Supplementary Information

Interactions of silver nanoparticles with antioxidant enzymes

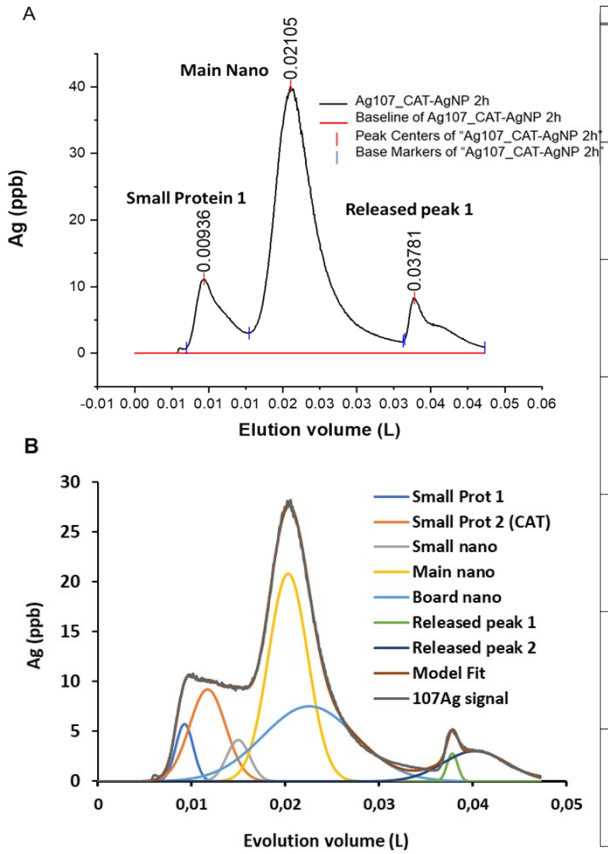
Wei LIU*, Isabelle Worms and Vera I. Slaveykova

University of Geneva, Faculty of Sciences, Earth and Environment Sciences. Switzerland

* Corresponding author; Email: wei.liu@unige.ch

Address: Department F.-A. Forel for Environmental and Aquatic Sciences, Uni Carl Vogt, 66 Blvd Carl-Vogt, CH 1211 Geneva, Switzerland

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_{inj}= 0.2 mL min^{-1} during 5 min. The focusing conditions were V_{foc} = 3.88 mL min^{-1}, V_{XF} = 2.7 mL min^{-1} and V_{out} = 1 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_{XF} = 0 ml min^{-1}. The runs were ended by a 10 min elution step at V_{XF} = 0 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.



