Electronic Supplementary Material (ESI) for Lab on a Chip.

- SUPPLEMENTARY INFORMATION -

Liposome manufacturing under continuous flow conditions: towards a fully integrated set-up with in-line control of critical quality attributes

Maryam Sheybanifard[a], Luis P. B. Guerzoni[b], Abdolrahman Omidinia-Anarkoli [b,c], Laura De Laporte [b,c,d], Johannes Buyel[e,f,g], Rut Besseling[h], Michiel Damen[h], Ad Gerich[h], Twan Lammers[a], Josbert M. Metselaar[a*]

[a] Department of Nanomedicine and Theranostics, Institute for Experimental Molecular Imaging, Uniklinik RWTH Aachen and Helmholtz Institute for Biomedical Engineering, Faculty of Medicine, RWTH Aachen University, 52074 Aachen, Germany

[b] DWI Leibniz Institute for Interactive Materials, Forckenbeckstrasse 50, 52074 Aachen, Germany

[c] Institute of Applied Medical Engineering, RWTH University, Pauwelsstraße 20, 52074 Aachen, Germany

[d] Institute for Technical and Macromolecular Chemistry, RWTH Aachen, Worringerweg 1-2, 52074 Aachen, Germany

[e] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstrasse 6, 52074 Aachen, Germany

[f] Institute for Molecular Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[g] Institute of Bioprocess Science and Engineering, University of Natural Resources and Life Sciences (BOKU), Muthgasse 18, A-1190 Vienna, Austria

[h] InProcess-LSP, Kloosterstraat 9, 5349 AB Oss, The Netherlands

[*] *Corresponding author: E-mail: jmetselaar@ukaachen.de*

Table S1

ANOVA	Size (50% v/v ethanol)	PDI (50% v/v ethanol)	Size (30% v/v ethanol)	PDI (30% v/v ethanol)
R^2	0.8885	0.9192	0.7364	0.0000
Adjusted R^2	0.8641	0.8984	0.6939	0.0000
Predicted R^2	0.8117	0.8538	0.5828	-0.1652
Lack of fit (p-value)	0.0103	0.0962	0.2234	0.0056
Model	Reduced cubic	Reduced cubic	Reduced cubic	Mean
Model significant?	Yes, p-value < 0.0001	Yes, p-value < 0.0001	Yes, p-value < 0.0001	-

Table S1. ANOVA analysis for the size and PDI responses in two conditions, namely in the presence of 50% v/v ethanol and 30% v/v ethanol.

Fabrication of Coaxial Millifluidic Mixer Used for Liposome Formation

A T-shaped tube fitting and clear PTFE tubing were used for the fabrication of the coaxial mixer. A blunt needle with ID of 0.6 mm was inserted to the straight channel from one side. The other side of the straight channel was connected to a PTFE tubing with an ID of 3 mm, where the needle was positioned in center as the inner channel. Luer lock connections were used to connect the inner and outer channels to the pumps.

Figure S1

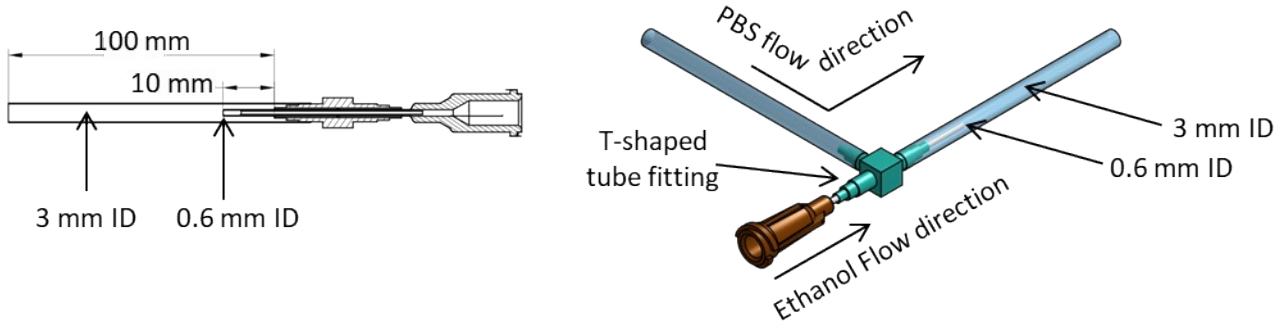

Figure S1. Schematic view of the fabricated coaxial millifluidic mixer.

