



Multiplexed Data Independent Acquisition for Comparative Proteomics

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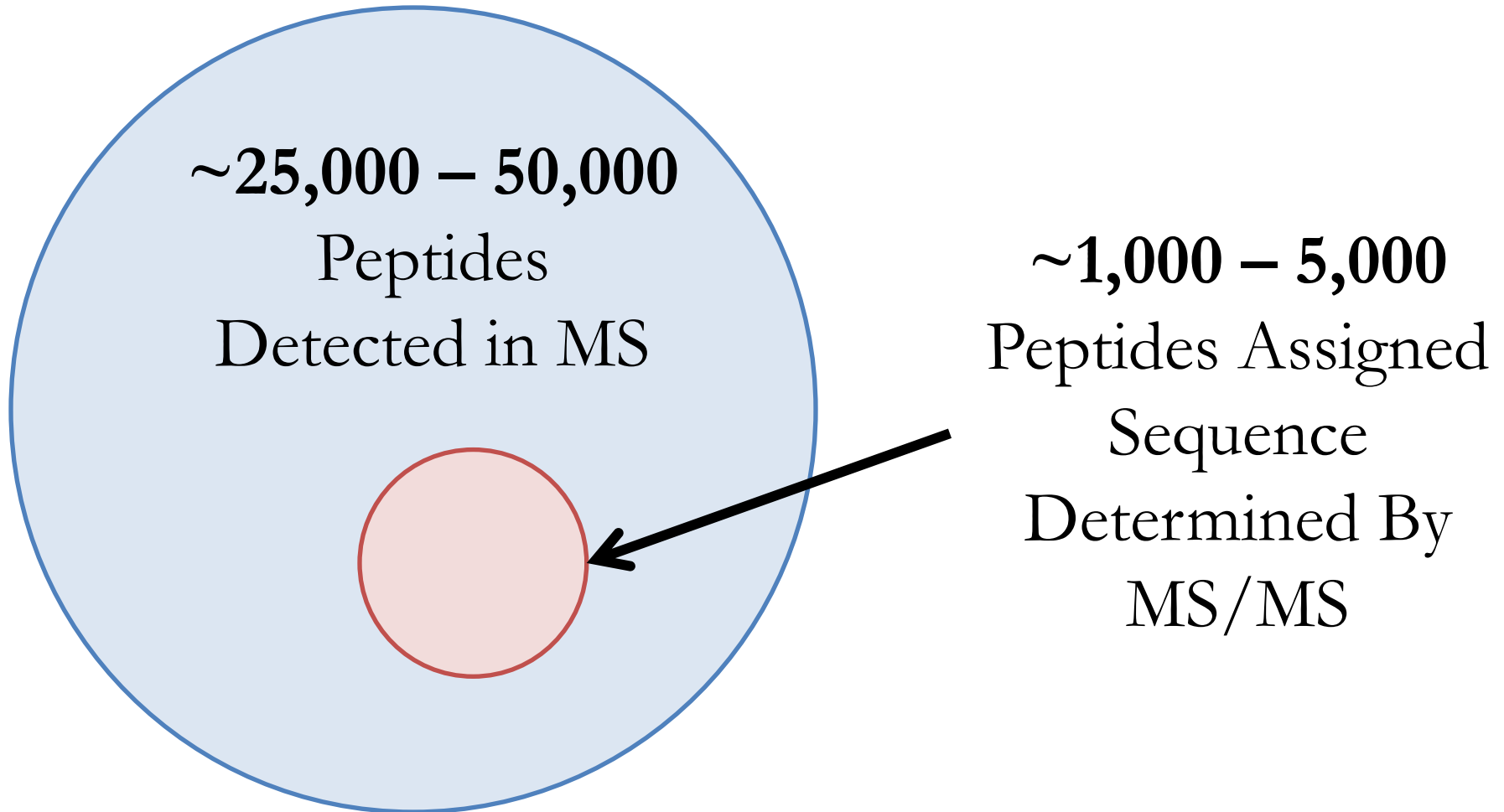
University of Washington

5/20/2012

Current Technology for Comparative Proteomics

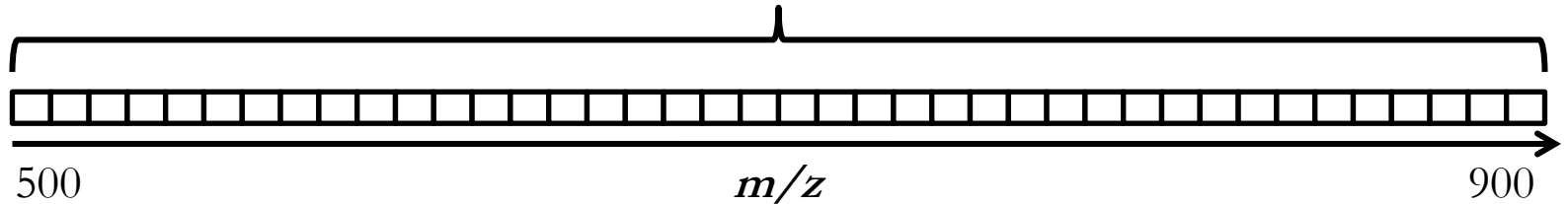
- Targeted:
 - *How much* does protein X increase/decrease?
 - For a small target list (<100 peptides)
 - Often requires extra steps
 - Retention time scheduling
 - Peptide transition refinement
- Discovery:
 - What proteins are changing in abundance?
 - For ~1,000 - 5,000 semi-randomly selected peptides
 - Data is not collected on the majority of peptides!

Many Peptides Are Missed By Data Dependent Acquisition



Data Independent Acquisition (DIA) to Increase Sequence Coverage

40 $10\ m/z$ -wide windows = 400 m/z

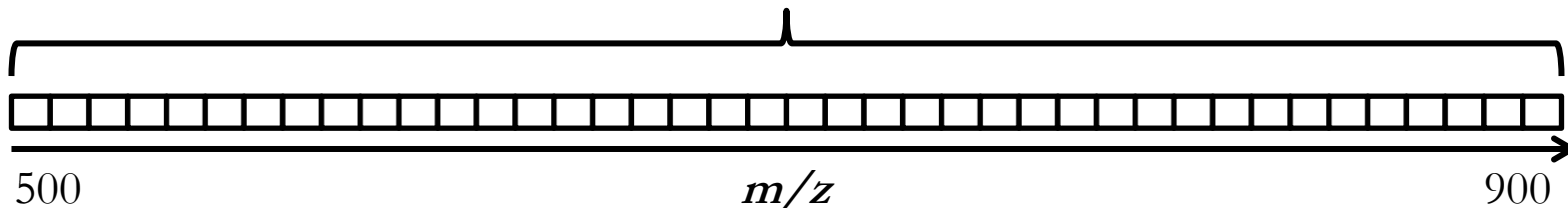


Scan 1

Scan 2

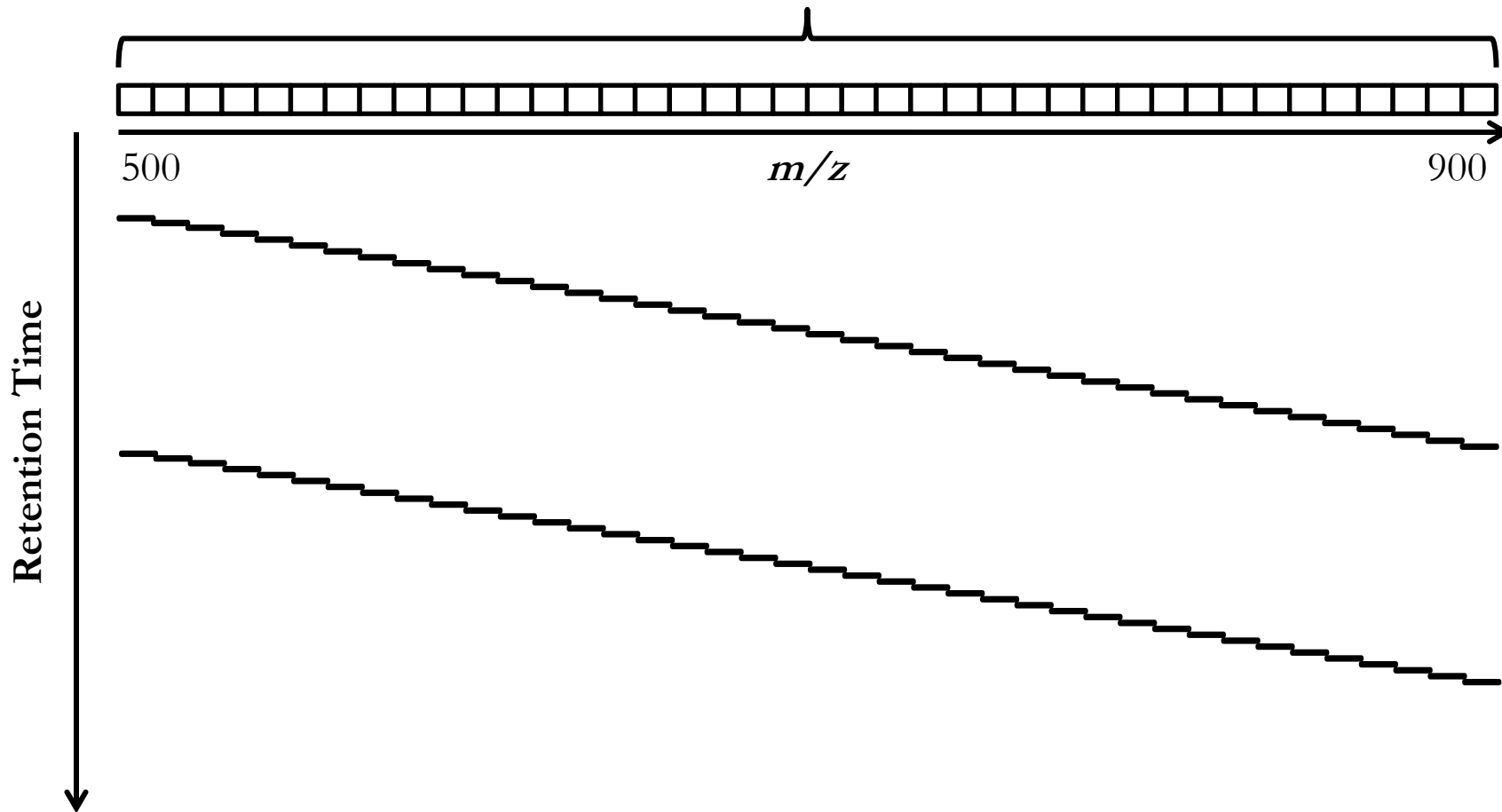
Data Independent Acquisition (DIA) to Increase Sequence Coverage

