Supporting Information

A Three-Dimensional Hydrodynamic Focusing Method for Polyplex Synthesis

Mengqian Lu,[1] Yi-Ping Ho,[2,3] Christopher L. Grigsby,[2] Ahmad Ahsan Nawaz,[1] Kam W. Leong,[2] and Tony Jun Huang[1*]*

[1] Department of Engineering Science and Mechanics, The Pennsylvania State University, University Park, PA 16802, USA

[2] Department of Biomedical Engineering, Duke University, Durham, North Carolina 27708, USA

[3] Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Denmark

* Address correspondence to kam.leong@duke.edu and junhuang@psu.edu

Concentration Distribution of DNA

The concentration distribution of DNA was simulated using the experimental parameters, as shown in Fig. S1. At various flow rates, the DNA solution was confined in both horizontal and vertical directions. As the flow rate increased, the size of the highly concentrated DNA region decreased, while the region of lower DNA concentration increased, indicating the enhancement of mixing. This simulation was carried out using a commercial computational fluid dynamics (CFD) simulation software (CFD-ACE+, ESI-CFD), by assuming the diffusion coefficient of the 390 kD pGFP as 0.5×10^{-12} m^2/s and the initial concentration as 1.905×10^{-9} M, without considering the reaction between the DNA and polymer.

