Electronic Supplementary Information

Safer-by-design biocide made of tri-thiol bridged silver nanoparticle assemblies

Marianne MARCHIONI,[a] Giulia VERONESI,[a,c] Isabelle WORMS,[a,d,≠] Wai Li LING,[e] Thomas GALLON,[a,b] Didier LEONARD,[f] Christelle GATEAU,[b] Mireille CHEVALLET,[a] Pierre-Henri JOUNEAU,[g] Laura CARLINI,[h] Chiara BATTOCCHIO,[h] Pascale DELANGLE,[b] Isabelle MICHAUD-SORET,*[a] and Aurélien DENIAUD*[a]

[a] Univ. Grenoble Alpes, CNRS, CEA, BIG-LCBM, 38000 Grenoble, France

[b] Univ. Grenoble Alpes, CEA, CNRS, INAC-SYMMES, 38000 Grenoble, France

[c] ESRF, The European Synchrotron. 71 avenue des Martyrs, 38000 Grenoble, France

[d] Univ. Grenoble Alpes, CEA, LITEN/DTNM/SEN/L2N, F-38054 Grenoble Cedex 09, France

[e] Univ. Grenoble Alpes, CEA, CNRS, IBS, F-38000 Grenoble, France

[f] Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, UMR 5280, 5, rue de la Doua, F-69100 Villeurbanne, France

[g] Univ. Grenoble Alpes, CEA, INAC-MEM, 38000 Grenoble, France

[h] Univ. Roma Tre, Dept. of Sciences, Via della Vasca Navale 79 - 00146 - Rome, Italy

[≠] Present address: Department F.-A. Forel for Environmental and Aquatic sciences, University of Geneva, 66 boulevard Carl Vogt CH-1211 Geneva 4.

Experimental Section

Chemicals. GSH was purchased from Sigma. Synthesis and characterization of the molecule L^{3S} was previously described.[1] L^{3S} was dissolved in HEPES buffer (10 mM, pH 7.5) / acetonitrile, 6/4, v/v.

AgNP synthesis. 20 nm diameter AgNPs coated with citrate were synthesized based on [2]. Briefly, AgNPs were produced by reduction of $AgNO_3$ by tannic acid in presence of citrate. An aqueous solution (100 mL) containing 2 mM sodium citrate and 50 nM tannic acid were heated at 90 °C under 800 rpm stirring. When this temperature was reached five sequential additions of $AgNO_3$ (200 µL) at 25 mM were done every 5 min. Upon AgNP synthesis the solution becomes yellow. AgNPs are finally recovered by 1 hour centrifugation at 9000 g. The pellet containing AgNPs was washed twice with an aqueous solution of 2 mM sodium citrate.

AgNP assembly process. AgNPs at a final concentration of 370 µM in Ag were mixed in HEPES-Citrate buffer (10 mM HEPES, 2 mM sodium citrate pH 7) with L^{3S} at various concentrations in order to reach S:Ag molar ratio of 1:1, 0.25:1 or 0.05:1. The solutions were then incubated at 22 °C, under 400 rpm stirring for the desired incubation time before Ag quantification, AgNP SPR signal or DLS measurements. The process was also followed by AF_4-ICP-MS. All these analyses were performed as described in [3]. The experiments performed with GSH and GSSG were done using the same protocol according to S:Ag ratio mentioned in the different figure legends.

AgNP assembly production. For large scale assembly production, a S:Ag molar ratio of 0.25:1 was chosen. After 90 min reaction at 22 °C, the reaction was stopped by addition of 100 µM iodoacetamide. The mixture was further incubated in the same conditions for 2 hours. Assembly populations of different average diameters were fractionated over discontinuous gradient centrifugation made of 50 µL of buffer containing 1.5 M sucrose, 100 µL with 1.15 M sucrose,

200 µL with 0.9 M sucrose and 200 µL with 0.7 M sucrose, on top of which assembly mixtures (500 µL) were loaded. The gradient was centrifuged 20 min at 4400 g at 4 °C. Assemblies of different sizes were visually identified by their different colors and recovered by pipetting layer by layer. Assemblies of average DLS diameter of 40, 60 and 200 nm were thus obtained. Each population of assemblies were then rinsed three times in HEPES-Citrate buffer by centrifugation.

