Simultaneous, single -particle measurements of size and loading give new insights into the structure of drug -delivery nanoparticles

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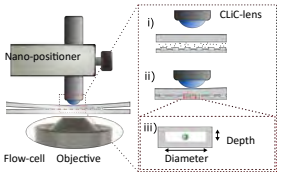
Convex Lens-induced Confinement (CLiC) Device

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.



CLiC allows us to:

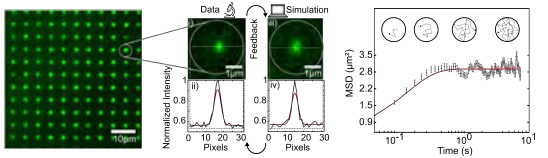
1. Trap and observe freely diffusing particles for long periods of time.

2. Watch reaction dynamics in real-time

CLiC instrument [in-set schematics: samples are trapped in nano features when flow-cell surfaces are deflected and brought into contract

[Kamanzi et al., ACS Nano 2021]

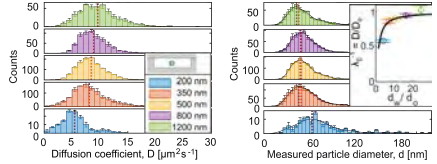
Particle sizing method:



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; and 4. Deducing hydrodynamic radius

[Kamanzi et al., ACS Nano 2021]

Validation: measurements on beads

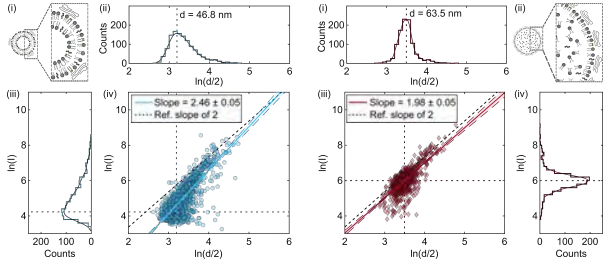


Size diffusion coefficients measurements on 48 nm beads under varying confinement depths. Obtained results are in aggreement with the known size to within 1nm

[Kamanzi et al., ACS Nano 2021]

Sizing and loading (intensity) for empty LNPs

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

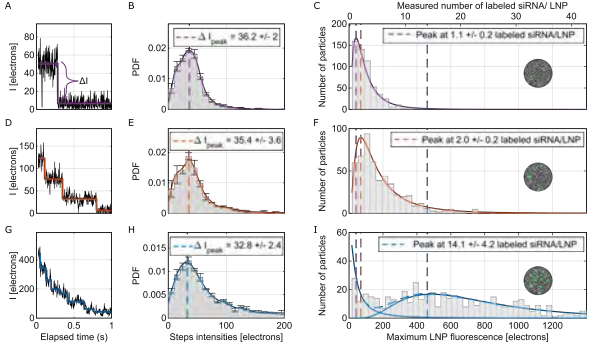


Correlation single particle intensities with sizes, for bilayer structured LNPs in pH 4 buffer (shown in blue) and for oil-droplet structured LNPs at pH 7.4 buffer (shown in red)

[Kamanzi et al., ACS Nano 2021]

Drug loading measurement with photo-bleaching

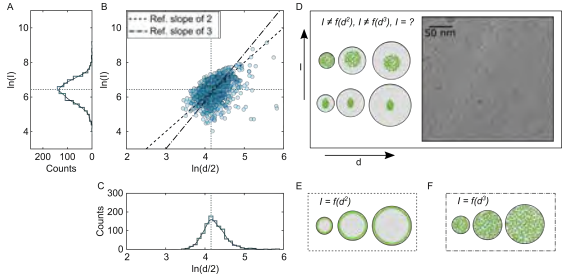
We use photo-bleaching measurements to measure drug loading with single molecule resolution. The figure shows measurements on samples with 3 different labeling ratios (1, 3, and 10 dyes per LNP)



[Kamanzi et al., ACS Nano 2021]

Size and loading for siRNA containing LNPs

We obtain structural information from the scaling of intensity measurements with particle sizes



Correlation single particle intensities with sizes, for si-RNA drug loaded LNPs. Here the dyes are found inside the particles, as they are covalently bonded to the drugs.

[Kamanzi et al., ACS Nano 2021]

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
* Measured si-RNA drug loading with single molecule resolution (repeated for 3 different labeling ratios)
* Measured LNP structures, by using the correlation in the size and loading measurements

Next steps:

* Investigate LNP dynamics, such as the pH driven fusion process,
* Investigate changes in particle properties over time
* Visualize and study drug release kinetics

Outlook:

* investigate drug release at the single molecule level
* Build a biophysical understanding of drug delivery properties

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