Characterizing the Conformational Fluctuations of DNA Under Physiological and Salt-Stabilized Conditions

Maya Pande, Anabel Chang, Dylan Heussman, Jack Maurer, Amr Tamimi, Peter H. von Hippel and Andrew H. Marcus
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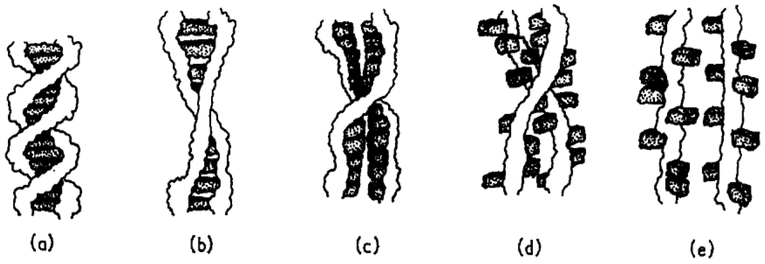

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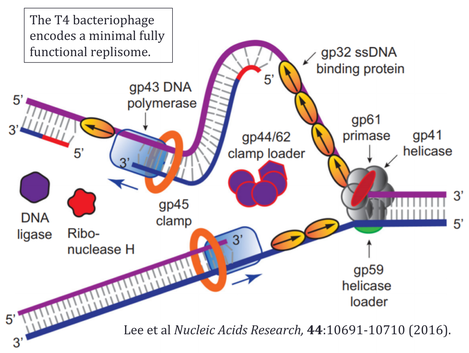

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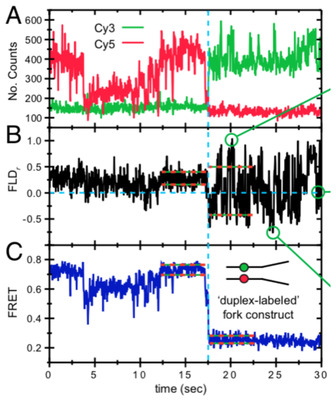

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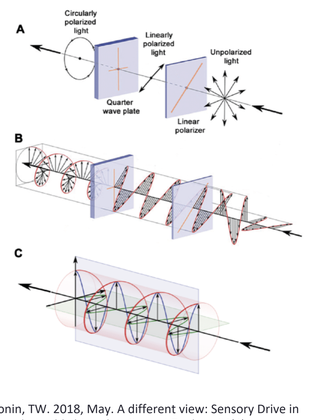

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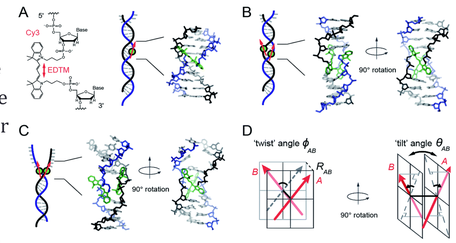

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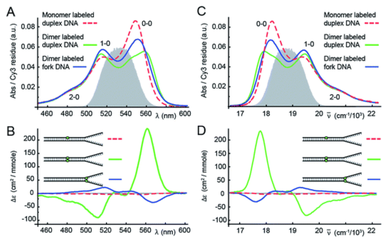

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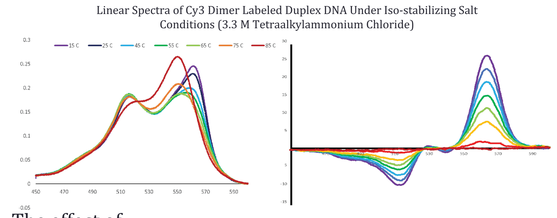

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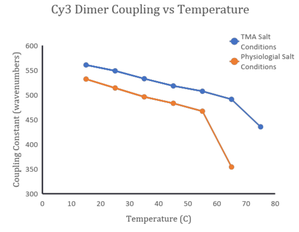

