Metalloid Reductase of Pseudomonas moravenis Stanleyae Conveys Nanoparticle Mediated Metalloid Tolerance

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ABSTRACT: A glutathione reductase (GSHR)-like enzyme in Pseudomonas moravenis stanleyae was previously implicated as underlying the bacterium’s remarkable SeO\(_3^{2−}\) tolerance. Herein, this enzyme is sequenced, recombinantly expressed, and fully characterized. The enzyme is highly adapted for selenodiglutathione substrates (\(K_m = 336 \mu M\)) compared to oxidized glutathione (\(K_m = 8.22 \text{ mM}\)). The recombinant expression of this enzyme in the laboratory strains of Escherichia coli conveys a 10-fold increase in IC\(_{90}\) for SeO\(_3^{2−}\), but not in the corresponding no-enzyme controls. The analyses of the structural homology models of the enzyme reveal changes in the parts of the enzyme associated with product release, which may underlie the Se substrate specialization. Combined, the observations of adaptation to Se reduction over oxidized glutathione reduction as well as the portability of this nanoparticle-mediated SeO\(_3^{2−}\) tolerance into other cell lines suggest that the P. moravenis GSHR may be better described as a GSHR-like metalloid reductase.

INTRODUCTION

The enzymatic conversion of soluble inorganic ions into insoluble forms is accomplished by enzyme centers such as those found in ferritin, magnetosomes, and silicateins. This enzymatic alteration in the solubility state facilitates the synthesis of biogenic inorganic materials. These naturally occurring catalysts, in concert with accessory proteins, can exhibit control over subsequent material composition, oxidation state, morphology, and structure. These natural precedents suggest that the intentional engineering of biological diversity could underlie the engineered diversity in biogenically synthesized inorganic materials. Such inorganic materials—synthesized by laboratory-evolved or engineered biomolecules (peptides, proteins, and nucleic acids) attract attention in catalysis, self-assembly, and in biocontrast (labeling) applications.4–8

Many self-contained biological systems, for synthesizing an inorganic nanostructure, will generally require an oxidoreductase activity, enabling the conversion of inorganic ions from soluble to insoluble oxidation states. Ferritins and DNA-binding proteins accomplish this with the ferroxidase enzymatic centers.9,10 Mercuric reductases accomplish this with substrate reduction through an active dithiol,11 which exhibits striking similarities to other enzymes such as lipoamide dehydrogenase.12

We recently reported on the ability of glutathione reductase (GSHR) to enzymatically reduce selenite (SeO\(_3^{2−}\)) to zerovalent red selenium in a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reaction.13 Similar, although diminished, activity was observed for the same enzyme in reducing tellurite (TeO\(_3^{2−}\)) to elemental Te. Our prior work identified the selenite reductase activity in Pseudomonas moravenis stanleyae. This microbe attracted our attention because it is found as an endophyte in the selenium-tolerant plant, Stanleya pinnata. When cultured independently in liquid media, it tolerates SeO\(_3^{2−}\) supplementation in liquid culture up to 10 mM. This is 10-fold more than the SeO\(_3^{2−}\) tolerance of most other microbes. We attributed the observed selenite reduction activity of P. moravenis to a GSHR-like enzyme on the basis of proteomic mass spectrometry of an in-gel in situ selenium reductase activity.

GSHRs generally belong to the family of pyridine nucleoside-dependent oxidoreductases. This enzyme family also, notably, includes another well-characterized metal-reducing enzyme—mercuric reductase.14 Within this enzyme family, the active sites are highly conserved. The typical active site peptide sequences are CXXXXC for type I and CXXC for type II enzymes.15 These classes of enzymes have demonstrated their ability to reduce a variety of metal ions to zerovalent forms, including Se, Hg, Te, Fe, Cr, and U.16–19 GSHR is reported to reduce Au(III) to the zerovalent form as well.20 Thus, the class of pyridine nucleoside-dependent oxidoreductases may repre-
sent an evolutionarily adaptable platform of inorganic ion reductases, with modifications to the enzyme, altering the metal-ion selectivity. Such a catalytic center, with alterable precursor selectivity, is of interest in biogenic inorganic nanoparticle (NP) synthesis.

