# Supporting Information

## Progress Toward Clonable Inorganic Nanoparticles

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Experimental Section

### Cell lysate preparation

*P. moraviensis* Stanleyae cells were grown for 24 h at 30 °C in 2,800-mL Fernbach flasks containing 1 L of LB-Miller medium supplemented with 10 mM $Na_2SeO_3$. Cells were harvested via centrifugation at 10,000 x g for 20 minutes and the pellet resuspended in 15 ml of ice-cold 25 mM Tris and 192 mM glycine buffer (pH 8.3) supplemented with 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) and 0.02% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate hydrate (CHAPS). Then, cells were treated with 0.2 mg $mL^{-1}$ lysozyme (Sigma-Aldrich, St. Louis) for 20 min in a 100 rpm shaking incubator at 30°C. Following lysozyme treatment, the cells were homogenized via a French pressure cell operated at 120 MPa. The procedure was repeated twice and this final homogenate was spun at 22,500 x g for 60 min at 4°C to remove unbroken cells and cell debris. These procedures were modified from Hunter.[1]

### SEM

*P. moraviensis* Stanleyae cells were grown for 24 h at 30 °C in a 125 mL Erlenmeyer flask containing 50 mL LB medium (Teknova) supplemented with 10 mM $HNaSeO_3$ (Alfa Aesar, 98+%). Cells were harvested via centrifugation at 4,000 rpm (3,220 rcf) for 20 minutes at 4 °C. Cells were then washed in 20 mM Tris (pH 7.4) (Fisher) three times followed by resuspension in 1 mL of fixing fixative solution (2% glutaraldehyde (25%, Sigma-Aldrich) and 2.5% formaldehyde); the fixing solution was allowed to react for 6 h at 4 °C. Post-fixation, the fixing

solution was removed by centrifugation and the pellet was washed five times in 20 mM Tris (pH 7.4). The cells were then resuspended in 1 mL 20 mM Tris (pH 7.4). Aliquots (2 μL) were mounted on 400 mesh Cu grids with a 50 nm C coating. Dry-mounted cells on TEM grids were loaded onto a STEM holder. STEM images were taken on a JEOL JSM-6500-F Scanning Electron Microscope at an accelerating voltage of 30 kV.

**EDS**

EDS was performed on *P. moreviensis* Stanleyae cells in the SEM as described above. EDS was collected on a Noran System 7 X-ray Microanalysis detector with a time interval of 1 s.

**Dry-mount cellular TEM**

*P. moraviensis* Stanleyae cells were grown for 24 h at 30 °C in a 125 mL Erlenmeyer flask containing 50 mL LB medium (Teknova) supplemented with 10 mM $HNaSeO_3$ (Alfa Aesar, 98+%). Cells were harvested via centrifugation at 4,000 rpm (3,220 rcf) for 20 minutes at 4 °C. Cells were then washed in 20 mM Tris (pH 7.4) (Fisher) three times followed by resuspension in 1 mL of fixing fixative solution (2% glutaraldehyde (25%, Sigma-Aldrich) and 2.5% formaldehyde); the fixing solution was allowed to react for 6 h at 4 °C. Post-fixation, the fixing solution was removed by centrifugation and the pellet was washed five times in 20 mM Tris (pH 7.4). The cells were then resuspended in 1 mL 20 mM Tris (pH 7.4). Aliquots (2 μL) were mounted on 400 mesh Cu grids with a 50 nm C coating. TEM images were taken on a JEOL JEM-1400 Transmission Electron Microscope at an accelerating voltage of 100 kV.

**3D EM**

<u>Cells harvested at 6, 9, and 12 hours post selenium inoculation</u>:

A single colony was picked from an LB agar plate of *P. moraviensis* Stanleyae and placed into 3 ml of LB culture media and incubated aerated at 160 RPM, overnight at 28 $^{o}$C. The cells were

harvested and rinsed 2 times with sterile phosphate-buffered saline (PBS) and then resuspended into 200 mL of fresh LB media. Half of the flasks were given a 10 mM final concentration of $HNaSeO_3$, the other half an equivalent amount of media as control. The cultures were shaken at 160 RPM at 28 $^oC$ and samples were collected 6, 9, and 12 hours post Se inoculation. They were high pressure frozen using a Wohlwend Compact 02 (Technotrade) high pressure freezer into aluminum planchettes. Samples were freeze-substituted in 0.2% osmium tetroxide, 0.1% uranyl acetate, and 5% water in acetone using the fast-freeze substitution method[3] over 3 hours. Samples were rinsed in acetone and embedded in epon (EMS) over several days. 200 nm sections were cut using a Ultracut UCT (Leica) microtome with a diamond knife (Diatome) and placed on formvar-coated copper slot grids. Sections were post-stained with 2% aqueous uranyl acetate and lead citrate. 15 nm gold fiducials were added.

Dual-axis tilt-series were acquired using SerialEM[4] on a Tecnai TF20 (FEI) transmission electron microscope from +/-60, 1 degree intervals on an Ultrascan digital camera (Gatan) at a pixel size of 0.91 nm. Tomograms were reconstructed using IMOD.[5] A total of 23 tomograms were collected which are detailed below.

