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Supplementary Information

Interactions of silver nanoparticles with antioxidant enzymes

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Supplementary information for AF4-MD-ICPMS measurements: A trapezoidal channel of 350 μm thickness and a 1 kDa cut-off regenerated cellulose membrane (RC, Postnova Analytics) for the accumulation wall were used. A carrier solution made of 10 mM HEPES pH 7 pre-filtered on 0.1 μm Teflon filters (Postnova Analytics) was employed. The samples were injected manually using a 0.1 mL sample loop at $V_{\text{inj}} = 0.2 \text{ mL min}^{-1}$ during 5 min. The focusing conditions were $V_{\text{foc}} = 3.88 \text{ mL min}^{-1}$, $V_{\text{XF}} = 2.7 \text{ mL min}^{-1}$ and $V_{\text{out}} = 1 \text{ mL min}^{-1}$; the elution step was operated during 20 min using a constant V_{XF} of 1 ml min^{-1} finished by a 2.5 min linear gradient to $V_{\text{XF}} = 0 \text{ ml min}^{-1}$. The runs were ended by a 10 min elution step at $V_{\text{XF}} = 0 \text{ mL min}^{-1}$. For online analysis of metallic species, the outflow of the last detector was splitted using a 3 ways peek connector, one way was directly linked to the peristaltic pump of the ICPMS detector and the other to the waste tank. The ICP-MS tune was performed using the MassHunter workstation software (Agilent technology) in the He ORS mode to avoid poly-atomic interferences. The calibration solutions containing increased metal concentrations from 0.025 ppb to 10 ppb were prepared daily by diluting a standard solution (ICP multi-element standard solution VI, Merck Millipore) in 2 % HNO_3 .

