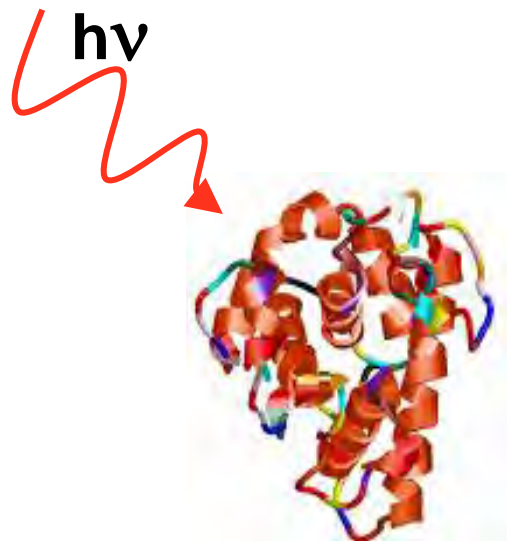
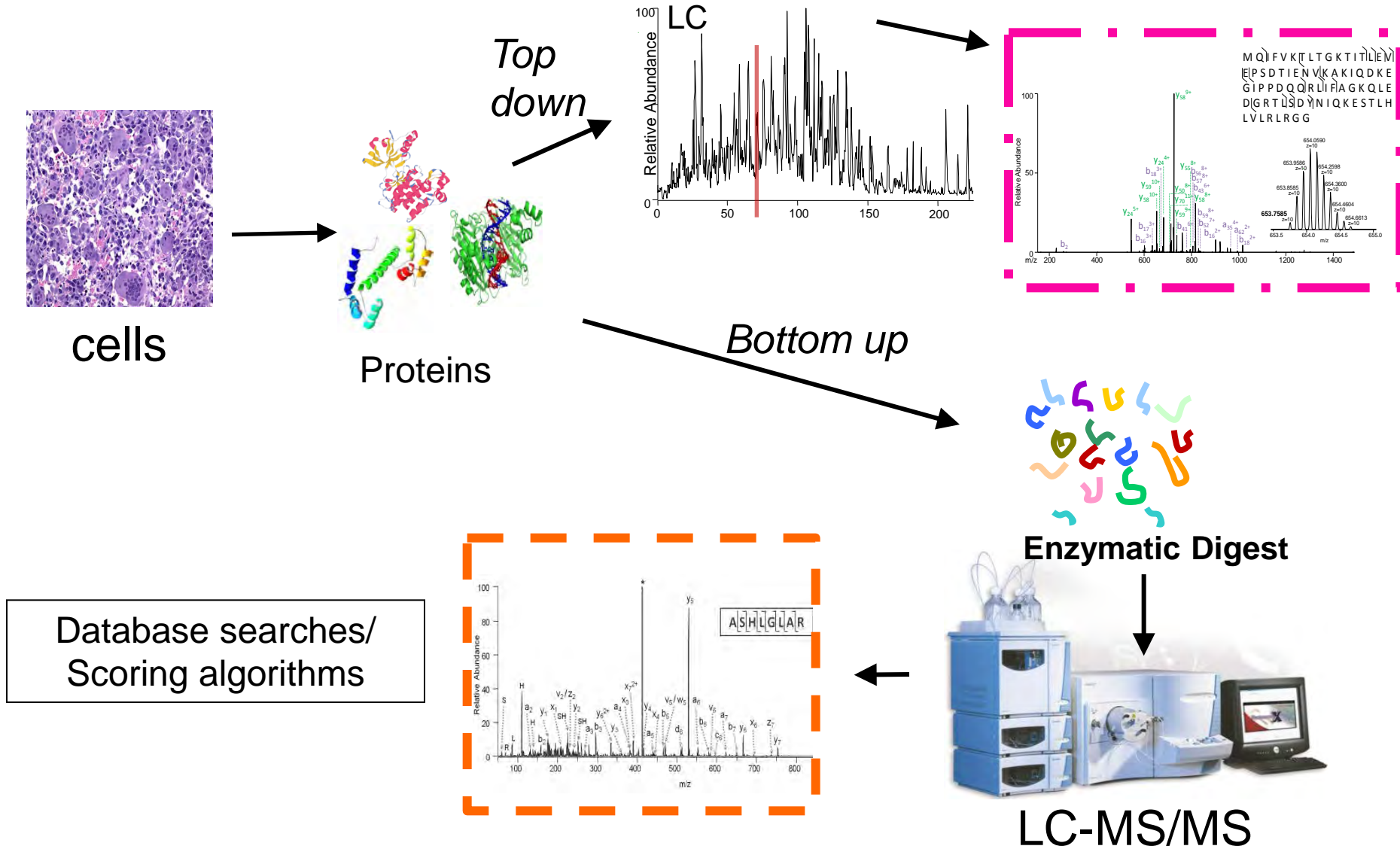

Development of Photodissociation for Analysis of Biological Molecules



Jenny Brodbelt

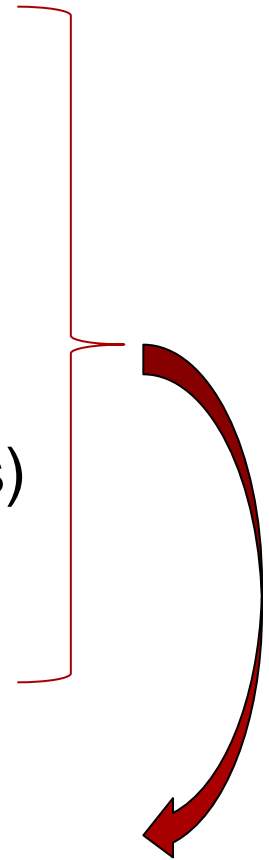
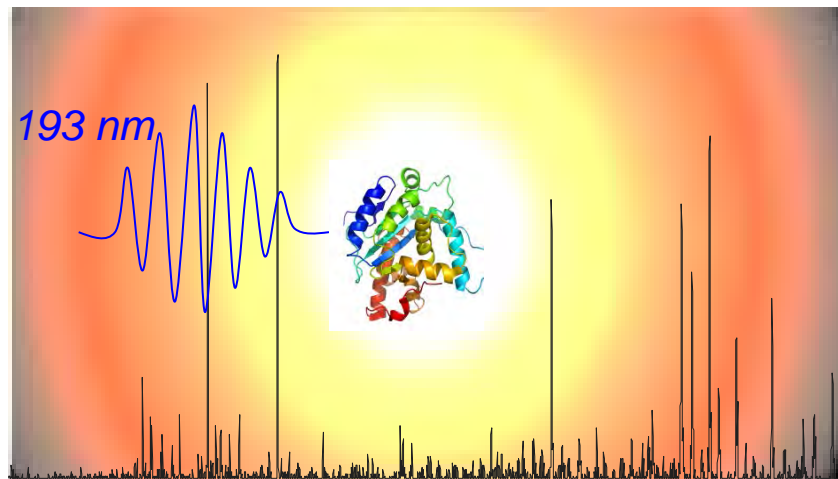
Department of Chemistry
University of Texas at Austin

The need for fast, efficient, robust ion activation methods



Ideal Attributes of an Activation Method

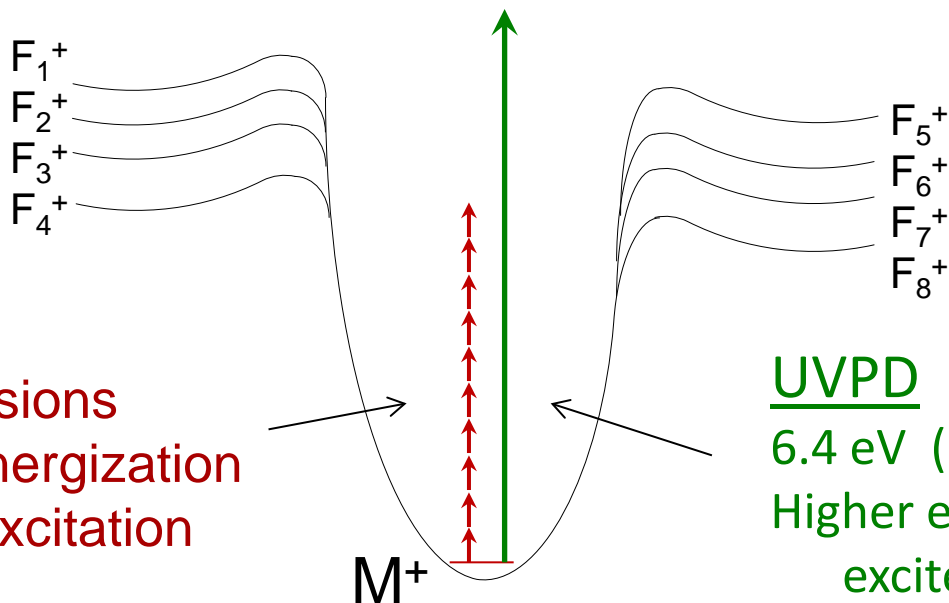
- Fast
- High energy deposition
- Large diversity of fragment ions
- Efficient
- Robust (little dependence on tuning conditions)
- Effective for positive/negative ions