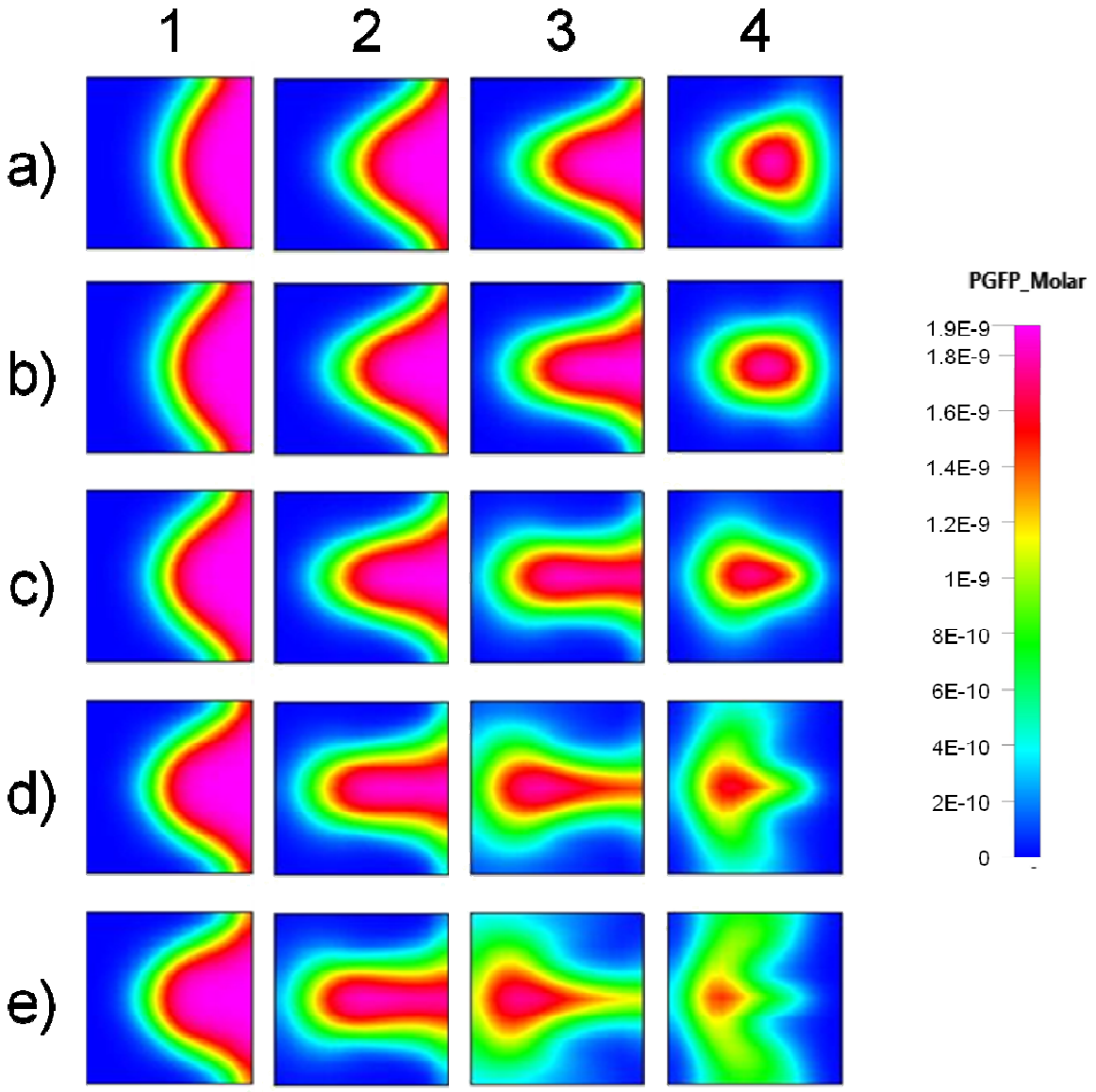

Figure S1. The DNA concentration distribution in cross-section areas at location 1, 2, 3, and 4 in Figure 1 at different flow rates. The total flow rate was a) 90 µL/min, b) 120 µL/min, c) 180 µL/min, d) 270 µL/min, and e) 360 µL/min.

Estimation of Shear Rate

The shear rate ($\dot{\gamma}$) was calculated as $\dot{\gamma} = \partial v / \partial h$, where v is the velocity of the fluid and h is the distance from the channel wall. We can expect the highest shear rate at the highest flow rate (*i.e.*, 360 μL/min). The velocity distribution was simulated using a computational fluid dynamic (CFD) simulation software (CFD-ACE+, ESI-CFD), as shown in Fig. S2. The highest shear rate happens near the channel wall, which was calculated as 8.1×10^4/s.

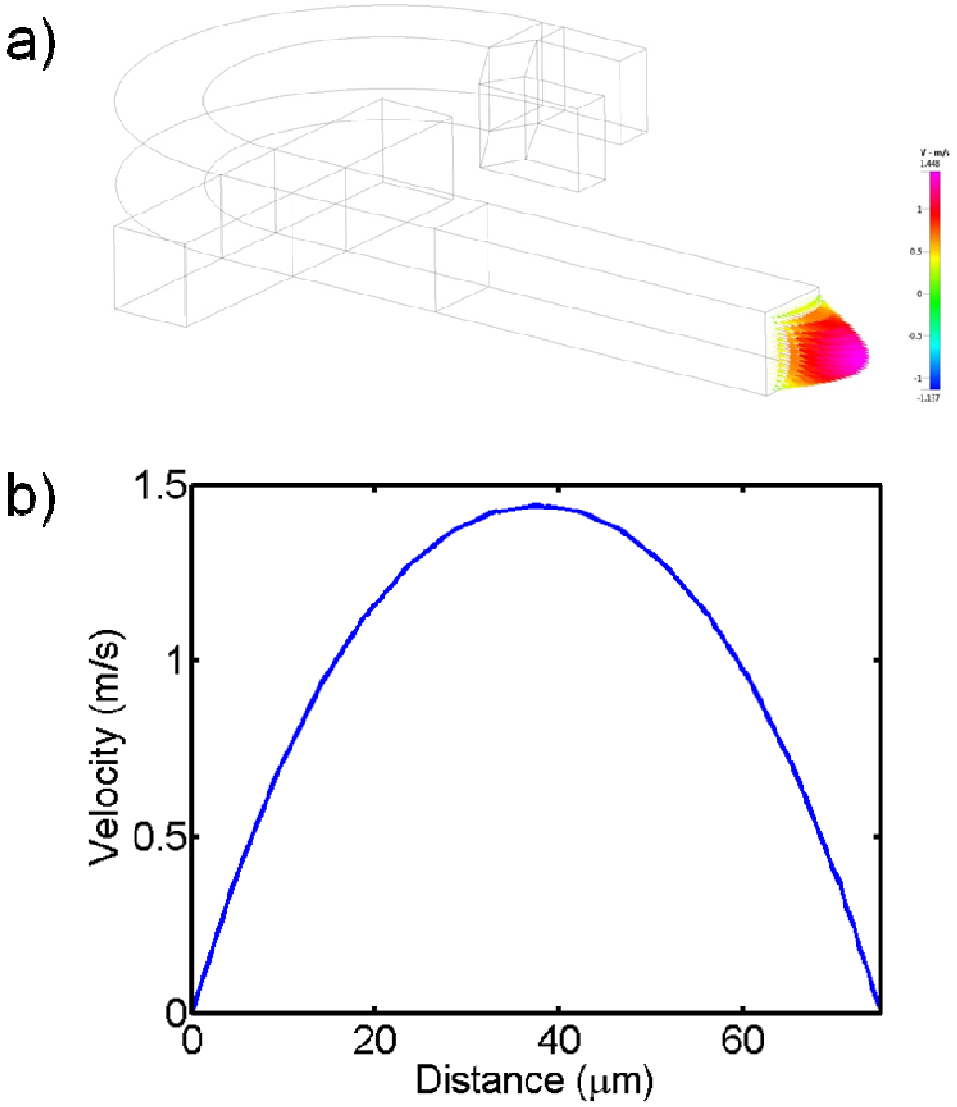

Figure S2. a) The velocity distribution of fluid velocity in a cross-section at a total flow rate of 360 μL/min. b) The velocity distribution at the center line in the cross-section in a).

