

Biomolecules in isolation: Challenges and benchmarks for theory

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Outline

- ***The interplay between experiment and theory***
- ***Our experimental approach***
- ***Isolated biomolecules: challenges and benchmarks***

Why do theory?

3

Experiment-theory interface in rovibrational spectroscopy

1. Born-Oppenheimer approximation
 - separates electronic and nuclear motion
2. Use a “model” Hamiltonian for the nuclear motions
 - Simplest: rigid rotor, harmonic oscillator
 - Can add vib-rotation interaction, centrifugal distortion, anharmonicity, . . .
3. Fit the model to the spectrum
 - The fitting parameters provide information on the molecule

 ***The most accurate molecular geometries come from rotational or vib-rotation spectroscopy***

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Experiment-theory interface for large biological molecules

Challenges:

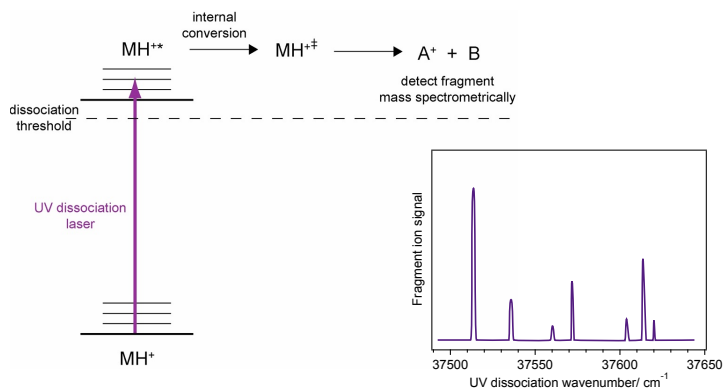
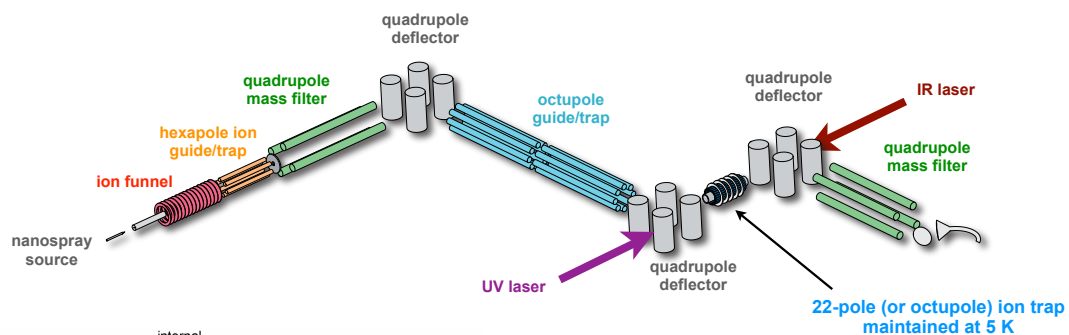
- No rotational resolution - only vibrational frequencies
- Many vibrational bands of the same type, problem of overlap
- Assignments of vibrational bands?
- Multiple stable conformations

1. Calculate the PES and find the lowest energy minima
 - first with classical force fields
 - refine quantum mechanically
2. Calculate vibrational spectrum for different minima
 - scale frequencies to account for anharmonicity
3. Compare with measured spectrum and select the structure that matches best.
 - No fitting procedure. No goodness of fit.

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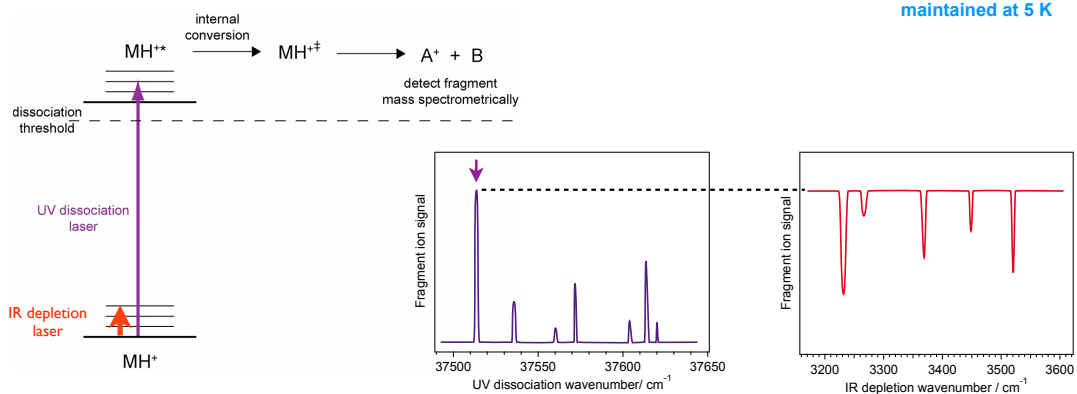
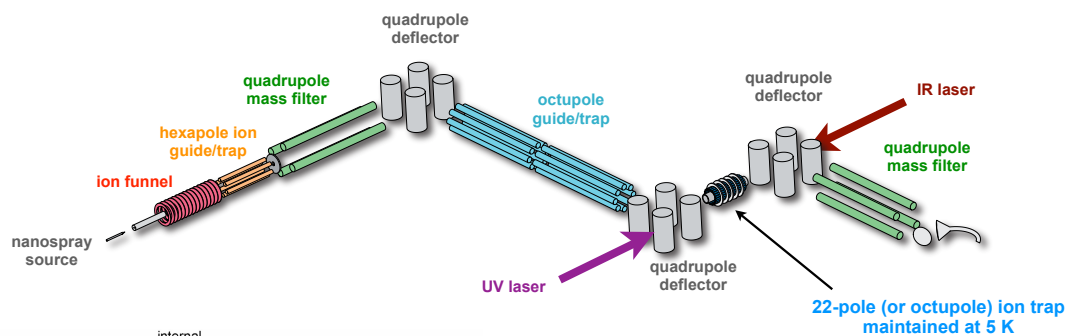
Our experimental approach

Cold-ion spectroscopy



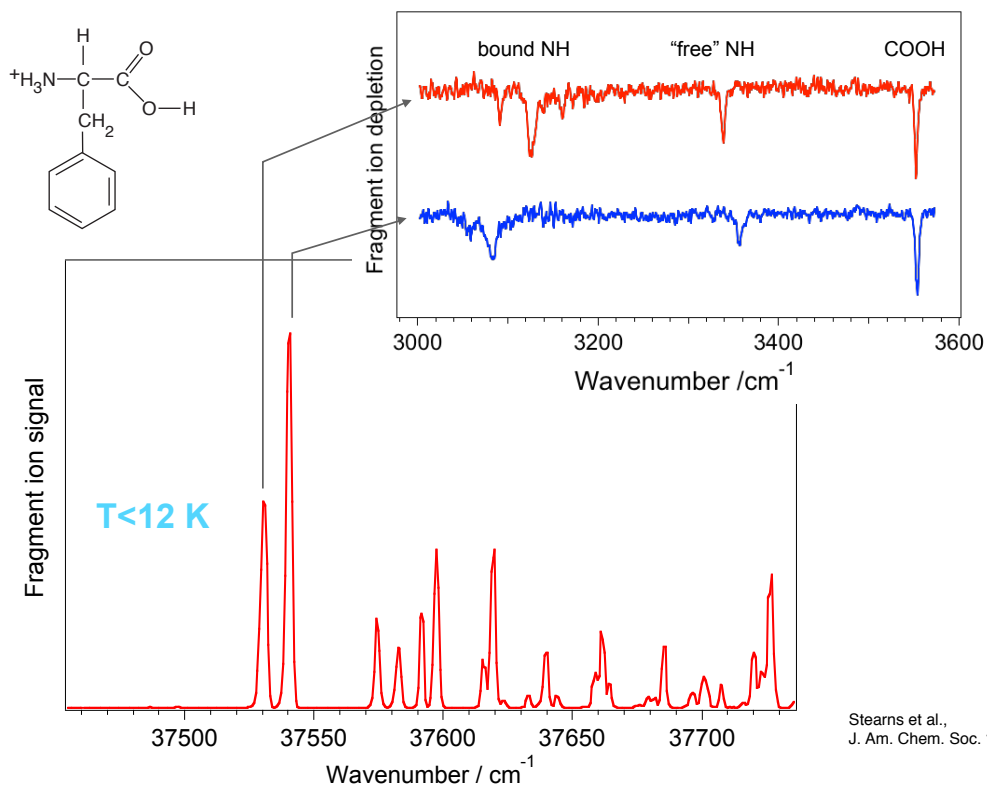
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Cold-ion spectroscopy