+6 hours control: 3 TS

+6 hours Se: 3 TS

+9 hours control: 3 TS

+9 hours Se: 3 TS

+12 hours control: 3 TS

+12 hours Se: 3 TS

36 hours control, dried: 1 TS

36 hours Se, dried: 1 TS

36 hours Se, resin: 1 TS

Cells harvested at 36 hours post Se inoculation:

Cells were grown as previously described in the dry-mount cellular TEM methods. Differences in the procedure were that the cells were grown for 36 hours and the media was changed every 12 hours. Media was exchanged by centrifuging cells at 4,000 rpm at 4 °C for five minutes. The supernatant was poured out and new LB was added and the cells were then resuspended and allowed to continue growing. Cells were fixed at 36 hours as described in the dry-mount cellular TEM methods.

Chemically fixed cells were rinsed 2 times in $dH_20$ and then went through a graduated dehydration series into 100% acetone. They were infiltrated with epon (EMS) over several days. 300 nm sections were cut using an Ultracut UCT (Leica) microtome with a diamond knife (Diatome) and placed on formvar-coated copper slot grids. Samples did not undergo post-stain. 10 nm fiducials were added.

Single-axis tilt-series were acquired using SerialEM[4] on a Tecnai TF30 (FEI) transmission electron microscope from +/-60, 1 degree intervals on an Ultrascan digital camera (Gatan) at a pixel size of 1.02 nm. Tomograms were reconstructed and modeled using IMOD.[5]

**Segmentation**

Reconstructions were sectioned using IMOD.[5] The outer cell walls were segmented every 5 tomographic slices on the XY plane. An isosurface was generated and the threshold lowered to determine a cutoff value for imodauto to differentiate cellular background from SeNP. Imodauto was set at a threshold of 1 (out of 255), which generated a model with both the gold fiducials and the SeNPs. The models were merged and the outline of the cell was meshed to generate the image.

**Native Polyacrylamide Gels**

Native polyacrylamide gels were used to check for selenite reduction capabilities utilizing the procedure described by Hunter.[6] Briefly, cell lysate was purified via HIC column then separated on a 8% non-denaturing polyacrylamide electrophoresis gel (Invitrogen, Carlsbad, CA) using a Novex® Tris-Glycine Native Running Buffer (Invitrogen). Following electrophoresis, gels were placed into zip-lock bags filled with Ar and assayed for selenite reduction capability. Assay was performed by incubation of the gel in a solution of 50 mM HEPES (pH 7.5), 200 mM $Na_2SeO_3$ and 1mM NADPH. Bands that turned red were excised for further study.

**PROTEIN MS/MS**

SDS-PAGE and In-Gel Trypsin Digestion for LC-MS/MS:

Bands of interest were excised from the gel and processed for in-gel trypsin digestion and LC-MS/MS as previously described.[7] Briefly, the gel pieces were washed with 200 µL of LC-MS Grade Water (Optima LC-MS, Fisher Scientific) for 30s and destained with 2 x 200 µL of 50% Acetonitrile (ACN; Optima LC-MS Grade)/50 mM ammonium bicarbonate at 60°C, with intermittent mixing. The pieces were dehydrated with 100% ACN and dried via vacuum centrifugation in a SpeedVac for 5 min. Proteins were reduced and alkylated, in-gel with 25 mM DTT in 50 mM ammonium bicarbonate (60°C for 20 min) and 55 mM IAA in 50 mM ammonium bicarbonate at room temperature in the dark for 20 min. Gel pieces were then washed with Optima water and dried. The dried gel pieces were rehydrated in 20 µL 12 ng/µL MS-grade Trypsin (ThermoPierce, San Jose, CA) /0.01% ProteaseMAX surfactant/50 mM ammonium bicarbonate mixture for 10 min at room temperature, overlaid with 30 µL 0.01% ProteaseMAX surfactant/50 mM ammonium bicarbonate and incubated at 50 °C for 1 h. Extracted peptides were transferred and the digestion halted by addition of 10% trifluoro-acetic acid to a final

concentration of 0.5%. Peptide extracts were dehydrated and stored at −20 °C prior to analysis by LC-MS/MS.

Peptides were purified and concentrated using an on-line enrichment column (Thermo Scientific 5 mm, 100 mm ID x 2 cm C18 column). Subsequent chromatographic separation was performed on a reverse phase nanospray column (Thermo Scientific EASYnano-LC, 3 mm, 75 mm ID x 100 mm C18 column) using a 30 minute linear gradient from 10%-30% buffer B (100% ACN, 0.1% formic acid) at a flow rate of 400 nanoliters/min. Peptides were eluted directly into the mass spectrometer (Thermo Scientific Orbitrap Velos). The instrument was operated in Orbitrap-LTQ mode where precursor measurements were acquired in the Orbitrap (60,000 resolution) and MS/MS spectra (top 20) were acquired in the LTQ ion trap with a normalized collision energy of 35kV. Mass spectra were collected over a m/z range of 400-2000 Da using a dynamic exclusion limit of 2 MS/MS spectra of a given peptide mass for 30 s (exclusion duration of 90 s). Compound lists of the resulting spectra were generated using Xcalibur 2.2 software (Thermo Scientific) with a S/N threshold of 1.5 and 1 scan/group.

