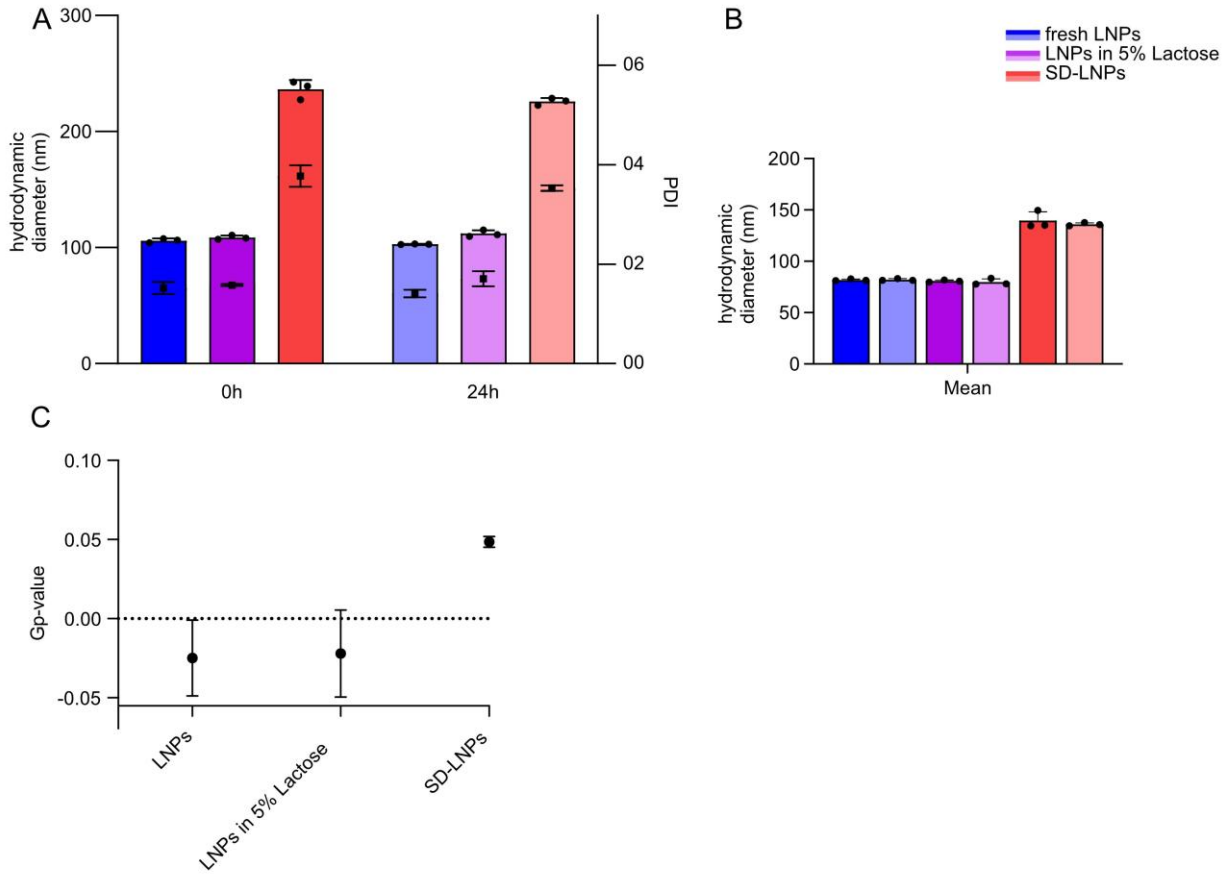
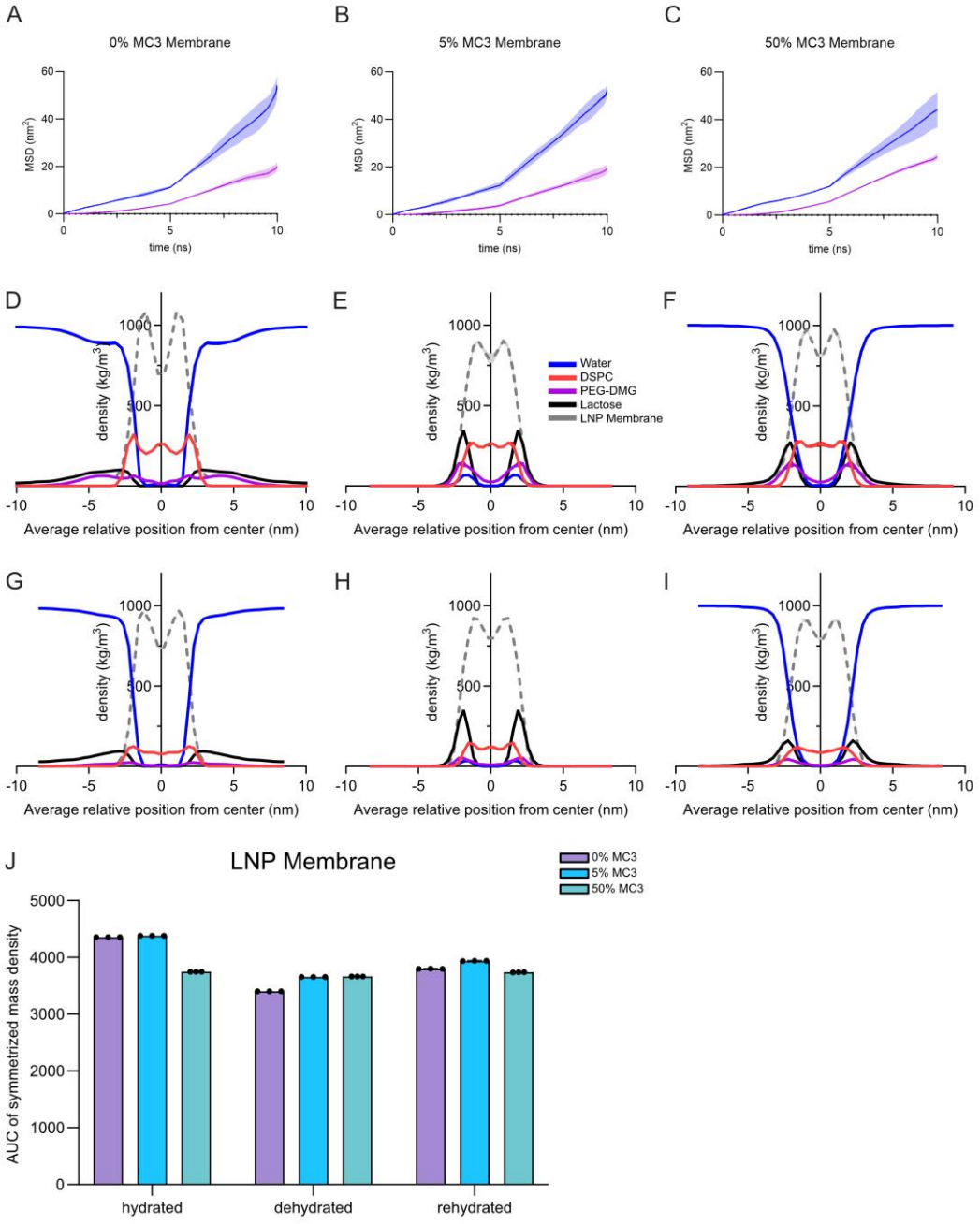
Supplementary Materials:

