FRET Measurements of Trapped Oligonucleotide Anions
Allison S. Danell and Joel H. Parks
ROWLAND INSTITUTE AT HARVARD, 100 EDWIN LAND BLVD., CAMBRIDGE, MA 02142

Presented at the 51st Annual ASMS Conference on Mass Spectrometry and Allied Topics
Montreal, 2003

This poster was originally presented in large format, but it was modified for easier reading/downloading from the web.
INTRODUCTION

Fraying, or unzipping, of double-stranded oligonucleotides ions has been studied with fluorescence resonance energy transfer (FRET) and mass spectrometry.

- Oligonucleotide anions derivatized with fluorophores introduced into quadrupole ion trap mass spectrometer
- Mass and fluorescence spectra correlated to conformational changes of anions during manipulation in gas phase
Analytes

- Oligonucleotides derivatized with fluorophores (Synthegen)
- RP-HPLC purification prior to shipment
- BODIPY dyes (Molecular Probes)
- N-succinimyldil ester groups react with amino functionalities present on 5' or 3' end of oligonucleotides

EXPERIMENTAL

- 50 µM complementary single strands in 50 mM NH$_4$OAc
- Annealed for 8 min. at ~ 90°C, then cooled over 2-3 hours
- Thermal denaturation of duplexes monitored with UV detection confirmed presence of duplexes in solution
- nESI solutions 5 - 10 µM in 50/50 MeOH/water or 60/20/20 acetonitrile/isopropyl alcohol/water, with 5 – 10 mM NH$_4$OAc
EXPERIMENTAL

Mass Spectrometry
• Custom-built quadrupole ion trap [1]
• Temperature-controlled trap assembly and He background gas
  — Electrodes and He inlet seated in copper housing
    ▪ Copper housing resistively heated with Watlow 965 controller
  — Heating to ~ 170°C with 1°C precision

Fluorescence
• Pulsed Nd-YAG laser (Molectron)
• Photomultiplier tube detector (Hamamatsu)
• Donor fluorescence detected (bandpass 535 to 580 nm)
Overlap Volume $\delta V_{hv}$

Fluorescence Detection

Electrospray

Brewster Angle Window

PMT

$\lambda F, NF$

$\lambda_{L}$

$532 \text{ nm}$

Attenuator

Spatial Filter

$25 \mu m$ pinhole Microscope Objective

Polarizing Beamsplitter

Half waveplate

Iris

50 cm Lens

Iris

Collimating lens

Shutter

Periscope

$I_{L} \sim 0.5 \text{ MW/cm}^2$

$150 \mu m$
FRET
• Method used to determine distances between donor and acceptor fluorophores.
• Förster distance: distance at which FRET rate reduces the donor fluorescence by factor of ~ 2
• Average distances between fluorophores can be estimated if Förster distance known

Oligonucleotide sequences were chosen
— to bias possibility that long-lived intermediate state may be present
— to bias possibility that fraying of duplex would occur from A·T ends (derivatized with fluorophores)
— to correlate with dissociation rates previously measured of similar, partially-complementary 7-mers [2]
RESULTS

Study of BODIPY-TMR-5’-AATTAATCCGCGCCG-3’ and 5’-CGGCCGGATTAATT-3’-BODIPY-TR

- Isolated 7- charge state of duplex (ds7-)
- 60 s irradiation period, 1 µJ pulse energy

(a) Single scan mass spectrum and (b) single scan donor fluorescence spectrum from isolated (ds)7- ion; 60 s irradiation, pulse energy 1.0 µJ.
(c) Representative spectrum of background PMT signal (no ions loaded).
• Temperature varied from 109 – 123°C
  — Above 130°C, fragmentation of ds\textsuperscript{7−} occurred in 60 s time period
  — Fragmentation primarily base loss
• Donor fluorescence from ds\textsuperscript{7−} compared to donor fluorescence from ss\textsuperscript{6−} (BODIPY-TMR-5′-AATTAATCCGGCCG-3′)
• Increase in fluorescence from ds\textsuperscript{7−} attributed to increased distance between the FRET donor and acceptor fluorophores
  — Indicates ions undergoing conformational change over the temperature range 109 – 123°C