Scanning transmission electron microscopy. Sample (5 µL) was deposited on a glow-discharged nickel grid coated with a carbon film (Mesh 300, Agar Scientific / S160N3) to avoid particle agglomeration and enable even dispersion of objects on the grid. The drop was then dried under air. STEM micrographs were taken on a Zeiss MERLIN microscope operated at 30 kV, using the solid-state bright-field detector. Micrographs were analyzed in the Fiji software.[4] For each sample, the number of NPs per assemblies was determined with a total counting of at least 600 individual NPs.

Cryo-transmission electron microscopy. A small volume (2-4 µL) of the sample was deposited on a glow-discharged holey carbon grid and vitrified using an FEI Vitrobot. Frozen grids were observed on an FEI F20 Tecnai microscope at 200 kV and images were recorded on an FEI Ceta detector.

Synchrotron radiation X-ray photoelectron spectroscopy. Synchrotron radiation X-ray Photoelectron Spectroscopy (SR-XPS) experiments were carried out at the BACH (Beamline for Advanced DiCHroism) beamline at the ELETTRA synchrotron facility (Basovizza, IT). XPS data were collected in fixed analyzer transmission mode (pass energy = 30 eV). A photon energy (PE) of 596 eV was used to acquire C 1s, S 2p, Ag 3d, O 1s and N 1s core-levels. The achieved resolving power was of 0.22 eV. Calibration of the energy scale was made by referencing to the gold Fermi-edge, and the metallic Ag $3d_{5/2}$ signal was always found at 368.20

eV, as expected.[5] XPS data analysis of S 2p and Ag 3d experimental spectra was performed via curve-fitting, by using a combination of Gaussian peaks, after subtraction of a Shirley background. The S $2p_{3/2}$ - S $2p_{1/2}$ and Ag $3d_{5/2}$ - Ag $3d_{3/2}$ doublets were fitted by using the same FWHM (full width half maximum) for the two spin-orbit components of the same signal, a spin-orbit splitting of 1.2 eV for S 2p and 6.0 eV for Ag 3d, and branching ratios S $2p_{3/2}$ / S $2p_{1/2}$ = 2, Ag $3d_{5/2}$ / Ag $3d_{3/2}$ = 3/2, respectively. In case of identifying presence of many chemically different species of the same element, the same FWHM value was used for all individual photoemission bands in order to reduce the number of refinement parameters then improving the reliability of the results.

Time-of-Flight Secondary Ion Mass Spectroscopy. ToF-SIMS analysis was carried out at Université Claude Bernard-Lyon 1 (Villeurbanne, France) using a Physical Electronics TRIFT III ToF-SIMS instrument operated with a pulsed 22 keV Au^+ ion gun (ion current of 2 nA) rastered over a 300 µm x 300 µm area. Ion dose was kept below the static conditions limit. Data were analysed using the WinCadence^{TM} software. Mass calibration was performed using hydrocarbon secondary ions.

Zêta potential. Zêta potential measurements were performed in a ZetaSizer (Malvern) at a 0.1 $g.L^{-1}$ silver concentration in DTS1060 cuvettes (Malvern) and at 25 °C. The final value is based on 200 acquisitions using the Smoluchowski model.[6]

Biocide assays. The *E. coli* strain K12 1655 was grown overnight in MD minimal medium (3.07 mM K_2HPO_4, 1.57 mM KH_2PO_4, 7.57 mM $NH_4(SO_4)_2$, 1.94 mM sodium citrate, 0.1 $g.L^{-1}$ glucose, 0.1 $g.L^{-1}$ magnesium sulfate, pH 7.4). For MIC determination, *E. coli* cells were then inoculated at 0.01 OD in 96-well plates in which assemblies or AgNPs were added in conditions allowing to screen for a wide range of Ag concentration. The plates were incubated at 37 °C with 900 rpm shaking in a Tecan reader plate and bacterial growth was continuously followed

by measuring absorbance at 600 nm every 15 min. For *B. subtilis* (strain 3610), the set up was similar except that bacterial cells were grown in LB media. Besides, growth delays were determined instead of MIC that require too high Ag concentration.

Hepatocyte culture and cytotoxicity. HepG2 cells were grown in MEM media supplemented with fetal calf serum, glutamine and antibiotics (penicillin/streptomycin) and exposed to citrate-coated AgNPs or assemblies at the indicated concentrations of total Ag and for 24h. After exposure, HepG2 cells were harvested, suspended in PBS and mixed in one volume of Trypan Blue. Total and viable cells were counted in a TC20 Automated Cell Counter (Bio-Rad).

