Supporting Information for:

Single-Step Synthesis of Hybrid Lipid Nanoparticles Using Microfluidic Rapid Mixing

By Pedro M. Valencia, Pamela A. Basto, Liangfang Zhang, Minsoung Rhee, Robert Langer, Omid C. Farokhzad, Rohit Karnik

1. Comparing NPs with microfluidics vs. NP with previously published method

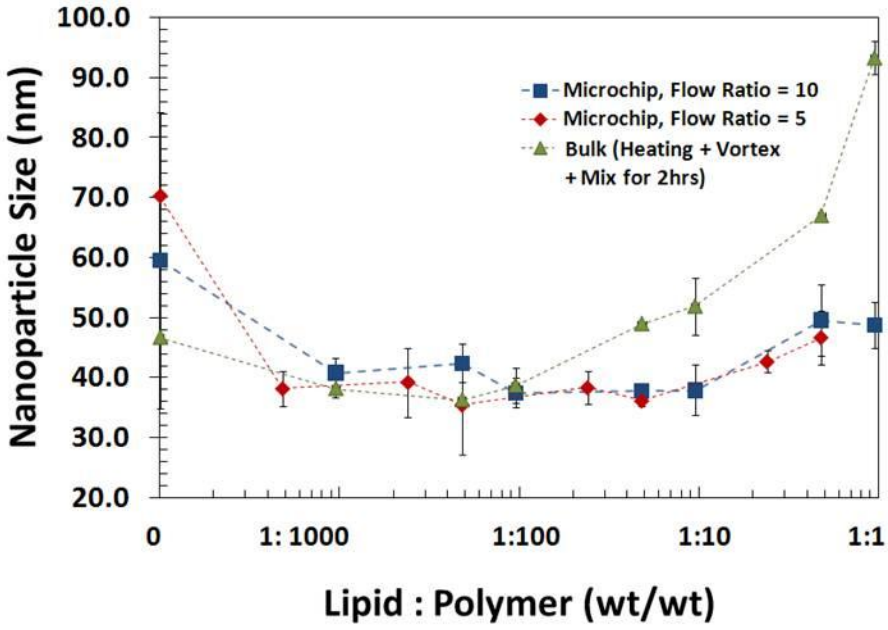

Figure S1. Changes in average NP size dependency on lipid:polymer (wt/wt) for a organic to aqueous flow ratio of 5 and 10. Average NP sizes at different lipid concentrations from NPs prepared using a prior method described L. Zhang, et al., 2008, are also included for comparison purposes. From 1:1 all the way to 1:100 lipid to PLGA ratio the NP size obtained from the microfluidic synthesis is smaller than those prepared in "bulk." As shown in Figure 2a, changes in flow ratio does not seem to have a significant effect on NP size.