**GSHR TEM**

Glutathione reductase Se nanoparticles were made by mixing 25 μL(66 μg) of glutathione reductase (from *S. cerevisiae*) with 500 μL 100 mM selenite ($HNaSeO_3$), 100 μL 10mM NADPH and 275 μL 10x PBS (pH 7.4). The solution was allowed to mix for an hour, at which point the solution turned red. The samples were centrifuged and the pellet washed with 1x PBS (pH 7.4). Particles were then mounted onto 400 mesh Cu grids with a 50 nm C coating (Electron

Microscopy Sciences). TEM images were taken on a JEOL JEM-1400 Transmission Electron Microscope at an accelerating voltage of 100 kV.

**$SeO_3$/$TeO_3$ assays**

Samples (1 ml) were made using a constant NADPH concentration (0.36 mM) in PBS. Varying selenite/tellurite concentrations were used (1 mM to 1 M). All blanks contained the same content as the reactions except NADPH. 5 μL (13 μg) of glutathione reductase was added to the reaction and $A_{340}$ measurements were obtained every 2 seconds for the first 10 minutes, every 10 seconds for the next 20 minutes, followed by every minute for the next 30 minutes. The decrease in absorbance at 340 nm was used to analyze the initial reaction rate.

**Protein Concentration Assay.**

Four 315 uL samples were created in 1x PBS, 10mM selenite, and equal amounts of Baker's yeast glutathione reductase from Sigma Aldrich. The samples had varying NADPH concentrations from 0 to 6mM and were allowed to react for approximately 20 hours. Once the reaction was completed the samples were centrifuged at 14000 rpm for 30 minutes to remove any synthesized nanoparticles. The supernatants were collected and a Bradford assay was performed in triplicate on the four samples in a 1:9 ratio of sample to reagent. The nanoparticle pellets were then washed with water two times to remove any free protein, followed by re-dispersion of the pellets in .005% SDS. These samples were then run through the assay in the same ratio also in triplicate. Two standard curves were made, one using GSHR in 1x PBS and one using GSHR in .005% SDS.

**SDS Gel Assay**

Seven 315 uL samples were created in 1x PBS, six in 4mM NADPH and a control with no NADPH. The six samples contained a range of selenite from 0 to 10mM while the control

contained no selenite. An equal amount of Baker's yeast glutathione reductase from Sigma Aldrich was added to each sample and allowed to react for approximately 20 hours. The samples were spun down at 14000 rpm for 30 minutes to remove any synthesized nanoparticles. Following this, a 4-15% SDS gel from Bio-rad was set up with each well containing 25 uL of a solution made from 25uL sample in 5 uL of SDS loading dye (Bio-Rad). The gel was run at 110 V for 75 minutes. After staining with Coomassie Brilliant Blue G-250 followed by de-staining, the gel was imaged with a Bio-Rad GelDoc XR. It should also be noted that another set of samples were created which contained 1/3 of the protein as the SDS samples. Three samples were created under the exact same conditions as stated before apart from the difference in added enzyme. The samples consisted of a control of GSHR in 1x PBS, and two samples containing 4mM NADPH. One of the cofactor samples contained no selenite and the other with a final concentration of 10mM. A 6% native gel was casted and six wells were loaded with 25uL of a solution made from 25uL sample and 5 uL SDS loading dye. The first three wells were filled with samples containing the lowered amount of added enzyme, while the last three wells contained samples with the added enzyme mentioned in the beginning of this section. This gel was run at 110 V for 1 hour and was stained with Coomassie Brilliant Blue G-250 following de-staining and imaged with the same Bio-Rad GelDoc XR.

References


1 W. J. Hunter, D. K. Manter, *Curr. Microbiol.* **2008**, *57*, 83–88.
2 J. M. Galloway, S. S. Staniland, *J. Mater. Chem.* **2012**, *22*, 12423–12434.
3 K. L. McDonald, R. I. Webb, *Journal of Microscopy* **2011**, *243*, 227–233.
4 D. N. Mastronarde, *J. Struct. Biol.* **2005**, *152*, 36–51.
5 J. R. Kremer, D. N. Mastronarde, J. R. McIntosh, *J. Struct. Biol.* **1996**, *116*, 71–76.
6 W. J. Hunter, *Curr. Microbiol.* **2014**, *69*, 69-74
7 S. V. Saveliev, C. C. Woodroofe, G. Sabat, C. M. Adams, D. Klaubert, K. Wood, M. Urh, *Anal. Chem.* **2013**, *85*, 907–914.


Supporting Figures


**Figure S1**. Photographs of *P. moraviensis* stanleyae liquid LB cultures. The culture on the left hand side is supplemented with 10mM $SeO_3^{2-}$. Upon initial growth, both cultures appear as the no-selenite control culture shown at right. We attribute the red-color of the culture to which selenite is added to the reduction of selenite to zerovelant red selenium.

