



Application of Data Independent Acquisition Techniques Optimized for Improved Precursor Selectivity

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University of Washington

6/8/2013

Acquisition Methods

Targeted

Selected Reaction
Monitoring (SRM)

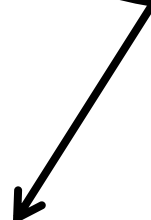
Peptide **Quantitation**

Data Independent
Acquisition (DIA)

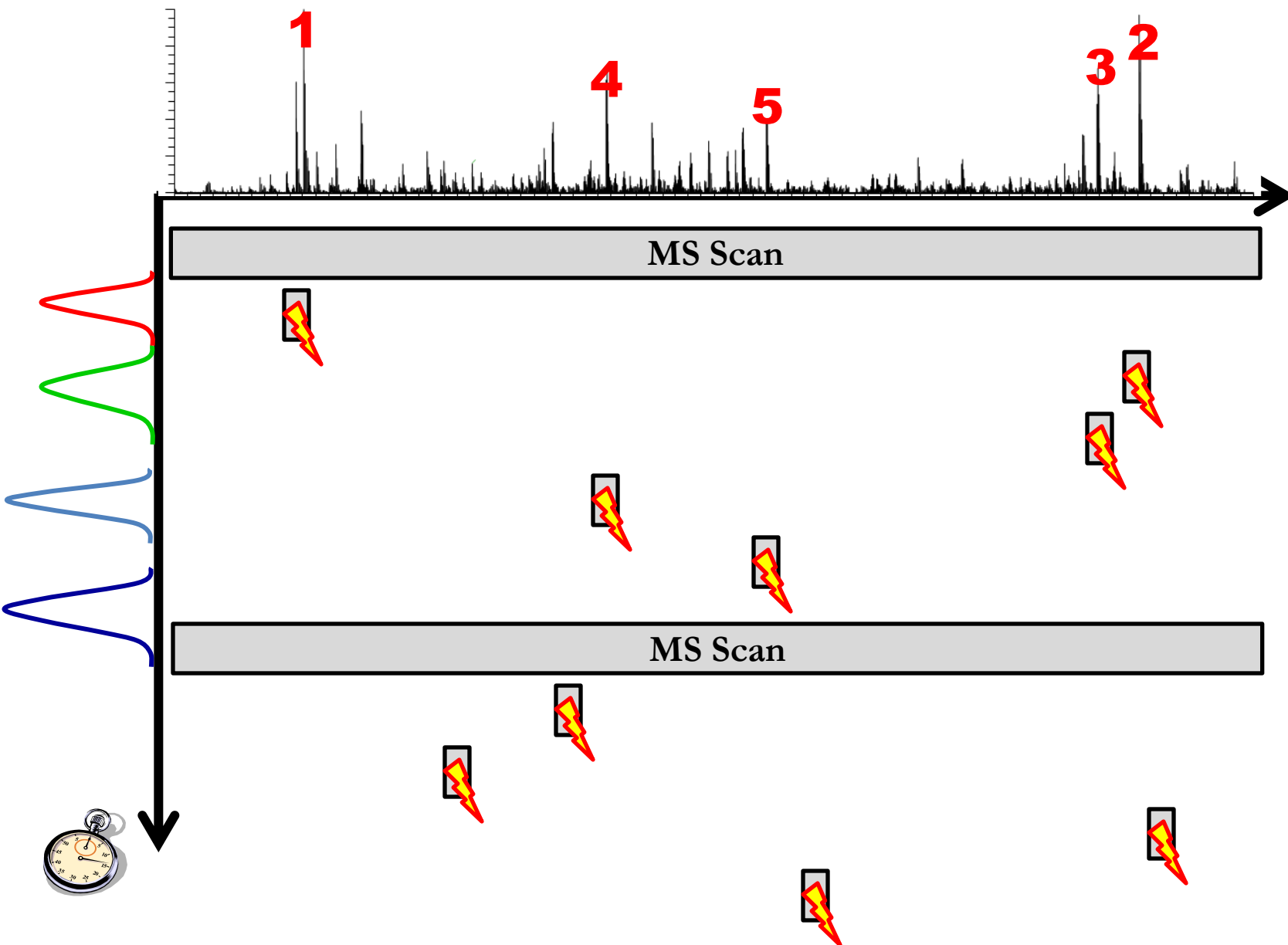
Discovery

Data Dependent
Acquisition (DDA)

Peptide **Identification**

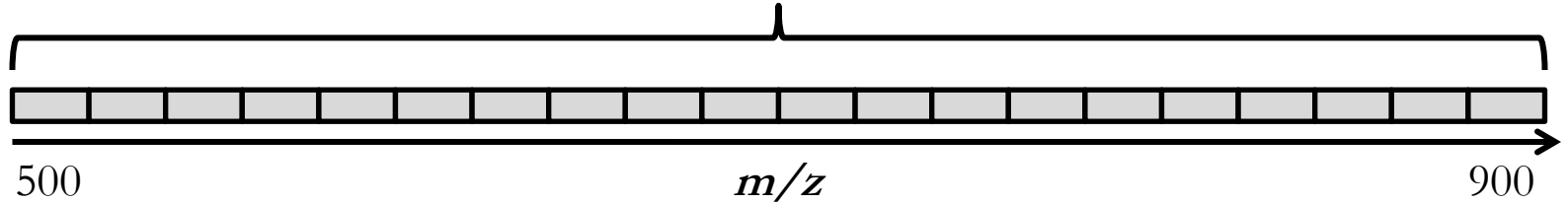


LC-MS/MS: Data Dependent



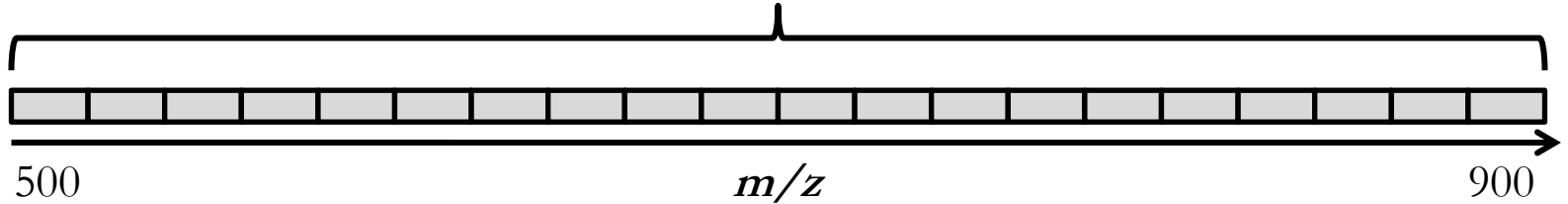
Data Independent Acquisition (DIA)

20 $20\ m/z$ -wide windows = 400 m/z



Data Independent Acquisition (DIA)

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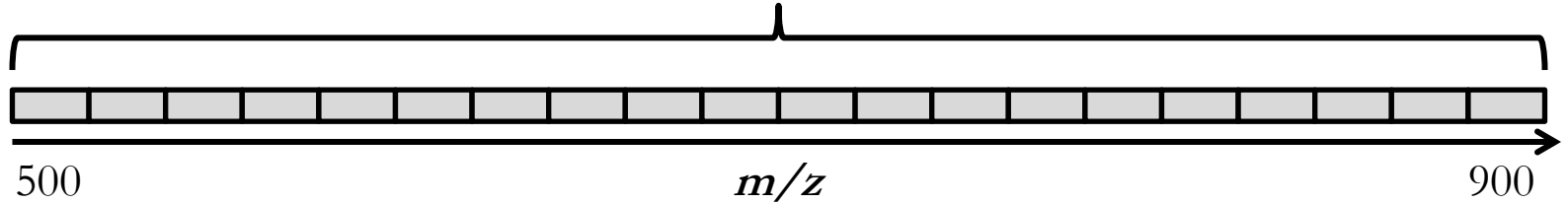


Scan 1



Data Independent Acquisition (DIA)

20 $20\ m/z$ -wide windows = **400 m/z**



Scan 1

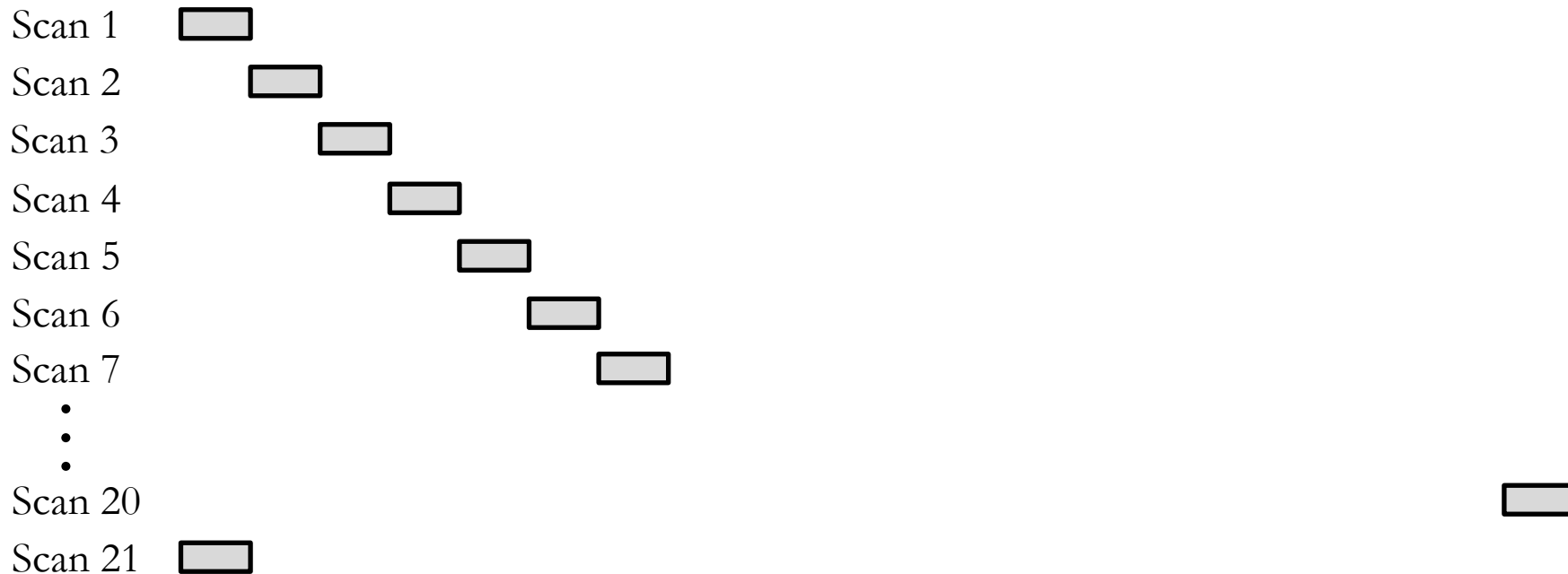
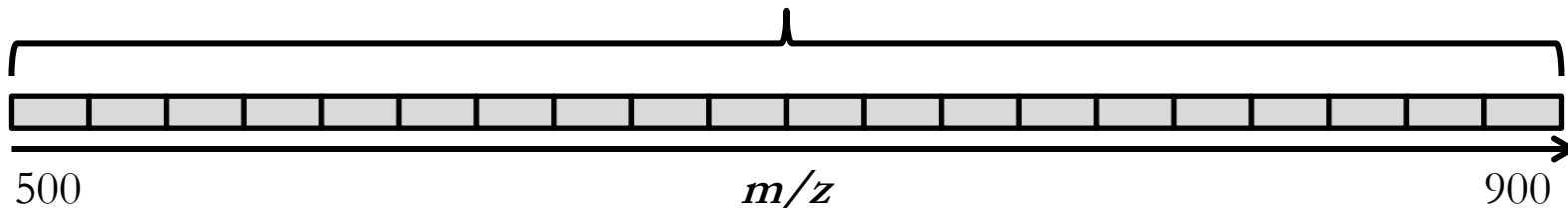


Scan 2



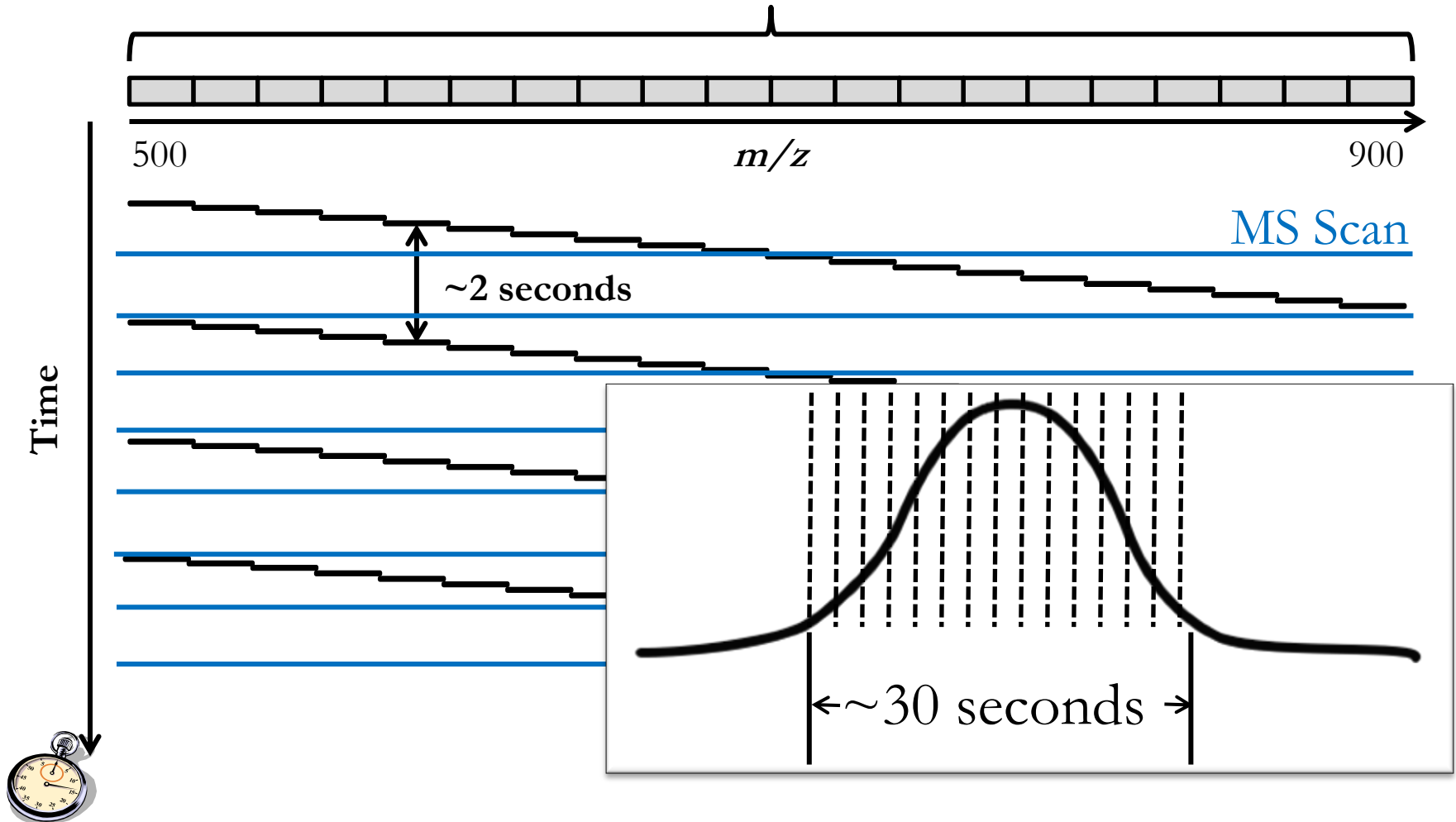
Data Independent Acquisition (DIA)

