

**Silver Accumulation in the Green Microalga *Coccomyxa actinabiotis*:
Toxicity, *in Situ* Speciation and Localization Investigated using
Synchrotron XAS, XRD and TEM**

Thomas Leonardo, Emmanuel Farhi, Stéphanie Pouget, Sylvie Motellier, Anne-Marie Boisson, Dipanjan Banerjee, Fabrice Rébeillé, Christophe den Auwer, and Corinne Rivasseau*

Supplementary Information:

Number of pages: 4

Number of figures: 2

Number of tables: 1

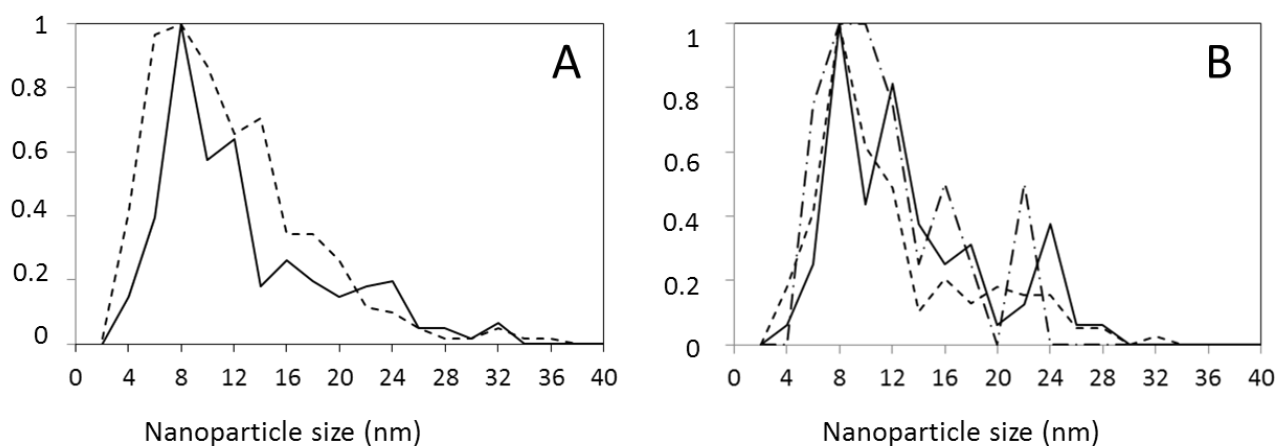
Supplementary Table S1: Identical to Table 1 with the list of all the Ag-Ag distances included in the fitting procedure of algae samples exposed to silver at 10⁻³ and 10⁻⁴ M.

Sample	Ag-S	Ag-Ag	S_0^2 , Δe_0 , R factor, χ^2_R
Algae exposed to 10 ⁻³ M Ag ⁺ (15 K)	0.7(1) S at 2.46(1) Å $\sigma^2 = 0.0057 \text{ Å}^2$	3.9(1) Ag at 2.87(1) Å $\sigma^2 = 0.0035 \text{ Å}^2$ 2.0 Ag at 4.06 Å $\sigma^2 = 0.0056 \text{ Å}^2$ 7.8 Ag at 4.98 Å $\sigma^2 = 0.0054 \text{ Å}^2$ 31.2 Ag at 5.36 Å* $\sigma^2 = 0.0039 \text{ Å}^2$ 3.9 Ag at 5.75 Å $\sigma^2 = 0.0062 \text{ Å}^2$ 7.8 Ag at 5.75 Å* $\sigma^2 = 0.0069 \text{ Å}^2$ 3.9 Ag at 5.75 Å* $\sigma^2 = 0.0069 \text{ Å}^2$	$S_0^2 = 0.9$ $\Delta e_0 = 2.53(30)$ R factor = 0.030 $\chi^2_R = 0.10$
Algae exposed to 10 ⁻⁴ M Ag ⁺ (15 K)	0.7(1) S at 2.46(1) Å $\sigma^2 = 0.0050 \text{ Å}^2$	3.6(1) Ag at 2.87(2) Å $\sigma^2 = 0.0032 \text{ Å}^2$ 1.8 Ag at 4.06 Å $\sigma^2 = 0.0056 \text{ Å}^2$ 7.2 Ag at 4.98 Å $\sigma^2 = 0.0049 \text{ Å}^2$ 28.8 Ag at 5.36 Å* $\sigma^2 = 0.0036 \text{ Å}^2$ 3.6 Ag at 5.75 Å $\sigma^2 = 0.0047 \text{ Å}^2$ 7.2 Ag at 5.75 Å* $\sigma^2 = 0.0064 \text{ Å}^2$ 3.6 Ag at 5.75 Å* $\sigma^2 = 0.0064 \text{ Å}^2$	$S_0^2 = 0.9$ $\Delta e_0 = 1.73(24)$ R factor = 0.019 $\chi^2_R = 0.19$

Numbers in italics are linked by geometry to the parameters of the first path. * indicates multiple scattering paths.

Supplementary Figure S1. Size distribution of nanoparticles in microalgae (Normalized particle number as a function of particle size, in nm).

(A) Size distribution depending on silver concentration in microalgae exposed to 10^{-2} M (solid line) and 10^{-4} M (dashed line) Ag^+ ; (B) Size distribution depending on subcellular localization in microalgae exposed to 10^{-2} M Ag^+ : chloroplast (solid line), mitochondria (dashed line) and vacuoles (dash-dotted line). The statistical analysis has been performed with 200 particles at 10^{-2} M and 70 particles at 10^{-4} M.



Supplementary Figure S2. Physiological impact of silver on *C. actinabiotis* microalgae exposed to various Ag^+ concentrations and recovery thereafter. Change in (A) the photosynthetic yield (photochemical yield F_v/F_m of photosystem II) and in (B) algal growth (cellular density) as a function of time. Microalgae at $2 \text{ g}_{\text{FW}}\cdot\text{L}^{-1}$ were exposed to Ag^+ in ultrapure water for 2 h and transferred into a silver-free growth medium, indicated by the “T” arrow on the graphs. The first value at time zero in Figure S2A corresponds to the photochemical yield just before silver addition. Silver addition is indicated by the “ Ag^+ ” arrow on the graph.

