Ion Mobility as a Probe for Molecular Structure & Oligomer States in Biological Assemblies

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Organization

1. Ion mobility
2. α-Synuclein (Parkinson’s Disease)
3. Aβ Assembly (Alzheimer’s Disease)
4. IAPP (Type 2 Diabetes)
5. Summary
Ion Mobility
Introduction

Ion mobility has many uses:

Physics
- Plasma analysis
- Ion-neutral interaction potentials

Chemistry
- Analytical applications
  - forensics/security
  - proteomics
  - others
- Electronic Structure/ Reactivity
  - Transition metals
  - Main group excited states
- Structure
  - Small Molecules
  - Clusters
  - Polymers
  - Biological Systems
    - peptide/proteins
    - nucleotides
    - aggregates
    - noncovalent complexes

examples coming!
Ion Mobility Method

ESI Source

Analyzer Region

Ion Funnel → Drift Cell → MS

Detector

mass spectrum

$M_n^{4n-}$

$M_n^{5n-}$

$M_n^{7_2n-}$

$M_n^{7_3n-}$

$M_n^{2n-}$

$m/z$

time

arrival-time distribution for $M_n^{4n-}$

$n = \text{oligomer order}$

$z = \text{charge} = -4n$

$z/n = -4$

closed conformation $\rightarrow$ fast

large $n \rightarrow$ fast

open conformation $\rightarrow$ slow

small $n \rightarrow$ slow
The velocity of an ion through the drift cell is proportional to the electric field

\[ v = K \cdot E \]

\( K = \text{ion mobility} \)

\[ K = f(T, p, q, \mu, \sigma) \]

The collision cross section of the ion, \( \sigma \), depends on its shape

\[ \sigma = f(\text{He-ion interaction} \& \text{ion shape}) \]

\[ t_a = \frac{\ell^2 T_0 p}{K_0 \rho_0 T V} + t_0 \]

slope \( \propto \frac{1}{K_0} \)

\[ \sigma = \frac{3ze}{16N} \left( \frac{2\pi}{\mu kT} \right)^{\frac{1}{2}} \frac{1}{K_0} \]
Assigning ATD Peaks: Injection Energy Studies

Human Tau 441 fragment SVQIVYKPVDSLK: m/z = 1520, z/n = +1
Cross Section: Experiment & Theory

Arrival Time Distribution

Mobility: $K \propto \frac{1}{t_a}$

Cross Section: $\sigma \propto \frac{1}{K}$

Experiment

Theory

Mobility: $K \propto \frac{1}{\sigma}$

Cross Section: trajectory or hard-sphere scattering

Model Structure
Amyloid Diseases & Their Worldwide Prevalence

- Aβ
  - Alzheimer’s Disease
  - ~27 Million

- α-Syn
  - Parkinson’s Disease
  - ~4 Million

- IAPP
  - Type 2 Diabetes
  - ~160 Million

- PrP
  - Human TSEs
  - < 10,000

www.alz.org • www.who.int/diabetes • www.parkinsons.org.uk • www.cdc.gov/ncidod/dvrd/prions
α-Synuclein
α-Synuclein Fibrillization and Parkinson’s Disease

- α-Synuclein is the primary component of Lewy bodies in all Parkinson’s patients.
- Two independent missense mutations, A30P & A53T (associated with early onset) form fibrils.
- Transgenic mice expressing human α-synuclein develop α-synuclein inclusions that correlate with onset of Parkinson’s disease phenotype.
ESI Mass Spectrum of [A30P]α-Synuclein

Aggregation enhanced at low pH

pH 2.5

pH 7.5
Cross Sections for Three Alloforms

- A30P larger
- Aggregation enhanced at low pH

Charge State vs. Cross Section (Å²)
ATDs for Three Alloforms: Effect of Charge State

IE = 50 eV

-7  -8  -9  -12

WT

A30P

A53T
A30P Mutant -13/2 tetramer dissociation via temperature and injection energy increase.
A30P tetramer and dimers larger than WT for all charge states.
Spermine

- Naturally occurring polyamine found in neuronal cells
- Known to be involved in neurodegenerative processes
- Increases the rate of aggregation and fibrillization of WT α-synuclein
- Does not induce significant secondary structure in WT α-synuclein
Mass Spectrum of [A53T]α-Syn + Spermine

