Supplementary Information

Cellular internalization of silver nanoparticles in gut epithelia of the estuarine polychaete *Nereis diversicolor*

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Number of figures: 3

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Properties of the AgNPs

The size of the citrate-capped silver nanoparticles AgNPs, 30 ± 5 nm, was verified by TEM (Figure 1a). The nature of the particles changed upon their dispersion in the artificial estuarine water (AEW) used in the experiments (salinity 16 ‰). The zeta potential of the AgNPs suspension in fresh water was -40 ± 3 mV while in AEW the value changed to -15 ± 2 mV. The change indicates that the suspension is more prone to aggregation (not as stable) in AEW than in fresh water. In addition, isolated AgNPs (Figure 1b) also showed specific peak corresponding to Ag in the X-ray energy dispersive analytical spectrum (Figure 1c).

Gut epithelial cells of *N. diversicolor*

Figure S2 shows the gut of unexposed *N. diversicolor* at progressively higher magnification (A – D

respectively). Typical of a polychaete, the gut epithelium has an enormous surface area for nutrient absorption (Figure S2A). The apical surfaces of the cells are covered by microvilli (Figure S3B), at the base of which cytoskeletal structures form a network, extending filaments into the microvilli. Large membrane bounded organelles were observed in the cytoplasm including mitochondria, lysosomes and peroxisomes (Figure S2B). In some parts of the gut epithelia, fenestrated cell surfaces appear increasing the surface for absorption (Figure S2C). With higher magnification the gut lumen between the epithelial cells can be clearly observed (Figure S2D).

AgNPs in the cell fractions

These operational procedures assume that metals contained within a particular fraction are biologically incorporated within that fraction. However, that assumption is less certain with AgNPs than with dissolved Ag, because the former are inorganic particles with an inherent size that will form a pellet at high enough centrifugation speed and/or duration. To assess this effect we conducted the exact same fractionation protocol with two additional sample sets: AgNPs alone in buffer solution (final concentration 0.66 µg ml^{-1}), and unexposed *N. diversicolor* homogenates (110 ± 20 mg fresh weight, fw) spiked with 2 µg AgNPs (final concentration 18 µg g^{-1}). The purpose of the AgNPs in buffer was to determine where the NPs fractionated at the different centrifugation speeds in a solution of similar pH and ionic strength to body fluids; and whether they were decomposed in the NaOH treatment. The aim of spiking the homogenate was to determine if the presence of the organic materials present in tissues changed this behaviour. The difference between these two controls and the exposed worms would then indicate if Ag was taken up in NP form and which fraction the NP was incorporated into.

Elevated Ag concentrations were first observed in the “organelle” fraction, suggesting that at least some of the nanoparticles remain small enough in the buffer that they do not form a pellet until centrifugation speeds exceed 1450 x g for 15 minutes. Silver also was enriched in the fraction

considered to contain separate metal rich granules (Figure S4) These are probably nanoparticles that aggregated at the pH and ionic strength of the Tris-Base buffer and thus formed a pellet after centrifugation at 145 x g for 15 minutes. The aggregates were not soluble in NaOH, consistent with the inorganic character of pure AgNPs.

The spiking of unexposed worm homogenates with AgNPs and subsequent fractionation, revealed Ag localised to the organelle fraction (as above) and the heat denatured proteins (HDP) fraction. This suggested that some of the AgNPs associate with the large biomolecules that precipitate in the presence of heat. Association with organic materials in the homogenate may have prevented formation of the largest aggregates because Ag was not detectable in the pellet after the first centrifugation, in contrast to the results with the buffer alone.

The highest percentages of Ag occurred in the MRG fraction in animals exposed to AgNPs and in the MTLP fraction in animals exposed to aqueous Ag in sediment (Figure S3).

