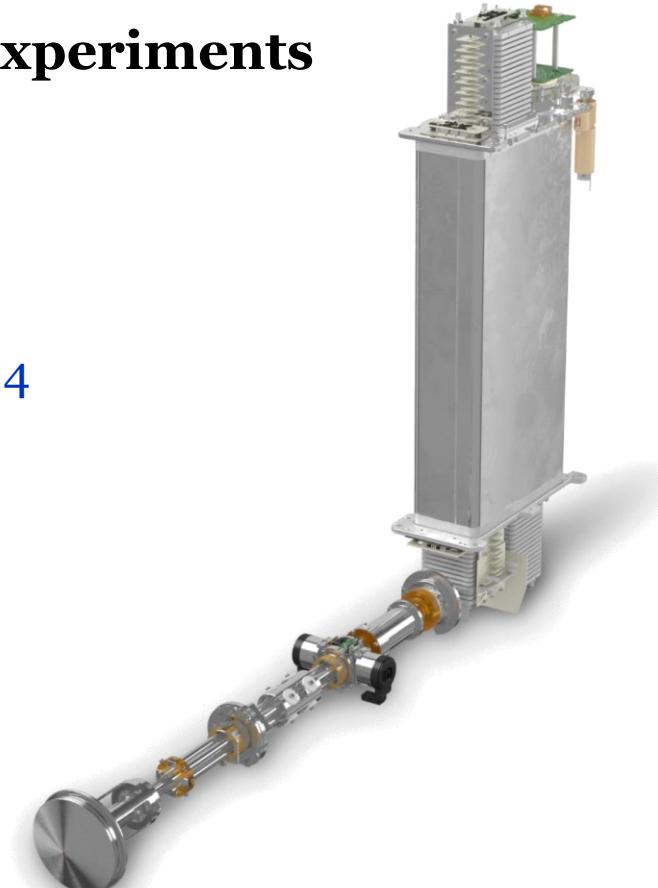


Non-ergodic dissociation of covalent molecules via IRMPD

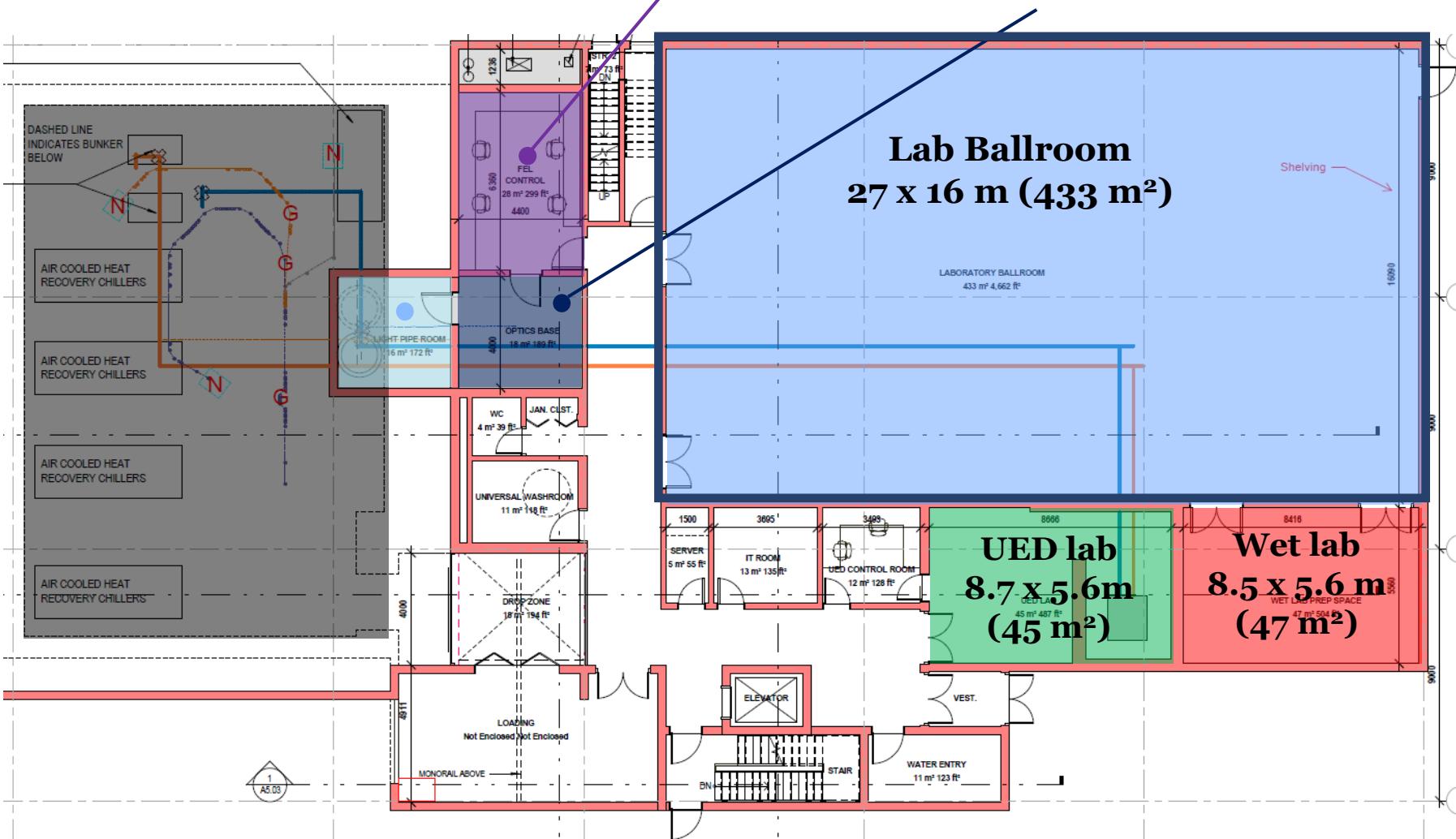
But first: WaterFEL user end-stations and planned experiments

Christian Ieritano (WaterFEL)

Infrared Free-Electron Lasers: The State of the Art 2024



WaterFEL facility



Lab Ballroom

10 user end-stations interfaced w/ FEL and/or external lasers

IR microscopy

Wet lab

Fumehood-equipped sample prep and storage area

UED lab

300 kV ultra-fast electron diffraction interfaced w/ FEL



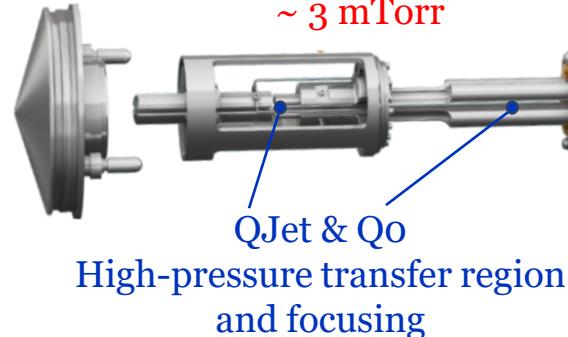
WaterFEL end station #1

SCIEX QTRAP 5500

ESI source with hybrid triple-quad/linear ion trap MS modified for UV-PD

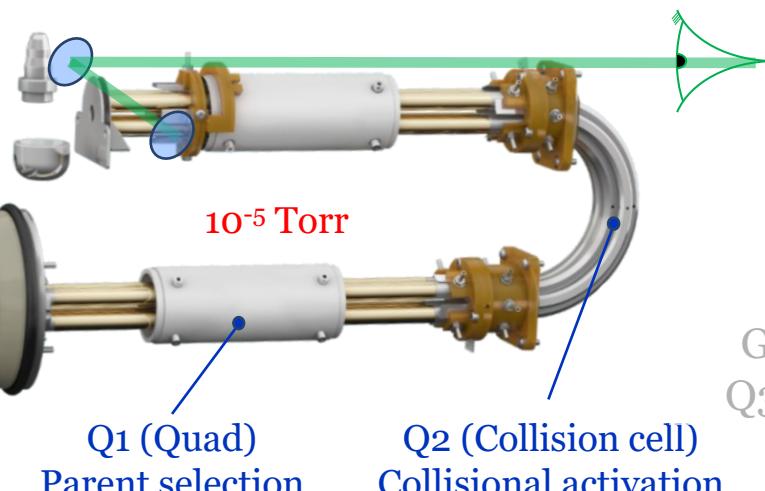


Orifice plate



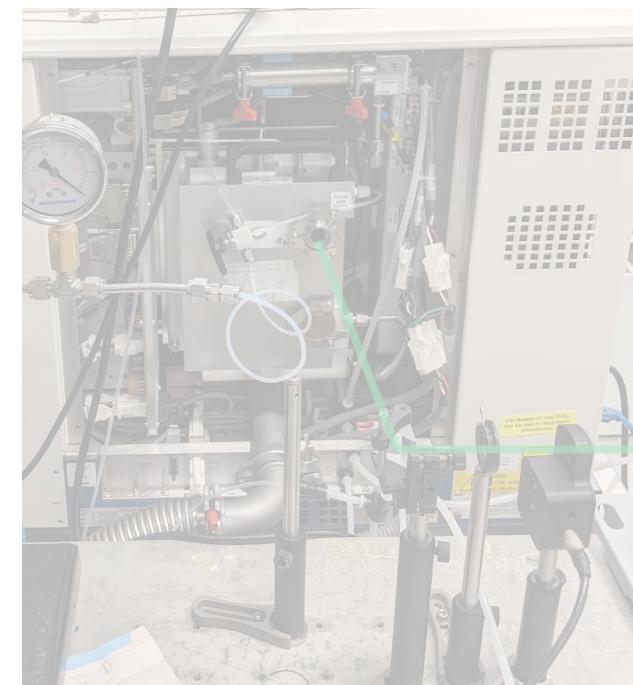
...and soon to be IRMPD!

Q3 (Linear ion trap)
Product ion detection & MS^n



3

Hole “drilled” into the ion chamber cover and affixed w/ UV-transparent lens



Gas mixer can pulse “alternatives” into Q3 (e.g., SF₆, solvent-entrained N₂, etc.)



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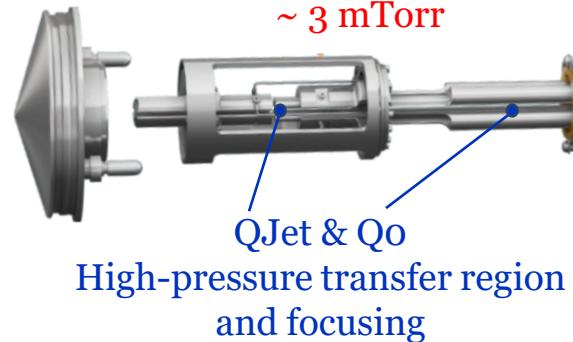
WaterFEL end station #1

SCIEX QTRAP 5500

ESI source with hybrid triple-quad/linear ion trap MS modified for UVPD

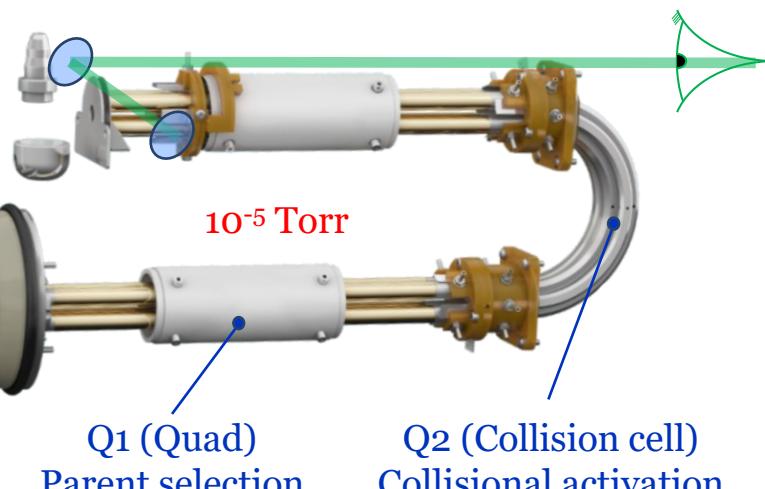


Orifice plate

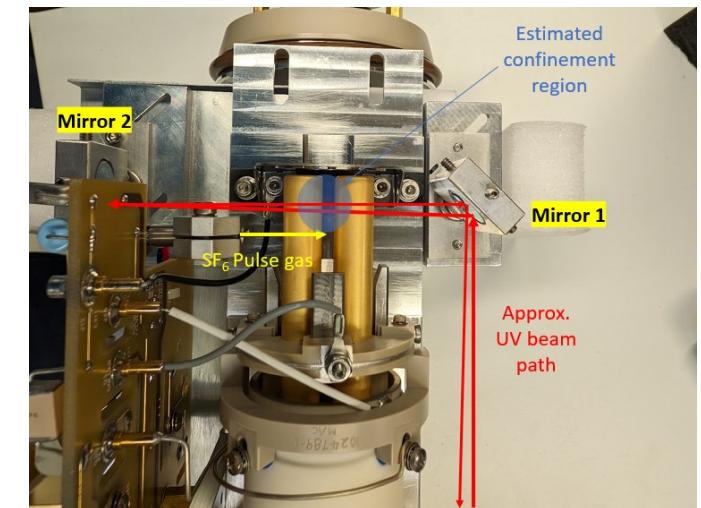
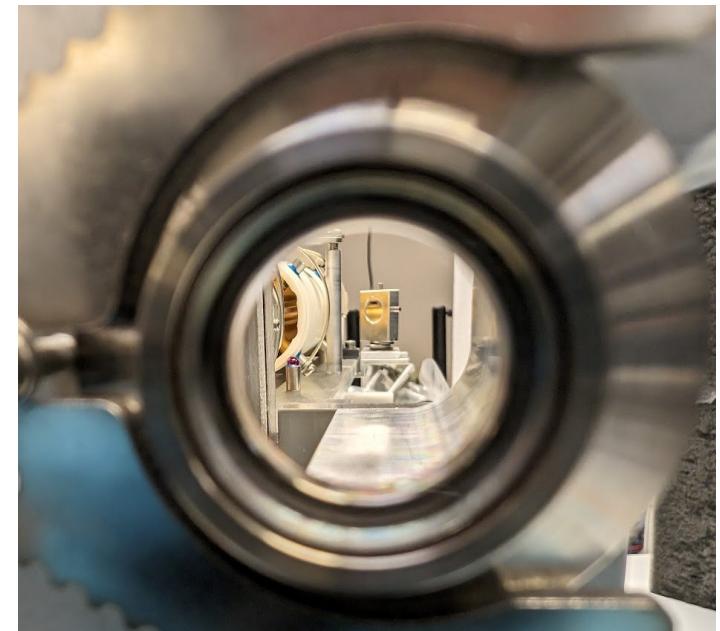


...and soon to be IRMPD!

