

JUNE 6<sup>TH</sup> 2022



# Multiple methods of dissociation and their applications on the UHMR

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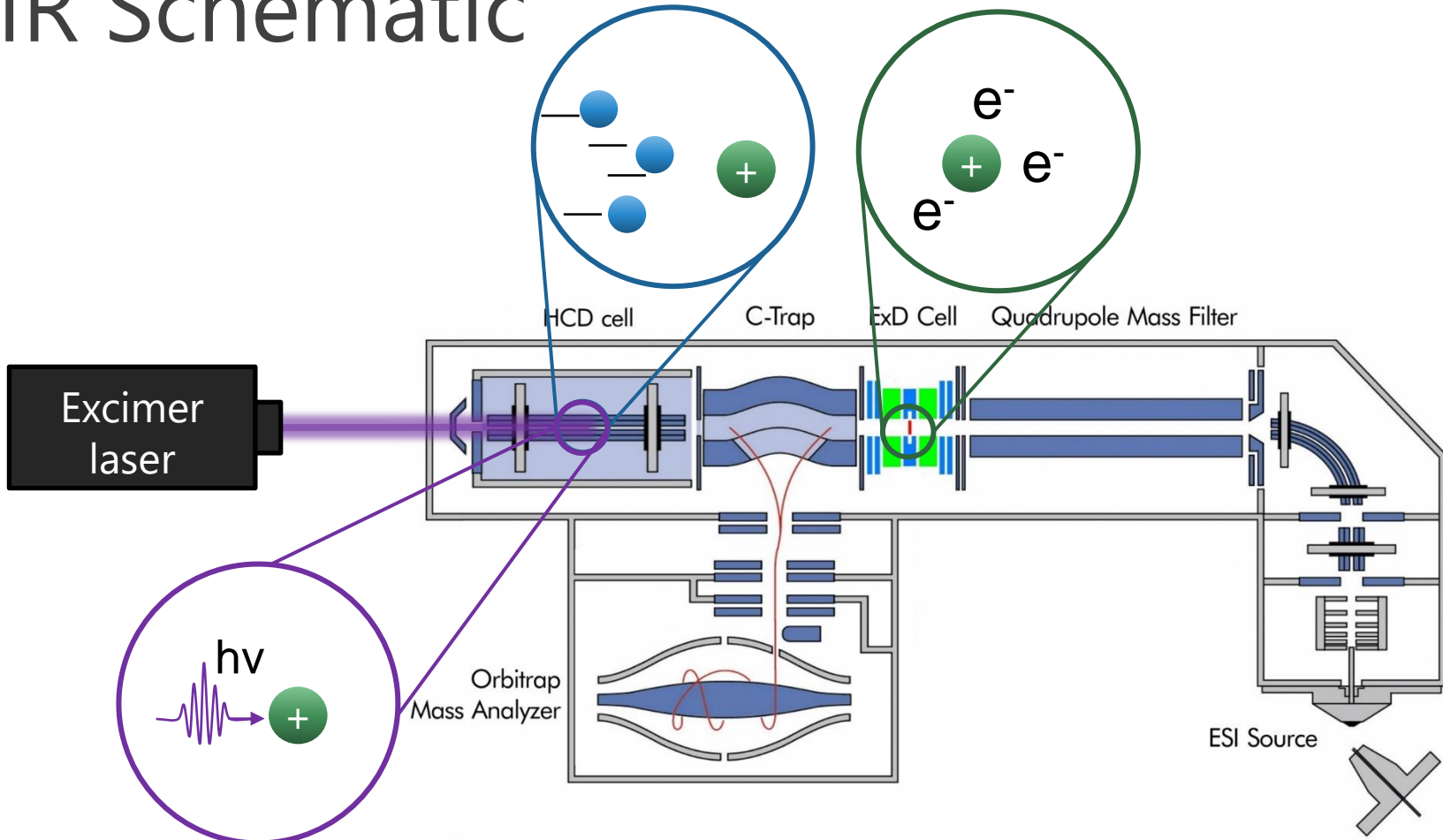
American Society for Mass Spectrometry

