

diaPASEF – concepts and data analysis

Skyline Webinar #21: Analysis of diaPASEF Data With Skyline

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Outline

- Primer on trapped ion mobility separations (TIMS)
- diaPASEF concept
- Data set for the tutorial – 3 species mixture

For background see:

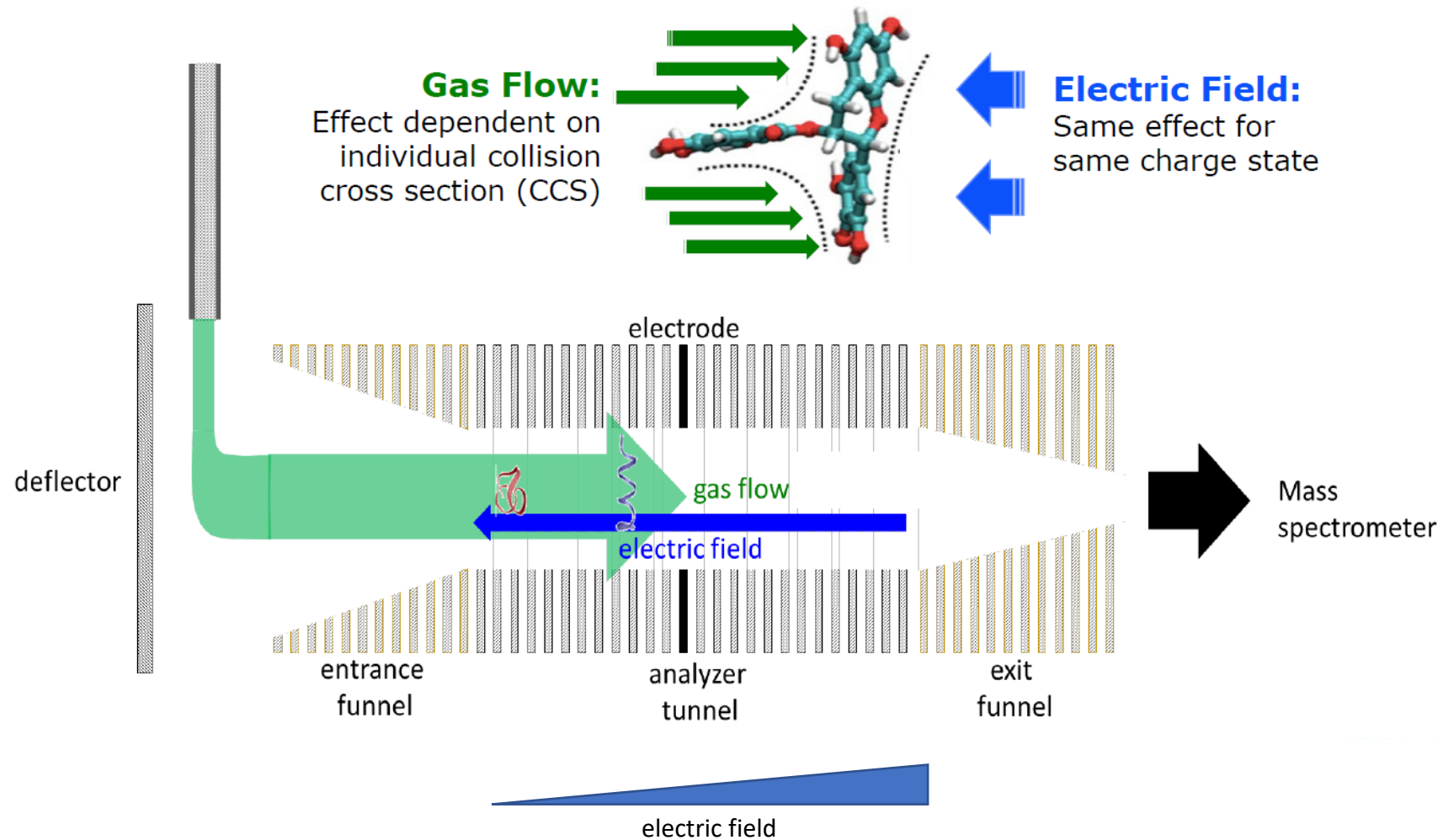
[Webinar #18](#)

[DIA Data Analysis revisited](#)

[Webinar #19](#)

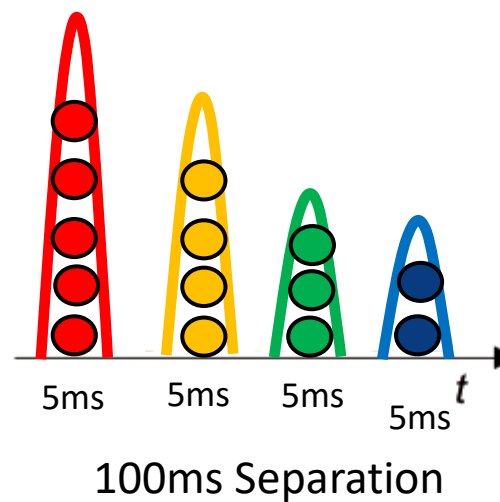
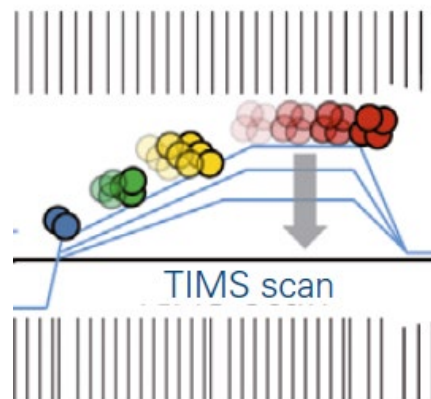
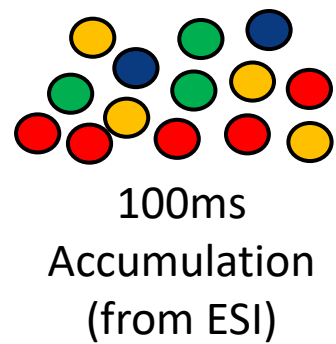
[Ion Mobility Spectrum Filtering](#)

Trapped ion mobility spectrometry (TIMS)