Q3 (Linear ion trap)
Product ion detection & MSⁿ



4

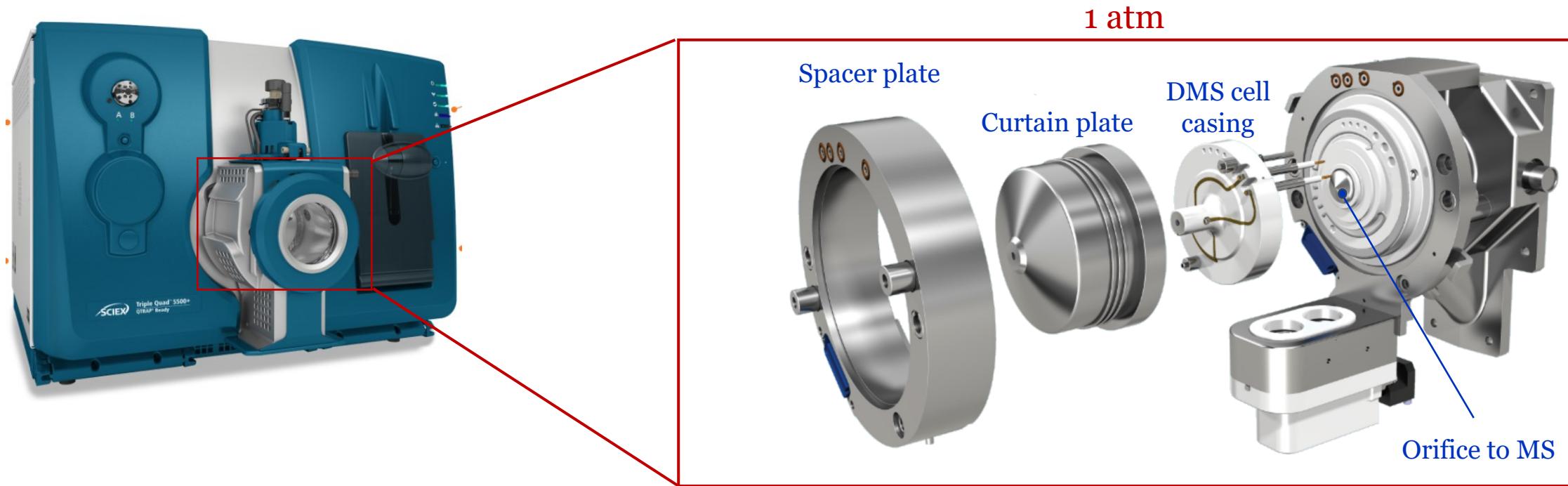


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WaterFEL end station #1

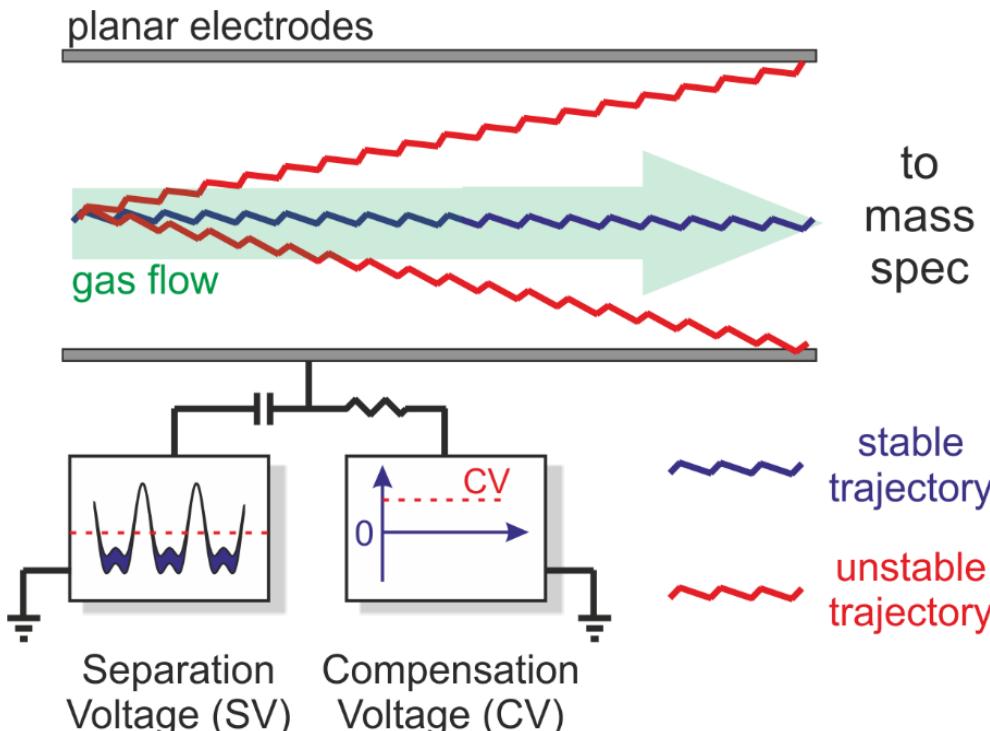
A differential mobility spectrometry (DMS) cell is interfaced between the ESI source and orifice to the MS



Time-independent separation dimension that is **orthogonal to, and compatible with**, external separation dimensions (e.g., liquid chromatography, capillary electrophoresis)

Why (and what is...) Differential Mobility Spectrometry (DMS)?

DMS is a non-linear form of ion mobility that harnesses the non-linear evolution of ion mobility with electric field to resolve analytes.

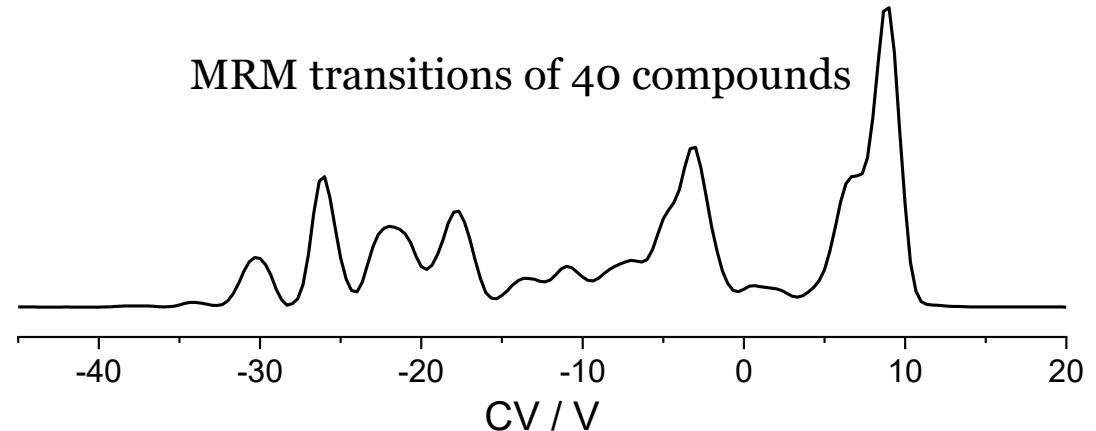


Hopkins, W. S. Mol. Phys. 2015, 113, 3151–3158.

Above the low field limit, ion mobility K varies non-linearly with the field strength

$$\alpha(E) = \frac{K(E)}{K(0)} - 1$$

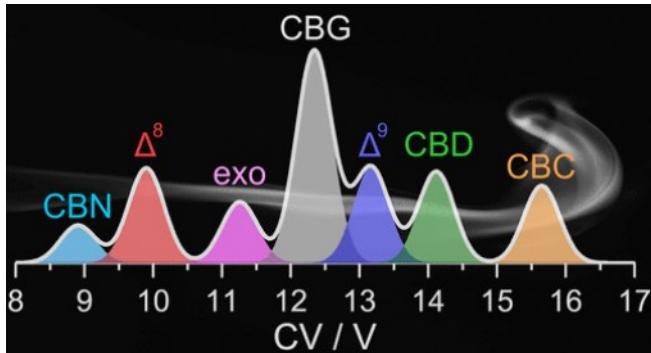
Scanning CV at a fixed SV gives an ionogram



For a specific SV, every analyte will elute from the DMS cell at a characteristic CV

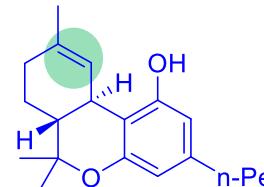
DMS can separate complex, isobaric mixtures

A silver bullet for cannabinoids

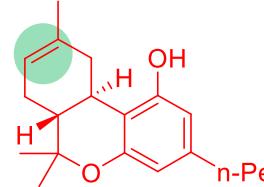


Anal. Chem. 2023, 95, 8668-8678.

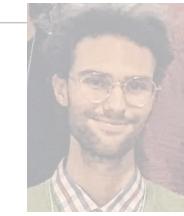
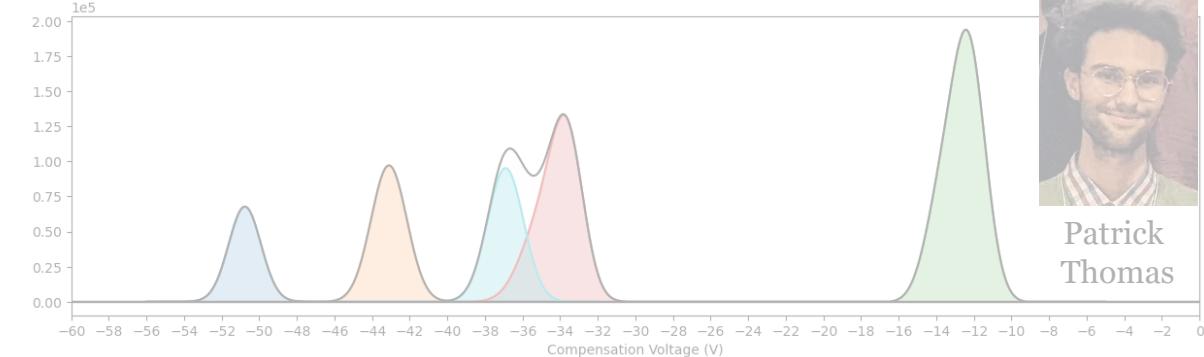
**Δ⁹-THC
Illegal**



**Δ⁸-THC
Kind of
legal?**



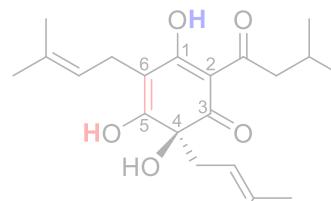
Opioids (Dangerous “feel-good” molecules)



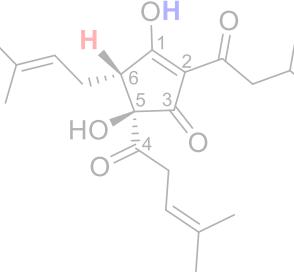
Patrick
Thomas

Humulones (Yummy beer molecules)

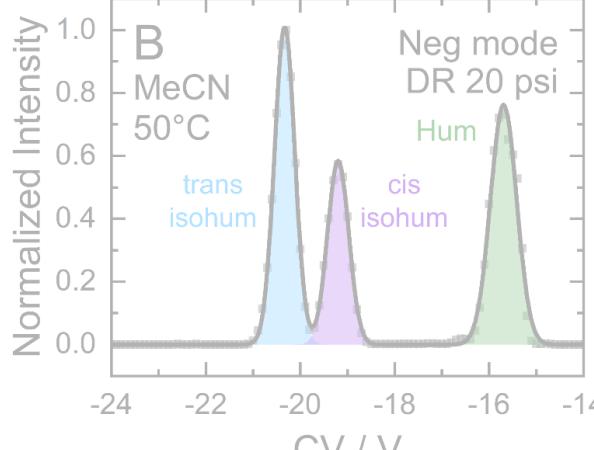
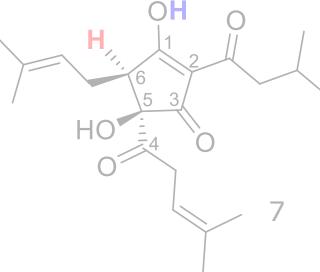
Humulone



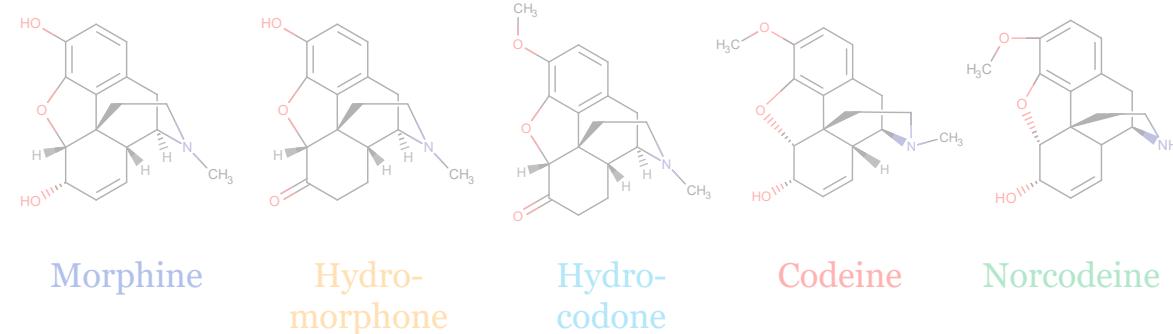
cis-isohumulone



trans-isohumulone



J. Am. Soc. Mass Spectrom. 2023, 34, 1315-1329



$\Delta CCS < 2.5\%$, so difficult to resolve via linear IMS

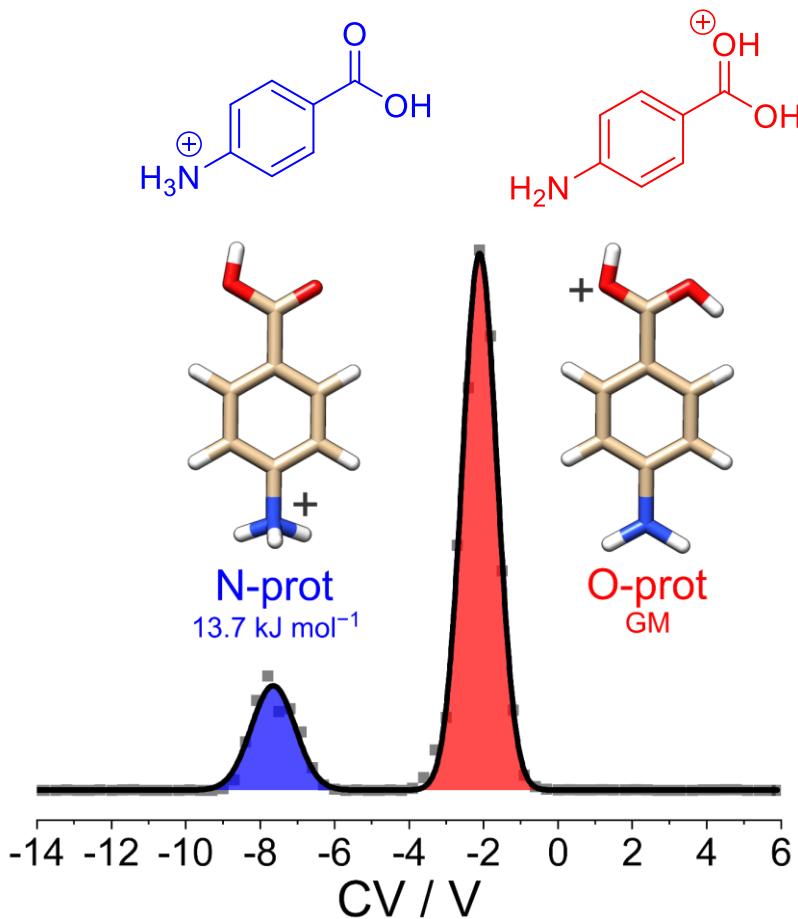


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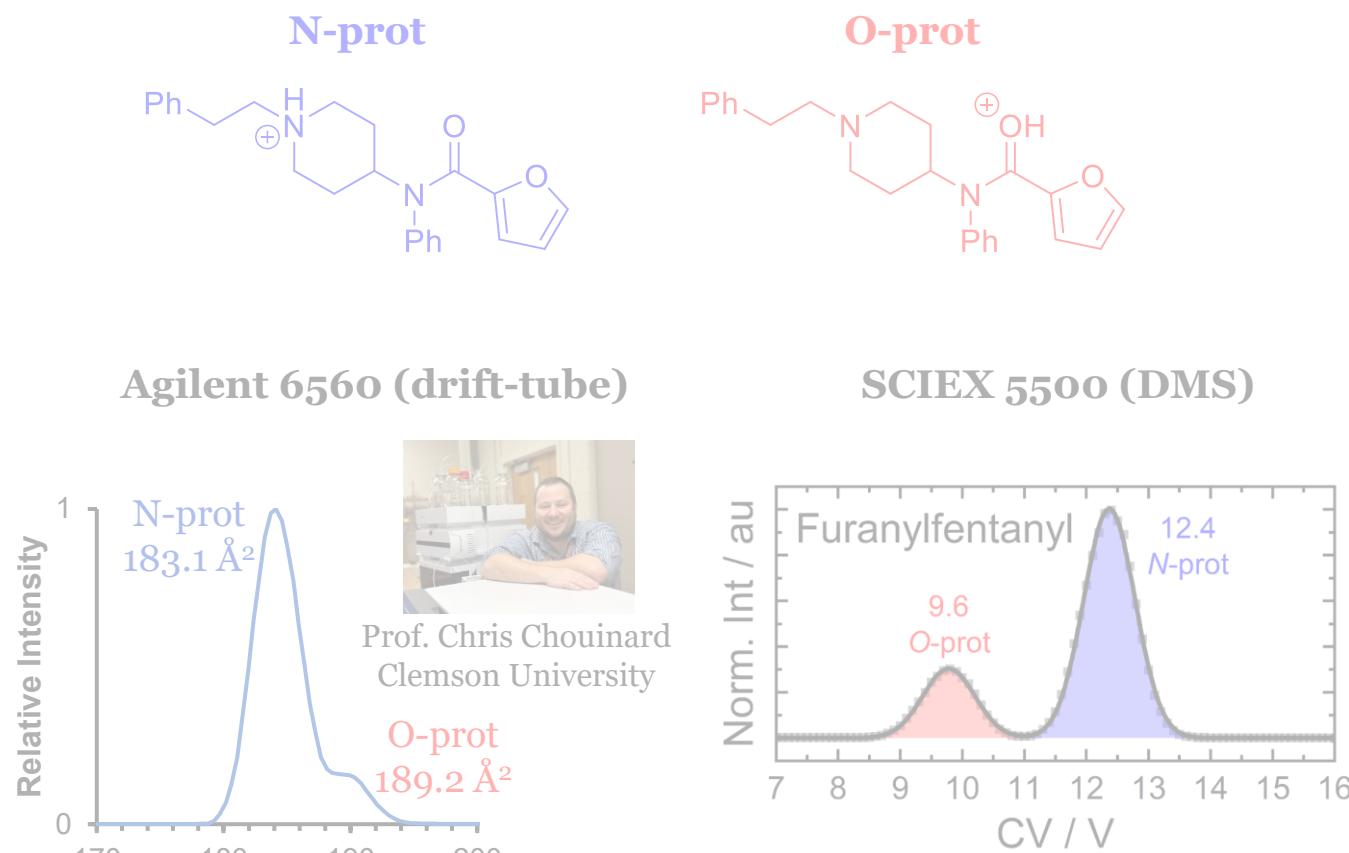
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DMS can disentangle spectroscopic “contaminants”

4-aminobenzoic acid: The poster child of prototropic isomers

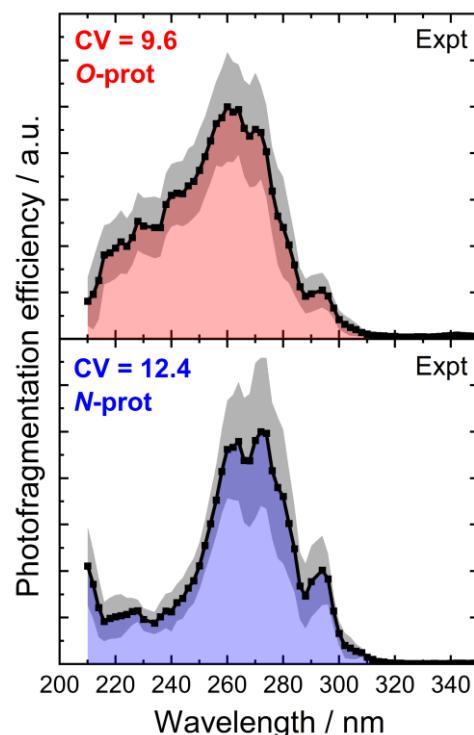
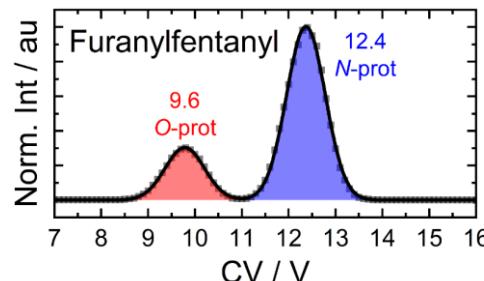


All fentanylts exhibit two peaks in IMS experiments – prototropic isomers!



