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Effects of differently coated silver nanoparticles on the photosynthesis of *Chlamydomonas reinhardtii*

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KEYWORDS: *Chlamydomonas reinhardtii*, silver nanoparticles, dissolved silver, bioavailability, coatings.

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ABSTRACT

Various factors have been invoked to explain the toxicity of silver nanoparticles (AgNP) to microorganisms including particle size, the nature of stabilizing coatings as well as the amount of dissolved silver occurring in AgNP suspensions. In this study we have assessed the effects of nine differently coated AgNP (chitosan, lactate, polyvinyl pyrrolidone, polyetheleneglycol, gelatin, sodium-dodecyl-benzenesulfonate, citrate, dexpanthenol and carbonate) and $AgNO_3$ on the photosynthesis of the freshwater algae *Chlamydomonas reinhardtii*. We have thus examined how AgNP effects to algae relate to particle size, measured dissolved silver (Ag_d) and bioavailable silver (Ag_{bioav}). Ag_{bioav} was indirectly estimated in toxicity experiments by cysteine-silver complexation at the EC_{50}. The EC_{50} calculated as a function of measured Ag_d concentrations showed for some coatings similar values to that of dissolved Ag, while other coated AgNP displayed lower EC_{50}. In all cases, excess cysteine completely prevented effects on photosynthetic yield, confirming the role of Ag_d as a cause of the observed effect on the photosynthesis. Toxicity was neither related to particle size nor to the coatings. For all differently coated AgNP suspensions, the EC_{50} values calculated as a function of Ag_{bioav} were comparable to the value of $AgNO_3$. Depending on the coatings Ag_{bioav} was comparable or higher than measured Ag_d.

INTRODUCTION

Because of their bactericidal properties [1], silver nanoparticles (AgNP) are present in numerous consumer products. During recent years, an increasing number of studies have demonstrated toxicity of AgNP to different microorganisms such as bacteria [2-4] or algae [5-7]. The toxicity of AgNP to microorganisms has been suggested to result from both the release of silver ions (Ag^+) and interactions between AgNP and cell membrane [2, 8-10]. The determining role of Ag^+ in explaining the observed toxicity of AgNP to microorganisms is experimentally supported by the fact that complexation of Ag^+ by thiol ligands, as well as anaerobic conditions prevent toxicity of AgNP [5, 7, 11-15]. Thus, risk assessments of AgNP need careful consideration of the contribution of Ag^+ to toxicity. Ionic silver might occur in AgNP suspensions, both as residual from particle synthesis and formed upon particle oxidation. Major factors expected to influence AgNP dissolution in aqueous solutions include pH, ionic strength, and the presence of ligands [16, 17].

AgNP are typically synthesized with different surface coatings to minimize particle agglomeration in aqueous systems [1]. Depending on their chemical nature, coatings might by complexation influence the release of Ag^+ ions from particles [18-21], or affect the stability of particles and indirectly, their toxicity to aquatic organisms [8, 22-24]. Moreover, intrinsic properties of coatings might contribute to observed effects of AgNP [25]. So far, no studies have systematically examined the contribution of size, dissolved silver and of several coatings on AgNP toxicity in a common approach.

In this study we have assessed the toxicity of differently coated AgNP and of dissolved Ag (provided as $AgNO_3$) on the freshwater algae *Chlamydomonas reinhardtii* and examined how effects related to particle size, dissolved silver (Ag_d) and bioavailable silver (Ag_{bioav}) which is the concentration that provokes toxicity. Considered coatings are representative of different chemical families (Table 1) and were selected considering their differences in Ag binding properties and molecular size.

We first assessed short-term effects on photosynthesis and measured Ag_d in experimental media used for toxicity testing. The concentration of Ag_{bioav} was assessed indirectly in toxicity experiments by modifying the speciation of Ag_d with cysteine. This use of the cysteine is supported by recent studies showing that the uptake of Ag in presence of cysteine is strongly reduced [15, 26]. Therefore effective concentrations on the base of dissolved and bioavailable silver were compared to infer the influence of coatings on bioavailability and toxicity of AgNP.

MATERIALS AND METHODS

AgNP characterization. AgNP were provided by NanoSys GmbH (Wolfhalden, Switzerland) as aqueous suspensions of 1 g/L total Ag (nominal concentrations). Original AgNP suspensions (see Table 1 for bulk concentrations) were kept in the dark. Experimental concentrations were prepared using 10 mM MOPS (3-morpholine propanesulfonic acid, buffered at pH 7.5). For cysteine experiments, fresh stock solutions of cysteine were prepared in nanopure water, and kept on ice to prevent oxidation. For exposure studies, $AgNO_3$ solutions and AgNP suspensions were freshly prepared before use. Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich. AgNP were characterized under experimental conditions (10 mM MOPS, pH 7.5) for size and ζ –zeta– potential by Dynamic Light Scattering (DLS) using a Zeta Sizer (Nano ZS, Malvern Instruments).

ICP-MS measurements. The silver concentration (isotope ^{107}Ag) was measured in acidified solutions (0.1 M HNO_3) by ICP-MS (Element 2 High Resolution Sector Field ICP-MS; Thermo Finnigan). Reliability of the measurements was controlled using a water reference solution (Standard Reference Material 1643e, National Institute of Standards & Technology, USA).

For total Ag measurements AgNP suspensions were digested with HNO_3 in a microwave oven (MLS 1200 Mega, Microwave Laboratory Systems). Recovery of Ag was 88 – 95 % of the nominal concentrations.

Dissolved silver present in AgNP suspensions was examined by centrifugal ultrafiltration (Millipore Amicon Ultra-4 3K) through a membrane with a nominal molecular weight limit of 3 kDa. Recovery measurements using $AgNO_3$ and the same experimental media were done, showing that between 90-99% of the silver was recovered after the Ultrafiltration process. Suspensions (5μM AgNP in 0.01 M MOPS at pH 7.5) were centrifuged for 30 min at 3000×g (Megafuge 1.0R, Heraeus Instruments). Concentration of dissolved Ag (Ag_d mostly Ag^+, see at Supporting Information -SI- Table S1) in the filtrate was expressed as % of the total Ag concentration after 1 or 2 h in the experimental media without algae (Table 1). Measurement of Ag_d in the AgNP suspensions were confirmed using diffusive gradients in thin films (DGT) [5, 27], and by ultracentrifugation. Bioavailable Ag (Ag_{bioav}) was on the basis of cysteine-silver complexation experiments (see below).

Speciation calculations. Calculations of the Ag_d speciation upon cysteine addition to $AgNO_3$ or AgNP were done using the software ChemEQL 3.0 [28] using the corresponding equilibrium constants, as described in Navarro et al. 2008 [5]. Detailed calculations and the list of stability constants and appropriate references are available in SI Tables S1 and S2 respectively.

Algal culture and photosynthetic yield measurements. *Chlamydomonas reinhardtii* (wild type, CC-125 137c mt+) were cultured under controlled conditions (25 °C, 90 rpm, 120 μE) in standard growth medium at pH 7.5 [29]. Cell number was measured by an electronic particle counter (aperture 50 μm; Z2 Coulter Counter; Beckman Coulter, Fullerton, CA). While growth is a more sensitive endpoint towards silver with EC_{50} values of 12 nM [30], photosynthetic yield was chosen as a toxicity endpoint because effects are measurable upon short-term exposure thus avoiding accumulation of secreted biomolecules. The algal photosynthetic yield of the photosystem II in light was measured by fluorometry using a PHYTO-PAM (Heinz Walz GmbH) equipped with an Optical Unit ED-101US/MP. The yield reflects the efficiency of the photochemical energy conversion process [31]. Fluorometry measurements were done at similar light intensities than those used during growth and exposure (140 μmol photons m^{-2} s^{-1} PAR radiation).

Effects of $AgNO_3$ and AgNP on photosynthetic yield. Exponentially growing algae in culture media were first centrifuged (2000×g, 10 min) and then resuspended in 10 mM MOPS at pH 7.5 to a final volume of 25 mL in glass Erlenmeyers and a density of $1.7x10^6$ cells mL^{-1}. Photosynthetic yield was not affected by the algal translocation into MOPS or by exposure to cysteine [5]. Toxicity of AgNP and $AgNO_3$ to photosynthesis was assessed by concentration-response experiments by exposing algae to increasing concentrations of $AgNO_3$ and AgNP (see SI Table S3 for a comprehensive list of the experiments and concentrations used). At least 3 concentration-response experiments per coating were done. AgNP and $AgNO_3$ toxicity to the algal photosynthetic yield was measured after short-term exposure (1 and 2 h) in order to minimize accumulation of algal products in the exposure media, and thus changes in the silver speciation. The photosynthetic values of all concentration-response experiments were represented as percentage of the respective controls. Values were plotted as a function of the total Ag or Ag_d concentration measured in AgNP suspensions, in the absence of algae in the suitable concentration range. The Ag_d bound to the algae was estimated to be negligible in comparison with the EC_{50} concentrations, as calculated based on previous studies (ref. 26), see calculations in SI. The Ag_d values were determined by centrifugal ultracentrifugation. Data were fitted to a four parameters logistic curve to determine EC_{50} values as described below in the Statistics section.