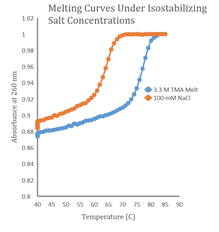

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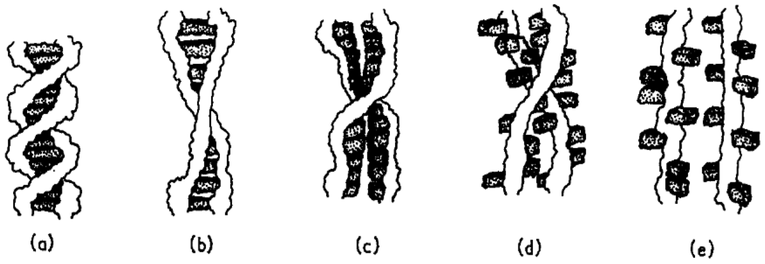

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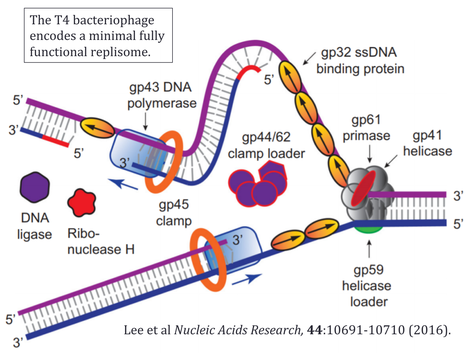

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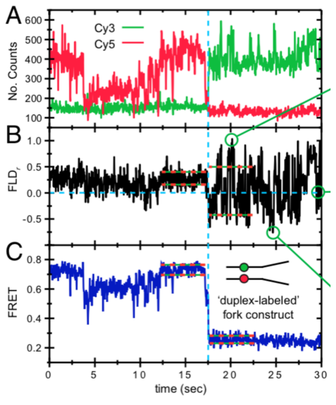

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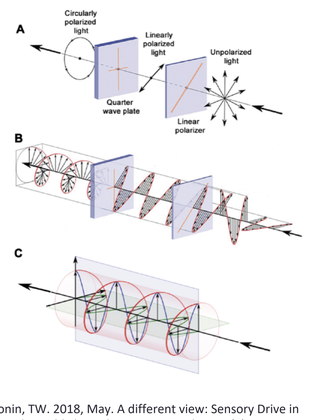

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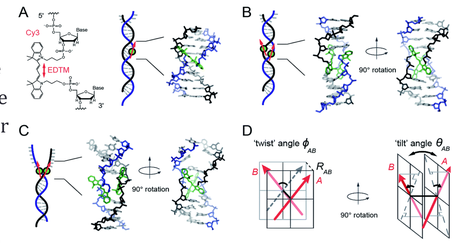

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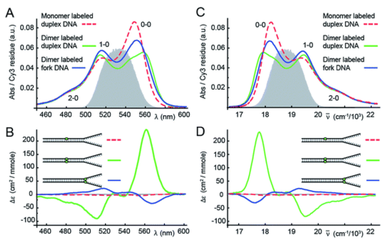

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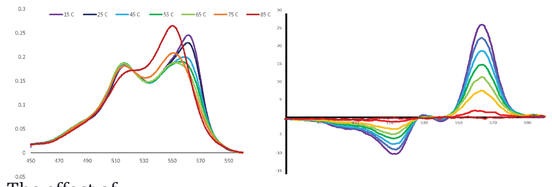

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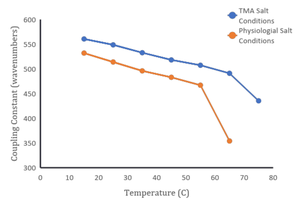

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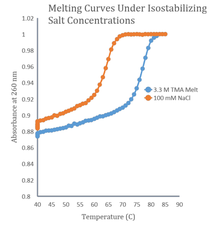

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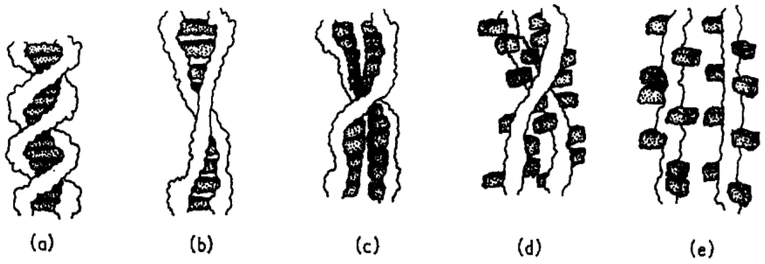