Energy Deposition: CID versus UV photodissociation

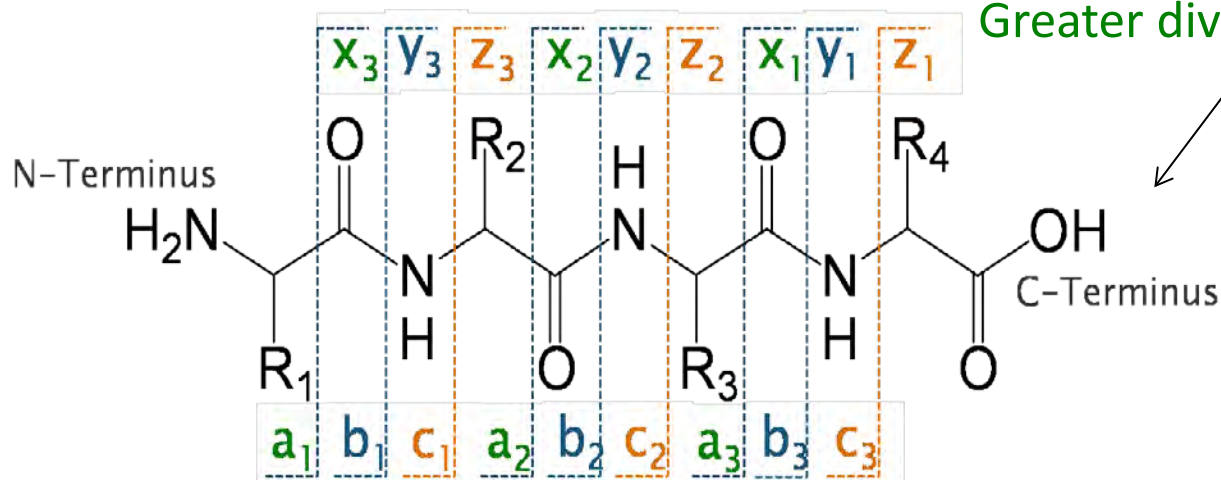
CID

Multiple collisions
 Step-wise energization
 Vibrational excitation



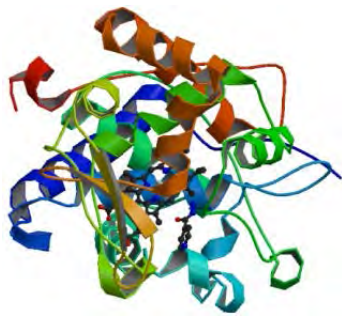
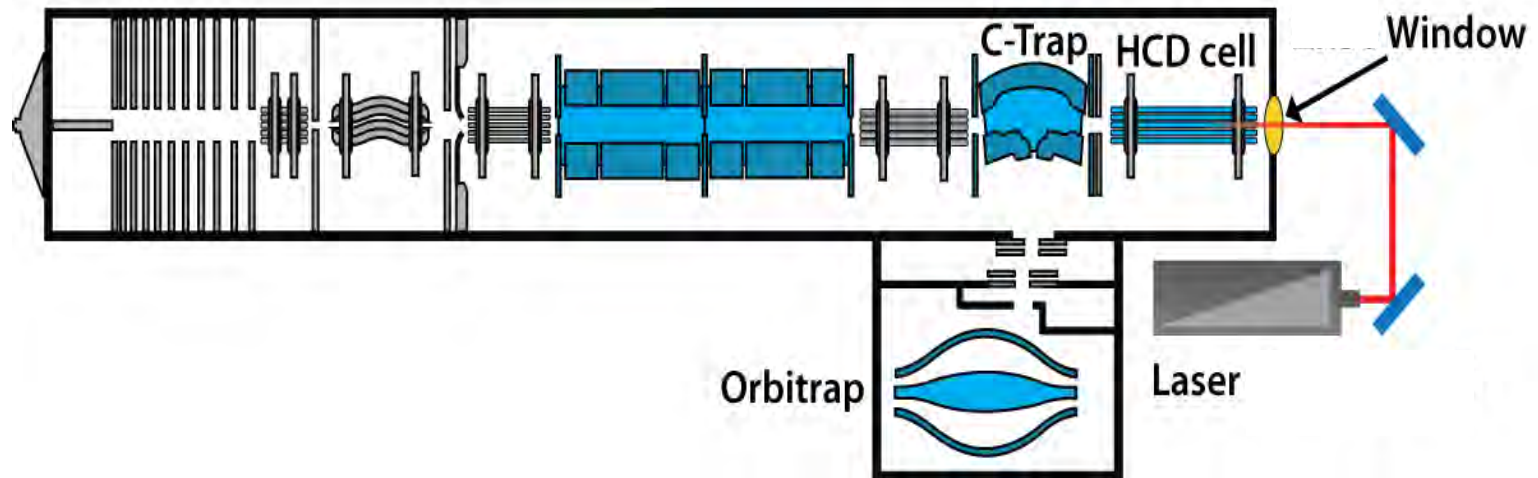
UVPD

6.4 eV (193 nm ArF laser)
 Higher energy deposition/
 excited electronic states
 Fast
 Greater diversity of fragment ions

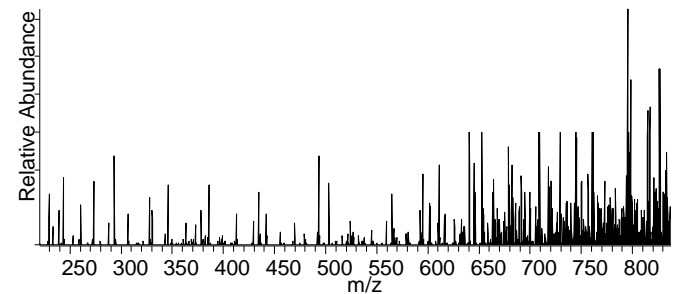
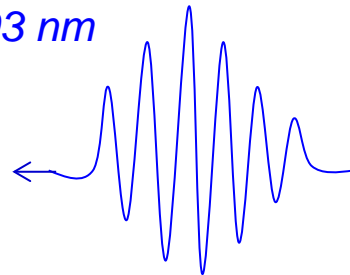