2. Change in size over time of nanoparticles made with different precursors at different conditions

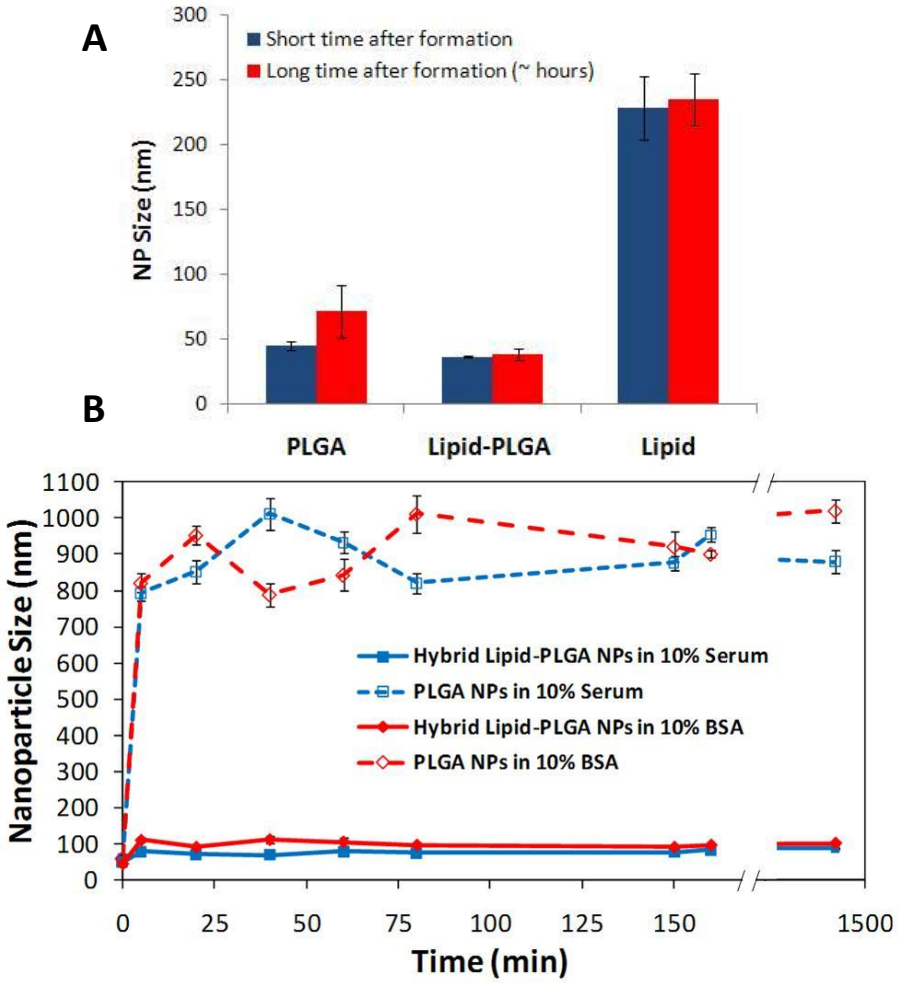

Figure S2. Effect of time on NPs made with different precursors and at different conditions. **(A)** NPs in water made with polymer only initially had a size of 40-50 nm and then increase to a size of 50-100 nm within a few hours of formation. NPs made with both lipid and polymer had a size of 40 nm, which remained essentially unchanged within several hours. NPs made with only lipid had a wide distribution of sizes ranging from 200-300 nm but the sizes did not change within several hours. **(B)** Hybrid Lipid-polymeric NPs were stable in both 10% BSA and 10% Serum at

37^{o}C. In contrast, PLGA NPs had a dramatic increase in size after they were incubated in BSA and Serum.

3. Investigating the effect of DSPE-PEG on lipid-PLGA NP stability

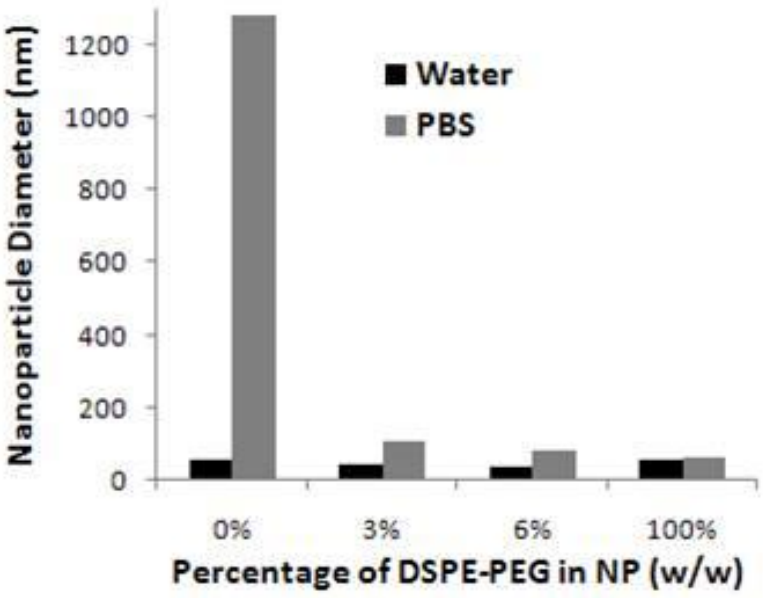

Figure S3. Effect of DSPE-PEG in stability of lipid-PLGA NP. When DSPE-PEG is absent, although there is a lipid layer around the polymeric core, the resulting NP is not stable in PBS. When the amount of DSPE-PEG is increased to 3% a slight increase in NP size is observed when NPs are suspended in PBS compared to water. A smaller increase in NP size is observed for 6% of DSPE-PEG and an insignificant increase in size is observed when only DSPE-PEG covers the polymeric core.