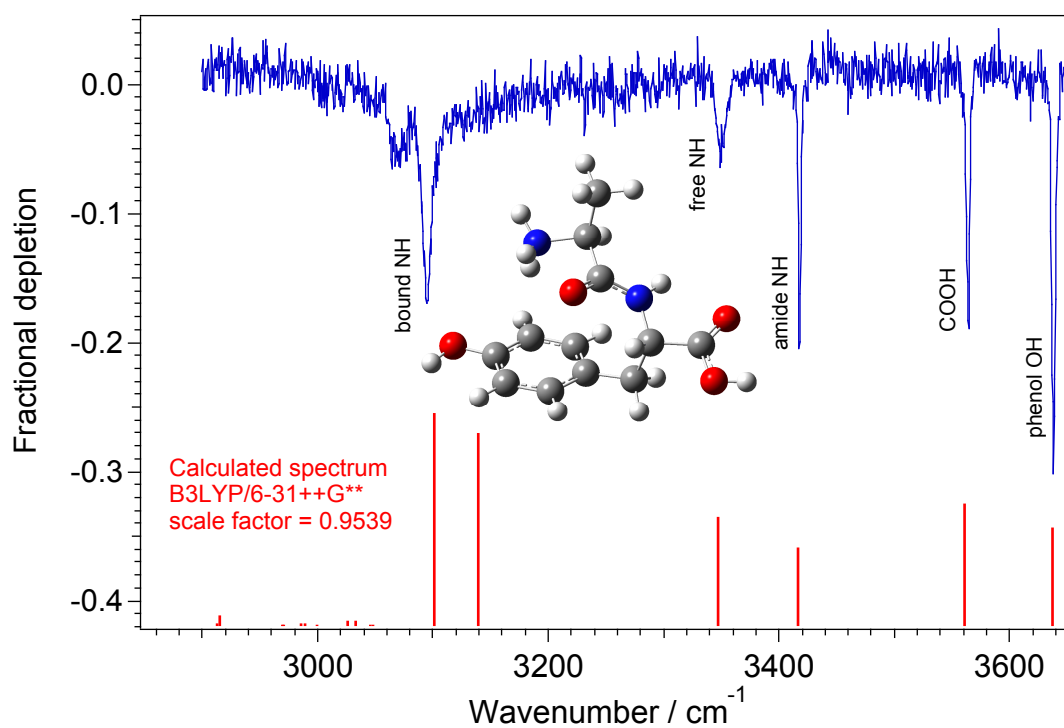
8

A simple example: phenylalanineH⁺



9

IR spectrum of protonated Ala-Tyr



10

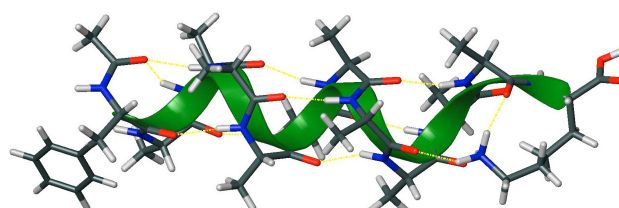
Example 1: helical peptides

Ion mobility studies of gas-phase helices

R. R. Hudgins and M. F. Jarrold,
J. Am. Chem. Soc. **121**, 3494 (1999).

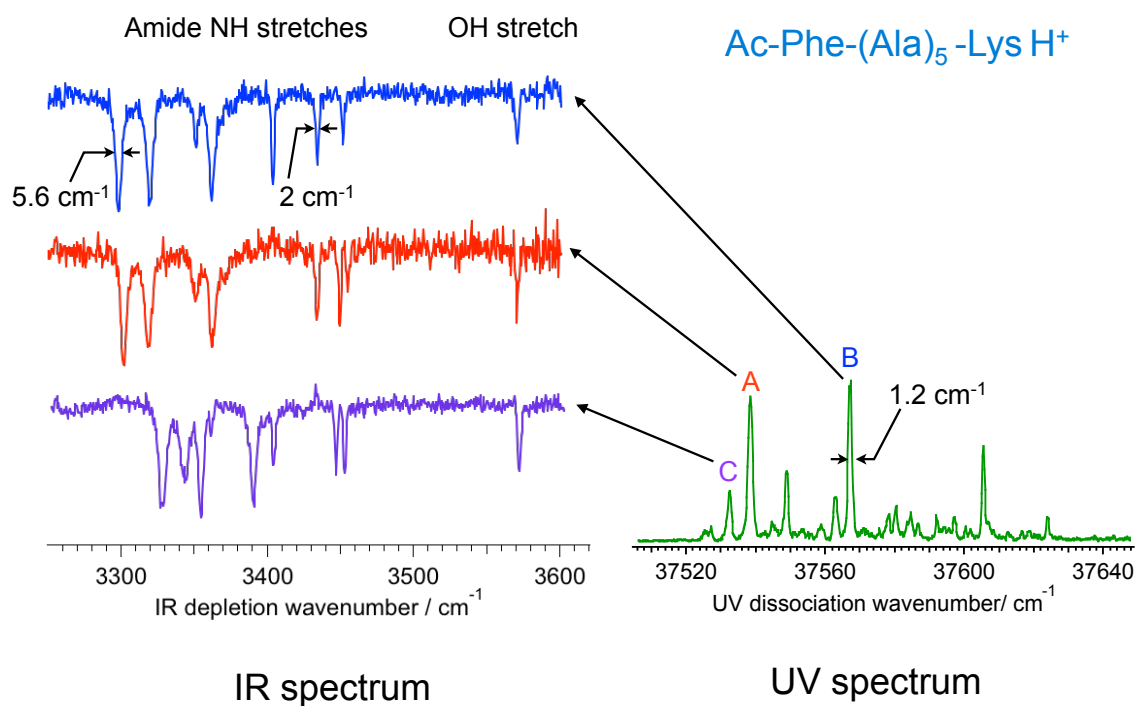
Ac-(Ala)_n-Lys H⁺ helical structures for n>5

Ac-Lys H⁺-(Ala)_n
(Ala)_n H⁺ } globular structures



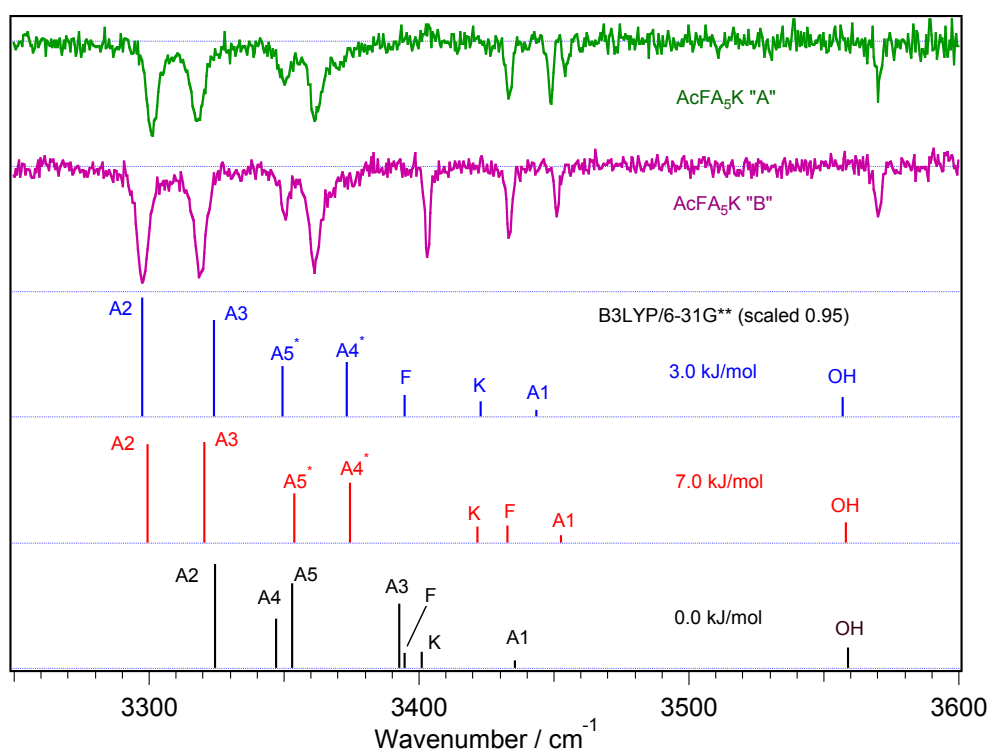
Ac-Phe-(Ala)_n-Lys H⁺

Conformer specific spectroscopy of gas-phase helices



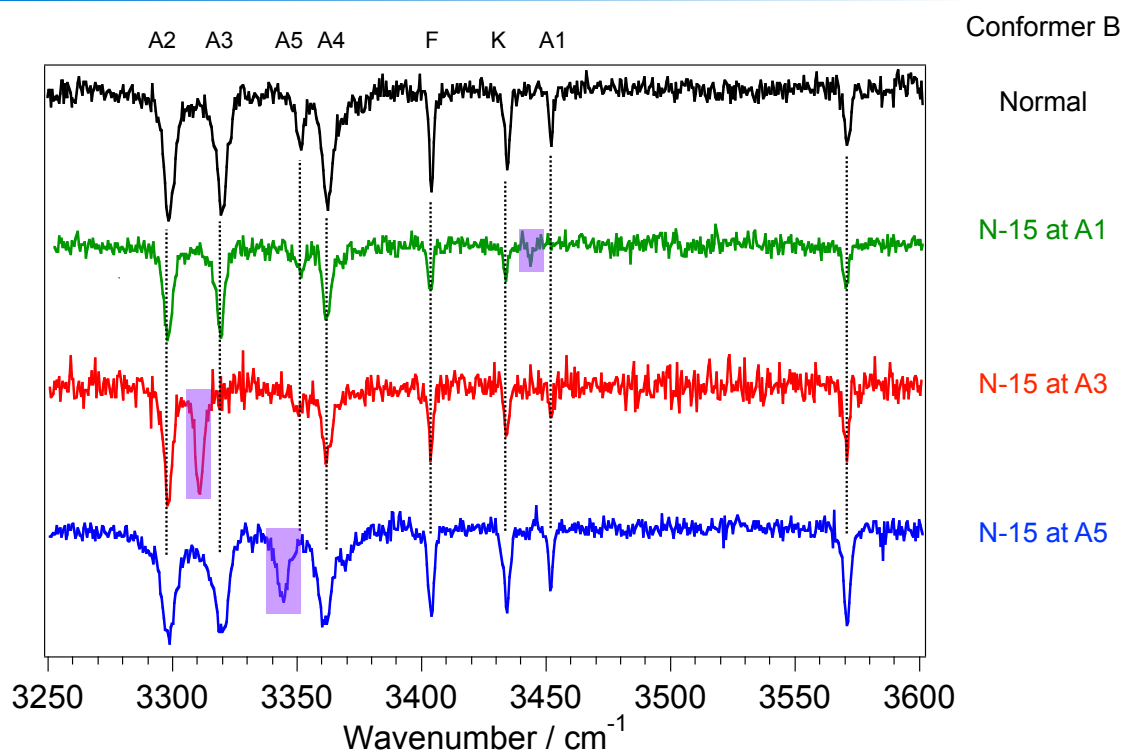
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Spectral assignments of Ac-Phe-(Ala)₅-Lys H⁺