Cysteine-silver complexation experiments. Complexation experiments were carried out in order to determine the concentration of bioavailable silver (Ag_{bioav}). Based on our previous results [5], cysteine concentrations similar to or higher than Ag_d (mostly Ag^+), were expected to complex Ag^+ and to decrease the free Ag^+ concentrations to levels which show no effect to photosynthesis (see detailed speciation calculation at SI Table S1 and S2). Different cysteine concentrations were first added to one concentration of the various AgNP which were in the range of the respective EC_{50}. After ten minutes pre-equilibration (time to reach equilibrium of cysteine-silver complexes) algae were added to the suspensions and their photosynthetic yield measured after 1 h of exposure. Up to 47 complexation experiments were done with various AgNP and cysteine concentrations (SI Table S3). The photosynthetic values were represented as percentage of the respective controls

and plotted as a function of cysteine concentration. Points were fitted to the best curve (maximizing R^2 value), using Sigma Plot 12 (© Systat Software Inc.). The models were then used to calculate the lowest amount of cysteine required to completely prevent the toxicity to photosynthesis. For each AgNP, the concentration of bioavailable Ag_{bioav} at EC_{50} (1h) was estimated as the product of the EC_{50} values (based on Ag_d) by the cysteine/Ag_d ratio ($Ag_{bioav} = Ag_d$ $EC_{50} \times Cys/Ag_d$). This approach is detailed in the discussion.

The potential effects of cysteine on the dissolution of Ag or the characteristics of AgNP were assessed by measuring dissolved Ag_d, as well as size and ζ- potential of AgNP in the presence of 500 nM cysteine which was the highest concentration used in the silver complexation experiments.

Statistics. Errors of measurements (total number of experiments and replicates are shown in SI Table S3) are expressed as standard deviation (SD). Concentration-response curves were fitted to a four parameters logistic curve using R and drc package [32], to obtain the corresponding EC_{50} values and standard error (SE). The same package was also used to perform comparison tests, using R "compPAR" function; the null hypothesis is that the ratio equals 1. The ratio was obtained by dividing EC_{50} value for $AgNO_3$ by EC_{50} for each AgNP. If the ratio significantly differs from 1, the null hypothesis should be rejected, meaning that values are significantly different ($p<0.05$). The p-values are adjusted using Bonferroni correction for multiple T-tests.

RESULTS

AgNP characterization. In the simple experimental medium used for toxicity experiments (10 mM MOPS, pH 7.5), the AgNP displayed average diameters ranging from 17 nm (CIT) to 456 nm (DEX), and ζ- potential from -3.5 mV (LAC) to -36 mV (CO_3) (Table 1). Dissolved silver (Ag_d), measured 1 h after dilution of the original AgNP suspensions in 10 mM MOPS and as percentage of measured total Ag, was between 12 and 19 % in the case of PEG, LAC, DEX and PVP and

around 1-3 % in the case of CO_3, CHI, CIT, GEL and NDB (according to both ultrafiltration and DGT). These percentages were similar after 2 h (Table 1).

Effects of dissolved Ag and AgNP on photosynthetic yield. All differently coated AgNP and dissolved Ag proved to be toxic, decreasing the photosynthetic yield of algae as a function of increasing total Ag concentrations (Figure 1). At the highest applied concentrations (between 20 and 50 μM as total silver) most AgNP completely inhibited photosynthesis within 1 h, while in the case of GEL, CIT and CHI coated AgNP, photosynthetic yield remained between 10-30 % of the control. Based on total Ag, the various AgNP strongly differed in their toxicity (Table 2A), with EC_{50} values ranging from 0.28 (DEX) to 5.25 μM (CIT). Dissolved $AgNO_3$ showed the highest toxicity to *C. reinhardtii* photosynthetic yield (Figure 1 and Table 2A), with an EC_{50} of 0.18 μM, up to 30 times lower than that of CIT coated AgNP.

Examination of effects of the AgNP on photosynthetic yield as a function of measured Ag_d, showed concentration-response curves and EC_{50} values to differ less than as a function of total Ag (Figure 2 and Table 2B). Compared to $AgNO_3$, the EC_{50} values expressed as function of Ag_d were slightly lower or similar for the CHI, LAC, PEG and PVP coated AgNP, but appeared to be significantly lower for the CO_3, DEX, NDB, CIT and GEL coated AgNP (Table 2B).

When expressed as function of measured total Ag, EC_{50} values were found to decrease with increasing particle size (Figure 3A). No correlations between the EC_{50} of the various AgNP and the corresponding average particle size have been found, whether expressed as total Ag or as Ag_d (Figure 3).

Silver-cysteine complexation experiments. After 1 h exposure at the highest applied concentration of cysteine (500 nM), the ζ- potential of most AgNP shifted to less negative values (SI Figure S2), while in the case of polymeric coatings (PEG, PVP and CHI), no effect was observed. The size of most AgNP was not affected by cysteine, and only a few showed a slight increase of the averaged size (SI Figure S2). Cysteine had only small effects on the Ag_d determined in most of the AgNP suspensions (SI Figure S3), with only the GEL AgNP showing a significantly higher Ag_d concentration (4 times).

Pre-equilibration of particle suspensions with cysteine resulted for all AgNP in a lowering of toxicity with increasing cysteine concentration (see examples with CO_3, PVP and CHI AgNP in Figure 4). At the highest concentration of 500 nM, cysteine suppresed toxicity of all AgNP completely, indicating absence of toxicity by the coatings.

Curves were modeled to calculate the minimal cysteine concentration that prevented toxicity to the photosynthetic yield and which is supposed to correspond to the Ag_{bioav} concentration (Table 3, for an example of calculation see SI Figure S1). For most AgNP, the amount of cysteine required to prevent toxicity was between 0.3-0.5 μM and was lower in case of NDB or CIT (0.07 or 0.13 μM, respectively). Using the ratio of cysteine concentrations to the measured Ag_d concentrations, toxicity values were corrected to obtain EC_{50} values as a function of Ag_{bioav} (Table 3). Compared to dissolved Ag , most AgNP displayed similar or slightly higher EC_{50} values, while DEX and NDB coated AgNP appeared to be more toxic.

DISCUSSION

AgNP toxicity as a function of total silver and of dissolved Ag_d. All AgNP were toxic to the photosynthetic yield of algae, with calculated EC_{50} values expressed as measured total Ag varying in the range 0.28 – 5.25 μM (Table 2A and Figure 1). These EC_{50} were all higher than the EC_{50} of dissolved Ag and thus suggested that various AgNP were less toxic than a similar amount of dissolved Ag. The range of the EC_{50} expressed as the measured dissolved Ag_d was narrower (0.029 – 0.19 μM, Table 2B and Figure 2), as expected considering the role of dissolved silver in AgNP toxicity to algae [5]. The size of the nanoparticles did not account for differences in toxicity (see Figure 3B), in contrast to the expected higher toxicity of smaller silver nanoparticles found in other studies [33, 34].

While for some of the AgNP the EC_{50} based on measured Ag_d became similar to that of $AgNO_3$, for other the Ag_d based EC_{50} were lower, indicating for these AgNP an apparently higher toxicity compared to $AgNO_3$. Factors to be considered for this apparent higher toxicity include intrinsic

toxicity of the coatings, direct nanoparticle effects, increased dissolution of NP in contact with the algae and underestimation of Ag_d.

Some of the coating compounds, in particular glycols (PEG), CHI, or NDB are toxic for algae [35-37] and bacteria [38] only at high concentrations with EC_{50} ranging from 120 to 1900 mg/L. However, considering the complete suppression of toxicity by cysteine, the various coatings were not affecting photosynthetic yield at the applied experimental concentrations. The suppression of toxicity by cysteine also indicated that direct effects of AgNP were unlikely. Another possibility to explain the apparent higher toxicity of some AgNP is that the concentration of Ag^+ ions did increase during the toxicity experiments upon contact of the particles with algae. As already hypothesized in a previous study that suggested a higher Ag_d based toxicity of carbonate coated AgNP compared to $AgNO_3$, the production and secretion of reactive oxygen species by algae might lead to increased dissolution [6, 39]; as an example, hydrogen peroxide is very efficient in dissolving AgNP [40]. Interestingly, a recent study showed that a close contact of AgNP with bacteria surfaces is required for bactericidal effects, also suggesting the release of Ag^+ from AgNP in close vicinity to the bacteria [13]. Last but not least, underestimation of Ag_d would also lead to the higher apparent toxicity of certain AgNP. The levels of Ag_d in the particle suspensions were measured in the absence of algae to avoid underestimation due to the efficient uptake of Ag^+ ions by algae [15, 26]. In the presence of algae, because of complexation of Ag^+ ions by coating molecules (e.g. in the case of proteins or retention of dissolved Ag^+ ions within a polymeric coating), Ag_{bioav} might be higher than measured Ag_d. However, the Ag^+ bound in polymeric species may be exchangeable with added cysteine, as well as with algal biotic ligands. If the AgNP are in contact with the algae cells, free Ag^+ is rapidly taken up and may then be supplied by dissociation from the complexes with the coating molecules.