4. NPs made at slow mixing conditions

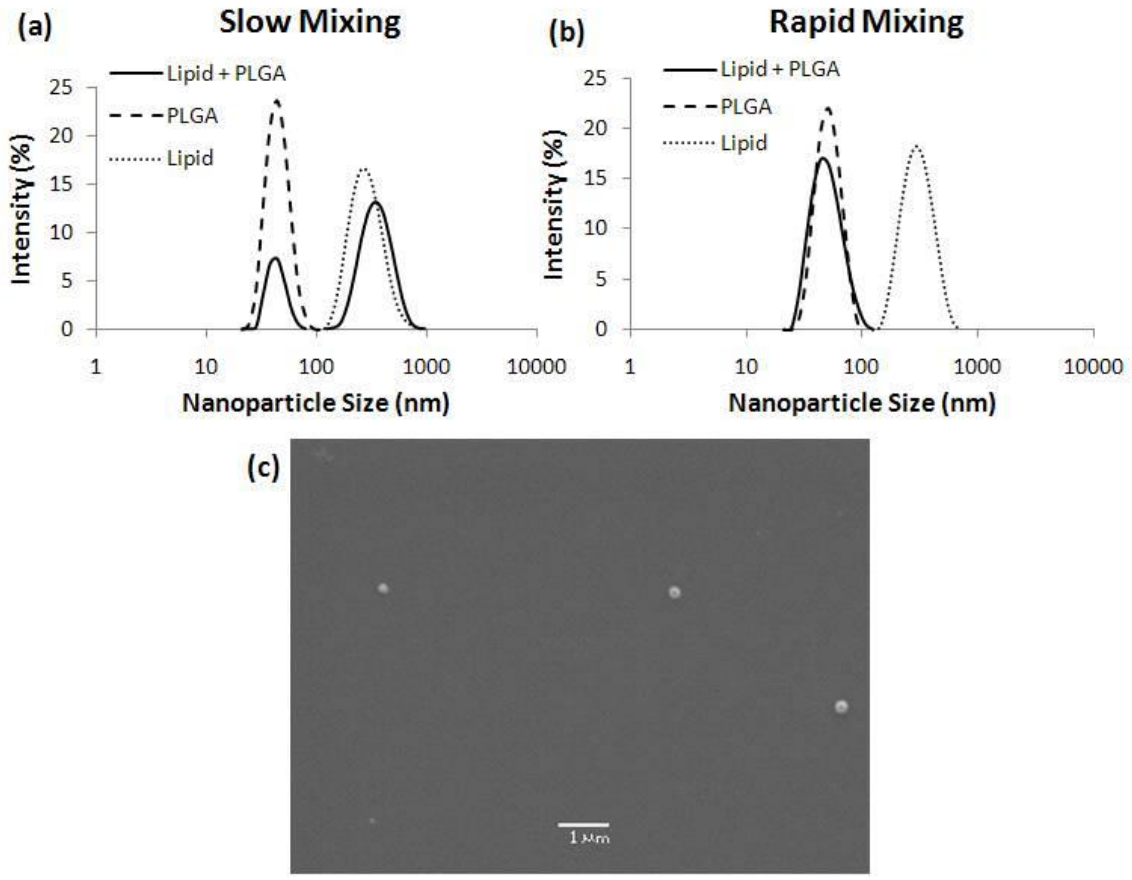

Figure S4. (a) Size distribution of NPs made at slow mixing conditions. (b) Size distribution of NPs made at rapid mixing using microfluidic channels. Solid line: NPs obtained from a mixture of PLGA and lipid at a lipid to polymer ratio of 1 : 10. Note that two distinct peaks are present at the characteristic size of polymeric NPs (50 nm) and liposomes (250 nm). Dashed line: NPs obtained when only PLGA is present. Dotted line: NPs obtained when only lipid is present. (c) SEM image of NPs made from a mixture of PLGA and lipid at a lipid to polymer ratio of 10 : 1. Because of the resolution limitations of SEM only NPs with size larger than about 150nm can be

observed. The NP sizes shown in this image are 170 nm, 250 nm, and 290 nm, and are characteristic of liposomes. Scale bar is 1 μm. Before imaging, NPs were sputtered with few nanometers of gold. Acceleration voltage used was 15 kV.

