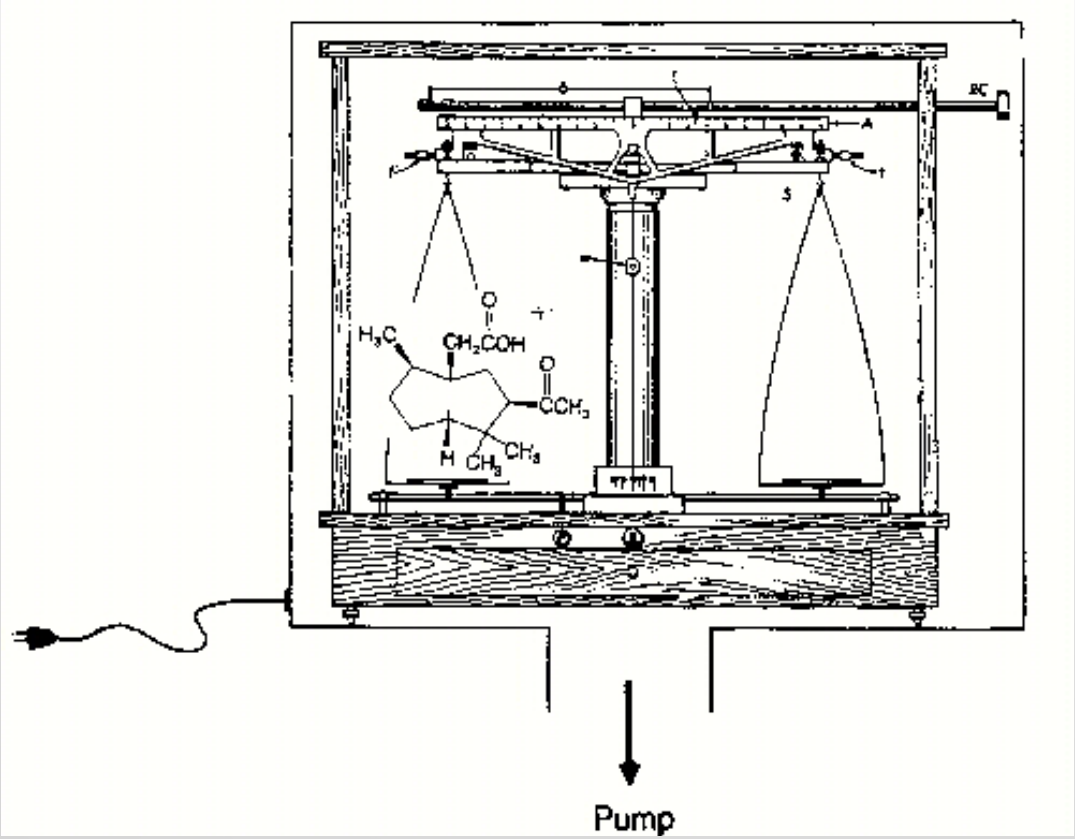
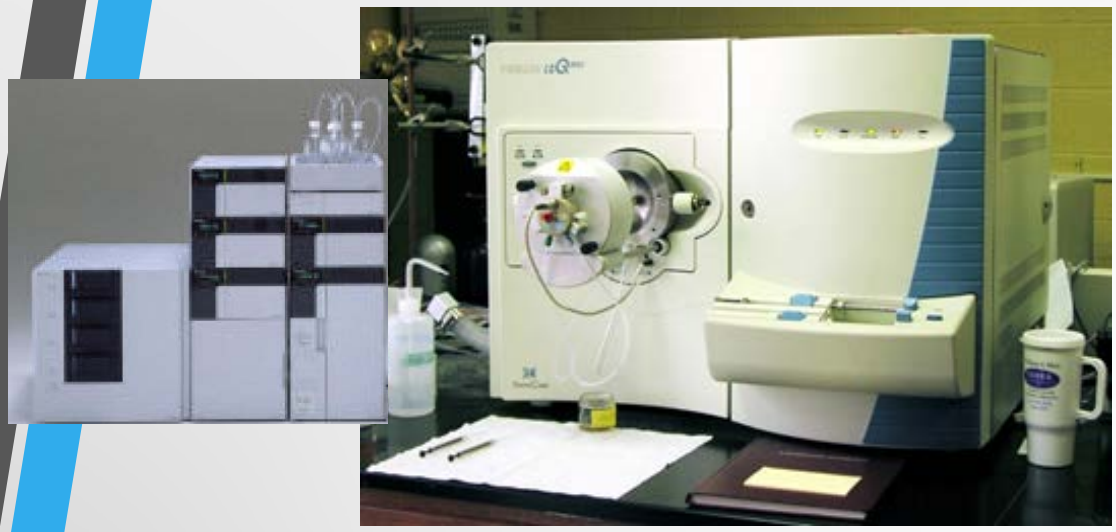
Research Opportunities in the Poutsma Group