In a prior study, we characterized the commercially sourced \textit{Saccharomyces cervisiae} GSHR for selenite reductase activity, showing the ability of the enzyme to oxidize NADPH while reducing \( \text{SeO}_3^{2-} \) to \( \text{Se}(0) \) NPs.\textsuperscript{13} In the present study, we characterize a homologous metalloid reductase from the seleno-specialist \textit{P. moraviensis}. We find that the substrate selectivity of the metalloid reductase \( (K_m) \) shows a substantially larger preference for GS-Se-SG relative to all other reported GSHR enzymes. These enzymatic properties can be partially rationalized in terms of sequence and the corresponding homology-modeled structure of the enzyme. We also observe that expressing this enzyme in the laboratory strains of \textit{E. coli} (BL21, SS320) results in an increased tolerance to \( \text{SeO}_3^{2-} \), as well as the presence of Se NPs in these cells. Overall, our data suggest that the enzyme may be best described as a GSHR-like metalloid reductase (GRLMR).

## RESULTS AND DISCUSSION

The altered substrate specificity of GRLMR enzymes, favoring selenodiglutathione (GS-Se-SG) over oxidized glutathione (GSSG) as a substrate, could underlie the remarkable \( \text{SeO}_3^{2-} \) tolerance of \textit{P. moraviensis} stanleyae. We therefore characterized the \textit{P. moraviensis} stanleyae GRLMR enzyme identified previously. The DNA sequence of the enzyme was acquired through a full-genome sequencing (ACGT Inc., Wheeling, IL). The sequencing was conducted using de novo paired-end sequencing.\textsuperscript{21} This revealed a genome in which 70.3% of the nucleobases have, at the most, a 1:1000 probability of misassignment. Figure S1 shows the "Quality Score" \( (Q \text{ score}) \) for each sequenced base with \( Q = -\log_{10}(e) \). The \( Q \) scores are derived from a phred-like error probability assessment of each individual nucleotide.\textsuperscript{22}

A basic local alignment search tool search of the genomic sequence, using the \textit{Pseudomonas R-28S} GSHR as a reference, identified one GSHR-like sequence, with 93% sequence homology. The sequence alignment of this GRLMR DNA using Serial Cloner shows a high similarity (98.00%) to \textit{Pseudomonas fluorescine}, \textit{E. coli}, \textit{S. cervisiae}, and \textit{H. sapiens} DNA. The sequence similarities are summarized in Table 1, and full alignments are shown in the Supporting Information, Figure S2. The DNA sequence, combined with the homology modeling of the structure, suggests that all of the structural domains of type I pyridine nucleotide-dependent oxidoeductases, including catalysis, dimerization, nucleotide binding, and substrate/product-binding domains,\textsuperscript{15,23,24} are present in this enzyme. Such a homology would suggest that the GRLMR enzyme would conduct substrate reduction for GSSG, and related molecules, in a fashion similar to previously mentioned GSHR enzymes.

### Table 1. Sequence and Structural Homology\textsuperscript{a}

|         | PM MTLR   | PF GSHR   | EC GSHR   | SC GSHR   | HS GSHR   |
|---------|-----------|-----------|-----------|-----------|-----------|
| PM MTLR | 98.00%/1.71 | 74.14%/1.23 | 60.78%/1.27 | 64.71%/1.31 |
| PF GSHR | 67.42%/1.44 | 68.31%/1.42 | 77.59%/1.05 | 75.47%/1.12 | 68.00%/1.07 |
| EC GSHR | 70.92%/1.56 | 73.48%/1.33 | 74.61%/1.17 | 81.48%/0.87 | 79.63%/0.85 |
| SC GSHR | 70.34%/1.44 | 70.63%/1.24 | 78.97%/1.07 | 75.77%/0.92 |
| HS GSHR | 70.34%/1.44 | 70.63%/1.24 | 78.97%/1.07 | 75.77%/0.92 |

\( ^a \) Sequence similarity (left value) and RMSD calculations (right values). PF—\textit{P. fluorescine}, EC—\textit{E. coli}, SC—\textit{S. cervisiae}, and HS—\textit{H. sapiens}.

