Dynamics of Trapped Oligonucleotides

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Outline

• Fluorescence of Gas Phase Biopolymers
  – FRET fluorescence and mass spectrometry measurements of trapped oligonucleotides
  – DNA duplex dissociation: intermediate states

• Sequential Charge Loss in Oligonucleotides
  – Charge-state dependence
  – Sequence dependence

• Conclusions and future Work
Fluorescence Analysis of Trapped Biopolymers

• Use fluorescence measurements to monitor conformational changes

• Fluorescence Resonance Energy Transfer
  – Correlate changes in fluorescence intensity with changes in average conformation
Electrospray Ionization Ion Trap Mass Spectrometer
Illumination and Detection Optics

- Periscope
- Shutter
- 150 μm
- 50 cm Lens
- Iris
- Collimating Lens
- 25 μm pinhole
- Microscope Objective
- Polarizing Beamsplitter
- Half waveplate
- Iris
- Brewster Angle Window
- Ion Detector
- Electrospray
- PMT
- L λF NF
- 532 nm
- $I_L \sim 0.5 \text{ MW/cm}^2$
Ion Cloud Fluorescence

Overlap Volume

\[ \delta V_{hv} \]

\[ N_{hv} = \int n(x, y, z) \exp\left(-\frac{x^2 + z^2}{a^2}\right) dV \]

\[ \frac{\delta V_{hv}}{V_{cloud}} \sim .03 - .15 \]

\[ P_{hv} \propto N_{hv} \]
FRET Pair

BODIPY TMR

BODIPY TR

Normalized Signal

Wavelength/nm

450 500 550 600 650 700

0 20 40 60 80 100
BODIPY Solvent Dependence

**BODIPY-TMR**
- T~20°C
- Normalized Intensity vs Wavelength (nm)
- **abs** and **em**

- Methanol (solid line)
- Hexane (dotted line)

**BODIPY-TR**
- T~20°C
- Normalized Intensity vs Wavelength (nm)
- **abs** and **em**

- Methanol (solid line)
- Pentane (dotted line)
BODIPY Temperature Dependence

BODIPY-TMR
Methanol
- $T = 19.7^\circ C$
- $T = 58.6^\circ C$

BODIPY-TR
Methanol
- $T = 19.8^\circ C$
- $T = 59.6^\circ C$
Fluorescence

0.9 μJ

~ 350 ions in δVhv

(5′-AAAAGCAAA-3′-BODIPY-TMR)⁴⁻

T=25°C
Fluorescence vs. $N_{\text{ions}}$

(BODIPY-TMR-5'-AATTAATCCGGCCG-3')$^6$-
Dissociation of DNA Duplexes

- Thermal Dissociation Rates → Watson-Crick Pairing
  - Schnier, P. D., Klassen, J. S., Strittmatter, E. F., Williams, E. R.

- Collision-induced Dissociation Rates → multistep process
  - Gabelica, V., De Pauw, E.
Intermediate States: Fraying/Bubble Dynamics

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

Fluorescence Mass Spectrometry
Dissociation Intermediate States

Model 14-mer sequence

D₅'TTAATTAGGCCGCGC₃'
A₃'AATTAATCCGCGCCG₅'

K₁₂(T)  K₂₃(T)

~ Two-State Transition

| 1⟩  | 2⟩  | 3⟩

K₁₂  K₂₁  K₂₃
Effect of $<R>$ on Donor Fluorescence

Donor Fluorescence

$\frac{<R_2>}{R_0} = 0.61$

Mass Spectrum Intensity of ss

$\frac{<R_2>}{R_0} = 1.0$

$\frac{<R_2>}{R_0} = 0.84$

$\frac{<R_2>}{R_0} = 1.35$
Nanospray Mass Spectrum

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

60/20/20 Acetonitrile/water/isopropanol

Solution Absorbance

\( \lambda = 260 \text{ nm} \)
Isolated 14-mer Duplex Fluorescence

(ds)$^7$-

$T=117$°C
60 s

$\sim 400$ ions in $\delta V_{hv}$
Threshold Fluorescence

![Graph showing the relationship between temperature and fluorescence intensity.]
Intermediate State Model: Data Comparison

- Duplex Fluorescence
- Single Strand Ion Abundance

\[ \langle R_2 \rangle \approx R_0 \approx 48 \text{ Å} \]
Nanospray Mass Spectrum

