Salt Bridges: Aggregation, Hydration, and Fragmentation of Peptides and Oligonucleotides

UCSB: Thomas Wyttenbach, Perdita Barran, Jennifer Gidden, Summer Bernstein, Dengfeng Liu, Mike Bowers

U. Arizona: Linda Breci, Vicki Wysocki

Heidelberg: Bela Paizs

$$ NSF (UCSB)

Structure of the talk

- Structure of small peptides
  H/D exchange vs. ion mobility
- Aggregation of peptides
- Salt bridges in oligonucleotides?

Structure of small peptides:
H/D exchange vs. ion mobility

Motivation

- Building accurate computer models for protein identification from MS/MS data requires knowledge of fragmentation mechanisms.
- Is the structure/conformation related to fragmentation pattern/mechanism?
- Does H/D exchange give information on peptide structure/conformation?
- Can H/D exchange and ion mobility data be structurally correlated?

Approach

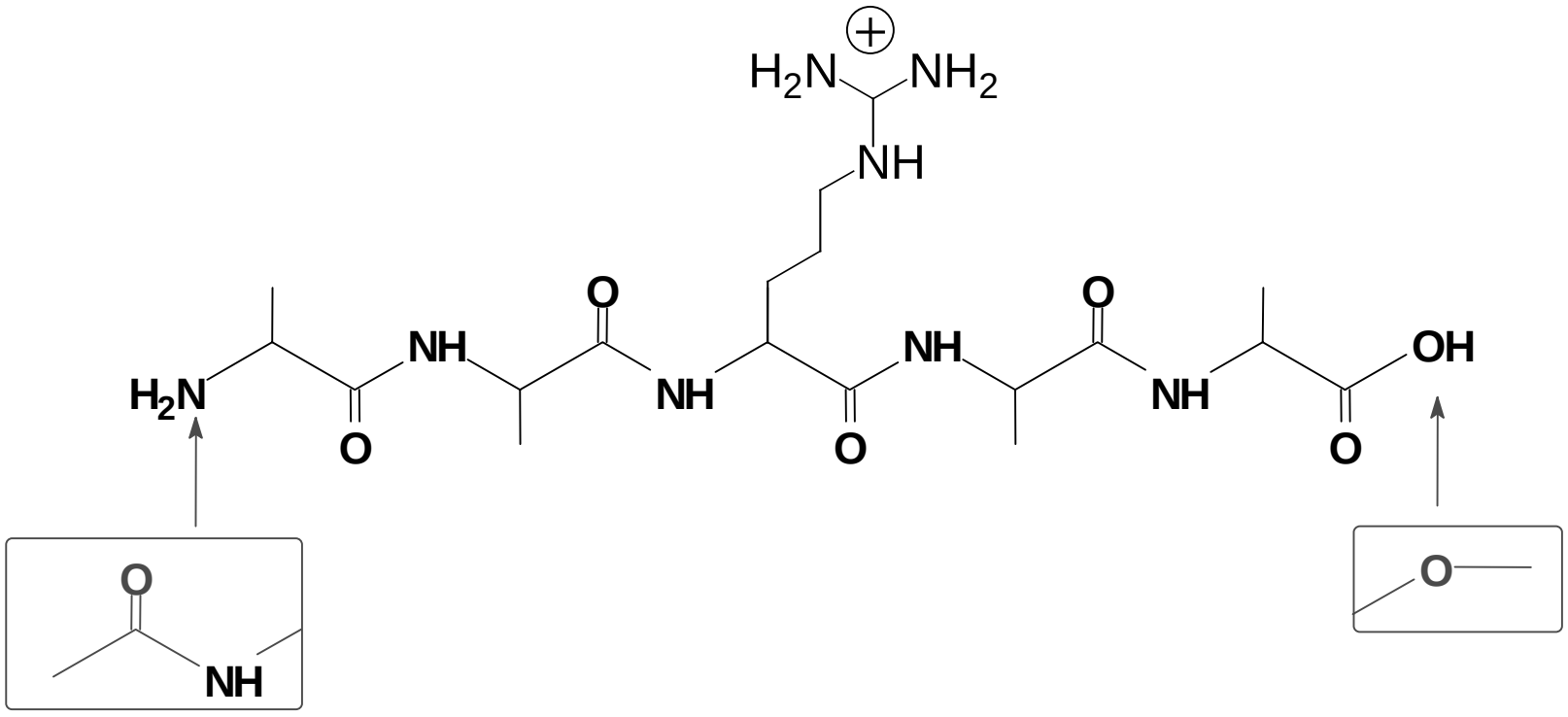
- Look at several series of peptides where important groups are systematically varied.
- Do H/D exchange and ion mobility studies on each group.
- Measure hydration energies.
- Do detailed ab initio/DFT calculations on selected systems.
- Here we will discuss RAAAA, AARAA, AAAAR but focus on AARAA.

AARAA

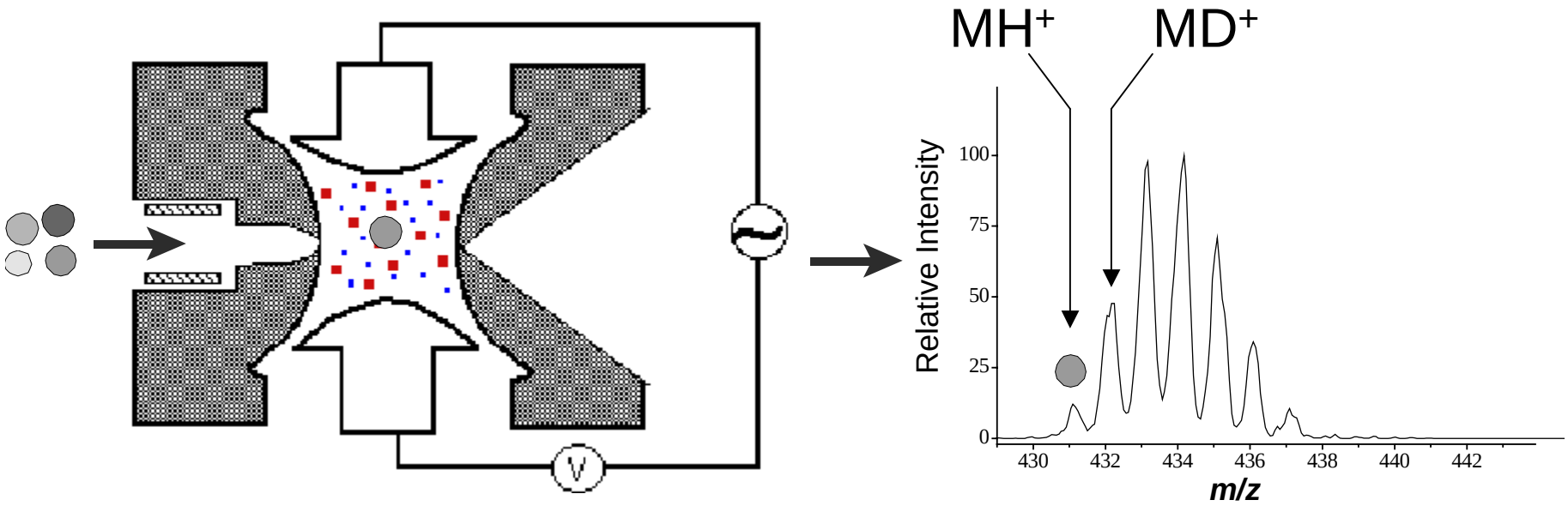
- University of Arizona, Tucson
- University of California, Santa Barbara
- German Cancer Research Center Heidelberg

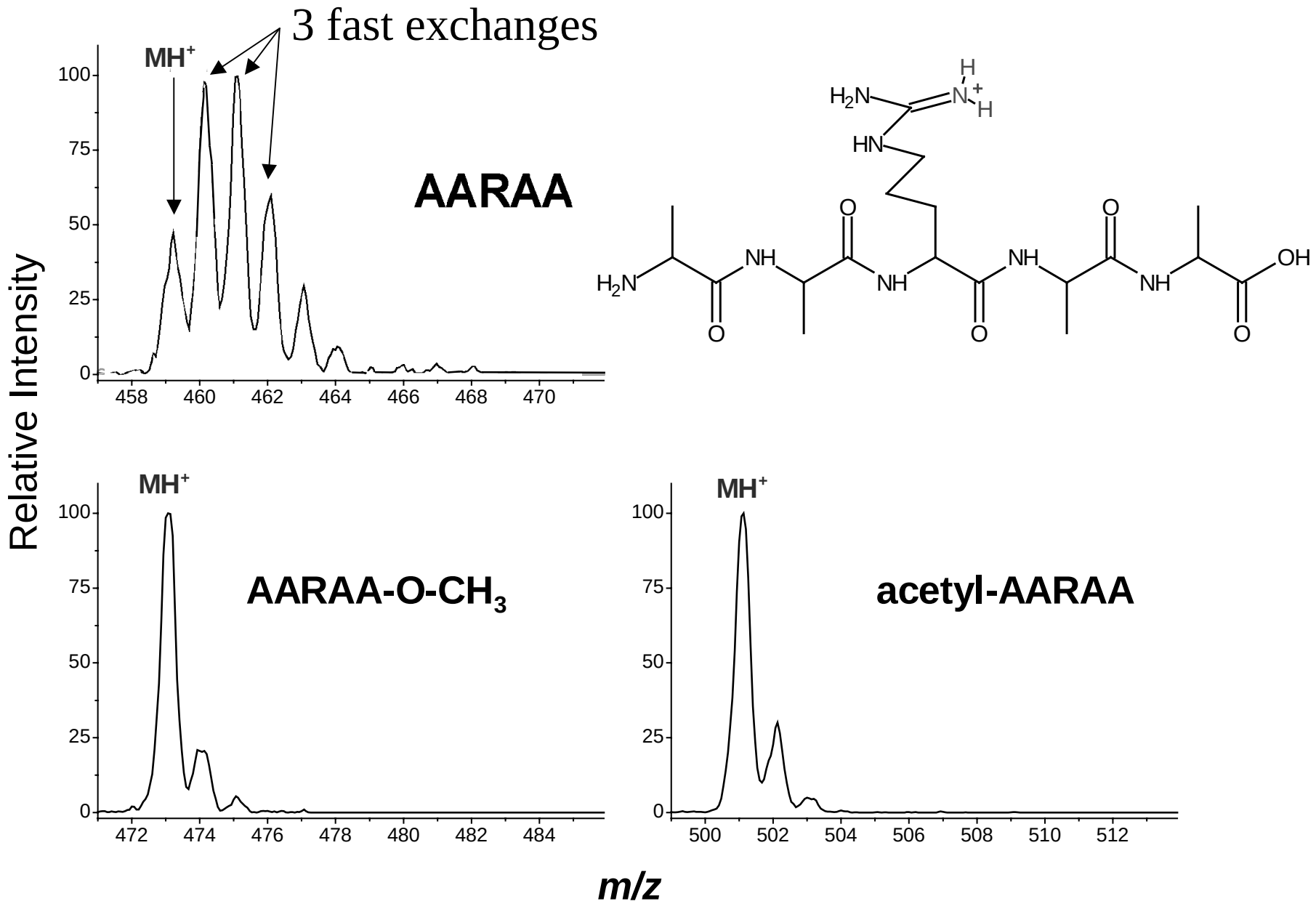
AARAA

with and without blocked termini

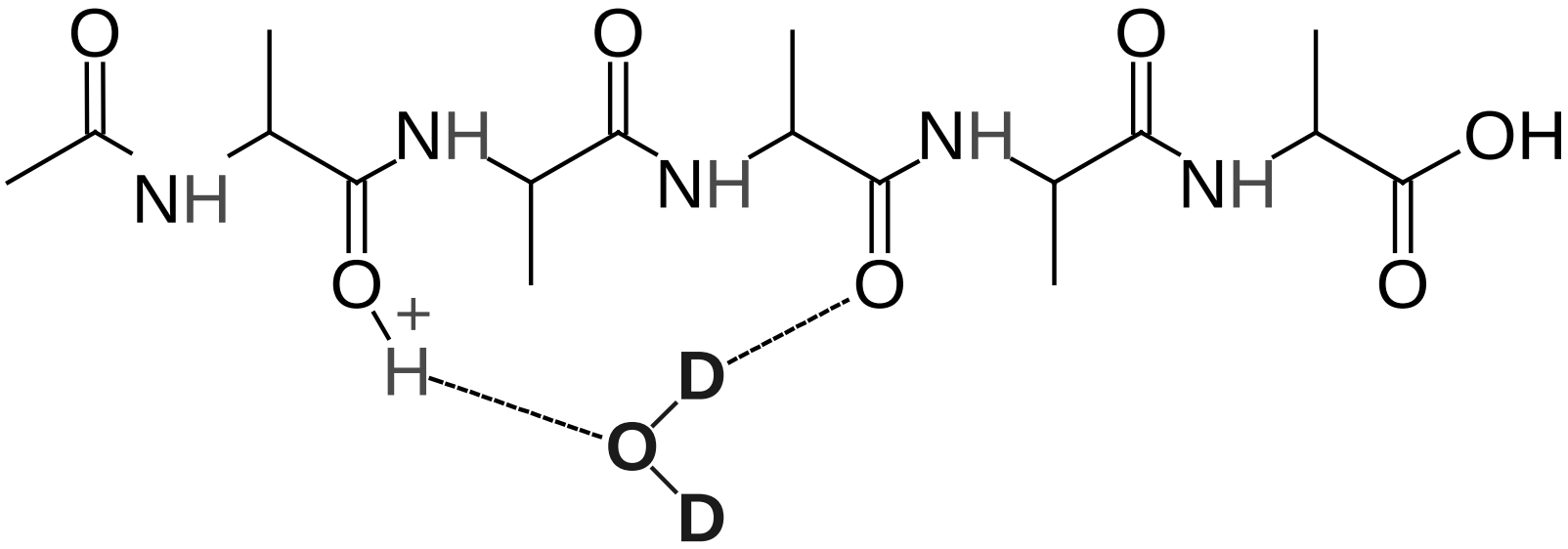


H/D exchange in the Ion Trap (UA)