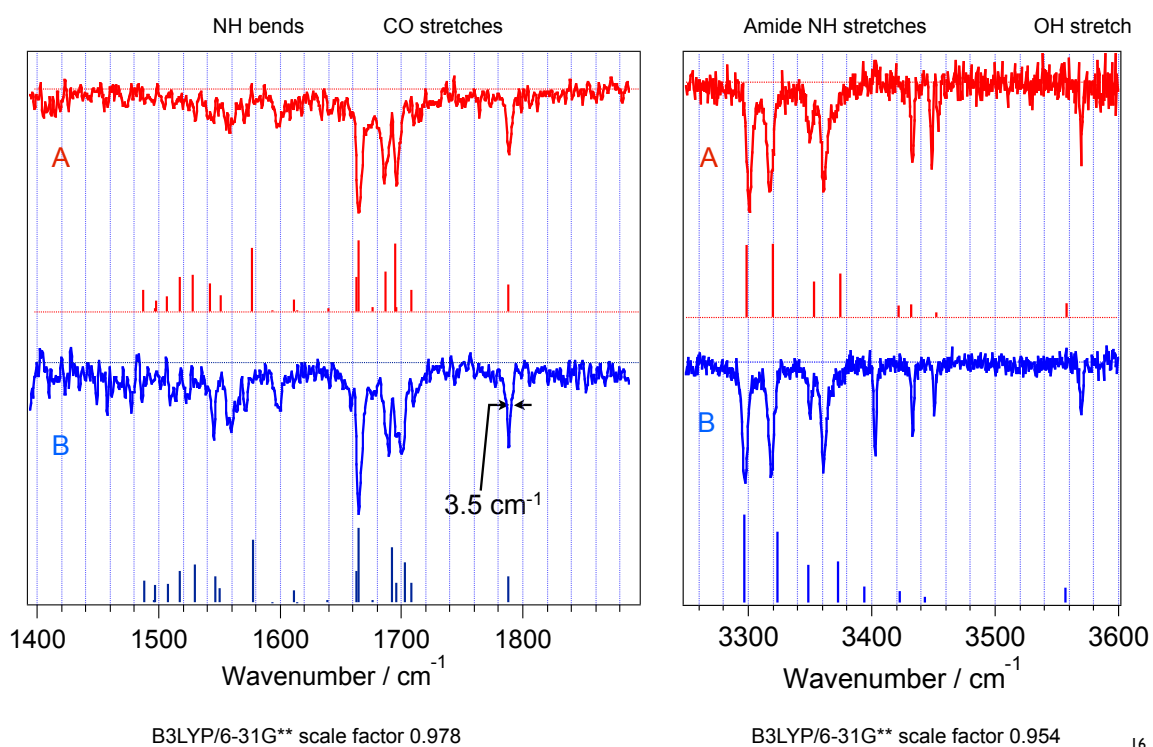
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Spectral assignments of Ac-Phe-(Ala)₅-Lys H⁺



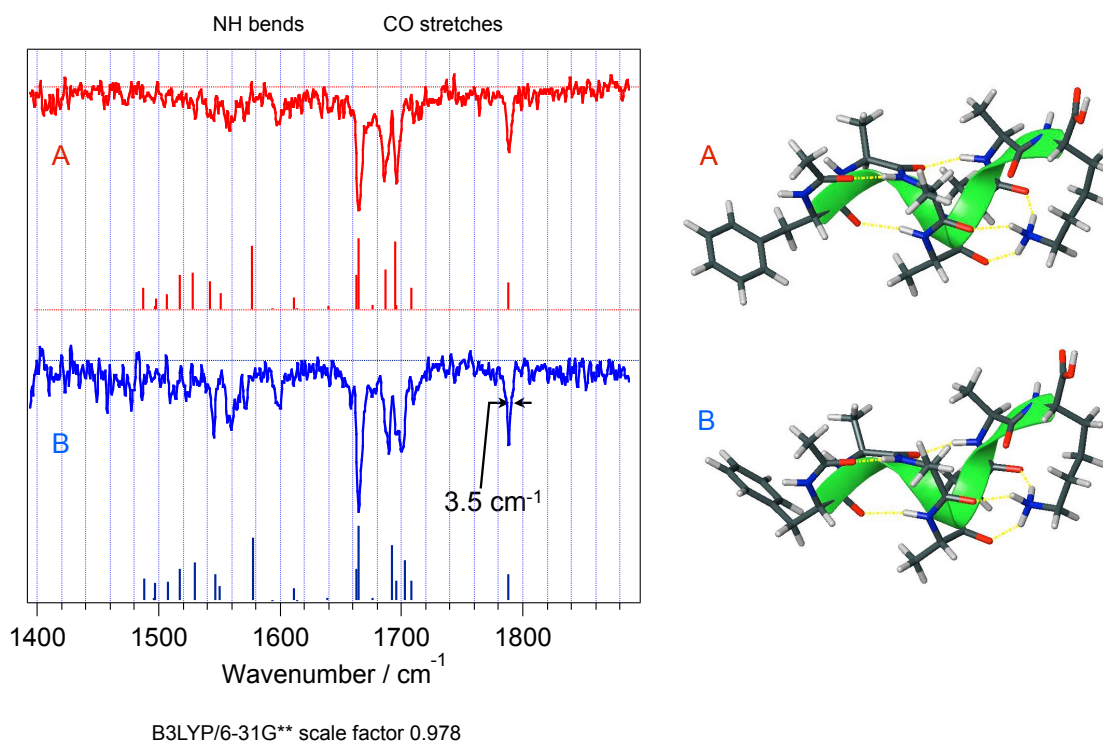
15

Extending into the amide I and II regions



16

Extending into the amide I and II regions



17

Example 1 conclusions

1. Need cooling and IR-UV to get single-conformer IR spectra
2. Need isotopic substitution to get band assignments

Challenge: do away with scale factors and calculate anharmonic vibrations

18

Example 2: Peptide sequence scrambling in mass spectrometry

19

A practical example

Peptide sequencing by MS: collision-induced dissociation (CID)

Do CID fragments cyclize and permute their sequence?

Example: [F — A — G — F — A — G — P — G]H⁺

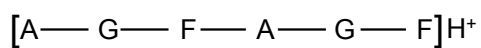
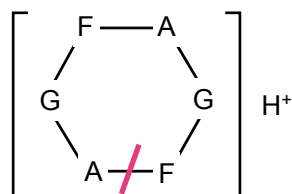
20

A practical example

Peptide sequencing by MS: collision-induced dissociation (CID)

Do CID fragments cyclize and permute their sequence?

Example: $[F - A - G - F - A - G]H^+$ b_6 -fragment



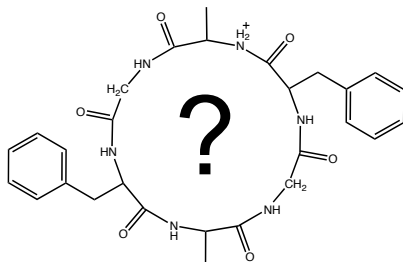
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A challenging example on a "small" system

Peptide sequencing by MS

Do CID fragments cyclize and permute their sequence?

Example: $[F - A - G - F - A - G]H^+$

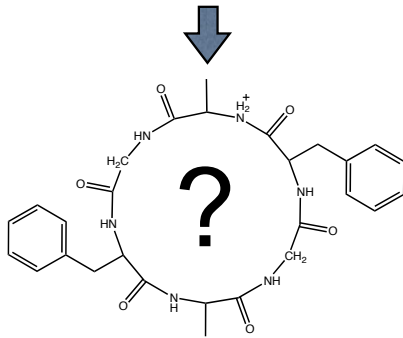
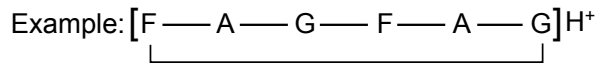


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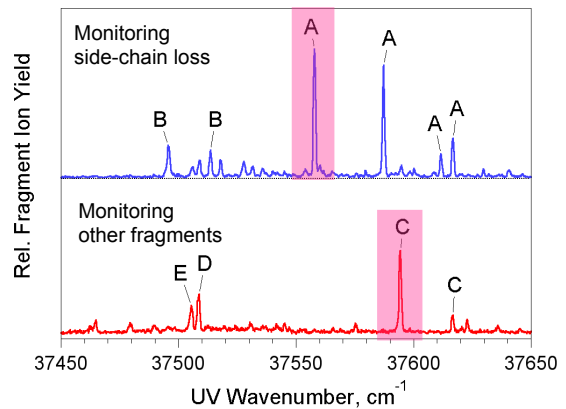
A challenging example on a "small" system

Peptide sequencing by MS

Do CID fragments cyclize and permute their sequence?