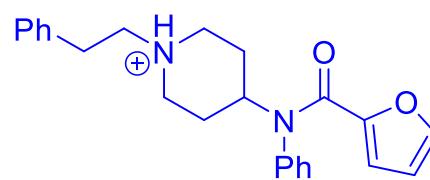
DMS can disentangle spectroscopic “contaminants”

DMS resolves analytes in space, so time synchronization with instrumentation & laser pulses is easy

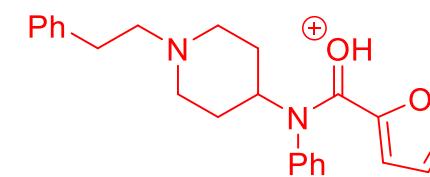


The extended absorption profile of the **O-prototropic isomer** is a consequence of the furan moiety being **conjugated with the site of protonation**

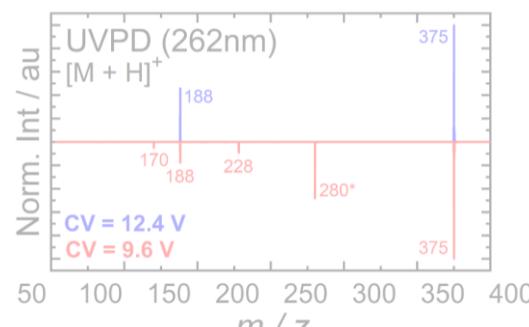
N-prot



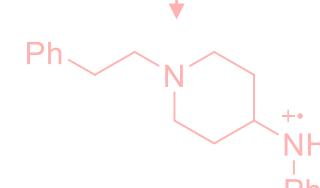
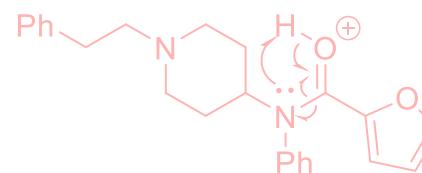
O-prot



C–N bond cleavage



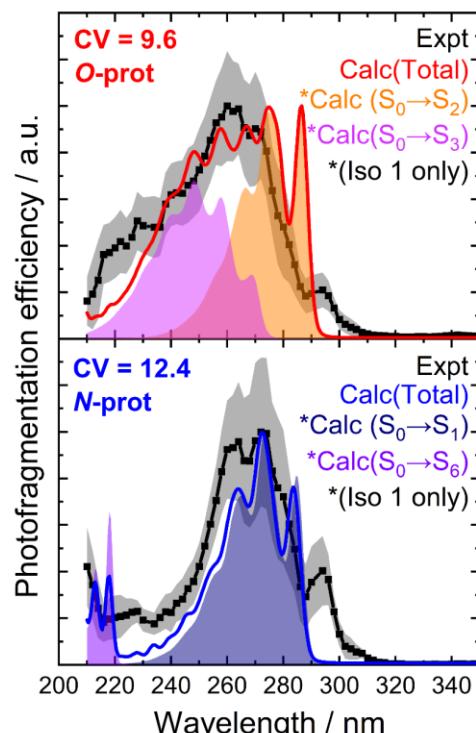
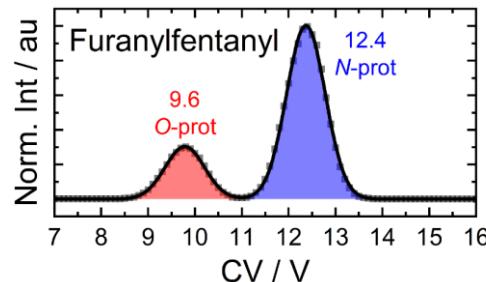
m/z 280 is exclusive to UVPD ($\pi \rightarrow \pi^*$)



m/z 280.19

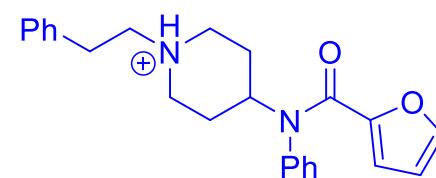
DMS can disentangle spectroscopic “contaminants”

DMS resolves analytes in space, so time synchronization with instrumentation & laser pulses is easy

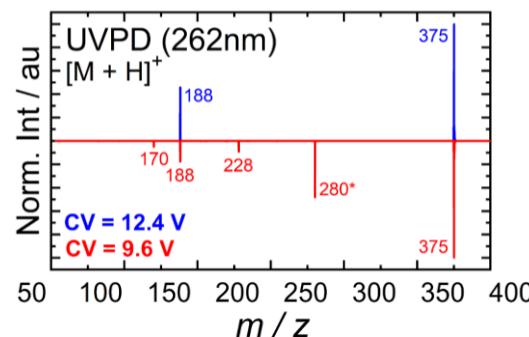
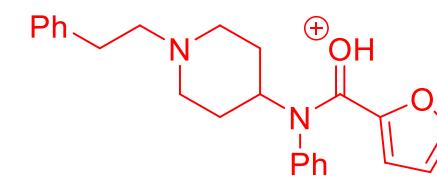


The extended absorption profile of the **O-prototropic isomer** is a consequence of the furan moiety being **conjugated with the site of protonation**

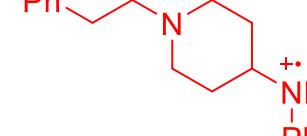
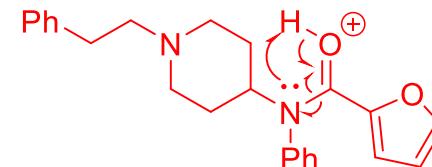
N-prot



O-prot



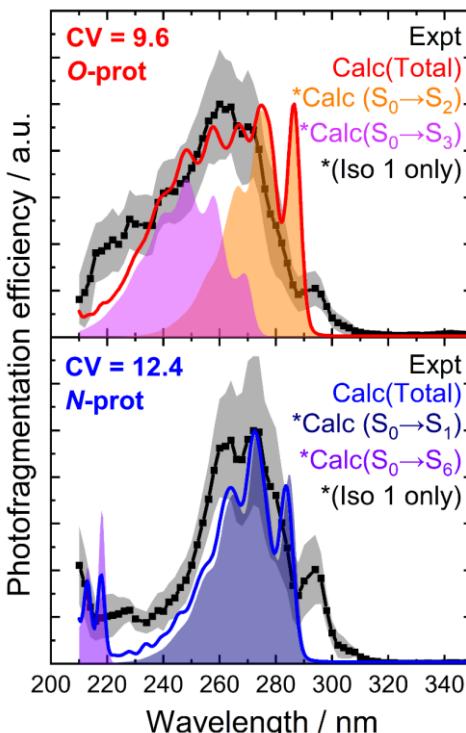
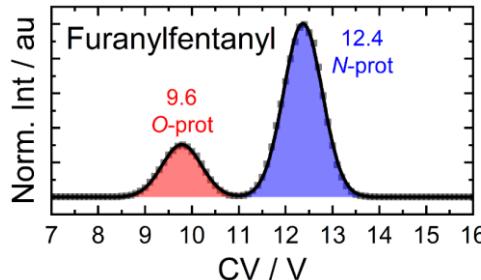
m/z 280 is exclusive to UVPD ($\pi \rightarrow \pi^*$)



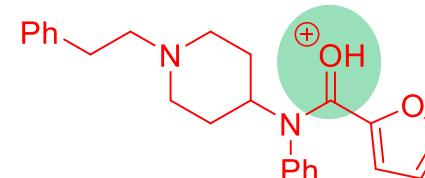
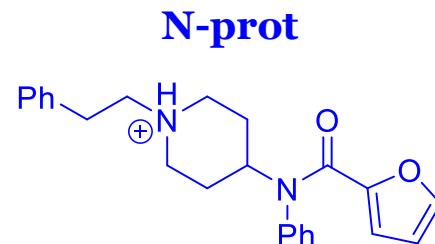
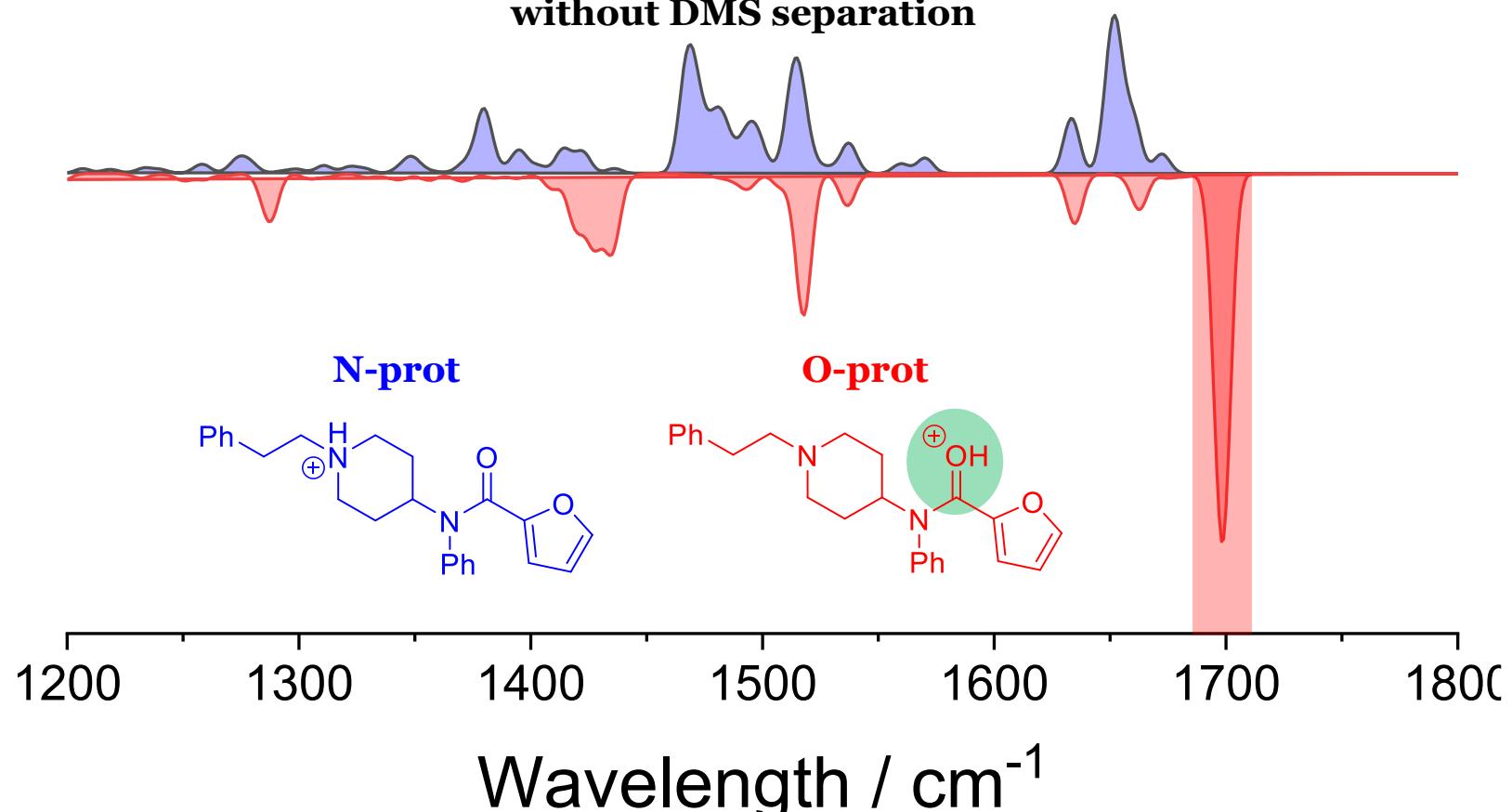
m/z 280.19

C–N bond
cleavage

DMS can disentangle spectroscopic “contaminants”



If this was IRMPD, users would see a ~ 3:1 weighting of the **N-protomer : O-protomer** without DMS separation



Alas, triple quads are limited to unit resolution

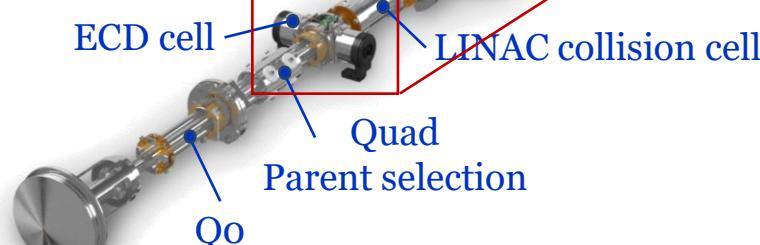
Proposed end-station #2:
SCIEX 7600 - QToF

213 or
266 nm



While not available commercially, the WaterFEL 7600 will have a DMS front end

ToF
Range of 40 to 40 kDa
Resolution > 42k
MS/MS at 133 Hz



ECD "Chimera" cell (RF ion trap)

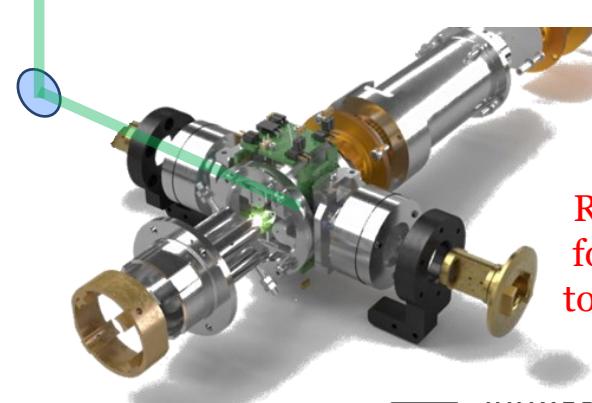
e⁻ filament 1

e⁻ filament 2

One of the filaments can be removed and replaced with a mirror for UVPD/IRMPD.