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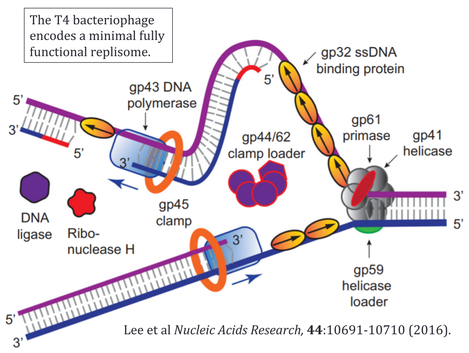

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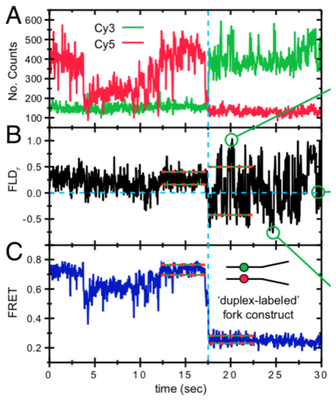

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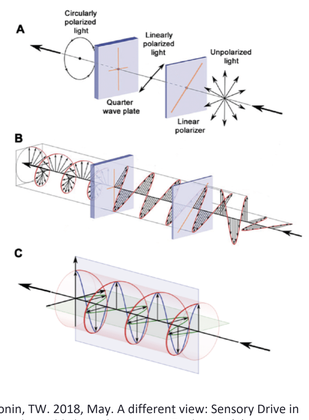

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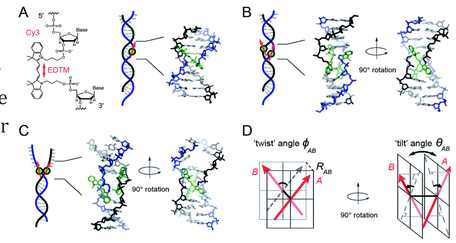

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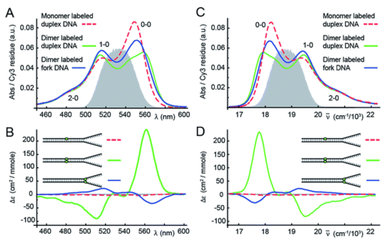

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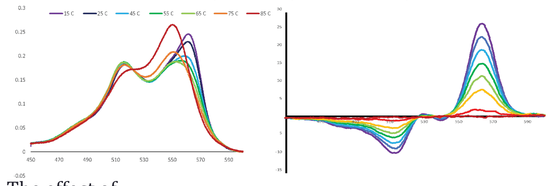

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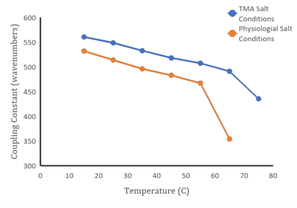

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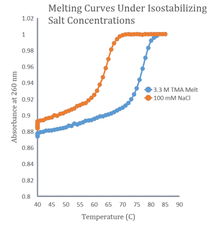

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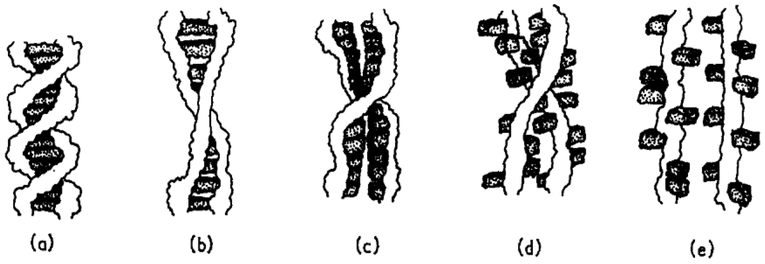

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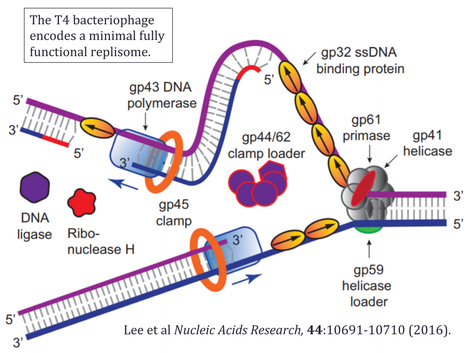