Figure S2

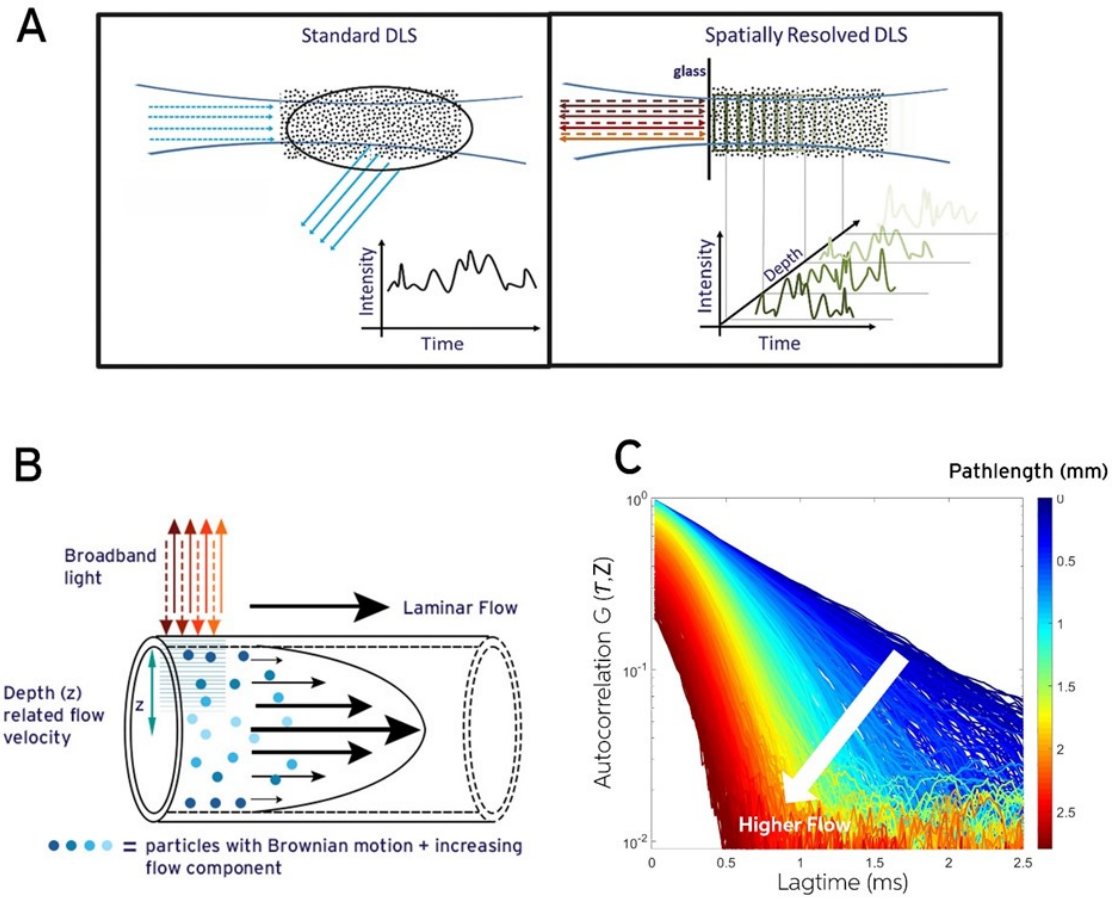

Figure S2. A) Schematic illustration of standard DLS measurement (left) and Spatially Resolved-DLS (right) B) Representation of particle motion during laminar pipe flow of a nanosuspension. Superimposed on the normal Brownian motion of nanoparticles is the Poiseuille laminar flow profile, both of which are spatially characterized using SR-DLS. The broadband illumination from the NFS probe and backscattering are also indicated. C) Autocorrelation functions measured simultaneously over a range of sample depths using SR-DLS, the white arrow indicates the increase in local velocity for larger depths. Image adapted from our previous publication [1].