C

			Value	Standard Error	t-Value	Prob>|t|	Dependency
Small Prot 1	Peak1(Ag107_CAT-AgNP 24h)	y0*	0	0	--	--	0
	Peak1(Ag107_CAT-AgNP 24h)	xc	0,00927	3,02E-05	307,39403	0	0,81145
	Peak1(Ag107_CAT-AgNP 24h)	w	0,00186	8,71E-05	21,38052	1,94E-72	0,93974
	Peak1(Ag107_CAT-AgNP 24h)	A	0,01343	0,00175	7,68166	8,40E-14	0,99417
	Peak1(Ag107_CAT-AgNP 24h)	sigma	9,31E-04	4,35E-05			
	Peak1(Ag107_CAT-AgNP 24h)	FWHM	0,00219	1,03E-04			
	Peak1(Ag107_CAT-AgNP 24h)	Height*	5,75347	0,49843			
Small Prot 2 (CAT)	Peak2(Ag107_CAT-AgNP 24h)	y0	0	0	--	--	0
	Peak2(Ag107_CAT-AgNP 24h)	xc	0,01173	1,33E-04	87,89958	0	0,99221
	Peak2(Ag107_CAT-AgNP 24h)	w	0,00385	1,95E-04	19,71346	2,26E-64	0,99031
	Peak2(Ag107_CAT-AgNP 24h)	A	0,04439	0,00411	10,78825	1,58E-24	0,99782
	Peak2(Ag107_CAT-AgNP 24h)	sigma	0,00193	9,77E-05			
	Peak2(Ag107_CAT-AgNP 24h)	FWHM	0,00453	2,30E-04			
	Peak2(Ag107_CAT-AgNP 24h)	Height*	9,19714	0,48488			
Small nano	Peak3(Ag107_CAT-AgNP 24h)	y0	0	0	--	--	0
	Peak3(Ag107_CAT-AgNP 24h)	xc	0,015	9,27E-05	161,86774	0	0,9499
	Peak3(Ag107_CAT-AgNP 24h)	w	0,00246	2,19E-04	11,20278	3,92E-26	0,97616
	Peak3(Ag107_CAT-AgNP 24h)	A	0,01285	0,00353	3,63483	3,07E-04	0,99812
	Peak3(Ag107_CAT-AgNP 24h)	sigma	0,00123	1,10E-04			
	Peak3(Ag107_CAT-AgNP 24h)	FWHM	0,00289	2,58E-04			
	Peak3(Ag107_CAT-AgNP 24h)	Height*	4,17225	0,79485			
Main nano	Peak4(Ag107_CAT-AgNP 24h)	y0	0	0	--	--	0
	Peak4(Ag107_CAT-AgNP 24h)	xc	0,02031	4,77E-05	426,09607	0	0,98703
	Peak4(Ag107_CAT-AgNP 24h)	w	0,00419	1,11E-04	37,93659	5,27E-149	0,99357
	Peak4(Ag107_CAT-AgNP 24h)	A	0,10944	0,00963	11,3693	8,68E-27	0,99957
	Peak4(Ag107_CAT-AgNP 24h)	sigma	0,0021	5,53E-05			
	Peak4(Ag107_CAT-AgNP 24h)	FWHM	0,00494	1,30E-04			
	Peak4(Ag107_CAT-AgNP 24h)	Height*	20,8201	1,29028			
Board nano	Peak5(Ag107_CAT-AgNP 24h)	y0	0	0	--	--	0
	Peak5(Ag107_CAT-AgNP 24h)	xc	0,02258	8,08E-04	27,9486	4,45E-104	0,99918
	Peak5(Ag107_CAT-AgNP 24h)	w	0,00988	6,25E-04	15,82157	5,64E-46	0,99633
	Peak5(Ag107_CAT-AgNP 24h)	A	0,09279	0,01538	6,03118	3,18E-09	0,9996
	Peak5(Ag107_CAT-AgNP 24h)	sigma	0,00494	3,12E-04			
	Peak5(Ag107_CAT-AgNP 24h)	FWHM	0,01163	7,35E-04			
	Peak5(Ag107_CAT-AgNP 24h)	Height*	7,49263	0,78257			
Released peak 1	Peak6(Ag107_CAT-AgNP 24h)	y0	0	0	--	--	0
	Peak6(Ag107_CAT-AgNP 24h)	xc	0,03782	2,18E-05	1737,8017	0	0,05804
	Peak6(Ag107_CAT-AgNP 24h)	w	0,00113	5,04E-05	22,4842	8,50E-78	0,53224
	Peak6(Ag107_CAT-AgNP 24h)	A	0,00396	2,00E-04	19,76998	1,21E-64	0,72877
	Peak6(Ag107_CAT-AgNP 24h)	sigma	5,67E-04	2,52E-05			
	Peak6(Ag107_CAT-AgNP 24h)	FWHM	0,00134	5,94E-05			
	Peak6(Ag107_CAT-AgNP 24h)	Height*	2,78278	0,09867			
Released peak 2	Peak7(Ag107_CAT-AgNP 24h)	y0	0	0	--	--	0
	Peak7(Ag107_CAT-AgNP 24h)	xc	0,04025	7,32E-05	550,06159	0	0,56704
	Peak7(Ag107_CAT-AgNP 24h)	w	0,00686	1,24E-04	55,13506	3,62E-214	0,58938
	Peak7(Ag107_CAT-AgNP 24h)	A	0,02605	4,88E-04	53,40439	2,89E-208	0,72373
	Peak7(Ag107_CAT-AgNP 24h)	sigma	0,00343	6,22E-05			
	Peak7(Ag107_CAT-AgNP 24h)	FWHM	0,00807	1,46E-04			
	Peak7(Ag107_CAT-AgNP 24h)	Height*	3,03224	0,04506			

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 calculated 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).

