Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Protein corona alters the mechanisms of interaction between silica nanoparticles and lipid vesicles

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Materials and Methods

Materials

DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) and Rh-DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt)), were purchased from Avanti Polar Lipids Inc. (Alabaster, Alabama, USA). Laurdan (6-Dodecanoyl-2-Dimethylaminonaphthalene) and DiO (3,3'-Dioctadecyloxacarbocyanine Perchlorate) were purchased from ThermoFisher Scientific Ltd. (Loughborough, Leicestershire, UK). Microscope µ-slide 8 well glass bottom chambers (Ibidi GmbH) were purchased from Thistle Scientific Ltd (Glasgow, UK). Indium tin oxide (ITO) coated glass slides (surface resistivity 8–12 V sq-1), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), sodium chloride, sucrose, lyophilised bovine serum albumin and colloidal silica nanoparticles Ludox SM-30 and Ludox TM-50 were purchased from Sigma-Aldrich Co. (Gillingham, UK).

Bovine serum albumin corona

To coat the surface of the SNPs with a BSA corona we suspended 10 mg/ml SNPs in a solution of 100 mg/ml BSA in the experimental buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). The SNPs were incubated at 4 ºC for 24 hours (unless otherwise specified).

Nanoparticle characterization

The size of the bare SNPs was initially determined by transmission electron microscopy (TEM) with a FEI Tecnai TF20 field emission gun (FEG) transmission electron microscope operating at 200 kV and fitted with a Gatan Orius SC600A CCD camera. A drop of the sample was placed on a continuous carbon-coated copper grid (EM Resolutions, Sheffield, UK). After being left to dry, this was transferred to the transmission electron microscope. Images were processed with Fiji by applying a threshold to obtain a binary image and using the automatic particle analysis to obtain the size distribution of the SNPs.

Dynamic light scattering (DLS) and Doppler electrophoretic light scattering (DELSA) measurements were performed with a Malvern Zetasizer Nano ZSP (Malvern Panalytical, Malvern, UK) equipped with a 633 nm helium-neon laser to determine the hydrodynamic size and ζ-potential of the SNPs, respectively. For DLS, the SNPs were incubated in the experimental

buffer for 1 h, 24 h and 48 h, then 200 µl of sample were transferred to a microcuvette and each sample was measured three times (unless otherwise specified) at a fixed 173° back-scattering angle. The hydrodynamic size is reported as the average of the three readings. For the SNPs@BSA the procedure was the same but the NPs were incubated in the solution containing BSA specified in the section above. To measure the ζ-potential, the SNPs were incubated for 24 h in experimental buffer (and buffer supplemented with BSA in the case of SNPs@BSA) and 1 ml of sample was introduced in a cuvette for zeta potential measurements (cuvette DTS1070, Malvern Panalytical, Malvern, UK). Each sample was measured three times at a fixed 173° back-scattering angle. The results were processed using the Malvern Zetasizer software.

GUV electroformation and confocal microscopy imaging

GUVs were prepared using the electroformation method [1] . First, 15 µl of lipid solution (0.7 mM DOPC + 0.5 mol% of Rh-DOPe or 1 mol% DiO) were deposited as a thin layer over the conductive side of two ITO coated glass slides and then dried under a nitrogen stream. The ITO slides were then assembled into an electroformation chamber each in contact with a copper tape and separated by a 1.6 mm Teflon gasket. The chamber was filled with a 300 mM sucrose solution (300 mOsm kg^{-1}) and connected to a function generator to apply an AC electric field. The frequency of the electric field was set at 10 Hz and the voltage was gradually increased from 1 V peak-to-peak (Vpp) to 5 Vpp over 15 minutes and maintained at 5 Vpp for two hours. Finally, the frequency was gradually reduced to 0.1 Hz over 10 minutes to facilitate the closure and detachment of GUVs from the slide. After electroformation, the GUVs were suspended in isotonic experimental buffer.

We used fluorescence confocal laser scanning microscopy to visualize the effect of the SNPs on the GUVs. The experiments were performed at room temperature on a Zeiss LSM-880 inverted laser scanning confocal microscope with a Plan-Apochromat 40×/1.4 Oil DIC M27 objective lens (NA = 1.4). The glass surfaces of the microscope slides passivated with a 5% BSA solution for 15 min then rinsed with Milli-Q water and to prevent GUVs from adhering and rupturing onto the glass. GUVs (200 µL) were deposited into a well of the microscope slide. Once the GUVs had sunk to the bottom of the well, 25 µg/mL SNPs were carefully added. DiO and Rh were excited with a 488 nm argon laser and a 561 nm diode pumped solid-state (DPSS) laser, respectively. The emission of DiO was recorded between 493 and 553 nm and the emission of Rh between 566 and 630 nm.