1. C. Schuurmans, J.-P. Wijgergangs, A. Gerich and R. Besseling, AZoNano, 2022, DOI: 10.13140/RG.2.2.18231.80805.

Figure S3

To properly mix the concentrated lipids with pure ethanol before the resulting ethanolic lipid solution enters the millifluidic device as illustrated above, we installed a static mixer (consisting of 8 internal mixing compartments) with one inlet for each stream and an outlet. The static mixer is a 316 stainless steel Sulzer static mixer SMX™ that was purchased from Sulzer Chemtech AG (Winterthur, Switzerland). PTFE tubing was used to direct each stream into the static mixer and out of it to the millifluidic device.

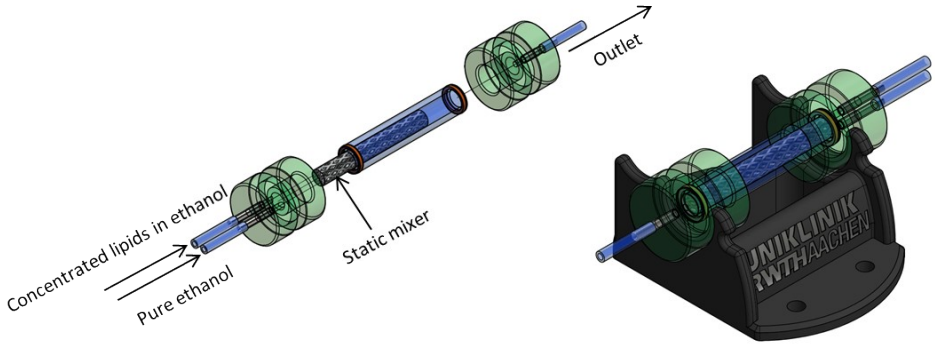

Figure S3. Schematic view of the static mixer for proper mixing of the concentrated lipids and pure ethanol.

Figure S4

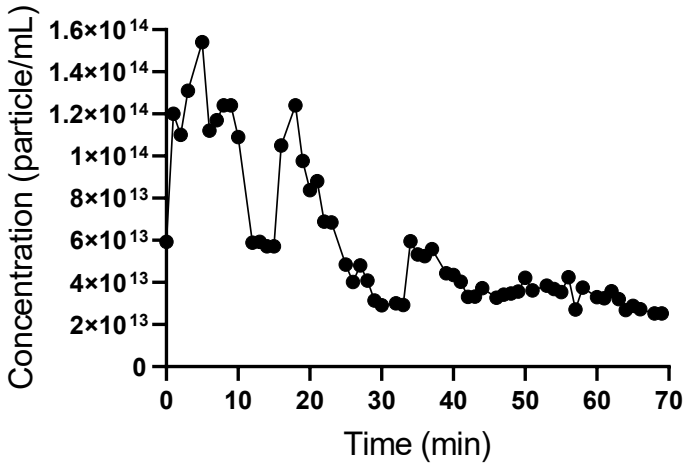

Figure S4. The concentration of liposomes decreases over time in the presence of 50% v/v ethanol/PBS. *Liposomes were formed with 70 mM lipid in ethanol and TFR 62 mL /min-1 and the sample was diluted 10,000 times with a mixture of PBS and ethanol (1:1 ratio). Data were obtained from NTA device.*