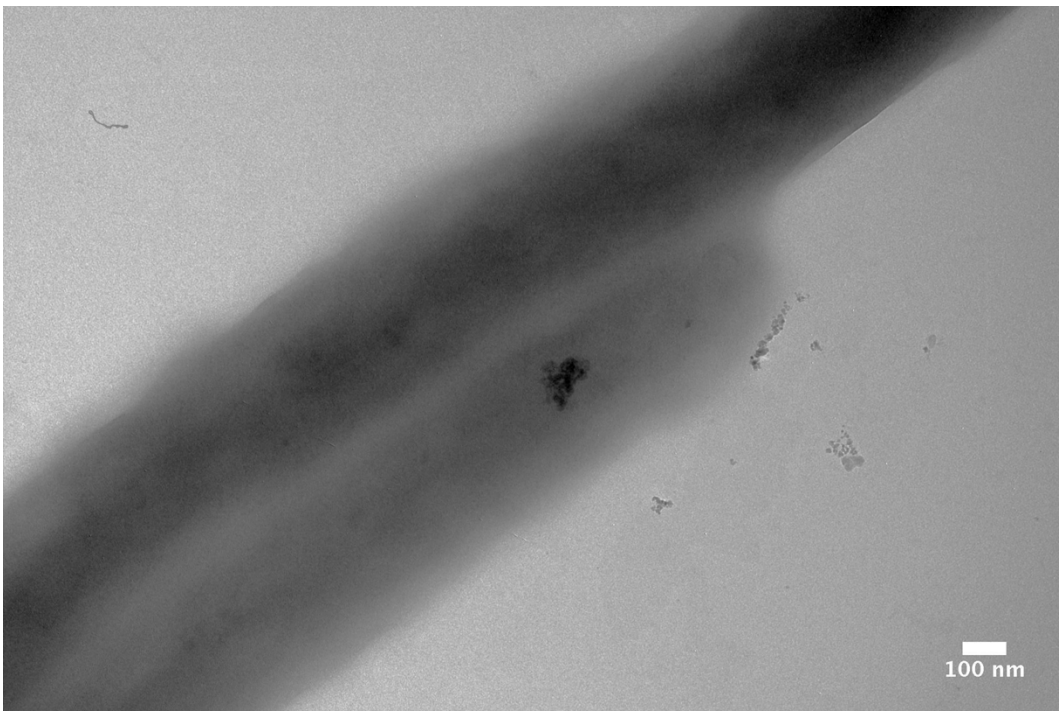



**Figure S2.** Representative micrograph showing irregular electron dense object

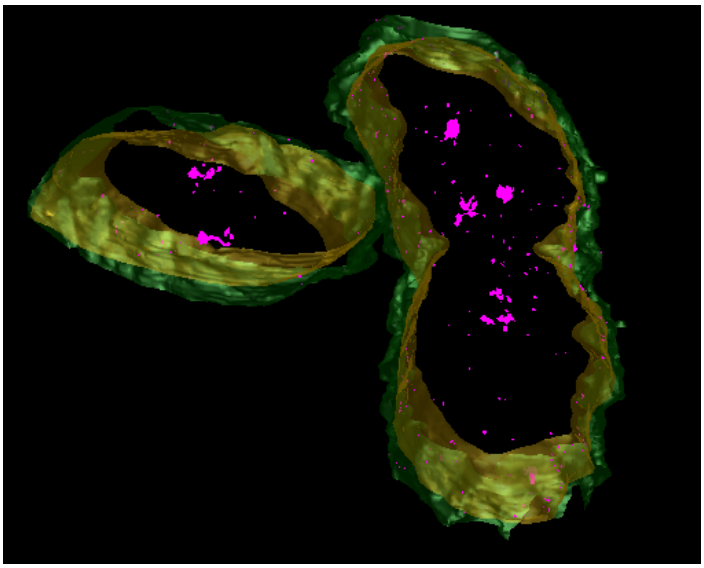


**Figure S3.** Electron tomographic reconstruction of *P. moraviensis* stanleyae with osmium staining. The outer membrane (green), inner membrane(yellow) and putative SeNP (pink) densities are segmented. Due to the presence of stain, the particle segmentation is ambiguous.

