Analytical Chemistry

SUPPLEMENTARY INFORMATION

Native Nano-electrospray Differential Mobility Analyzer (nES GEMMA) Enables Size Selection of Liposomal Nanocarriers Combined with Subsequent Direct Spectroscopic Analysis

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Information on applied chemicals, liposome preparation and instrumentation as well as sample preparation and AFM height maps of liposomes with different deposition methods are given.

Materials and Methods

Chemicals: Ammonium acetate (NH_4OAc, ≥ 99.99 %), ammonium hydroxide (ACS reagent), boric acid (pro analysis, *p.a.*), DMSO (≥ 99.9 %) and cytarabine (Cytosine β-D-arabinofuranoside, ≥ 90 %) were purchased from Sigma Aldrich (Steinheim, Germany). Benzoic acid (> 99 %) was obtained from Fluka (Buchs, Switzerland). Chloroform (Spectronorm) was obtained from VWR (Roncello, Italy), Methanol (LiChrosolv) and sodium hydroxide pellets from Merck (Darmstadt, Germany). Nitrogen gas was obtained from Messer (Gumpoldskirchen, Austria). The lipids L-α-phosphatidylcholine, hydrogenated (Soy) (HSPC), 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (18:0 PE, DSPE), 1,2-distearoyl-sn-glycero-3-phosphoethanol-amine-N-[methoxy(polyethylene glycol)-2000 (DSPE-mPEG2000) and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, AL, USA obtained via Instruchemie, Delfzyl, The Netherlands). Atto633-COOH was purchased from Atto Tec (Siegen, Germany). Millipore (Billerica, MA, USA) grade water was employed (18.2 MΩcm resistivity at 25°C). NH_4OAc (40 mM, pH 8.4) filtered through a 0.2 µm pore size syringe filter (surfactant free cellulose acetate membrane from Sartorius, Göttingen, Germany) was used for vesicle preparation and as aqueous electrolyte. Cytarabine was either employed from a 40 mM or a 50 mM stock in 40 mM NH_4OAc, pH 8.4, respectively.

Liposome preparation: Dry lipids were weighed to 50 mL round bottom glass flasks previously cleaned with methanol: chloroform, 1: 3 mixture [v: v] and dried (i) via nitrogen gas and (ii) subsequent deposition into vacuum (desiccator). Lipids were dissolved in methanol:chloroform (1: 3 mixture [v: v]) and a thin, regular film was formed under a constant stream of nitrogen gas. The lipid film was further dried in a desiccator for approx. 2 hours. Hydration of the lipid film was either performed with (i) 1 mL NH_4OAc or (ii) 1 mL NH_4OAc including cytarabine (40 mM or 50 mM as indicated). This yielded dispersions of 10 mM total lipid concentration. Dispersions were vortexed and heated in a water bath (approx. 65 °C) until

the lipid films had fully detached from the flask surfaces. Subsequently, dispersions were extruded for 21 times through two pre-wetted 100 nm pore size, polycarbonate membranes (Avanti Polar Lipids) applied in the same membrane orientation to obtain small unilamellar liposomes. Liposome stock solutions were stored in brown glass vials at 4°C at least over night until further use.

Native nES GEMMA measurements: 4.0 pounds per square inch differential pressure (psid, approx. 28 kPa) and 0.1 liters per minute (Lpm) CO_2 and 1.0 Lpm compressed, particle-free air were employed for transport of analytes through the capillary through the neutralization chamber and to the nDMA unit. Particle-free air was additionally dried (Donaldson Variodry Membrane Dryer Superplus obtained via R. Ludvik Industriegeräte, Vienna, Austria) prior to application. Individual measurements were carried out between 5 and 184 nm EM diameter upon application of 2.5 Lpm sheath flow inside the nDMA. Four respective measurements (150 s scan time, 30 s to reset the high voltage of the nDMA to starting values, respectively, raw particle counts per detector channel were used) were combined via their median to yield a corresponding spectrum.

