Characterizing Conformational Fluctuations of DNA

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INTRODUCTION

Local fluctuations of the sugar-phosphate backbones of DNA (a form of DNA 'breathing') play key roles in protein-DNA assembly and enzymatic function. These fluctuations contribute towards the formation of DNA structures which can be recognized by proteins during replication, allowing access to the interior bases and sequence independent binding.

We aim to characterize the dominant structures and their fluctuations involved in functionally relevant DNA topologies, most notably ss-ds DNA fork junctions.

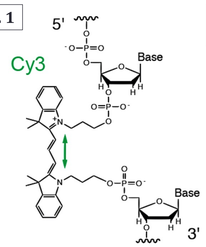
Can we characterize local backbone fluctuations of ss-ds DNA forks and primer-template junctions using photon correlation single-molecule fluorescence spectroscopy?

MATERIALS & METHODS

Labeled DNA Replication Fork

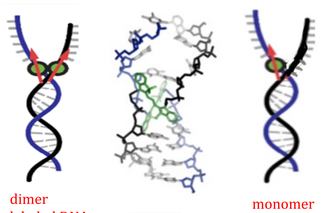
DNA is "internally" labeled by insertion of a fluorescent dye into the sugar-phosphate backbone.

Fig. 1



L. Kringle, et al., *J. Chem. Phys.*, 2018, **148**, 085101.

DNA fork constructs are prepared, which contain a $(Cy3)_2$ dimer or a Cy3 monomer at specific positions relative to the fork junction.



dimer labeled DNA fork construct

Fig. 2

monomer labeled DNA fork construct

Single Molecule Polarization-Sweep Spectroscopy

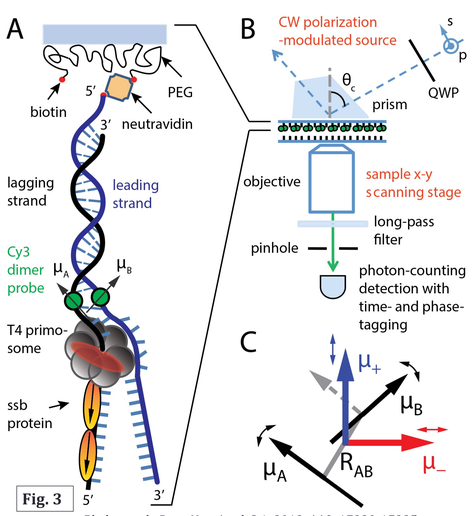


Fig. 3

Phelps et al., *Proc. Nat. Acad. Sci.*, 2013, **110**, 17320-17325.

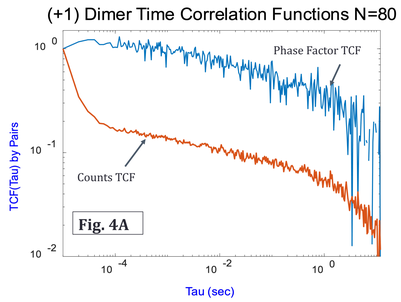
Fig. 3. (A) Experimental setup for single-molecule measurements of backbone fluctuations at the DNA fork junction using Cy3 dimer probes.

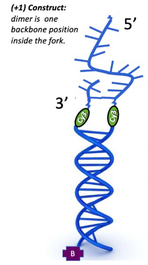
(B) The plane polarization direction of a continuous wave 532 nm laser is rotated from 0 to 2π at 1MHz frequency. When a fluorescent photon is detected, information about the phase of the laser polarization angle, as well as the photon arrival time, is recorded.

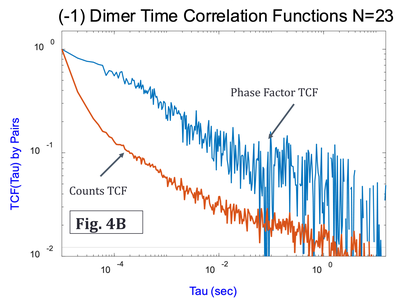
(C) Differential absorption in the two polarization directions is due to the symmetric and antisymmetric excitons being orthogonally polarized to one another.

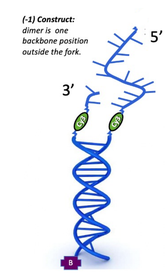
RESULTS

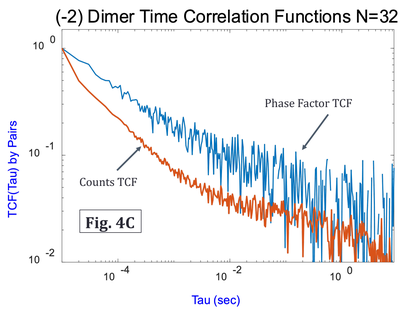
Single Molecule Polarization-Sweep Spectroscopy Experimental Time Correlation Functions (TCFs)

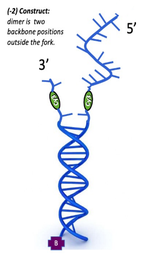












Simple model of the Polarization Sweep Signals of the Cy3 Dimer and Monomer labeled DNA fork constructs:

$$I_{Dim} = \frac{(A+B)}{2}\left[1 + \frac{B-A}{A+B}\cos(\varphi + \varphi_0)\right] \qquad I_{Mono} = C\left[1 + v\cos(\varphi + \varphi_0)\right] \qquad v = \text{a 'visibility'}$$

