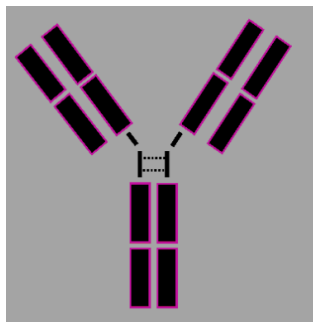


16.15 - 16.45

WP7. Demonstrated effectiveness of product ion isotopic distribution deconvolution



Project Review Meeting

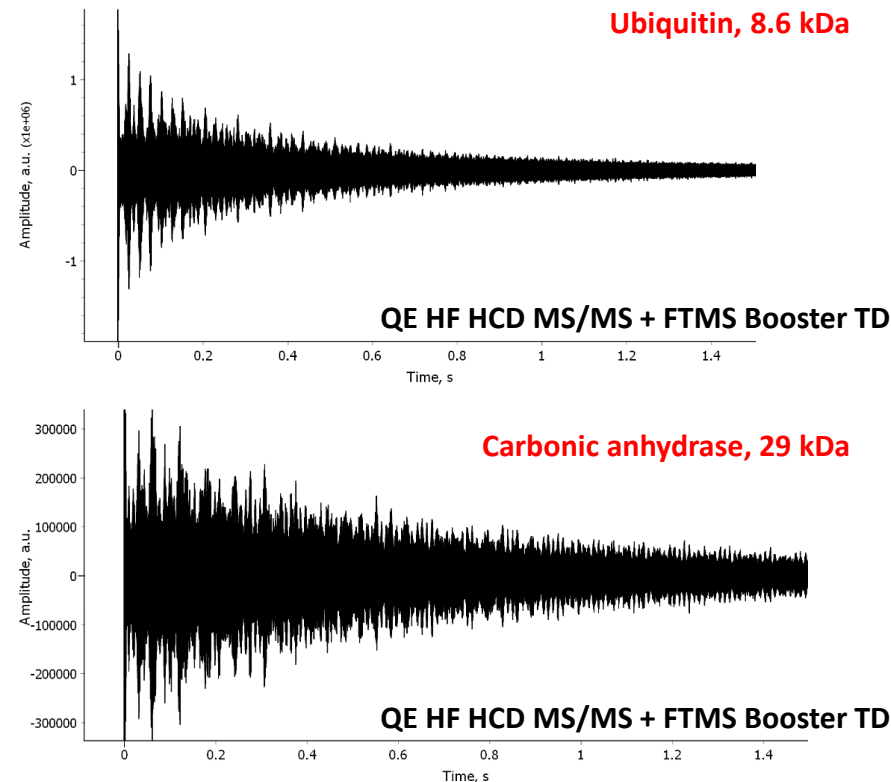
Project number: 829157

*M1. Demonstrated effectiveness of
product ion isotopic distribution deconvolution*

Brussels, February 6th 2020

Top Down Deconvolution via Product Ion Decay Rates

- Top Down spectra are often congested
 - isotopic distributions overlaid
- Spectra contain ions across a very broad mass and charge state range
- All ion signals decay with time
 - on the scale of 1-3 seconds
- Signals of product ions from the same isotopic envelope will decay at the same rate
- Different isotopic envelopes (charge state and/or mass differences) decay differently
- **Information on product ion signal decay rate could help deconvolve complex top-down spectra**



Approaches to Reveal Product Ion Decay

1. Sliding window transient processing

- Transient is split into many shorter transient sections, using a sliding window
- Each shorter transient is processed, in magnitude mode FT, to yield top-down mass spectra
- Protein sequence coverages from different windows are combined together

2. Asymmetric apodization window processing

- Absorption mode FT is applied to the original extended-duration transient
- The apodization function parameters (e.g., F-parameter) are varied to create apodization asymmetry
- Protein sequence coverages obtained with different F-values are combined together

3. Decay rate-based mass spectra fractionation

- Decay rates are calculated for each peak in a mass spectrum, via inverse F
- A 3D “mobilogram” shows product ion abundance as a function of decay rates – ion deconvolution
- Product ion annotation and sequence maps are constructed with additional information on decays

Approach 1. Sliding window processing

Lead: SPS



Experimental data: SPS

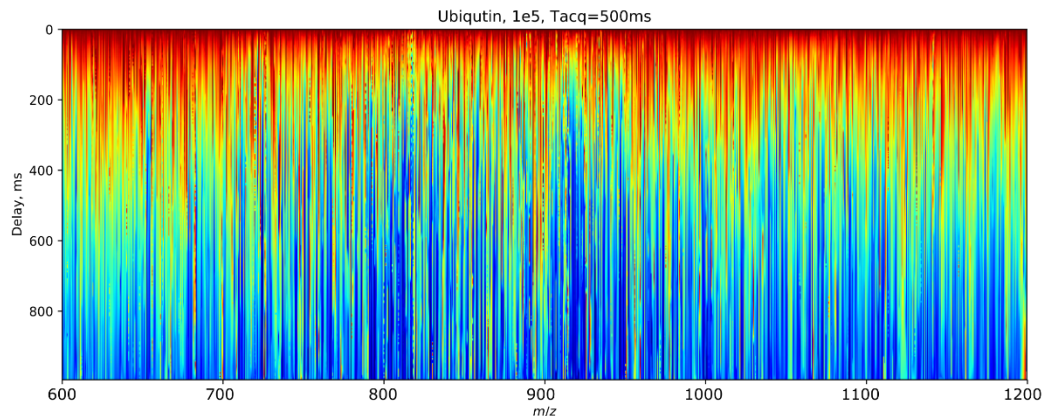


QE HF FTMS

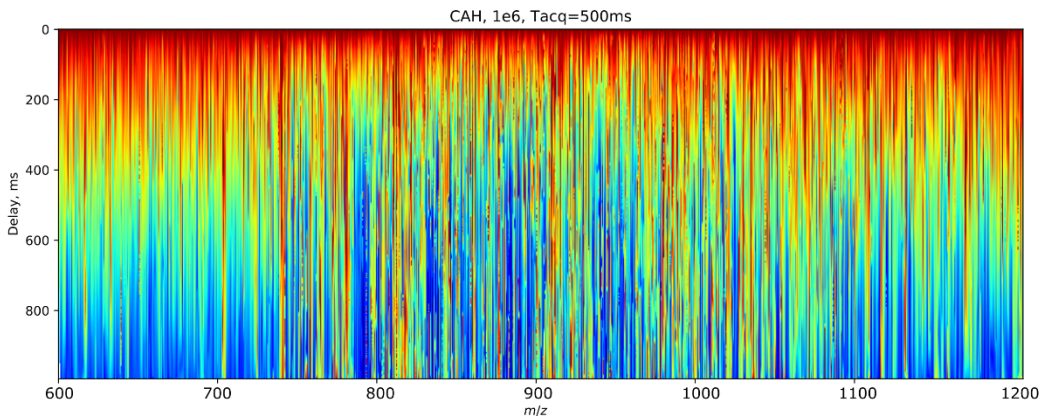
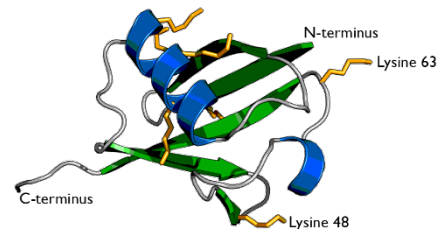
HCD MS/MS

FTMS Booster TD

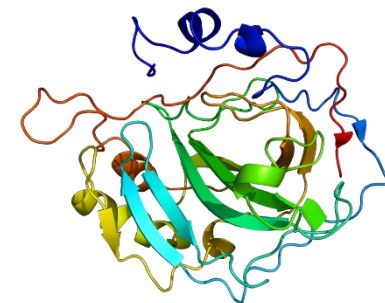
«Mobilograms»