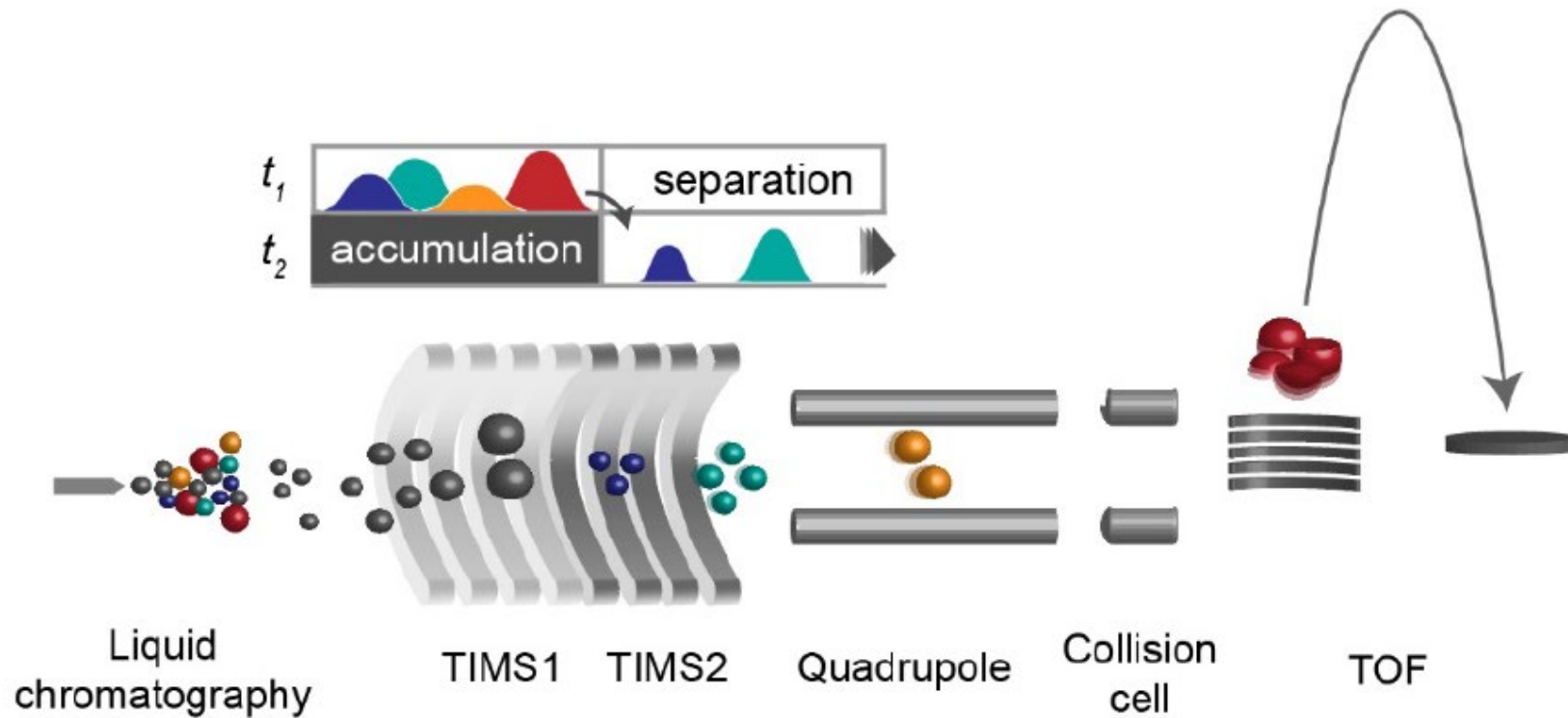


Fernandez-Lima, Francisco, Desmond A. Kaplan, J. Suetering, and Melvin A. Park. "Gas-Phase Separation Using a Trapped Ion Mobility Spectrometer." *International Journal for Ion Mobility Spectrometry: Official Publication of the International Society for Ion Mobility Spectrometry* (2011)

TIMS separates but also focuses ions



timsTOF Pro instrument



ddaPASEF

Meier, F, et al. *Journal of Proteome Research* (2015)

Meier, F., et al. *Molecular & Cellular Proteomics* (2018)

Outline

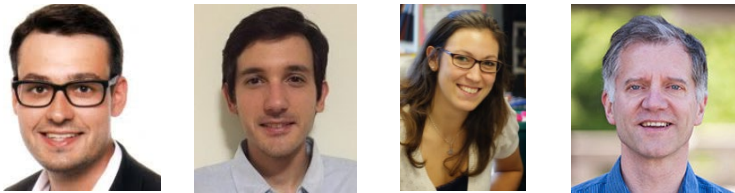
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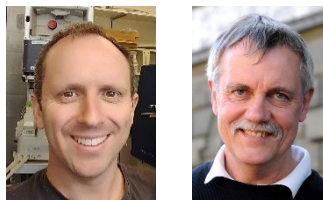
diaPASEF collaboration

Goal: Combine efficiency and sensitivity/selectivity of PASEF with the deterministic quantification capability of DIA



Florian Meier, Andreas-David Brunner,
Isabell Bludau, Eugenia Voytik, Matthias
Mann

Max Planck Institute of Biochemistry



Ben Collins, Ruedi
Aebersold

Queen's University
Belfast and ETH Zurich



Hannes Röst, Max Frank, Annie Ha
Donnelly Centre for Cellular and Biomolecular
Research
University of Toronto

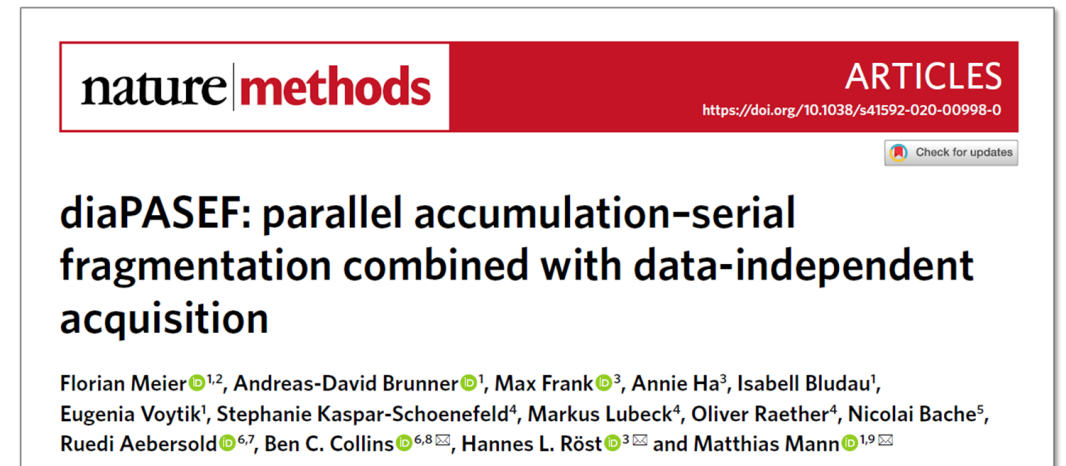


Stephanie Kaspar-Schönefeld,
Markus Lubeck,
Heiner Koch, Scarlet Koch, Oliver
Räther