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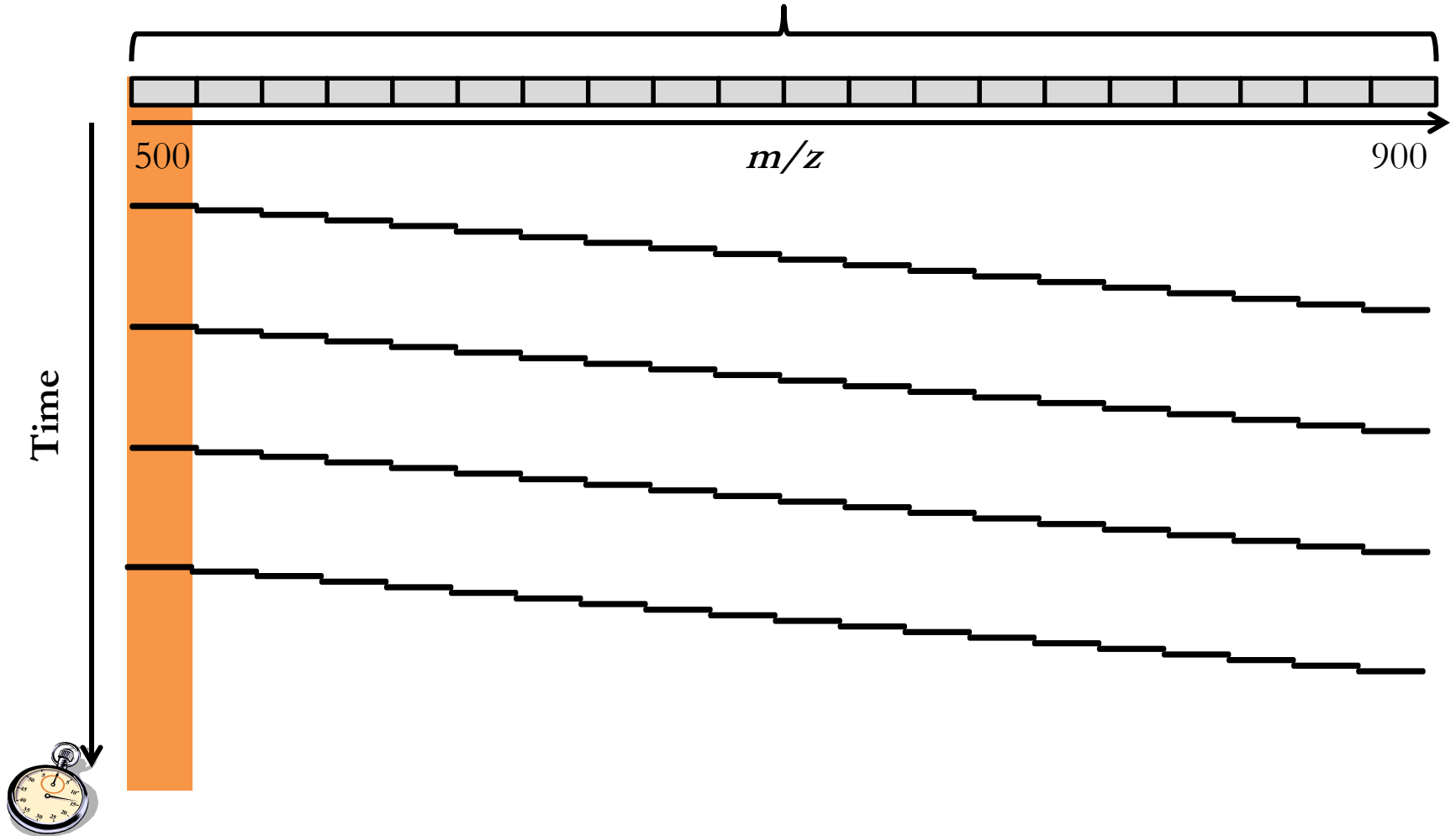
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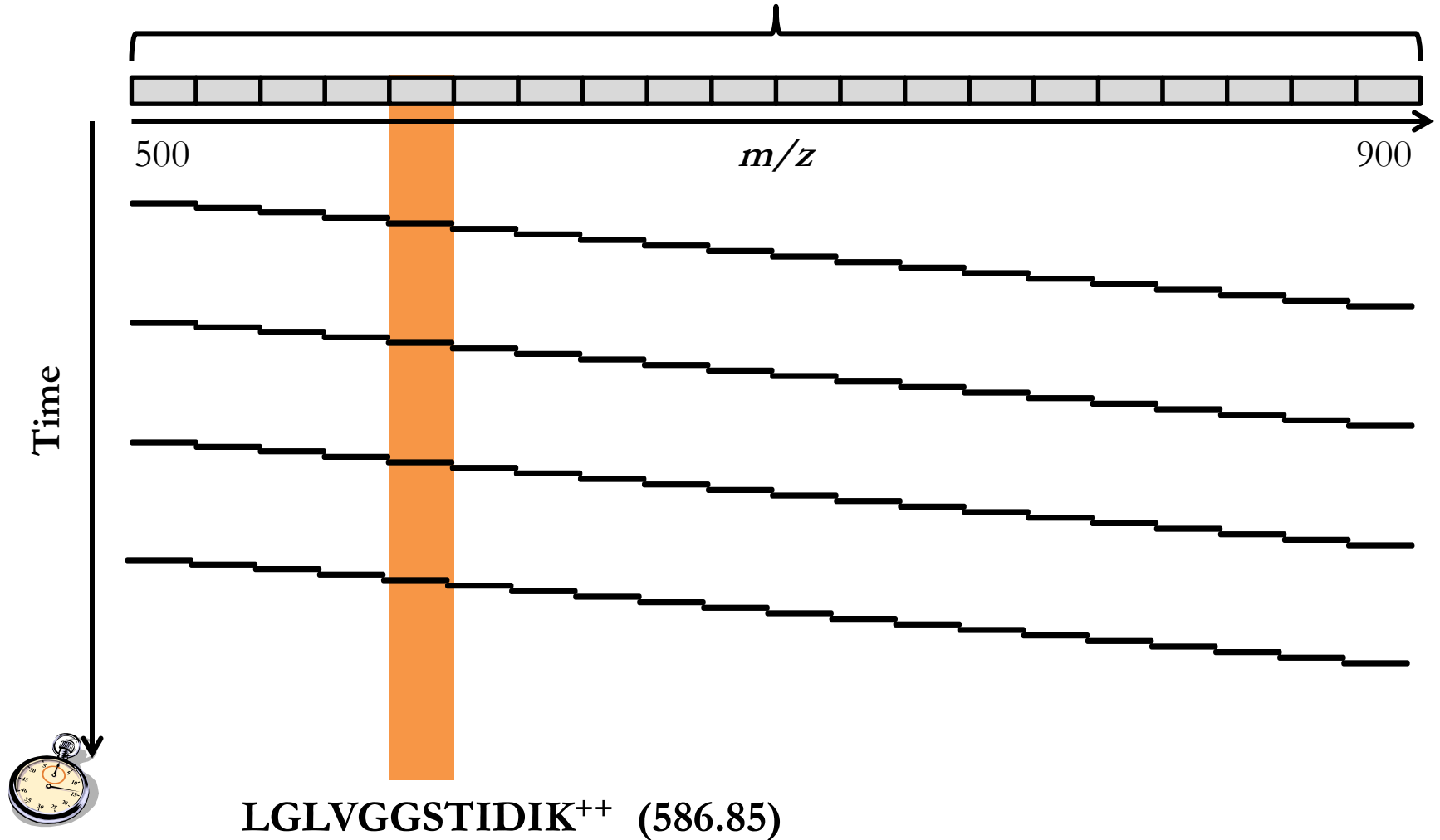
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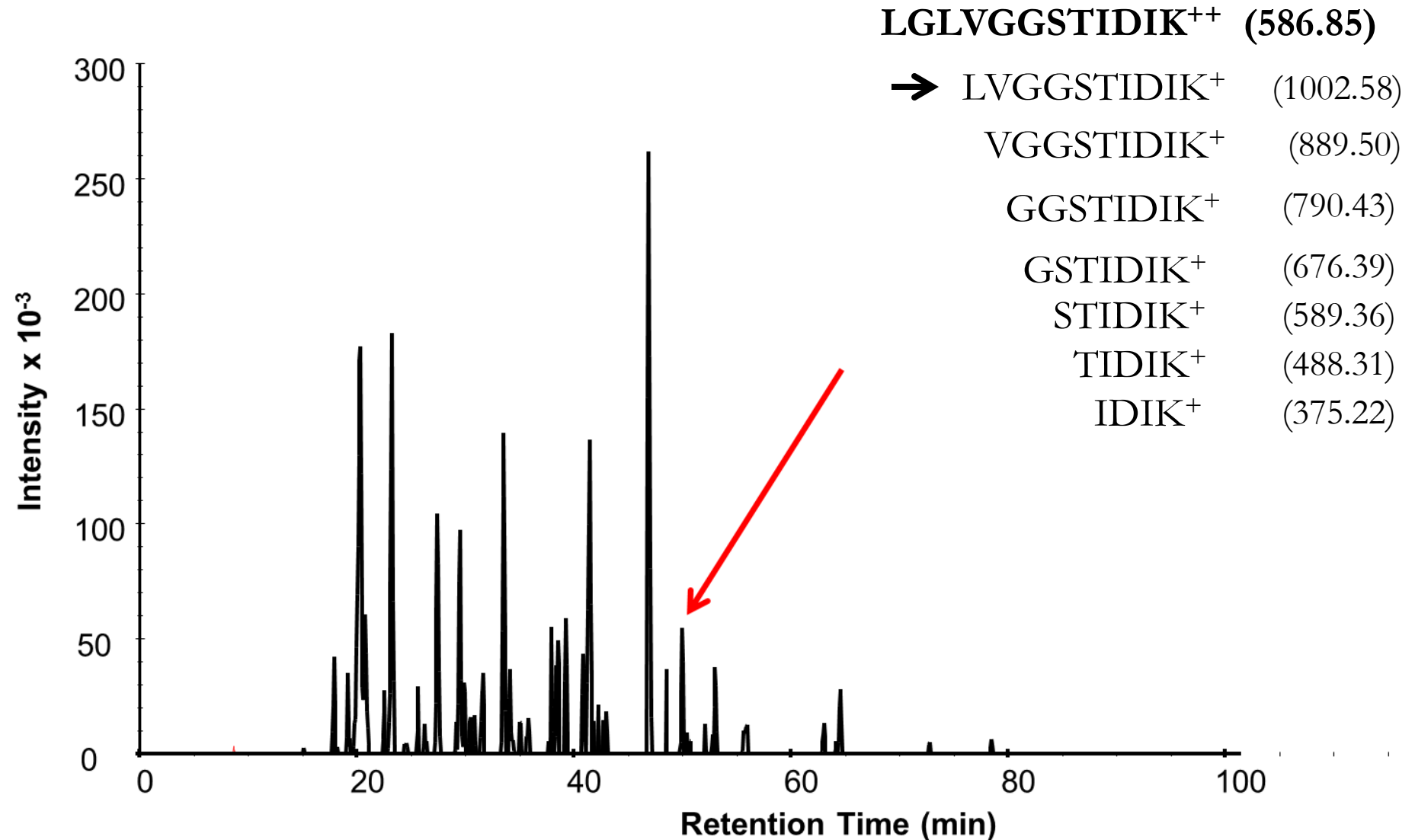


Data Independent Acquisition (DIA)

20 $20\ m/z$ -wide windows = $400\ m/z$



Data Independent Acquisition (DIA)



Data Independent Acquisition (DIA)

LGLVGGSTIDIK⁺⁺ (586.85)

LVGGSTIDIK⁺ (1002.58)

→ VGGSTIDIK⁺ (889.50)

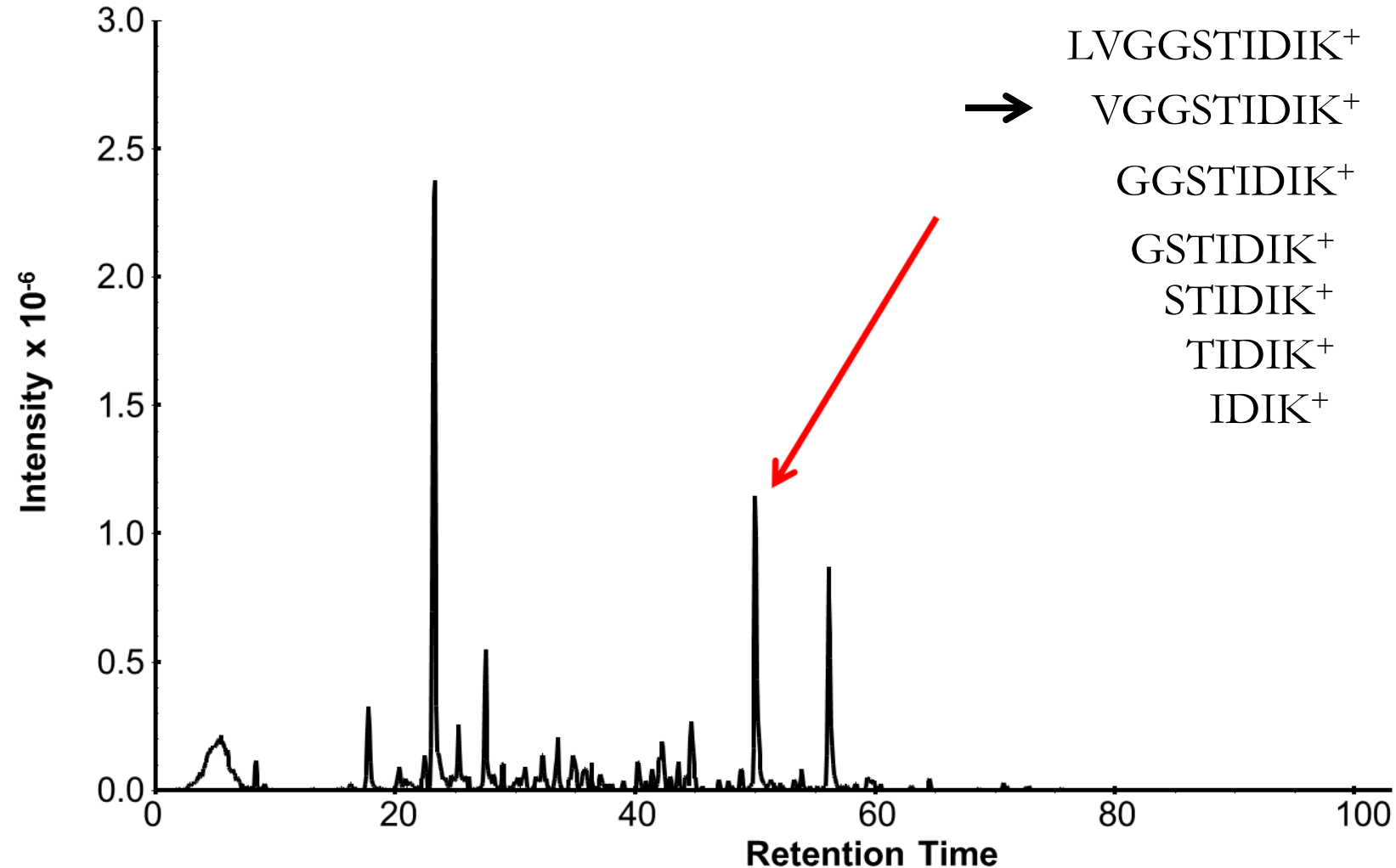
GGSTIDIK⁺ (790.43)