Estimation of the apparent shrinking rate of GUVs

To observe the shrinking of the GUVs after exposure to SNPs-18 and calculate the apparent shrinking rate we acquired time series confocal images. We then used the radial profile angle plugin (http://questpharma.u-strasbg.fr/html/radial-profile-ext.html) of Fiji to analyse the change in radius and membrane fluorescence intensity of the GUVs over time. From the values of the radius we estimated the surface area of the GUV at each time point assuming it is spherical. Finally we calculated the apparent shrinking rate (*R*) as:

$$R = \frac{S_0 - S_t}{t}$$

Where S_0 and S_t are the initial and final estimated surface areas of the GUVs, respectively, and t is the time passed when the GUV reaches its final state.

Laurdan spectral imaging

GUVs labelled with Laurdan (0.7 mM DOPC + 0.5 mol% Laurdan) were prepared by electroformation. Laurdan is a polar-sensitive fluorophore that shows maximum fluorescence at 490 nm or 440 nm depending on the membrane hydration [2, 3]. Thus, an emission peak at 490 nm implies a hydrated and disordered membrane, while an emission maximum at 440 nm indicates a more ordered membrane where lipids are tightly packed and less water molecules are located near the dye. The fluorescence intensity at 440 nm (I_{440}) and 490 nm (I_{490}) is used to calculate a quantitative indicator of membrane order called generalized polarization (GP) [2, 3], which increases with the order of the membrane:

$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}}$$

To acquire spectral images of the GUVs we used the lambda mode of a Zeiss LSM880 confocal microscope equipped with a 32-channel GaAsP detector array, which allows split the fluorescence emission spectrum of a dye into spectral intervals recorded in individual detection channels. Laurdan was excited with the 405 nm diode laser and the fluorescence detection range was set between 410 nm and 550 nm with a spectral step of 8.9 nm per channel. Snapshots of Laurdan labelled GUVs were acquired before and after exposure to NPs. The images were analysed with the GP plugin of Fiji [4], setting 440 nm and 490 nm as maximum emission wavelengths to calculate the GP values.

Supplementary figures

Figure S1.Transmission electron microscopy images of SNPs and analysis of their size distribution. a) SNPs-18 (Ludox SM) have an average diameter of 17.8 ± 4.8 nm. b) SNPs-30 (Ludox TM) have an average diameter of 30.8 ± 3.9 nm. (The authors acknowledge Dr Nicole Hondow from the School of Chemical and Process Engineering, University of Leeds, for collecting the TEM images)

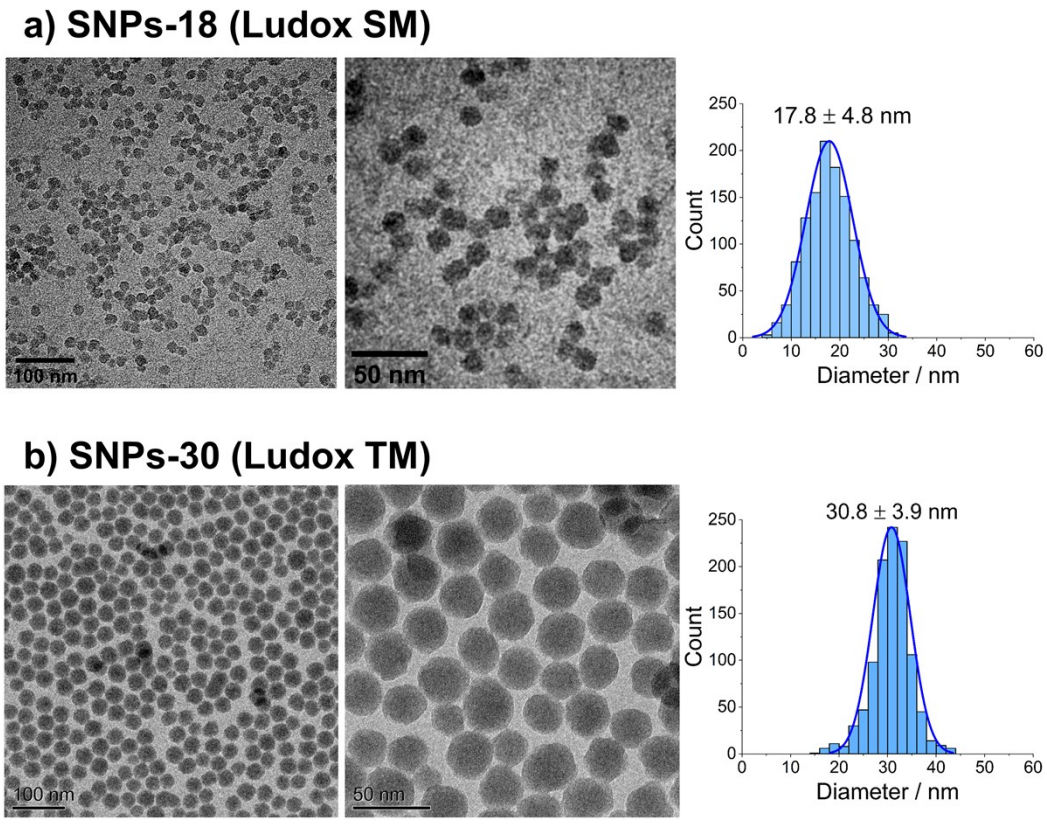


Figure S2. Hydrodynamic diameter and ζ-potential of SNPs with and without BSA corona. a) Hydrodynamic diameter of bare SNPs-18 (blue), SNPs-18@BSA (magenta) and free BSA control (grey) after 1 h, 24 h and 48 h of incubation in the buffer. b) Hydrodynamic diameter of bare SNPs-30 (blue), SNPs-30@BSA (magenta) and free BSA control (grey) after 1 h, 24 h and 48 h of incubation in the buffer. c) ζ-potential of SNPs with and without BSA corona as well as free BSA control. The plot shows the mean ± SD of 3 independent measurements.