40 $10\ m/z$ -wide windows = 400 m/z



Data Independent Acquisition (DIA) to Increase Sequence Coverage

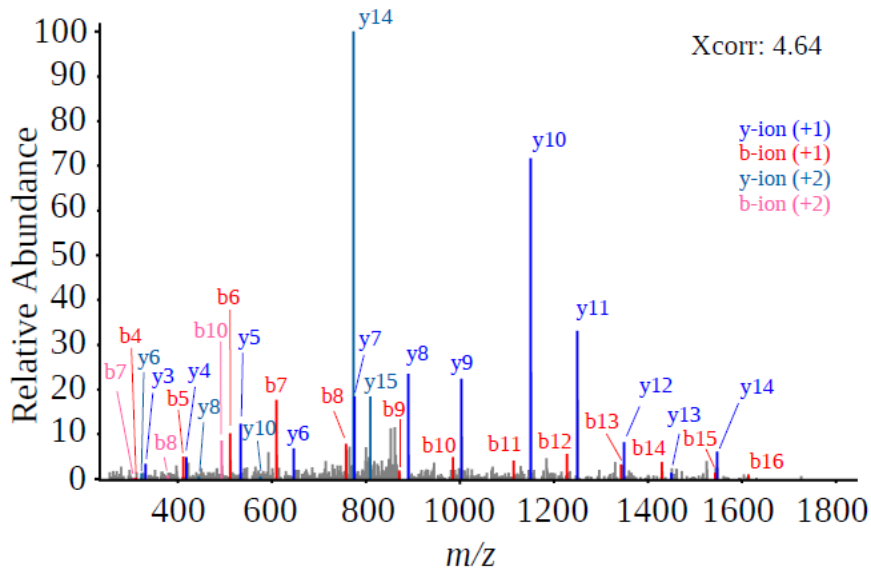
40 $10\ m/z$ -wide windows = 400 m/z



DIA Lacks the Specificity of DDA

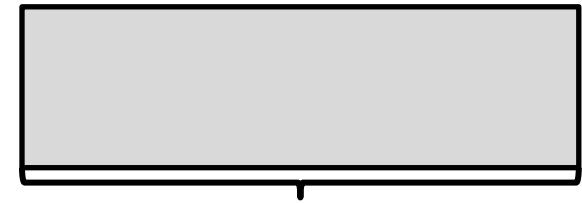
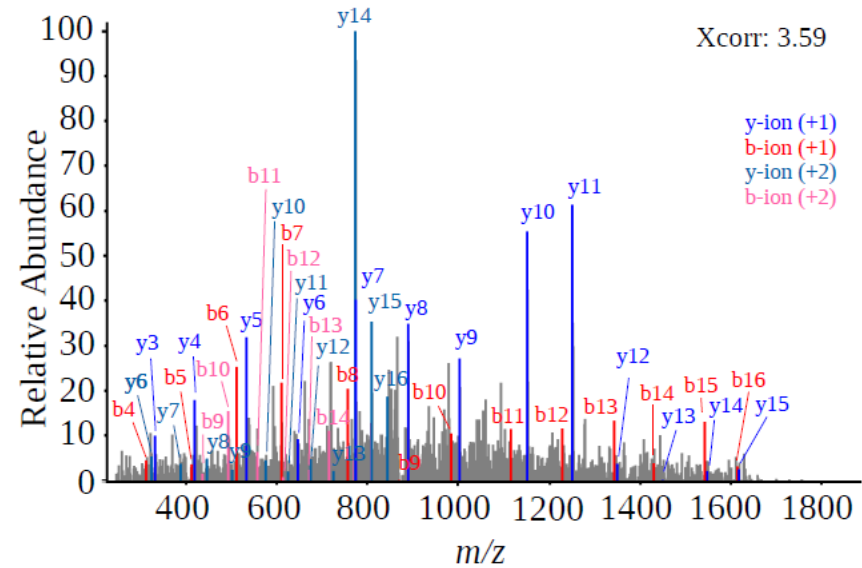
Sample: *S. cerevisiae* lysate (soluble) Peptide: R.AAAPTVVFLDELDSIAK.A

2 m/z Isolation Window



2 m/z

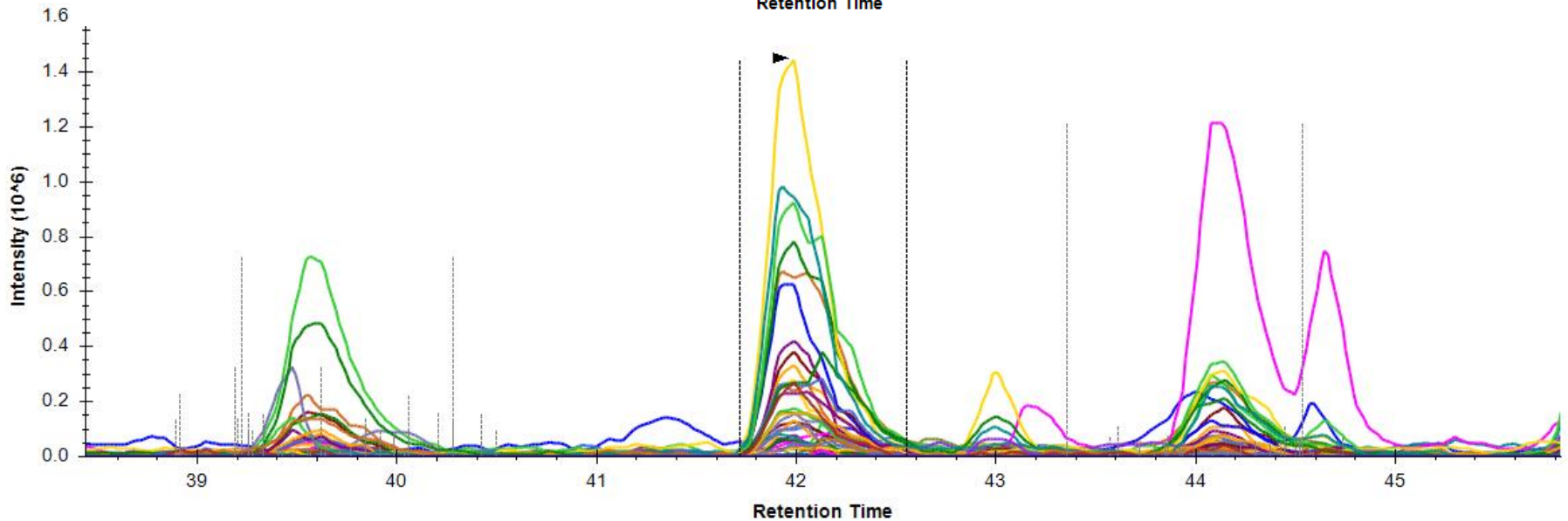
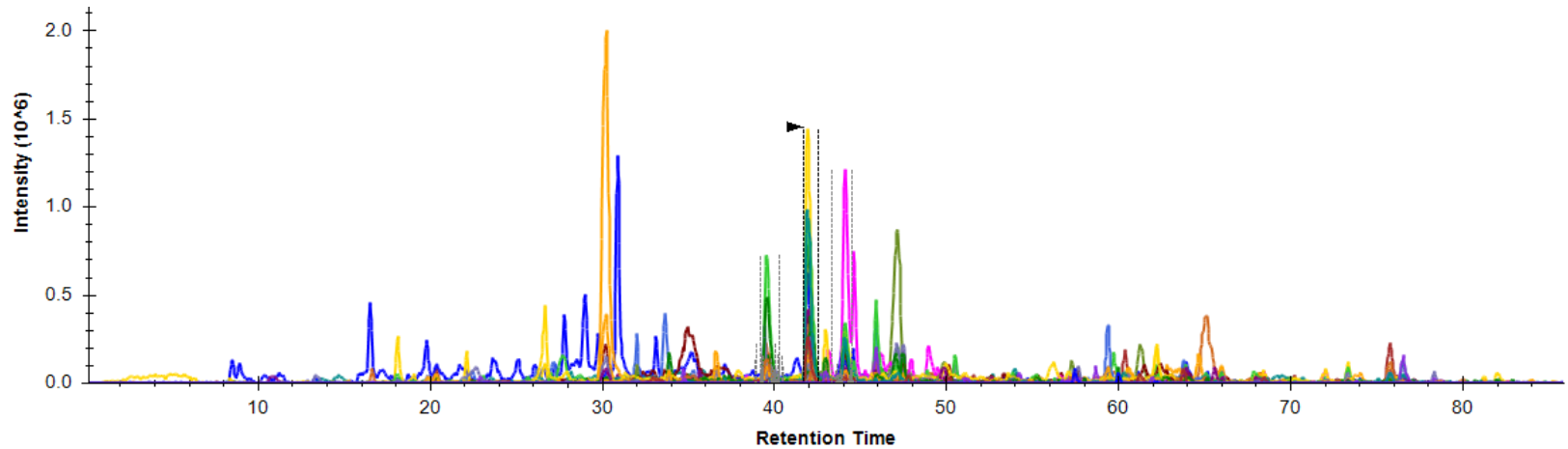
10 m/z Isolation Window



10 m/z

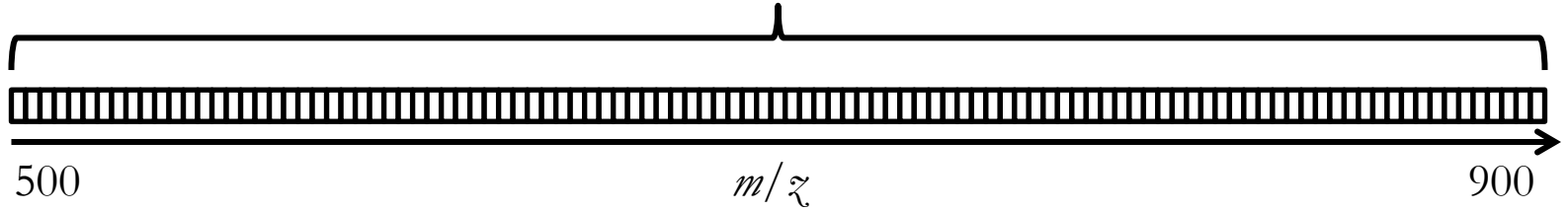
DIA Interference/ Low Specificity

FEIELLSLDDDSIVNHEQDLPK *S. cerevisiae* lysate (soluble) 10 m/z wide window DIA (Q-Exactive)