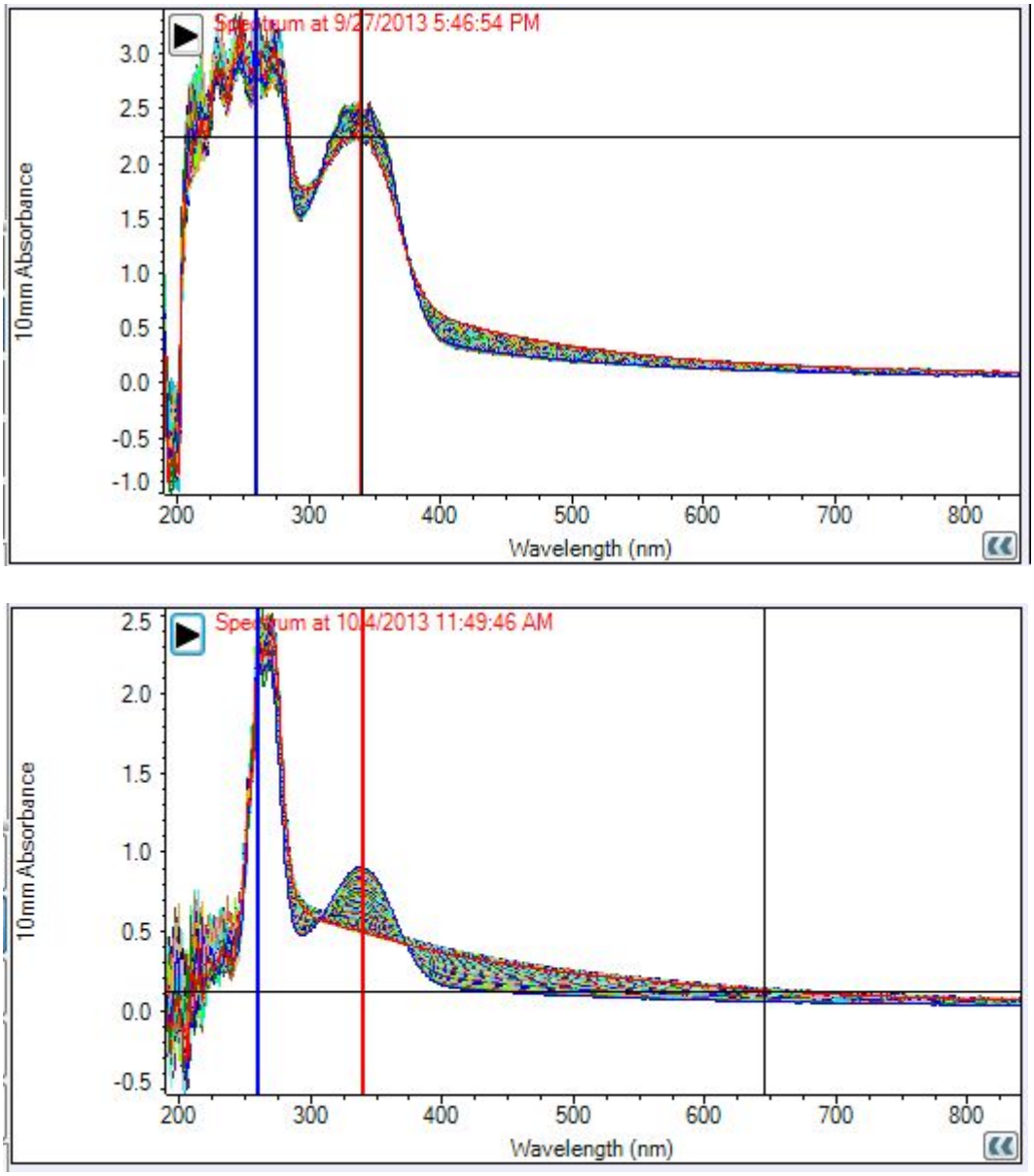



**Figure S4.** Example spectroscopic data showing enzymatic consumption of NADPH as judged by diminishment over time of the peak at 340nm that arises from NADPH. Monitoring of this consumption (or lack therof) allowed claims of substrate specificity and the Lineweaver-Burk plots shown in Figure S4.

