Supplemental Data

RNA-Seq analysis of the effects of metal nanoparticle exposure on the transcriptome of *Chlamydomonas reinhardtii*

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ADDITIONAL INFORMATION ON PARTICLE SIZE DETERMINATION METHODS

Analytical ultracentrifugation (AUC). Sedimentation velocity experiments were performed in an OPTIMA XL-I analytical ultracentrifuge (Beckman Coulter) at 20^{o}C. Interference detection optics (wavelength 675 nm) were used for the $nTiO_2$ and nZnO, while absorbance (wavelength 420 nm) and fluorescence (wavelength 488 nm) were used for nAg, and QD, respectively. A rotational speed of ranging between 35000 and 50000 rotations per minute were employed, depending on the NP sample and 300 scans were taken. NP concentrations ranging from 5 to 46 mg L^{-1} were employed, depending upon the NP composition and AUC detector. The sedimentation velocity data obtained from the analytical ultracentrifuge were analyzed using the Sedfitdata analysis program (1). Particle densities of 3.9 (TiO_2), 5.6 (ZnO), 10.5 (Ag) and 2 (QD) kg m^{-3} were employed in order to obtain particle size distributions from the experimentally determined sedimentation coefficients.

Fluorescence correlation spectroscopy (FCS). A Leica TCS SP5 laser scanning microscope was employed, which makes use of an argon ion laser for fluorescence excitation (488 nm) and an avalanche photodiode detector for fluorescence intensity fluctuation determinations. Diffusion times of unknown components were measured and interpreted based upon an instrument defined autocorrelation function. Diffusion coefficients were determined by calibrating the size and the shape of the confocal volume with R110, which has a known D of 4.4×10^{-10} m^2 s^{-1} (2). The diffusion times of 1 mg L^{-1} of $nTiO_2$ and nZnO were determined under the same conditions as in the exposure solutions by labeling the particles with a small quantity (1×10^{-8} M) of R6G, which has a known D of 4.0×10^{-10} m^2 s^{-1} (2). The raw data was interpreted using ISS

Vista FCS software (version 3.6 RC Build 3637). Hydrodynamic diameters, d_H, of the $nTiO_2$ and nZnO (or their aggregates) have been calculated using the Stokes-Einstein equation:

$$d_h = {}^{kT}/_{3\pi\eta D} \qquad \text{(Equation S1)}$$

where k is the Boltzmann constant, T is the absolute temperature, and η is the solution viscosity.

Atomic force microscopy (AFM). An AFM Digital Instruments extended, Dimension 3100NanoscopeIIIa was used to quantify NP diameters from a 0.125 mg L^{-1} dispersion of the QD in the exposure media. Two different methods were used to attach the QD to freshly cleaved muscovite mica sheets: (i) the adsorption method, where mica sheets were suspended for 20 min. in the dispersion containing the QD, followed by their gentle immersion in deionized water, and (ii)the drop deposition technique, where 5 μL of the QD sample was pipetted onto freshly cleaved mica and then allowed to dry overnight in an enclosed Petri dish.Size distribution histograms were based upon the height measurements of at least 20 AFM images taken from different areas of the mica.

References

1. **Schuck P.** 2000. Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. Biophys. J. **78:**1606-1619.
2. **Gendron P-O, Avaltroni F, Wilkinson KJ.** 2008. Diffusion Coefficients of Several Rhodamine Derivatives as Determined by Pulsed Field Gradient-Nuclear Magnetic Resonance and Fluorescence Correlation Spectroscopy. J. of Fluorescence **18:**1093-1101.