UV photo fragment spectra

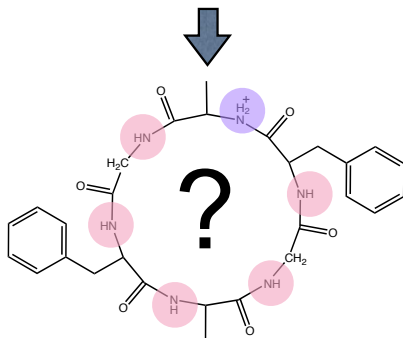
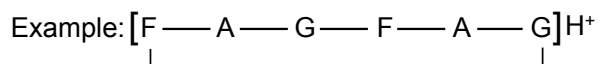


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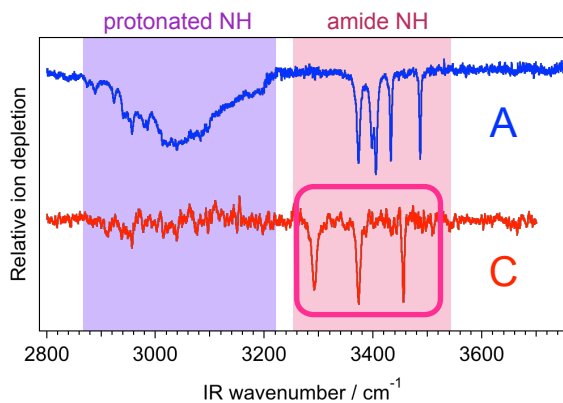
A challenging example on a "small" system

Peptide sequencing by MS

Do CID fragments cyclize and permute their sequence?



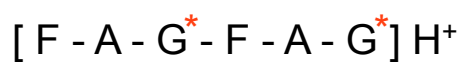
Conformer-specific vibrational spectra



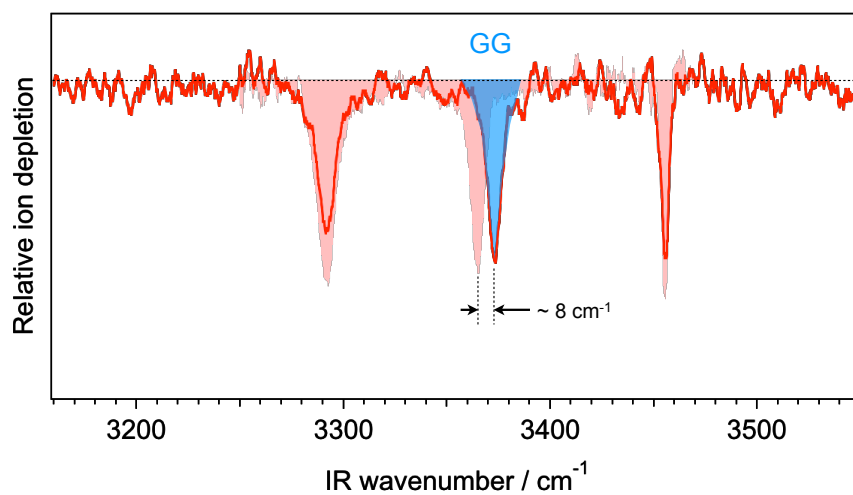
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Isotopic substitution for spectroscopic assignments

Conformer C



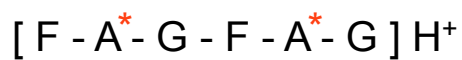
* denotes N-15 substitution on the amide NH



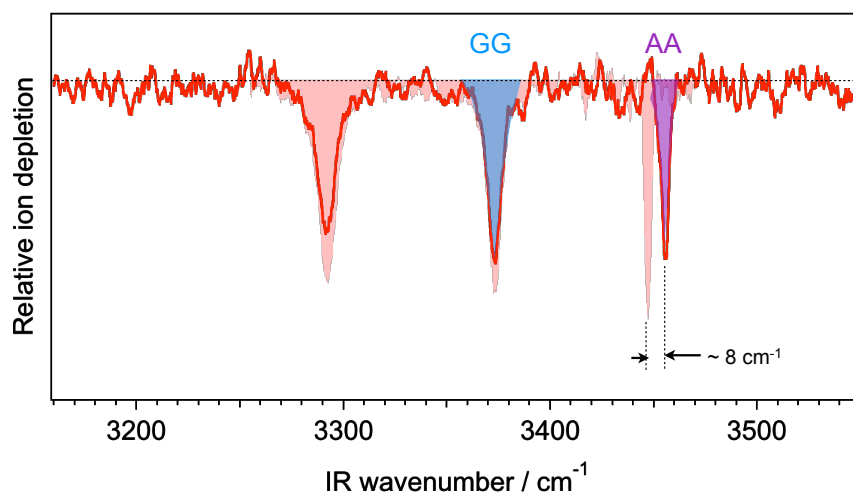
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Isotopic substitution for spectroscopic assignments

Conformer C



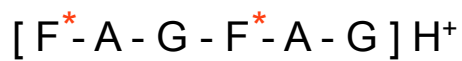
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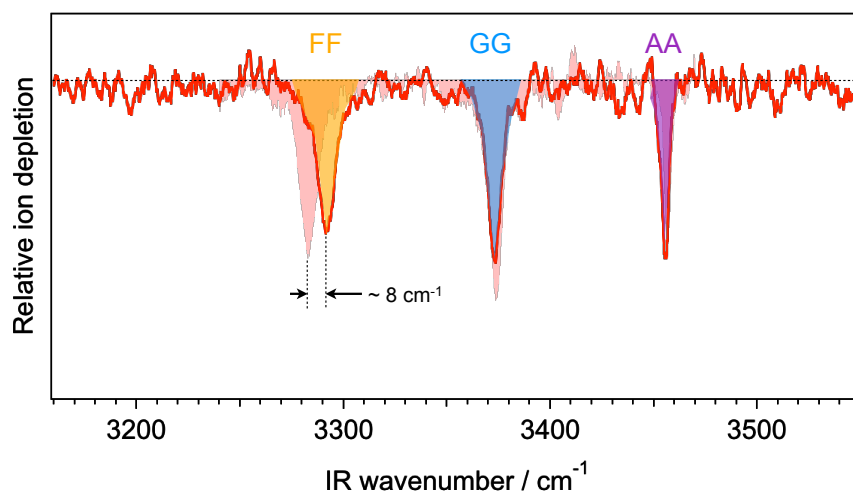
26

Isotopic substitution for spectroscopic assignments

Conformer C



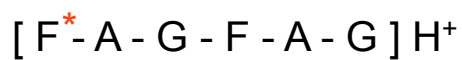
* denotes N-15 substitution on the amide NH



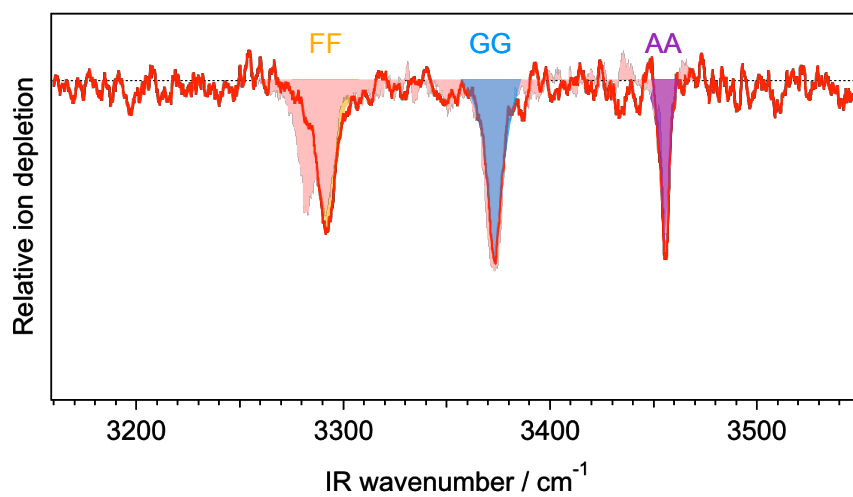
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Isotopic substitution for spectroscopic assignments

Conformer C



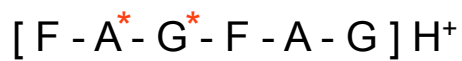
* denotes N-15 substitution on the amide NH



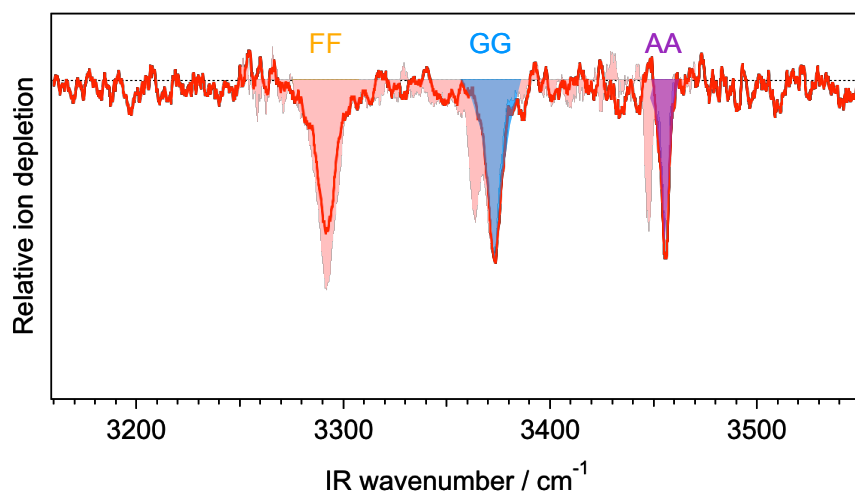
28

Isotopic substitution for spectroscopic assignments

Conformer C



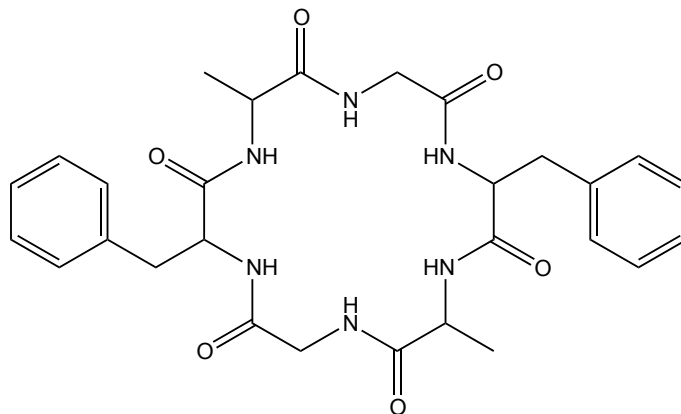
* denotes N-15 substitution on the amide NH