***Supplementary Table 1*: Number of lipids and lactose molecules per system.** Membranes were simulated as bilayers; the numbers are given for the total amount of lipids in the system.

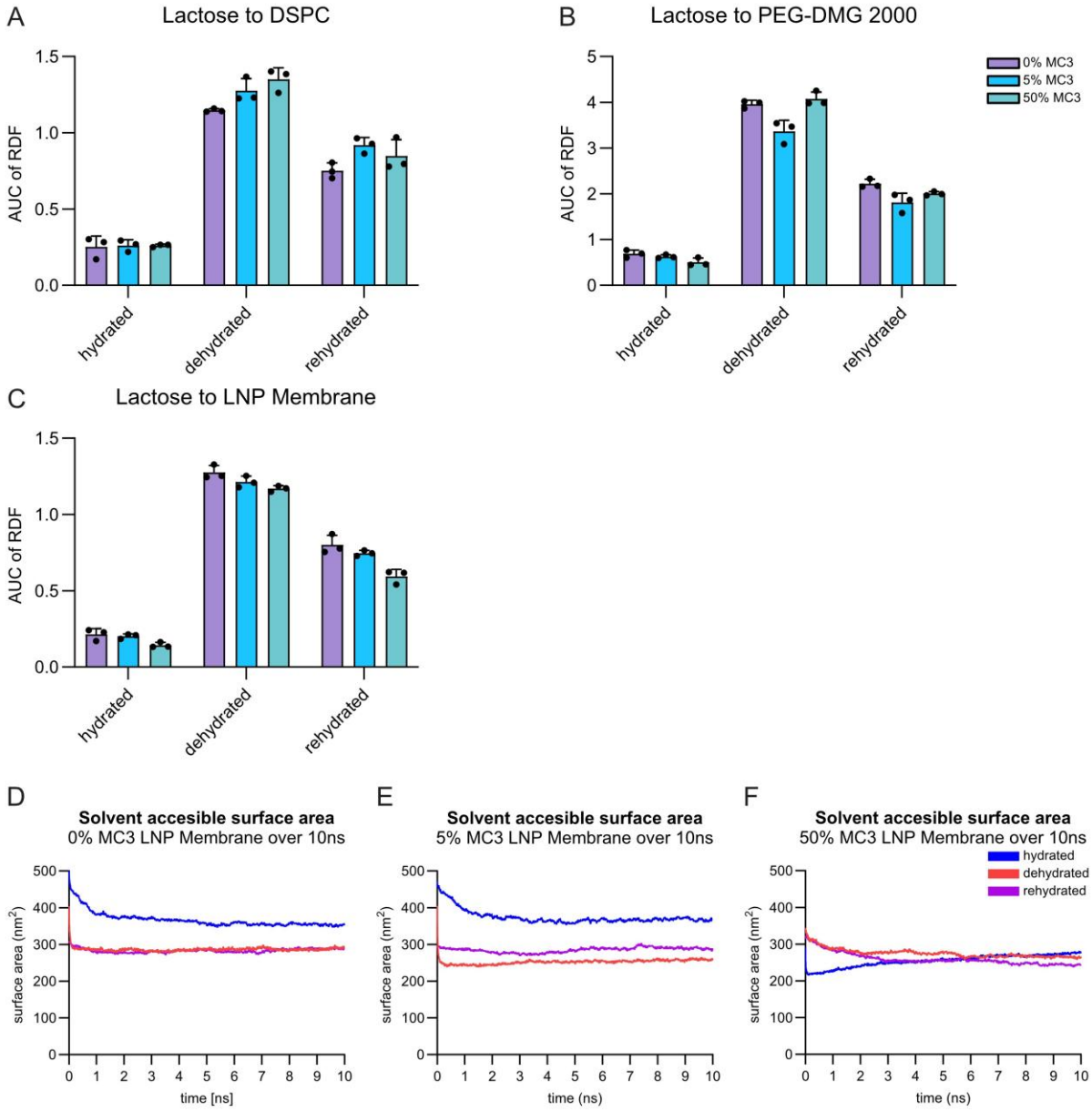
	D-Lin-MC3-DMA	Cholesterol	DSPC	PEG-DMG 2000	Lactose
50% MC3 hydrated	100	76	20	2	69
50% MC3 dehydrated	100	76	20	2	69
50% MC3 rehydrated	100	76	20	2	69
5% MC3 hydrated	10	146	38	6	65
5% MC3 dehydrated	10	146	38	6	65
5% MC3 rehydrated	10	146	38	6	65
0% MC3 hydrated	0	154	40	6	69
0% MC3 dehydrated	0	154	40	6	69
0% MC3 rehydrated	0	154	40	6	69



