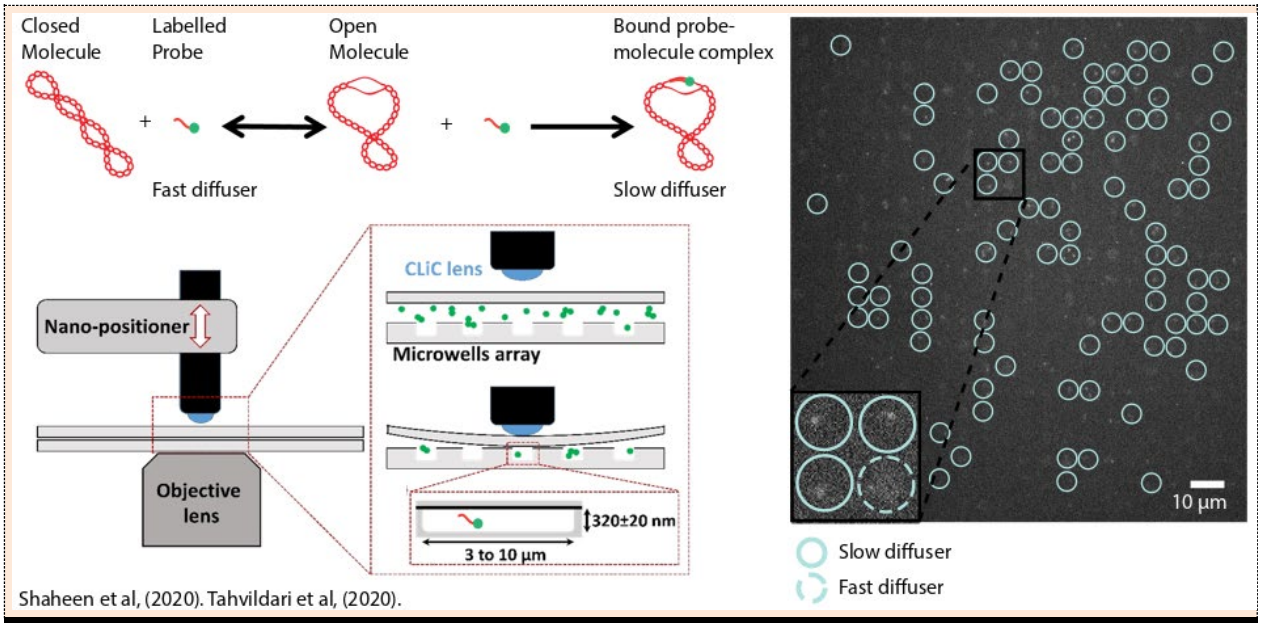


# High-throughput, single-molecule, CLiC analytics of nucleic acid binding kinetics, and applications to oligotherapeutics development

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Typical biomolecular assays rely on measuring populations which can overlook fundamental details only detectable on the scale of individual molecules. In contrast to ensemble studies, single-molecule studies identify heterogeneous sub-populations and detect rare events. Understanding such heterogeneity is critical to understanding and treating many diseases.

Ideally, a single-molecule platform would be simple in terms of required hardware, experimental design and data analysis. Towards this end, we developed high-throughput, single-molecule *Convex Lens-induced Confinement* (CLiC) analytics to enable direct imaging, manipulation, and quantification of biomolecules. CLiC enables long observation times (minutes to hours) and uses *untethered and freely diffusing* biomolecules. Furthermore, CLiC provides large numbers of observations yielding *high statistics and high signal-to-noise*. Finally, because it allows reagent exchange during observations, CLiC can *mimic the crowded and confined conditions in cells*.

In this work, we investigate structural heterogeneity at the single-molecule level caused by supercoiling of plasmids, as well as its impact on the binding/unbinding of probes to targets on the plasmids. Using CLiC, we assayed the impact of several biophysical variables (supercoiling, crowding, oligos-probe sequence, etc) on kinetic interaction parameters (on/off rates, site opening/closing rates), and related our observations to statistical physics theory of DNA.

Recently, we have extended the CLiC DNA-binding assay to interrogate and quantify the interaction kinetics of oligonucleotide therapeutics such as ASOs to RNA targets, to understand the mechanisms and efficacies of emerging classes of genetic medicines, and help engineer better drugs. Excited to share our approaches and findings with the UBC nanomedicine community.