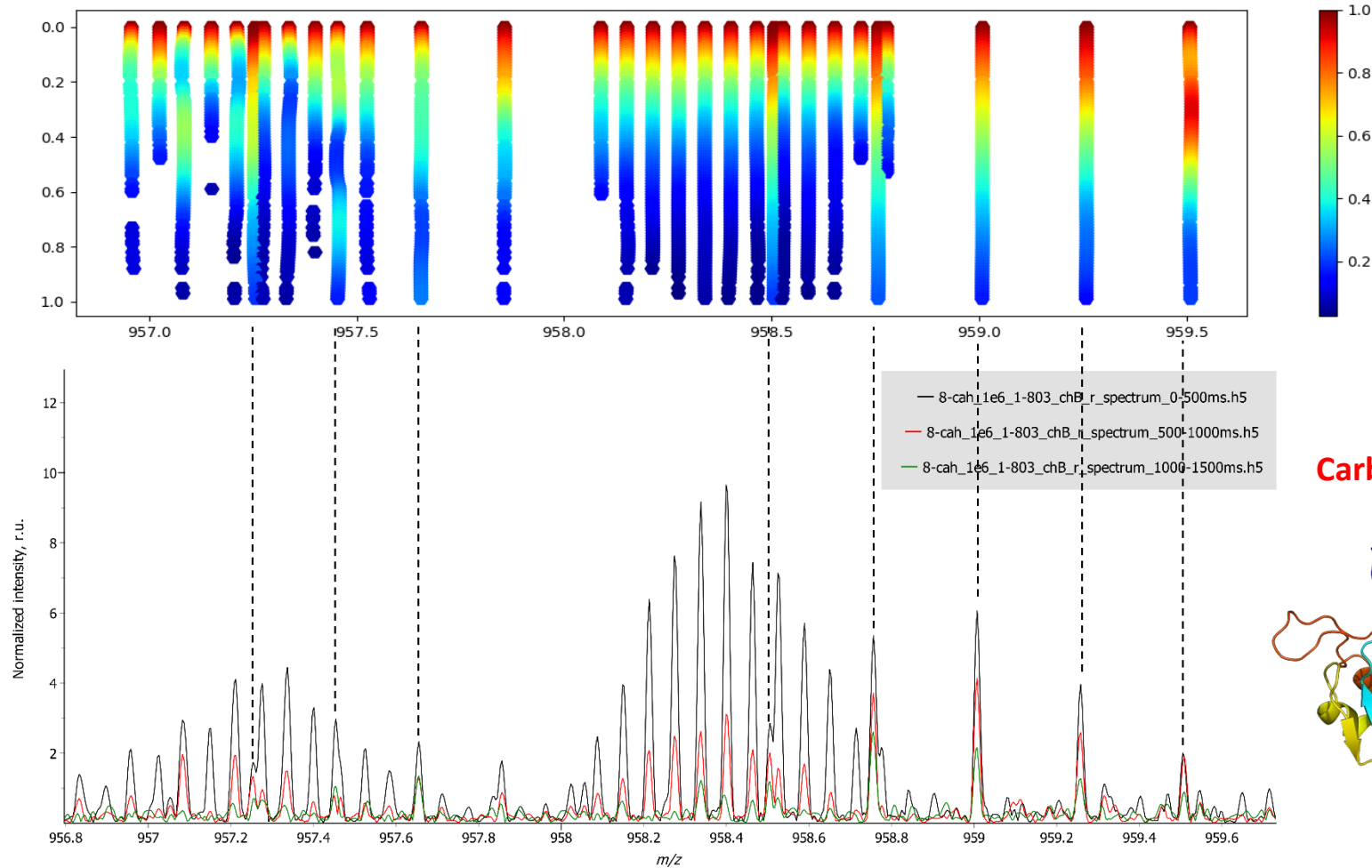
Ubiquitin, 8.6 kDa



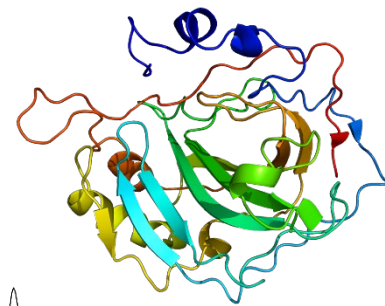
Carbonic anhydrase, 29 kDa



CAH, 1e6, Tacq = 500 ms (mFT)

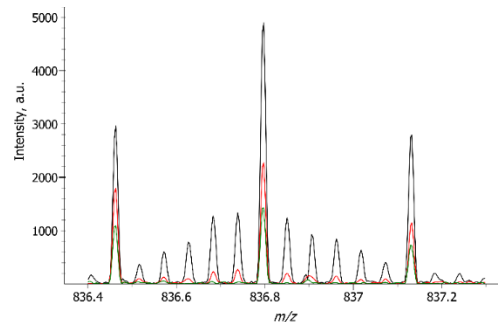
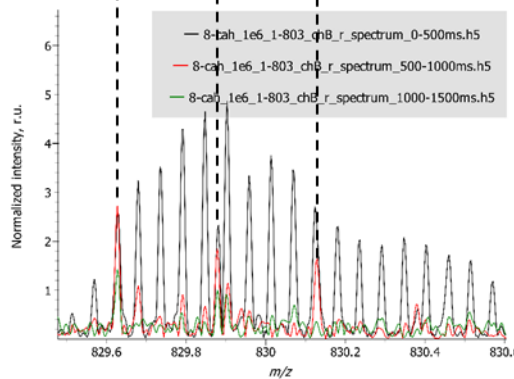
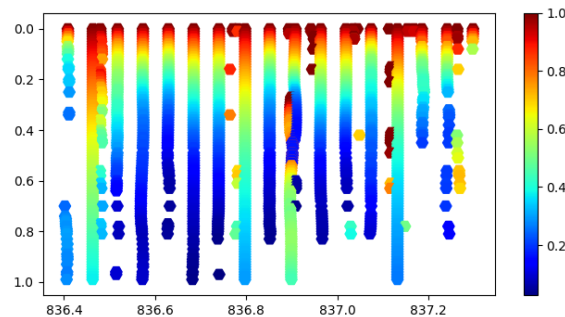
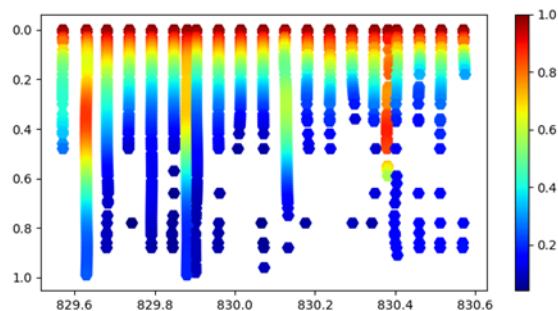


Carbonic anhydrase



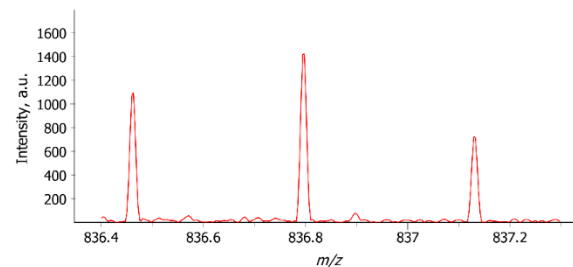
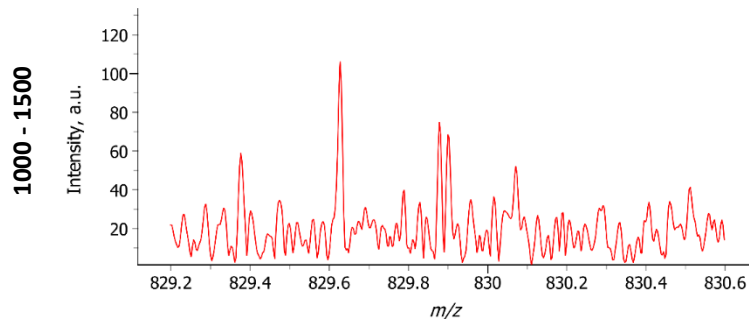
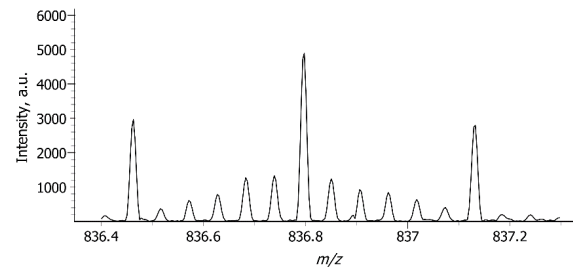
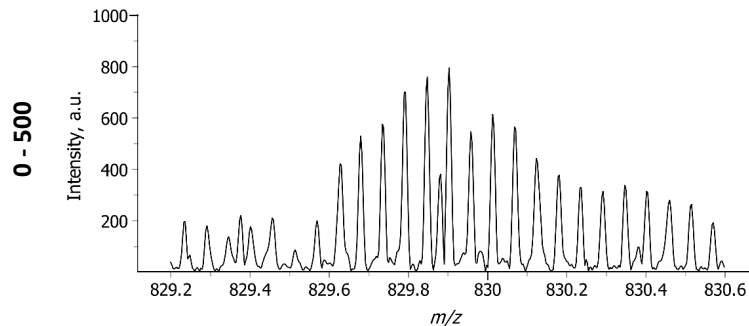
Deconvolving Overlapping Isotopes

CAH, 1e6, Tacq = 500 ms (mFT)

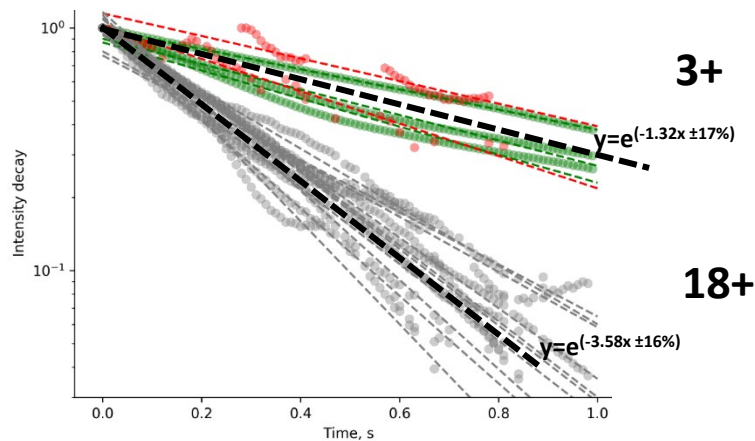
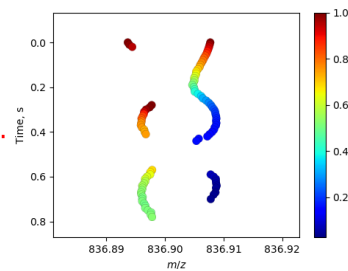
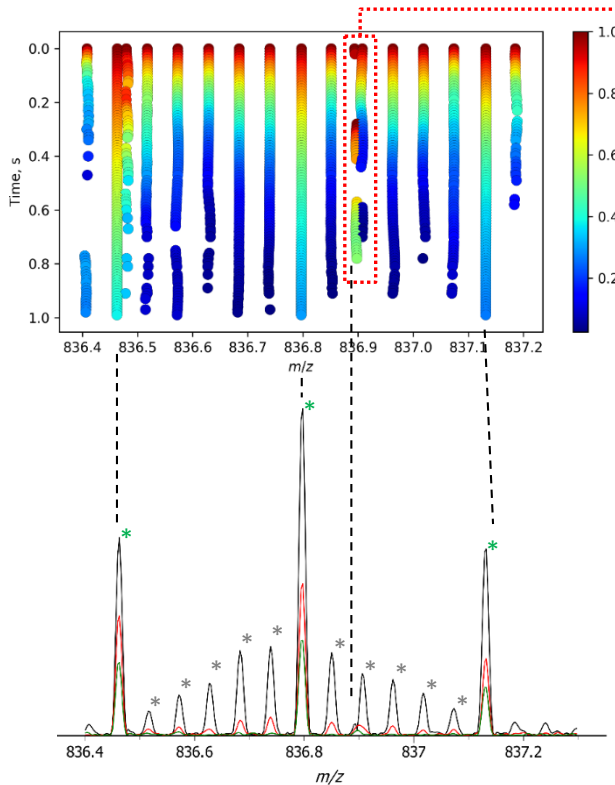


