# Supplementary Information for:

# Interaction of nanoparticles with lipid films: the role of symmetry and shape anisotropy

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#### **Small Angle X-ray Scattering**

SAXS measurements were carried out on a S3-MICRO SAXS/WAXS instrument (HECUS GmbH, Graz, Austria) which consists of a GeniX microfocus X-ray sealed Cu K $\alpha$  source (Xenocs, Grenoble, France) of 50 W power which provides a detector focused X-ray beam with  $\lambda = 0.1542$  nm Cu K $\alpha$  line. The instrument is equipped with two one-dimensional (1D) position sensitive detectors (HECUS 1D-PSD-50 M system), each detector is 50 mm long (spatial resolution 54 µm/channel, 1024 channels) and cover the SAXS Q-range (0.003< Q <0.6 °Å –1). The temperature (25°C) was controlled by means of a Peltier TCCS-3 Hecus. The analysis of SAXS curves was carried out using Igor Pro (Kline, 2006). SAXS measurements on AuNPs aqueous dispersions, were carried out in sealed glass capillaries of 1.5 mm diameter. The concentration of AuNSs in water was  $1.1 \cdot 10^{15}$  particles/mL.

To analyze AuNSs scattering profile we chose a model function with a spherical form factor and a Schulz size distribution (Kotlarchyk and Chen, 1983) from the NIST package SANS Utilities. This model calculates the scattering for a polydisperse population of spheres with uniform scattering length density. The distribution of radii is a Schulz distribution given by the following equation:

$$f(R) = (z+1)^{z+1} x^{z} \frac{exp[-(z+1)x]}{R_{avg}\Gamma(z+1)}$$

where  $R_{avg}$  is the mean radius,  $x = R/R_{avg}$ , z is a parameter related to the polydispersity and  $\Gamma(x)$  indicates the Gamma function. The form factor is normalized by the average particle volume, using the 3rd moment of R:

$$\langle V \rangle = \frac{4\pi}{3} \langle R^3 \rangle = \frac{4\pi}{3} \langle R \rangle^3 \frac{(z+3)(z+2)}{(z+1)^2}$$

The scattering intensity is:

$$I(Q) = \left(\frac{4\pi}{3}\right)^2 N_0(\Delta\rho)^2 \int_0^\infty f(R) R^6 F^2(QR) dR$$

where  $N_0$  is the total number of particles per unit volume, F(QR) is the scattering amplitude for a sphere and  $\Delta \rho$  is the difference in scattering length density between the particle and the solvent.

The SAXS profile of AuNRs (at a concentration of  $2.4 \cdot 10^{14}$  particles/mL) was fitted by the Cylinder poly radius model from the NIST package SANS Utilities; this model calculates the form factor for a polydisperse right circular cylinder with uniform scattering length density and a Schulz polydispersity of the cylinder length is considered. The function calculated is the orientationally averaged cylinder form factor which is then averaged over a Schulz distribution of the cylinder length 2*H*. The size averaged form factor is thus:

$$P(Q) = \int_0^\infty \int_0^{\pi/2} f(2H) F^2(Q\alpha) \sin \alpha \, d\alpha \, d(2H)$$

where f(2H) is the normalized Schulz distribution of the length 2*H*. The limits of the integration are adjusted automatically to cover the full range of length. The scattering amplitude, F, is:

$$F(Q\alpha) = 2V_{cyl}(\rho_{cyl} - \rho_{solv})j_0(QH\cos\alpha)\frac{j_1(QR\sin\alpha)}{(QR\sin\alpha)}$$

Where  $j_1(QR \sin \alpha)$  is the first order Bessel function,  $V_{cyl} = 2\pi HR^2$ ,  $j_0(QH \cos \alpha) = \frac{\sin(QH\cos\alpha)}{QH\cos\alpha}$ , with  $\alpha$  defined as the angle between the cylinder axis and the scattering vector (Q) and  $\rho_{cyl}$  and  $\rho_{solv}$  the scattering length density of the nanorod and the solvent respectively. The integral over  $\alpha$  averages the form factor over all possible orientations of the cylinder with respect to Q.

#### **Transmission Electron Microscopy (TEM)**

Transmission electron microscopy (TEM) images were acquired with a STEM CM12 Philips electron microscope, at CeME (CNR Florence Research Area, Via Madonna del Piano, 10 - 50019 Sesto Fiorentino). The sample was placed on a 200 mesh carbon-coated copper grid.

