Supplementary Information

Silver nanoparticle toxicity and association with the alga *Euglena gracilis*

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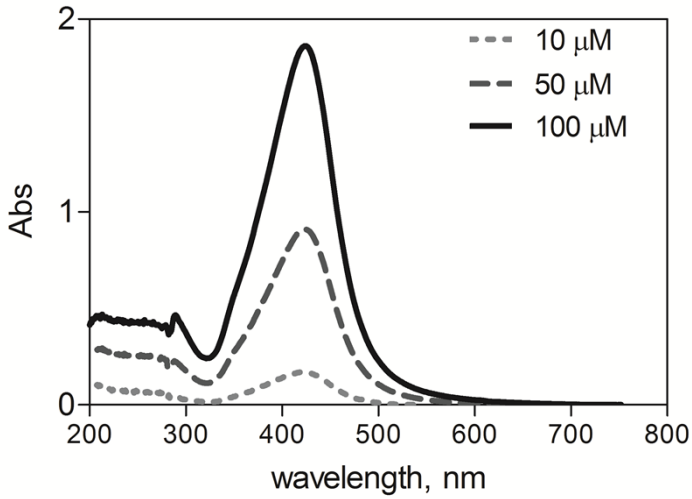

Fig. S1 UV-vis spectra of 10, 50, 100 μM AgNP measured after 1 h dilution in 10 mM MOPS, pH 7.5.

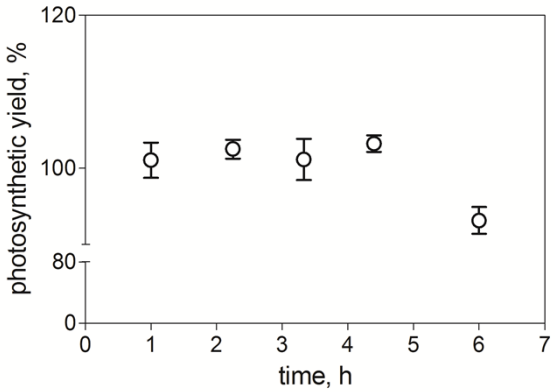

Fig. S2 Photosynthetic yield of *E. gracilis* in 10 mM MOPS, pH 7.5 as a function of time.

Table S1 Recalculated AgNP EC50 values based on different percentages of measured dissolved silver

Dissolved silver	**Time**	**AgNP EC_{50}, nM**	**95% CI, nM**	***p* value[a]**
0.5%	1 h	10	8-11	<0.0001
	2 h	7	6-9	<0.0001
1.7%	1 h	32	26-39	<0.0001
	2 h	16	14-19	<0.0001
3.5%	1 h	65	53-80	0.0036
	2 h	52	45-60	<0.0001

[a] Data analyzed using *F*-test. $p < 0.001$, AgNP significantly different from $AgNO_3$