Fluorescence intensities from ds\textsuperscript{7−} and ss\textsuperscript{6−}. 
• Model of intermediate states of dissociation has been formulated [3]
  – Based on dissociation rates previously determined for (AATTAAT)$_2$ and (CCGGCCG)$_2$ to describe dissociation of 14-mer duplex in 2 steps [2]
  – Assumes average distance $\langle R^2 \rangle$ of $\sim 48\text{Å}$ (2x length of 7 base pair A·T)
  – Assumes Förster distance of $\sim 50\text{Å}$ from solution phase measurements [4]
• Compared experimental fluorescence data with calculated fluorescence based on model
  – Normalized data to calculated curve but no curve fitting applied
• Comparison supports presence of a conformational state in which only part of duplex has begun to fray from A·T end

(a) Model of donor fluorescence and single strand ion abundance during dissociation of 14-mer duplex (parameters in text). (b) comparison of experimental (squares) and theoretical (solid line) data.
Study of BODIPY-TR-5'-'-AAAAAAAGGCCGGC-3' and 5'-GCCGGCCTTTTTTT-3'-BODIPY-TMR

- Isolated 7- charge state of duplex (ds7-)
- 90 s irradiation period, 0.7 μJ pulse energy
- Temperature varied from 75 – 160°C
  - Above 140°C, fragmentation of ds7- occurred in 90 s time period
  - Fragmentation primarily cleavage between A·T and G·C portions of strands
- Donor fluorescence from ds7- compared to donor fluorescence from ss6- (5'-GCCGGCCTTTTTTT-3'-BODIPY-TMR)
- Donor fluorescence does not change upon fragmentation
  - Indicates separation between fluorophores large across the temperature range shown
• Average distance between fluorophores estimated ~ 55Å  
  – Consistent with unpairing of A·T section of duplex  
• Estimated average distance and fragmentation pattern both support conclusion that separation between fluorophores was large over temperature range studied

Fluorescence intensities from ds7- and ss6-.

Isolation and thermal dissociation spectra of ds7-; 20 min. at 160°C.
Preliminary experiments with BODIPY-TMR-5'-TTTTTTTTTTTTTTTTTTTTT-3'-BODIPY-TR

- Single-stranded oligonucleotide labeled with donor fluorophore at 5' end and acceptor fluorophore at 3' end
- Fluorescence intensities corrected for decaying population of ions due to electron autodetachment [5]
  - Reduced ion population at end of 60 s irradiation period
- Varied charge state of isolated ions and measure fluorescence
  - Expect conformational changes with change in charge state
  - Fluorescence intensity changes suggest changes in distance between donor and acceptor fluorophores
  - cw excitation (system currently being installed) will reduce uncertainty associated with measurements

Fluorescence intensities from dual-labeled T\textsubscript{14}. 

![Fluorescence intensity vs. charge state graph](image)
SUMMARY

- Fluorescence and mass spectra have been acquired as a function of temperature from 14-mer duplexes derivatized with FRET fluorophores.
- Fluorescence intensities were used to estimate the distance between the fluorophores.
  - Mass spectra acquired at the end of each irradiation period were crucial for verification that duplex ions were intact.
  - Estimated fluorophore separations indicate the duplexes frayed at the A·T ends of the duplexes.
- Preliminary studies of dual-fluorophore-labeled oligonucleotide anions have been performed.
  - Measured donor fluorescence intensity as a function of charge state.
- These studies provide a basis for further experiments which can describe oligonucleotide dynamics more quantitatively.

References

[5] Electron autodetachment, $M^{n^-} \rightarrow M^{(n-1)^-}$, of oligonucleotides has been observed recently in our trap; presented MPR#342.