Deconvolving Overlapping Isotopes

CAH, 1e6, Tacq = 500 ms (mFT)



Deconvolving Overlapping Isotopes



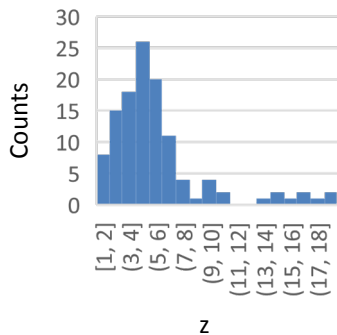
Sequence Coverage Maps

CAH, 1e6, mFT, MASH Suite

0 - 500

```

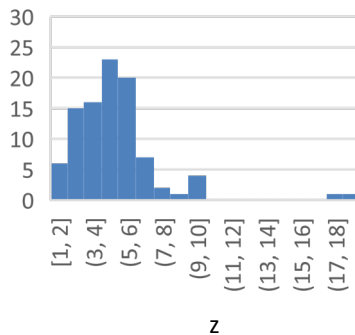
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51 G E A T S R R M V N N G H S F N V I E Y D D S Q D K 75
76 A V L K D G P L T G T Y R L V Q F H F H W G S S D 100
101 D Q G S E H T V D R K K Y A A E L H L V H W N T K 125
126 Y G D F G T A A Q Q P D G L A V V G V F L K V G D 150
151 A N P A L Q K V L D A L D S I K T K G K S T D F P 175
176 N F D P G S L L P N V L D Y W T L Y P G S L T T P L P 200
201 L L E S V T W I V L L K E P I S V S S Q Q M L K F R 225
226 T L N F N A E L G E L P L L L M L L A N W R P A Q P L K 250
251 N R Q V R G F P K
  
```



500 - 1000

```

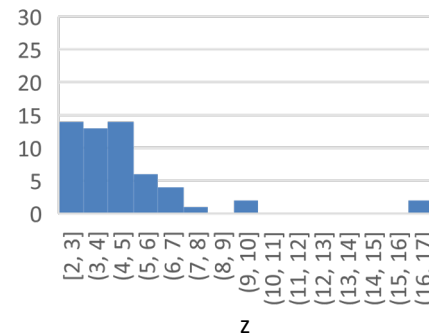
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151 A N P A L Q K V L D A L D S I K T K G K S T D F P 175
176 N F D P G S L L P N V L D Y W T L Y P G S L T T P L P 200
201 L L E S V T W I V L L K E P I S V S S Q Q M L K F R 225
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251 N R Q V R G F P K
  
```



1000 - 1500

```

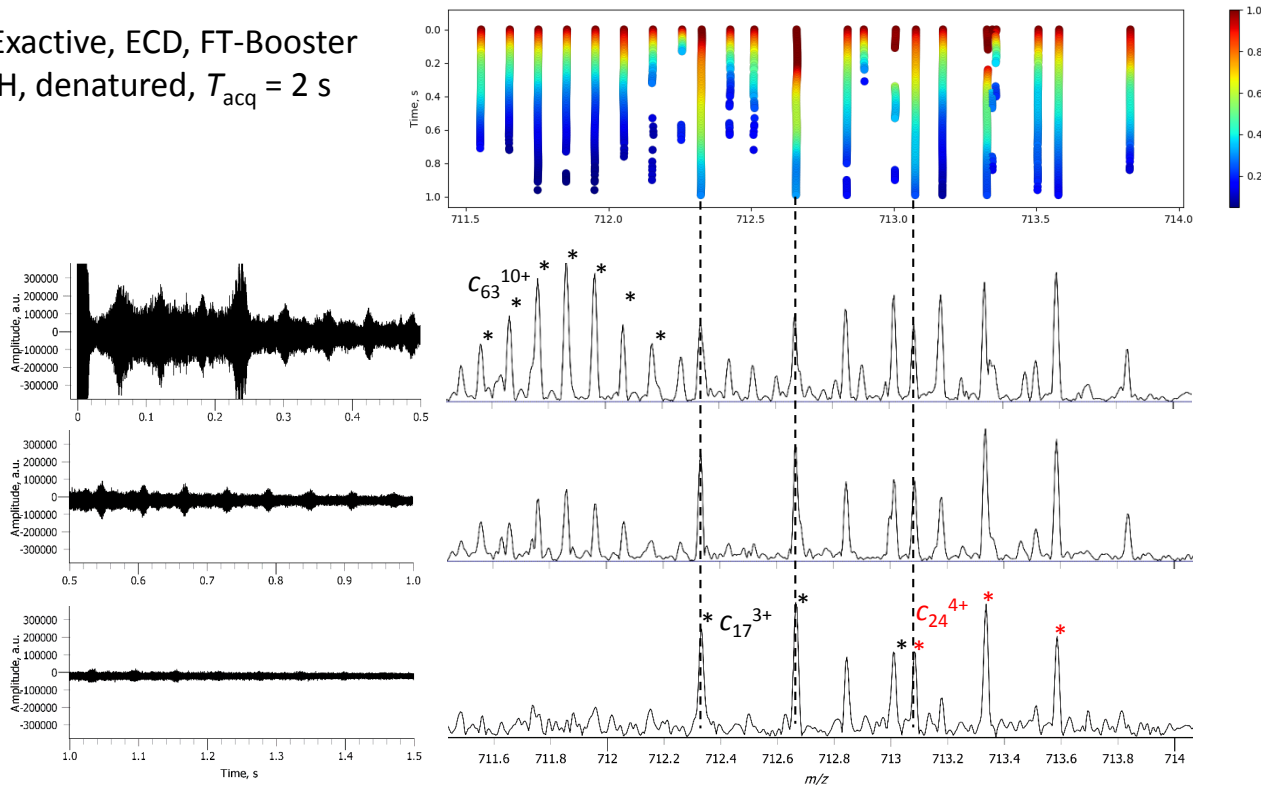
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226 T L N F N A E L G E L P L L L M L L A N W R P A Q P L K 250
251 N R Q V R G F P K
  
```



Summary: minor increase in sequence coverage for up to 29 kDa proteins & HCD MS/MS

