REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)* 03/11/2015	**2. REPORT TYPE** Conference Publication	**3. DATES COVERED** *(From - To)* 2014-2015

4. TITLE AND SUBTITLE
Direct Measurement of the Isomerization Barrier of the Isolated Retinal Chromophore

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jonathan Dilger, Lihi Musbat, Mordechai Sheves, Anastasia V. Bochenkova, David E. Clemmer and Yoni Toker

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Surface Warfare Center, Crane Division
Code WXRP, Building 3323
300 Highway 361
Crane, IN 47522-5001

8. PERFORMING ORGANIZATION REPORT NUMBER
NSWCCR/RDTR-15/57

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Naval Innovative Science and Engineering Program

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT A. Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Ion mobility spectroscopy (IMS) allows one to differentiate between different isomers of a given molecular ion according to their collisional cross-section. Using two stages of IMS (IMS-IMS) one can select a specific isomer, collisionally heat it and follow its isomerization pathways. Recently it has been shown that this technique allows one to determine internal energy barrier for isomerization. Here we apply the technique to the important case of the retinal protonated Schiff base (RPSB). Photoisomerization of the RPSB is the primary in animal vision. We find that the energy barrier for a single *cis-trans* isomerization is 0.64 ± 0.05 eV, which is significantly lower than that observed for the reaction within opsin proteins. Thus the protein has a significant role in increasing the barrier energy for thermal isomerization relative to the gas phase which lacks interaction with the RPSB counterion and steric constraints. High barrier energy is mandatory for efficient vision processes, otherwise thermal noise would overwhelm the signal originating from the photochemical isomerization.

15. SUBJECT TERMS
ion mobility spectrometry, mass spectrometry, retinal, chromophore, isomerization energy

16. SECURITY CLASSIFICATION OF: Unclassified			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** Jonathan Dilger
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	1	**19b. TELEPHONE NUMBER** *(include area code)* 1-812-854-1452

XXIX International Conference on Photonic, Electronic, and Atomic Collisions (ICPEAC2015) IOP Publishing
Journal of Physics: Conference Series **635** (2015) 032034 doi:10.1088/1742-6596/635/3/032034

Direct Measurement of the Isomerization Barrier of the Isolated Retinal Chromophore

Jonathan Dilger[A,B], **Lihi Musbat**[C], **Mordechai Sheves**[D],
Anastasia V. Bochenkova[E], **David E. Clemmer**[B] **and Yoni Toker** [C, 1]

[A] Spectrum Warfare Systems Department, NSWC Crane Division Crane, IN 47522 (USA)
[B]Department of Chemistry, Indiana University, Bloomington, Indiana 47405, USA
[C]Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat-Gan 290002, Israel
[D]Chemistry Department, Weizmann Institute of Science, Rehovot 978007, Israel
[E]Department of Physics and Astronomy, Aarhus Universi-ty, DK-8000 Aarhus C, Denmark

Synopsis Energy barrier Heights for isomerization of the isolated retinal chromophore were measured using two stages of ion mobility spectroscopy (IMS-IMS).

Ion mobility spectroscopy (IMS) allows one to differentiate between different isomers of a given molecular ion according to their collisional cross-section. Using two stages of IMS (IMS-IMS) one can select a specific isomer, collisionally heat it and follow its isomerization pathways (See Fig. 1). Recently it has been shown that this technique allows one to determine internal energy barrier for isomerization [1].

Here we apply the technique to the important case of the retinal protonated Schiff base (RPSB) [2]. Photoisomerization of the RPSB is the primary in animal vision. We find that the energy barrier for a single *cis-trans* isomerization is 0.64±0.05 eV, which is significantly lower than that observed for the reaction within opsin proteins. Thus the protein has a significant role in increasing the barrier energy for thermal isomerization relative to the gas phase which lacks interaction with the RPSB counter-ion and steric constraints. High barrier energy is mandatory for efficient vision processes, otherwise thermal noise would overwhelm the signal originating from the photochemical isomerization.

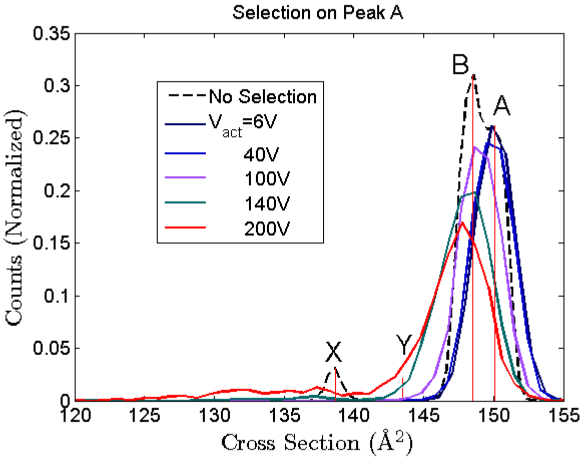


Figure 1. Results of selection and activation when the selection is applied to peak A (the *all-trans* isomer), and activation is performed for different activation voltages, V_{act}. The dashed line corresponds to the IMS of the RPSB with no selection.

References

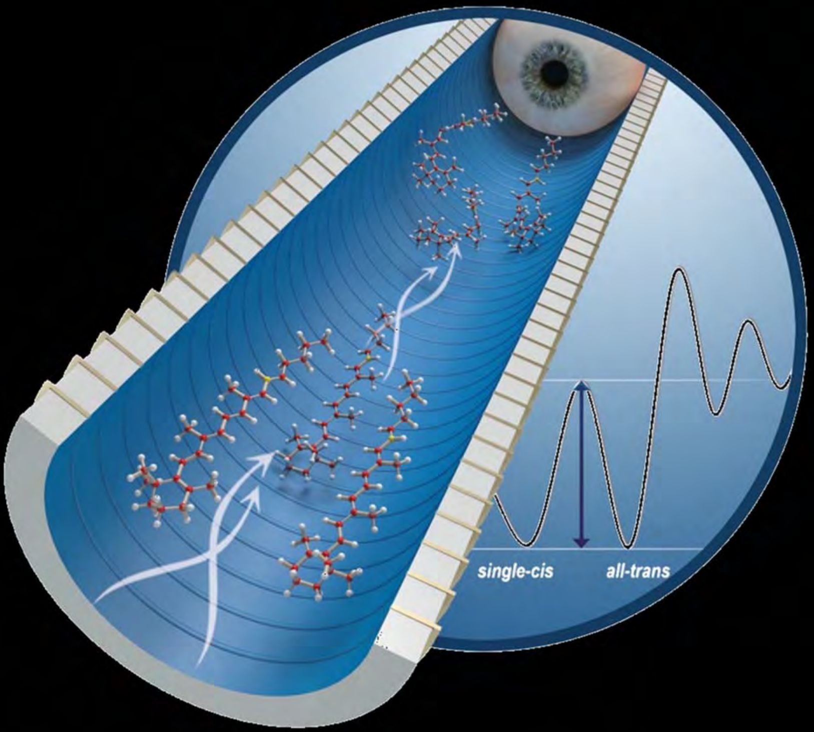
[1] N. A. Pierson, S. J. Valentine, D. Clemmer 2015 Int. J. Mass. Spectrom, **377**, 646-654.
[2] J. Dilger. Y. Toker *et al*. 2015 Ang. Chemie Int. Ed. **127**, 4830-4834.

---

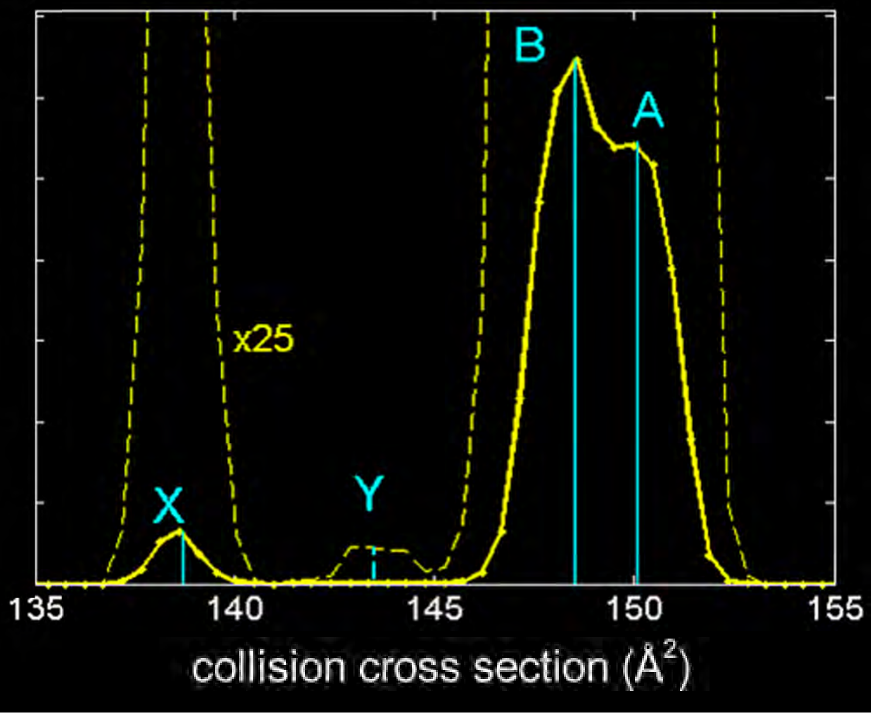
[1] E-mail: yonitoker@gmail.com

Direct Measurement of the Isomerization Barrier of the Isolated Retinal Chromophore

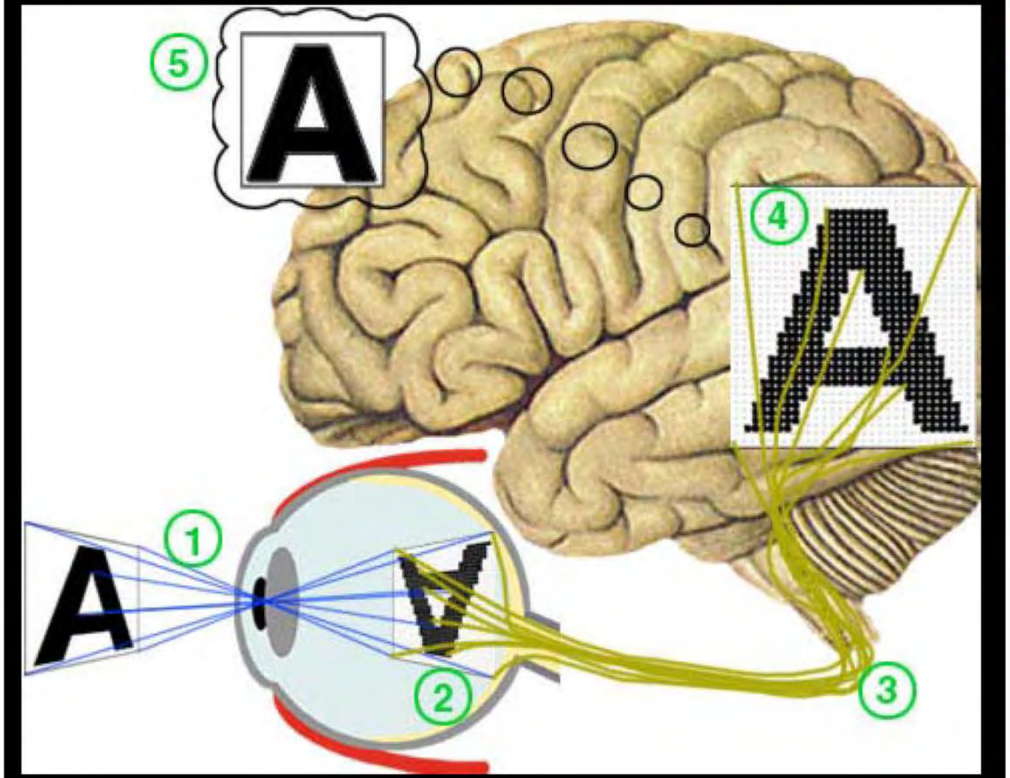
Dr. Jonathan M. Dilger
XXIX ICPEAC
July 23, 2015

