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SUPPORTING INFORMATION

A sensitive whole-cell biosensor for the simultaneous detection of a broad-spectrum of toxic heavy metal ions

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Experimental Procedures

Bacterial strains and growth conditions

Bacterial strains and plasmids used in this study are listed in Table S3. Bacterial strains were routinely grown at 37ºC in Luria Broth (LB) or LB-agar plates, except for metal-induction assays. Kanamycin, chloramphenicol or ampicillin was used at 25, 10 or 100 µg ml^{-1} respectively. All reagents and chemicals were from Sigma-Aldrich, except the Luria-Bertani culture media that was from Difco. Analytical grade (ACS reagent, ≥98.0% purity or higher) heavy metal salts were used in all experiments. Oligonucleotides listed in Table S4 were provided by Life Technology.

Genetic and molecular biology techniques

Gene disruptions, point mutations and FLP-directing removing of antibiotic resistance cassette in *Escherichia coli* or *Salmonella enterica* were carried out essentially as previously described.[1, 2] P1 or P22 transduction were used to transfer the constructions between different *E. coli* or *S. enterica* strains, respectively.[3] All constructs were verified by DNA sequencing.

Transgenic *E. coli* MC1061 *golT-golS*$_{S77C}$ strain were constructed as follows using Lambda Red-mediated recombination.[4] First, a ~4000 pb fragment containing the whole *golT-golS*$_{S77C}$ locus linked to the *cat* gene (see Fig. 1A, in the text) was amplified from the chromosome of the *Salmonella* PB7657 strain (Table S3) using the P1-gol and P2-cat oligonucleotides (Table S4). This fragment was used to transform *E. coli* W3110 strain carrying pKD46 plasmid as previously described.[1] The resulting W3110 derivative has the *Salmonella golTS*$_{S77C}$ operon inserted in the *ampH-sbmA* intergenic region (at nucleotide position 395486 in the W3110 genome).

The *golS*$_{S77C+L}$ allele was generated in *Salmonella* by PCR overlap extension essentially as described[2] but using total DNA from PB4922 (*golS*$_{L}$) as template.

Metal induction assays

Metal induction assays were done as described.[1] Briefly, cells were grown to mid exponential phase at 37°C in SM9 minimal media supplemented with 0.2% glucose and 0.5% casamino acids. After that, the indicated metal salt was added to the bacterial culture and incubation was continued in the same conditions for 3 additional hours before emitted fluorescence was determined. *E. coli* strains were additionally supplemented with 2 µg ml^{-1} thiamine. Stock solutions (1 or 0.5 M) of $KAu(CN)_2$, $CuSO_4 \cdot 5H_2O$, $ZnCl_2$, $CdCl_2$, $HgCl_2$, $Pb(NO_3)_2$, $AgNO_3$ or $NiSO_4 \cdot 6H_2O$ were prepared using sterile distilled water and stored at 4 °C. Working dilutions were made in order to apply a 10 µl aliquot onto each test tube.

When indicated, cell cultures grown as described above were centrifuged, resuspended in half their original volume in fresh 2X SM9 medium, and mixed in a 1:1 ratio to tap water supplemented with metals as described in the text or in the legends to Fig.3 and S4.

Emitted fluorescence was recorded using a Synergy 2 Multi-Mode Microplate Reader (BioTek) and the 485±20 and 528±20 nm filters for excitation and emission wavelengths, respectively. Fluorescence (F) of each sample (measured in instrument's arbitrary units) was normalized against optical density at 600 nm (OD_{600}) and the fluorescence of the same strain carrying the pPROBE-NT vector as previously described.[1] Biosensor response to metal was expressed as Induction coefficients (IC), that is the ratio between the normalized F value of the sensor bacteria exposed to the metal, and the normalized fluorescence of the biosensor cultured without metal added (background fluorescence). Background fluorescence of 171.46±22.71 and 126.19±43.42 arbitrary units was determined for *Salmonella* and *E. coli*, respectively.