(fun with mass spectrometers)

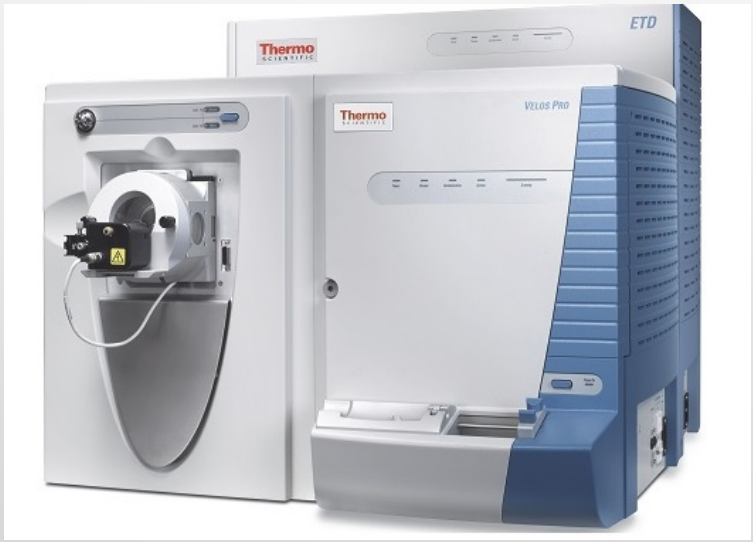


Mass Spectrometers



LCQ-DECA ion trap with Shimadzu UPLC

TSQ-quantum ultra (QQQ)



LTQ-ETD with Eksigent nano-HPLC

On-going Projects

- Gas-phase proteomics research (using the rules)
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  - custom solid-phase synthesis of peptides
  - mass spectrometer fragmentation studies
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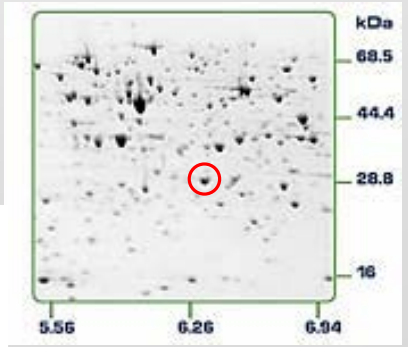
Proteomics

- proteomics is the study of proteins
- one might wish to determine the identity and concentration of all the proteins expressed by an organism
- or one can do a differential study of targeted proteins

differentiate

extract tissue, harvest proteins

separate proteins



identify protein, digest protein

separate peptides

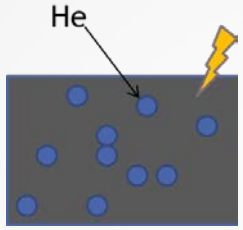
VALIHAK
TNRSCILGR
YGGWL

to MS

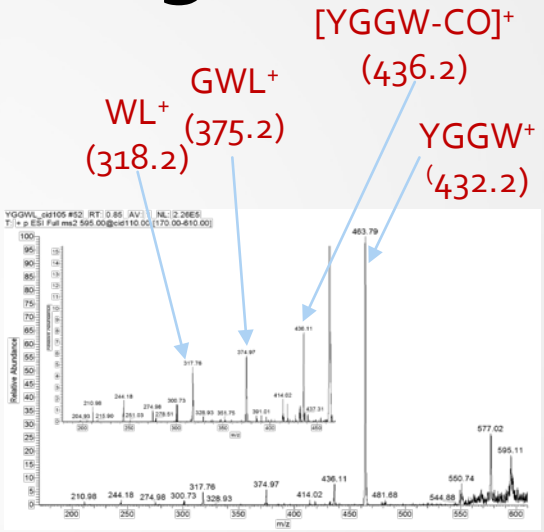
MS-based peptide sequencing

$YGGWL^+$

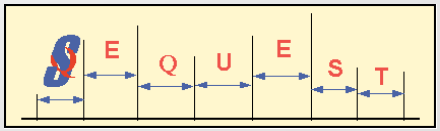
select parent record mass (595.6)