Top-down protein analysis by photodissociation

Orbitrap: high performance mass spectrometer



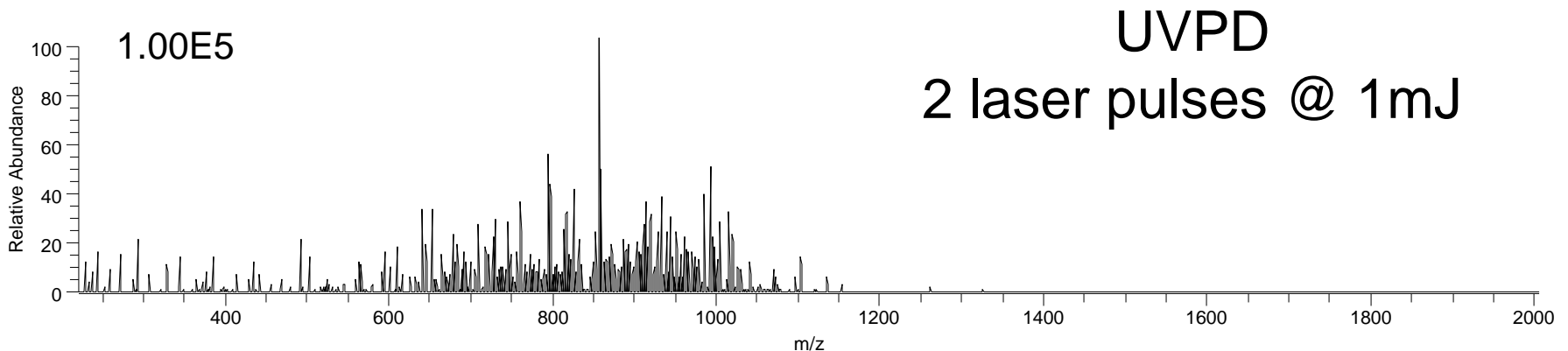
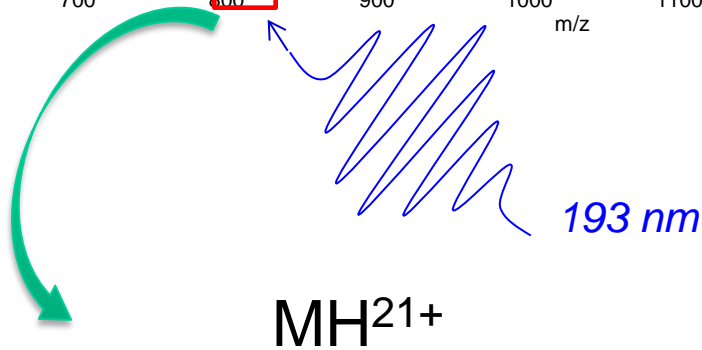
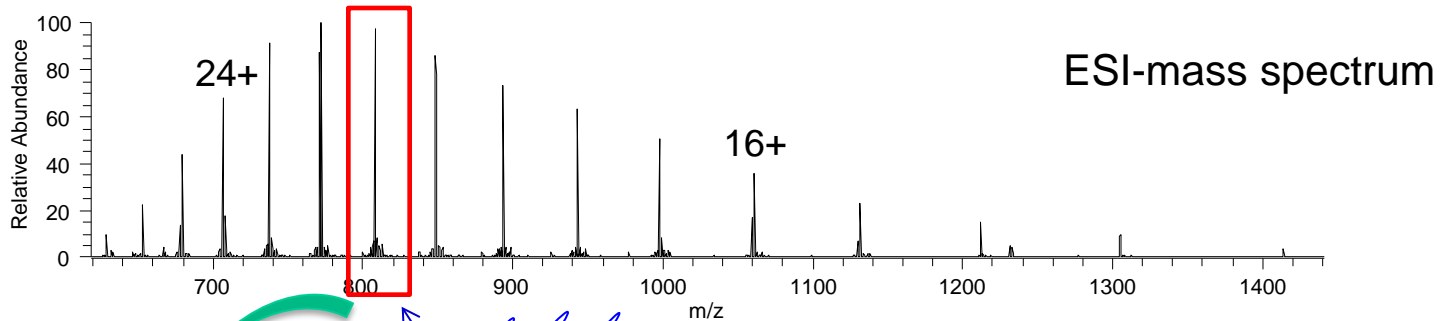
193 nm



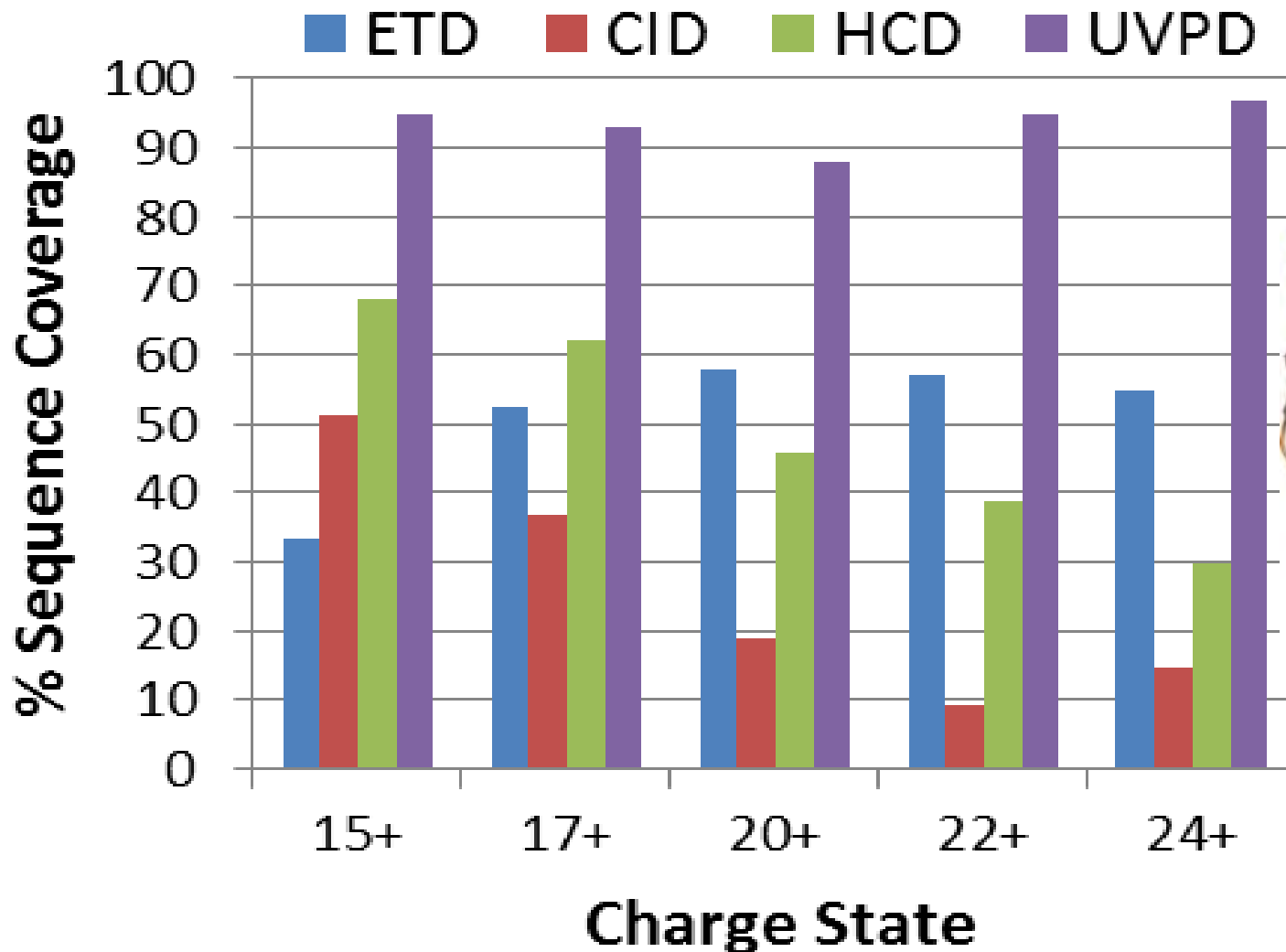
Need high resolution/high accuracy for confident assignment of multi-charged fragment ions

UVPD of Myoglobin

153 residues (17 kDa)