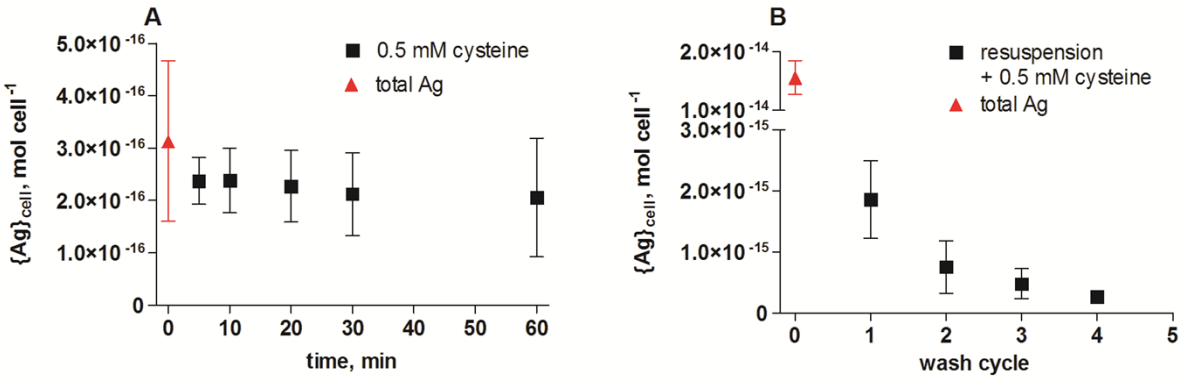

Fig. S3 Preliminary experiments for establishing the wash protocol for the uptake studies. Algae were exposed to 50 nM $AgNO_3$ and 5 µM AgNP for 1 h. To remove surface adsorbed silver ions in $AgNO_3$ exposure, algae were centrifuged (1200 × g, 5 min), resuspended in fresh MOPS (10 mM, pH 7.5) containing 0.5 mM cysteine, and then gently stirred. Between 5-60 min, algae were filtrated for metal analysis. Algae without washing were also measured to determine total silver. The metal analysis showed that the cysteine wash after as little as 5 min resulted in a constant level of cell-associated silver $\{Ag\}_{cell}$ and therefore was selected as optimal washing protocol for $AgNO_3$ (A). To remove the surface adsorbed AgNP, algae were centrifuged and resuspended in MOPS. After 1-4 wash cycles, algae were resuspended in cysteine-MOPS and stirred for 5 min to remove adsorbed silver ions. Algae washed for different cycle number as well as unwashed algae were filtrated for metal analysis. The $\{Ag\}_{cell}$ showed to remain constant after two wash cycles followed by a 5 min cysteine-MOPS wash (B).

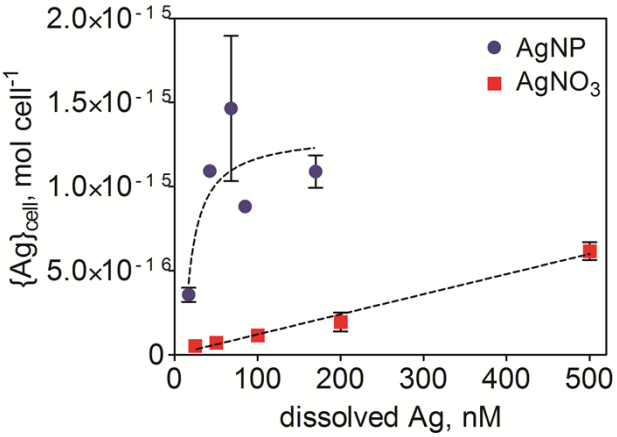

Fig. S4 Modelling of uptake results.

Fitting model for AgNP: $Y=Min\{Ag\}_{cell} + (Max\{Ag\}_{cell} - Min\{Ag\}_{cell})/(1+10\text{^}((Log\ X\text{`}-X)))$

Slope: 2.97e-018 ± 1.87e-018

R square: 0.61

Fitting model for $AgNO_3$: Y=kX + b

Slope: 1.2e-018 ± 7.72e-023

R square: 0.99

Table S2 Calculation of bioconcentration factors (BCF) for $AgNO_3$, possible number of AgNP per cell, and dissolution of AgNP based on $\{Ag\}_{cell}$ measured in the uptake experiments.

Treatment	$\{Ag\}_{cell}$, mol cell^{-1}	$\{Ag\}_{cell}$, mol L_{cell}^{-1}	$^{*}[Ag]_{out}$, mol L^{-1}	BCF, L L_{cell}^{-1}	No. of NP	Dissol. %
25 nM $AgNO_3$	5.31E-17	3.49E-05	2.49E-08	1713		
50 nM $AgNO_3$	7.32E-17	4.71E-05	4.99E-08	1080		
100 nM $AgNO_3$	1.17E-16	8.07E-05	9.98E-08	898		
200 nM $AgNO_3$	1.95E-16	1.39E-04	2.00E-07	756		
500 nM $AgNO_3$	6.17E-16	1.44E-04	4.99E-07	339		
1 μM AgNP	3.57E-16	1.86E-04			68	35
2.5 μM AgNP	1.14E-15	4.18E-04			215	44
4 μM AgNP	1.53E-15	5.13E-04			289	37
5 μM AgNP	8.76E-16	4.47E-04			166	18
10 μM AgNP	1.08E-15	3.48E-04			205	11

* $[Ag]_{out}$ is the silver concentration in the medium after subtracting the silver taken up by cells

BCF were calculated as the ratio between cell-associated silver and silver remaining in the exposure medium, based on Equation 1.

The $\{Ag\}_{cell}$ measured in AgNP exposures include silver from the AgNP ($\{Ag\}_{AgNP}$) and the dissolved Ag ($\{Ag\}_{d}$) in AgNP suspensions. For each AgNP exposure concentration (1.7% dissolved Ag), $\{Ag\}_{d}$ was calculated according to the linear uptake model established in the uptake experiments with $AgNO_3$ (Equation 2). Then the fraction derived from $\{Ag\}_{d}$ in AgNP suspensions was subtracted. The $\{Ag\}_{cell}$ after subtraction ($\Delta\{Ag\}_{cell}$) was calculated to correspond to 68~289 AgNP per cell, based on the mean nanoparticle size of 47 nm, silver density (ρ_{Ag}) of 10.49 g cm^{-3}, and silver mass (M_{Ag}) of 107.8682 g mol^{-1} (Equation 3).

