JUNE 6TH 2022

TEXAS
The University of Texas at Austin

Multiple methods of dissociation and their applications on the UHMR

American Society for Mass Spectrometry

70th Annual Conference

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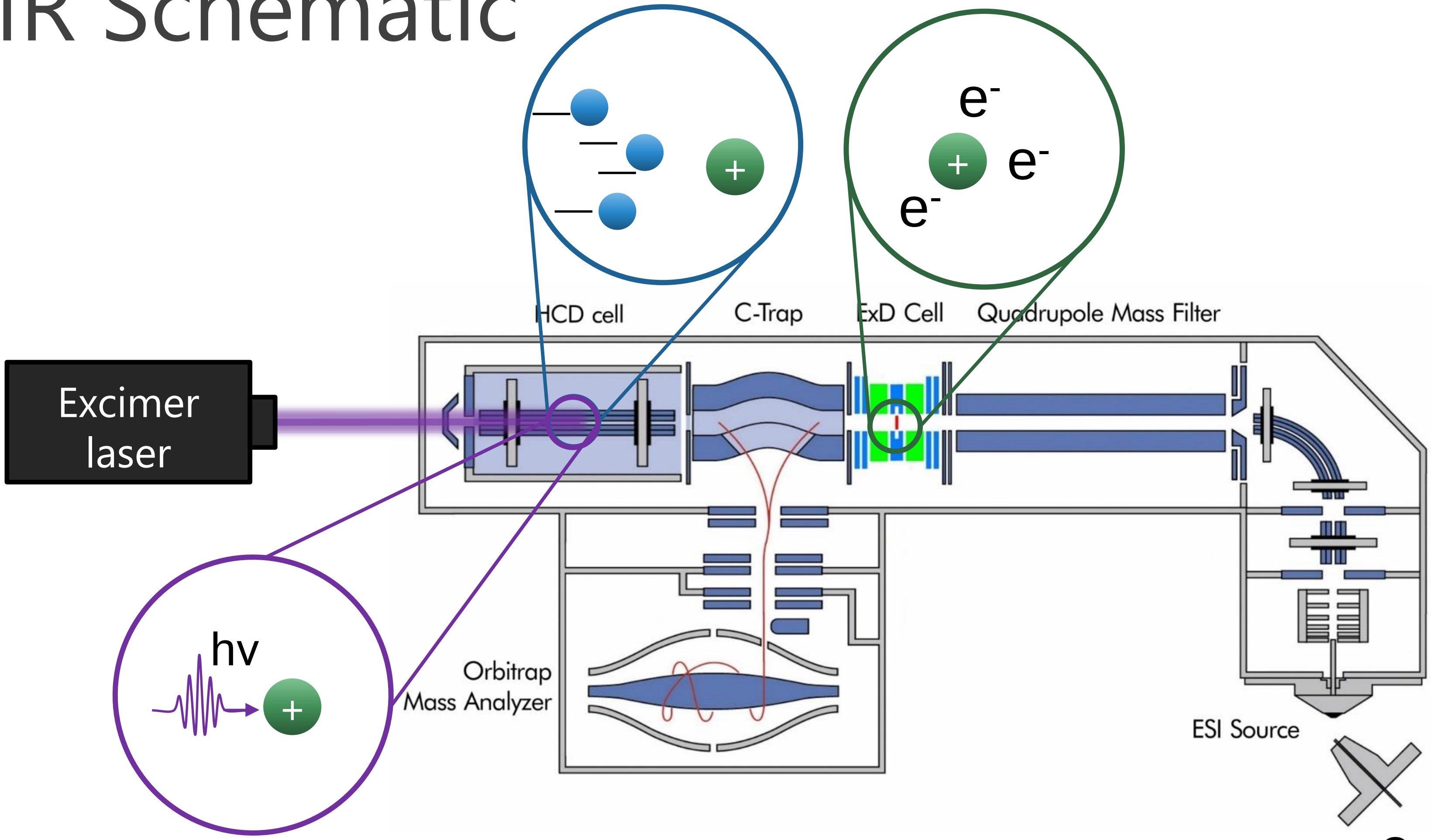
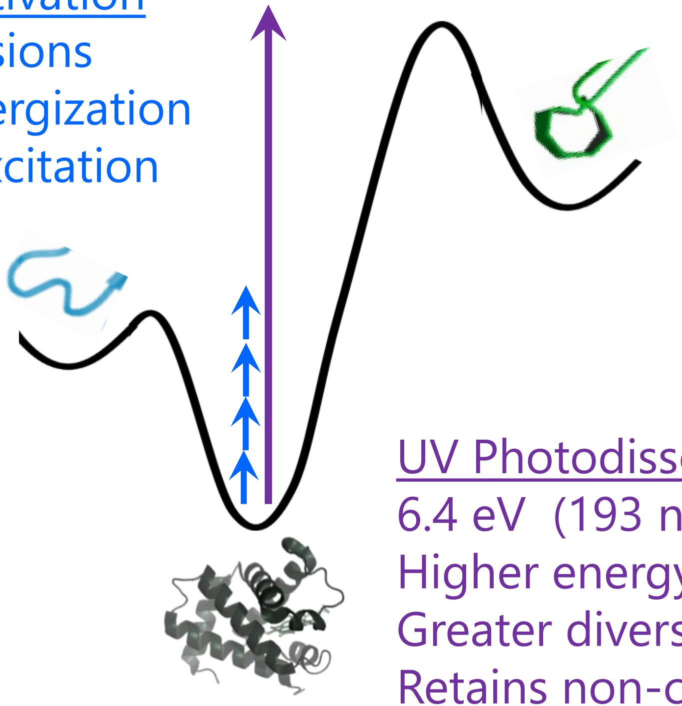
UHMR Schematic

