) the cell pellet of the *sufS* mutant that was exposed to 500 nM Hg for 3 hours, (B) the cell pellet of the *iscS* mutant that was exposed to 500 nM Hg for 3 hours, (C) the freeze-dried cell pellet of wild-type *E. coli* that was exposed to 50 nM Hg and 1 mM Cys for 3 hours, and (D) an aqueous solution of 25 µM $Hg(NO_3)_2$ and 50 µM H_2Cysteine at pH = 5. The red line is the best-fit result of a linear combination fit, which included β-HgS, α-HgS, $Hg(Cys)_{2(aq)}$ at pH=3, and $Hg(Cys)_{2(aq)}$ at pH=11.6 as references.

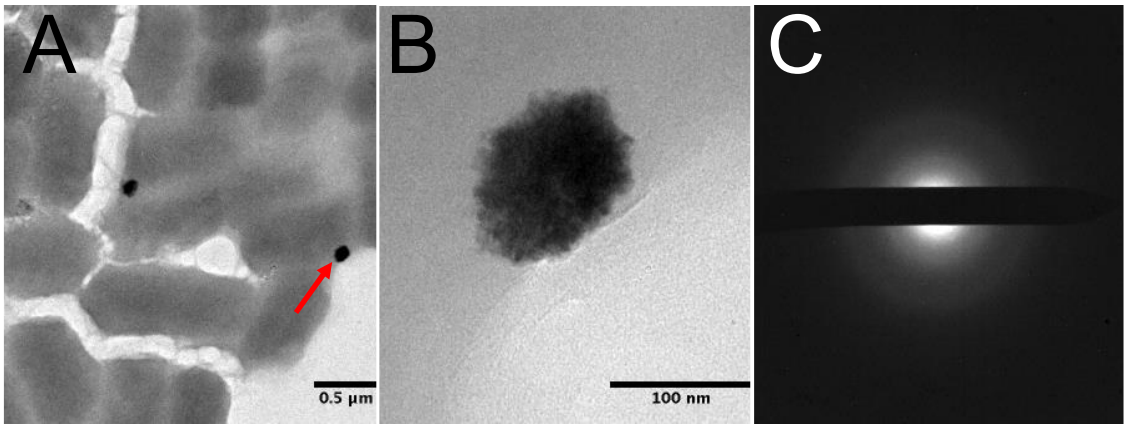

Figure S4: (A,B) TEM image of HgS particles associated with the wild-type strain exposed to 500 nM Hg + 1 mM cysteine for 3 hours. The image in panel B is a magnification of the arrow-marked HgS particle in panel A. (C) Selected area electron diffraction (SAED) pattern of the HgS particle in panel B. The diffuse rings and lack of sharp rings or spots in the SAED pattern indicate that the HgS particle is amorphous.

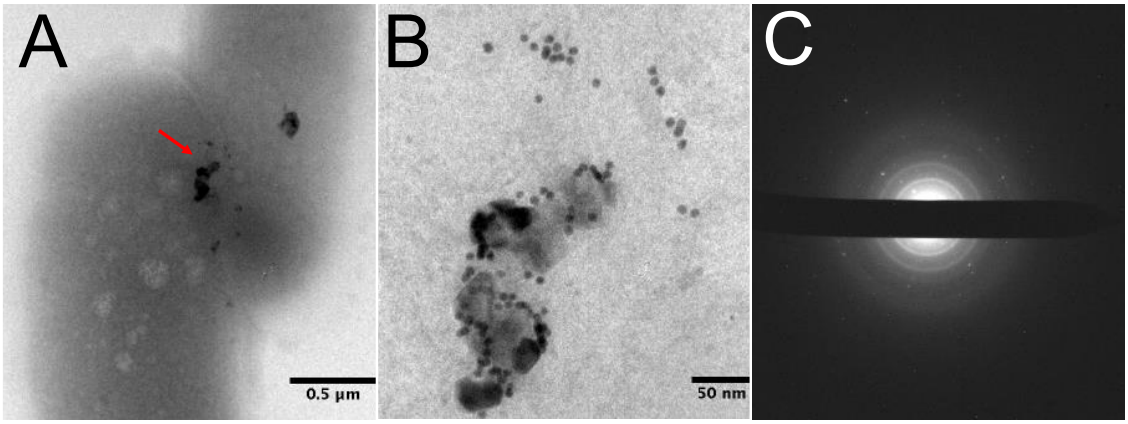

Figure S5: (A,B) TEM image of HgS particles associated with the *decR* mutant strain exposed to 500 nM Hg + 1 mM cysteine for 3 hours. The image in panel B is a magnification of the arrow-marked HgS particle in panel A. (C) Selected area electron diffraction (SAED) pattern of the HgS particle in panel B. The SAED patterns contains rings, indicating that the HgS particles are polycrystalline. The SAED pattern was indexed in Table S4 and fits the d-spacings of α-HgS. The source of the diffraction spots in the SAED pattern is unknown.

Table S4: Indexed Diffraction Pattern from Figure S5C

	Experimental			From literature[8]	
	Ring radius (1/nm)	d-spacing (nm)	d-spacing (Å)	d-spacing of α-HgS (Å)	Miller indices
Ring 1	3.03	0.330	3.30	3.36	(1 0 1)
Ring 2	3.53	0.283	2.83	2.86	(1 0 2)
Ring 3	5.06	0.198	1.98	1.98	(1 0 4)
Ring 4	6.07	0.165	1.65	1.73/1.68/1.68	(1 1 3)/(2 0 2)/(1 0 5)

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