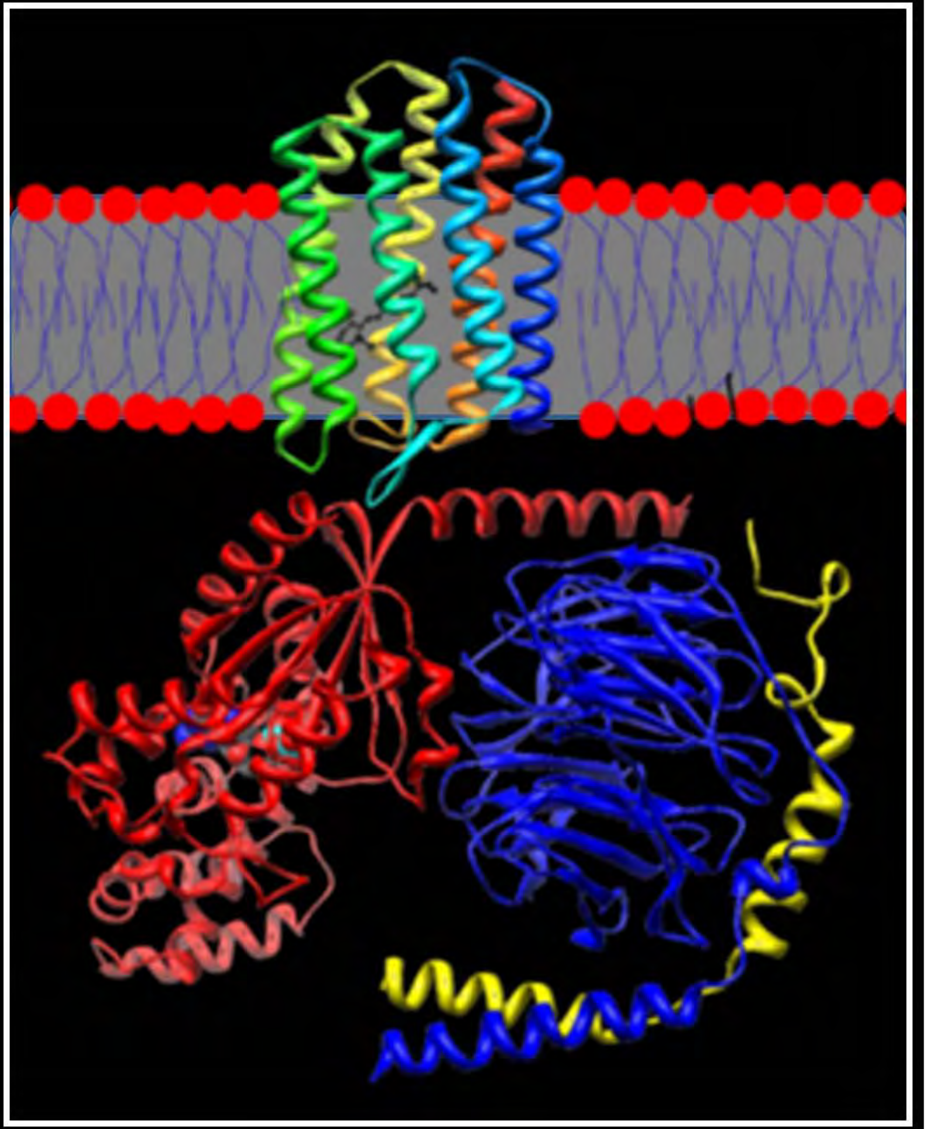


Retinal: $[RPSB+H]^+$



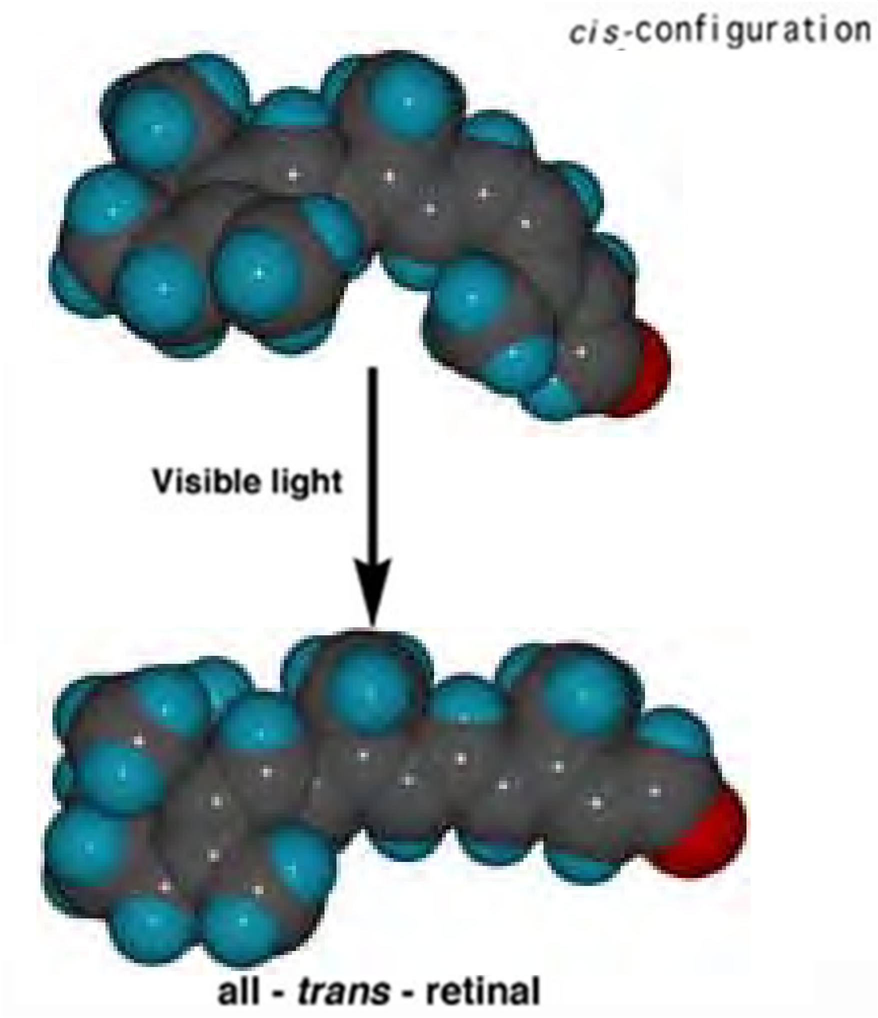
Vision

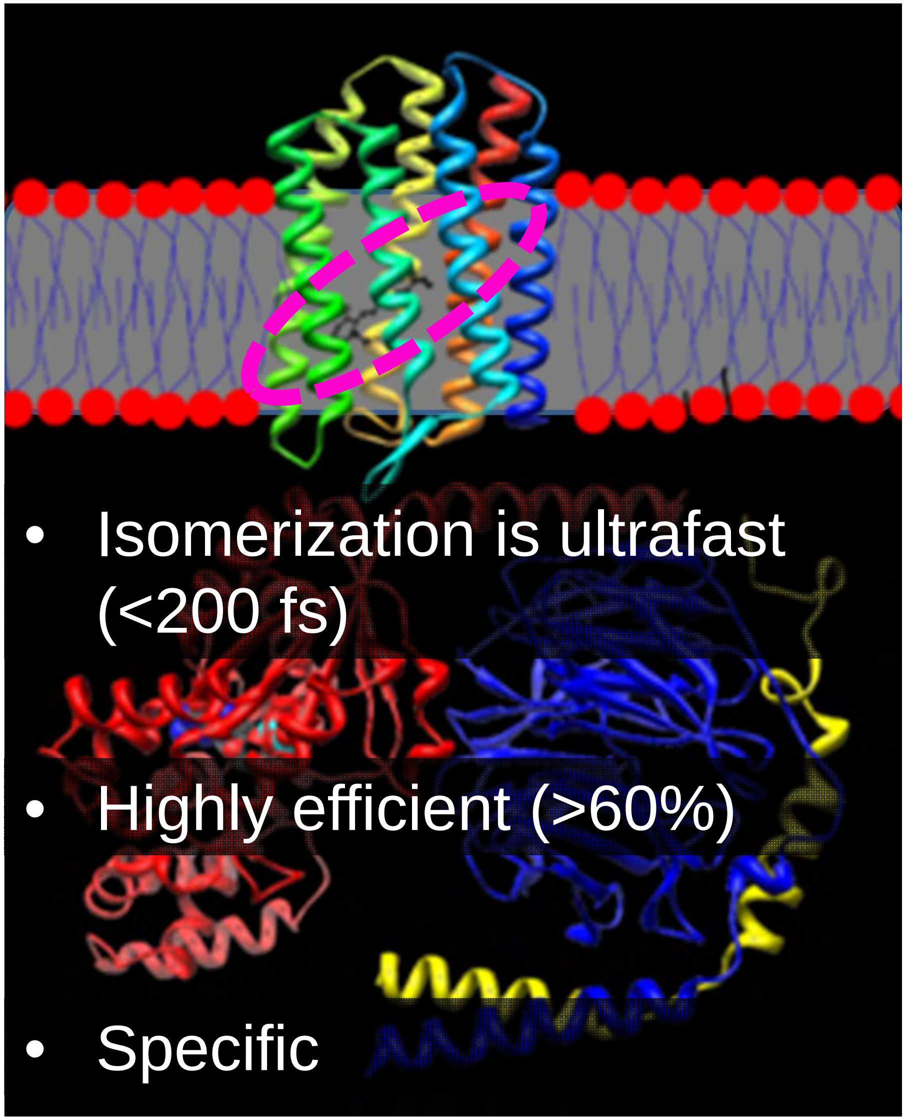




“Vision and Light-Induced Molecular Changes”,
www.chemistry.wustl.edu/~edudev/LabTutorials/Vision/Vision.html

Light-induced Retinal Isomerization



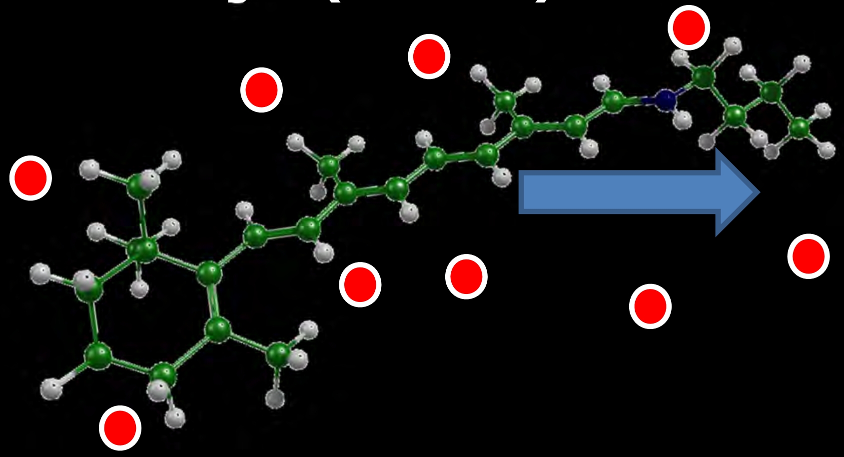


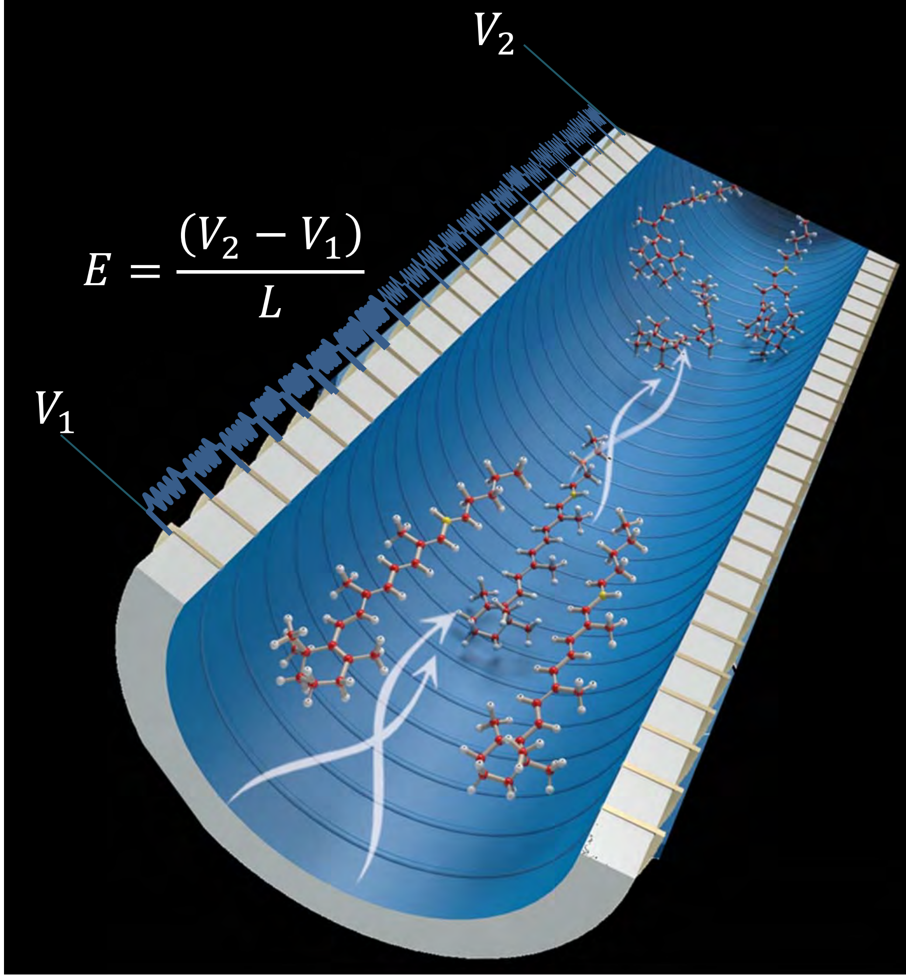
- Isomerization is ultrafast (<200 fs)
- Highly efficient (>60%)
- Specific

“Vision and Light-Induced Molecular Changes”,
www.chemistry.wustl.edu/~edudev/LabTutorials/Vision/Vision.html

Ion Mobility Spectrometry (IMS)

IMS pulls molecular ions with a small electric field (typically ~10 V/cm) through a drift tube filled with gas





$$E = \frac{(V_2 - V_1)}{L}$$

The ions are accelerated by the electric field, and slowed down by the collisions, reaching an average drift velocity

$$v_d = \frac{L}{t_d} \qquad K = \frac{v_d}{E}$$

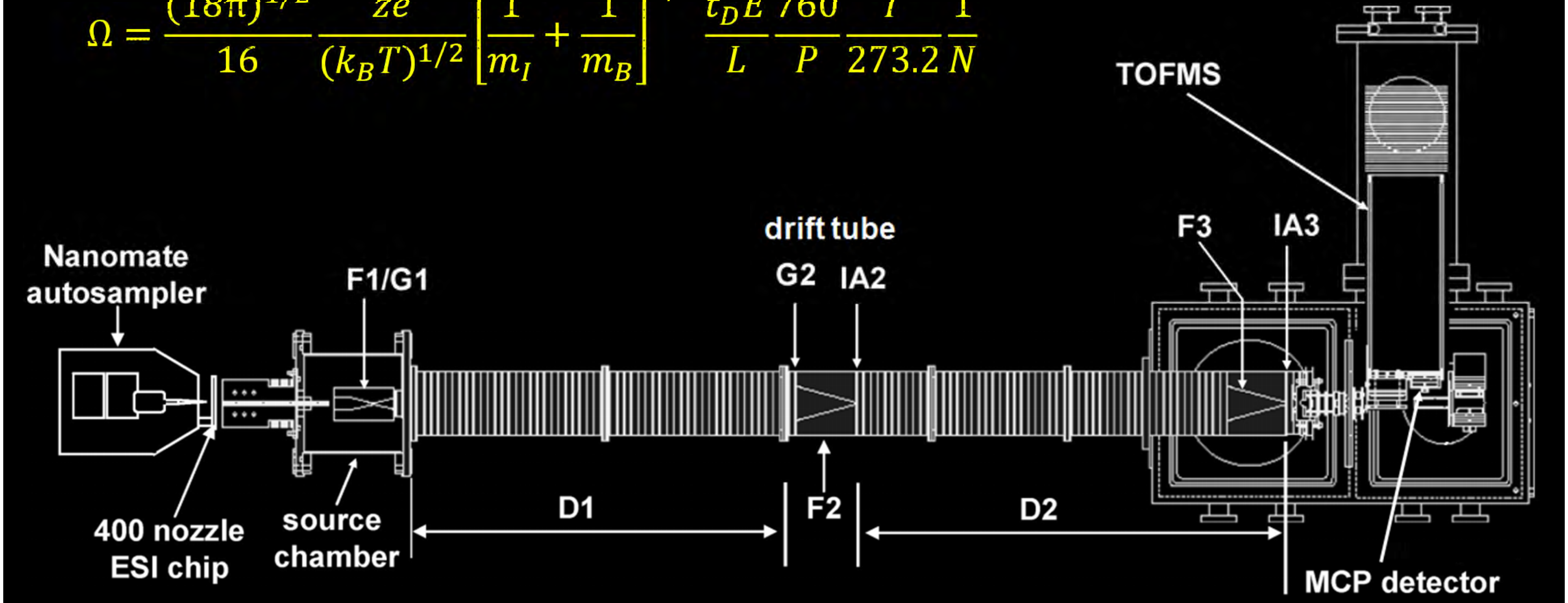
$$\mathrm{K} = \frac{1}{\Omega}\frac{\sqrt{18\pi}}{16}\frac{ze}{\sqrt{k_B T\,\mu}}\frac{1}{\mathrm{N(T,P)}}$$

Theory and Instrumentation

$v_D = KE$ Ion Mobility

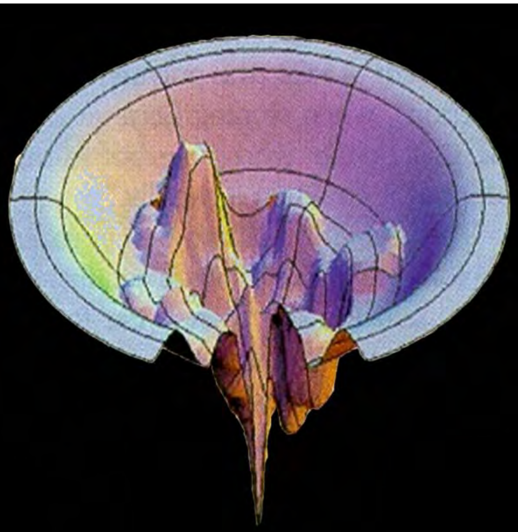
Collision cross section

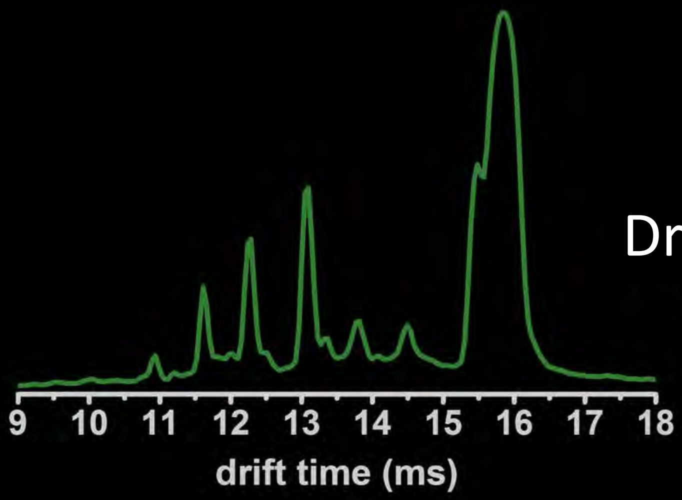
$$\Omega = \frac{(18\pi)^{1/2}}{16}\frac{ze}{(k_B T)^{1/2}}\left[\frac{1}{m_I}+\frac{1}{m_B}\right]^{1/2}\frac{t_D E}{L}\frac{760}{P}\frac{T}{273.2}\frac{1}{N}$$



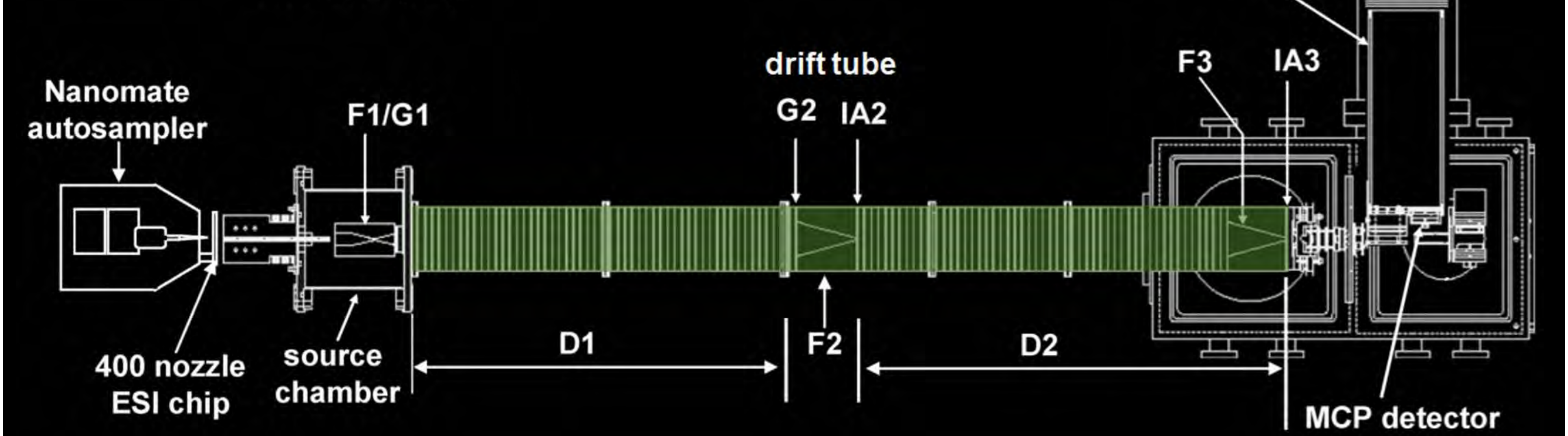
Revercomb, H. E.; Mason, E. A. *Anal. Chem.* **1975**, *47*, 970–983
Koeniger, et al. *Anal. Chem.* **2006**, *78*, 4161-4174