70<sup>th</sup> Annual Conference

**AMANDA HELMS**

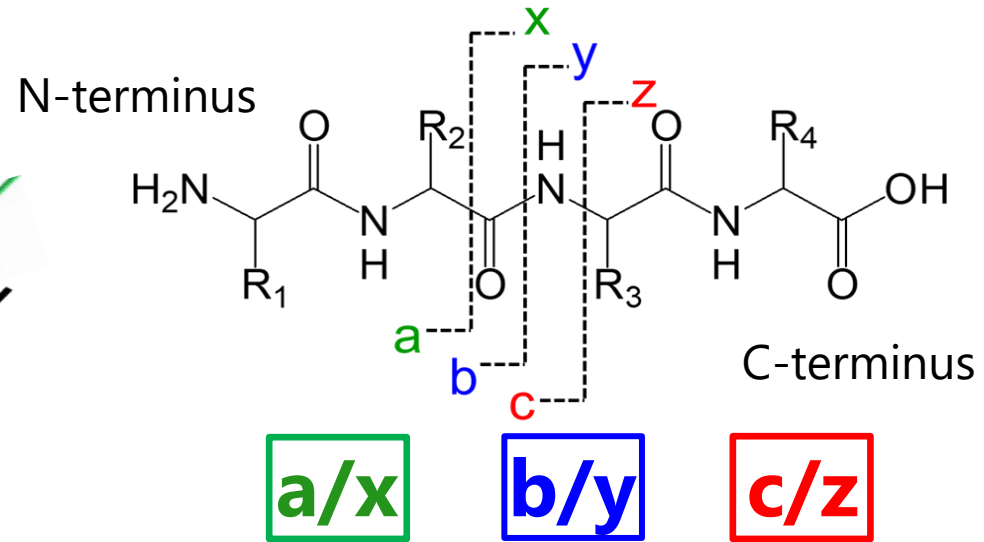
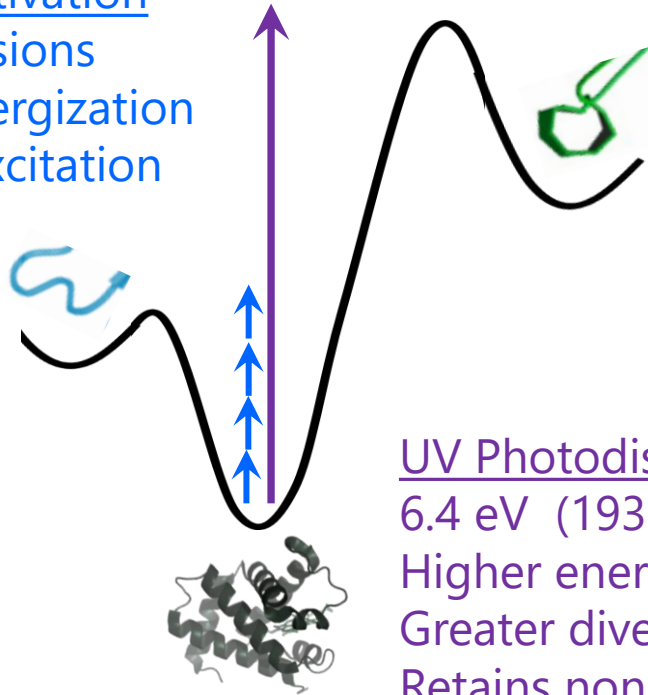
**PhD Candidate, Brodbelt Group, The University of Texas at Austin**

# UHMR Schematic



# Energy Deposition: CID versus UVPD

Collisional activation  
Multiple collisions  
Step-wise energization  
Vibrational excitation



UV Photodissociation

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

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N M F R K L A A E C F G T F C L V F G G I C G I S A V L 25
26 P A G F P E L G I G F I A G V A I L A F G L T V I 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 P I V G G I I L G L L I Y R T 225
226 L L E K R L D A S G E N L Y F Q C
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5% sequence coverage

UVPD 3 mJ 1 pulse

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

```
N M F R K L A A E C F G T F C L V F G G I C G I S A V L 25
26 P A G F P E L G I G F I A G V A I L A F G L T V I L T M 50
51 A F
76 F P
101 L I
126 P G
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 P I V G G I I L G L L I Y R T 225
226 L L E K R I D A S G E N L Y F Q C
```