Bruker Daltonics



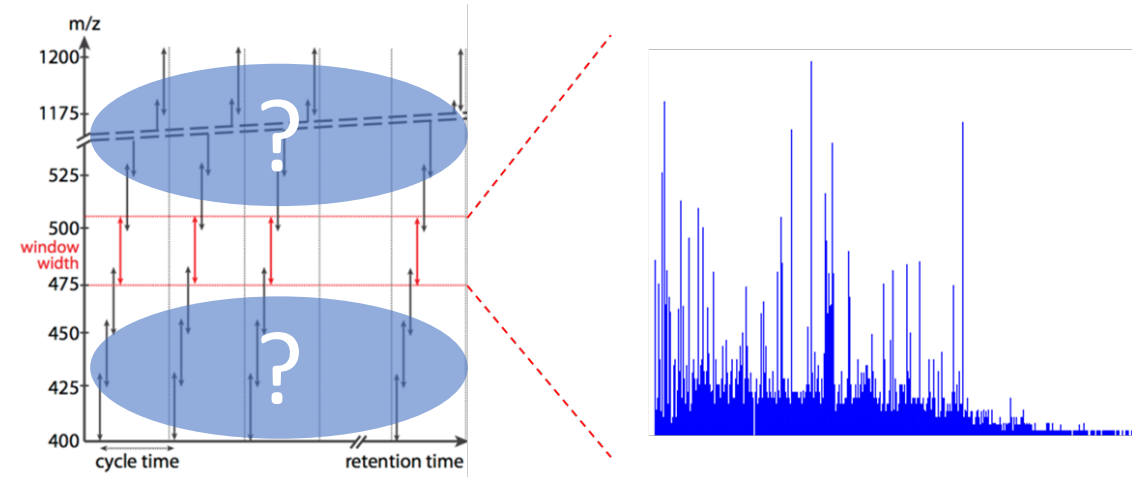
Nicolai Bache
Evosep



Problem statement(s)

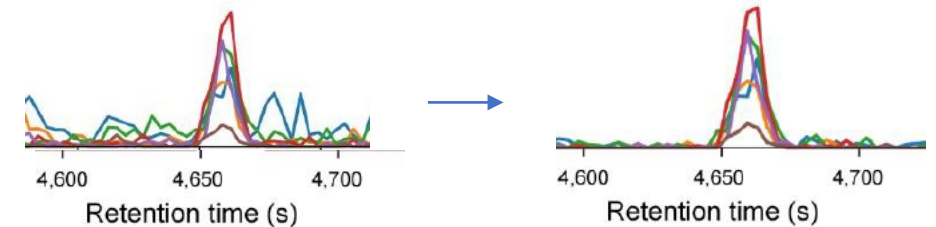
1) Global efficiency of DIA/SWATH at the mass selective quadrupole is low

