

FRET Measurements of Trapped Oligonucleotide Anions

Allison S. Danell and Joel H. Parks

ROWLAND INSTITUTE AT HARVARD, 100 EDWIN LAND BLVD., CAMBRIDGE, MA 02142

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INTRODUCTION

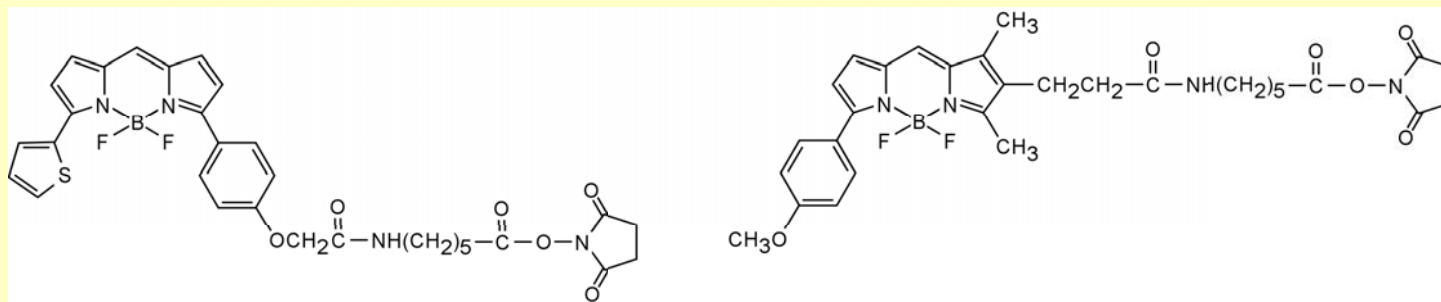
Fraying, or unzipping, of double-stranded oligonucleotides ions has been studied with fluorescence resonance energy transfer (FRET) and mass spectrometry.

- 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

EXPERIMENTAL

Analytes

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



BODIPY-TMR (donor)

BODIPY-TR (acceptor)

- 50 μM complementary single strands in 50 mM NH_4OAc
- Annealed for 8 min. at $\sim 90^\circ\text{C}$, 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_4OAc