GSTIDIK⁺ (676.39)

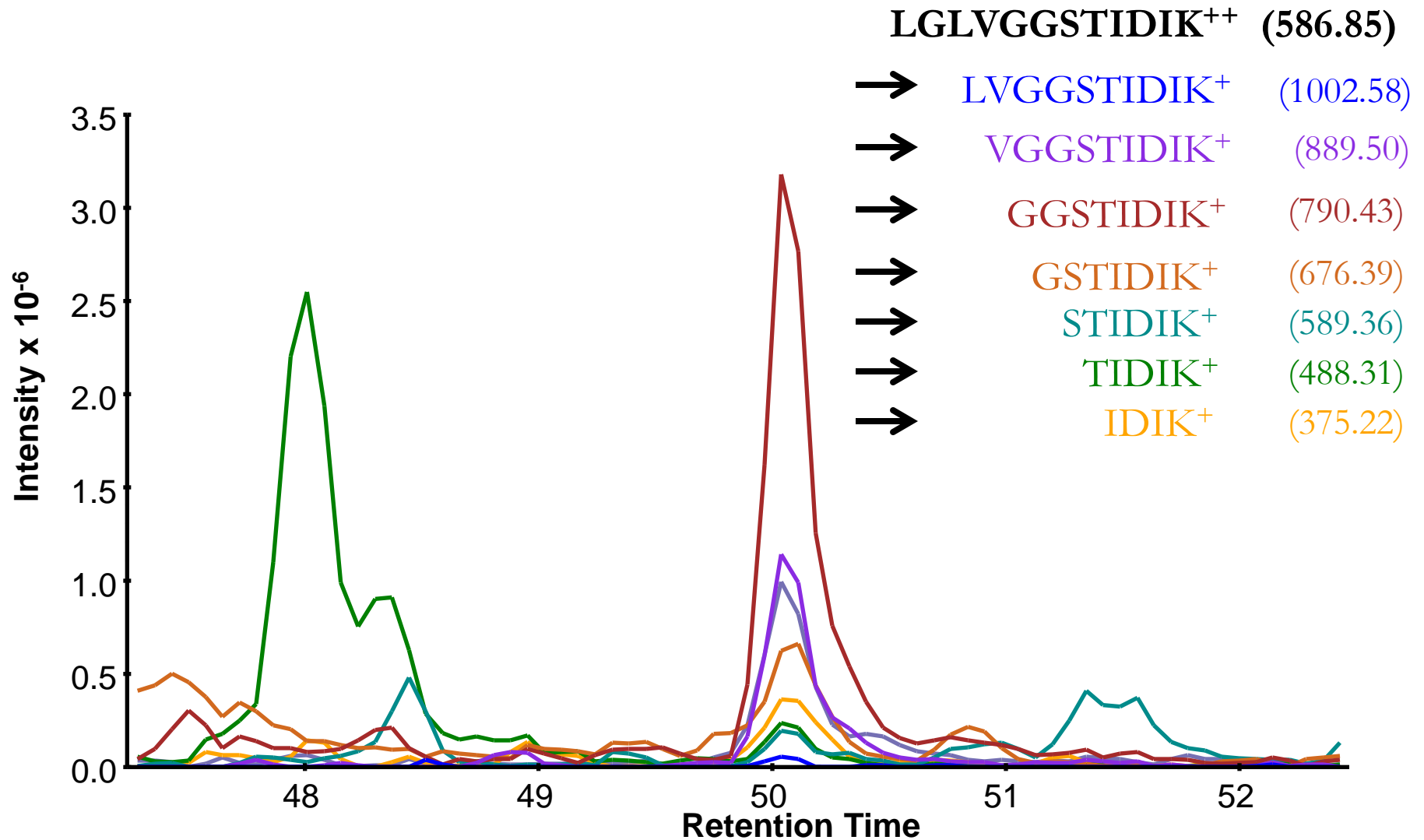
STIDIK⁺ (589.36)

TIDIK⁺ (488.31)

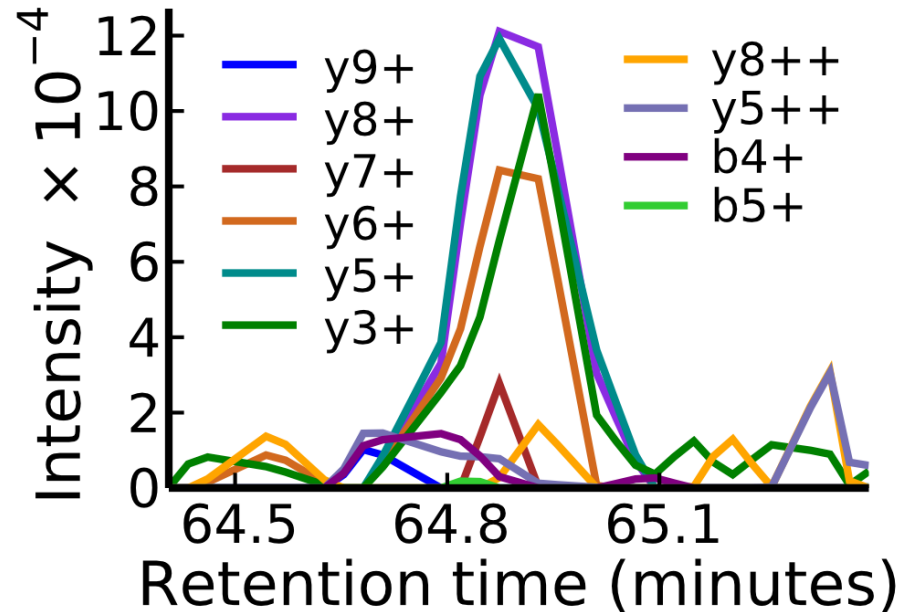
IDIK⁺ (375.22)



Data Independent Acquisition (DIA)

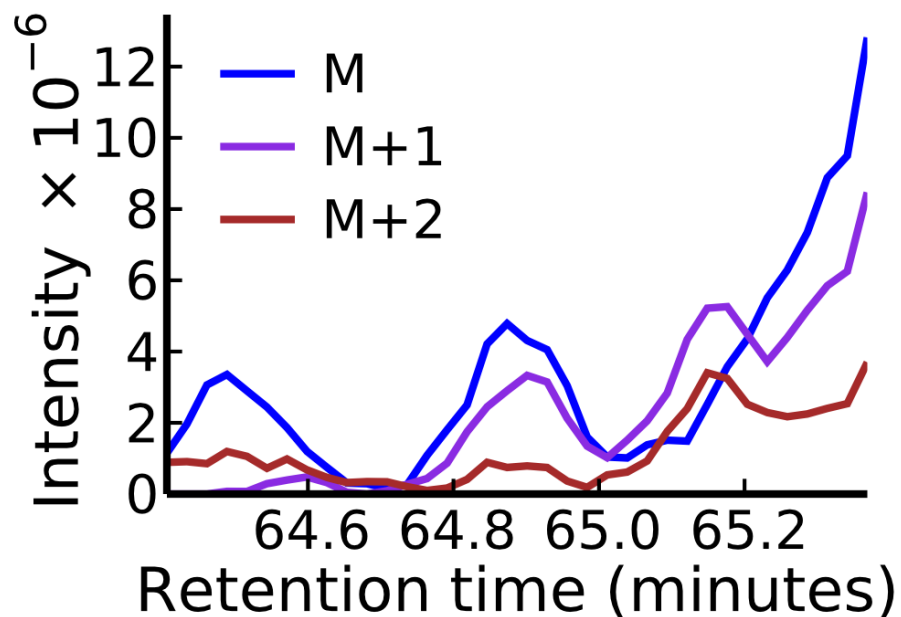


MS/MS

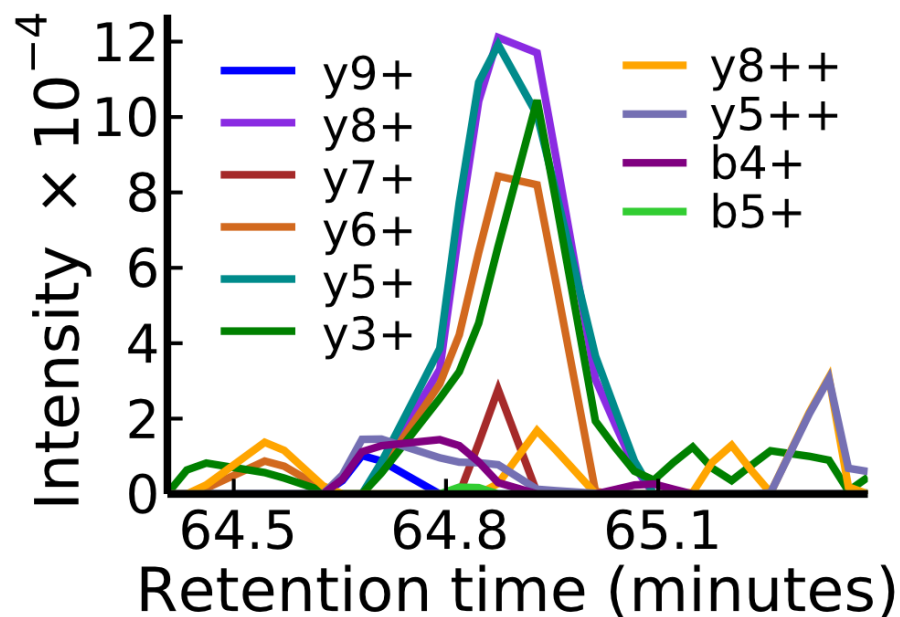


1.02 femtomoles of Bovine Serum Albumin
(LVNELTEFAK++) in 1.2 ug of *S. cerevisiae* lysate

MS

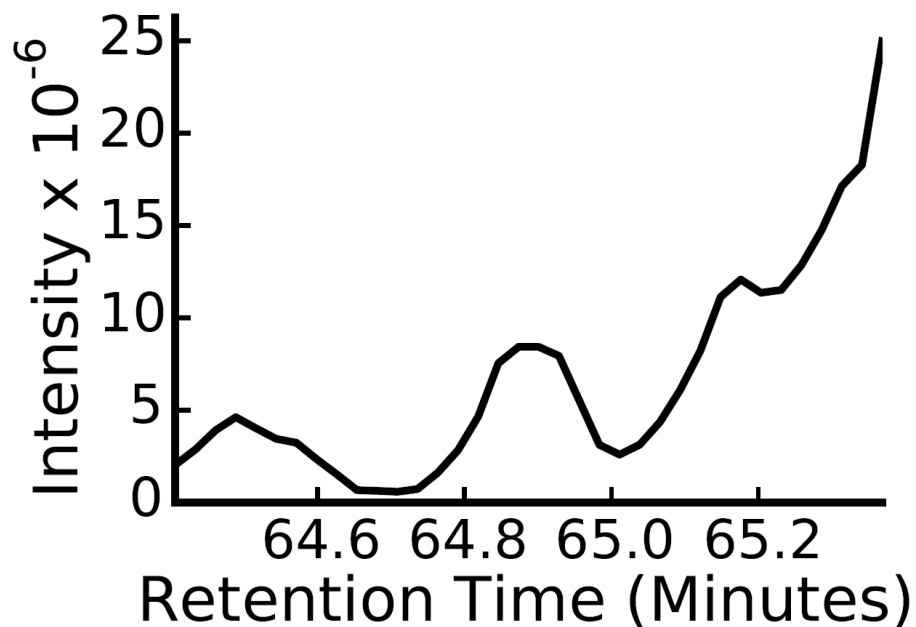


MS/MS

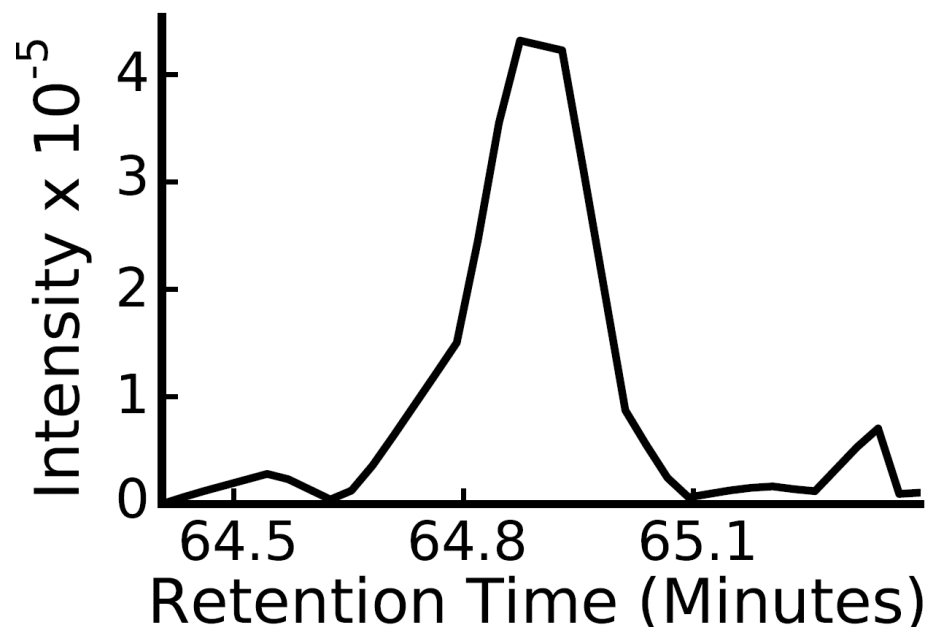


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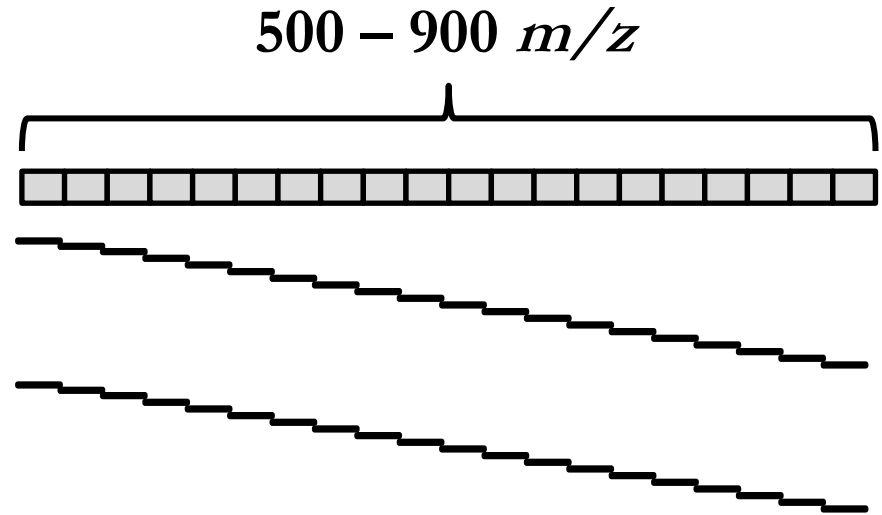
MS/MS