Multiplexed DIA

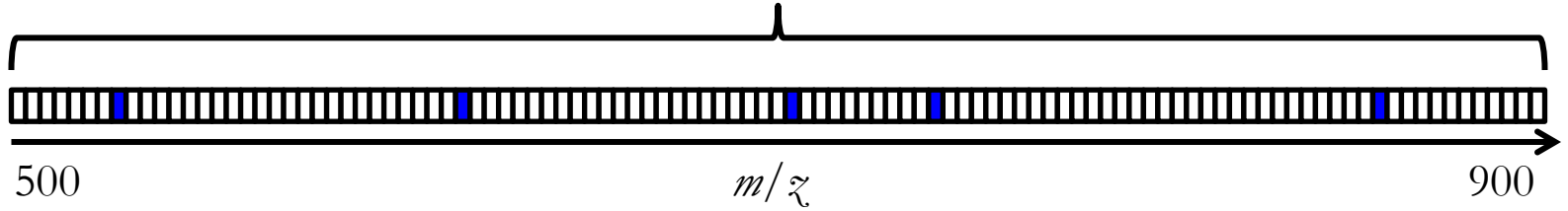
100 $4\ m/z$ -wide windows = 400 m/z



Scan 1

Multiplexed DIA

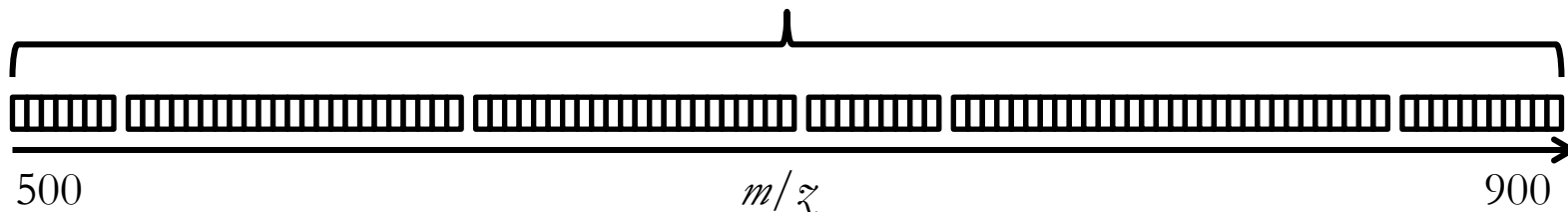
100 $4 m/z$ -wide windows = 400 m/z



Scan 1

Multiplexed DIA

100 $4 m/z$ -wide windows = 400 m/z



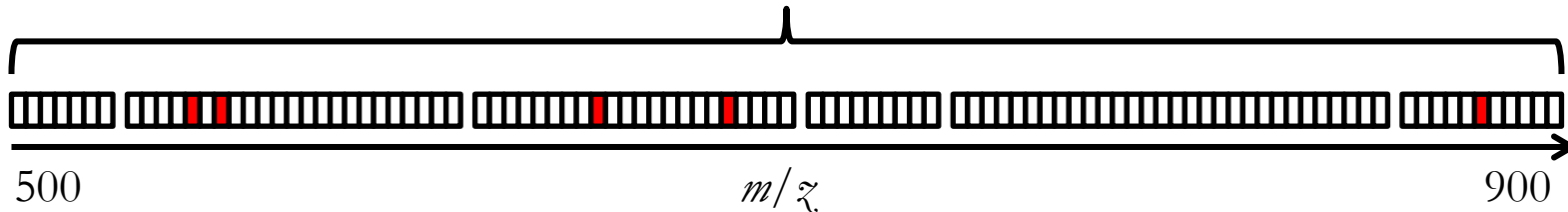
Scan 1



Scan 2

Multiplexed DIA

100 $4 m/z$ -wide windows = 400 m/z

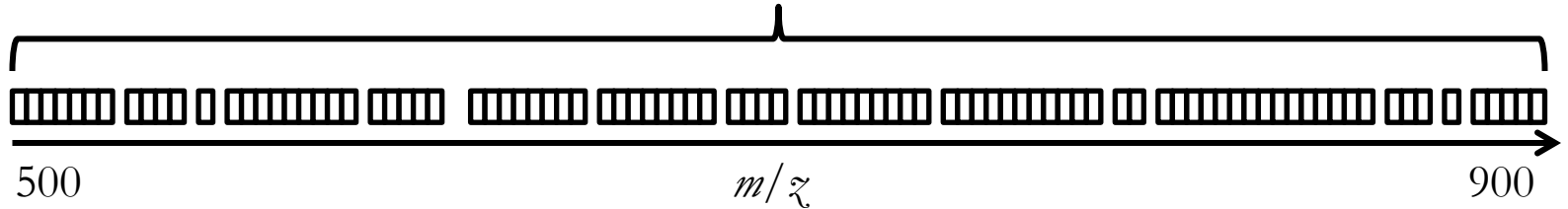


Scan 1

Scan 2

Multiplexed DIA