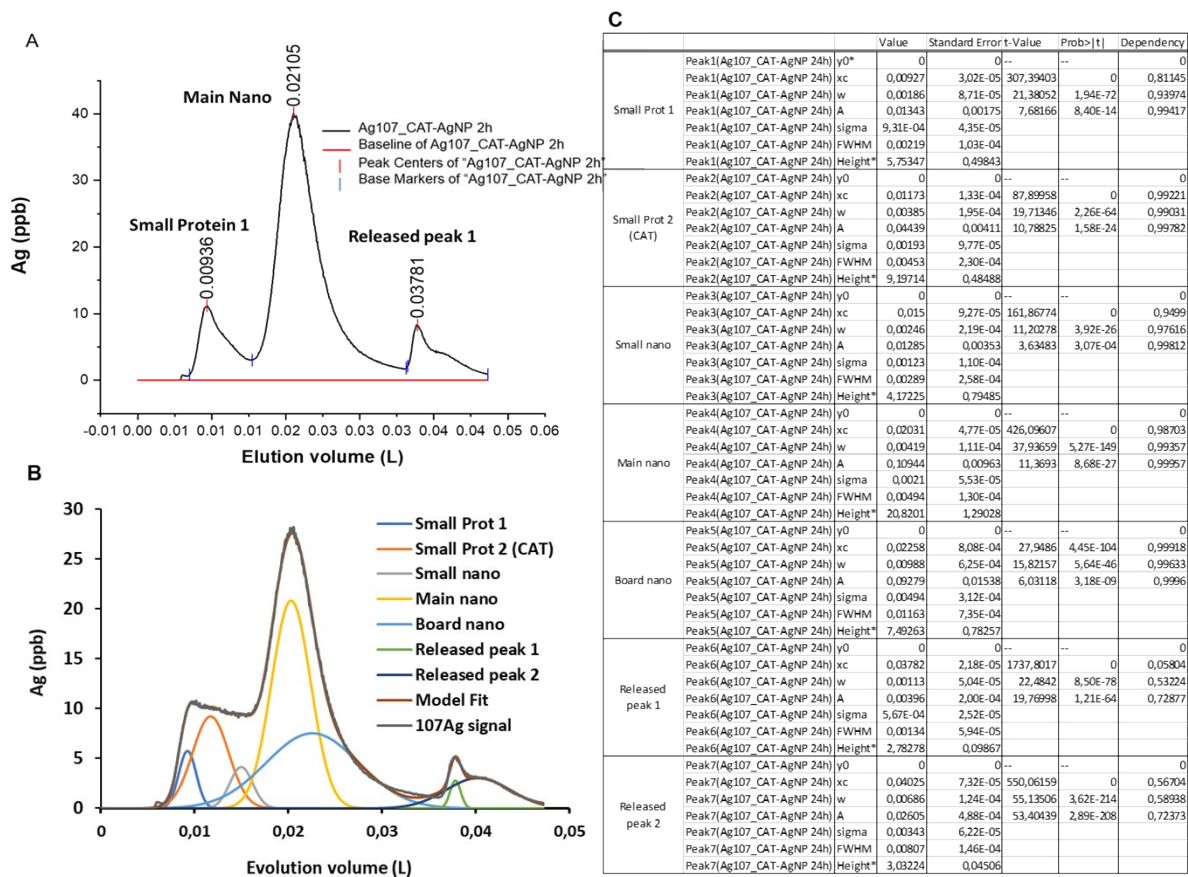


Fig SI-1. Peak assignment of Ag fractograms obtained by AF4-ICP-MS after 2h (A) or 24h (B) of incubation of CAT with AgNPs. Peak deconvolution used to quantify the amount of silver in each Ag-containing size fraction and % of Ag that is not as a nanoparticle form (e.g. dissolved Ag). For (A) only the peak 2 was attributed to remaining AgNPs. For (B) a more complex deconvolution of the signal needs to be done, and peaks 3,4,5 were used to calculate the remaining AgNPs in the systems. Dissolution was determined by subtracting the total concentration of AgNPs to the total concentration injected. (C) Characteristic of Gauss modelled peak variables used to evaluate the area of Ag species quantities from (B).

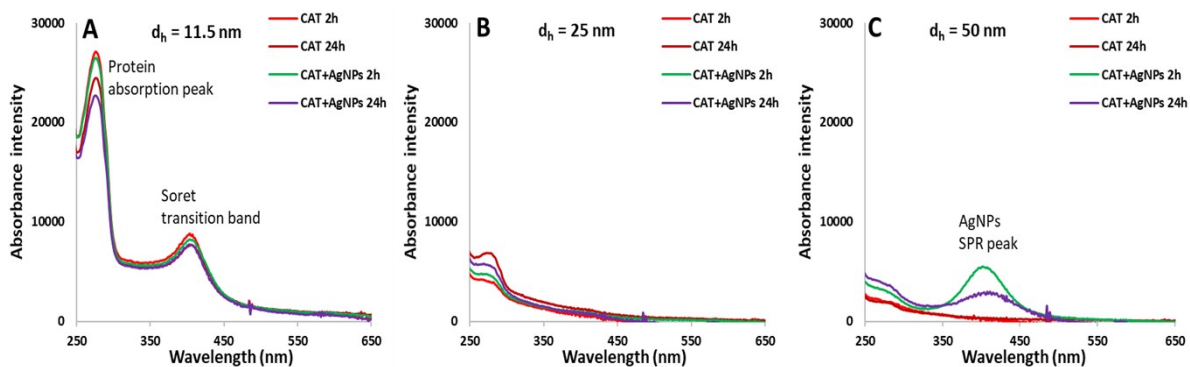


Figure SI-2. UV-visible spectra obtained online by AF4-DAD for 5 μM CAT suspension in the absence and presence of 35 μM AgNPs, for material with different hydrodynamic diameters: at the CAT maximum adsorption peak, 11.5 nm (A), between CAT and AgNPs peaks, 25 nm (B) and at the maximum adsorption peak of AgNPs, 50 nm (C). CAT $t = 2$ h (bright red) and $t = 24$ h (dark red) and in the presence of AgNPs after 2 h (green) and 24 h (purple) of interaction.

