Electron Autodetachment and Fluorescence Measurements of Trapped Oligonucleotides

Allison S. Danell and Joel H. Parks

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

Presented at the 52nd Annual ASMS Conference on Mass Spectrometry Nashville, 2004

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

INTRODUCTION

Oligonucleotide anions have been studied in an ion trap mass spectrometer by observing fluorescence resonance energy transfer (FRET) and electron autodetachment.

- Both methods are being used to try to understand more about ionic structures and conformational features of oligonucleotides.

EXPERIMENTAL

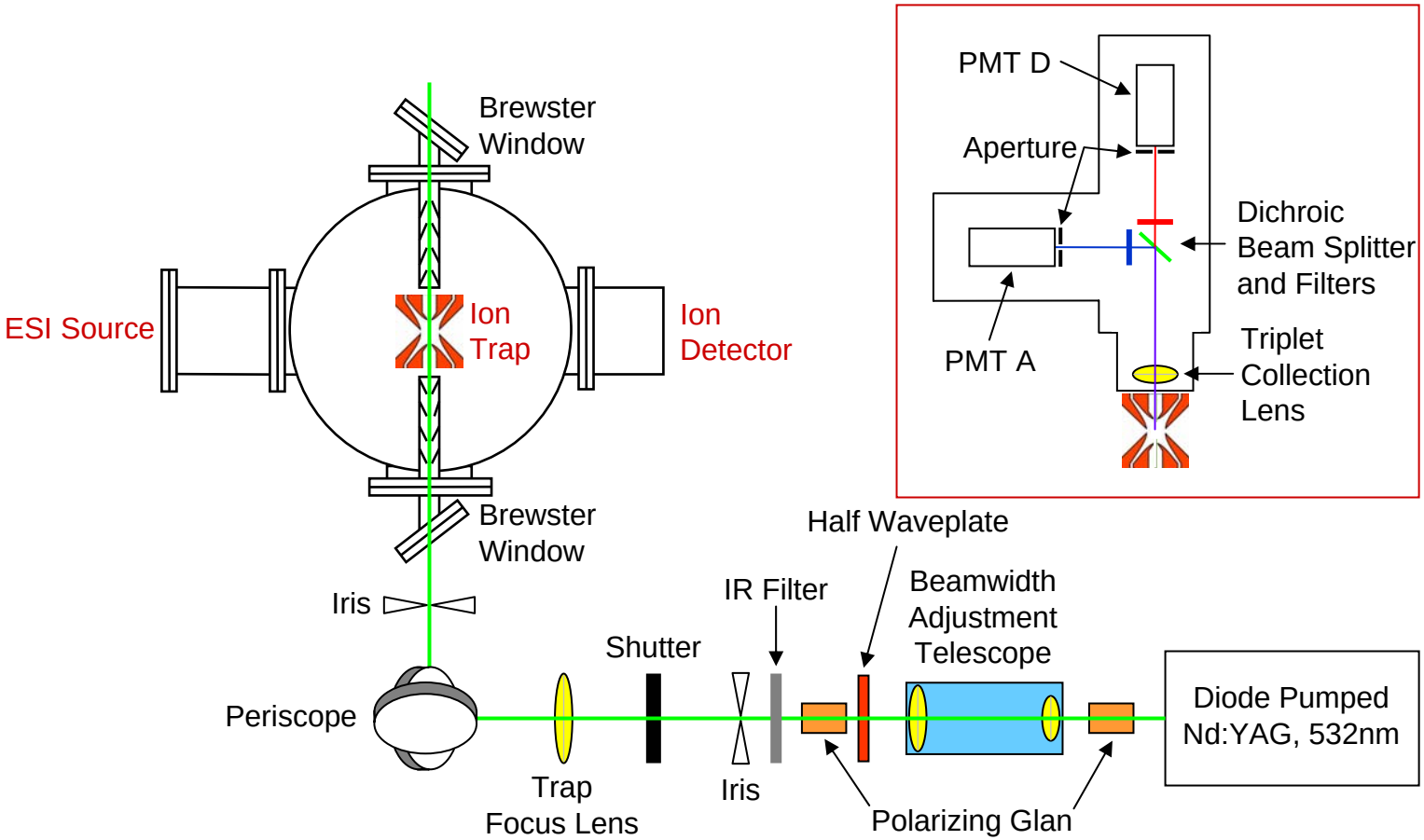
Mass Spectrometry

- 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

- 532 nm cw Nd:YAG laser (Spectra Physics)
- Two-channel detection system (GaAs photomultipliers, Hamamatsu)
- Dye filters (Chroma) isolate fluorescence bandwidths of each fluorophore
- Counts recorded on counter board (NI) operating with 50 nsec resolution

EXPERIMENTAL



Instrument Diagram

EXPERIMENTAL

Nonradiative Analytes

- Oligonucleotides commercially synthesized (Synthegen)
- Desalted prior to shipment

Radiative Analytes

- Oligonucleotides commercially synthesized and derivatized with fluorophores (Synthegen)
- BODIPY® fluorophores (Molecular Probes)
  - Analogs of Tetramethylrhodamine (TMR) and Texas Red (TR)
- RP-HPLC purification prior to shipment

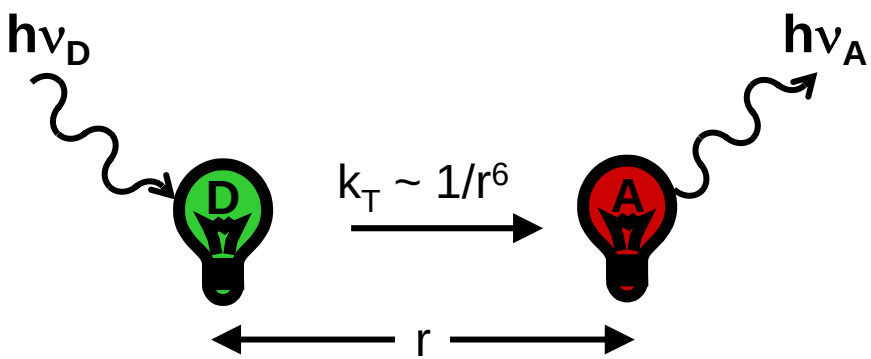
Procedure

- nESI solutions 15 μM in 70/20/10 methanol/water/trifluoroethanol *or* 15 μM in 80/20 acetonitrile/water/2.5 M acetic acid/25mM imidazole
  - Complementary single strands first annealed at 100μM in 100 mM ammonium acetate at 90°C for 8 min.
- Ions of interest isolated with either
  - rf ramping and single frequency resonant ejection
  - rf ramping and SWIFT ejection
- For fluorescence experiments
  - Isolated ions irradiated 100 ms at $q_z = 0.50$
  - Beam FWHM ~220μm, power 10 mW
  - 2.5 V dc bias applied to ring electrode to center ion cloud on beam