Supplementary figures

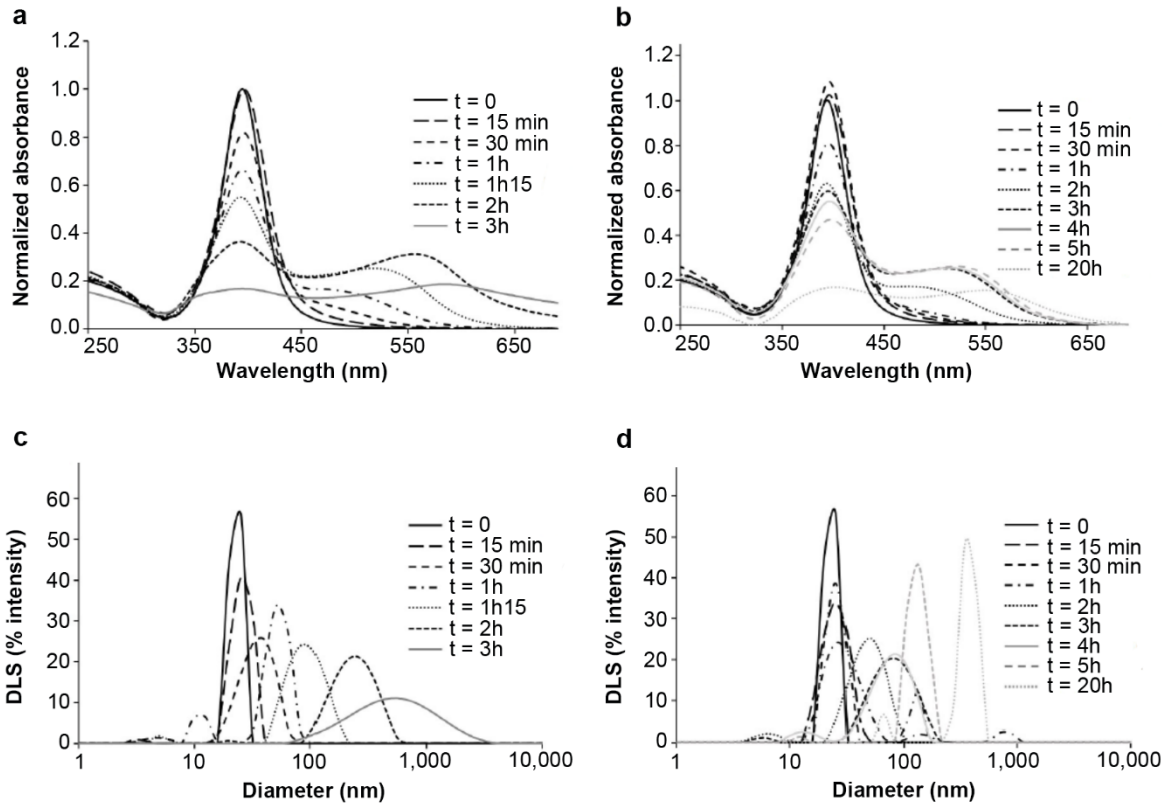


Fig. S1. L^{3S}-induced AgNP assemblies at various S:Ag ratio. (a) time-dependent analysis of AgNP SPR spectra in the presence of L^{3S} between 0 and 3 hours of incubation in 0.25:1, S:Ag molar ratio. (b) time-dependent analysis of AgNP SPR spectra in the presence of L^{3S} between 0 and 20 hours of incubation in 0.05:1, S:Ag molar ratio. (c) time-dependent evolution of the hydrodynamic diameter distribution of the species in a solution containing AgNP and L^{3S} at a 0.25:1, S:Ag molar ratio and between 0 and 3 hours of incubation. (d) time-dependent evolution of the hydrodynamic diameter distribution of the species in a solution containing AgNP and L^{3S} at a 0.05:1, S:Ag molar ratio and between 0 and 20 hours of incubation. In all experiments, t=0 corresponds to AgNPs alone in solution.