3 fast exchanges
MH+
AARAA
Relative Intensity
100
75
50
25
0
458
460
462
464
466
468
470
H2N
HN
NH
OH
O
MH+
AARAA-O-CH3
472
474
476
478
480
482
484
MH+
acetyl-AARAA
500
502
504
506
508
510
512
m/z

D_2O: Relay Mechanism for H/D exchange



Campbell, Rodgers, Marzluff, Beauchamp,

JACS, 1995, 12840-12854

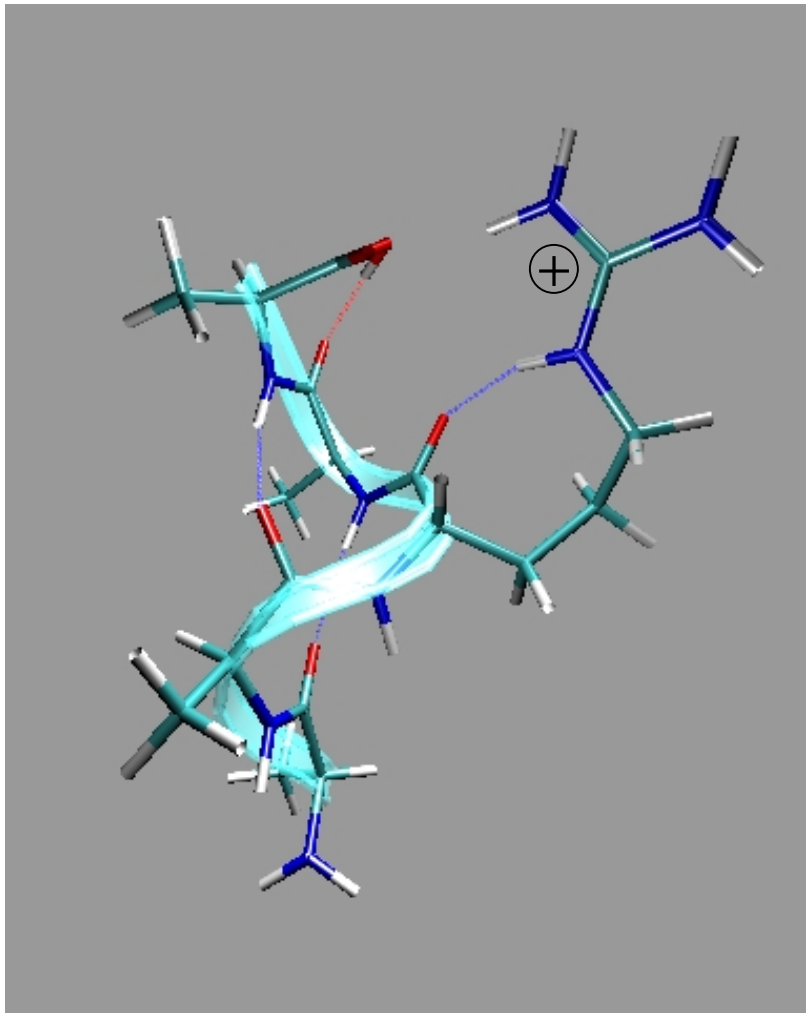
	AARAA	AARAA-OMe	Ac-AARAA
Exchange	YES	NO	NO

Both termini involved

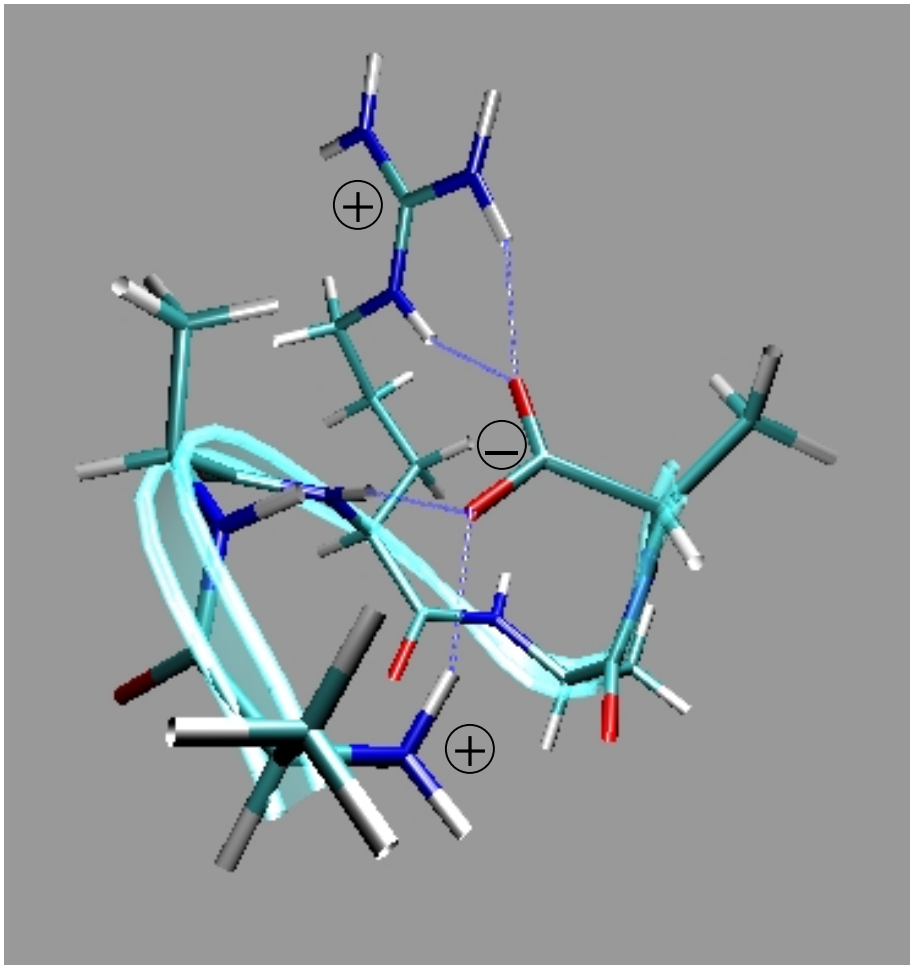
Salt bridge?

[AARAA]H$^+$

Lowest energy structure (AMBER)

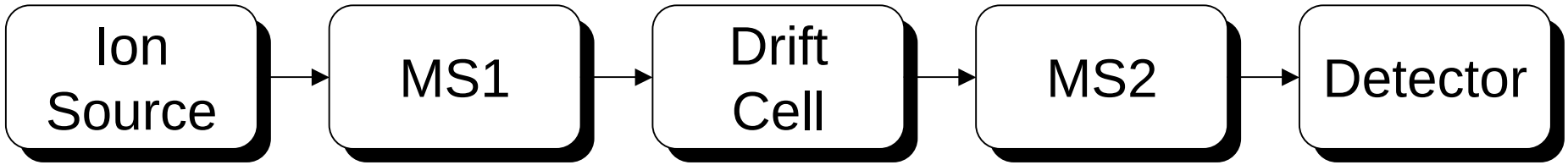


Charge solvation

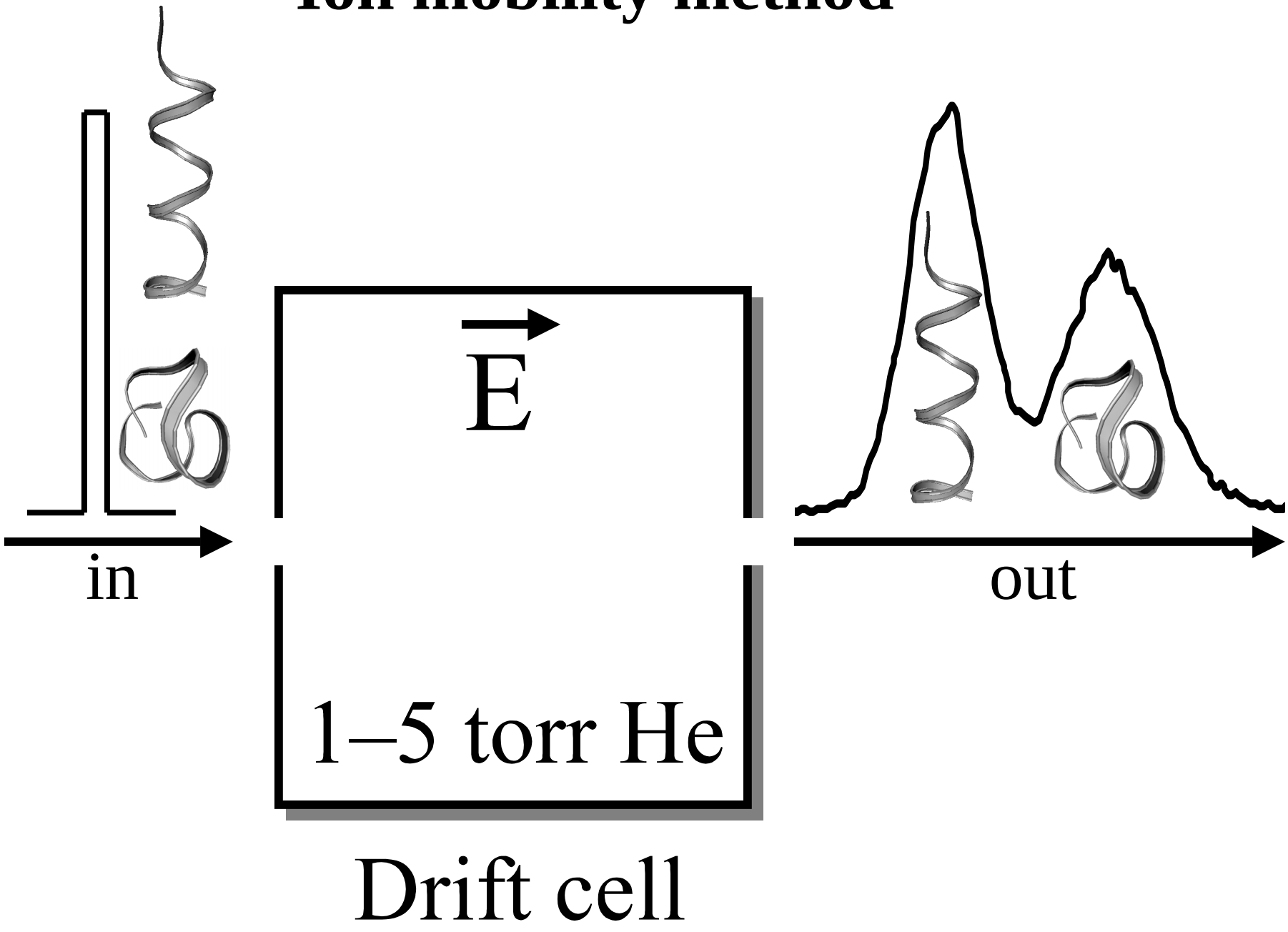


Zwitterion (salt bridge)

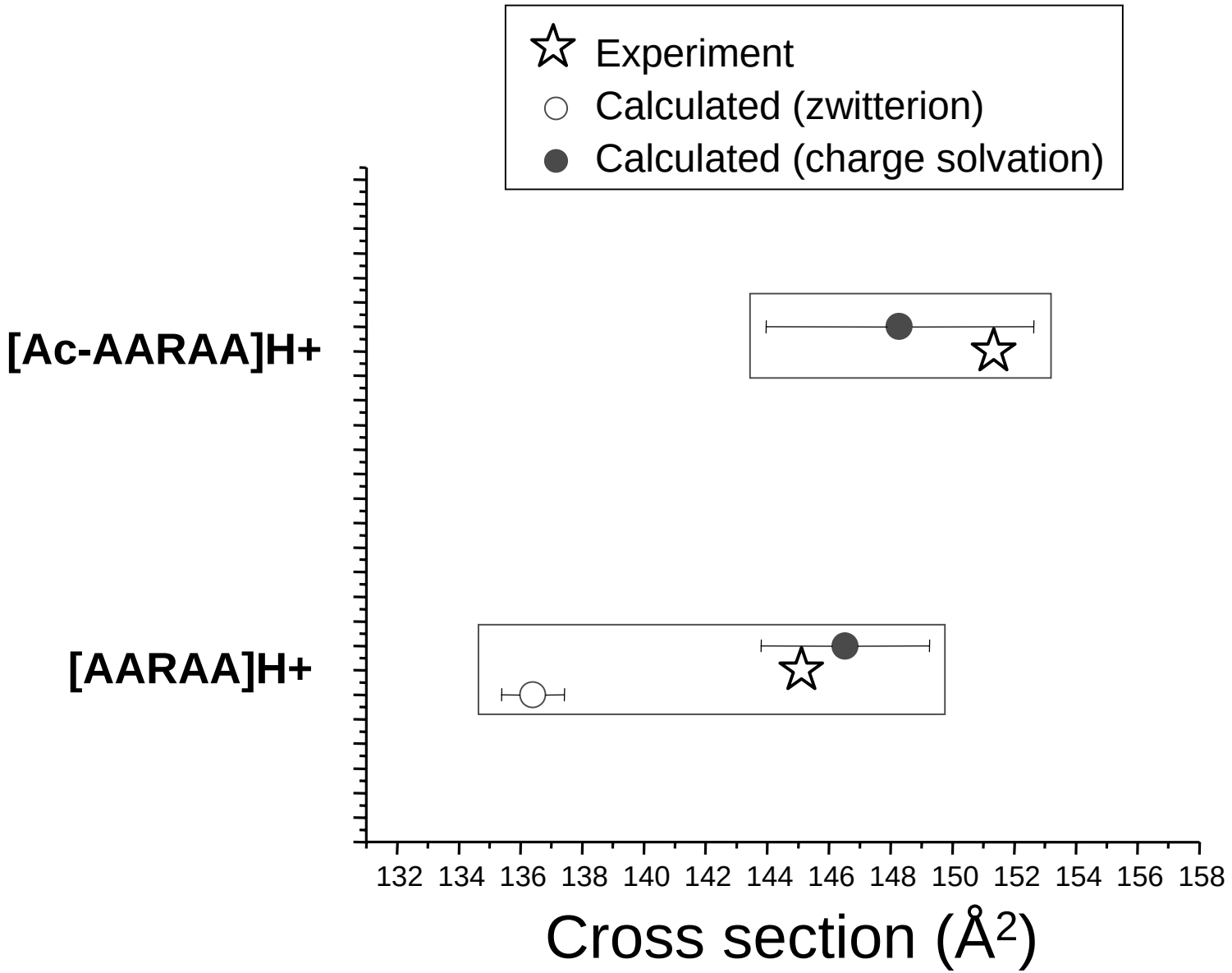
Ion mobility method (UCSB)



Ion mobility method



Ion Mobility (UCSB)



Peptide	σ (Experiment)	σ (AMBER Calculation)	
		Neutral Terminus	Zwitterions Terminus
$[RAAAA]H^+$	136.43	139.38	136.38 (★)
$[AARAA]H^+$	145.11	146.52 ★	136.40
$[AAAAR]H^+$	147.51	148.77 ★	139.79
$[AcRAAAA]H^+$	144.16	144.25	--------
$[AcAARAA]H^+$	151.38	148.29	--------
$[AcAAAAR]H^+$	151.84	151.64	--------

H/D-exchange: salt bridge (?)