Indirect estimation of bioavailable Ag. The titration experiments with cysteine were used to estimate the concentration of Ag_{bioav} for comparison with measured Ag_d. Cysteine as a very strong ligand for Ag^+ is expected to form complexes with free Ag^+ present at the beginning of the exposure and with Ag^+ adsorbed or complexed to the coatings by competition with ligands such as

citrate or functional groups of peptides in gelatin. If Ag^+ is released from AgNP in close vicinity to the algae, these Ag^+ ions are also likely to be rapidly complexed by cysteine (Cys). Formation of AgCys and $Ag(Cys)_2$ complexes is expected, thus with a ratio of Cys/Ag in the range 1 – 2, depending on the concentrations of Cys and Ag^+ [41]. It was assumed that the concentration of cysteine that suppressed toxicity corresponded to the bioavailable Ag concentration within this ratio. Interactions of cysteine with the nanoparticles surfaces appeared to be of minor importance, as shown by only slight changes of ζ- potential and nanoparticle size in the presence of cysteine (SI Figures S2 and S3).

The calculated cysteine concentrations suppressing toxicity, the ratios of cysteine to measured Ag_d and the calculated bioavailable Ag at EC_{50} are presented in Table 3. In the case of $AgNO_3$, the Cys/Ag_d ratio of 1.1 confirmed the speciation calculations for these conditions (SI Table S1 and S2). For the AgNP coated with CHI, LAC, PVP, DEX and NDB, the Cys/Ag_d ratios were in the range 1.0 to 2.6, which can be considered as similar to the ratio for $AgNO_3$. For these coatings, measured Ag_d concentrations under abiotic conditions were in reasonable agreement with the Ag_{bioav} concentrations at which algae were exposed. In the case of other coatings (CO_3, CIT, GEL and PEG), the higher ratios indicated that Ag_{bioav} was higher than the measured Ag_d. Based on the estimated Ag_{bioav} concentrations (Table 3) the EC_{50} were in a narrow range for most coatings, namely 0.22 - 0.58 μM, and thus close to the EC_{50} for $AgNO_3$, but were lower in the case of NDB and DEX coated AgNP.

The processes leading to high Cys/Ag_d ratios need to be considered in detail. The high ratio Cys/Ag_d (8.2) in the case of GEL indicated that measured Ag_d was likely underestimated, due to complexation of Ag^+ to gelatin proteins, in particular to methionine or cysteine groups [42]. Ag bound to larger proteins would not pass through the ultrafiltration membranes with a cutoff of 3 kDa. Ag^+ complexed to the GEL-coating molecules may then be released by cysteine addition and may also be exchanged with the biotic ligands [18] of algae. The excess of cysteine required to prevent toxicity suggests that there was competition between cysteine and proteins as Ag_d ligands. The calculated Ag_{bioav} at EC_{50} was within the range of other coatings. The fact that GEL coated

AgNP were the only ones showing a noticeable increase of measured Ag_d upon cysteine interactions (4 fold increase, SI Figure S3), supports all the previous points. GEL coated AgNP did not provoke a complete photosynthetic inhibition, even at higher concentrations, also likely due to the effect of complexation of Ag^+ by proteins (Figure 2).

The CO_3 coated AgNP presented the highest Cys/Ag_d ratio (14.7), and the lowest EC_{50} value as Ag_d. Carbonates are not good silver ligands, so no Ag_d was expected to be complexed to the AgNP coating. These results, together with a strong decrease of EC_{50} from 1 to 2 h (SI Table 5S), suggest some Ag dissolution from AgNP as a result of biological interactions. A tentative explanation, for a better dissolution in this case, is that dissolution of AgNP by ROS produced by the algae is more efficient in the case of CO_3, which is the coating least adequate to scavenge ROS, in comparison to the organic compounds. Another factor may be the small size of the CO_3 ions, which may facilitate a close contact and subsequent surface interactions between AgNP and algal cells and their by-products [13]. Furthermore, specific interactions of the carbonate coating with carbonic anhydrases are also possible. However, the calculated EC_{50} based on Ag_{bioav} (Table 3) was also within the range of the other coatings.

In the case of citrate, the Cys/Ag_d ratio was 3.7 and thus also indicated that complexation of Ag^+ by citrate and possibly dissolution occurred. Citrate may complex Ag^+ and thus retains the ions at the AgNP surfaces [18, 19, 23], and possibly leads to renewed reduction of dissolved Ag^+ to Ag^0 [43, 44]. The incomplete inhibition of photosynthesis at high concentrations of CIT coated AgNP may also be due to decreased Ag^+ concentration in the presence of citrate (Figure 2).

In the case of PEG, the ratio Cys/Ag_d (3.1) also indicated some additional interactions, although PEG is not a good ligand for Ag^+. The Ag^+ can be retained by this polymeric coating close to the nanoparticle surface and be released in the presence of cysteine, as well as by interactions with algae.

In the case of NDB and DEX, lower EC_{50} based on Ag_d, as well as based on the Cys/Ag_d ratio indicate that measured Ag_d was similar to Ag_{bioav}, but that increased toxicity occurred. These two molecules are not likely to strongly complex Ag^+. However, both NDB as a surfactant and DEX as

298 a solvent may increase membrane permeability, thus facilitating Ag^+ uptake or even leading to the
299 uptake of AgNP [45, 46].

300 **Role of dissolved Ag^+ and of AgNP coatings**. These results thus confirm the predominant role
301 of dissolved Ag for toxicity of AgNP, as also demonstrated in other studies with algae [5, 26], as
302 well as with nematodes [23] and bacteria [12]. Furthermore, Ag uptake in *C. reinhardtii* from AgNP
303 has been shown to be dependent on dissolved Ag, and no evidence of substantial AgNP uptake
304 was found [15, 26]. The role of AgNP coatings in toxicity to algae appears thus to be only minor in
305 acting as an intermediate reservoir of Ag^+ which may subsequently be released, and in modulating
306 interactions of AgNP with algae, which may lead to further release of Ag^+.

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314 **SUPPORTING INFORMATION AVAILABLE**

315 This paper includes supporting data as: speciation calculations and the corresponding constants,
316 a complete list of the experiments, tables containing size and ζ- potential measurements for all
317 nanoparticles, the ratio between EC_{50} (calculated as a total Ag) after 1 and 2 hours of exposure. An
318 examples of the calculation of the cysteine required to abolish the toxicity of AgNPs and of the
319 Ag_d adhered by the algae cells, and three figures showing the influence of Cysteine on ζ- potential
320 and size of differently coated AgNPs and in the Ag dissolution from the different AgNPs are also
321 provided. All this information is available free of charge via the Internet at http://pubs.acs.org.

FIGURE LEGENDS

Figure 1. Concentration-response curves of photosynthetic yield after 1 h of exposure to differently coated AgNP and to $AgNO_3$ as a function of measured total Ag. The dashed line indicates 50% of the control value. Photosynthetic values are expressed as the percentage of the control.

Figure 2. Concentration-response curves of photosynthetic yield after 1 h of exposure to differently coated AgNP, as a function of measured dissolved Ag_d. The dashed line indicates 50% of the control value. Photosynthetic values are expressed as the percentage of the control.

Figure 3. EC_{50} values expressed as measured total Ag (A) and as measured Ag_d (B) as a function of particle size.

Figure 4. Effect of different concentrations of cysteine on the effect of three differently coated AgNP (CO3, PVP, CHI; concentrations are indicated in the legend) on photosynthetic yield. Photosynthetic values are expressed as the percentage of the control.

SUPPORTING INFORMATION

Effects of differently coated silver nanoparticles on the photosynthesis of *Chlamydomonas reinhardtii*

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Table S5. Ratio between EC_{50} (calculated as a total Ag) after 1 and 2 hours of exposure.
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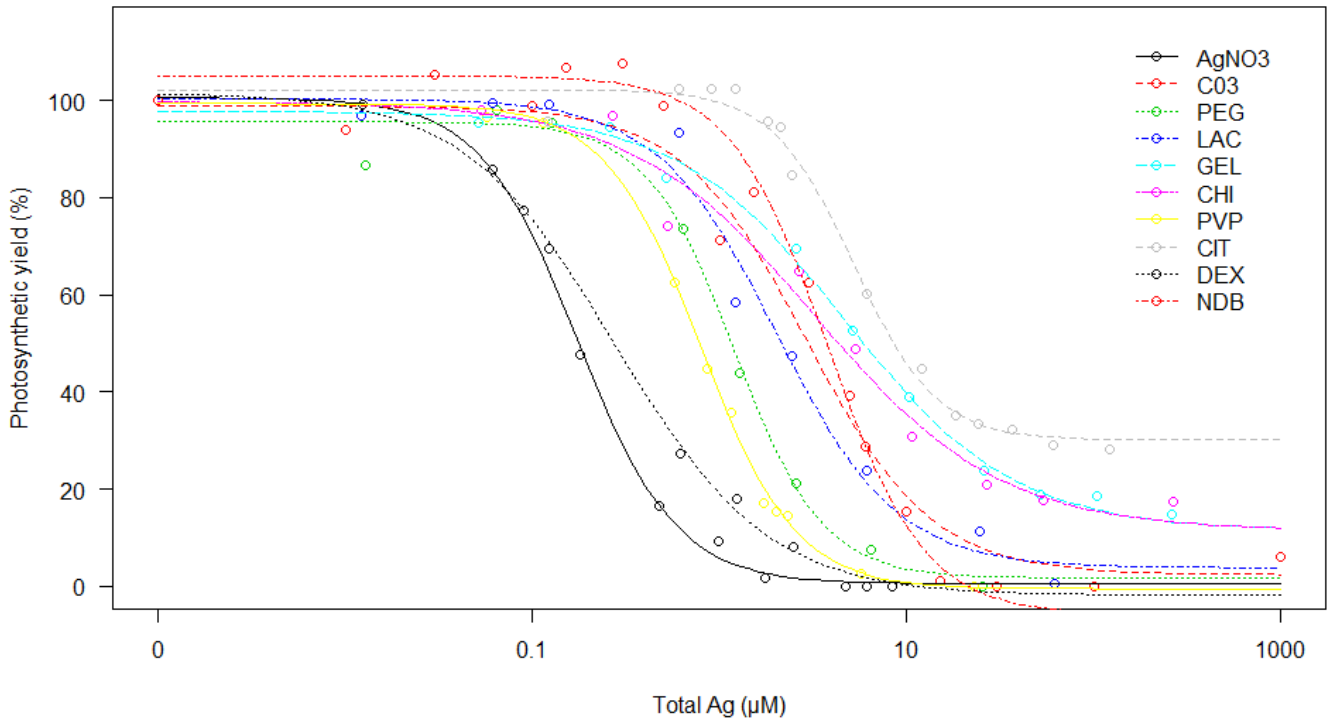
Figure S2. Influence of Cysteine on ζ- potential and size of differently coated AgNPs.
Figure S3. Influence of cysteine on Ag dissolution from the different AgNPs
Calculation S1. Ag_d adhered by the algae