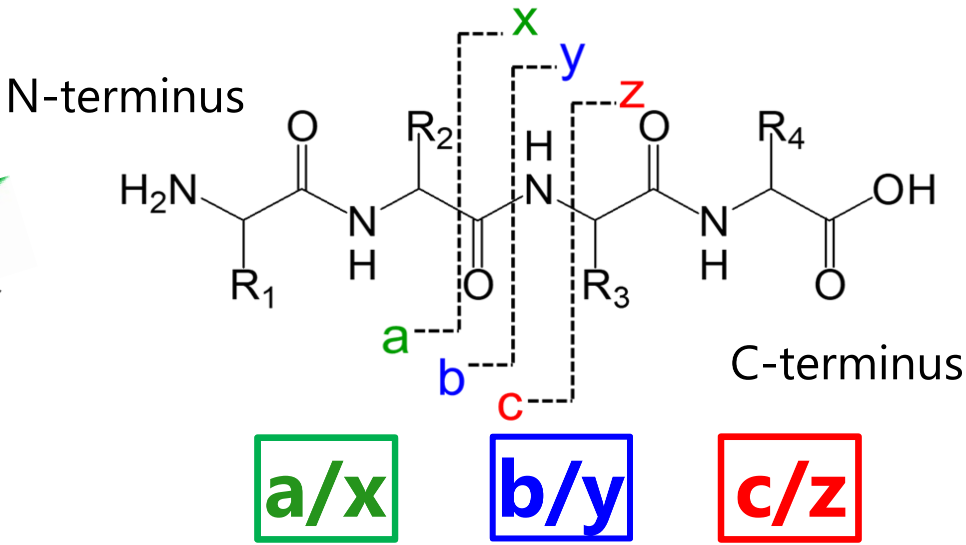


https://e-msion.com/

Energy Deposition: CID versus UVPD

<u>Collisional activation</u>
Multiple collisions
Step-wise energization
Vibrational excitation





<u>UV Photodissociation</u>
6.4 eV (193 nm ArF laser)
Higher energy deposition → excited electronic states
Greater diversity of fragment ions
Retains non-covalent interactions and PTMs

Aquaporin Z

Membrane protein channel that is responsible for osmoregulation
Tricky to study using native MS