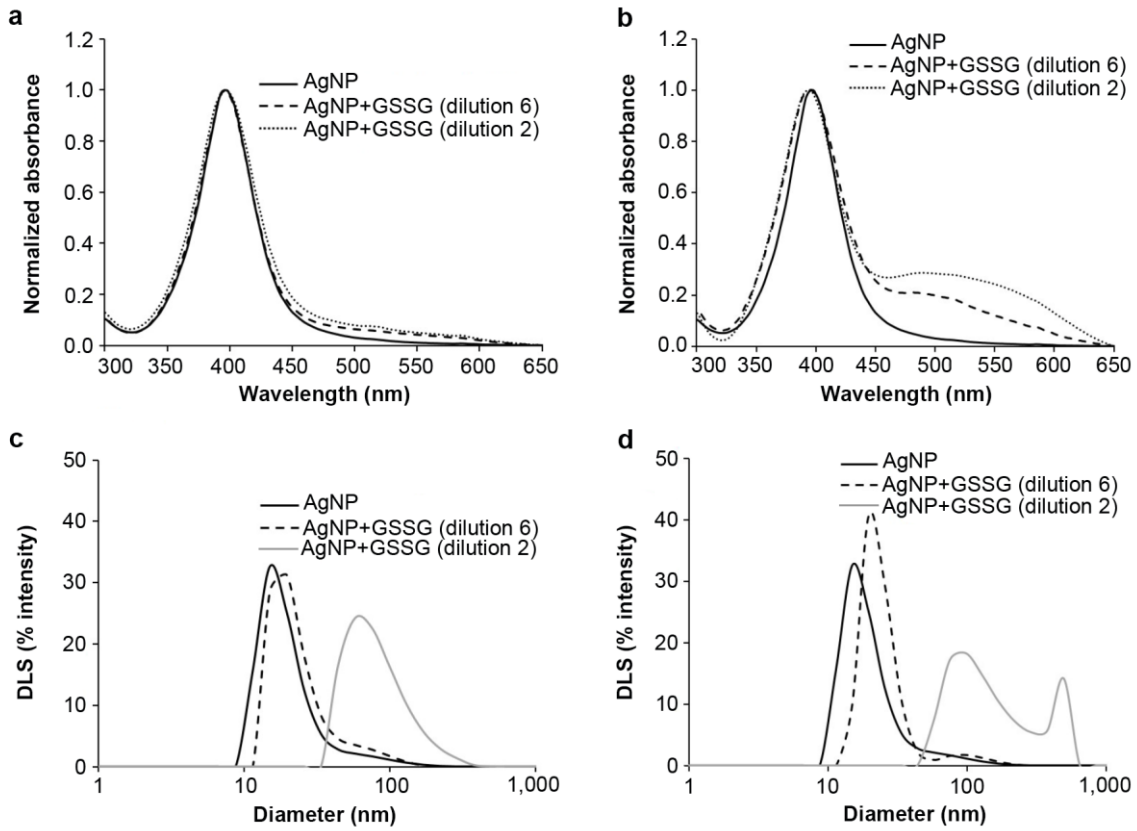


Fig. S2. AgNP agglomeration induced by oxidized glutathione. (a) AgNPs were incubated with oxidized glutathione (GSSG) for 30 min (a, c) or 1 hour (b, d) in presence of iodoacetamide (100 µM). The mixtures are then analyzed diluted two or six fold. SPR spectra of the different samples were recorded (a, b) and showed the appearance of higher wavelengths peaks in presence of GSSG that are partially lost upon 6-fold dilution. Hydrodynamic diameter distribution of the species in solution (c, d) showed the formation of large size species in presence of GSSG that are lost upon 6-fold dilution. GSSG induces the agglomeration of AgNPs. The optical properties of agglomerates are similar to those of assemblies but agglomerates are unstable upon dilution contrary to assemblies. The reaction have been performed at a 0.25:1, S:Ag molar ratio. Besides, the reactions were performed in presence of iodoacetamide to prove that this agglomeration is independent of reduced thiolate. “dilution 2” and “dilution 6” stands for 2-fold and 6-fold dilution of the sample, respectively.