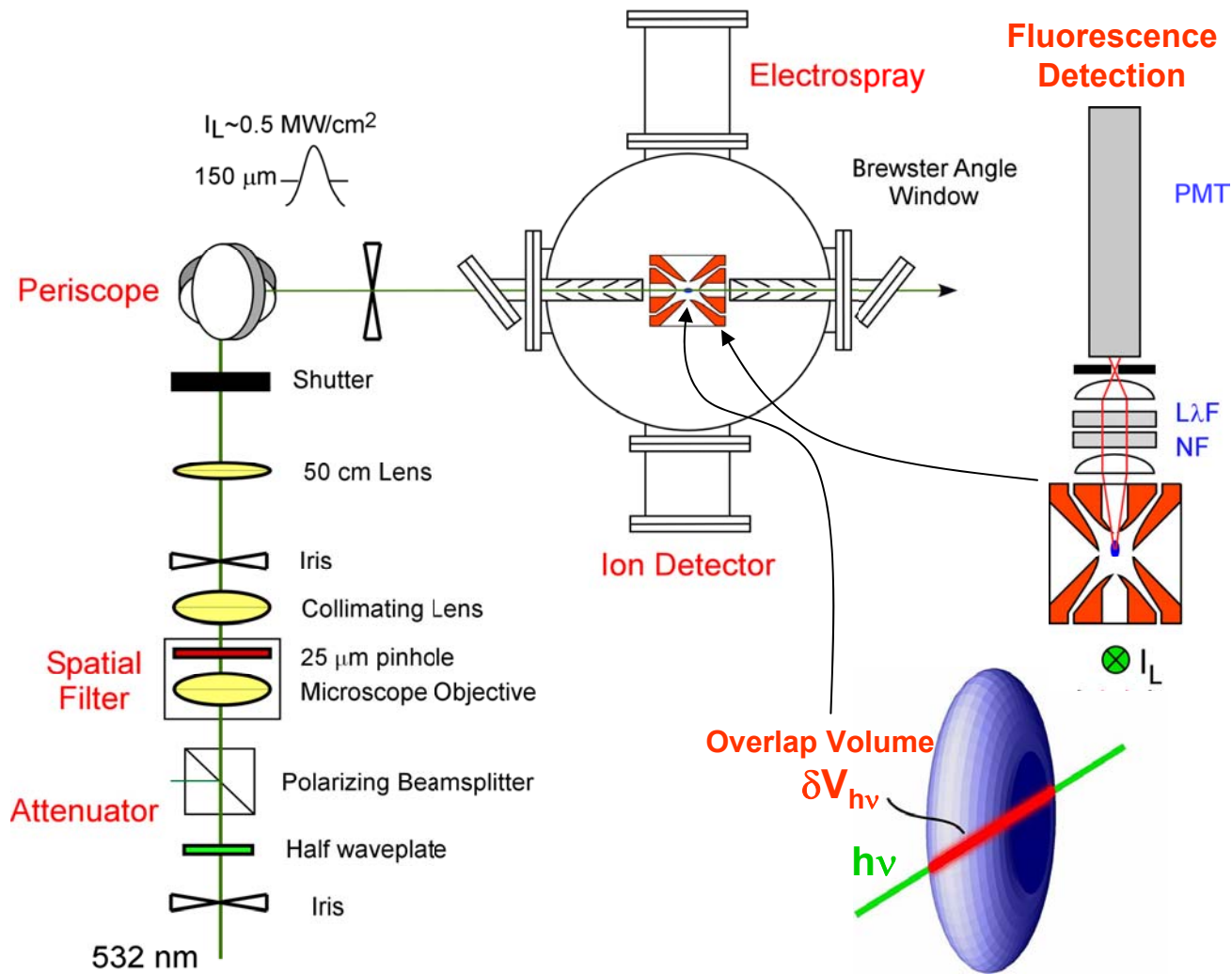
EXPERIMENTAL

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 $\sim 170^{\circ}\text{C}$ with 1°C precision

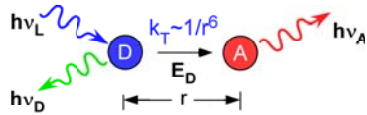
Fluorescence

- Pulsed Nd-YAG laser (Molelectron)
- Photomultiplier tube detector (Hamamatsu)
- Donor fluorescence detected (bandpass 535 to 580 nm)



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



Schematic of FRET

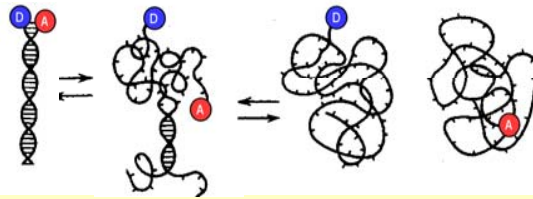


Illustration of duplex fraying before complete dissociation

BODIPY-TMR-5' -AATTAATCCGGCCG-3'
BODIPY-TR-3' -TTAATTAGGCCGGC-5'

BODIPY-TR-5' -AAAAAAGGCCGGC-3'
BODIPY-TMR-3' -TTTTTTTCCGGCCG-5'