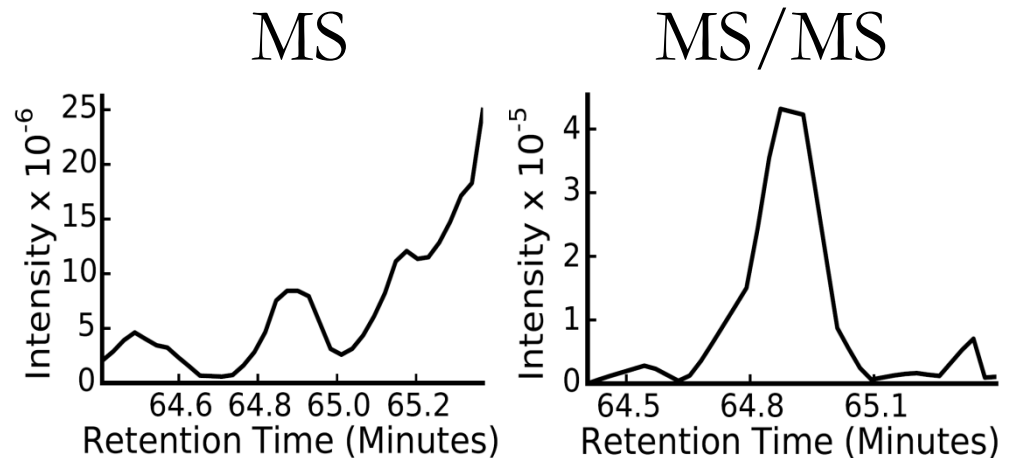
1.02 femtomoles of Bovine Serum Albumin
(LVNELTEFAK++) in 1.2 μg of *S. cerevisiae* lysate

Theoretical Benefits of DIA

- Comprehensive Sampling
 - Reproducibility



- Improved Quantitation



Isolation Window Width

DDA



2 m/z

Vs.

DIA



10 m/z

Vs.