Metal diffusion assays were performed in LB agar plates inoculated by spreading 100 µl of an overnight culture of the biosensor bacteria. Sterile cellulose filter paper discs (Whatman) containing the indicated metal salt or sterile water were placed on the agar surface. After overnight incubation at 37°C, the fluorescent haloes were observed on UV or blue-ligth transilluminator.

Data fitting and calculation

Focus on the average behaviour of a bacterial population at steady state, we fitted the experimental data of the *Salmonella* and *E. coli* $GolS_{S77C}$-based fluorescence biosensors to a transfer function based on an environment-responsive promoter-based sensor as was previously reported by Wang et al.[5] The applied transfer function at the steady state is:

$$f(I) = IC = k\,[\alpha + I^n/(K_M{}^n + I^n)]$$

where I is the concentration of the metal salt, k, the maximum expression level due to induction, α, a constant relating to the basal level of the promoter due to leaky expression, while K_M and n are the Hill constant and coefficient, respectively, relating to the promoter–regulator/inducer interaction.

The experimental data of the response of the biosensors to each metal ion were fitted to the above transfer function using the GraphPad Prism 6 (trial version, http://www.graphpad.com/demos/). The estimated function parameters and the corrected coefficient of determination (R^2) are listed in Table S1 and S2.

The limit of determination (LOD) for each metal was determined using the function equation as the concentration of metal ions that induced the reporter expression to a value equal to 2 (FB + 3 SD)/ FB (where FB and SD are the mean background

fluorescence and the standard deviation, respectively).[6] The Hill equation and the k value estimated for each metal ion (Table S1 and S2) were used to calculate the concentration of the metal ion that produced the maximal induction in fluorescence (CMI). Microsoft Excel or the free Walframalpha software (http://www.wolframalpha.com) were used for data management and calculations. The calculated LOD and CMI values for each metal ion are indicated in Table S1 and S2.

Table S1. The best fits for metals that act as inducers of the *E. coli* $GolS_{S77C}$-based biosensor with 95% confidence

Inducer metal	k (IC)*	n[†]	K_M (μM)[‡]	α[§]	R^{2} [¥]	LOD (nM) [‖]	CMI (μM) [±]
Au	577.1 ± 58.5	3.20 ± 0.28	0.228 ± 0.025	0.0025 ± 0.0003	0.984	42.7	1.495
Hg	288.5 ± 21.6	2.86 ± 0.24	0.022 ± 0.002	0.0043 ± 0.0005	0.960	4.4	0.147
Pb	62.8 ± 8.6	3.43 ± 0.66	0.098 ± 0.015	0.0220 ± 0.0038	0.976	39.6	0.296
Cd	87.4 ± 14.7	3.48 ± 0.63	0.764 ± 0.122	0.0153 ± 0.0036	0.980	283.9	2.529

* Maximum expression level due to induction.
[†] Hill coefficient relating to the promoter–regulator/inducer interaction. It controls the steepness of the switch between no-activation to full-activation.
[‡] Hill constant relating to the promoter–regulator/inducer interaction. It is equal to the concentration of the metal ion needed to activate by 50% the overall expression.
[§] Constant relating to the basal level of expression due to the promoter leaky.
[¥] Corrected coefficient of determination.
[‖] Limit of determination.
[±] Maximal induction in fluorescence.