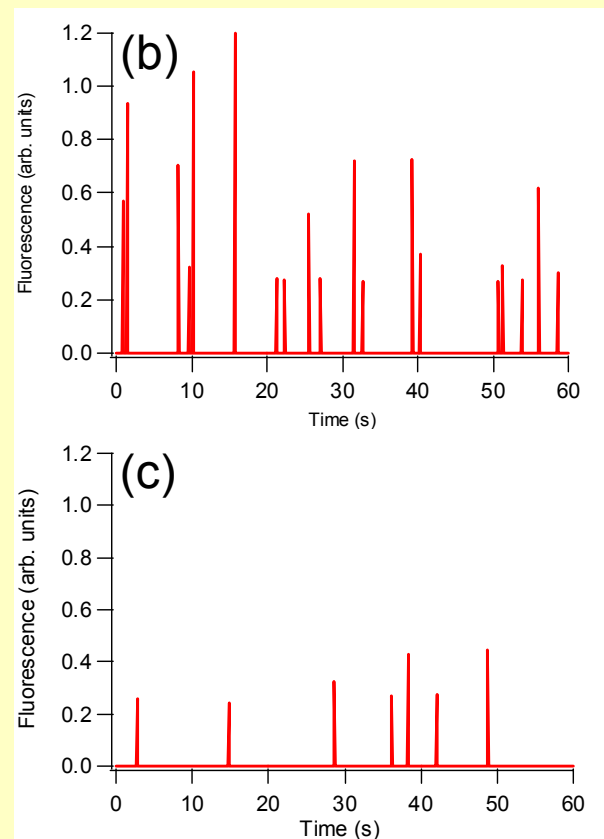
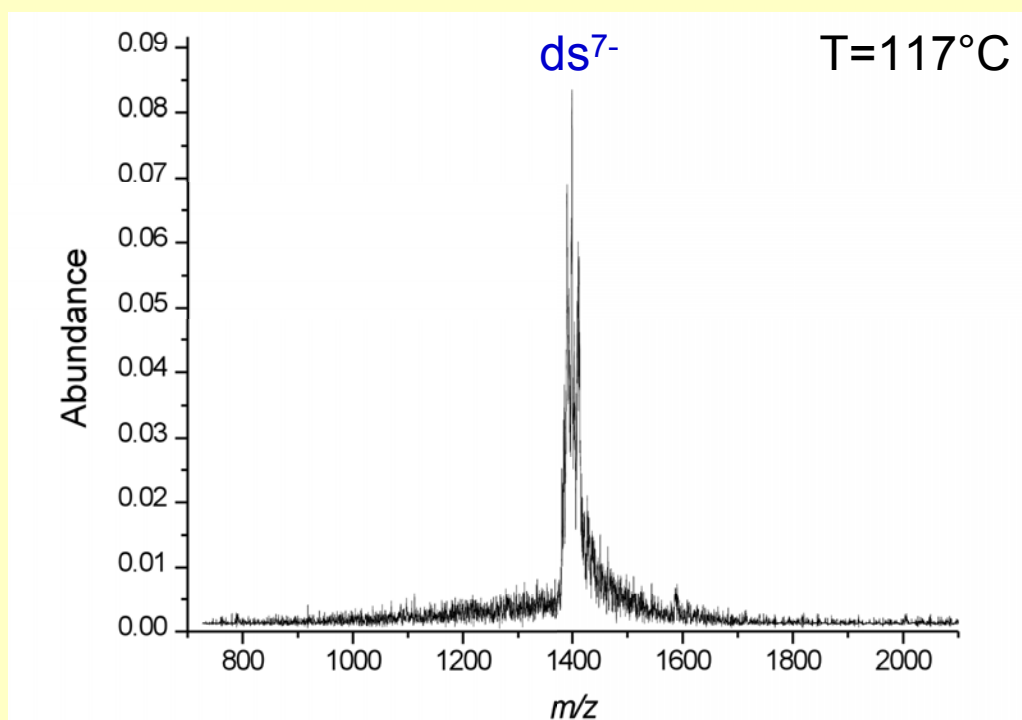
Duplex sequences chosen