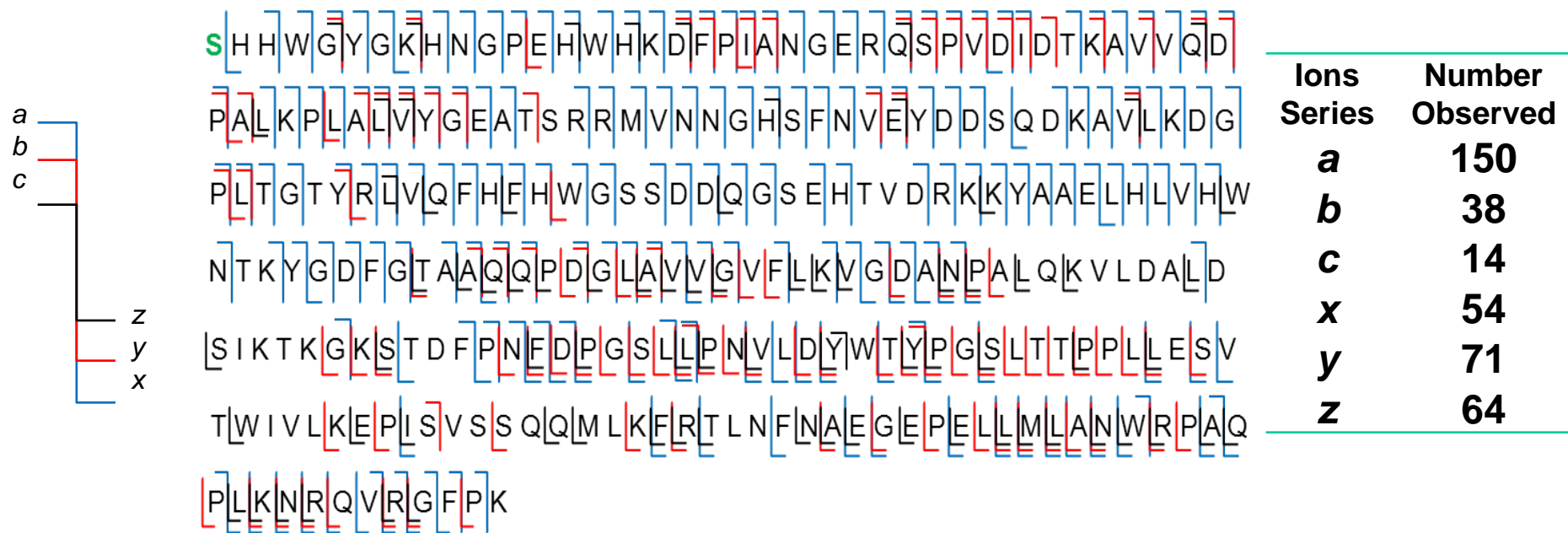
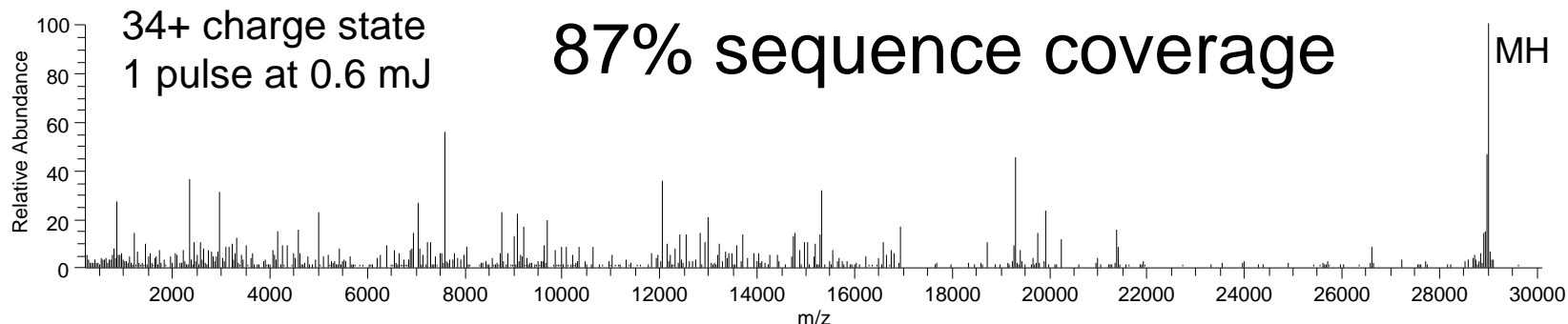


Sequence coverage obtained for myoglobin



UVPD of Carbonic Anhydrase

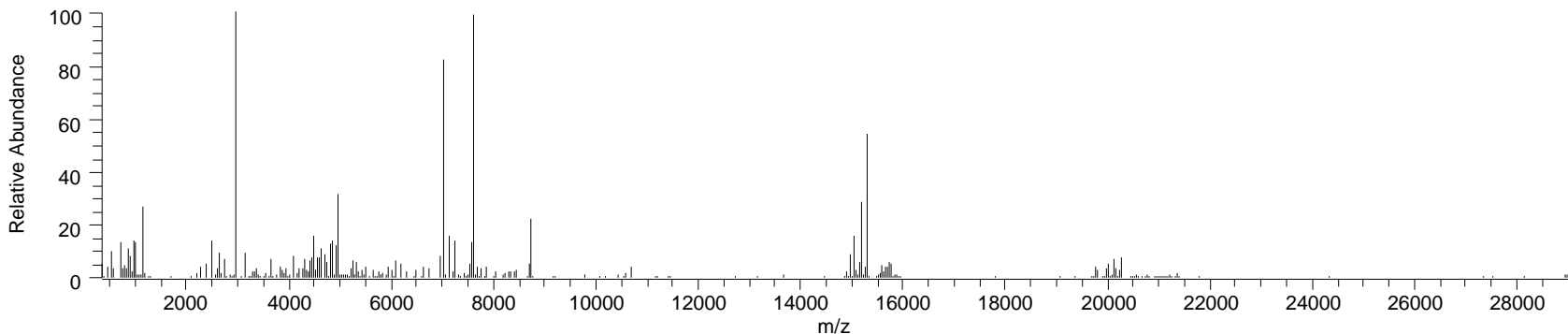
259 residues (29 kDa)