- $1 / \#$ precursor windows
- ~1-3% for commonly used schemes

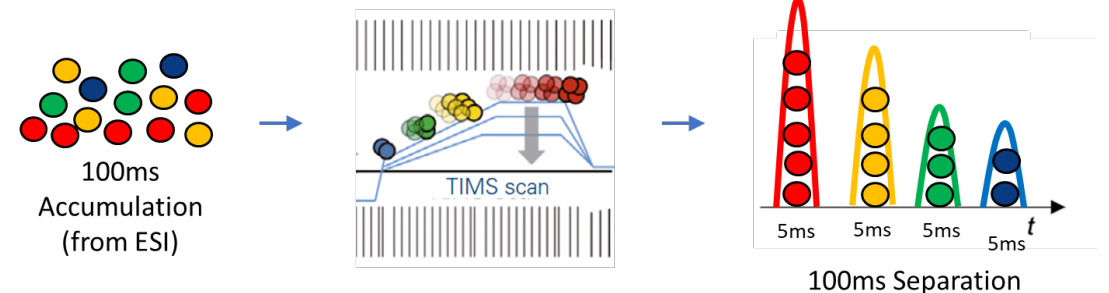


2) In DIA/SWATH selectivity is (sometimes) limiting

- Mobility separation should help

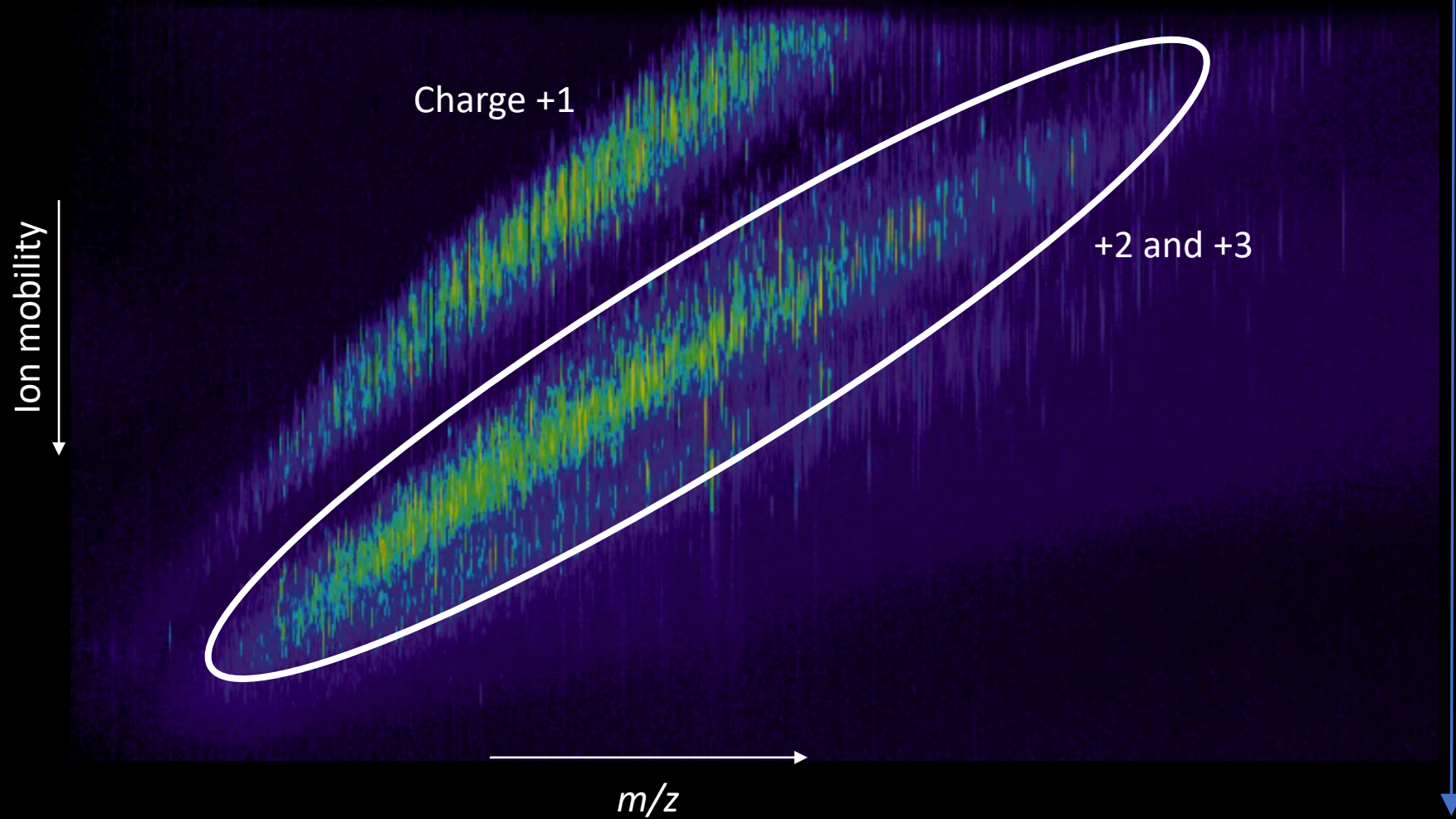


3) Ion stacking benefit of PASEF not yet implemented in DIA mode



120 min HeLa run
Summed MS1 signal

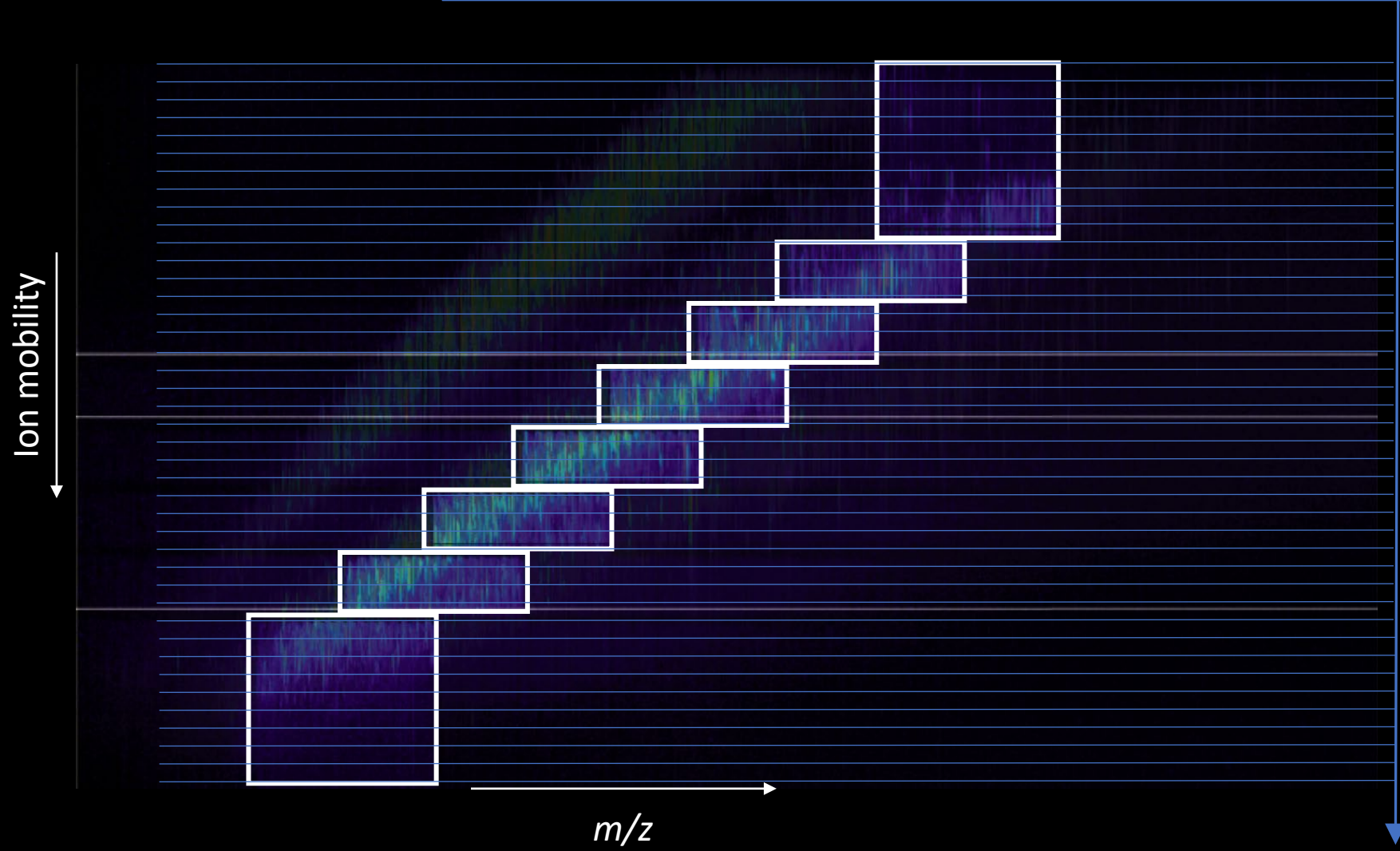
~1,000 TOF pushes (MS1) in one ion mobility cycle 100ms