- 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

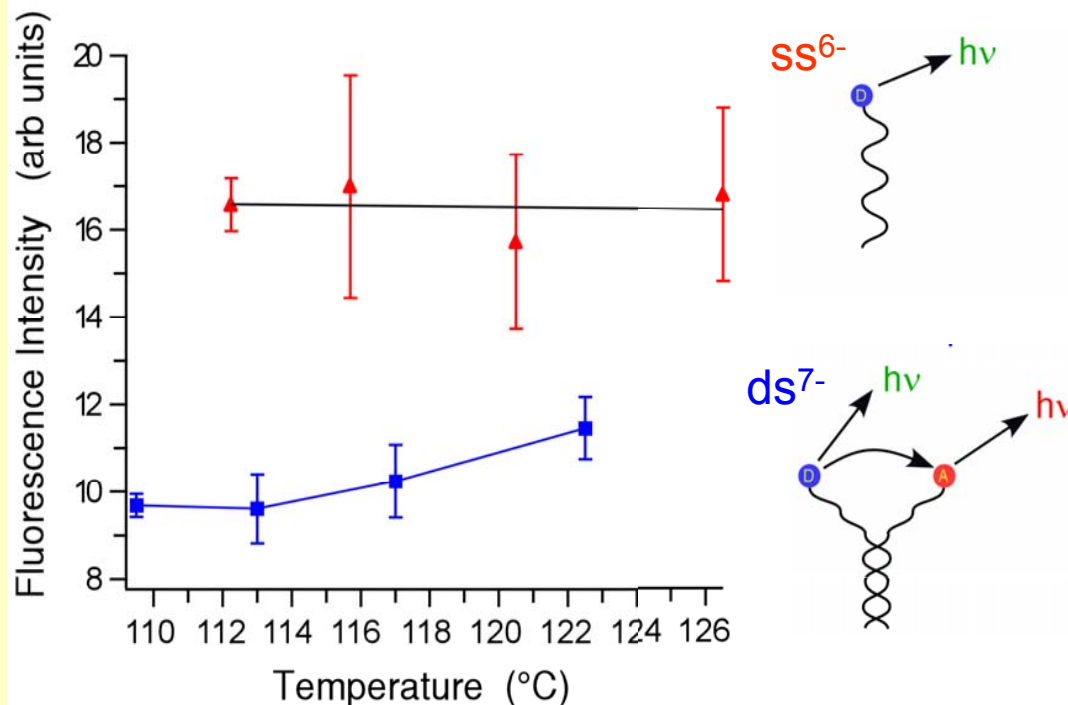
Study of BODIPY-TMR-5'-AATTAATCCGGCCG-3' and 5'-CGGCCGGATTAATT-3'-BODIPY-TR

- Isolated 7- charge state of duplex (ds^{7-})
- 60 s irradiation period, 1 μ J pulse energy



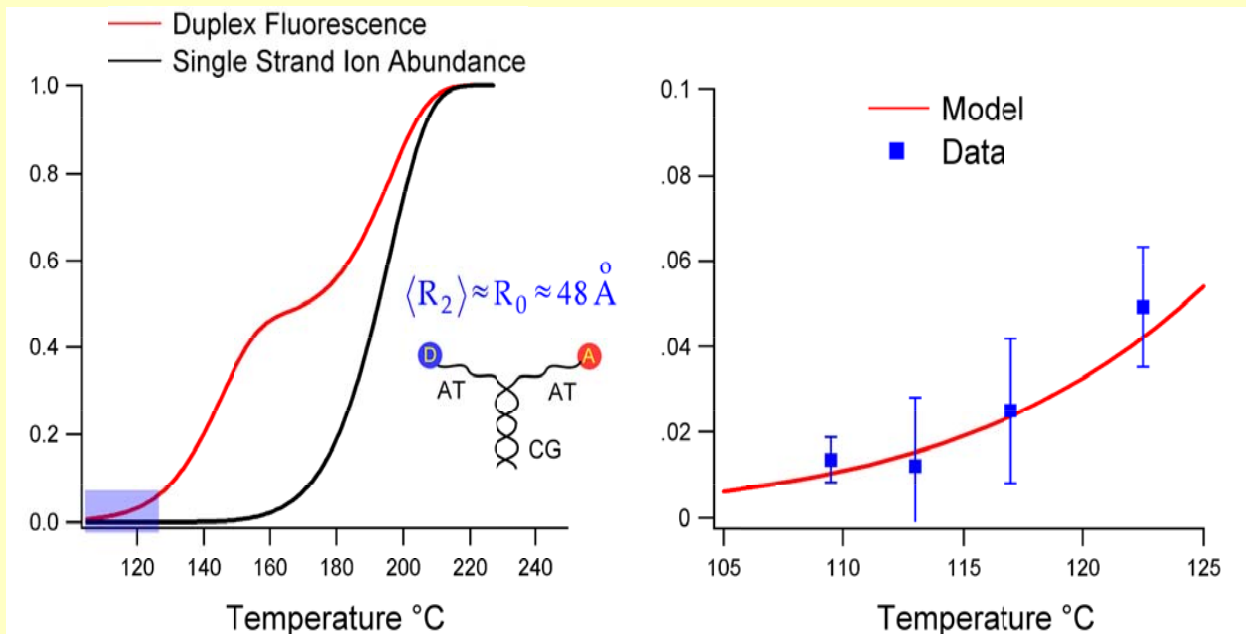
(a) Single scan mass spectrum and (b) single scan donor fluorescence spectrum from isolated (ds)⁷⁻ ion; 60 s irradiation, pulse energy 1.0 μ J.
(c) Representative spectrum of background PMT signal (no ions loaded).