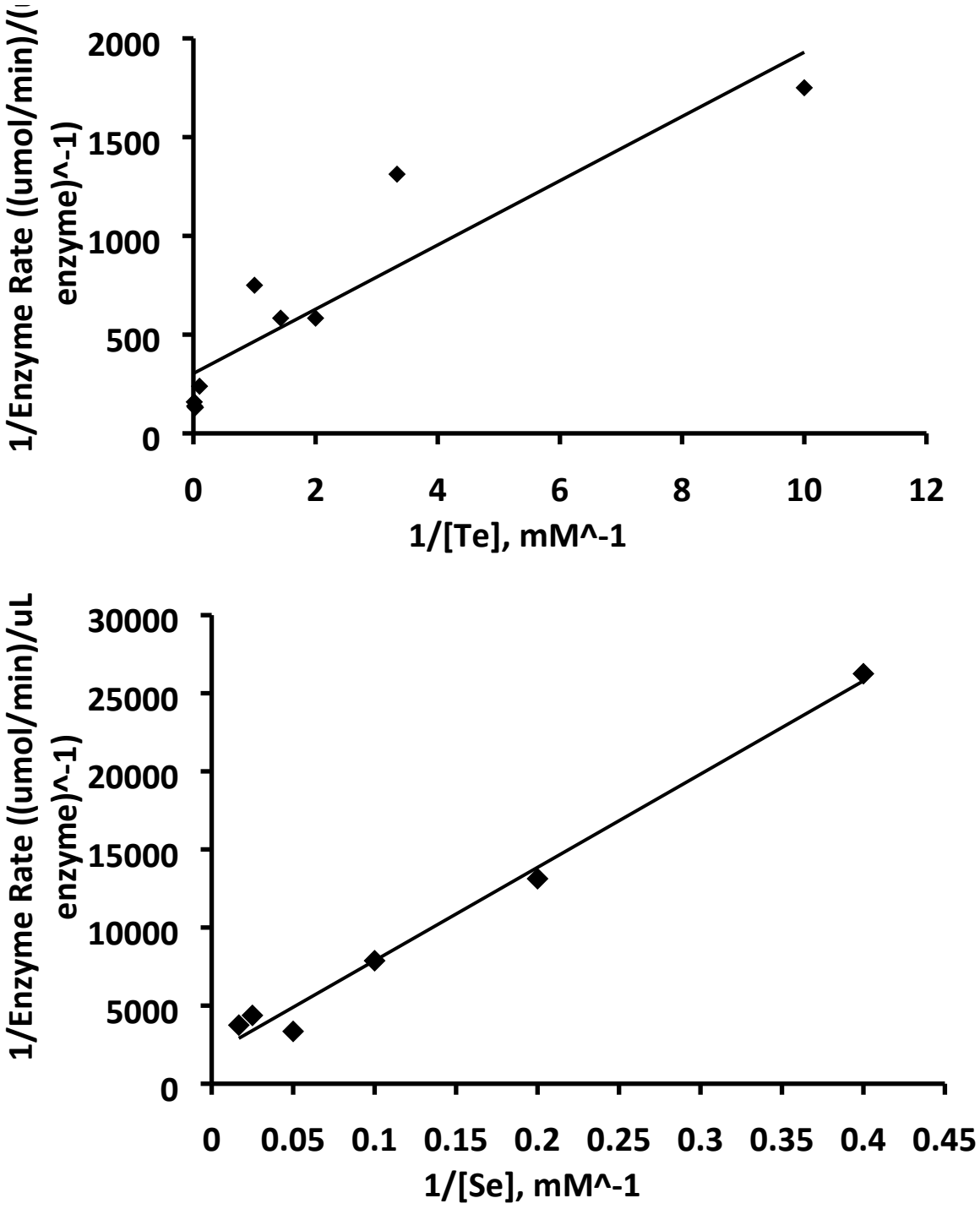



**Figure S5.** Lineweaver -Burk plots for $TeO_3^{2-}$ and $SeO_3^{2-}$ reduction by GSHR.

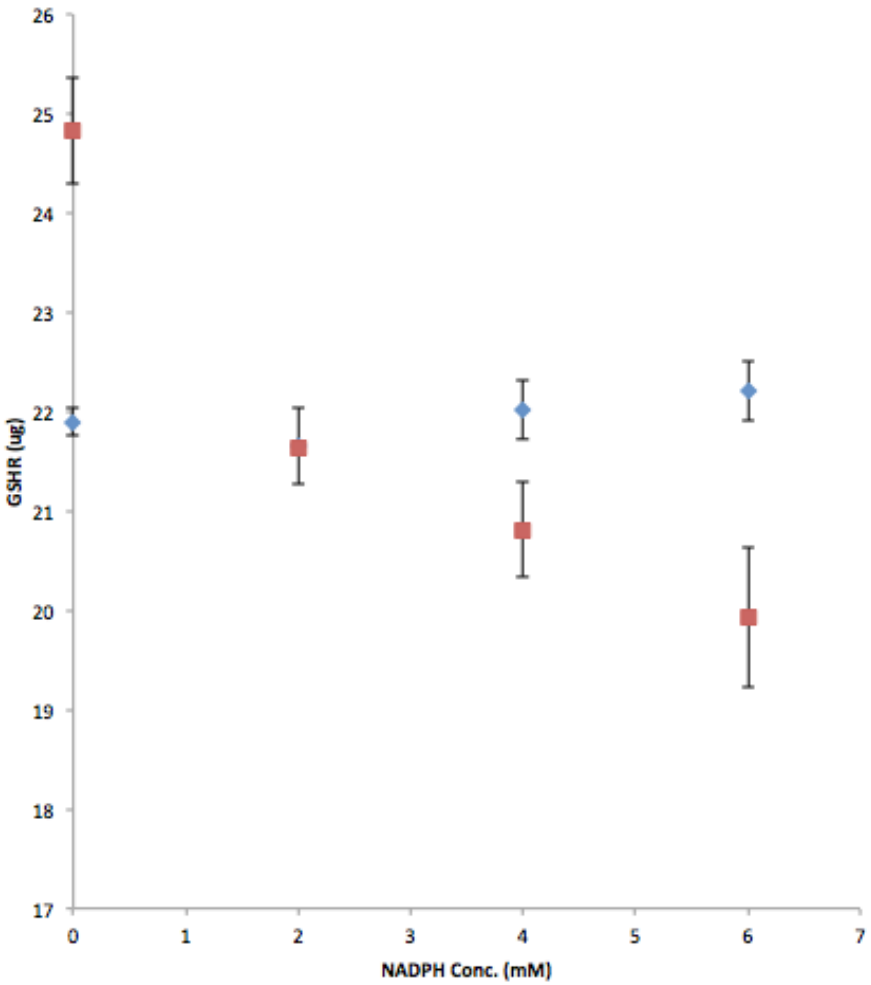



**Figure S6.** The results of a Bradford assay of a fixed amount of GSHR exposed to varying amounts of NADPH, with $SeO_3^{2-}$ either present at 10mM concentration (red squares) or absent (blue diamonds). When $SeO_3^{2-}$ is present (red squares) the enzyme vanishes from the assay in an NADPH dependent manner.