100 $4\ m/z$ -wide windows = 400 m/z



Scan 1

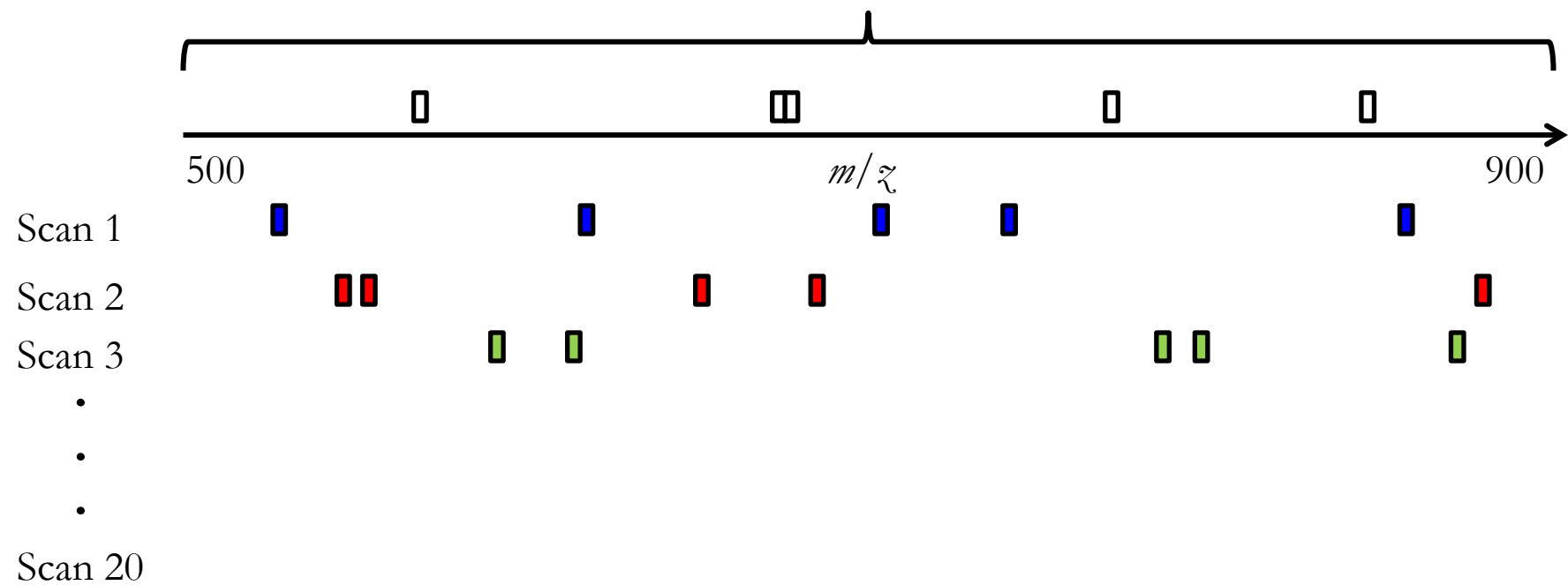
Scan 2

Scan 3



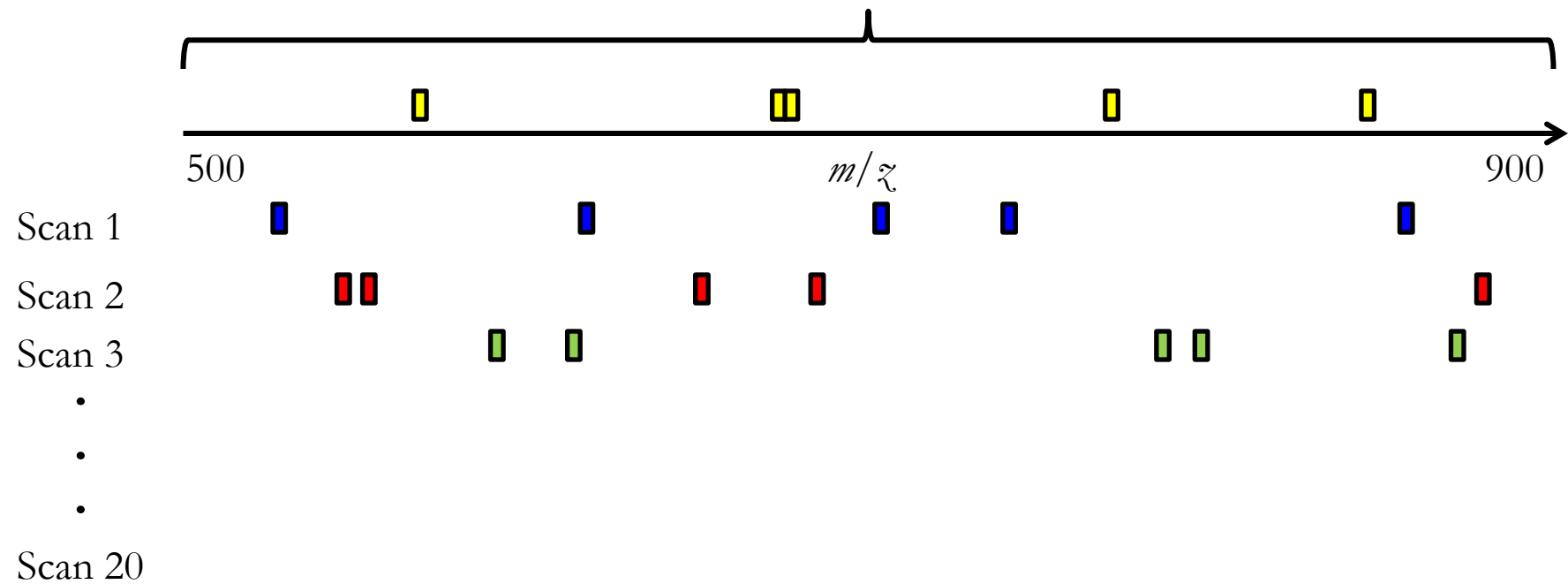
Multiplexed DIA

100 m/z -wide windows = 400 m/z



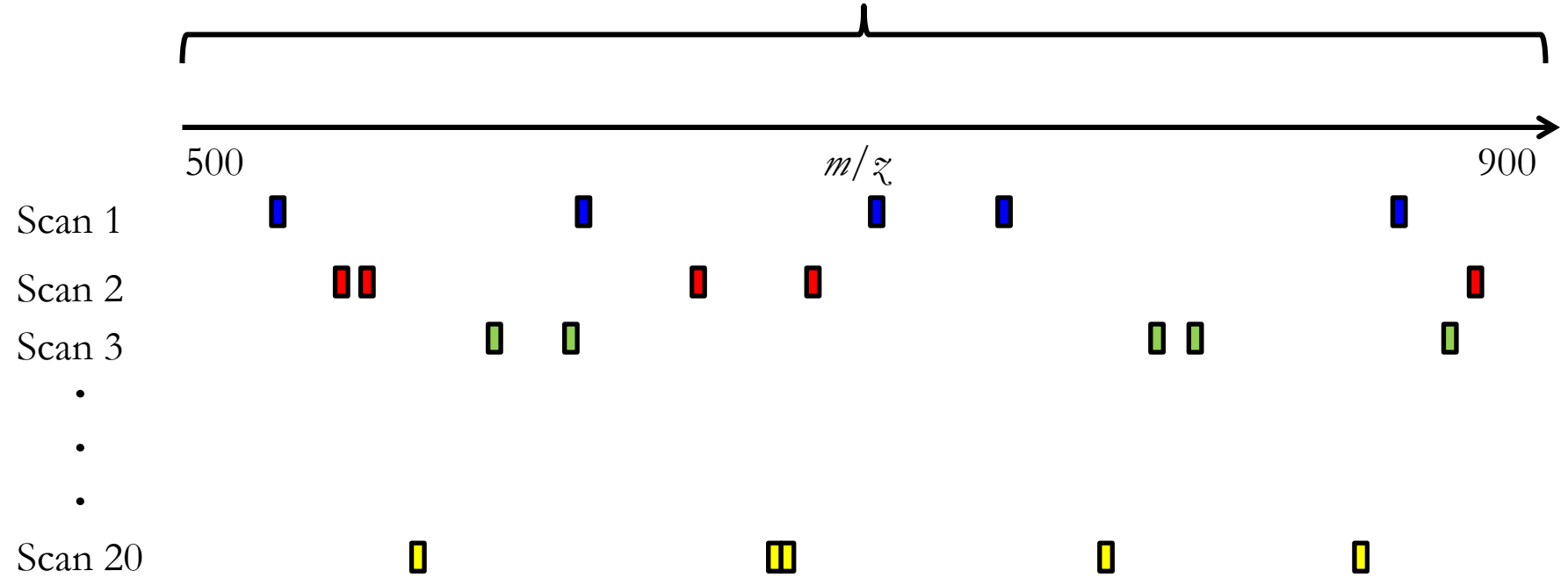
Multiplexed DIA

100 m/z -wide windows = 400 m/z



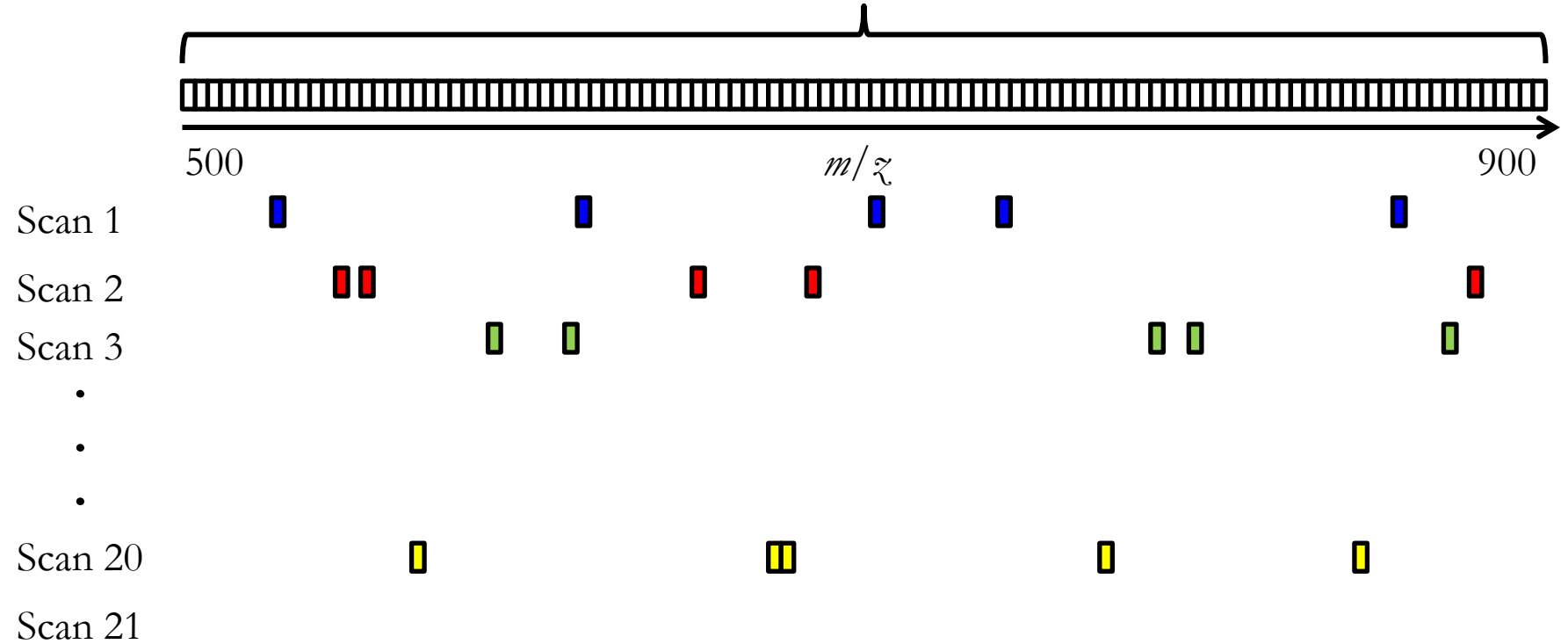
Multiplexed DIA

100 m/z -wide windows = 400 m/z



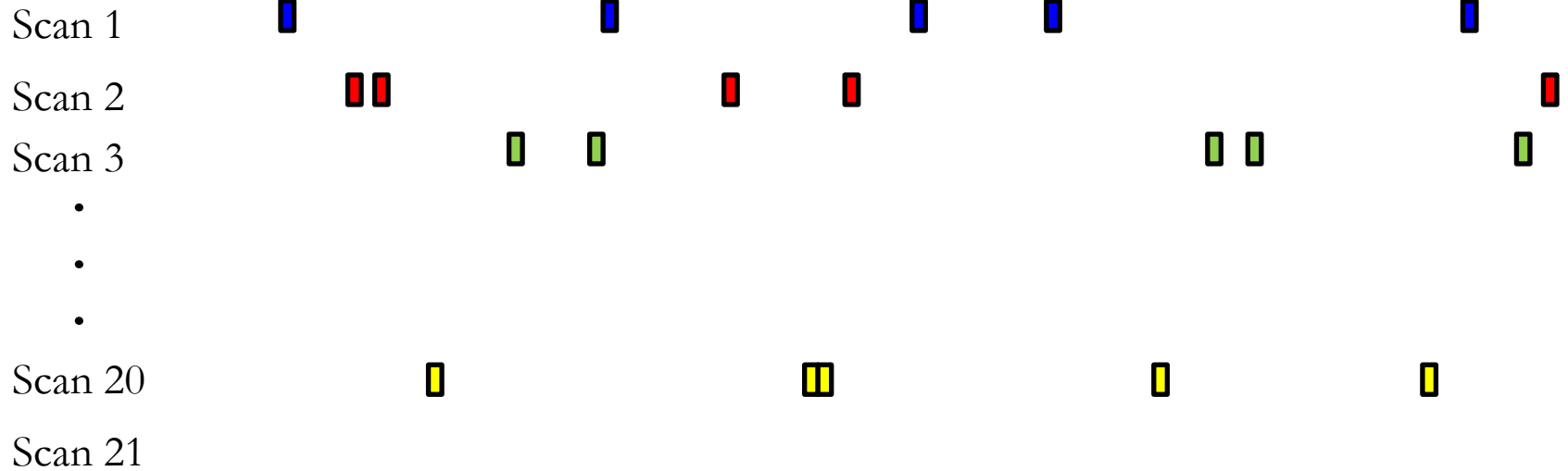
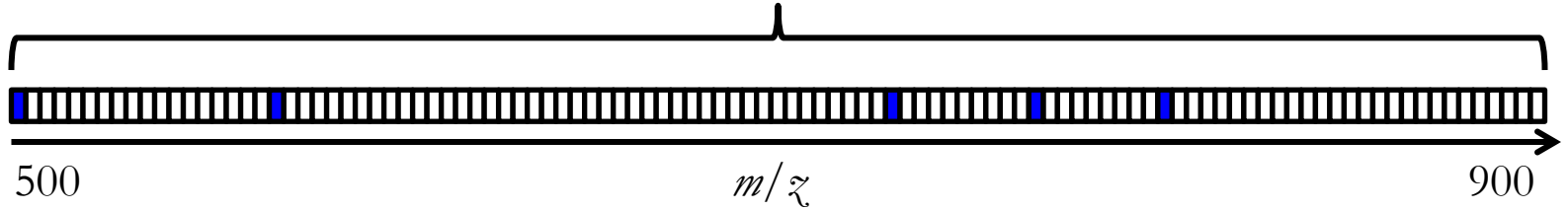
Multiplexed DIA

