# *(Supplemental Information)*

# Characterization of Nanoscale Loaded Liposomes Produced by 2D Hydrodynamic Flow Focusing

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*Summary:*

Number of pages: 8, Number of Figures: 5, Number of Tables: 6.

# CFD Parameters and Simulations

Stationary Steady state studies are governed by "Incompressible Navier-Stokes" (1) and "Continuity" (2) equations where the final forms assuming incompressible Newtonian fluids, are presented below:

$$\rho \left[ \frac{\delta u}{\delta t} + (u \cdot \nabla) u \right] = -\nabla p + \mu \nabla^2 + F \tag{1}$$

$$\rho \left[ \nabla \cdot (u) \right] = 0 \tag{2}$$

Where: $u$ is the flow velocity, $\rho$ is the fluid density, $p$ is the pressure, $\mu$ is the dynamic viscosity and $F$ represents outer forces. The outlet boundary was set to a function of pressure, with $p = 0$ Pa.

For mass transport of dissolved (solute) species, concentration gradients will cause diffusion. In addition, convection will also contribute to the flux of chemical species by bulk fluid motion. Therefore, we were interested in solving for the combined effect of both convection and diffusion. For a dilute species:

$$N_i = -D_i \nabla C_i + c_i u \tag{3}$$

$$\frac{\delta c_i}{\delta t} + (\nabla \cdot N_i) = R_i \tag{4}$$

Where: $c_i$ is the species concentration ($mol.m^{-3}$), $N_i$ is the molar flux ($m^{-2}s^{-1}$), $R$ is a net volumetric source for the species concentration: $R > 0$ assumes that a chemical reaction is creating more of the species, and $R < 0$ that a chemical reaction is destroying the species. $D_i$ is the diffusion coefficient of the species ($m^2/s$).

With water as the working fluid, the flow focusing device is characterized by the Flow Rate Ratio (FRR), which is defined as follows:

$$FRR = \frac{Q_1 + Q_2}{Q_1} \tag{5}$$

Where: $Q_1$ and $Q_2$ represent flow rate of the lipid stream dissolved in ethanol and the water flow rates respectively ($\mu$L/min).

Table S.1: Computational Parameters used for 2D CFD Modeling and their Values.

| **Parameters** | **Value(s)** |
|---|---|
| Flow Rate at Inlet 1 | $Q_1$inlet = 10 [$\mu$L/min] = cst |
| Flow Rate at Inlet 2 | $Q_2$inlet = 40-480 [$\mu$L/min] |
| Fluid Density | $\rho_0$= 1,000 [$kg/m^3$] |
| Fluid Viscosity | $\mu_1$= 0.00195 [$Pa.s$] |

Table S.2: Experimental Values for the width of the focused stream at channel junction (in $\mu m$) and corresponding FRRs and flow Rate values (in $\mu m$).

| **FRR** | $Q_2$ [$\mu L/mn$] | $Q_1$ [$\mu L/mn$] | $w_f$ [$\mu m$] |
|---|---|---|---|
| 5 | 10 | 40 | 9.00 |
| 10 | 10 | 90 | 4.50 |
| 15 | 10 | 140 | 3.00 |
| 20 | 10 | 190 | 2.25 |
| 25 | 10 | 240 | 1.80 |
| 30 | 10 | 290 | 1.50 |
| 35 | 10 | 340 | 1.29 |
| 40 | 10 | 390 | 1.13 |
| 50 | 10 | 490 | 1.00 |

Table S.3: Computational Parameters used for Mesh Modeling (in 2D) and their Values.

| **Parameters** | **Value(s)** |
|---|---|
| Mesh Elements | $N = 146,302$ |
| Maximum Element Size | 0.0025 [$m$] |
| Minimum Element Size | 2.32 x $10^{-4}$ [$m$] |
| Maximum Element Growth Rate | 1.08 |
| Curvature Factor | 0.25 |
| Resolution of Narrow Regions | 1.00 |