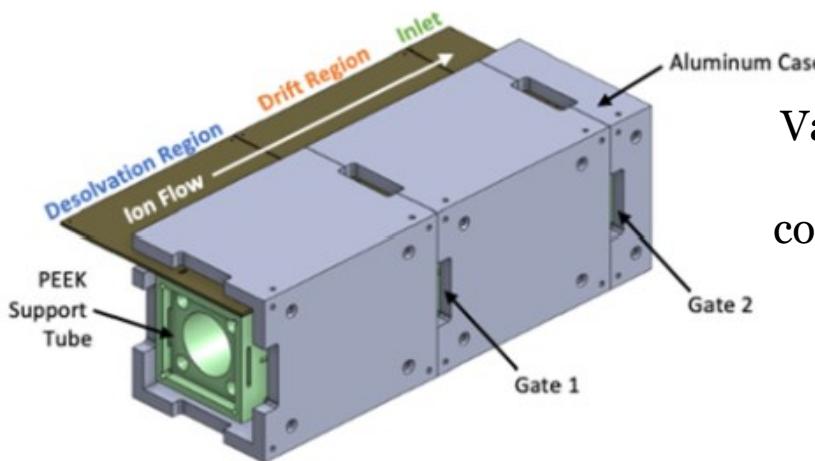
IRMPD of ECD fragments

Removal of e⁻ filament 2 for pump/probe coupled to external laser (UV/Vis)



Plug-and-play IMS devices

Open-source ‘lego-block’ enclosures for non-DMS ion mobility platforms



Variable temperature drift tube module compatible w/ solvent modifiers

J. am. Soc. Mass Spectrom. 2024, Accepted. <https://doi.org/10.1021/jasms.4c00183>

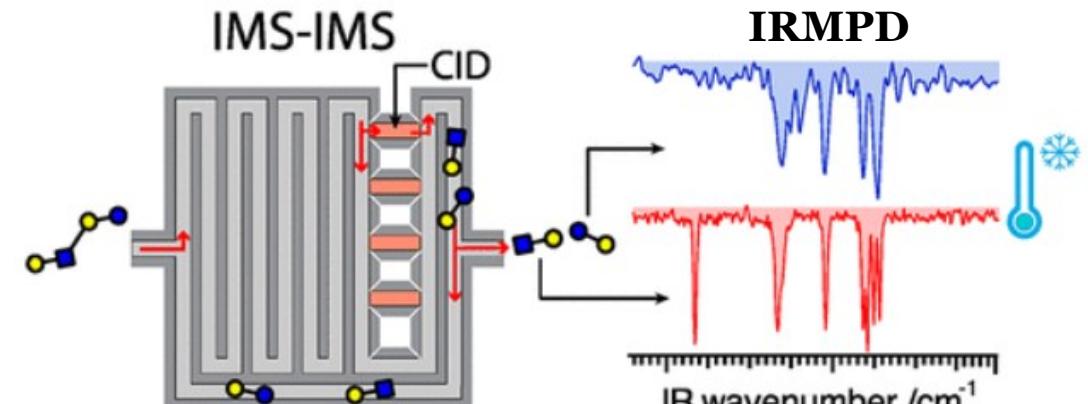


Prof. Brian Clowers
(WSU)



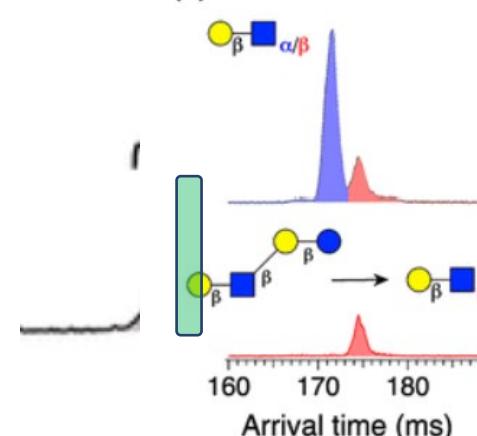
Dr. Haley Schramm
(WSU/UW)

SLIMS: structures for lossless ion manipulation.
Multi-lap racetracks for ions

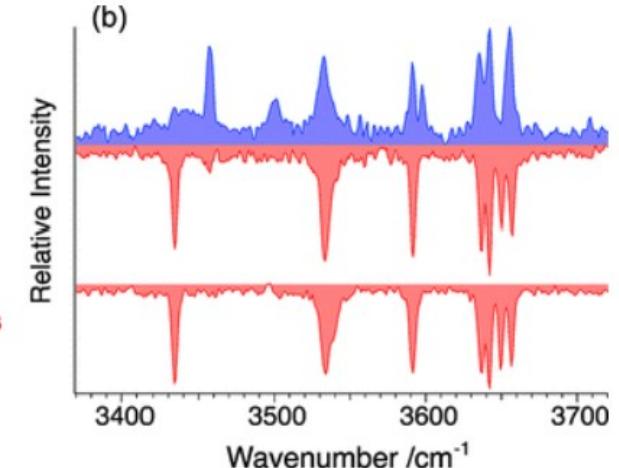


Anal Chem. 2023, 95, 25, 9623–9629

(a)

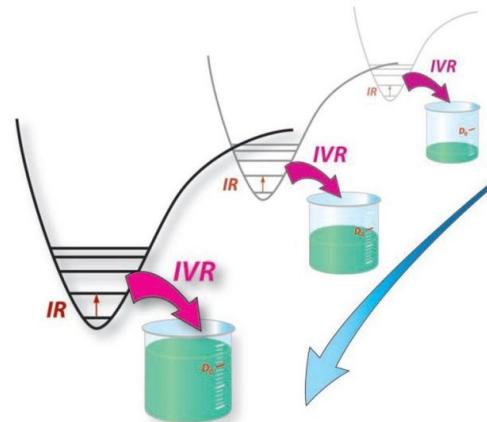


(b)

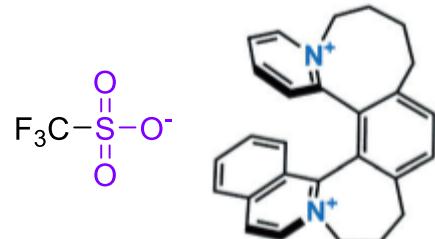
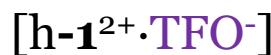


The quest to find non-ergodic behaviour in IRMPD

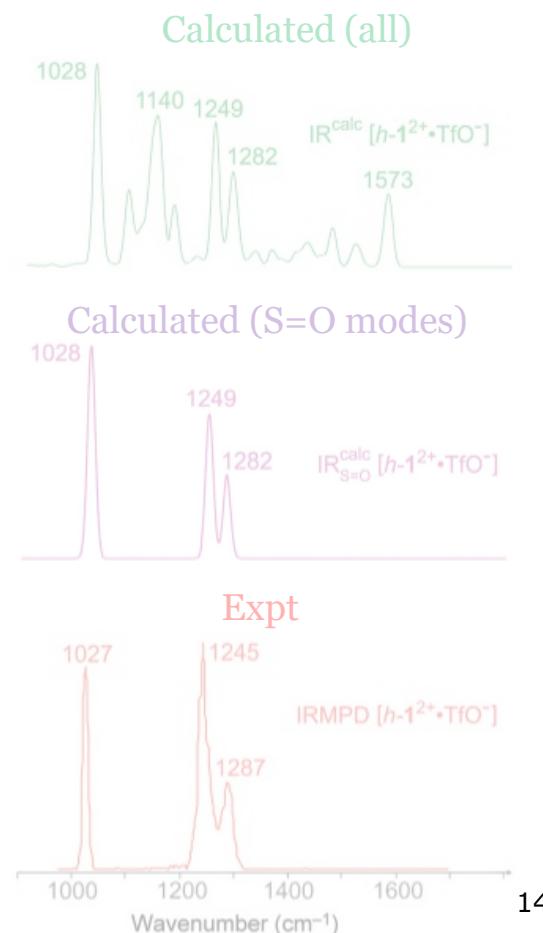
IVR is generally thought to partition energy statistically throughout the molecule's rovibrational degrees of freedom



Polfer, N. C., Oomens, J. *J. Mass Spectrom. Rev.* **2009**, 28, 468.

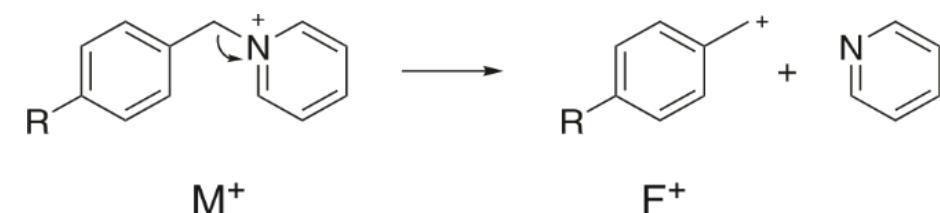


Angew. Chem. Int. Ed. **2012**, 51, 10050 – 10053.



14

Incomplete IVR or ion heating?
(*a la* thermometer ions)

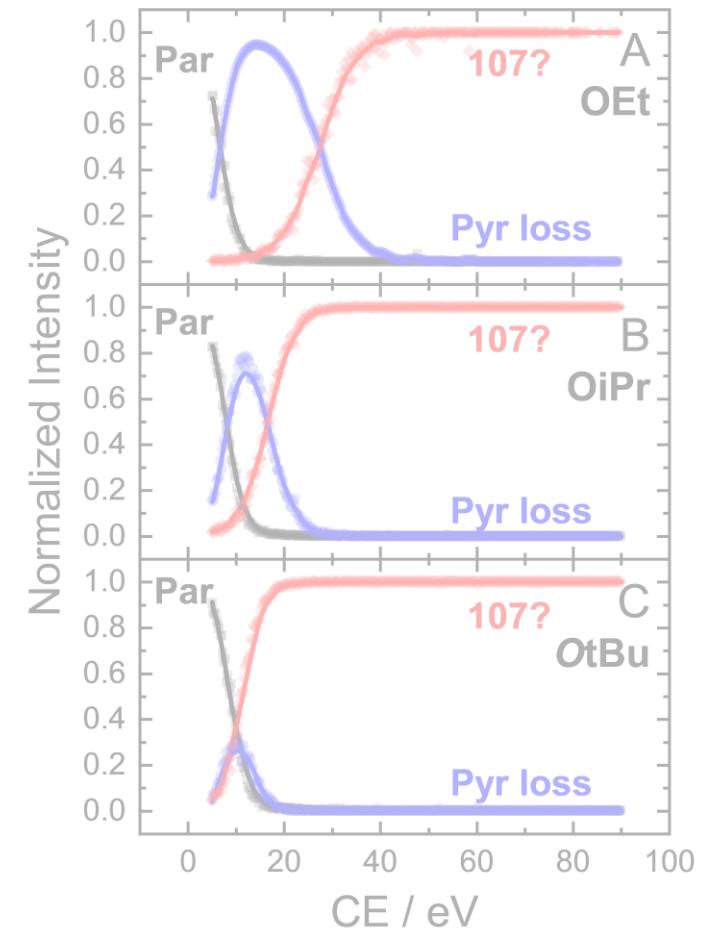
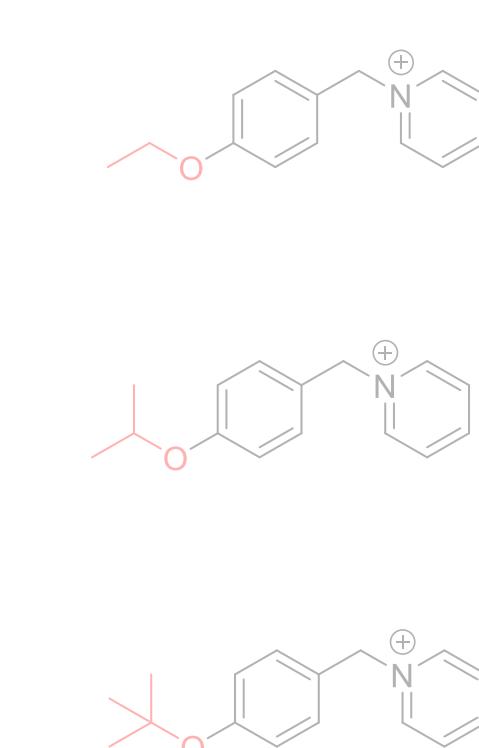
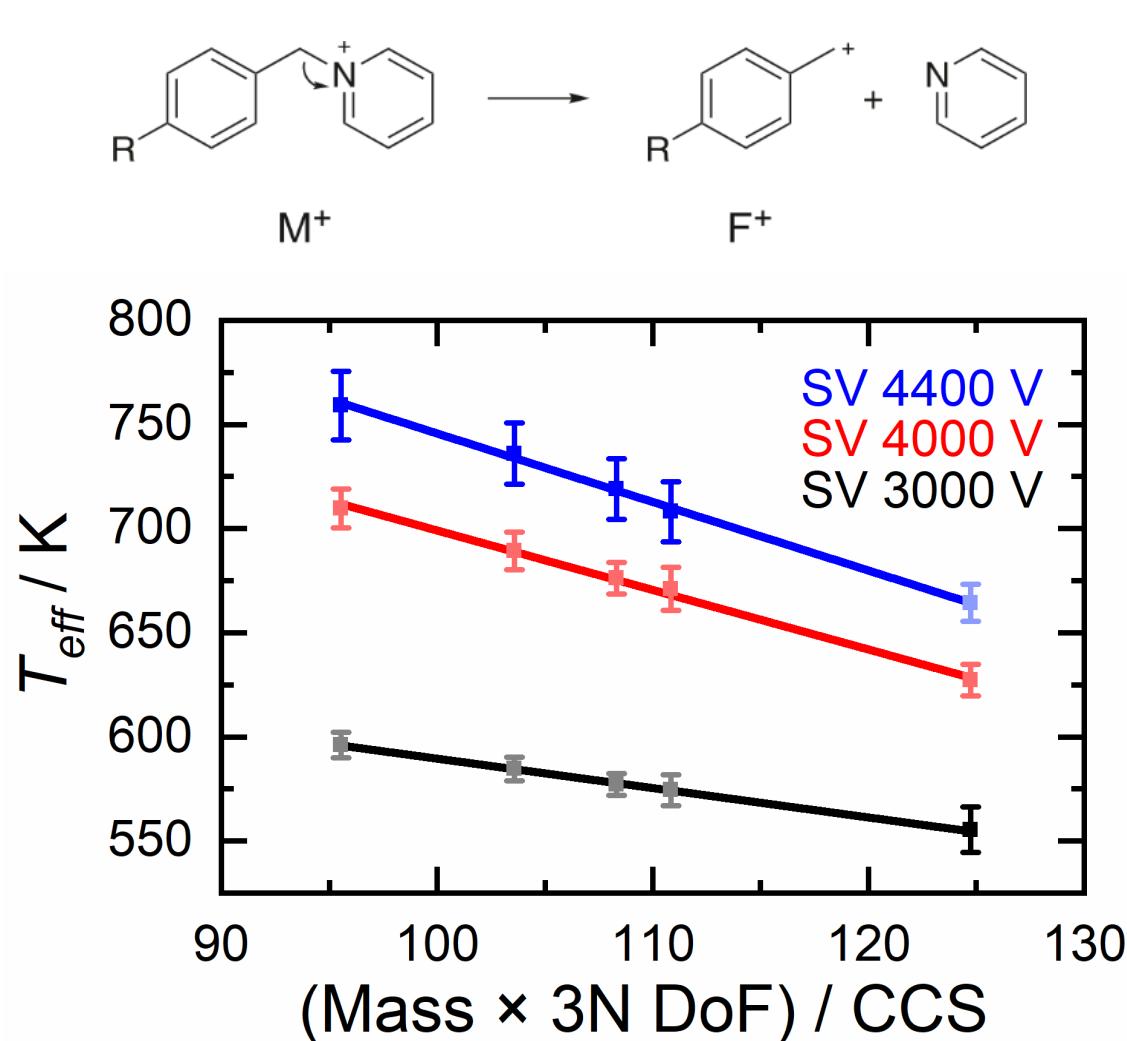


Fragmentation occurs **exclusively** through the loss of pyridine, meaning any build-up of internal energy results in a **single reaction that can be detected via MS**.

Non-ergodic behaviour only seen in weakly bound adducts, making it difficult to disentangle the possibility of non-statistical energy partitioning.