UVPD 3 mJ 1 pulse

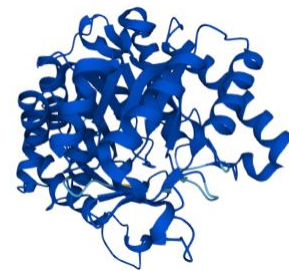
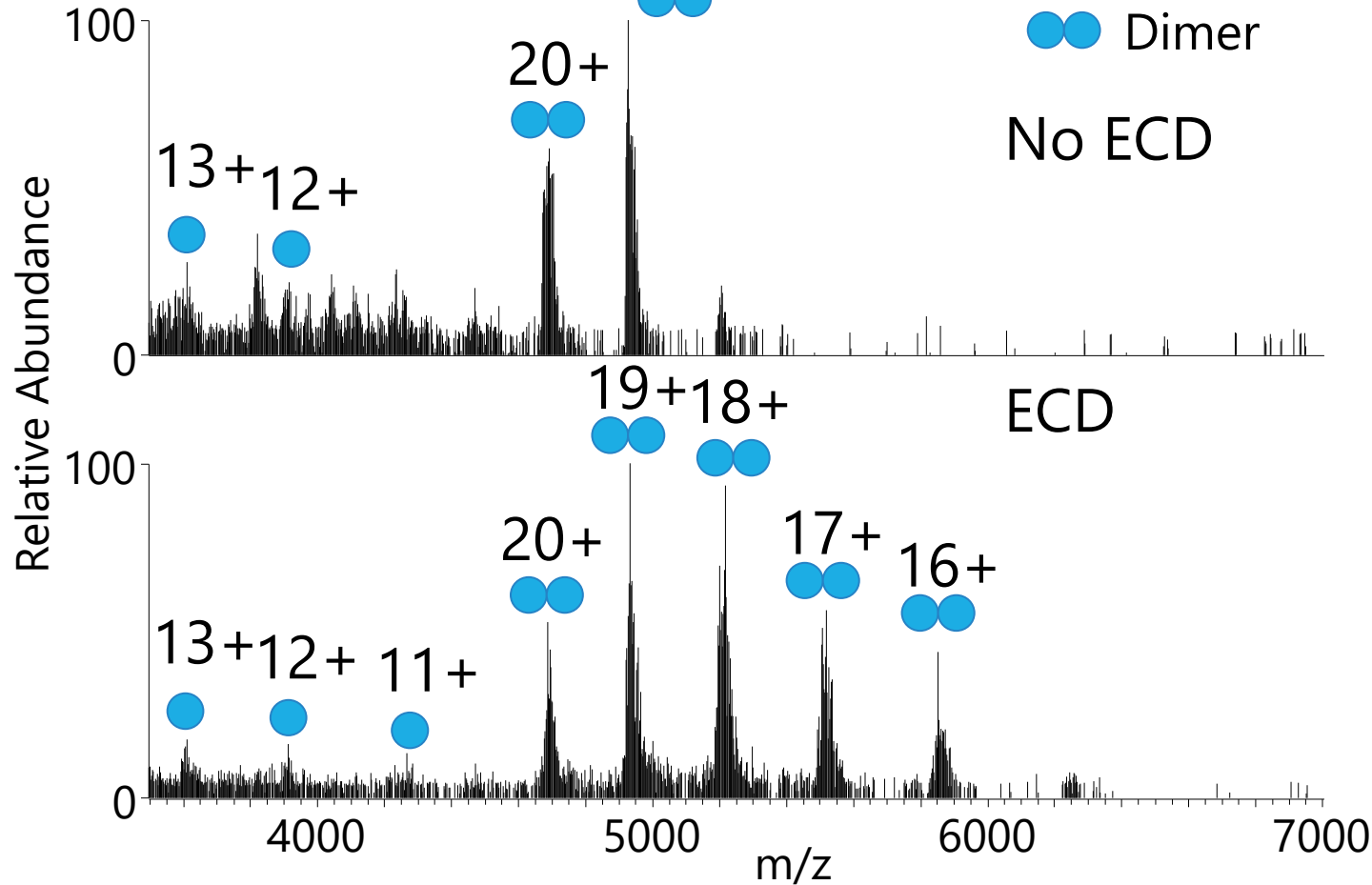
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N M F R K L A A E C F I G T F I C L V F G I G C G S A V L 25
26 P A I G I F I P I E L G I I G F I A I G V A L A F I G L T I V L T M 50
51 A F I G I R 75
76 F P I L Y 100
101 L I H S 125
126 P G I I H 150
151 G I A T D K F I A P I A G F I A P I A I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