![Figure 1. Substrate activity assays: (a) GRLMR, (c) GSHR, with the corresponding Lineweaver-Burke plots: (b) GRLMR. (d) GSHR.](image-url)
reported GSHR reduction pathways via an active-site dithiol. This GRLMR shows 19% DNA sequence homology to mercuric reductase from *Pseudomonas aeruginosa*.

Homology modeling using intensive parameters on the Phyre 2 server suggests that the structures of the *Pseudomonas*-derived GSHR and GRLMR enzymes are homologous to the other GSHR enzymes, despite modest DNA sequence divergence. Table 1 shows the root-mean-square deviation (RMSD) of the atomic position values for a set of GSHR homology models and/or crystal structures. Overall, the RMSD values for these structures are similar, suggesting an overall structural homology between GRLMR, and GSHR from *P. fluorescences*, *E. coli*, *H. sapiens*, and *S. cervisiae*.

Under the hypothesis that the GRLMR enzyme has altered selectivity relative to other characterized GSHRs, we characterized the enzyme kinetics of both GRLMR and *S. cervisiae* GSHR. We expressed GRLMR recombinantly in the *E. coli* BL21 cells. Following a 6×-histidine tag purification, we determined the Michaelis–Menten constants (*Km*) for both GRLMR and the commercially sourced *S. cervisiae* GSHR (Sigma, G3664). The *Km* value of each enzyme was determined for each of the three substrates: SeO$_3^{2−}$, GSSG, and GS-Se-SG. It has been previously reported that the GSHR enzymes have the ability to reduce these three substrates, with the proposed mechanisms for the GSH-based substrates. While SeO$_3^{2−}$ (Alfa Aesar, 12585) and GSSG (Sigma, G4376) are commercially available, we synthesized GS-Se-SG according to the previous published methods.

The enzymatic rates for both enzymes with both the GSSG and GS-Se-SG substrates are plotted in Figure 1, panels (a,c). The data are plotted as NADPH cofactor consumption, observed experimentally as the depletion of a spectroscopic peak characteristic of NADPH (but not NADP$^+$) at 340 nm. The decay rate, as measured at 340 nm, was converted to a normalized reaction rate. Lineweaver–Burke plots (shown as insets) were generated for each enzyme to determine $V_{max}$ and *Km* for the corresponding substrates. We chose to utilize the Lineweaver–Burke analysis over the more current method of nonlinear regression because our data was not accurately modeled using this technique. Points after 0.2 mM for GS-Se-SG were not used for the determination of $K_m$ because of particle scattering altering the observed rate. Without having the data points near the asymptote, the nonlinear regression analysis suffered from large errors and major variations in the calculated constants. When nonlinear regression was used for the analysis of all GSSG data points, the reciprocal plots showed an uneven distribution, indicating that the enzyme kinetics were not modeled correctly using this method. Upon further investigation, we discovered that the reported $K_m$ values for SC GSHR were calculated using the Lineweaver–Burke analysis. Using the Lineweaver–Burke analysis granted us consistency across our data analysis and allowed for comparison with the literature, giving us the most accurate and comparable values relative to the other common kinetic analyses. The determined $K_m$ values for each enzyme/substrate combination are shown in Table 2.

For the GRLMR enzyme, we observe a remarkable specialization of the enzyme for GS-Se-SG over GSSG. Specifically, the $K_m$ value of GRLMR is 25 times more favorable for GS-Se-SG as compared to GSSG. This difference in $K_m$ for the two substrates strongly implies that the enzyme is specialized for Se reduction over GSSG reduction. For $S. cervisiae$ GSHR, we determined similar $K_m$ values for GSSG and GS-Se-SG substrates, with the enzyme showing a slightly greater affinity for GSSG. The values we find for both substrates are consistent with the previous findings from other research groups. The $K_m$ value of GRLMR for SeO$_3^{2−}$ has not been previously reported, to our knowledge. The physiological relevance of the $K_m$ value for SeO$_3^{2−}$ is questionable, as Se salts such as SeO$_3^{2−}$ are converted to GS-Se-SG via GSH reduction in vivo. We established here the $K_m$ value for SeO$_3^{2−}$ for both GRLMR and *S. cervisiae* GSHR because we identified GRLMR on the basis of the SeO$_3^{2−}$ reductase activity, but note that the value is sufficiently unfavorable compared to the GSH-based substrates that it is unlikely to be physiologically relevant.