338 **Figure 1.**

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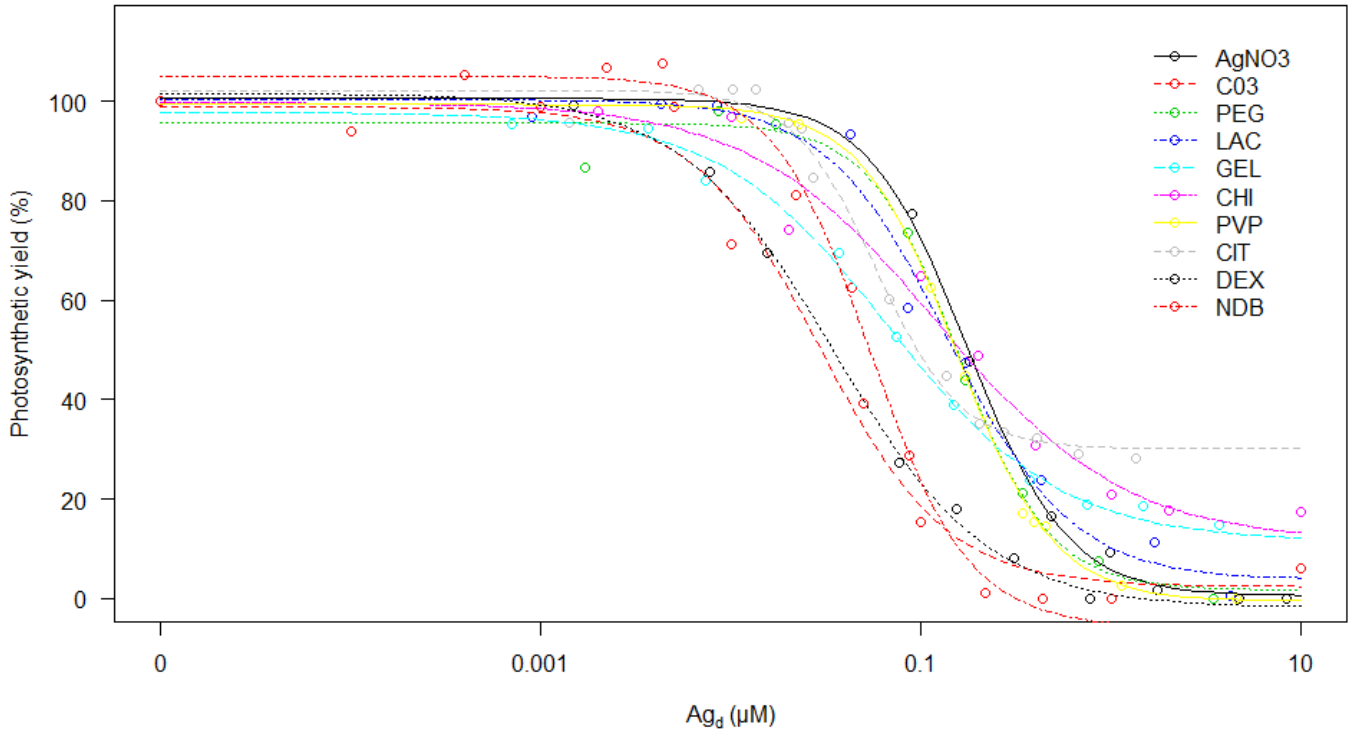

340 **Figure 2.**

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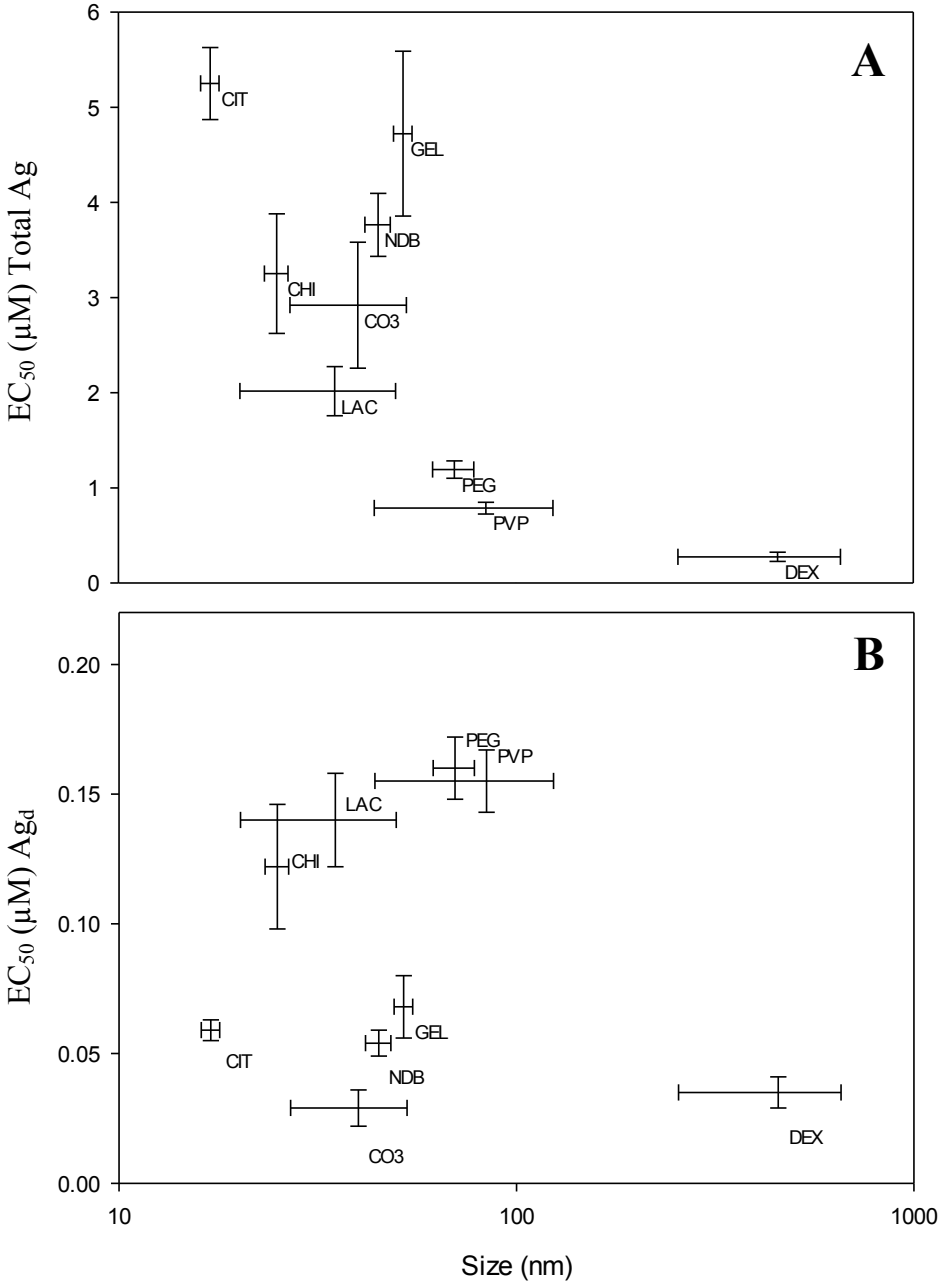

