Enhancing Biomolecular Force Spectroscopy

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Background:

Scaffolded DNA origami consists of a several kilobase (kb) single stranded (ss) plasmid that is annealed into a three-dimensional structure using short ss nucleotides (~50 base). Existing CAD software can program the positions of cross-linking staples, allowing for countless unique structures to be created. The structure is then built by mixing the sequenced DNA scaffold strand with a specific set of staple strands, heating the sample to denature any base pairing interactions, then slowly cooling the reaction (hours to days). This slow cooling ensures that the base pairing interactions reach their lowest energy configuration.

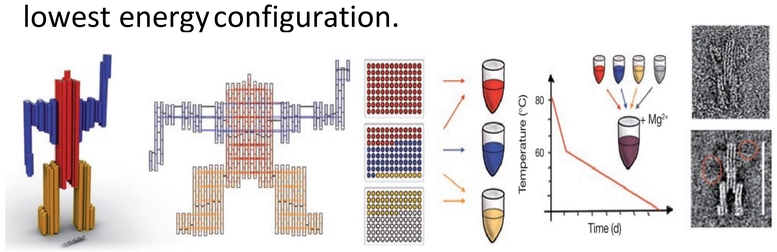


Figure 1: Visualizes the general process of creating DNA Origami Structures

Introduction:

Force experiments on biomolecules are subject to limitations when applying high forces (~60pN or higher) and low forces (~1pN or lower). Due to the physical properties of dsDNA, forces in excess of 60pN cause a structural rearrangement in the DNA. This rearrangement causes DNA to deviate drastically from the worm-like chain model. While using low forces, thermal noise associated with force spectroscopy experiments can bury low force information. We propose one possible solution in which DNA origami can be used as a tool, allowing us to extend the useful range of forces available while conducting force spectroscopy experiments on biomolecules. By incorporating a lever-arm between the dsDNA handle and the molecule of interest, we can amplify or suppress the force felt by the molecule while remaining in the optimal experimental force range of the force spectroscopy instrument.

Preparing Experiment

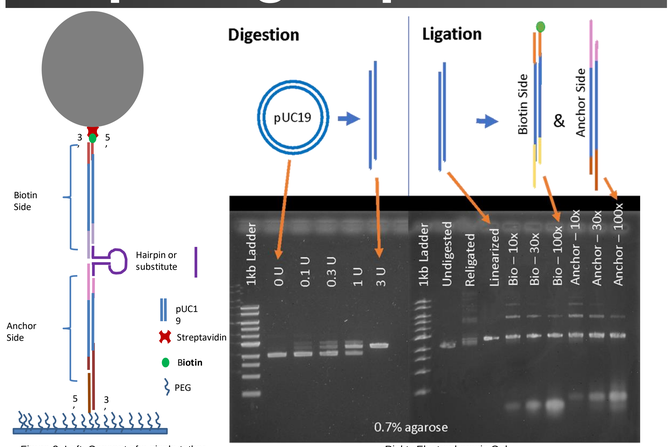


Figure 2: Left- Concept of a single tether needed for this experiment

Right- Electrophoresis Gel

Annealing hairpin to DNA tether for flow cell

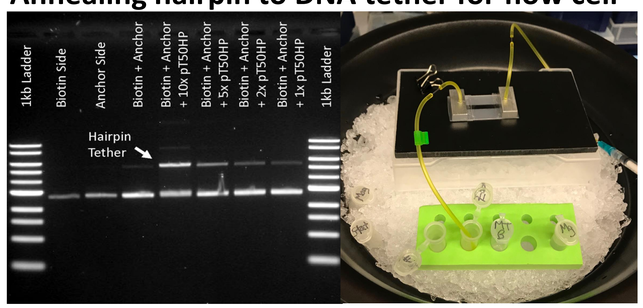


Figure 3: Left- Electrophoresis Gel

Right- Example of flow cell used In experiment

Magnetic beads (Mag) anneal to DNA tethers: Calibration Curve is used to establish baseline for pointwise data in order to verify Mag is attached to single tether.

Figure 4: Determining 'tethered' magnetic beads and stationary beads attached to the flow Cell

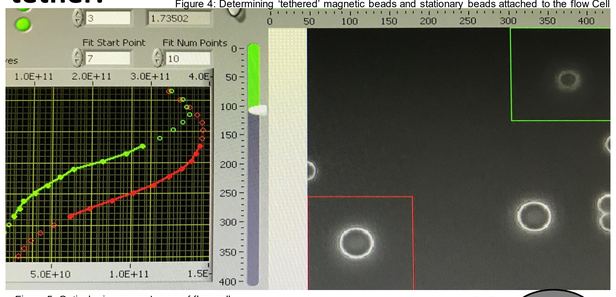
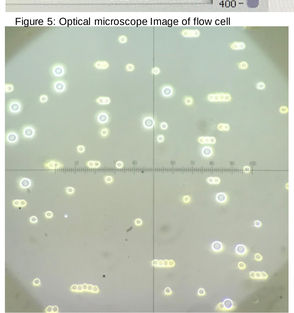


