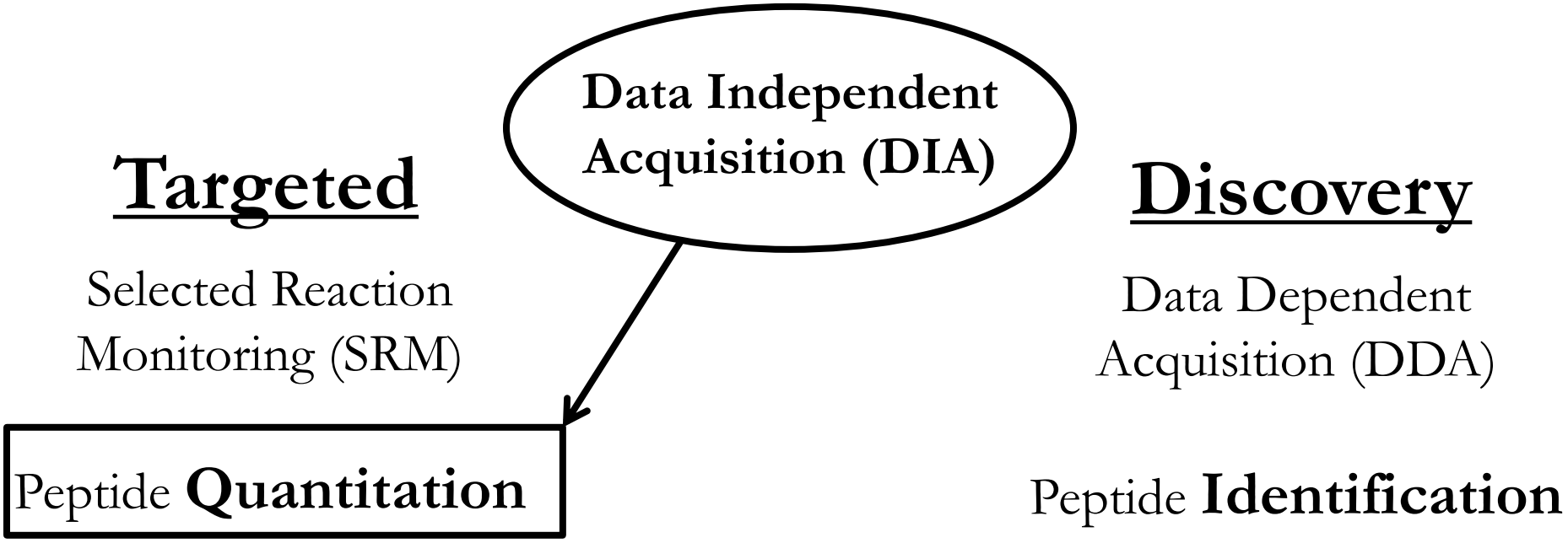
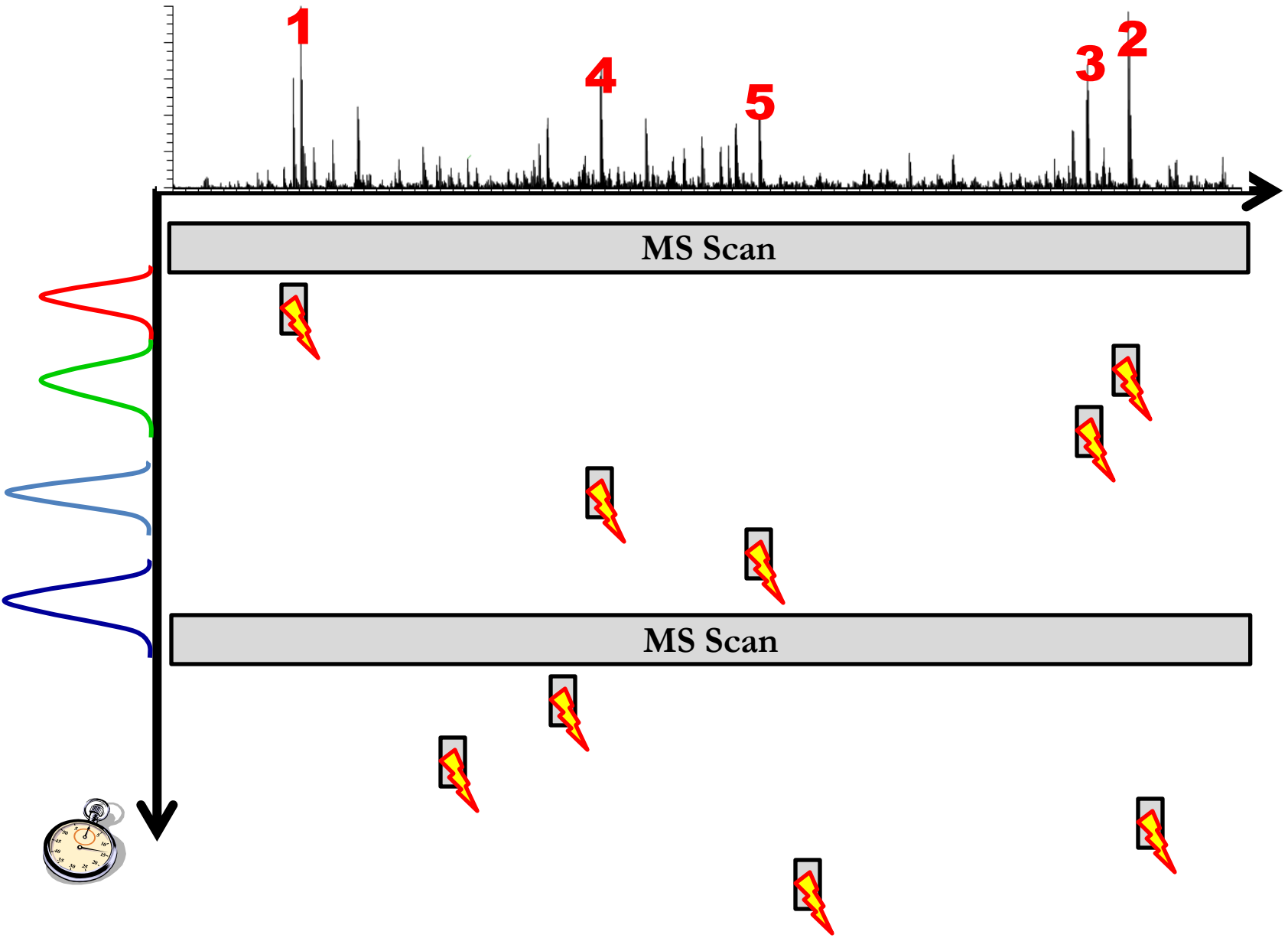
Application of Data Independent Acquisition Techniques Optimized for Improved Precursor Selectivity

Jarrett D. Egertson, Ph.D.
MacCoss Lab
Department of Genome Sciences
University of Washington
6/8/2013

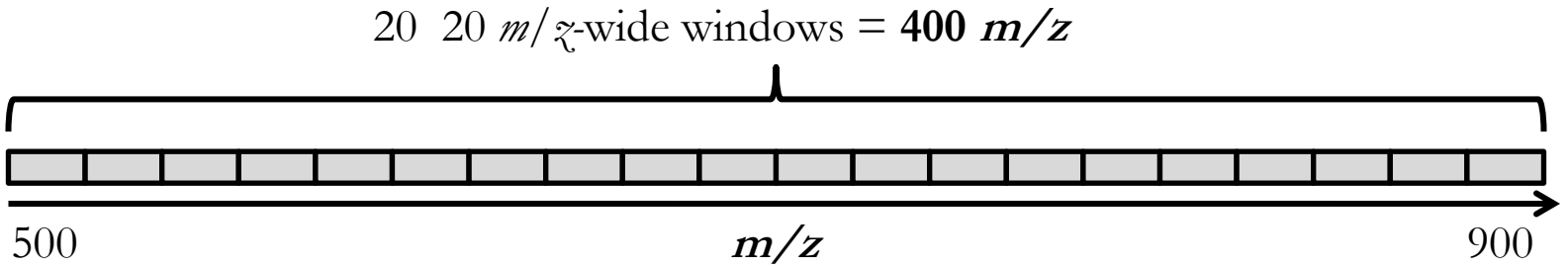
Acquisition Methods



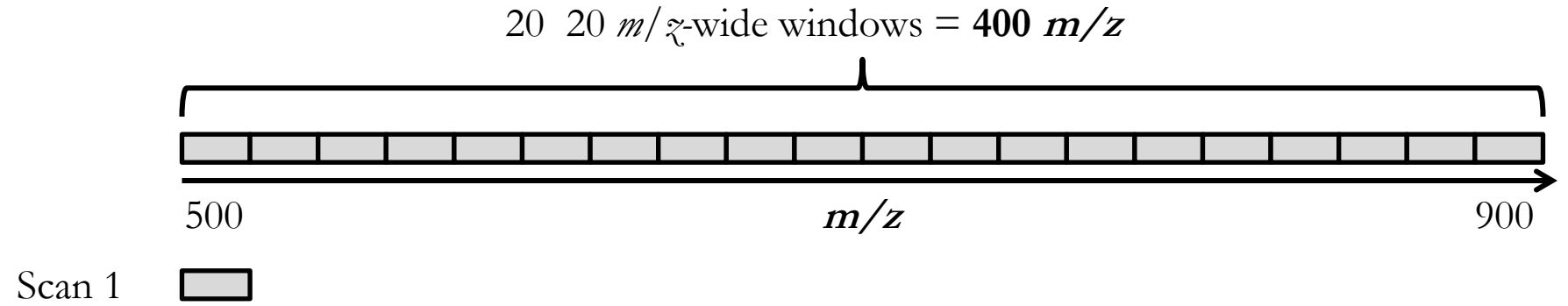
LC–MS/MS: Data Dependent



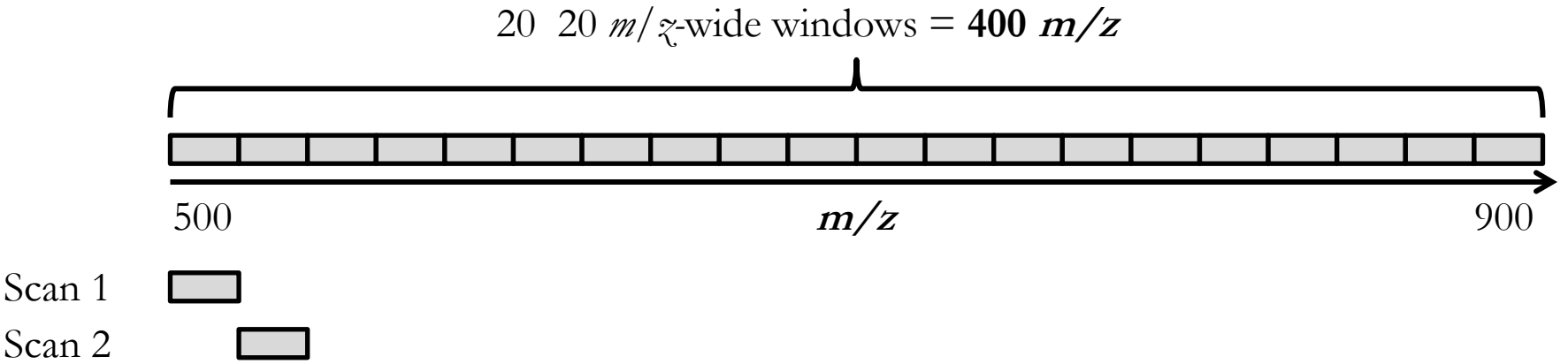
Data Independent Acquisition (DIA)



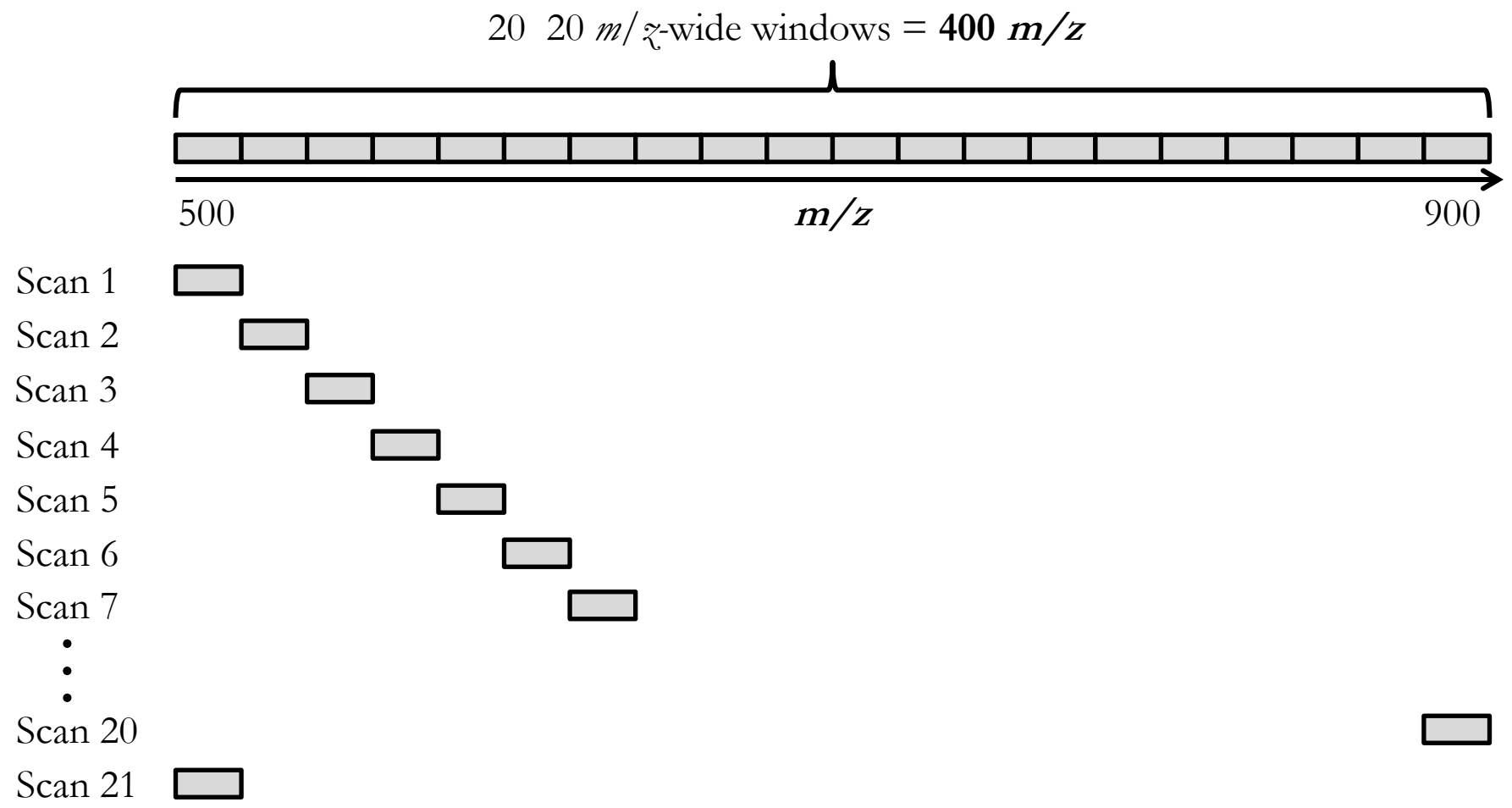
Data Independent Acquisition (DIA)