$(AARAA)H^+ + H_2O$

Ion mobility: no salt bridge

$(AARAA)H^+$

Calculations

(Heidelberg)

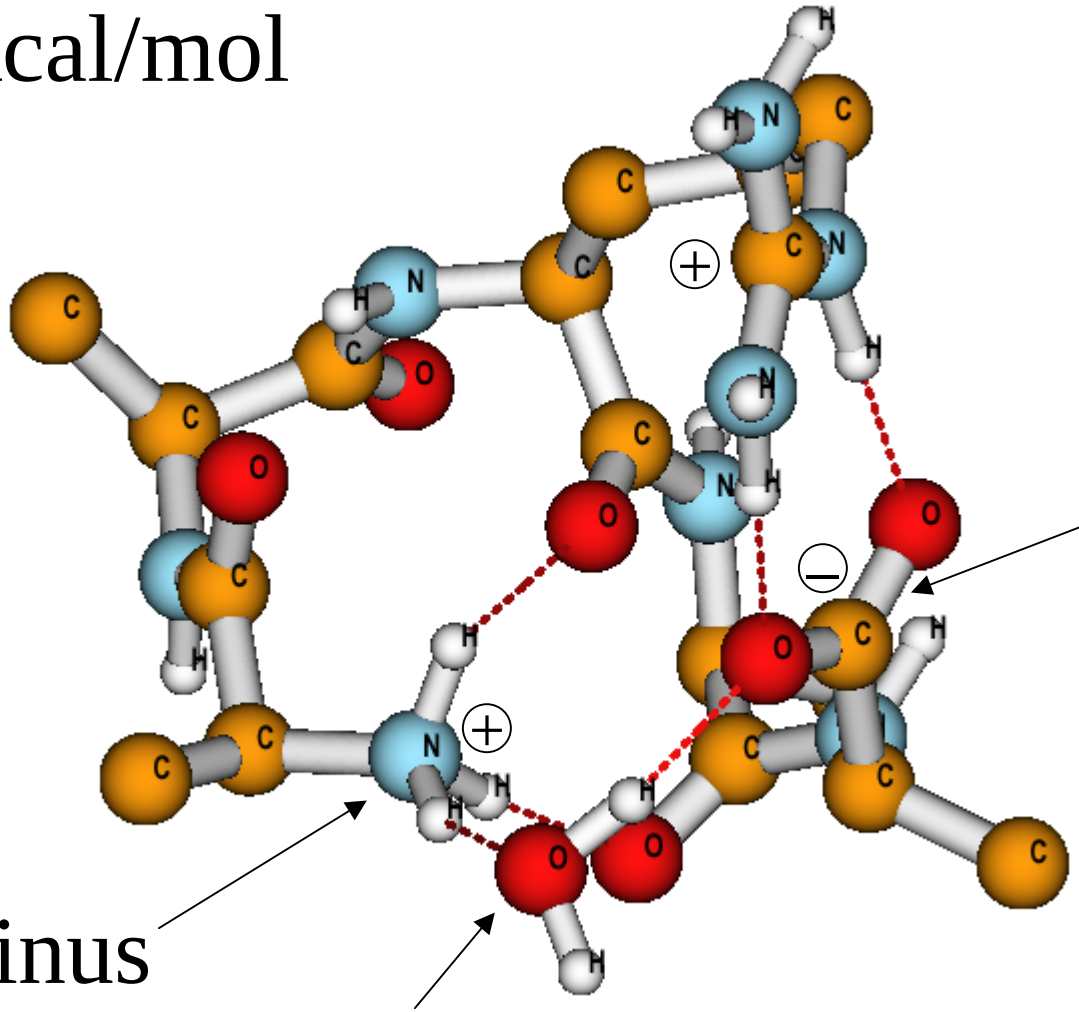
B3LYP/6-31g(d)

Structure	**Energy (kcal/mol)**	
	(AARAA)H^+	(AARAA)H^+ + H_2O
Charge solvation	0.0	0.3
Zwitterion (–NH_3^+)	2.3	0.0
Zwitterion (>C=OH^+)	12.4	—

$(AARAA)H^+ + H_2O$

Zwitterion setup for relay mechanism:

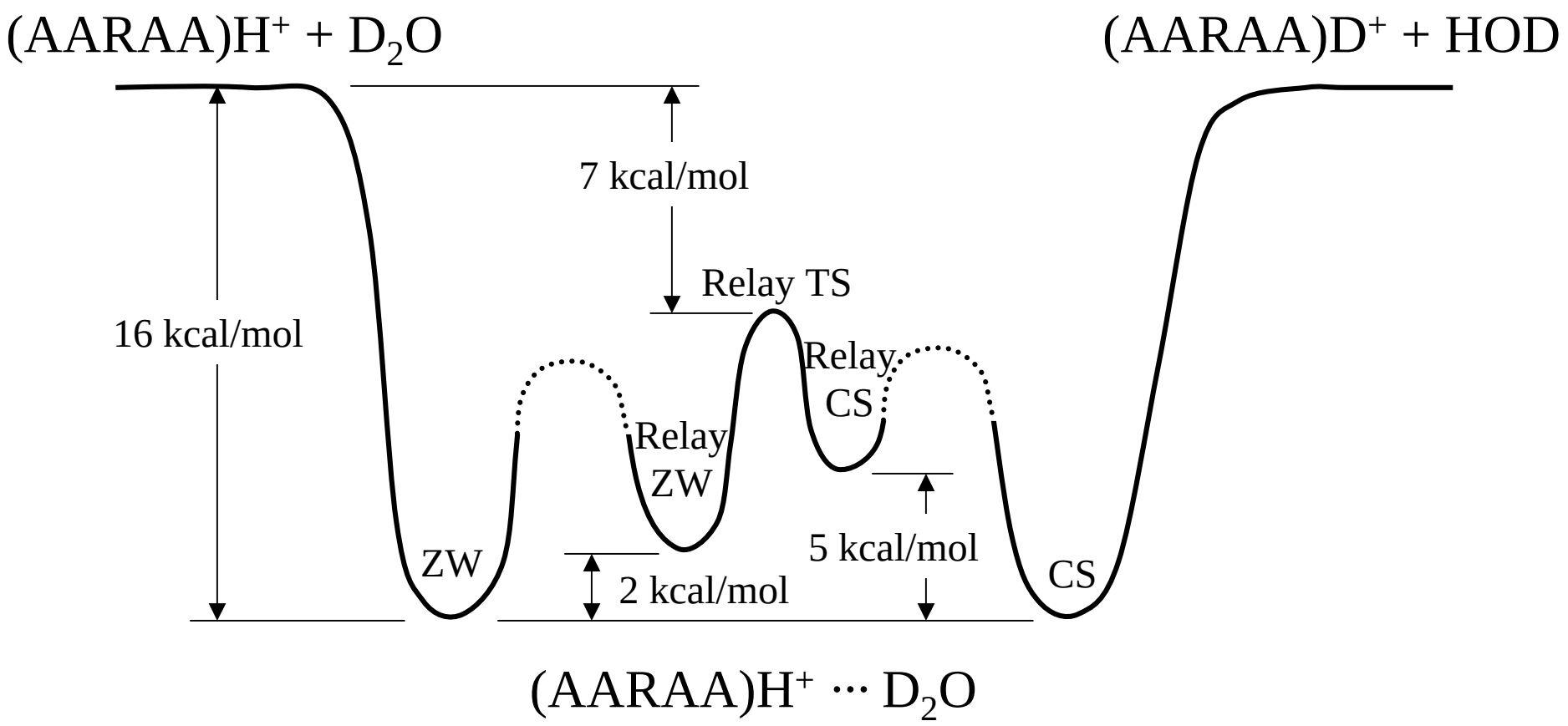
–14.1 kcal/mol



C-terminus

N-terminus

H_2O


(AARAA)H+ + D2O
(AARAA)D+ + HOD
7 kcal/mol
Relay TS
16 kcal/mol
Relay
CS
Relay
ZW
ZW
5 kcal/mol
2 kcal/mol
CS
(AARAA)H+ ··· D2O

Water binding energy

$(AARAA)H^+ \cdots H_2O$

Theory (Heidelberg): 16 kcal/mol

Experiment (UCSB): 10 kcal/mol

(BSSE correction? Larger basis set?)

Summary

H/D-exchange: salt bridge (?)

$(AARAA)H^+\cdots H_2O$

Ion mobility: no salt bridge

$(AARAA)H^+$

Theory:

- no salt bridge for $(AARAA)H^+$
- salt bridge for $(AARAA)H^+\cdots H_2O$
- low TS for H/D-exchange for all 3 N-terminus hydrogens from salt bridge form
- no exchange possible for blocked termini since TS for proton transfer to >C=O groups high in energy

Peptide aggregation

- Important in certain diseases
  - Alzheimers
  - Mad Cow (TSE)
  - Diabetes type II
- Important for chaperone formation
- Observed as (common) occurrence in electrospray of peptides
- Energetics and mechanism not yet known

Self aggregation of peptides in ESI

- Beauchamp et al.: penta-, hexa-peptides
- Clemmer et al.: BK, Insulin chain A, ala_{12}
- Jarrold et al.: ac-lys-ala_{19}, ac-$(gly\text{-}ala)_7$-lys
- Bowers et al.: BK, LHRH, neurotensin

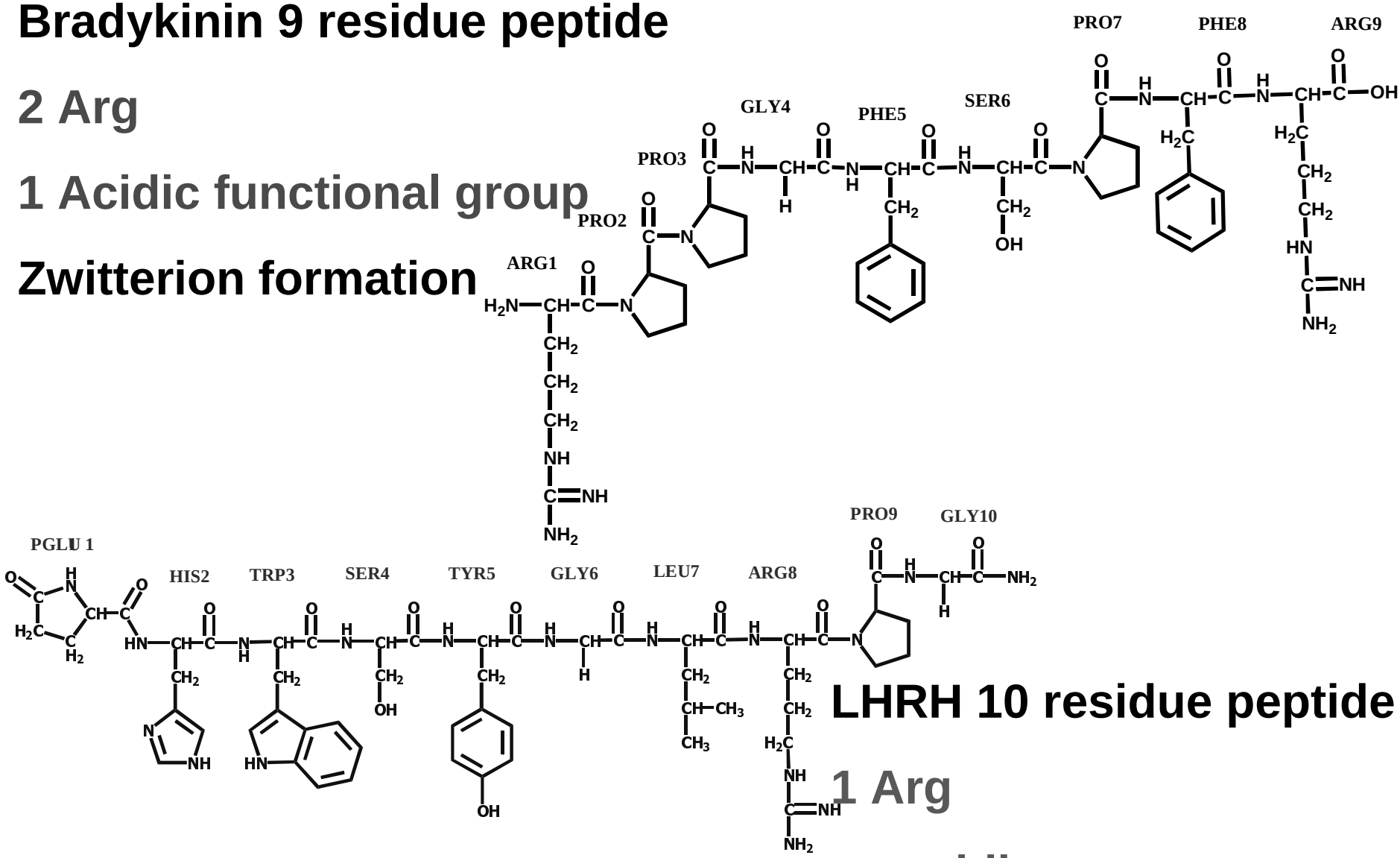
- But little detailed understanding of factors that control this aggregation