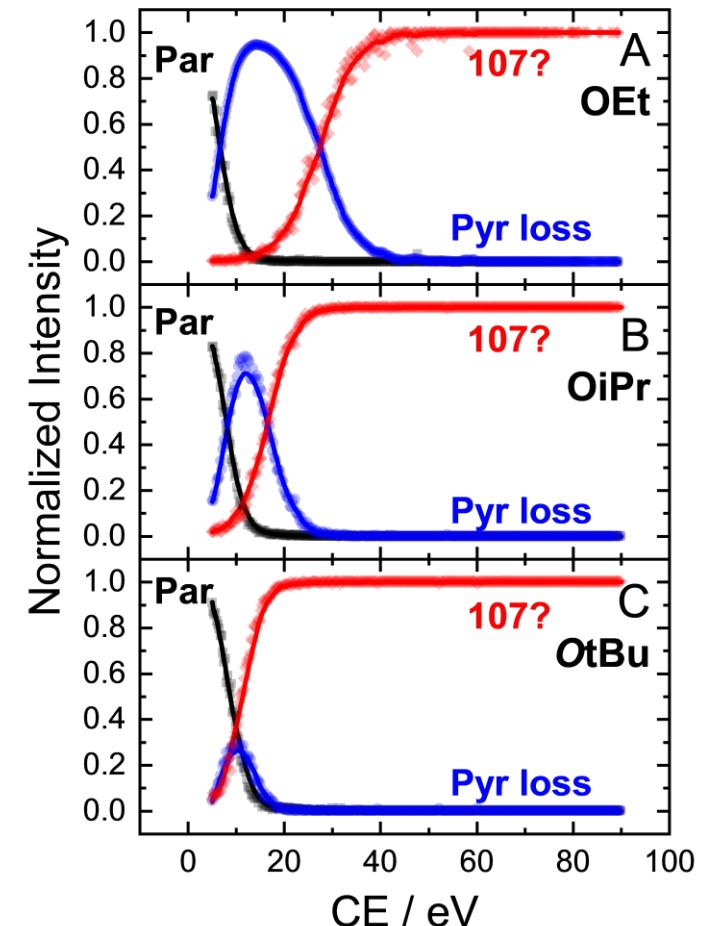
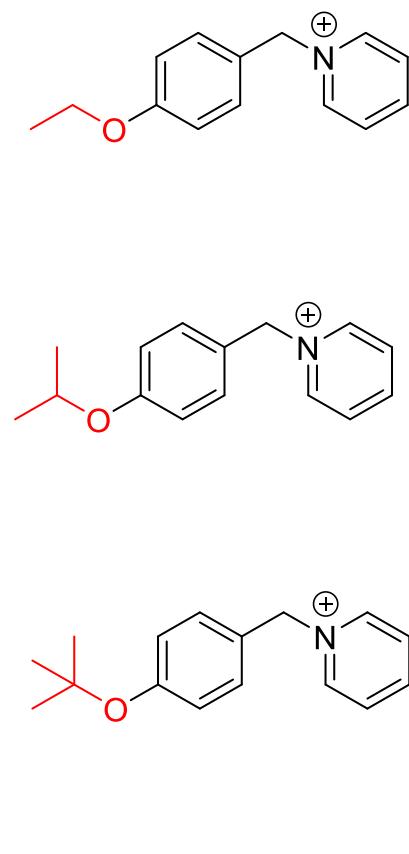
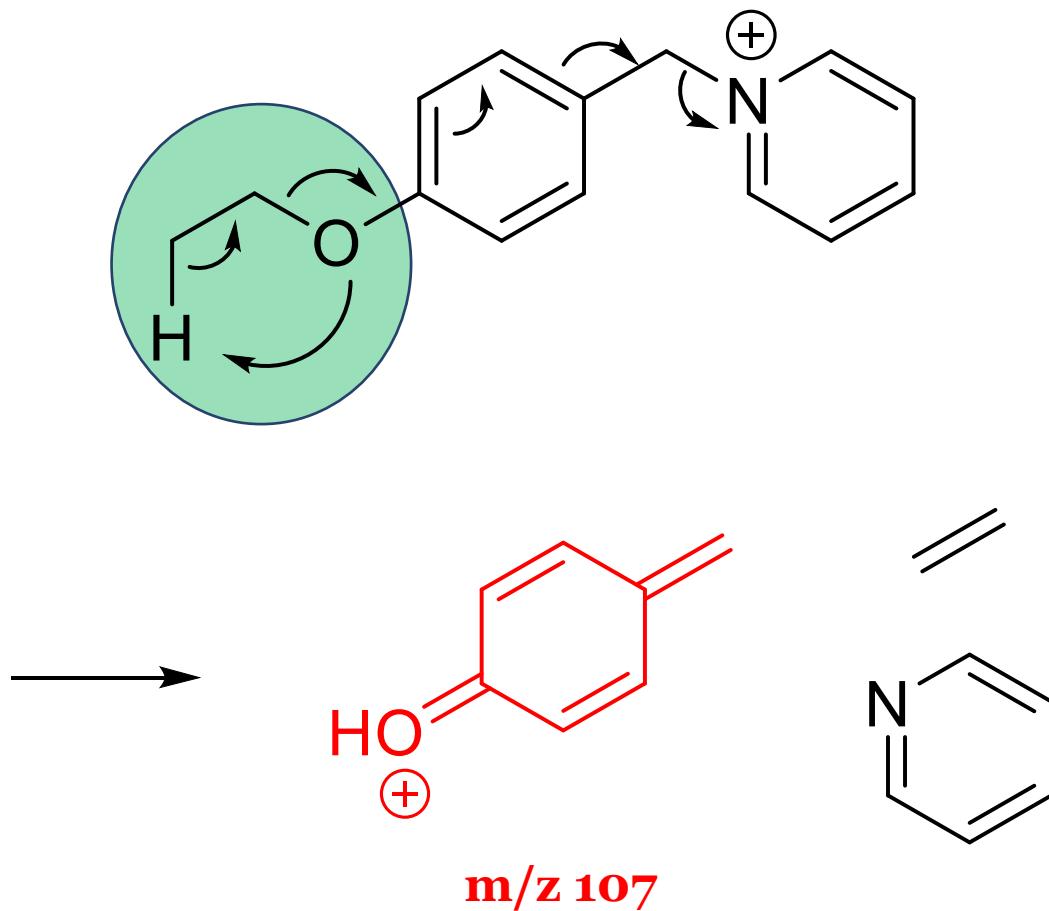
The dawning of a cool idea (or a “selectively hot” idea)

In our 2019 assessment of DMS temperatures using benzylpyridinium thermometers, we noticed something strange...



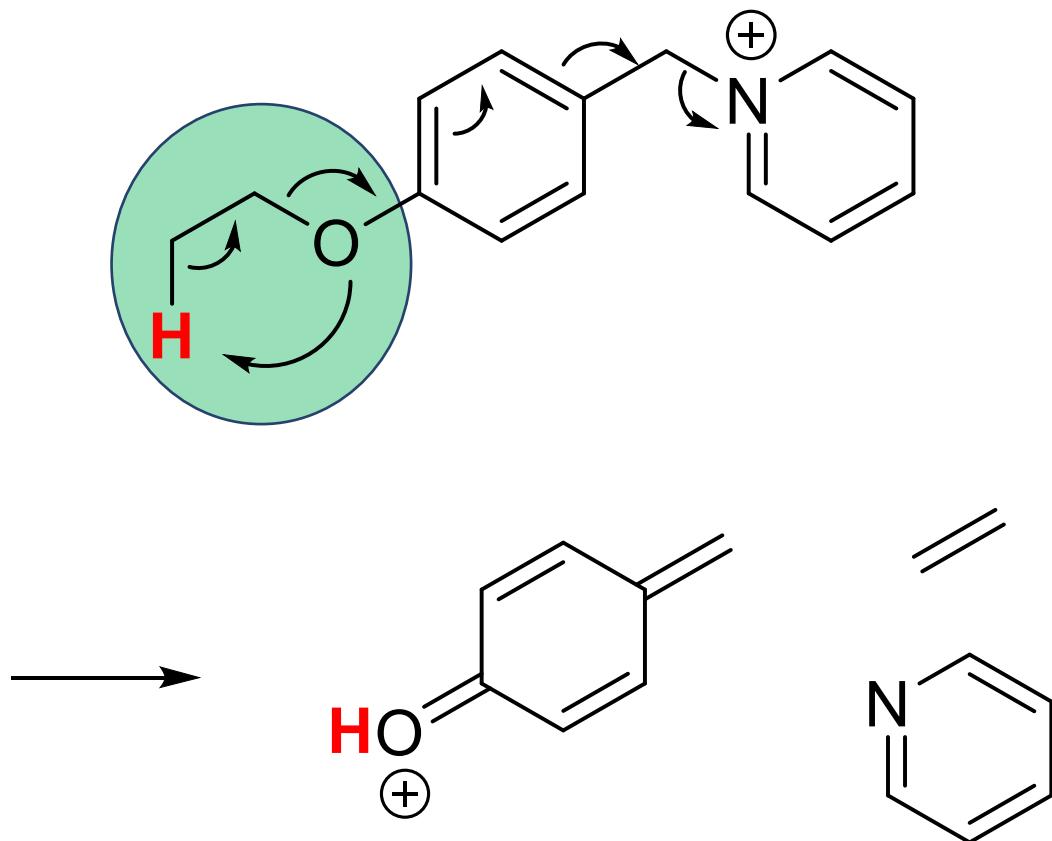
What is m/z 107?

m/z 107 being common to all three systems with a β -proton suggests an E_i -like elimination within the alkoxy chain

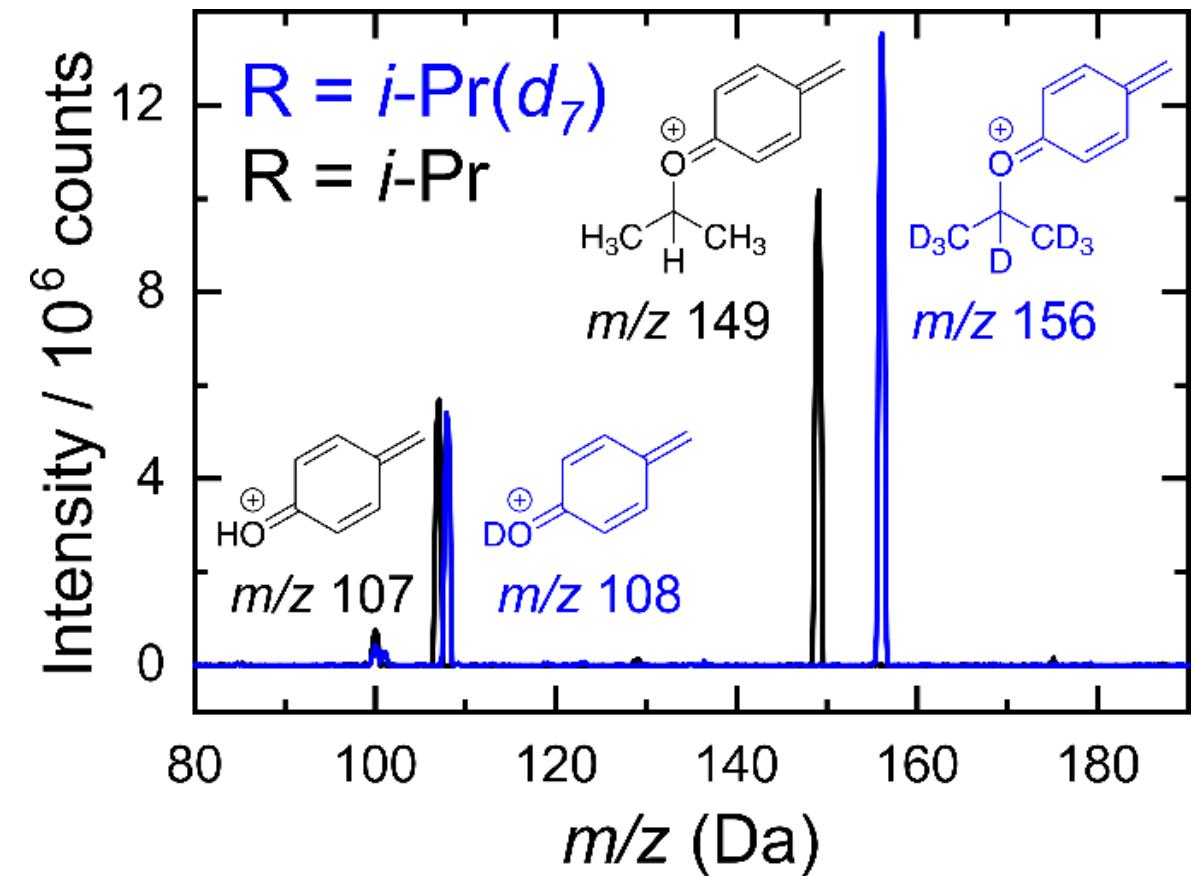


What is m/z 107?

m/z 107 being common to all three systems with a β -proton suggests an E_i-like elimination within the alkoxy chain

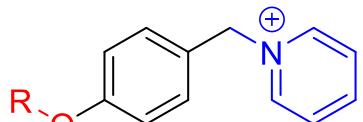


Intramolecular mechanism necessitates retention of the β -proton within the m/z 107 fragment



What does this have to do with non-ergodicity in IRMPD?

Pyr loss
 $\Delta G \sim 110 \text{ kJ/mol}$

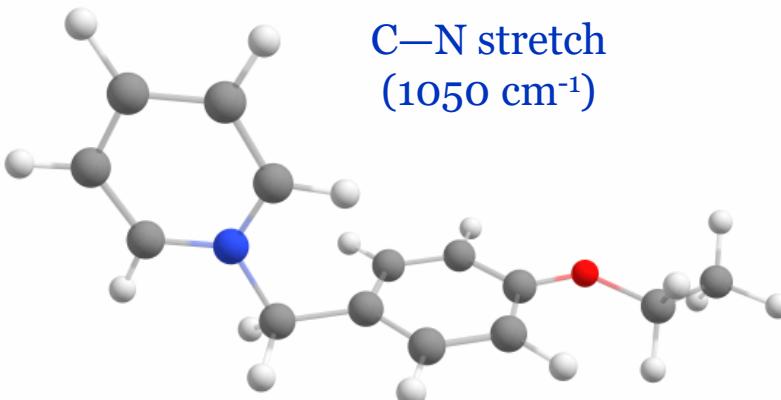


E_i
 $\Delta G \sim 90 - 183 \text{ kJ/mol}$

Benzylpyridiniums with alkoxy chains containing β -protons fragment in **two distinct ways** with the reaction coordinate being intrinsically coupled to a **normal mode**

Any Pyr or C—N vib. mode

C—N stretch
 (1050 cm^{-1})

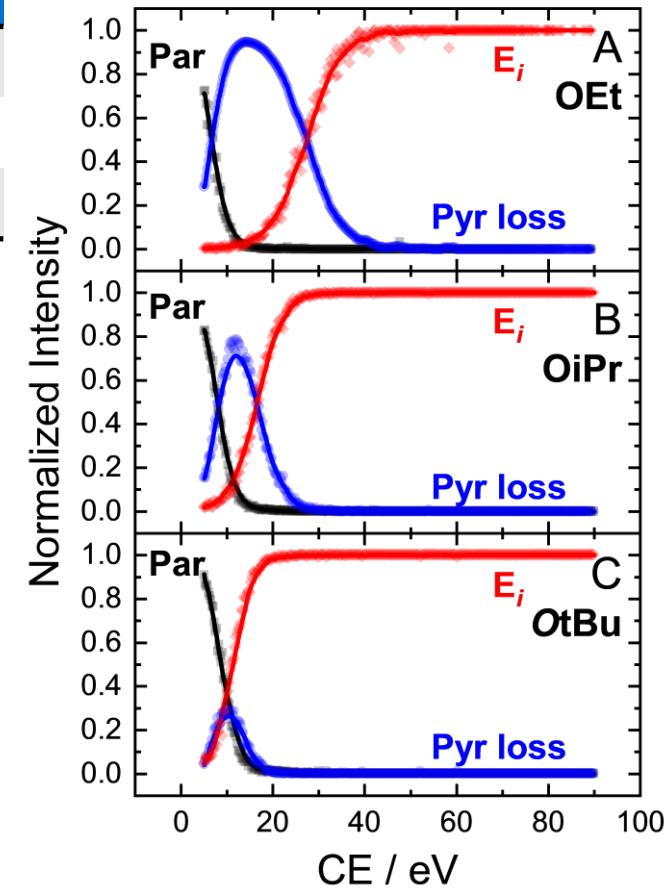
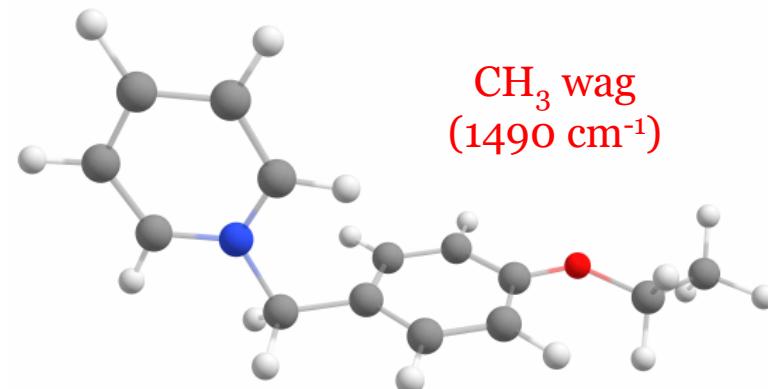


R	$\Delta G^\ddagger (\text{Pyr loss}) / \text{kJ mol}$	$\Delta G^\ddagger (E_i) / \text{kJ mol}^{-1}$
OEt	118	183
OiPr	110	142
OtBu	105	90