The constants A and B describe the overlap intensities of the plane-polarized laser with the absorption spectra of the symmetric and anti-symmetric excitons of the dimer. The constant C is the overlap of the laser with the monomer spectrum. The angle φ_0 describes the orientation of the probe chromophore relative to the laser polarization. The time correlation functions (TCFs) in **Fig. 4A – 4C** compare the 'counts' and 'phase-factor' signals measured for the +1, -1, and -2 labeled DNA constructs..

$$I_{counts} = \frac{1}{T_w}\sum_{j=1}^{N_w}\delta(t - t_j) = \frac{N_w}{T_w} \qquad I_{phase-factor} = \frac{1}{T_w}\sum_{j=1}^{N_w}\delta(t - t_j)e^{-i\theta_j} \qquad TCF = \langle \delta I_{C(P-F)}(t)\delta I_{C(P-F)}(t+\tau)\rangle$$

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FUTURE DIRECTION

Single Photon Phase-Tagging For Single Molecule Fourier Transform Electronic Spectroscopy (FTES)

- From the phase- and time-tagged data, the time-dependent overlap spectrum of a single molecule can be determined.
- The smFTES method can be used to monitor structural changes of a dimer labeled DNA construct in real time at the single molecule level. By also rotating the polarization of the incident broadband pulses, we can obtain the single molecule dichroism spectrum.

The smFTES experimental setup.

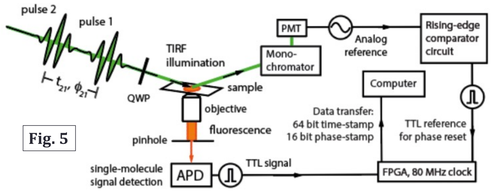
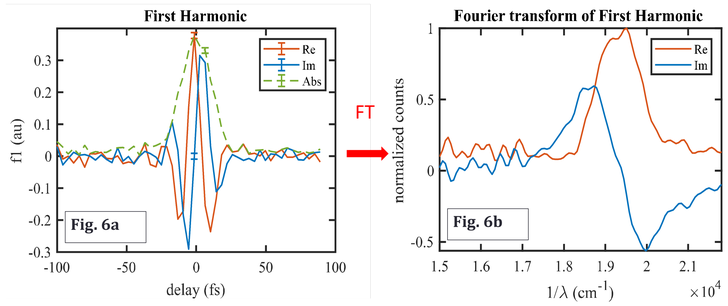


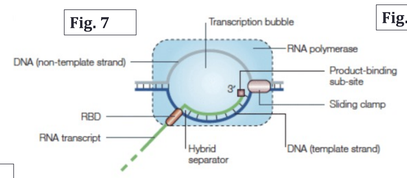
Fig. 5



The smFTES approach can be used to track the spectral evolution of our Cy3 dimer probe. This would reveal the local conformation changes of the DNA backbones within nucleic acid-protein complexes. For example, the method could be used to study structural changes within the transcription-elongation complex (TEC) of **Fig. 7**, which is stable and long lived relative to experimentally accessible timescales available using this approach. A list of processes occurring in the TEC is shown in **Fig. 8.** along with the timescales associated with each rate of reaction.

Transcription elongation complex

Fig. 7



Greive, S. J., & Von Hippel, P. H. (2005). Thinking quantitatively about transcriptional regulation. *Nature Reviews Molecular Cell Biology*, *6*(3), 221–232. https://doi.org/10.1038/nrm1588

Processes occurring in the TEC and associated timescales

Fig. 8

Reaction pathway	Rate constant	Comments
Elongation	30–100 sec^{-1}	Favoured in the presence of saturating rNTP concentrations. Increased rates are induced by antitermination complexes.
Pyrophosphorolysis	~30–100 sec^{-1}	Favoured in the presence of high PP_i and low rNTP concentrations.
Arrest	1 hr^{-1} (or more)?	Favoured at specific sequences with weak RNA–DNA hybrids.
Pause	~0.1–1 sec^{-1} ~0.02–0.04 sec^{-1}	Long pauses (class I or II) occur at specific template sequences.
Misincorporation	0.05–1 min^{-1}	Favoured in the absence of the next required rNTP and saturating concentrations of other rNTPs.
Editing	0.1–1 sec^{-1} (?)	Requires back-sliding and cleavage factors GreA or GreB, or SII.
Termination	~0.1–1 sec^{-1}	At intrinsic or Rho-dependent terminator sites on the DNA template.

CONCLUSIONS

- Use of Cy3 monomers and dimers as probes are useful for studying structure and conformational fluctuations in biological systems such as dimer labeled DNA forks and junctions in the transcription-elongation complex.
- Refinement of current single molecule polarization sweep will characterize the dynamics of the sugar-phosphate backbones near DNA fork junctions.
- Single-photon phase-tagging techniques can be used to capture structural changes occurring at the single molecule level. This information could help to elucidate bimolecular mechanisms in protein-nucleic acid complexes.