The value of $V_{max}$ for the GS-SG and GS-Se-SG substrates track in tandem up to the initial concentrations of 0.20 mM. Deviations begin near a concentration of 0.30 mM, where the rate for GS-Se-SG has reached its maximum velocity, whereas the rate for oxidized glutathione continues to increase until roughly 0.60 mM substrate concentration. The maximum velocity observed for GSSG is approximately 14 times greater than the $V_{max}$ for GS-Se-SG, but this occurs at a larger substrate concentration for GSSG, accounting for the lower affinity. It is possible that the fall-off in rate that we observe at higher GS-Se-SG concentrations is artifactual, arising from the interference in the optical assay by the selenium NPs produced during the experiment.

Overall, the $K_m$ values we find suggest a strong substrate preference for GS-Se-SG for the GRLMR enzyme. This is in contrast to the other characterized GSHR enzymes, where there is essentially no differentiation between the substrates. The cellular concentrations of GSSG would typically be much higher than GS-Se-SG at sublethal amounts of selenium in vivo; thus, the SC GSHR enzyme would turn over GSSG much more frequently and does not lead to selenium resistance as in the *P. moraviensis* system. To our knowledge, this is the first finding of an enzyme specialized for reduction of GS-Se-SG over any other substrate. Such a specialized enzyme could be used for nanomaterial development such as biogenic quantum dots and, most notably, as a starting point for the development of a functionalized clonable NP.

We hypothesized that if GRLMR conveys SeO$_3^{2−}$ tolerance to *P. moraviensis*, then the recombinant expression of this enzyme may convey a similar tolerance to the host organism for GRLMR expression. To evaluate this hypothesis, we transformed the lab expression strains of *E. coli*, BL21 and SS320, with the isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible expression vector described above. For the GRLMR enzyme-expressing *E. coli*, we determined selenite tolerance as the concentration of SeO$_3^{2−}$ that kills 90% of the cells (IC$_{90}$). We also examined the cells microscopically for the presence or absence of SeNPs.

| enzyme/substrate | GRLMR $K_m$ (μM) | SC GSHR $K_m$ (μM) | GRLMR $V_{max}$ (μM/min) | SC GSHR $V_{max}$ (μM/min) |
|------------------|-----------------|--------------------|---------------------------|----------------------------|
| GSSG 8.22 mM     | 103             | 2.62               | 0.107                     | 0.137                      |
| GS-Se-SG 336 μM  | 133             | 0.187              | 0.137                     |                            |

*aV_{max}$ reported in (μM/min)/(μg of enzyme).

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day, the colony-forming units (cfus) were counted. IC90 was calculated as the percentage of cfus present relative to an identical control supplemented with 1.0 μM SeO3 2−. We determined 1.0 μM SeO3 2− as necessary for maximizing the number of observed cfus. We hypothesize that such a supplementation is necessary because the overexpression of a SeO3 2−-reducing enzyme in these cells reduces and makes unavailable the essential amount of Se needed as a micro-nutrient for optimum growth.30

Figure 2 shows relative growth inhibition as a function of selenite concentration. The log 10[SeO3 2−] gives a linear concentration dependence for SeO3 2− growth inhibition. This allows the determination of the inhibitory concentration of selenite that eliminates 90% of cell growth (IC90). We found an IC90 of 21.3 ± 9.80 mM under the conditions of GRLMR overexpression, whereas an IC 90 of 1.89 ± 0.46 mM is observed in a corresponding control experiment. This result is at least somewhat cell-line-independent. When GRLMR is recombinantly overexpressed in E. coli SS320, we observe an IC90 of 18.3 ± 19.50 mM. GRLMR scavenges the cells of the micronutrient levels of selenium which can stunt growth and cause cell death. This inherently causes large deviations in cfu formation from run to run. Even with the large error bars, a statistically significant increase in SeO3 2− tolerance is induced by the presence of the GRLMR plasmid, and we believe that the SS320 data is justified because of the similarly observed tolerance in the BL21 cell line.