On the other hand, assuming that the $\Delta\{Ag\}_{cell}$ corresponded to the uptake of dissolved Ag only, the amount of Ag needed to be present as dissolved Ag ($[Ag]_{exp}$) was estimated according to Equation 2. The dissolution of AgNP was then calculated as the percentage of dissolved Ag to AgNP exposure concentration, based on Equation 4. As a result, 11-44% of AgNP should have dissolved in the exposure medium.

Equation 1. Calculation of BCF. $[Ag]_{exp}$ is the exposure concentration of $AgNO_3$.

$$BCF\left(\frac{L}{L_{cell}}\right) = \frac{\{Ag\}_{cell}\left(\frac{mol}{L_{cell}}\right)}{[Ag]_{out}\left(\frac{mol}{L}\right)} = \frac{\{Ag\}_{cell}\left(\frac{mol}{L_{cell}}\right)}{[Ag]_{exp}\left(\frac{mol}{L}\right) - \{Ag\}_{cell}\left(\frac{mol}{cell}\right) \times \frac{No.\ of\ cells}{L}}$$

Equation 2. Linear uptake model of dissolved Ag ($\{Ag\}_d$) upon exposure to different concentrations of $AgNO_3$.

$$\{Ag\}_d\left(\frac{mol}{cell}\right) = [Ag]_{exp}(nM) \times 10^{-18} + 3 \times 10^{-18}$$

Equation 3. Calculation of nanoparticle number per cell (a). $\Delta\{Ag\}_{cell}$ representing the $\{Ag\}_{cell}$ after subtraction the $\{Ag\}_d$ in AgNP suspensions. (b) Calculation of M_{AgNP} based on d_{AgNP} = 47 nm, V_{AgNP}, and M_{AgNP}, representing diameter, volume and mass of single AgNP, ρ_{Ag} (silver density) = 10.49 g cm^{-3}, and M_{Ag} (silver mass) = 107.8682 g mol^{-1}.

(a)

$$\frac{No.\ of\ AgNP}{cell} = \frac{\Delta\{Ag\}_{cell}\left(\frac{mol}{cell}\right) \times M_{Ag}\left(\frac{g}{mol}\right)}{M_{AgNP}\left(\frac{g}{AgNP}\right)}$$

(b)

$$M_{AgNP}\left(\frac{g}{AgNP}\right) = V_{AgNP}\left(\frac{m^3}{AgNP}\right) \times \rho_{Ag}\left(\frac{g}{m^3}\right) = \left(\frac{d_{AgNP}}{2}\right)^3 \times \pi \times \frac{4}{3} \times \rho_{Ag}\left(\frac{g}{m^3}\right)$$

Equation 4. Calculation of nanoparticle dissolution. $[Ag]_{exp}$ is calculated based on Equation 2. $[AgNP]_{exp}$ is the exposure concentration of AgNP.

$$\%\ of\ AgNP\ dissolution = \frac{[Ag]_{exp}(nM) \times 10^{-3}}{[AgNP]_{exp}(\mu M)} \times 100$$