5. Determining mixing time in channel

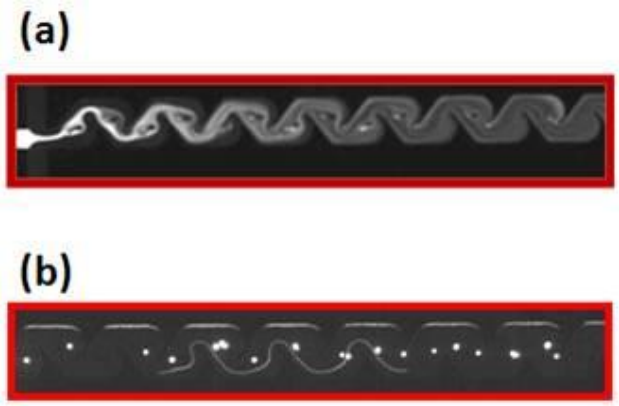

Figure S5. (a) Using fluorescent dye as the input in the organic solvent stream, complete mixing occurs at 1.4 cm in the Tesla channels. (b) Observation of fluorescent bead transport used to calculate mixing time within the channel design. First the track length of the bead at a specific exposure time is calculated from the image. From the track length and exposure time, the fluid velocity was calculated. In this study the track length used to calculate the fluid velocity was the average of 50 track lengths measured from 50 different images. In addition, other different fluid velocities at different exposure times were measured and no significant variability was found. The mixing time of ~10 ms was determined by using the average fluid velocity times the distance at which complete mixing was observed.

6. Self-assembly of lipid-QDs NPs inside microfluidic channel

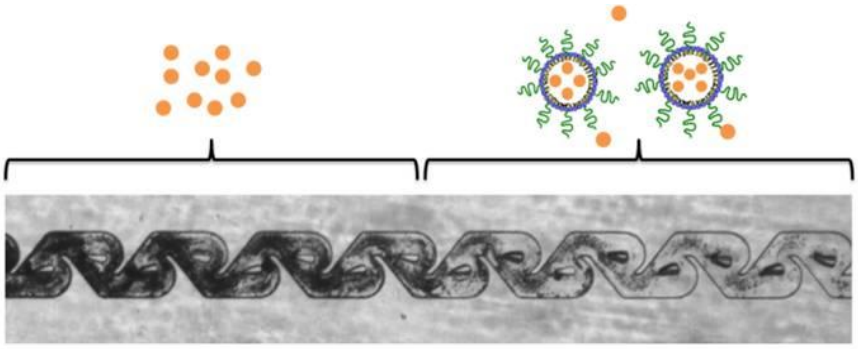

Figure S6. Light microscope screen capture of entire Tesla channel (60 µm x 20 µm) illustrating areas of QD aggregation followed by lipid coverage in the formation of hybrid-QD NPs.

7. Estimation of Mixing Timescales

$$V = \frac{Q}{A} = \frac{60\mu L/\min}{50\mu m \times 60\mu m} = 0.333\ m/s \quad (1)$$

$$w_f \approx \frac{2w}{3(1+1/R)} \approx 5.56\mu m \quad (2)$$

$$Pe = \frac{V \cdot w_f}{D} \approx 1851 \quad (3)$$

$$time\,to\,travel\,one\,Tesla\,structure = \frac{flow\,velocity(V)}{travel\ path\,length} \approx 1.1ms \quad (4)$$

$$\tau_{mix,water+ACN,diffusion} \approx \frac{w_f^2}{4D} \approx \frac{w^2}{9D}\frac{1}{(1+1/R)^2} = \frac{(5\times10^{-8})^2}{9(1\times10^{-9})}\frac{1}{(1+1/0.1)^2} \sim 2.3ms \quad (5)$$

$$\tau_{mix,water+ACN,convection} \sim 0.286\times\frac{w_f^2}{D}\times\left(\frac{Pe}{L/w}\right)^{-2/3} \sim 0.23ms \quad (6)$$

For D = 10^{-9} m^2/s, w = 50 µm, R = 0.1, Eq (5) predicts a time for solvent exchange of water and acetonitrile purely by diffusion of ~2.3 ms. Conversely, for L = 2.5 mm, Eq (6) predicts a time

for solvent exchange of water and acetonitrile purely by convection of ~0.23 ms. Note that the folding and stretching of the focused stream enforced by Tesla structures is based on different mechanism of convective mixing while Eq (6) was developed for convective droplet mixing [1]. However, Eq (6) can be a still good measure for rough estimates. Since the transport in the Tesla mixer is a combinatory process of diffusion and convection, the real PLGA core formation time can be estimated between 0.2-2 ms, which requires the stream traveling less than 1 Tesla structure (convection extreme case) or up to 2 sequential Tesla structures (diffusion extreme case).