29

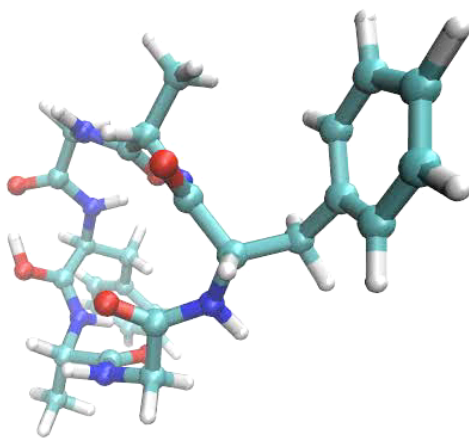
A symmetrical structure . . .

Conformer C



30

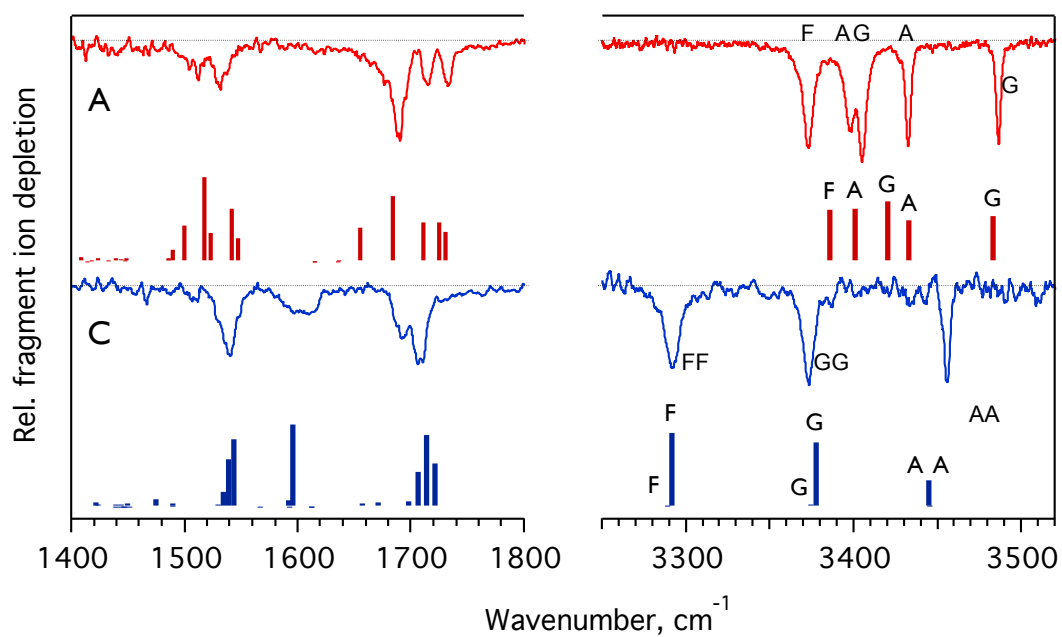
Quantum simulated annealing of conformer C



O. Aseev, M. A. S. Perez, U. Rothlisberger, and T. R. Rizzo, J. Chem. Phys. Lett. **6**, 2524 (2015).

31

Comparison with calculated structures

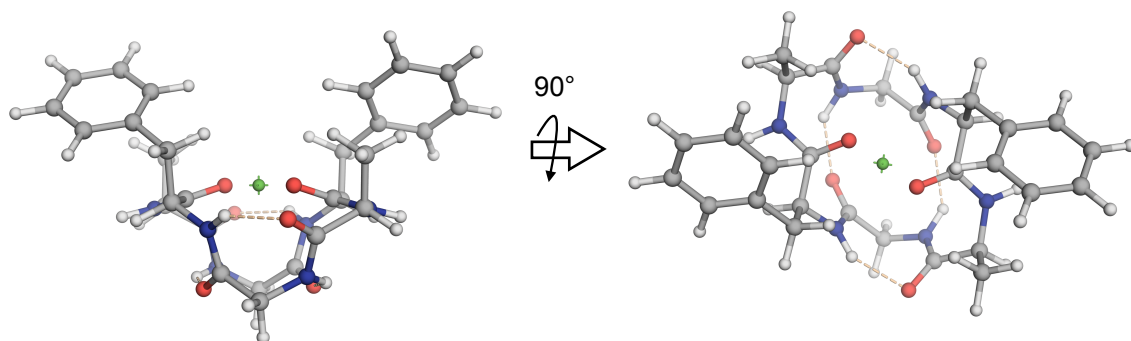


Computational details:

Conformational search - simulated annealing (SA) using AIMD based on DFT (B3LYP and a 6-31G)
Optimized energies - DFT M11//6-31G(d, p)

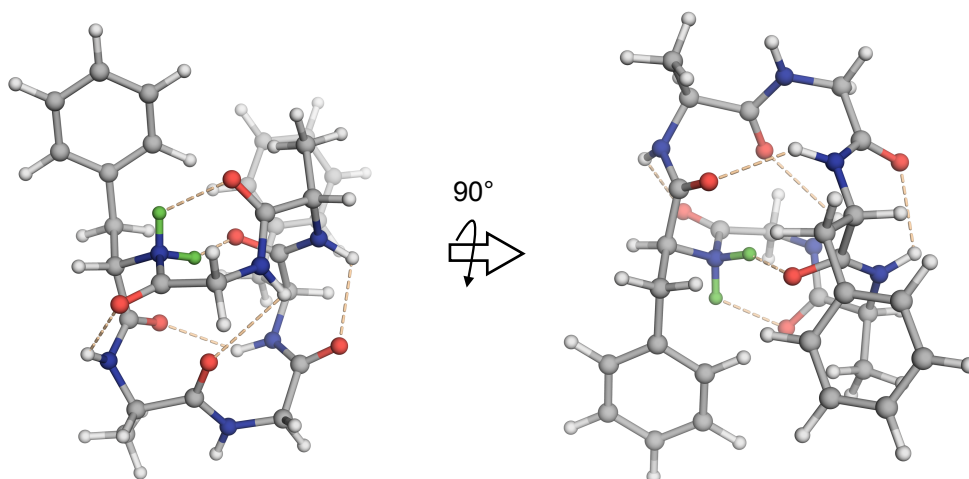
32

Conformer C: A symmetrical structure . . .



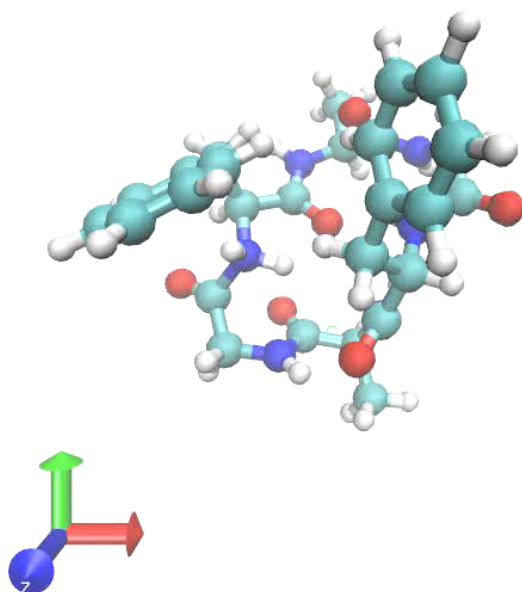
33

Conformer A: A cyclic, non-symmetrical structure



34

Conformer A: A cyclic, non-symmetrical structure



Example 2 conclusions

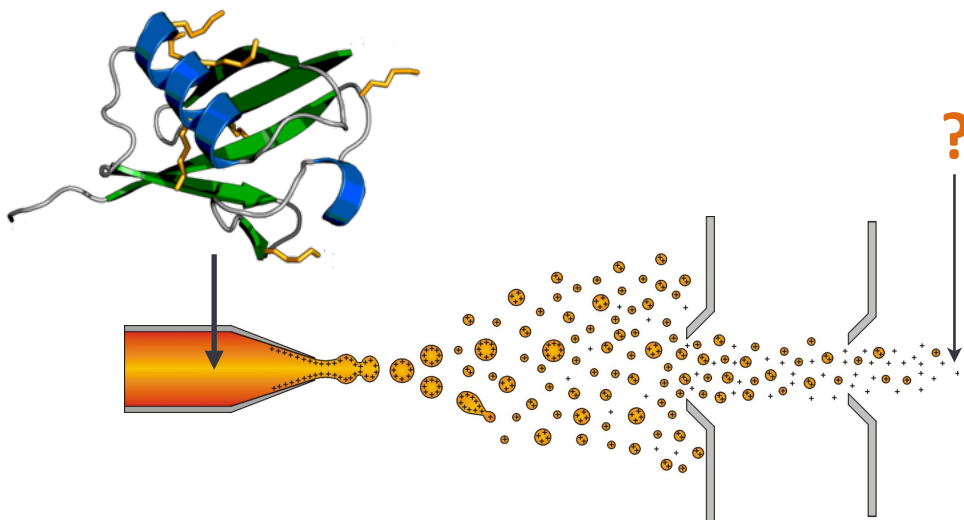
1. Conformational searches using FF's must assume protonation site. Cannot deal with mobile protons.