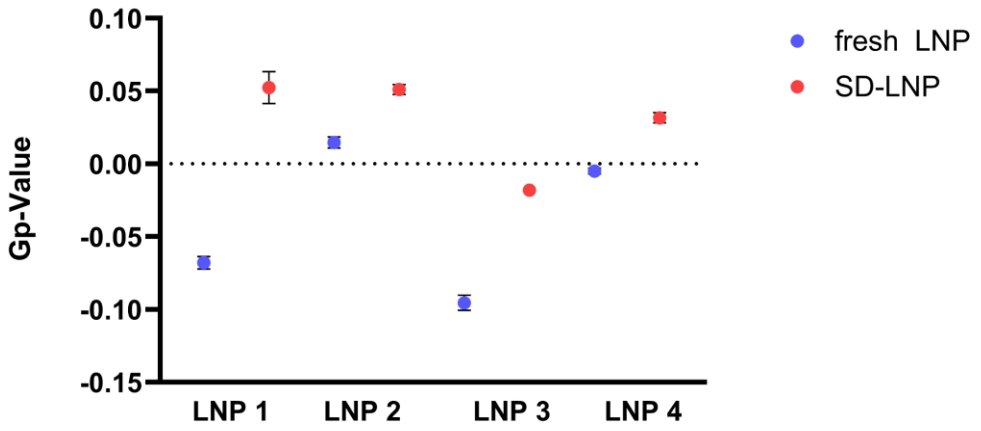
Supplementary Figure 1: Hydrodynamic diameter of fresh and redispersed Onpattro like LNPs before (dark) and after (lighter) 24 h dialysis against PBS, measured via A) dynamic light scattering (DLS) or B) measured via NTA. C) Gp-value of Laurdan stained Onpattro like LNPs, LNPs in 5% Lactose and after spray drying. Data points indicate mean ± s.d, with individual replicates in bar diagrams shown as points.



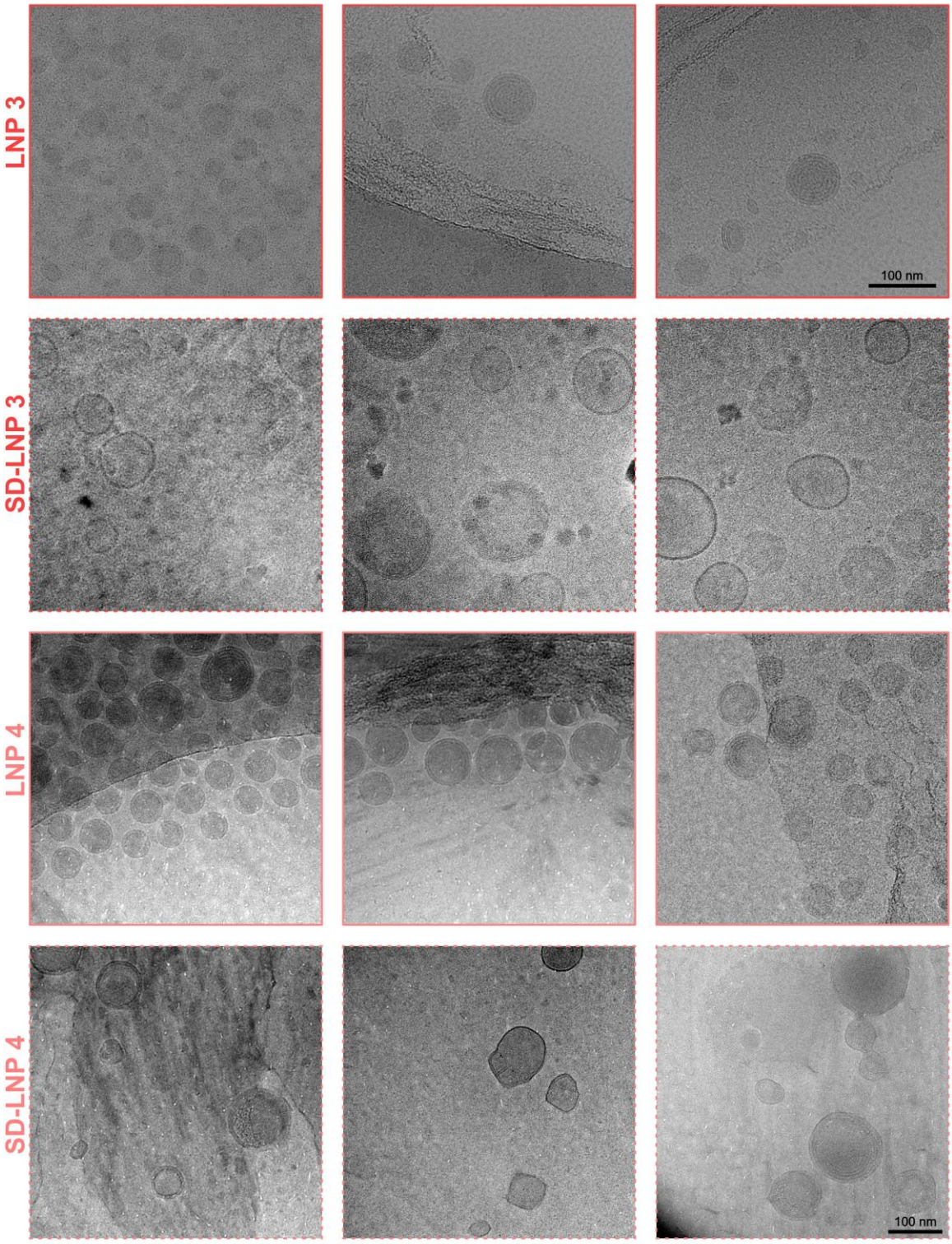
***Supplementary Figure 2*: Additional simulation data:** A–C) Mean square displacement (MSD) of lactose molecules obtained from NPT simulations. Data are shown as mean ± standard deviation (shaded area around the lines). D–F) Symmetrized mass-density profiles of LNP membrane models containing 0% MC3 at different hydration states: (D) hydrated LNP membrane formulation before spray drying (SD), (E) SD LNP membrane formulation (dehydrated), and (F) rehydrated SD LNP membrane formulation. G–I) Symmetrized mass-density profiles of LNP membrane models containing 50% MC3 at different hydration states: G) hydrated, H) dehydrated (spray-dried), and I) rehydrated. J) Area under the curve (AUC) of the symmetrized mass-density profiles. Data points indicate mean ± s.d, with individual replicates in bar diagrams shown as points.