- Temperature varied from 109 – 123°C
 - Above 130°C, fragmentation of ds⁷⁻ occurred in 60 s time period
 - Fragmentation primarily base loss
- Donor fluorescence from ds⁷⁻ compared to donor fluorescence from ss⁶⁻ (BODIPY-TMR-5'-AATTAATCCGGCCG-3')
- Increase in fluorescence from ds⁷⁻ 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 ds⁷⁻ and ss⁶⁻.

- 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 $\sim 48\text{\AA}$ (2x length of 7 base pair A·T)
 - Assumes Förster distance of $\sim 50\text{\AA}$ 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

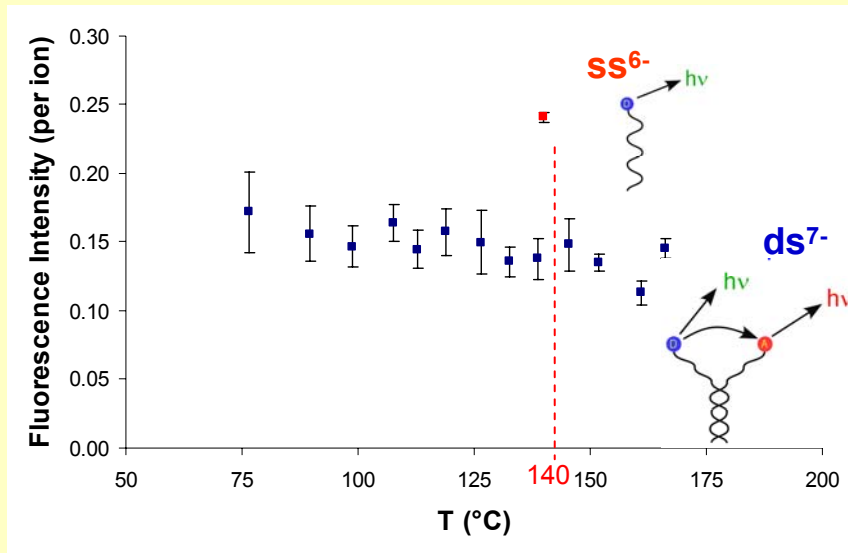


(a) Model of donor fluorescence and single strand abundance during dissociation of 14-mer duplex (parameters in text). (b) comparison of experimental (squares) and theoretical (solid line) data.

