**Supplementary Information**

**Three-layered silver nanoparticles to trace silver nanoparticle dissolution and accumulation by a green alga**

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1. **Culture media**

**Supplementary Table S1:** Final concentrations of major salts and other trace elements in modified high salt medium (MHSM from Fortin et al., 2014) used for *Chlamydomonas reinhardtii* culture. The ionic strength was 10.5 meq/L.

|  |  |  |  |
| --- | --- | --- | --- |
| MHSM Major salts  | [mM] | Other molecules | [µM] |
| NH4HPO4 | 0.94 | H3BO3 | 3.0 |
| MgSO4; 7 H2O | 0.08 | MnCl2; 4H2O | 2.1 |
| Ca(NO3)2; 4H2O | 0.07 | FeCl3; 6 H2O | 0.6 |
| KH2PO4 | 0.05 | Na2EDTA 2H2O | 1.0 |
| K2HPO4 | 0.08 | Cu | 70.3 |
| KNO3 | 1.50 | Mo | 30.0 |
| KCl | 1.50 | Co | 10.9 |
| NaOH | 0.10 | Zn | 24.3 |

All labware were soaked for at least one day in 7.5% nitric acid (HNO3), 7.5% hydrochloric acid (HCl; volume/volume (v/v)) and rinsed five times with deionized water and two times with ultrapure water (18 MΩ cm). To maintain axenic cultures, Erlenmeyer flasks were sterilized with the culture media (without trace elements and sodium hydroxide (NaOH)) at 121oC for 15 minutes in an autoclave. After sterilization and cooling, NaOH and trace elements were added. Culture media was inoculated using sterilized pipet tips under a laminar flow hood. A sterile piece of cotton (USP Sterile Cotton Roll, U.S. Cotton) was put in the opening of each flask to prevent biological contamination. Cultures were held in an environmental growth chamber (Thermo Scientific) at 20oC (± 1) under constant fluorescent light (1000 lux). Flasks were rotated at the rate of 100 rotations per minute (VWR Standard Orbital Shaker; Model 3500).

1. **Hydrodynamic size and zeta potential**

**Supplementary Figure S1:** The hydrodynamic size of the core-shell Ag@Au@Ag nanoparticles was measured using dynamic light scattering (DLS) in ultrapure water (31 ± 11 nm (± SD)). The zeta potential was -47 mV. The polydispersity index was 0.19. The measurement was made during 70 seconds and the count rate was 207 kcounts per second (measured with Zetasizer Nanoseries, Malvern).



1. **Exposure media**

**Supplementary Table S2:** Concentrations of major salts in the moderately hard water (MHW; US EPA, 2002) used for exposure. The ionic strength was 10.5 meq/L.

|  |  |  |
| --- | --- | --- |
| Salts | [Salt] (mg/L) | [Salt] (µM) |
| NaHCO3 | 96 | 1143 |
| CaSO4; 2H2O | 60 | 348 |
| MgSO4 | 60 | 285 |
| KCl | 4 | 54 |

**4. Gold internal reference material**

We created an internal reference material for Au to optimize the digestion procedure and ensure adequate recovery of Au in tissues. It was prepared by exposing the pond snail *Lymnaea stagnalis* to 100 nM of Au (added as AuCl4) for 12 days, with media renewal every other days. Gold concentrations in water were thus relatively constant throughout the exposure (more than 90% of the original concentration (data not presented)). Gold concentration in the in-house reference material was 5.3 ± 0.2 ug g-1 (*n* = 5) among digestion batches. Standardization of this concentration to account for the exposure duration yielded an Au uptake rate that fits Au uptake rates previously measured (Fig. S2).

Gold is among the least reactive chemical element. It resists attack of individual acids and is generally insoluble. The in-house Au reference material was used to optimize our digestion procedure and ensure proper recovery of Au. We observed that a one to one ratio of HCl and HNO3 yielded the same Au recovery as aqua regia (3:1 ratio, respectively). We also observed an important loss of gold when the digested samples were filtered onto 0.45 µm filters (Acrodisc® LC 13 mm Syringe Filter, Life Sciences, Pall) prior to analysis (to remove any particles, i.e. silica from diatoms). Furthermore, we observed that heating the sample after adding HCl (see section *Sample Digestion and analysis*) did not improve recovery.



**Supplementary Figure S2:** Gold uptake rates in snails exposed to two aqueous concentrations of AuCl4 (closed symbols). The concentration measured in the internal reference material and standardized for the 12 day exposure is shown by the open symbol.