Table S2. The best fits for metals that act as inducers of the *Salmonella* $GolS_{S77C}$-based biosensor with 95% confidence

Inducer metal	k (IC)*	n[†]	K_M (μM)[‡]	α[§]	R^{2} [¥]	LOD (nM) [‖]	CMI (μM) [±]
Au	545.8 ± 82.9	3.25 ± 0.48	0.270 ± 0.045	0.0042 ± 0.0009	0.959	31.3	1.452
Hg	376.0 ± 32.7	4.20 ± 0.44	0.019 ± 0.002	0.0041 ± 0.0005	0.975	4.5	0.070
Pb	215.3 ± 16.1	2.57 ± 0.20	0.231 ± 0.024	0.0056 ± 0.0007	0.984	34.1	1.726
Cd	168.6 ± 23.2	2.96 ± 0.47	0.966 ± 0.172	0.0069 ± 0.0015	0.944	200.8	5.145

* Maximum expression level due to induction.
[†] Hill coefficient relating to the promoter–regulator/inducer interaction. It controls the steepness of the switch between no-activation to full-activation.
[‡] Hill constant relating to the promoter–regulator/inducer interaction. It is equal to the concentration of the metal ion needed to activate by 50% the overall expression.
[§] Constant relating to the basal level of expression due to the promoter leaky.
[¥] Corrected coefficient of determination.
[‖] Limit of determination.
[±] Maximal induction in fluorescence.

Table S3. Bacterial strains and plasmids used in this study.

Strain	Relevant genotype or properties	Refs. or source
Salmonella enterica serov. Typhimurium		
14028s	Wild type	ATCC®-14028™
PB4922	14028s *golS*$_L$-*cat*	[7]
PB10266	14028s *ΔzntA golS*$_{S77C}$-*cat*	[8]
PB10507	14028s *golS*$_{S77C\text{-}L}$-*cat*	This work
Eschericha coli		
MC1061	*hsdR2 hsdM*$^+$ *hsdS*$^+$ *araD139 Δ(ara-leu)7697 Δ(lac)X74 galE15 galK16 rpsL (StrR) mcrA mcrB1*	[9]
PB10375	MC1061 Δ*zntA*	This work
PB10340	MC1061 *golTS*$_{S77C}$-*cat*	This work
PB10379	MC1061 Δ*zntA golTS*$_{S77C}$-*cat*	This work
PB11227	MC1061 ΔzntA *golTS*$_{S77C\text{-}L}$-*cat*	This work
Plasmids		
pPROBE-NT	rep$_p$ BBR1 Km^R promoter less *gfp*	[10]
pPB-GFP	pPROBE-NT derived plasmid with the *golB* promoter inserted upstream the *gfp* gene	[1]
pKD3	Ori R6K, Cm^R, Amp^R	[4]
pKD4	Ori R6K, Km^R, Amp^R	[4]
pCP20	*flp*, Amp^R, Cm^R	[11]
pKD46	P$_{araB\text{-}γ\text{-}β\text{-}exo}$, Amp^R	[4]

Table S4. Oligonucleotides

Primer name	Sequence (5'-3')	Purpuse
zntA-EC-P1-F	TCCTTCGGTTAATGAGAAAAAACTTAACCGGAGGATGCCGTG TAGGCTGGAGCTGCTTCG	λ Red deletion of *E. coli zntA*
zntA-EC-P2-R	GAGGGGACCGATCGCGCTCAATGTTGCGATCGGTTTGCCCA TATGAATATCCTCCTTA	λ Red deletion of *E. coli zntA*
P1-gol	CGCCGCATGGACGGAGGTCAATGACGCCGCACACAGCACG AATTTTGAATGTTCTACGCATG -	λ Red insertion of the *golT-golS*$_{S77C}$ *cat* locus in *E. coli*
P2-cat	TATGGACACCACCGTTGAAACGTAGTCTGCTTTTTTCTGCATT ACACGTCTTGAGCGAT	λ Red insertion of the *golT-golS*$_{S77C}$ *cat* locus in *E. coli*
golS$_{S77C}$-R	GCGTTTGACGTCAGCGCATTGCCGCGACT	Mutagenesis of *golS*
golS(wt)-F	ATGAGGAGGAGCGTCATGAACATCG	PCR overlap extension and λ Red insertion of fragment encoding the *golS*$_{S77C+L}$ allele in the *Salmonella* chromosome
RvP1-golB-R	GTGAACTCCTTTTGTGTGGGAACTG	PCR overlap extension of fragment encoding the *golS*$_{S77C+L}$ allele
golB-P1-F	CACTGGCAAGGTCCAGACTGGCAACAGTTCCCACACAAAAG GAGTTCACTGTGTAGGCTGGAGCTGCTTC	PCR overlap extension of fragment encoding the *golS*$_{S77C+L}$ allele
golB-P2-R	TGGCTAGCGTATCGCGACCGGCCTGTCGCCAGACCGATCGC CATTGACGACATATGAATATCCTCCTTA	PCR overlap extension and λ Red insertion of fragment encoding the *golS*$_{S77C+L}$ allele in the *Salmonella* chromosome