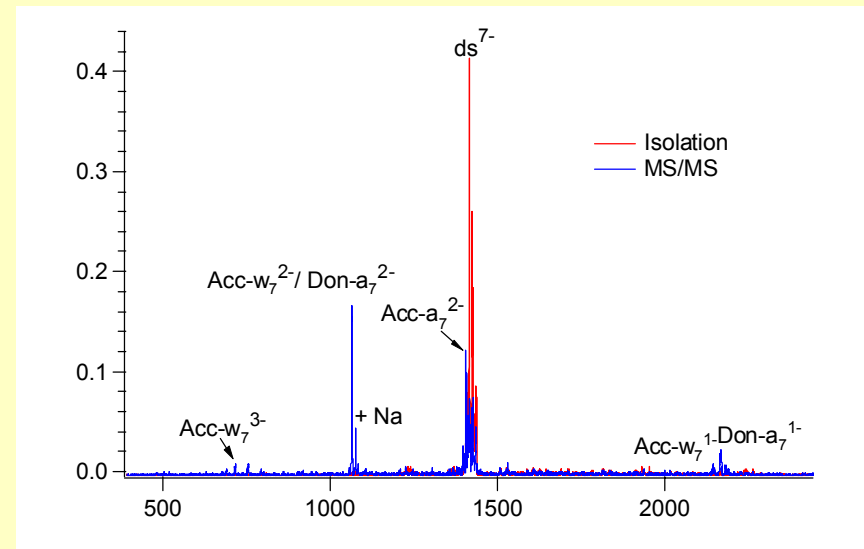
Study of BODIPY-TR-5'-AAAAAAGGCCGGC-3' and 5'-GCCGGCCTTTTTTT-3'-BODIPY-TMR

- Isolated 7- charge state of duplex (ds^{7-})
- 90 s irradiation period, 0.7 μ J pulse energy
- Temperature varied from 75 – 160°C
 - Above 140°C, fragmentation of ds^{7-} occurred in 90 s time period
 - Fragmentation primarily cleavage between A·T and G·C portions of strands
- Donor fluorescence from ds^{7-} compared to donor fluorescence from ss^{6-} (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 $\sim 55\text{\AA}$
 - 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



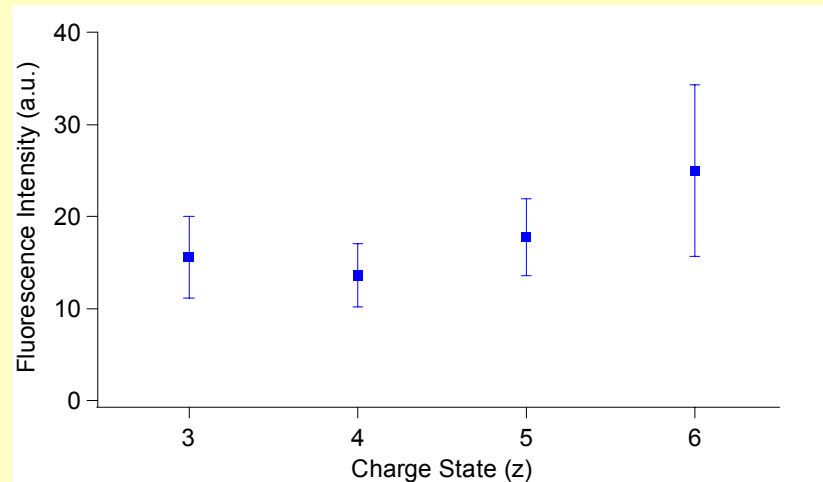
Fluorescence intensities from ds^{7-} and ss^{6-} .



Isolation and thermal dissociation spectra of ds^{7-} ; 20 min. at 160°C .

Preliminary experiments with BODIPY-TMR-5'-TTTTTTTTTTTTTTT-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 T₁₄-

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

- [1] Khoury, J. T.; Rodriguez-Cruz, S. E.; Parks, J. H. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 696-708.
- [2] Schnier, P. D.; Klassen, J. S.; Strittmatter, E. F.; Williams, E. R. *J. Am. Chem. Soc.* **1998**, *120*, 9605-9613.
- [3] Danell, A. S.; Parks, J. H. *Int. J. Mass Spectrom.* **2003**, *in press*.
- [4] Parkhurst, K. M.; Parkhurst, L. J. *Biochemistry.* **1995**, *34*, 293-300.
- [5] Electron autodetachment, $M^{n-} \rightarrow M^{(n-1)-}$, of oligonucleotides has been observed recently in our trap; presented MPR#342.