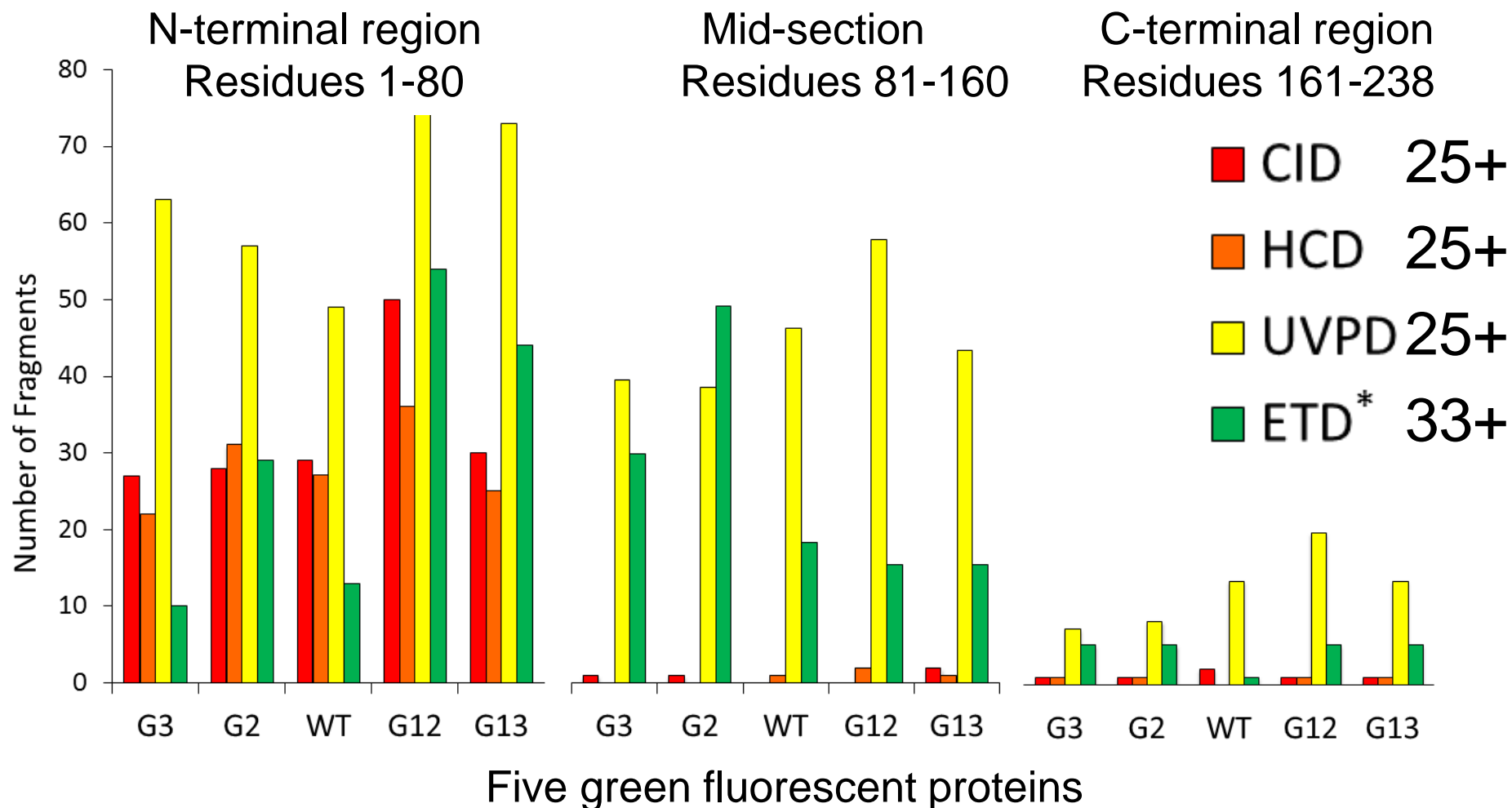
High Energy CID of Carbonic Anhydrase

34+ 30% coverage

b1 -S-H-H-W-G-Y-G-K-H-N-G-P-E-H-W-H-K-D-F-P}I}A}N}G-E-R-Q}S}P}V} y230
b31 -D}I}D}T-K-A}V}V}Q}D}P}A}L-K-P-L}A}L}V}Y}G-E-A-T-S-R-R-M-V-N- y200
b61 -N-G-H-S-F}N}V}E}Y-D-D-S-Q-D-K-A-V-L-K-D-G-P-L-T-G-T-Y-R-L-V- y170
b91 -Q-F-H-F-H-W-G-S-S-D-D-Q-G-S-E-H-T-V-D-R-K-K-Y-A-A-E-L-H-L-V- y140
b121 -H-W-N-T-K-Y-G-D-F-G-T}A}A}Q}Q}P-D}G}L}A}V}V-G-V-F-L-K-V-G-D- y110
b151 -A-N-P-A-L-Q-K-V-L-D-A-L-D-S-I-K-T-K-G-K-S-T-D-F-P-N-F-D-P-G- y80
b181 -S}L}L}P-N}V}L}D}Y}W}T}Y}P-G-S}L}T}T}T}P}P-L}L}E}S-V}T}W}I}V-L- y50
b211 -K}E}P-I}S-V}S-S-Q-Q}M-L-K-F-R-T-L-N-F-N}A}E}G}E}P}E}L}L}M}L- y20
b241 }A}N}W-R-P-A-Q-P-L-K-N-R-Q-V-R-G-F-P-K- y1



Distribution of N-terminal fragment ions for CID, HCD, UVPD, and ETD



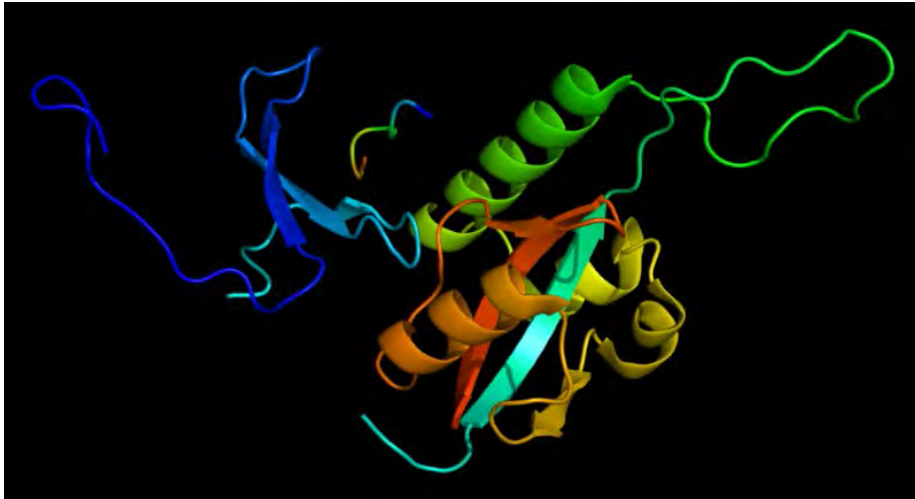
UVPD gives fragmentation deeper into the protein sequence
(Same trend is observed for C-terminal fragment ions)



Two key questions:

- 1) Post-translational modifications?
- 2) High-throughput strategies?

Pin1: Peptidyl-prolyl cis-trans isomerase (regulates phosphorylation of Ser/Thr-Pro motifs)



- Pin1 oxidation/dysfunction has been linked to Alzheimer's disease
- Pin1 activity is reduced in the early stage of Alzheimer's disease through modification by **oxidation**

GSHG**M**ADEEKLPPGW**E**KAM**S**RSSGRVYYFNHITNASQW
ERPSGNSSSGGKNGQGEPARVR**C**SHLLVKHSQSRRPSS
WRQEKITRTKEEALELINGYIQKIKSGEEDFESLASQFSD**C**S
SAKARGDLGAFSRGQ**M**QKPFEDASFALRTG**E**MSGPVFTD
SGIHILRTE

Possible oxidation sites

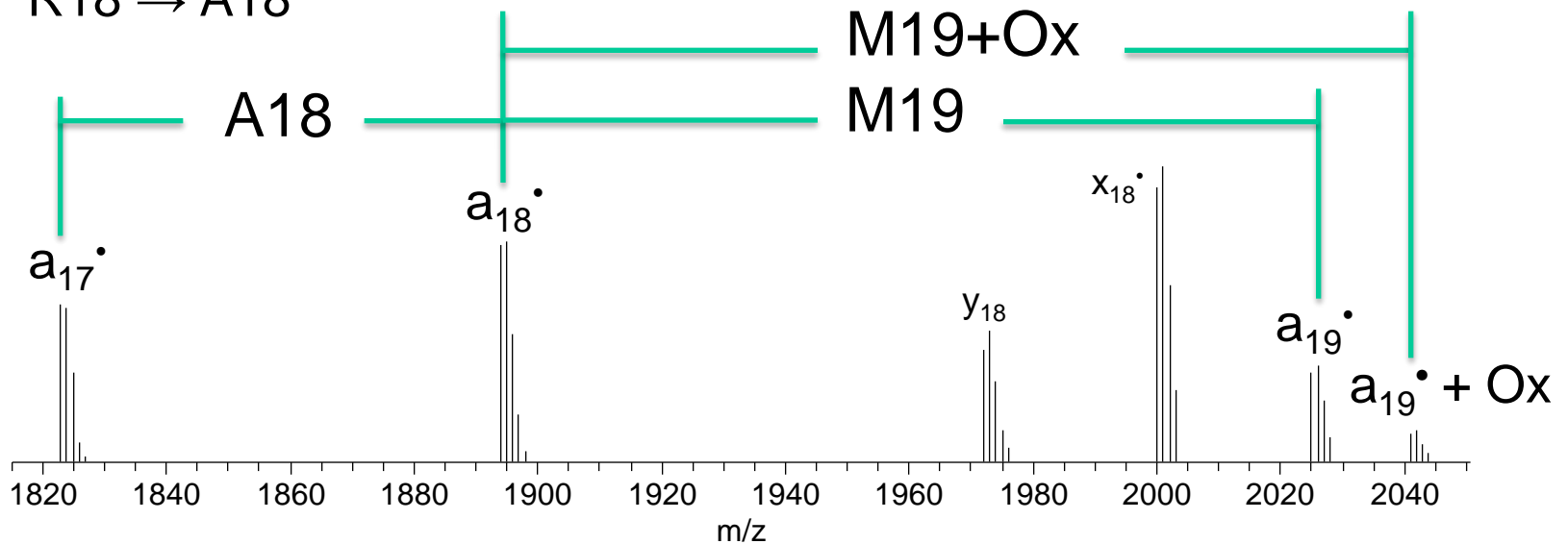
- Collaboration with Prof. Jessie Zhang (UT-Austin)

Pin1

GSHGMADEEKLPPGWEK**AM**SRSSGRVYYFNHITNASQWERPS
GNSSSGGKNGQGEPARVRCHELLVKHSQSRRPSSWRQEKITRTK
EEALELINGYIQKIKSGEEDFESLASQFSDCSSAKARGDLGAFSRG
QMOKPFEDASFALRTGEMSGPVFTDSGIHILRTE

