

Extended materials and methods

Robin Capomaccio, Isaac Ojea Jimenez, Pascal Colpo, Douglas Gilliland, Giacomo Ceccone, François Rossi, and Luigi Calzolari.

Gold nanoparticle synthesis

The gold nanoparticles were made by first heating 100 mL of a 0.5 mM HAuCl₄ solution to 97°C in a microwave reactor (Discover S by CEM). After 5 minutes temperature stabilization, 2.5 mL of a 0.1 M solution of sodium citrate was injected with vigorous stirring into the HAuCl₄ solution and the reaction mixture was kept at 97 °C for 20 min. The final suspension was cooled down to room temperature. The nanoparticles have been concentrated 5 times by centrifugation at 12000g during 25 minutes at 4°C with a centrifuge 5430R from Eppendorf (Hamburg, Germany)

Preparation of the samples

HSA fatty acid free, globulin free was purchased from Sigma Aldrich diluted in phosphate buffer (1mM, pH 7.4). Gold nanoparticles were diluted in presence or absence of HSA. In presence of HSA, the AuNPs/HSA ratios were: 1/50, 1/100, 1/200, 1/400 or 1/1000. AuNP-HSA complexes at different nanoparticle-protein ratios were formed by adding variable volume of 15µM and 150µM of the stock HSA solution to AuNP concentrated at 2nM (molarity of particle).

Dynamic light scattering in batch mode

Dynamic light scattering (DLS) measurements have been done in batch mode with a DLS Zetasizer from Malvern. The temperature of analysis has been set up at 25°C. The cell used for batch mode were PMAA disposable cuvettes. The time of equilibration has been set up at 120 seconds. The number of runs per measurement is determined automatically and two measurements are completed per sample. The delay between the measurements is set up at 30seconds. Data are reported as Z-average or as intensity based particle size distribution using cumulant analysis.

Transmission electron microscope

AuNP were visualized using a transmission electron microscope TEM (JEOL 2100, Japan) at an accelerating voltage of 200 kV. The samples were prepared by placing a drop (5 μ L) onto ultrathin Formvar-coated 200-mesh copper grids (Tedpella Inc.) and left to dry in air at 4°C. For each sample, the size of at least 100 particles was measured to obtain the average and the size distribution. Digital images were analyzed with the ImageJ software and a macro performing smoothing (3x3 or 5x5 median filter), manual global threshold and automatic particle analysis provided by the ImageJ. The macro can be downloaded from <http://code.google.com/p/psa-macro>. The circularity filter of 0.8 was used to exclude agglomerates that occurred during drying.

The asymmetrical flow field-flow fractionation and dynamic light scattering in flow mode measurement

The asymmetrical flow field-flow fractionation (AF4) system used was a Postnova AF2000 equipped with a PN3212 spectrophotometer UV detector (tuned at 525 nm and 500 nm), and a PN3412 fluorescence detector (excitation wavelength tuned at 280 nm and emission wavelength tuned at 340 nm), all from Postnova (Postnova Analytics, Landsberg, Germany).. Phosphate buffer (1mM, pH 7.4) has been used as carrier and cross flow. The channel was set up with a 350 μ m spacer and using a regenerated cellulose membrane with 10 kDa cut-off. The detector flow was set at 0.5 mL.min⁻¹. The injection step has been done at 0.25 mL per minute for 5 minute. The elution time came after a transition step of 1 minute with a cross flow of 2 mL.min⁻¹ for 5min then the cross flow is set up to decrease to 0 mL.min⁻¹ with a linear gradient in 10 minutes. DLS Zetasizer from Malvern was used online with the AF4. The flow cell is a Quartz cuvette ZEN0023 from Malvern. The temperature of analysis has been set up at 25°C and every run last 5 seconds. Samples were collected with a fraction collector from Postnova.

Differential centrifugation sedimentation measurement

Differential centrifugation sedimentation data have been collected using a sucrose gradient 8-24% (w/w) with a CPS disc centrifuge model DC24000 (CPS instruments, inc.) running at 22000 rpm .

Circular dichroism

Circular dichroism spectra were collected using either a Jasco CD J810 or a Chirascan CD instrument. A 10 mm pathlength low volume quartz cell has been used to analyse low concentration of AuNP-HSA in complex. For each sample nine scans have been acquired and averaged. The CD spectra of free HSA and AuNP-HSA samples have been baseline corrected by subtracting the CD spectrum of the buffer (for free HSA) or the CD spectrum of AuNP at the same concentration of the AuNP-HSA samples. The baseline-corrected spectra have

been smoothed with Savitzky-Golay smoothing function of 9 points. The secondary structure analysis of the CD data has been performed with the DichroWeb analysis software using the CONTINLL algorithm and the set 3 for the database (37 proteins).