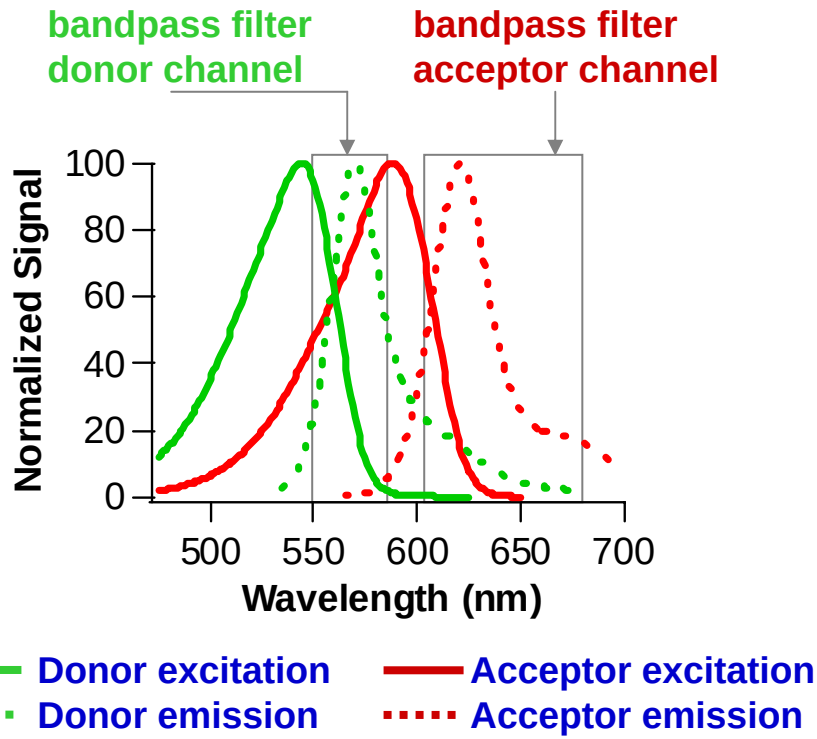
FRET

Background

- Excited **donor** fluorophore transfers energy to **acceptor** fluorophore
- Donor emission and acceptor absorption curves must overlap
- Method used to determine distances between donor and acceptor fluorophores [2]
  - Acceptor emission observed only if donor within close proximity