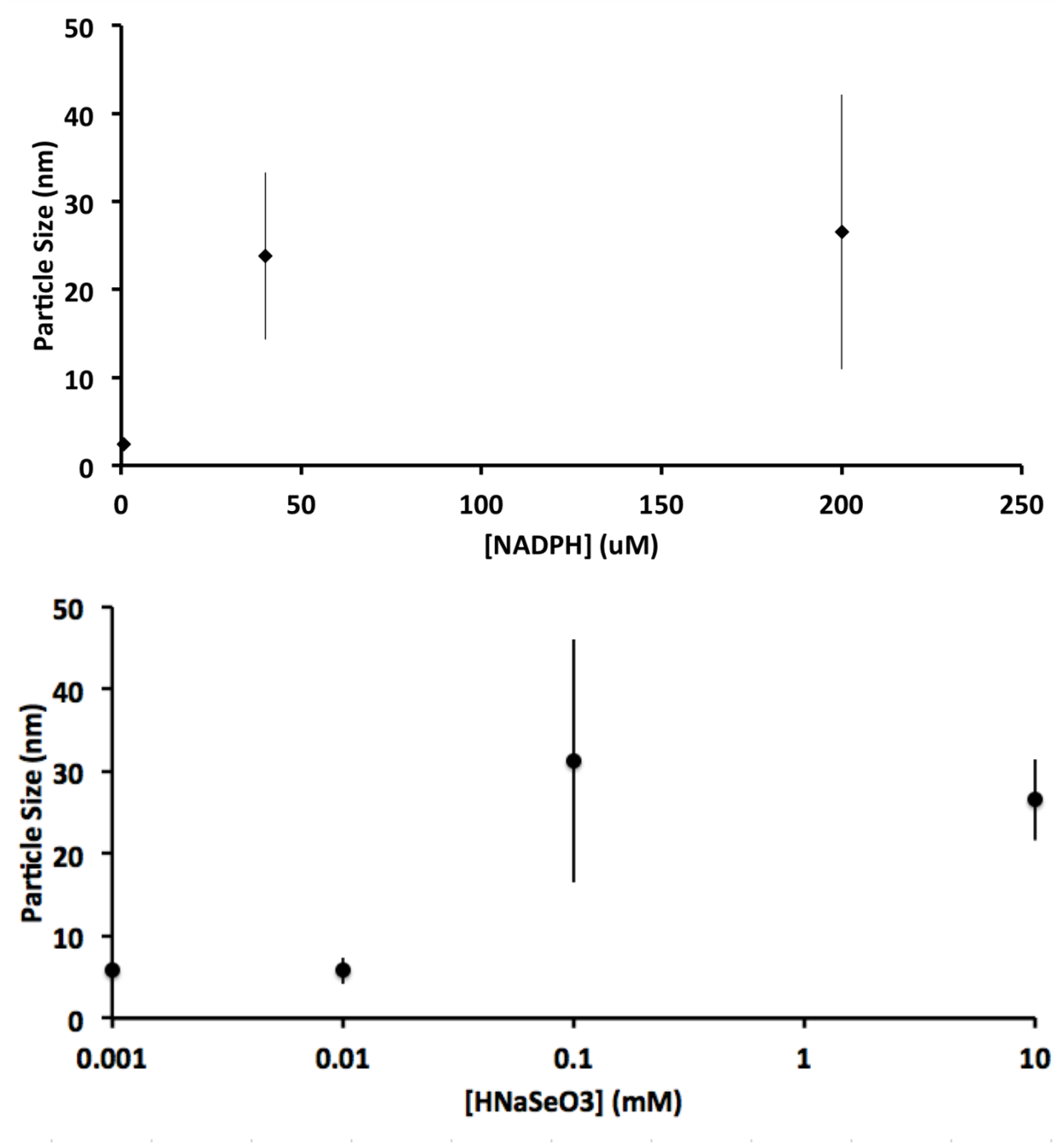
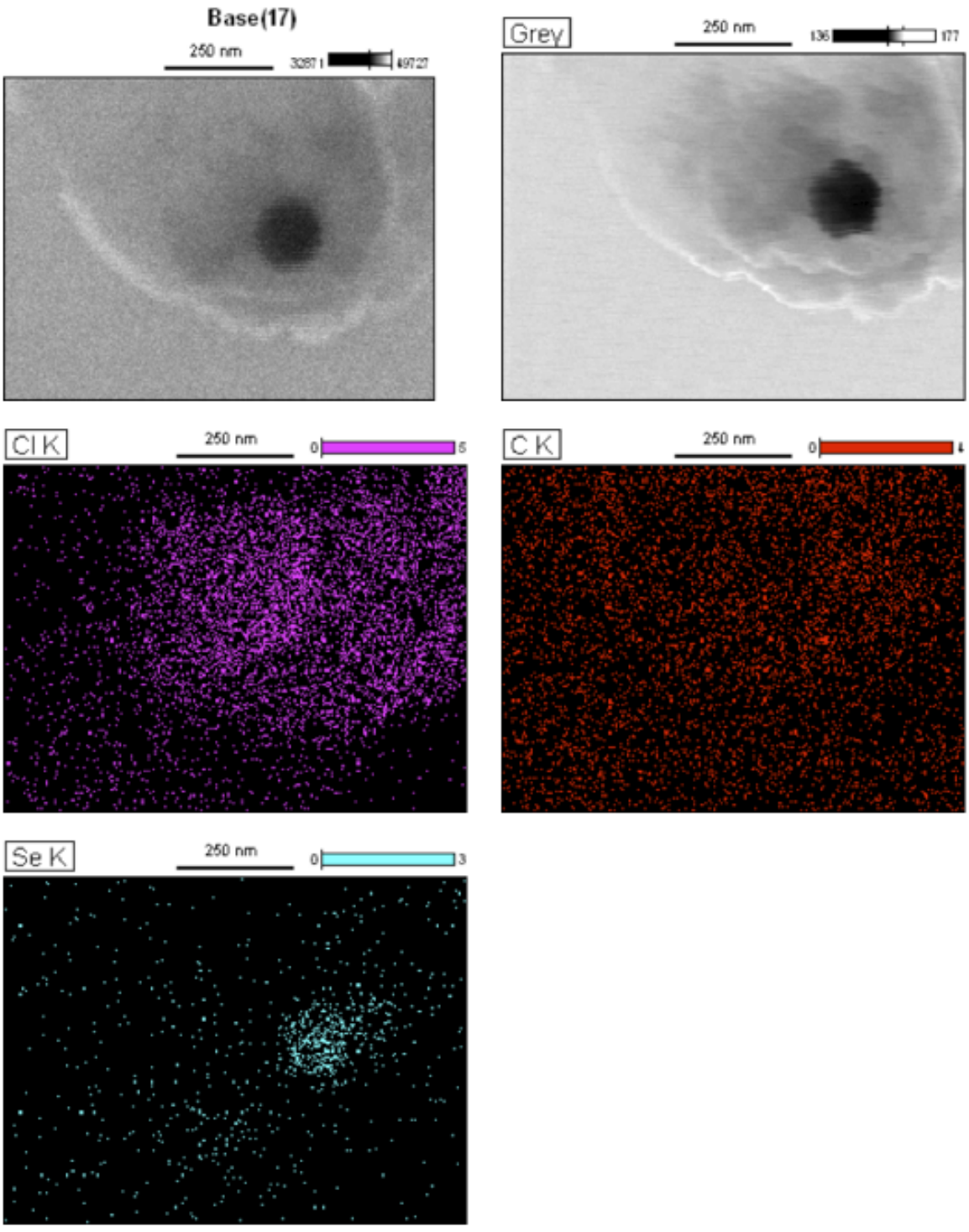