Bradykinin 9 residue peptide

2 Arg

1 Acidic functional group

Zwitterion formation

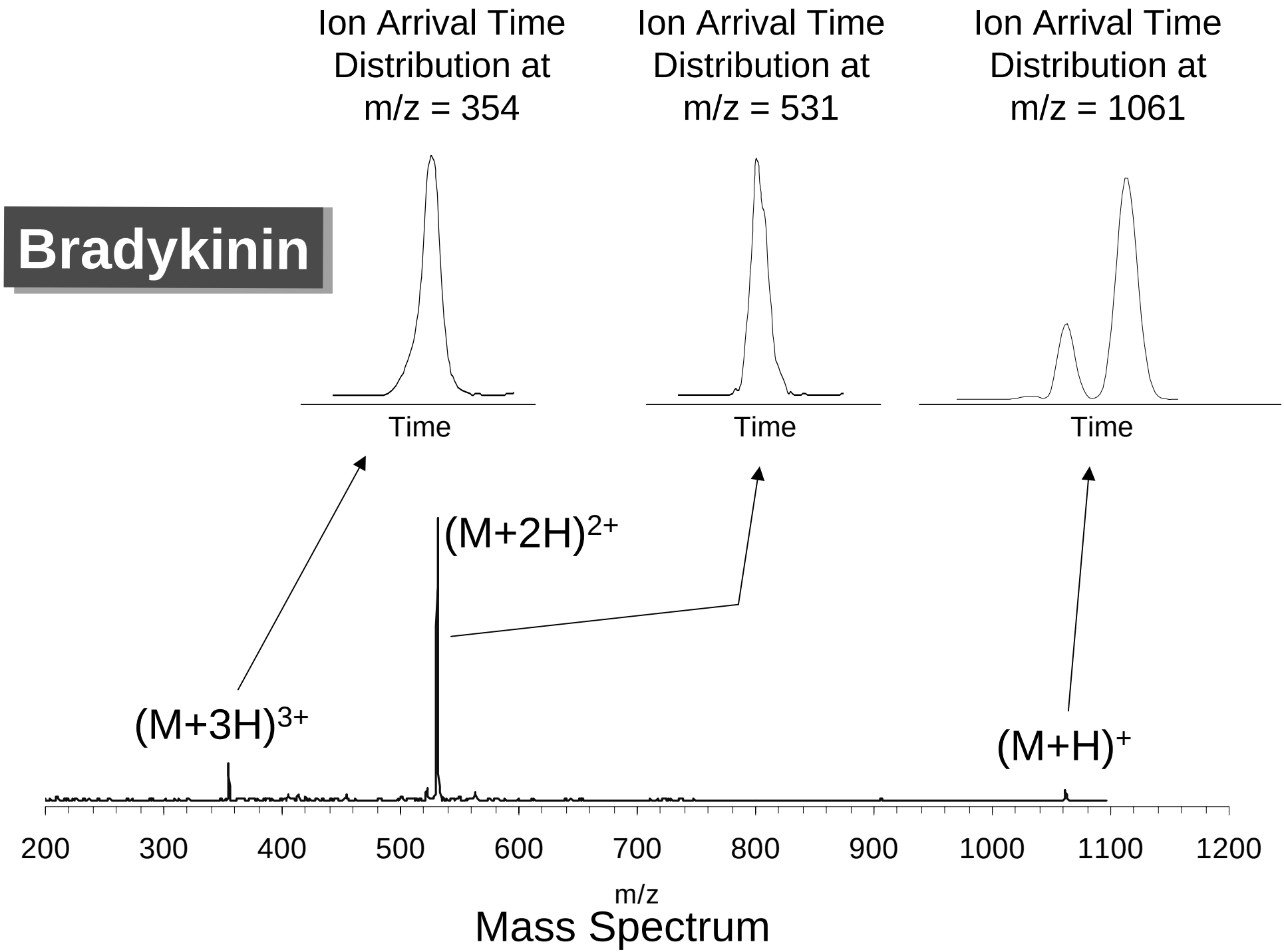


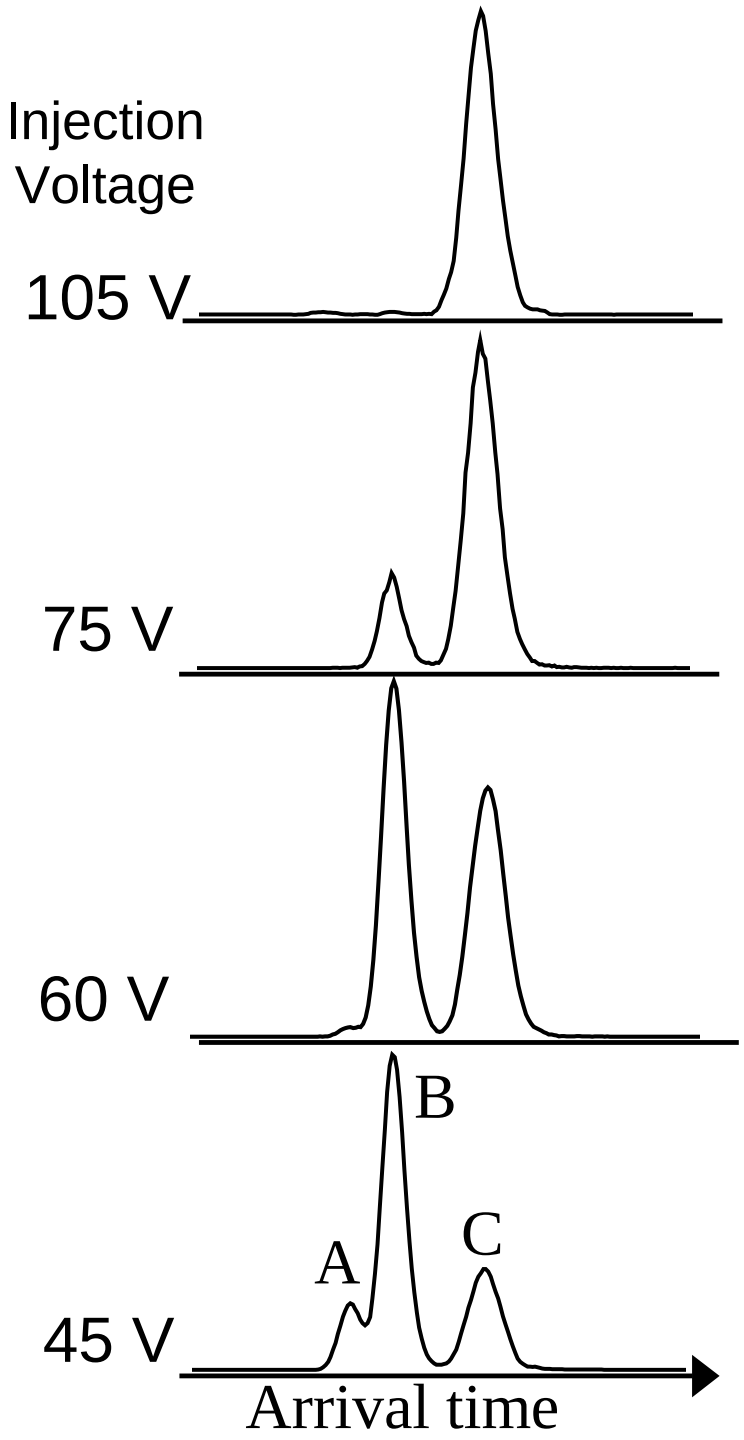
LHRH 10 residue peptide

1 Arg

no acidic group

Blocked Termini


Ion Arrival Time Distribution at m/z = 354
Ion Arrival Time Distribution at m/z = 531
Ion Arrival Time Distribution at m/z = 1061
Bradykinin
Time
Time
Time
(M+2H)2+
(M+3H)3+
(M+H)+
200
300
400
500
600
700
800
900
1000
1100
1200
m/z
Mass Spectrum



Conclude:

A = $(3BK+3H)^{3+}$

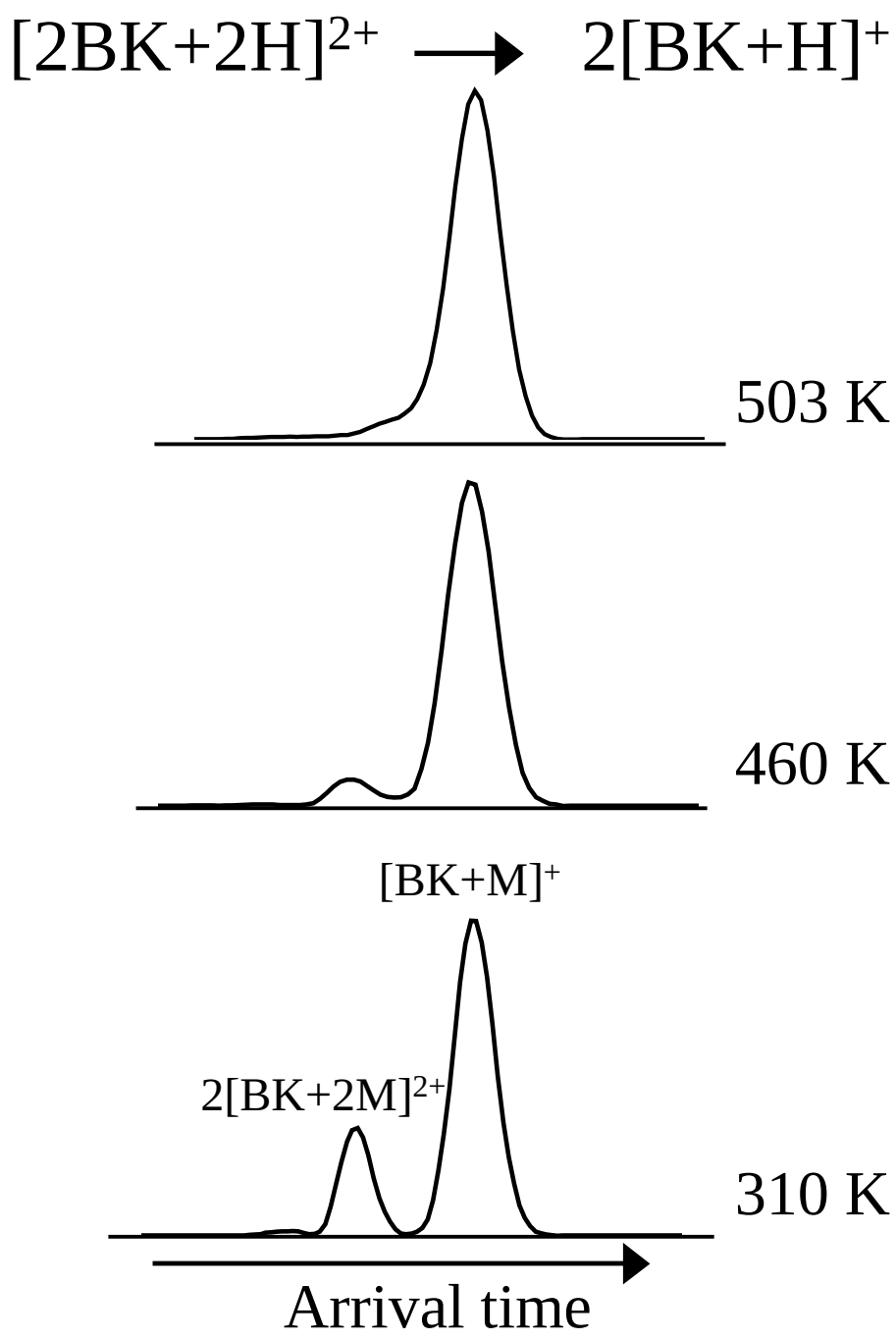
B = $(2BK+2H)^{2+}$

C = $(BK+H)^{+}$

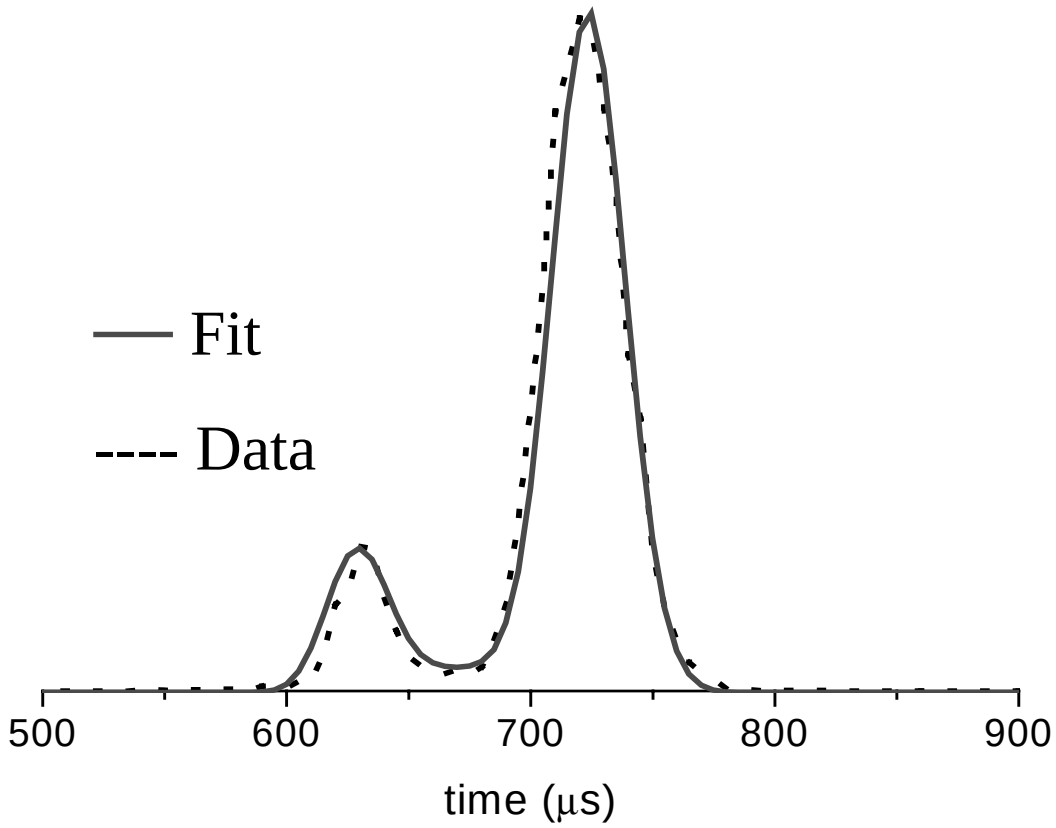
Increased Cell Temperature

$(2M+2H)^{2+} \longrightarrow 2(M+H)^{+}$ dissociation

- Fitting the ATDs yield rate constants
- Binding energies (E_a) are obtained from an Arrhenius type of analysis