100 $4 m/z$ -wide windows = 400 m/z



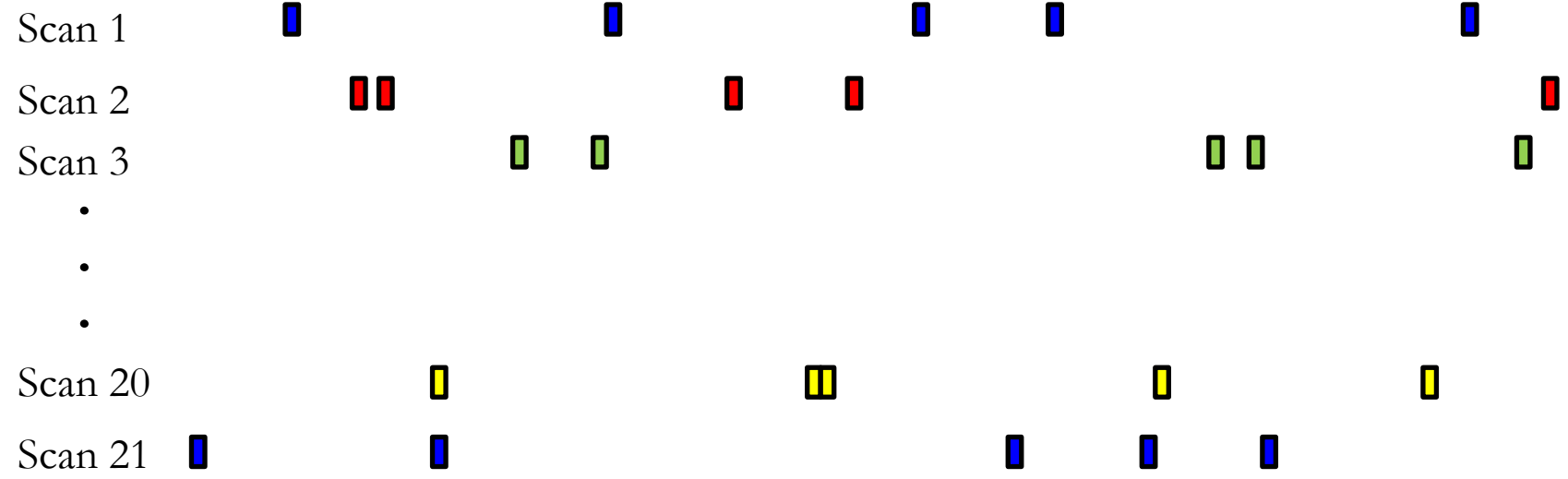
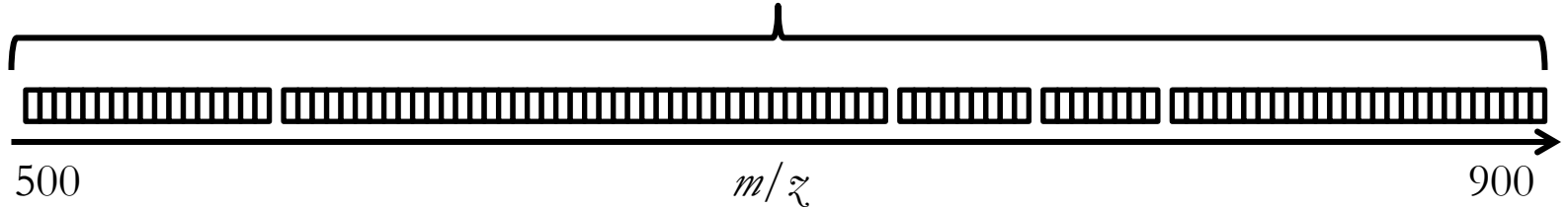
Multiplexed DIA