IE = 50 eV

[A53T]α-syn, no spermine

[α-syn]^{6-}

[α-syn + spermine]^{6-}
ATDs for -6 Charge State

Free Protein

1457 Å²
1807 Å²

Free Protein in Complex Solution

1430 Å²
1783 Å²

Protein•Spermine Complex

1430 Å²
1770 Å²

σ(-10) = 2600 Å²
Spermine Binding at pH 7.5

α-Synuclein has net charge near -10

Isoelectric point = 4.4

Spermine exists as a polycation with a charge of +4

Expected complex: $[\alpha\text{-syn + spermine}]^{6-}$
Spermine Ligation

\[ \alpha\text{-synuclein} \]

\[ + \quad \left( \begin{array}{c} \_\_\_\_ \end{array} \right)^{+4} \]

spermine

Change in charge state and conformation
Effect of Spermine on Aggregation

with spermine

slow

without spermine

fast

with spermine
Effect of Spermine on Aggregation

Positively charged metals facilitate aggregation by inducing collapse into a partially folded intermediate.

Acc. Chem. Res. 2006, 39, 628
α-Synuclein Conclusions

- pH affects the charge state distributions but not cross sections for all three proteins
- A30P mutant is much larger than WT and A53T mutant at low charge states
- Addition of spermine drastically changes the charge state distribution to favor the solution phase complex charge of -6
- Formation of spermine complex redistributes existing structures to favor more compact families
- Abundance of spermine-induced compact structures may facilitate aggregation
Aβ Assembly
APP protein

β-secretase

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIGLMVGGVV40IA42

γ-secretase

90% Aβ40
<10% Aβ42
Aβ42 Oligomerization Mechanism

Paranuclei

Protofibrils (β)

Large oligomers

Fibrils (β)

Bitan et al. PNAS 2003
Objectives

1. Quantify the soluble oligomer distributions
2. Understand how the oligomers are assembled
3. Determine key structure elements controlling oligomerization
Aβ42 wild type – Monomer Experimental Data

Bernstein et al. JACS 2005
Aβ42 wild type – Monomer Theoretical Calculations

Implicit water → REX → Dehydrated

No water → REX → MIN → Gas Phase
Aβ42 wild type – Monomer Structures

Calculated Gas Phase Structure

Calculated Dehydrated Structure

Baumketner et al. Protein Sci. 2006
Aβ42 wild type – Oligomer Experimental Data

Where are ?

No Octamer!

Bernstein et al. JACS 2005
Primary Structure Elements Controlling Aβ Oligomerization

- **Pro\(^{19}\)**
  - Substitution by proline abolishes fibril formation

- **Met\(^{35}(O)\)**
  - Oxidation may be important for toxicity and/or oligomerization

- **DAEFRHDSGY\(^{10}\)EVHHQKLVFF\(^{20}\)AEDVGSNKGA\(^{30}\)IIGLMVGGVV\(^{40}\)IA**

- **CHC**
  - Residues in/near the central hydrophobic core (CHC) whose substitutions are associated with familial, early-onset AD

- **Aβ40 v. Aβ42**
  - Essential residues for paranucleus formation and/or self-association
Evidence for Aggregation – Positive Ion Mode

Evidence for Aggregation – Positive Ion Mode

[Pro^{19}]Aβ_{42}

ATD Comparison

ATDs for $z/n = -5/2$

<table>
<thead>
<tr>
<th></th>
<th>wild type</th>
<th>A21G</th>
<th>F19P</th>
<th>Met$_{35}(O)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ42</td>
<td>[Graph]</td>
<td>[Graph]</td>
<td>[Graph]</td>
<td>[Graph]</td>
</tr>
<tr>
<td>Aβ40</td>
<td>[Graph]</td>
<td>[Graph]</td>
<td>[Graph]</td>
<td>[Graph]</td>
</tr>
</tbody>
</table>

Drift time (μs)
Preliminary Conclusions

- $\alpha\beta_{42} \text{ wt is the only alloform to } [(\alpha\beta)_6]_2$

  No octamer formed
  \[ (\alpha\beta)_6 + (\alpha\beta)_6 \rightarrow [(\alpha\beta)_6]_2 \]

- No $[(\alpha\beta)_6]_3$ is formed

  \[ [(\alpha\beta)_6]_2 + (\alpha\beta)_6 \xrightarrow{?} [\beta\text{-sheet}]? \]

