Effects of DNA looping behavior using smFRET

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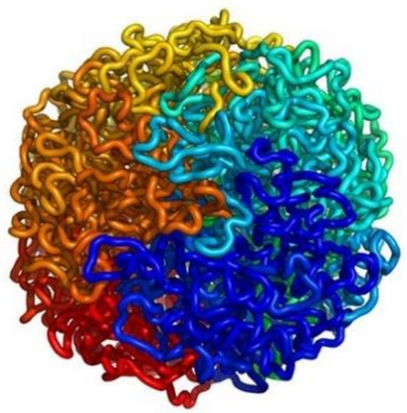
Introduction

This study developed a single-molecule-based assay to track the looping (circularization) of individual double-stranded DNA (dsDNA) molecules in real time. DNA encodes our genetic information through a unique combination of four nucleotides: Adenine (A), Thymine (T), Cytosine (C), and Guanine (G). Base pairing among these nucleotides forms dsDNA molecules in a stable double-helical form. The goal of my research is to understand how small-scale defects in DNA can disrupt local helical structures, along with higher-order structures, required for accurate large-scale organization of the genome. Future work will prove how defects alter the looping behavior.

Significance of Experiment

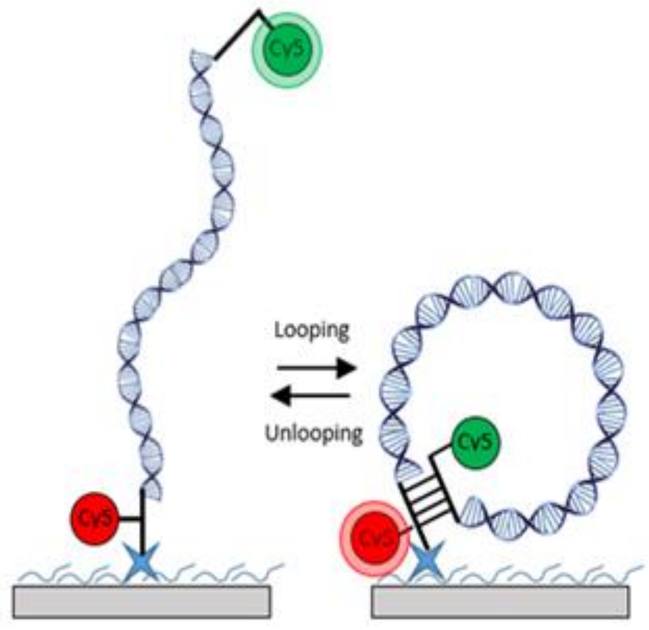
In addition to forming stable dsDNA, the genome must also achieve a precise three-dimensional architecture through twisting and bending of dsDNA; however, the mechanical properties (i.e., bending propensity) of dsDNA are sensitive to sequence, such that sequence defects can alter genome topology, resulting in genomic misfolding that has been linked to many disorders like cardiovascular diseases, cancers, schizophrenia, and limb development disorders.