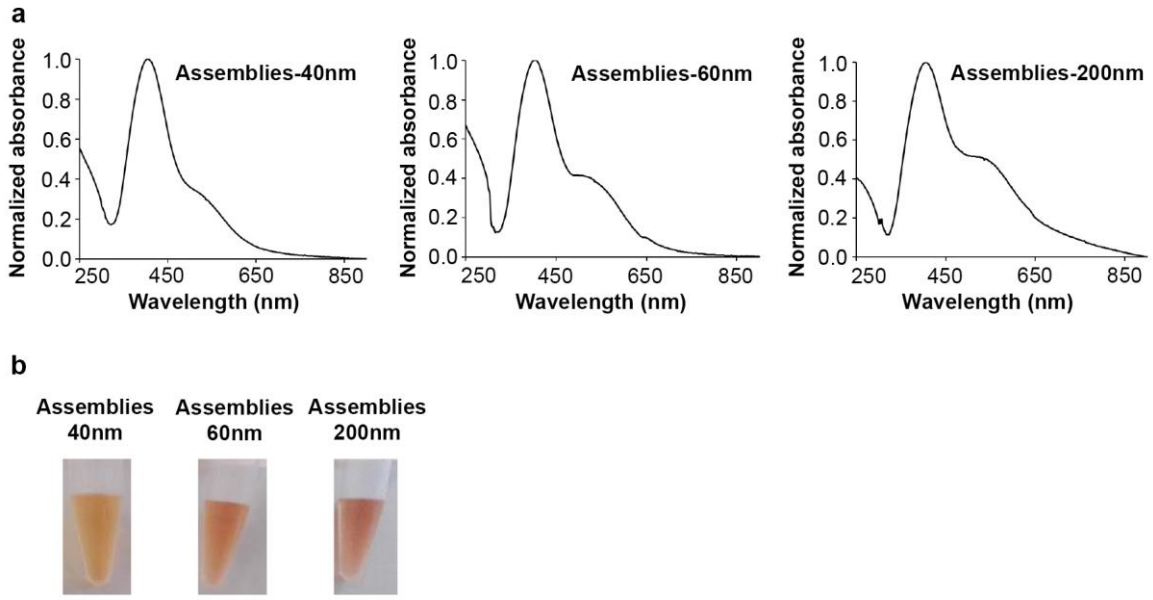


Fig. S3. Characterization of the different populations of assemblies. SPR spectra (a) and picture of the solution (b) of the different population of assemblies.

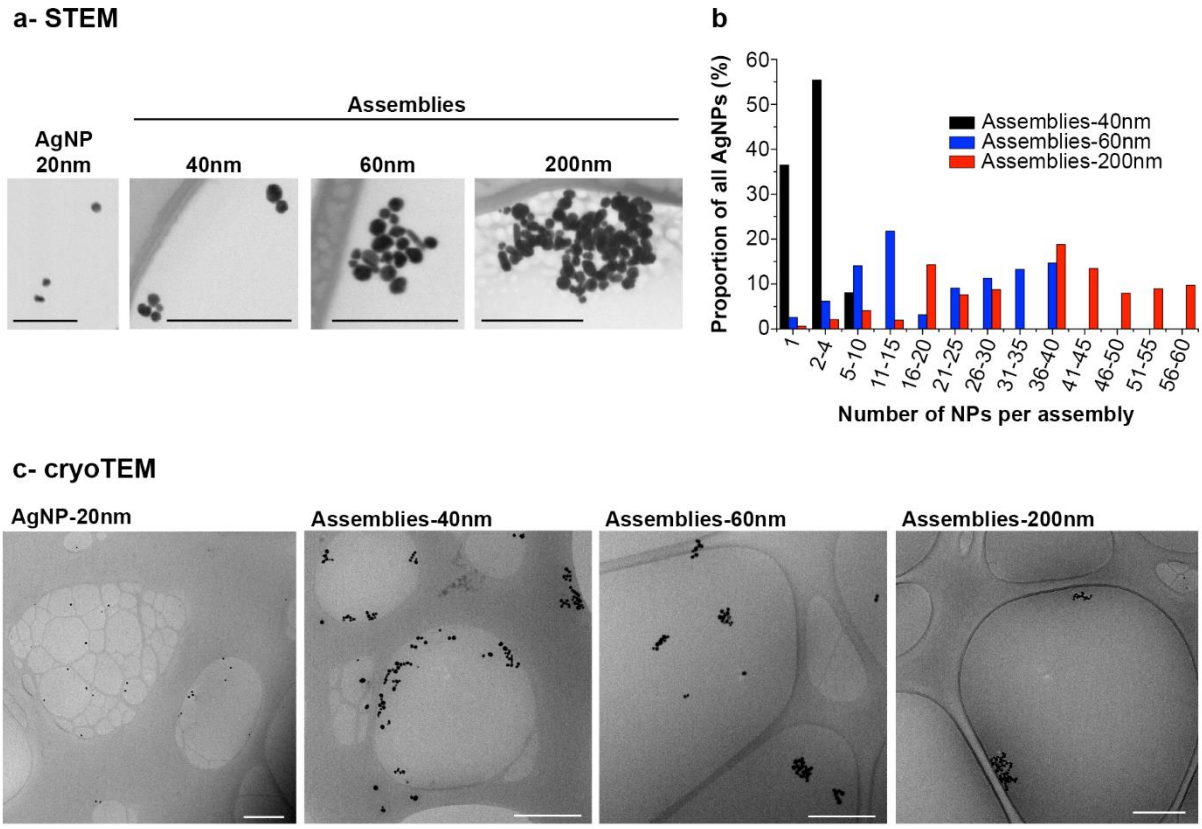
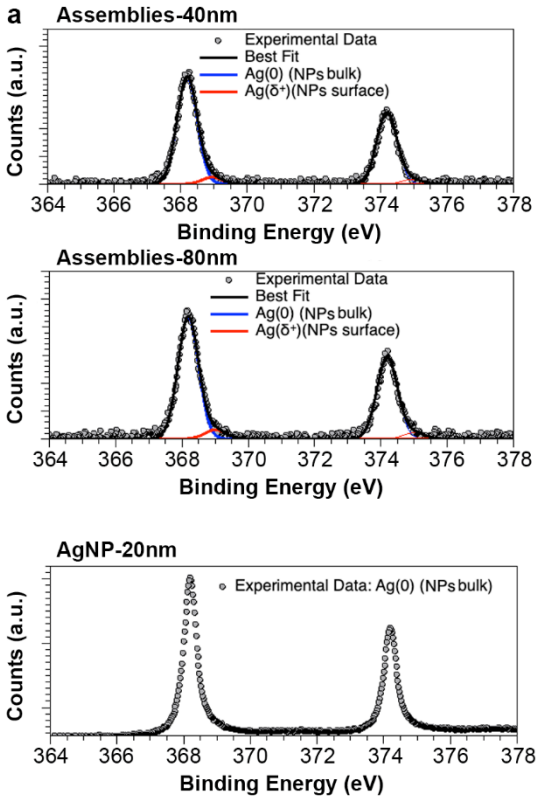