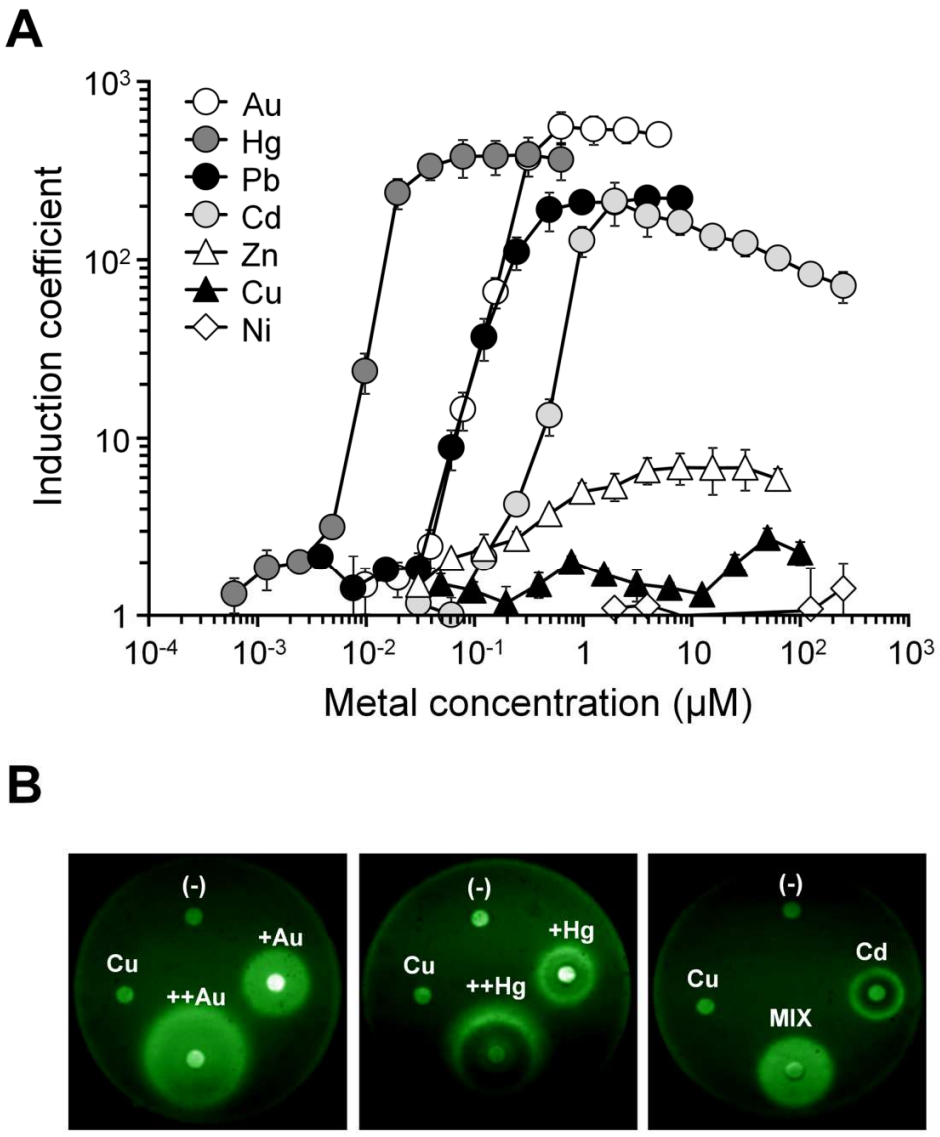

Fig. S1. Metal detection by the *Salmonella* $GolS_{S77C}$-based biosensor. (A) Dose–response curve. The response to $KAu(CN)_2$ (Au), $HgCl_2$ (Hg), $Pb(NO_3)_2$ (Pb), $CdCl_2$ (Cd); $ZnCl_2$ (Zn) or $CuSO_4$ (Cu) was plotted against the final concentration of metal in SM9 culture medium. Induction coefficient, IC, is the ratio between the normalized fluorescence measured in the presence and absence of metal. The data represent the mean±SD of at least five independent determinations done in triplicate. (B) Diffusion assays on agar plates. Sterile cellulose filter paper discs soaked up with 2 or 20 μM $KAu(CN)_2$ (+Au or ++Au), 0.2 or 2 μM $HgCl_2$ (+Hg or ++Hg), 200 μM $CuSO_4$ (Cu), 20 μM $CdCl_2$ (Cd), a mixture containing 0.5 μM $KAu(CN)_2$ 0.05 μM $HgCl_2$ 50 μM $CuSO_4$ 5 μM $CdCl_2$ (MIX) or sterile water (-) were placed on the surface of a SLB-agar plate inoculated with the reporter bacteria. After incubation overnight, the fluorescent haloes were evidenced in a UV-light transilluminator.