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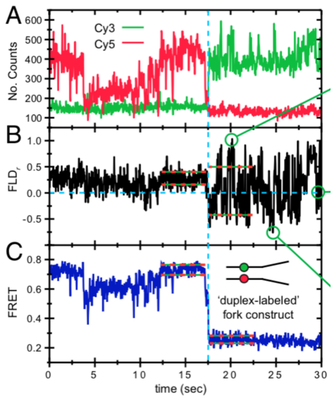

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The measurements are analyzed using a MATLAB program through integrated histograms to visualize smFLD and smFRET trajectories on millisecond time scales, and time correlation functions to evaluate fluctuations of the sugar-phosphate backbone on tens-of-microsecond time scales.

PROJECTS

In our experiments, we used a variety of techniques to analyze the structure of DNA with the overall goal of better understanding the conformations it can take. Our studies were focused in two areas:

1. Understanding the mechanisms of DNA breathing. Duplex and fork DNA undergo thermal fluctuations under physiological conditions that allow for the binding of proteins.
2. Conducting experiments on the stabilizing and destabilizing properties of salt solutions on DNA. We also looked to understand the method by which iso-stabilizing salts stabilize the conformation of DNA even under varied temperatures.

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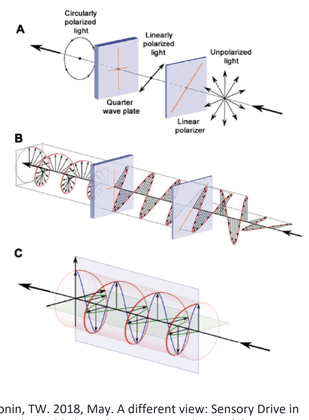

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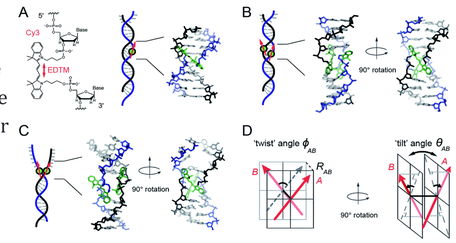

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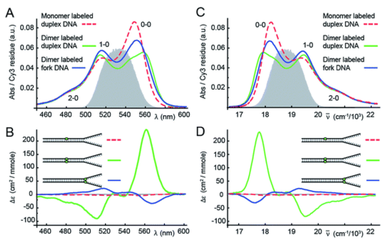

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ISO-STABILIZING SALTS MODIFY THE FREE ENERGY LANDSCAPE OF CY3 LABELED DNA

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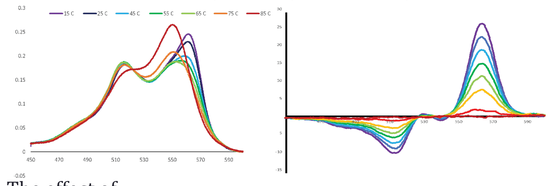

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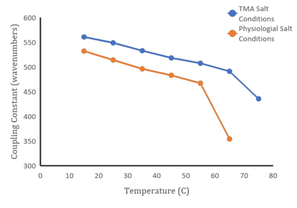
Cy3 Dimer Coupling vs Temperature



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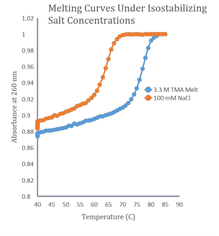

Future studies may focus on fork rather than duplex DNA. Tetramethylalkyl-ammonium is known to raise the melting temperature of DNA. By measuring the CD and absorption over larger temperature ranges or increasing coupling between the two Cy3 labeled sites, we may be able to learn about the structural changes occurring during melting and the stabilizing properties of TMA.