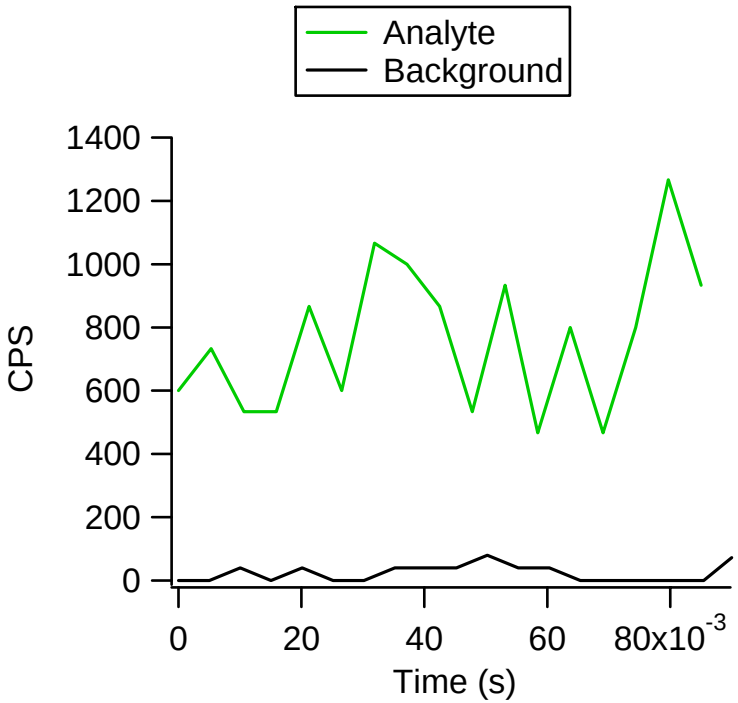
Schematic of FRET



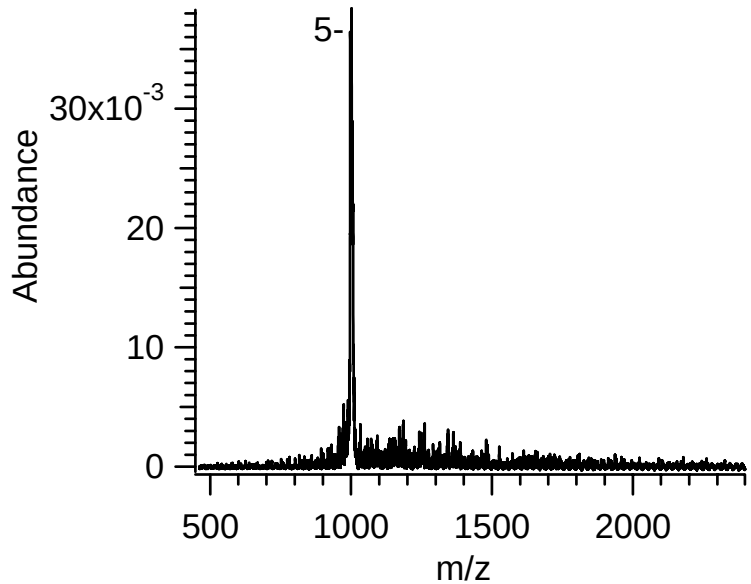
FRET

Initial analysis of labeled oligonucleotides

- Fluorescence and mass spectra of TMR-labeled oligonucleotide shown below
- Mass spectra acquired immediately after irradiation with laser



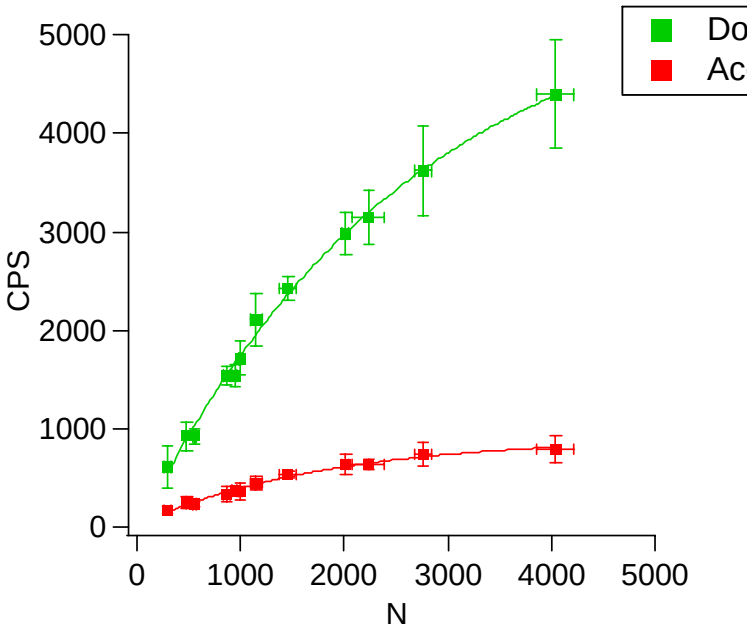
Fluorescence from TMR-AATTAATCCGGCCG following isolation of z = -5 ion



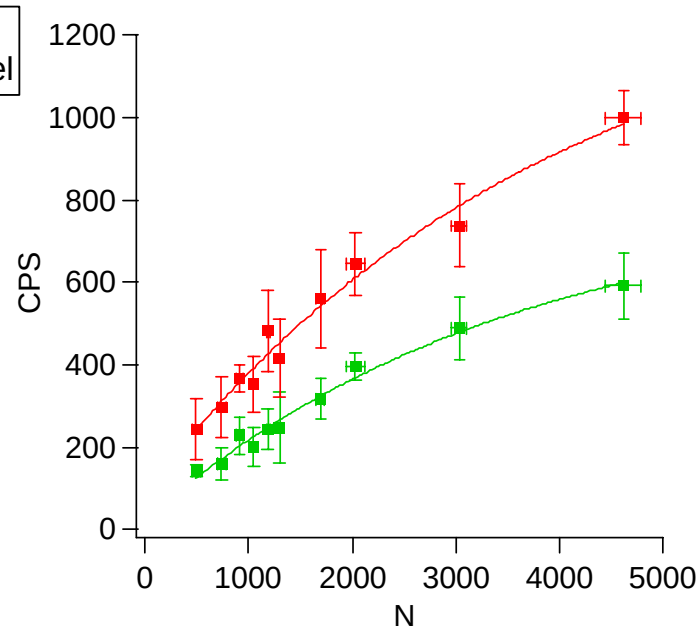
MS of TMR-AATTAATCCGGCCG following isolation and irradiation of z = -5 ion

FRET

- Fluorescence of donor- and acceptor-labeled oligonucleotides measured as a function of number of ions (N)
- Fluorescence CPS increased nonlinearly, as expected due to space charging in the ion trap [1]
  - Exponential curves fit to data
- Excitation wavelength excites acceptor fluorophore
  - Acceptor emission detected on donor detection channel



