Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information: Serum Albumin enhances the Biomembrane Activity of ZnO nanoparticles

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Materials & Methods

Materials

NanoSun ZnO NPs were kindly supplied by Dr. Claus Svendson of the E.U. NANOFATE consortium and were originally manufactured by Micronisers Pty. Ltd. (Australia). Bovine Serum Albumin (BSA) was sourced from Sigma Aldrich. 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rh-DOPE) were obtained from Avanti Polar Lipids in chloroform. Alexa Fluor 647 dextran (10 kDa; A647-10k) was purchased from Invitrogen. Laurdan was sourced from Cambridge Bioscience.

Preparation of ZnO samples

For stock samples without BSA, NanoSun ZnO NPs (10 mg) were dispersed in 1.0 mL deionized water by vortexing (1 min.) and then sonicating (approx. 15 min.) until the NPs were well suspended. For stock samples with BSA, 10 mg NanoSun ZnO NPs were added to 1.0 mL of 100 mg/mL BSA by vortexing until the particles were stably suspended (approx 2 – 3 min.). ZnO stock solutions were made fresh on the day of the experiment and shaken well immediately before use in experiments.

Dynamic Light Scattering (DLS)

Particle size distributions were obtained using a Malvern Zetasizer Nano ZS instrument. Data manipulation was performed by the DTS Nano software to determine the particle size distribution. Samples were diluted to 25 µg/mL ZnO in 300 mM sucrose for DLS analysis. Samples were sonicated for 30 minutes prior to DLS analysis. Size distributions were obtained from the average of five runs.

Transmission Electron Microscopy (TEM)

NP dispersions were directly imaged by TEM. 3.5 µL of NP sample was placed on the TEM grid (glow discharge treated carbon film on a copper grid). Grids were blotted then plunge frozen in liquid ethane. This produces an electron transparent thin film of the vitrified dispersant with the NP dispersion trapped within. It has been shown that when the specimens are warmed under the vacuum conditions of a TEM the solution devitrifies and sublimes leaving the dispersion of NPs on the carbon film unaltered.[1] TEM was performed using a using a Phillips FEI Tecnai TF20 field emission gun TEM operating at a gun voltage of 200 kV.

Electroformation of Giant Unilamellar Vesicles (GUVs)

GUVs were created using the electroformation method. Lipid mixtures in chloroform were made up of 1.0 mM DOPC and 0.5 mol% lipophilic fluorophore (Rh-DOPE or Laurdan). 50 µL of the mixture was spread dropwise on the Pt wires in the Teflon housing of a custom-built electroformation chamber using a Hamilton syringe, taking care to not allow droplets to merge by sliding along the wire. The chamber was placed under vacuum for at least 1 h to remove residual chloroform. Two glass slides were treated with a 10% (w/v) BSA solution for 10 – 20 min before rinsing thoroughly with deionised water then blowing dry under a nitrogen stream. The electroformation chamber was assembled with the BSA-coated glass facing the Pt wires and rubber spacers between the glass and Teflon housing. Two aluminium plates were then screwed into place on the outside of the glass plates to hold the chamber together. The chamber was carefully filled with a 300 mM sucrose solution with a syringe to ensure minimal air bubbles. A 10 V a.c. field was applied across the wires: 10 Hz (30 min), 3 Hz (15 min), 1 Hz (7 min) and 0.5 Hz (7 min). Al foil was placed over the chamber during electroformation in order to protect the sample from the light. The solution was then removed from the chamber, while gently rocking it back and forth to ensure all GUVs were removed from the Pt wires. GUVs were stored in a plastic vial covered with Al foil.

Extrusion of Large Unilamellar Vesicles (LUVs)

LUVs were formed by the extrusion method. 300 µL DOPC in chloroform (25 mg/mL) and < 0.5 mol% (lipid equivalent) Laurdan in chloroform were mixed in a glass vial before being dried down overnight into a thin film under vacuum. The lipid film was the rehydrated with 1.0 mL buffer A (125 mM NaCl, 10 mM HEPES, pH 7.4) and vortexed until all the lipid was suspended in solution. The sample was fully frozen in liquid nitrogen before thawing in warm water. The vortex-freeze-thaw cycle was repeated five times in total. The lipid suspension was then extruded through a polycarbonate membrane with 100 nm pores for a minimum of 11 passes using a mini-extruder (Avanti Polar Lipids). The LUV sample was then stored in a plastic vial wrapped in foil to protect from the light.

Lipid concentration was measured using a total phosphorous assay. Briefly, 6 reference samples ranging from 0 µM to 0.228 µM phosphorous were created in test tubes using a phosphorous standard solution. 6 sample test tubes each contained 10 µL of the LUV sample. All 12 tubes were then treated identically. 450 µL 8.9 N sulphuric acid was added to the tubes which were then heated at 215 °C in a heating block for 25 min. The samples were cooled before adding 150 µL 30% hydrogen peroxide and returning the tubes to the heating block for a further 30 min. The samples were again cooled before adding 3.9 mL deionised water, 0.5 mL 2.5% (w/v) ammonium molybdate tetrahydrate and 0.5 mL 10% (w/v) ascorbic acid. The tubes were vortexed for a few s before heating in a hot water bath at 100 °C for 7 min. Once cool the sample absorbances were measured at 820 nm using a UV-vis spectrophotometer (Perkin Elmer Lambda 900) and the LUV samples were compared to the calibration curve from the known reference samples to calculate the lipid concentration.

Confocal Fluorescence Microscopy

Confocal fluorescence microscopy experiments were conducted on a Zeiss LSM510. Samples were prepared on glass bottom dishes (MatTek P35G-1.5-14-C) that had been pretreated with a 10% BSA solution to prevent GUVs adhering to the glass coverslip. GUV samples were diluted in 330 mM sucrose or microscope buffer (165 mM NaCl, 10 mM HEPES, pH 7.4) which caused the GUVs to settle onto the coverglass due to the denser encapsulated sucrose solution. ZnO NPs and Alexa 647 dextran were added depending on the specific experiment.

Multiphoton Fluorescence Imaging of Laurdan

Multiphoton imaging of GUVs containing the Laurdan probe was done using the Olympus FV1000 with IX61 inverted body. Multiphoton excitation was from a Maitai Ti:Saphire laser mode-locked at 780 nm (0.5 W, 12 µs pulses) and a quarter waveplate was used to circularly polarize the incident light to ensure even excitation of the probe in all regions of the vesicle equator. Images were captured using a 60x 1.45 N.A. oil immersion lens. Emitted light was split using a 50/50 beamsplitter and intensity images were collected in two channels: 400 – 460 nm and 470 – 530 nm. GP intensity images were created using ImageJ software and GP intensity histograms were analyzed in Origin.

Fluorescence Spectroscopy

Fluorimetry experiments using the Laurdan probe in LUVs were conducted on a Spex Fluoromax 3 Spectrofluorometer. The excitation wavelength was set at 360 nm and emission intensities were measured in the range 400 nm to 560 nm. LUV samples were prepared at a concentration of 100 µM lipid (diluted with buffer A). Control experiments using ZnO NPs only were using to subtract background from the Laurdan emission spectra.

1. N. Hondow, R. Brydson, P. Wang, M. D. Holton, M. R. Brown, P. Rees, H. D. Summers and A. Brown, *Journal of Nanoparticle Research*, 2012, **14**, 977.