We note that the presence of the recombinantly expressed enzyme results in the liquid cultures taking on the red color characteristic of the red allotrope of zerovalent selenium, whereas cultures grown with SeO3 2− without the recombinantly expressed enzyme do not take on this color. To illustrate this, Figure 3 shows the cell cultures expressing GRLMR (left panel) or the green fluorescent protein (GFP) (right panel) in the presence of SeO3 2− supplementation at 5.0 mM, after 3 h of exposure. This “bulk color” change suggests that the cells expressing the recombinant enzyme may also be forming Se(0) NPs, just as we previously observed for the P. moravensis strain.

The examination of cells by scanning electron microscopy (SEM) revealed the presence of selenium NPs in cells expressing the recombinant enzyme and grown in Se-supplemented media. Figure 4 shows the scanning transmission electron micrographs of glutaraldehyde-fixed dry-mounted BL21 E. coli cells expressing GFP or GRLMR after growth in SeO3 2−-supplemented media. Both GFP and GRLMR cells show dark inclusions, with more inclusions observed in the GRLMR-expressing cell line. The dark
inclusions seen in both cell lines near the cell walls are most likely the cellular nuclei because of their cellular placement and single appearance per cell. Electron-dispersive spectroscopy (EDS) mapping confirms that the dark inclusions are Se-rich for the GRLMR cells, whereas any inclusions observed in the GFP-expressing cells show no evidence of Se presence. The corresponding EDS spectra further confirm these conclusions by the presence of signature selenium peaks being present for the GRLMR cells and the absence of these peaks for the GFP cells. Complete EDS mapping for the control and GRLMR cells can be seen in Figure S6. The intracellular Se-rich NPs have an amorphous surface similar to our previously reported results, suggesting a partial cytosol-exposed surface. This appears to be unique to our enzyme-synthesized particles because inorganic/bioinorganic methods tend to make smooth-surfaced particles and require capping agents for stabilization. The transportability of enzymatic function to foreign cell lines further demonstrates the ability for this enzyme’s potential application as a clonal NP contrast generator.

Previously, we observed that some fraction of NPs synthesized by *S. cervisiae* GSHR were associated with the particle fraction. We examined GRLMR for a similar behavior. Selenium particles were synthesized in vitro and were separated from the solution using centrifugation. The analysis of the protein content in the solution and the particles were determined by Bradford assay (Bio-Rad). Overall, approximately 10% of the enzyme is associated with the selenium NPs. This is a smaller fraction than we observed for *S. cervisiae* (~18%), suggesting that the *P. moravenis*-derived enzyme is more efficient at turning over or releasing the particles that they create. Overall, this contributes to the picture of GRLMR being specialized for conveying Se tolerance.

Differences in the product-/substrate-binding pocket of this family of enzymes may underlie any observed differences in substrate specificity and enzyme activity. The key residues in the product-/substrate-binding areas of GSHR are shown in Table 3, and the models of these are shown in Figure S3. The structural alignment between GRLMR and *P. fluorescences* reveals an RMSD of 1.71 Å for the full enzyme and 1.56 Å for the product-/substrate-binding pocket. These values show the largest deviation between any of the enzymes considered here, but to the best of our knowledge, no crystal structure of *Pseudomonas* GSHR has been obtained which would affect the generated structure models.

The product-/substrate-binding pocket for GSHR contains a set of evolutionarily conserved residues, most notably including a cysteine that is implicated in glutathionylation regulatory mechanisms. There are three residues that dominate the binding interaction, one on an α-helix, and two on parallel β-strands (βLα, βR). Comparing our *Pseudomonas* enzymes, we see that they contain the same key residues; α-Ser, βL-Lys, and βR-Glu (Figure 5).33

This absence of a sulfur-containing residue (Figure 1) in the *P. moravenis* GRLMR suggests that the enzyme is not subject to the glutathionylation regulatory mechanism well-established for canonical GSHR enzymes such as *S. cervisiae* GSHR. Glutathionylation of enzymes is a common post-translation modification for proteins in signaling pathways and survival gene modification. This reversible post-translation modification is the binding of glutathione to an unpaired cysteine residue. Such a modification alters the enzyme activity, presumably as a regulation mechanism.9 In the case of *S. cervisiae* GSHR, glutathionylation at C239 (Figure 5) inhibits the enzyme. Chemically blocking the glutathionylation pathway is shown to increase the GSHR activity by a factor of 2.1. Overall, the absence of the possibility of a glutathionylation regulation mechanism for the GRLMR enzyme suggests that it is distinct from the other GSHR enzymes.