TMR-AATTAATCCGGCCG (z = -5)



CGGCCGGATTAATT-TR (z = -5)

FRET

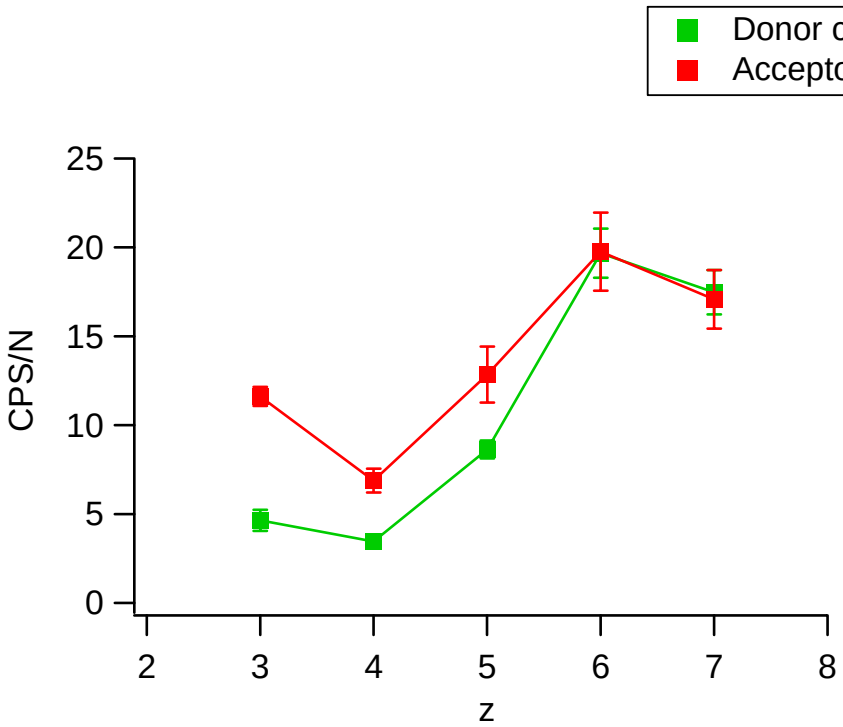
TMR-TTTTTTTTTTTTTT-TR as a function of charge

- *Hypothesis:*
  - Increasing number of charges on TMR-T_{14}-TR will cause:
    - TMR-T_{14}-TR to adopt elongated structure to reduce Coulombic interactions
    - Distance between donor and acceptor to increase
    - Donor fluorescence to increase and acceptor fluorescence to decrease
- *Observed (data next panel):*
  - Donor fluorescence increased as charge increased
  - Acceptor fluorescence increased also

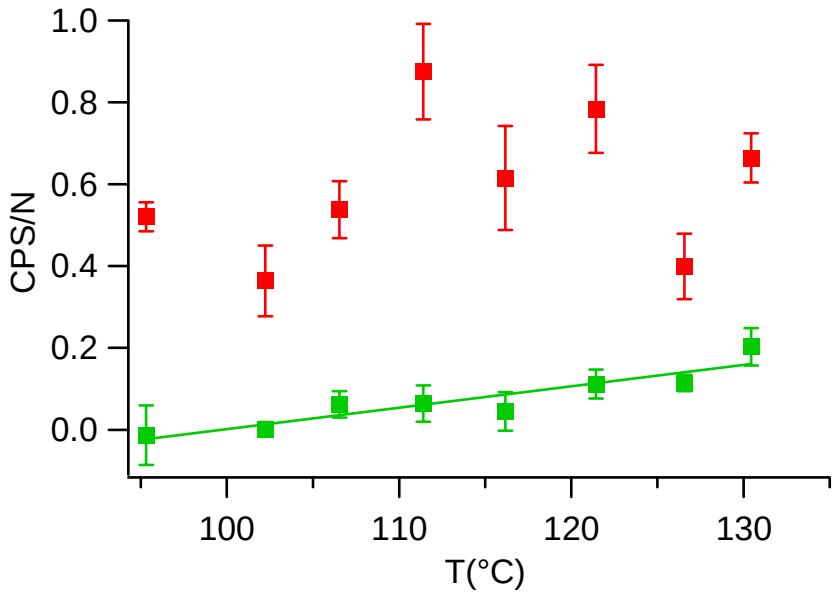
Double-stranded TMR-AATTAATCCGGCCG/CGGCCGGATTAATT-TR as a function of temperature