Single Residue Mutation
R18 → A18

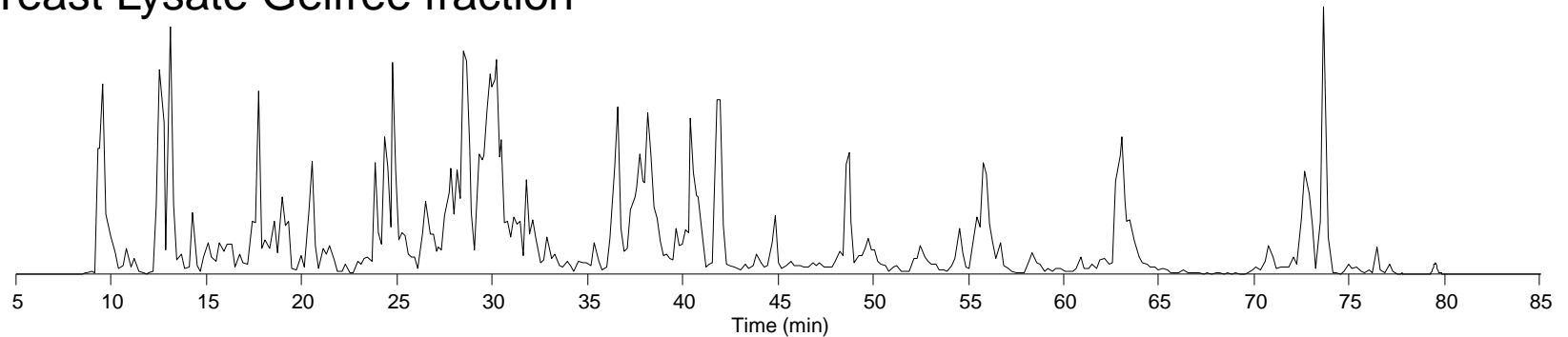
Partial M19 Oxidation



Charge deconvoluted UVPD spectrum of MH²²⁺

UVPD for High Throughput Analysis of Complex Mixtures

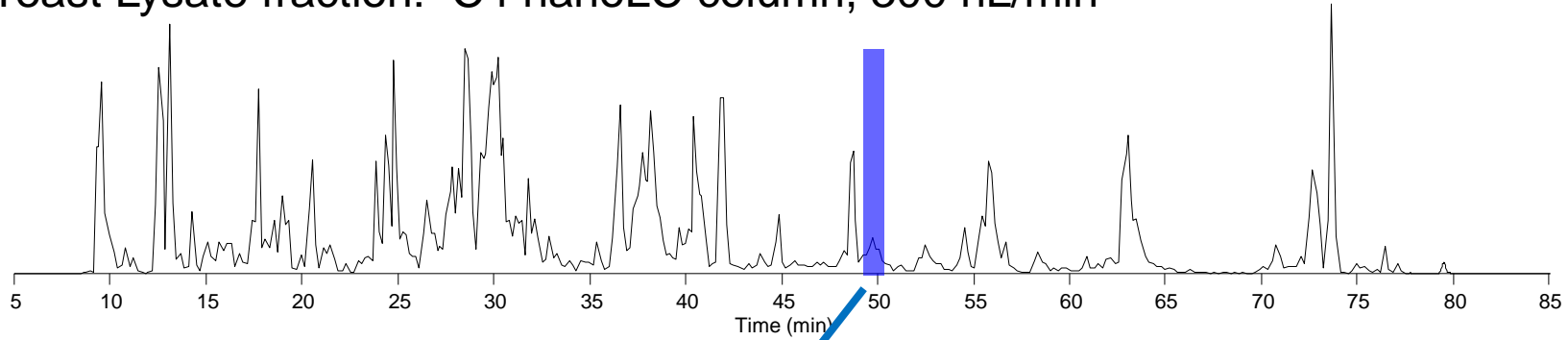
Yeast Lysate Gelfree fraction



2 cm C4 trap column, 15 cm C4 analytical column, 300 nL/min

UVPD for High Throughput Analysis of Complex Mixtures

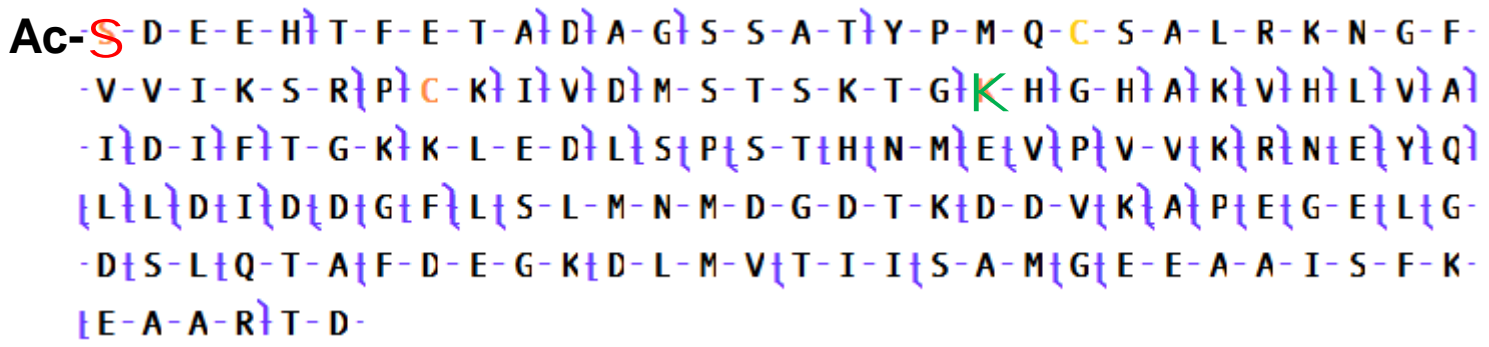
Yeast Lysate fraction: C4 nanoLC column, 300 nL/min



Eukaryotic translation initiation factor 5A (eIF-5A, 17kDa)

N-terminal acetylation and phosphorylated serine

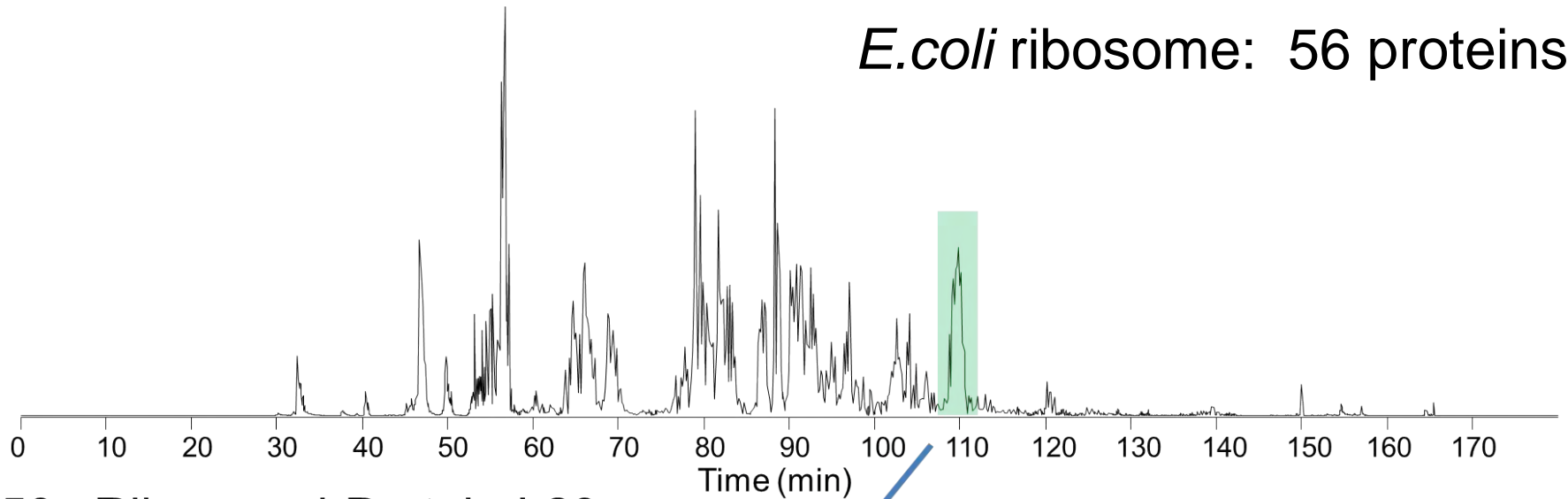
Hypusine (N-epsilon-(4-aminobutyl)lysine)