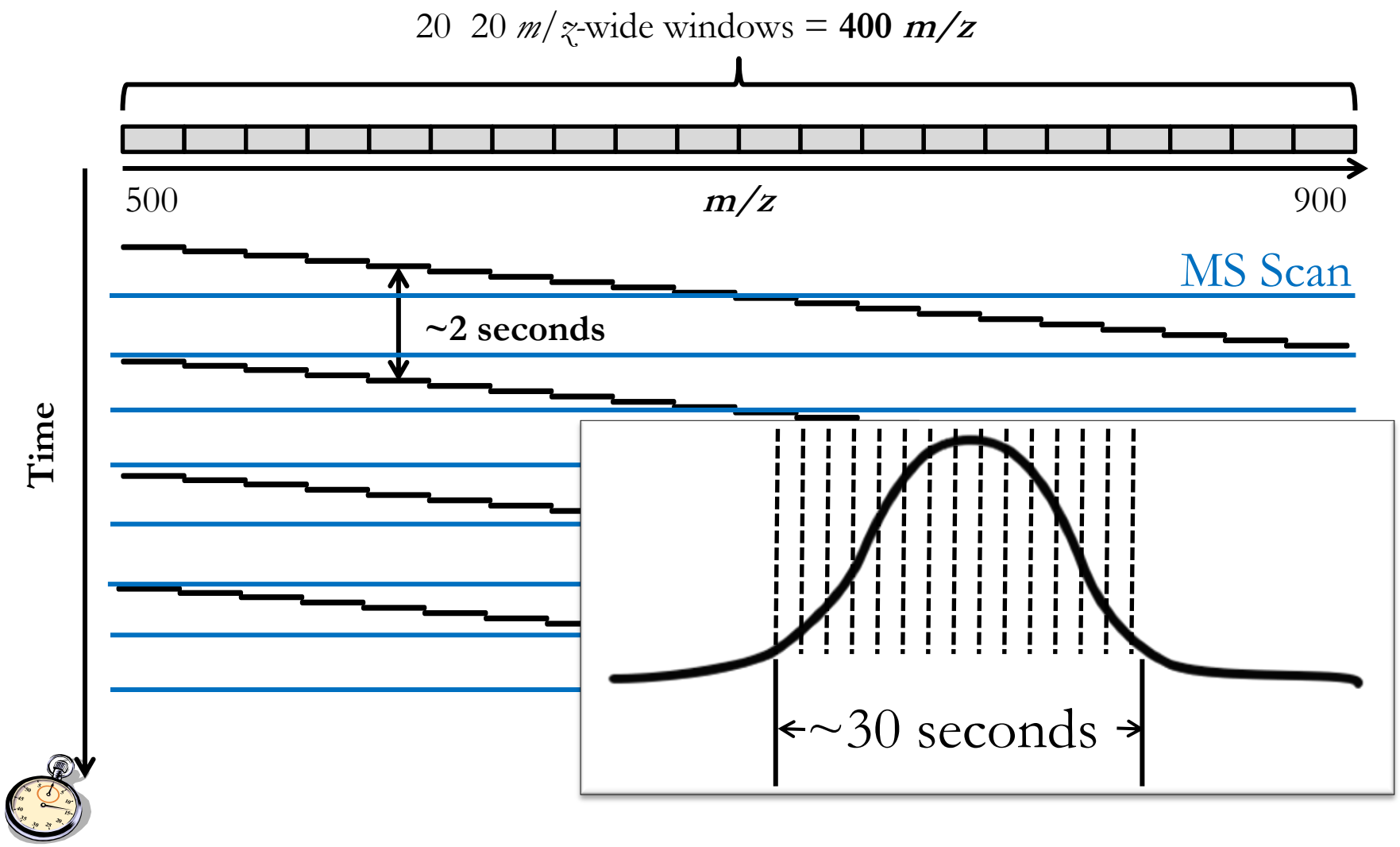
Data Independent Acquisition (DIA)



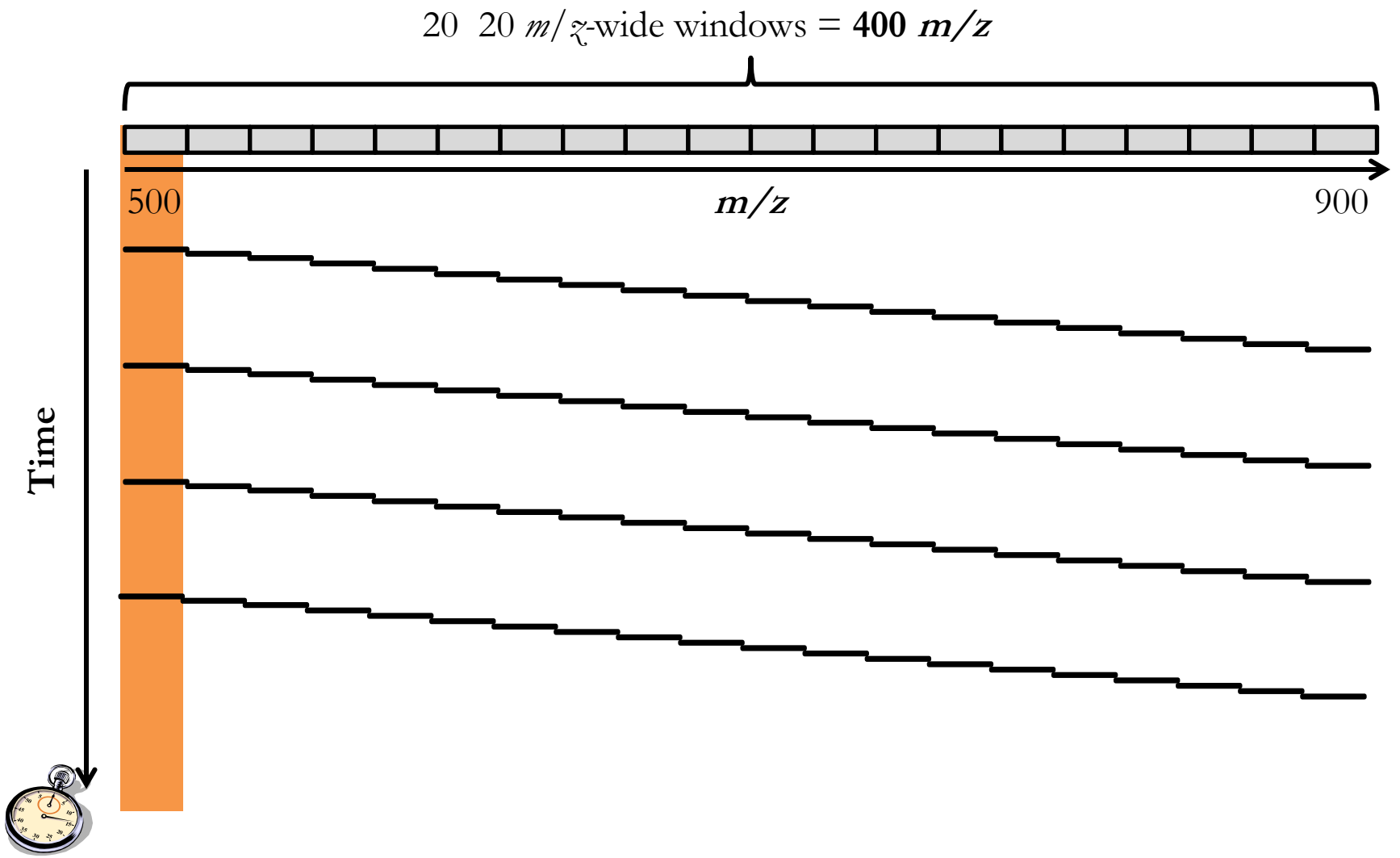
Data Independent Acquisition (DIA)



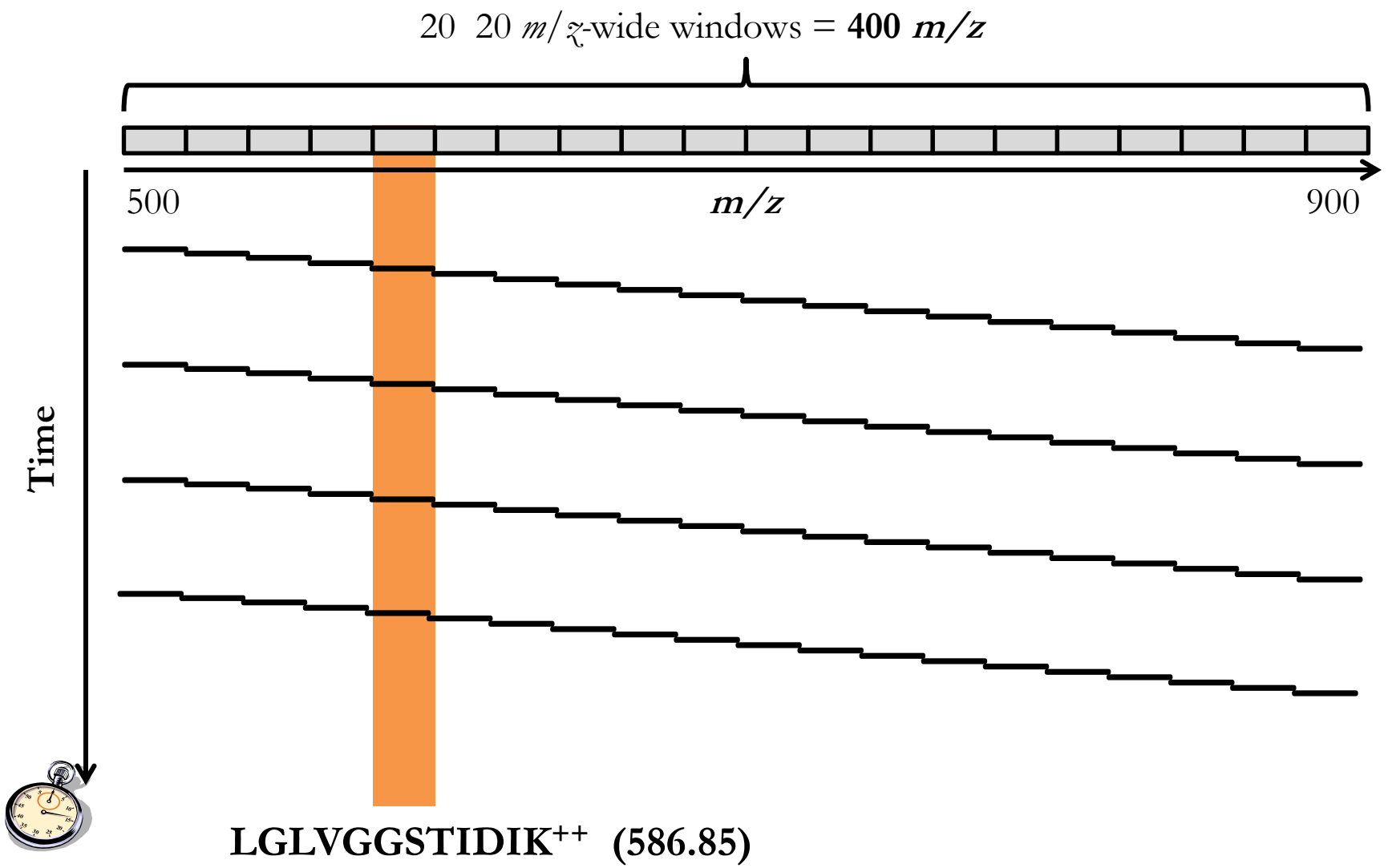
Data Independent Acquisition (DIA)



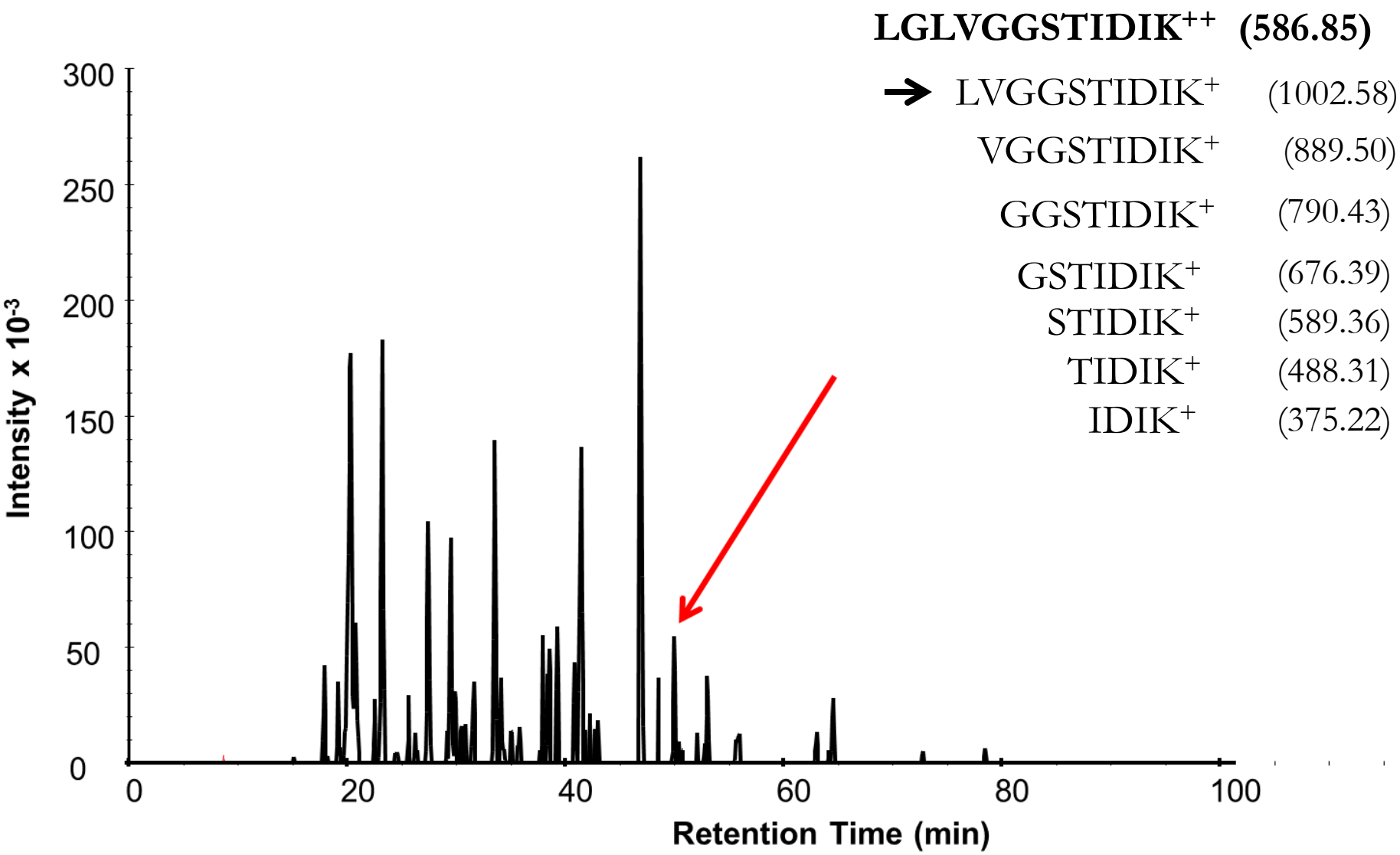
Data Independent Acquisition (DIA)



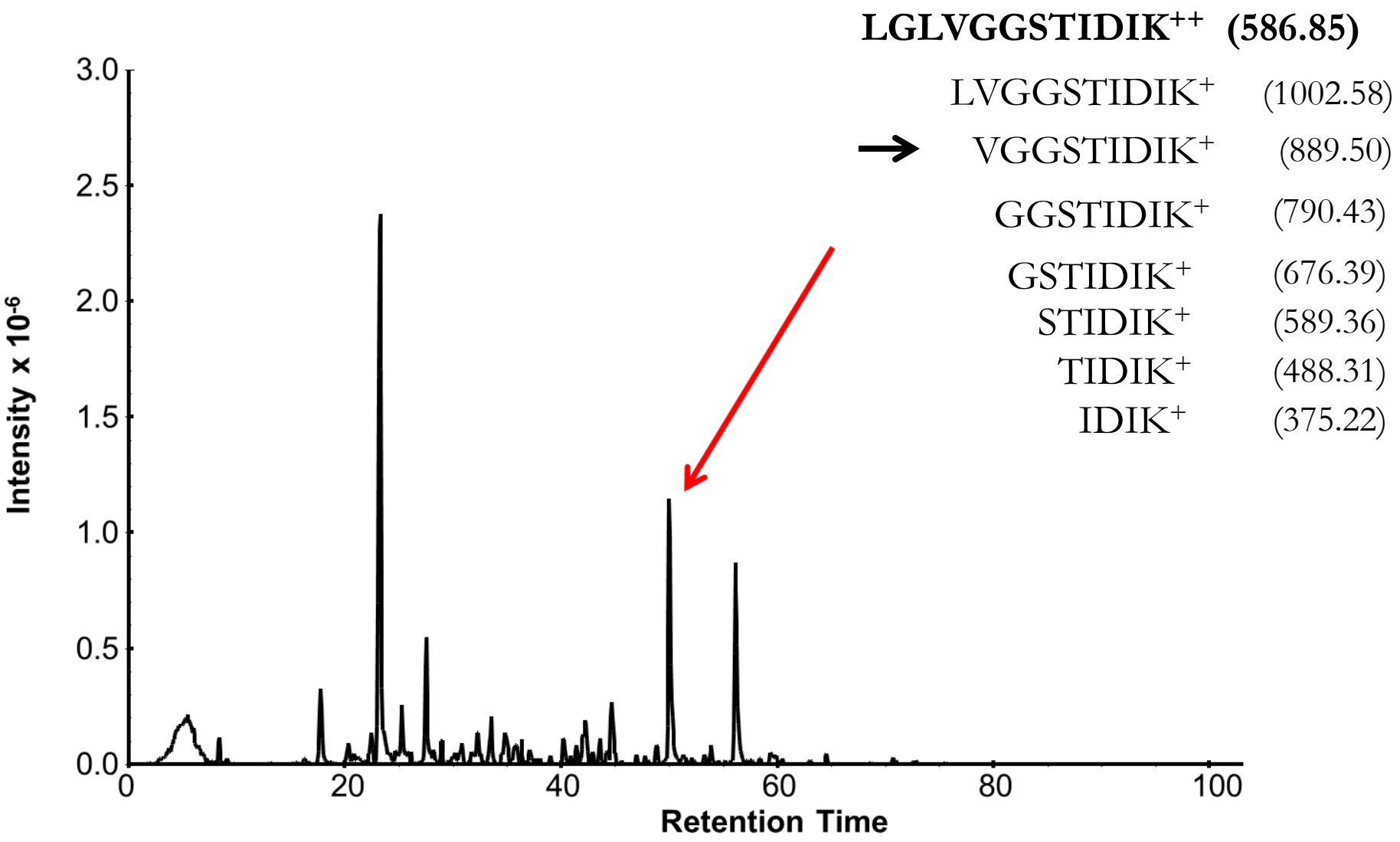
Data Independent Acquisition (DIA)