- *Hypothesis:*
  - Increasing temperature in ion trap will cause:
    - Double-stranded species to fray at A/T end
    - Distance between donor and acceptor will increase
    - Donor fluorescence to increase and acceptor fluorescence to decrease
- *Observed (data next panel):*
  - Donor fluorescence increased as temperature increased
  - Acceptor fluorescence was scattered over range of temperature

FRET



TMR-T_{14}-TR



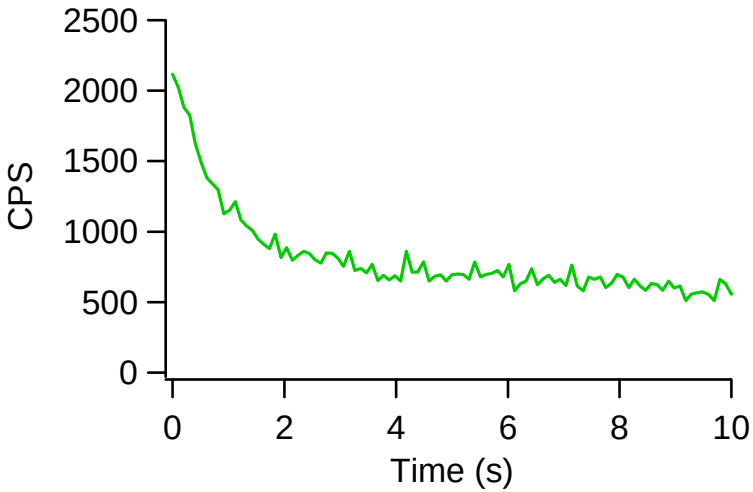
ds^{7-} of TMR-AATTAATCCGGCCG and CGGCCGGATTAATT-TR

FRET

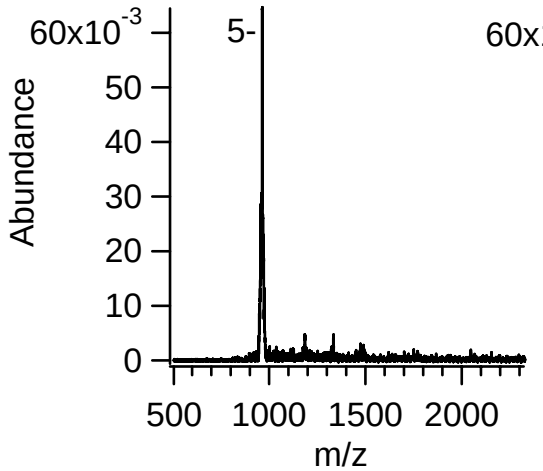
Discussion of FRET results

- FRET process is occurring
  - Acceptor fluorescence intensities indicate energy transfer from excited donor
    - Reported acceptor fluorescence intensities corrected for direct excitation of acceptor from laser
- Gas phase absorption/emission behavior unknown
  - Laser excitation of acceptor indicates blue-shifted absorption curve
  - Detection of acceptor fluorescence on donor detector channel indicates blue-shifted emission curve
- Alternative channels to FRET exist for energy release from excited donor
  - Electron autodetachment [3, *ibid.*] is at least one alternate channel
    - Observable in mass spectra
    - Product ions do not fluoresce (data next panel)
  - Intramolecular charge transfer could be another channel
    - Excited donor fluorophore could transfer charge to oligonucleotide
    - If fluorophore structure altered, fluorescence behavior unknown