* $[(\alpha\beta)_6]_2$ toxic oligomer?
Some Assembly Required

Paranucleus → Protofibril → Fibril
Building Blocks
Simple Monomer Hard Sphere Approximation

$\sigma_{\text{expt}}(A\beta 42)_2 = 1256 \text{ Å}^2$

We can vary the distance (d) between the centers of the two spheres in the dimer model.
<table>
<thead>
<tr>
<th>Hexamers</th>
<th>Cross Section (Å²)</th>
<th>Aβ42</th>
<th>[A21G]Aβ42</th>
</tr>
</thead>
<tbody>
<tr>
<td>linear</td>
<td></td>
<td>3450</td>
<td>3372</td>
</tr>
<tr>
<td>ring</td>
<td></td>
<td>3100</td>
<td>3100</td>
</tr>
<tr>
<td>close-packed</td>
<td></td>
<td>2578</td>
<td>2020</td>
</tr>
</tbody>
</table>

σ_{exp} = 2898 \quad σ_{exp} = 2928
Dihexamers

Cross Section (Å²)

Aβ42

planar

5824

stacked

4562

$\sigma_{exp} = 4307$
Significance of Tetramer

- Aβ42 wt forms an “open” tetramer
  - reactive to form larger oligomers
- All other alloforms form “closed” tetramers
  - unreactive
Why no T

Top & bottom may be very different

since expect large oligomers and poor binding surface
Biological Conformation


Expressed human APP in transgenic mice

<table>
<thead>
<tr>
<th>Time (mo.)</th>
<th>Memory Defects</th>
<th>Neuronal Plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 6</td>
<td>no memory loss</td>
<td>no plaques</td>
</tr>
<tr>
<td>6 – 14</td>
<td>memory defects</td>
<td>no plaques</td>
</tr>
<tr>
<td>&gt; 14</td>
<td>memory defects</td>
<td>plus plaques</td>
</tr>
</tbody>
</table>

At 6 – 14 months, only 56 kDa Aβ assembly observed. \((\text{Aβ}_{42})_{12}\)

Consistent with our observation

\[ (\text{Aβ}_{6})_2 \text{ but not } (\text{Aβ}_{6})_3 \]

Toxic species?
Proposed Assembly Mechanism

M → D → Te → Paranucleus → Toxic?

β-Oligomers
Protofibrils

β-Fibrils

M → D → Te → β-Fibrils

slow fibril formation

Aβ42 wt
Aβ40 wt, etc.
Mix Aβ40 + Aβ42
1:1

→ Aβ42 is aggregating

• 40/42 ~5/1

• 40/42 oligomer observed
  • Expected 1:2:1
Mix Aβ40 + Aβ42
1:1
26AIAβ42 → Aβ42

26-O-acyl-isoAβ42

Gly25 esterification to β-hydroxyl group of Ser26

DAEFRHDSGYEVHHQKLVFFAEDV

pH 7.4

Aβ42

DAEFRHDSGYEVHHQKLVFFAEDV

NKGAIIGLMVGGVVIA
DAEFRHDSGYEVHHQK[L^{17}VFFA_{21}EDVG^{26}SNKG_{30}IIGLMVGVV^{40}]A_{42}

26-AIA\beta_{42}

\frac{A}{A_0} = e^{-t/\tau}

\text{pH 7.4} \quad 26-AIA\beta_{42} \quad \circlearrowleft_{\text{fast}} \quad A\beta_{42} \quad \tau_{1/2} \sim 2.8 \text{ min.}
26-AIAβ42 $\xrightarrow{\text{Q}}$ Aβ42

pH 7.4

20 minutes

-3

-4

2 hours

-3

-5/2

-2

m/z

1000 1200 1400 1600 1800 2000 2200 2400

200 400 600 800

ATD for $z/n = -5/2$
ATDs for $z/n = -5/2$

26-AIAβ42 $\xrightarrow{\tau} Aβ42$

Aβ42  Bernstein et al. JACS 2005
1:1
\( \text{A} \beta_{40} : 26-\text{AIA} \beta_{42} \)

1:1
\( \text{A} \beta_{40} : \text{A} \beta_{42} \)
Diabetes Type 2 Mellitus

Type 2 diabetes comprises 90% of people with diabetes around the world.