Aggregation kinetics over 4 hours

During the transfection, the HEK293T cells were incubated with the synthesized polyplexes for 4 hours. Therefore, we studied the aggregation kinetics of the polyplexes over this period. As shown in Fig. S3, over 4 hours, the polyplexes synthesized by the 3D-HF method show smaller size than the ones synthesized by the bulk mixing method.

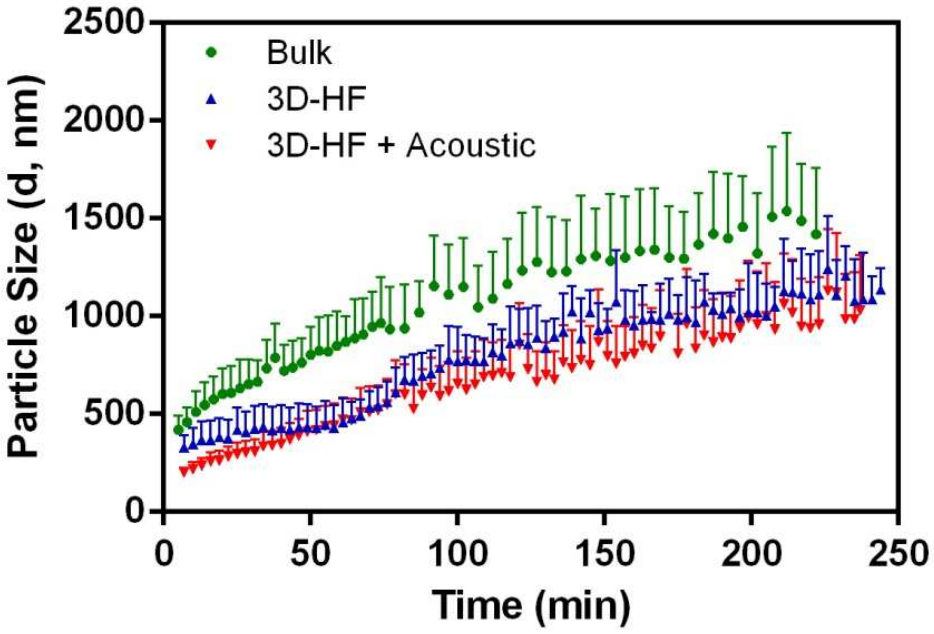

Figure S3. Aggregation kinetics over 4 hours after synthesis.

Agarose gel electrophoresis of DNA

The integrity of the DNA following exposure to microfluidic processing and acoustic perturbation was confirmed experimentally. Plasmid DNA (pmax-GFP) solutions were handled per the nanoparticle generation protocol, but with Opti-MEM medium introduced *via* inlets B, C, and D in place of the Opti-MEM/polymer solution. The products were collected and analyzed by agarose gel electrophoresis. Experimental samples and untreated controls (~ 50 ng) were loaded onto agarose gels (2% w/v) containing 50 μg/mL ethidium bromide in Tris-Borate-EDTA (TBE) buffer. The gels were run at 120 volts for 3 hours prior to UV transillumination and imaging with an Alpha-Innotech FluorChem instrument. As shown in Fig. S4, each sample produced a single band, corresponding to the presence of intact plasmid DNA only. No additional bands were observed, confirming that 3D-HF with acoustic mixing causes no significant DNA fragmentation or degradation under the conditions used here for nanoparticle synthesis.

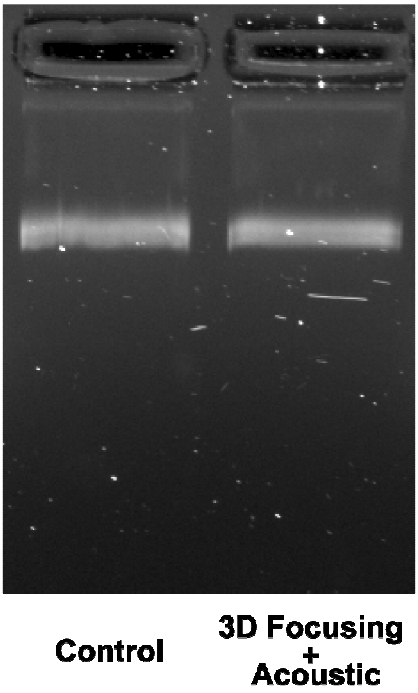

Figure S4. Gel electrophoresis shows the integrity of DNA following exposure to microfluidic processing and acoustic perturbation.