#### **Dynamic Light Scattering (DLS)**

DLS measurements were performed using a Malvern Panalytical Zetasizer Nano ZS90 instrument which does DLS measurements at a fixed scattering angle of 90°. A 4 mW laser of 633 nm wavelength is used as light source, the lag times of

the correlator start from 25 ns as shortest and go up to 8000 s, using a maximum number of 4000 channels. After checking monomodality with a CONTIN fit, the ACFs were analyzed through the cumulant fitting stopped to the second order, allowing an estimate of the hydrodynamic diameter and the polydispersity of AuNSs and AuNRs, which were found equal to:  $25.6 \pm 0.2$  and  $37.6 \pm 0.1$  nm (hydrodynamic diameter) with a 0.3 and 0.21 (PDI), respectively.

#### **Z-Potential**

Zeta potential measurements were performed using a Zeta Potential Analyzer (Zeta Plus, Brookhaven Instruments Corporation, Holtsville, NY). Zeta potentials were obtained from the electrophoretic mobility u, according to Helmholtz-Smoluchowski equation:  $\zeta = (\eta \epsilon) \times u$  with  $\eta$  being the viscosity of the medium,  $\epsilon$  the dielectric permittivity of the dispersing medium. The Zeta Potential values are reported as averages from ten measurements.

#### Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

This analysis was kindly done by Dr. Mirco Severi, in order to define the concentration of NPs dispersions, with an ICP-AES Varian 720-ES. For the analysis, 200  $\mu$ L of NPs dispersion were placed in a vial, then the solvent was evaporated under slight nitrogen flow. The sample, consisting of a dry film of nanoparticles, was diluted to 5 mL with 0.1% super pure nitric acid, obtained by

distillation under boiling, then, to the sample, 1 ppm of Ge was added, as internal standard; the sample thus treated was analysed.

The operating conditions for the ICP-AES analysis have been optimized to obtain the maximum signal intensity and are as follows:

Instrument: Varian 720-ES

Power R.F: 1.20 KW

Flow rate of Argon Plasma: 16.5 L min<sup>-1</sup>

Auxiliary Argon flow rate: 1.50 L min<sup>-1</sup>

Argon nebulizer flow rate: 0.75 L min<sup>-1</sup>

Replicated reading time: 5 seconds

Instrument stabilization time: 30 seconds

Sample introduction settings:

Sample uptake: 30 seconds

Flow rate: 1 mL min<sup>-1</sup>

Rinse time: 70 seconds

Fast Pump (sample delay / rinse): active

Smart rinse: active

Replicates: 3

From the ICP-AES data, it results that the quantity of gold in 200  $\mu$ L of AuNSs and AuNRs dispersions is equal to 259  $\mu$ g and 278  $\mu$ g; as a result, the concentrations of Au in AuNSs and AuNRs is 1.3 mg/mL and 1.4 mg/mL,

respectively. The AuNSs and AuNRs concentration per ml was subsequently evaluated considering the size of AuNPs determined through SAXS and is equal to 3.2·10<sup>15</sup> and 7.4·10<sup>14</sup> particles/mL for AuNSs and AuNRs, respectively.

# Evaluation of the spacing parameter of cubic and lamellar films through NR The unit cell spacing (d) of cubic and lamellar films was evaluated from NR data (i.e., with hkl Miller indices, hkl, (100) and (200)), using the Q-position of the Bragg reflection peaks of cubic and lamellar phases. In particular, the lamella phase features two prominent Bragg peaks located at 0.099 and 0.192 Å<sup>-1</sup>, corresponding to the first two reflexes of the lamellar, $L_{\alpha}$ , phase (i.e., with Miller indices, hkl, (100) and (200)). The unit cell spacing was calculated using the qposition of the maximum Bragg reflection peaks $d = 2\pi h/Q_h$ , yielding a value of $6.4\pm0.1$ nm as the mean between the two peak positions. The reflectivity profile of the GMO lipid film presents two clearly distinguishable Bragg peaks at q values 0.093 and 0.115 Å<sup>-1</sup>, corresponding to Miller indices (110) and (111) of the Pn3m inverse cubic phase. The lattice spacing d, can be calculated from $d = 2\pi (h^2 + k^2 + l^2)^{1/2} / Q_{100}$ (Sands, 1969), yielding a value of 9.51±0.05 nm as the mean between the two peak positions.