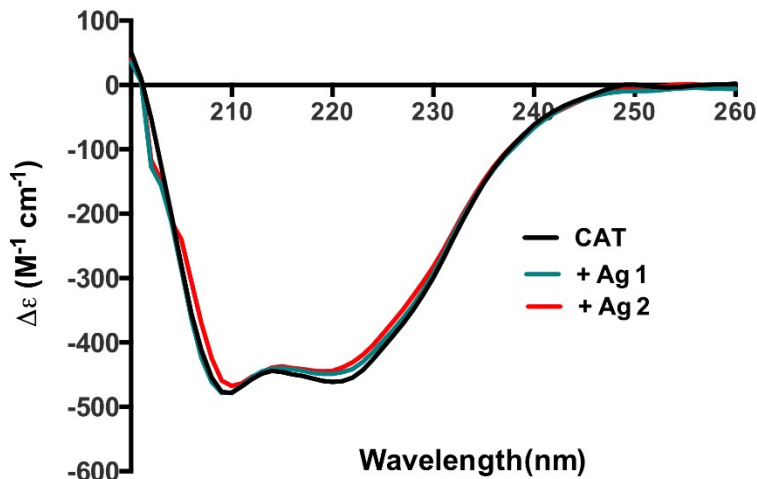


Figure SI-3. CD spectral evolution of 1 μM CAT expressed in $\Delta\epsilon$ ($\text{M}^{-1} \text{cm}^{-1}$) in the presence of AgNO_3 at 2.8 μM (Ag1) and 4.3 μM (Ag2) corresponding the dissolved fraction of 10 μM AgNPs at 2h (28%) and 24h (43%).

Table SI-1. Calculated contents of the enzymes secondary structures in the absence and presence of AgNPs.

Condition	Content of secondary structure (%)			
	α -Helix	β -sheet	β -Turn	Random coil
Native CAT 1 μ M	33.1	8.7	25.0	33.2
CAT+AgNPs 10 μ M (t0)	28.7	13.9	23.7	33.6
CAT+AgNPs 10 μ M (24h)	22.7	11.1	26.2	39.9
CAT+AgNO ₃ 2.8 μ M	34.1	6.9	23.5	35.4
CAT+ AgNO ₃ 4.3 μ M	32.2	12.9	19.0	35.8
Native SOD 9 μ M	9.6	19.0	40.6	30.8
SOD+AgNPs 9 μ M	9.3	17.7	41.2	31.9
SOD+AgNPs 18 μ M	7.7	21.6	38.4	32.3
SOD+AgNPs 27 μ M	7.8	19.9	40.2	32.0
SOD+AgNPs 36 μ M	8.0	21.0	38.9	32.1

The % of different secondary structure was calculated using fitting-analysis of CD spectra (**Fig. 5**) as reported by Chen et al ¹. AgNO₃ concentrations corresponded to the dissolved fraction of AgNPs at 2h (28%) and 24h (43%).

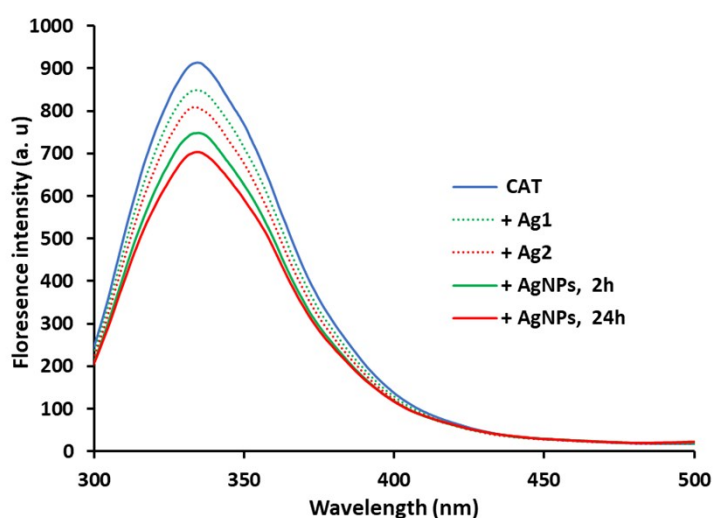


Figure SI-4. Fluorescence spectra of 1 μ M CAT in the absence and the presence of 10 μ M AgNPs at 2 and 24h, and AgNO₃ at 2.8 (Ag1) and 4.3 μ M (Ag2) corresponding the dissolved fraction of AgNPs at 2h (28%) and 24h (43%).

Reference

- 1 Chen, Y. H., Yang, J. T. & Chau, K. H. Determination of the helix and beta form of proteins in aqueous solution by circular dichroism. *Biochemistry* **13**, 3350-3359 (1974).