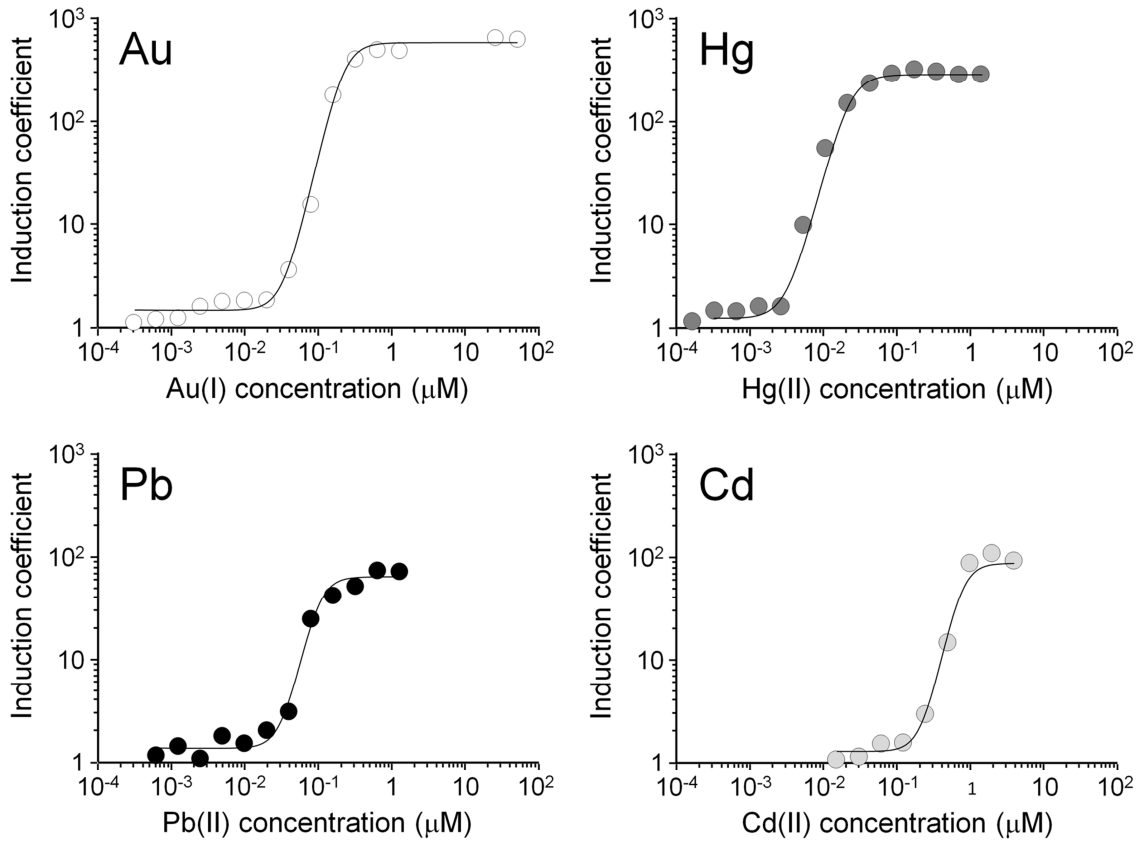

Fig. S2. Response of the *E. coli* GolS$_{S77C}$-based biosensor to Au, Hg, Pb or Cd ions. The experimental data (symbols) were individually fitted to the transfer function (solid lines) described in Experimental Procedures. The calculated parameters are depicted in Table S1.