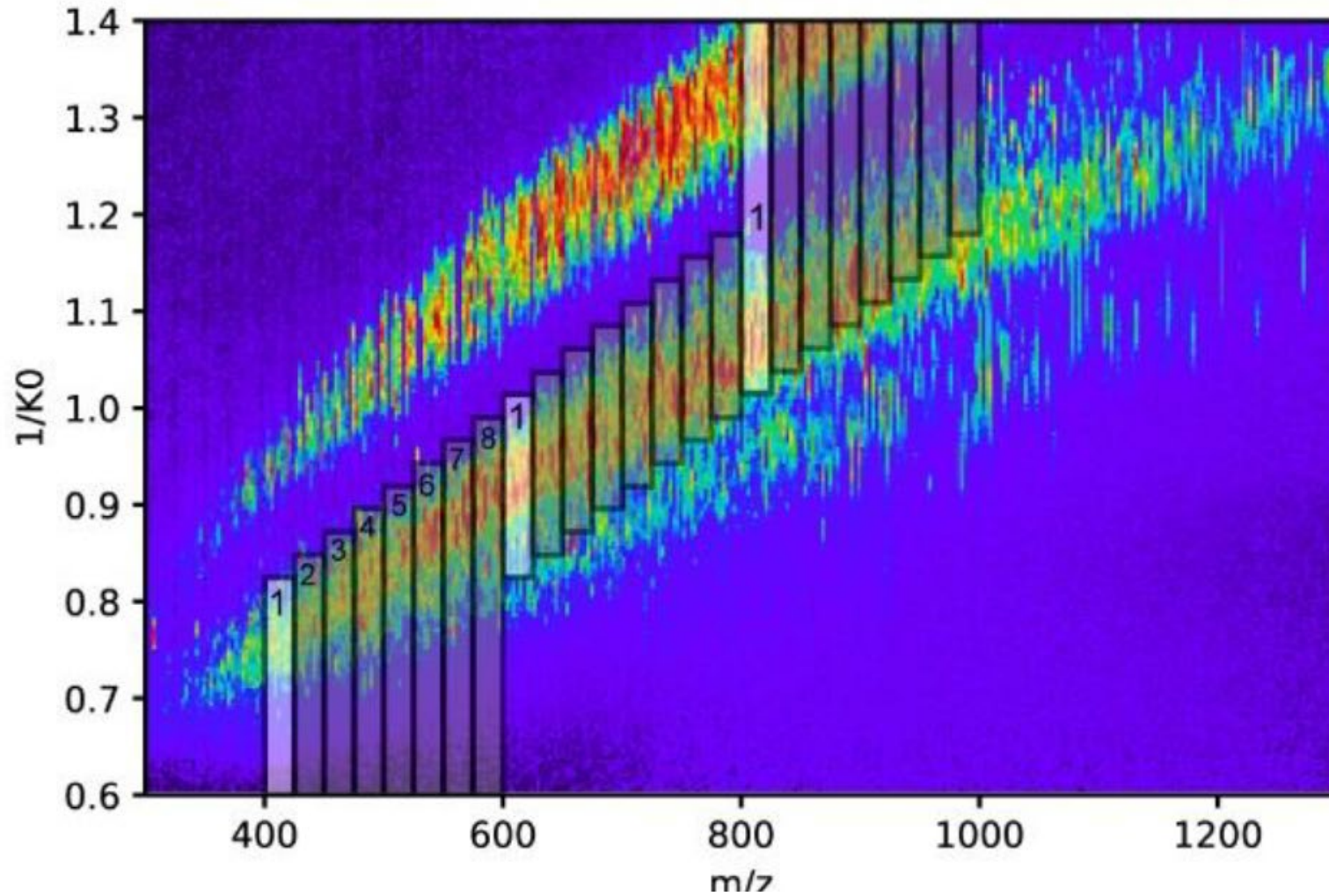
120 min HeLa run

Overlay

~1,000 TOF pushes (MS2) in one ion mobility cycle 100ms



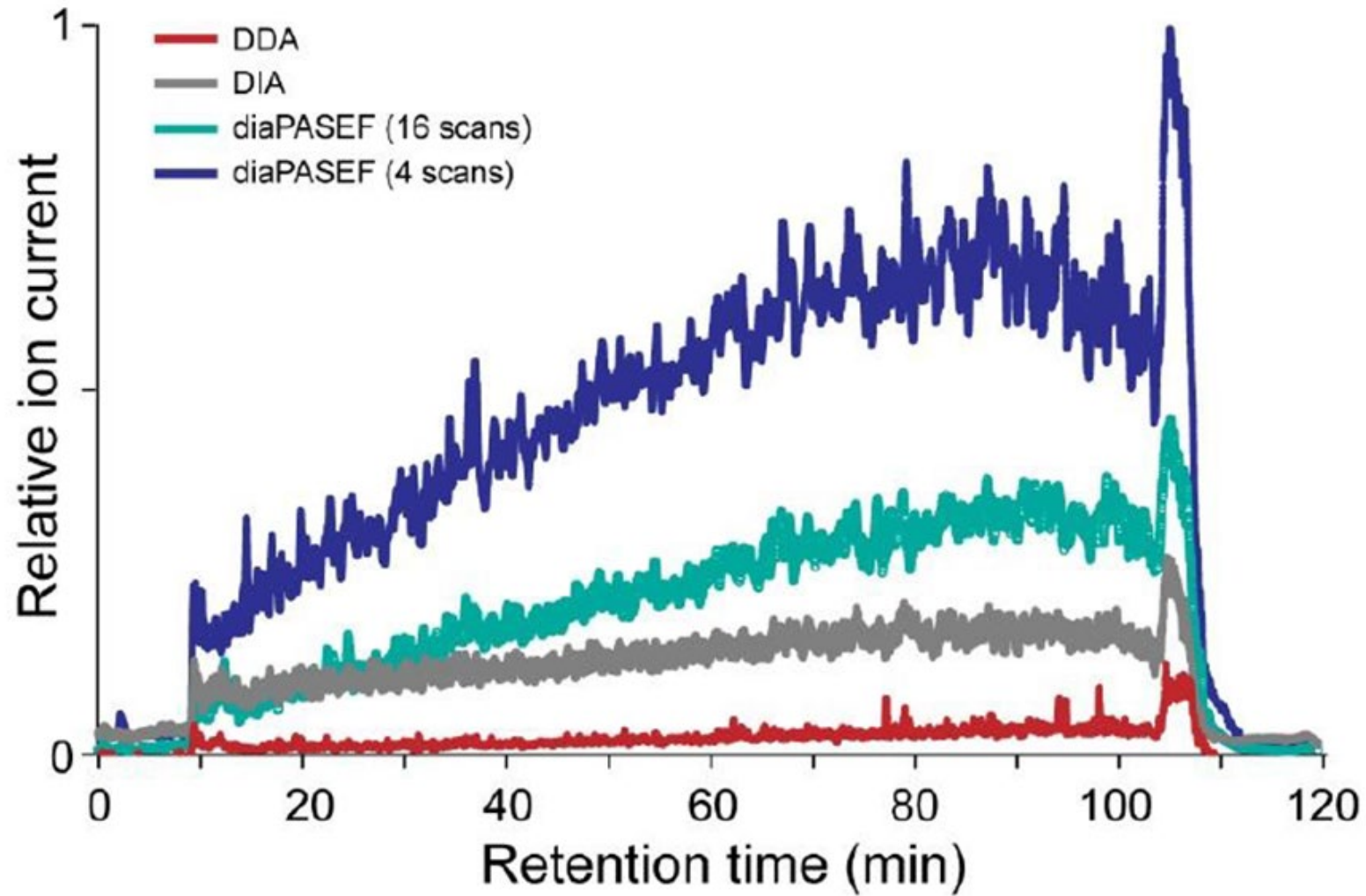
Balance between ion utilization efficiency, selectivity, and precursor coverage



high speed diaPASEF

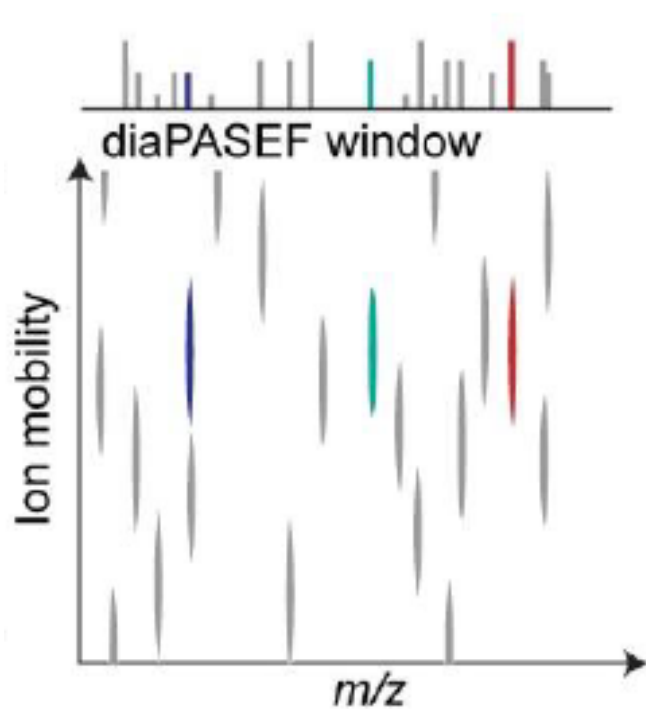
- 24 x 25 m/z windows
- 3 IM windows / 100 ms IM cycle
- 8 IM cycles to cover precursors ~ 0.8 sec