Fig. S4. Electron microscopy characterization of the different assemblies. (a) representative STEM micrographs of AgNPs alone and of the different populations of assemblies. Scale bars correspond to 200 nm. (b) statistical analysis of the number of particles per assembly in the different populations of assemblies based on STEM micrographs. The percentage of the total number of AgNP counted for an assembly population is given for each fraction. For each assembly population, at least 600 NPs have been counted. (c) large field cryo-TEM micrographs of AgNPs alone and of the different populations of assemblies. Scale bars correspond to 500 nm.

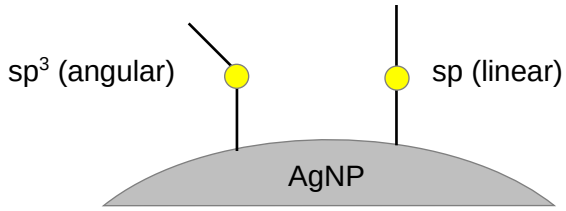
a



b

Assemblies	$Ag3d_{5/2}$ BE (eV)	FWHM (ev)	Atomic %*	Assignment
40 nm	368.20	0.71	93.4	Ag(0)
	368.96	0.71	6.6	$Ag(\delta^+)$
80 nm	368.20	0.73	93.2	Ag(0)
	369.02	0.73	6.8	$Ag(\delta^+)$

Fig. S5. Ag 3d XPS analysis. (a) SR-XPS Ag 3d core level spectra of 40 nm assemblies, 80 nm assemblies, and unassembled AgNPs of 20 nm diameter. AgNP assemblies show two pairs of spin-orbit components (Ag $3d_{5/2}$ and Ag $3d_{3/2}$, with intensity ratio Ag $3d_{5/2}$ / Ag $3d_{3/2}$ = 3/2, and doublet separation of 6.0 eV). Spectral assignments are based on Ag $3d_{5/2}$ component BE values. The main signal (Ag $3d_{5/2}$ = 368.2 eV, red curves) is attributed to metallic silver atoms at the NP core, while the signal at higher BE values (Ag $3d_{5/2}$ = 369.0 eV, blue curves) is due to partially positively charged silver atoms at the NP surface, $Ag(\delta^+)$, chemically interacting with thiol end-groups of the ligand L^{3S}.[5,7] (b) semiquantitative analysis and spectral assignment. * the statistic error in semiquantitative XPS analysis is of about 5% of the estimated value.[8]