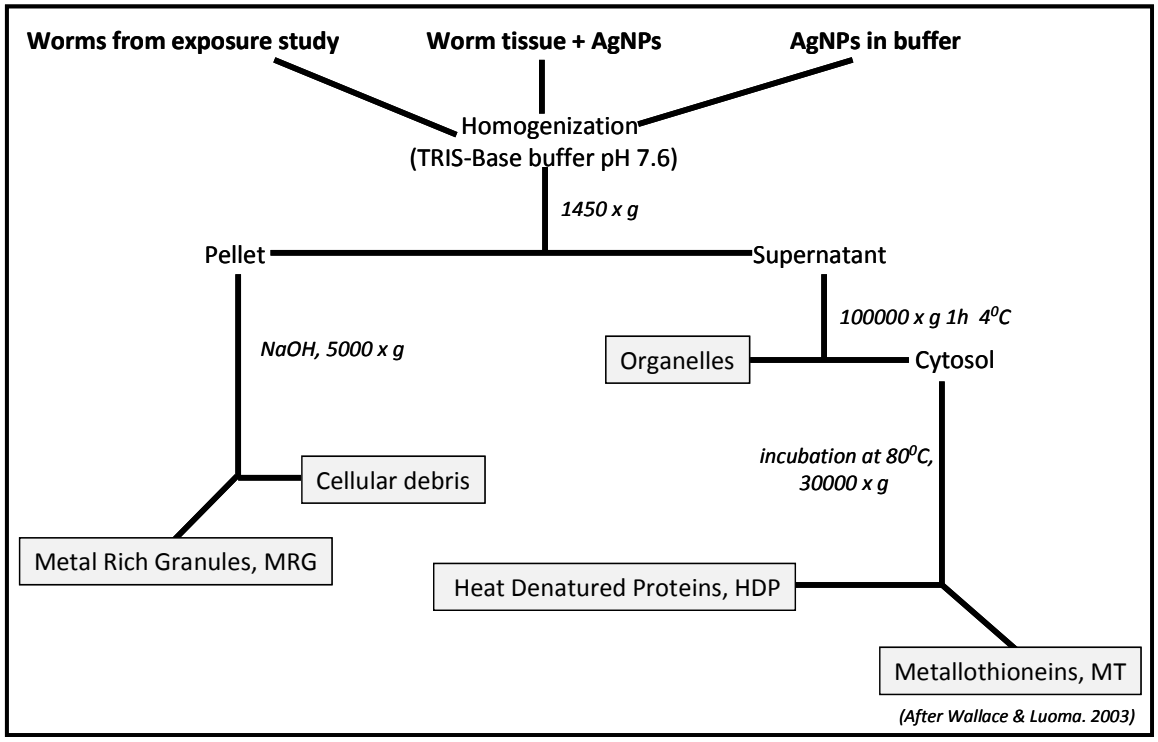

Figure S1. Scheme of the subcellular fractionation procedure. Tissue from *N. diversicolor* exposed to control, AgNPs and aqueous Ag in sediment as well as controls (Worm tissue + AgNPs and AgNPs alone followed a serial centrifugation and incubation steps in order to obtain different enriched subcellular fractions).