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

5% sequence coverage

26% sequence coverage

# Enolase (46 kDa) 19+



Application of ECD promotes

- Larger charge state distribution
- Reduction of baseline noise

# Enolase 19+ MS/MS

## UVPD 2 mJ 1 pulse

```

N  A V S K V Y A R S V Y D S R G N P T V E V E L I T T 25
26  E I K I G I V I F I R S I V P I S I G I A I S I T I G V H E I A L E M R I D 50
51  G D K S K W M G K G V L H A I V K N V N I D V I A P A 75
76  F V K A N I D V K D Q K A V D D F L I S L D G T A 100
101 N K S K L G A N A I L G V S L A A S R A I A A A E K 125
126 N V P L Y K H L A D L S K S K T S P Y V L P V P F 150
151 L N V L N G G S H A G G A L A L Q E F M I A P T G 175
176 A K T F A E A L R I G S E V Y H N L K S L T K K R 200
201 Y G A S A G N V G D E G G V I A P N I Q T A E E A L 225
226 D L I V D A I I I K I A A G H D I G K I K I G L D C A S S 250
251 E F F K D G K Y D L D F K N P N S D K S K W L T G 275
276 P Q L A D L Y H S L M K R Y I P I V S I E D I P F A E 300
301 D D W E A W S H F F K T A G I Q I V A D D L T V T 325
326 N P K R I A T A I E I K K A A D A L L L K V N Q I G 350
351 T L S E S I K A A Q D S F A A I G W I G V I M I V S H I R S 375
376 G E T I E D T I F I I A I D L I V V G L R T I G Q I K I T G I A I P 400
401 I A R I S I E I R I L I A K I L I N Q L L R I E E I E L G D N A V F 425
426 A G E N F H H G D K L C

```

12% sequence coverage

## ECuypD 2 mJ 1 pulse

```

N  A V S K V Y A R S V Y D S R G N P T V I E I V I E I L I T I T 25
26  E I K I G I V I F I R S I I V I P I S I G I A I S I T I G I V H E I A I L I E I M R I D 50
51  G D K S K W M I G K G V L H A I V K N V N I D V I A I P I A 75
76  F V K A N I D V K D Q K A V D D F L I S L D G T A 100
101 N K S K L G A N A I L G V S L A A S R A I A A A E K 125
126 N V P L I Y K H L A D L S K S K T S P Y V L P V P F 150
151 L I N V L N G G S H A G G A L A L Q E F M I A P T G 175
176 A K T F A E A L R I G S E V Y H N L K S L T K K R 200
201 Y G A S A G N V G D E G G V I A P N I Q T A E E A L 225
226 D L I V D A I I I K I A A G H D I G K I K I G L D C A S S 250
251 E F F K D G K Y D L D F K N P N S D K S K W L T G 275
276 P Q L A D L Y H S L M K R Y I P I V S I E D I P F I A E 300
301 D D W E A W S H F F K T A G I Q I V A D D L T V T 325
326 N I P K R I A T A I E I K K A A D A L L L K I V I N Q I G 350
351 T L S E S I K A A Q D S F I A A I G W I G V I M I V S H I R S 375
376 I G E T I E D T I F I I A I D L I V V G I L I R I T I G Q I I K I T I G I A I P 400
401 I A I R I S I E I R I L I A I K I L I N Q L L R I I E E I E L G D N A V F 425
426 A G E N F H H G D K L C

```

20% sequence coverage

# EChcD for proteins separated by capillary electrophoresis (CE)

ThP171

## Apo-myoglobin (8+)

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

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

HCD CE 100, 22% coverage

EChcD CE 50, 28% coverage

More sequence coverage and better coverage of center of protein