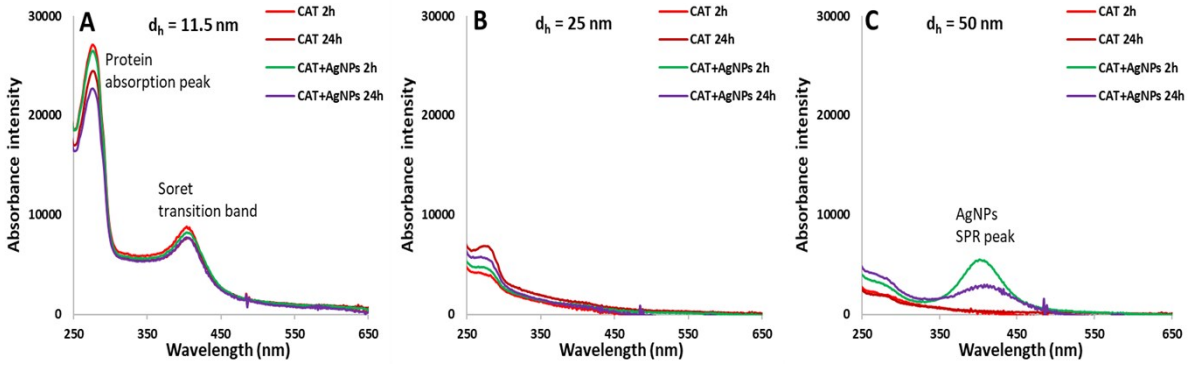


Figure SI-2. UV-visible spectra obtained online by AF4-DAD for 5 µM CAT suspension in the absence and presence of 35 µM AgNPs, for material with different hydrodynamic diameters: at the CAT maximum adsorption peak, 11.5 nm (A), between CAT and AgNPs peaks, 25 nm (B) and at the maximum adsorption peak 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.

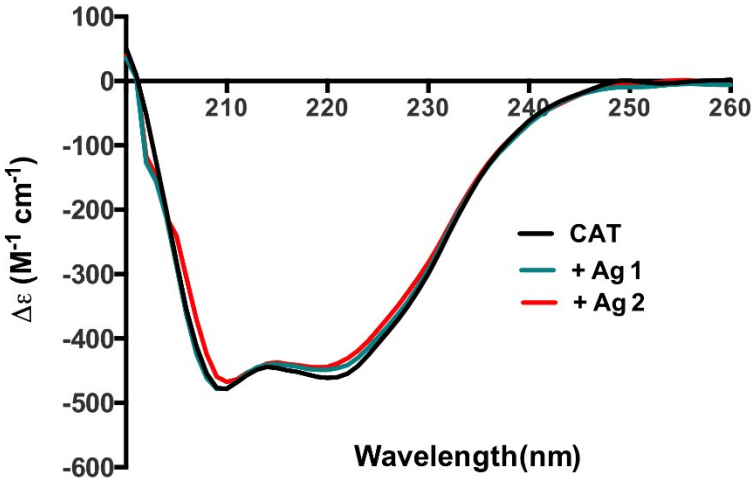


Figure SI-3. CD spectral evolution of 1 µM CAT expressed in Δε (M^{-1} cm^{-1}) in the presence of $AgNO_3$ at 2.8 µM (Ag1) and 4.3 µM (Ag2) corresponding the dissolved fraction of 10 µM AgNPs at 2h (28%) and 24h (43%).

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 µM	33.1	8.7	25.0	33.2
CAT+AgNPs 10 µM (t0)	28.7	13.9	23.7	33.6
CAT+AgNPs 10 µM (24h)	22.7	11.1	26.2	39.9
CAT+$AgNO_3$ 2.8 µM	34.1	6.9	23.5	35.4
CAT+ $AgNO_3$ 4.3 µM	32.2	12.9	19.0	35.8
Native SOD 9 µM	9.6	19.0	40.6	30.8
SOD+AgNPs 9 µM	9.3	17.7	41.2	31.9
SOD+AgNPs 18 µM	7.7	21.6	38.4	32.3
SOD+AgNPs 27 µM	7.8	19.9	40.2	32.0
SOD+AgNPs 36 µM	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]. $AgNO_3$ concentrations corresponded to the dissolved fraction of AgNPs at 2h (28%) and 24h (43%).

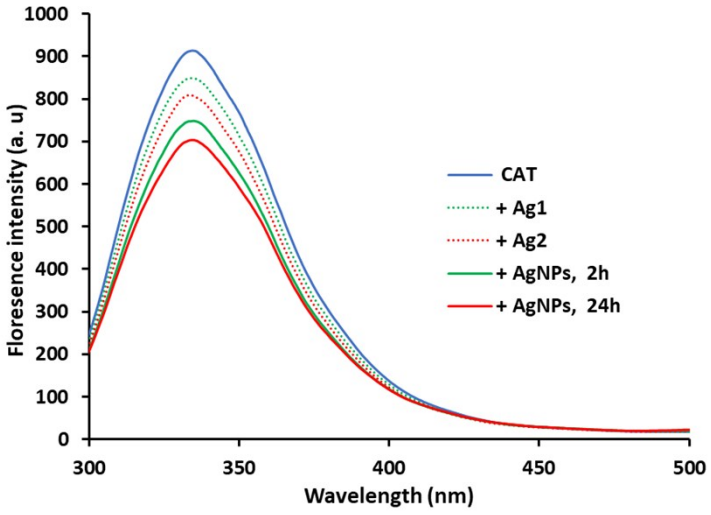


Figure SI-4. Fluorescence spectra of 1 µM CAT in the absence and the presence of 10 µM AgNPs at 2 and 24h, and $AgNO_3$ at 2.8 (Ag1) and 4.3 µM (Ag2) corresponding the dissolved fraction of AgNPs at 2h (28%) and 24h (43%).

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).