- 180 million people worldwide have diabetes. This number is likely to more than double by 2030.
  - 20.8 million people in the US, or 7% of the population, have diabetes.
- 1.1 million people worldwide died from diabetes in 2005. Diabetes accounts for 5% of all deaths globally each year, a rate that is likely to increase by more than 50% in the next 10 years without urgent action.

WHO: [www.who.int/diabetes](http://www.who.int/diabetes)
ADA: [www.diabetes.org](http://www.diabetes.org)
The image illustrates the conversion process of IAPP. It starts with proIAPP, which is cleaved by prohormone convertases PC1/3 & PC2 to form 67-AA peptide. This 67-AA peptide then undergoes the addition of a 2-7 disulfide bridge and becomes amidated at the C-terminus, resulting in the 89-AA peptide with amino-terminal signal sequence. The 89-AA peptide is further converted into the mature IAPP.
Fibrillization & pH

Human IAPP\textsubscript{1-37}  
IAPP\textsubscript{8-37}  
→ Fibrils form faster at pH 8.8 than pH 4.0

Histidine Side Chain pKa 6.0

Protonated at pH 4.0  
Deprotonated at pH 8.8

Note: Inside of islet granule pH 5.5, intercellular space pH 7.4

Abedini & Raleigh *Biochemistry* 2005, 44, 16284
Traveling Wave Ion Mobility MS

Prototype built by Waters Corp. (Milford, MA) for their Synapt High Definition MS System

T-Wave Technology: A new method for separating ions based on their mobility
Human vs. Rat IAPP

Human IAPP\(_{(8-37)}\)

Rat IAPP\(_{(8-37)}\)

no aggregates

20 \(\mu\)M
pH 7.4
Effect of Incubation on Human IAPP

Human IAPP\textsubscript{(8-37)}

20 \text{\mu M pH 7.4}

+1.5 hrs

+3

+4

+5/2

+2

large oligomeric species appear after incubation

+\sim 15 hrs

m/z

700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000

2100 2200 2300 2400 2500 2600 2700 2800 2900 3000 3100 3200 3300 3400 3500
How do we slow down aggregation?

Observation: Fibrillization rate is reduced at low pH

Try: Higher concentration, lower pH
Effect of Incubation on Human IAPP at 100 μM & pH 4.0

Human IAPP(8-37)

100 μM pH 4.0

0

+90 min
Aggregation of Rat IAPP at 100 μM & pH 4.0
Assigning ATD Peaks with High-Resolution MS

- MS for fast ATD peak
  - 0.25 amu splitting
  - +4 Dimer

- MS for slow ATD peak
  - 0.5 amu splitting
  - +2 Monomer

+2 ATD
Comparison of Human & Rat ATDs at Low Charge States

Human IAPP (8-37)

Rat IAPP (8-37)

100 μM pH 4.0
IAPP Observations to Date

- Human IAPP oligomerizes very much faster than rat.
- Oligomerization of hIAPP is reduced at low pH. Why? Oligomerization of rIAPP appears unaffected by pH.
- hIAPP has at least two structural forms of dimer, trimer and tetramer. What are they? rIAPP has only a single form.

Approach

- Measure cross sections on IMS-MS instrument.
- Model to get idea of structures.
- Injection-energy studies.
- Temperature dependent studies.
Summary

- Information about solution conformations obtained for
  - α-Synuclein
  - Aβ monomer and aggregates
  - G-quadruplex assemblies

- Absolute cross section measurements allow structural information to be obtained

- Spermine induces compact α-synuclein structures
  - promotes aggregation

- (Aβ)$_{12}$ appears to be transitional species and may be toxic oligomer

- hIAPP aggregates very rapidly
  - several dimer, trimer and higher-order aggregate structures

- NH$_4^+$ and ligand attachment stabilize G-quadruplex structures
多謝！

Thanks for your attention!
G-Quadruplex Formation
Self-Assembly of d-Guanosine

Aggerholm et al. J. Mass Spectrom. 2003, 38, 87
- Observed “magic number” quartet adducts with alkali ions
- Did not use NH$_4^+$ adducts
ESI Mass Spectrum of d-Guanosine in NH₄OAc

ESI Mass Spectrum of d-Guanosine in NH$_4$OAc

How will DNA strands self-assemble?
Motivation

- Formed from telomere repeats that cap chromosomes
- Over 360,000 DNA segments have G-quadruplex potential in the human genome
  - Common G-quartet motif
  - Different loop structure
  - Often found in promoter region of oncogenes

Stabilization of quadruplexes by drugs can alter gene expression and telomerase functions
Human Telomere – (TTAGGG)\textsubscript{n}

...TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG...