Challenge: perform conformational searches quantum mechanically

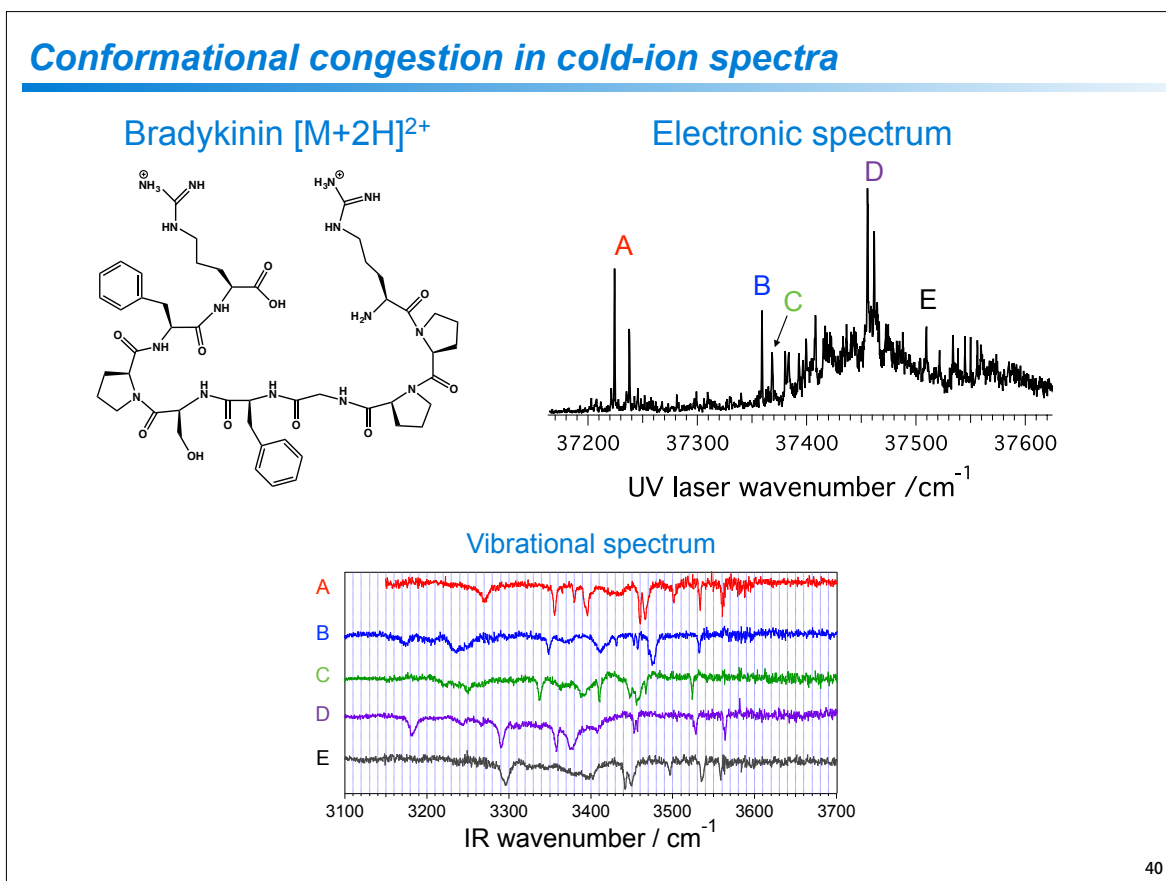
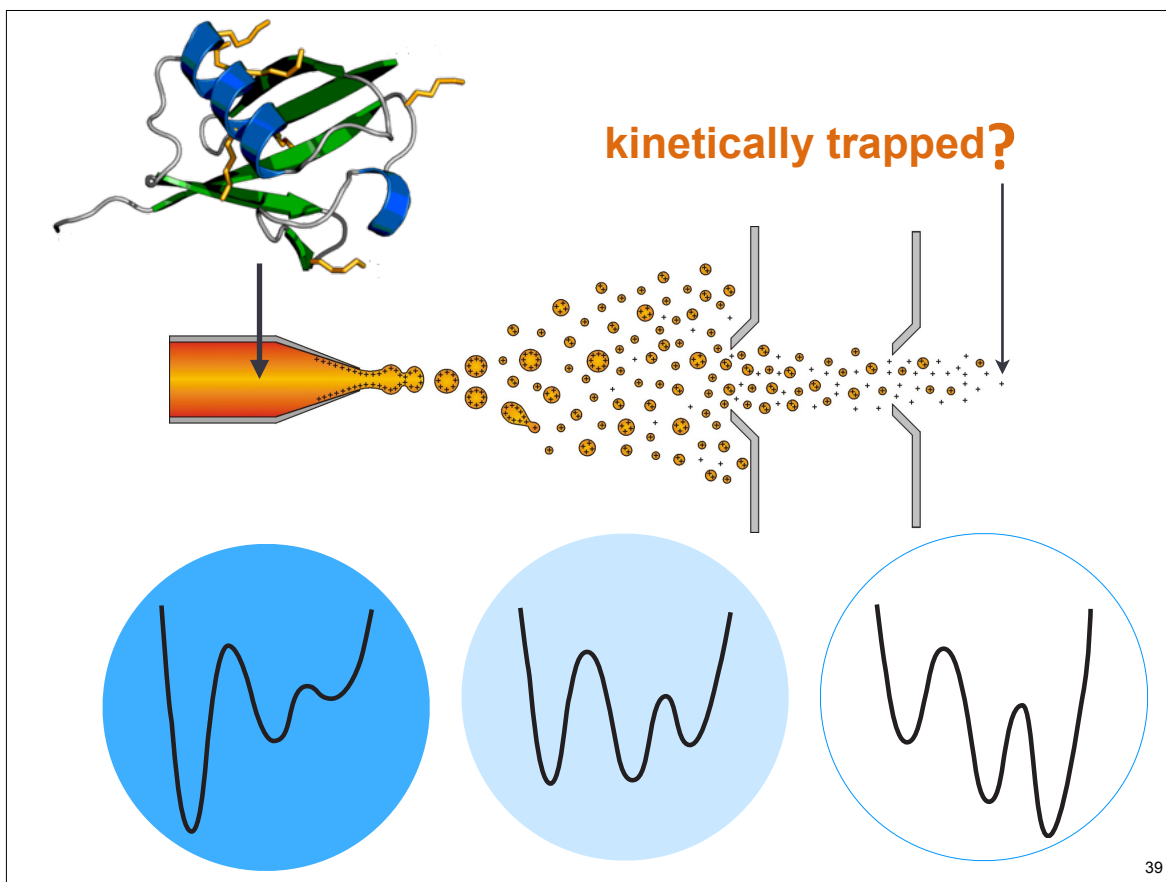
Example 3: Kinetic trapping of molecules produced by electrospray

37

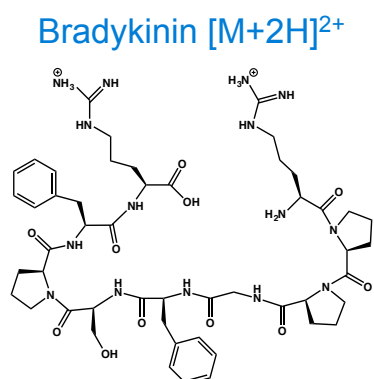
What is the relationship between the
structures of (bio)molecules produced by
electrospray and those in solution?



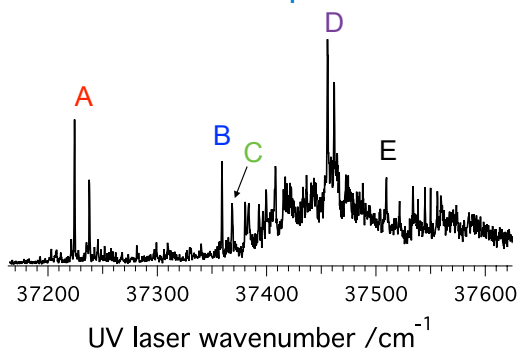
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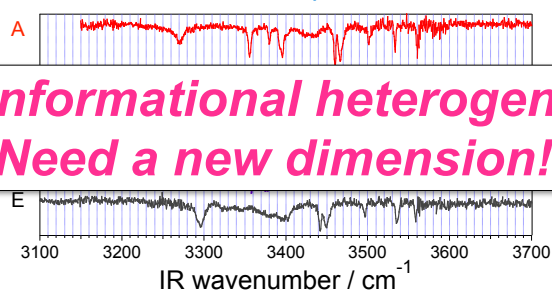
Conformational congestion in cold-ion spectra