1 pulse per scan, 6 averages, RP = 60,000 at *m/z* 400

LC-UVPD-MS for higher throughput protein identification

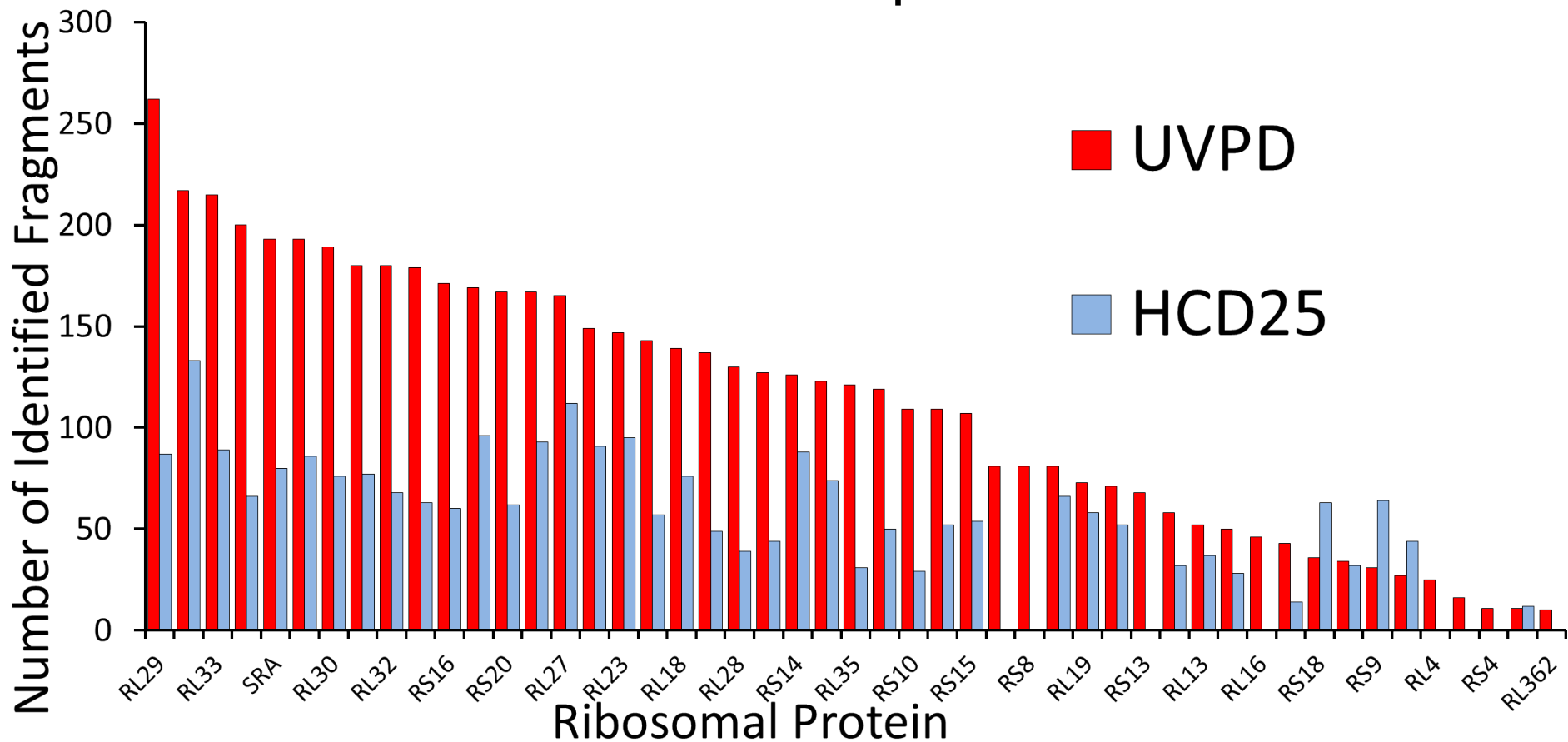
E.coli ribosome: 56 proteins



50s Ribosomal Protein L29

c1 -M t K t A t K - E t L t R t E t K t S t V t E t E t L t N t T t E t L t L t N t L t L t R t E t Q t z39
t F t N t L t R t M t Q t A t A t S t G t Q t L t Q t Q t S t H t L t L t K t Q t V t R t R t D t V t
t A t R t V t K t T t L t L t N t E t K t A t G t A -