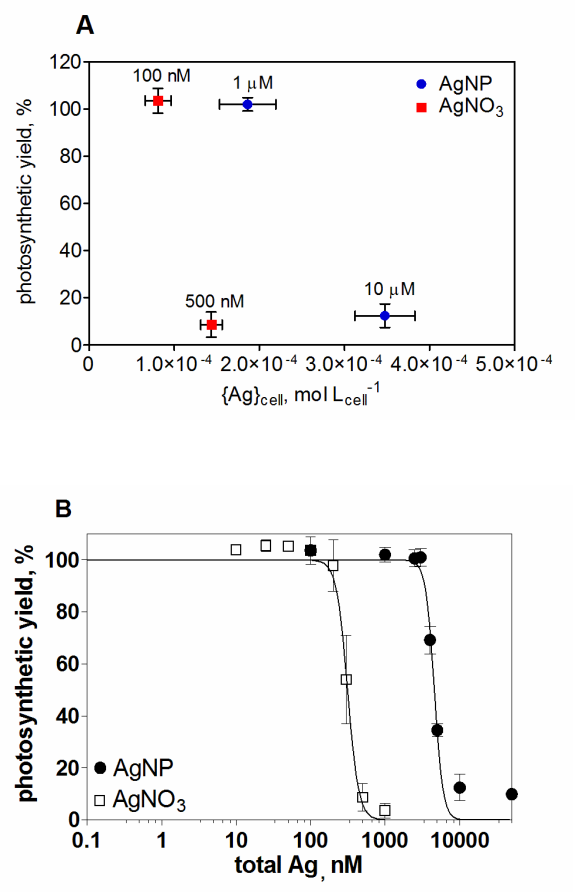

Fig. S5 Photosynthetic yield plotted as a function of $\{Ag\}_{cell}$. The photosynthetic yield was measured at a cell density of 1.0×10^5 cell/mL upon 1 h exposure to $AgNO_3$ and AgNP (B). The estimated EC_{50}s were 312 nM for $AgNO_3$ and 77 μM for AgNP. $\{Ag\}_{cell}$ were obtained from uptake experiments. For 100 nM $AgNO_3$, the $\{Ag\}_{cell}$ was 8.1×10^{-5} mol L_{cell}^{-1} and the photosynthetic yield of algae was close to control (105%). At a higher $AgNO_3$ exposure concentration, 500 nM, the photosynthetic yield decreased to 8.7% of control cells while the $\{Ag\}_{cell}$ reached 1.4×10^{-4} mol L_{cell}^{-1}. In case of 1 μM AgNP, the measured $\{Ag\}_{cell}$ was 1.9×10^{-4} mol L_{cell}^{-1}, yet the photosynthetic yield remained uninhibited (102%). The decrease of photosynthetic yield to 12% was observed when $\{Ag\}_{cell}$ reached 5.1×10^{-4} mol L_{cell}^{-1} upon exposure to 10 μM AgNP.