collision induced dissociation



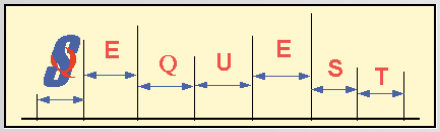
product ions



fingerprint matching

Peak Mass (Da)		Sequence
1871.9	**1-18**	**acetyl-GEEDVQALVVDNGSGNVK**
928.45	19-28	AGVAGDDAPR
1186.67	29-39	SVFPSIVGRPK
2522.16	40-62	NPGIMVGMEEKDAFVGDEAQTKR
800.53	62-68	RGILTLK
1946.91	**69-84**	**YPIEHGIVTNWDDMEK**
1515.73	85-96	IWHHTFYNELR
1926.03	96-113	AAPEEHPVLLTEAPLNPK
3169.49	148-177	TTGIVLDSGDGVSHTVPIYEGYALPHAIMR
644.4	178-183	LDLAGR
1012.5	184-191	DLTEYLMK
595.7	192-196	YGGWL
1046.51	197-206	GYGFSTSAEK
1129.65	207-215	EIVRDIKEK
1645.72	216-228	LCYIALNFDEEMK
1776.88	239-254	SYELPDGNIITVGNER
1796.92	255-269	FRCPEALFQPSFLGK
1617.83	270-284	EAAGIHTTTFNSIKK
1033.56	284-291	KCDVDIRK
2245.08	292-312	DLYGNIVLSGGTTMYEGIGER
1177.61	316-326	DITTLAPSTMK
1136.72	327-336	IKVVAPPERK
1517.68	360-372	EEYDESGPSIVHR

peptide identification



protein identification

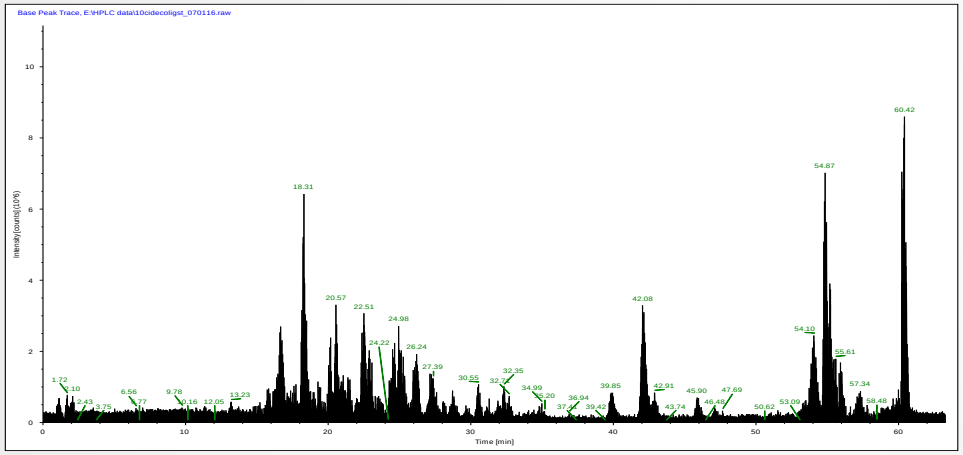
Actual real-life samples

- *M. Smegmatis* infection study
  - Many novel bactcteriophage viruses can infect the soil bacterium *M. smegmatis.*
  - *M. Smegmatis is a* non-pathogenic model system for *M. tuberculosis*
  - we freeze *M. Smegmatic* cells at varying time points after infection by novel pages that were discovered in the PhageLab Freshman Biology Lab experience.
  - We lyse the cells, harvest the proteins, and digest with trypsin.
  - We the perform shotgun proteomics experiments aim to identify all of the proteins being expressed by the bacterium and the virus at varying time points.
  - By determining which viral proteins are expressed at different times after infection, we can begin to classify sequenced proteins of unknown function as regulatory (early time points) or structural (late time points)
  - In addition to the information gleaned from the viral proteins, we can also begin to look for bacterial responses to infection.