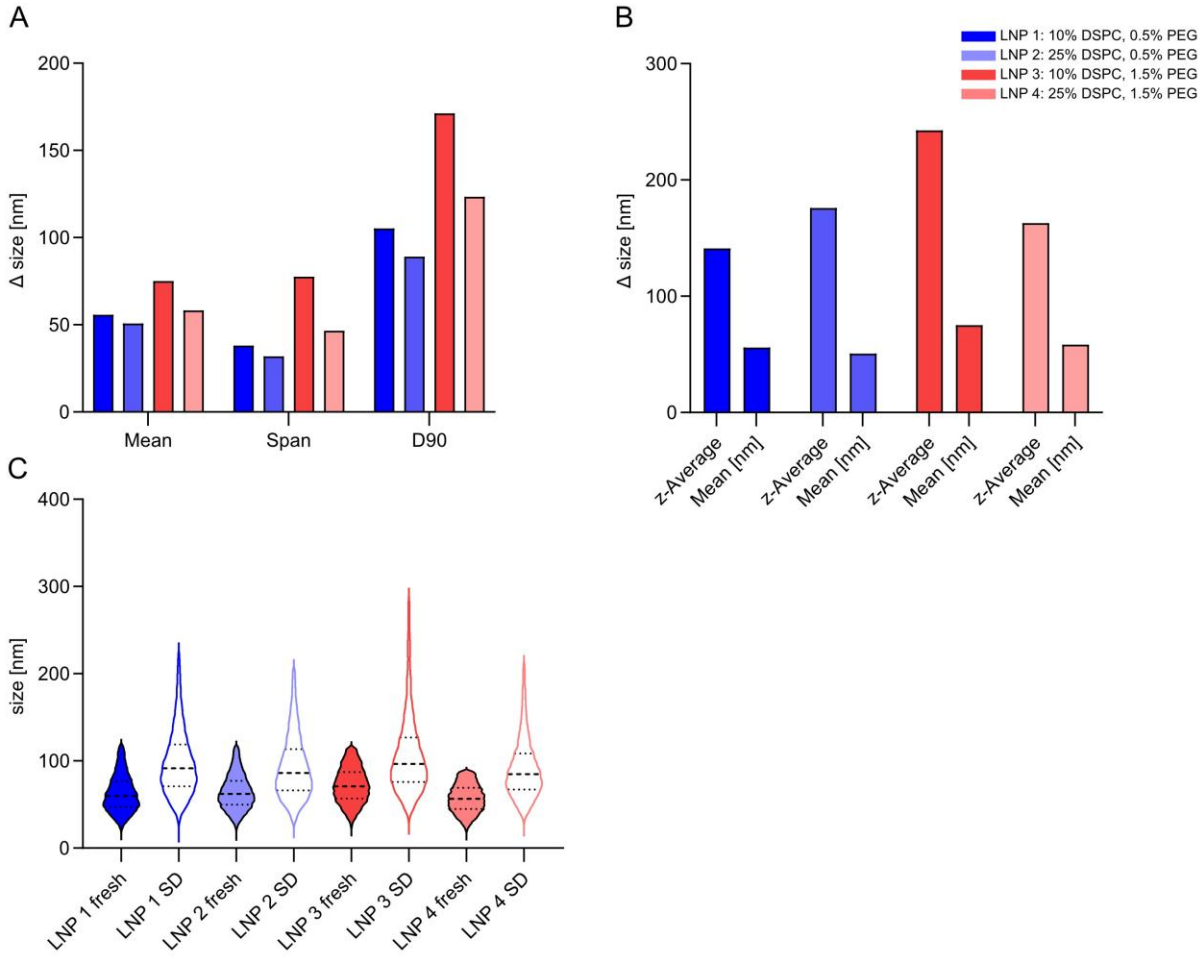
***Supplementary Figure 3*: Additional MD analysis of LNP membrane models at different hydration states.** A) Area under the curve (AUC) of the radial distribution function (RDF) of lactose towards DSPC. B) AUC of the RDF toward PEG-DMG-2000 C) AUC of the RDF of lactose towards the LNP Membrane. D-F) Mean solvent accessible surface area (SASA) of the simulated LNP membranes over time. AUC values were integrated from 0 to 1nm from the corresponding RDF curves. Data are shown as mean ± s.d., with individual replicates in bar plots displayed as points.



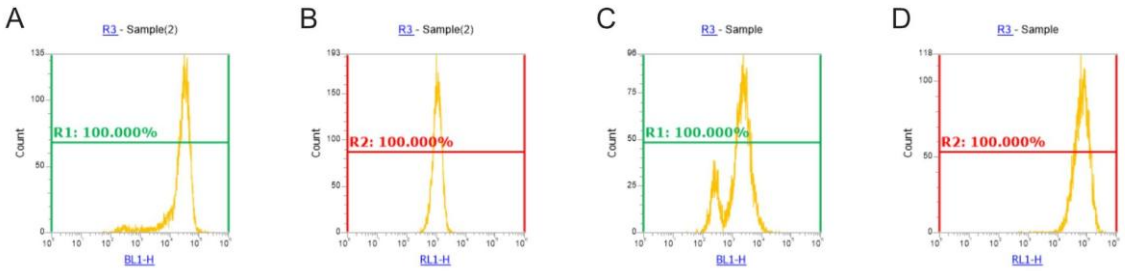
***Supplementary Figure 4*:** Gp-value of the four different LNP compositions before and after spray-drying Data points indicate mean of three measurement replicates performed ± s.d.