Figure S5

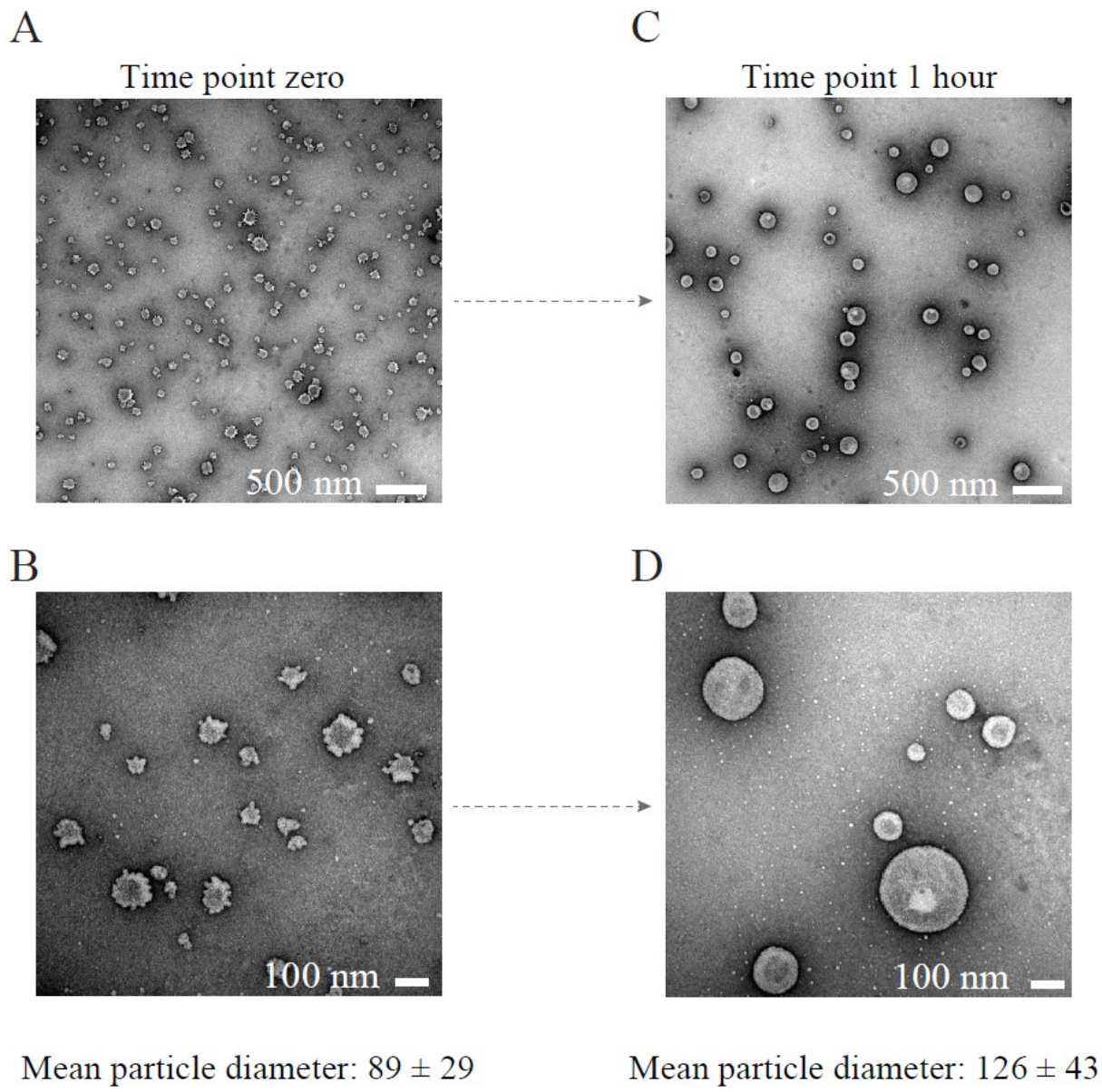

Figure S5. *TEM images obtained from liposomes at time point zero (A and B) and after 1 hour (C and D). Liposomes were formed with 70 mM lipid in ethanol and TFR 62 mL min^{-1}. The presented size of particles is the mean size of ≥ 40 particles that were calculated in the representative TEM image of each set (i.e., A and C) using ImageJ 1.43u software, Java 1.6.0_12).*