BODIPY-TR-5'-AAAAAAAGGCCGGC-3'
BODIPY-TMR-3'-TTTTTTTCCGGGCCG-5'

70/20/10 Methanol/Water/Trifluoroethanol
Duplex Fluorescence

Fluorescence Intensity (per ion)

0.7 µJ
90 s

T (°C)

SS-Donor

DS-7 Duplex
20 min.
T 160°C
$q_z (ds^7^-)$ 0.27

Acc-$a_7^2$ - Don-$a_7^2$

Acc-$w_7^2$ - Don-$w_7^2$

Acc-$w_7^3$ + Na

Don-$w_7^2$ + Na

Acc-$w_7^1$ - Don-$a_7^1$

BODIPY-TR-5'-AAAAAAAGGCCGGC-3'

BODIPY-TMR-3'-TTTTTTTCGGCCG-5'

Dissociation conformation

FRET

MS/MS

Isolation

MS/MS
Observation of Charge Loss

- No indication of heavy ion fragmentation
- No pressure dependence
- Constant total ion number (MS, fluorescence)
Sequential Charge Loss Model

$\text{(T}_7\text{)}^{4-} \rightarrow \text{(T}_7\text{)}^{3-} \rightarrow \text{(T}_7\text{)}^{2-}$

**Loss Rates**

$T = 123 \, ^\circ\text{C}$

$k_{4-3} = 1.41(\pm0.03)x10^{-3} \, (\text{s}^{-1})$

$k_{3-2} = 0.60(\pm0.05)x10^{-3} \, (\text{s}^{-1})$

$k_2 = 0.59(\pm0.03)x10^{-3} \, (\text{s}^{-1})$
(T_7)^n - Temperature Dependence

\[ \ln(k) \] vs. \[ T \text{ (°C)} \]

- \[ k_{43} \] (blue line)
- \[ k_{32} \] (red line)
Multi-Charged Anions: Repulsive Coulomb Barriers

Stable Anions

\[(\text{PtCl}_4)^{2-}\]

Metastable Anions

\[[\text{CuPc(SO}_3)_4]^{4-}\]

\[k_{\text{Detachment}} = F(b_n, e_n)\]

Sequence Dependent Charge Loss

$3^- \rightarrow 2^-$

$T = 102^\circ\text{C}$

$\text{(T}_7\text{)}^3^- \quad \text{(A}_7\text{)}^3^- \quad \text{(ATATATA)}^3^-$

$k_{32} = 3.6 (\pm 0.1) \times 10^{-4}$

$k_{32} = 19.2 (\pm 0.07) \times 10^{-4}$

$k_{32} = 5.6 (\pm 0.3) \times 10^{-4}$
Base Stacking

Base-Base Interactions:
- Electrostatic: Polar Bonds
- Charge Induced Moments
- Van der Waals

Solution Calculation
\[ \Delta G_{\text{stack}} \text{(kcal/mole)} \]

- AA  -0.8
- AU  -0.5
- TT  -0.4

Florian et al.

Weiner et al.
MD Simulation: $C_7^{3-}$

300 K, 2 ns
Temperature Dependence

\[ \ln(k_{32}) \]

vs.

\[ T \ (°C) \]

Graph showing the temperature dependence of \( \ln(k_{32}) \) with different data points and trend lines for different samples.
Charge Loss Model

sequence of repulsive coulomb barriers

Relative Rates: \( \{ e_{4-3} > e_{3-2} > e_{2-1}, b_{4-3} > b_{3-2} > b_{2-1} \} \rightarrow k_{4-3} > k_{3-2} > k_{2-1} \)

Absolute Rates: at 300K \( E_v \sim 18.75 \text{ eV} \gg b_{4-3}, b_{3-2}, b_{2-1} \)

?? \( K_{RRKM}(E_v, b_{i \downarrow}) \) relative to \( k_{4-3}, k_{3-2}, k_{2-1} \)
Research Plans

• FRET Fluorescence Measurements
  – DNA duplex intermediate states
  – DNA hairpin closure rates
  – Alpha helix stability
  – Trp-cage unfolding

• Oligonucleotide Charge Loss
  – Mechanism
  – Sequence sensitivity
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.

• Sequential charge loss in oligonucleotides exhibits a strong dependence on sequence → base stacking.