Deconvolving Overlapping Isotopes

Q Exactive, ECD, FT-Booster
CAH, denatured, $T_{\text{acq}} = 2$ s



Summary: potential for higher value for ECD/ETD MS/MS and larger proteins

Approach 2. Asymmetric apodization window processing

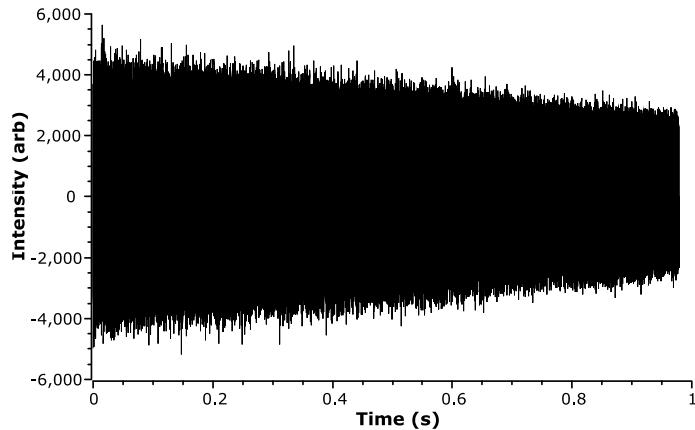
Lead: NTU



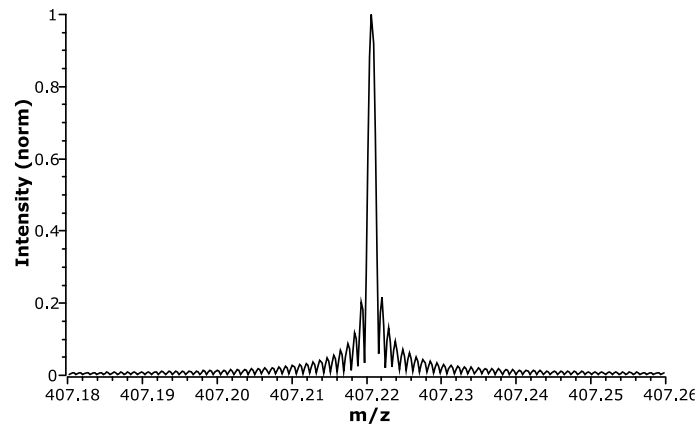
Experimental data: SPS



Apodization

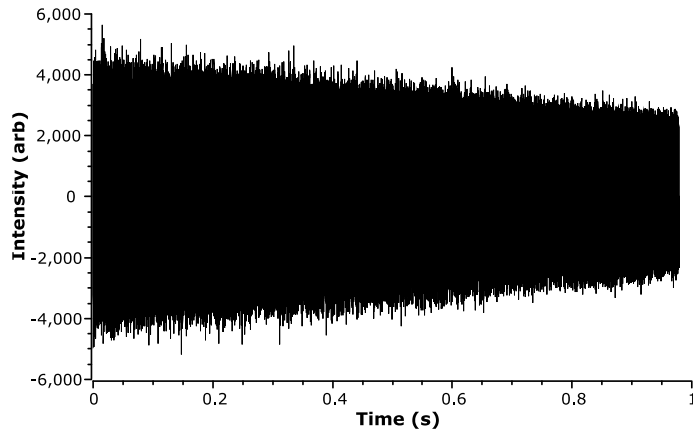


- Raw transient

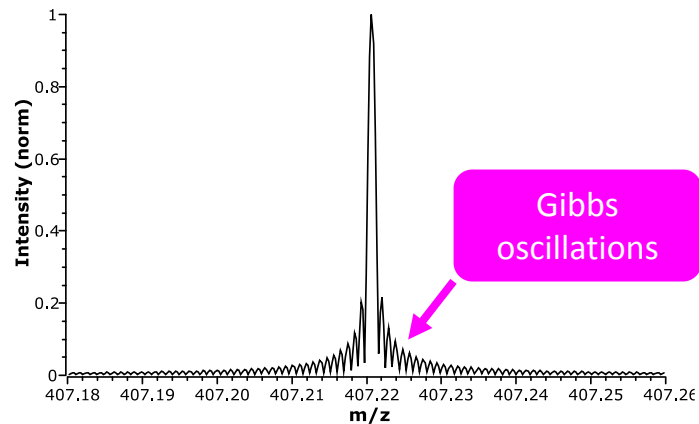


- Resulting spectrum
- Zoomed onto a single peak

Apodization

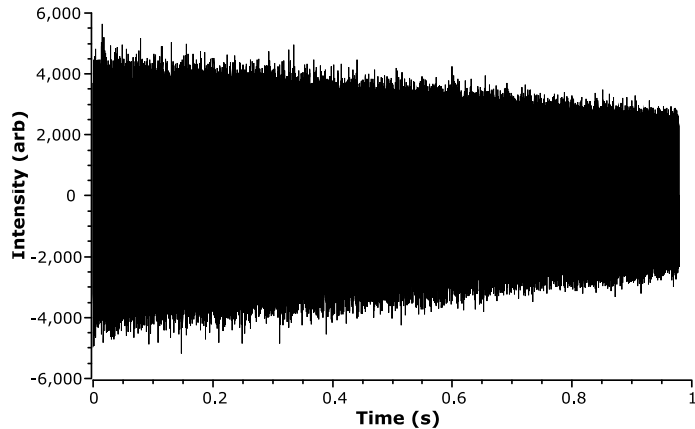


- Raw transient

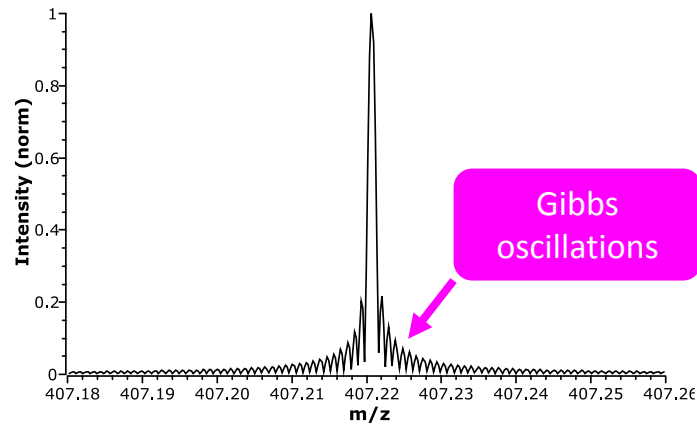


- Resulting spectrum
- Zoomed onto a single peak

Apodization

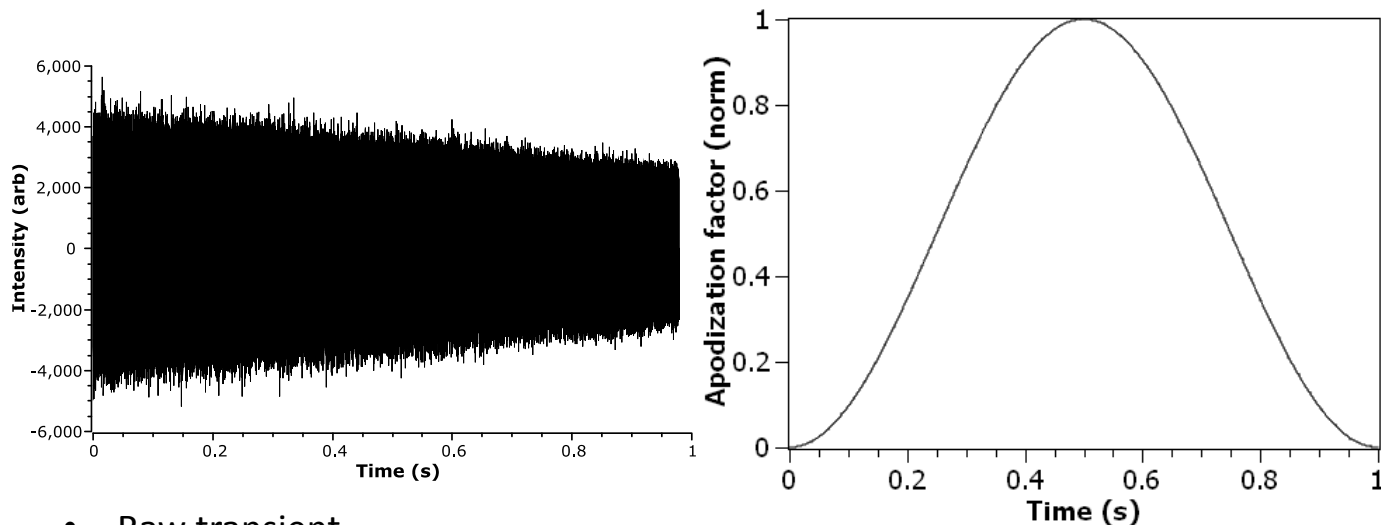


- Raw transient
- Squared off ends of transient cause multiple false peaks in spectrum



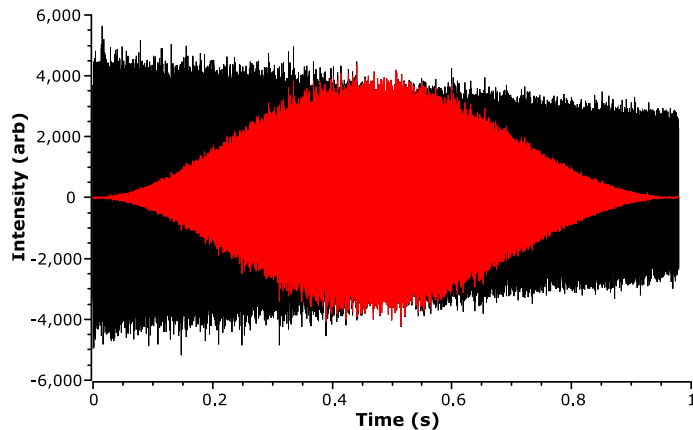
- Resulting spectrum
- Zoomed onto a single peak

Apodization

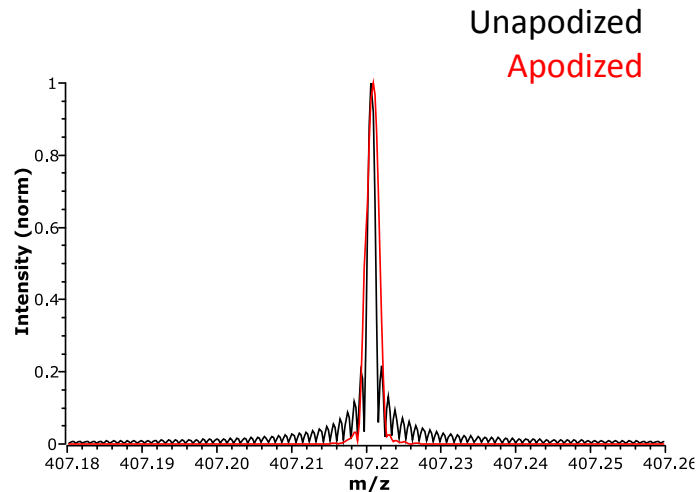


- Raw transient
- Squared off ends of transient cause multiple false peaks in spectrum
- Multiply transient by apodization function