Characterizing the Conformational Fluctuations of DNA Under Physiological and Salt-Stabilized Conditions

Maya Pande, Anabel Chang, Dylan Heussman, Jack Maurer, Amr Tamimi, Peter H. von Hippel and Andrew H. Marcus
Institute of Molecular Biology, Center for Optical, Molecular and Quantum Science,
Department of Chemistry and Biochemistry, University of Oregon, Eugene, OR 97403

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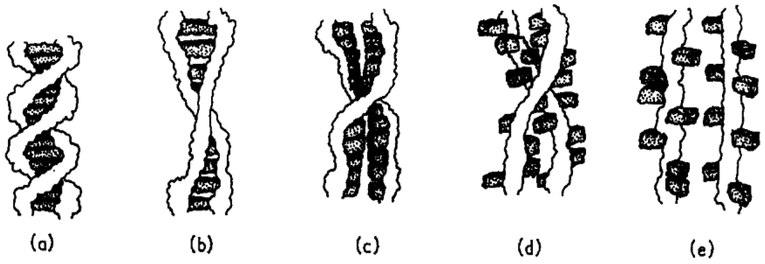
DNA "BREATHING"



McConnell, von Hippel *J. Mol. Biol.*, **50**: 298 (1970).

At physiological temperatures, DNA undergoes local thermal fluctuations, or "breathing", in which the double-stranded (ds) DNA unwind and base pairs unstack. Breathing occurs on sub-millisecond time scales, and are studied in the fork and duplex regions of dsDNA using time-resolved single-molecule measurements.

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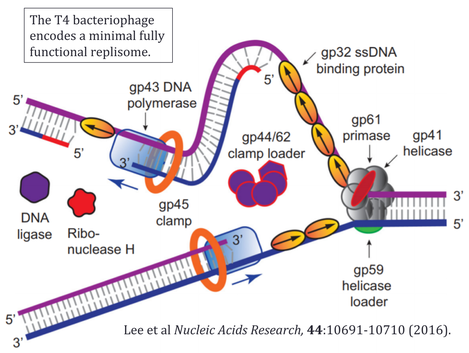

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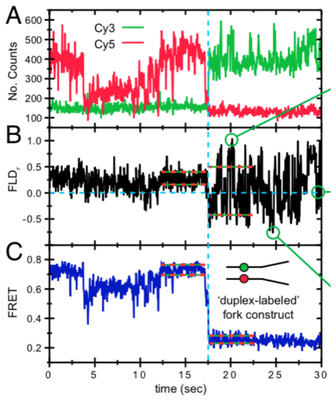

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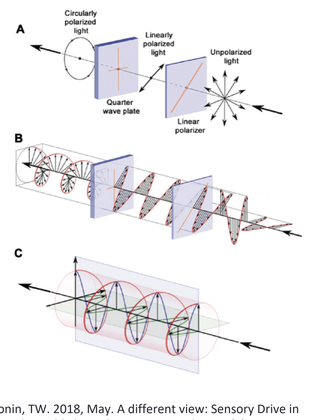

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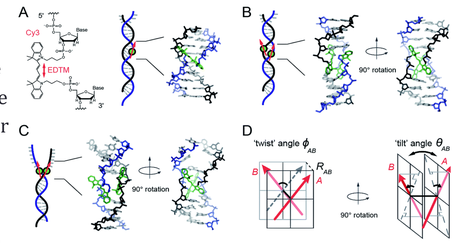

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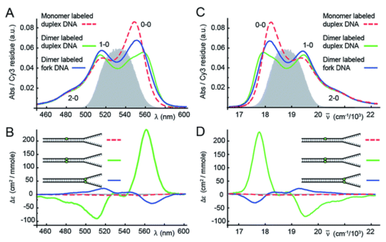

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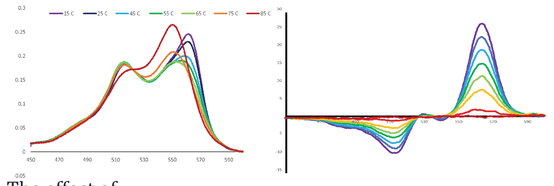

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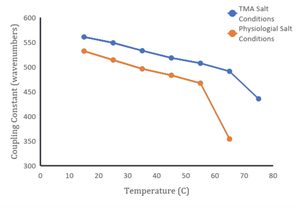

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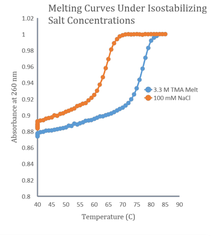

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