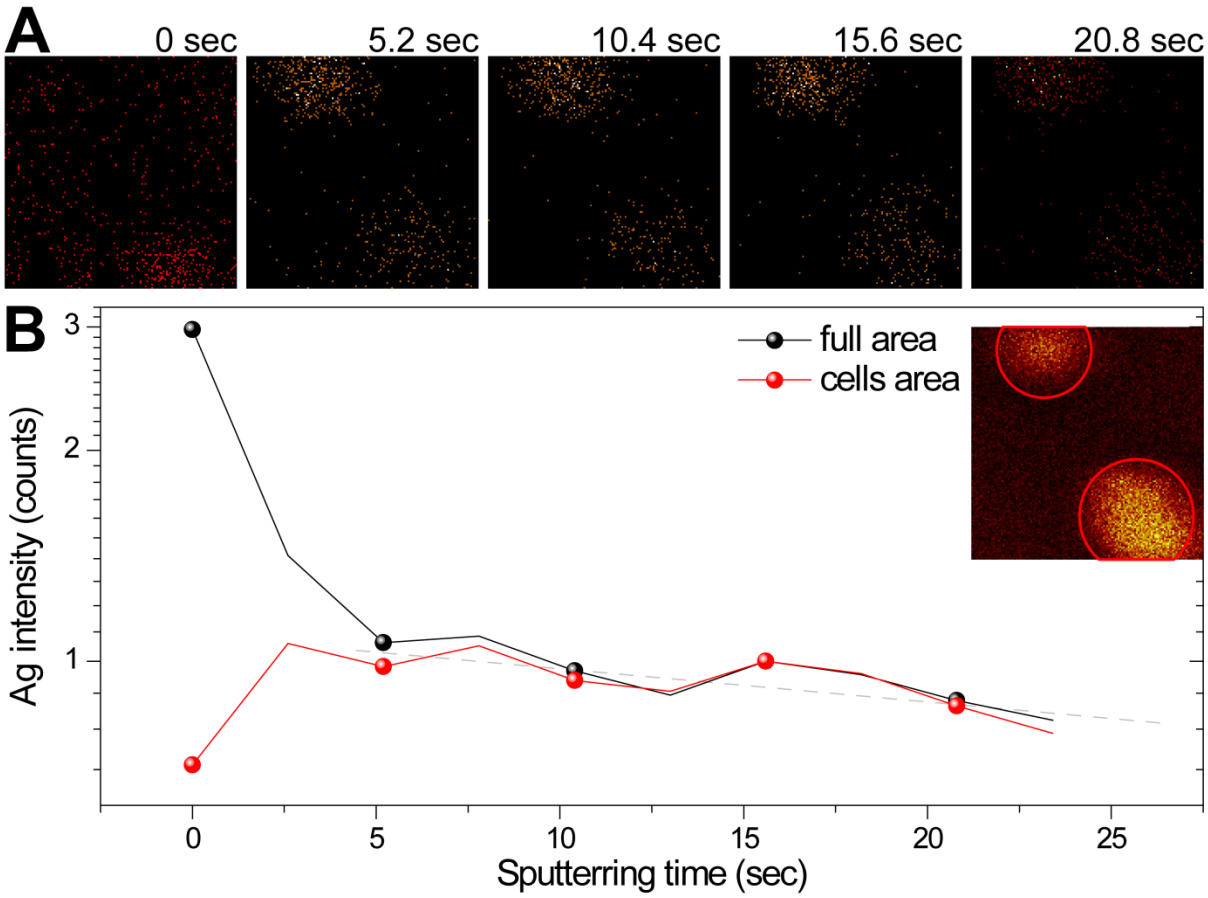

Fig. S6 ToF-SIMS in-depth sputtering on cells after 1 h of exposure to 5 µM AgNP. Silver maps ($^{107}Ag^+$ + $^{109}Ag^+$) of the topmost surface, after 5.2, 10.4, 15.6 and 20.8 sec of sputtering (A). Surface area: 150 × 150 µm^2. Silver intensity as a function of the sputtering time, with the full area (black line) and cell area (red line) analyzed. The carbon map shows the position of the cells and the areas taken into account for the intensity plot (insert).

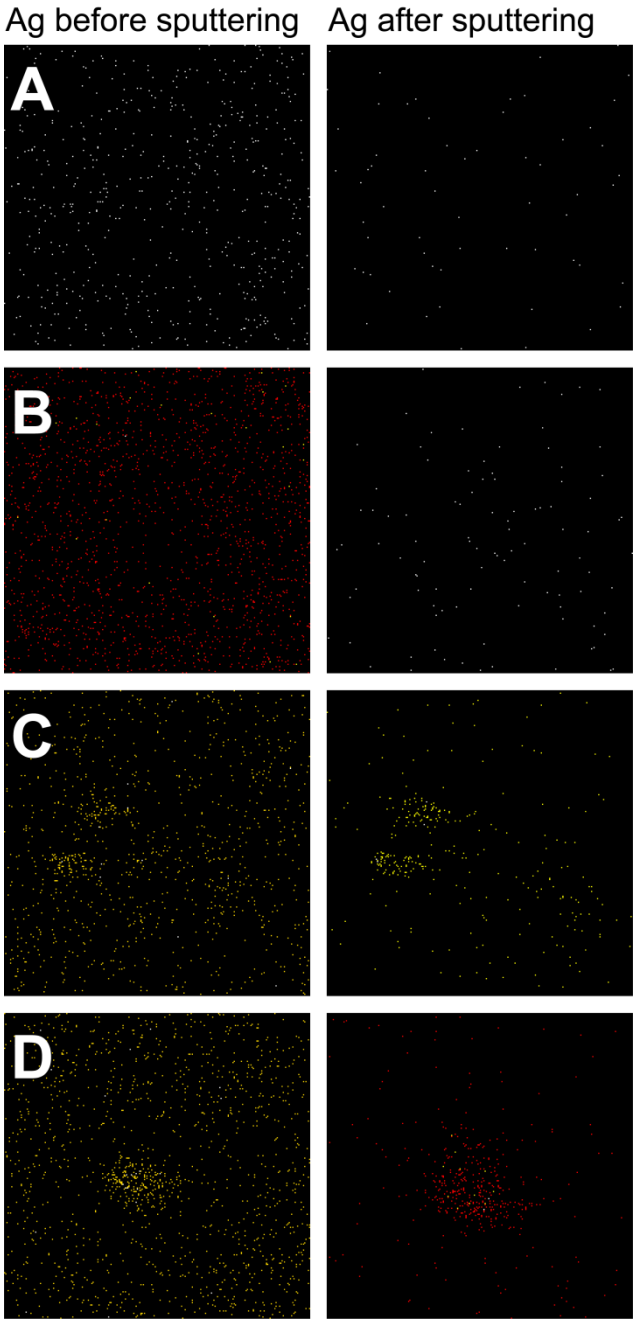

Fig. S7 ToF-SIMS chemical analysis of silver before and after sputtering of the control cell (A), cell exposed to 250 nM $AgNO_3$ (B), 1 µM (C) and 5 µM AgNP (D). Surface area: 150 × 150 μm^2.