IMS-MS: Bradykinin (BK)



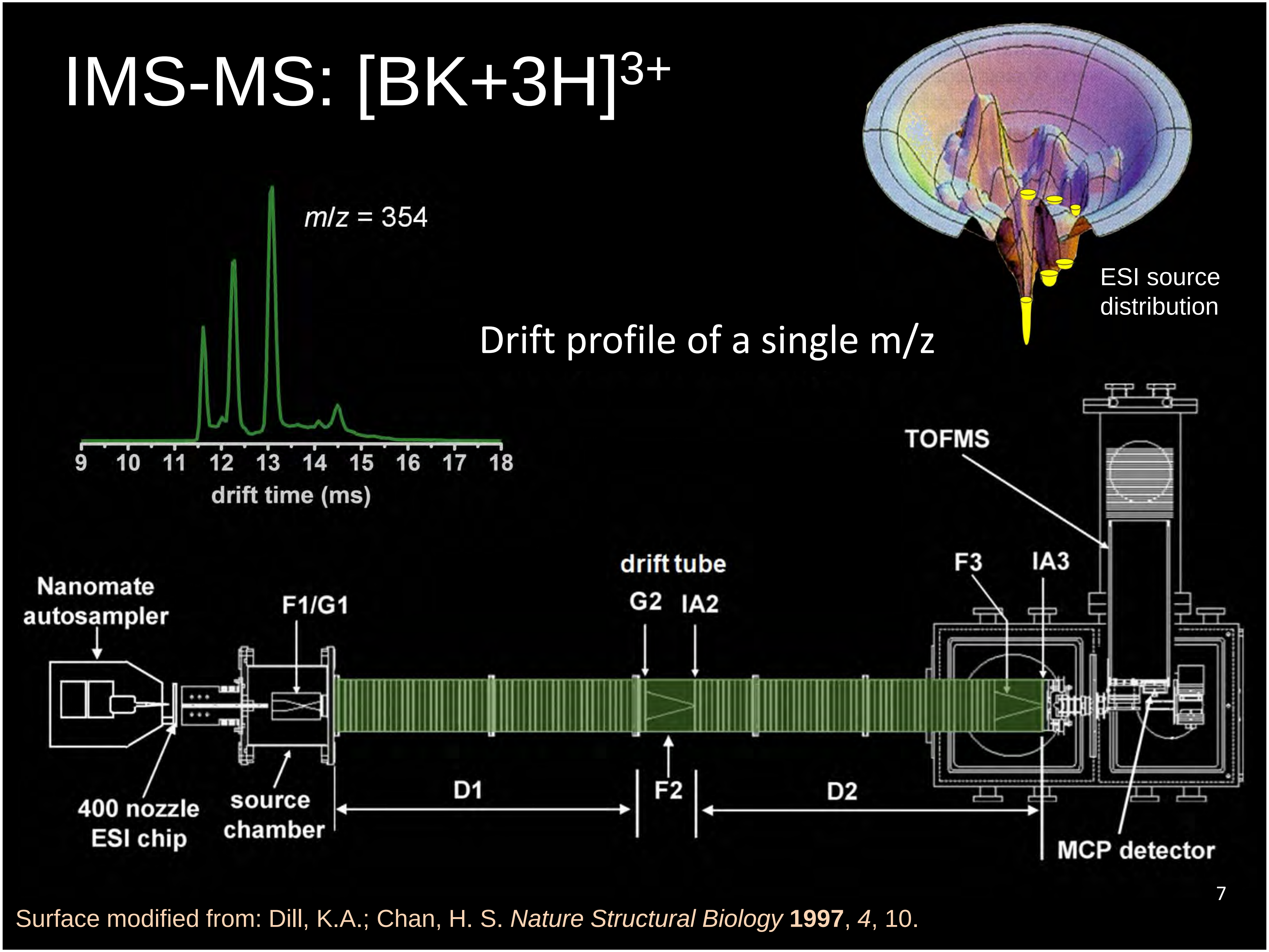


Drift profile of all ions



Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

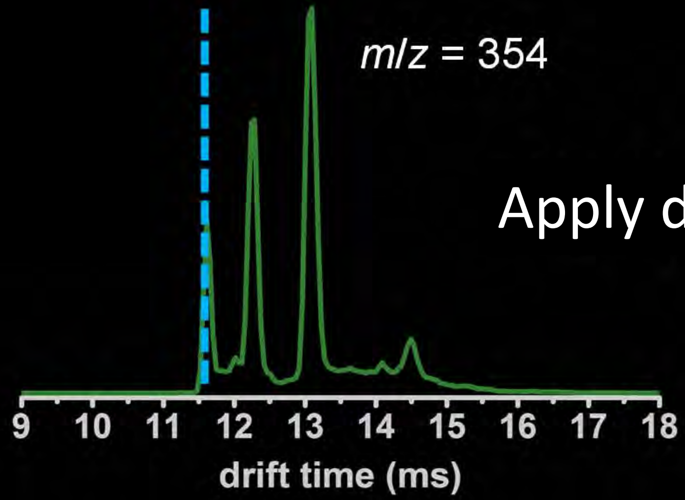
IMS-MS: $[BK+3H]^{3+}$



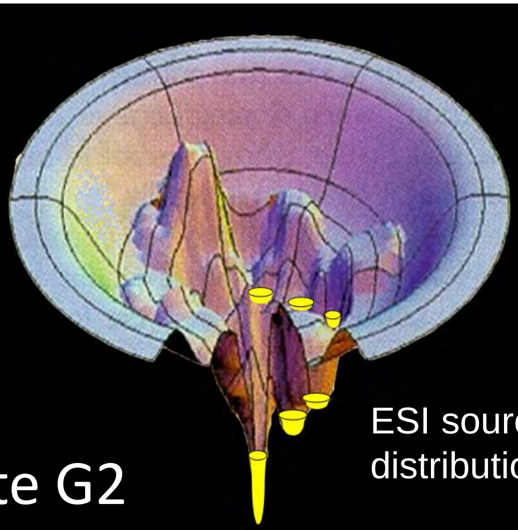
Drift profile of a single m/z

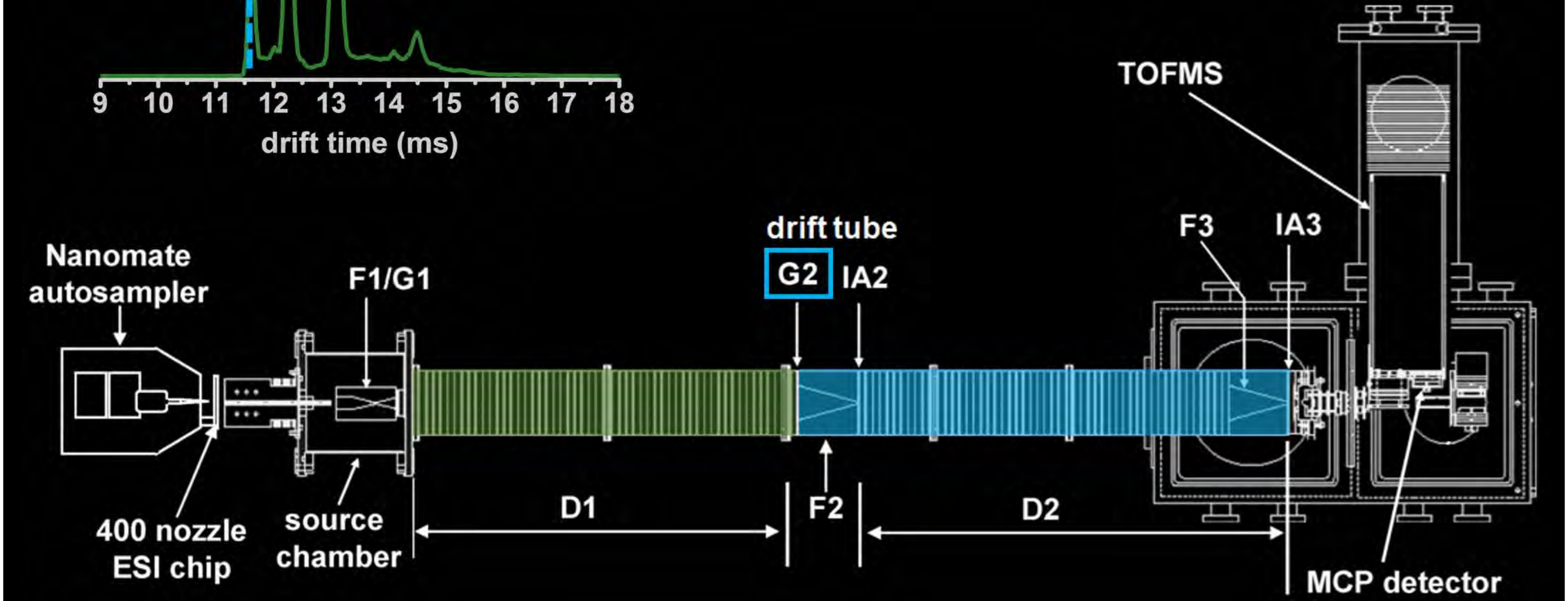
Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

IMS-MS: [BK+3H]$^{3+}$



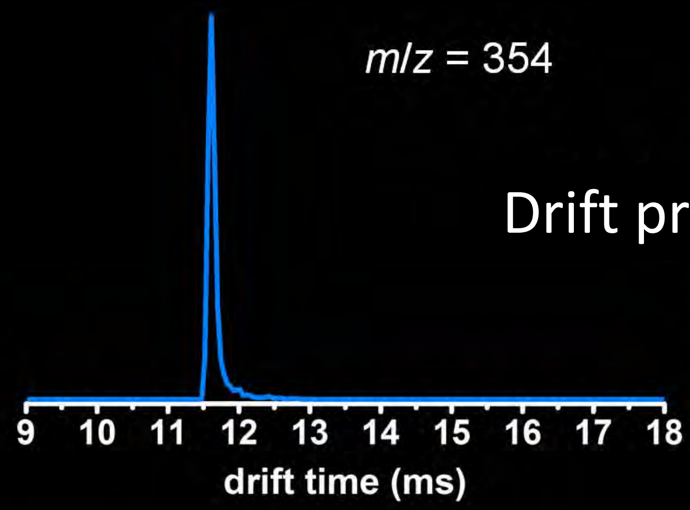
Apply delay pulse at ion gate G2



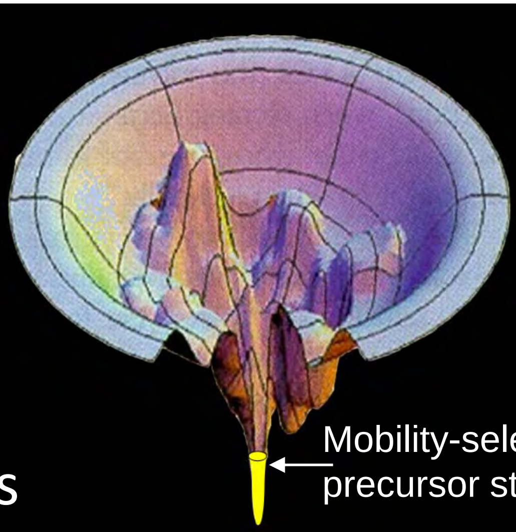


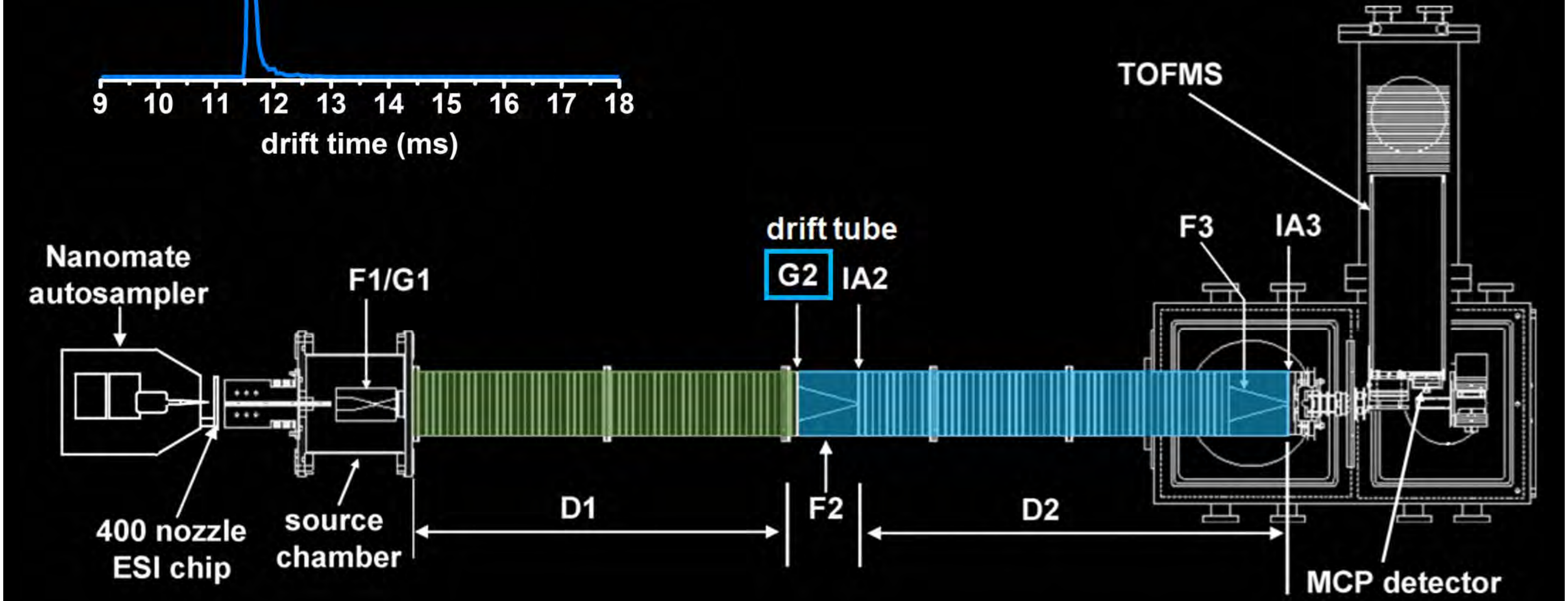
Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