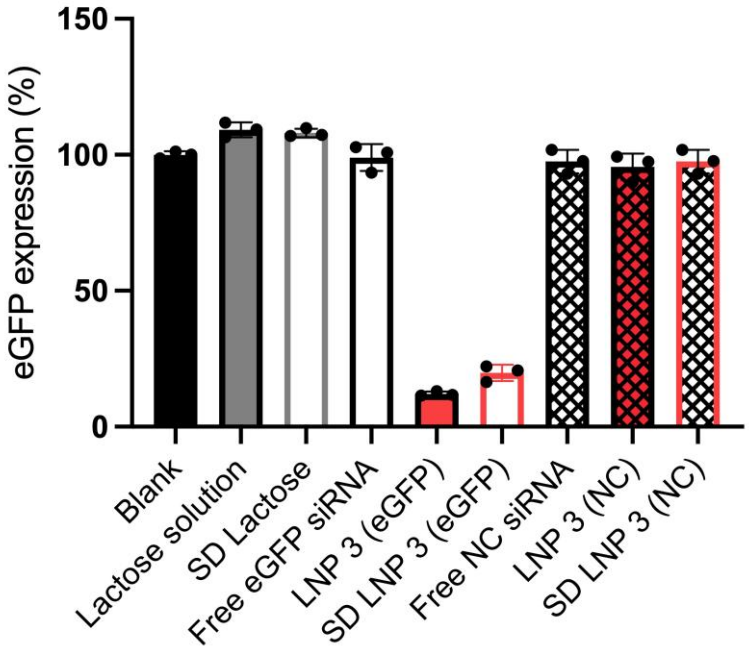
***Supplementary Figure 5*: cryo-TEM micrographs.** First column shows full micrographs from Figure 3 E-H. The others are additional micrographs to give an overall view of the morphology of the different LNPs. LNPs were concentrated towards a siRNA concentration of 1000 ng/µl.



***Supplementary Figure 6*: NTA measurements:** A) Difference between fresh LNPs and spray dried LNPs for Mean, Span and D90 B) Comparison between measurement results from DLS (Z-Average) and NTA measurements Mean, regarding particle size C) Population within D90 of all four formulations. Data points indicate Mean of 5 measurements for NTA data and Mean of 3 measurements for DLS data. For the particle population all points from the measurements within the D90 of the measurement were plotted.



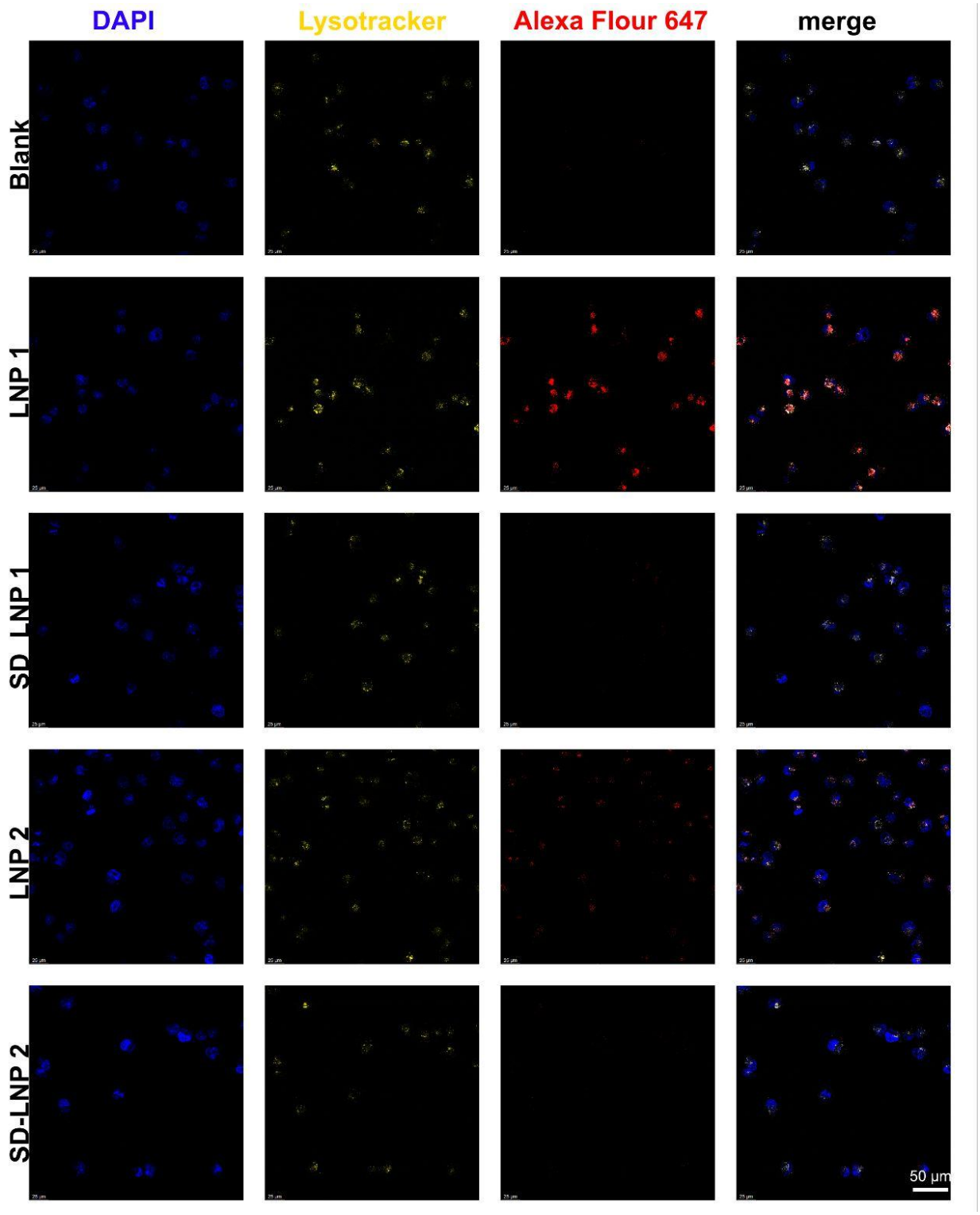
Supplementary Figure 7: **Histogram plots of eGFP knockdown and cellular uptake in H1299-eGFP cells.** For siRNA cellular uptake and eGFP knock down measurements, 638 nm and 488 nm excitation laser were used and detected with RL-1H and BL-1H filters simultaneously. Blank samples were treated with 100 µL PBS (panel A and B). For sample wells, cells were incubated with LNPs containing 80 pmol of AF647-labled eGFP-siRNA. Treatment shown exemplarily for fresh LNP 2 formulations after 4 h of incubation (panel C and D).