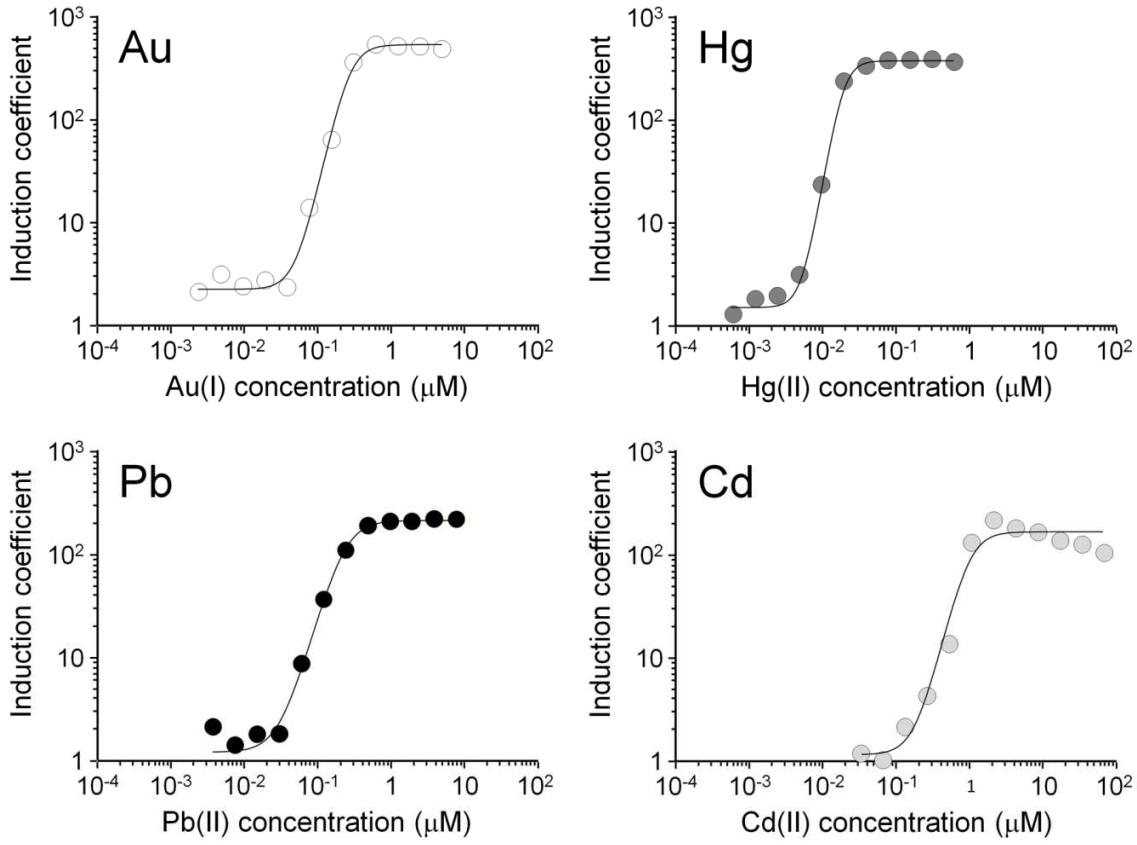

Fig. S3. Response of the *Salmonella* GolS$_{S77C}$-based biosensor to Au, Hg, Pb or Cd ions. The experimental data (symbols) were individually fitted to the transfer function (solid lines) described in Experimental Procedures. The calculated parameters are depicted in Table S2.