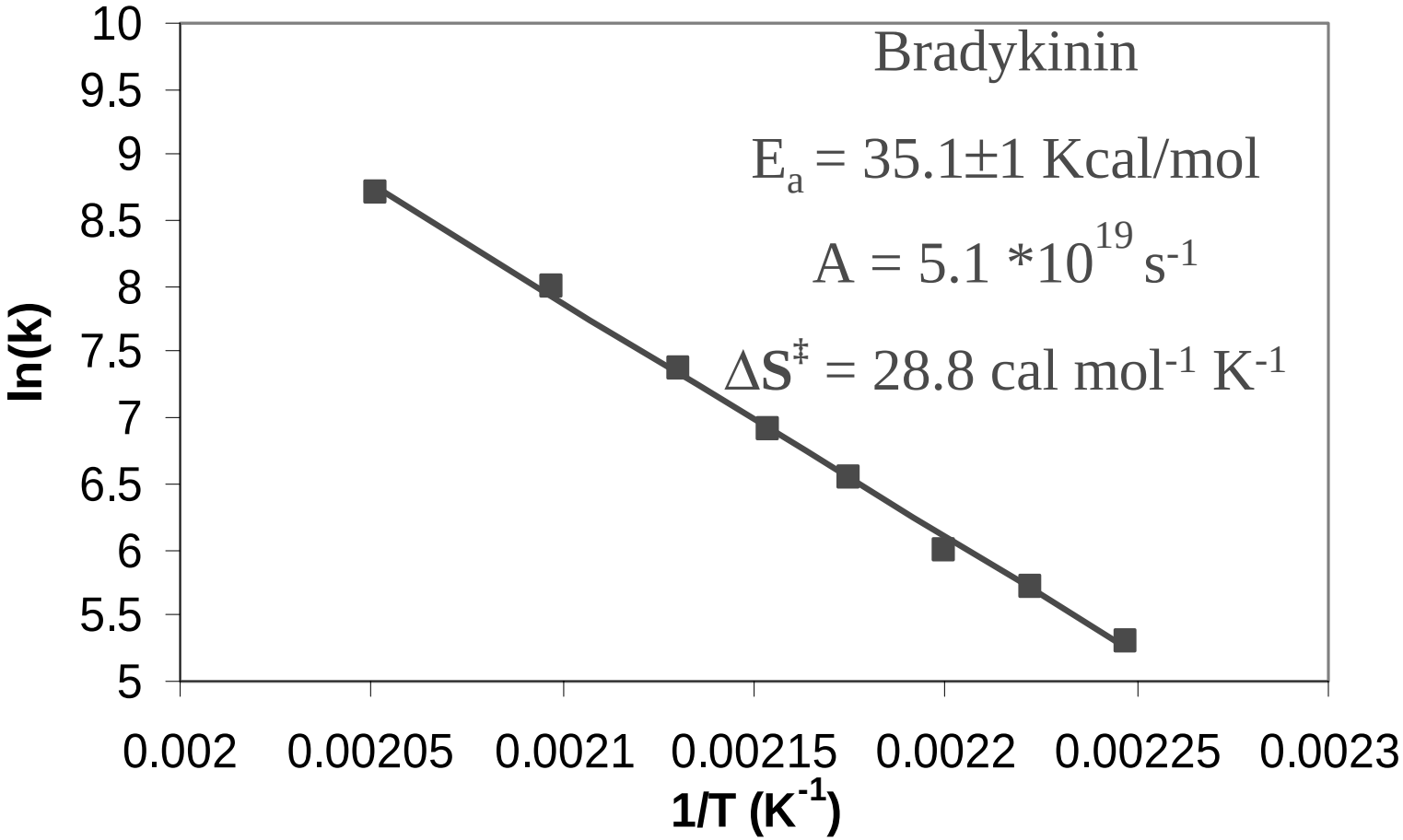

[2BK+2H]2+
2[BK+H]+
503 K
460 K
[BK+M]+
2[BK+2M]2+
310 K
Arrival time

BK Fit

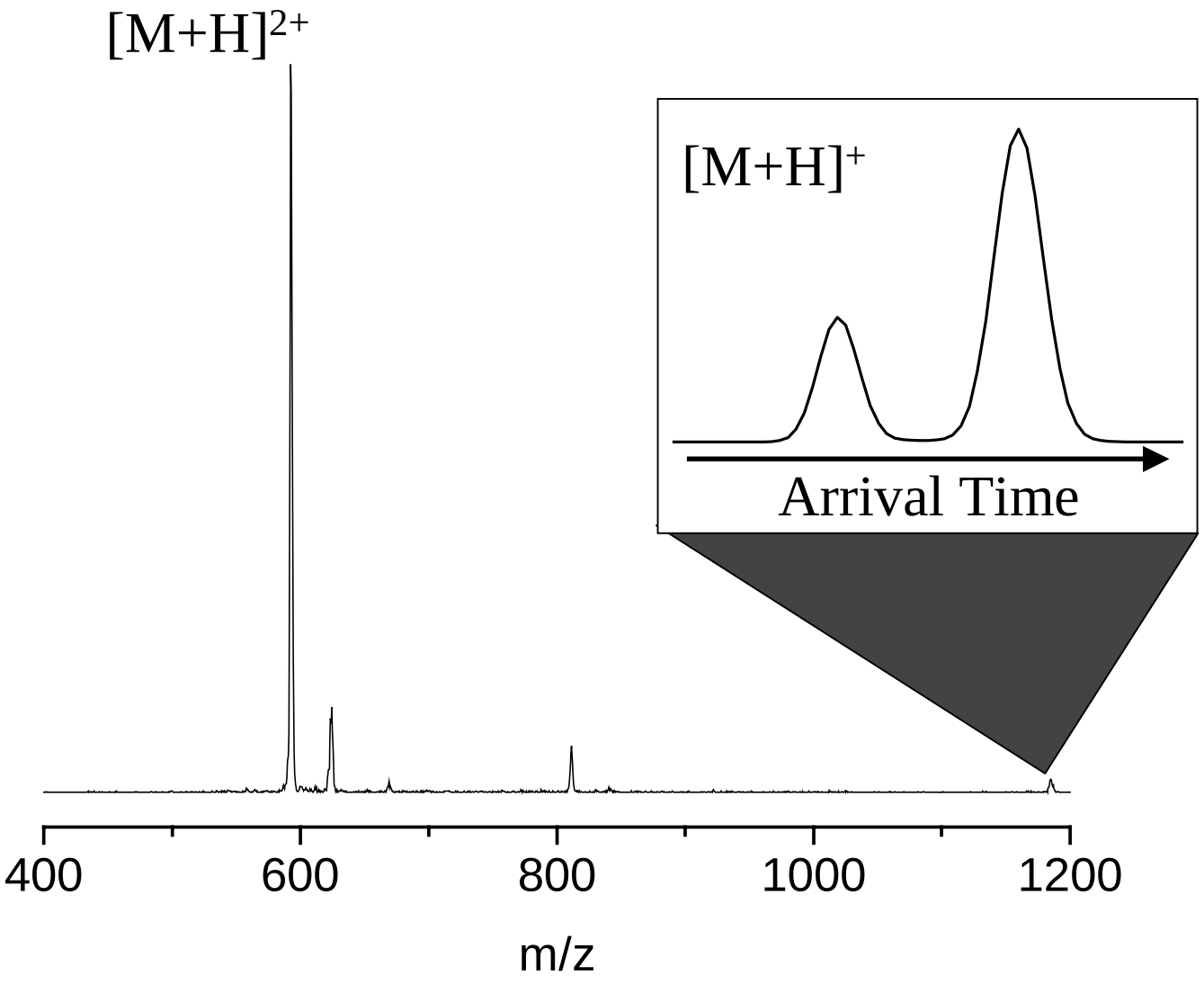


Arrhenius Plot

$(2M+2H)^{2+} \longrightarrow 2(M+H)^{+}$

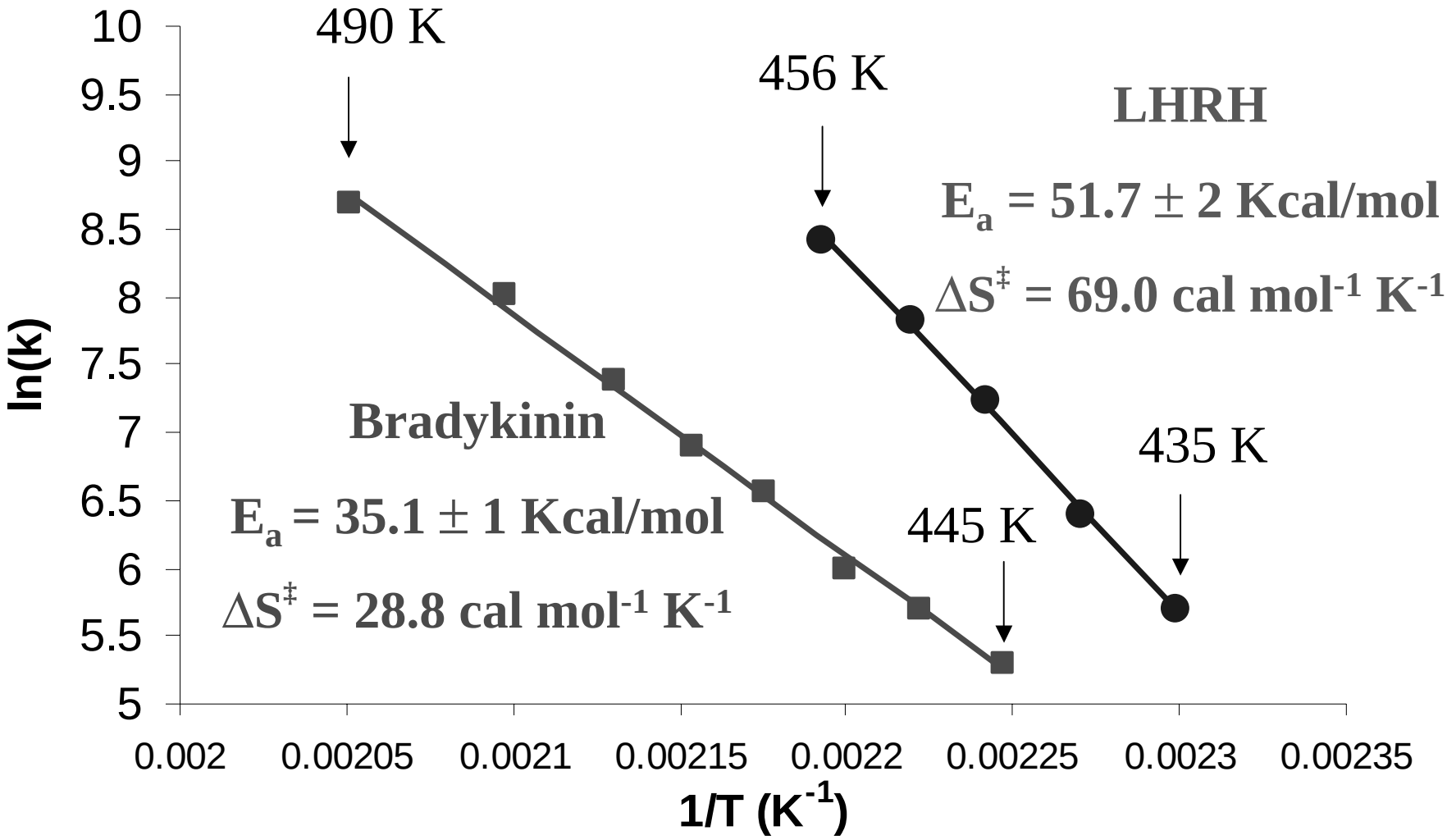


LHRH



Arrhenius Plot

$$(2M+2H)^{2+} \longrightarrow 2(M+H)^{+}$$

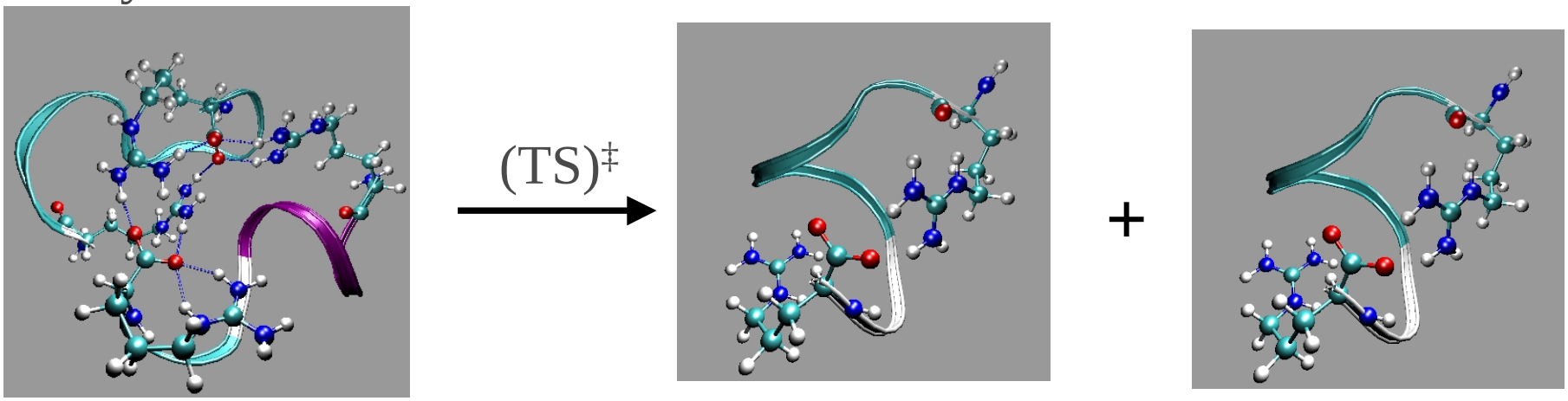


Molecular modeling

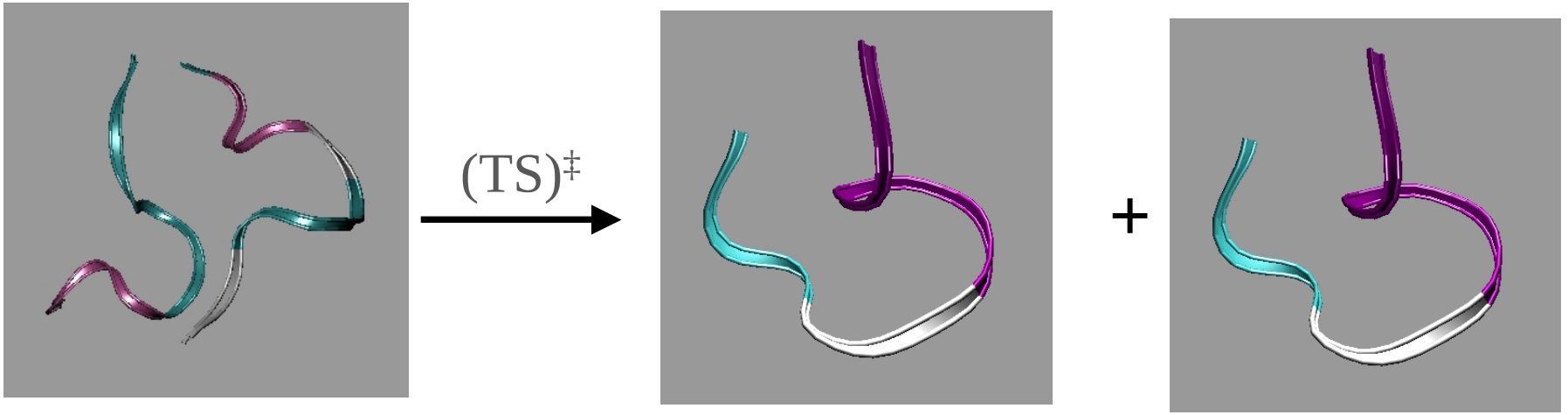
- Simulated annealing
- Dynamics simulations (300 K)