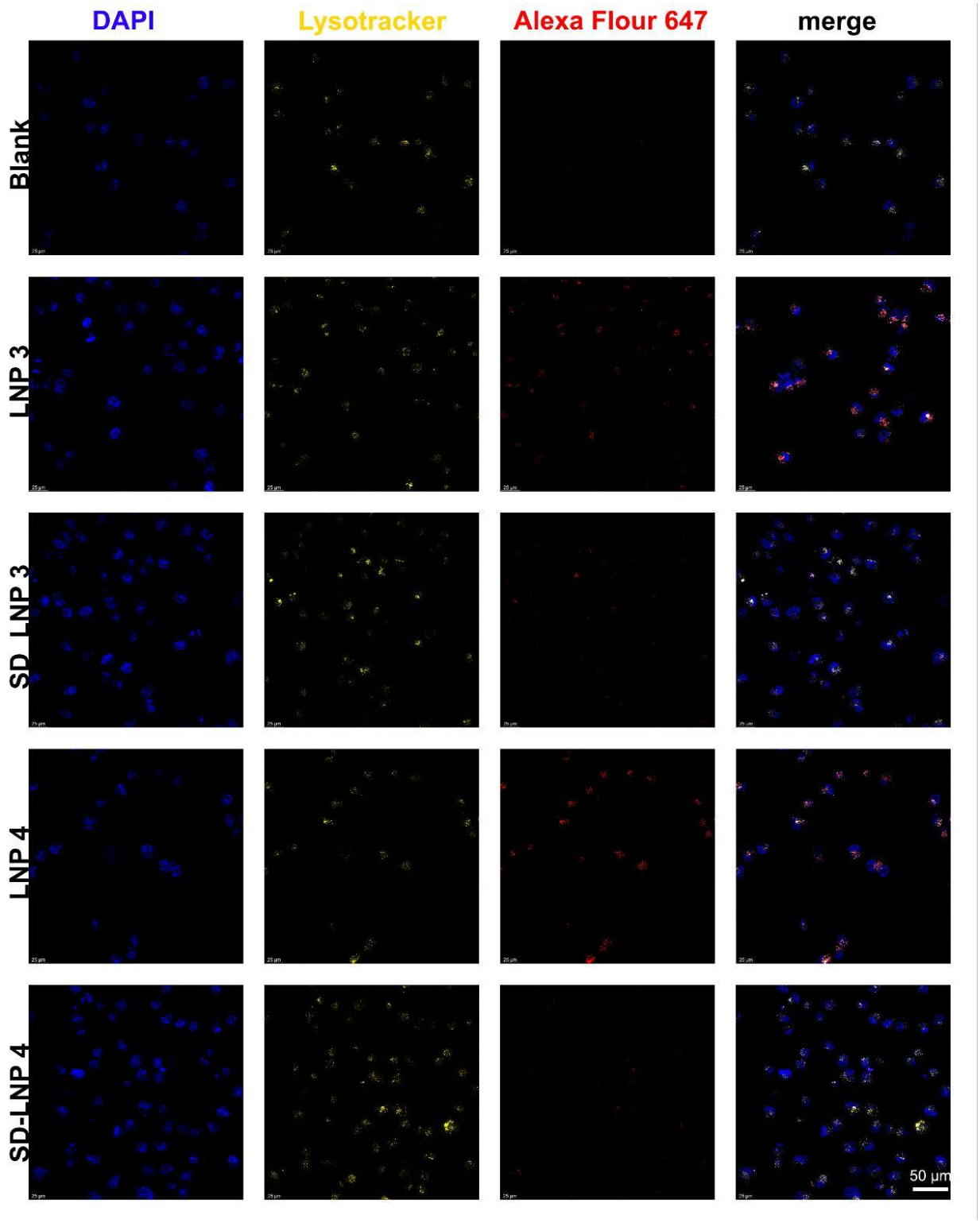
Supplementary Figure 8: **Monitoring of specific eGFP knockdown in H1299-eGFP cells.** For 24 h eGFP knock down measurements, 488 nm excitation laser was used and detected with BL-1H filter. Blank samples were treated with 100 µL PBS. For sample wells, cells were incubated with fresh and reconstituted SD LNP 3 containing 80 pmol of eGFP-siRNA. As a control for the specific knock down effect of eGFP-siRNA encapsulating LNPs, wells were transfected with fresh and reconstituted SD lactose solution 5% (m/V), 80 pmol of free siRNA in PBS, 80 pmol of free scrambled siRNA (NC) in PBS and fresh and SD reconstituted LNP 3 encapsulating 80 pmol of scrambled siRNA. Measurements were performed in technical triplicates; data points indicate mean ± s.d..

Supplementary Table 2: **Knockdown halftime of fresh and SD LNP 1-4** Results of fitting the eGFP expression data to a four-parameter logistic regression model, with the time point corresponding to 50% maximal expression defined as $T_{1/2}$.

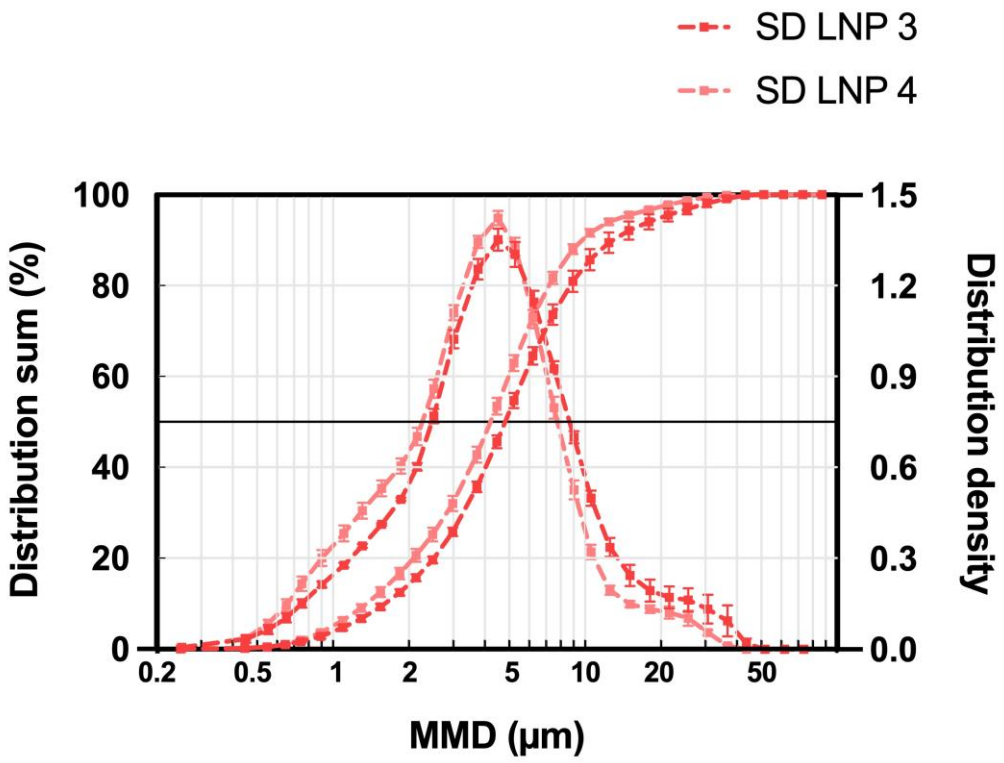
	Fresh LNPs		SD LNPs	
	$T_{1/2}$ (h)	**R^2**	**$T_{1/2}$ (h)**	**R^2**
LNP 1	5.80	0.9928	16.7	0.9391
LNP 2	8.20	0.9814	19.8	0.9694
LNP 3	5.90	0.9697	6.00	0.9896
LNP 4	7.90	0.9641	7.70	0.9836