343
344 **Figure 3.**
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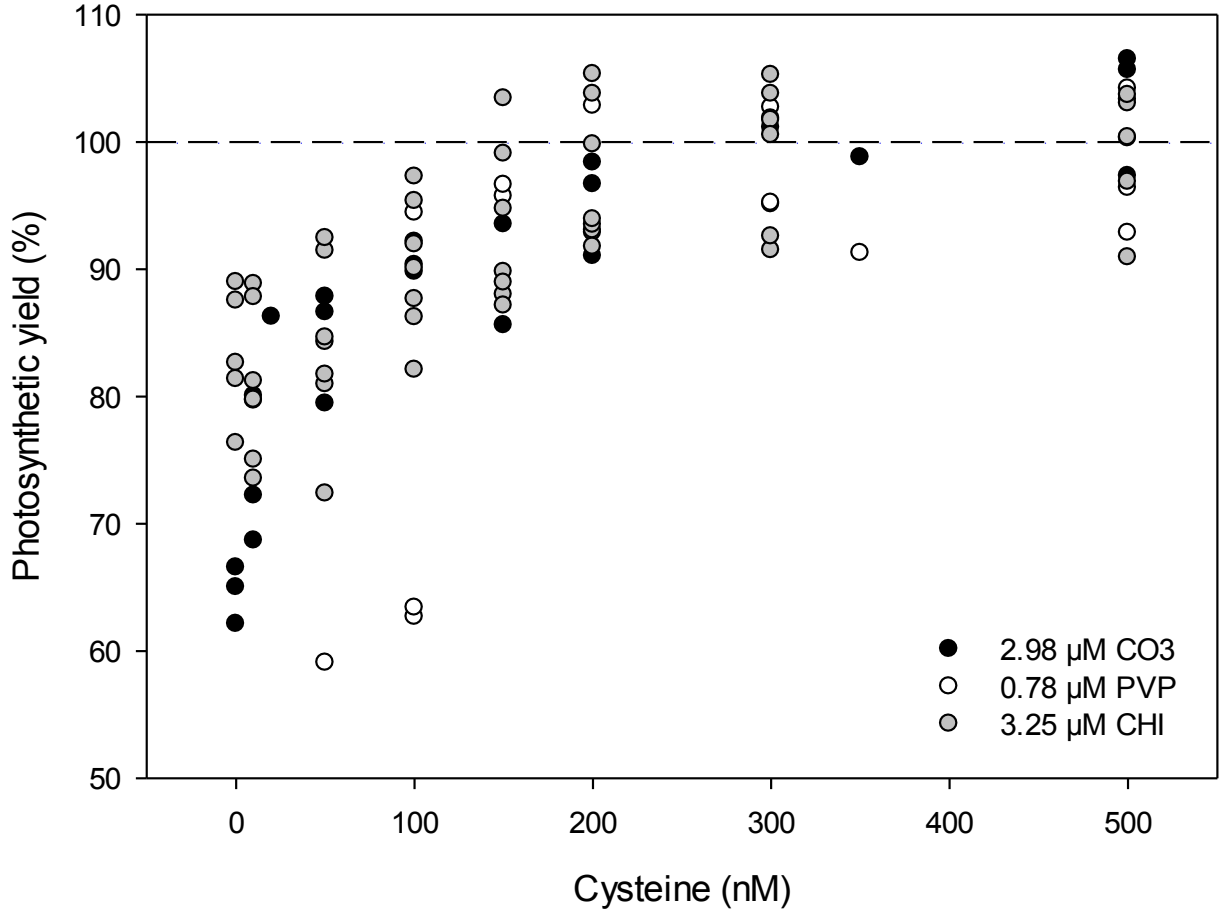

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348 **Figure 4.**

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TABLES

Coating	Chemical family	Total Ag (mM)	Size (nm)	ζ Pot. (mV)	% of Ag_d 1h	% of Ag_d 2h
Carbonate (CO3)	Carbonates	9.27	40 ± 0.5	-36 ± 3.2	0.7 ± 0.1	0.8 ± 0.1
Chitosan (Chi)	Polypeptide+sugar	4.93	25 ± 1.7	-5.1 ± 1.7	3.7 ± 0.2	4.9 ± 0.1
Citrate (Cit)	Carboxylic acid	11.25	17 ± 0.9	-29 ± 3.3	1.1 ± 0.04	1.4 ± 0.03
Dexpanthenol (Dex)	Peptide (imides)	11.42	456 ± 200	-3.8 ± 2.3	12.5 ± 0.69	11.5 ± 0.18
Gelatine (Gel)	Polypeptide	4.79	52 ± 2.8	-6.8 ± 3.0	1.4 ± 0.1	2.4 ± 0.1
Lactate (Lac)	Carboxylic acid	11.35	35 ± 14.8	-3.5 ± 1.5	9.2 ± 2	13.2 ± 3.8
Na-dodecylbenzenesulfonate (NDB)	Detergent	2.80	45 ± 3.3	-29 ± 2.0	1.9 ± 0.1	4.1 ± 2.5
Polyetheleneglycol (PEG)	Hydroxi-alcohol polymer	11.88	70 ± 8.3	-5.5 ± 2.4	16.2 ± 2.4	15.5 ± 2.3
Polyvinyl poly pyrrolidone (PVP)	Hydrocarbons (alkenes)	10.67	84 ± 40.0	-6.3 ± 3.1	19.6 ± 0.6	19.8 ± 1.5

Table 1. AgNP characteristics. Total Ag measured in AgNP stock solutions, average AgNP size, ζ- potential, and Ag_d (as percentage of total Ag) measured in AgNP suspensions 1 and 2 h after dilution in 10 mM MOPS, pH 7.5.

A. Total Ag				**B. Dissolved Ag**		
AgNP	EC_{50} (µM) at 1h	EC_{50} Ratio	p-value	EC_{50} (µM)	EC_{50} Ratio	p-value
$AgNO_3$	0.18 ± 0.03	-	-	0.176 ± 0.029	-	-
CO3	2.92 ± 0.66	6.01E-02	0.000	0.029 ±0.007	6.0	0.004
CHI	3.25 ± 0.62	5.40E-02	0.000	0.122 ±0.024	1.4	0.240
CIT	5.25 ± 0.38	3.34E-02	0.000	0.059 ±0.004	2.9	0.000
DEX	0.28 ± 0.05	6.35E-01	0.021	0.035 ±0.006	5.1	0.001
GEL	4.72 ± 0.86	3.72E-02	0.000	0.068 ±0.012	2.6	0.016
LAC	2.02 ± 0.26	8.71E-02	0.000	0.140 ±0.018	1.2	0.348
NDB	3.76 ± 0.33	4.66E-02	0.000	0.054 ±0.005	3.2	0.000
PEG	1.19 ± 0.09	1.47E-01	0.000	0.160 ±0.012	1.1	0.626
PVP	0.79 ± 0.06	2.23E-01	0.000	0.155 ±0.012	1.1	0.529

Table 2: EC_{50} values (1 h) and standard error of all AgNP calculated based on measured total (A) and measured Ag) concentrations (B), and ratio of EC_{50} of $AgNO_3$ to EC_{50} of each AgNP. The result of the statistical comparisons of EC_{50} values (as a function of Ag_d) of $AgNO_3$ relative to coated AgNP is shown in the last column; if the EC_{50} ratio significantly differs from 1 (p-value < 0.05), those EC_{50} values are significantly different.

AgNP	Total Ag (µM)	Ag_d (µM)	Cysteine (µM)	Ratio Cys/Ag_d	Corrected EC_{50} (Ag_{bioav})
AgNO3	0.50	0.500	0.595	1.19	0.21
CO3	2.98	0.021	0.306	14.67	0.43
CHI	3.25	0.120	0.316	2.63	0.32
CIT	3.20	0.035	0.130	3.69	0.22
DEX	1.52	0.190	0.369	1.94	0.07
GEL	5.17	0.072	0.599	8.28	0.56
LAC	2.02	0.186	0.327	1.76	0.25
NDB	3.77	0.072	0.072	1.01	0.05
PEG	1.19	0.193	0.593	3.08	0.48
PVP	0.78	0.153	0.270	1.74	0.27

Table 3. Total Ag concentrations at which algae have been exposed, measured concentration of Ag_d , estimated cysteine concentration that completely prevents the toxicity of each AgNP, ratio of cysteine to measured Ag_d and EC_{50} values corrected as function of estimated Ag_{bioav}. This has been calculated multiplying the EC50 values as a function of Ag_d (from Table 2B) by the cysteine/Ag_d ratio.