Electronic spectrum

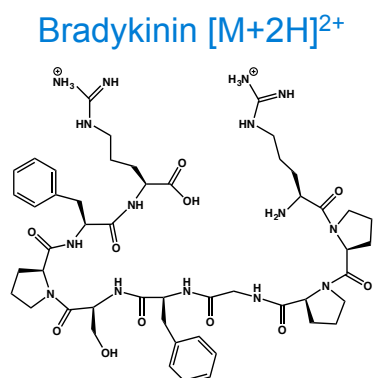


Vibrational spectrum

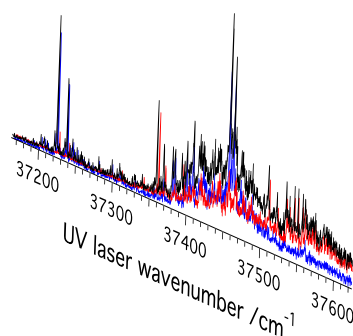


41

Conformational congestion in cold-ion spectra



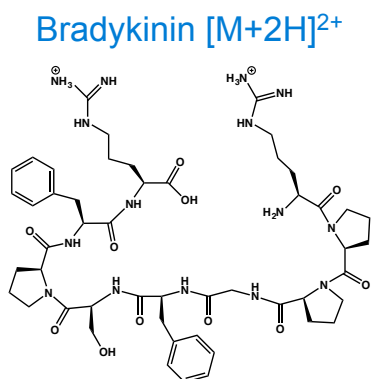
Electronic spectrum



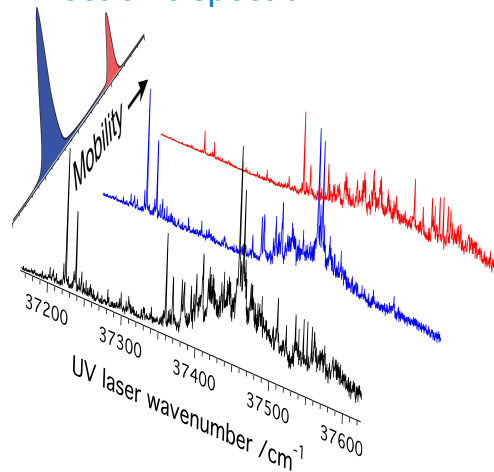
**Conformational heterogeneity
→ Need a new dimension!**

42

Conformational congestion in cold-ion spectra



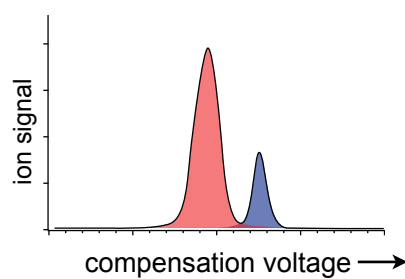
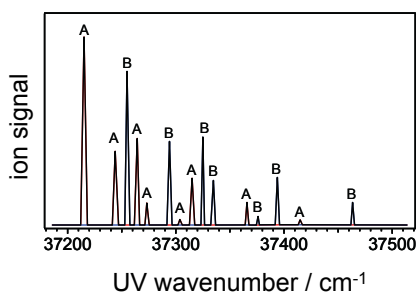
Electronic spectrum



**Conformational heterogeneity
→ Need a new dimension!**

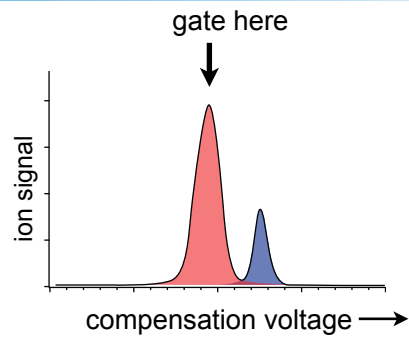
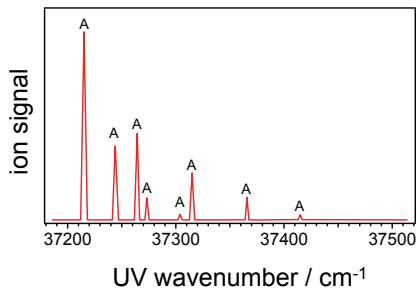
43

Ion mobility as a conformational filter for spectroscopy



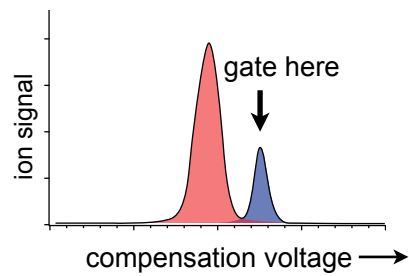
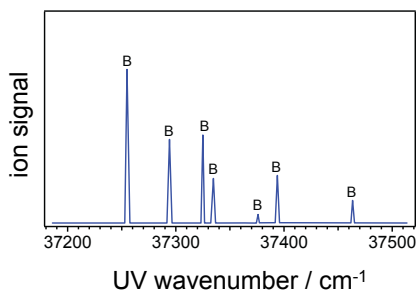
44

Ion mobility as a conformational filter for spectroscopy



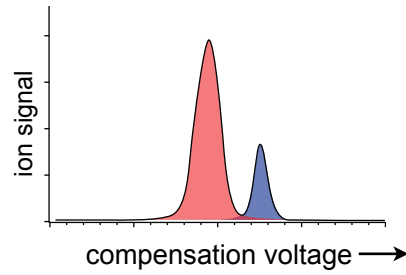
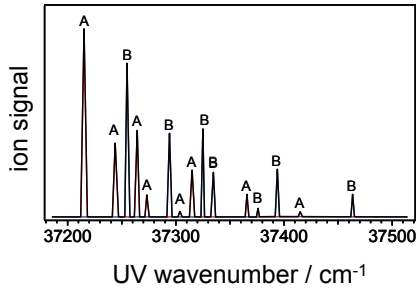
45

Ion mobility as a conformational filter for spectroscopy



46

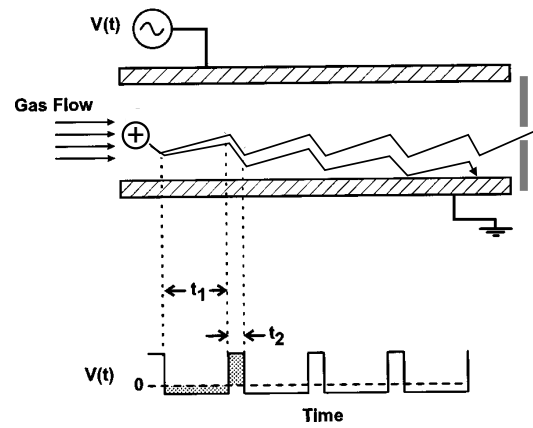
Ion mobility as a conformational filter for spectroscopy



Field Asymmetric Ion Mobility Spectrometry (FAIMS)

Differential ion mobility spectrometry

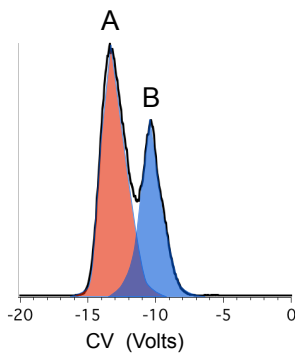
if mobility at high field \neq mobility at low field