#### Evaluation of the spacing parameter of cubic films through GISANS

In order to obtain the lattice parameter of the Pn3m cubic architecture (in the presence and in the absence of AuNSs), we used the GISANS images shown in the main text (Figure 3). We performed horizontal line cuts from the 2D GISANS data, along selected Qz values, obtaining the Intensity *vs* Qy plots, reported in Figure S2. The Qy values corresponding to the intensity's maxima were then used to determine the Q value of a spot on the  $Q_y/Q_z$  GISANS plot, according to following equation:

$$Q^2 = Q_y^2 + Q_z^2$$

The lattice parameter (d) is then evaluated from the following equation:  $d = 2\pi (h^2 + k^2 + l^2)^{1/2} / Q_{hkl}$  (with h, k and l miller indices identifying a Bragg reflection peak).  $Q_{hkl}$  is taken as the mean between the Q-values obtained for the different intensity's spots of each horizontal line cut.



**Figure S1:** a) Left part: GISANS 2D plot of the GMO/water film; the horizontal lines represent the directions along which the line cuts are performed. Right part: horizontal Intensity vs Qy line cuts obtained from the 2D GISANS plot along different Qz.



Analysis of NR profiles of cubic and lamellar films interacting with AuNRs

**Figure S2:** Neutron Reflectometry profiles of (a) GMO cubic films and (b) GMO/DOPC lamellar films upon interaction with AuNRs. Three contrasts were measured for GMO/AuNRs system: D<sub>2</sub>O (SLD 6.34e-6 Å<sup>-2</sup>), cmAu (SLD 4.6e-6 Å<sup>-2</sup>, where Au is contrast-matched), cmSi (SLD 2.07e-6 Å<sup>-2</sup>, where Si is contrast-matched), while two contrasts were measured for GMO/AuNRs system (D<sub>2</sub>O, cmAu). It was possible to analyze both GMO (a) and GMO/DOPC (b) systems according to a five-layers model, accounting for the formation of a lipid bilayer. For GMO the bilayer thickness is 34 +/-3 nm, while for GMO/DOPC the bilayer thickness is 40 +/- 4 nm, consistent with the parameters expected from the literature ((Chang *et al.*, 2016)). The bilayer thickness was obtained by analyzing the data with motofit (Nelson, 2006), considering scattering length density values of: 2.07e-6 Å<sup>-2</sup> (silicon layer), 3.41e-6 Å<sup>-2</sup> (Silicon oxide layer), 2.17e-6 Å<sup>-2</sup> (mixed GMO/DOPC polar headgroup layer). In both cases, the layer appear homogeneous, with high coverage of the substrate (inferred from the low hydration degree of the systems <10%)

## CLSM images of AuNSs clusters on lipid films



**Figure S3:** Representative transmission CLSM images of GMO cubic films incubated with AuNSs. The orange arrows in the figure highlight the presence of multiple round-shaped black spots attributable to the presence of AuNSs clusters.

#### Stability of lipid films upon water flow

A possible effect of lipid removal caused by the liquid flow through the sample cell had been investigated by means of Neutron Reflectivity (OFFSPEC, ISIS Neutron and Muon Source (United Kingdom)). In particular, the Reflectivity of a GMO lipid film in D<sub>2</sub>O, adsorbed onto silicon, has been acquired before (black profile in Figure S.4) and after rinse with D<sub>2</sub>O (green profile in Figure S.4), flushed into cell at the same flow rate used for the injection of AuNPs, i.e. 0.1 ml/min. The difference in the intensity of black and green profiles is due to the different acquisition time, i.e. 5 hours vs 30 minutes, respectively. However, in spite of the shorter acquisition time, the green profile shows a well distinct pattern of Bragg reflexes, which is similar to the one highlighted in the black profile and identifies a cubic Pn3m arrangement. This demonstrates that the lipid films are structurally stable and fully preserved upon liquid flow through the sample cell.



**Figure S4:** Specular reflectivity profiles of a GMO film acquired in  $D_2O$  before and after rinse with  $D_2O$ , together with the reflectivity of the bare silicon in  $D_2O$ .

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