Preliminary Results

E. coli sample: 15 minutes after infection with T7 phage

>3800 proteins, >20,000 peptides



time	# proteins	representative proteins
15 min	21	DNA primase/helicase, DNA-directed DNA polymerase peptidoglycan hydrolase gp 16
30 min	27	endonuclease I portar protein
45 min	30	bacterial RNS polymerase inhibitor exonuclease inhibitor of dGTPase terminase (large and small subunits)
60 min	26	capsid assembly scaffolding protein nucleotide kinase tail tubular protein

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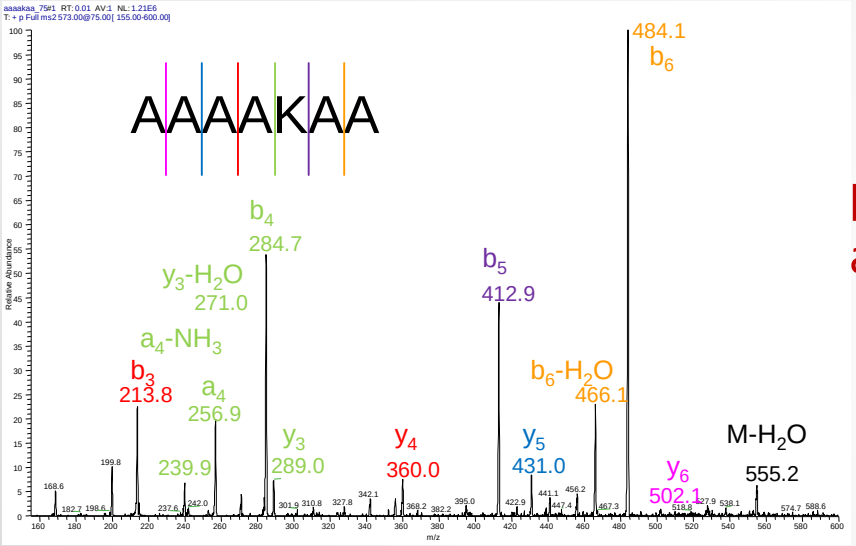
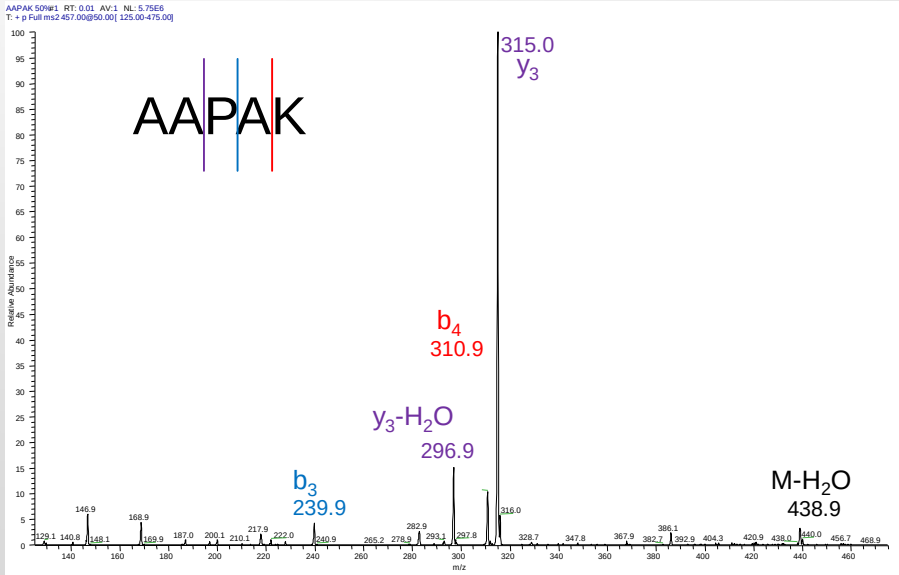
Selective Cleavages