Apodization

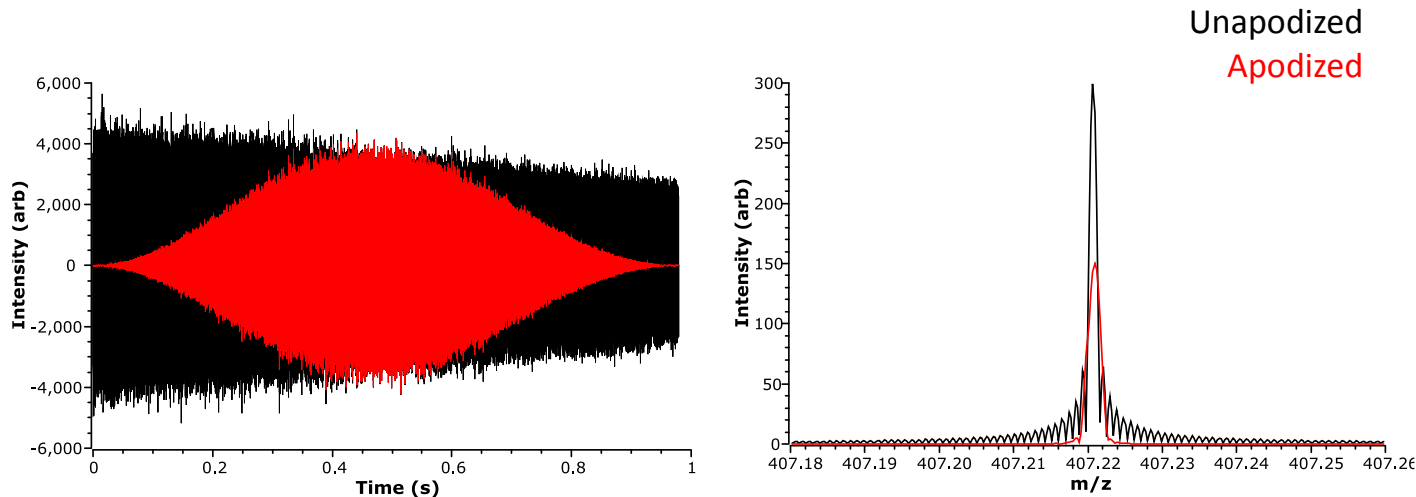


- Apodize transient
- Full bell (Hann)
- No sharp edges
- No Gibbs oscillations



- But..
- Slight loss in resolution

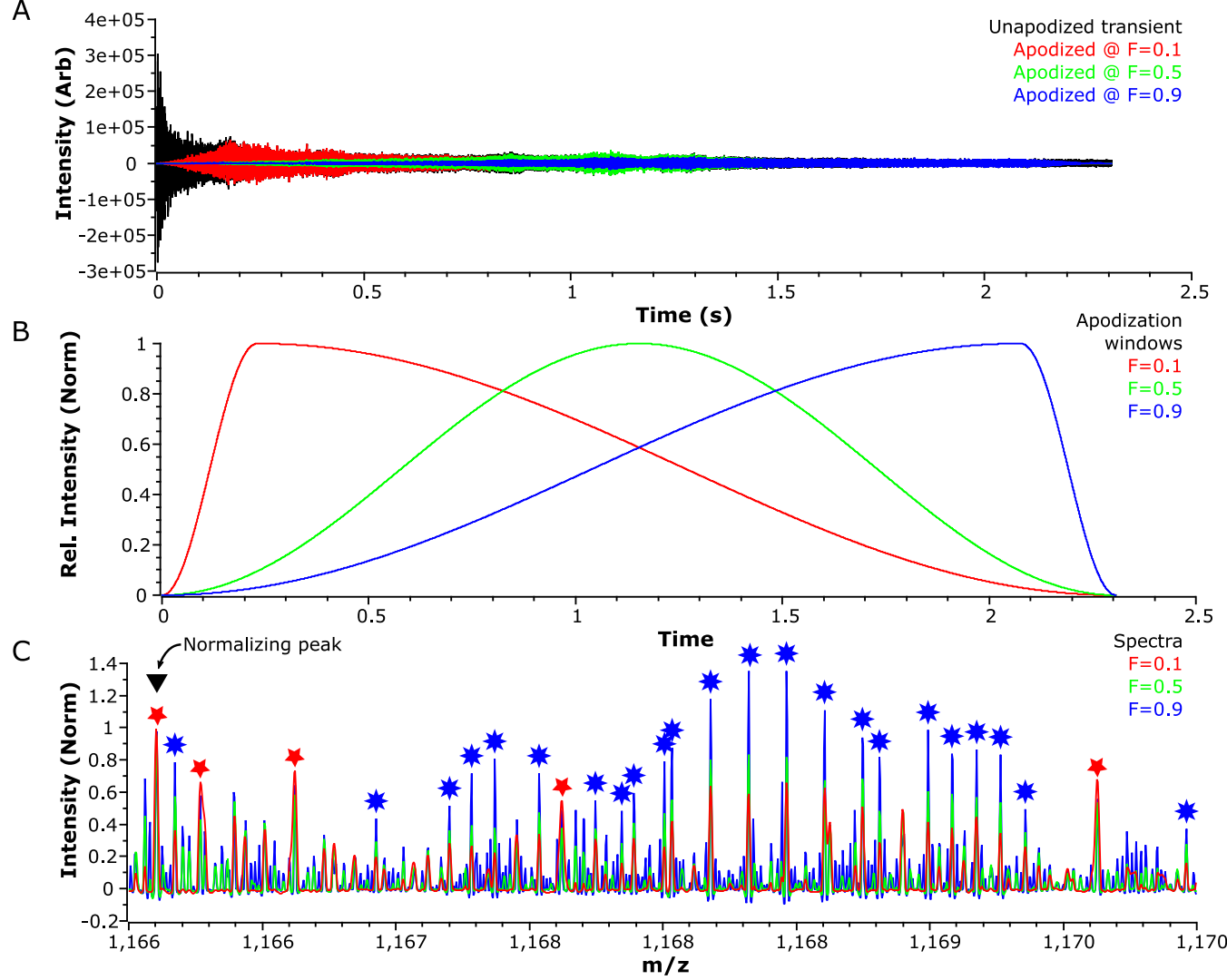
Apodization



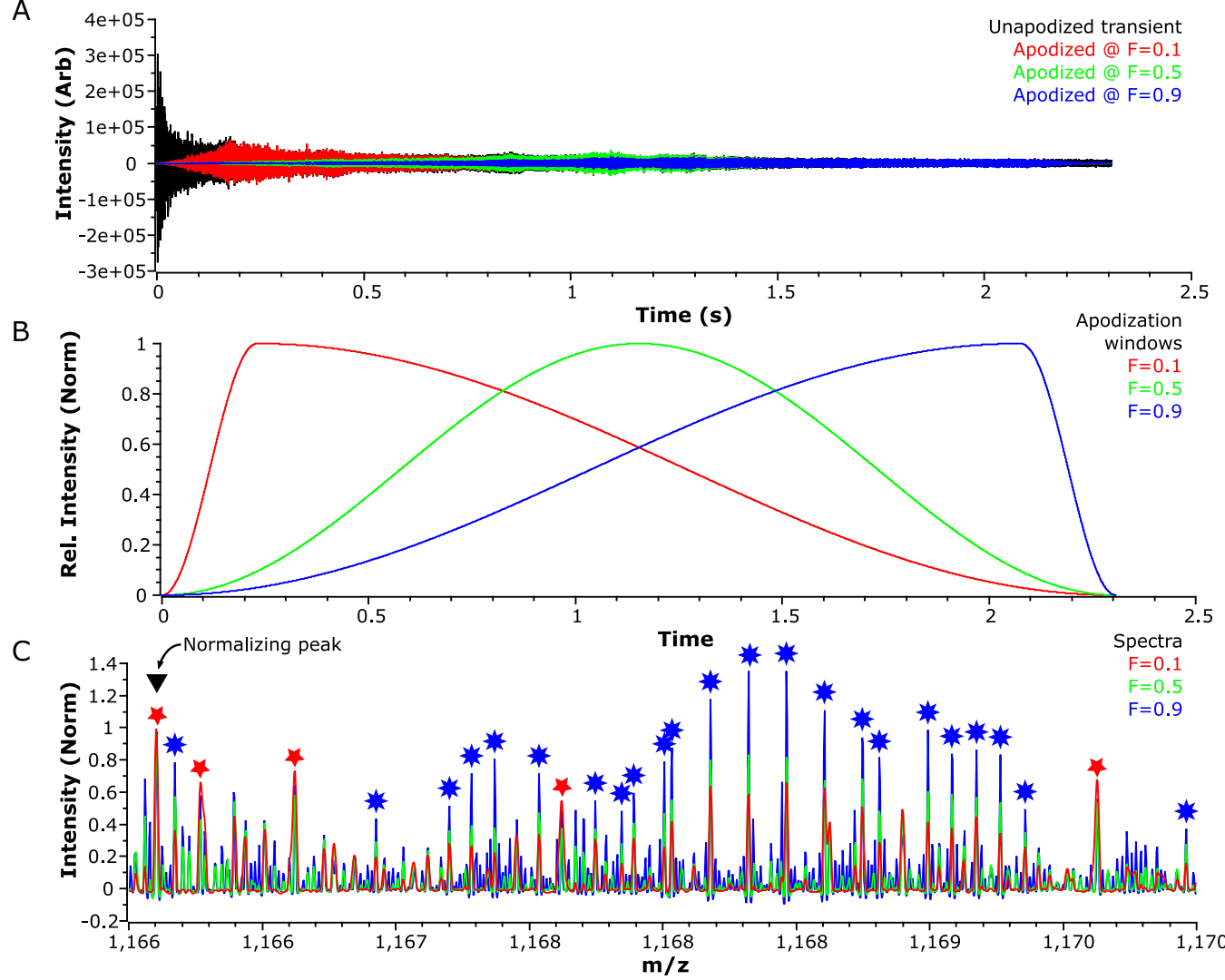
- Apodize transient
- Full bell (Hann)
- No sharp edges
- No Gibbs oscillations