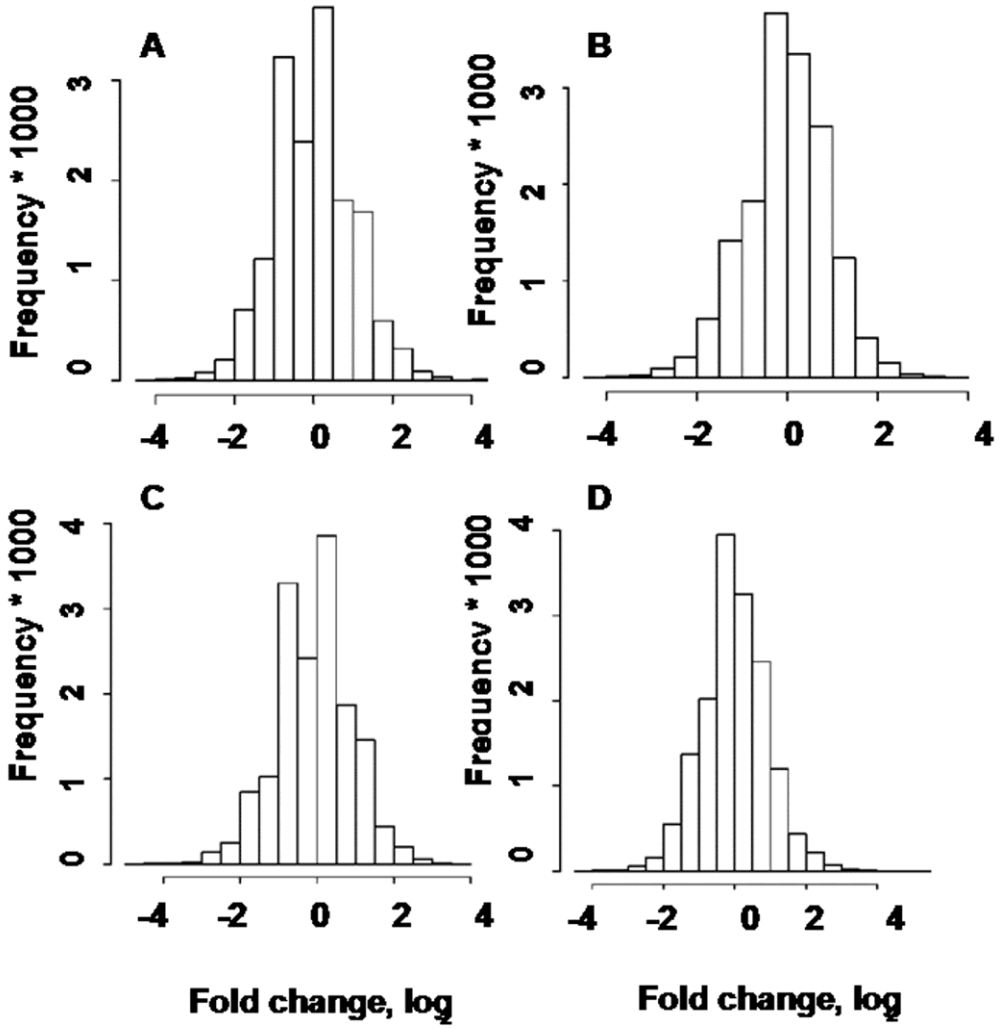
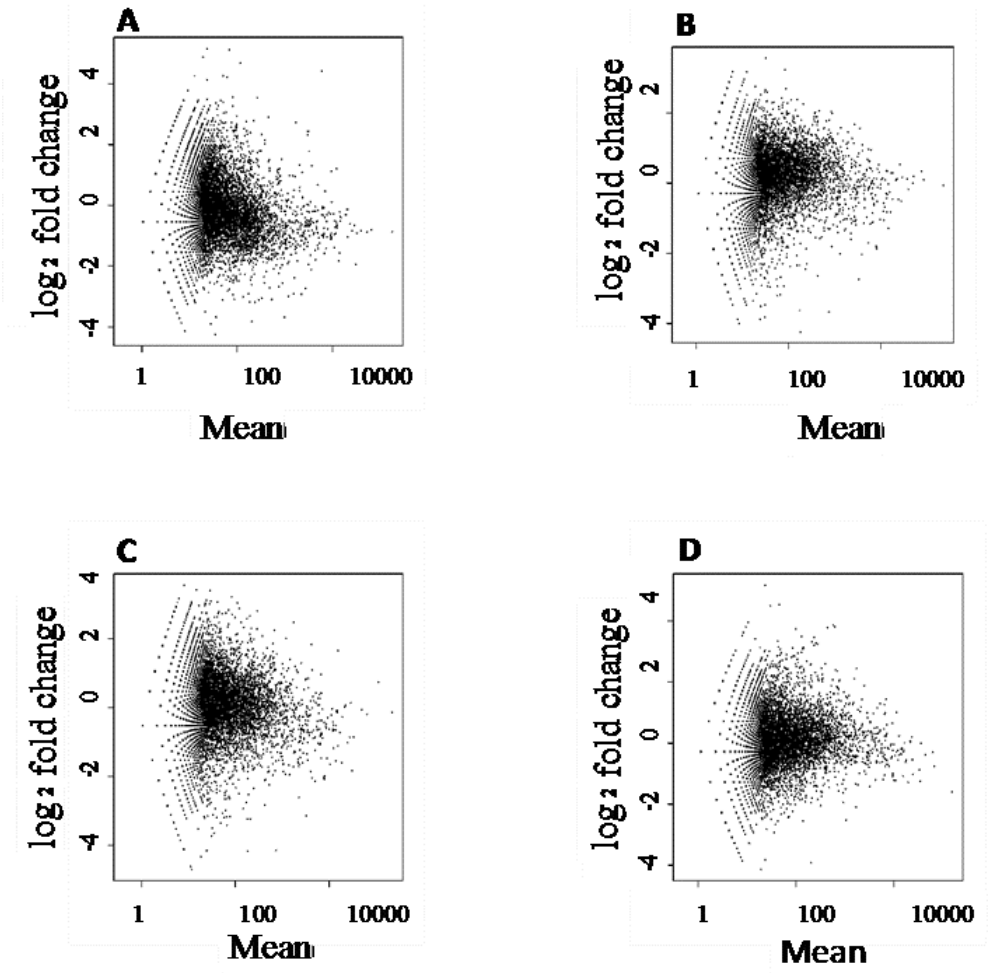