(improving the rules)



peptides normally fragment randomly along peptide backbone

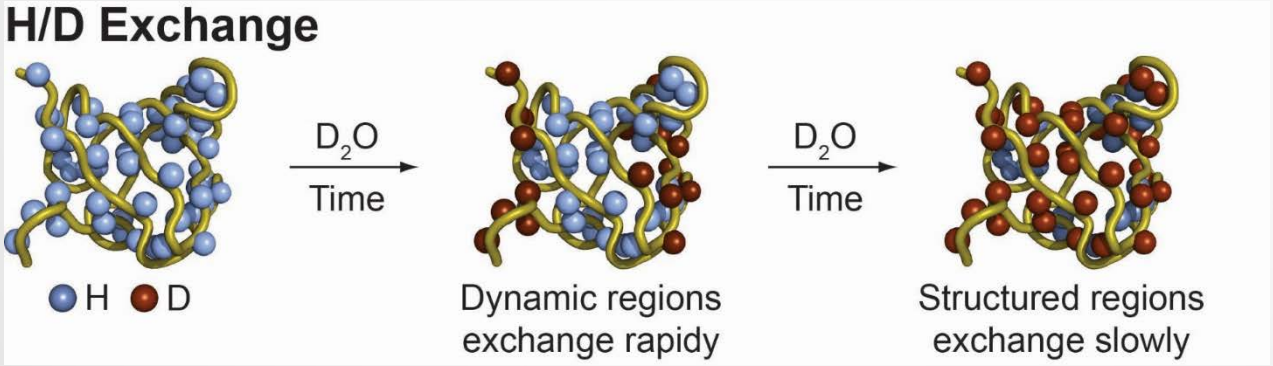
some residues such as proline produce selective cleavages that can confound searching algorithms



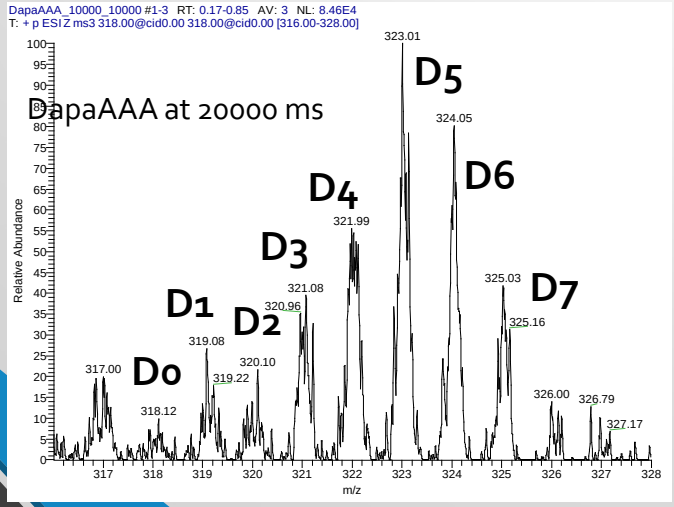
Hydrogen-deuterium Exchange

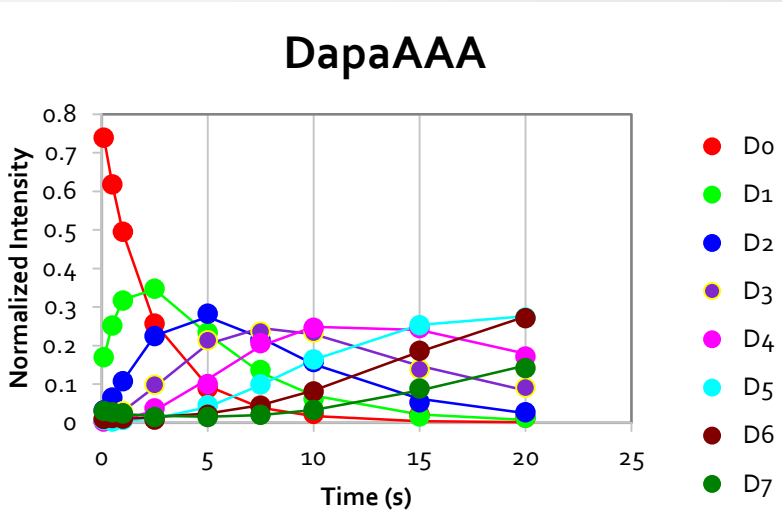
(understanding the rules)

the rate at which a gas-phase ion substitutes H for D gives an indication of the availability of exchangeable hydrogens, and thus and indirect indication of structure