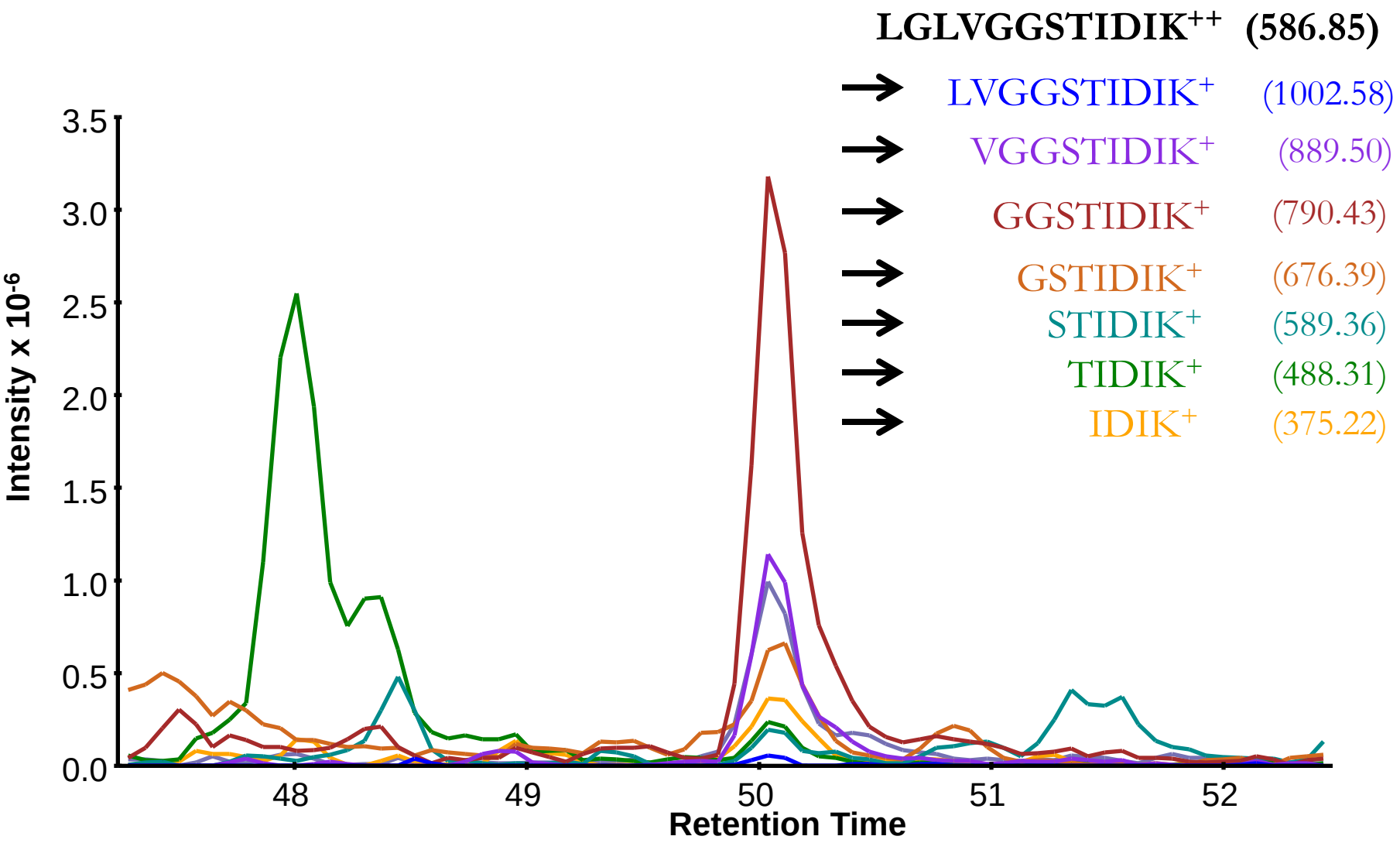
Data Independent Acquisition (DIA)



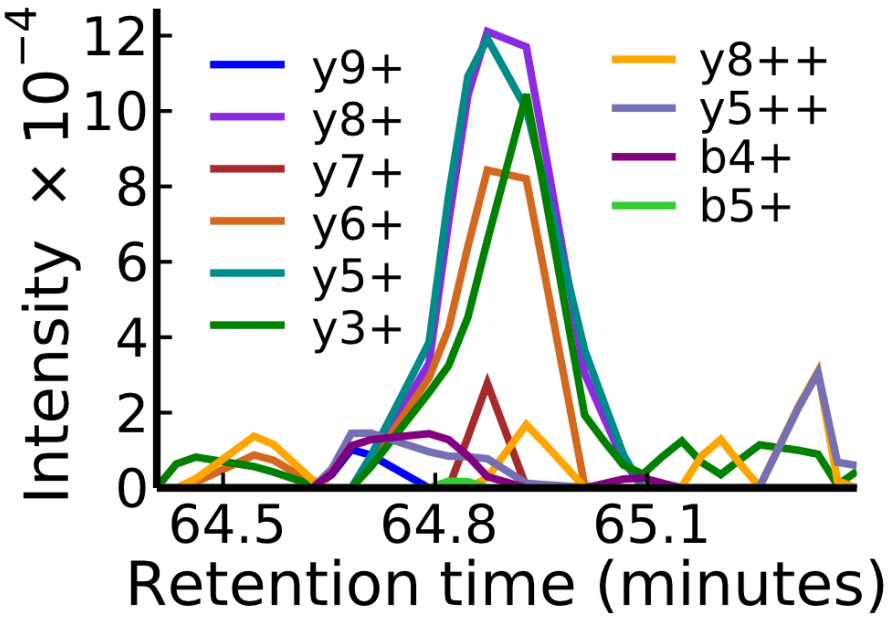
Data Independent Acquisition (DIA)



Data Independent Acquisition (DIA)

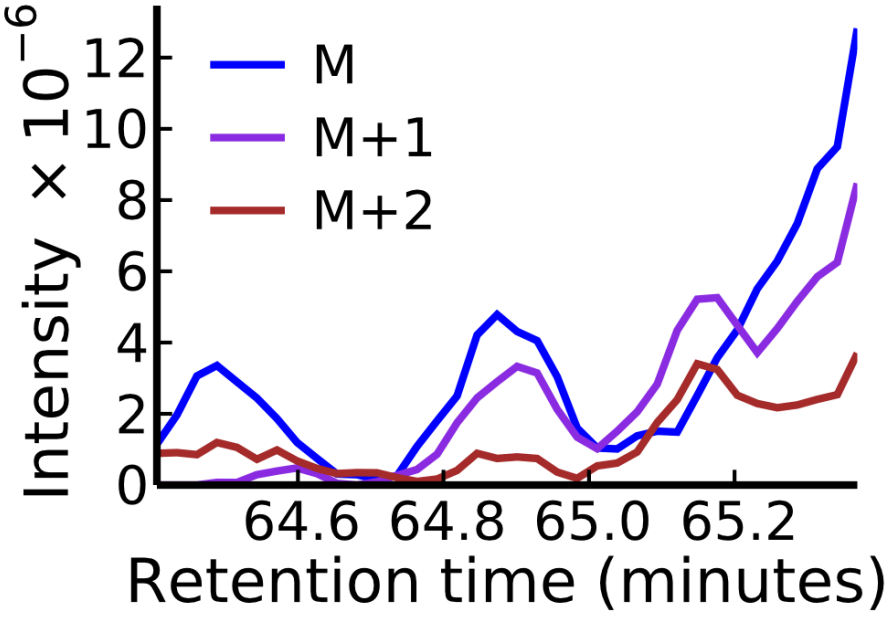


MS/MS

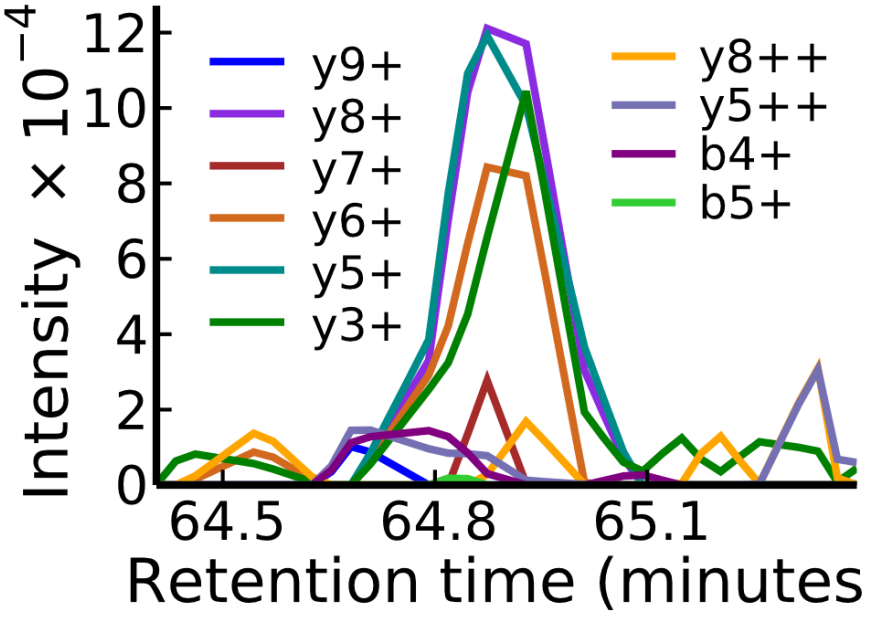


1.02 femtomoles of Bovine Serum Albumin (LVNELTEFAK++) in 1.2 ug of *S. cerevisiae* lysate

MS

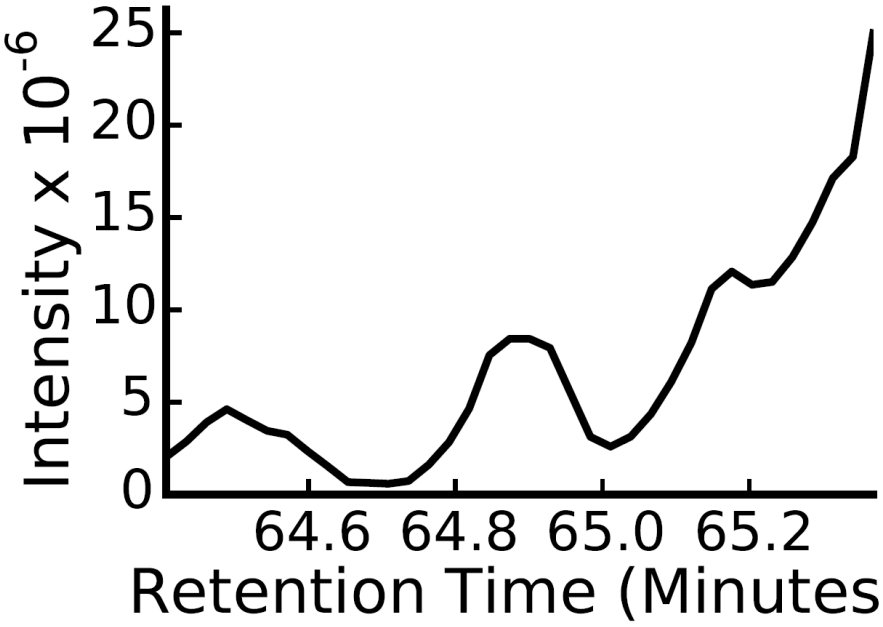


MS/MS

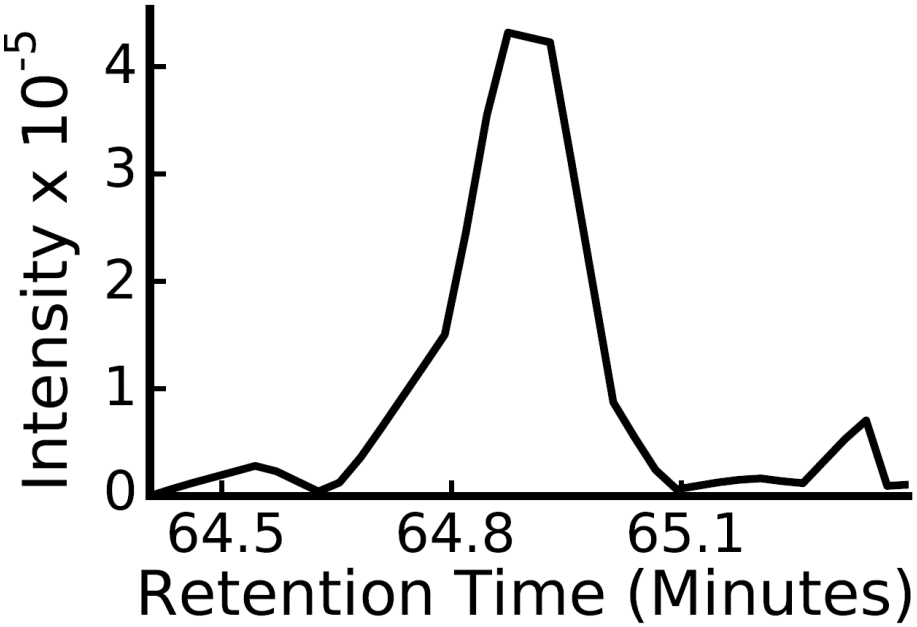


1.02 femtomoles of Bovine Serum Albumin (LVNELTEFAK++) in 1.2 ug of *S. cerevisiae* lysate

MS



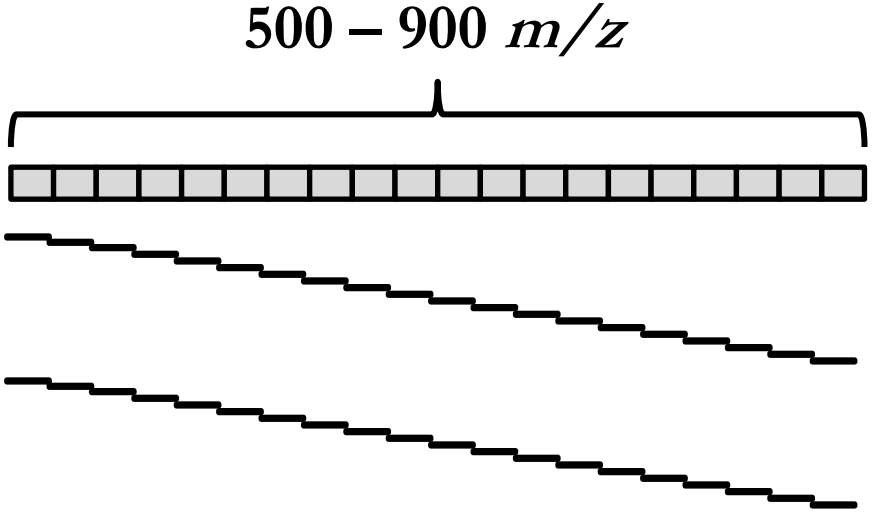
MS/MS



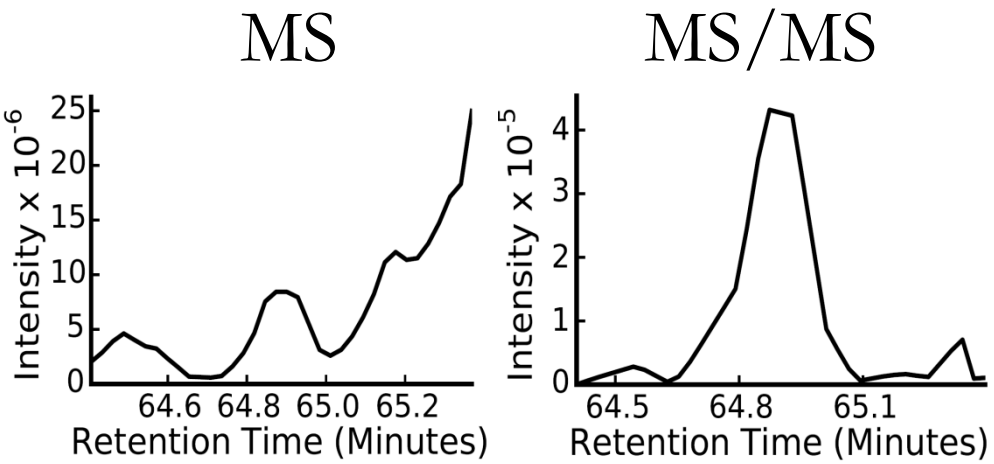
1.02 femtomoles of Bovine Serum Albumin (LVNELTEFAK++) in 1.2 ug of *S. cerevisiae* lysate

Theoretical Benefits of DIA

- Comprehensive Sampling
  - Reproducibility



- Improved Quantitation



Isolation Window Width

DDA

2 ***m/z***

Vs.