UVPD outperforms collision-based methods for most ribosomal proteins



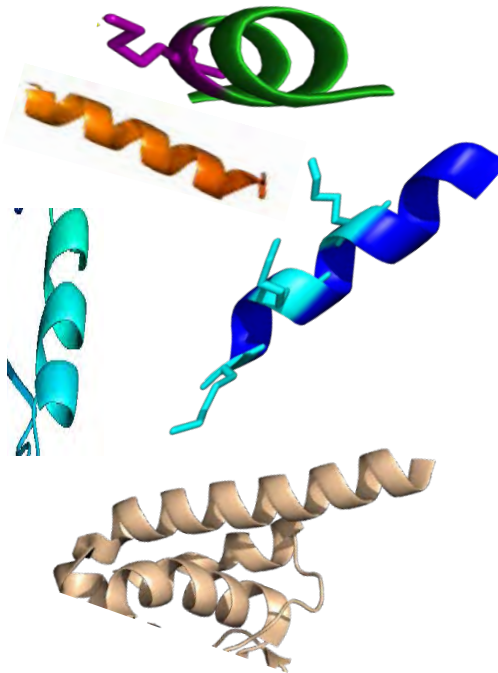
Three Strategies for Proteomics

Top-Down



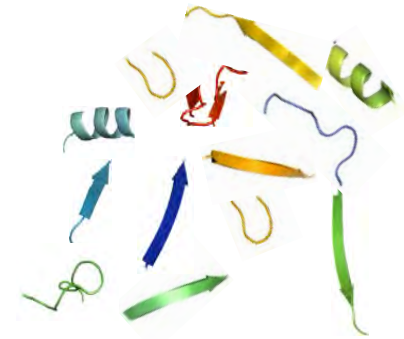
Intact proteins

Middle-Down



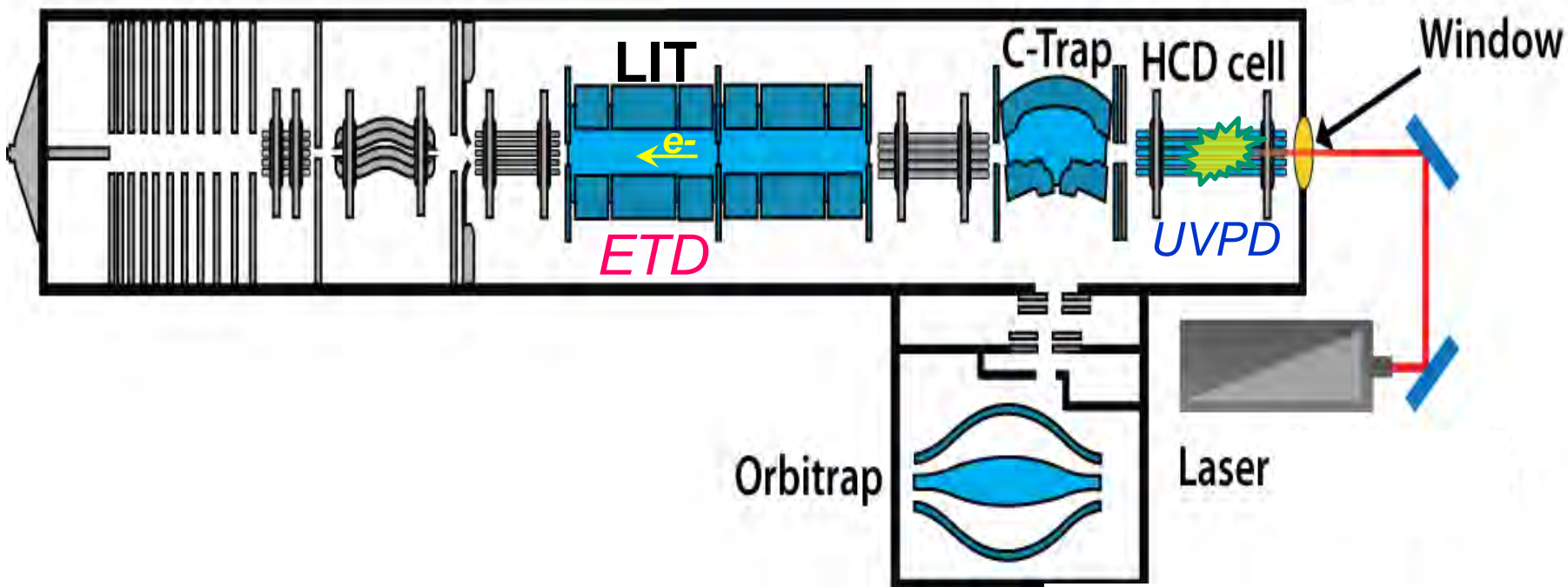
Large peptides

Bottom-Up



Peptides

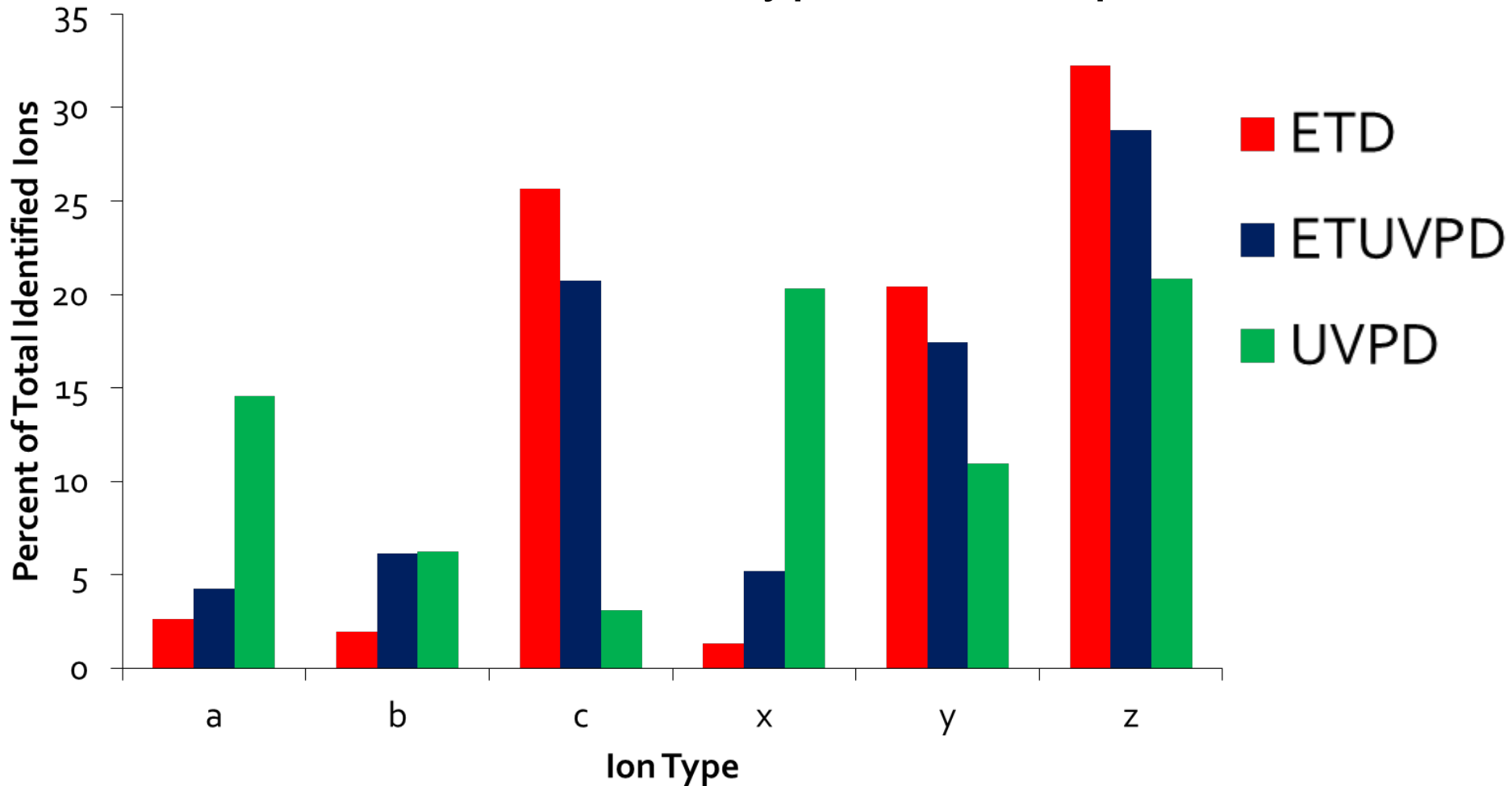
Integration of ETD and UVPD



ETD: 15 - 100 ms

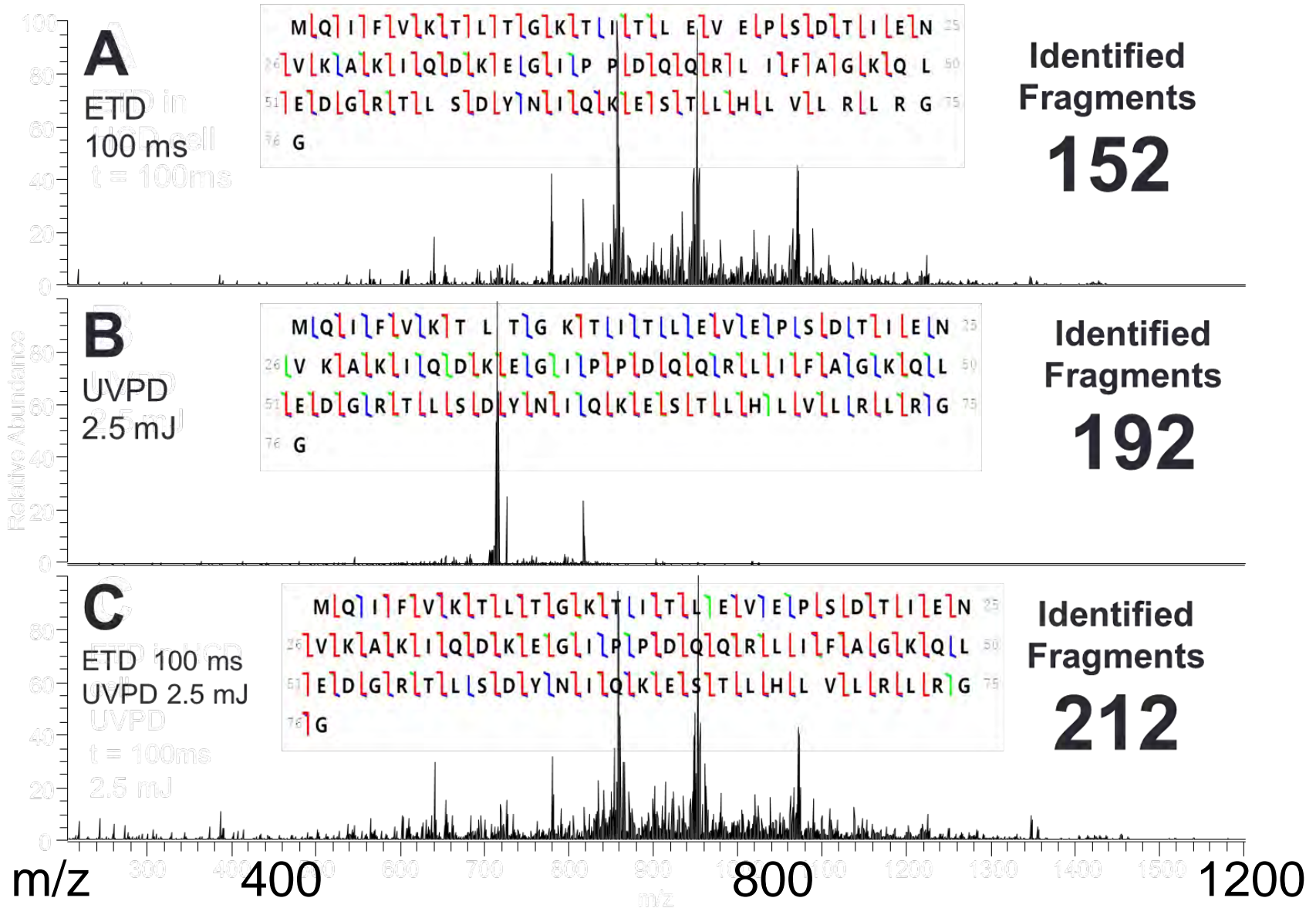
UVPD: excimer laser (193 nm) → one 5 ns pulse

Distribution of ion types for ubiquitin



ETUVPD gives a “balance” of ETD and UVPD fragment ions

Comparison of number of identified fragment ions





Photodissociation offers:

Fast, high energy deposition

Fragment ion diversity: a,b,c, x,y,z

Promising strategy for top-down proteomics

Acknowledgements

Broadbelt group

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- Scott Robotham



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Dr. Walt Fast

Dr. Hua Xu

Dr. Neil Kelleher

Dr. Adrian Keatinge-Clay

Thermo Scientific



National Science Foundation
WHERE DISCOVERIES BEGIN



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National Institutes of Health