DNA Origami Nanocalipers as a Mesoscale Probe of Chromatin Fibers

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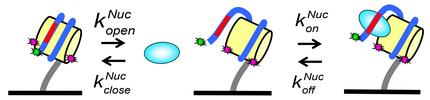
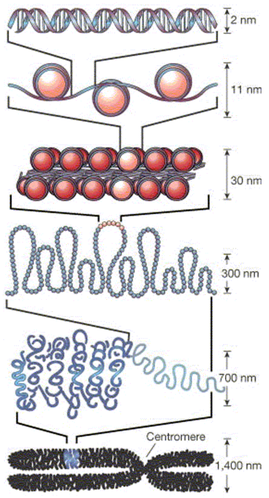
Single Molecule Biophysics

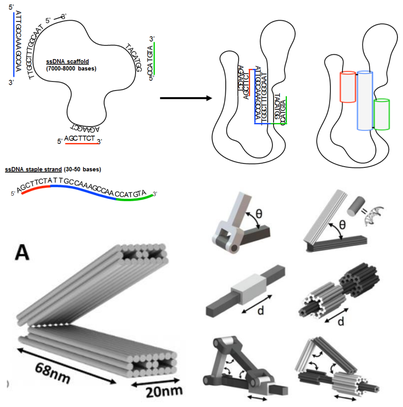
- By examining the dynamics of single molecules, we can piece apart complicated biological mechanisms
- The biophysical mechanisms of gene regulation are not yet well understood due to their scale and complexity
- We aim to develop new tools to probe these regulatory mechanisms



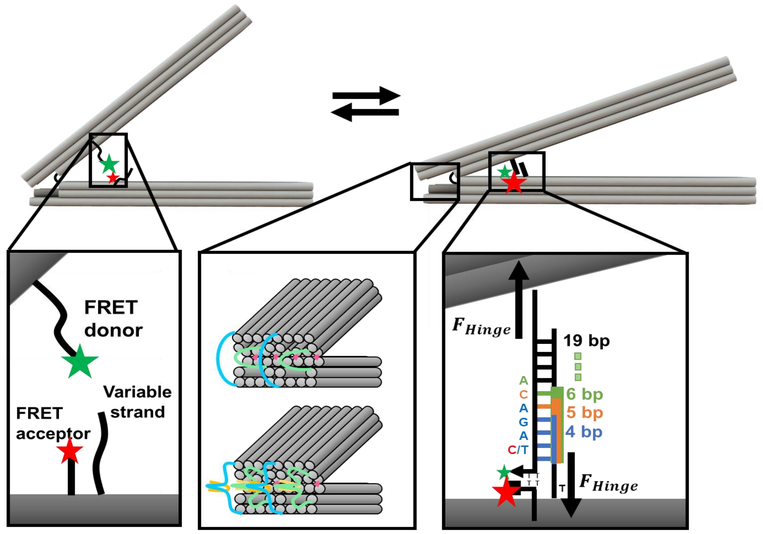


DNA Origami Nanotechnology

- Programmed self-assembly of a 7000-8000 nucleotide "scaffold" strand (single stranded DNA)
- Guided by ~200 shorter (30-50 nt) single-stranded "staples"
- Assembly of scaffold and staples by thermal gradient "folds" dynamic and static components

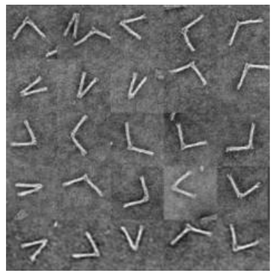


Sensor Mechanism and Design

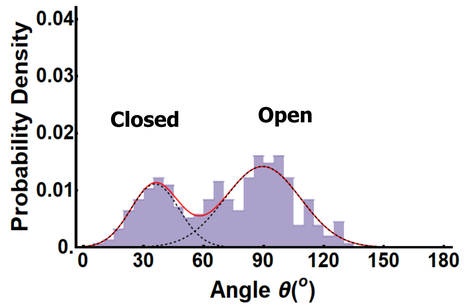


Ensemble Characterization

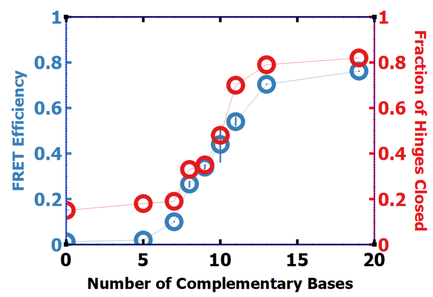
Electron Microscope (EM)