Increased ion utilization efficiency compared to standard DIA (or DDA)



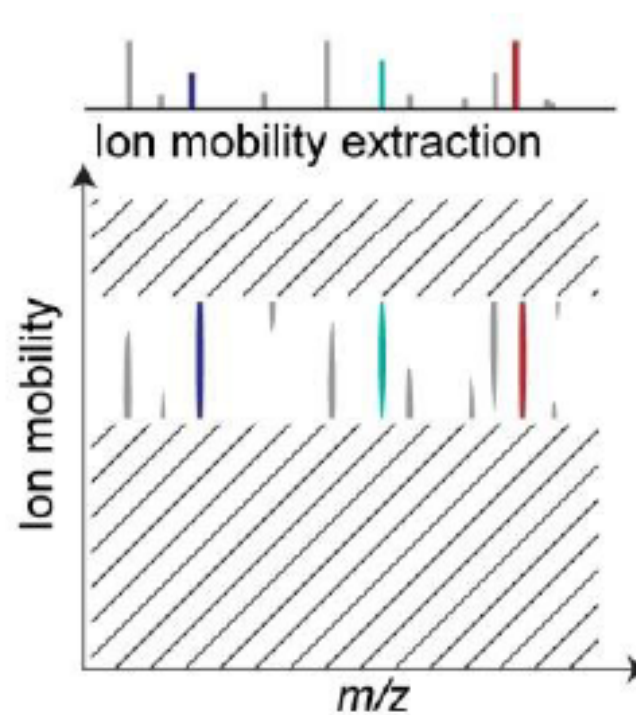
Data analysis strategy adapted for diaPASEF

Selective extraction in the ion mobility domain



Targeted data
extraction
→

OpenSWATH

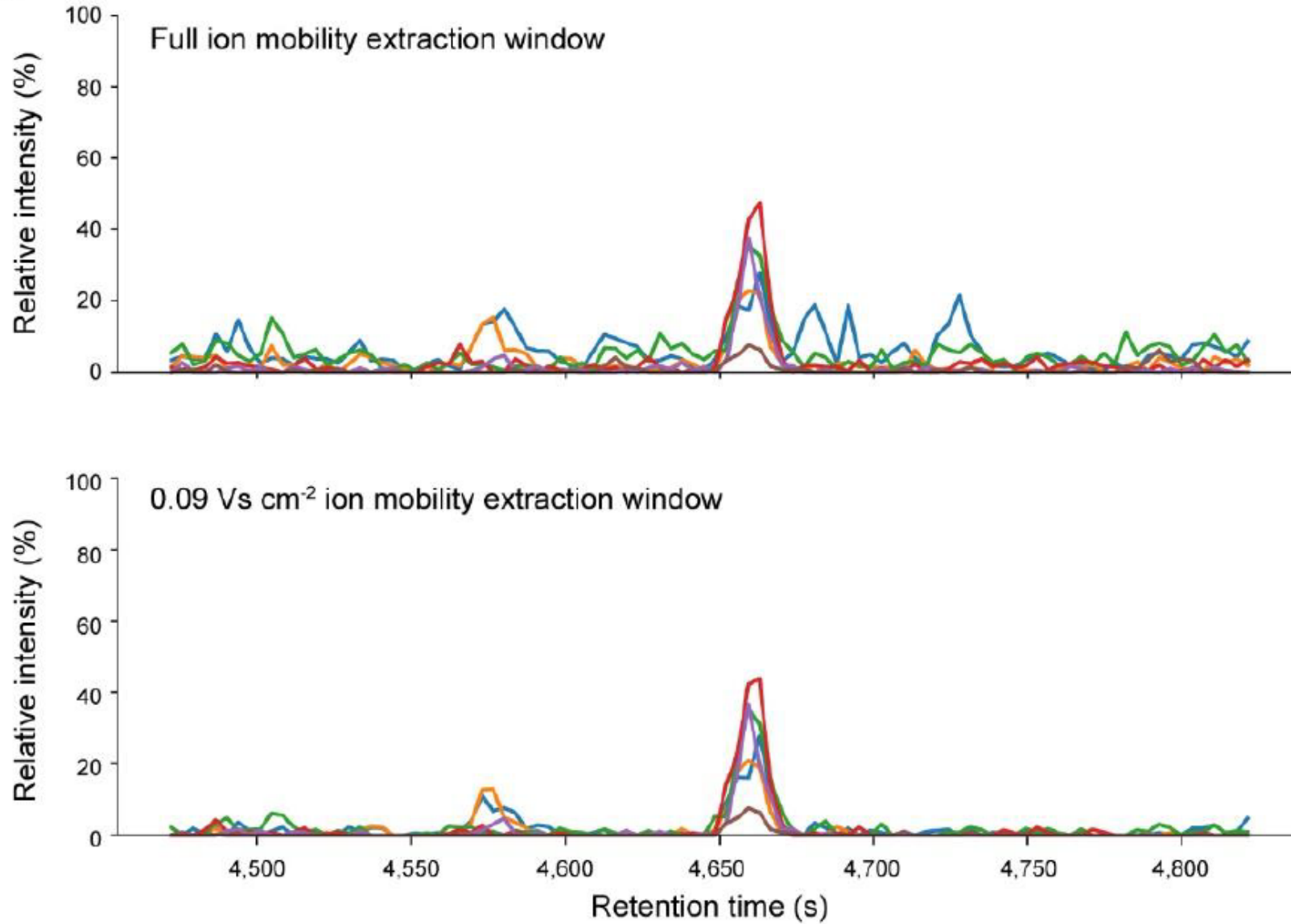


Now also:

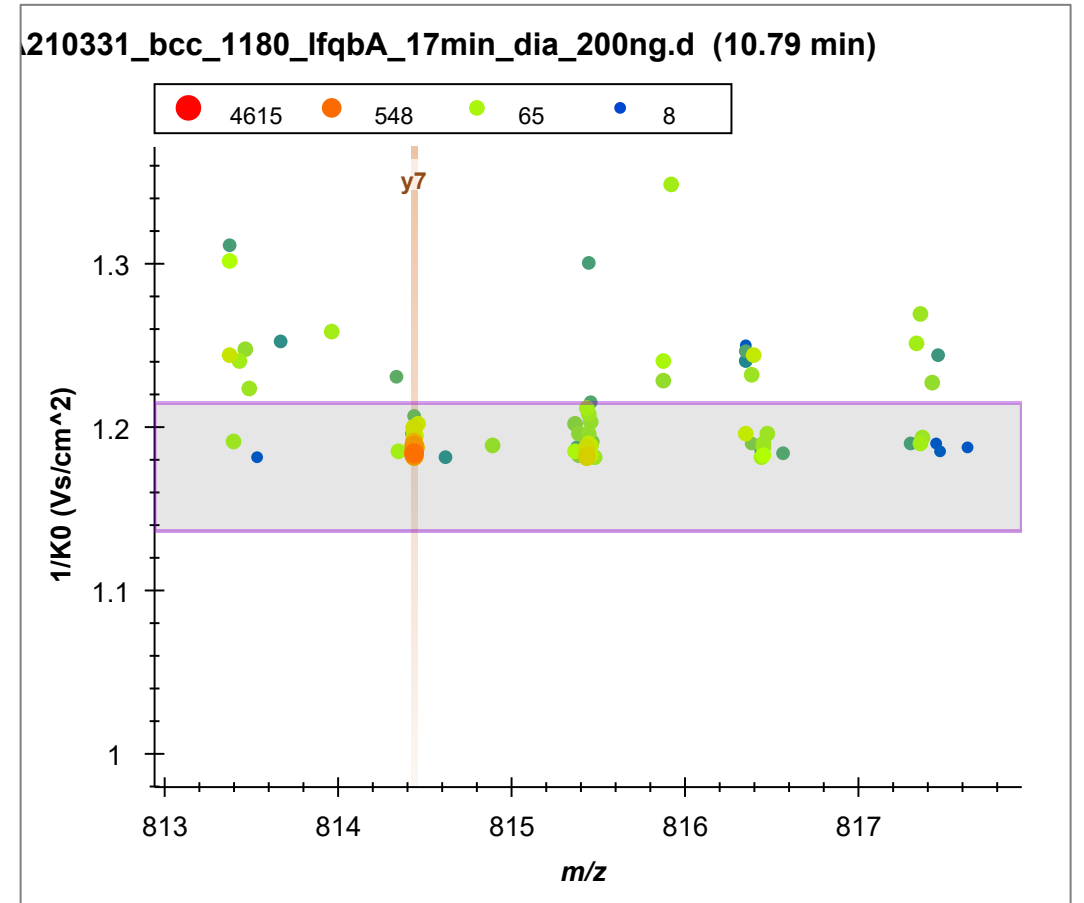
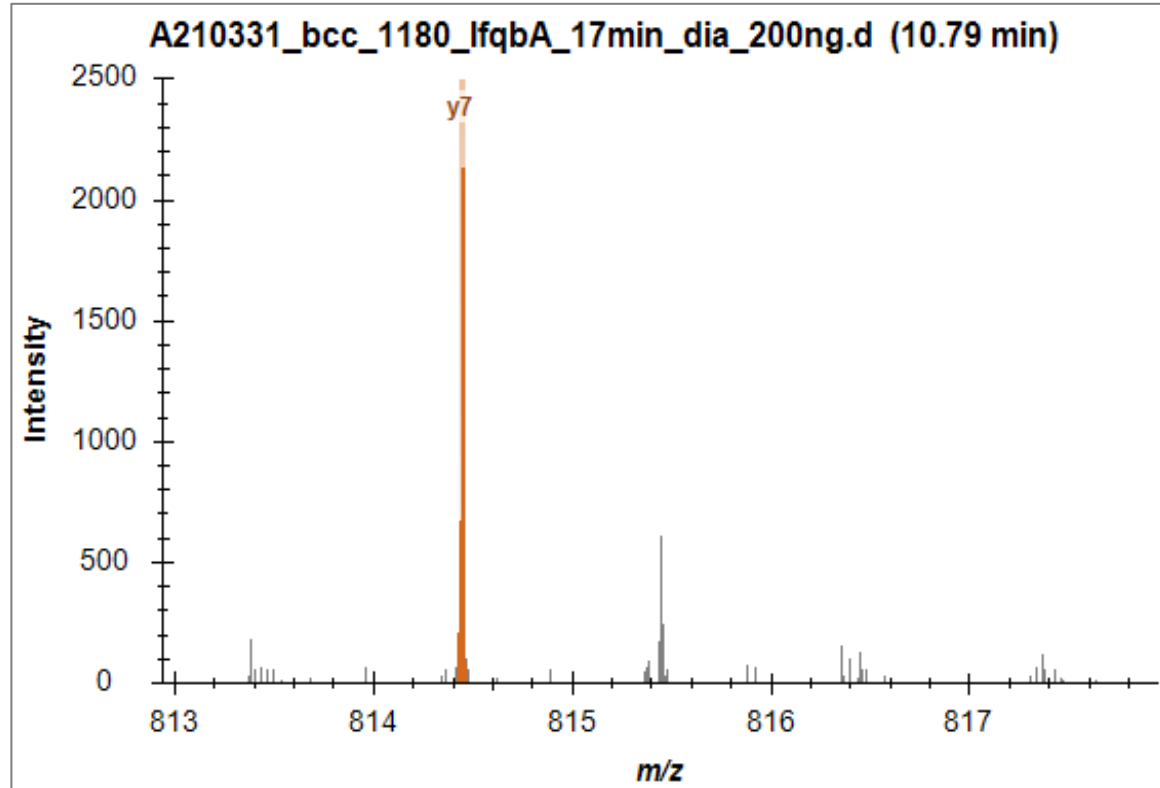


And several others...

Does selective extraction in ion mobility dimension make a difference?



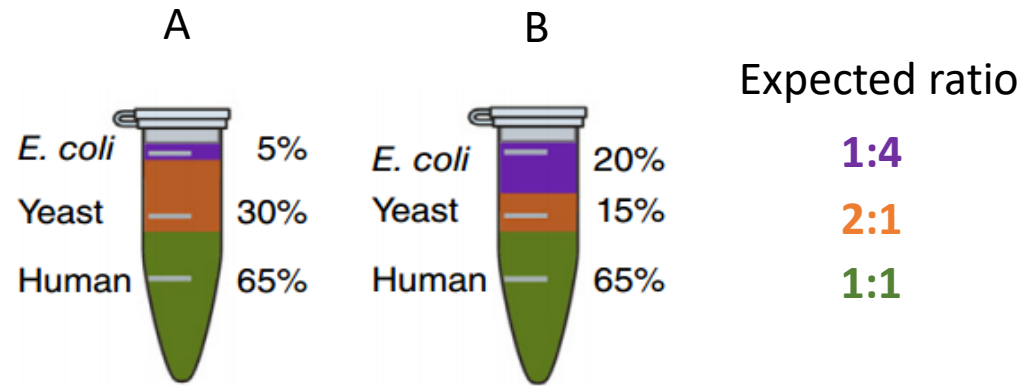
Skyline – ion mobility resolved visualization