CE measurements: CE separations were carried out at 16 kV (27 kV/m field strength) at 22.5 °C after 2 min flushing of the fused silica capillary (50 µm inner / 375 µm outer diameter; total/effective length $L_{tot/eff}$ = 59.9/51.6 cm from Agilent Technologies) with BGE (preconditioning). Postconditioning was performed via 1 M NaOH and water (2 min each). Sample buffer was 36 mM NH_4OAc, pH 8.4 including DMSO (final dilution $1:4 \times 10^3$) and 20 µg/mL benzoic acid as internal standards. Samples were injected for 9 s at 40 mbar and included cytarabine at indicated concentrations. Liposomes were desalted as for ENAS collection experiments and applied after a total 1:6.7 dilution. Sonication of samples (already including the internal standards) was performed in an ultrasonic bath for 30 min. Obtained electropherograms were aligned to the peaks of the internal standards and time corrected areas

were taken to calculate concentration values for encapsulated material. Analyte detection was at 200, 205 and 260 nm UV absorption (205 nm data was employed for calculation and display).

Buffer exchange of samples: 10 µL of a respective liposome preparation were applied to 490 µL of 40 mM NH_4OAc, pH 8.4 on top of a 10 kDa MWCO spin filter (polyethersulfone membrane, VWR, Vienna, Austria). Subsequent centrifugation was performed at 1.1×10^4 g until most of the liquid had passed the membrane. Care was exercised, not to dry down the vesicles. Buffer was replenished on top of the filter, the eluate discarded and the spinning repeated. This procedure was carried out for in total three times (buffer was replenished twice). The retentate was recovered from the membrane, the membrane was washed with buffer and retentate and washing solution were combined.

Instrumentation: For FTIR transmission measurements, a Vertex 80v (Bruker, Germany) spectrometer equipped with a MCT (mercury cadmium telluride) detector with liquid nitrogen cooling was employed for transmission measurements in a flow cell (27 µm path length) connected to a syringe pump (ALADDIN-300 World Precision Instruments) with a 500 µL Hamilton glass syringe.

FTIR-ATR measurements were performed using a Tensor 37 (Bruker, Ettlingen, Germany) spectrometer equipped with a DLaTGS (deuterated L-alanine doped triglycine sulfate) detector operated at room temperature and a single-reflection diamond ATR crystal (Platinum ATR module, Bruker). For FTIR transmission as well as FTIR-ATR measurements, 100 scans were co-added for one spectrum with a spectral resolution of 2 cm^{-1}. OPUS 7.2 software was used for spectrum acquisition and instrument control.

AFM height maps: Liposomes were collected on substrates using nES GEMMA collection to avoid sample destruction as is the case for liposomes that were deposited on the substrate and let dry at room temperature (see Figure S1). The different sample height indicates intact liposomes after nES GEMMA collection, while the height of the structures with the second

deposition method more likely reflects collapsed vesicles forming a phospholipid bilayer.

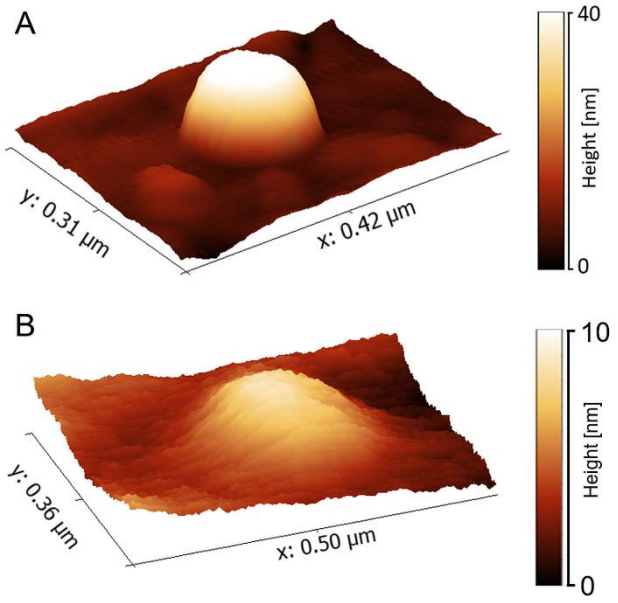

Figure S1. AFM height maps of drug filled liposomes deposited via nES GEMMA (A) and a simpler deposition method consisting of placing a droplet on the substrate before letting it dry in an unforced way at room temperature (B)