IMS-IMS-MS



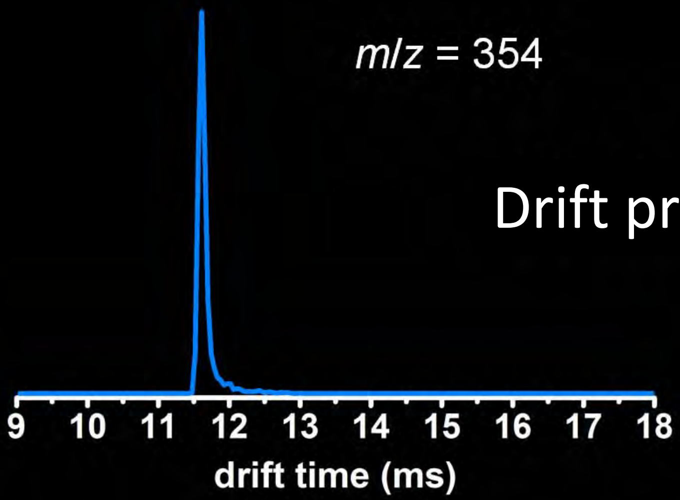
Drift profile of selected ions


Mobility-selected precursor state

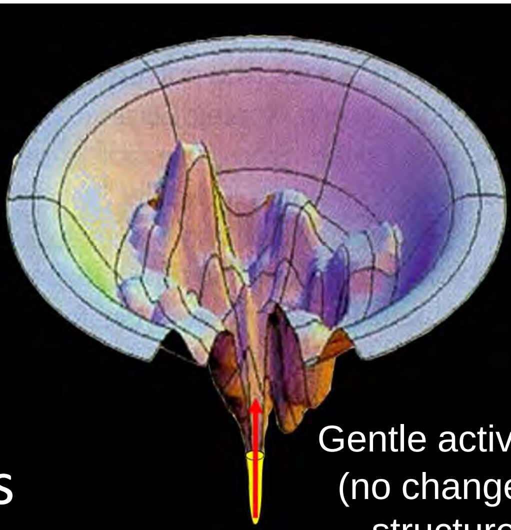


Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

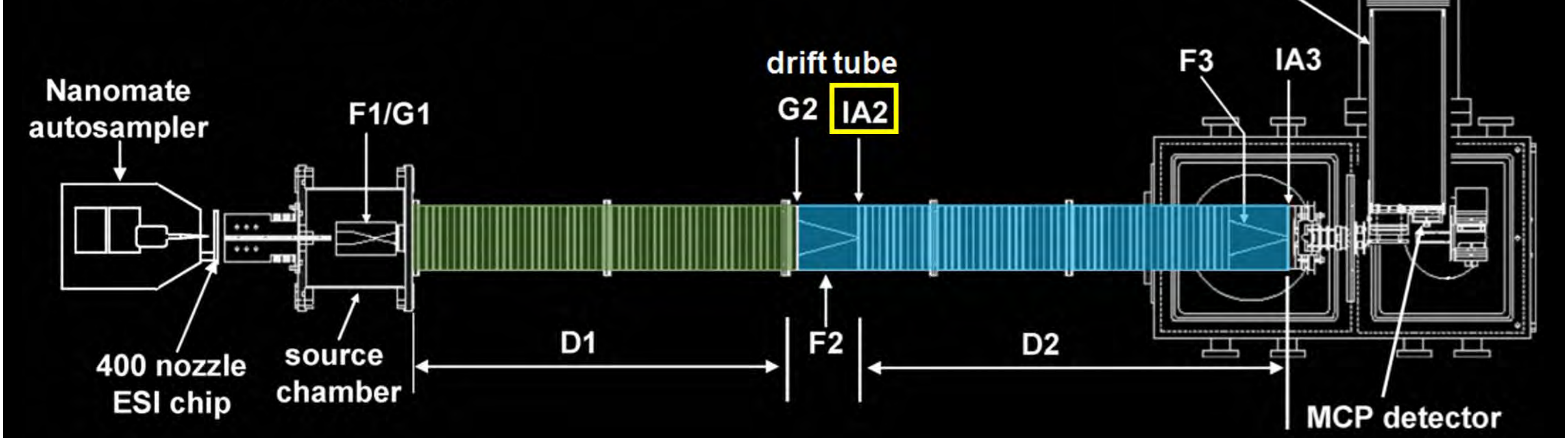
IMS-IMS-MS



Drift profile of selected ions

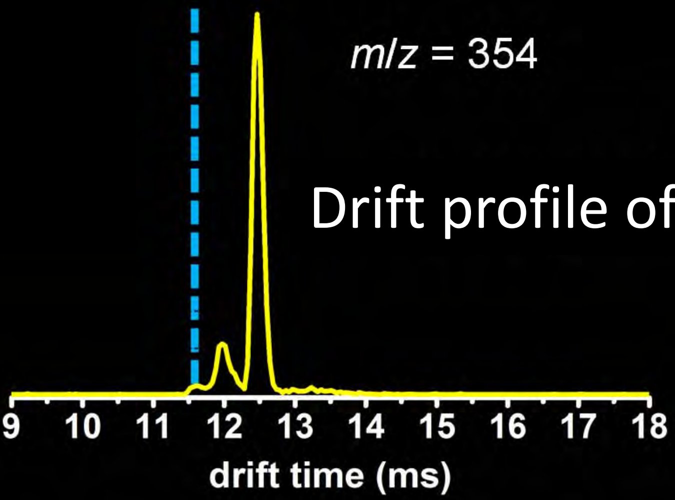


Gentle activation (no change in structure)

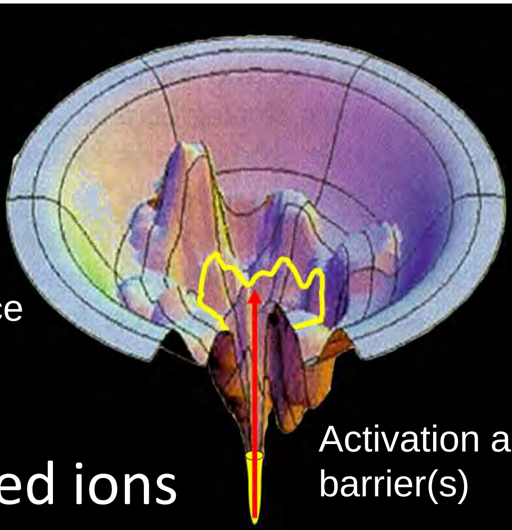


Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

IMS-IMS-MS

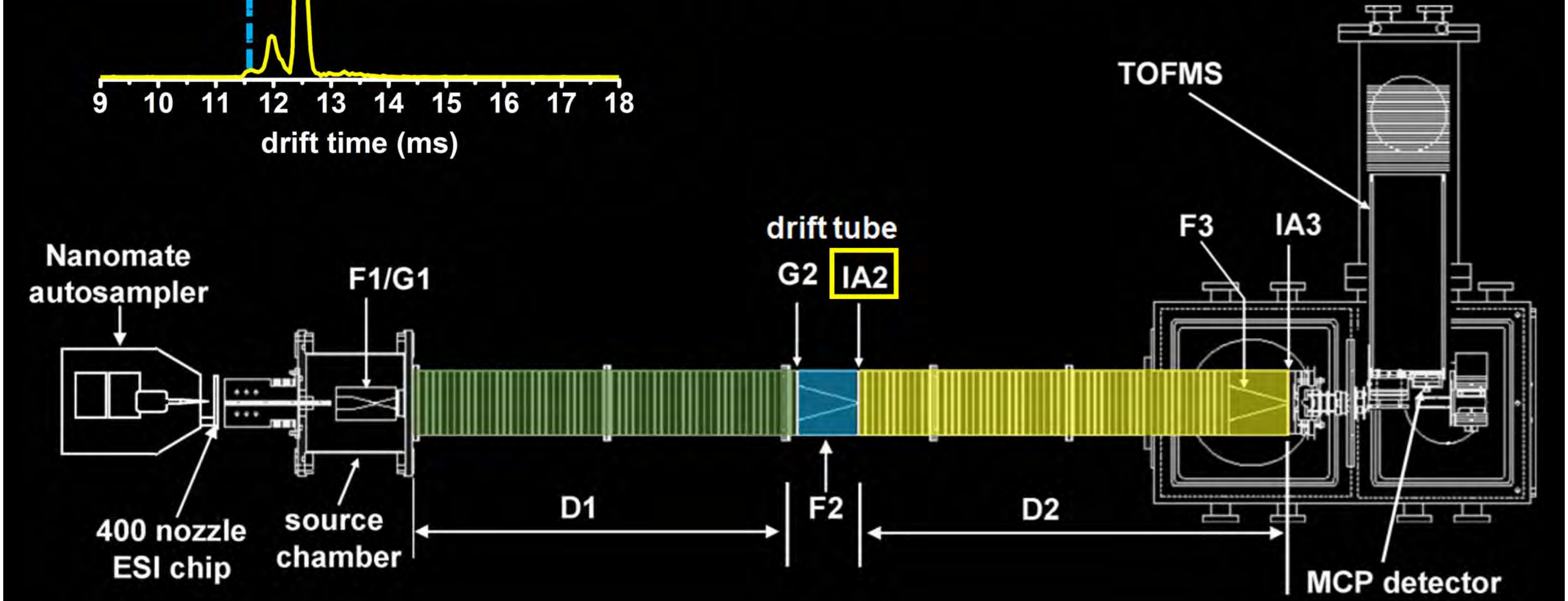


Drift profile of collisionally-activated ions



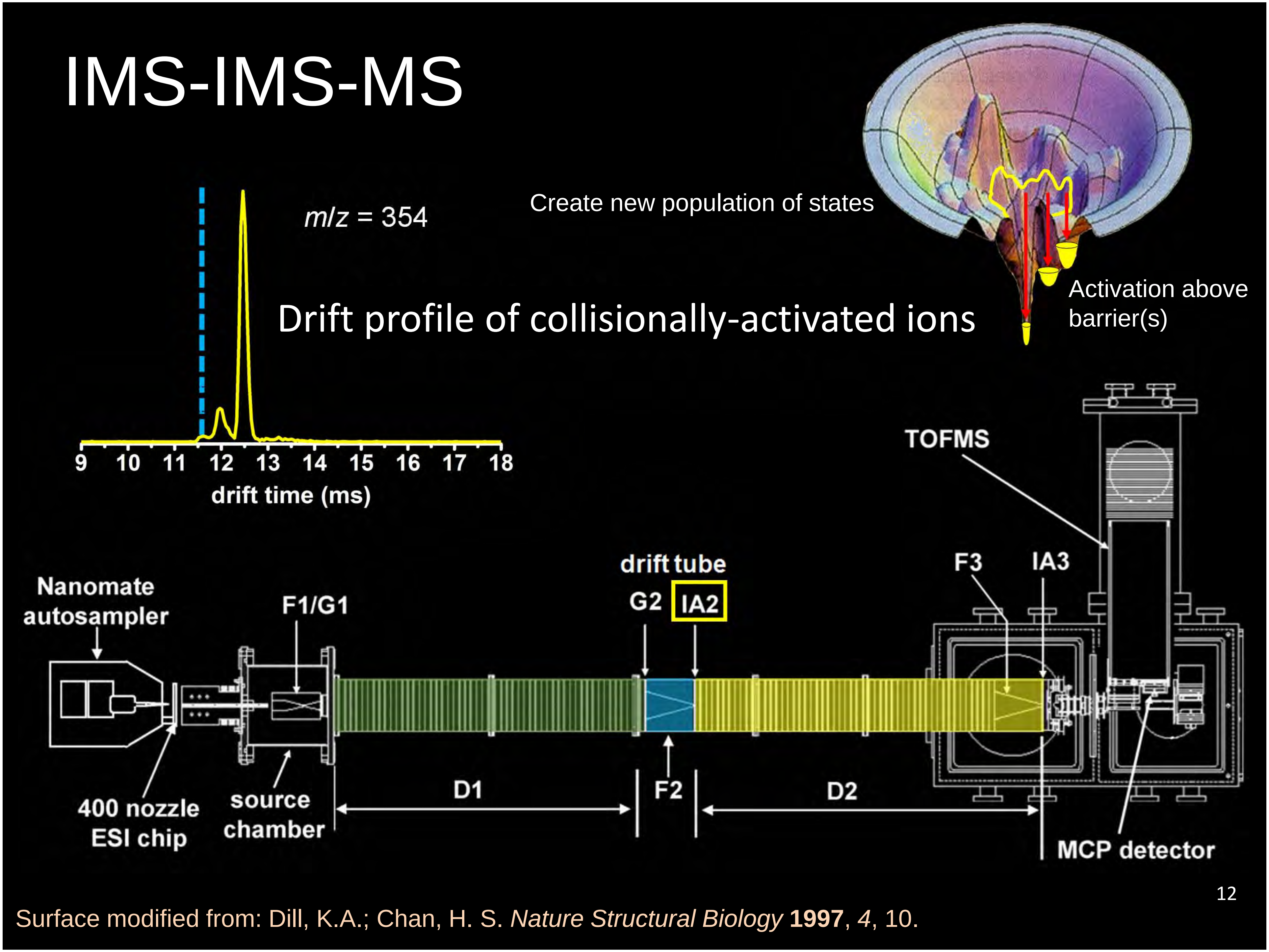
Sample conformational space

Activation above barrier(s)



Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

IMS-IMS-MS



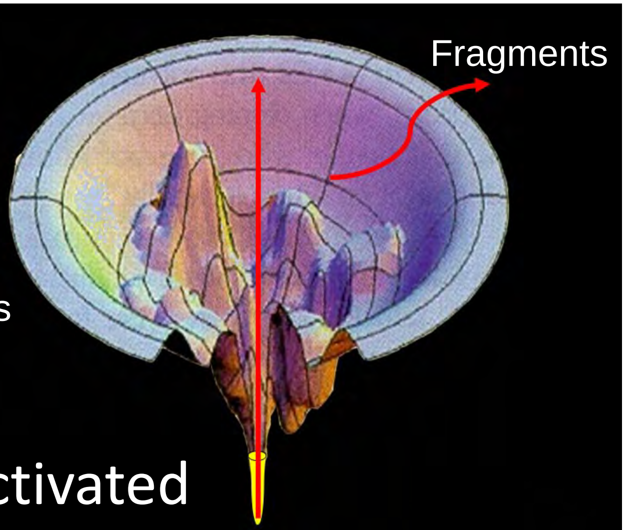
Create new population of states

Drift profile of collisionally-activated ions

Activation above barrier(s)

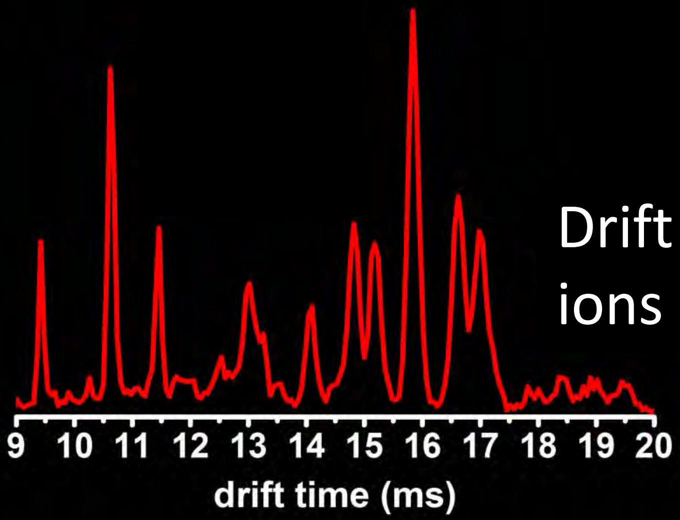
Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