100 $4 m/z$ -wide windows = 400 m/z



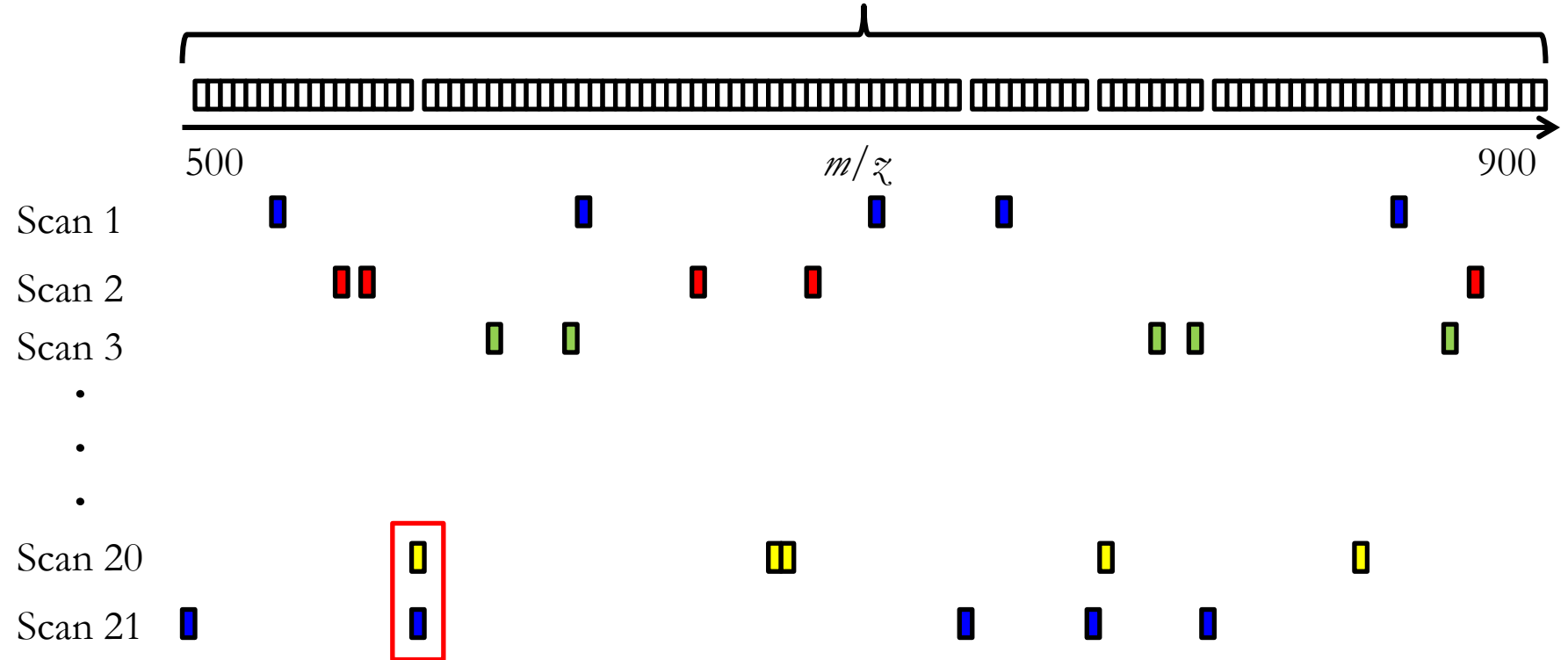
Multiplexed DIA

100 $4\ m/z$ -wide windows = 400 m/z

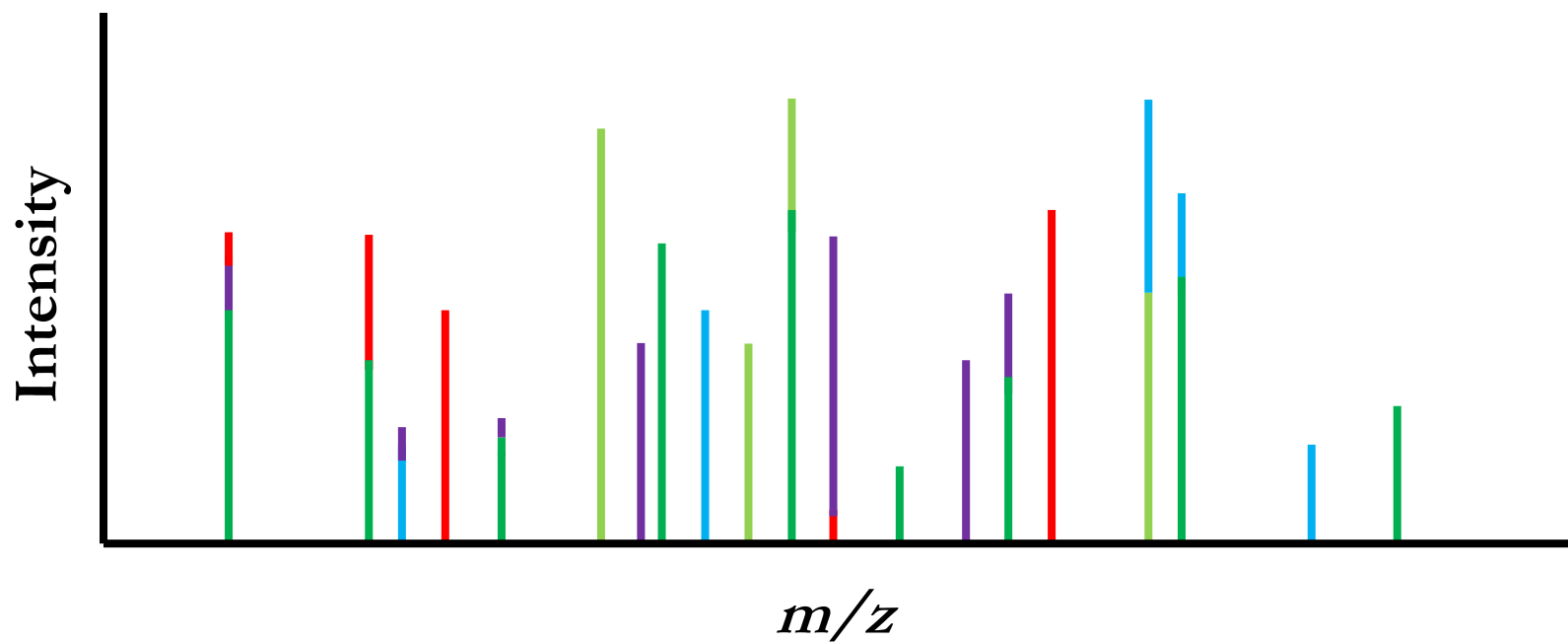


Multiplexed DIA

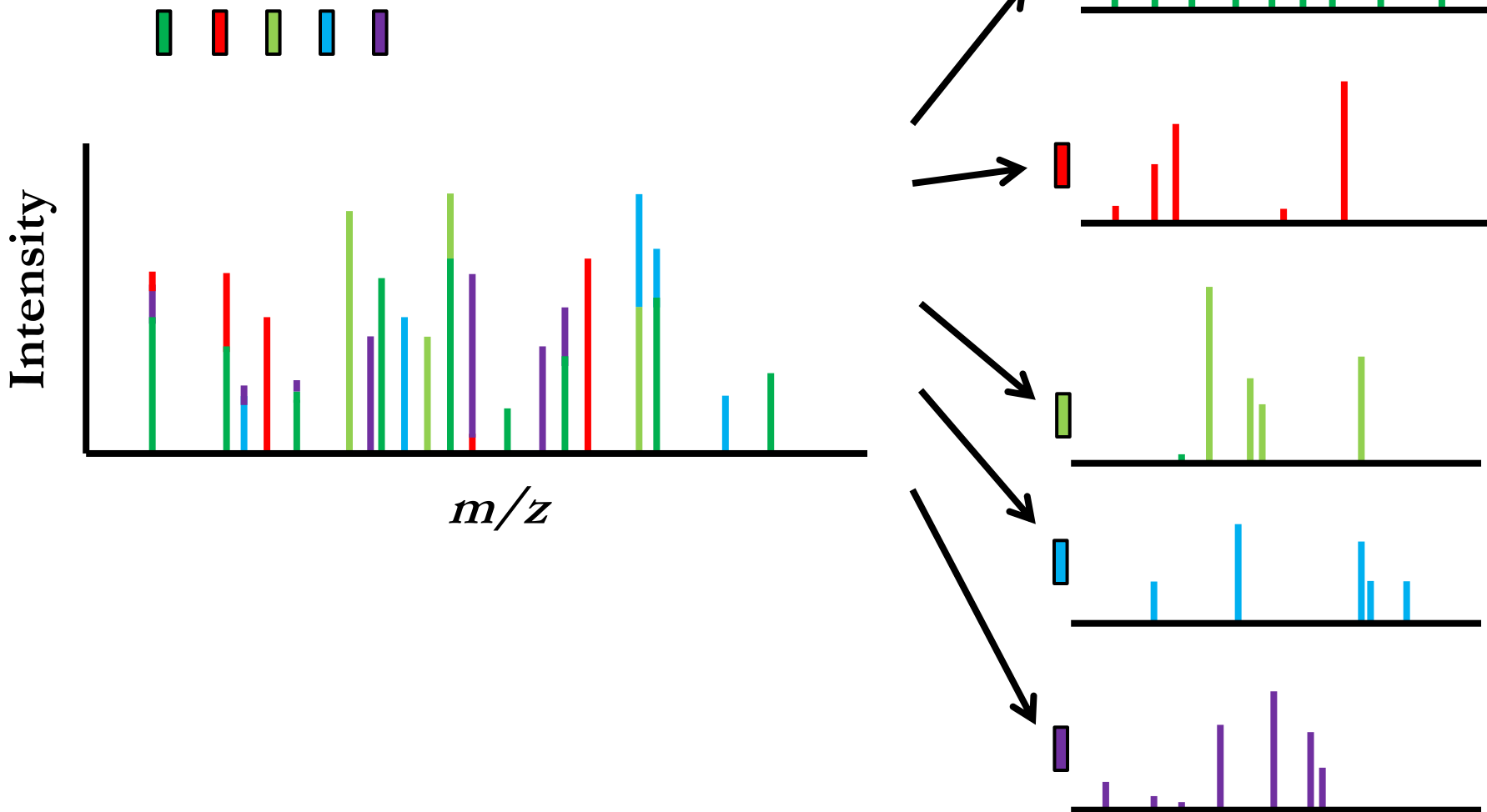
100 $4 m/z$ -wide windows = 400 m/z



Demultiplexing

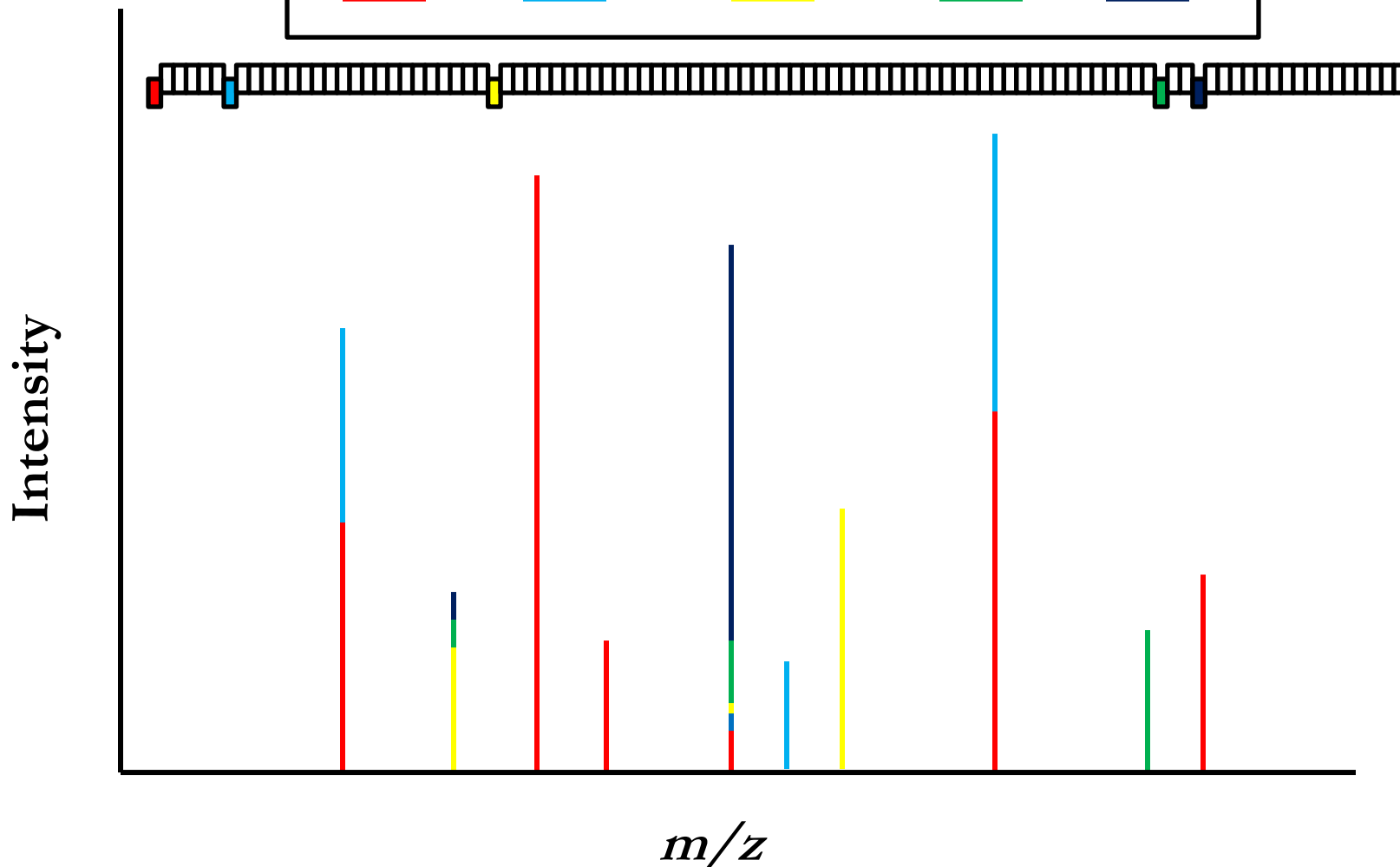


Demultiplexing



Demultiplexing

Isolation Windows				
1	7	28	81	84



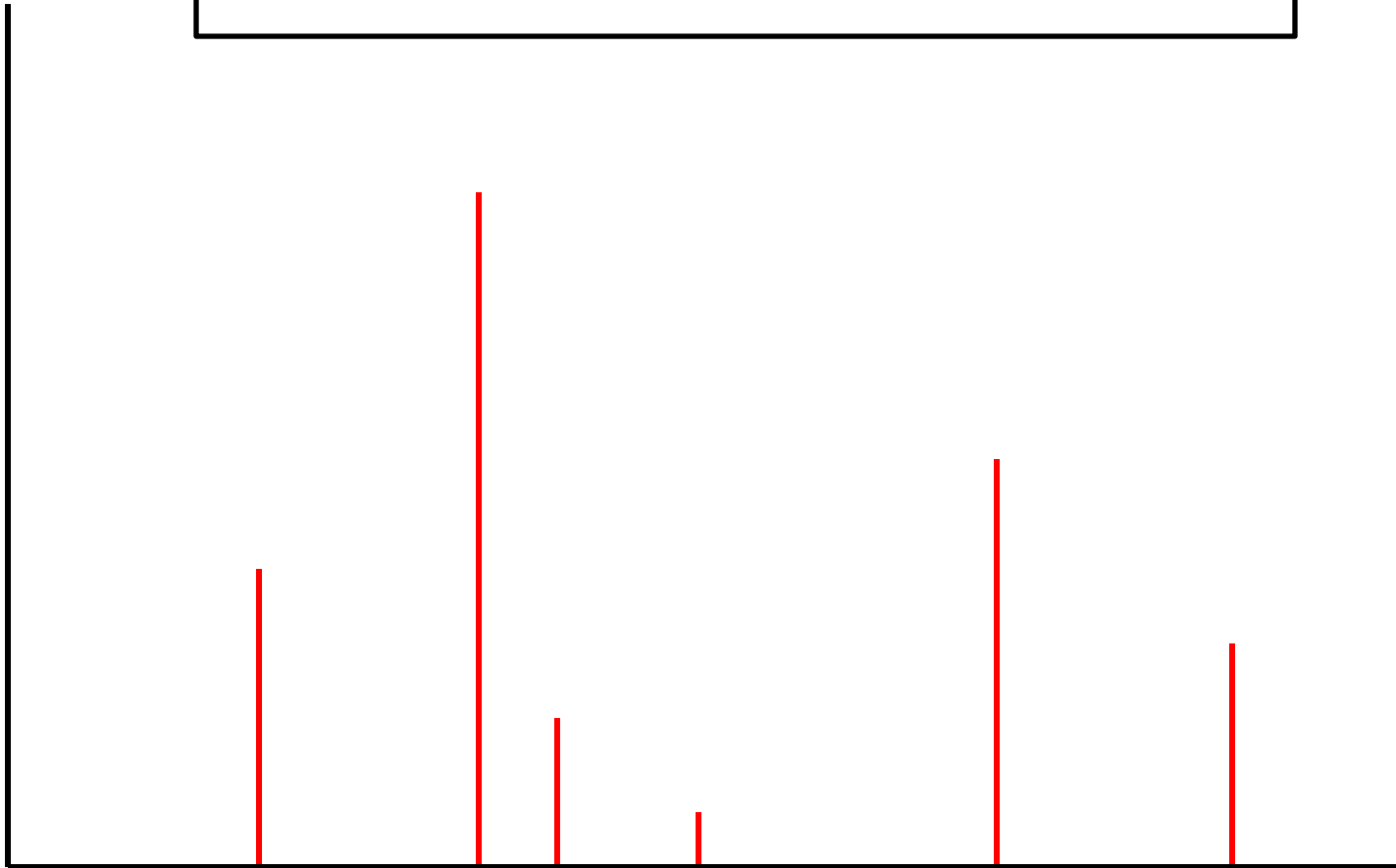
Demultiplexing

Isolation Windows

1



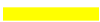




Intensity



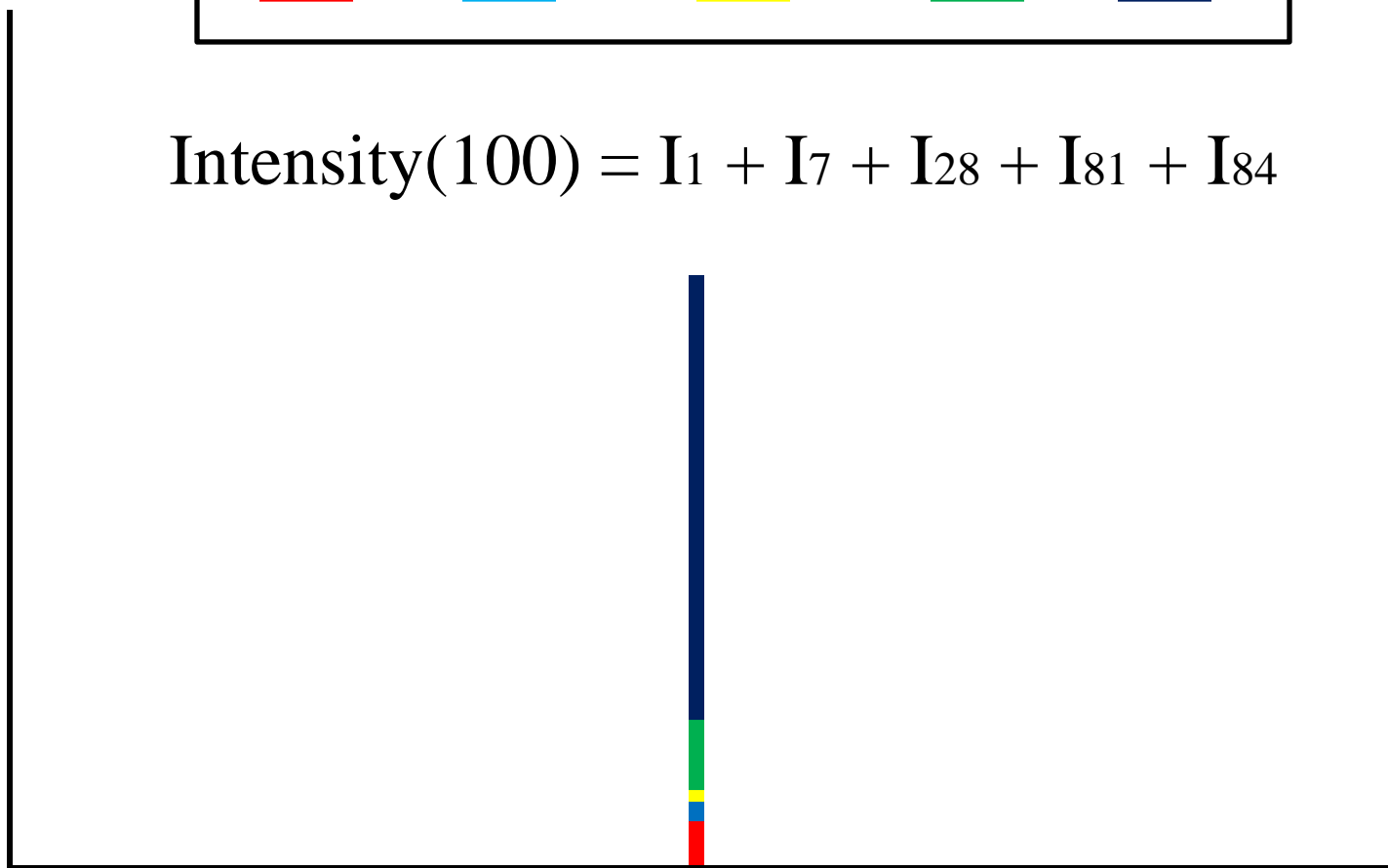
m/z

Demultiplexing

Isolation Windows				
1	7	28	81	84
				

$$\text{Intensity}(100) = I_1 + I_7 + I_{28} + I_{81} + I_{84}$$

Intensity



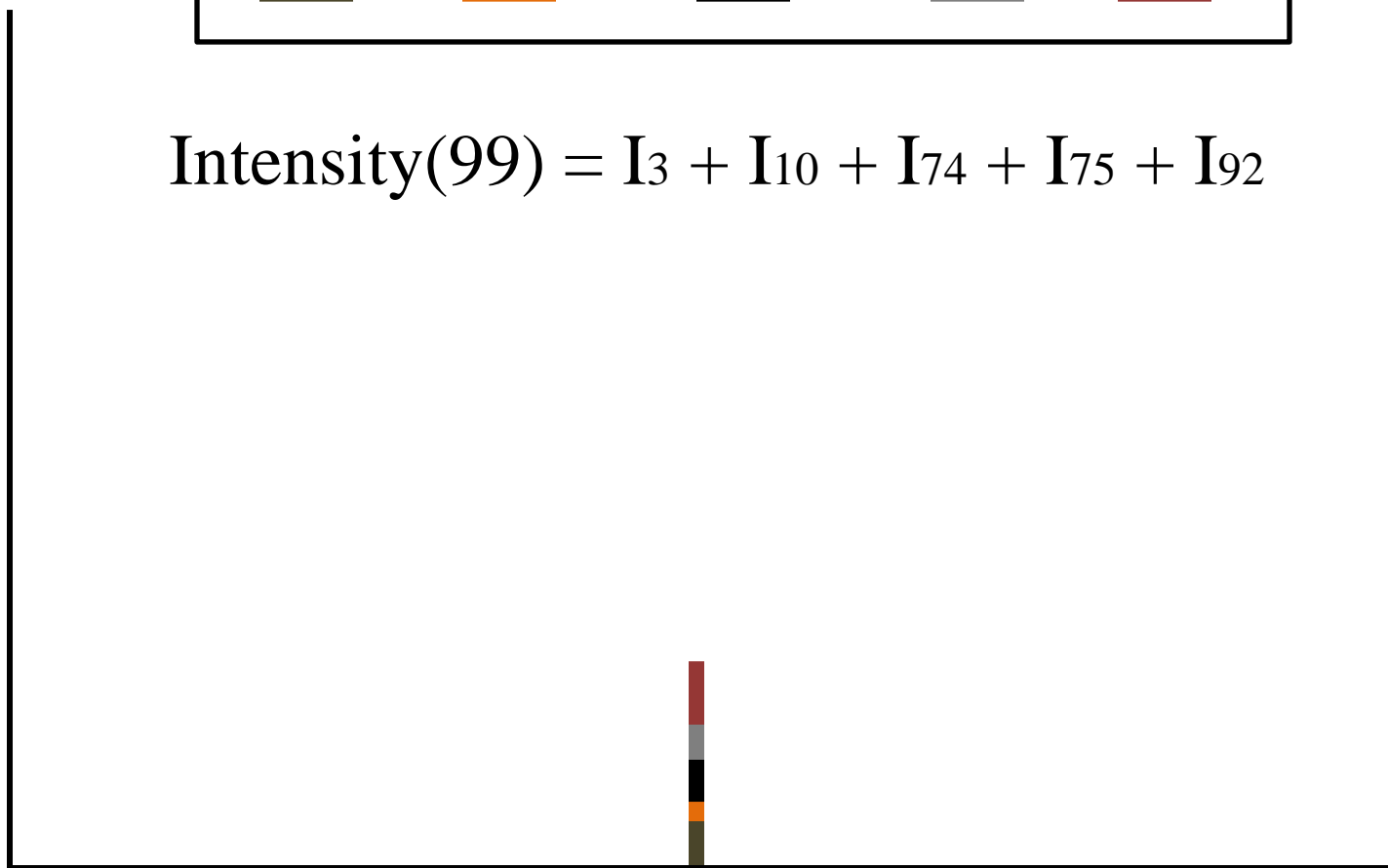
m/z

Demultiplexing

Isolation Windows				
3	10	74	75	92

$$\text{Intensity}(99) = I_3 + I_{10} + I_{74} + I_{75} + I_{92}$$

Intensity



m/z

Demultiplexing

$$\text{Intensity}(99) = I_3 + I_{10} + I_{74} + I_{75} + I_{92}$$