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Species	Stoich. Matrix	Log K	Conc. (mol/l)	Log conc.
Ag+	1 0 0	0.00E+00	4.52E-11	-1.03E+01
AgCysH	1 1 1	2.27E+01	5.00E-07	-6.30E+00
Ag(CysH)2-	1 2 2	3.67E+01	1.10E-11	-1.10E+01
Cys2-	0 1 0	0.00E+00	7.12E-12	-1.12E+01
CysH-	0 1 1	1.08E+01	1.39E-08	-7.86E+00
CysH2	0 1 2	1.91E+01	8.61E-08	-7.07E+00
CysH3+	0 1 3	2.08E+01	1.34E-13	-1.29E+01
H+	0 0 1	0.00E+00	3.10E-08	-7.51E+00
Components	Mode	Initial Conc.	In or out of system	Case of
Ag+	total	5.00E-07	----	AgNO3
Cys2-	total	6.00E-07	----	
H+	free	3.10E-08	7.17E-07	

Species	Stoich. Matrix	Log K	Conc. (mol/l)	Log conc.
Ag+	1 0 0	0.00E+00	1.64E-09	-8.78E+00
AgCysH	1 1 1	2.27E+01	2.98E-07	-6.53E+00
Ag(CysH)2-	1 2 2	3.67E+01	1.08E-13	-1.30E+01
Cys2-	0 1 0	0.00E+00	1.17E-13	-1.29E+01
CysH-	0 1 1	1.08E+01	2.29E-10	-9.64E+00
CysH2	0 1 2	1.91E+01	1.41E-09	-8.85E+00
CysH3+	0 1 3	2.08E+01	2.20E-15	-1.47E+01
H+	0 0 1	0.00E+00	3.10E-08	-7.51E+00
Components	Mode	Initial Conc.	In or out of system	Case of
Ag+	total	3.00E-07	----	AgNO3
Cys2-	total	3.00E-07	----	
H+	free	3.10E-08	3.32E-07	

Species	Stoich. Matrix	Log K	Conc. (mol/l)	Log conc.
Ag+	1 0 0	0.00E+00	7.75E-12	-1.11E+01
AgCysH	1 1 1	2.27E+01	6.00E-08	-7.22E+00
Ag(CysH)2-	1 2 2	3.67E+01	9.26E-13	-1.20E+01
Cys2-	0 1 0	0.00E+00	4.98E-12	-1.13E+01
CysH-	0 1 1	1.08E+01	9.74E-09	-8.01E+00
CysH2	0 1 2	1.91E+01	6.03E-08	-7.22E+00
CysH3+	0 1 3	2.08E+01	9.36E-14	-1.30E+01
H+	0 0 1	0.00E+00	3.10E-08	-7.51E+00
Components	Mode	Initial Conc.	In or out of system	Case of
Ag+	total	6.00E-08	----	CIT-AgNP
Cys2-	total	1.30E-07	----	
H+	free	3.10E-08	2.21E-07	

Species	Stoich. Matrix	Log K	Conc. (mol/l)	Log conc.
Ag+	1 0 0	0.00E+00	2.71E-11	-1.06E+01
AgCysH	1 1 1	2.27E+01	5.40E-08	-7.27E+00
Ag(CysH)2-	1 2 2	3.67E+01	2.15E-13	-1.27E+01
Cys2-	0 1 0	0.00E+00	1.28E-12	-1.19E+01
CysH-	0 1 1	1.08E+01	2.51E-09	-8.60E+00
CysH2	0 1 2	1.91E+01	1.55E-08	-7.81E+00
CysH3+	0 1 3	2.08E+01	2.41E-14	-1.36E+01
H+	0 0 1	0.00E+00	3.10E-08	-7.51E+00
Components	Mode	Initial Conc.	In or out of system	Case of
Ag+	total	5.40E-08	----	NDB-AgNP
Cys2-	total	7.20E-08	----	
H+	free	3.10E-08	1.19E-07	

Table S1. Ag^+ speciation calculations examples using ChemEQL 3.0 (Müller, 1996) First scenario show that a slight excess of cysteine concentrations (0.6 cysteine vs 0.5 $AgNO_3$), already resulted in extremely low Ag^+ concentrations (4.5E-11). In the second case, even at an equimolar scenario (0.3 for both compounds), the amount of Ag^+ is around 1E-9.

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Reaction	log K	Reference
$Ag^+ + Cys^{2-} + H^+ \leftrightarrows AgCysH$	22.7	1
$Ag^+ + 2\ Cys^{2-} + 2\ H^+ \leftrightarrows Ag(CysH)_2^-$	36.7	
$Ag^+ + H_2O \leftrightarrows AgOH + H^+$	-12	2
$Ag^+ + 2\ H_2O \leftrightarrows Ag(OH)_2^- + 2\ H^+$	-24	
$Cys^{2-} + H^+ \leftrightarrows CysH^-$	10.8	
$Cys^{2-} + 2\ H^+ \leftrightarrows CysH_2$	19.1	
$Cys^{2-} + 3\ H^+ \leftrightarrows CysH_3^+$	20.8	

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Table S2. Stability constants used to calculate the Ag^+ concentrations at equilibrium with cysteine. Constants for AgCysH and $Ag(CysH)_2$ are calculated from (1) after J. Kramer (personal communication, 2007).

Exp. Number	AgNP coating	(AgNP) µM	(Cysteine) nM
Exp 1	all AgNP	2	
Exp 2	PEG	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 3	PEG	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 4	LAC	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 5	LAC	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 50	
Exp 6	LAC	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 50	
Exp 7	PEG	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 8	PEG	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 9	GEL	0.1, 0.5, 1, 2, 5, 20, 50, 100	
Exp 10	GEL	0.1, 0.5, 1, 2, 5, 20, 50, 100	
Exp 11	GEL	0.1, 0.5, 1, 2, 5, 20, 50, 100, 200, 500	
Exp 12	CHI	0.1, 0.5, 1, 2, 5, 20, 50, 100	
Exp 13	CHI	0.1, 0.5, 1, 2, 5, 20, 50, 100	
Exp 14	CHI	0.1, 0.5, 1, 2, 5, 20, 50, 100	
Exp 15	PVP	0.05, 0.1, 0.5, 1, 2, 5, 20, 50	
Exp 16	PVP	0.1, 0.5, 0.75, 1, 1.5, 1.75, 2, 5	
Exp 17	PVP	0.1, 0.5, 0.75, 1, 1.5, 1.75, 2, 5	
Exp 18	Lac,Gel,Chi, PVP,PEG	2.16, 4.2, 2.84, 0.78, 1.3 respectively	500
Exp 19	CO3	10	0, 10, 20, 50,100,150,200, 350, 500
Exp 20	GEL	10	0, 10, 20, 50,100,150,200, 350, 500
Exp 21	PVP	10	0, 10, 20, 50,100,150,200, 350, 500
Exp 22	CO3	2.98	0, 10, 20, 50,100,150,200, 350, 500
Exp 23	PVP	0.78	0, 10, 20, 50,100,150,200, 350, 500
Exp 24	PEG	1.3	0, 10, 20, 50,100,150,200, 350, 500
Exp 25	PVP	0.78	0, 10, 20, 50,100,150,200,300, 350, 500
Exp 26	PEG	1.3	0, 10, 20, 50,100,150,200,300, 350, 500
Exp 27	CO3	2.98	0, 10, 20, 50,100,150,200,300, 350, 500
Exp 28	PVP	0.78	0, 10, 20, 50,100,150,200,300, 350, 500
Exp 29	CO3	2.98	0, 10,50,100,150,200, 300, 500
Exp 30	CO3	2.98	0, 10,50,100,150,200, 300, 500
Exp 31	PVP	0.78	0, 10,50,100,150,200, 300, 500
Exp 32	PEG	1.3	0, 10,50,100,150,200, 300, 500
Exp 33	LAC	2.16	0, 10,50,100,150,200, 300, 500
Exp 34	CHI	2.84	0, 10,50,100,150,200, 300, 500
Exp 35	CIT	0.1, 0.5, 0.75, 1, 1.5, 1.75, 2, 5	
Exp 36	CIT	0.1, 0.5, 0.75, 1, 1.5, 1.75, 2, 5	
Exp 37	CIT	0.1, 0.5, 0.75, 1, 1.5, 1.75, 2, 5	
Exp 38	DEX	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 39	DEX	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 40	DEX	0.01, 0.05, 0.1, 0.5, 1, 2, 5, 20	
Exp 41	CHI	2.84	0, 10,50,100,150,200, 300, 500
Exp 42	CHI	2.84	0, 10,50,100,150,200, 300, 500
Exp 43	LAC	2.16	0, 10,50,100,150,200, 300, 500
Exp 44	CHI	2.16	0, 10,50,100,150,200, 300, 500
Exp 45	CHI	2.84	0, 10,50,100,150,200, 300, 500
Exp 46	CHI	2.84	0, 10,50,100,150,200, 300, 500
Exp 47	CHI	2.84	0, 10,50,100,150,200, 300, 500
Exp 48	LAC	2.16	0, 10,50,100,150,200, 300, 500
Exp 49	DEX	0.29	0, 10,50,100,150,200, 300, 500
Exp 50	DEX	0.29	0, 10,50,100,150,200, 300, 500