47

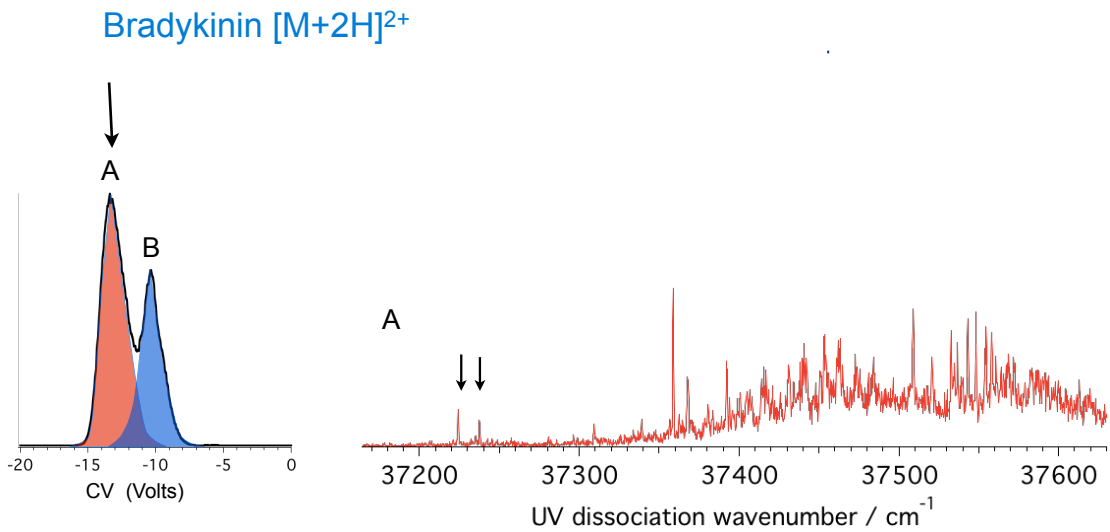
Using FAIMS as a conformational filter

Bradykinin $[\text{M}+2\text{H}]^{2+}$



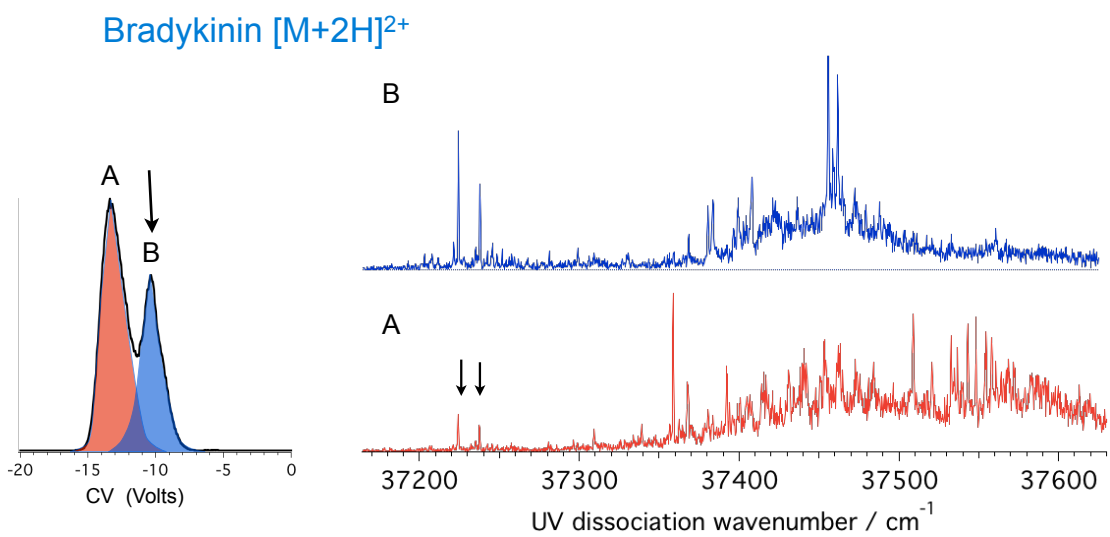
48

Using FAIMS as a conformational filter



49

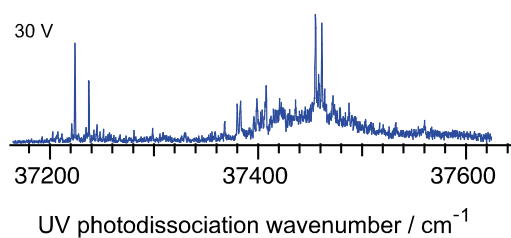
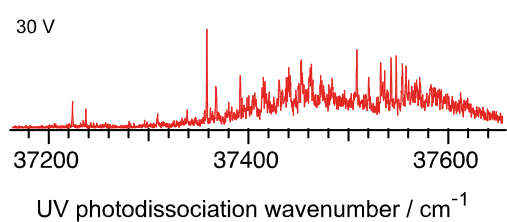
Using FAIMS as a conformational filter



50

Conformational isomerization

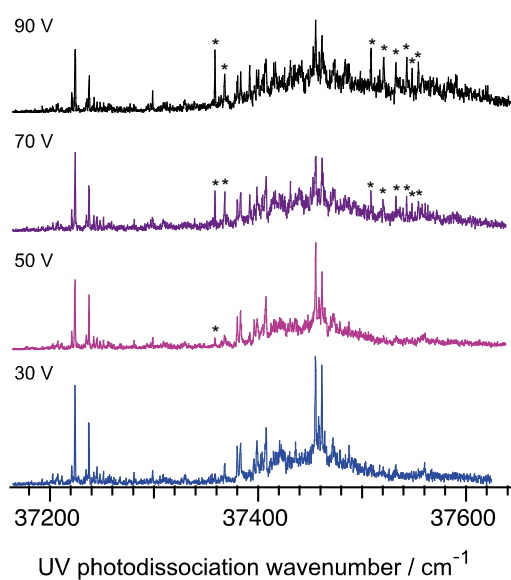
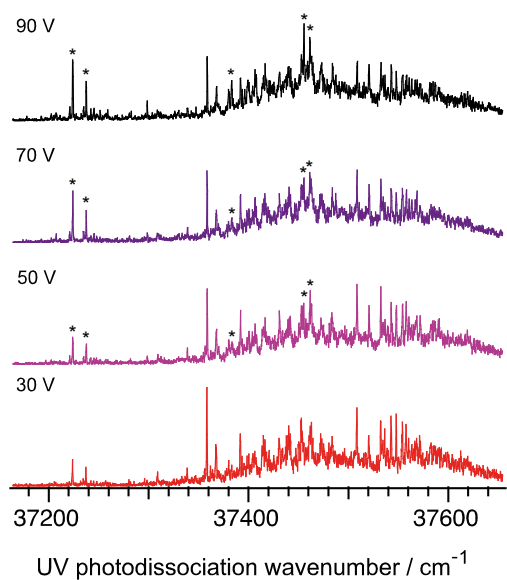
“quasi-equilibrium” conformer distribution



51

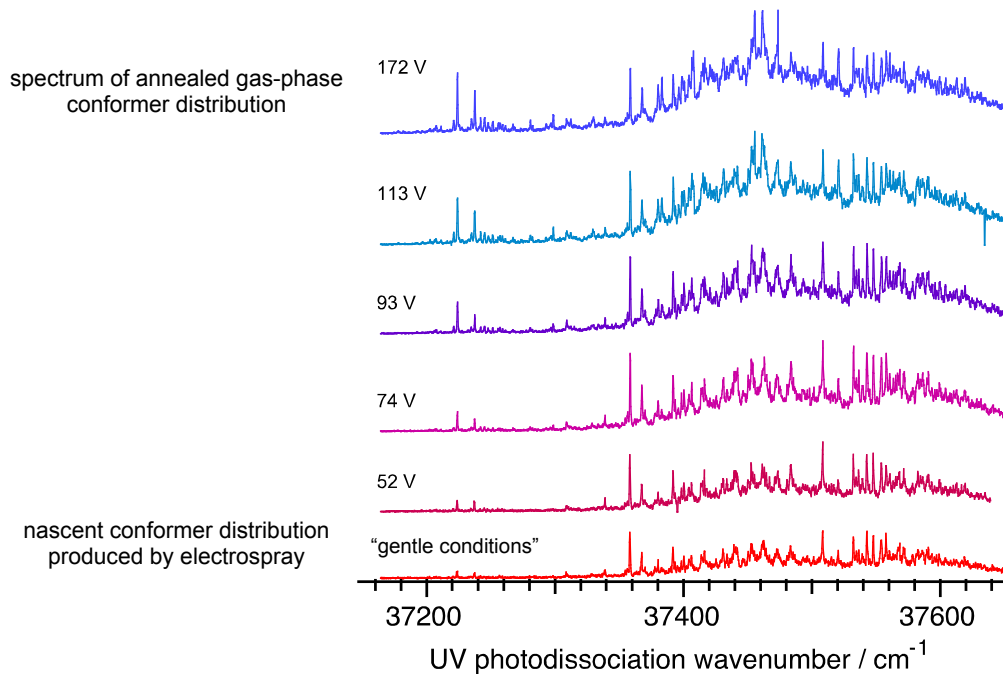
Conformational isomerization

“quasi-equilibrium” conformer distribution



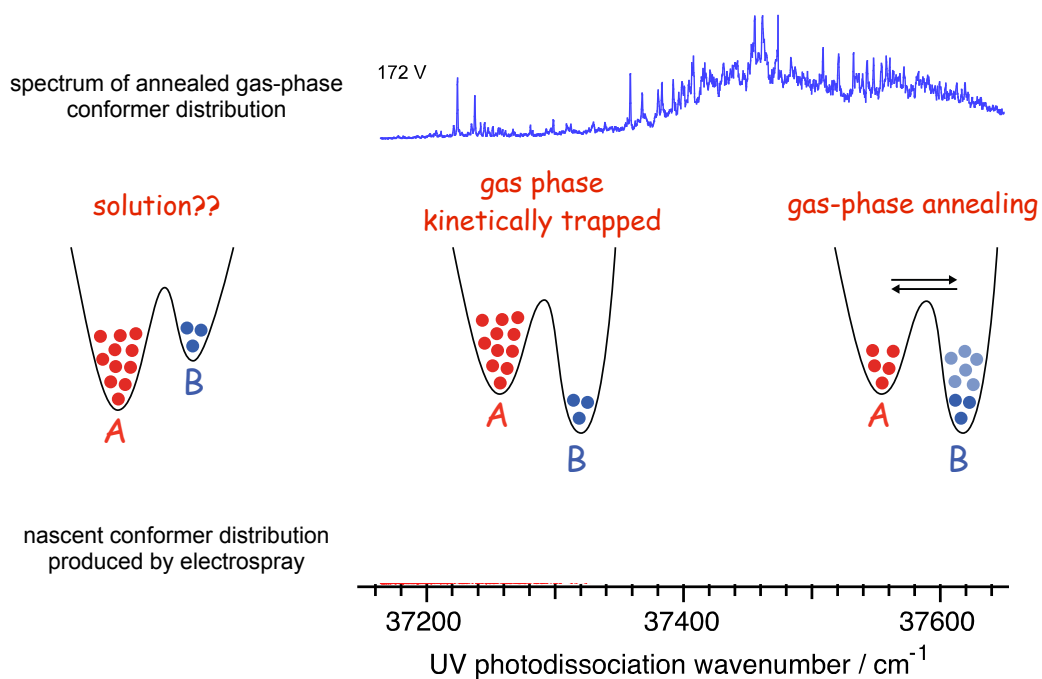
52

Kinetic trapping of conformations



53

Kinetic trapping of conformations



54

Example 3 conclusions

1. Additional conformation separation techniques needed to simplify the spectra of large molecules.

Challenge: finding high-energy, kinetically trapped species in conformational searches

55

Challenges for theory of isolated biomolecules

- ➔ Anharmonic vibrational calculations
- ➔ Conformational searching at the QM level
- ➔ Ability to find kinetically trapped conformations

56

Acknowledgements



Jaime Stearns



Oleg Aseev



George Papadopoulos



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Marta Perez

Funding:

