#### SUPPORTING INFORMATION

### In situ protein corona study by scattering correlation spectroscopy: a comparative study between spherical und urchin-shaped gold nanoparticles

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This document contains informations and figures regarding gold nanoparticles (GNP) extinction spectra before and after incubation with proteins measured by UV-Vis spectroscopy. GNP size characterization by transmission electron microscopy. Scattering cross-correlation curves of GNP incubated with different concentrations of proteins (HSA, lysozyme and  $\alpha$ -2-hs-glycoprotein). Diffusion time values obtained from the fitting of the cross-correlation curves and the corresponding R<sub>H</sub> values of GNP before and after incubation with proteins. Calculated values of  $\gamma$  coefficients for each nanoparticle and protein.



1) GNS and GNU extinction spectra before and after incubation with proteins measured by UV-Vis spectroscopy:

Figure S1: Extinction spectra of GNP incubated with different concentrations of proteins. GNS incubated with (a) HSA, (b) Lysozyme and (c) α2-hs-Glycoprotein. (d) GNU incubated with HSA.



2) GNP size characterization by transmission electron microscopy (TEM):

Figure S2: GNP size characterization by TEM: (a) TEM images of GNS (left) and GNU (right). (b) Size measurements using ImageJ, the arrows correspond to the measured area of GNS (left) and GNU (right). GNP size measurements by ImageJ, the dashed line corresponds to the mean size (c). GNP size measurement final results (mean size  $\pm$  SD).

## **3)** Normalized SCS curves of GNS and GNU incubated with different concentrations of HSA:

For ease comparison, the cross-correlation curves were normalized to 1 at 0.1 ms. Some high fluctuations of G(t) were recorded at higher times ( $10^2$  ms), which were due to high fluctuations of the scattering signal coming from some aggregated GNP (<10% of the total amount of GNP).





Figure S3: Cross-correlation curves of GNS incubated with different concentrations of HSA.

Figure S4: Cross-correlation curves of GNU incubated with different concentrations of HSA.

Table S1: Diffusion time values obtained from the fitting of the cross-correlation curves and the corresponding R<sub>H</sub> values of GNS and GNU incubated with different concentrations of HSA.

	GNS		GNU	
HSA	$ au_{ m D}$	RH	$ au_{ m D}$	RH
	(±0.10ms)	(±0.2nm)	(±0.15ms)	(±0.3nm)
1	6.15	22.5	10.61	38.7
2	-	-	10.62	38.8
5	6.15	22.5	10.70	39.0
10	6.23	22.8	10.68	38.9
30	6.75	24.7	-	-
50	6.92	25.3	11.25	41.0
60	7.01	25.6	-	-
100	7.1	25.9	12.79	46.7
200	7.23	26.3	13.64	49.8
300	7.24	26.4	14.07	51.3
400	7.24	26.4	14.14	51.6
500	7.25	26.5	-	-

$$R_{\rm h} = \left(\frac{4 \ k \ T}{6\pi\eta}\right) \frac{\tau_D}{\omega_{xy}^2}$$

Equation used to calculate the hydrodynamic radius:

$$\Rightarrow \begin{cases} water \ viscosity \ \eta = \ 9.10^{-4} \ Kg.m^{-1}.s^{-1} \\ T = 296 \ ^{\circ}K(room \ temp.) \ ;Boltzmann \ constant \ k = 1,38 \ . \ 10^{-23} J.k^{-1} \\ The \ lateral \ waist \ \omega_{xy} = (470 \ \pm 2)nm \end{cases}$$

The focal volume dimensions were measured by fluorescence correlation spectroscopy using Alexa Fluor 633. The authors already reported these measurements in reference 50. At  $\lambda_{exc}$  =633 nm. The lateral waist is  $\omega_{xy} = (470 \pm 2)$  nm, the axial waist is  $\omega_z = (1.50 \pm 0.01) \mu m$  and an effective volume of &.8 fL.

$$R_h = \left(\frac{4 \ k \ T}{6\pi\eta}\right) \frac{\tau_D}{\omega_{xy}^2}$$

Equation used to calculate the hydrodynamic radius:

As reported in references 31 and 50, the Equation giving the calculation of the fractional error in  $R_h$ :

 $\frac{\delta R_h}{\langle R_h \rangle} = \frac{\delta \tau_D}{\langle \tau_D \rangle} - 2 \frac{\delta \omega_{xy}}{\langle \omega_{xy} \rangle}$ 

Experiments were performed at room temperature (T = 296 K). Temperature fluctuations were at most 0.5 K and therefore the error propagation of T was neglected.

For GNS, the maximum value of the standard deviation of the time diffusion was about  $\pm 0.10$  ms (in the case of HSA concentration between 1 to 50  $\mu$ M, for other concentrations this value was three times less). The calculation of the maximum standard deviation of R<sub>h</sub> by using the above equation gave the value of  $\pm 0.2$  nm.



4) Normalized SCS curves of GNS incubated with different concentrations of Lysozyme:

# Figure S5: Cross-correlation curves of GNU incubated with different concentrations of lysozyme.

	GNS		
Lysozyme	$ au_{ m D}$	RH	
	(±0.03ms)	(±0.2nm)	
1	6.15	22.5	
10	6.24	22.8	
20	6.35	23.2	
30	6.52	23.8	
40	6.57	24	
50	6.73	24.6	
60	6.79	24.8	
70	6.86	25.0	
80	7.06	25.8	
100	7.26	26.5	
200	7.53	27.5	
400	7.59	27.7	

Table S2: Diffusion time values obtained from the fitting of the cross-correlation curves and the corresponding  $R_H$  values of GNS incubated with different concentrations of lysozyme.

### 5) Normalized SCS curves of GNS incubated with different concentrations of α-2hs-glycoprotein:

Table S3: Diffusion time values obtained from the fitting of the cross-correlation curves and the corresponding  $R_H$  values of GNS incubated with different concentrations of  $\alpha$ -2-hs-glycoprotein.

	GNS		
α-2-hs-glycoprotein	$ au_{ m D}$	RH	
	(±0.03ms)	(±0.2nm)	
1	6.15	22.5	
2	6.16	22.6	
3	6.17	22.6	
4	6.70	24.5	
5	7.28	26.6	
6	7.75	28.3	
7	8.16	29.8	
8	8.25	30.1	
9	8.33	30.4	
10	8.36	30.5	



Figure S6: Cross-correlation curves of GNU incubated with different concentrations of α-2hs-glycoprotein.

### **6)** Calculated values of the fitting parameter γ:

Table S4: The coefficient  $\gamma$  calculated values, representing the ratio between the GNP volume (V<sub>0</sub>) and the protein volume (V<sub>p</sub>). For the calculations, V<sub>0</sub>(GNS) = 65500 nm<sup>3</sup> and V<sub>0</sub>(GNU) = 268000 nm<sup>3</sup>.

	$V_p (nm^3)$	γ <sub>GNS</sub>	γgnu
HSA	~185	0.003	0.0007
Lysozyme	~ 38	0.0006	-
α-2-hs- Glycoprotein	~ 190	0.003	-