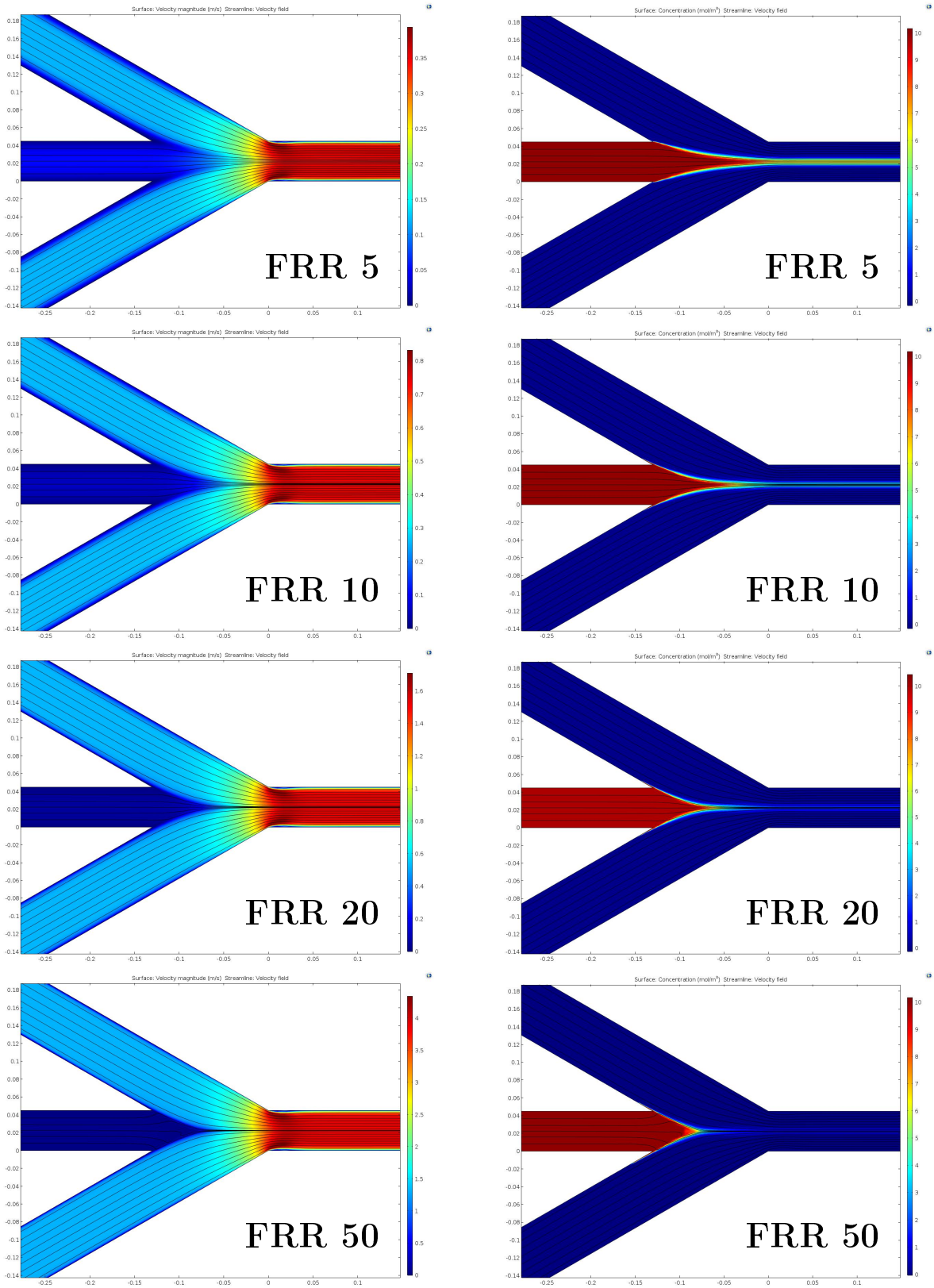



Figure S.1: CFD Simulations showing the 2D Velocity (left) and Concentration profiles (right) at the flow focusing junction of the microfluidic device at the given FRRs.