EChcD CE50

N M F R K L A A E C F G T F C L V F G G C G S A V L 25
26 P A G F P E L G I G F A G V A L A F G L T V L T M 50
51 A F A V G H I S G G H F N P A V T I G L W A G G R 75
76 F P A K E V V G Y V I A Q V V G G I V A A A L L Y 100
101 L I A S G K T G F D A A A S G F A S N G Y G E H S 125
126 P G G Y S M L S A L V V E L V L S A G F L L V I H 150
151 G A T D K F A P A G F A P I A I G L A L T L I H L 175
176 I S I P V T N T S V N P A R S T A V A I F Q G G W 200
201 A L E Q L W F F W V V P I V G G I I G G L I Y R T 225
226 L L E K R D A S G E N L Y F Q C

5% sequence coverage

UVPD 3 mJ 1 pulse

N M F R K L A A E C F G T F C L V F G G C G S A V L 25
26 P A G F P E L G I G F A G V A L A F G L T V L T M 50
51 A F A V G H I S G G H F N P A V T I G L W A G G R 75
76 F P A K E V V G Y V I A Q V V G G I V A A A L L Y 100
101 L I A S G K T G F D A A A S G F A S N G Y G E H S 125
126 P G G Y S M L S A L V V E L V L S A G F L L V I H 150
151 G A T D K F A P A G F A P I A I G L A L T L I H L 175
176 I S I P V T N T S V N P A R S T A V A I F Q G G W 200
201 A L E Q L W F F W V V P I V G G I I G G L I Y R T 225
226 L L E K R D A S G E N L Y F Q C