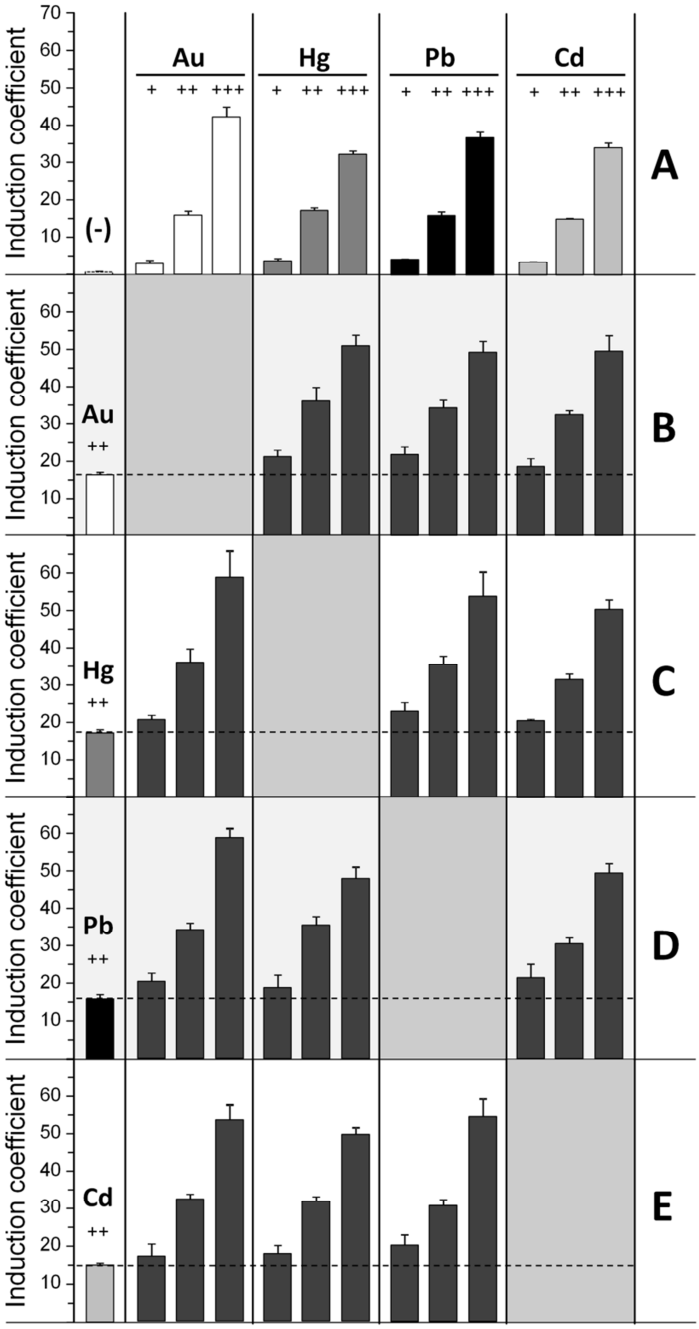

Fig. S4. Analysis of artificially contaminated tap water samples using the *E. coli* $GolS_{S77C}$-based biosensor. Water samples were supplemented only with increasing amounts of one metal (panel A) or with two metals (panel B-E) in different ratios. Final concentrations in mixture were 40, 80 or 100 nM for $KAu(CN)_2$ (Au) or $Pb(NO_3)_2$ (Pb); 4, 8 or 10 nM for $HgCl_2$ (Hg) or 250, 500 or 650 nM for $CdCl_2$ (Cd). The + symbol stands for the relative amount of metal added. (-) means no other metal added. The data represent the mean±SD of IC values from at least four independent measurements done in triplicate.