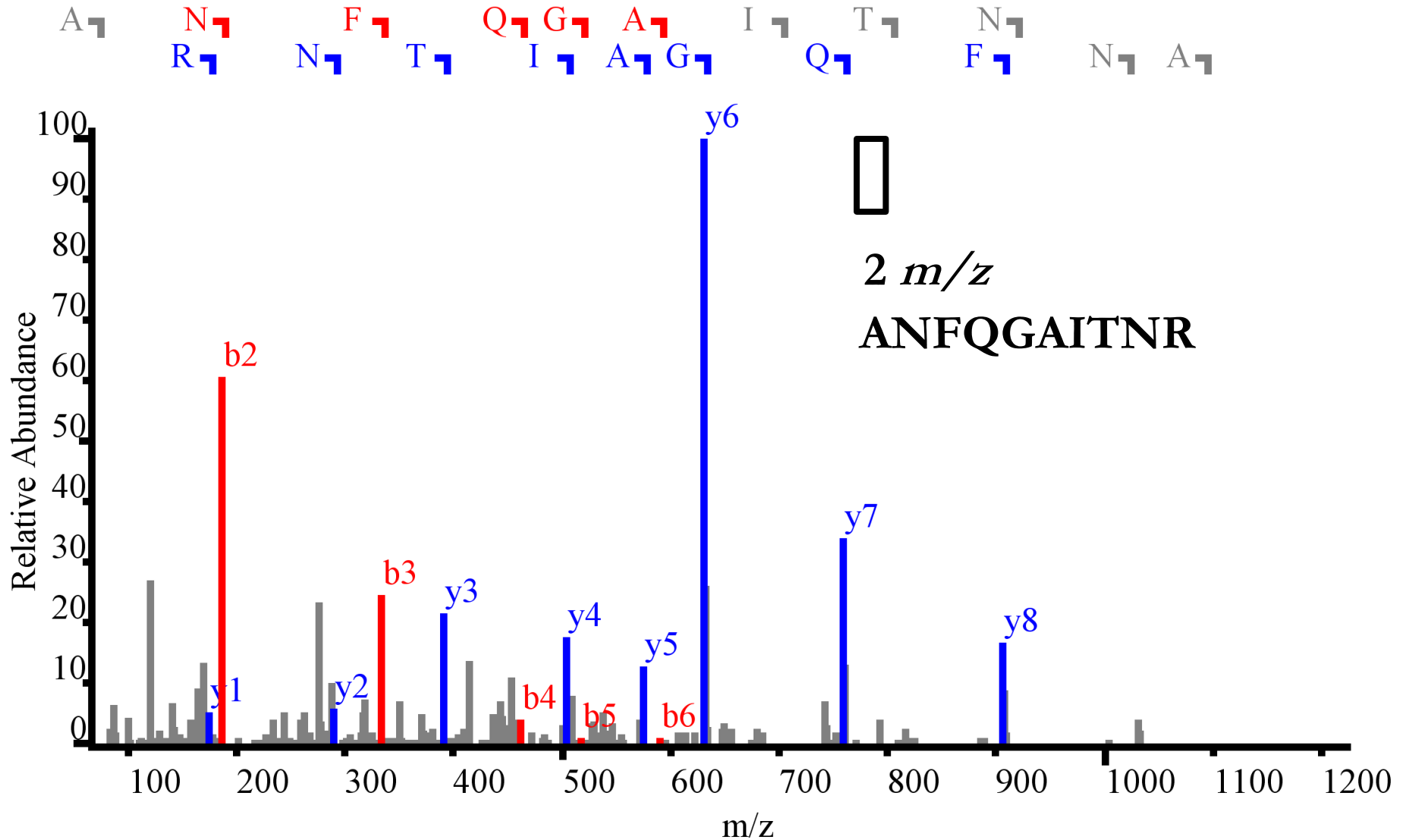


20 m/z

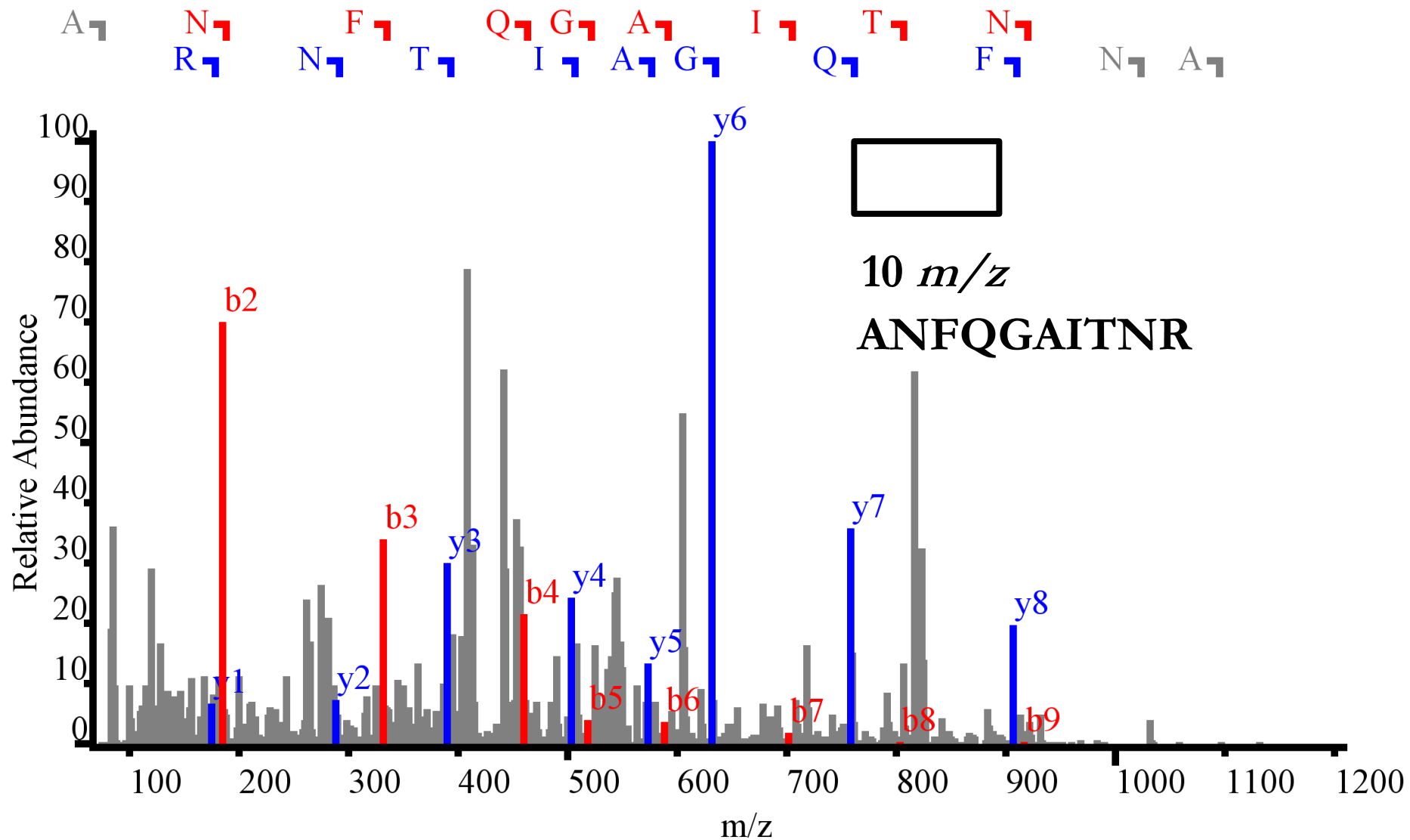
Lower precursor selectivity

- More peptides co-fragmented
- More complex MS/MS spectra
- More interference

Precursor Selectivity

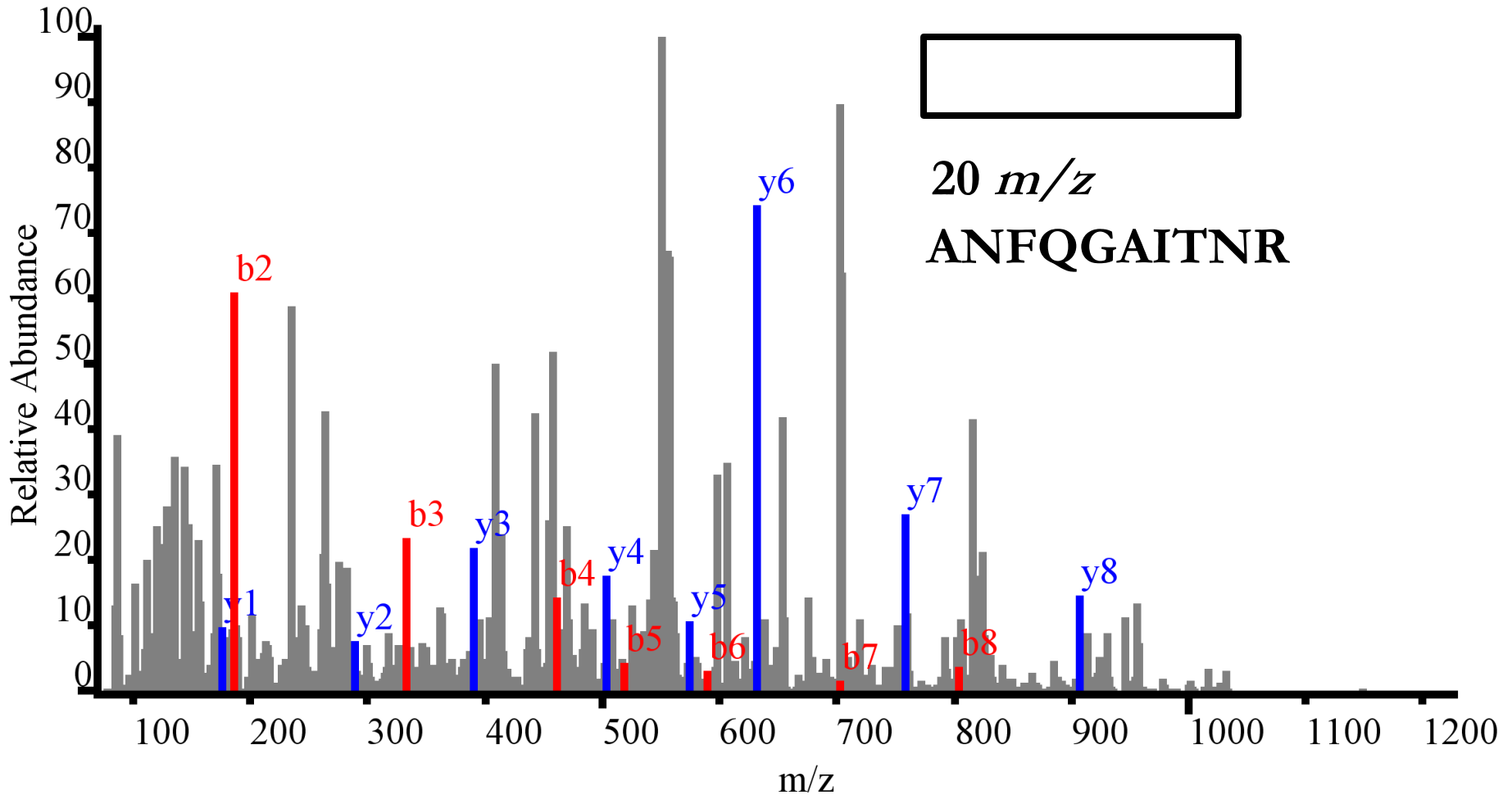


Precursor Selectivity

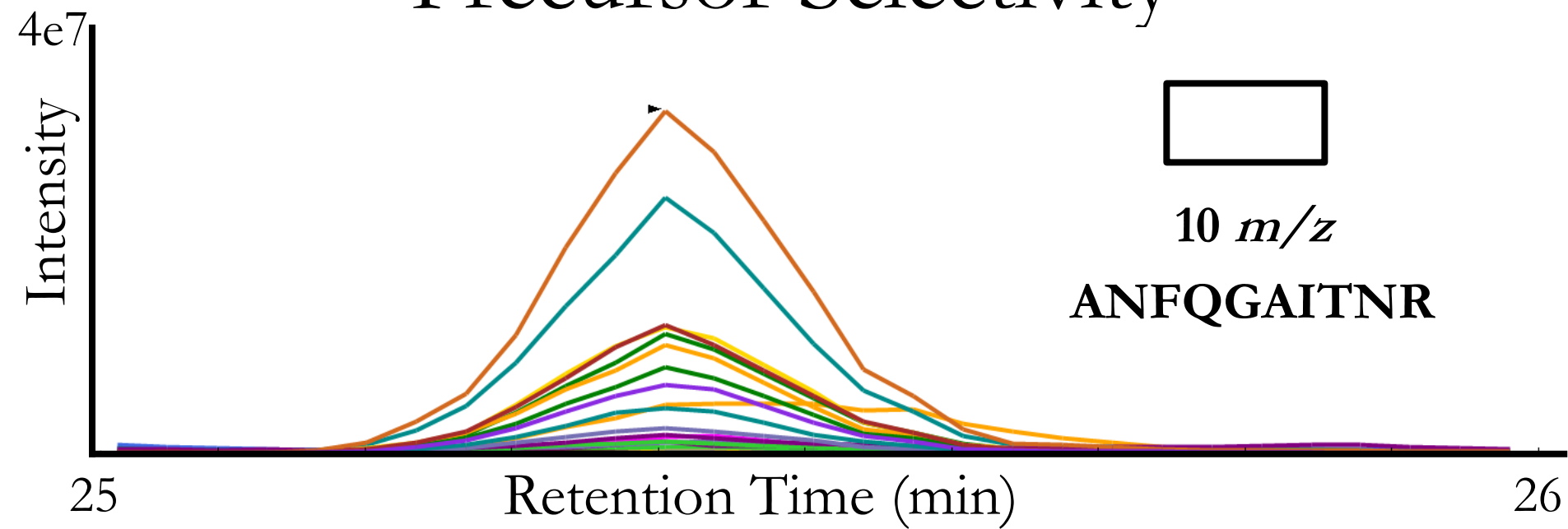


Precursor Selectivity

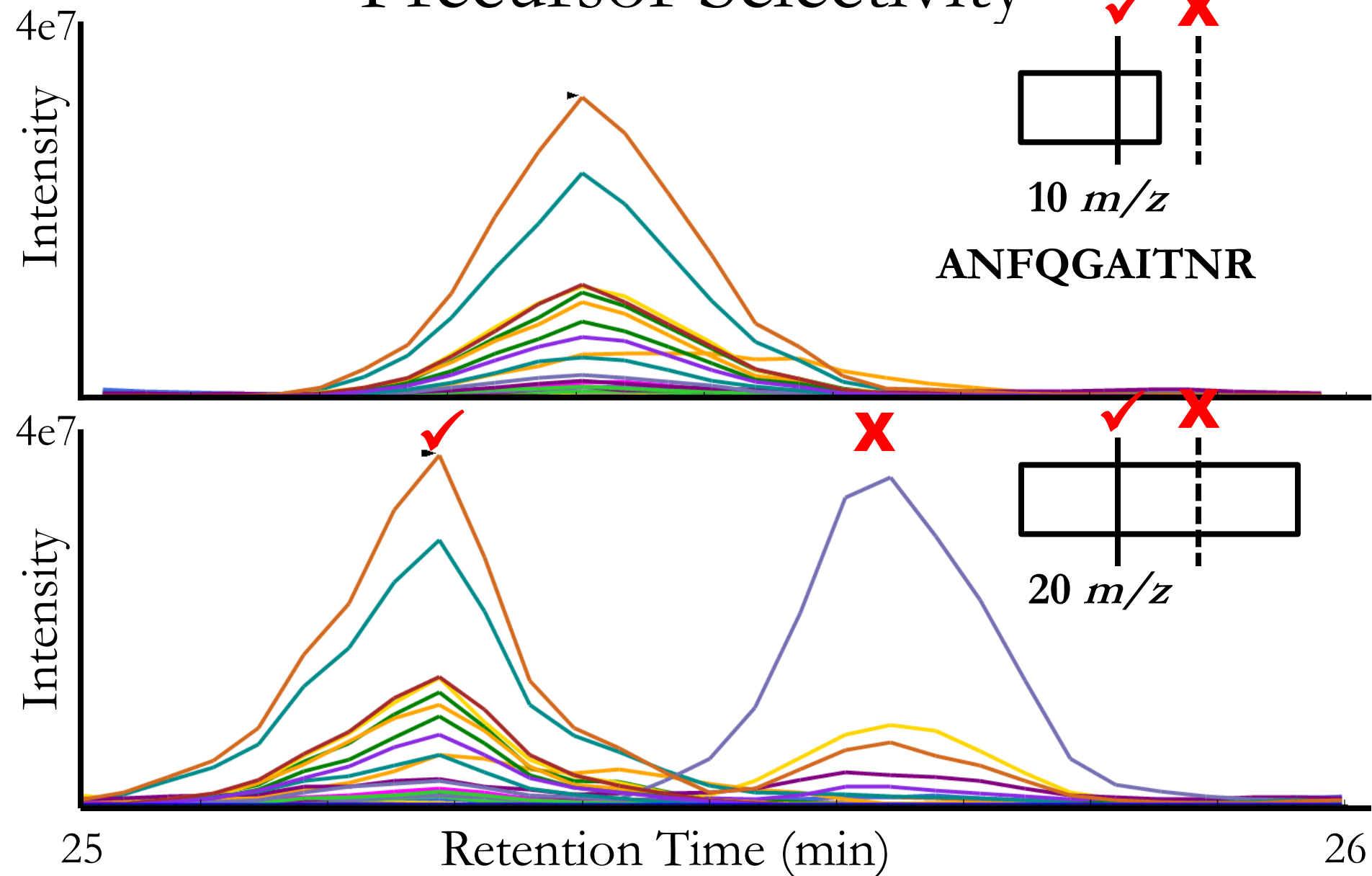
N R F Q G A I T N
R N T I A G Q F N A



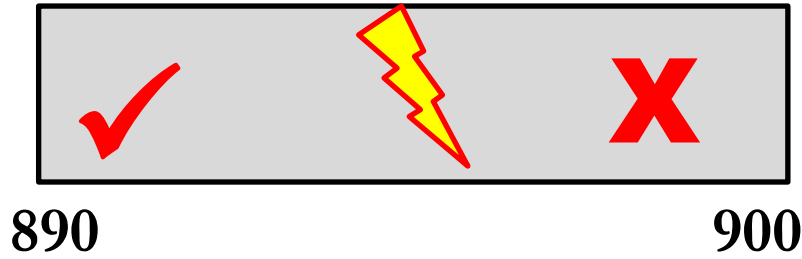
Precursor Selectivity



Precursor Selectivity

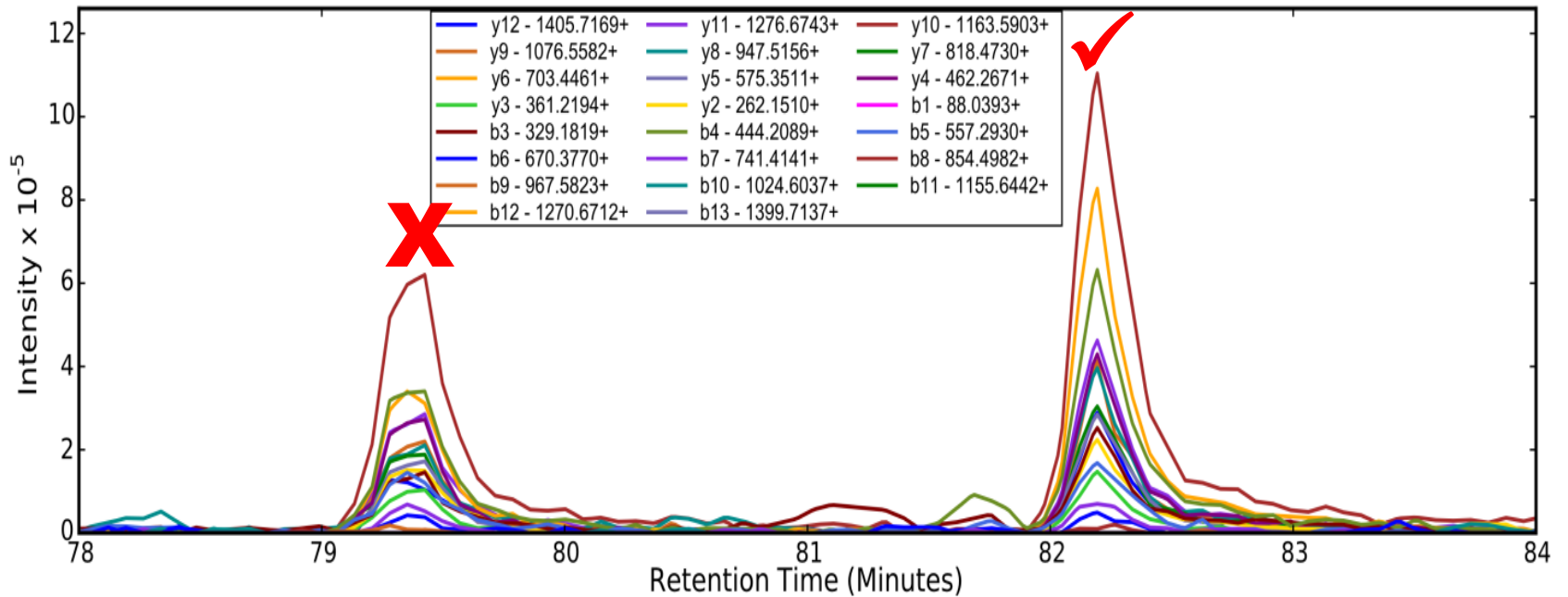


Precursor Selectivity

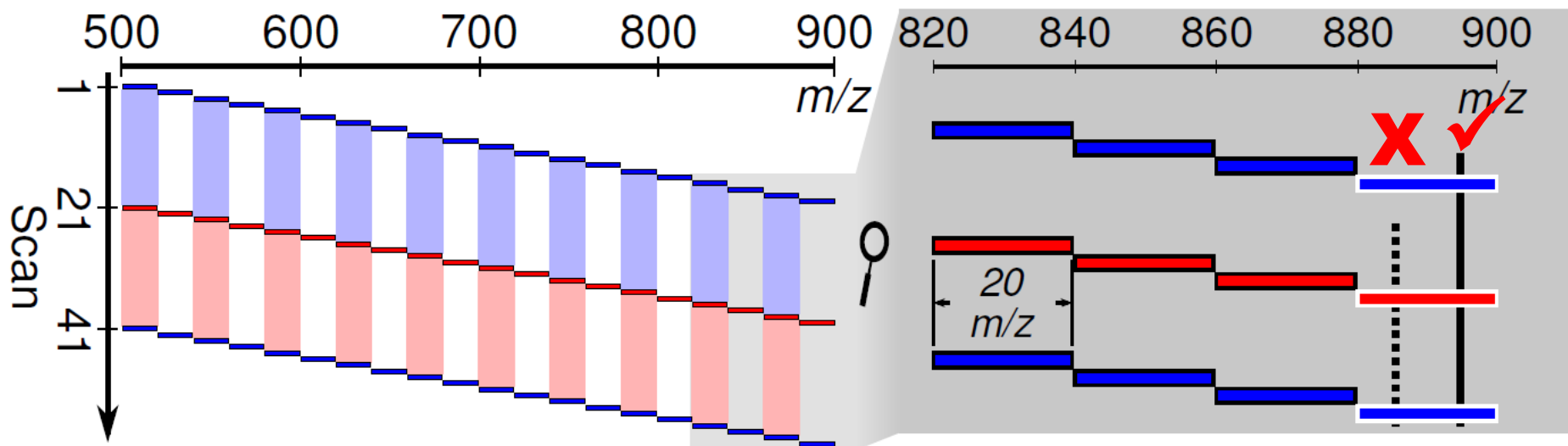


SLQDIIAILG**M**DELSEEDKLTVSR+++
(897.8 m/z)