Figure S1 – Histograms of fold changes in gene expression for the four NPs; A) – nAg; B) – QD; C) – $nTiO_2$; D) – nZnO. The distribution of the number of genes (frequency) as a function of (log_2) fold change.



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95 **Figure S2 –** Scatterplot of fold change as a function of mean for each NP; A) – nAg ;

96 B) – QD; C) – $nTiO_2$; D) – nZnO.

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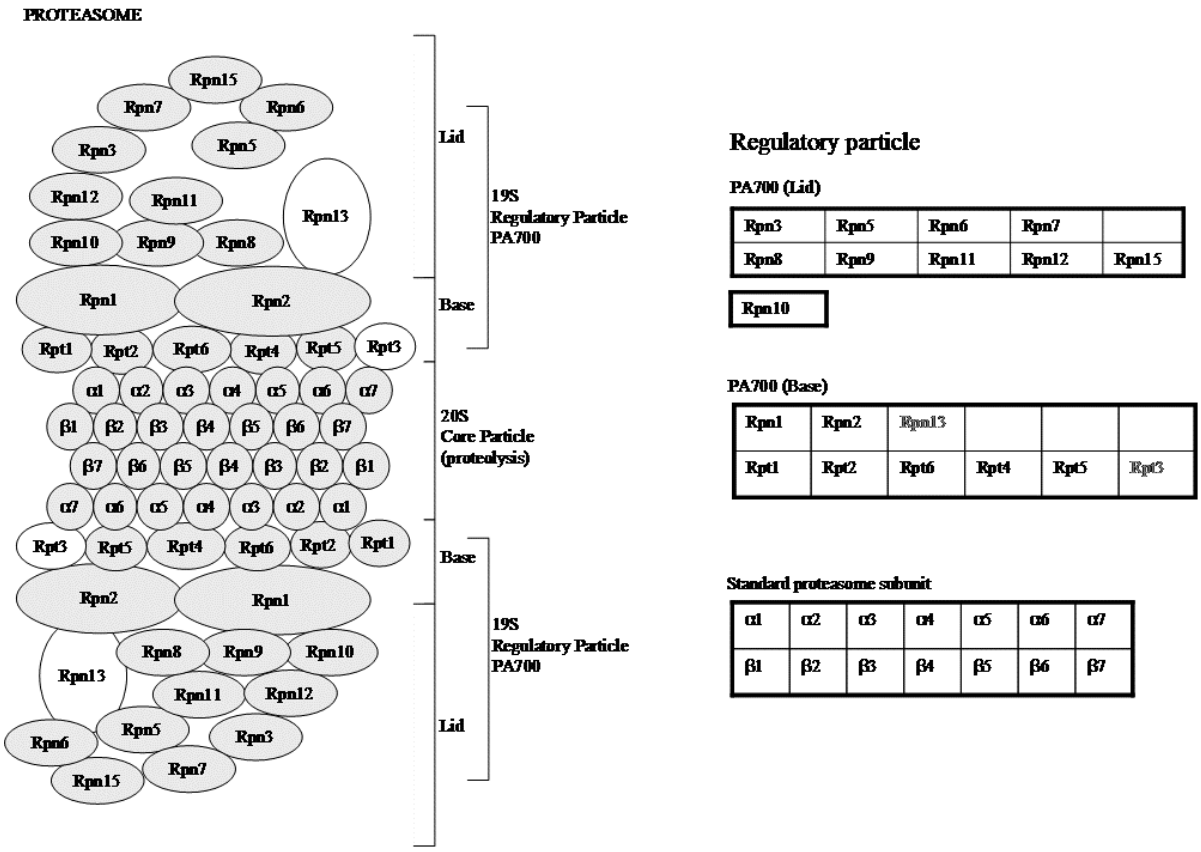

Figure S3 – Proteasome subunit-encoding transcripts elevated by exposure to NPs. Transcripts elevated following exposure to QD, nZnO, or $nTiO_2$ are highlighted in grey.