DIA

10 ***m/z***

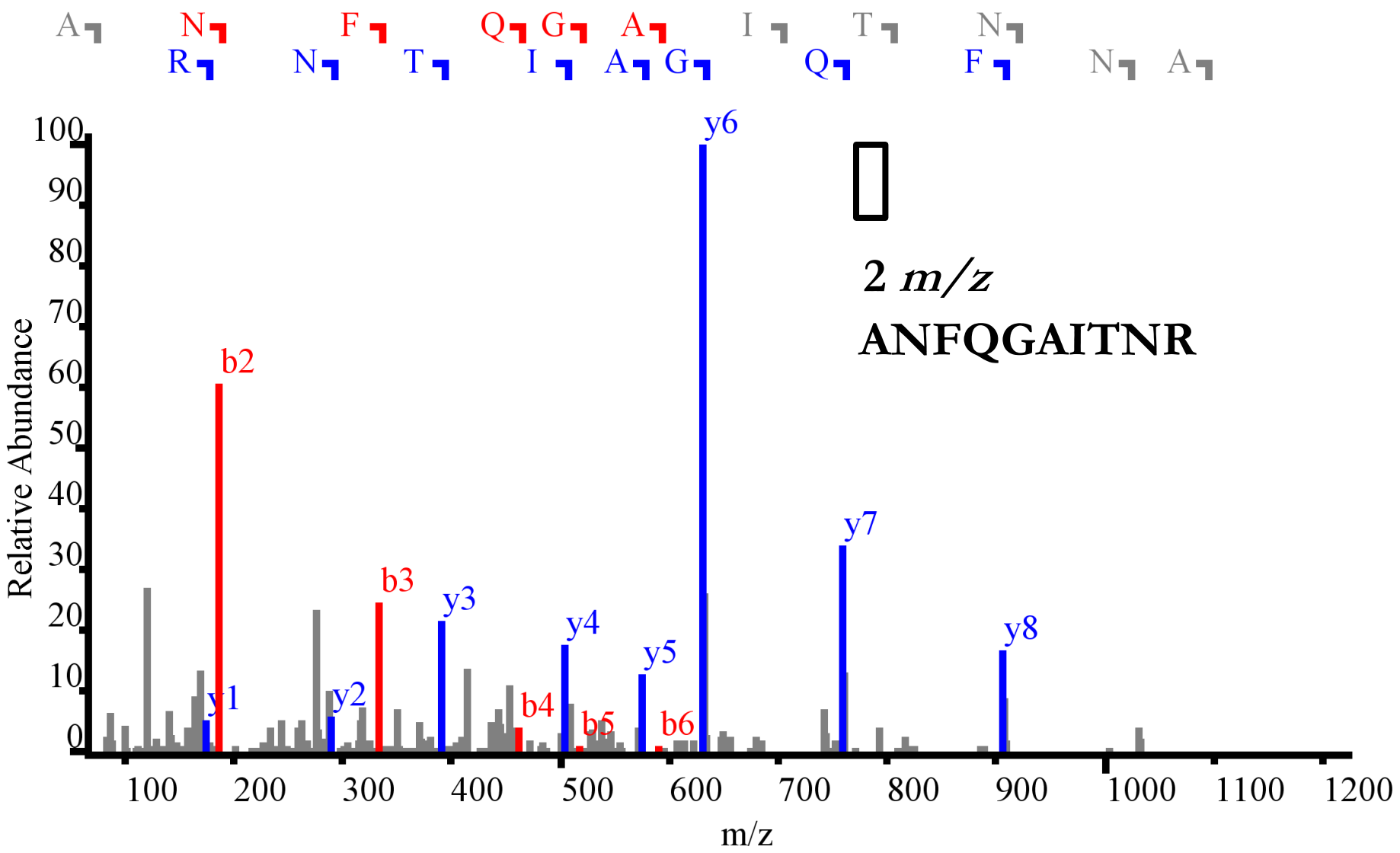
Vs.