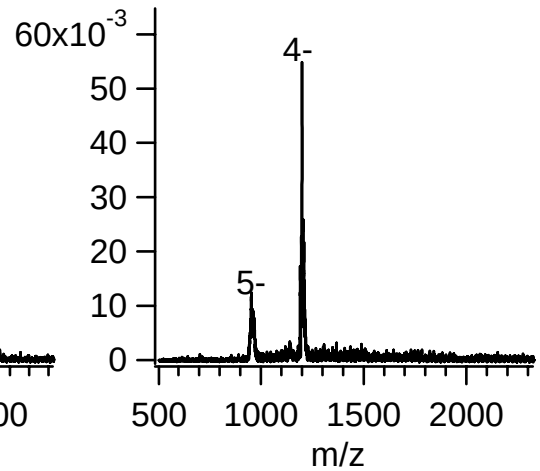
FRET



10 s irradiation of $(TMR\text{-}T_{14})^{5-}$



MS of $(TMR\text{-}T_{14})^{5-}$ at t = 0 s



MS of $(TMR\text{-}T_{14})^{5-}$ at t = 5 s

Electron Autodetachment

Background

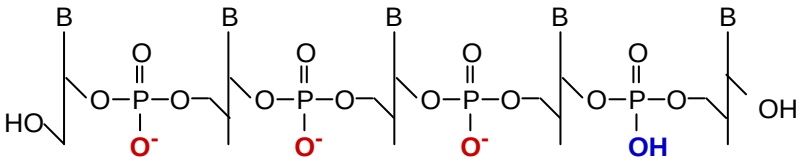
- Electron autodetachment from multiply-charged oligonucleotide anions has been observed in an ion trap [3]
  - Observe $M^{n-} \rightarrow M^{(n-1)-}$
  - Total ion population conserved
  - Rates increase with temperature and with charge state
  - Rates vary as a function of base sequence

Electron Autodetachment

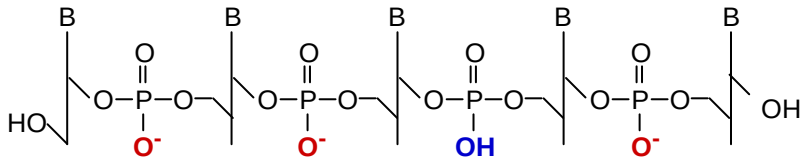
AAAAA as a function of charge

- *Hypothesis:*
  - Can distribute negative charges in one way for $(AAAAA)^{4-}$ and two for $(AAAAA)^{3-}$
    - Negative charges believed to reside on phosphate groups
  - Electrons will autodetach at one rate for $(AAAAA)^{4-}$ and two rates for $(AAAAA)^{3-}$
- *Observed (data next panel):*
  - $(AAAAA)^{4-}$ electron autodetachment data best fit by single exponential
  - $(AAAAA)^{3-}$ electron autodetachment data best fit by double exponential
- Fits support hypothesis that one population of $(AAAAA)^{4-}$ exists and two populations of $(AAAAA)^{3-}$ exist
- Propose $(AAAAA)^{3-}$ structure (a) shown above corresponds to "fast" rate population
  - Closer proximity of three charges → larger Coulombic interaction

(a)