DLPNO-CCSD(T)/Def2-TZVPP//ωB97X-D3/Def2-TZVPP

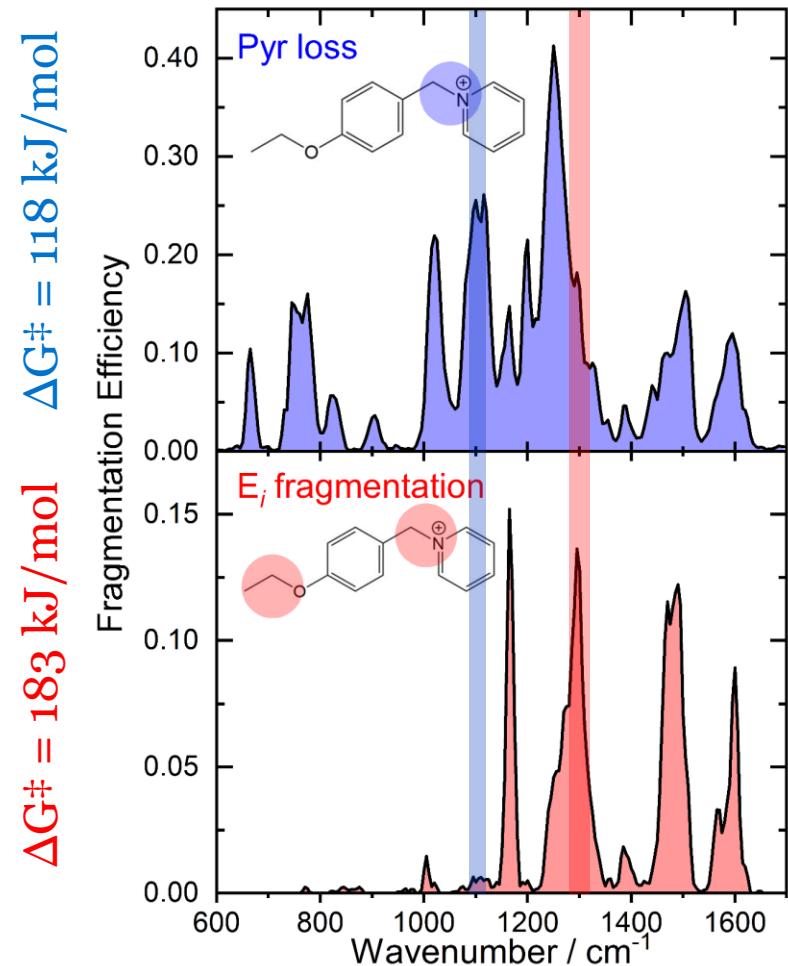
any C—O or alkoxy sp³ CH vib. mode

CH₃ wag
 (1490 cm^{-1})

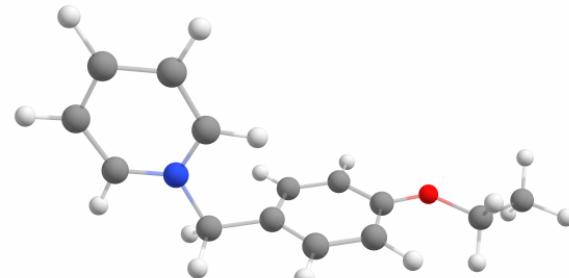


Non-ergodic dissociation of alkoxy BPs induced by IRMPD

E_i product is essentially absent between $600 - 1100 \text{ cm}^{-1}$

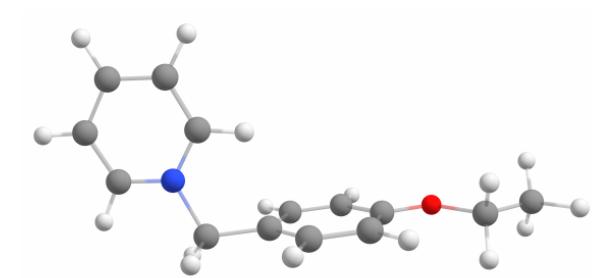


$\nu = 1100 \text{ cm}^{-1}$ (C–N stretch)



Minimal E_i pathway observed when
C–N stretch is probed

$\nu = 1300 \text{ cm}^{-1}$

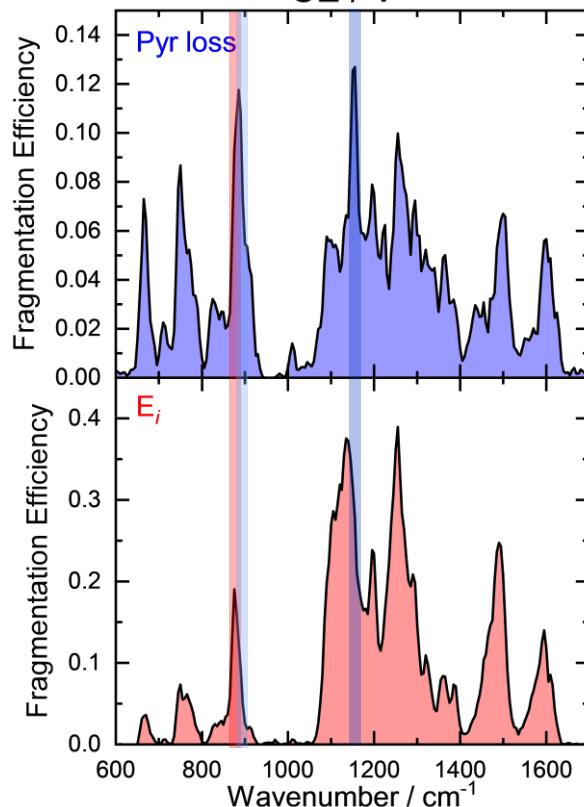
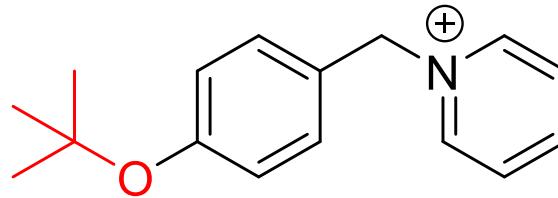
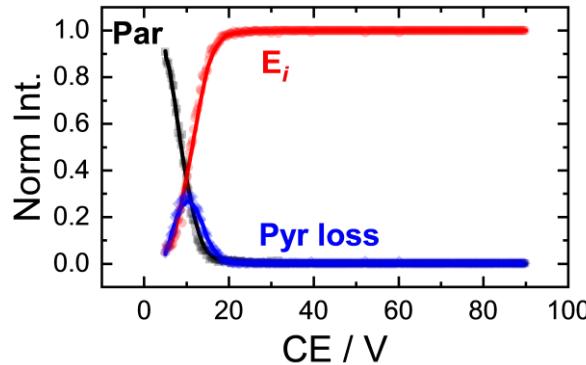


Some Pyr loss observed when alkoxy
sp³ C–H modes are probed

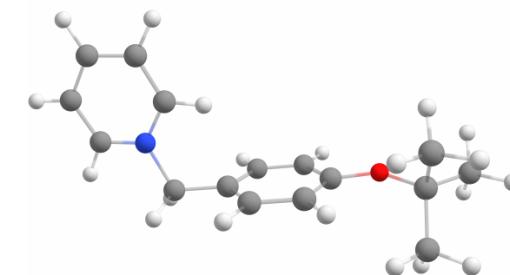
Problem: the E_i pathway is 80 kJ/mol higher in energy than Pyr loss and initially requires Pyr loss (CID suggests stepwise mechanism).

R	ΔG^\ddagger (Pyr loss) / kJ mol	ΔG^\ddagger (E_i) / kJ mol ⁻¹
OEt	118	183

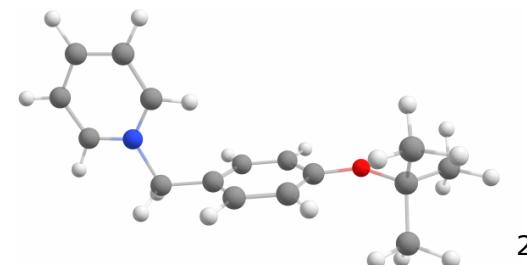
What happens when E_i has a lower barrier than Pyr loss?



$\nu = 885 \text{ cm}^{-1}$ ($\text{sp}_3 \text{ CH}$)

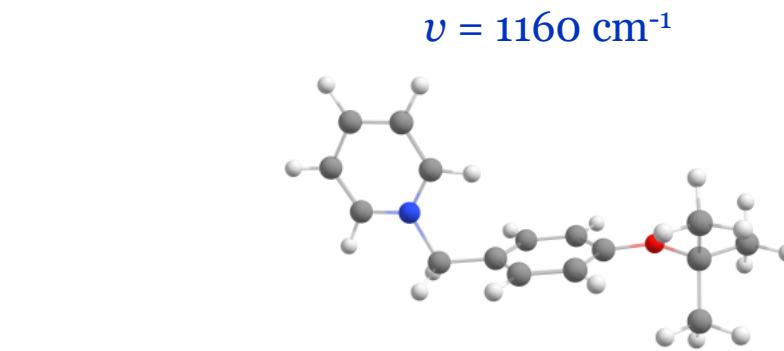


$\nu = 890 \text{ cm}^{-1}$ (pyr $\text{sp}^2 \text{ CH}$;
5 cm⁻¹ higher!)



CID dominated by E_i , whereas IRMPD is a combination of Pyr loss and E_i

Pyr loss: 110 kJ/mol
 E_i : 90 kJ/mol



Pyr C—N stretch has no matching peak in the lower energy E_i pathway, but overlaps with other absorptions in the E_i pathway

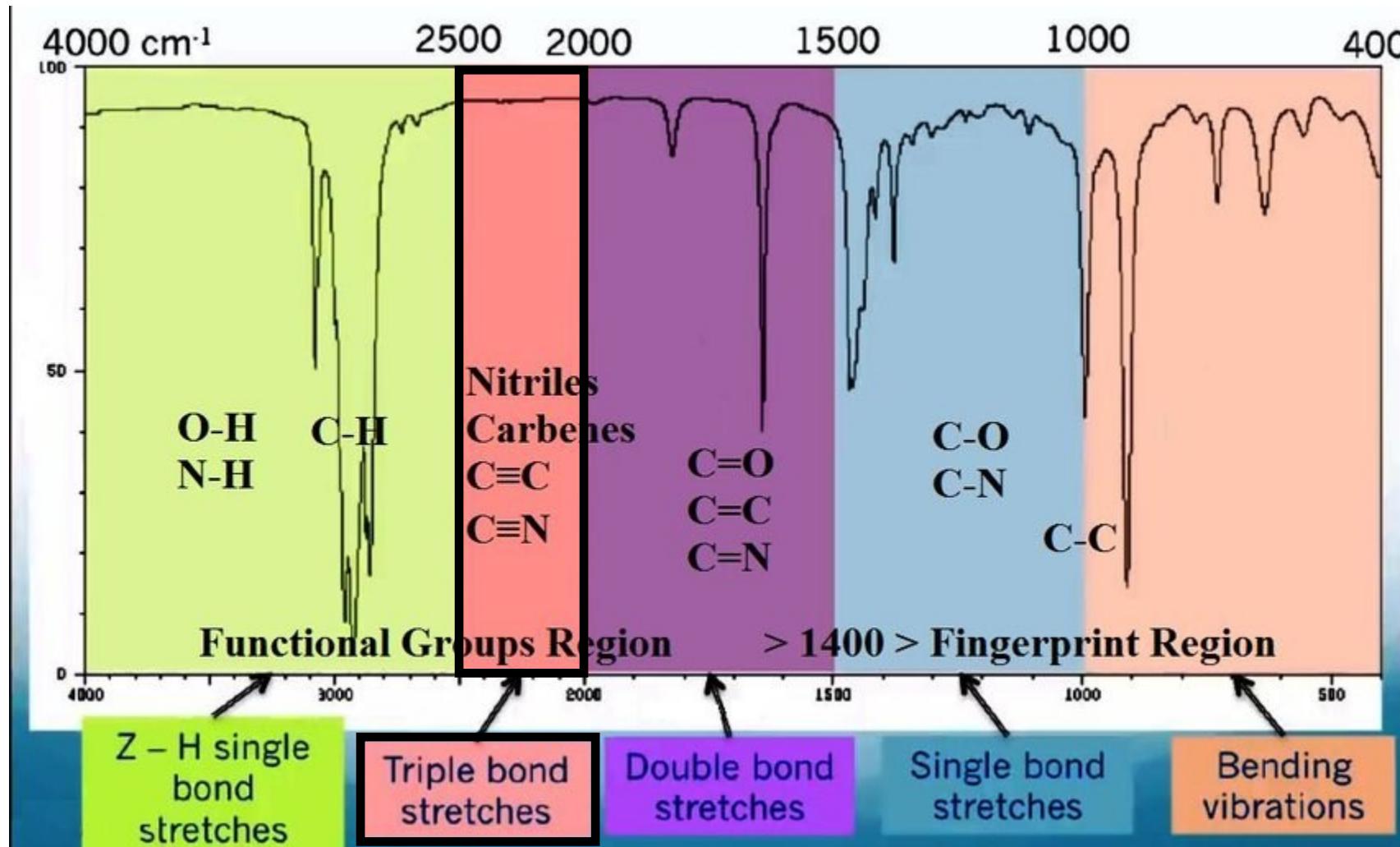
It's a shame that most vib. modes are so closely spaced, but we can change that



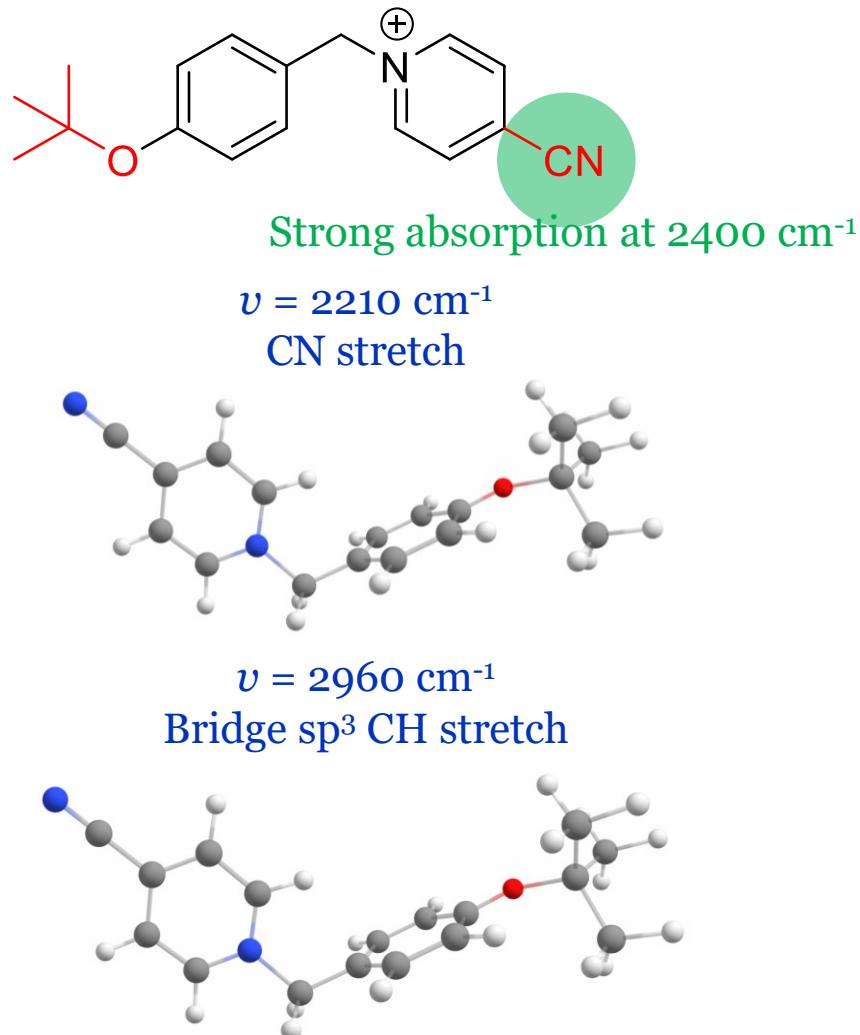
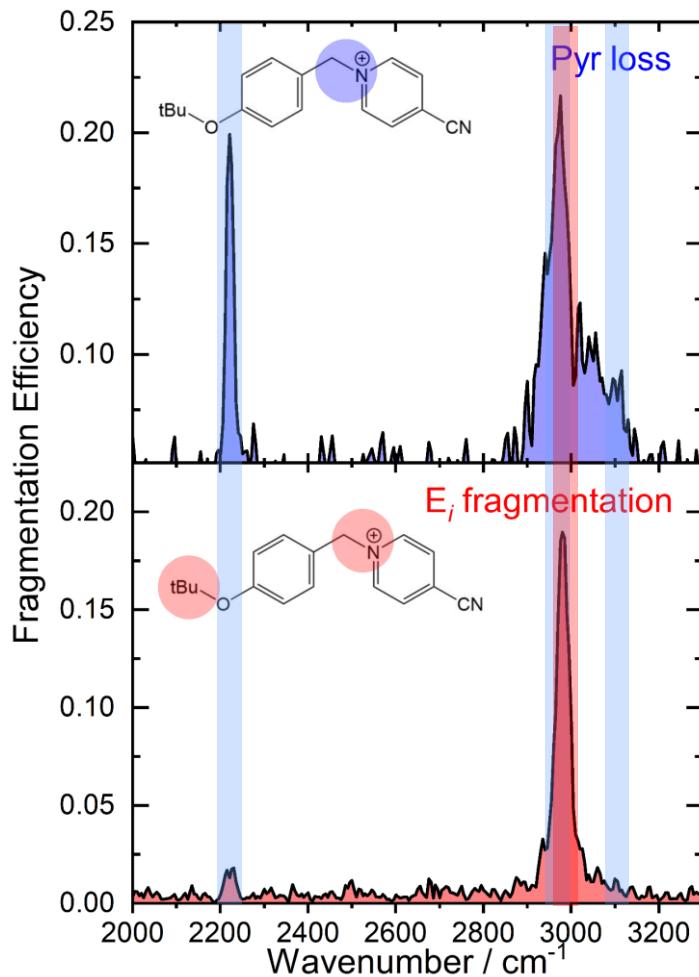
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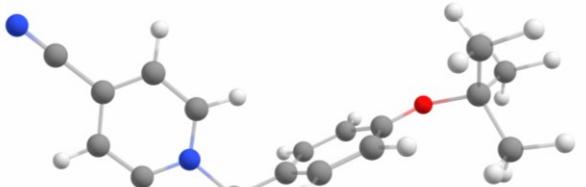
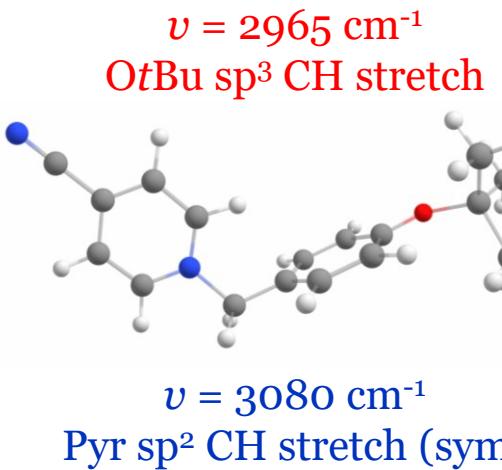
Characteristic IR absorptions



