Non-equilibrium Structural Dynamics of Supercoiled DNA Plasmids

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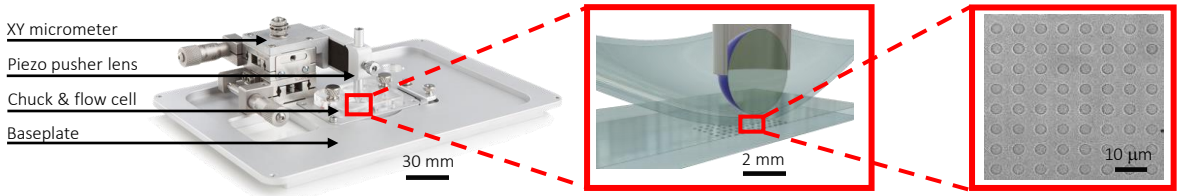
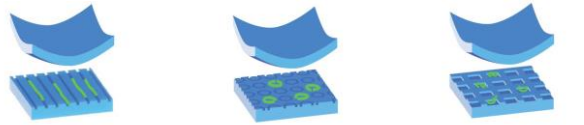
1) Convex Lens-induced Confinement (CLiC) microscopy



Feature	TIRF	ABEL	STLV	CLiC
Single-molecule				
High throughput				
Complete interactions				
Large concentration range				
Untethered molecules				

- **Access a wide range of applications** – using a variety of features (pits, posts, rings).
- **Discover heterogeneity** – from a distribution of individuals, not ensemble averages.
- **Establish mechanistic insights** – from entire histories of interacting molecules.
- **Extract binding kinetics** – from on/off rates, cooperativity effects.
- **Emulate cell-like conditions** – such as crowding, confinement and ionic strength.



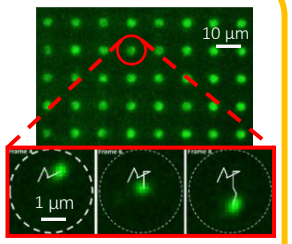
Channels *ex: genome mapping* | **Rings** *ex: end-to-end ligation* | **Wells** *ex: interaction kinetics*

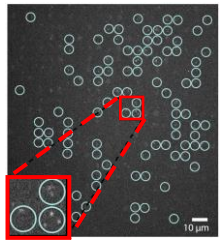
CLiC patent issued: Leslie and Cohen, Harvard, 2018-08-14

CLiC Modalities

Narrative

- Wide-field imaging of many molecules interacting over time.
- Enables long, direct measurements of a system states in response to changing conditions
- This is key to understanding out-of-equilibrium reactions such as RNA cleavage, and the nanoparticle-release kinetics of drugs.





Snapshot

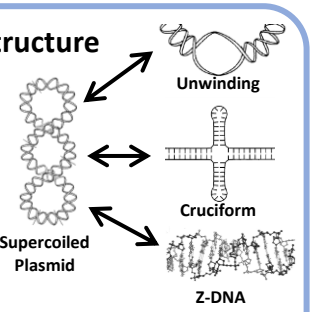
- Confocal or wide-field imaging of single molecules.
- This method identifies different populations in a sample and quantifies their relative proportion.
- By comparing snapshots we can quantify interaction rates and affinities.

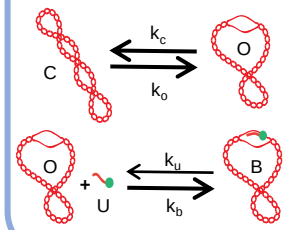
Leslie et al, Anal. Chem. (2010); Berard et al, PNAS (2014); Berard et al, APL (2016)

2) Supercoiling and Structure

$$L_k = T + W$$

- Supercoiling occurs when a strand of DNA is over- or under-twisted.
- The torsional energy of supercoiled DNA drives sequence-specific structural transitions.
- Examples of secondary structures include left-handed DNA (Z-DNA), cruciforms, and strand separation.





- In this work we study supercoil-induced strand separation.
- This can be understood by modelling transitions in a 3-state system: **C** closed plasmid, **O** open plasmid, and **B** bound plasmid.
- Kinetics are inferred by comparing the model to experimental data.