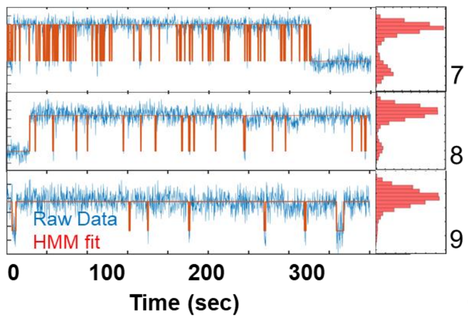
Angle Histogram from EM



Ensemble FRET and EM



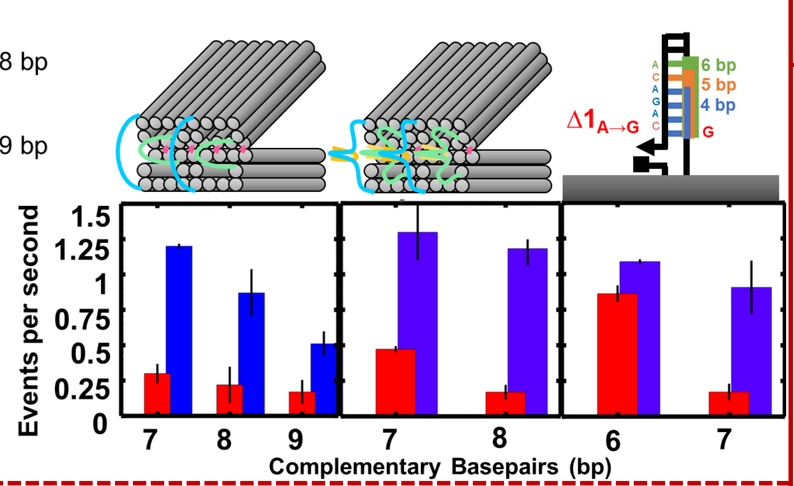
Single Molecule Measurements of Nanocalipers



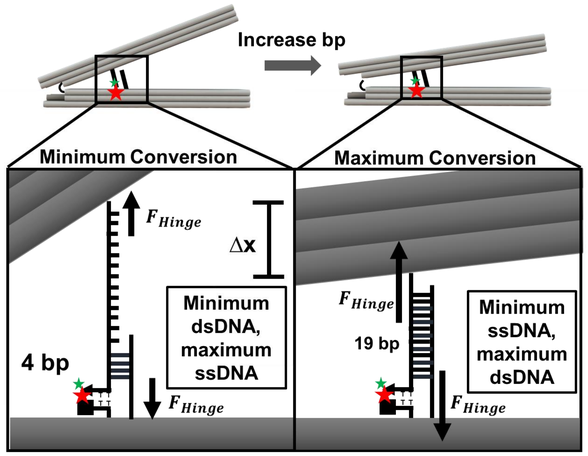
- Individual molecules (above) are fit to ideal Markov paths
- Dwell times are collected and fit to single exponential rate models (right)

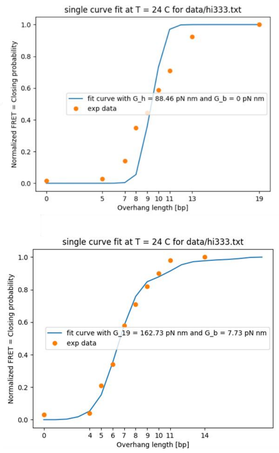
Vertex Design Variants 3.3.0 3.3.3

Strut Variant

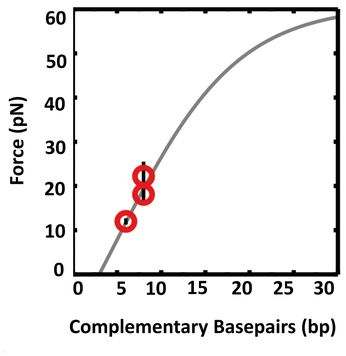


Modeling Nanocaliper-Strut Interactions





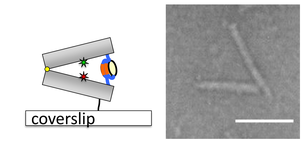
Observed vs. predicted forces



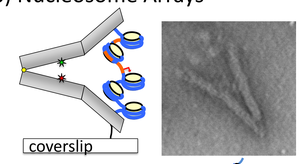
FUTURE WORK

GOAL: develop a device to probe site-specific chromatin dynamics at 10-100nm length scales:

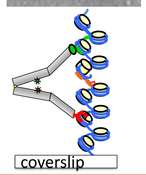
(a) Single nucleosomes



(b) Nucleosome Arrays



(c) Site-specific within array



CONCLUSIONS

- We are able to faithfully monitor nanocaliper state by fluorescent reporter
- Nanocaliper dynamics are sensitive to both pivot location design and strut length
- Multiple opening and closing rates indicate potentially complicated dynamics

REFERENCES

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