The selenium metabolism literature highlights several examples of species within the *Pseudomonas* genus with remarkable tolerance to Se. In many cases, GSHR enzymes are implicated in the tolerance. GSHR was responsible for reducing selenite and tellurite to insoluble NPs using the O-2 strain of *Pseudomonas maltophilia*.42 GSHR and thioredoxin reductase are responsible for selenite and selenate reduction in *Pseudomonas seleniipraecipitans*.43 The highly conserved sequence across species within the *Pseudomonas* genus, including conservation in the product/Substrate binding pocket, is suggestive that the ability to handle the normally toxic amounts of SeO3−2 may be a general feature of the *Pseudomonas* genus. This Se tolerance may arise from the nature of GSHRs in this genus.

Table 3. Key Glutathione Pocket Residues

| enzyme     | PM MTLR | PF GSHR | EC GSHR | SC GSHR | HS GHSR |
|------------|---------|---------|---------|---------|---------|
| key product-binding site residues | α-Ser   | α-Ser   | α-Met   | α-Cys   | α-Met   |
| βL-Lys     | βL-Lys  | βL-Lys  | βL-Lys  | βL-Lys  | βL-Lys  |
| βR-Glu     | βR-Glu  | βR-Glu  | βR-Glu  | βR-Asn  | βR-Ser  |

Figure 5. Red—α residue, teal—β residues, and yellow—GSH. (a) Yeast GHSR substrate-binding pocket with bound GSH. (b) *E. coli* GSHR substrate-binding pocket. (c) GRLMR substrate-binding pocket.
CONCLUSIONS
In summary, we have characterized GRLMR from the bacterium *Pseudomonas moraviensis* stanleyae. The kinetic studies showed an overall decrease in substrate affinity for GRLMR relative to the *S. cervisiae* GSHR, but an overall increased affinity for GS-Se-SG over GSSG. The trans- 
portability of the gene was tested by transforming the lab 
strain *E. coli* with GRLMR. Selenite tolerance increased 10-fold 
compared to the cells without the gene, and elemental red 
selenium was formed when GRLMR was present. SEM/EDS 
further confirmed this by showing the selenium particles 
associated with the cells containing the gene. Product 
association experiments showed a decrease in product 
retention when compared to the *S. cervisiae* GSHR, which 
ultimately allows for increased product release and contributes to 
the overall Se tolerance.

MATERIALS AND METHODS

Identification/Isolation. An LB agar plate with colonies of 
the original *P. moraviensis* cell line was submitted for full- 
genome sequencing. The DNA sequence from the most closely 
related enzyme identified by MALDI-MS was used to identify 
the sequence of our enzyme of interest. This sequence was 
cloned into a pD441-CH *E. coli* vector, and a standard heat- 
shock protocol was used to transform BL21 *E. coli*.

Standard protein purification was conducted by growing 
cells in 1 L of LB to an optical density at 600 nm (OD600) of 
0.6 and inducing protein expression with 1 mM IPTG for 2 h. 
The cells were collected and resuspended in 25 mL of lysis 
buffer and lysed by tip sonication. The soluble cell lysate 
was collected and nickel-agarose beads were used to isolate and 
wash our expressed protein.

GS-Se-SG Synthesis. The protocol from Ganther was 
followed for the synthesis of selenodiglutathione. To 24 mL 
of 0.1 M HCl, 400 moles of HNaSeO3 was added and cooled 
to 4 °C. Another solution of 0.1 M GSH was cooled to 4 °C 
and added quickly to the selenite solution. The mixture was 
allowed to react at 4 °C for 20 min. A 2.5 mL of 2 M NaOAc 
was added to obtain a final pH of 4.5. A C18 column was used to 
separate the products using pH 2.0 HCl. Thin-layer 
chromatography was used to check the contents of the 
lyophilized fractions. The isolated GS-Se-SG was identified and 
the amount quantified using UV–vis absorption at 263 nm. 