Figure S6

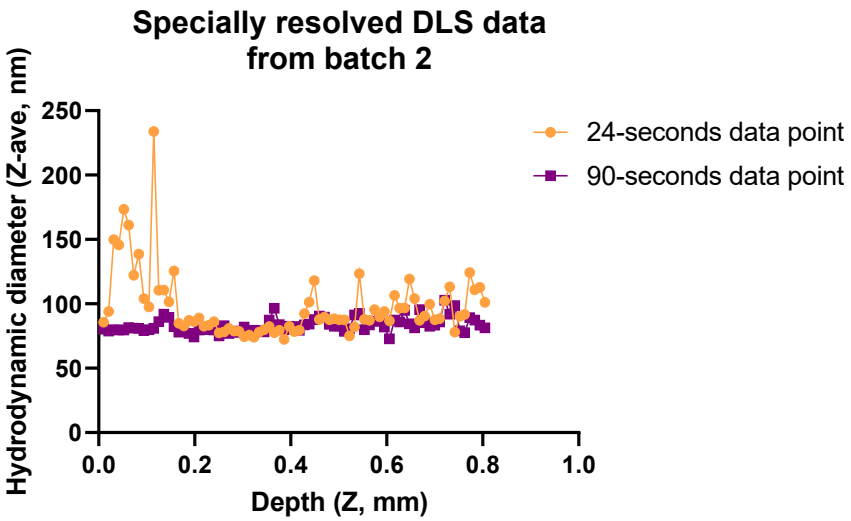

Figure S6*. spatially resolved liposome size as function of depth in the flow cell for Batch 2, for a reduced quality measurement (time point 24 s) and for an optimal quality measurement (time point 90 s).*

Figure S7

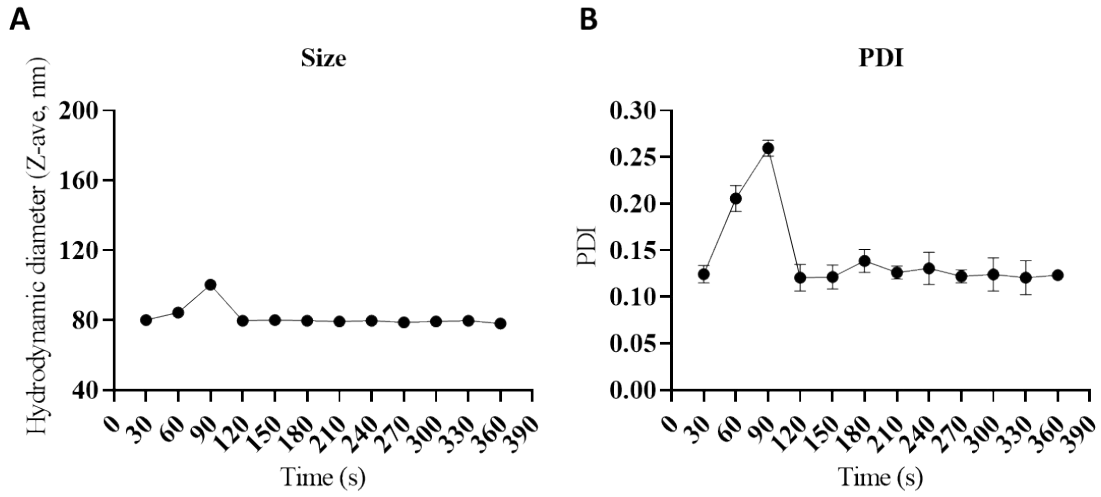

Figure S7. Stability of the liposome size (A) and PDI (B) after running the setup for 360 seconds.

Liposomes were formed with 70 mM lipid in ethanol and TFR was set at 62 mL min^{-1}.