20 ***m/z***

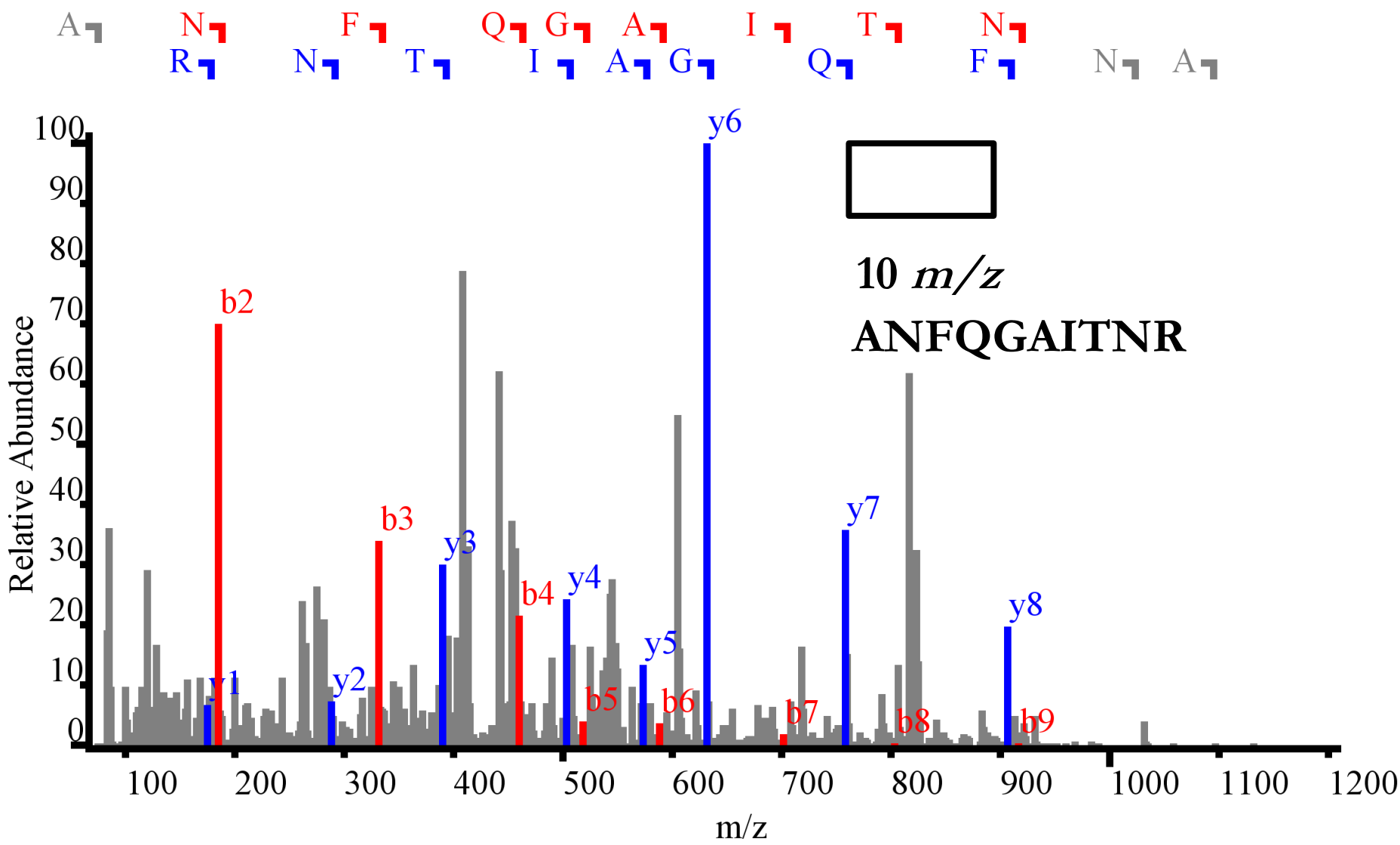
Lower precursor selectivity

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

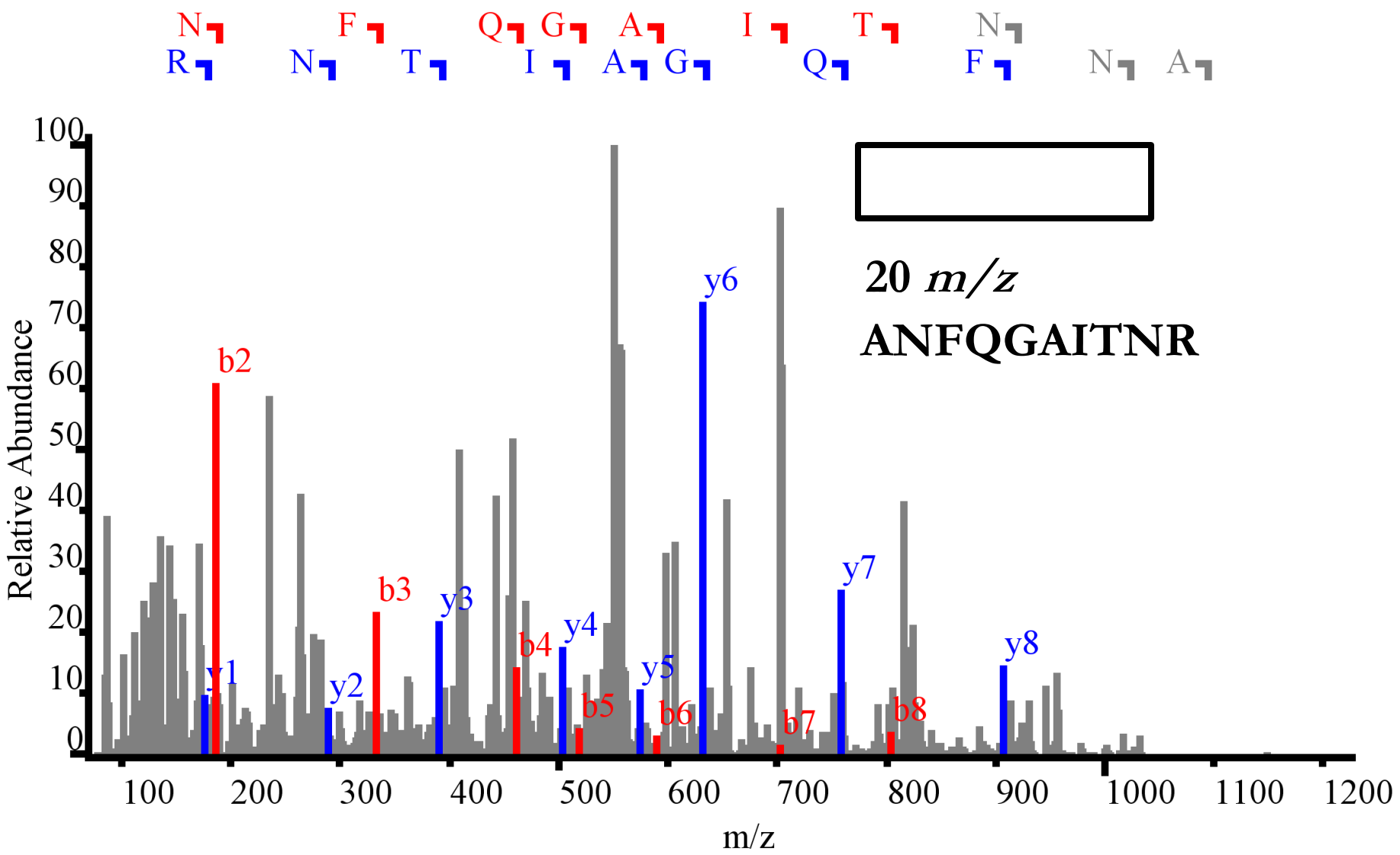
Precursor Selectivity



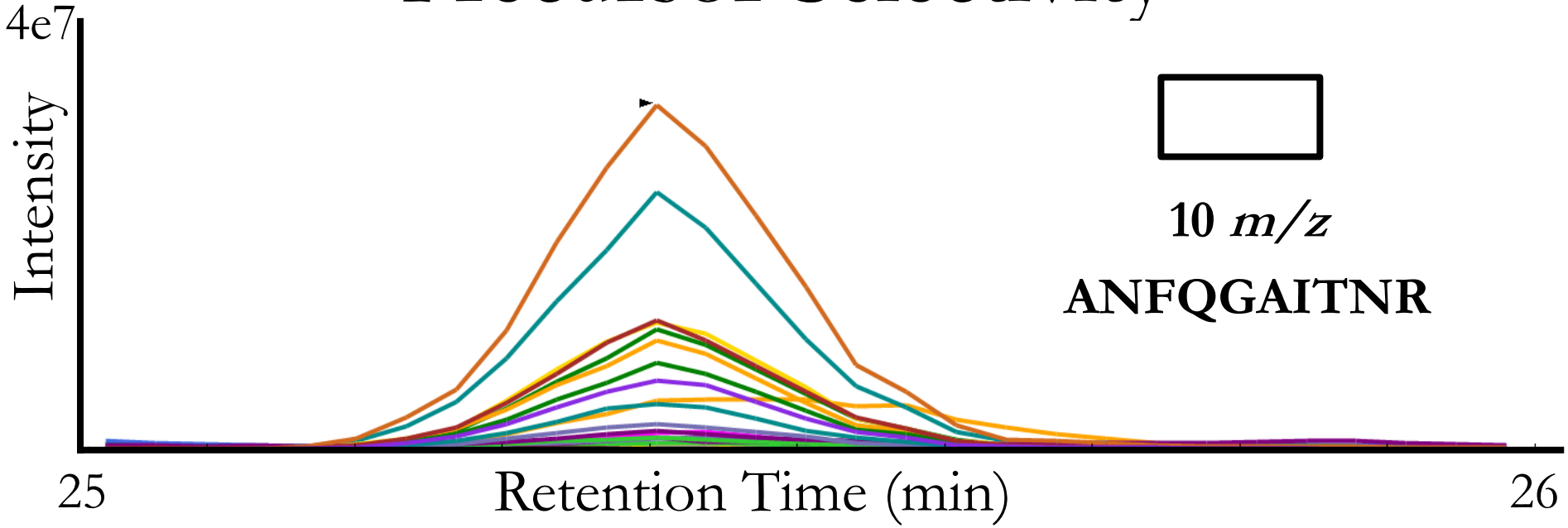
Precursor Selectivity

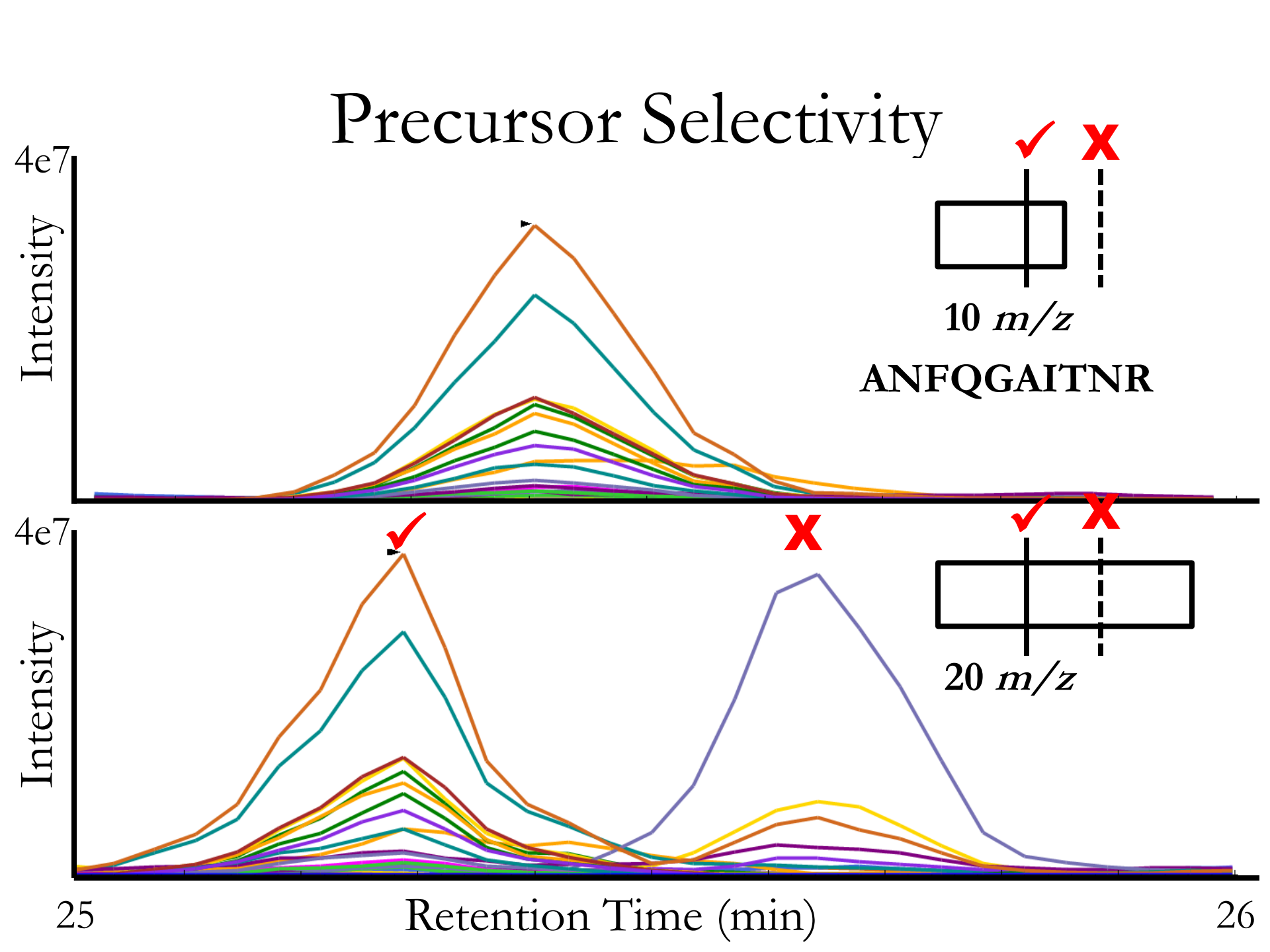


Precursor Selectivity

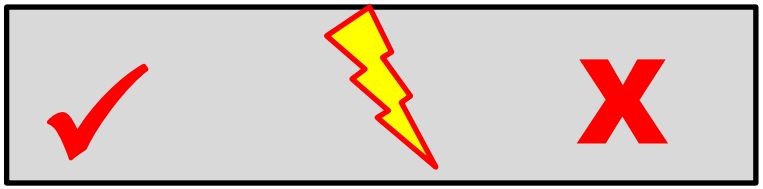


Precursor Selectivity




Precursor Selectivity
4e7
Intensity
10 m/z
ANFQGAITNR
4e7
Intensity
20 m/z
25
Retention Time (min)
26

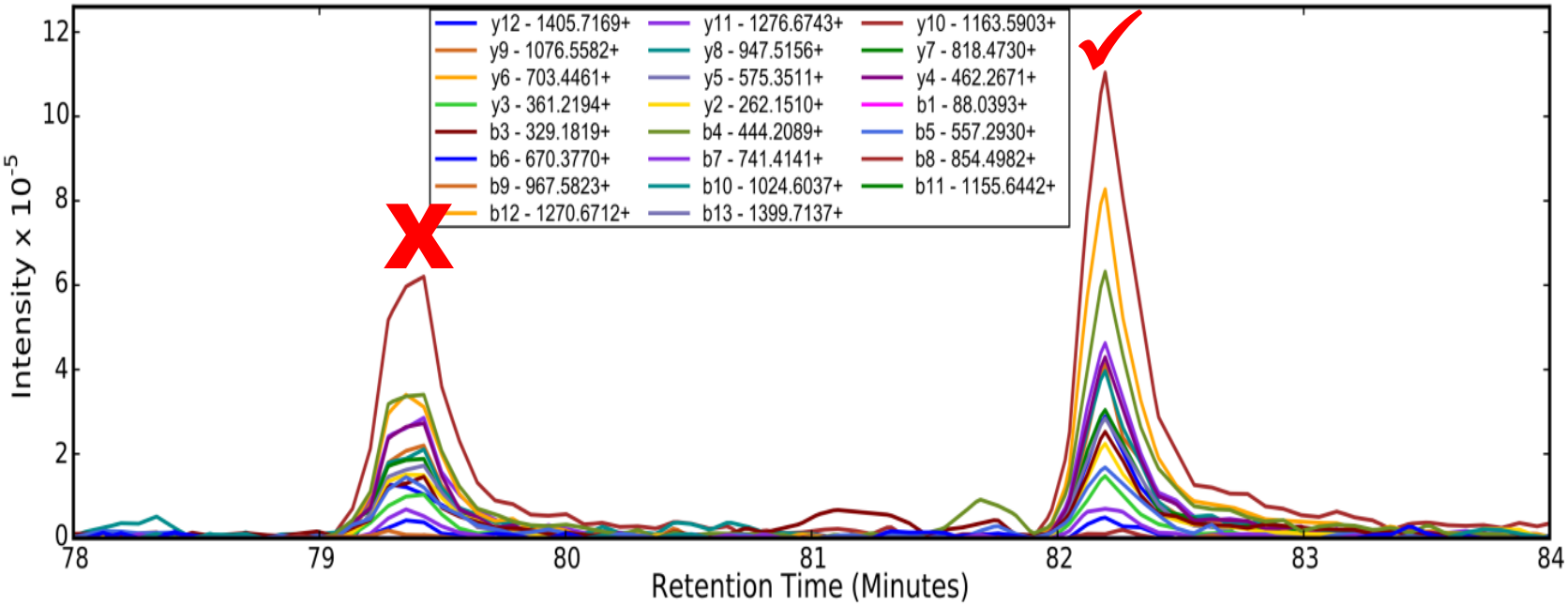
Precursor Selectivity



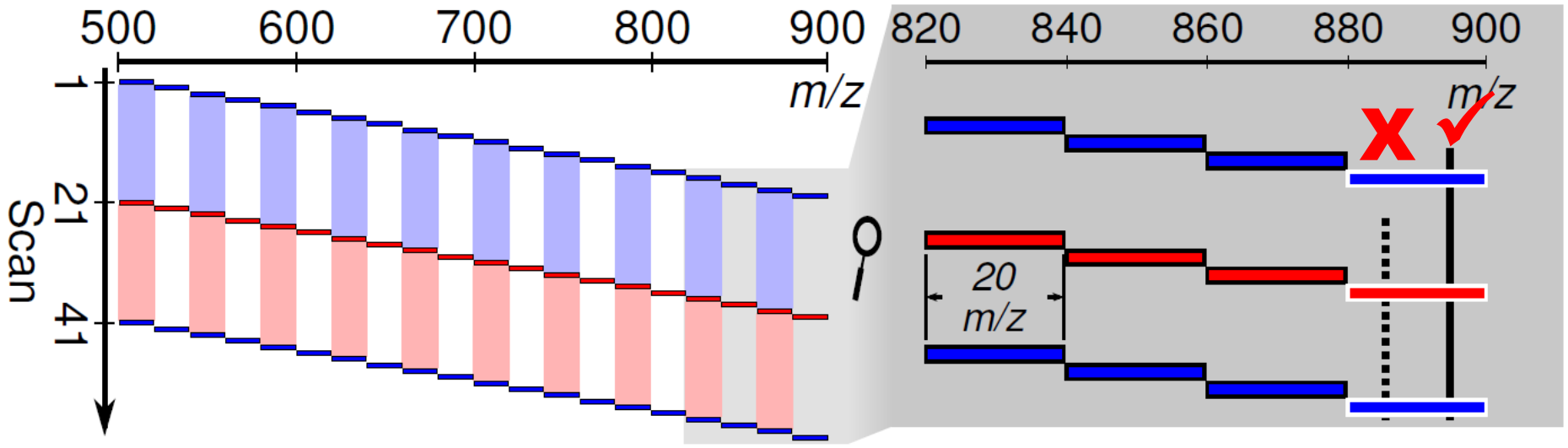
890 **900**

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

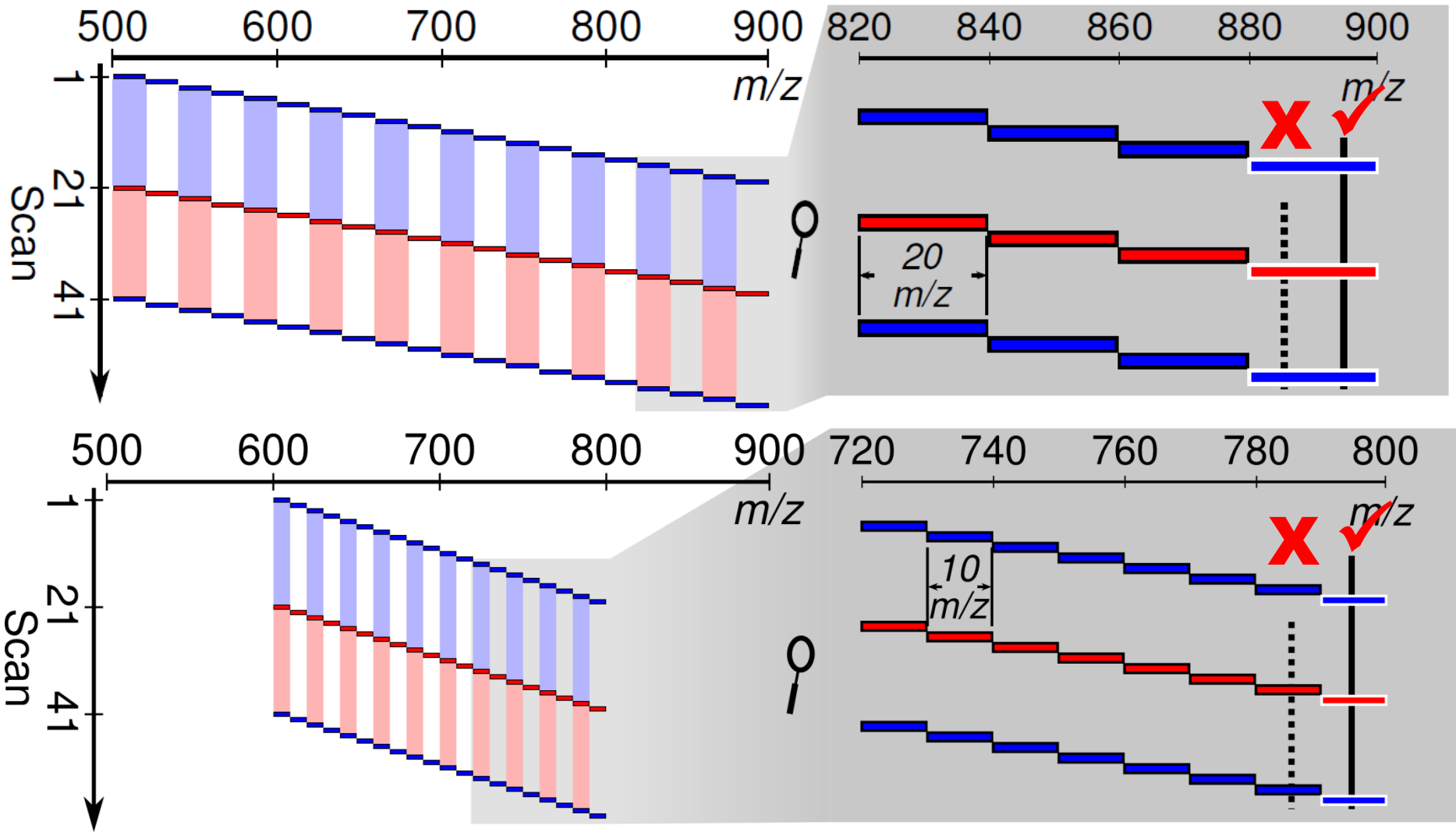
SLQDIIAILGMDELSEEDKLTVSR+++ (892.47 *m/z*)



