Fluorescence imaging of formation of triple helical DNA

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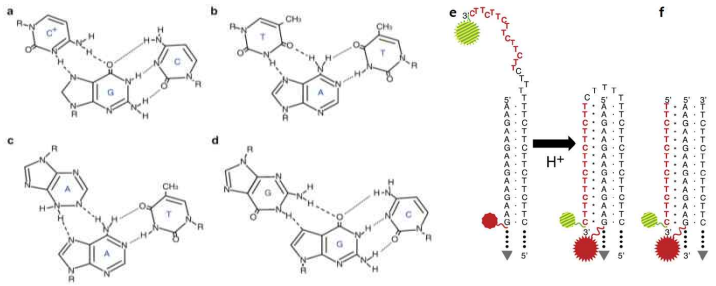
Abstract

Triple helical DNA (DNA triplex) is an exotic conformation of DNA, which is known to exist for special sequences under specific pH and salt conditions. *In vivo* formation of DNA triplex is thought to be responsible for a certain genetic disease such as *Friedreich Ataxia*. This conformation has also drawn much attention over decades because of its potential for sequence-specific DNA targeting.

Here we aim at directly monitoring triplex formation between a duplex and a single-stranded oligonucleotide in real time via single-molecule FRET technique. This technique not only enables us to detect triplex formation but also provides detailed information about relative orientation of these DNA strands, which further confirms the geometry of DNA triplex. We also investigate the effect of pH, salt, and molecular stoichiometry on dynamics of triplex formation. The results obtained here would provide crucial insight into nucleic acid interactions and molecular design for triplex-based nano-device and reporters.

DNA triplex

- **Structure of triple helical DNA**



(a-d) Triads in Triplex
- Base pairing between the middle and the right bases: Watson-Crick (dotted) of duplex
- Base pairing between the left (TFO: triplex-forming oligonucleotide) and the middle bases (purine): (reverse) Hoogsteen (dashed)
- (a) & (b): pyrimidine (Y) motif triplex (C^+: protonated cytosine induced at low pH)
- (c) & (d) purine (R) motif triplex.

(e-f) Sample design (In our study, we only study pyrimidine motif triplex.)
- (e) intramolecular triplex: $(TCC)_5$ tail serves as TFO [intraY]
- (f) intermolecular triplex: Separate 3rd strand (TFOCy3) binds to duplex to form a triplex. [interY]

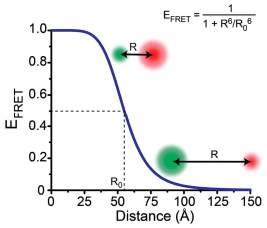
Single-molecule FRET technique

- **FRET (Fluorescence Resonance Energy Transfer)**: in FRET, via induced-induced dipole interaction, the energy of a donor dye is transferred to a nearby (within a few nm) acceptor dye and instead of the donor, the acceptor emits fluorescence.

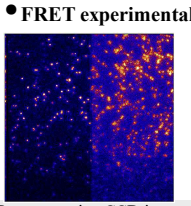
FRET efficiency (FE) is given by

$$E = \frac{I_{acceptor}}{I_{donor} + I_{acceptor}} = \frac{1}{1+(R/R_0)^6}$$

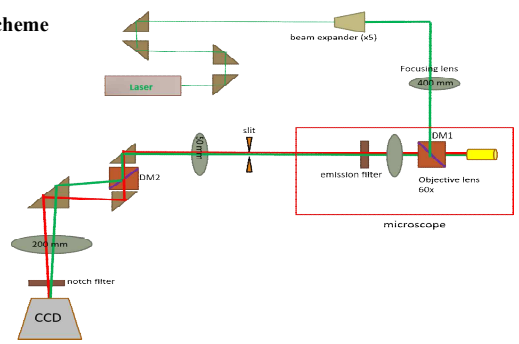
(I_{donor} : intensity of donor
$I_{acceptor}$: intensity of acceptor
R: distance between donor & acceptor
R_0: characteristic distance
at which 50% energy is transferred ~ 60Å)



- **FRET experimental scheme**

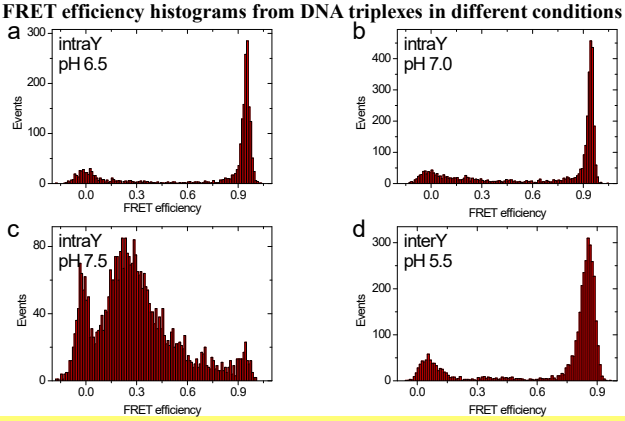


Representative CCD image of smFRET experiment (left) donor signal (right) acceptor signal



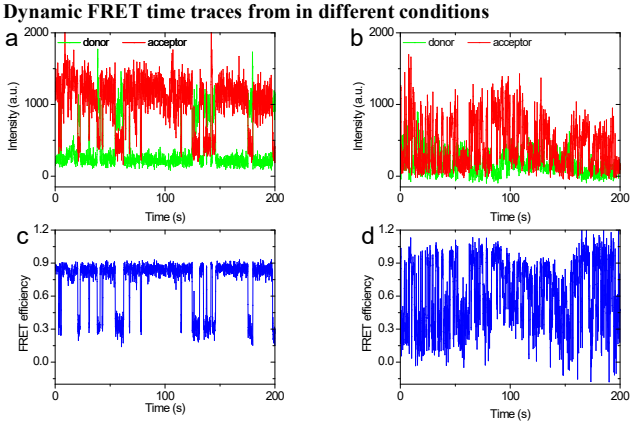
Results & Conclusion

- **FRET efficiency histograms from DNA triplexes in different conditions**



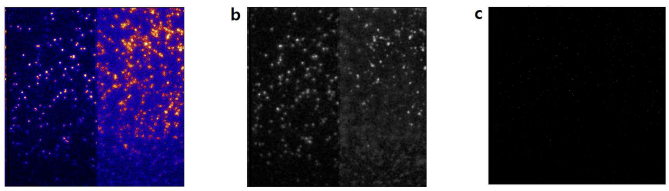
- Conformation of intraY sample for various pH: (a) pH 6.5, (b) pH 7.0, (c) pH 7.5
- (d) interY can also form triplex @ 10 mM Mg^{2+} and pH = 5.5.
  - FE ~ 0.9: folded triplex structure; FE ~0.1: unfolded structure.

- **Dynamic FRET time traces from in different conditions**



- Time trace of donor and acceptor intensities from intra Y: (a) pH 7.0 (b) pH 7.5
- FRET time trace from intraY: (c) pH 7.0 (d) pH 7.5

- Localization of dye positions in fluorescence image (intraY @ pH 6.5)



- (a) Raw image (b) modified image after background subtraction and removal of speckles (c) localization image of dyes via QuickPalm plugin by ImageJ.

- We observed triplex formation in both intra- and intermolecular geometries.
- Triplex formation is sensitive to pH: low pH facilitates triplex formation.
- We observed dynamic interconversion in folded-unfolded transition of triplex.
- We demonstrate localization of dyes by open-source imageJ program for future applications.

References

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Acknowledgement

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