# Liposome Synthesis

Table S.4: Experimental Values for the time required to synthesize 1 mL of loaded/unloaded liposomes at corresponding FRRs.

| **FRR** | **Time** |
|---|---|
| 5 | 25 min 00 s |
| 10 | 11 min 07 s |
| 15 | 07 min 09 s |
| 20 | 05 min 16 s |
| 25 | 04 min 10 s |
| 30 | 03 min 27 s |
| 35 | 02 min 56 s |
| 40 | 02 min 34 s |
| 50 | 02 min 02 s |

Table S.5: Composition of samples used for the DDAB: DPPC: EtOH and DOPG: DPPC: EtOH liposomes synthesis experiments.

| **DDAB [mg]** | **DPPC[mg]** | **Weight Ratio** |
|---|---|---|
| 0.00 | 5.00 | Control |
| 5.00 | 5.00 | 1:1 |
| 0.50 | 5.00 | 1:2 |
| 1.00 | 5.00 | 1:5 |
| 0.50 | 5.00 | 1:10 |
| 0.25 | 5.00 | 1:20 |

Table S.6: Composition of samples used for the DDAB: DPPC and DOPG: DPPC liposomes synthesis experiments.

| **DOPG [mg]** | **DPPC[mg]** | **Weight Ratio** |
|---|---|---|
| 0.00 | 5.00 | Control |
| 2.50 | 5.00 | 1:2 |
| 1.00 | 5.00 | 1:5 |
| 0.50 | 5.00 | 1:10 |
| 0.25 | 5.00 | 1:20 |
| 0.01 | 5.00 | 1:50 |

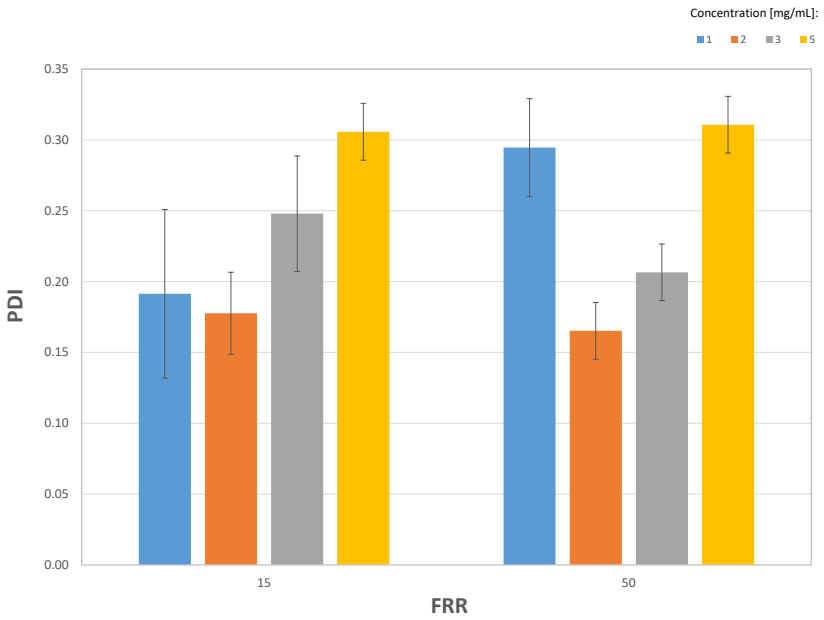



Figure S.2: Polydispersity index (PDI) of the synthesized liposomes as a function of the FRR and DPPC concentration [mg/mL]. The PDI values are the means of 30 repeat measurements (standard deviations in brackets) measured 5 times on 6 different concentrated liposome samples.