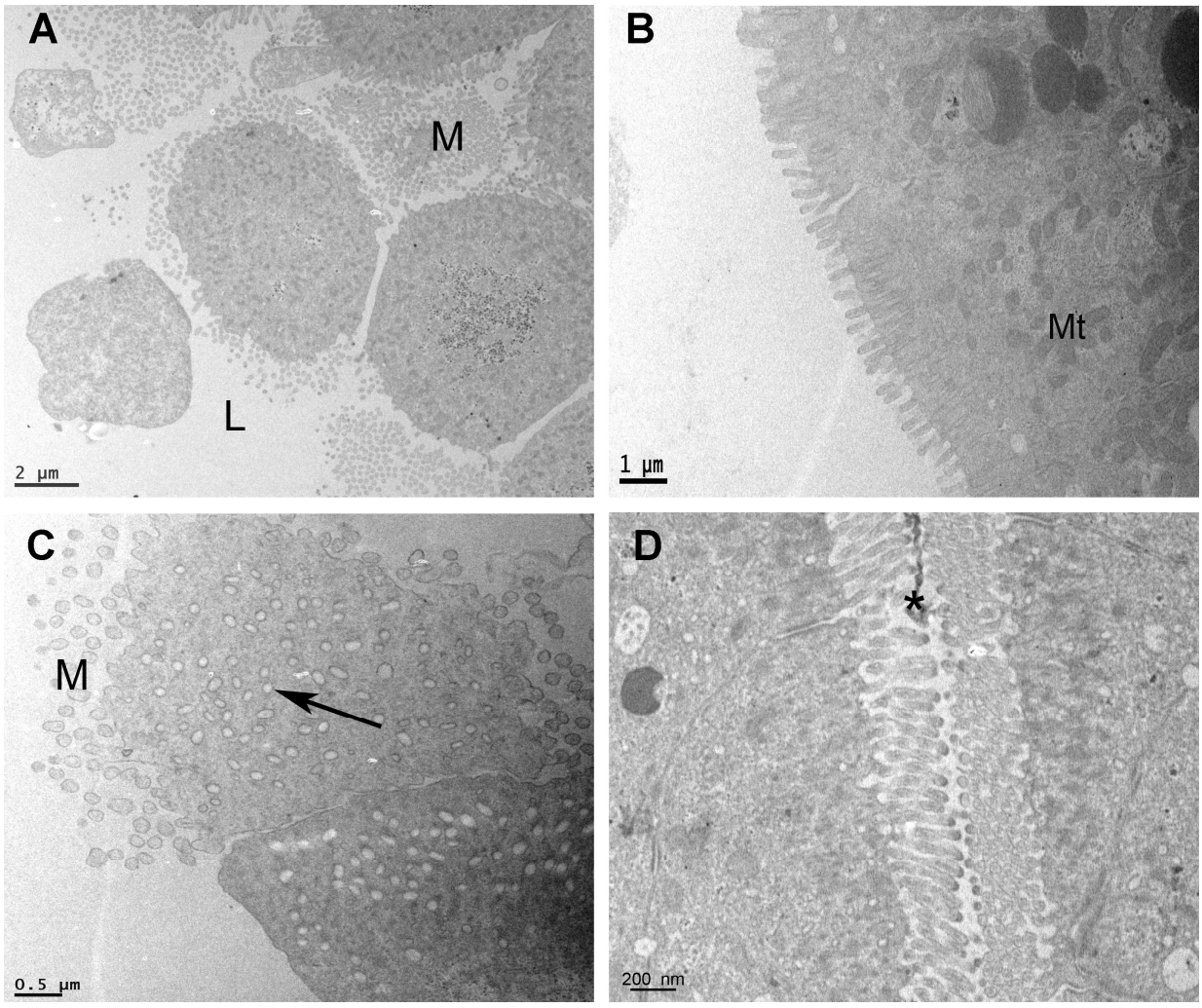

Figure S2. Transmission electron microscopy (TEM) of gut epithelial cells of *Nereis diversicolor*. (A) Low magnification showing the high surface area for absorption in the gut epithelia. (B) Gut epithelial cells covered by microvilli (asterisk). (C) Cytoplasm showing the presence of mitochondria and lysosomes and/or peroxisomes (P). (D) Fenestrated microvillus-like appendages of the epithelium cell (Arrow). M, microvilli; L, lumen.