$$\text{Intensity}(100) = I_1 + I_7 + I_{28} + I_{81} + I_{84}$$

Intensity

10
Unknowns

m/z

Demultiplexing

$$\text{Intensity}(99) = I_3 + I_{10} + I_{74} + I_{75} + I_{92}$$

$$\text{Intensity}(100) = I_1 + I_7 + I_{28} + I_{81} + I_{84}$$

2

Knowns

10

Unknowns

Intensity

m/z

Demultiplexing

100 Scans

5 Duty Cycles

~15 seconds

$$\begin{array}{l} \text{Intensity}(50) = I_3 + I_{11} + I_{34} + I_{35} + I_{90} \\ \vdots \\ \text{Intensity}(99) = I_3 + I_{10} + I_{74} + I_{75} + I_{92} \\ \text{Intensity}(100) = I_1 + I_7 + I_{28} + I_{81} + I_{84} \\ \vdots \\ \text{Intensity}(150) = \underbrace{I_{17} + I_{44}}_{100 \text{ knowns}} + \underbrace{I_{52} + I_{55} + I_{99}}_{100 \text{ unknowns}} \end{array}$$

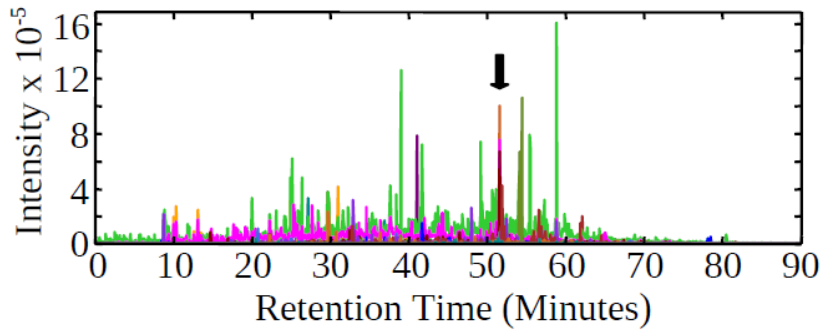
Solve by non-negative least squares optimization



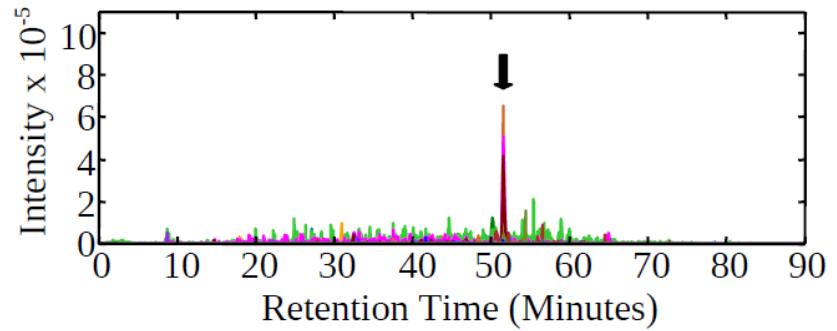
Demultiplexing

GPLVLEVETYR detected in *S. cerevisiae* lysate (soluble); MSX 5 x 4 m/z isolation windows per scan (Q-Exactive)