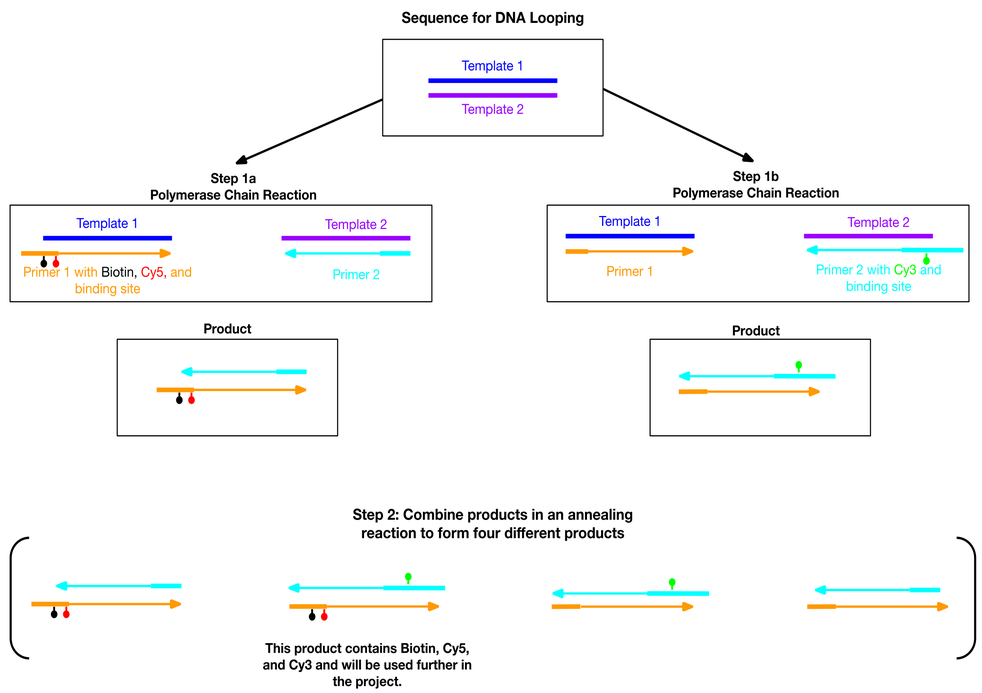
Fractal globule model of genome. Adapted from Lieberman-Aiden et al. 2009.

Nanometric, Single-molecule Looping Assay

The fluorescent dyes Cy3 and Cy5 can interact via resonance energy transfer to function as a spectroscopic ruler that reports distance changes up to 10 nm. The distance between the Cy3 and Cy5 dyes modulates the emission wavelength and intensity. The maximum observed intensity occurs for λ = 640 – 690 nm (red) when the dsDNA is in its looped conformation, and the maximum observed intensity occurs for λ = 540 – 590 nm (yellow green) when the dsDNA is in its unlooped conformation.

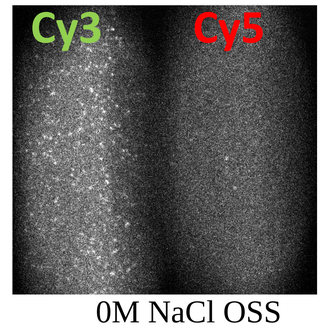


PCR-Based Strategy to Construct Looping DNA

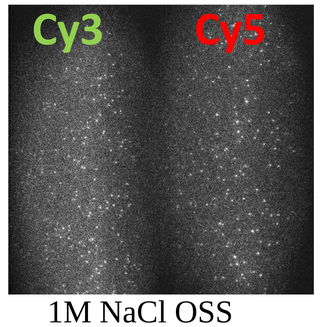


Results and Discussion

Sample images showing spatially separated Cy3 and Cy5 emission channels. Detection of DNA looping by smFRET with different loop states producing distinct FRET states, which appears as increased Cy5 signal.

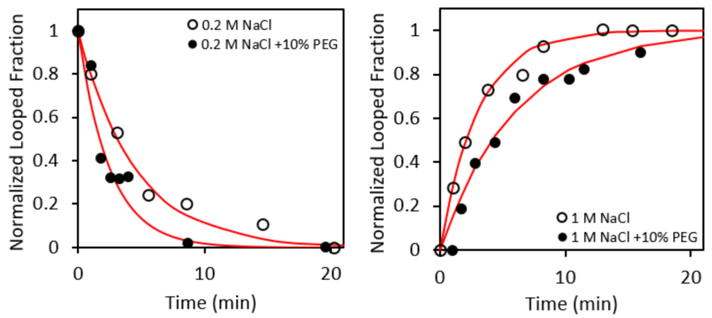


0M NaCl OSS



1M NaCl OSS

Time courses were measured to determine the unlooping and looping rates for different environmental and ionic conditions. The presence of molecular crowders accelerates unlooping and slows loop formation



Acknowledgements

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