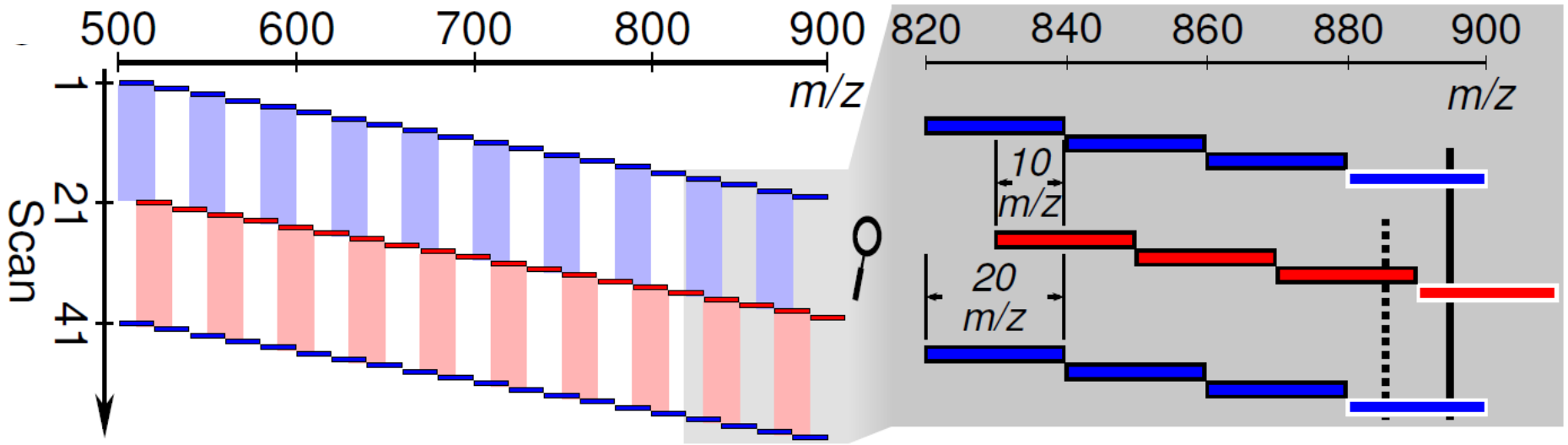
Improving Precursor Selectivity



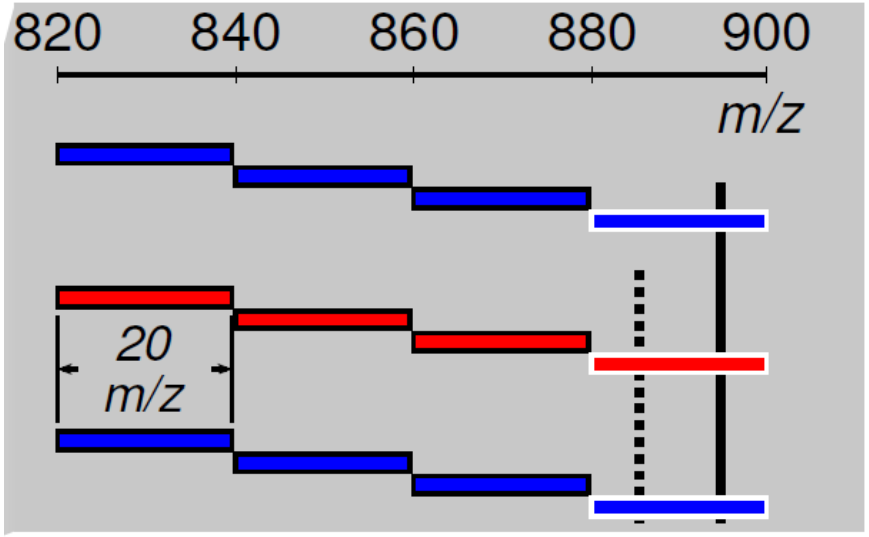
Improving Precursor Selectivity

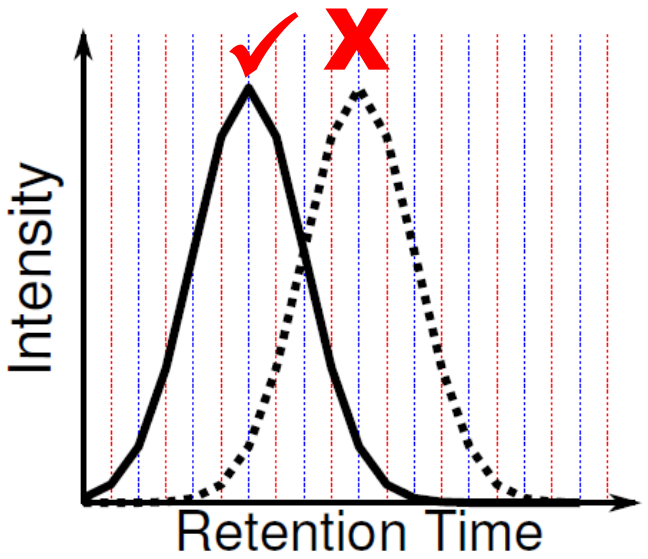


Improving Precursor Selectivity

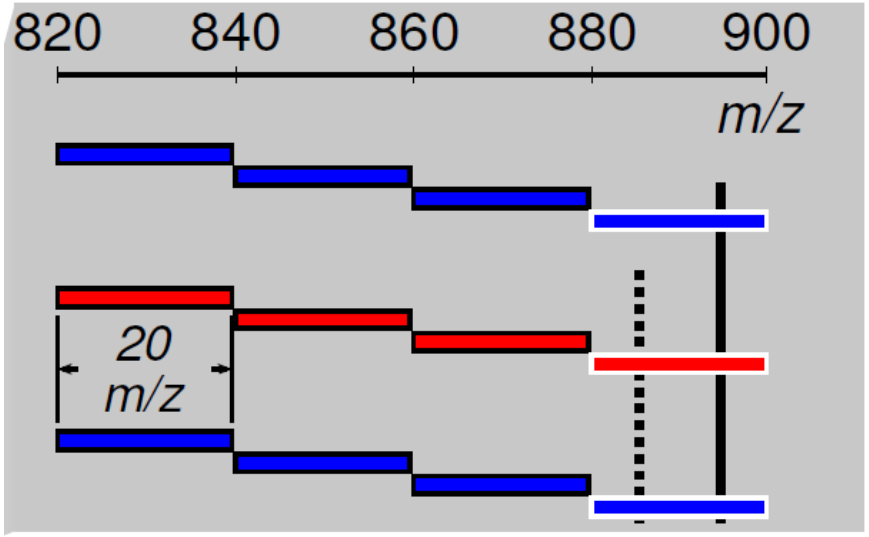


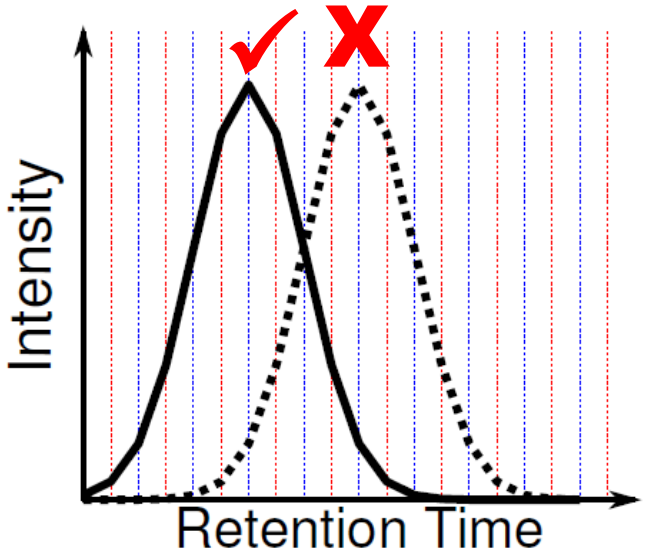
Improving Precursor Selectivity

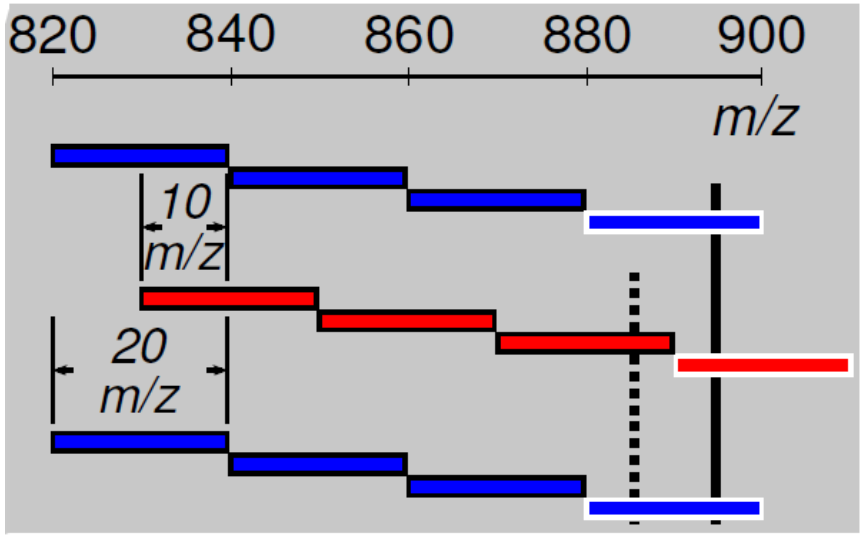


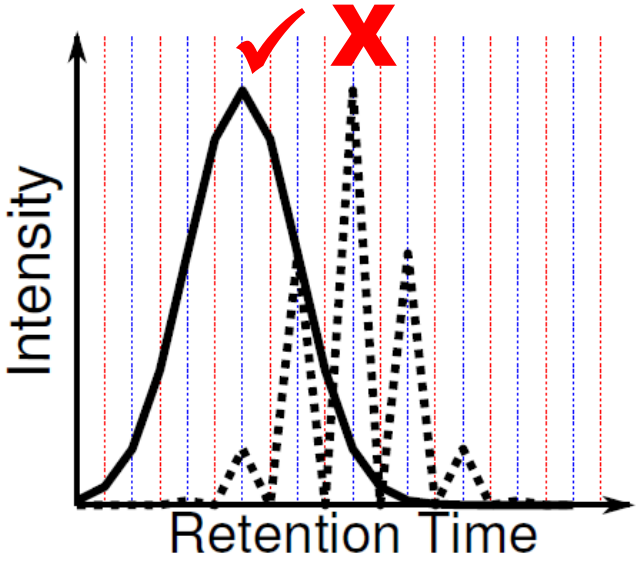


Improving Precursor Selectivity

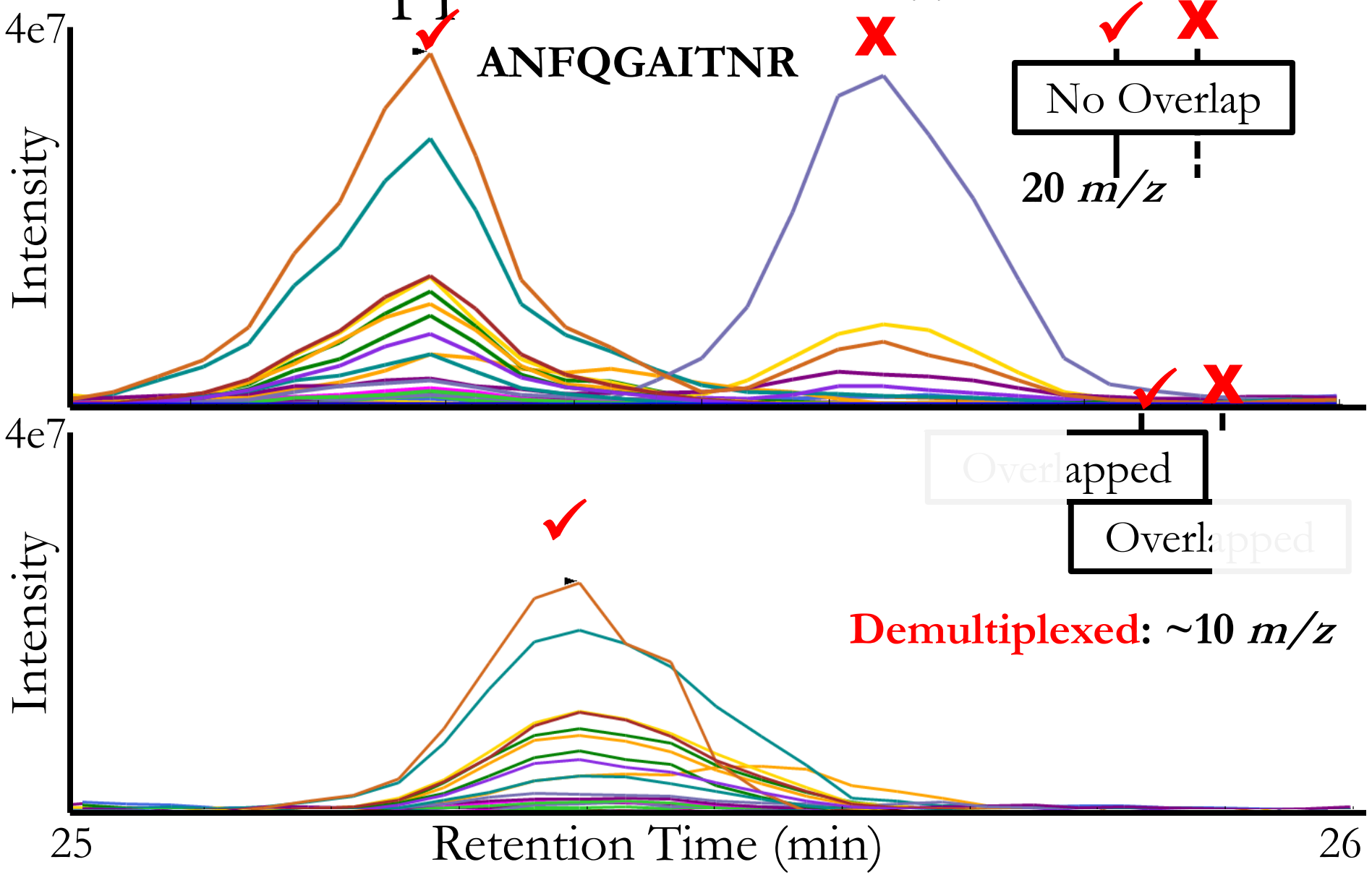




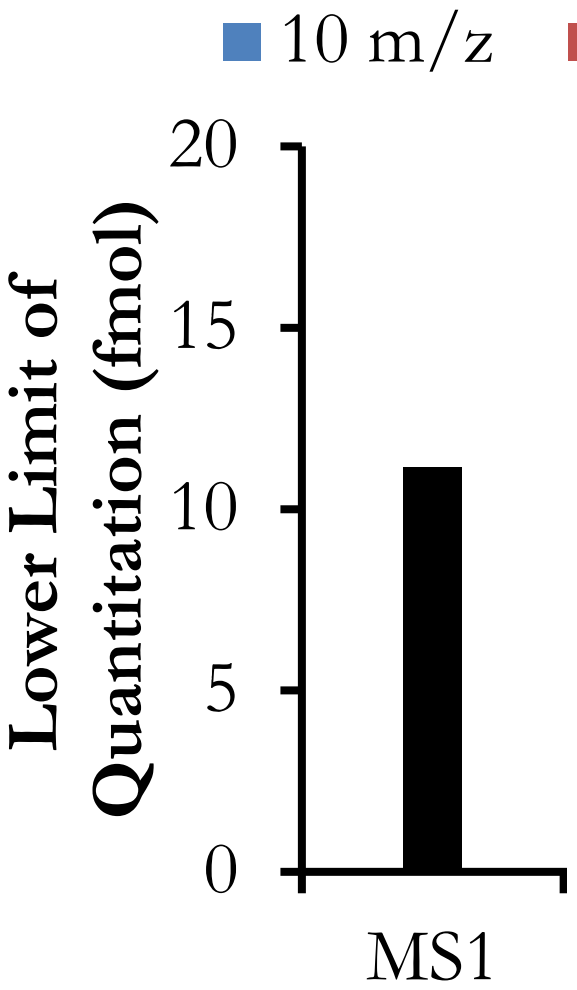