Mixing of Lipid molecules with PLGA molecules is slower than that of water molecules by an order of magnitude, either by convection or by diffusion. Thus, complete lipid coverage of NP takes longer timescales (>10 ms). At the same time, diffusion of synthesized NPs is much slower at least two orders of magnitude, and thus we can safely assume that NPs should hardly diffuse into the water stream during the transportation course in the Tesla mixer.

8. Estimation of self-assembly timescales of PLGA cores

$$C_{PLGA} \approx \frac{(1mg/mL)}{(70000g/mol)} 6.02\times10^{23} mol^{-1} = 8.6\times10^{21} m^{-3} \quad (7)$$

$$m_{PLGA\_CORE} \approx \frac{4\pi}{3}(5\times10^{-5})^3(0.5g/cm^3) = 1.68\times10^{-17} g \quad (8)$$

$$n_{PLGA\_CORE} = \frac{(1.68\times10^{-17} g)}{(70000g/mol)}(6.02\times10^{23} mol^{-1}) \sim 144 = \frac{4\pi}{3} l^3 C_{PLGA} \quad (9)$$

diffusion length scale ~ $l \approx 1.6\times10^{-7}\ m = 160\,nm$ (10)

$$\tau_{core\ formation} = \frac{l^2}{D} \approx \frac{(1.6\times10^{-7}\ m)^2}{1\times10^{-11}\ m^2/s} = 2.56\,ms \quad (11)$$

9. Estimation of the timescale for minimal lipid coverage on polymeric core for NP stabilization after complete mixing

Average M.W. of lipids: $330\times0.86 + 2850\times0.14 \approx 733$ (12)

In case of 1:1000,

Lipid supply: $\frac{(0.1\mu g/mL)}{(733 g/mol)} = 1.36\times10^{-7} mol/L \quad \left\{ \begin{matrix} 1.14\times10^{-7} mol/L: & lecithin \\ 0.22\times10^{-7} mol/L: & DSPE-PEG \end{matrix} \right\}$

Assuming the density of a PLGA core ~ $0.5 g/cm^3$, the core consists of $\sim 3.4\times10^{-22} mol$ of PLGA molecules. The concentration of assembled PLGA cores in the stream is then $\sim 5\times10^{-8} mol/L$.

For a unit time (1 minute),

# of PLGA cores:# of DSPE-PEG molecules ~ 1:4.

Roughly translated, four or more DSPE-PEG molecules can sufficiently stabilize NPs against long-term aggregation.

When stabilized,

$$lipid\ surface\ density(\sigma) \sim 8\times10^{14} m^{-2} \quad (13)$$

$$lipid\ concentration(C_{lipid}) \sim (0.22\times10^{-4} mol/m^3)(6.02\times10^{23} mol^{-1}) \sim 1.3\times10^{19} m^{-3} \quad (14)$$

$$\frac{4\pi}{3}(R_D^3 - R^3)C_{lipid} = 4\pi R^2\sigma \quad (15)$$

Assuming that $R_D^3 >> R^3$, the diffusion length scale ~ $R_D \sim 4.2\times10^{-7} m = 420nm$ (17)

$$\tau_{stabilization} \approx \frac{(R_D - R)^2}{D} = \frac{(4\times10^{-7} m)^2}{2\times10^{-10} m^2/s} = 0.8 ms \quad (18)$$

Similarly, for lipid : PLGA ratios of 1:100, 1:10, and 1:1, $\tau_{stabilization}$ ~ 0.14 ms, 0.025 ms, and 0.0024 ms respectively.

These calculations indicate that coverage by lipid layer that is sufficient to prevent long-term aggregation of the NPs occurs on a very rapid timescale after completion of mixing; thus, the timescale of coverage is essentially limited by the mixing timescale. If the lipid molecules must diffuse from the aqueous stream to the focused stream across a distance of 1 um, the timescale for minimum coverage of the PLGA cores in the case of 1:1 ratio of lipid : PLGA is on the order of 1 ms. Therefore, the timescales for self-assembly of the PLGA cores are on the same order of magnitude for those of minimal lipid coverage, at least for high lipid : PLGA ratios. However, complete lipid coverage requires complete mixing, which occurs on a timescale of 10 ms.

Reference:

[1] Rhee, M.; Burns, M. A. *Langmuir*, **2008**, *24* (2), 590–601