Km. 1 mL reactions were conducted in 1× phosphate- 
buffered saline with 0.1 mM NADPH and 15 μg of either 
purified enzyme or *S. cervisiae* GSHR purchased from Sigma. 
The substrates tested were GSSG, GS-Se-SG, and selenite. 
Their concentrations were varied between reactions, and the 
depletion of the NADPH peak at 340 nm was monitored every 
2 s after the contents were mixed.

Transportability. IC50’s were determined by standard 
plating experiments. In short, cultures of BL21 cells containing 
either a plasmid with GRLMR or a generic reporter gene were 
grown overnight in LB at 37 °C. The following morning, 100 
μL of this starter culture was added to 2.5 mL of fresh LB and 
grown for roughly 2.5 h to reach an OD600 of 0.6. Various 
amounts of selenite were added to each culture, and exposure 
was continued for 24 h. After the exposure, the cells were 
diluted 10-fold, and 20 μL of each dilution was plated in 
triplicate on 1× Kanamycin LB agar. The plates were put in an 
oven at 37 °C, and colonies were grown overnight and counted 
the following day.

Scanning Transmission Electron Microscopy (STEM). A volume of 3 mL of BL21 cells containing either a metalloid 
reductase gene or GFP reporter gene was grown separately in 
10 mL culture tubes overnight containing LB medium 
(Teknova) supplemented with Kanamycin at 25 μg/mL. The 
following morning, the culture was added to a 125 mL 
Erlenmeyer flask containing LB medium supplemented with 
Kanamycin (25 μg/mL). The cells were grown for 2.5 h, and 
100 mM Na2SeO3 (Alfa Aesar, 98+%) was added to reach a 
final concentration of 5 mM. The cells were collected by 
centrifuging for 20 min at 4000 rpm and 4 °C after 3 h of 
growth with selenite. The cells were washed with 20 mM Tris 
(pH 7.4) (Fischer) three times followed by resuspension in 1 
ml of fixing solution (2% glutaraldehyde (25% Sigma-Aldrich) 
and 2.5% formaldehyde); the fixing solution was allowed to 
react for 12 h at 4 °C. The fixing solution was centrifuged and 
the pellet was washed five times in 20 mM Tris (pH 7.4). The 
cells were resuspended in 1 mL of 20 mM Tris (pH 7.4). The 
alisquots (4 μL) were mounted on 400 mesh Cu grids with 50 
nm C coating and washed two times with H2O. The dry- 
mounted cells on transmission electron microscopy grids were 
loaded onto a STEM holder. The STEM images were taken 
with a JEOL JSM-6500-F scanning electron microscope at an 
accelerating voltage of 15 kV.

EDS. EDS was performed on the *P. moraviensis* stanleyae 
cells with SEM, as described above. EDS was collected on a 
NORAN System 7 X-ray microanalysis detector with a time 
interval of 1 s.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00826.

Genome sequencing scores, sequence alignment, generated model templates, glutathione-binding sites, GS-
Se-SG UV–vis, Km assay UV–vis, and SEM/EDS (PDF)

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The manuscript was written through contributions of all 
authors. All authors have given approval to the final version of 
the manuscript.

Funding

NIH R01 GM112225 A1. NSF CAREER 1455099.

Notes

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

The authors thank Zachary Butz for many conversations on technical aspects of this work.

ABBREVIATIONS

DPS, DNA-binding protein; GSHR, glutathione reductase; NADPH, nicotinamide adenine dinucleotide phosphate; GRLMR, glutathione reductase-like-metalloid reductase; BLAST, basic local alignment search tool; RMSD, root-mean-square deviation; NP, nanoparticle; IPTG, isopropyl β-D-1-thiogalactopyranoside; cfus, colony-forming units; GFP, green fluorescent protein; SEM, scanning electron microscopy; EDS, electron-dispersive spectroscopy; LB, lysogeny broth; MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; OD, optical density; TLC, thin-layer chromatography; PBS, phosphate-buffered saline

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