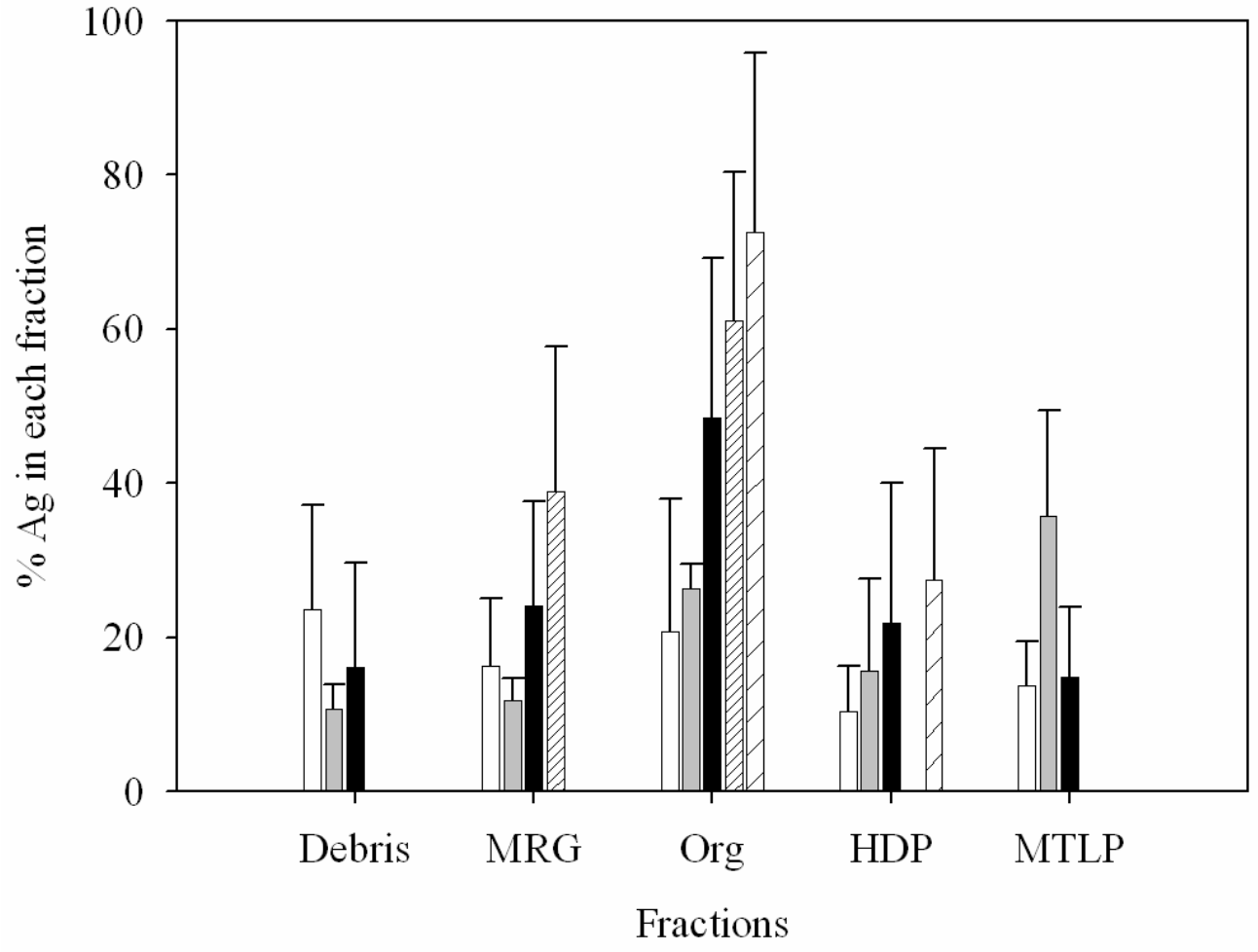

Figure S3. Percentages (%) of Ag concentration in different subcellular fractions of *Nereis diversicolor*, buffer alone, or homogenates of *N. diversicolor* tissues spiked with Ag NPs. Abbreviations: MRG, metal rich granules, Org, organelles; HDP, heat denatured proteins; MTLP, metallothionein-like proteins. White columns, control worms; grey columns, worms exposed to spiked sediment with aqueous Ag; black columns, worms exposed to spiked sediments with AgNPs; dense striped columns, AgNPs in buffer; striped columns, AgNPs in homogenised tissue.