Exp 51	DEX	0.29	0, 10,50,100,150,200, 300, 500
Exp 52	CIT	3.53	0, 10,50,100,150,200, 300, 500
Exp 53	CIT	3.53	0, 10,50,100,150,200, 300, 500
Exp 54	CIT	3.53	0, 10,50,100,150,200, 300, 500
Exp 55	CIT	3.53	0, 2, 5, 10, 20, 50,100, 200
Exp 56	CIT	3.53	0, 2, 5, 10, 20, 50,100, 200
Exp 57	CIT	3.53	0, 2, 5, 10, 20, 50,100, 200
Exp 58	DEX	0.29	0, 2, 5, 10, 20, 50,100, 200
Exp 59	DEX	0.29	0, 2, 5, 10, 20, 50,100, 200
Exp 60	DEX	0.29	0, 2, 5, 10, 20, 50,100, 200
Exp 61	NDB	0.1, 0.5, 1, 5, 20, 50, 100, 200	
Exp 62	NDB	0.1, 0.5, 1, 5, 20, 50, 100, 200	
Exp 63	NDB	0.1, 0.5, 1, 5, 20, 50, 100, 200	
Exp 64	NDB	3.77	0, 10,50,100,150,200, 300, 500
Exp 65	NDB	3.77	0, 10,50,100,150,200, 300, 500
Exp 66	NDB	3.77	0, 10,50,100,150,200, 300, 500
Exp 67	LAC	0, 0.02, 2, 40	
Exp 68	DEX	1	0, 10,50,100,150,200, 300, 500
Exp 69	DEX	1	0, 10,50,100,150,200, 300, 500
Exp 70	DEX	1	0, 10,50,100,150,200, 300, 500
Exp 71	GEL	5	0, 20,50,100,150,200, 350, 500
Exp 72	GEL	10	0, 10,50,100,150,200, 300, 500
Exp 73	GEL	10	0, 10,50,100,150,200, 300, 500
Exp 74	GEL	10	0, 10,50,100,150,200, 300, 500
Exp 75	CIT	0, 0.5, 1, 2 ,5, 10, 15, 20, 30	
Exp 76	CIT	0, 0.5, 1, 2 ,5, 10, 15, 20, 30	
Exp 77	CIT	0, 0.5, 1, 2 ,5, 10, 15, 20, 30	
Exp 78	CIT	0, 0.5, 1, 2 ,5, 10, 15, 20, 30	
Exp 79	CIT	0, 0.5, 1, 2 ,5, 10, 15, 20, 30	
Exp 80	CIT	0, 0.5, 1, 2 ,5, 10, 15, 20, 30	
Exp 81	CIT	0, 0.5, 1, 5, 10, 30, 50, 100	
Exp 82	CIT	0, 0.5, 1, 5, 10, 30, 50, 100	
Exp 83	CIT	0, 0.5, 1, 5, 10, 30, 50, 100	

Table S3. List of experiments, detailing AgNP (nominal) and cysteine concentrations used; every experiment was done using 3 replicates. In experiments using cysteine, a control algal treatment without AgNP was used; the rest of algae were exposed to the fix AgNP concentration shown on the third column and to the increasing cysteine concentrations shown in the fourth column. Different AgNP and cysteine concentrations were used in order to obtain the better concentration-response curve possible for modeling purposes.

Coating	Time	(conc) µM	Size ave	St dev	PdI	St dev	ζ	stdev
CO3	1	920	40.2	0.5	0.4	4.2E-03	-36.0	3.2
CO3	47	920	44.7	1.5	0.3	4.9E-03	-35.3	3.4
CHI	1	2.7	25.7	1.7	0.4	2.3E-03	-5.1	1.7
CHI	2	2.7	23.1	0.6	0.4	1.3E-02	-7.5	1.3
CIT	1	5.2	17.7	0.9	0.6	3.2E-02	-29.4	3.3
CIT	2	5.2	20.5	1.4	0.6	1.8E-01	-26.6	2.4
DEX	1	0.2	456.1	200.1	0.7	7.5E-02	-3.9	2.3
DEX	2	0.2	994.9	999.7	0.8	1.8E-01	-1.0	0.7
GEL	1	10	52.3	2.8	0.3	4.7E-02	-6.8	3.0
GEL	2	10	49.1	1.1	0.3	1.5E-03	-9.6	5.8
GEL	1	50	40.8	0.5	0.2	4.7E-03	-32.1	2.4
LAC	1	10	35.8	14.8	0.2	9.3E-02	-2.6	0.1
LAC	2	10	44.8	4.8	0.4	2.1E-02	-5.9	1.6
LAC	1	1	87.7	55.4	0.6	2.2E-01	-11.5	1.5
LAC	1	5	50.5	1.8	0.6	1.1E-01	-3.9	2.5
NADBS	1	3.7	45.9	3.4	0.4	1.1E-01	-29.5	2.0
NADBS	2	3.7	58.8	3.4	0.4	3.1E-02	-17.6	2.1
PEG	1	10	70.9	8.3	0.3	7.8E-02	-5.5	2.5
PEG	2	10	82.8	15.8	0.4	2.2E-01	-5.6	1.3
PEG	1	1	115.0	21.7	0.7	5.6E-02	-3.1	0.4
PEG	1	5	30.1	1.1	0.5	1.2E-01	-1.3	1.3
PVP	1	2.7	71.7	23.3	0.5	1.7E-01	-6.3	3.2
PVP	2	2.7	97.0	12.7	0.4	1.8E-01	-2.6	0.4

Table S4. Size in nm (including PDI) and ζ- potential measurements. Standard deviations have been calculated using, at least, three replicates. Time units are hours.

Total Ag			
AgNP	EC_{50} (µM) at 1h	$\frac{EC_{50}1h}{EC_{50}2h}$	p-value
$AgNO_3$	0.176 ± 0.029	1.1	0.561
CO3	2.920 ± 0.662	3.3	0.049
CHI	3.252 ± 0.628	1.2	0.616
CIT	5.250 ± 0.378	0.7	0.016
DEX	0.276 ± 0.049	2.0	0.044
GEL	4.723 ± 0.866	0.9	0.729
LAC	2.017 ± 0.258	1.2	0.305
NDB	3.765 ± 0.331	0.7	0.004
PEG	1.193 ± 0.092	1.6	0.004
PVP	0.788 ± 0.062	1.3	0.099

Table S5. The second column shows EC_{50} at 1 hour (error term is standard error) for all AgNP; the third column shows the ratio between EC_{50} (calculated as a total Ag) after 1 and 2 hours of exposure. The p-values < 0.05 indicate ratios significantly different from 1 and thus time-related differences in toxicity expressed as a function of total Ag. While algae exposed to $AgNO_3$, and AgNP coated with CHI, GEL, LAC show very similar EC_{50} after 1 and 2 h of exposure; AgNP coated with CO3, CIT, DEX, NDB, PEG and PVP show a lower EC_{50} after 2 h.