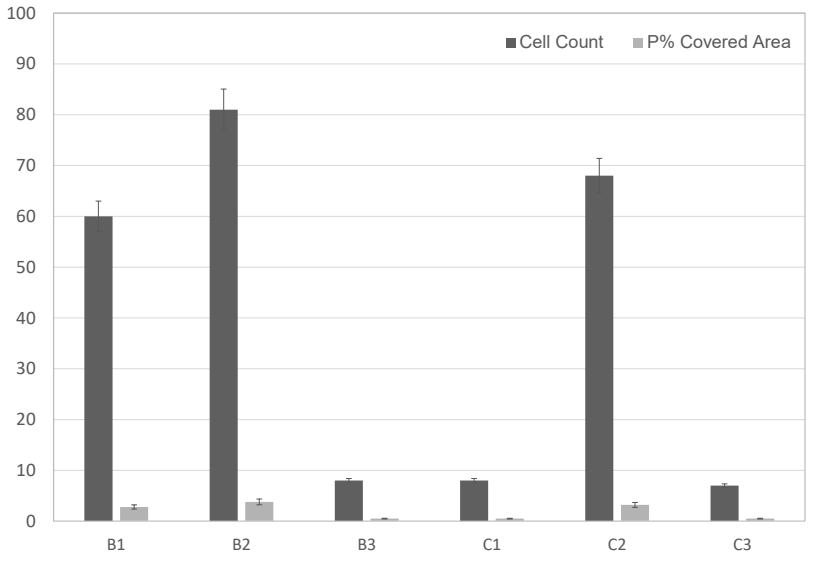
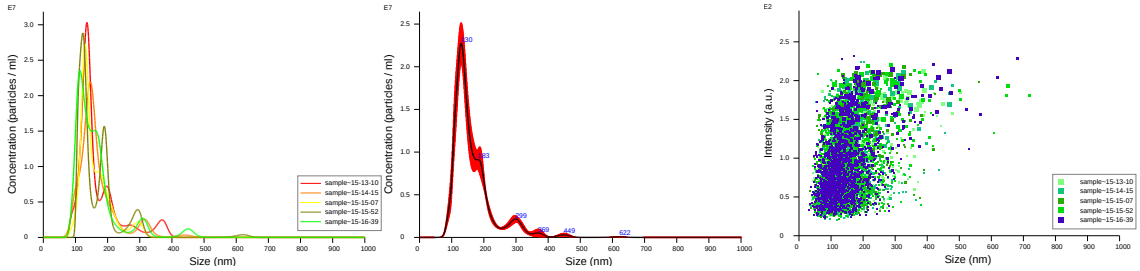
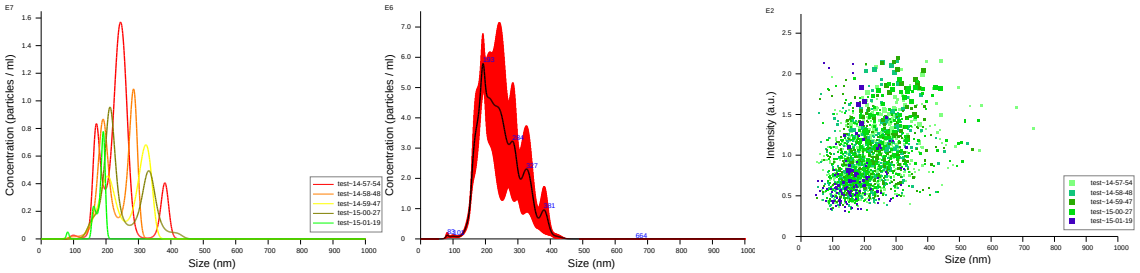



Figure S.3: Automatic particle counting averaged over the microscopy areas ($B_{1\text{-}3}$, $C_{1\text{-}3}$) displayed in Fig. 7. Error bars are ± standard errors.