Antiparallel (basket)

Antiparallel (chair)

Parallel (propeller)

Mixed (para/antipara)
Procedure

1. Carefully anneal potential DNA quadruplex segment

2. Prepare ESI spray solution (in 100 mM NH₄OAc – pH 7.4)

3. Take CD of solution

4. Spray solution
   ◦ Mass spectrum
   ◦ ATDs

5. Compare MD simulated structures / cross sections
Example for Simple Telomere Repeats

\[ T_1 \quad TTAGGG \quad 4 \]
\[ T_2 \quad (TTAGGG)_2 \quad 2 \]
\[ T_4 \quad (TTAGGG)_2 \quad 1 \]

\[ T_1: \text{parallel quadruplex} \quad \text{min} = 240 \text{ nm} \quad \text{max} = 265 \text{ nm} \]

\[ T_2: \text{antiparallel quadruplex} \quad \text{min} = 260 \text{ nm} \quad \text{max} = 295 \text{ nm} \]

\[ T_4: \text{antiparallel/parallel mixture} \quad \text{min} = 240 \text{ nm} \quad \text{max} = 265, 295 \text{ nm} \]
### Results for $T_1$, $T_2$ and $T_4$

<table>
<thead>
<tr>
<th></th>
<th>cross section ($\text{Å}^2$)</th>
<th>Experiment</th>
<th>Theory</th>
<th>Solution CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1$</td>
<td></td>
<td>805</td>
<td>740 (globular)</td>
<td>parallel</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>800 (parallel)</td>
<td></td>
</tr>
<tr>
<td>$T_2$</td>
<td></td>
<td>790, 838</td>
<td>745 (globular)</td>
<td>parallel/anti mixture</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>845 (parallel)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(~90%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>785-797 (anti)</td>
<td>(~10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_4$</td>
<td></td>
<td>789</td>
<td>743 (globular)</td>
<td>anti</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>842 (parallel)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>790 ± 1 (anti)</td>
<td></td>
</tr>
</tbody>
</table>

Questions of Interest

1. Do we always preferentially form quadruplexes from strands with G-repeats?

2. What is the role of positive ions in stabilizing quadruplexes?

3. What role do ligands (drugs?) play in stabilizing quadruplexes?

4. Are solution structures maintained under solvent-free conditions?
Systems We Will Study

\[ T_{3.5} \quad \text{GGG(TTAGGG)₃} \quad \{ \text{human telomere repeats} \} \]

\[ T_{6} \quad (TTAGGG)_6 \]

\[ \text{Pu22} \quad \text{GAGGGTGGGGAGG} \quad \{ \text{c-myc oncogene promoter region} \} \]

\[ \text{Pu27} \quad \text{TGGG[Pu22]G} \]
Stabilization Studies

A. $\text{NH}_4^+$ between G-quartet layers

B. Ligands:

- MMQ1, $R = \text{NH(CH}_2\text{)}_2\text{CH}_3$
- MMQ3, $R = \text{NH(CH}_2\text{)}_3\text{N(CH}_3\text{)}_2$
- TMPyP4
- BOQ1
- PIPER
Molecular Dynamics

1. Start with PDB structure if available

2. Construct various structures if no PDB structure
   - parallel
   - antiparallel
   - mixed

3. Two ns of 300 K dynamics

4. Compute cross sections of various structures

5. Compare with experimental cross sections of lowest charge states
Results on Bare Quadruplexes

Mass spectrum of $T_6$:

ATDs of $T_6$:

- $[T_6]^{5-}$: $989 \pm 9 \text{ Å}^2$
- $[T_6]^{6-}$: $1010 \pm 10 \text{ Å}^2$
- $[T_6]^{7-}$: $1074 \pm 3 \text{ Å}^2$
- $[T_6]^{8-}$: $1405 \pm 6 \text{ Å}^2$

G-quartets intact \[\leftrightarrow\] G-quartets broken
Theoretical Placement of Extra G’s in $T_6$ and Pu27

Extra G’s on end

Extra G’s in loop
# Theoretical Cross Sections (Å²)