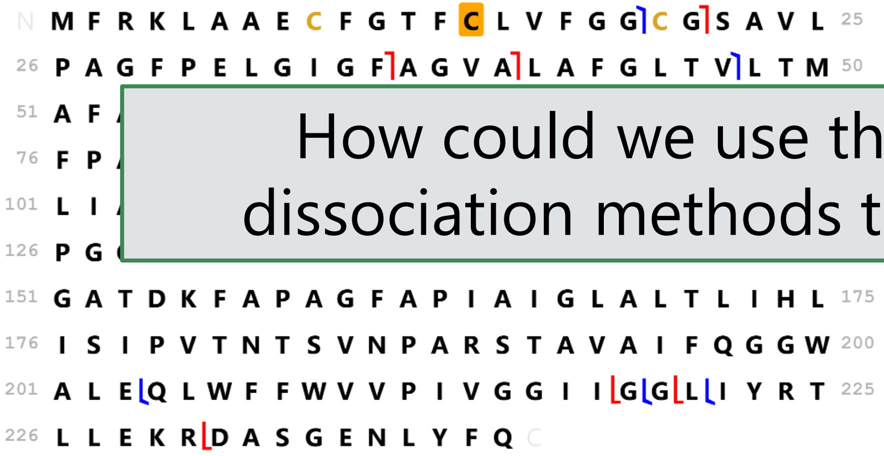
26% sequence coverage

Aquaporin Z

Membrane protein channel that is responsible for osmoregulation

Tricky to study using native MS

EChcD CE50



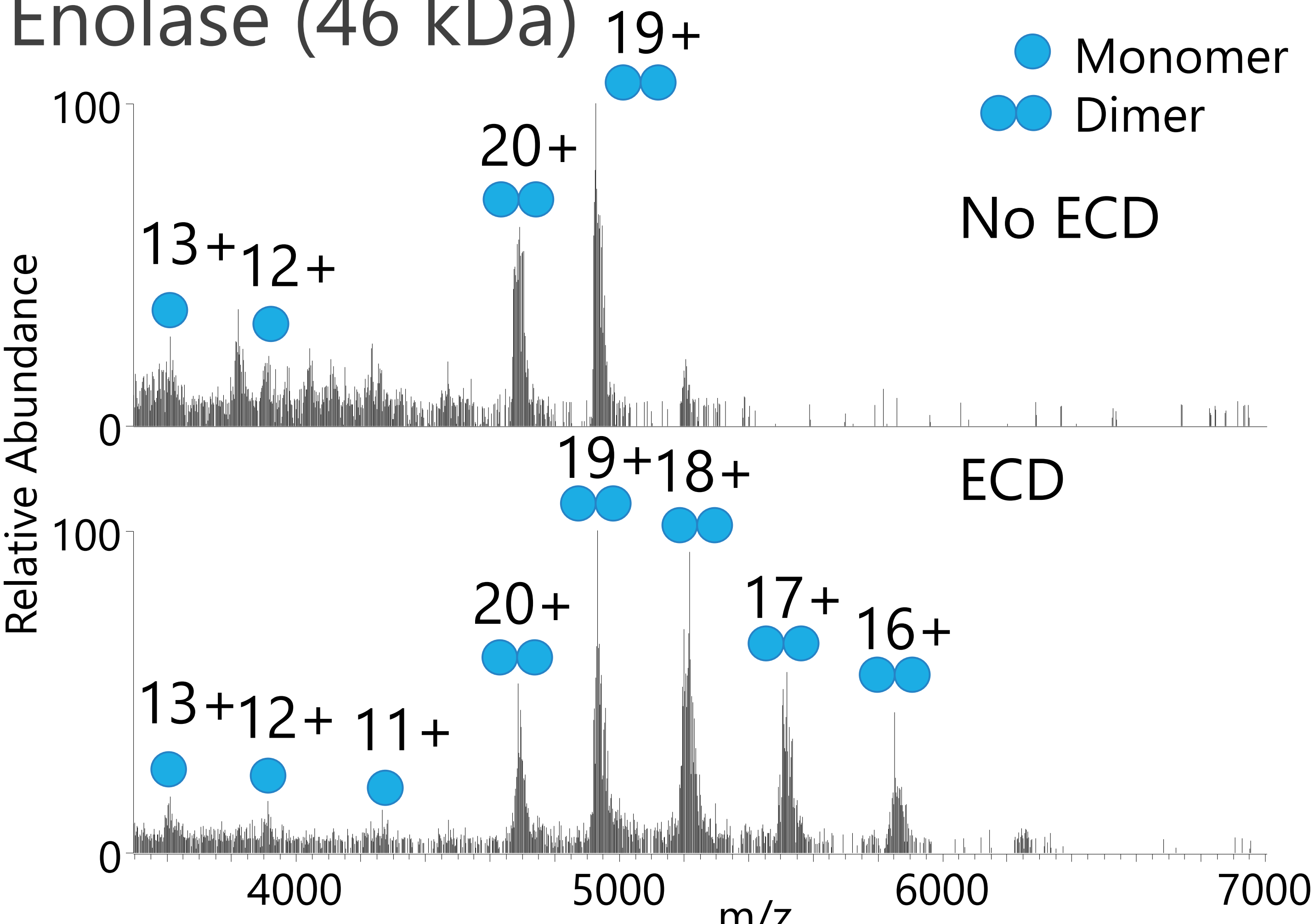
5% sequence coverage

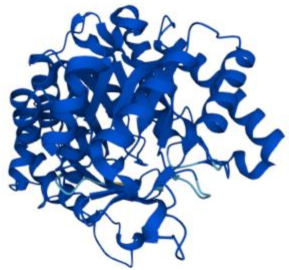
UVPD 3 mJ 1 pulse

26% sequence coverage

How could we use the combination of both dissociation methods to enhance our analyses?