$(2M+2H)^{2+} \xrightarrow{(TS)^{\ddagger}} 2(M+H)^{+}$

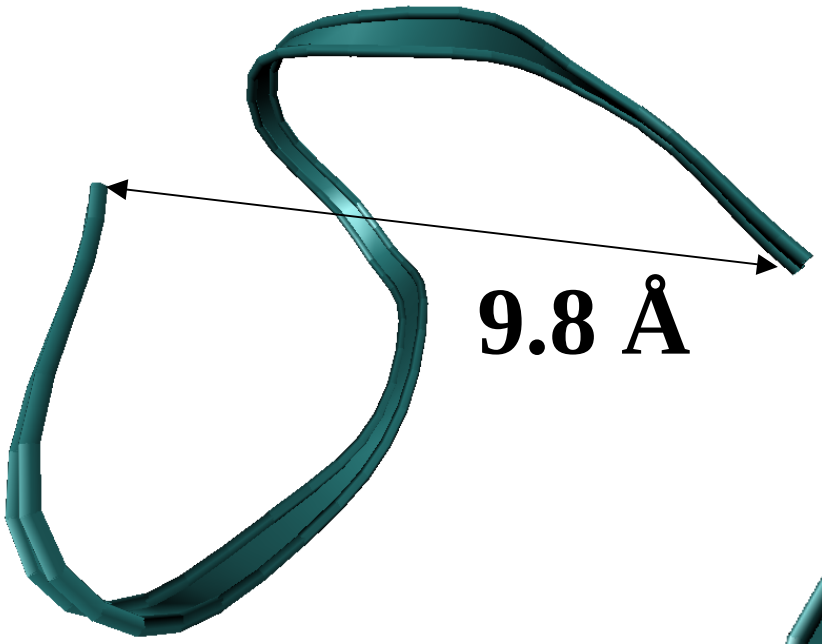
Bradykinin

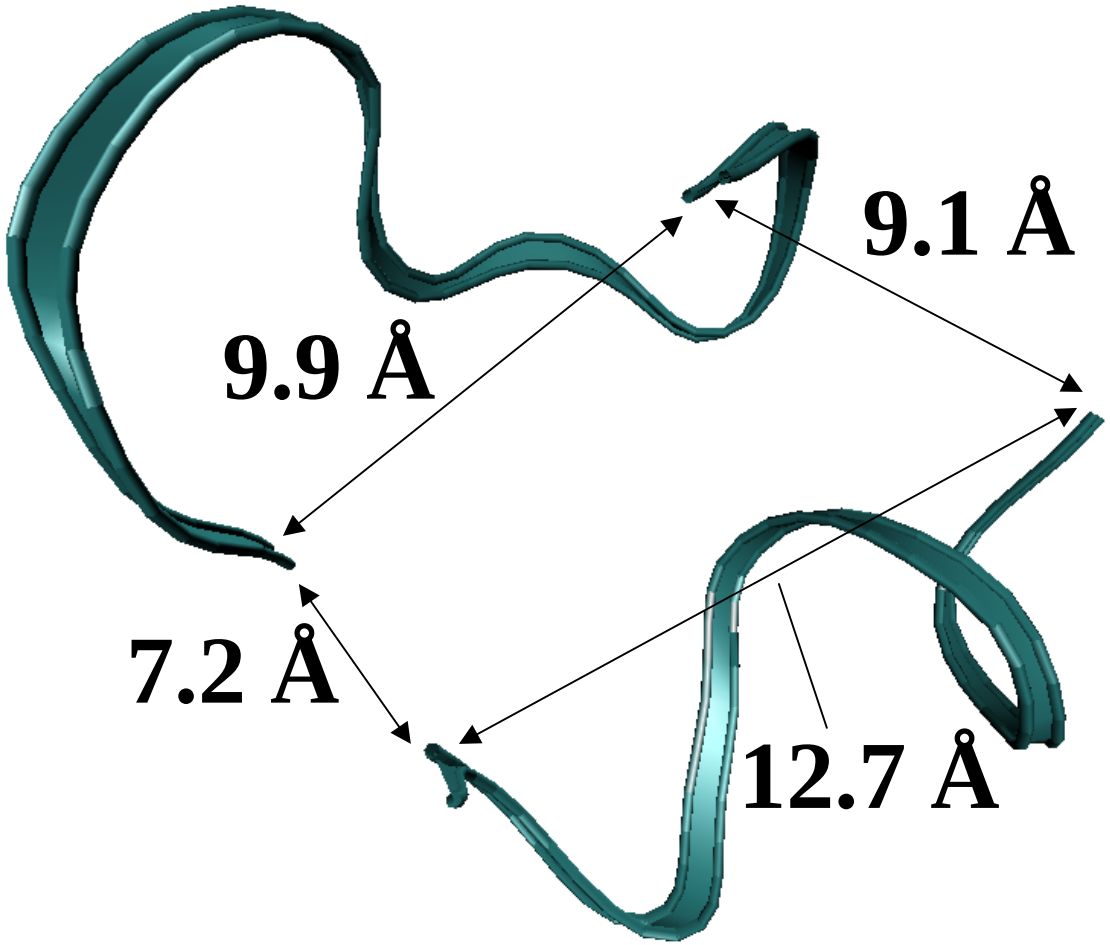


LHRH



BK Monomer vs. Dimer

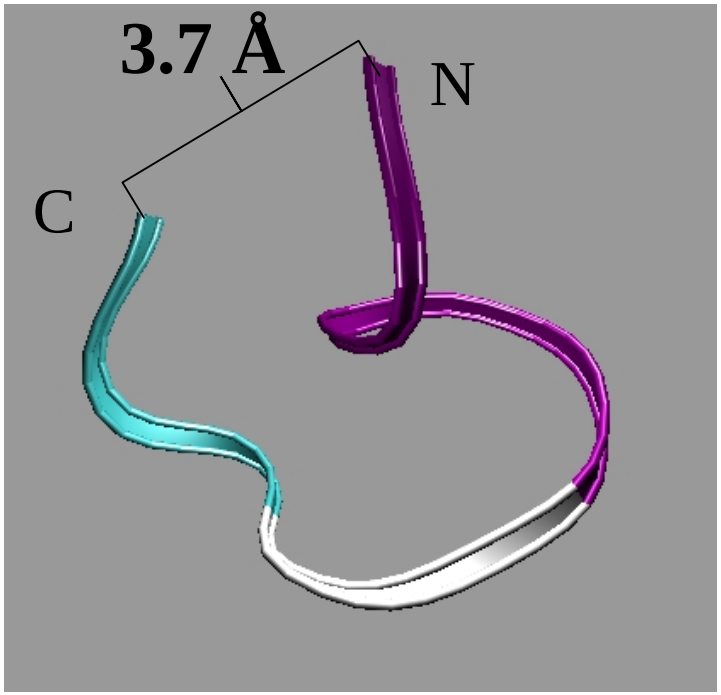


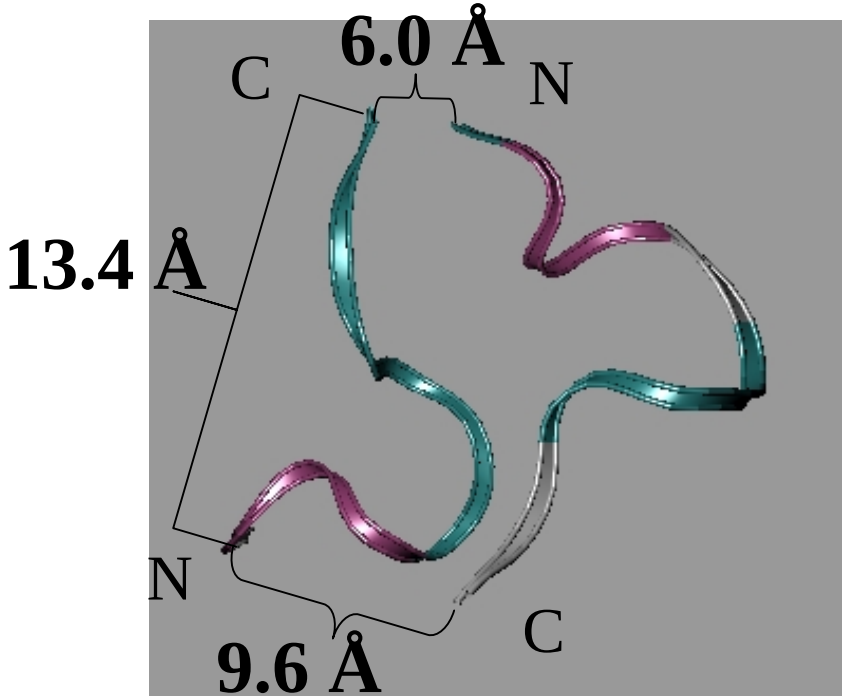


Monomers only slightly rearranged in dimer

Electrostatic binding between charged groups dominates

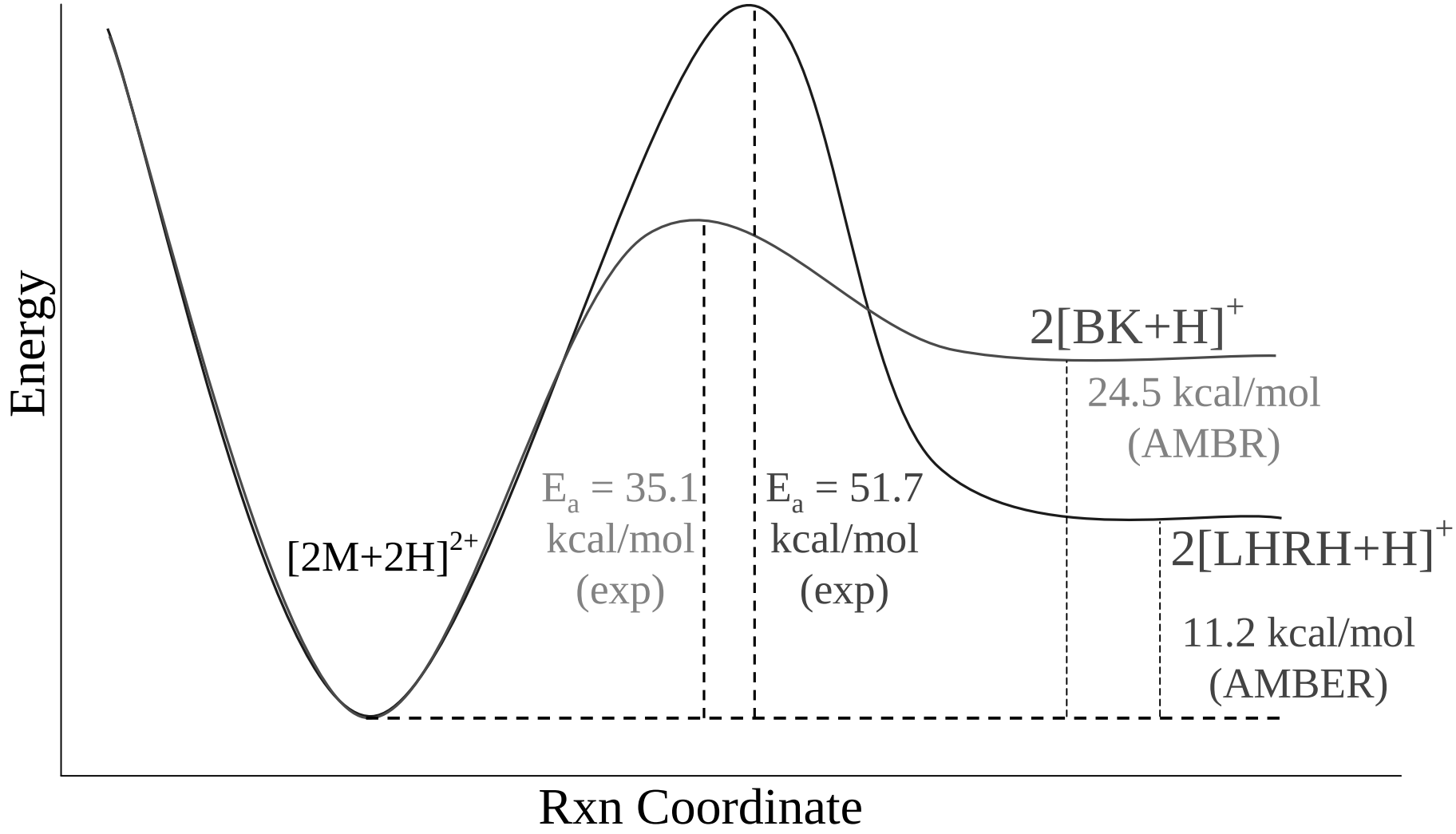
LHRH Monomer vs. Dimer





- Intermolecular interactions in dimer force open the backbone of the monomer
- Causes repulsion of the 2 N-termini and attraction between opposing N and C termini

Hypothesized Potential Energy Surface



Do our calculated structures agree with experiment? Compare Cross Sections ($Å^2$)

	Experiment	Theory*	$\Delta\sigma$ (%)
$[LHRH+H]^+$	257	261	+1.5
$[2LHRH+2H]^{2+}$	409	417	+1.9
$[BK+H]^+$	240	242	+0.8
$[2BK+2H]^{2+}$	384	376	–2.1

* minimized structures (lowest 10 kcal/mol)

Conclusions

- Peptides tend to form aggregates of the form $(zM+zH)^{z+}$.
- Energy barriers for dimer dissociation are of the order of 30-50 kcal/mol both for zwitterion (BK) and charge solvation structures (LHRH).
- Entropies (and energies) of activation indicate a major structural change occurs in TS for LHRH (i.e. a loose TS) while the TS for BK is much more dimer like (i.e. tighter).

Are salt bridges involved in the fragmentation of oligonucleotides?

Mass spectrometric analysis of DNA crucial for obtaining rapid information on small samples

Problem: DNA fragments during the sampling process

Solution: Understand the fragmentation mechanism

Proposed mechanism:

1. protonation of base
2. weaken / break base–sugar bond
3. eliminate base and induce backbone fragmentation

Proton affinities of the 4 DNA bases:

G > A C >> T

Hillenkamp, Gross:

Look at fragmentation of oligonucleotides with T and G bases

should see preferential loss of G

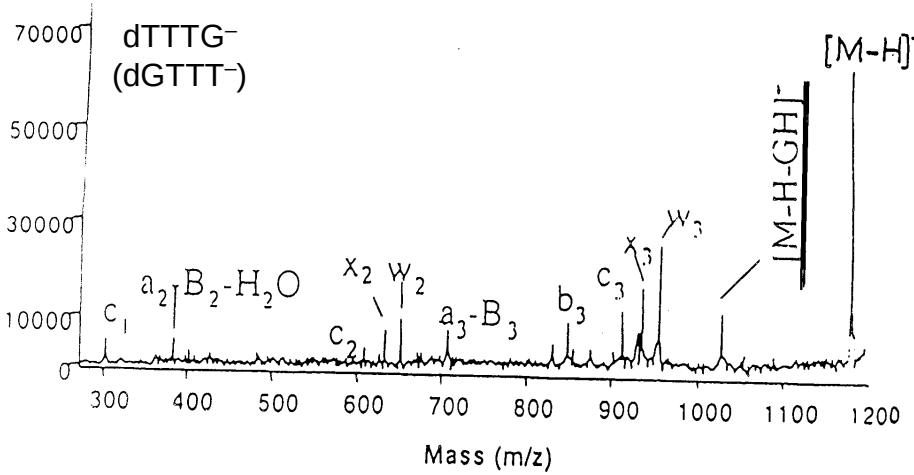
dGTTT, dTGTT, dTTGT, dTTTG

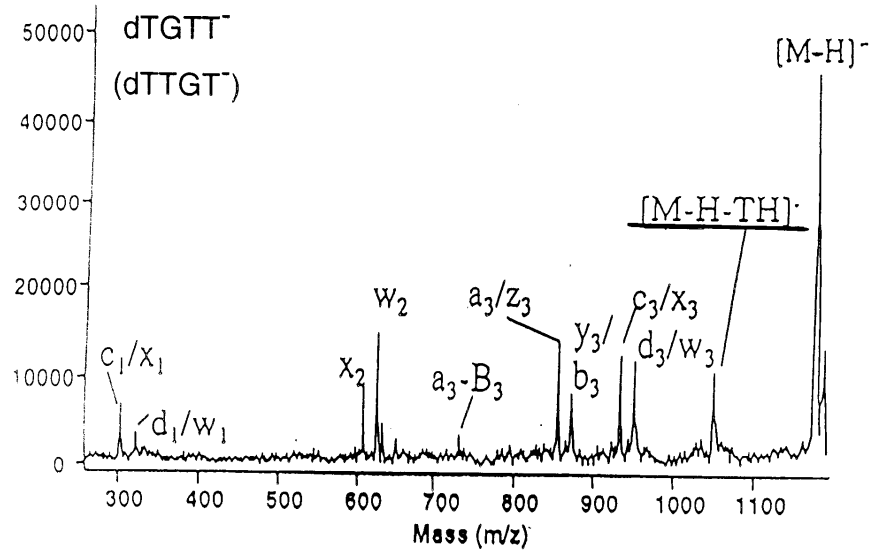
positive ions: see loss of G in all cases