Overlapped Isolation Windows



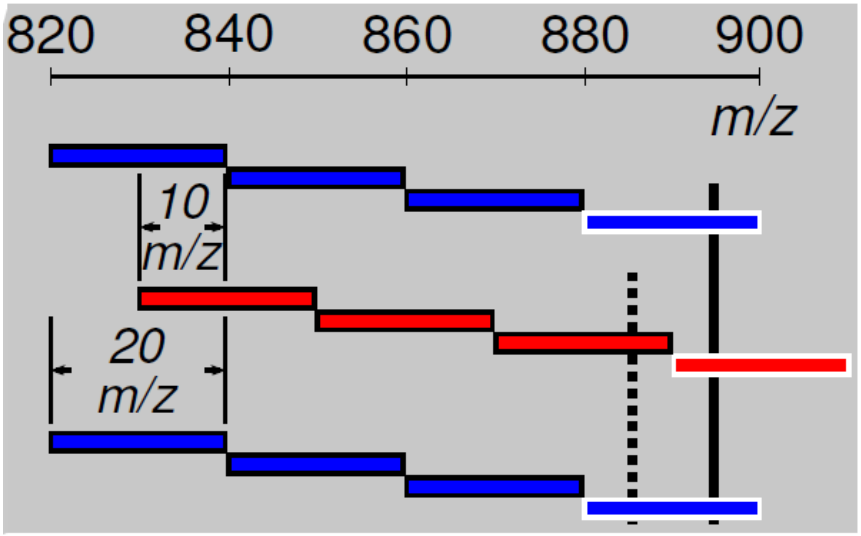
Improved Quantitation

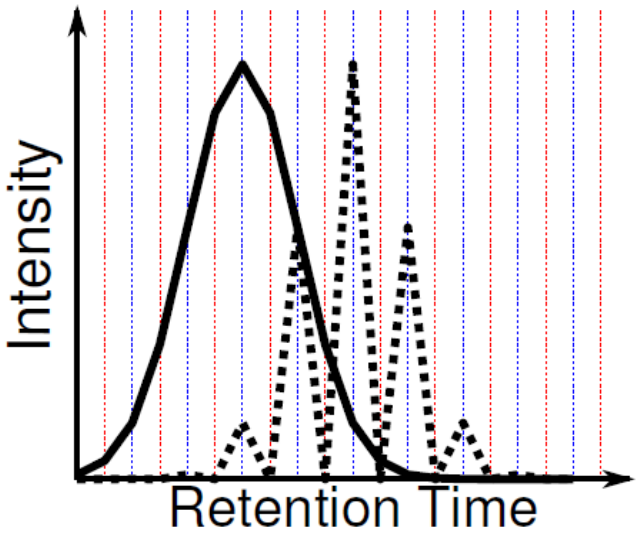


21 Peptides Spiked Into Yeast Lysate Quantified

Dario Amodei

Conclusions

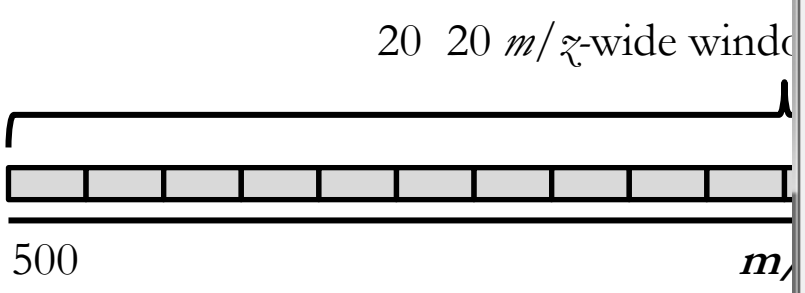


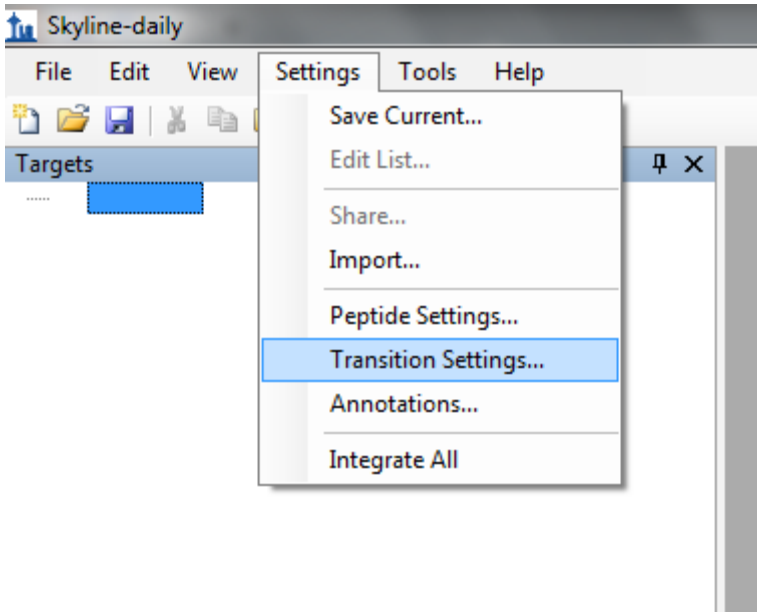


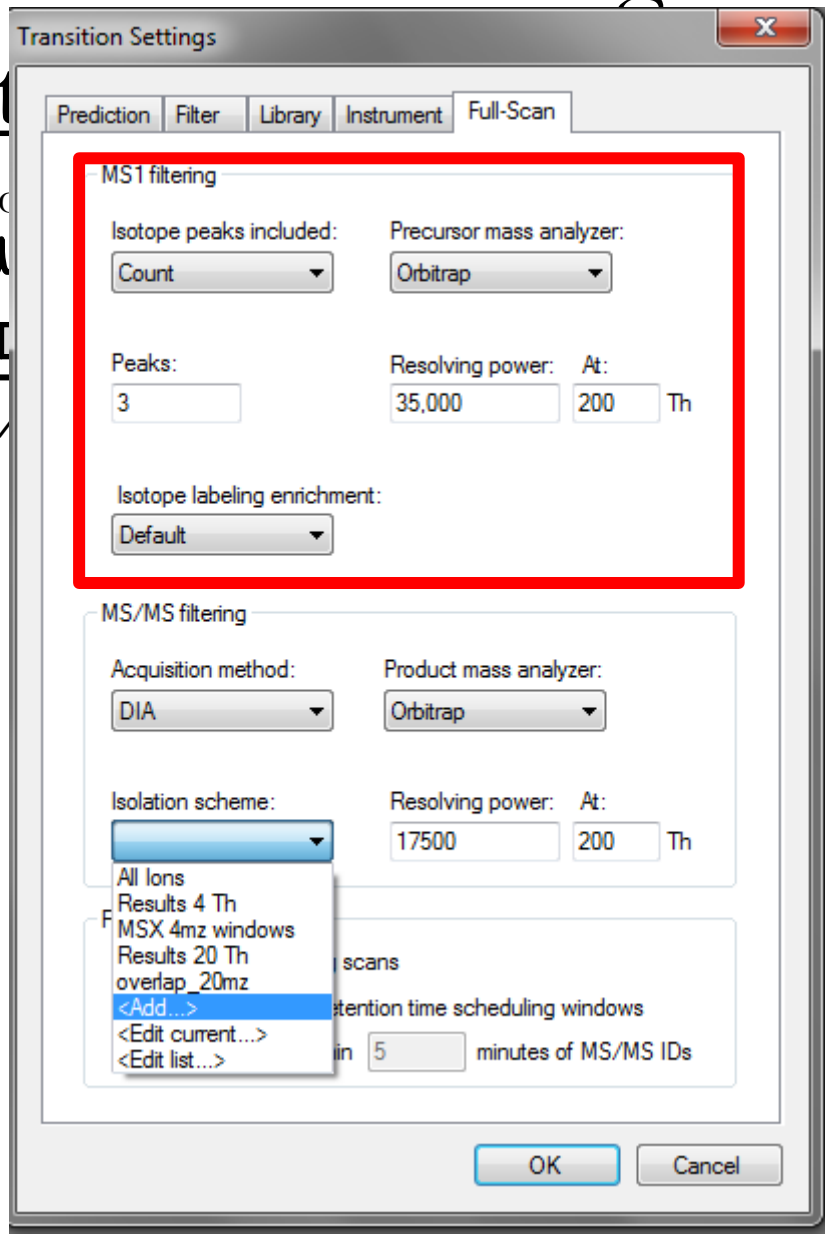
<u>Overlapping Windows Improves Selectivity and Sensitivity of DIA</u>

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

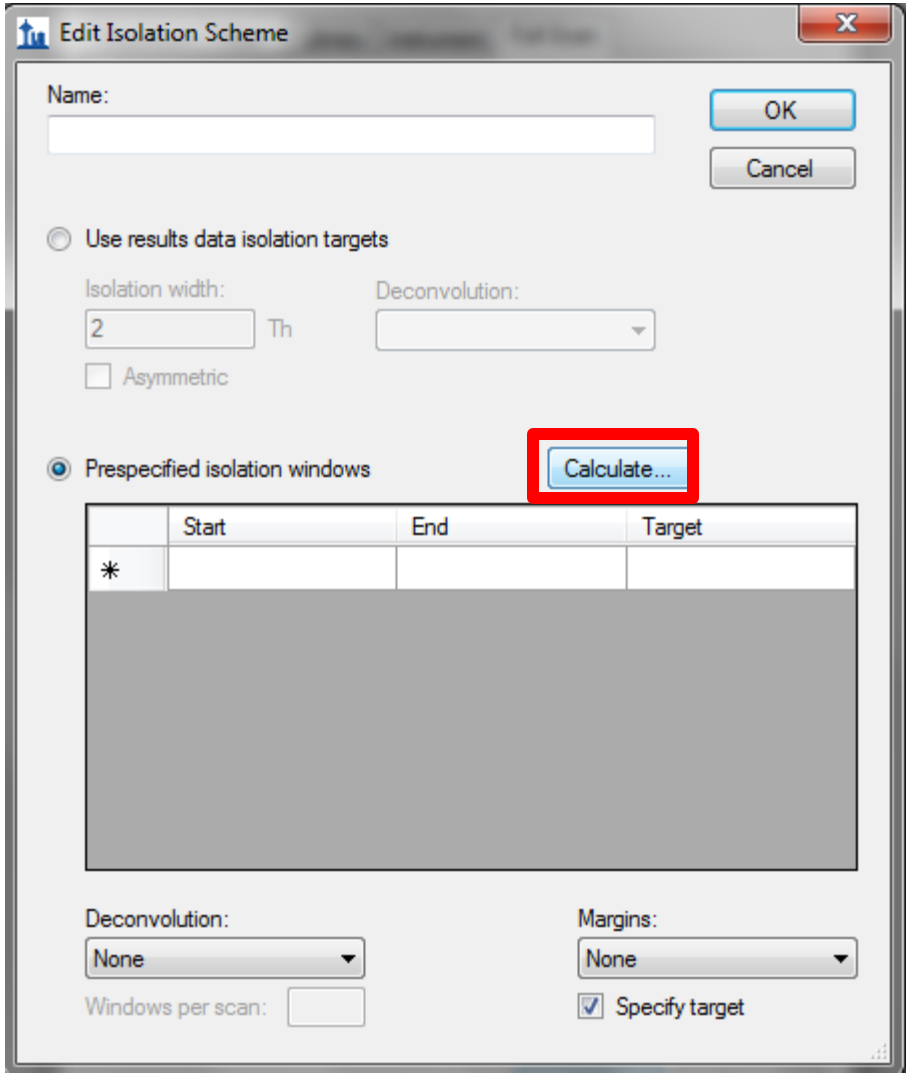
Generating a DIA Method Using Skyline: Generat