- But..
- Slight loss in resolution
- And loss in sensitivity

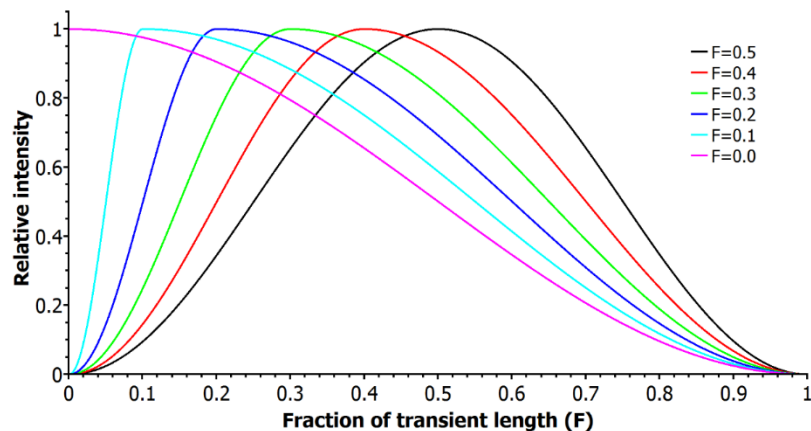
- Transient length = T
- Apodization F is the fraction of T when window is at maximum
- So, when $F = 0.1$, apod max is 10% of the way along the transient



- Transient length = T
- Apodization F is the fraction of T when window is at maximum
- So, when $F = 0.1$, apod max is 10% of the way along the transient
- **Red star** – peaks emphasized early in transient
- **Blue star** – peaks emphasized late in transient



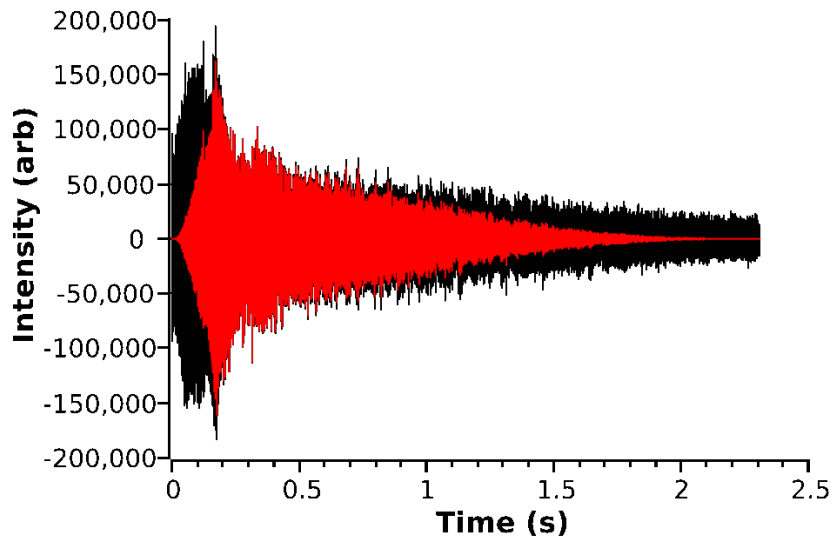
Transient decay ion deconvolution



- By changing symmetry of apodization
- Do see modest improvement in coverage
- But – user curation still needed to confirm benefit

1^b S H I H W I G I Y I G I K I H I N I G I P E I H I W I H I K I D I F I P I 240
 y I I A I N I G I E I R I Q I S I P I V I D I I I D I T I K I A I V I V I Q I D I 220
 41 P I A I L I K P L A I L I V I Y I G I E A T S R R M V N 200
 61 N G H S I F I N I V I E I Y D I D I S Q D K A V L K D 180
 81 G P L T G T Y R L V Q F H F H W G S I S D 160
 101 D Q G S E H T V D R K K Y A A E I L H L V 140
 121 H W N T K Y G D F G T I A I A I Q I Q I P D G I L A 120
 141 V V G V F L K V G D A N P A L Q K V L D 100
 161 A L D S I K T K G K S T D F P N F D P G 80
 181 S L L P N V L D Y I W T I Y I P G S L T I T I P I P 60
 201 L I L I E I S V I T I W I I V I L I K I E I P I I S I V I S I S I Q I Q 40
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 241 A I N I W I R I P I A I Q I P I L I K I N I R I Q I V I R I G I F I P I K 1

Transient decay ion deconvolution



1^b S H H W G Y G K H N G P E H W H K D F P 240
 21 I A N G E R Q S P V D I D T K A V V Q D 220
 41 P A L K P L A L V Y G E A T S R R M V N 200
 61 N G H S F N V E Y D S Q D K A V L K D 180
 81 G P L T G T Y R L V Q F H F H W G S S D 160
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 201 L L E S V T W I V L L K E P I S V S S Q Q 40
 221 M L K F R T L N F N A E G E P E L L M L 20
 241 A N W R P A Q P L K N R Q V R G F P K 1

- By changing symmetry of apodization
- Do see modest improvement in coverage
- But – user curation still needed to confirm benefit

Sequence coverage at different F values



- F value refers to position of apodization maximum as related to transient length
- Red frags seen in F=0.1
- Other frags added if seen in spectra produced using other F values, as indicated by colour scale



Benefit of this approach

- Improved MS resolution
 - Produces absorption mode spectra
 - Transients are longer than are used in approach 1
- But
 - Longer transients mean more computation

Approach 3. Decay Rate Calculations

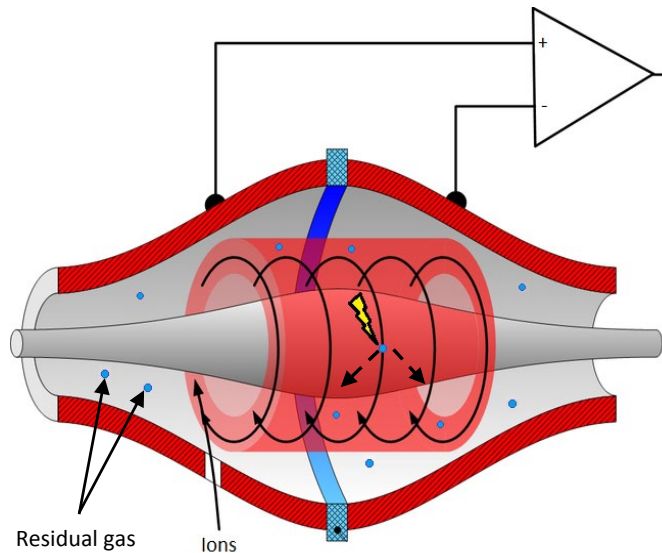
Lead: KI



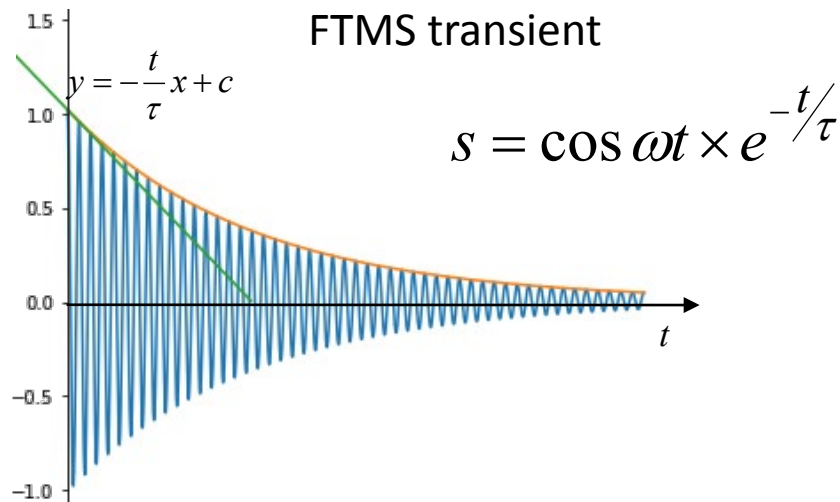
Experimental data: SPS



Background: Exponential Decay of FTMS Transient



Modified from wikipedia art of Admttool according to CCA-Share-Alike 3.0

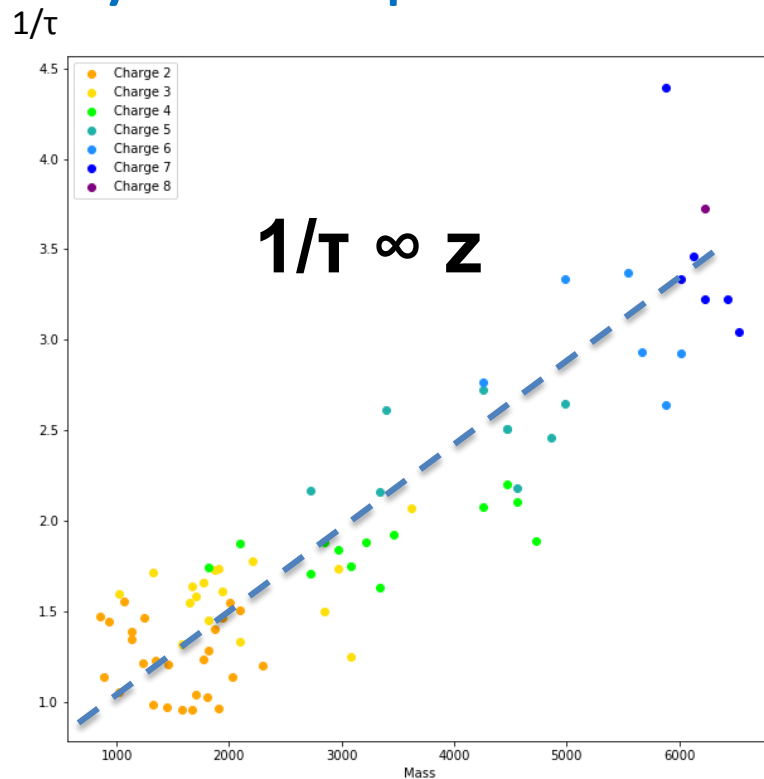


The usual assumption:

$1/\tau \approx$ probability of collision with residual gas $\approx$
 $\approx$ cross-section of molecule $\approx$ (molecular mass) $^{2/3}$

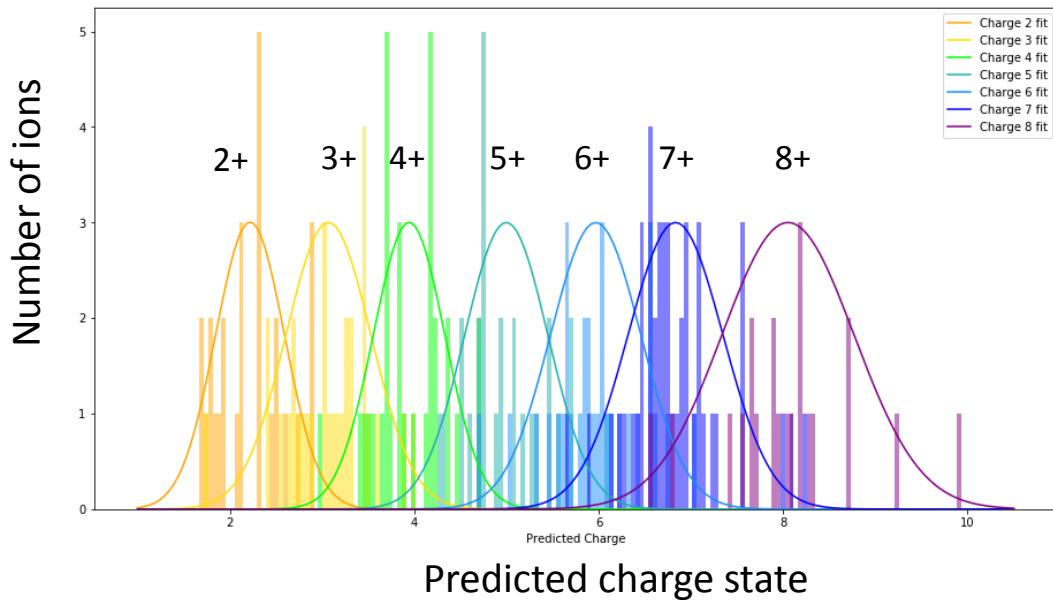
Ions with larger mass M have higher cross section –
 thus, decay of FTMS transient should be faster for them

Decay Analysis – Experimental Verification



Monoisotopic peaks $1/\tau$ decay constant vs molecular mass

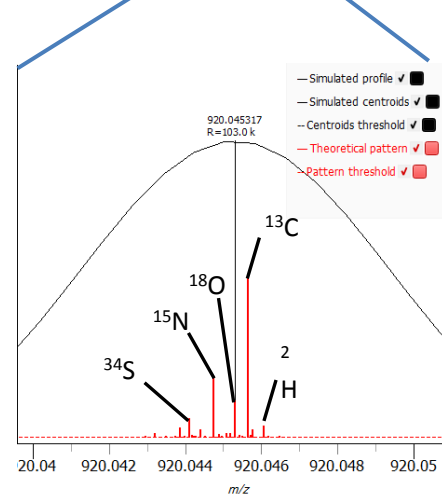
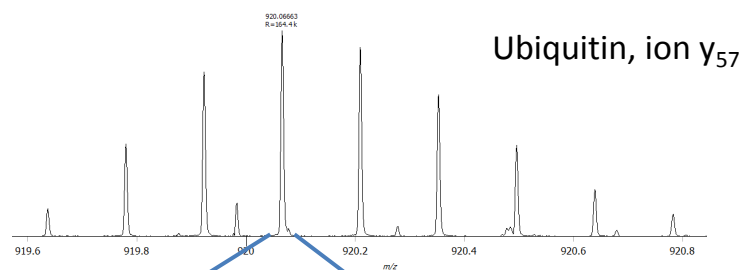
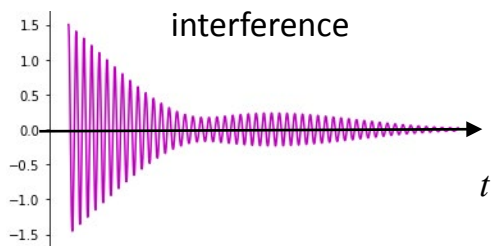
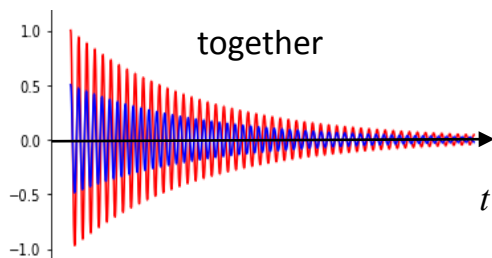
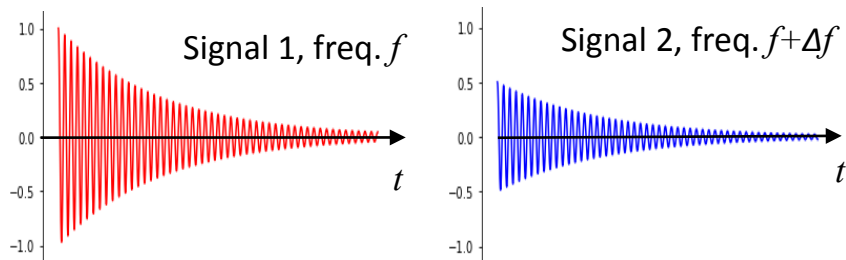
Decay Analysis – Resolution Determination



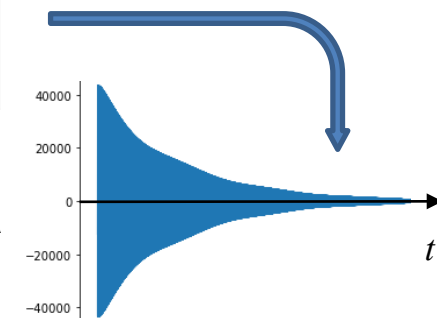
Resolution ≈ 7.1 for charge states 2+ to 8+ of ubiquitin

This is great, but what limits the resolution?

Isotopic Fine Structure as a Limiting Factor

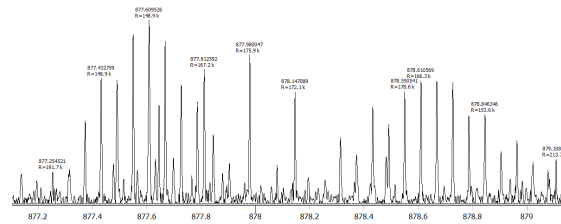
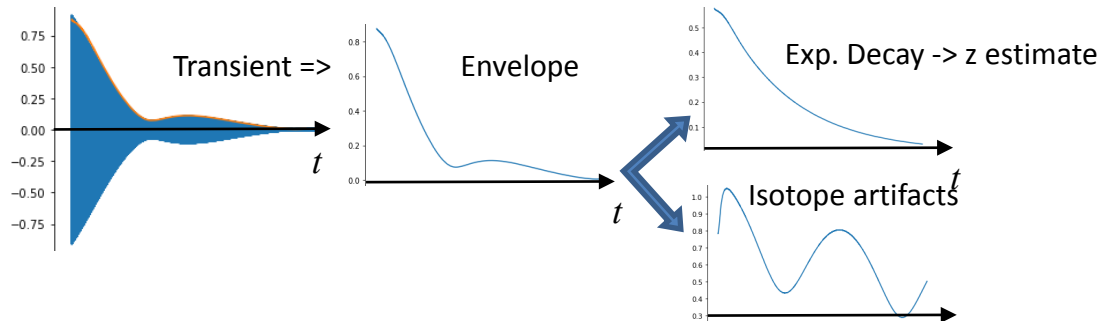


Exponential decay with fine isotopic structure



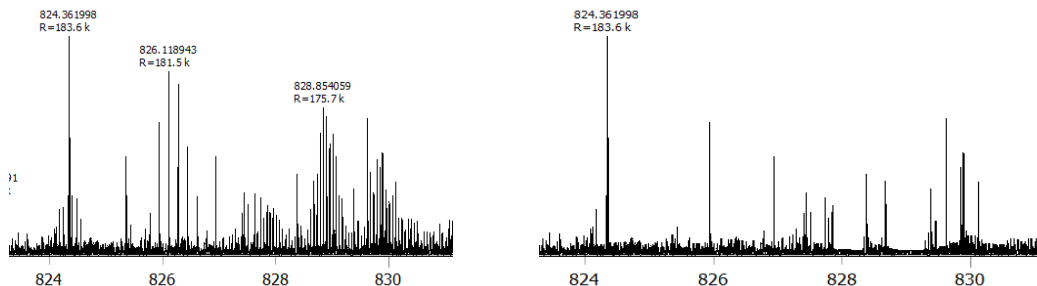
Approaches to Cope with IFS Limitation

1. Math modeling to split exponential decay and fine structure artifacts



This approach could be applied for analysis of complex spectra with multiple isotope distributions overlap

2. Remove fine isotopic structure using monoisotopic molecules: Natural 98.7% ^{12}C => artificial 99.9% ^{12}C



This approach requires growing cells or organisms in monoisotopic media. KI is now aiming at obtaining monoisotopic media for growth of hybridoma cells producing mAbs. Already obtained monoisotopic *E. Coli* and *C. elegans*.

Current Conclusions: M1

Demonstrated effectiveness of product ion isotopic distribution deconvolution.