4'-cyano-4-OtBu Benzylpyridinium



Pyr loss: 80 kJ/mol
E_i: 90 kJ/mol



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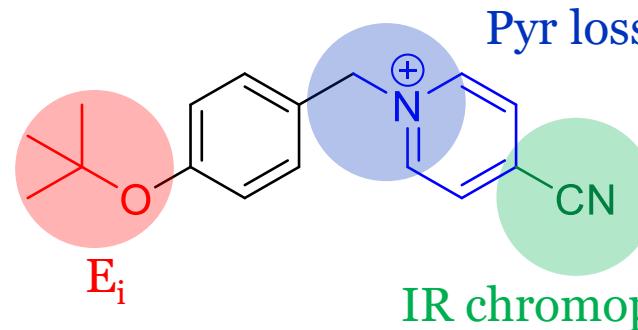
Concluding remarks

WaterFEL end stations



User facilities only works when built with
input from future users

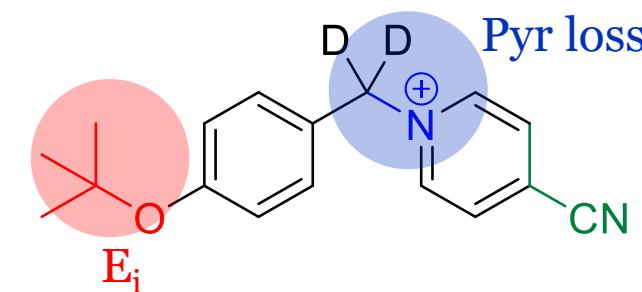
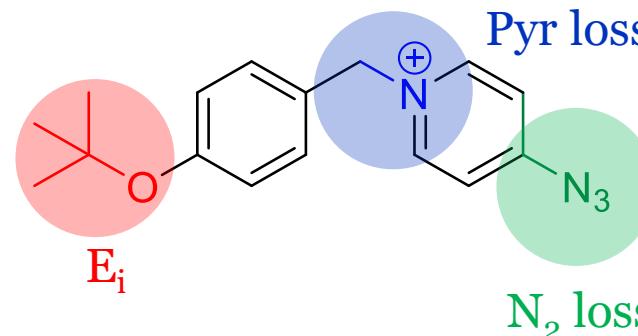
Non-ergodicity in IRMPD



Perhaps the most compelling evidence to date regarding non-statistical distribution of internal energy via IVR

3 breakpoints?

Reduced sp³ CH contamination?



Stay tuned!

Acknowledgements

WaterFEL team



Prof. W. Scott
Hopkins



Dr. Mike
Ditty



Dr. Alan Todd
(Stockphoto)



Prof. German
Sciani



Christine
Hancock

FELIX team



Prof. Jon
Marteens



Dr. Giel
Marteens



Dr. Jos
Ooomens

Wet lab space



Prof. Mike
Chong



Dr. Rosie
Chong

FHI colleagues



Prof. Wieland
Schöllkopf



Prof. Gerhard
Meijer

SCIEX Gurus



Dr. J. C. Yves
Le Blanc



Dr. Brad
Schneider



Dr. Mircea
Guna

Acknowledgements

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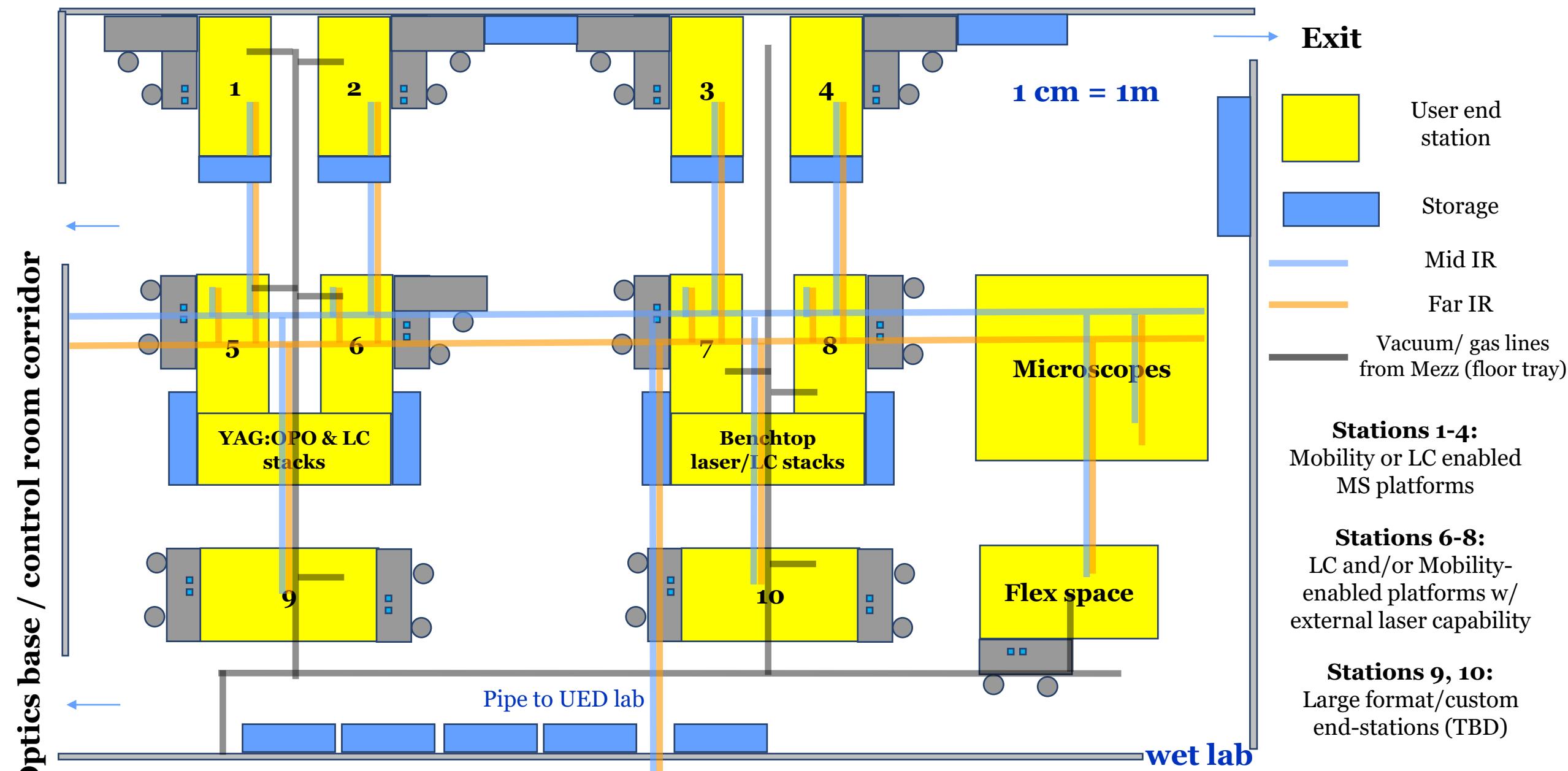


Dr. Brad
Schneider



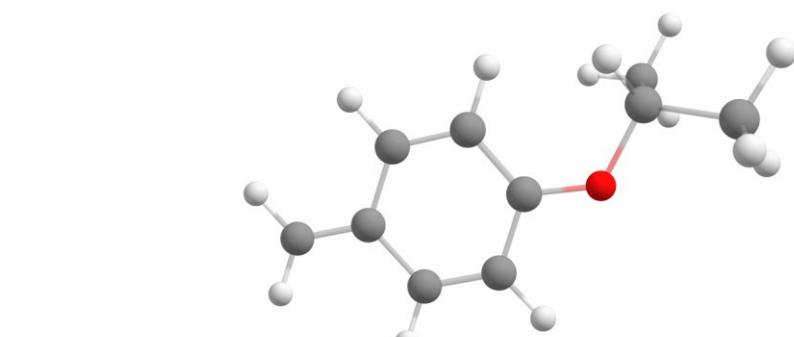
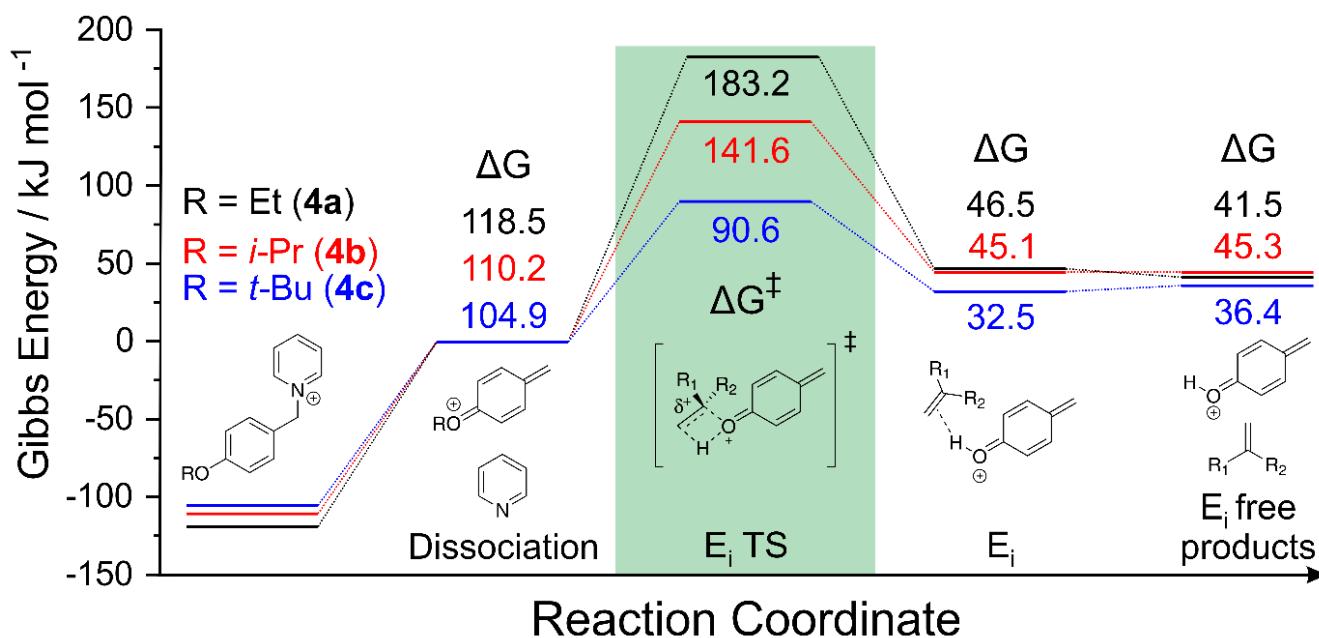
Dr. Mircea
Guna

WaterFEL Lab Ballroom

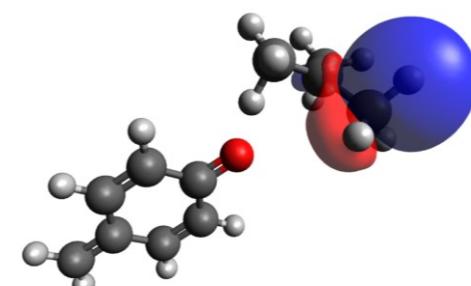


Finally understanding what was going on

Mapping the reaction coordinate using the nudged elastic band confirms our proposed fragmentation mechanism



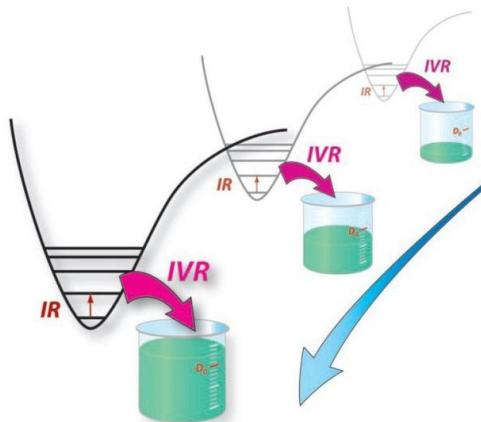
Hyperconjugation in the TS



OEt: 24.0 kJ mol⁻¹
OiPr: 206.0 kJ mol⁻¹
OtBu: 759.8 kJ mol⁻¹

Non-ergodic behaviour in IRMPD

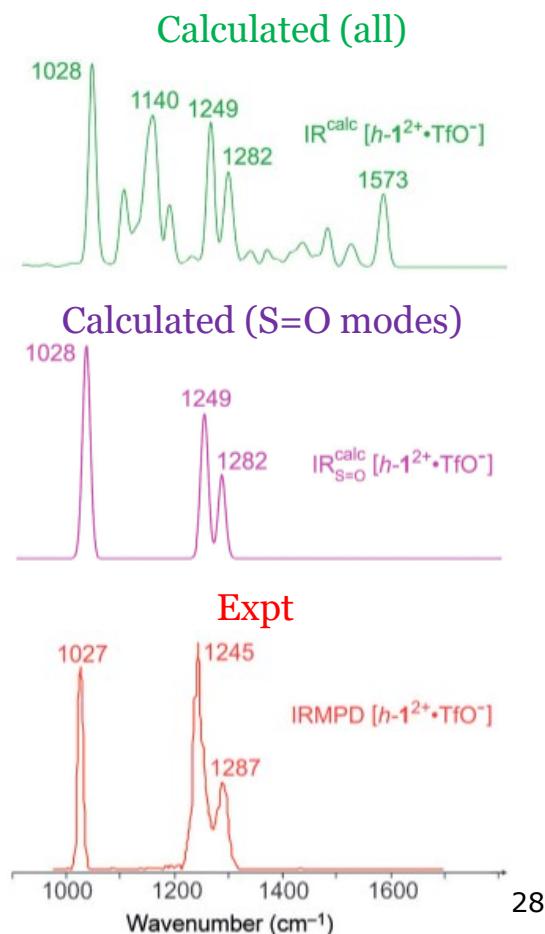
IVR constitutes a slow uptake of internal energy, and is generally thought to partition energy statistically throughout the molecule's rovibrational degrees of freedom



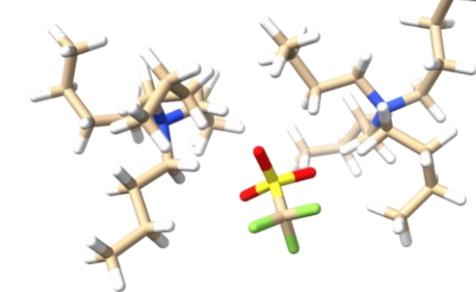
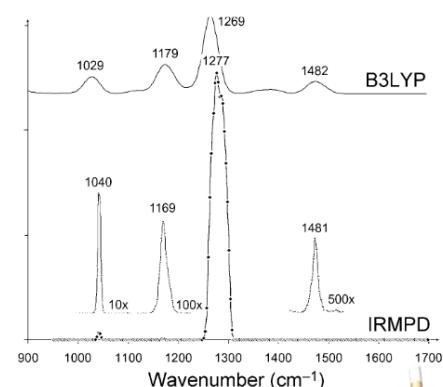
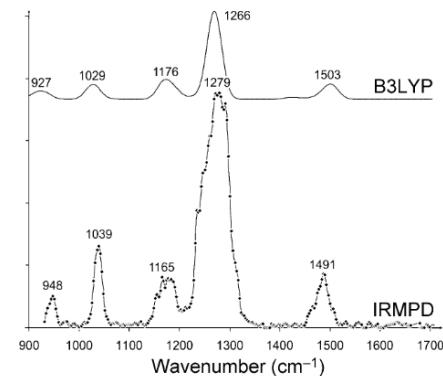
Polfer, N. C., Oomens, J. *J. Mass Spectrom. Rev.* **2009**, 28, 468.