IMS-IMS-MS

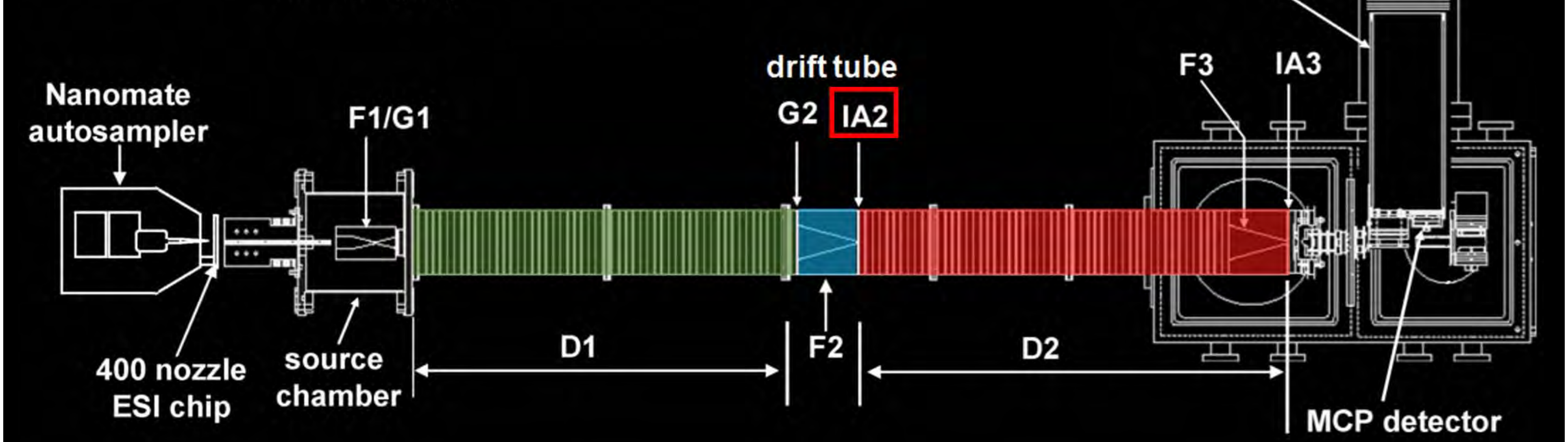


High activation voltages

→ Dissociation

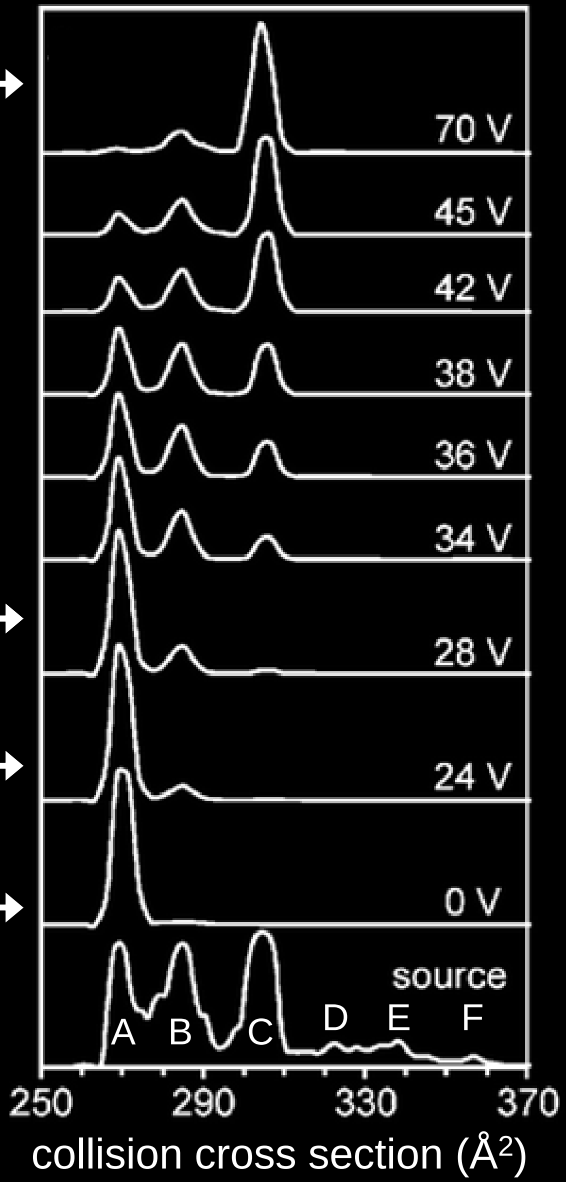


Drift profile of harshly-activated ions



Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

Voltage-Resolved Selection and Activation of Conformer A

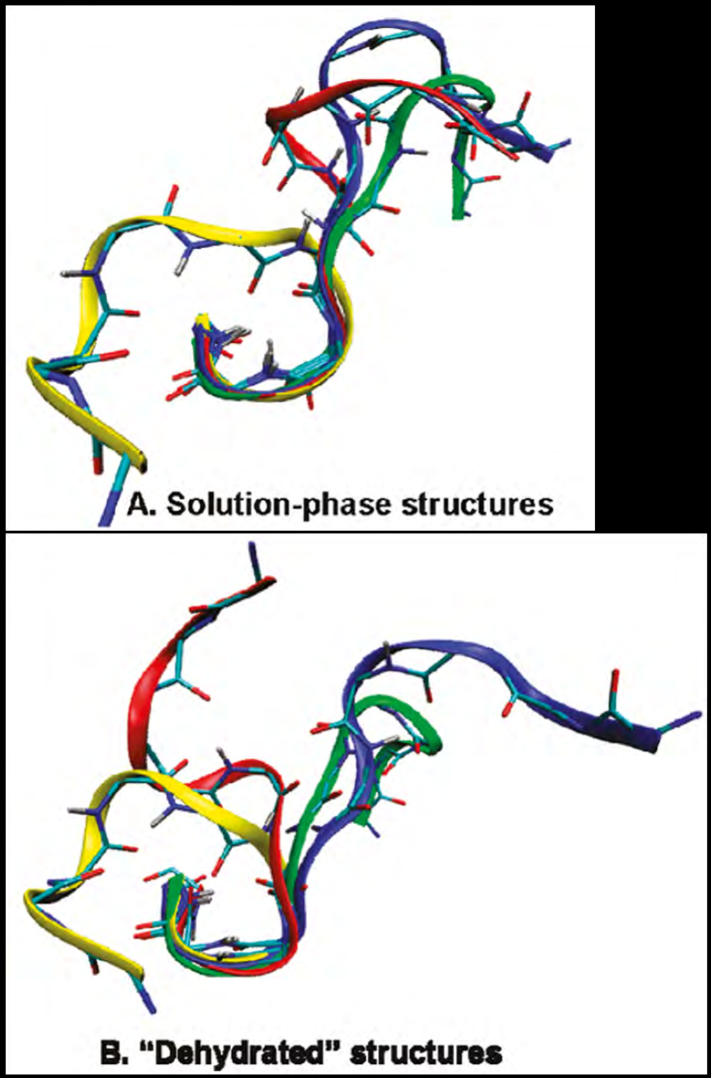


Annealed gas-phase distribution →

Threshold C formation →

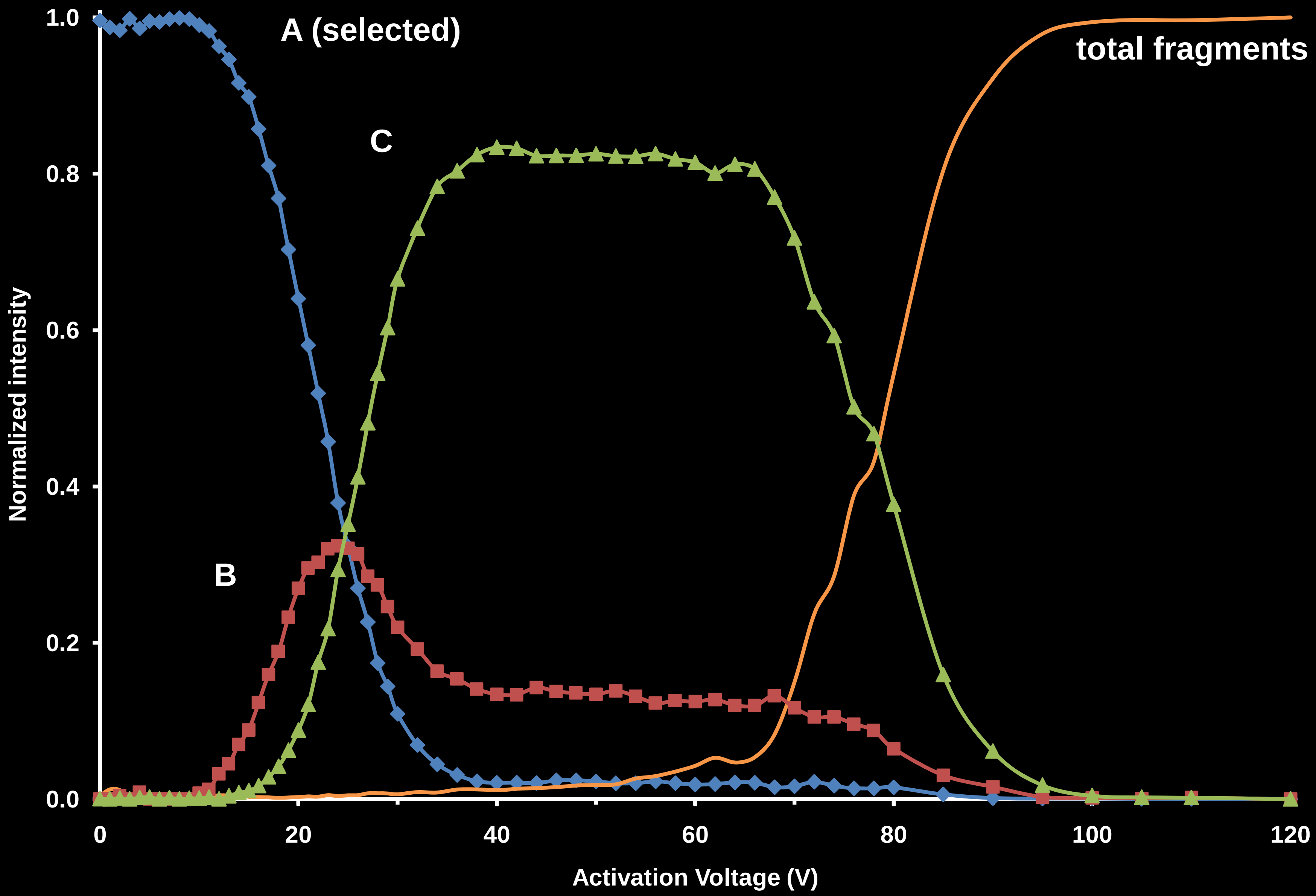
Threshold B formation →

Selection A →



Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783
Pierson, N.A.; Chen, L.; Valentine, S.J.; Russel, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810-13813

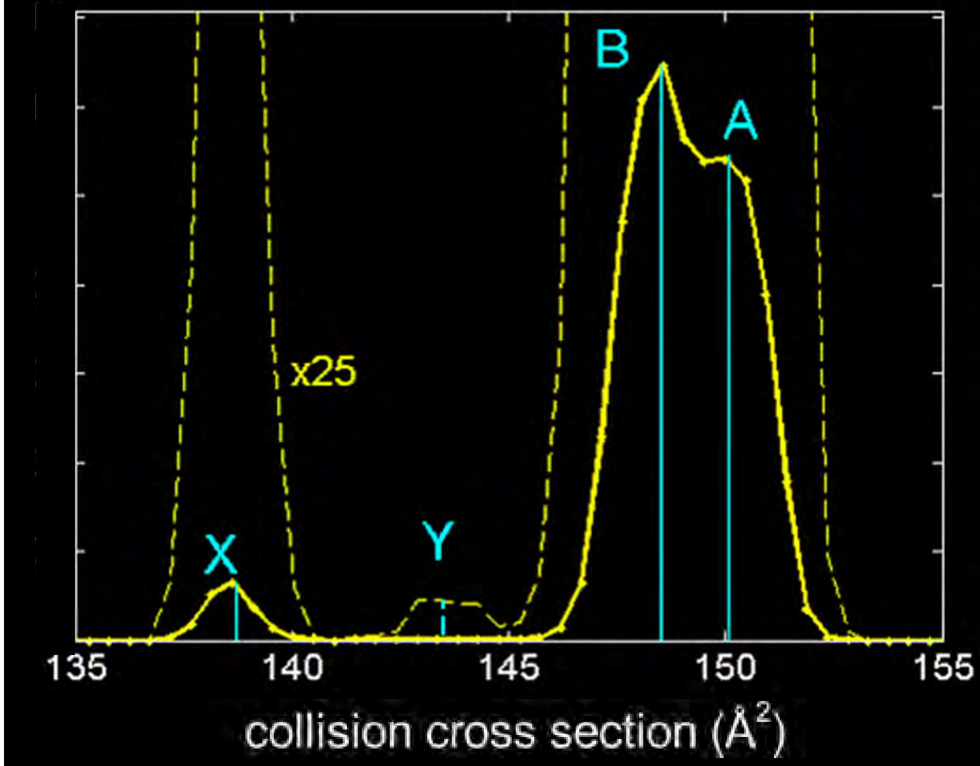
Voltage-Resolved Selection and Activation of Conformer A



Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783

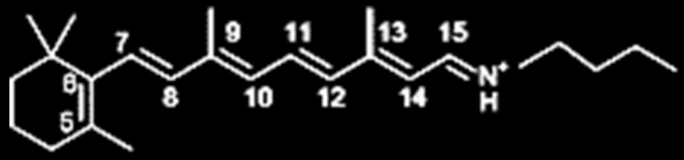
IMS-MS: $[RPSB+H]^+$

Retinal: $[RPSB+H]^+$
~340 amu



Peak A

- largest measured ccs
- calculated ccs of the *trans*-RPSB within 5%

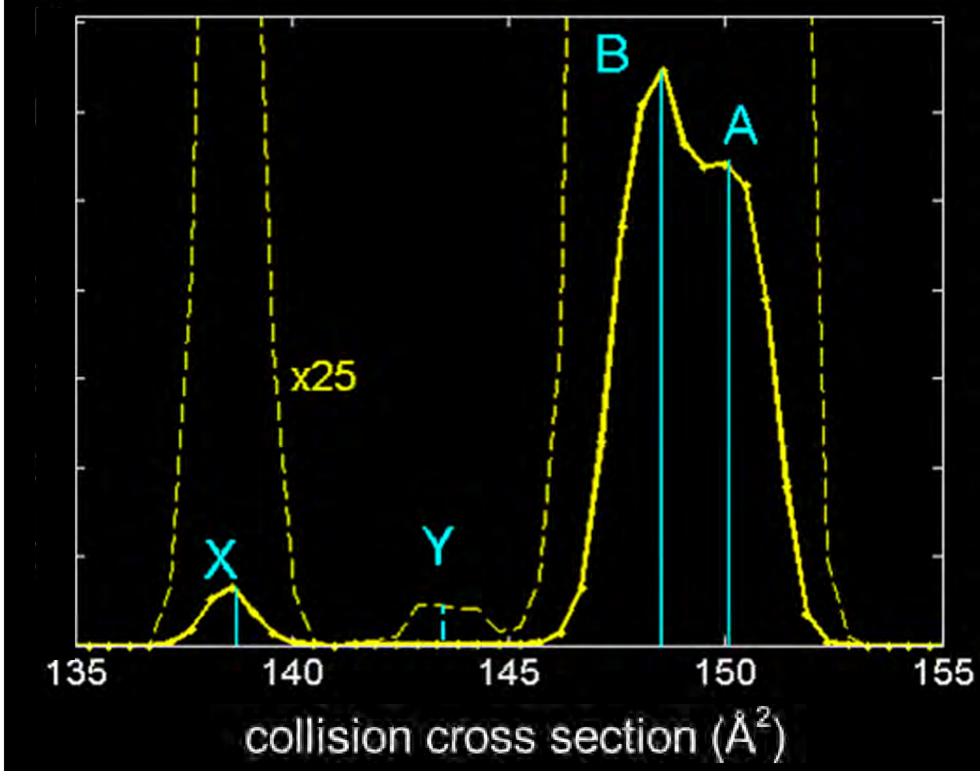


all-*trans*

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

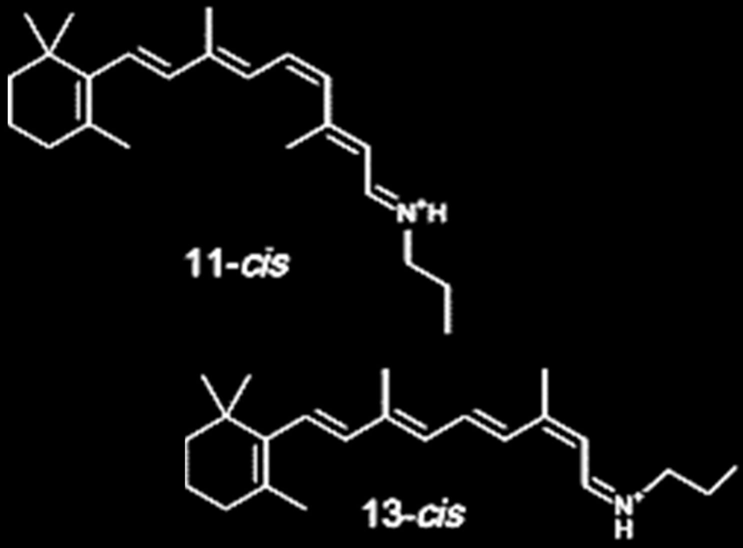
IMS-MS: $[RPSB+H]^+$

Retinal: $[RPSB+H]^+$
~340 amu



Peak B

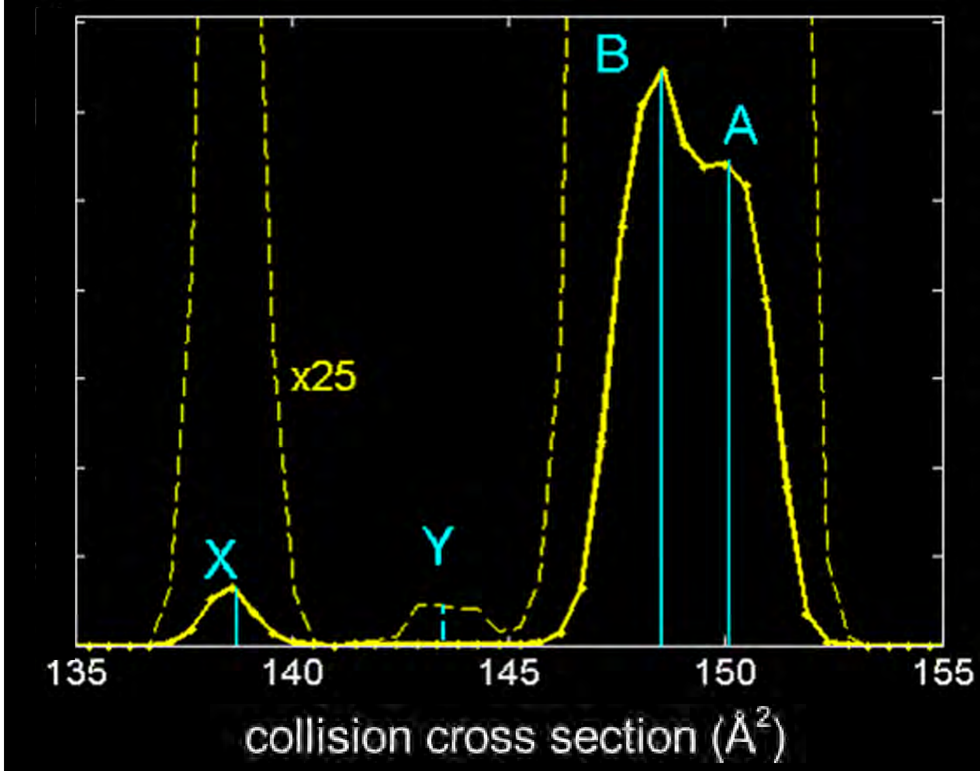
- most abundant peak in the distribution
- calculated ccs agree with multiple *cis*-RPSB geometries



Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

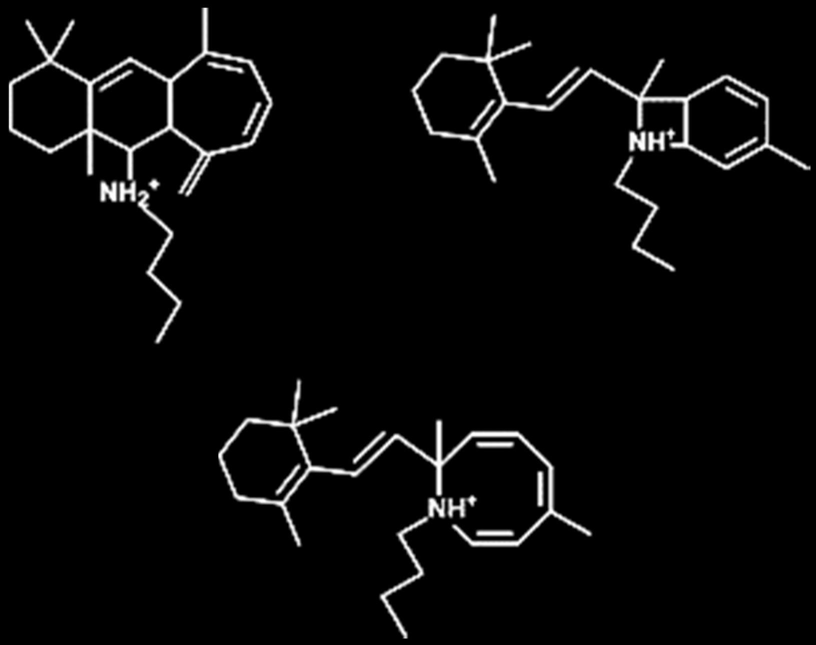
IMS-MS: $[RPSB+H]^+$

Retinal: $[RPSB+H]^+$
~340 amu



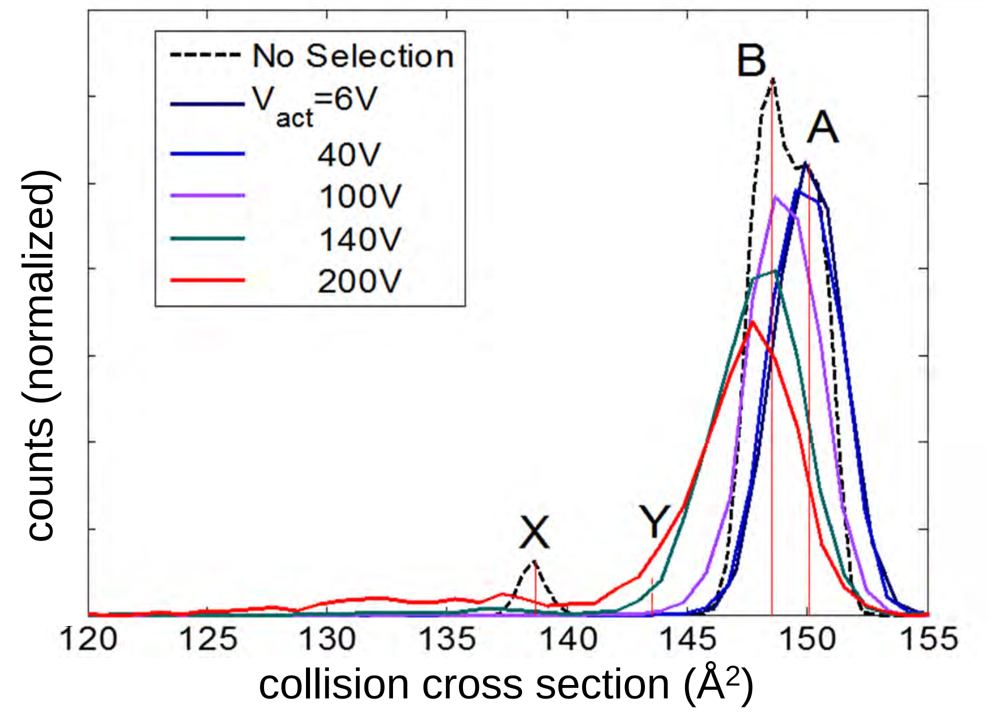
Peaks X & Y

- multiple-*cis* bonds
- potentially cyclized



Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

IMS-IMS-MS: Peak A of $[RPSB+H]^+$

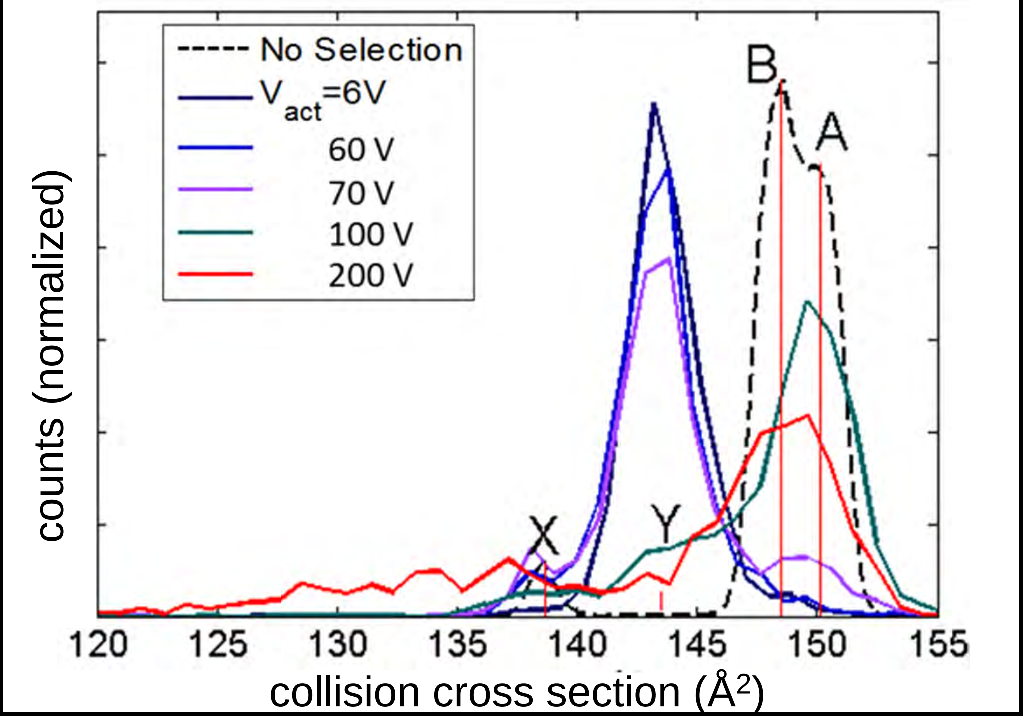


Activations were scanned in 10 V increments

Annealed gas-phase distribution shows a broad peak centered around Peak B

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

IMS-IMS-MS: Peak Y of $[RPSB+H]^+$



Activations were scanned in 10 V increments

Annealed gas-phase distribution shows a broad peak centered around Peak B

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

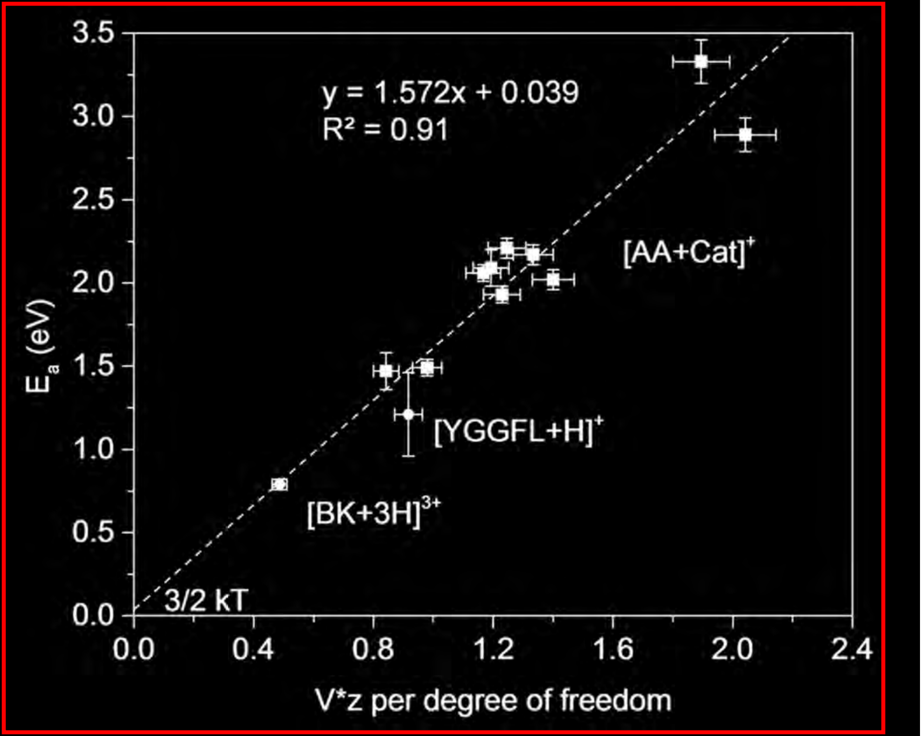
Measuring Activation Energies by IMS–IMS

We observe pre-dissociation state-to-state transitions, but at what energies?

- Use of an external IA2 voltage-to-eV calibration by measuring well-characterized dissociation events

“Thermometer ion” systems:

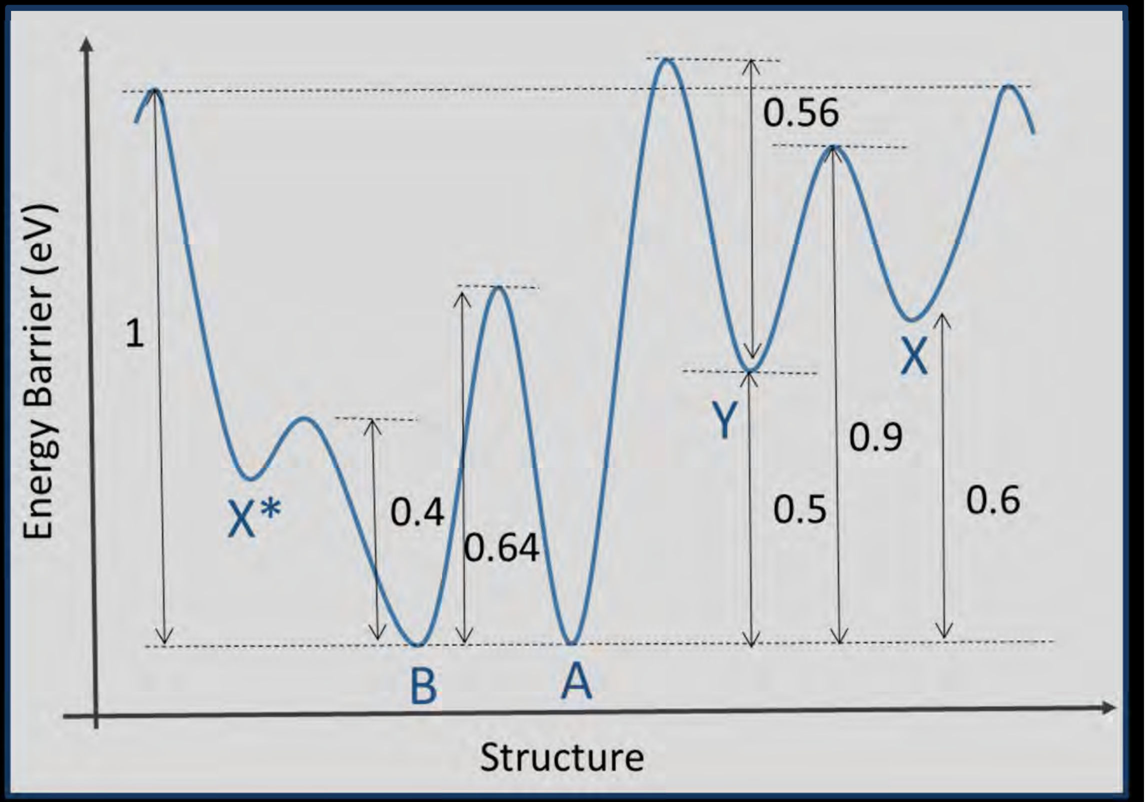
Ion	E_a, eV	IA2 Voltage · z
[Pro+K]$^+$	1.49 (±0.05)	47 (±2.4)
[Met+K]$^+$	1.47 (±0.11)	48 (±2.4)
[Asp+Na]$^+$	2.02 (±0.06)	63 (±3.2)
[Asn+Na]$^+$	2.17 (±0.06)	64 (±3.2)
[Glu+Na]$^+$	2.06 (±0.05)	63 (±3.2)
[Gln+Na]$^+$	2.21 (±0.06)	71 (±3.6)
[Pro+Na]$^+$	1.93 (±0.05)	59 (±3.0)
[Met Na]$^+$	2.09 (±0.11)	68 (±3.4)
[Pro Li]$^+$	2.89 (±0.10)	98 (±4.9)
[Met Li]$^+$	3.33 (±0.13)	108 (±5.4)
[YGGFL+H]$^+$	1.21 (±0.25)	209 (±10.5)
[BK+3H]$^{3+}$	0.79 (±0.03)	219 (±3.7)



Pierson, N.A.; Clemmer, D.E. *Int. J. Mass Spectrom.* **2015**, *377*, 646–654

Measuring Activation Energies by IMS–IMS

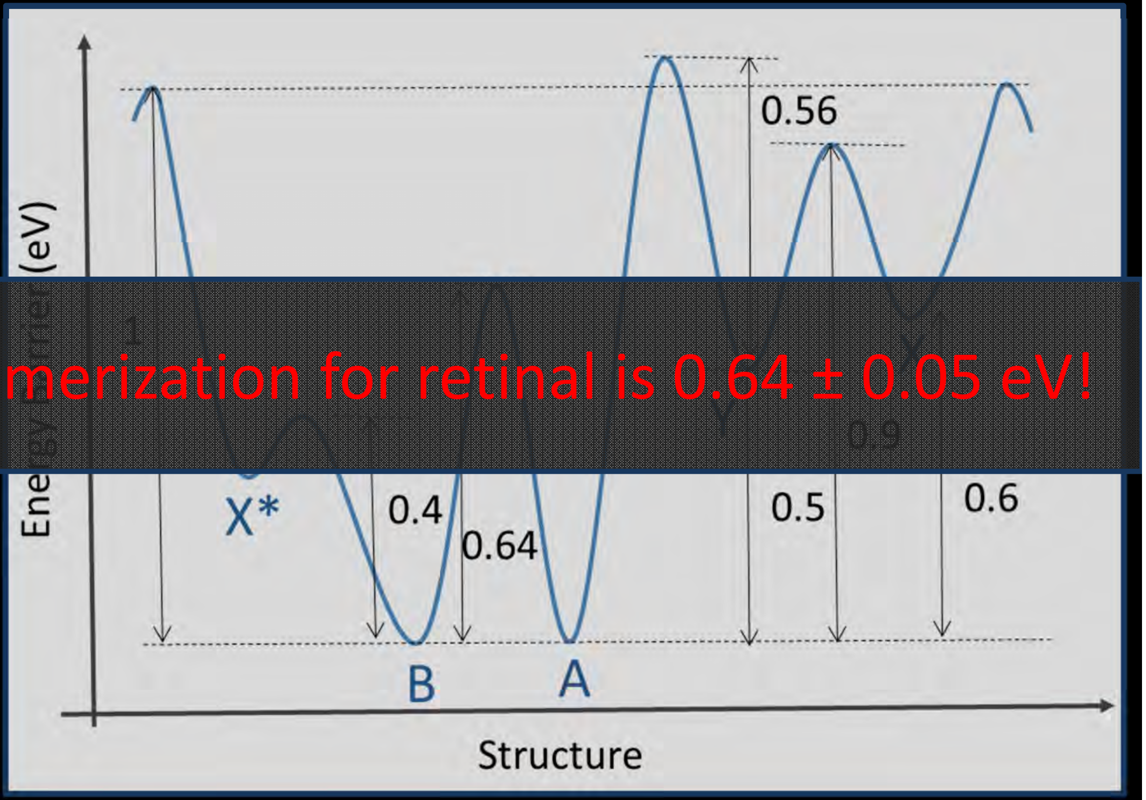
Process	Barrier Energy (eV)
A→X	0.9 (±0.05)
B→X	0.4 (±0.05)
Y→A	0.56 (±0.05)
Y→B	0.56 (±0.05)
Y→X	0.5 (±0.05)
X→A	0.5 (±0.05)
X→B	0.5 (±0.05)
A→B	0.64 (±0.05)
A→B	0.64 (±0.05)
A→*frag*	1.0 (±0.05)
B→*frag*	1.0 (±0.05)
Y→*frag*	0.5 (±0.05)
X→*frag*	0.4 (±0.05)



Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

Measuring Activation Energies by IMS–IMS

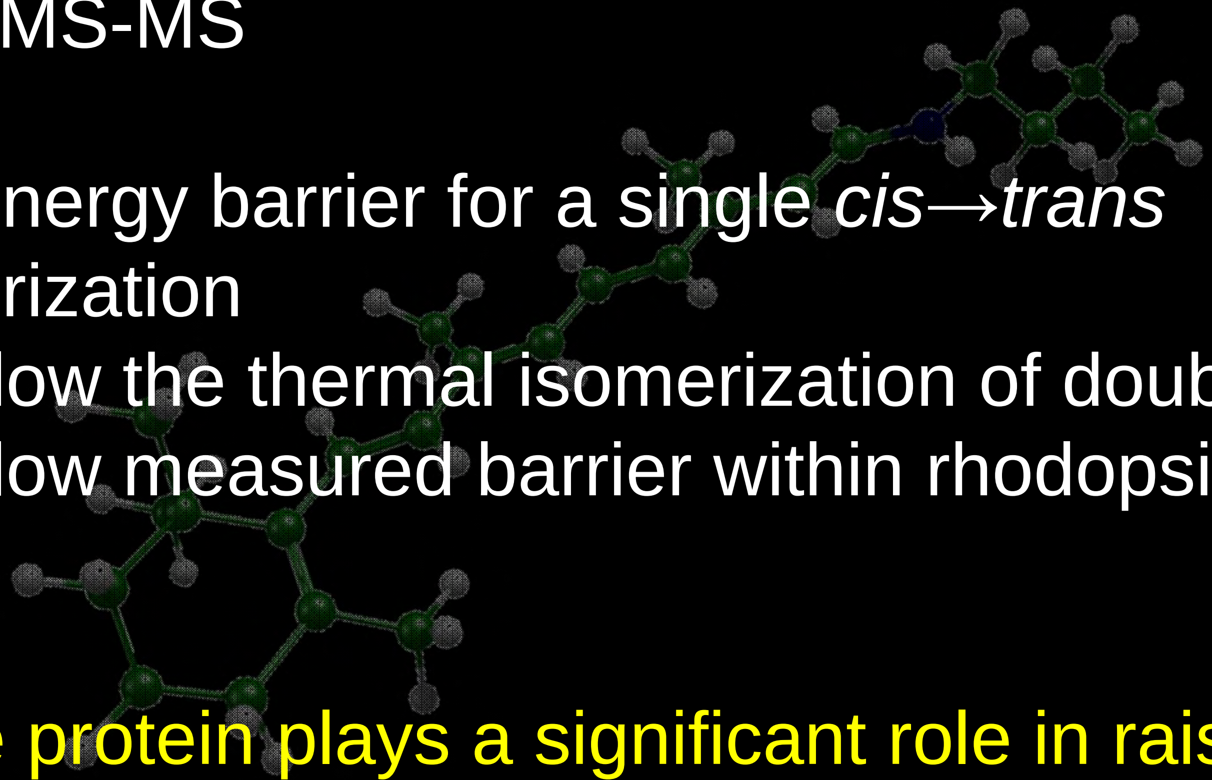
Process	Barrier Energy (eV)
A→X	0.9 (±0.05)
B→X	0.4 (±0.05)
Y→A	0.56 (±0.05)
Y→B	0.56 (±0.05)
Y→X	0.5 (±0.05)
X→A	0.5 (±0.05)
X→B	0.5 (±0.05)
A→B	0.64 (±0.05)
A→B	0.64 (±0.05)
A→*frag*	1.0 (±0.05)
B→*frag*	1.0 (±0.05)
Y→*frag*	0.5 (±0.05)
X→*frag*	0.4 (±0.05)



Thus, a ***single*** *cis-trans* isomerization for retinal is 0.64 ± 0.05 eV!

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

Summary



- Isomerization barriers of isolated $[RPSB+H]^+$ via IMS–IMS-MS
- Low energy barrier for a single *cis*→*trans* isomerization
  - Below the thermal isomerization of double bonds
  - Below measured barrier within rhodopsin proteins

Thus, the protein plays a significant role in raising the energetic barrier for photoisomerization of retinal

Acknowledgements

Bar-Ilan University:
Dr. Yoni Toker
Lihi Musbat

Indiana University:
Prof. David Clemmer
Nicholas Pierson
Matthew Glover

Aarhus University:
Anastasia Bochenkova
S. Brøndsted Nielsen

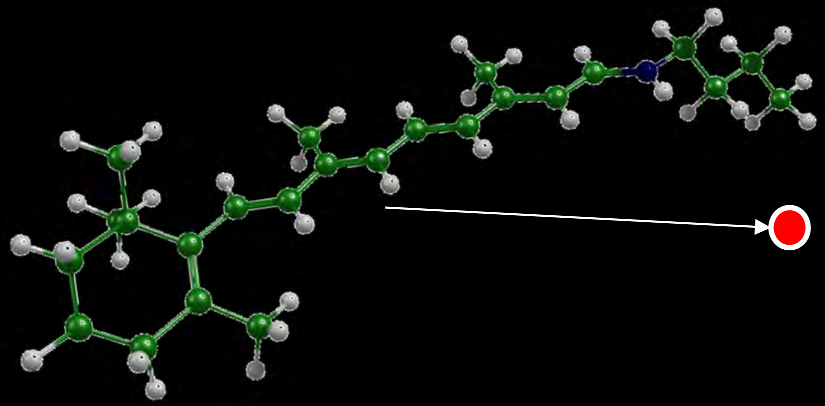
Weizmann Institute:
Mordechai Sheves

Funding:
NSWC Crane NISE/ 219
Indiana University METACyt

Backup Slides

Ion Mobility Spectrometry (IMS)

Study the shape of a molecule through collisions with neutral atoms/molecules



- Neutral atoms are as small as the features we want to study

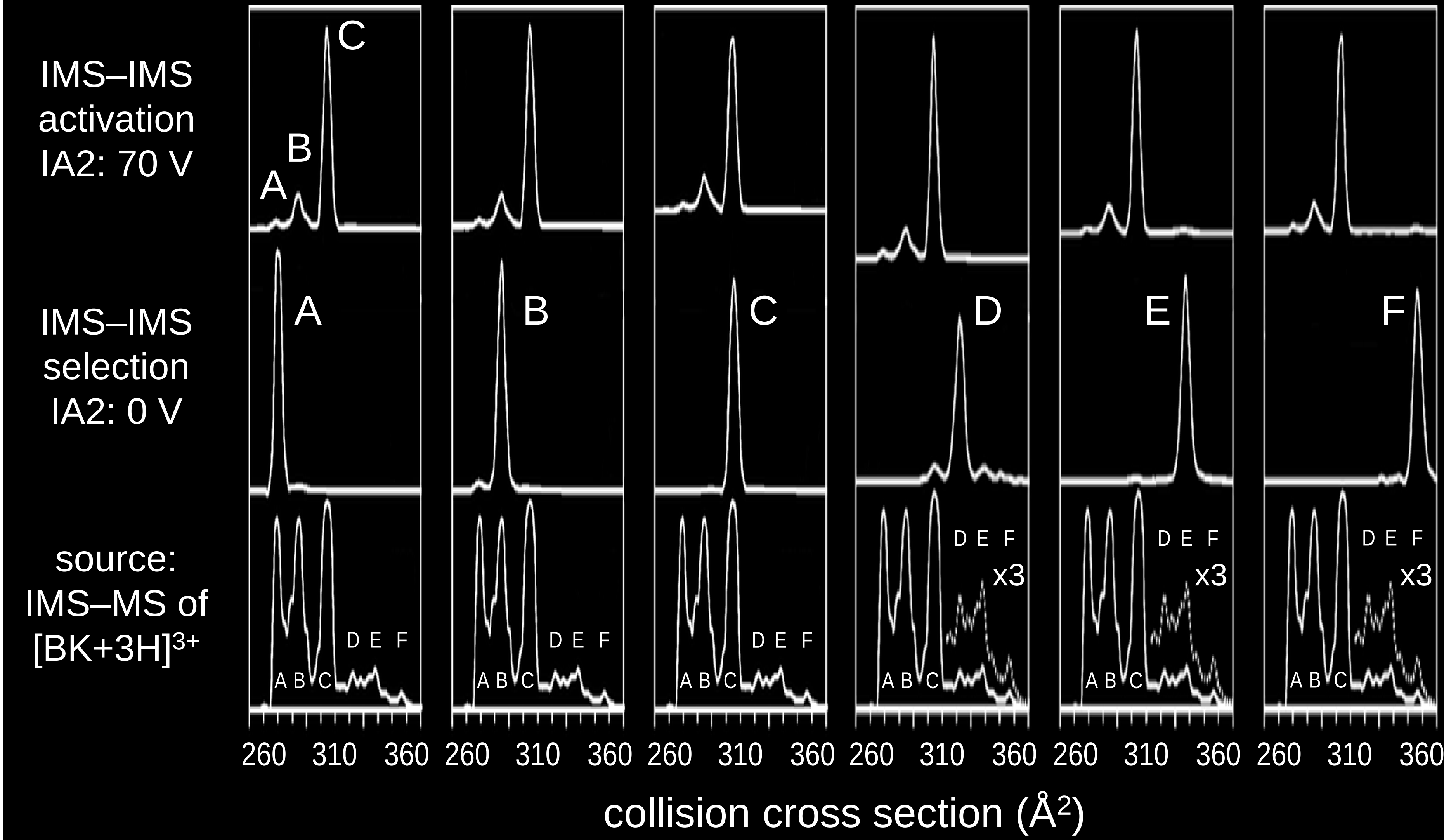
- Collision energy can be set to be very small

In order to study the structure

- And can also set to be large

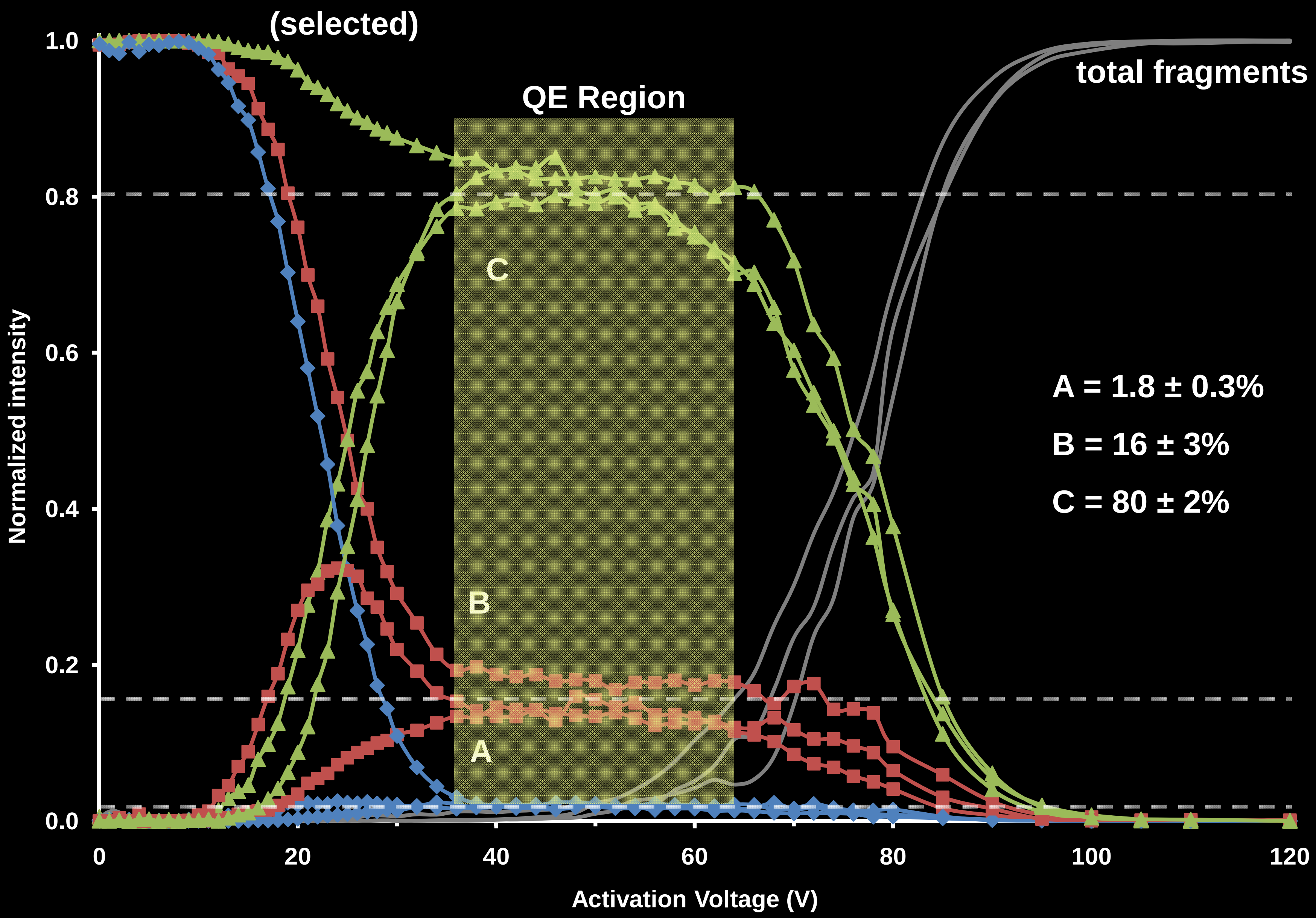
In order to heat the molecule, cause it to isomerize, and eventually break apart.