**Figure S7.** Top panel shows how particle size changes as [NADPH] cofactor is varied. Bottom panel shows distribution of particle sizes (y-axis) as a function of [$SeO_3^{2-}$] concentration in the assay (x-axis).




Data Type: Counts  Mag: 120000  Acc. Voltage: 30.0 kV  Detector: Pioneer

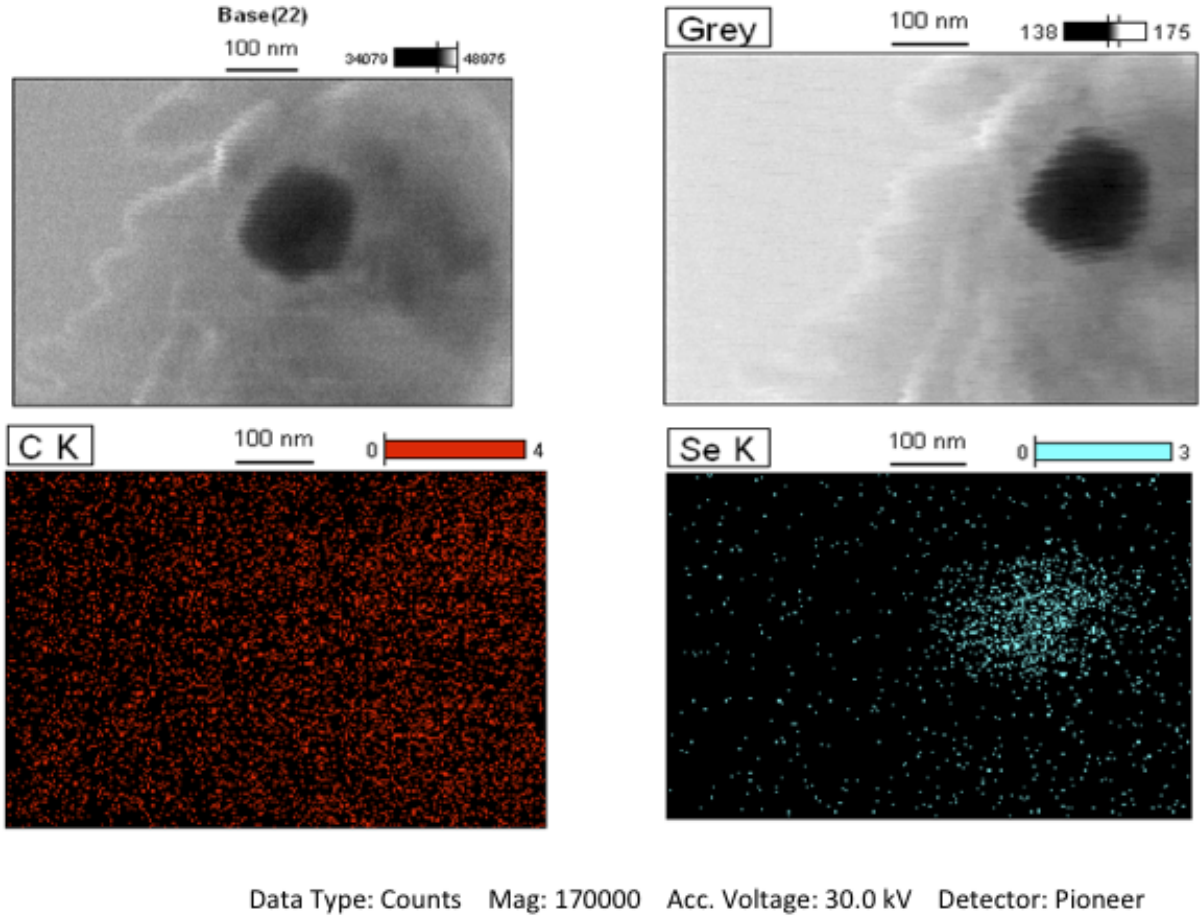



**Figure S8.** Raw EDS data taken indicating locations of detected energies of elements. The K-edge of chlorine, selenium, and carbon dominates the EDS spectrum. We interpret these images as follows: Carbon localizes throughout the image, due to the carbon support film on which the cells are mounted. Chlorine localizes within the cell boundaries, accounting for physiological saline within the cell. Selenium localizes within the putative SeNP.