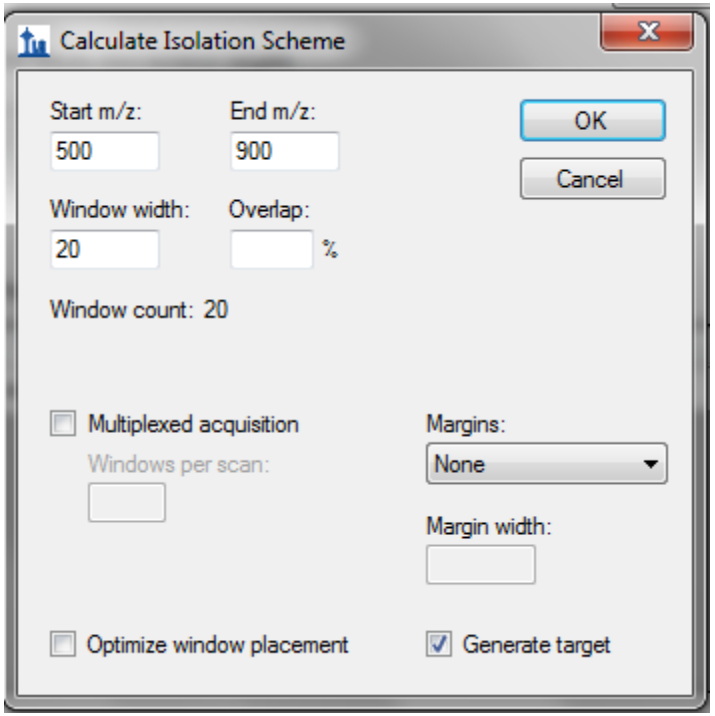




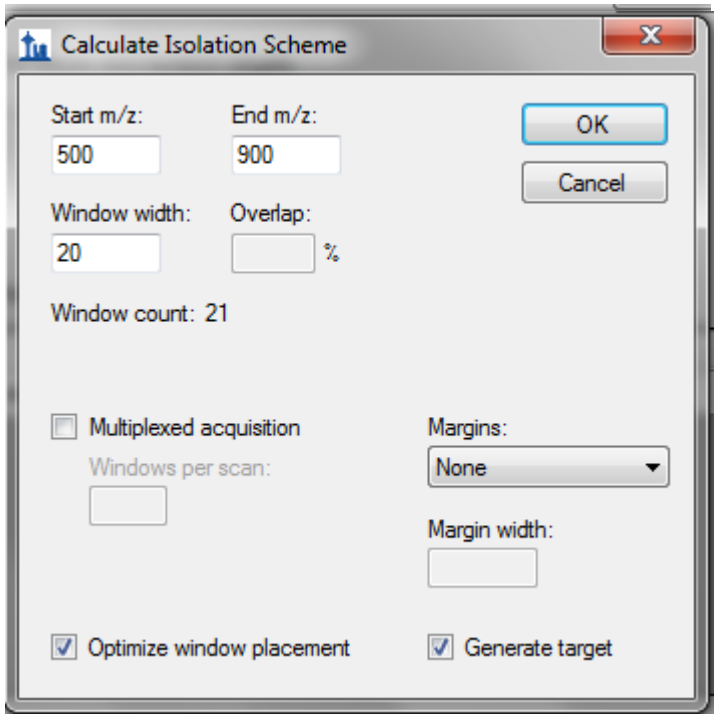


Generating a DIA Method Using Skyline: Generate a Target List



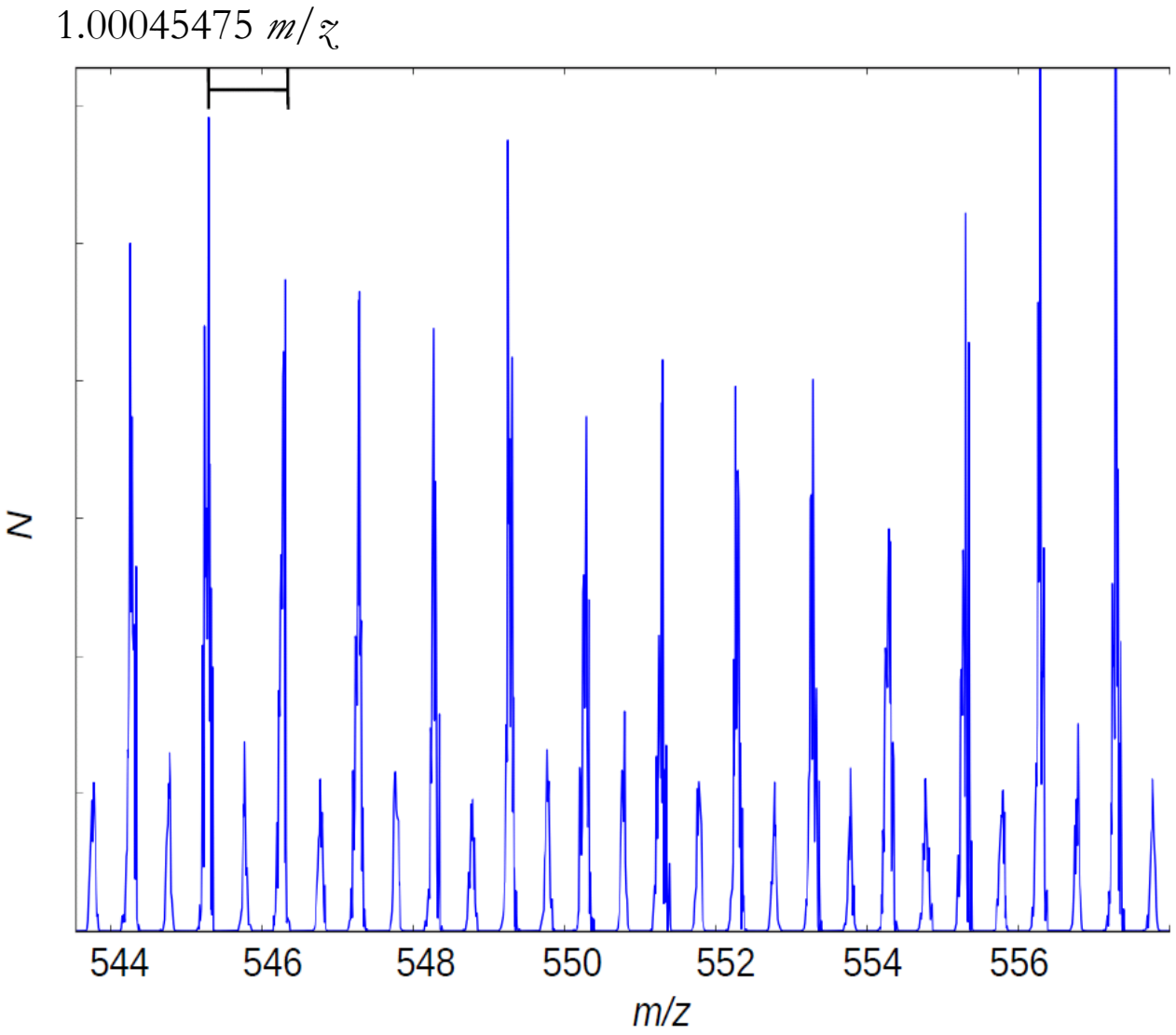


Generating a DIA Method Using Skyline: Generate a Target List



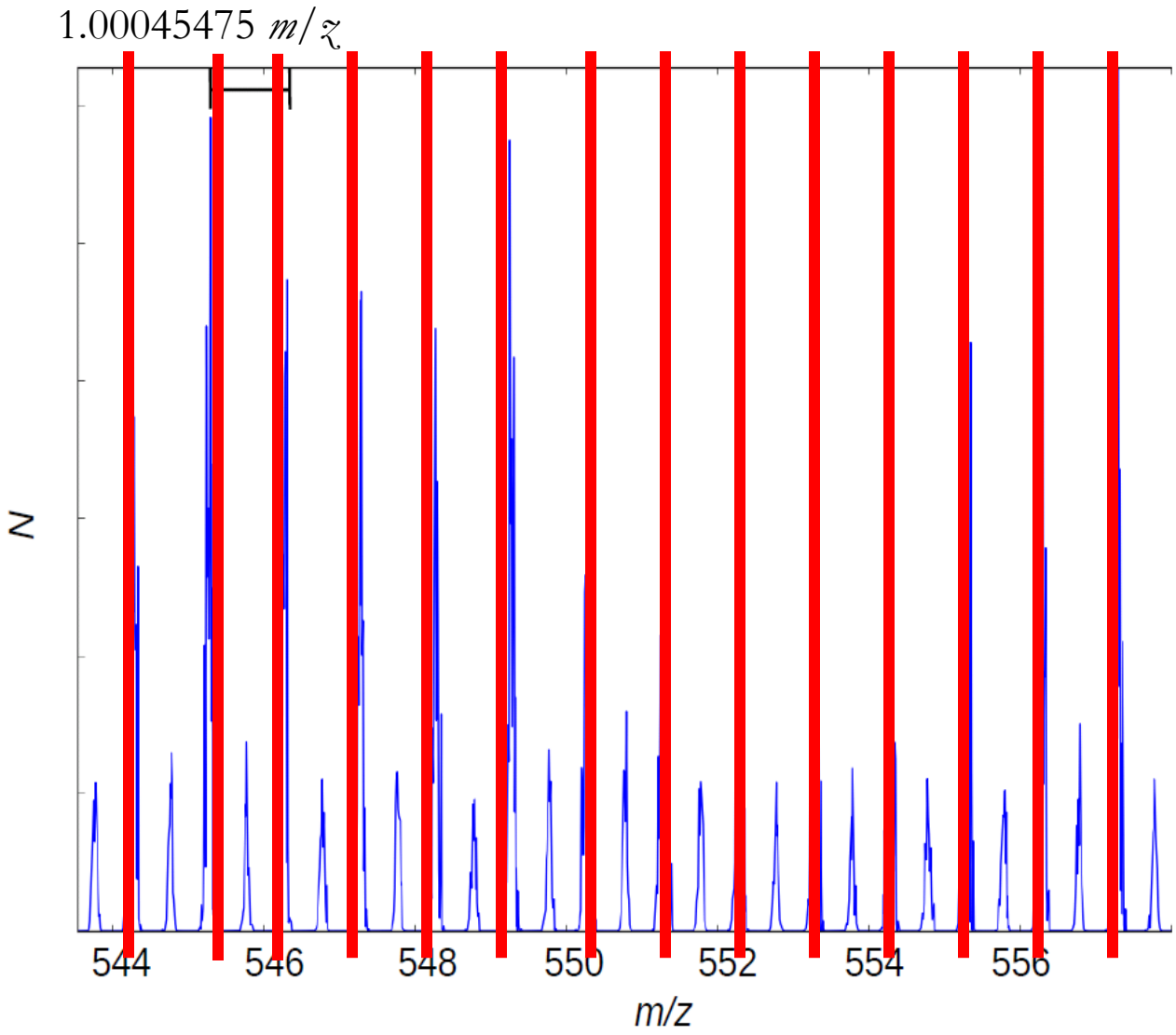
Generating a DIA Method Using Skyline: Generate a Target List

	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



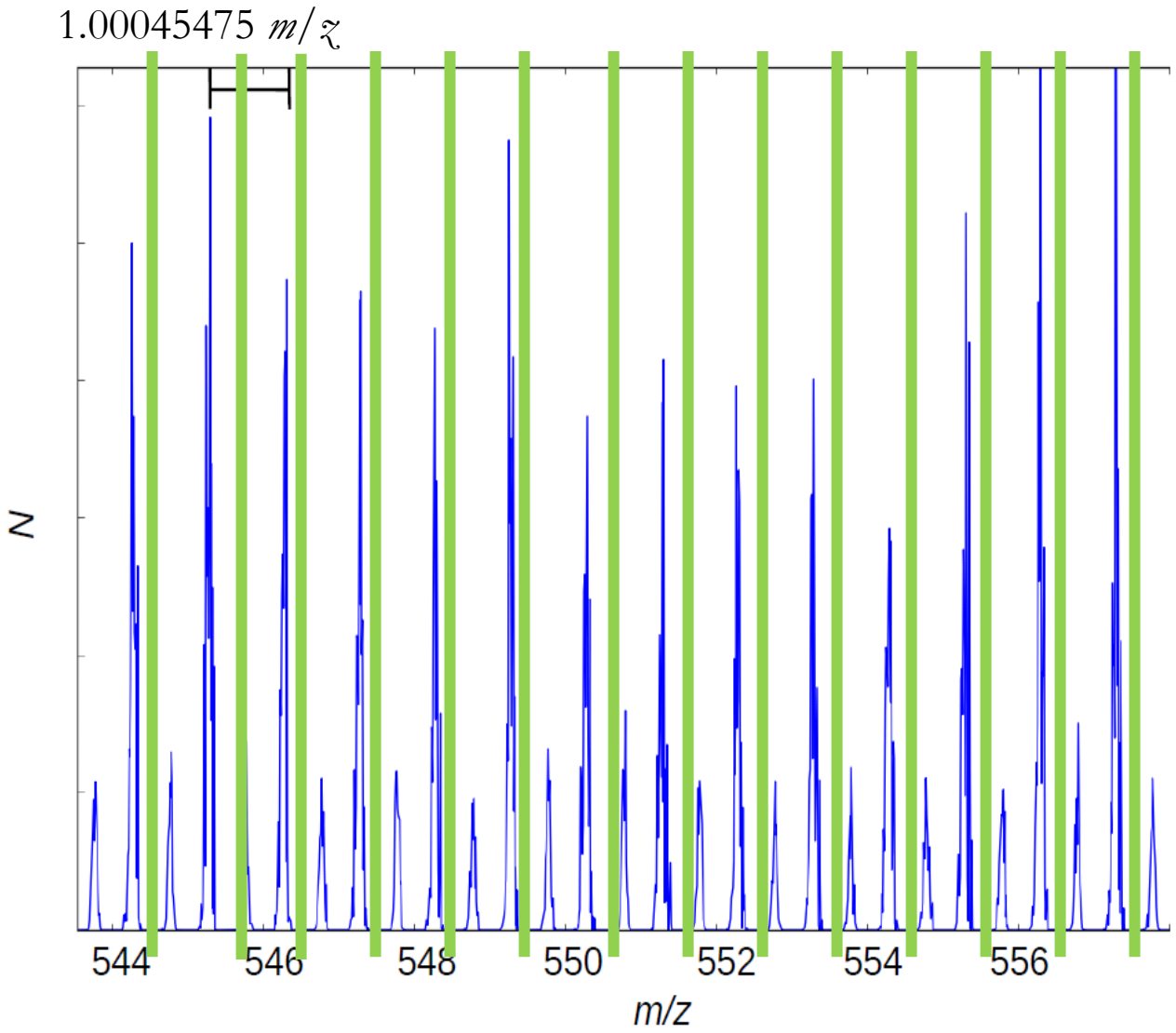
Generating a DIA Method Using Skyline: Generate a Target List

	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

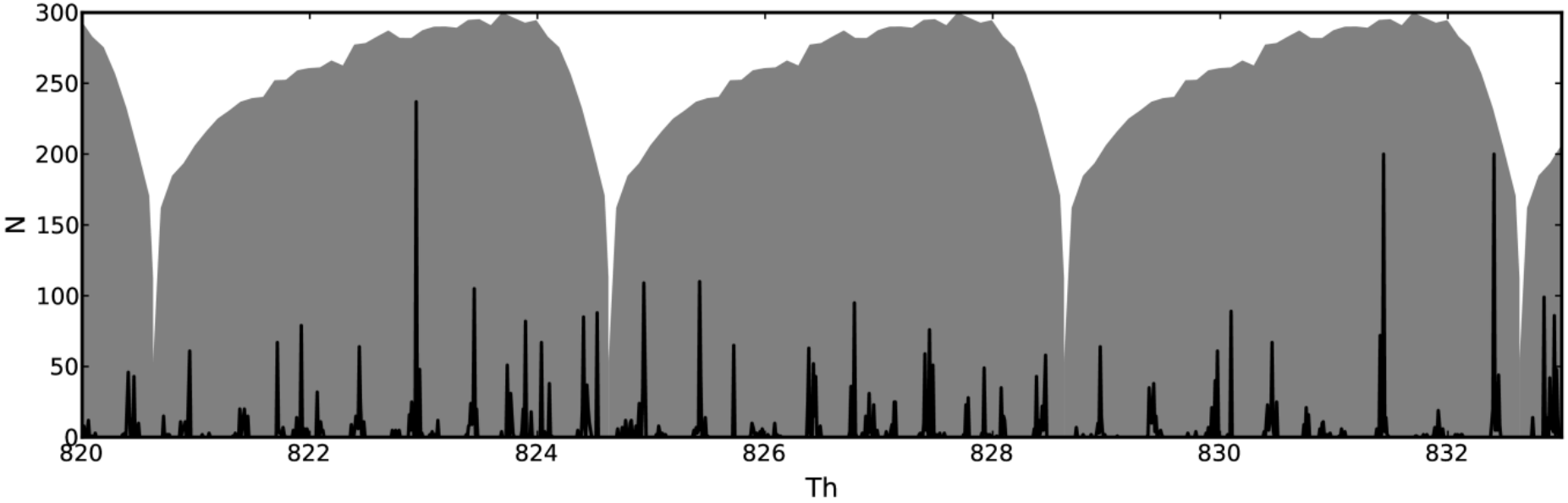


Generating a DIA Method Using Skyline: Generate a Target List

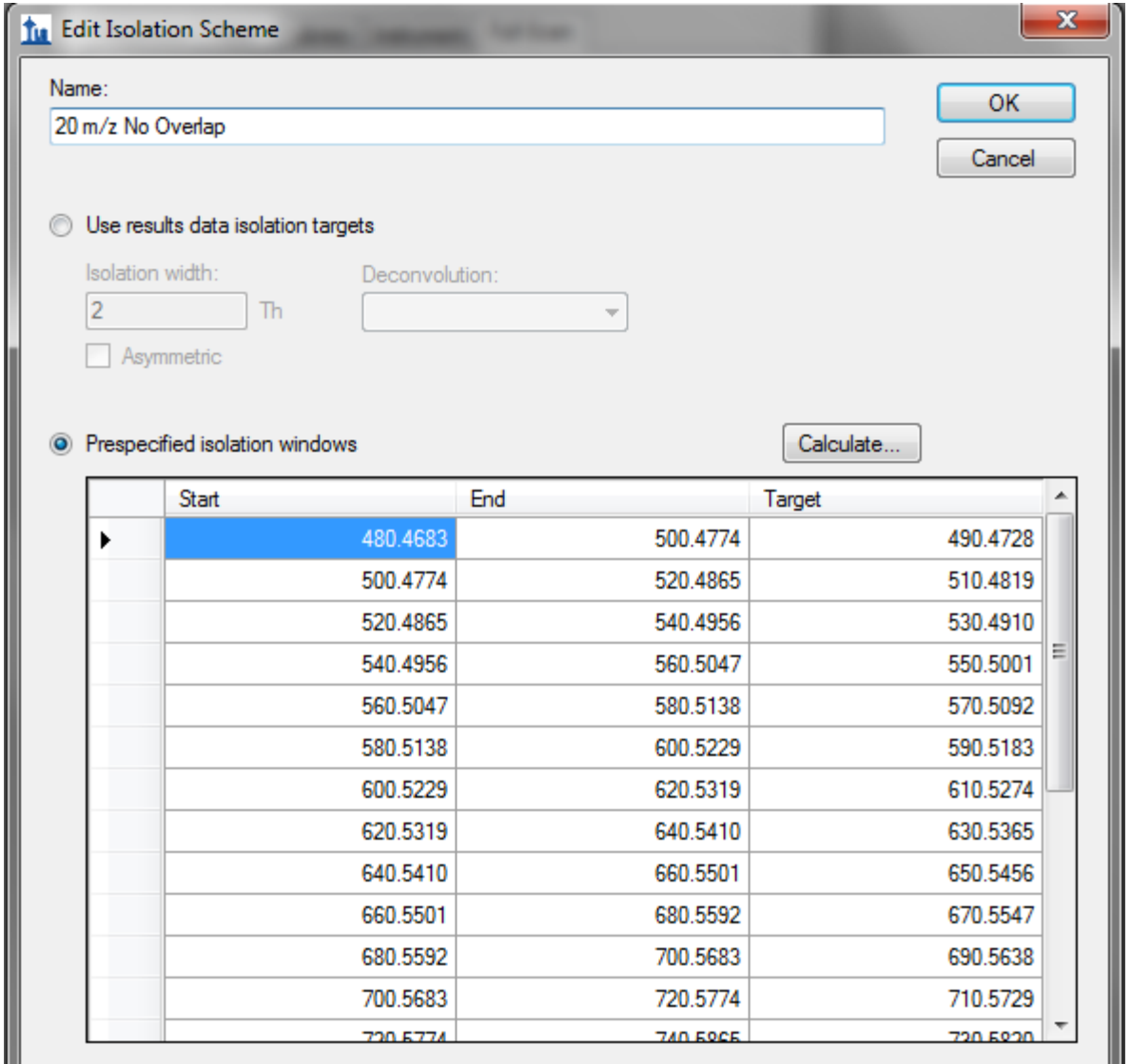
	Mass	Excess
H	1.00078	0.00078
C	12	0.0
O	15.9949	0.9949
N	14.0031	0.0031
S	31.9721	0.9721



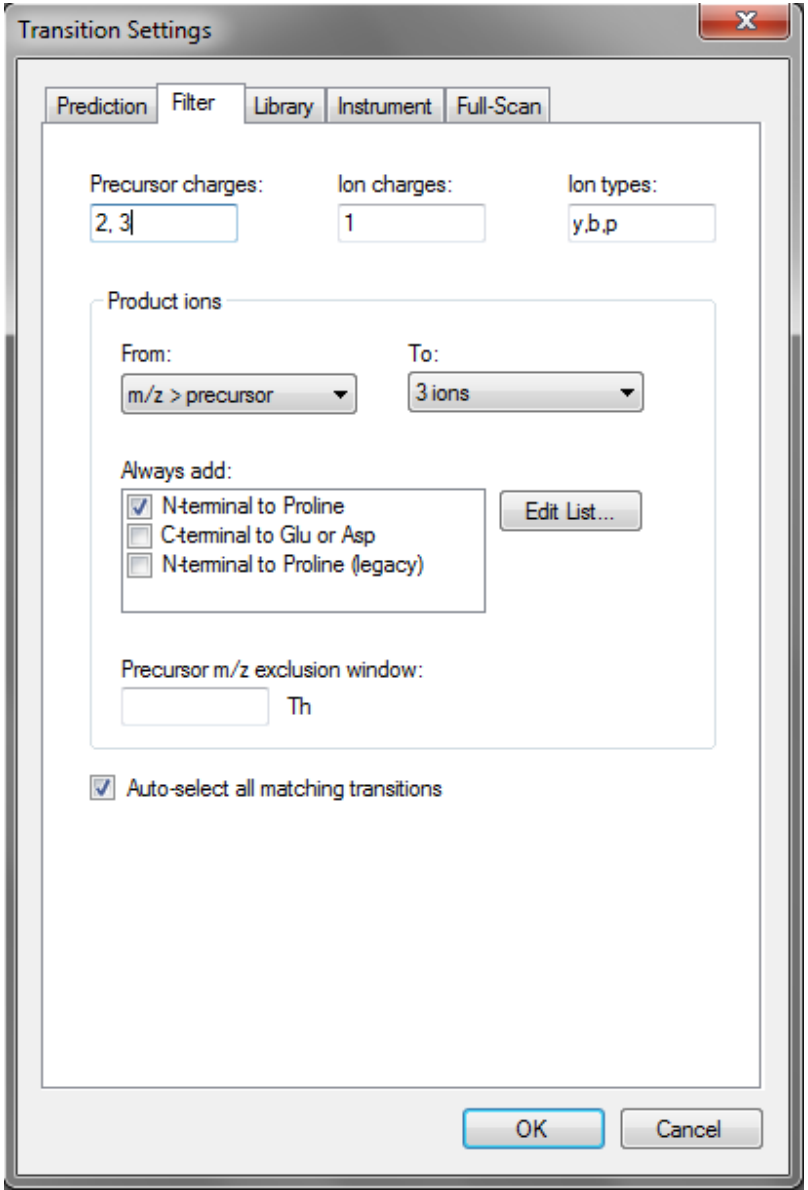
Generating a DIA Method Using Skyline: Generate a Target List



Generating a DIA Method Using Skyline: Generate a Target List



Importing Data: Filtering Settings



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

ersity

k

ntific

hann

Andreas Kuehn

Reiko Kiyonami

Yue Xuan

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