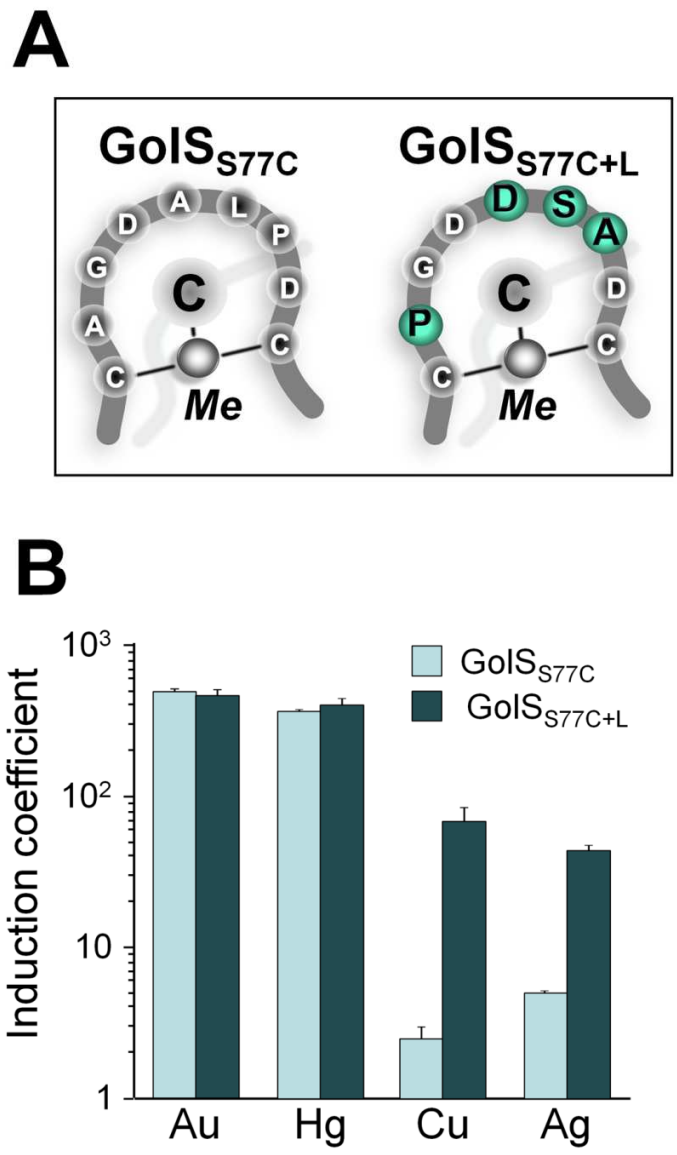

Fig. S5. The GolS$_{S77C+L}$-based biosensor platform (A) Schematic representation of the metal binding site in the GolS$_{S77C}$ and GolS$_{S77C+L}$ sensors. The amino acid residues replaced at the metal-binding loop region are highlighted. (B) Response to 1 μM $KAu(CN)_2$ (Au), 1 μM $HgCl_2$ (Hg), 100 μM $CuSO_4$ (Cu) or 10 μM $AgNO_3$ (Ag) added to the culture media of the GolS$_{S77C+L}$- and GolS$_{S77C}$-based biosensor. Culture and incubation with metals were done as described in Fig. 2.

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