Gas-Phase Distributions of Bradykinin



Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783

Selection and Activation of Conformers A, B, and C



A = 1.8 ± 0.3%

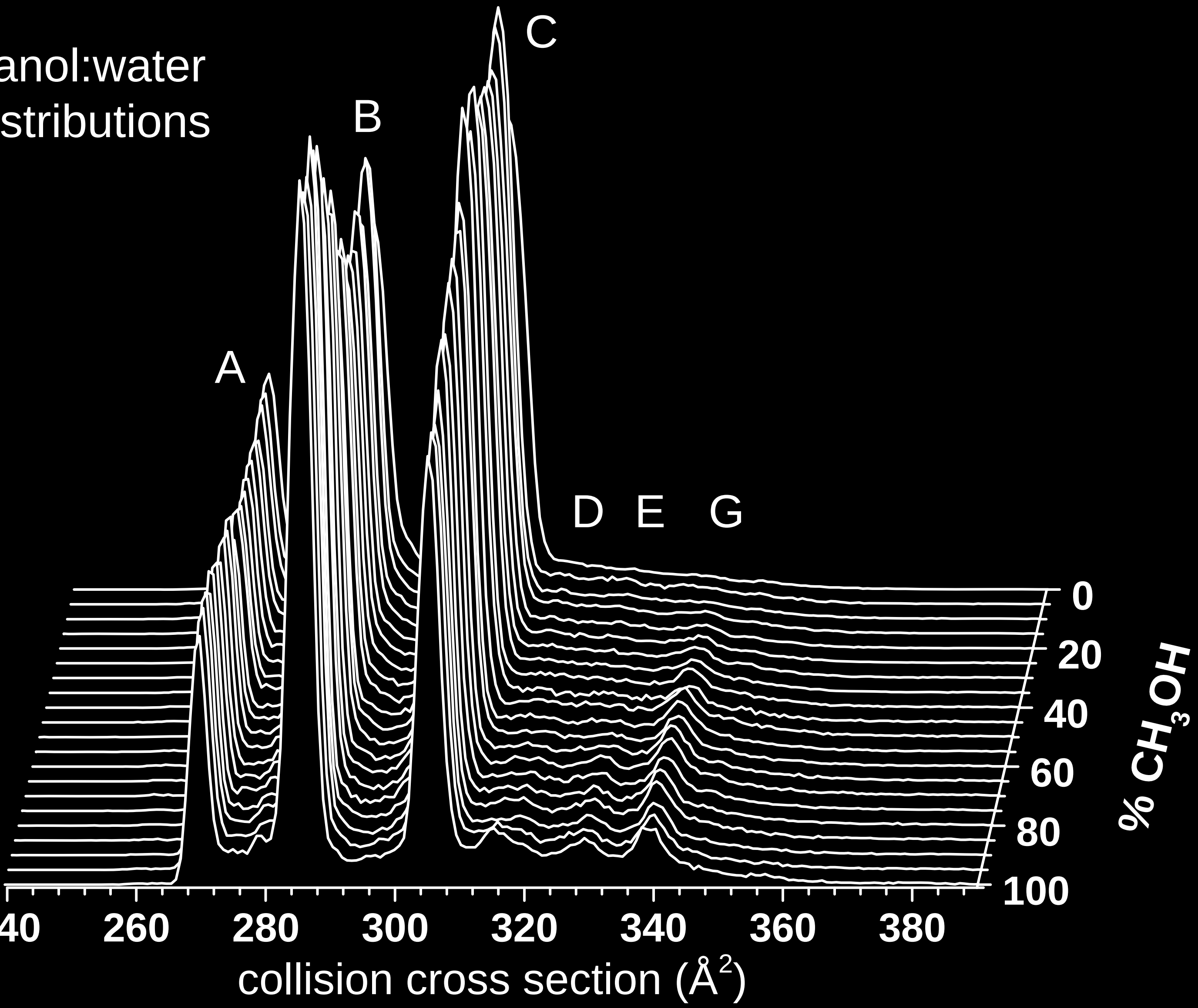
B = 16 ± 3%

C = 80 ± 2%

Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783

ESI Solution Studies

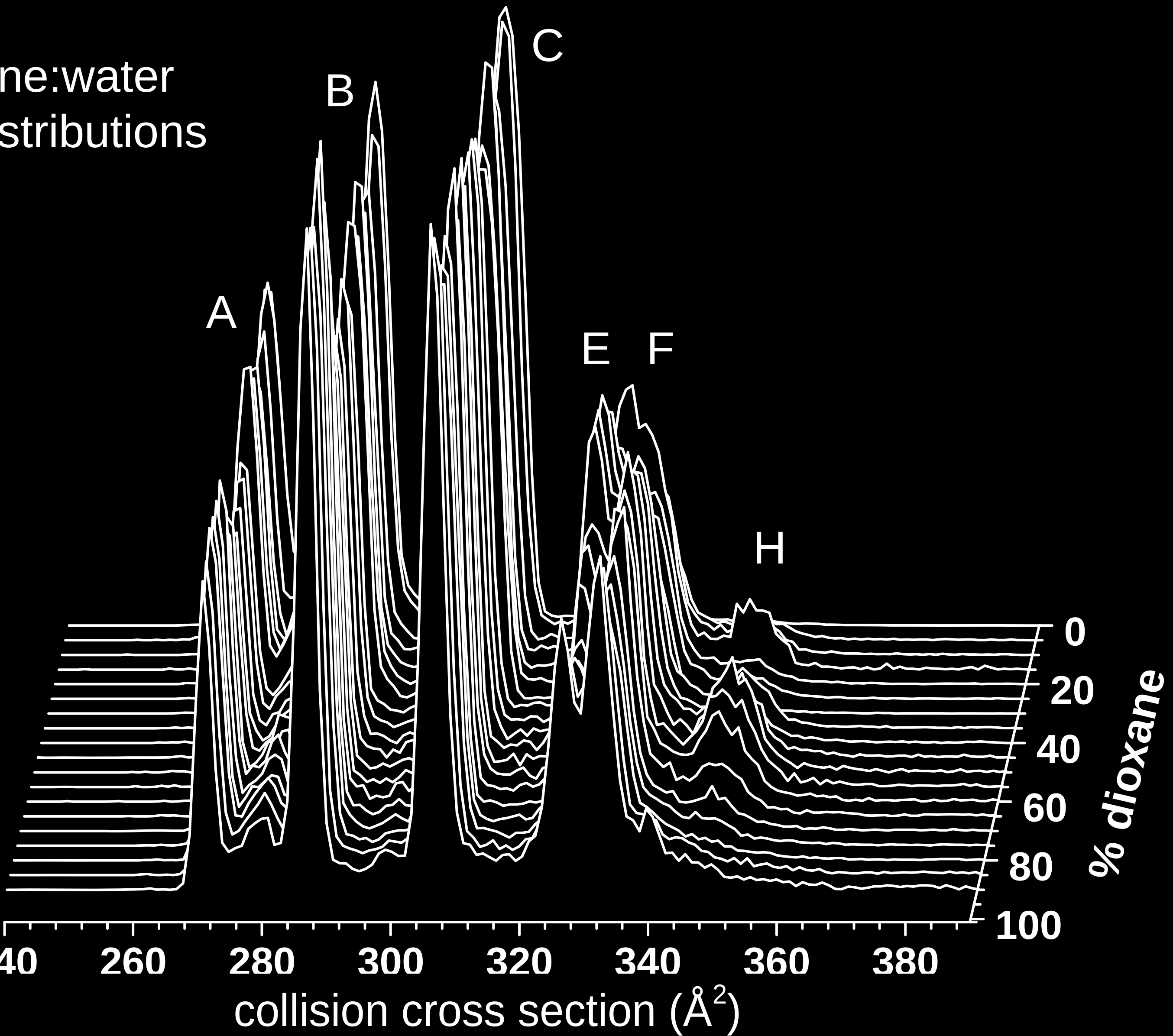
BK in methanol:water
$[M+3H]^{3+}$ distributions



Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

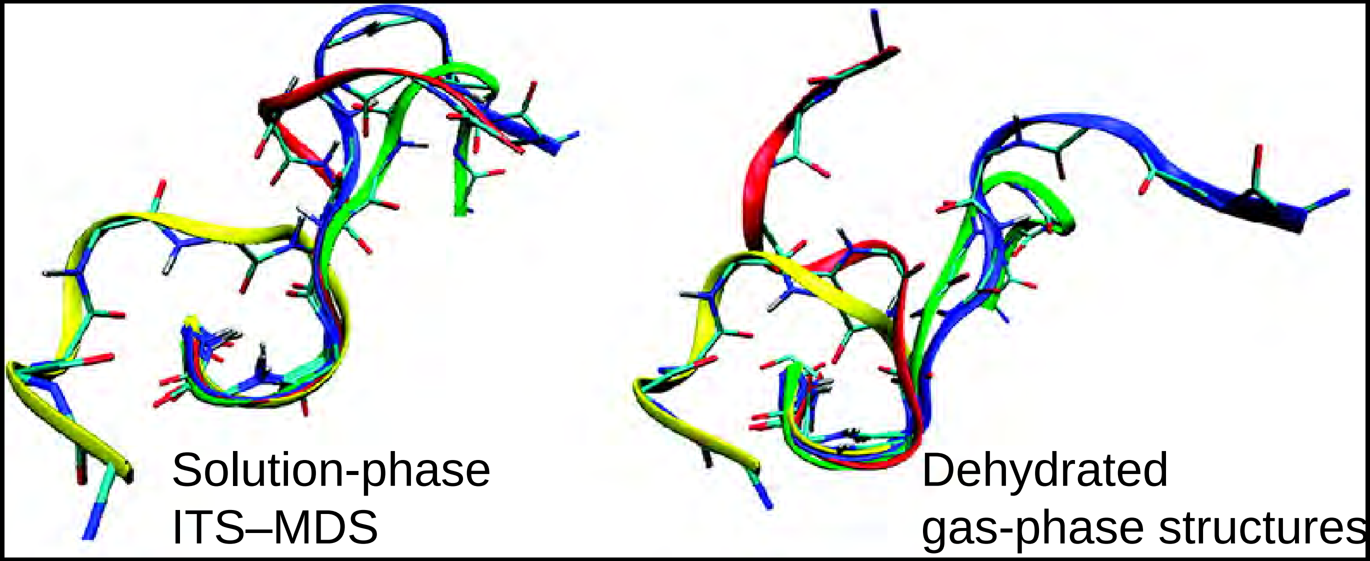
ESI Solution Studies

BK in dioxane:water
$[M+3H]^{3+}$ distributions



Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

Findings from BK Solution Scans

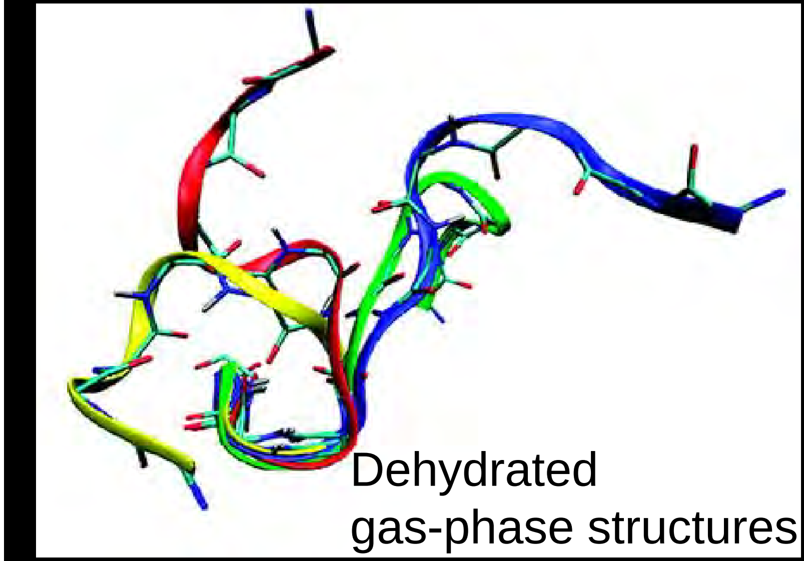


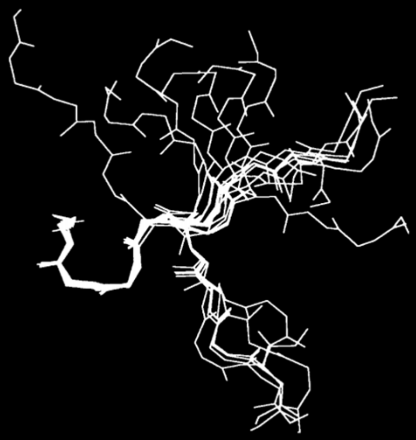
- Amino-terminal “unstructured” region consists of multiple, stable conformations

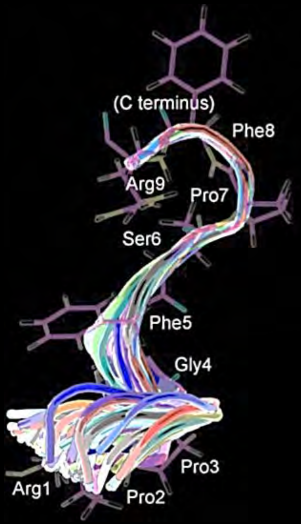
- Highly complementary to solution-phase characterization methods

Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

Findings from BK Solution Scans


Dehydrated
gas-phase structures


Young, J. K.; Hicks, R. P. *Biopolymers* **1994**, *34*, 611–623

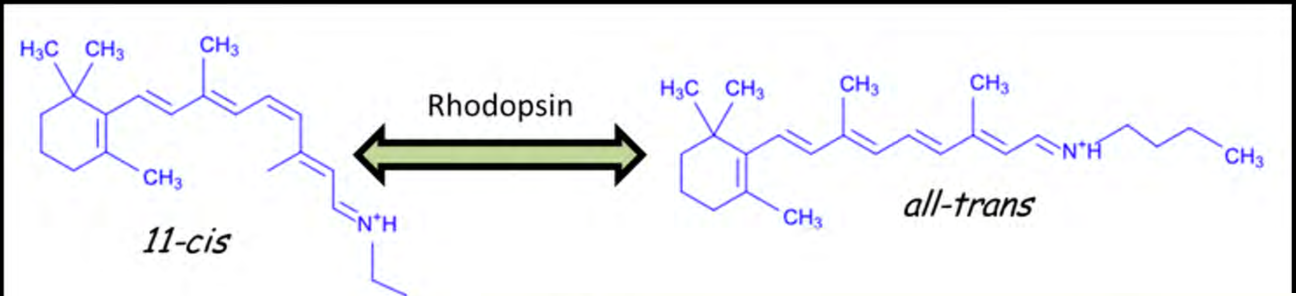


Lopez, J. J. et al. *Angew. Chem. Int. Ed.* **2008**, *47*, 1668 –1671

- Amino-terminal “unstructured” region consists of multiple, stable conformations
- Highly complementary to solution-phase characterization methods

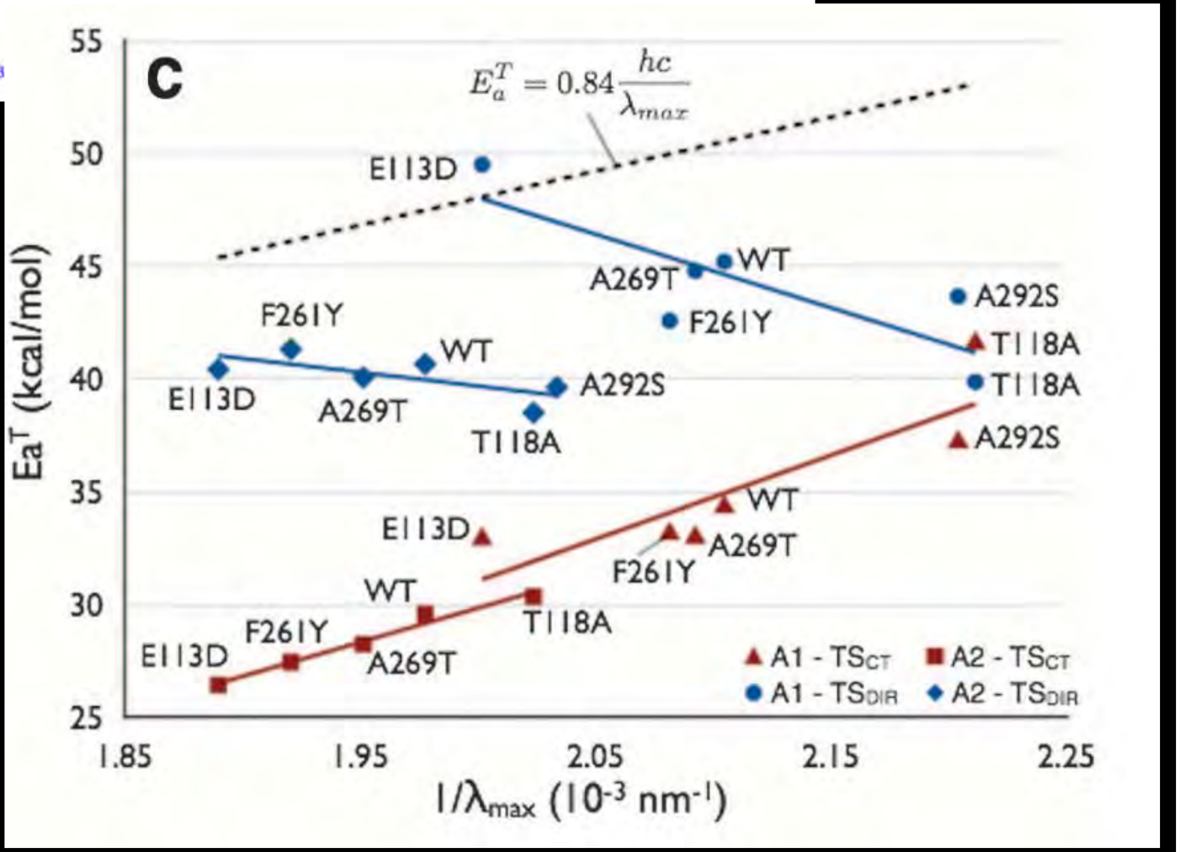
Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

Barlow Correlation



Experiment – Barrier energy in protein is ~1-1.2 eV, and inversely correlated with the absorption wavelength

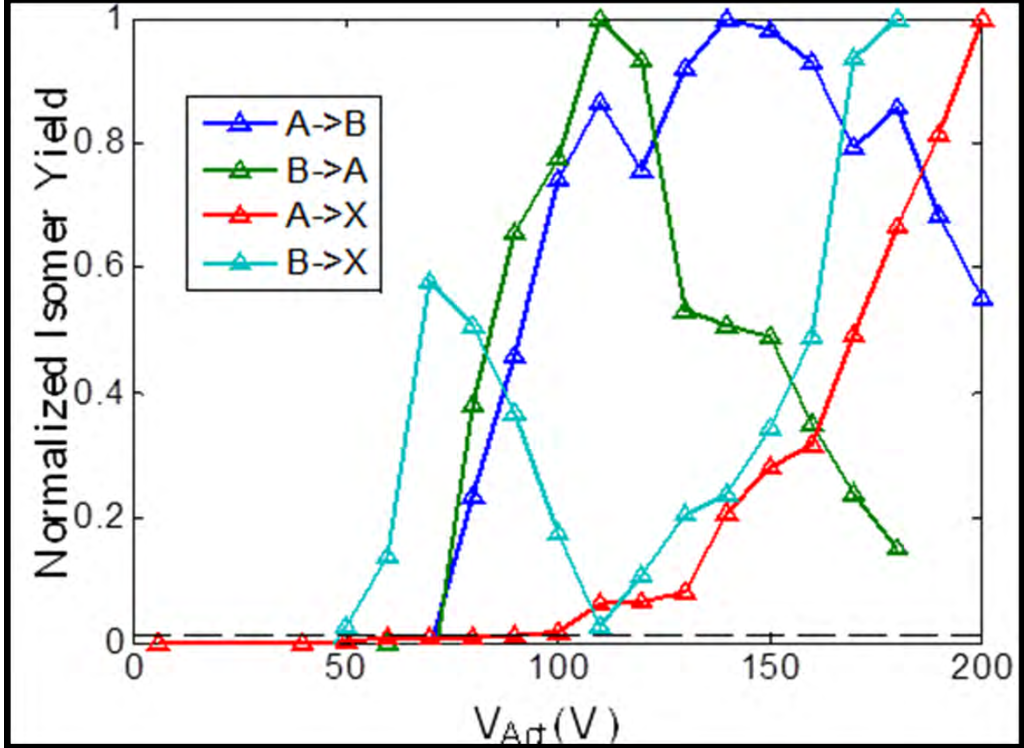
Theory – ~1-2eV



Barlow R.B.; Birge R.R.; Kaplan E.; Tallent J.R. *Nature* **1993**, *366*, 64–66

Gozem S.; Schapiro I.; Ferre N.; Olivucci M. *Science* **2012**, *337*, 1225-1228

IMS-IMS-MS: $[RPSB+H]^+$



- State-to-state conformational transitions
- Fragmentation pathway through a more compact, intermediate species

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834