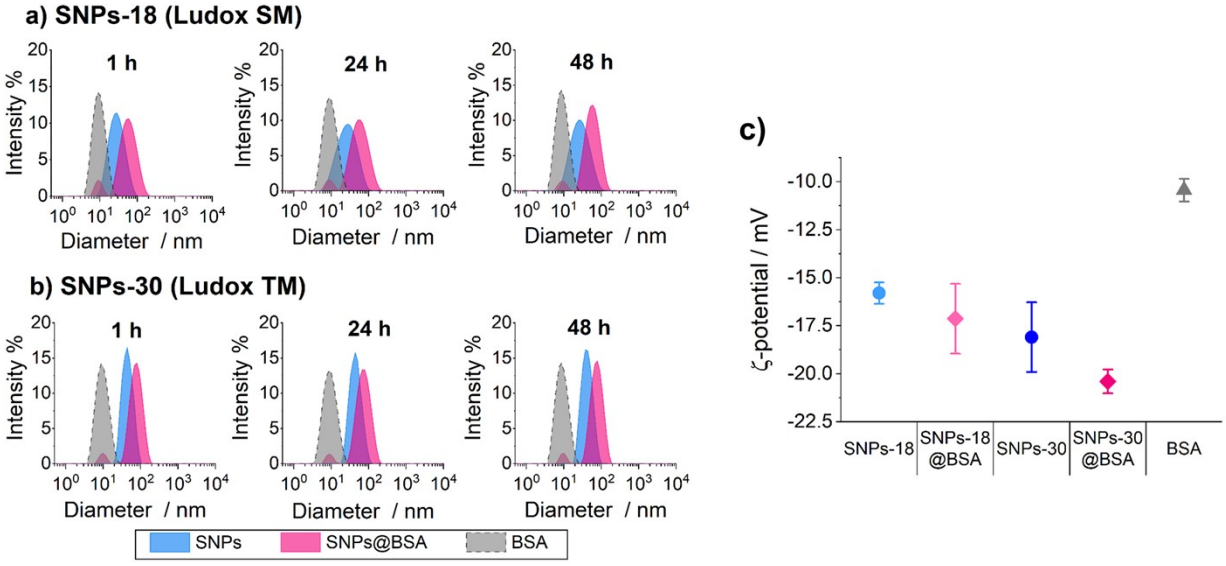
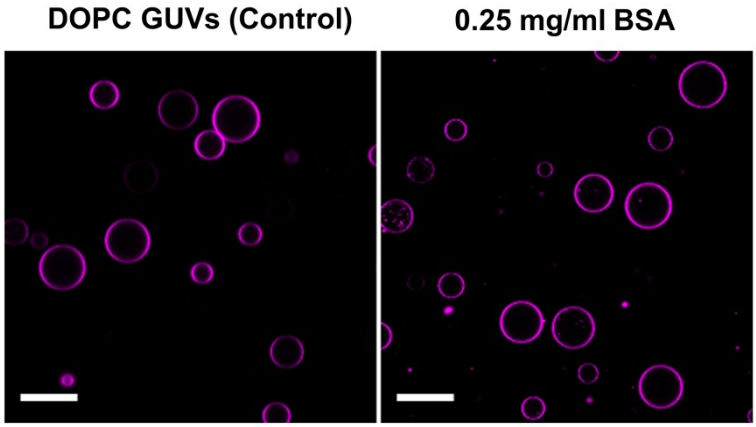


Table S1. Summary of hydrodynamic sizes and ζ-potential of the different SNPs and SNPs@BSA

	SNPs-18	SNPs-18@BSA	SNPs-30	SNPs-30@BSA
Hydrodynamic diameter 1h	24.9 ± 0.9 nm	58.8 ± 2.6 nm	43.0 ± 1.4 nm	82.9 ± 3.1 nm
Hydrodynamic diameter 24h	24.5 ± 1.1 nm	52.4 ± 1.8 nm	42.6 ± 1.6 nm	80.1 ± 4.4 nm
Hydrodynamic diameter 48h	23.7 ± 2.7 nm	61.5 ± 2.6 nm	38.8 ± 0.8 nm	81.0 ± 4.0 nm
ζ-potential	-15.8 ± 0.4mV	-17.1 ± 1.8 mV	-18.2 ± 1.8 mV	-20.4 ± 0.2 mV

Figure S3. Confocal microscopy images of DOPC GUVs labelled with 0.5 mol% Rh-DOPE before (left) and after (right) incubation with 0.25 mg/ml BSA for 30 minutes.



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