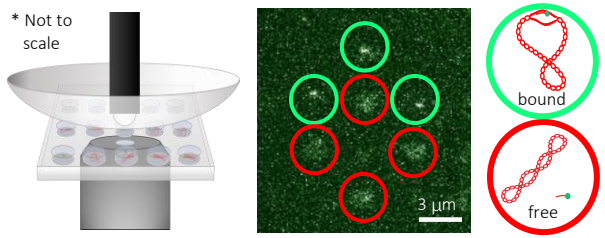
Shaheen (2020) in preparation

Sinden (1994) DNA Structure and Function, Academic Press

3) Oligo-plasmid Binding Experiment

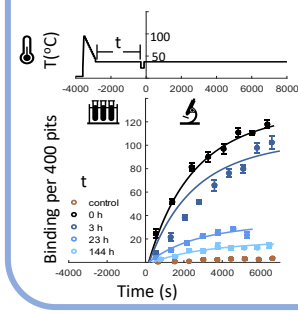
- Diffusion is inversely related to the size of a molecule.
- Introduce short (20b), complimentary oligo probe.
- Oligo can only be localized when bound to plasmid.
- Count the number of bound oligos using CLiC.

$$D = \frac{kT}{6\pi\eta R_g}$$



4) Influence of Temperature Perturbation

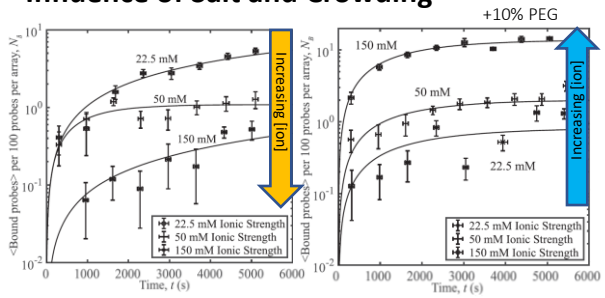
- Non-equilibrium dynamics were modelled by heating the plasmid from 37°C to 95°C.
- Plasmid is held at 95°C for a short time and then gradually cooled back to 37°C.
- Oligo-plasmid binding is measured as a proxy for unwinding at 37°C for various time lags after the 95°C perturbation.
- We observed that the number of unwound plasmids relaxes to an equilibrium value over the course of 144 hours.
- Chemical rate constants and the concentrations of plasmid states are estimated from the data using Markov chain Monte Carlo methods.



k_o [s^{-1}]	(2.4±0.1) x 10^{-5}
k_c [s^{-1}]	(8.1±0.6) x 10^{-8}
k_b [$M^{-1}s^{-1}$]	(3.1±0.3) x 10^{+5}
O(0) [%]	2.9±0.2
O(∞) [%]	0.34±0.01

Shaheen (2020) in preparation

Influence of Salt and Crowding

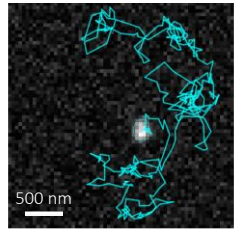


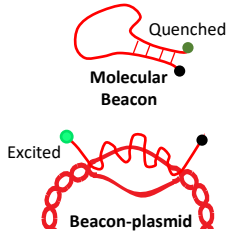
- DNA stability increases with ion concentration.
- We measure reduced oligo binding at higher ion concentration.
- This is expected, since the enhanced stability increases the melting temperature of the unwinding site.
- Binding increases with crowding.
- Introduction of a crowding agent reverses ion trend.
- This reversal was an unexpected result that warrants further investigation.

Scott (2019) Nucleic Acids Research

Future Investigations

- The combined effects of ion concentration and crowding need further investigation.
- Model of oligo diffusion could change as a function of salt and crowding, changing reaction rates.
- Gel electrophoresis of plasmids as a function of salt and crowding.





- A molecular beacon is a fluorophore-quencher system on an oligo with hairpin structure.
- Using a beacon complimentary to the unwinding site could allow for substantial increase in oligo [U].
- With an excess of molecular beacons the experiment would not be diffusion-limited.

Tyagi (1996) Nature

Acknowledgements