Angew. Chem. Int. Ed. **2012**, 51, 10050 – 10053.



Incomplete IVR or ion heating (*a la* thermometer ions)?



Non-ergodic behaviour only seen in weakly bound adducts.
Inconclusive, in my opinion. Needs to be demonstrated on a covalently bound system, which has yet to be observed.

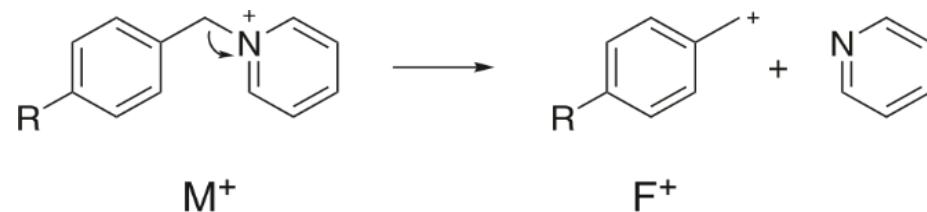


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The dawning of a hot idea

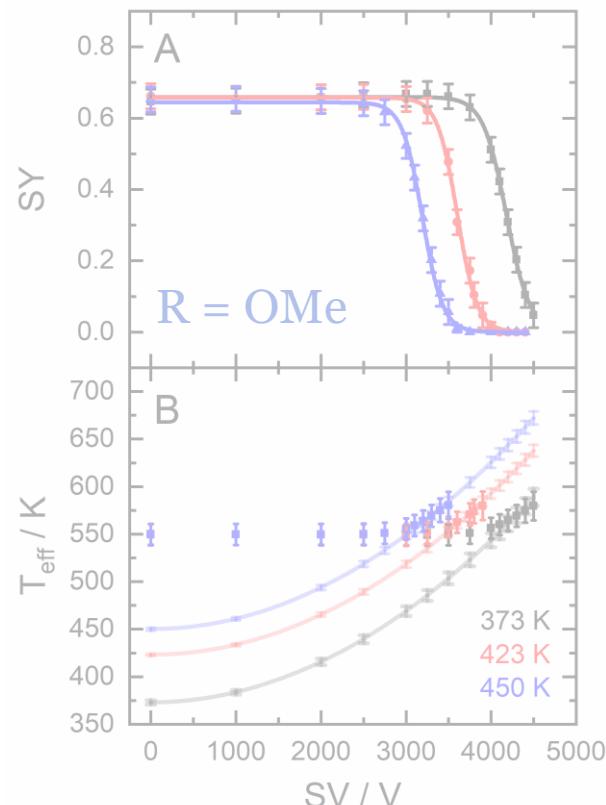
Benzylpyridiniums are chemical “thermometers” that can tell you how hot your instrument is.



Fragmentation occurs **exclusively** through the loss of pyridine, meaning any build-up of internal energy results in a **single reaction that can be detected via MS**

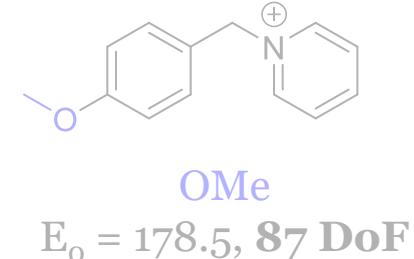
R	$E_o / \text{kJ mol}^{-1}$	Pyr loss Observed?
OMe	178.5	Yes
Me	219.0	No
F	230.6	No
Cl	228.7	No
H	241.2	No
CN	264.4	No

How hot are ions in the DMS cell?



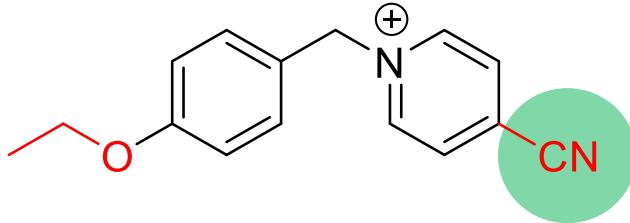
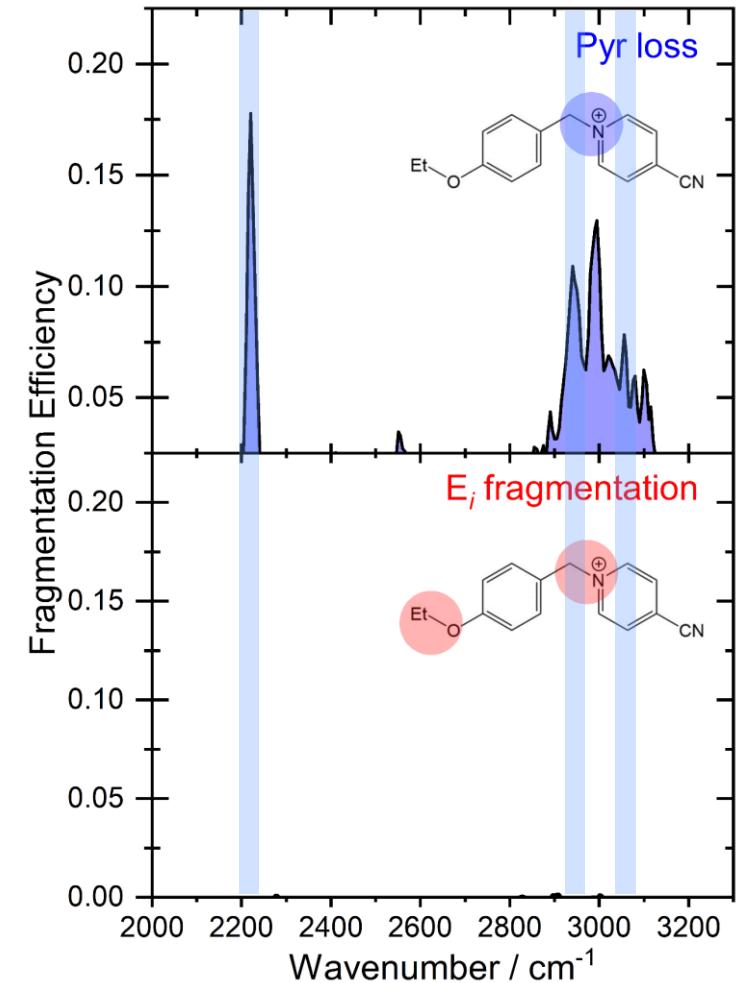
(in a nutshell)

The DMS isn't as hot as we thought – fragmentation of M^+ was only observed for the most sensitive benzylpyridinium ion



Hypothesis: If we extend the OMe chain, E_o wouldn't change, but T_{eff} would decrease given the increased number of rovibrational states

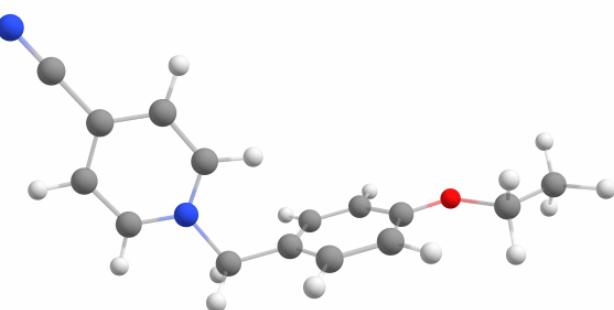
What happens when you add another IR chromophore?



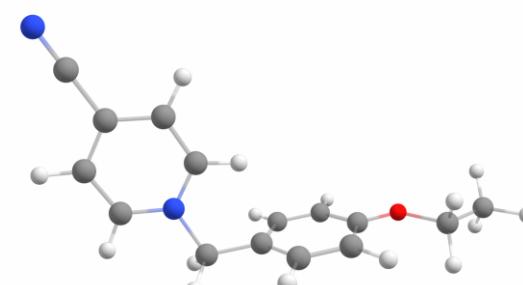
Strong absorption at 2400 cm⁻¹

Adding the cyano chromophore to the “naked” region of the mid-IR yields exclusive fragmentation to the higher energy Pyr pathway

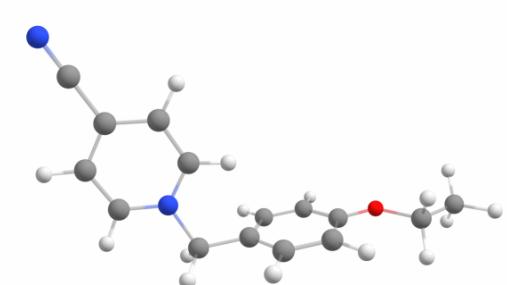
$\nu = 2210 \text{ cm}^{-1}$
CN stretch



$\nu = 2930 \text{ cm}^{-1}$
Bridge sp³ CH stretch

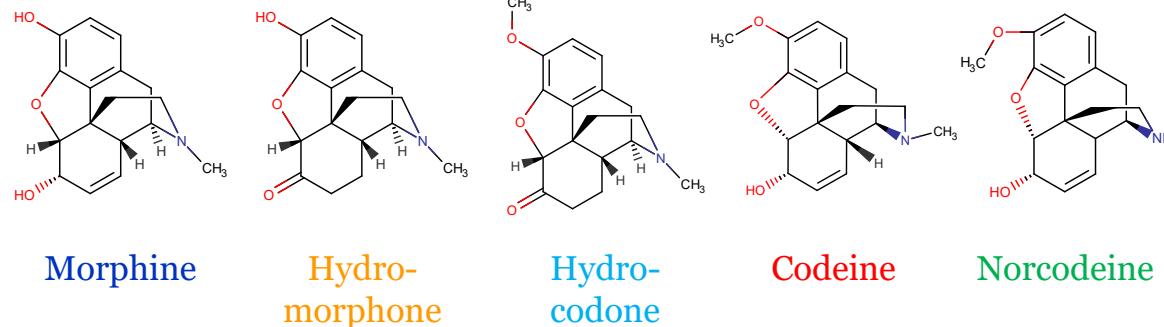
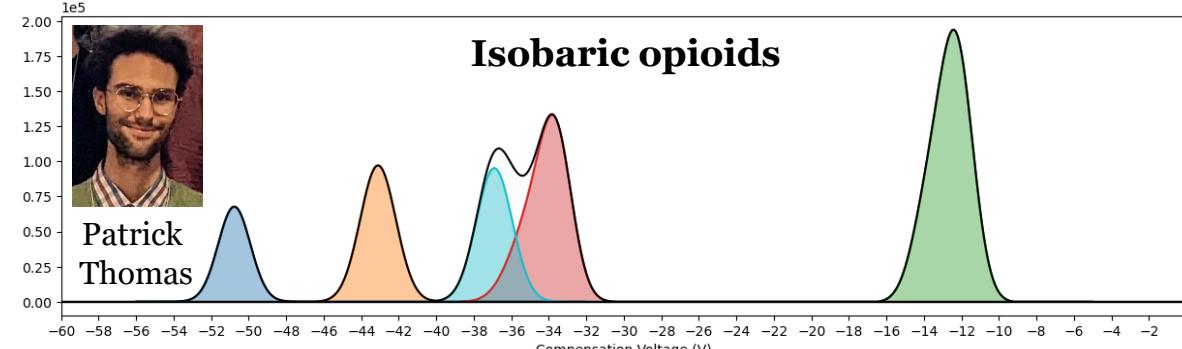
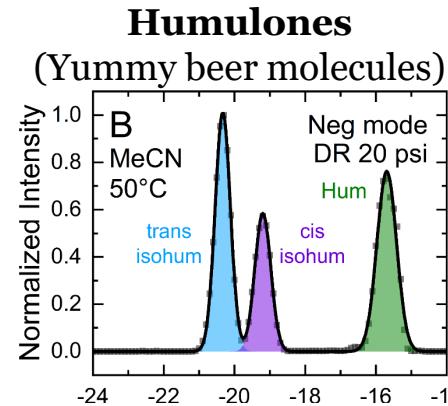
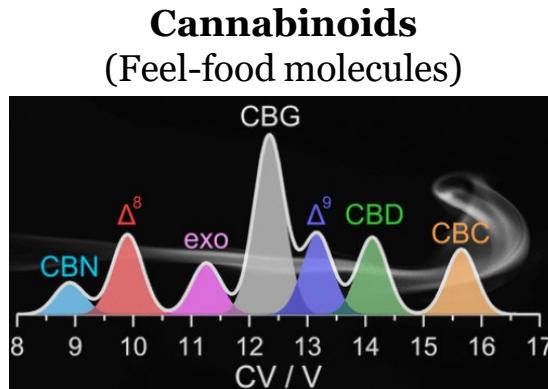


$\nu = 3080 \text{ cm}^{-1}$
Pyr sp² CH stretch (sym)

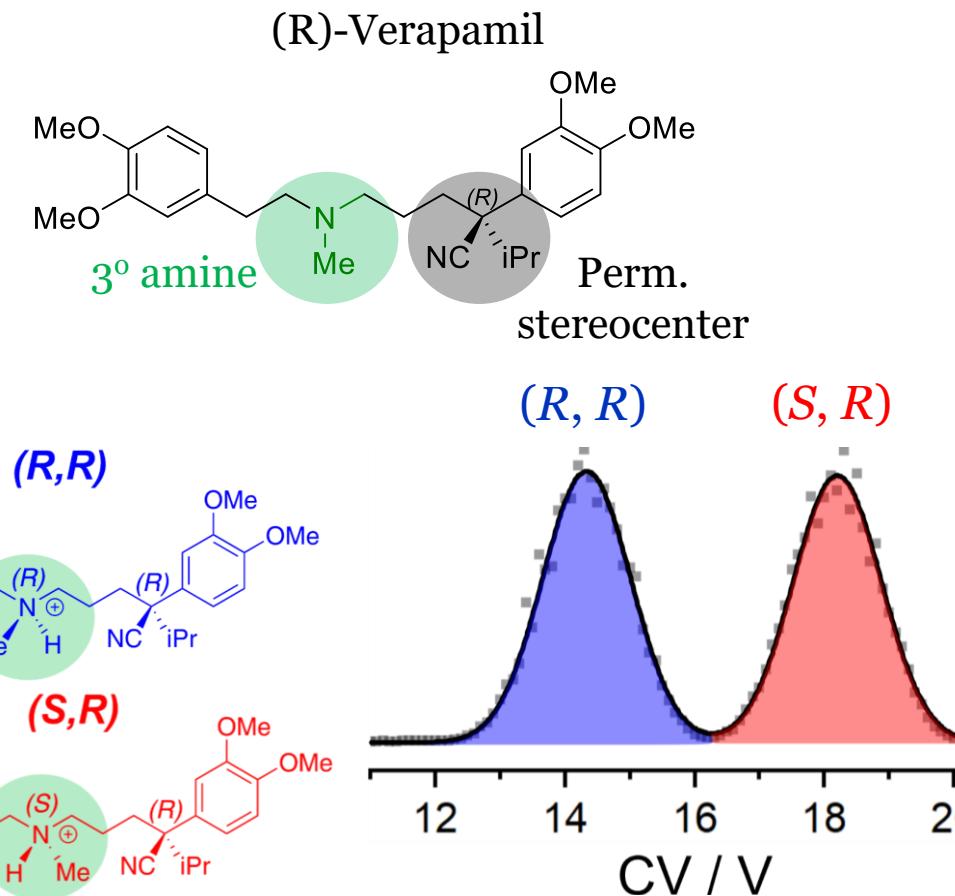


DMS can separate complex mixtures

Complex separations of isobaric compounds



Diastereomers formed via protonation during ESI



WaterFEL lab ballroom

Optics base / control room corridor