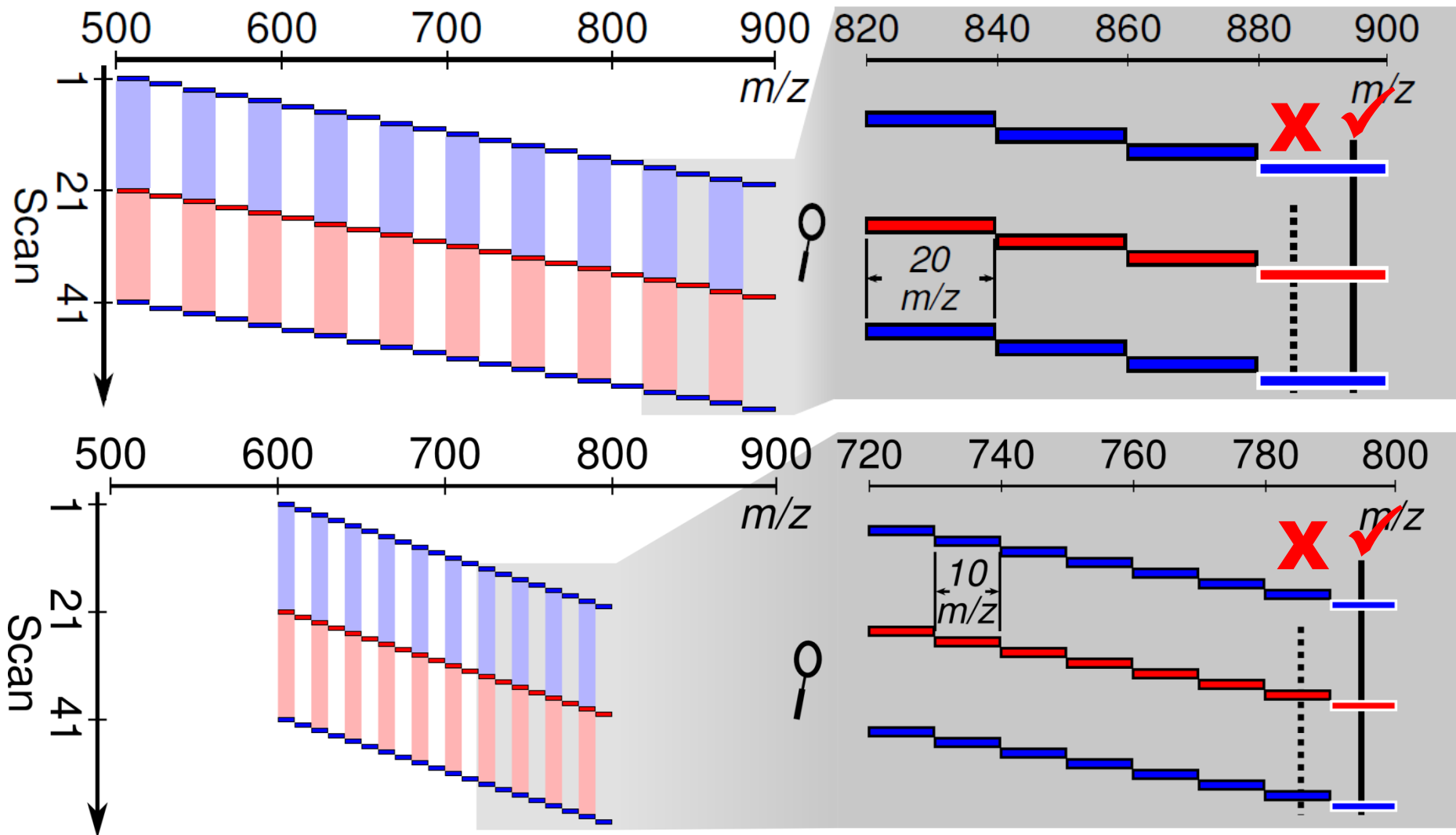
SLQDIIAILG**M**DELSEEDKLTVSR+++
(892.47 m/z)



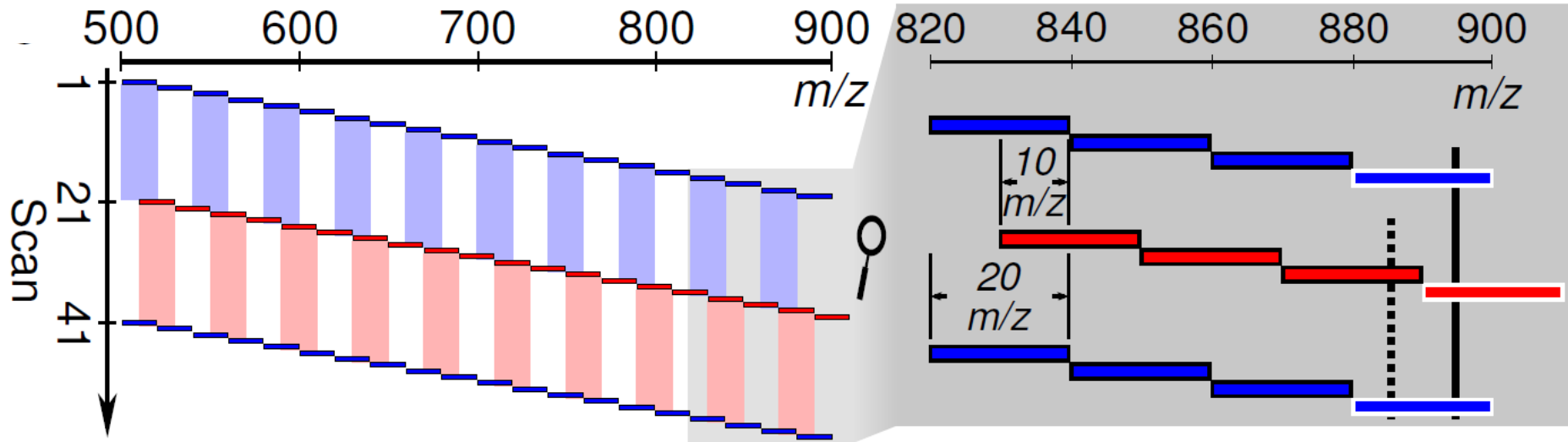
Improving Precursor Selectivity



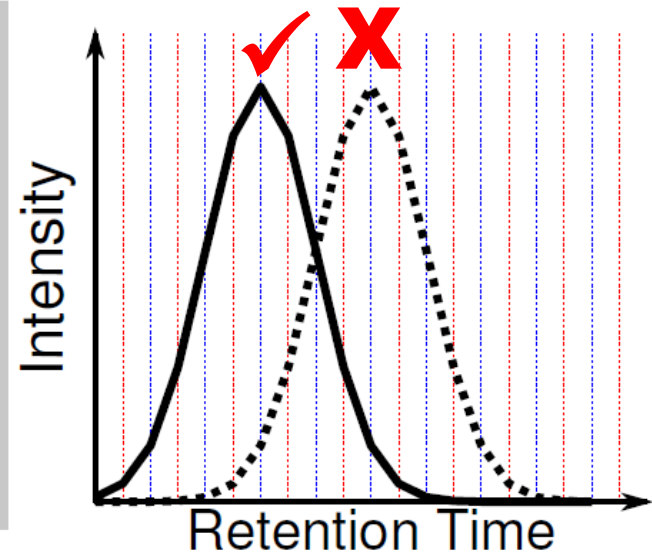
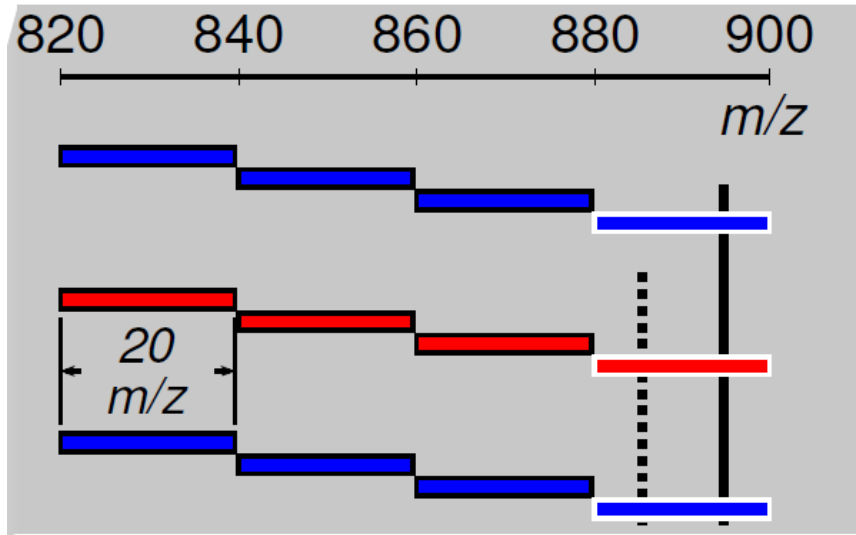
Improving Precursor Selectivity



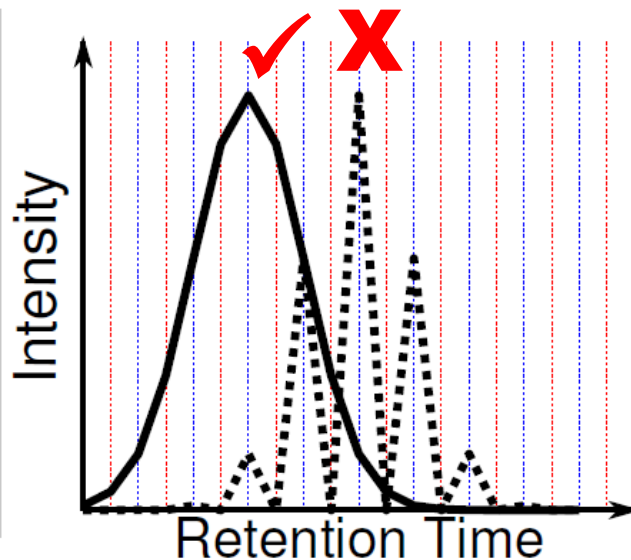
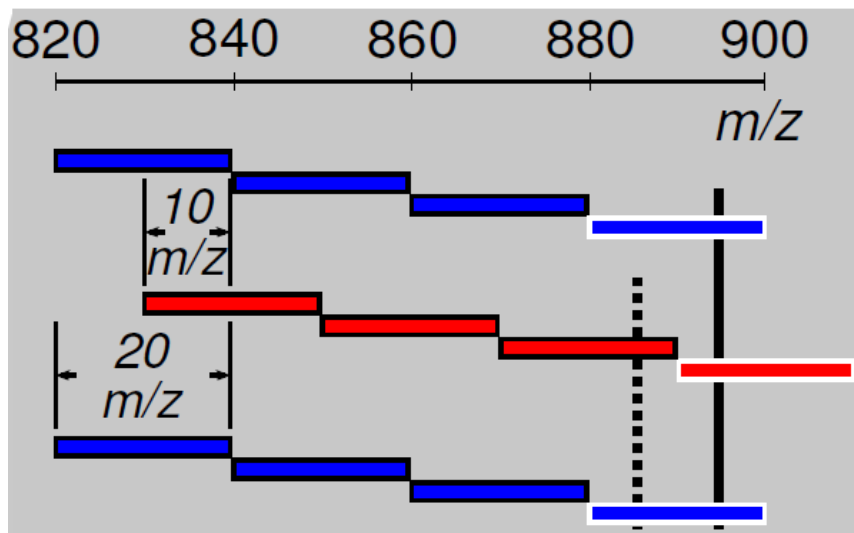
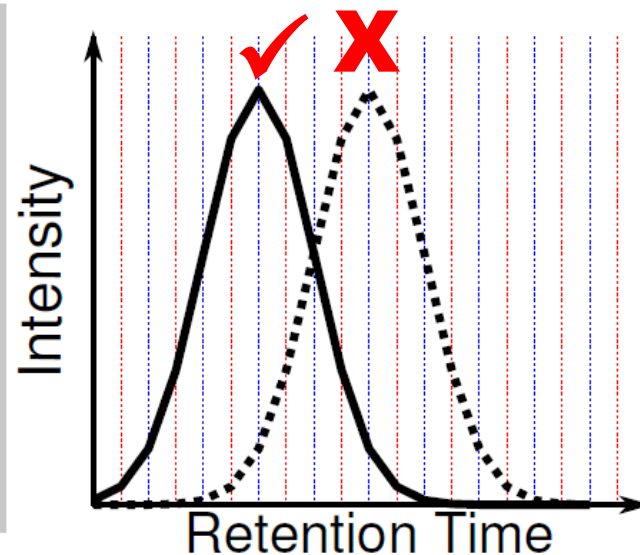
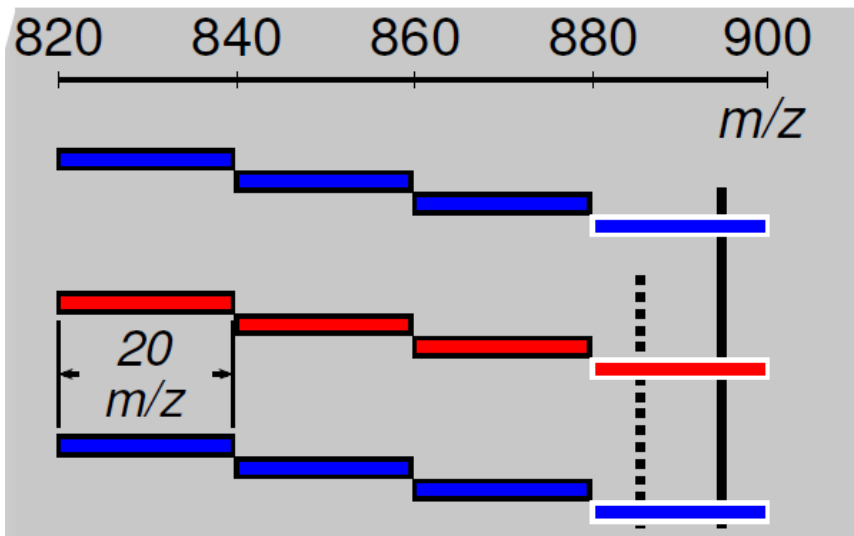
Improving Precursor Selectivity



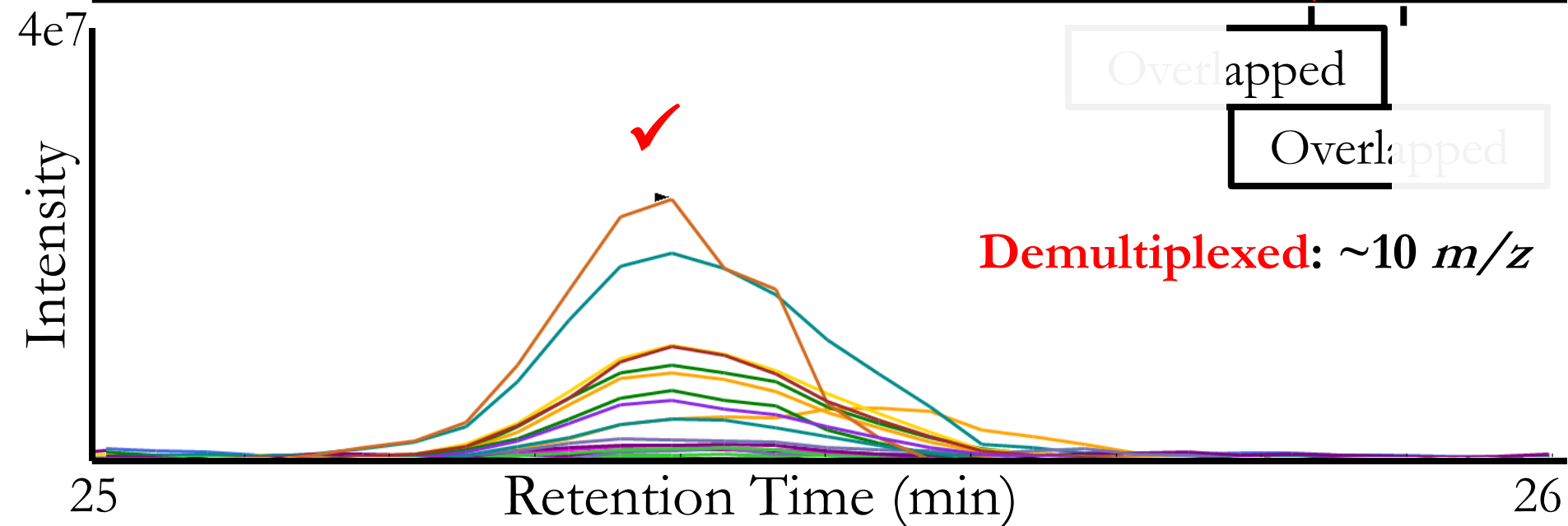
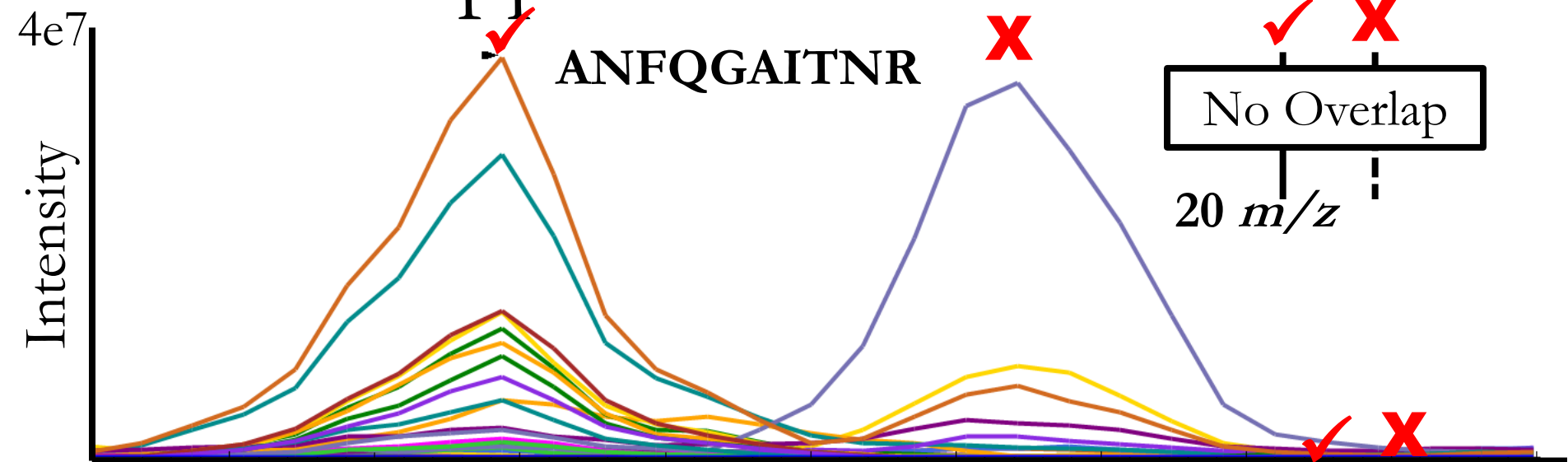
Improving Precursor Selectivity



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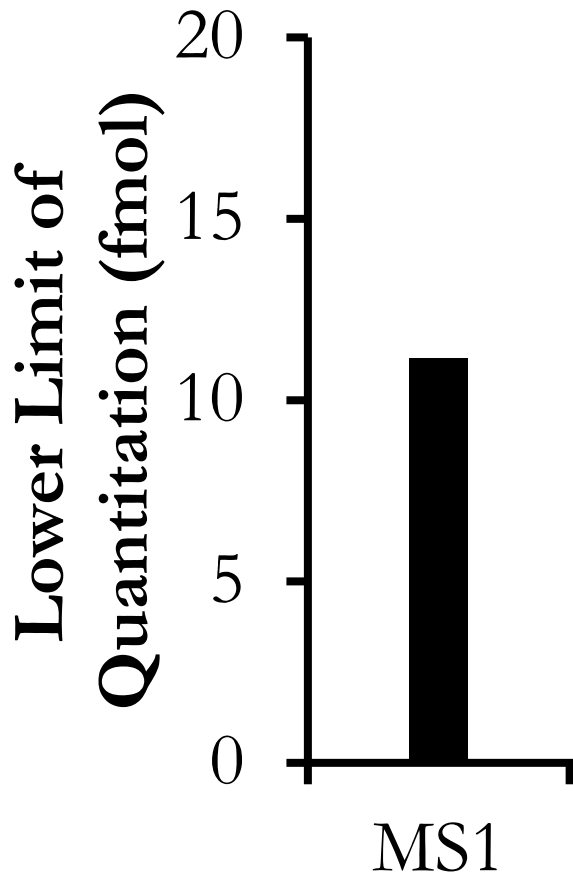


Overlapped Isolation Windows



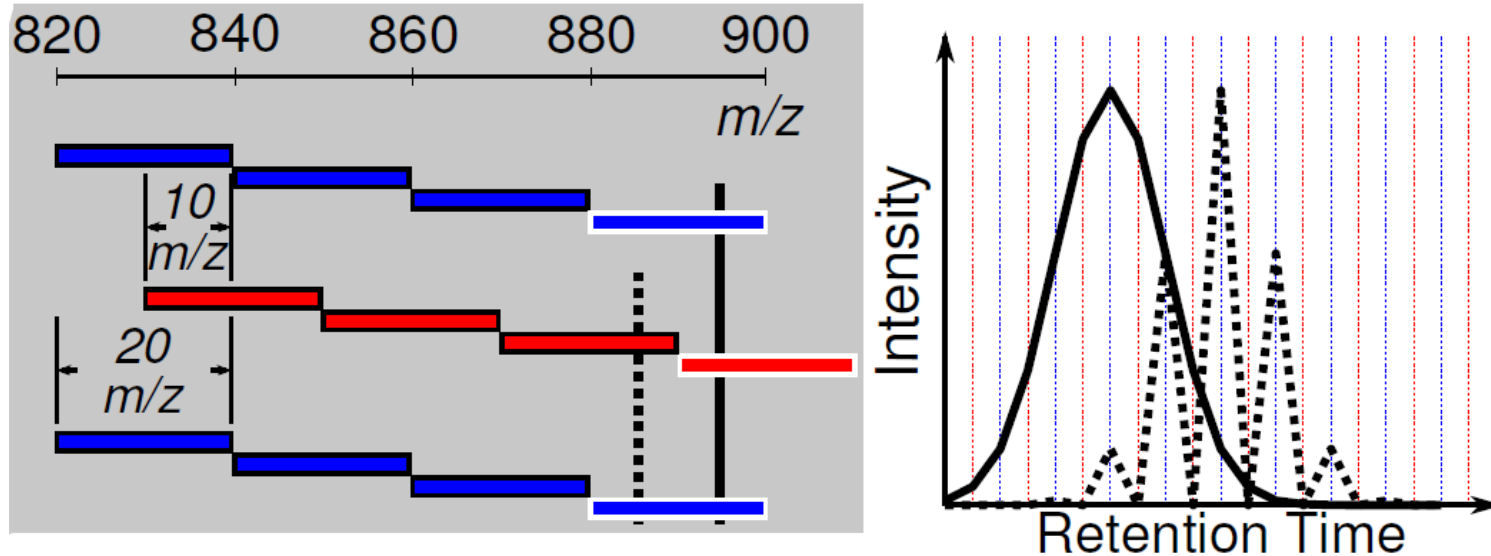
Improved Quantitation

■ 10 m/z ■ Demultiplexed ■ 20 m/z



21 Peptides Spiked Into
Yeast Lysate Quantified

Conclusions

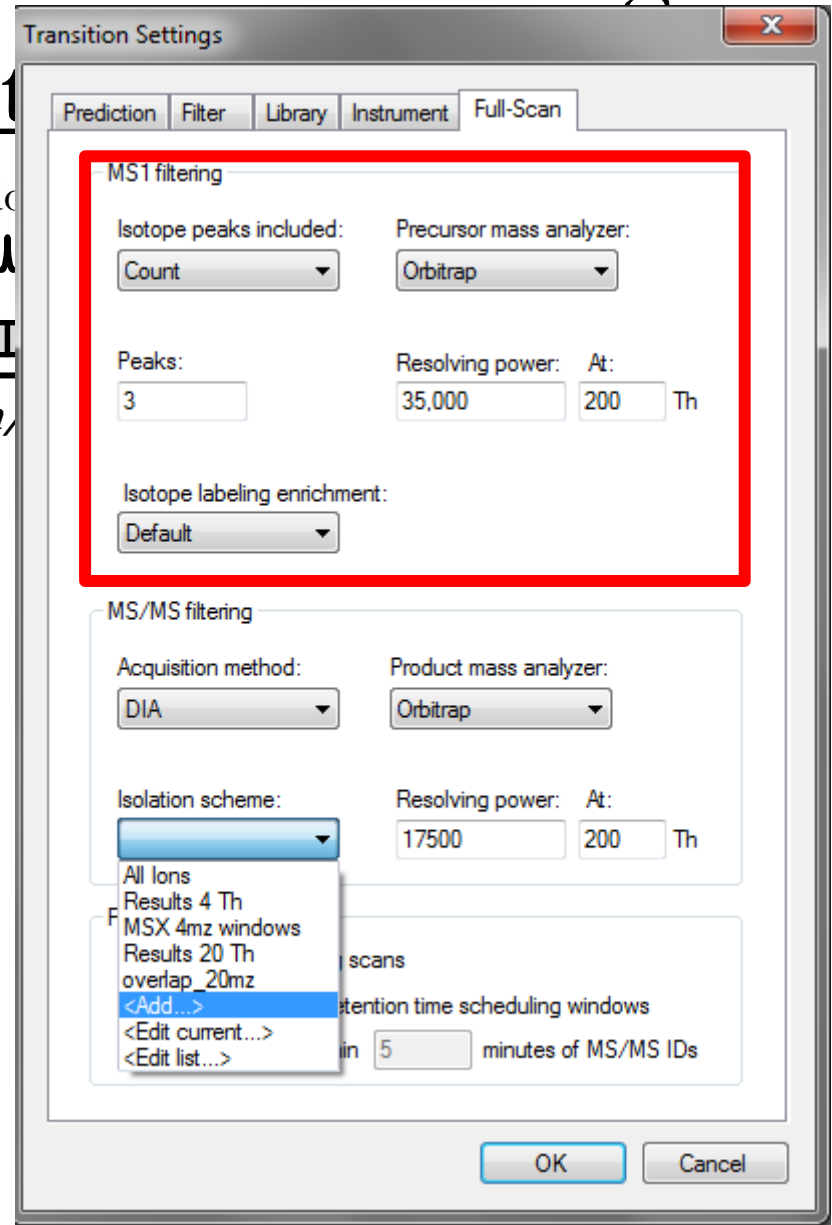
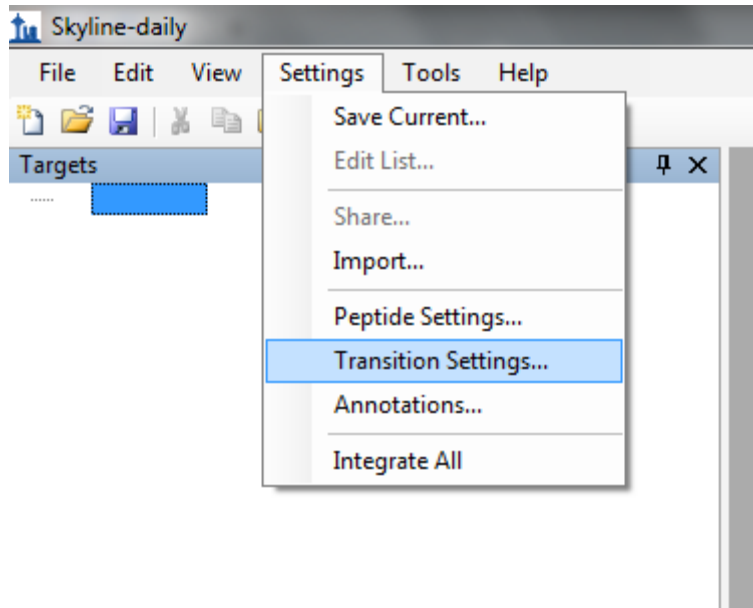
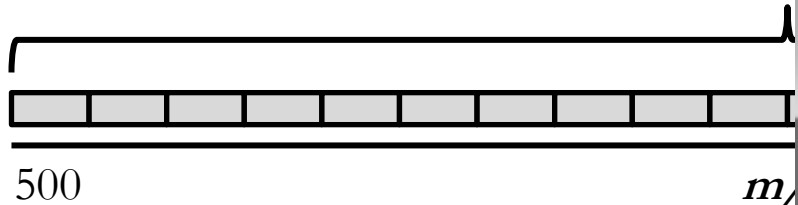


Overlapping Windows Improves Selectivity and Sensitivity of DIA

- **Easily applicable** to virtually any DIA-capable instrument
- De-multiplexing implemented in **Skyline** (multi-vendor support)
- These experiments can be done **now** with Skyline-daily

Generating a DIA Method Using Skyline: Generat

20 20 m/z -wide window



Generating a DIA Method Using Skyline: Generate a Target List

Edit Isolation Scheme

Name:

Use results data isolation targets

Isolation width: Th Deconvolution:

Asymmetric

Prespecified isolation windows

	Start	End	Target
*			

Deconvolution:

Margins:

Windows per scan:

Specify target

Calculate Isolation Scheme

Start m/z: End m/z:

Window width: Overlap: %

Window count: 20

Multiplexed acquisition

Windows per scan:

Margins:

Margin width:

Optimize window placement Generate target

Generating a DIA Method Using Skyline: Generate a Target List

The image shows a dialog box titled "Calculate Isolation Scheme" with a close button (X) in the top right corner. The dialog contains the following fields and options:

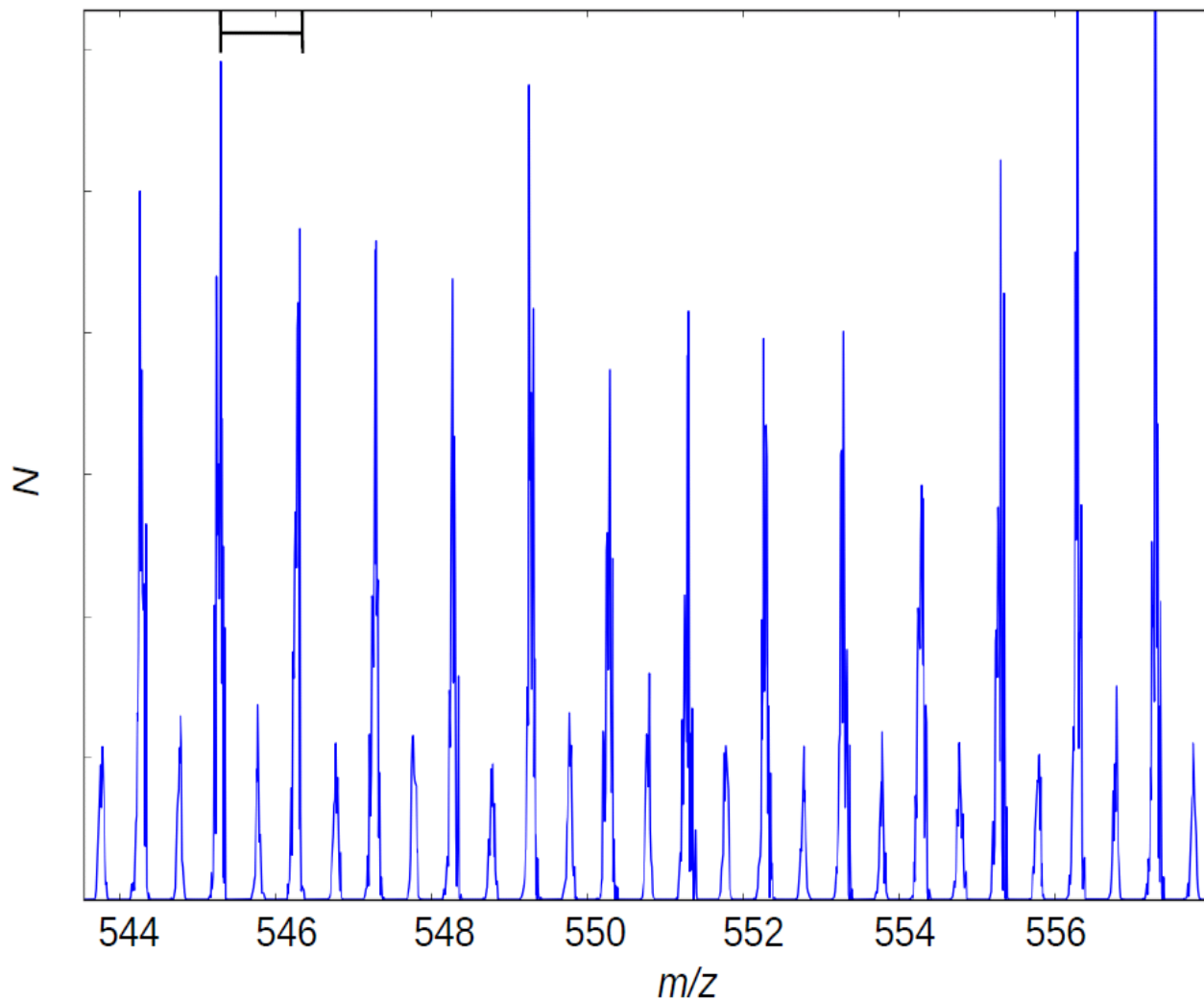
- Start m/z: 500
- End m/z: 900
- Window width: 20
- Overlap: [] %
- Window count: 21
- Multiplexed acquisition
- Windows per scan: []
- Margins: None (dropdown menu)
- Margin width: []
- Optimize window placement
- Generate target

Buttons: OK, Cancel

Generating a DIA Method Using Skyline: Generate a Target List

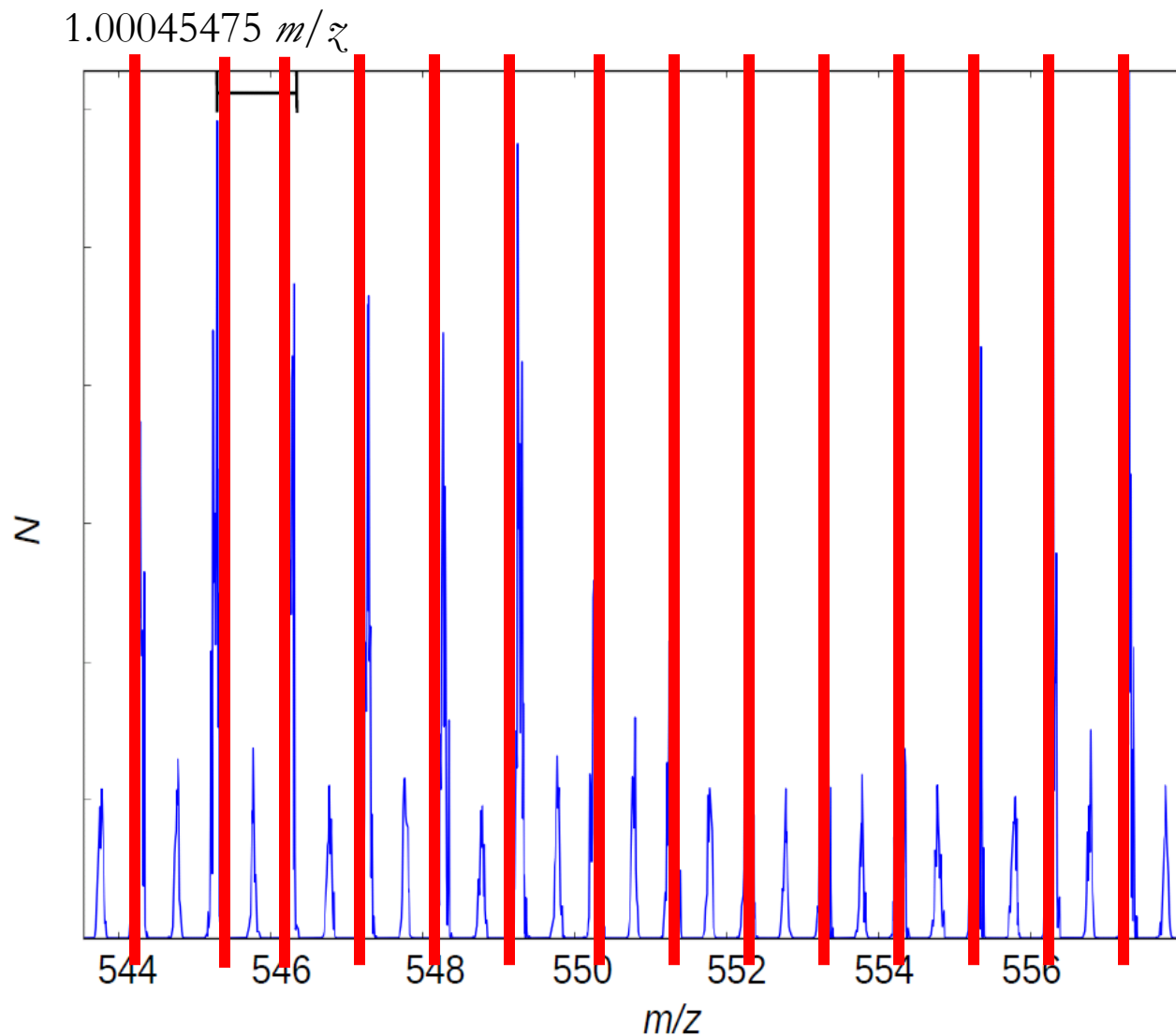
1.00045475 m/z

	Mass	Excess
H	1.00078	0.00078
C	12	0.0
O	15.9949	0.9949
N	14.0031	0.0031
S	31.9721	0.9721



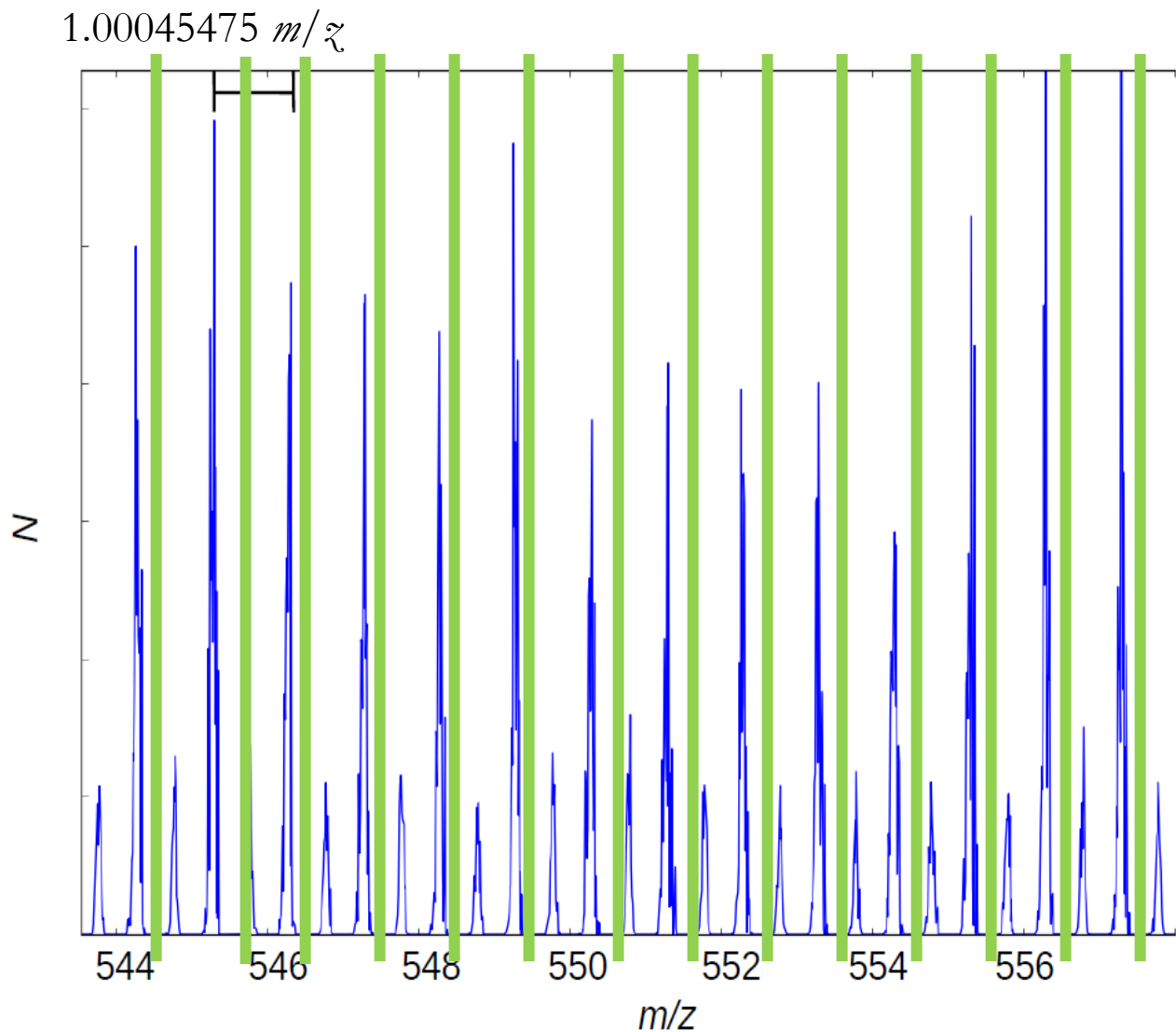
Generating a DIA Method Using Skyline: Generate a Target List

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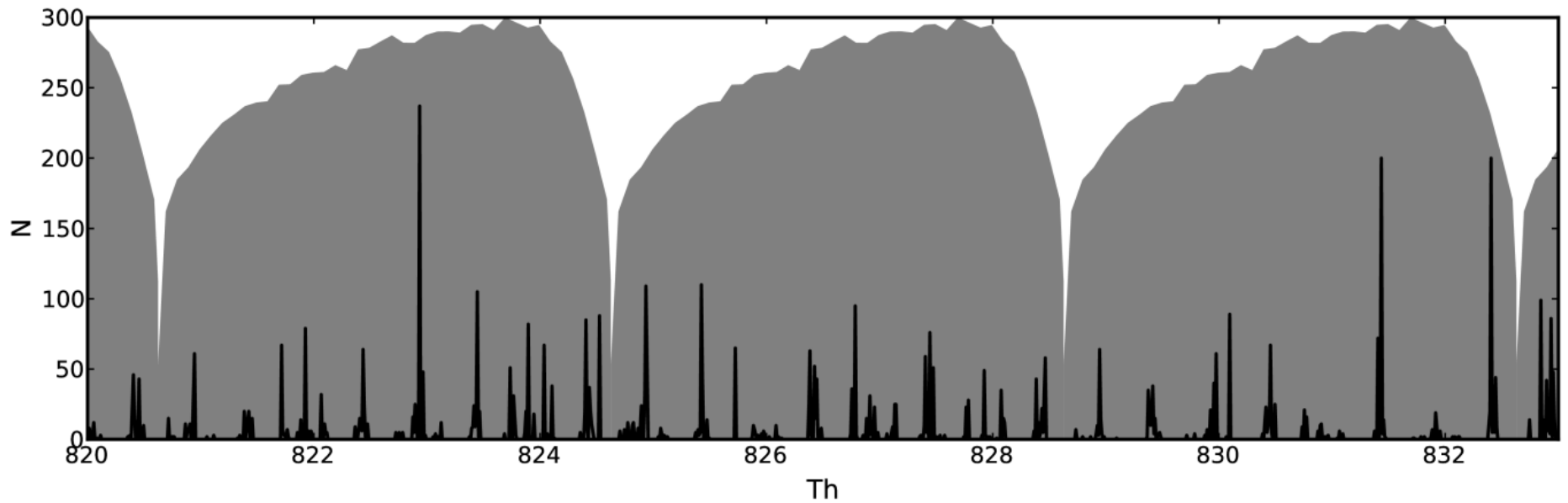


Generating a DIA Method Using Skyline: Generate a Target List

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Generating a DIA Method Using Skyline: Generate a Target List



Generating a DIA Method Using Skyline: Generate a Target List

Edit Isolation Scheme

Name:

Use results data isolation targets

Isolation width: Th Deconvolution:

Asymmetric

Prespecified isolation windows

	Start	End	Target
▶	480.4683	500.4774	490.4728
	500.4774	520.4865	510.4819
	520.4865	540.4956	530.4910
	540.4956	560.5047	550.5001
	560.5047	580.5138	570.5092
	580.5138	600.5229	590.5183
	600.5229	620.5319	610.5274
	620.5319	640.5410	630.5365
	640.5410	660.5501	650.5456
	660.5501	680.5592	670.5547
	680.5592	700.5683	690.5638
	700.5683	720.5774	710.5729
	720.5774	740.5865	730.5820

Importing Data: Filtering Settings

Transition Settings

Prediction Filter Library Instrument Full-Scan

Precursor charges: 2, 3 Ion charges: 1 Ion types: y,b,p

Product ions

From: m/z > precursor To: 3 ions

Always add:

- N-terminal to Proline
- C-terminal to Glu or Asp
- N-terminal to Proline (legacy)

Edit List...

Precursor m/z exclusion window: Th

Auto-select all matching transitions

OK Cancel

Acknowledgements

University of Washington

Mike MacCoss

Brendan M

Don M

Gennifer M

Richard J

Sonia Ting

& the rest of the
lab

Stanford University

Dario Amodei

Parag Mallick

Dario's Poster: Tuesday June 11th

(#512) 10:30 AM – 2:30 PM

Jarrett's Talk: Monday, June 10th

8:30-8:50AM Exhibit Hall A

Andreas Kuehn

Reiko Kiyonami

Yue Xuan

University

x

ntific

mann