How to convert DCS and AF4-DLS data into density

The raw data of DCS have been extracted and analysed with Origin Pro 7.5 using the following equations :

$$SI-1 \quad D = \sqrt{\frac{\ln \frac{R_f}{R_0} \times 18\eta}{t (\rho_p - \rho_f) \omega^2}}$$

Where D is the hydrodynamic diameter, and in this specific case the Z-average found with AF4-DLS

R_0 and R_f are the starting and the ending of the gradient, respectively.

ρ_p , ρ_f : density of the particle and of the fluid, respectively.

ω : rotational speed in radians

t: time of sedimentation.

This equation can be simplified to

$$SI-2 \quad D = \frac{K}{\sqrt{t \times (\rho_p - \rho_f)}}$$

Where K is replacing all the constants and can be determined by injecting a calibrant.

Modelling the protein layer for AuNP-HSA complex

The protein layer formed around each nanoparticle contains also water molecules that form the first hydration layer around each protein molecule, and are necessary to keep proteins folded and functional. The general consensus is that the hydration is 30% of the protein mass.

Mass of hydrated HSA: $1,42 \times 10^{-19}$ g

Number of HSA per NP = (mass protein layer)/(mass HSA hydrated)

The mass of a nanoparticle M_{AuNP} has been calculated by using the radius measured by TEM (R_{AuNP}) and the density ($19.3\text{g}\cdot\text{cm}^{-3}$) of the bulk gold (equation 1). In fact, gold nanoparticles have the same crystalline structure¹ of bulk gold. The mass of the complex ($M_{complex}$) has been calculated by the same way using the R_h found by DLS and the density found by DCS $\rho_{complex}$ (Equation 2).

The thickness of the corona T_c can be calculated by using the hydrodynamic diameter found for the different ratio of AuNP/HSA R_h and removing the hydrodynamic radius of the AuNP alone R_{hAuNP} . By doing this, we take in account the hydrodynamic layer formed around each nanoparticle that is about 2 nm thick (equation 3).

The coverage density $\rho_{coverage}$ is calculated with equation 4

The mass of the hydration (M_h) layer is calculated with equation 5 and the density of the hydration layer (ρ_h) and the thickness is coming from the density of the coverage of the AuNP alone. The radius of the particle in complex protein without hydration layer will be called R_{corona} .

The mass of the HSA (M_{HSA}) can be calculated with equation 6.

The number of HSA (N_{HSA}) per particle is calculated with equation using the hydrated mass of one molecule of HSA ($M_{HSAhydrated}$).

The diameter of the AuNP and the protein corona (D_c) is calculated with equation 8

Equation 1:

$$M_{AuNP} = \left(\frac{4}{3} \times \pi R_{AuNP}^3 \right) \times 19.3$$

Equation 2:

$$M_{complex} = \frac{4}{3} \times \pi R_h^3 \times \rho_{complex}$$

Equation 3:

$$2T_c = R_h - R_{hAuNP}$$

Equation 4:

$$\rho_{coverage} = \frac{(M_{complex} - M_{AuNP})}{\left(\frac{4}{3} \times \pi R_h^3 - \frac{4}{3} \times \pi R_{AuNP}^3 \right)}$$

Equation 5:

$$M_h = \left(\frac{4}{3} \times \pi R_h^3 - \frac{4}{3} \times \pi R_{corona}^3 \right) \times \rho_h$$

Equation 6:

$$M_{HSAs} = M_{corona} - M_{AuNP} - M_h$$

Equation 7:

$$N_{HSAs} = \frac{M_{HSAs}}{M_{HSA_{hydrated}}}$$

Equation 8:

$$D_c = AuNP_D + 2T_c$$

TEM

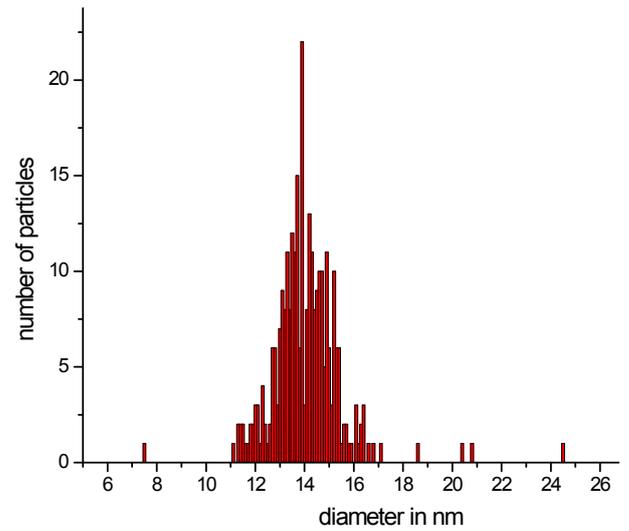
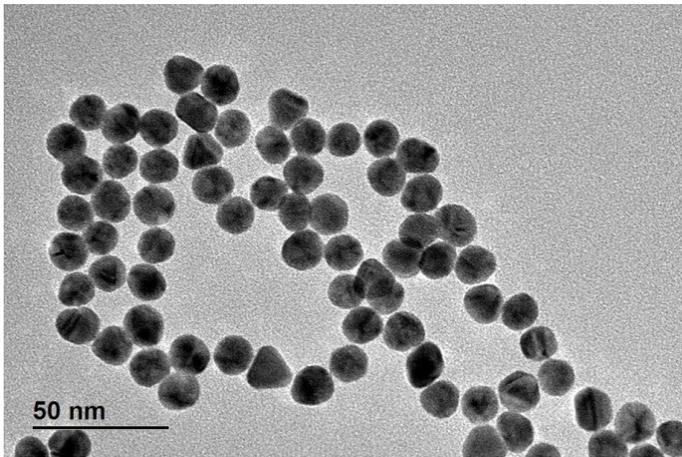
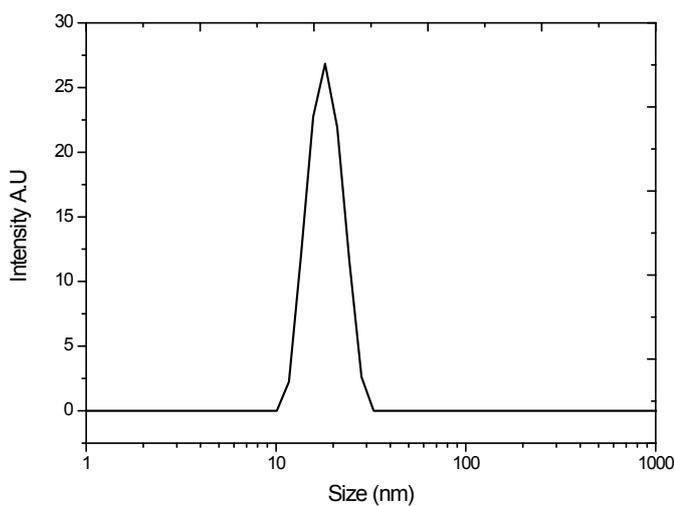


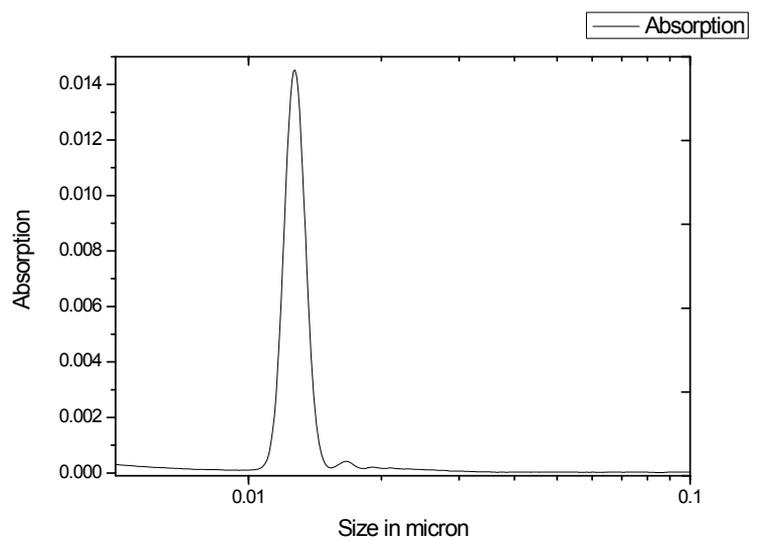
Figure S1 TEM Image and size population analysis

TEM image shows a monodisperse solution of gold nanoparticles with around 296 particles analysed the mean size is 14.01 nm. Most of the particles appear spherical and are compatible with other analysis techniques such as differential centrifugal sedimentation (DCS) or dynamic light scattering (DLS).

Hydrodynamic diameter



A)



B)

Figure S2. Size characterization by DLS (A) and DCS (B)

The naked particles appear to be a bit larger with the DLS with a Z-average of 17.8 nm and a bit smaller, 13 nm in DCS (if 19.3 g/mL is used as density for gold nanoparticles) compared to the TEM measurement (14.01 nm). Both techniques used the hydrodynamic diameter which makes appear the particles bigger in DLS and smaller in DCS due to the difference of density.

Ratio HSA/AuNPs	Z-average in nm	Std deviation
0/1	18.5	1.4
50/1	21.5	0.8
100/1	22.2	1.0
200/1	22.7	0.6
400/1	24.4	1.3
1000/1	25.3	0.7

Ratio	Density of the complex
0	9.6
50	6.9
100	6.2
200	5.9
400	5
1000	4.6

Table S1: Measured density and size of the different AuNP-HSA complexes.

1. V. Petkov, Y. Peng, G. Williams, B. Huang, D. Tomalia and Y. Ren, *Physical Review B*, 2005, **72**, 195402.