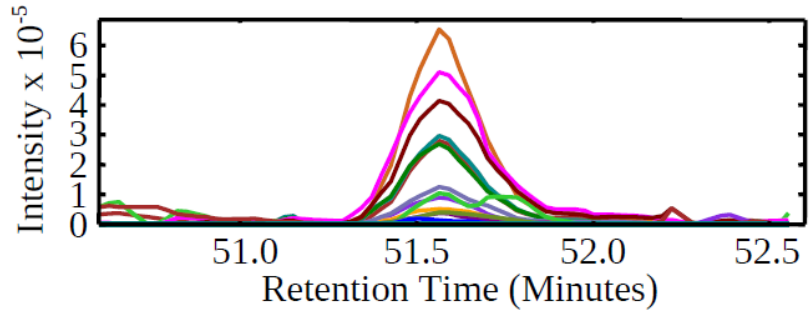
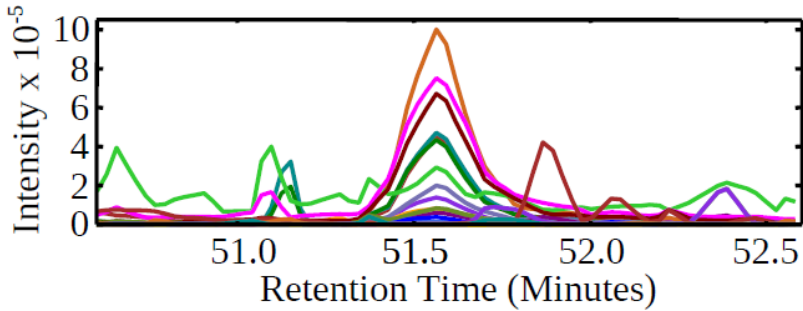
Not De-Multiplexed



De-Multiplexed

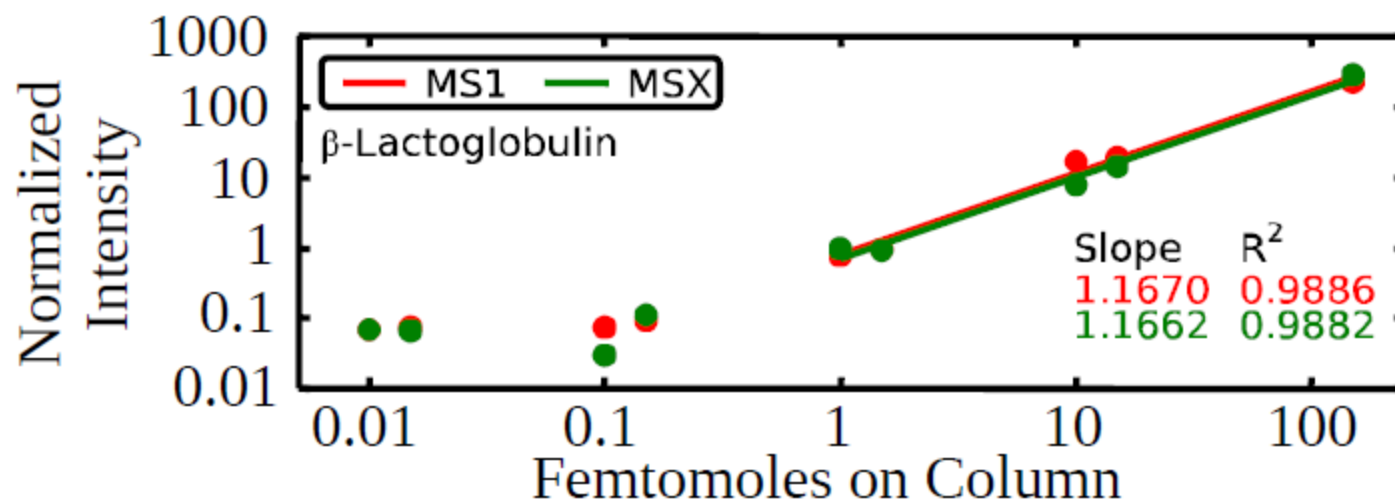
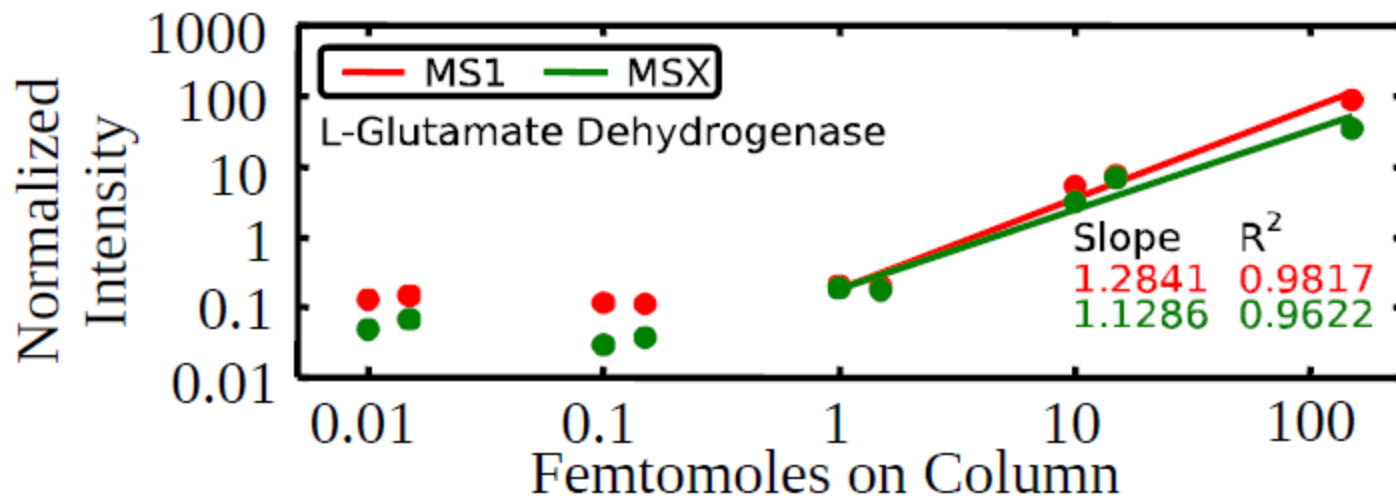


- y10
- y9
- y8
- y7
- y6
- y5
- y4
- y3
- y2
- y1
- b1
- b2
- b3
- b4
- b5
- b6
- b7
- b8
- b9
- b10



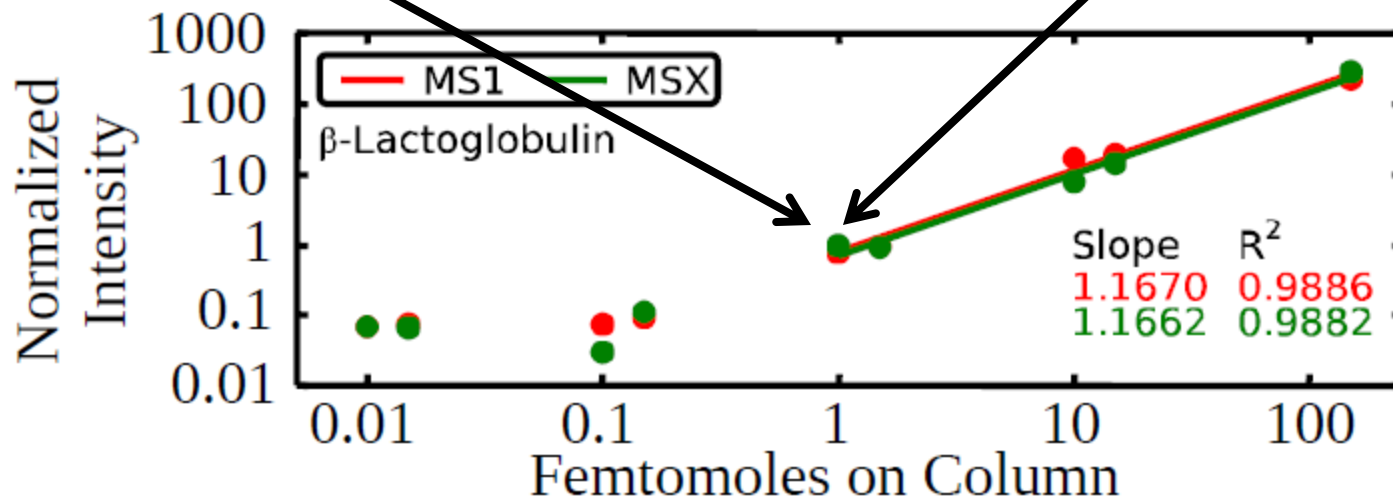
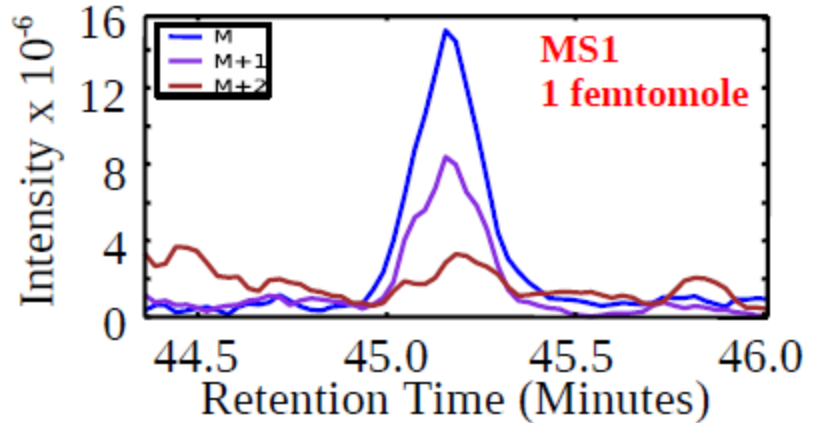
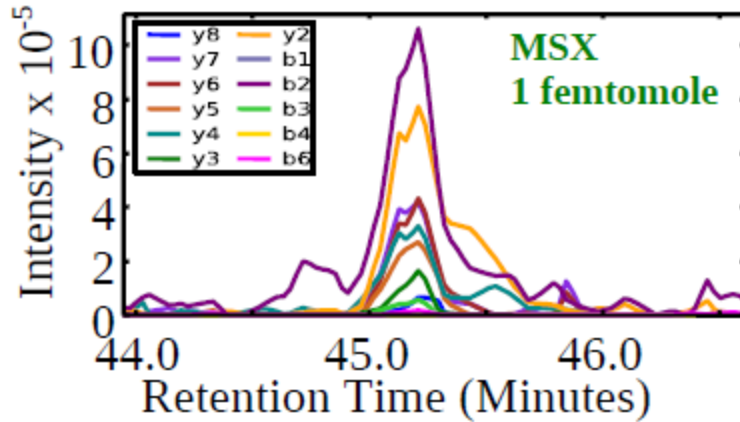
Sensitivity Similar to MS1 Quantification

Bovine proteins spiked into *S. cerevisiae* lysate (soluble fraction)



Sensitivity Similar to MS1 Quantification

Bovine proteins spiked into *S. cerevisiae* lysate (soluble fraction)



Conclusions

- DIA data can be multiplexed by mixing precursors prior to fragment ion analysis
- MSX de-multiplexing and isolation list export will be included in Skyline v1.3 (<http://skyline.maccosslab.org>)
- A firmware patch is needed to implement this method on the Q-Exactive
 - Markus Kellmann (markus.kellmann@thermofisher.com)

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