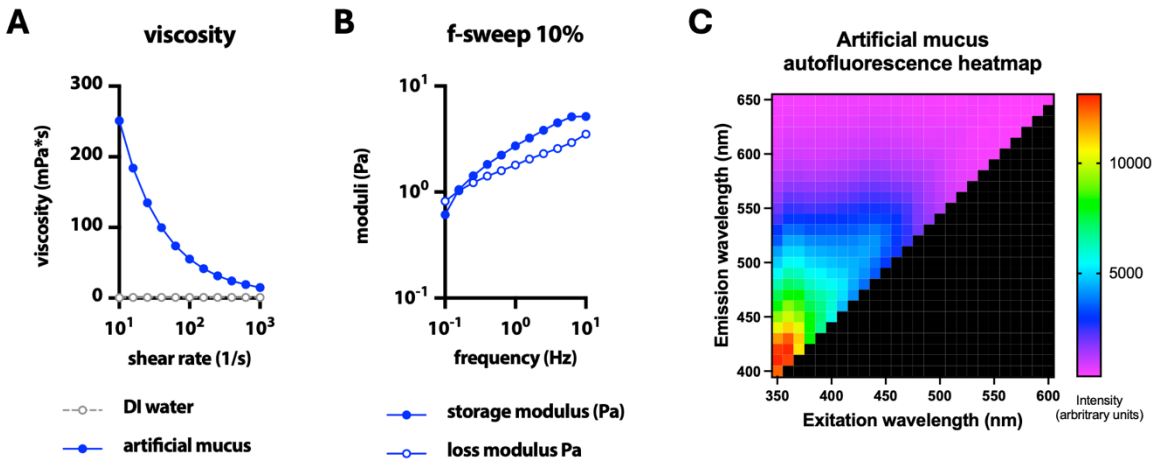
***Supplementary Figure 9*:** Cellular distribution of AF647-labeled LNPs in H1299-eGFP cells was visualized by confocal microscopy 4 hours post-transfection. Cells were transfected with 32 pmol siRNA using fresh or spray-dried LNP formulations 1 and 2, while blank samples received PBS. Nuclei were stained blue with DAPI, LysoTracker Red is shown in yellow, and AF647-labeled siRNA in red. Images were merged for analysis.



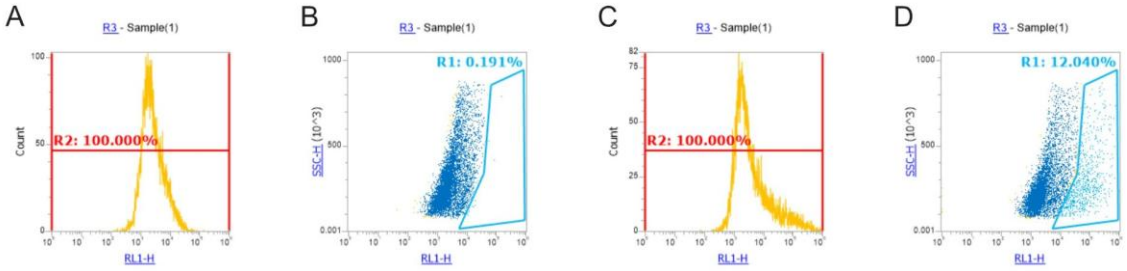
***Supplementary Figure 10*:** Cellular distribution of AF647-labeled LNPs in H1299-eGFP cells was visualized by confocal microscopy 4 hours post-transfection. Cells were transfected with 32 pmol siRNA using fresh or spray-dried LNP formulations 3 and 4, while blank samples received PBS. Nuclei were stained blue with DAPI, LysoTracker Red is shown in yellow, and AF647-labeled siRNA in red. Images were merged for analysis.