we can measure rates for H/D exchange in our ion trap mass spectrometer

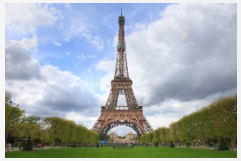


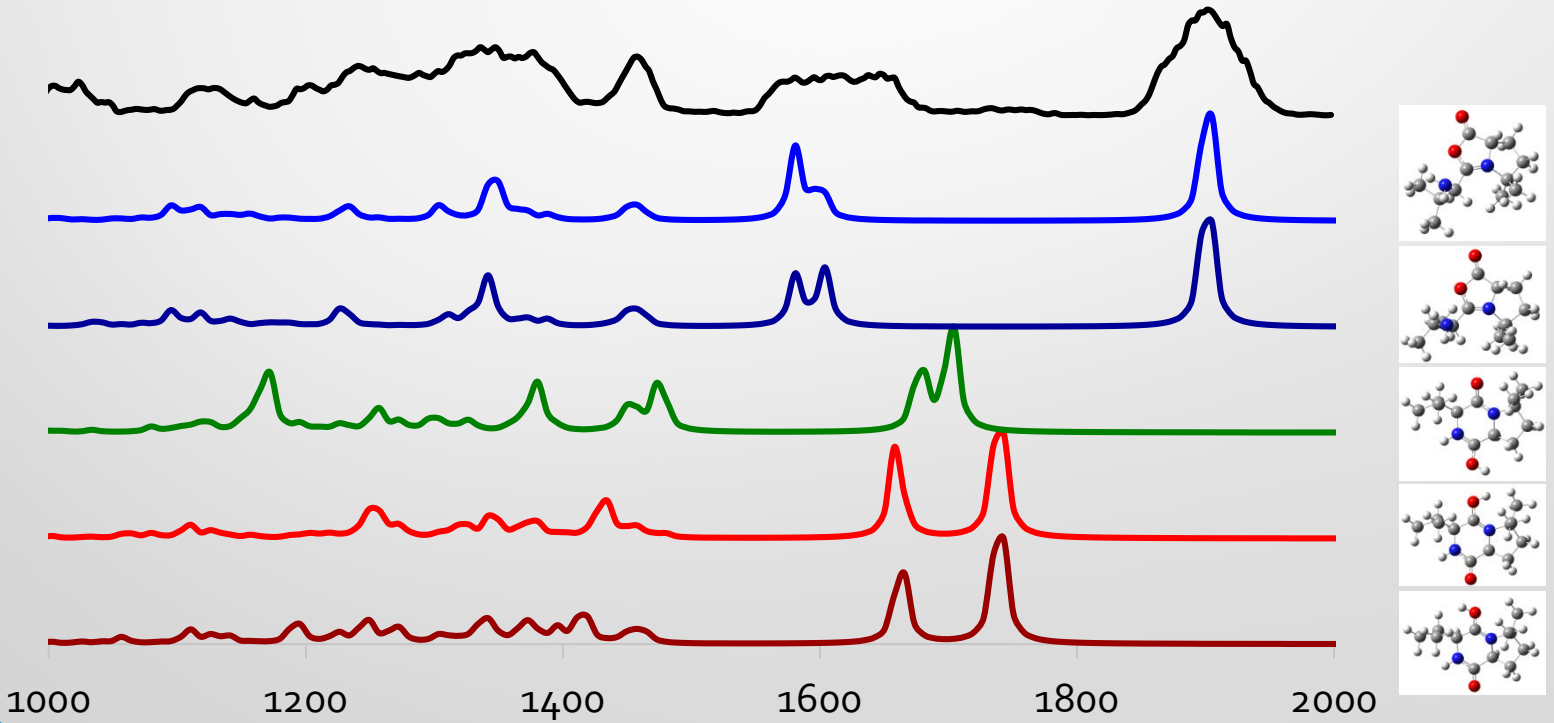


IRMPD: Vibrational Spectroscopy

(understanding the rules)

- CLIO (Orsay) and FELIX (Nijmegen) FEL's
- infrared action spectroscopy of ions
- gives direct information of structure





