Combining FRET and Mass Spectrometry to Study the Dynamics of Double-stranded Oligonucleotide Anions

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Overview

- Fluorescence Measurements
  - Trapped fluorophore ions
  - FRET
  - Fluorescence of trapped oligonucleotides

- Progress towards study of dissociation of DNA duplexes with FRET and MS
  - Intermediate state model
  - Duplex fluorescence/MS data

- Conclusions and future work
Biopolymer Dynamics in Gas Phase

- Observe dynamics in the absence of bulk water
  - Electrostatics
  - Hydrodynamic effects

- Correlating changes in fluorescence intensity of trapped biopolymer ions with changes in structure

- Dependence on:
  - Temperature
  - Level of Hydration
  - Charge State
  - Background Denaturants
Gas phase analyses of DNA duplexes

Electrospray ionization of DNA duplexes

- Doktycz, M. J., Habibi-Goudarzi, S., McLuckey, S. A.
  Anal. Chem. 1994, 66, 3416-3422
- Gale, D. C., Goodlett, D. R., Light-Wahl, K. J., Smith, R. D.

BIRD © dissociation rates of duplexes to single strands

- Schnier, P. D., Klassen, J. S., Strittmatter, E. F., Williams, E. R.
  J. Am. Chem. Soc. 1998, 120, 9605-9613

CID © dissociation may be multistep process

- Gableica, V., De Pauw, E.
Dissociation of DNA duplexes

- Heat trapped duplexes to induce dissociation

Mass Spectrometry

Schnier, P. D., Klassen, J. S., Strittmatter, E. F., Williams, E. R.
J. Am. Chem. Soc. 1998, 120, 9605-9613
Dissociation of DNA duplexes

- Modify single strands with fluorophores
- Heat trapped duplexes to induce dissociation
- Monitor changes in fluorescence

Fluorescence  Mass Spectrometry
Fluorescence Apparatus

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

150 $\mu$m beam width

$2\omega/3\omega$

Nd:YAG Laser

$4\omega$

$20 \text{ ns}$ pulse width

$\Delta \Omega/4\pi \sim 5 \times 10^{-3}$

Detection Optics

PMT

L$\lambda F$

NF

$|L|$
Laser - Ion Cloud Geometry

\[ I \sim N_{hv} = n_{ion} \delta v_{hv} \]

\[ \frac{\delta v_{hn}}{v_{cloud}} \sim 0.03 - 0.07 \]
Laser-Induced Fluorescence of Trapped Ions

- Minimize scatter **during** excitation to achieve zero background
- Necessary for short fluorescent lifetimes (~2-5 ns)
  
  Enables:
  
  - Large dynamic range for threshold studies
  - Small N (~10-50) for FCS

Space Charge Limited Fluorescence

Fluorescence Intensity (arb. units)

N Stored Ion Number

q_z = 0.61 140K
q_z = 0.61 300K
q_z = 0.38 140K
q_z = 0.38 300K
Fluorescence Analysis of Biopolymer Dynamics

- Use fluorescence measurements to monitor conformational changes of biopolymers in gas phase

- Fluorescence Resonance Energy Transfer
  - Correlate changes in fluorescence intensity with changes in average conformation

![Diagram](image-url)
FRET Pair

BODIPY TMR

BODIPY TR

Normalized Signal vs. Wavelength/nm
Intermediate State Model

Model Sequence: AATTAATCCGGCCG

$K_{12}$ $K_{23}$

~ Two-State Transition
Effect of \(<R>\) on Donor Fluorescence

\[<R>/R_0 = 0.61\]

\[<R>/R_0 = 1.0\]

\[<R>/R_0 = 0.84\]

\[<R>/R_0 = 1.35\]
Nanospray Mass Spectrum

TR - AATTAATCCGGCCG
TMR - TTAATTAGGCCGGC

60/20/20 Acetonitrile/Water/Isopropanol
Isolated Duplex Fluorescence

T = 117°C

~400 ions in δν_{hv}
Threshold Fluorescence: D-7 Duplex

![Graph showing fluorescence intensity vs. temperature](image)

- **SS-Donor**
- **D-7 Duplex**
Summary/Conclusions

- Fluorescence Measurements of trapped biopolymers provide sufficient sensitivity for dynamics studies.

- FRET fluorescence correlated with mass spectrometry shows promise as a probe of the intermediate states of DNA duplexes.
Research Plans

- Fluorescence Measurements
  - DNA duplex intermediate states
  - Bubbles in ~30 complementary pairs
  - Hairpin closure rates
  - DNA non-covalent complexes

- Correlation Fluorescence Spectroscopy
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