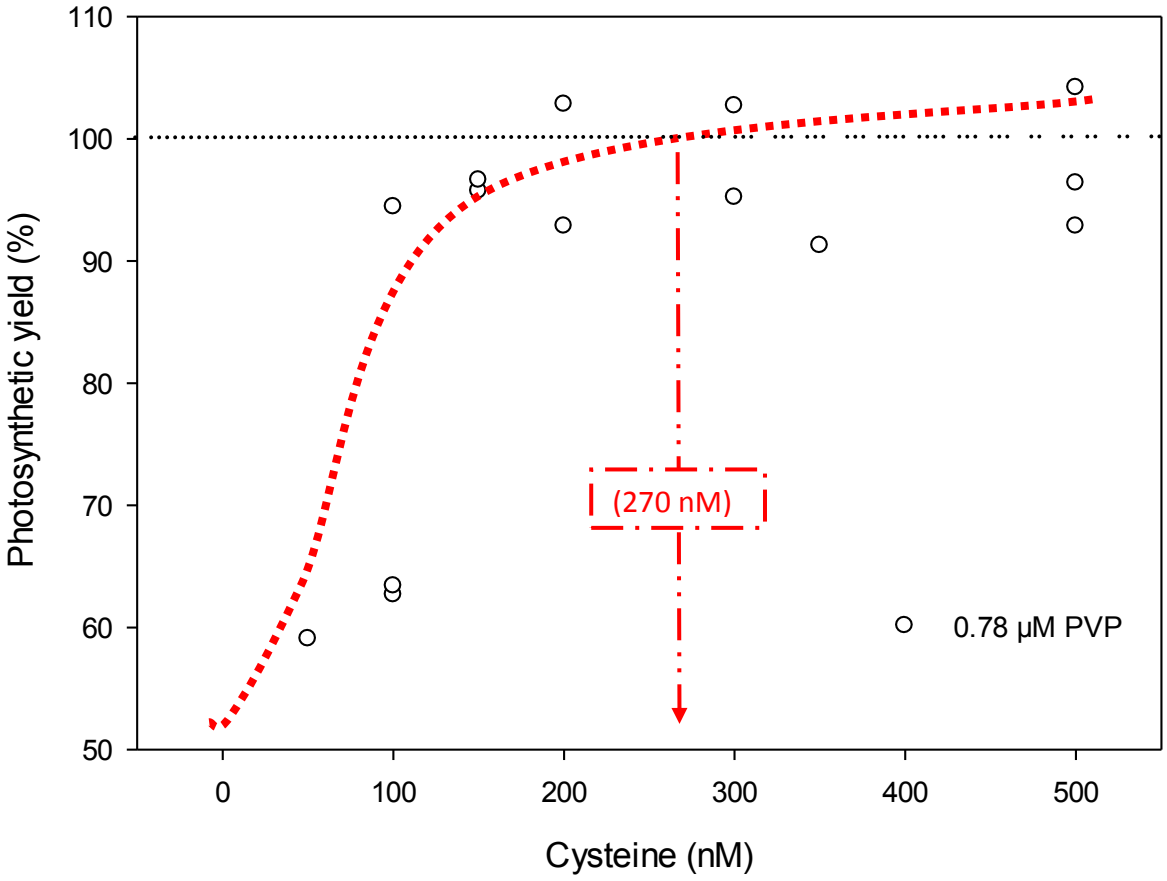


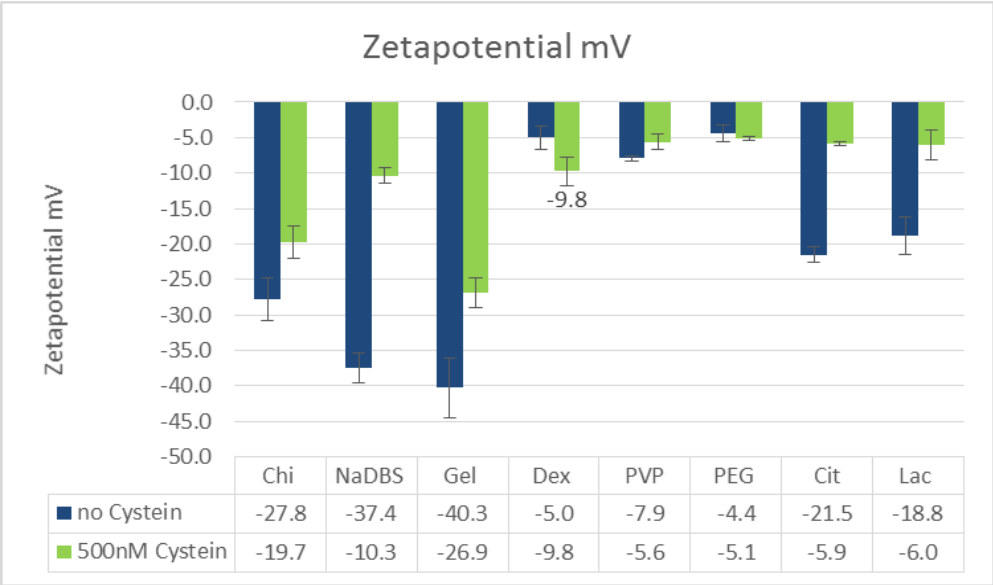
Figure S1. Example of the calculation of the amount of cysteine required to prevent the toxicity of a certain amount of PVP coated AgNP to the photosynthetic yield of *C. reinhardtii*. Graph shows the effect of increasing concentrations of cysteine to the photosynthetic yield. In this example, toxicity data from PVP coated AgNPs were fitted to a four parameters logistic model, that was the one showing the best fitting:

Algae exposed to 0.783 µM of AgNP coated with PVP				
y = min + (max-min)/(1 + (x/EC50)^(-Hillslope))		Using DataFit (Oakley)		
Model fit R sqr = 0.91				
(Cys nM)	Yield	Parameter	Value	StdErr
270	100	min	25.25	4.663
		max	110	7.036
		EC50	84.04	14.42
		Hillslope	1.725	0.5055

The PVP toxicity data model is show using a red dotted line in Figure S1. The calculated parameters are shown in the table. These were later used for estimating the (Cys nM) that resulted in a value of 100% of the photosynthetic yield:

Photosynthetic yield 100 = 25.25 + (110-25.5)/(1 + ((cysteine)/84.04)^(-1.725))

In this case, the amount of cysteine that prevents the toxicity to the photosynthesis (i.e. value of 99.7 % of photosynthesis vs. control) is 270 nM.



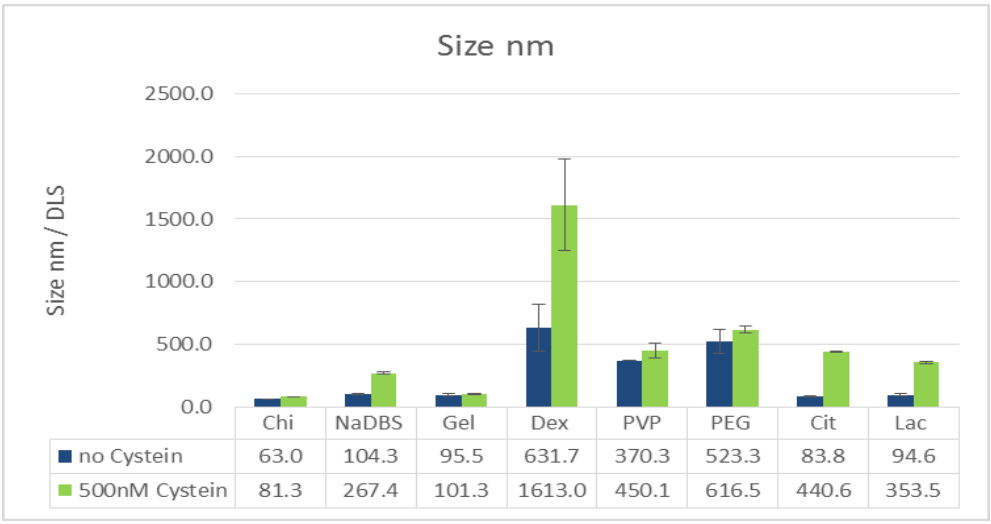
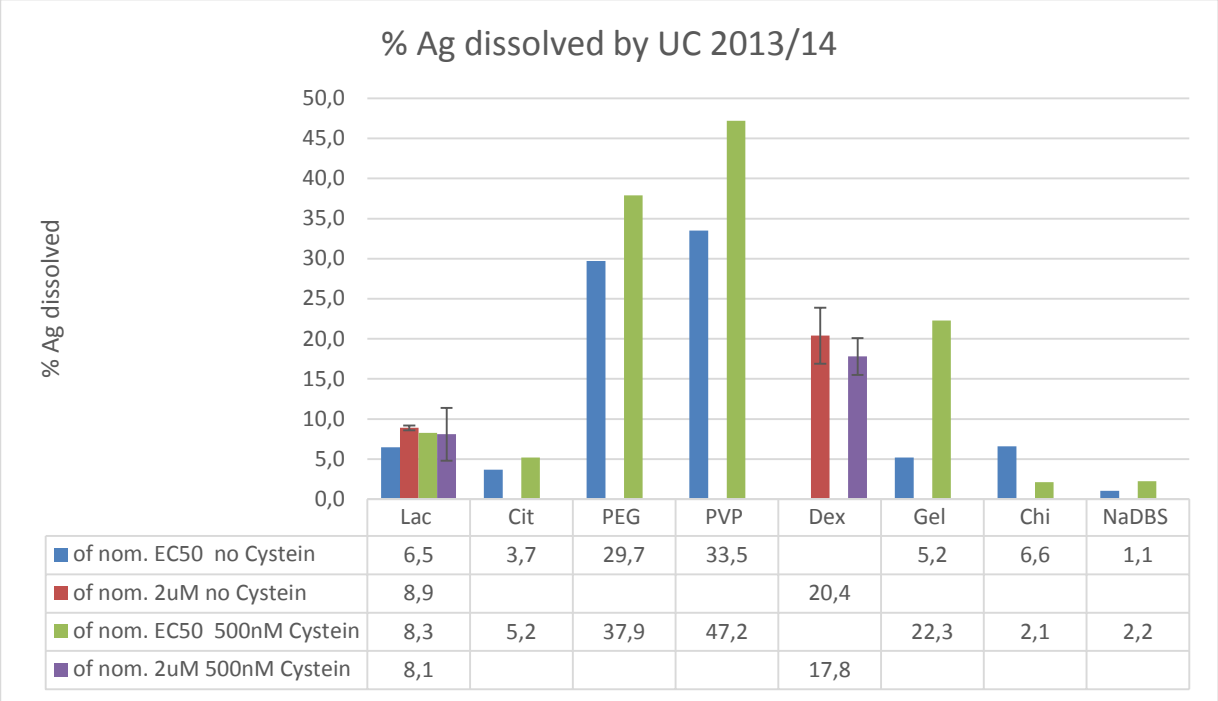


Figure S2. Influence of cysteine on ζ- potential and size of differently coated AgNP assessed at total Ag concentrations corresponding to the EC_{50} values. These measurements have been carried out 2 years after the original experiments. Thus absolute values were not considered in this new version of the manuscript.



	Lac	Cit	PEG	PVP	Dex	Gel	Chi	NaDBS
of nom. EC50 no Cystein	6,5	3,7	29,7	33,5		5,2	6,6	1,1
of nom. 2uM no Cystein	8,9				20,4			
of nom. EC50 500nM Cystein	8,3	5,2	37,9	47,2		22,3	2,1	2,2
of nom. 2uM 500nM Cystein	8,1				17,8			

Figure S3. Influence of cysteine on Ag dissolution from the different AgNPs used in this study. Previous data obtained for 5 µM carbonate coated AgNP and different concentrations of cysteine shown that after 1 h dissolution is increased at concentrations equal or higher to 1 µM cysteine. The methods used here are those detailed in the paper; the Ag_d present in AgNPs suspensions (at the corresponding EC_{50} values or at 2 µM as indicated in the legend of the last graph) with and without cysteine (500 nM) was measured by Centrifugal ultrafiltration followed by ICP-MS.

These measurements have been carried out 2 years after the original experiments. Even if the absolute values were not considered in this new version of the manuscript, the influence of cysteine in particle dissolution has been included in the discussion.

Calculation S1. Ag_d ADHERED BY THE ALGAE

Example of calculation with data from Piccapietra's paper (ref. 26):

For 2 µM AgNP intracellular Ag is $5x10^{-4}$ mol L_{cell}^{-1}

With the cell volume V_{cell} = 100 fL, Ag(intracellular) = $5x10^{-17}$ mol/cell

With $1.5x10^{8}$ cells /L

Ag(cells) = $7.5x10^{-9}$ mol/L

The cysteine wash would account for a factor 2-3 for dissolved Ag. Adsorbed AgNP are not relevant in this calculation, as they may contribute to Ag-uptake by dissolution in contact with the cells.