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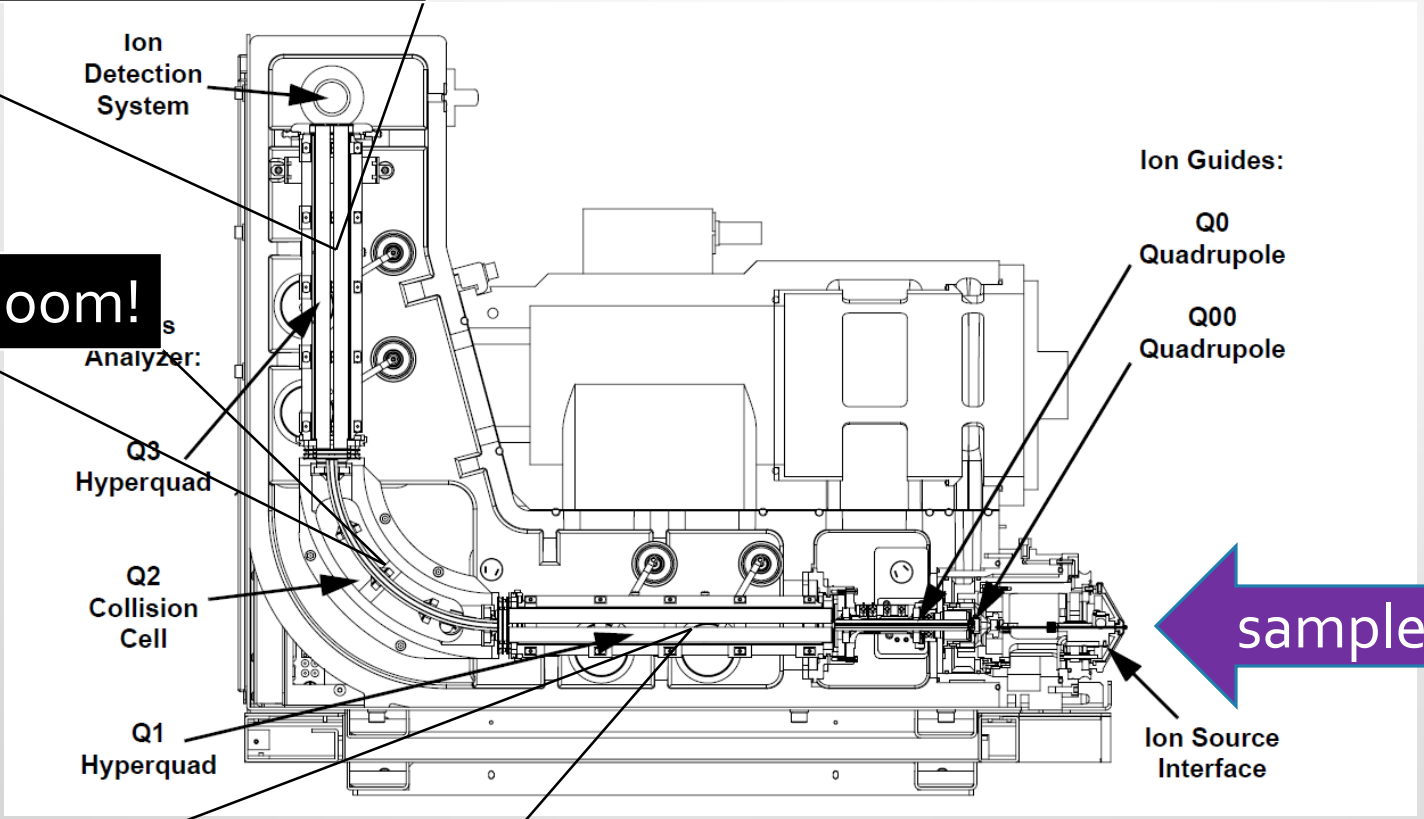
ESI-QQQ instrument kinetic method studies
(the basis for the rules)