Figure 5: Optical microscope Image of flow cell



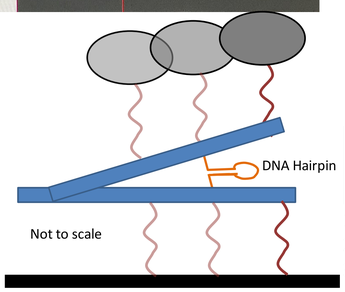


Figure 6: Conceptual aim of experiment, incorporate DNA origami hinge

Magnetic Tweezers Experiments

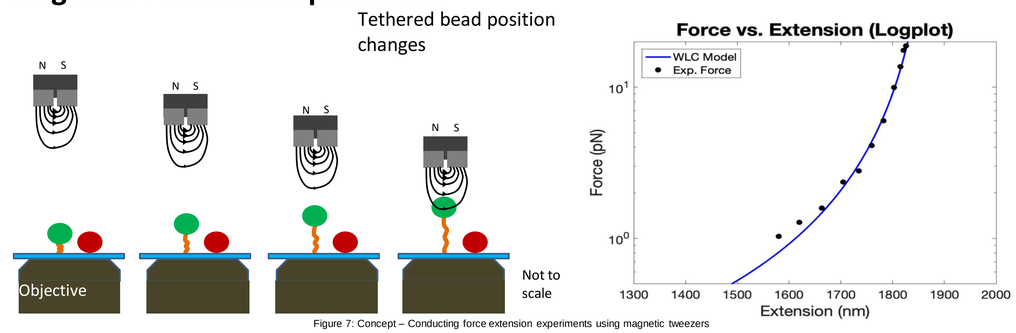


Figure 7: Concept – Conducting force extension experiments using magnetic tweezers

Results:

Characterization of a DNA hairpin rupture force

Force Extension data is obtained with a fixed magnet position at various heights above the sample. Fitting to the Worm-Like Chain model confirms single tethers. Extension and retraction of each tether reveals a hairpin opening between 30-40 pN and closing around 8 pN. With a larger number of data sets, we will determine the opening and closing force distributions of this hairpin. This will give us a baseline characterization for this hairpin which will then be incorporated into a DNA origami hinge.

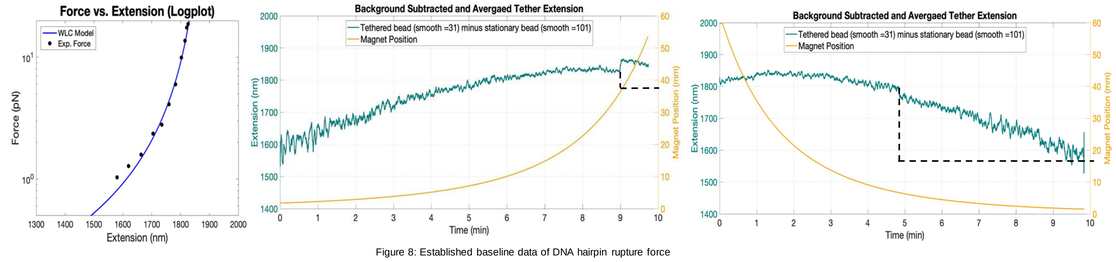


Figure 8: Established baseline data of DNA hairpin rupture force

Future Work:

We will embed the characterized hairpin into the DNA origami hinges designed by Castro lab and monitor the opening and closing force distributions of the hairpin. By altering the attachment points of both the hairpin and the DNA tether arms, we will show that a DNA origami hinge can be used to alter input force needed to rupture the hairpin. The figure on the right, for example, shows an arrangement that should allow us to apply less force to the bead and tether arms to open the hairpin.

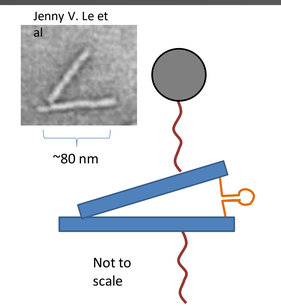


Figure 9: Top Right – TEM (Transmission Electron Mircroscopy) Image of Dynamic Hinge
Bottom Left – Concept of Dynamic DNA Orgiami Hinge

References:

1. Castro, A primer to scaffolded DNA origami (2011).
2. Nataša Vučemilovid-Alagid, Kratky-Porod model (2013).
3. Jenny V. Le, Yi Luo, Michael A. Darcy, Christopher R. Lucas, Michelle F. Goodwin, Michael G. Poirier, Probing Nucleosome Stability with a DNA Origami Nanocaliper. (2017).