but for negative ions …

MALDI PSD Mass Spectra for

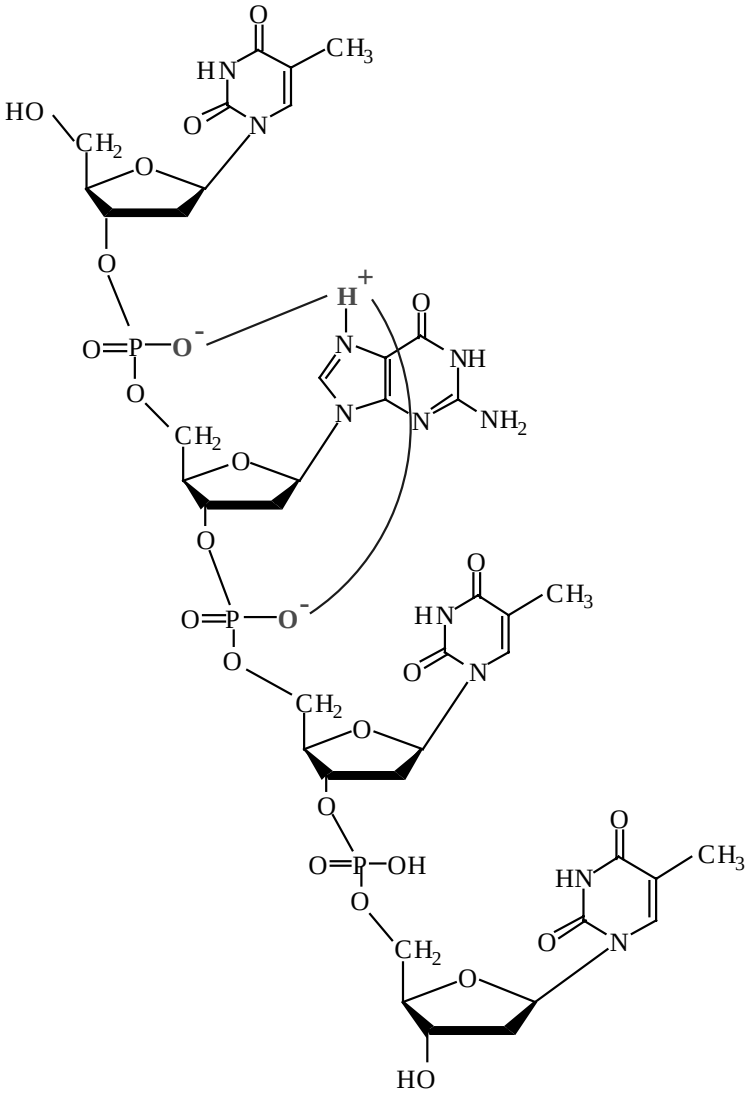
Deprotonated Tetranucleotides





J. Gross, F. Hillenkamp, K.X. Wan, M.L. Gross
J. Am. Soc. Mass Spectrom. **2001**, *12*, 180.

ossible explanation: dTGTT and dTTGT form salt bridges

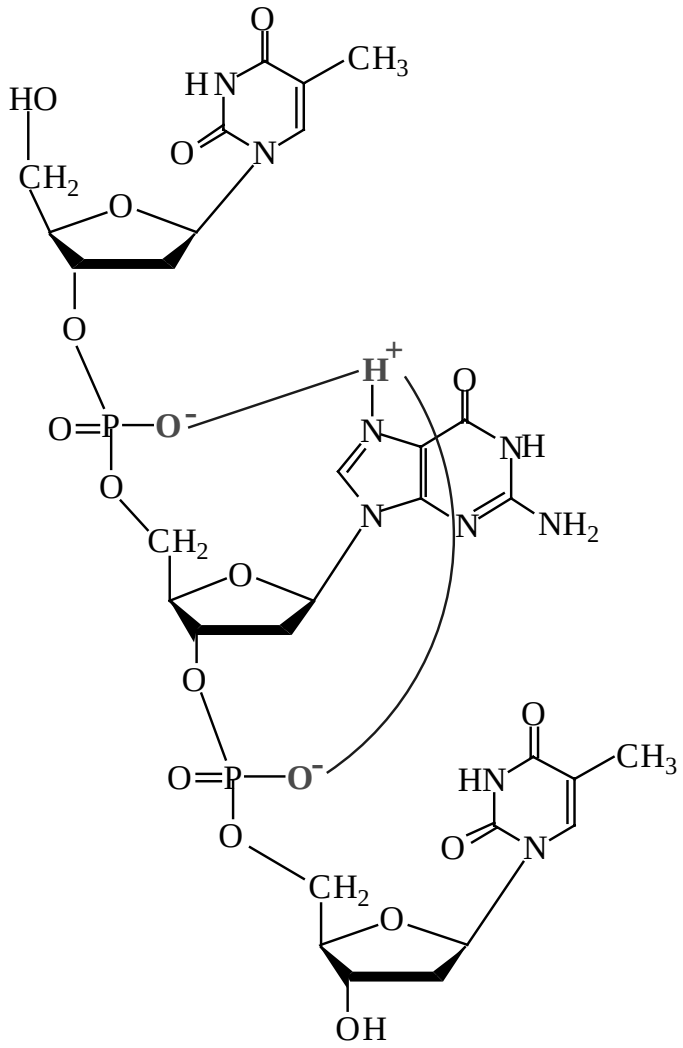


J. Gross, F. Hillenkamp, K.X. Wan, M.L. Gross

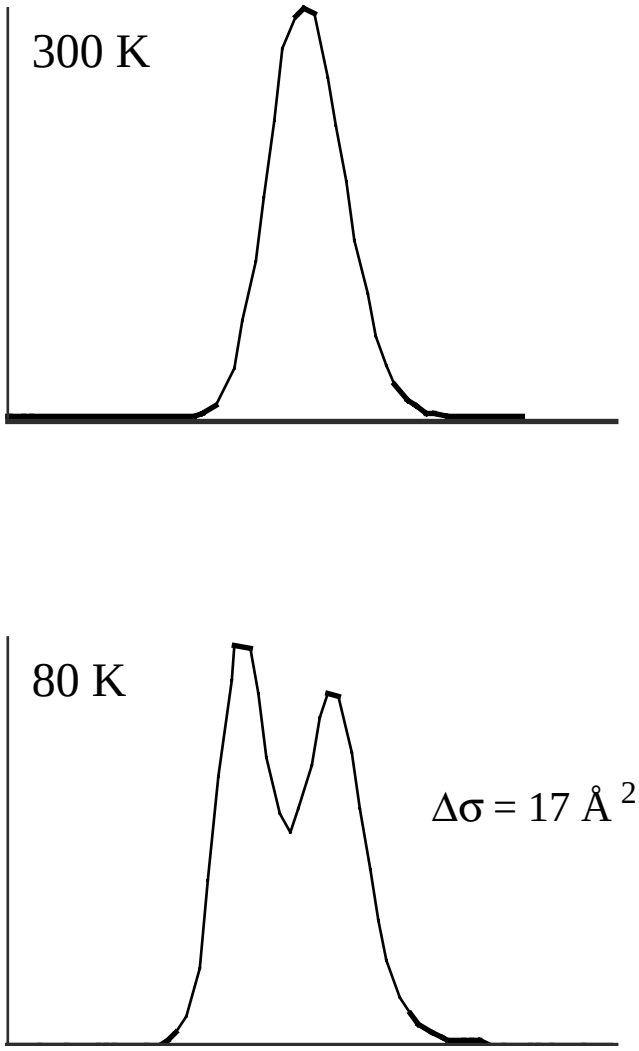
J. Am. Soc. Mass Spectrom. **2001** , *12*, 180

Look at a simpler model with same possibility of salt bridge formation

Is dTGT $^{-}$ a salt bridge?

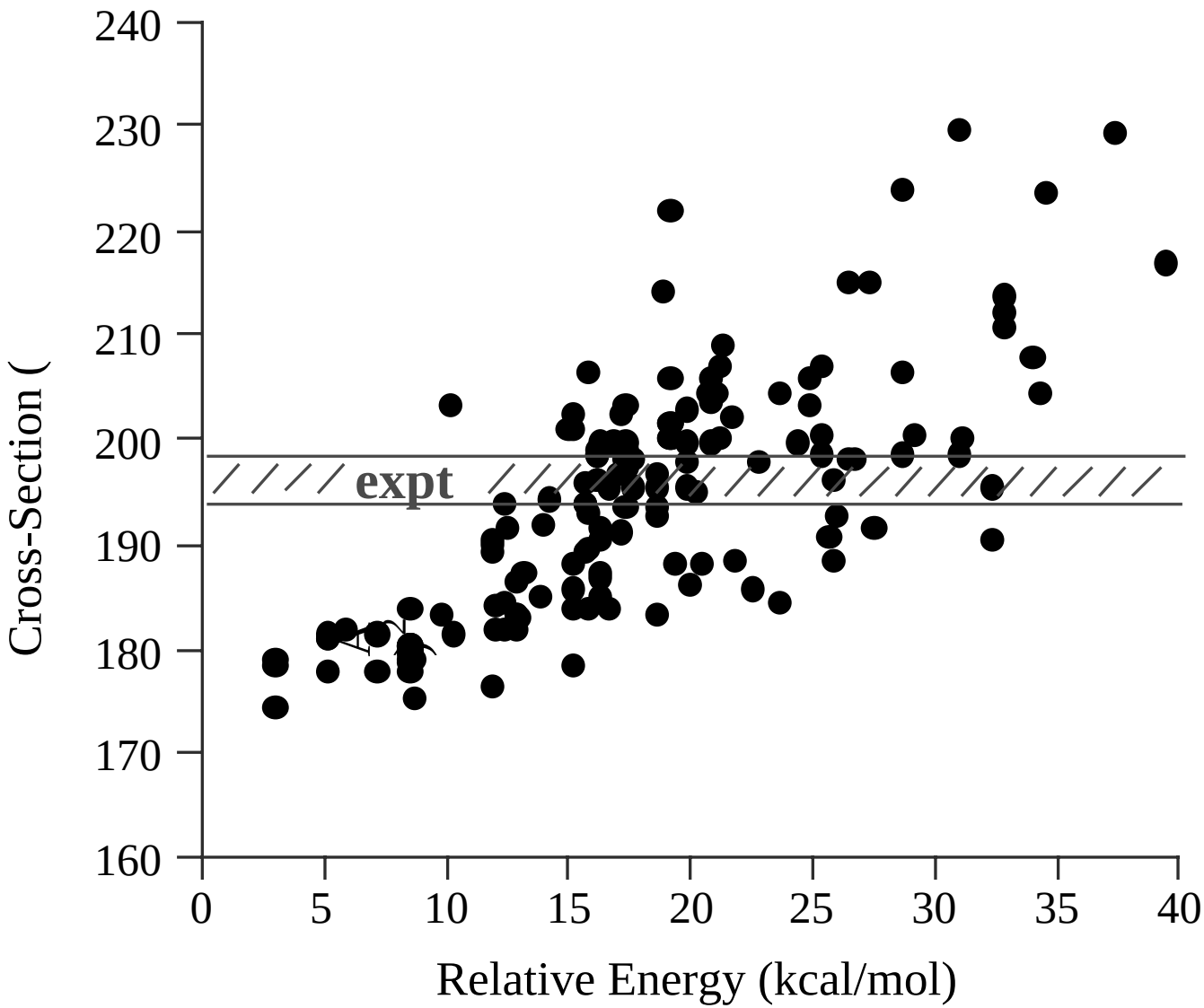


ATDs for dTGT $^-$



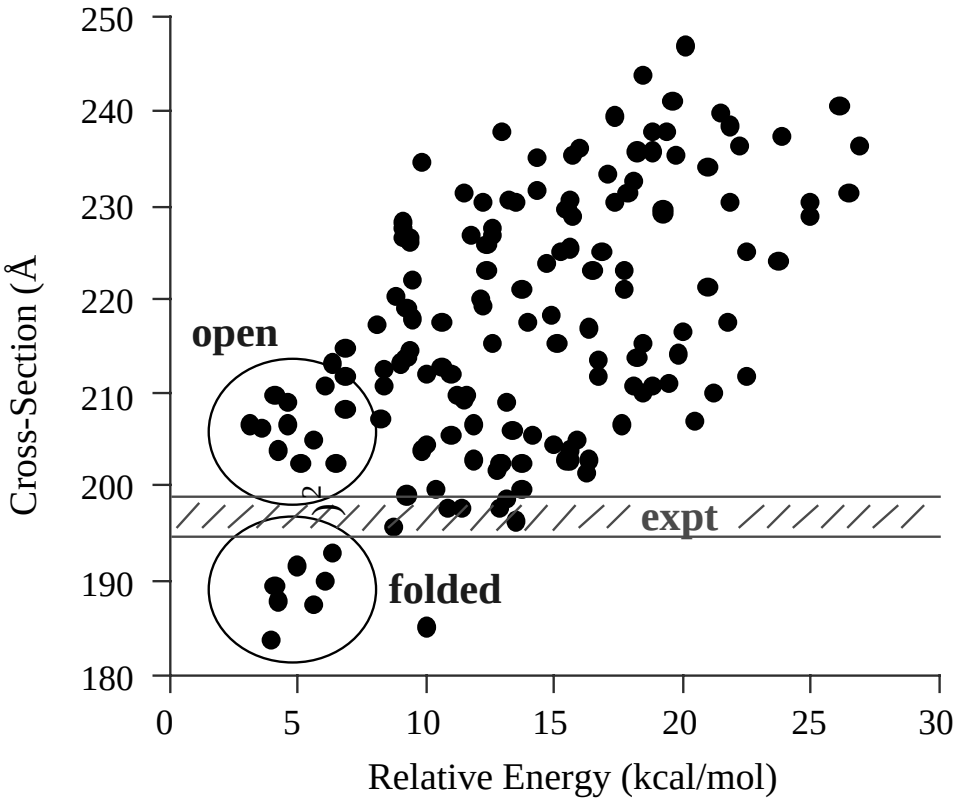
Scatter Plot for dTGT -

(salt bridge)



Scatter Plot for dTGT $^-$

(singly deprotonated)



2 families of low-energy conformers

"folded" smaller cross-section

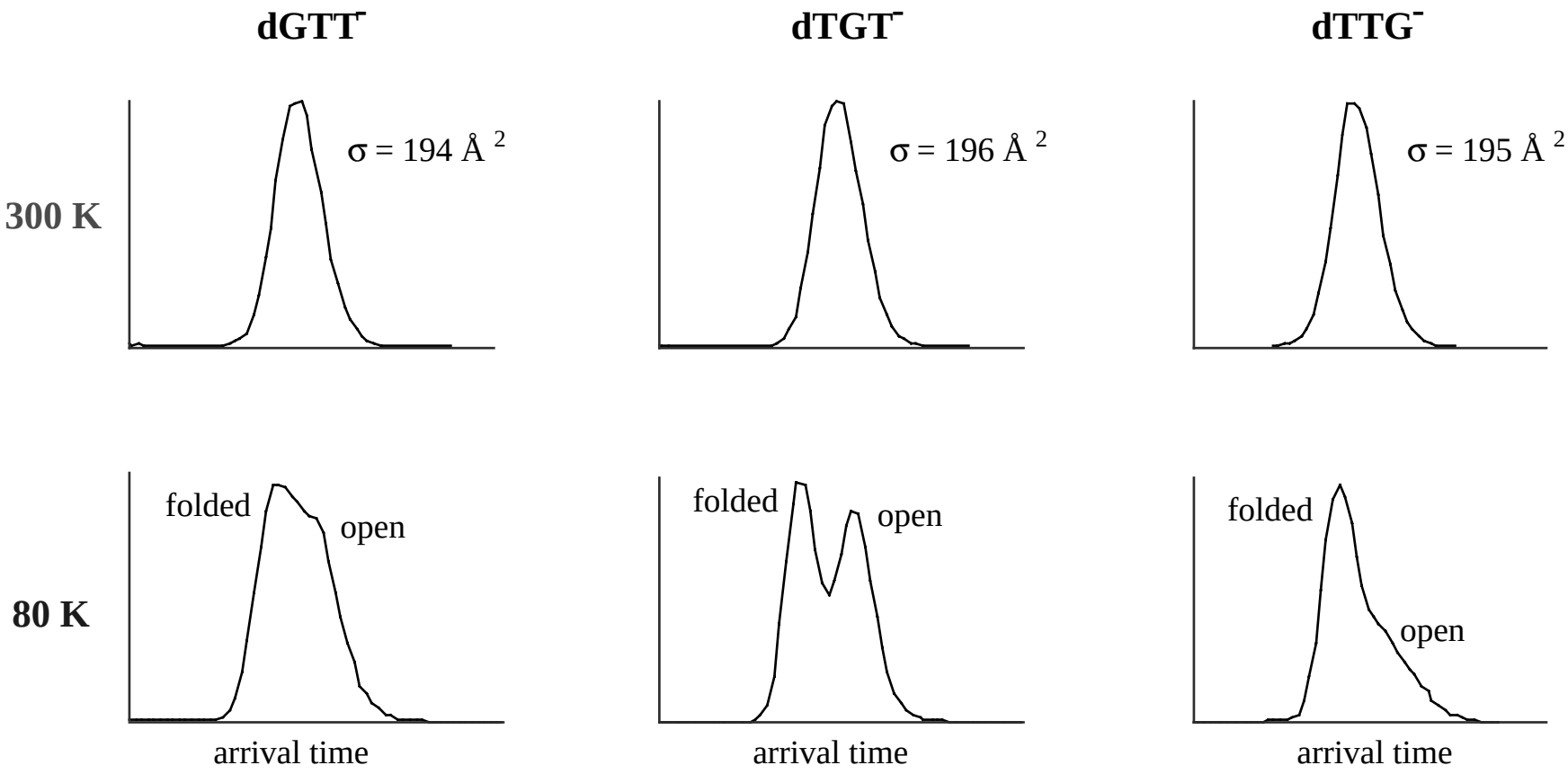
"open" larger cross-section

$\Delta\sigma \sim 18$ Å ^{2}

$\Delta\sigma_{expt} \sim 17$ Å ^{2}

Similar ATDs for dGTT $^-$ and dTTG $^-$

(which should <u>not</u> be salt bridges)



Conclusions :

- dTGT$^-$ not a salt bridge

  ∴ dTGTT$^-$ and dTTGT$^-$ also not salt bridges

  ∴ Gross / Hillenkamp mechanism may not be correct

  (or salt bridge is type of transition state)

- Preliminary calculations indicate salt bridge structure is higher in energy

- Hydration Studies being initiated

Final conclusions: salt bridges in the gas phase

- Small peptides and oligonucleotides are generally not salt bridges.
- However, salt bridges can be energetically close to charge solvation structures.
- Salt bridges are stabilized (compared to CS) by additional solvation.
- Salt bridges are important intermediates (H/D exchange, fragmentation).
- Little structural change occurs within a salt bridge unit when units aggregate.