[peptide-H^+ or base-H^+

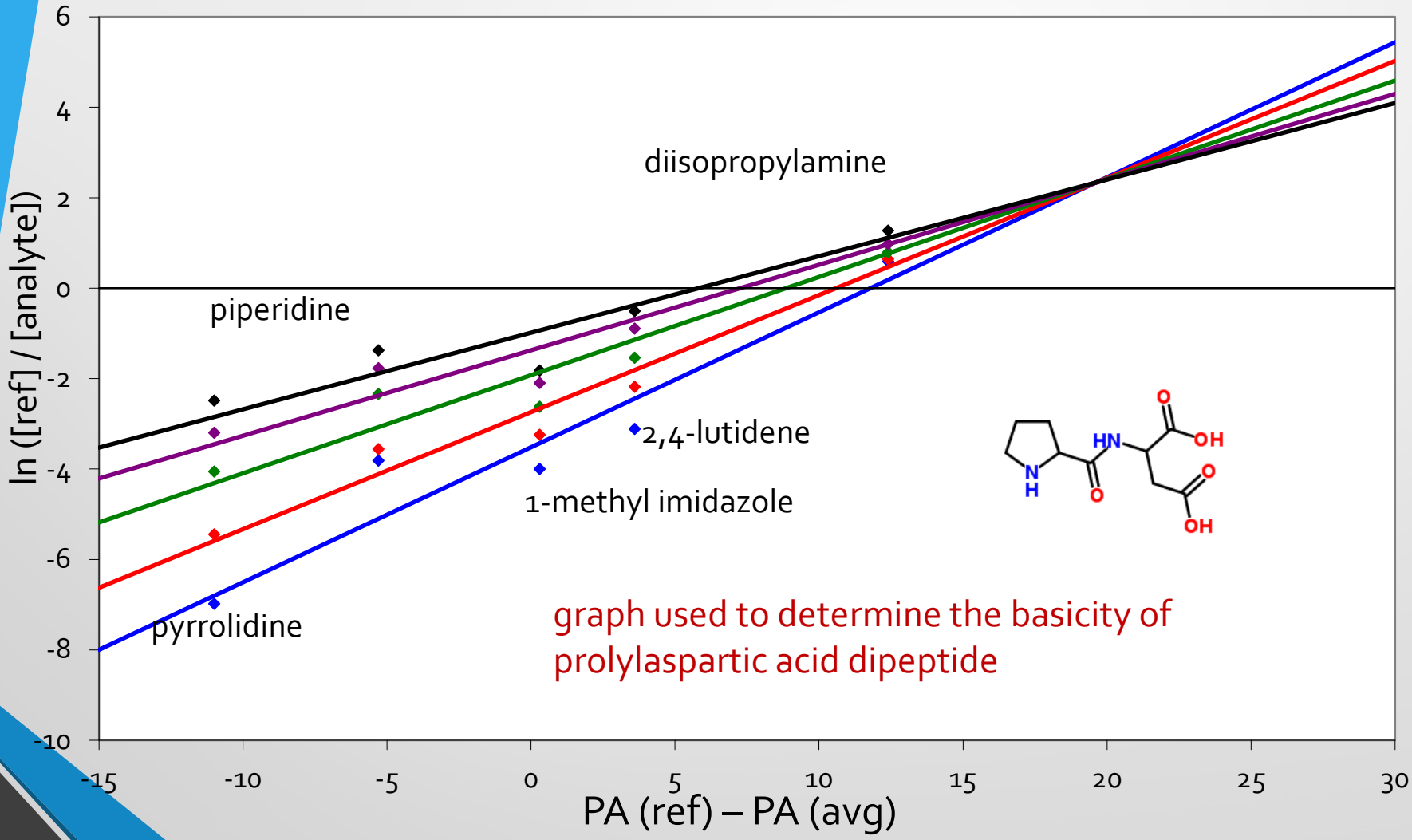


Boom!

[$peptide_1$----H^+---$base_2$]$^+$

measuring the fragment intensities of competitive cleavage of proton-bound dimer ions gives us acid-base properties for peptides

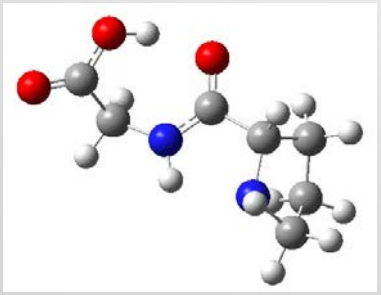
Pro-Asp: kinetic method plot



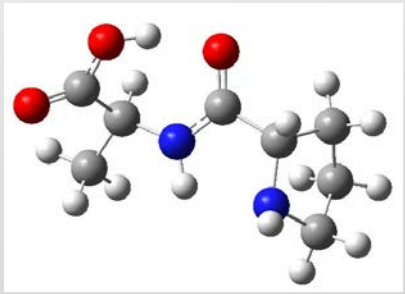
graph used to determine the basicity of prolylaspartic acid dipeptide

Computational Studies

- We use computational chemistry to support and guide our experimental work.
- Collaborator: Prof. Jennifer Poutsma (ODU)



Pro-Gly



Pro-Ala