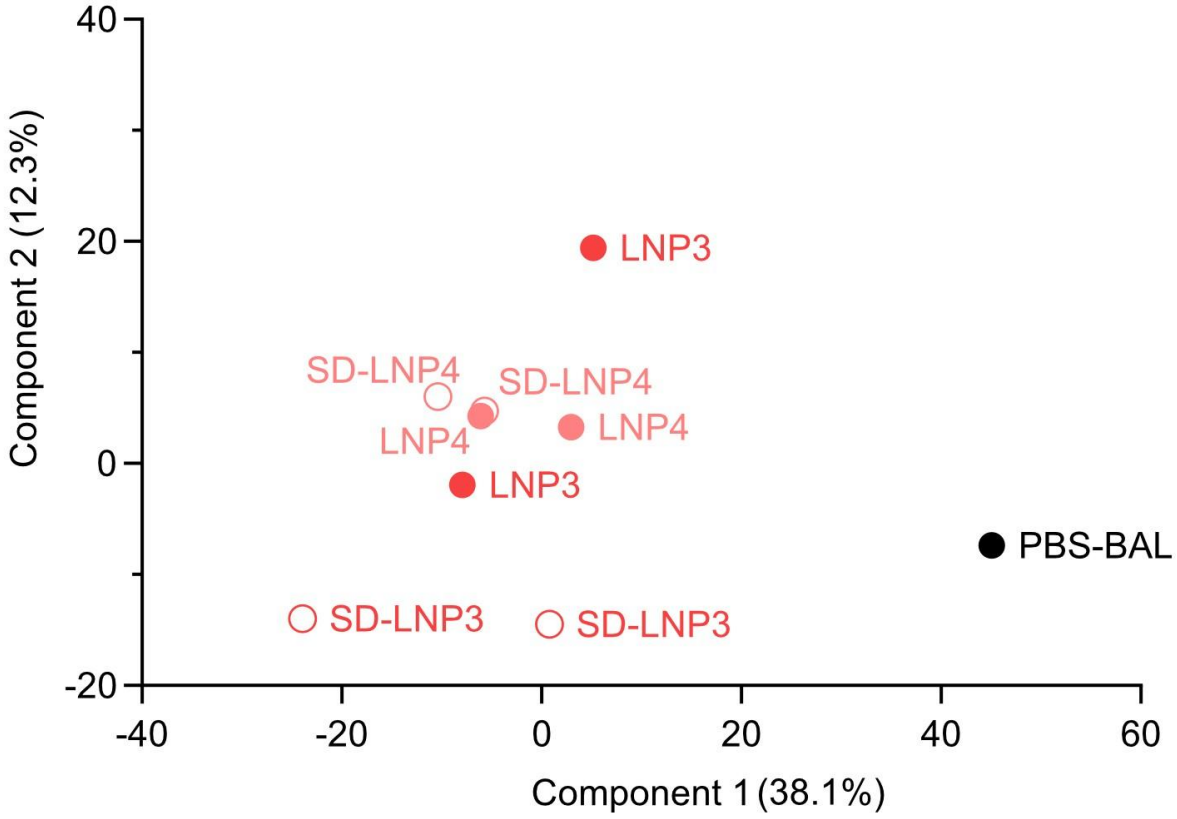
Supplementary Figure 11: Particle size distribution and distribution sum of SD LNP 3 and SD LNP 4 powder was assessed by laser diffraction using a inhaler module operating at a 4 kPa pressure drop difference mimicking a human inhalation manoeuvrer. Powder was discharged from the capsule based HandiHaler. SD LNP 3 and LNP 4 exhibited a mass median diameter (MMD) of 4.86 ± 0.13 µm and 4.26 ± 0.13 µm respectively. Measurements were performed in technical triplicates.



Supplementary Figure 12: **Rheological characterization of artificial mucus.** (A) Artificial mucus exhibits typical shear-thinning behaviour, with viscosity decreasing from approximately 250 mPa·s to 15 mPa·s over a shear rate range of 10–1000 s^{-1}. (B) Similar to native mucus, it shows gel-like behaviour, indicated by G′ > G″ across a broad range of tested frequencies. (C) Artificial mucus displays wavelength-dependent autofluorescence, which should be considered when performing fluorescent tracking in mucus samples.



Supplementary Figure 13: Gating strategy: Histogram and dot plots illustrate cellular uptake of fluorescent labeled siRNA in Calu-3 cells grown at the air-liquid interface. Intracellular uptake was measured using a 638 nm excitation laser and detected with the RL-1H filter. Blank samples were treated with 100 µL of PBS (Panels A and B). Sample wells were incubated with LNPs containing 160 pmol of AF647-labeled siRNA. Representative data for fresh LNP 2 formulation after 6 hours of incubation are shown (Panels C and D), depicting the percentage of transfected cells gated in R1.



Supplementary Figure 14: Principal component analysis (PCA) reveals distinct clustering based on LNP formulation (LNP3 vs. LNP4) and processing status (fresh vs. SD), as well as separation from the BALF-only control.

Supplementary Methods:

Monitoring of specific in vitro eGFP knock down in H1299-eGFP cells

H1299-eGFP cells were cultured in RPMI 1640 media supplemented with 10% foetal bovine serum (FBS), 1% penicillin-streptomycin and 0.4% G418. Cells were maintained, grown and incubated in a humidified incubator at 37 °C and 5% CO2. 30,000 cells were seeded in 24-well plates and incubated for 24 h before transfection. Blank samples were treated with 100 µL PBS. For sample wells, cells were incubated with 100 µL of fresh and reconstituted SD LNP 3 containing 800 pmol/mL of eGFP-siRNA. As a control for the specific knock down effect of eGFP-siRNA encapsulating LNPs, wells were treated with fresh and reconstituted SD lactose solution 5% (m/V), 80 pmol of free siRNA in PBS, 80 pmol of free scrambled siRNA (NC) in PBS and fresh and SD reconstituted LNP 3 encapsulating 80 pmol of scrambled siRNA. 24 h post transfection, the medium was removed, and cells were washed with PBS and trypsinised. The reaction was stopped by adding 200 µl of growth medium. The cell suspension was collected and centrifuged at 400 g for 5 min. Afterwards, supernatant was aspirated, cells washed with PBS, centrifuged, and finally suspended in 400 µl PBS with 2 mM EDTA. The eGFP knockdown was measured using flow cytometry (Attune NxT, Thermo Fisher Scientific, Waltham, MA, USA), with excitation at 488 nm, detected with BL-1H filter. Data were collected from 10,000 viable gated cells per sample. Knockdown efficiency is expressed as % eGFP expression relative to blank controls. Measurements were performed in technical triplicates, data points indicate mean ± s.d..

Particle size distribution of spray dried LNP powder

The particle size distribution of SD LNP 3 and SD LNP 4 dry powder was assessed by laser diffraction using the HELOS/KR with an integrated INHALER module (Sympatec, Clausthal-Zillerfelf, Germany). 5 to 8 mg powder was filled in hydroxypopylmethylcellulose capsules (Kapselwelt, Hude, Germany), loaded into the HandiHaler (Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany). Capsules were pierced, discharged and dry dispersed at a pressure drop difference of 4 kPa mimicking a human inhalation manoeuvrer. Data analysis was performed using the PAQXOS Software. Measurements were performed in technical triplicates. The mass median diameter (MMD) is presented as average size (µm) ± s.d..