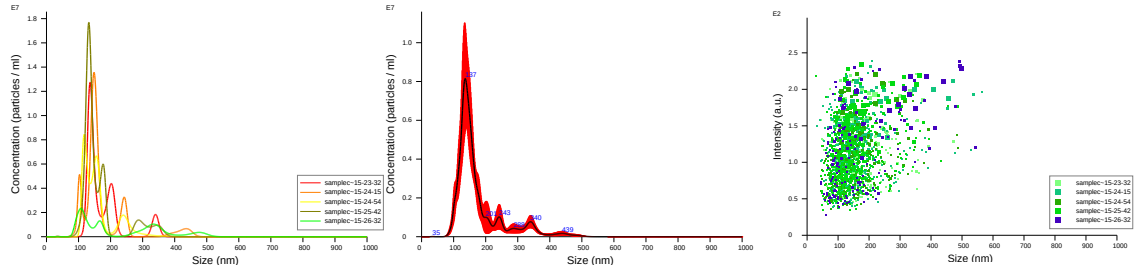
# NTA Results



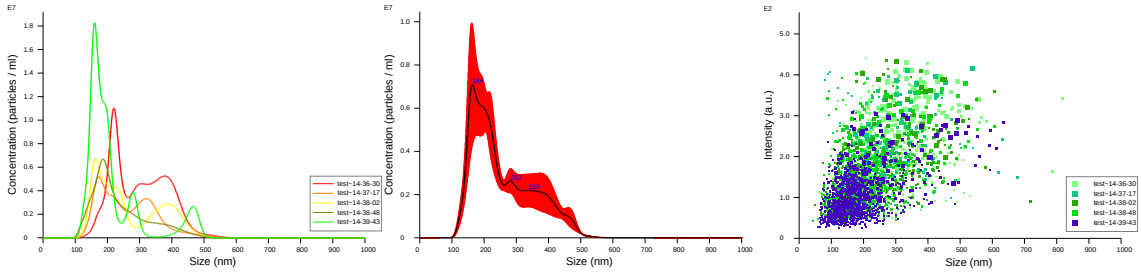

(a) (b)



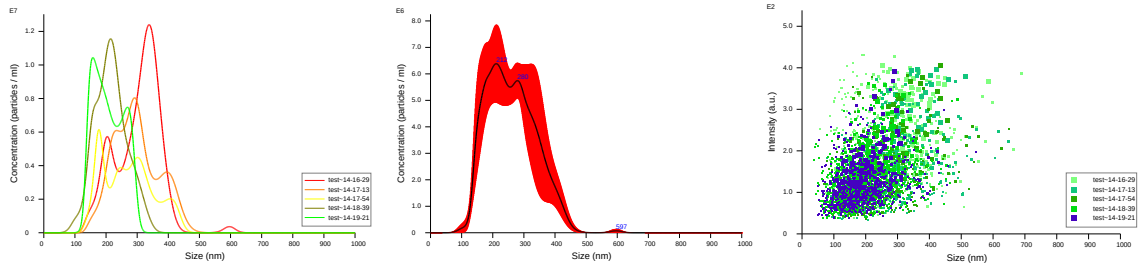

(c) (d)



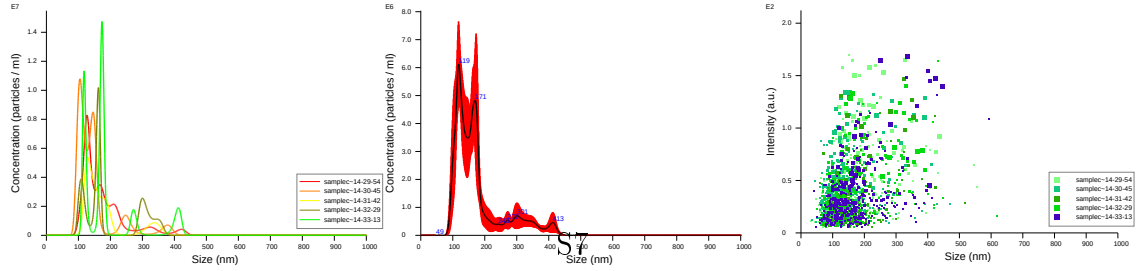

(e) (f)




(g) (h)




(i) (j)




(k) (l)

Figure S.4: NTA Measurements (n=5) of liposome particle concentration (E8 *particles/mL*) (left) and average concentration (right) as a function of size (nm) (a, c , e, g, i and k). Averaged Intensity/Size graphs (n=5) for liposomes samples (b, d, f, h, j and l). For: $C_{DPPC}$= 5 mg/mL; FRR=15 (a, b), FRR=30 (c, d) FRR= 50 (e, f) and at $C_{DPPC}$= 10 mg/mL; FRR=15 (g, h), FRR= 30 (i, j), and FRR= 50 (k, l). Samples were diluted 200 times prior to NTA measurement.

Play/Pause | FRR 30 | FRR 50

Figure S.5: NTA video capture of liposomes particles moving under Brownian motion. Lipid concentration is fixed at $C_{DPPC}$= 5 mg/mL. Two different FRR conditions are shown (FRR= 30 and 50 respectively).