<table>
<thead>
<tr>
<th></th>
<th>([\Gamma_{3,5}]^4)</th>
<th>([\Gamma_{3,5}]^5)</th>
<th>([\Gamma_6]^5)</th>
<th>([\text{Pu22}]^4)</th>
<th>([\text{Pu27}]^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>688</td>
<td>718</td>
<td>989</td>
<td>701</td>
<td>810</td>
</tr>
<tr>
<td>Theoretical</td>
<td></td>
<td>end</td>
<td>loop</td>
<td>end</td>
<td>loop</td>
</tr>
<tr>
<td>Antiparallel (basket)</td>
<td>700</td>
<td>713</td>
<td>1005</td>
<td>1060</td>
<td>755</td>
</tr>
<tr>
<td>Antiparallel (chair)</td>
<td>700</td>
<td>712</td>
<td>1003</td>
<td>1065</td>
<td>753</td>
</tr>
<tr>
<td>Parallel (propeller)</td>
<td>790</td>
<td>802</td>
<td>1083</td>
<td>1092</td>
<td>706</td>
</tr>
<tr>
<td>Mixed (para/antipara)</td>
<td>737</td>
<td>740</td>
<td>1047</td>
<td>1045</td>
<td>735</td>
</tr>
<tr>
<td>Mixed (mostly para)</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>Globular</td>
<td>660</td>
<td>662</td>
<td>1055</td>
<td>1049</td>
<td>743</td>
</tr>
</tbody>
</table>

Quadruplexes formed in all cases
### Comparison with Solution

<table>
<thead>
<tr>
<th>System</th>
<th>Solution NMR/CD</th>
<th>Solvent-Free IMS/Modeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>$4 \times T_1$</td>
<td>parallel</td>
<td>parallel</td>
</tr>
<tr>
<td>$2 \times T_1$</td>
<td>mixed</td>
<td>mixed</td>
</tr>
<tr>
<td>$T_{3.5}$</td>
<td>antiparallel</td>
<td>antiparallel</td>
</tr>
<tr>
<td>$T_4$</td>
<td>antiparallel</td>
<td>antiparallel</td>
</tr>
<tr>
<td>$T_6$</td>
<td>antiparallel</td>
<td>antiparallel</td>
</tr>
<tr>
<td>Pu22</td>
<td>parallel</td>
<td>parallel</td>
</tr>
<tr>
<td>Pu27</td>
<td>parallel</td>
<td>parallel</td>
</tr>
</tbody>
</table>

IMS structures reflect **solution** structures.
Stability Studies

Strategy – pick transitional charge states

\[ T_6 \]

- \([T_6]^{5-}\): 989 ± 9 Å²
- \([T_6]^{6-}\): 1010 ± 10 Å²
- \([T_6]^{7-}\): 1074 ± 3 Å²
- \([T_6]^{8-}\): 1405 ± 6 Å²

\[ Pu22 \]

- \([Pu22]^{4-}\): 701 ± 9 Å²
- \([Pu22]^{5-}\): 696 ± 8 Å²
- \([Pu22]^{6-}\): 757 ± 9 Å²
- \([Pu22]^{7-}\): 807 Å²
- \([Pu22]^{8-}\): 900 Å²

\[ Pu27 \]

- \([Pu27]^{5-}\): 810 ± 10 Å²
- \([Pu27]^{6-}\): 846 ± 6 Å²
- \([Pu27]^{7-}\): 949 ± 16 Å²
- \([Pu27]^{8-}\): 1080 Å²
NH₄⁺ Stabilization

(a) [Pu22]⁵⁻

(b) [Pu27]⁶⁻

(c) [T₆]⁷⁻

N = Native (quadruplex)
D = Denatured (partial or total quadruplex destruction)
Ligand Stabilization

\[
\begin{align*}
[T_6+2\text{NH}_4]^7- & \quad [T_6+2\text{NH}_4+1\text{Ligand}]^7- & \quad [T_6+2\text{NH}_4+2\text{Ligands}]^7- \\
\text{MMQ1} & \quad \text{MMQ3} & \quad \text{TMPyP4}
\end{align*}
\]

Arrival time (μs)

650 700 750 800 850 900

D

N
Injection Energy Effects

$[T_6 + 2NH_4]^7-$

$[T_6 + 2NH_4 + PIPER]^7-$

Arrival time (μs)

40 V 60 V 80 V

Injection Energy