In general, we have achieved milestone M1 by developing the allied approaches and demonstrating the effectiveness of product ion isotopic distribution deconvolution by decay rates.

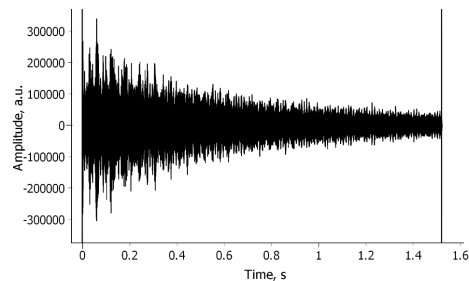
However, to increase the practical value and importance of this method, we will continue developing our data processing and data analysis software (within the frames of the follow-up Tasks) and will apply the optimized methods to experimental data acquired, over the course of the next 6-12 months, from the completed TopSpec platforms (equipped with FTMS Booster TD devices).

Outlook

- Fundamentally, the hypothesis has been verified and results are publishable
- Increased protein size (up to 150 kDa, mAb) may provide higher value
- Deconvolving more complex top-down spectra (new radical reactions) may provide higher value
- Results are to be compared with ultra-high-resolution (UHR) complementary approach
- Ion mobility by hardware (attached to omnitrap) is to be added into the consideration
- Sample production in isotopically-depleted media would lead to reduced complexity of isotopic envelopes and thus improve spectra deconvolution

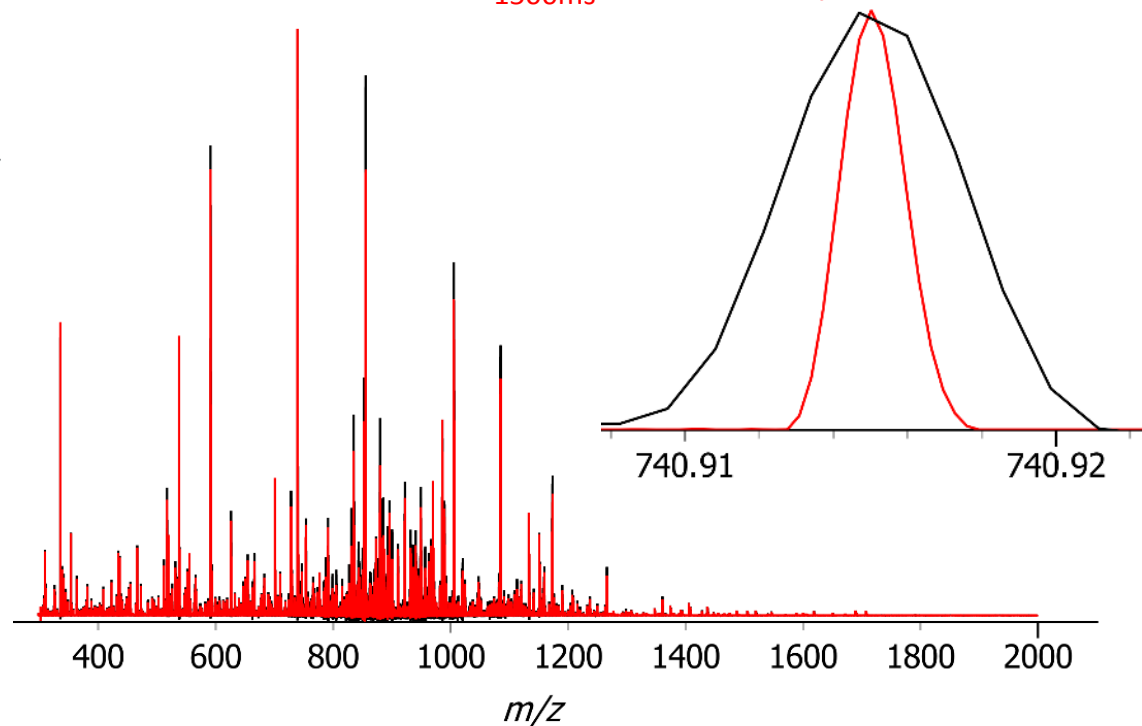
UHR Orbitrap Top-Down FTMS

Carbonic anhydrase (29 kDa) analysis with HCD on QE HF (aFT)



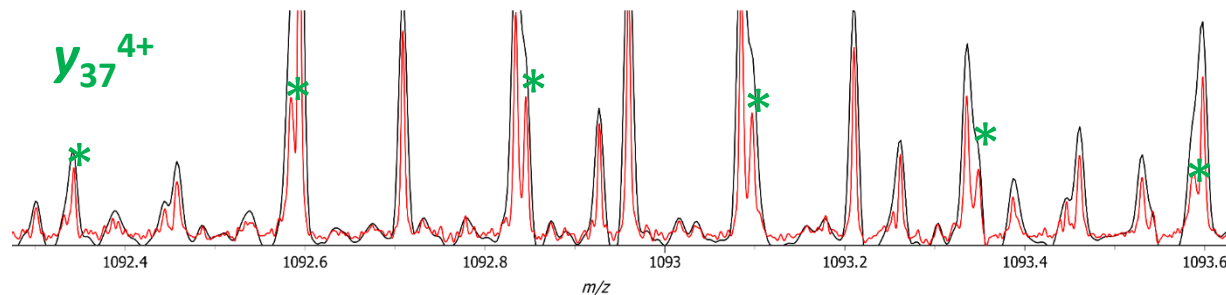
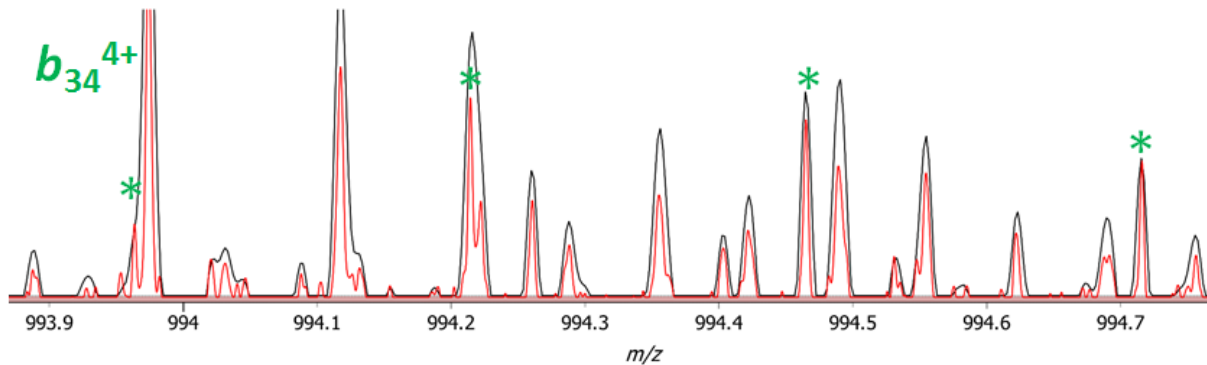
$R_{500\text{ms}} = 129'000$ (245k @ 200 m/z)

$R_{1500\text{ms}} = 354'000$ (670k @ 200 m/z)



Primary Structure Confirmation: UHR

- Ultra-high resolution (UHR) top/middle-down FTMS (Booster)



Higher sequence coverage, confidence in assignment

UHR Orbitrap Top-Down MS

UHR OT TD MS: improves sequencing, needs bioinformatics

$T_{\text{acq}} = 500 \text{ ms}$ vs $T_{\text{acq}} = 1500 \text{ ms}$

Carbonic anhydrase sequence coverage:

33.6 % vs **39.1 %**

N **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 25
26 R Q S P V D I D T K A V V Q D P A L K P L A L V Y 50
51 G E A T S R R M V N N G H S F N V E Y D D S Q D K 75
76 A V L K D G P L T G T Y R L V Q F H F H W G S S D 100
101 D Q G S E H T V D R K K Y A A E L H L V H W N T K 125
126 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 150
151 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 175
176 N F D P G S L L P N V L D Y W T Y P G S L T T P P 200
201 L L L E S V T W I V L K E P I S V S S Q Q M L K F R 225
226 T L N F N A E G E P E L L M L A N W R P A Q P L K 250
251 N R Q V R G F P K C

Decay Rates vs. UHR Performance

CAH, 1e6, mFT, MASH Suite

All_chunks

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N 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 25
26 R Q S P V D I D T K A V V Q D P A L K P L A L V Y 50
51 G E A T S R R M V N N G H S F N V E Y D D S Q D K 75
76 A V L K D G P L T G T Y R L V Q F H F H W G S S D 100
101 D Q G S E H T V D R K K Y A A E L H L V H W N T K 125
126 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 150
151 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 175
176 N F D P G S L L P N V L D Y W T Y P G S L T T P P 200
201 L L E S V T W I V L L K E P I S V S S Q Q M L K F R 225
226 T L N F N A E G E P E L L M L A N W R P A Q P L K 250
251 N R Q V R G F P K C
  
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Full 1500

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N 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 25
26 R Q S P V D I D T K A V V Q D P A L K P L A L V Y 50
51 G E A T S R R M V N N G H S F N V E Y D D S Q D K 75
76 A V L K D G P L T G T Y R L V Q F H F H W G S S D 100
101 D Q G S E H T V D R K K Y A A E L H L V H W N T K 125
126 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 150
151 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 175
176 N F D P G S L L P N V L D Y W T Y P G S L T T P P 200
201 L L E S V T W I V L L K E P I S V S S Q Q M L K F R 225
226 T L N F N A E G E P E L L M L A N W R P A Q P L K 250
251 N R Q V R G F P K C
  
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UHR FTMS & Ion Mobility Could be the Targets in TopSpec