**5. Calculations of Ag and Au recovery during exposure**

Both [AgT] and [Au] in the MHW declined during exposure due to uptake. Comparison of the amounts of Ag and Au lost from the water during exposure to the amounts of Ag and Au recovered with the algae after exposure (Supp. Eq. S1) shows that 91% and 96% of the Ag and Au, respectively (*n* = 24), were recovered with the algae. About 9% and 4% of the Ag and Au, respectively, were adsorbed on the Erlenmeyer flasks after exposure or in residual water.

Loss of Ag and Au in water during the exposure was determined by subtracting the measured initial and final Ag and Au quantities, that is for Ag,

 (Supp. Eq. 1)

Where AgH2O-Before (nM) is the Ag concentration in MOD water in Erlenmeyer flask before inoculation of algae. AgH2O-After (nM) is the Ag concentration in 0.45 µm filtrate after exposure. Agalgae is the measured Ag concentration with the digested algae and 0.45 µm filter.

**6. Loss by adsortion on 3 kDa membrane**

 A substantial proportion of dissolved Ag did not cross the 3 kDa membrane when the Ag concentrations as AgNO3 ranged from 10 to 40 nM (Figure S3; equation 2 in main text). We calculated that 12 ± 2 % (*n* = 4) of the Ag added as AgNO3 did not cross the 3 kDa membrane. Speciation calculations performed using MINTEQ 3.1 revealed that 10% of the Ag was complexed by chlorides in MHW (dissolved AgCl complexes). To determine if the AgCl complexes could be retained on the 3 kDa membrane, we replaced KCl by KNO3 in the formulation of the MHW (Supp. Table S2). As observed with KCl, 13% of the Ag did not cross the 3 kDa membrane when AgNO3 was added to MHW made with KNO3 instead of KCl. These results suggest that Agloss by adsorption on the 3 kDa membrane is not negligible at those concentrations. When using environmentally relevant Ag exposures, pre-conditioning the 3 kDa membranes before filtering samples therefore appears important in future studies.

Colloids or algal exudates larger than 3 kDa and smaller than 0.45 µm were present in the media after 10 min of algal exposure (seen by UV-visible measurements in this study). As shown in Figure S3, algal exudates lowered the [Ag] that passed through the 3 kDa membrane filters with the highest impact observed at the lowest [AgNO3]. Using equation 2 (main text), we calculated that the loss by adsorption of the Ag-exudate complexes was nearly 55% (Figure S3) at the lowest [AgNO3] (about 10 nM). The regression shown in Figure S3 was used to calculate the [AgD] in the media after exposure in function of the measured AgD concentration.



**Supplementary Figure S3:** Proportion (%) of Ag loss onto 3 kDa membrane as a function of the AgNO3 concentrations in the media in presence or absence of algal exudates (see eq. 2 in the main document for detailed calculation).

**7. Loss by adsorption on 0.45 µm membrane**

Filtration of 100 mL of media with added Ag NPs (10 to 40 nM) led to a 10% loss onto the 0.45 µm membrane. When media contained algal exudates, there was a 35% loss of NPs (Ag and Au) from sorption to the 0.45 µm membrane (Figure S4). From these results, we estimated that 35% of the Ag and Au were removed from *C. reinhardtii* uptake rate results.



**Supplementary Figure S4:** Loss of Ag as NPs on 0.45 µm filter in the presence or absence of algal exudates in MOD water as a function of the total Ag concentrations before filtration (0.45 µm).

**8. Ultra violet-visible sprectrum**



**Supplementary Figure S5:** Ultra-violet/visible spectrum of Ag@Au@Ag nanoparticles before exposure (top panel) of *Chlamydomonas reinhardtii* and in the filtrate after exposure (lower panel) at a total Ag exposure of 100 (first from bottom), 500 (orange, 2nd from bottom), 1000 (yellow 3rd from bottom), 2000 nM (top green).

**9. Ag to Au ratios after 24 hours**



**Supplementary Figure S6:** Ratios of AgNP/Au (closed circles) after 5 minutes in moderately hard water and AgNP/Au after 24 hours (open squares) as a function of the total Ag concentrations (AgT).

**10. Ag toxicity to algae**

The shape and number of algal cells were similar at the beginning and at the end of our exposure period of 10 minutes (data not presented), suggesting that the Ag NP exposures were not lethal to *C. reinhardtii* during this short period of exposure. That is, 186 nM of AgT is required to reduce 50 % of the *C. reinhardtii* photosynthetic yield (Piccapietra et al., 2012) and our maximum exposure concentration was 50 nM.