Scheme S1. Schematic representation of the different binding geometries of thiols at the surface of an Ag nanoparticle arising from different hybridizations of the atomic orbitals of S (yellow circles, electron configuration [Ne] 3s2 3p4) and Ag (electron configuration [Kr] 4d10 5s1).

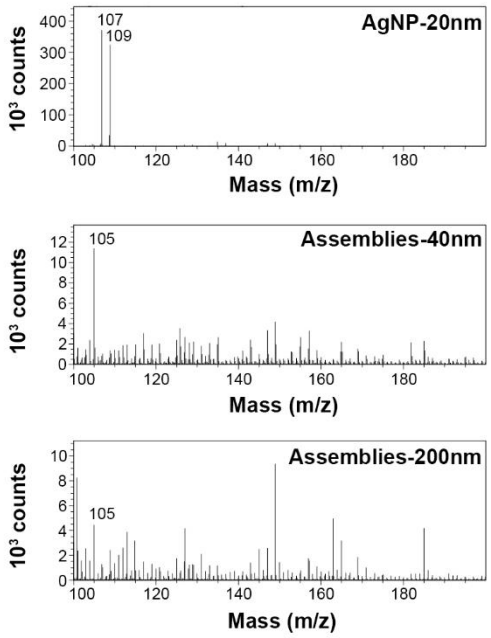


Fig. S6. Tof-SIMS analysis. ToF-SIMS positive mode spectra of AgNPs and of 40 and 200 nm assemblies in the m/z range of 100-200. Each sample was deposited on a mica slide and analyzed by ToF-SIMS. Ag was only detected for the AgNP sample as Ag^+ (peak at 107 and 109).

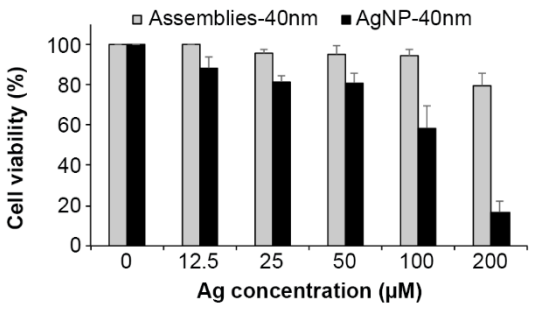


Fig. S7. Comparison of the toxicity of 40 nm AgNPs and assemblies on hepatocytes. Hepatocyte cells were exposed to 40 nm citrate-coated AgNPs or assemblies of 40 nm for 24h before being harvested. Total and viable cells were counted in a TC20 Automated Cell Counter (Bio-Rad) and using Trypan Blue.

Supplementary references

1 A. M. Pujol, C. Gateau, C. Lebrun and P. Delangle, *J. Am. Chem. Soc.*, 2009, **131**, 6928–6929.

2 N. G. Bastús, F. Merkoçi, J. Piella and V. Puntes, *Chem. Mater.*, 2014, **26**, 2836–2846.

3 M. Marchioni, T. Gallon, I. Worms, P.-H. Jouneau, C. Lebrun, G. Veronesi, D. Truffier-Boutry, E. Mintz, P. Delangle, A. Deniaud and I. Michaud-Soret, *Environ. Sci. Nano*, 2018, **5**, 1911–1920.

4 J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat. Methods*, 2012, **9**, 676–682.

5 A. V. Naumkin, A. Kraut-Vass, S. W. Gaarenstroom and C. J. Powell, *NIST*, 2013.

6 A. Sze, D. Erickson, L. Ren and D. Li, *J. Colloid Interface Sci.*, 2003, **261**, 402–410.

7 I. Venditti, G. Testa, F. Sciubba, L. Carlini, F. Porcaro, C. Meneghini, S. Mobilio, C. Battocchio and I. Fratoddi, *J. Phys. Chem. C*, 2017, **121**, 8002–8013.

8 J. E. Castle, *Surf. Interface Anal.*, 1984, **6**, 302–302.