Enolase (46 kDa)





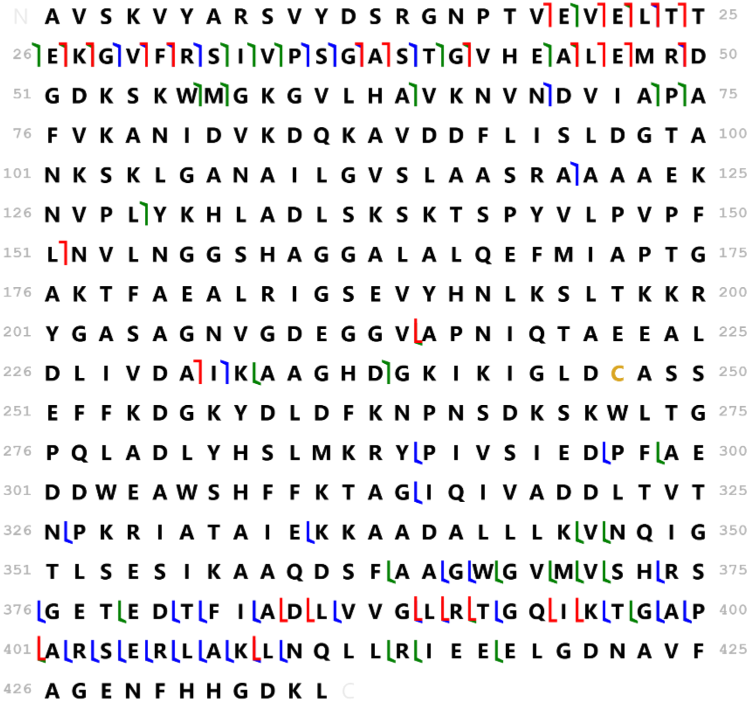
Application of ECD promotes
- Larger charge state distribution
- Reduction of baseline noise

Enolase 19+ MS/MS

UVPD 2 mJ 1 pulse

12% sequence coverage

ECuvpD 2 mJ 1 pulse

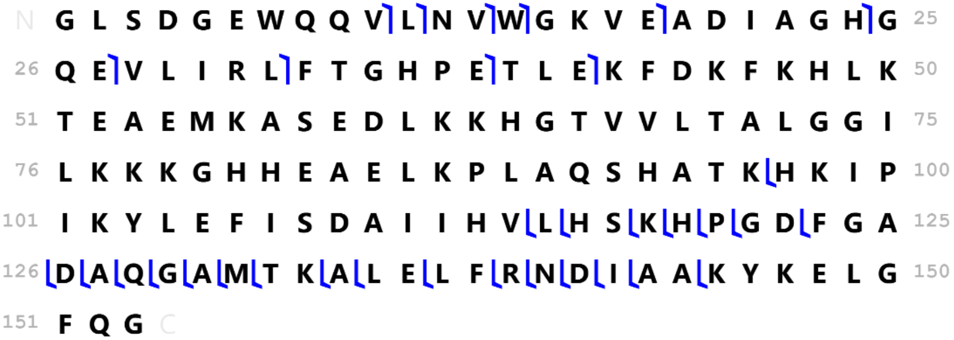


20% sequence coverage

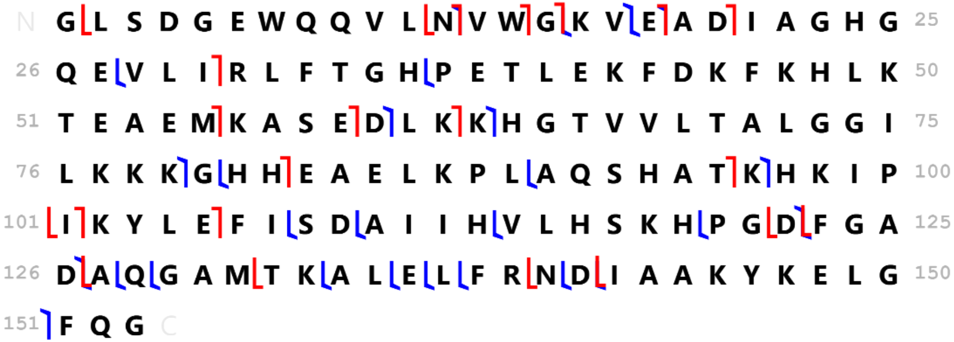
EChcD for proteins separated by capillary electrophoresis (CE)

ThP171

Apo-myoglobin (8+)



HCD CE 100, 22% coverage



EChcD CE 50, 28% coverage

More sequence coverage and better coverage of center of protein