High-throughput, single-particle, CLiC analytics of lipid nanoparticles for drug delivery: understanding size, loading, and release kinetics

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Motivation:

Genetic medicines (ASO, siRNA, mRNA-based drugs) represent promising therapeutic advancements, but delivery is still a major problem. Lipid nanoparticles (LNPs) have great potential as delivery vehicles. Hence there is a need for detailed quantitative analytics (i.e. structure, loading, release kinetics), as well as relating these properties to therapeutic action.

Materials: Lipid Nanoparticles

We use lipid nanoparticles composed of ionizable cationic lipids, phospho-lipids, cholesterol and PEG-lipids. Each of these components is optimized to control important properties of the LNPs.
We are interested in:
1. Understanding the different structures of these nanoparticles under different conditions
2. How these structures affect delivery/release of drugs in target cells
3. How pH affects dynamics of these nanoparticles
4. Directly visualizing their formation through fusion

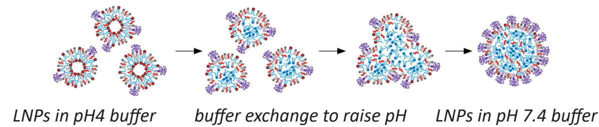


Figure 1. Schematic of LNPs [***Fig.4 of J. A. Kulkarni, et al., ACS Nano 2018, 12, 4787–4795***]

Convex Lens-induced Confinement (CLiC) Imaging

This single-particle fluorescence imaging technique allows real-time tracking of the trajectory of a freely diffusing single molecule or substrate for extended time periods

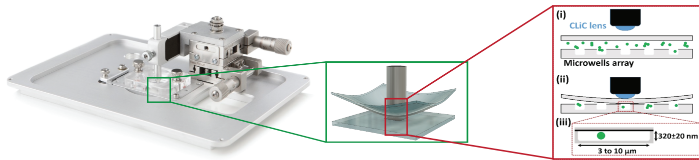


Figure 2. CLiC instrument [in-set schematic: ***Tahvildari et al., in preparation (2020)***]

CLiC allows us to:

1. Trap and observe freely diffusing particles for long periods of time.

2. Watch reaction dynamics in real-time

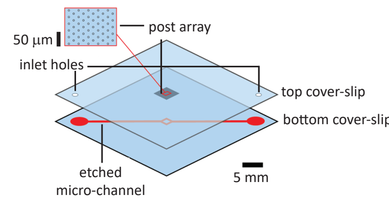
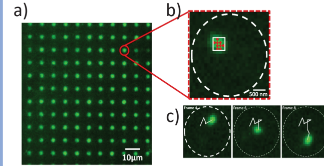


Figure 3. Flow-cell with a micro-channel for reagent exchange [***Henkin et al. Analytical Chemistry, 88(22), 11100–11107 (2016)***]

CLiC Nanoparticle Analytics:



We measure size and intensity distributions of these particles by:
1. Tracking particle positions and recording fluorescent intensities vs. time
2. Plotting mean square displacements (MSD) vs. time
3. Fitting data to a 2D confined diffusion model
4. Deducing hydrodynamic radius (RH)

Figure 4. a) Array of micro-pits with particles. b) Single micro-pit containing a freely diffusing particle c) Successive frames showing the trajectory of a diffusing particle [***Tahvildari et al., in preparation (2020)***]

Results: Particle sizing

We obtain structural information by correlating intensity and size distribution of the particles.

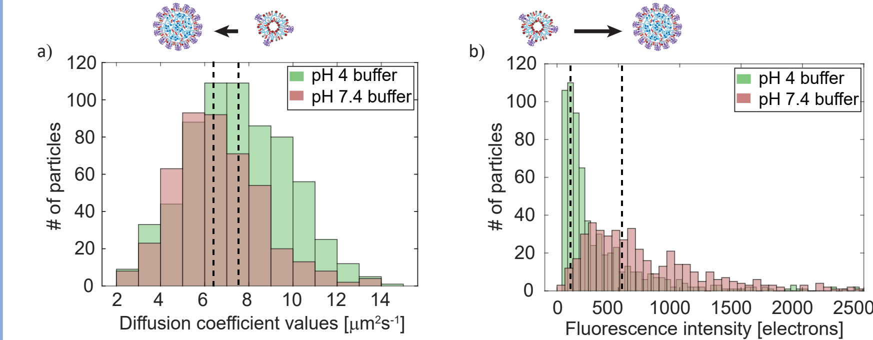


Figure 5. Distribution of a) Particle sizes as well as b) single particle intensities for different buffer conditions (green - sodium acetate pH 4 buffer, pink - 1xPBS buffer at pH 7.4). Preliminary data.

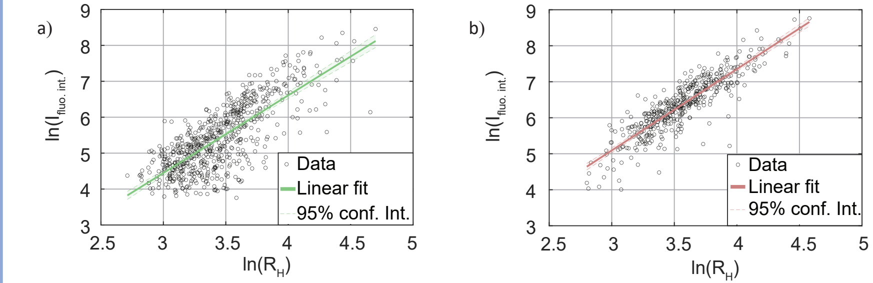


Figure 6. Correlation single particle intensities with sizes, for a) pH 4 buffer and b) pH 7.4 buffer

[***Kamanzi et al., in preparation (2020)***]

Results: Real-time Imaging of LNP Fusion

Visualizing LNP fusion, as buffer pH is raised from pH 4 to 7.4.

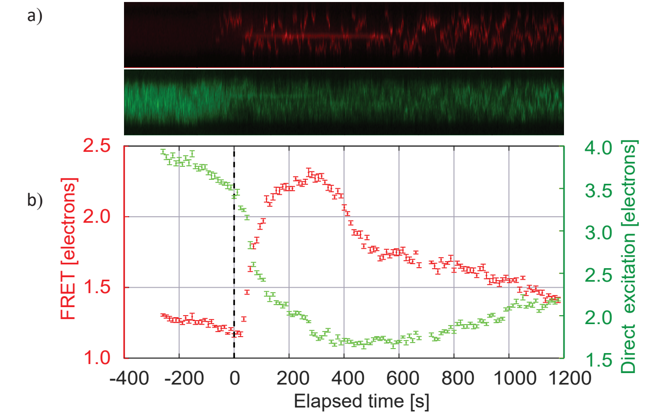


Figure 7. a) Kymogram of fusing particles, showing FRET channel in red and direct excitation in green b) Fluorescence intensities of the fusing particles, showing the onset of fusion as buffer pH is raised

[***Kamanzi et al., in preparation (2020)***]

Conclusion:

Detailed investigations of lipid nanoparticle properties vs. biophysical parameters
* Measured single-particle distributions of sizes, intensities (dye loading) vs. pH
* Obtained intensity-scaling with size which is consistent with dye loaded on the surface

Real-time imaging of the LNP fusion, with single-particle resolution and reagent-exchange
* Measured fusion time scales

Next steps:

* Study loading of LNPs with labeled genetic medicines such as siRNA
* Visualize and study drug release kinetics
* Perform microscopy experiments in cell like conditions, and in living cells

Outlook:

* Build a biophysical understanding of drug delivery properties

Acknowledgements:

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