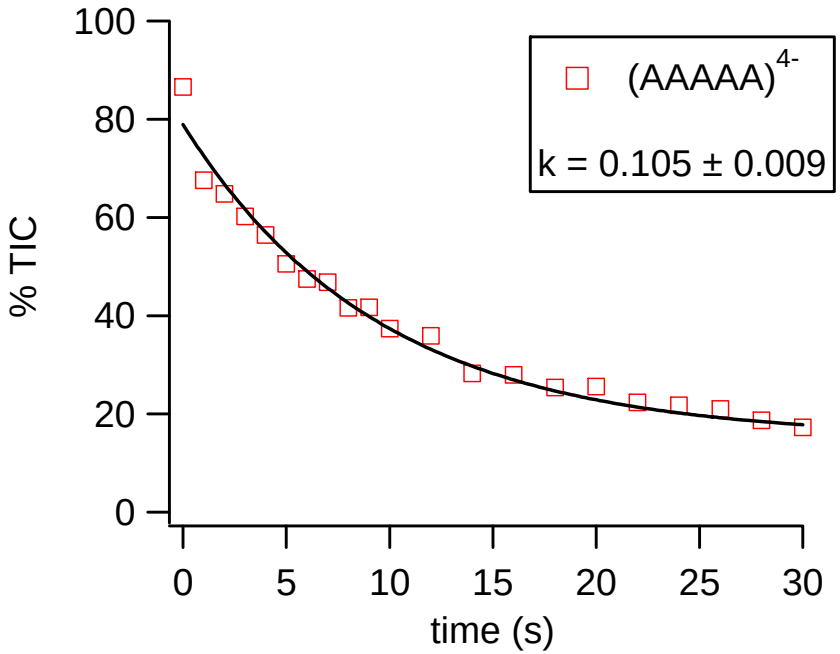


(b)

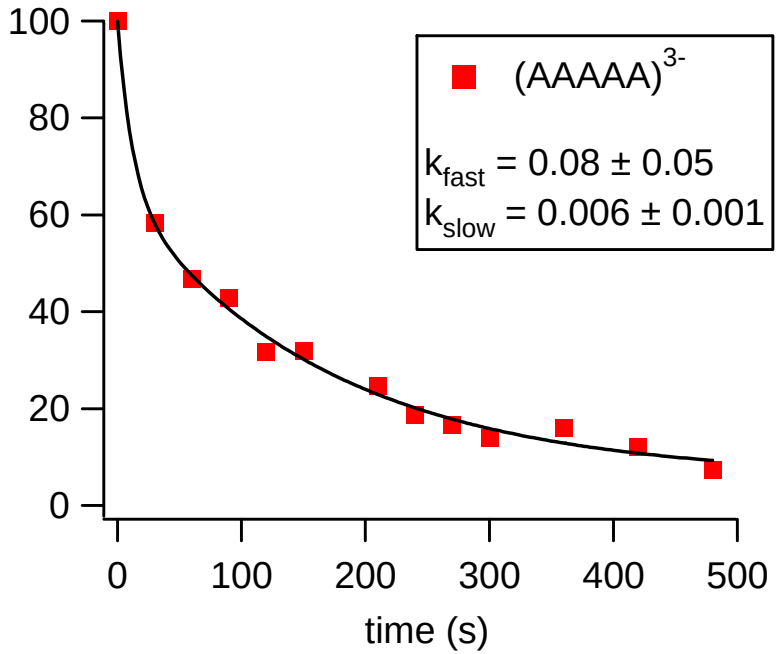


Two possible distributions of three negative charges on synthetic 5-mer oligonucleotide

Electron Autodetachment



$(AAAAA)^{4-} \rightarrow (AAAAA)^{3-}$
$q_z(4-) = 0.50$ at T = 45°C



$(AAAAA)^{3-} \rightarrow (AAAAA)^{2-}$
$q_z(3-) = 0.38$ at T = 45°C

Summary

Fluorescence Resonance Energy Transfer

- FRET observed with two-channel photon detection in a dual-labeled oligonucleotide and a duplex of singly-labeled oligonucleotides
  - Absorption and emission behavior of fluorophores differ from condensed phase
  - Complexity of energy distributions may prevent efficient energy transfer to acceptor
- Future Work
  - Measurement of gas phase absorption/emission spectra
  - Different bandpass filters
  - Minimize electron autodetachment during fluorescence

Electron Autodetachment

- Rates indicate two populations exist of 5-mers with z = 3
  - Molecular modeling collaborators investigating structures with electrons localized on phosphate groups or mobile along backbone

References

[1] Khoury, J. T.; Rodriguez-Cruz, S. E.; Parks, J. H. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 696-708.

[2] Selvin, P. R. *Nature Struct. Biol.* **2000**, *7*, 730-734.

[3] Danell, A. S.; Parks, J. H. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1330-1339.