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Data set for the webinar demo and tutorial

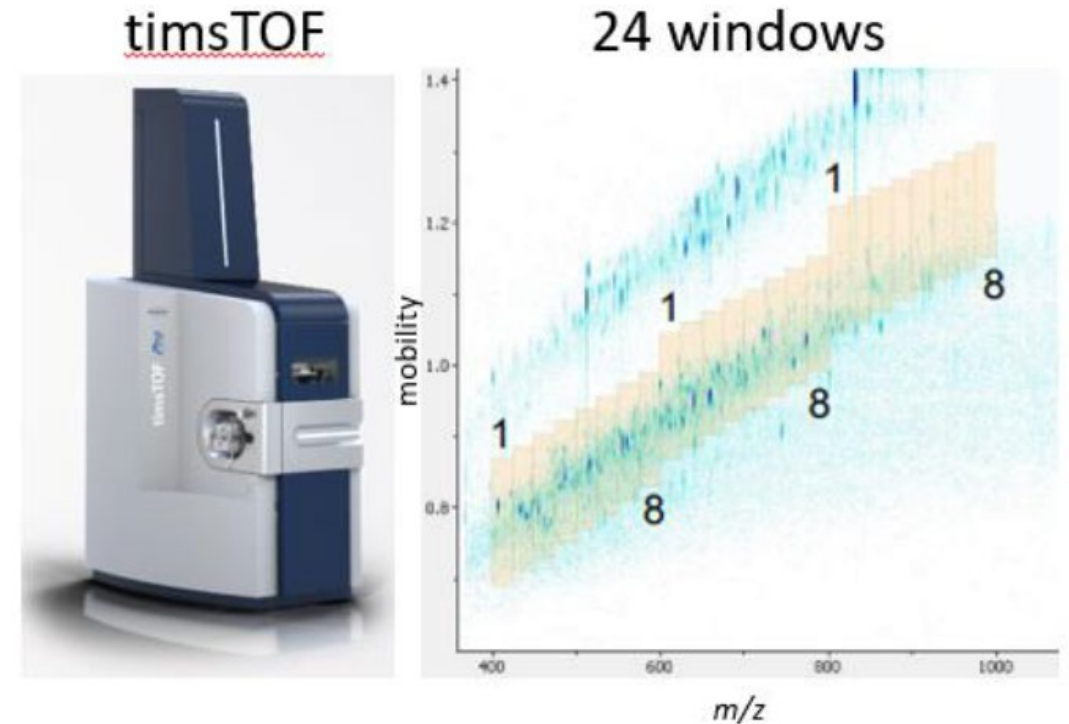


- 3 x diaPASEF technical replicates
- 17 Minute gradient
- 200ng injected (5x less than Navarro et al)

- 2 ddaPASEF files (1 of each condition)

(A/B labelling is opposite from Navarro et al)

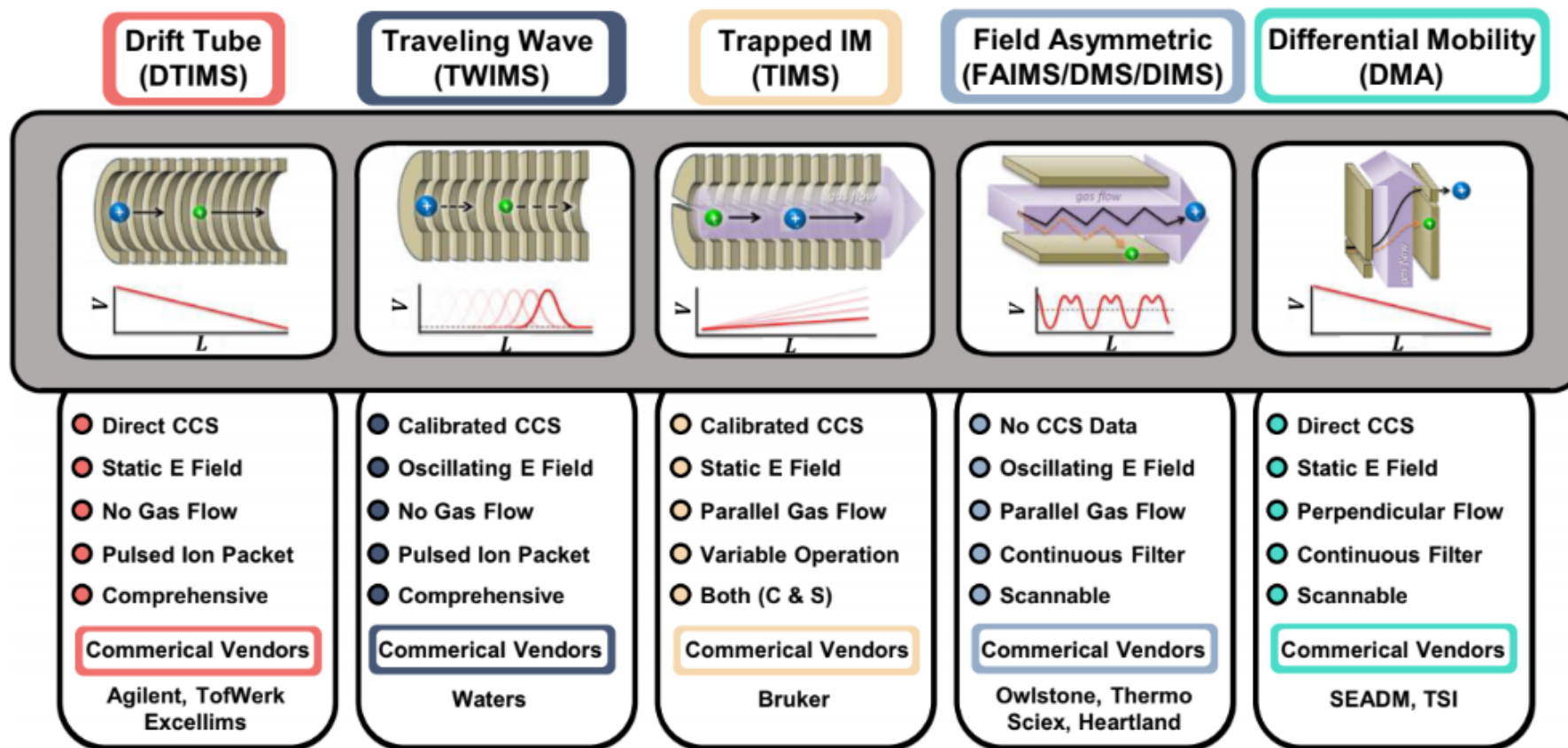
Navarro, P. et al. 'A Multicenter Study Benchmarks Software Tools for Label-Free Proteome Quantification'. *Nature Biotechnology* (2016)



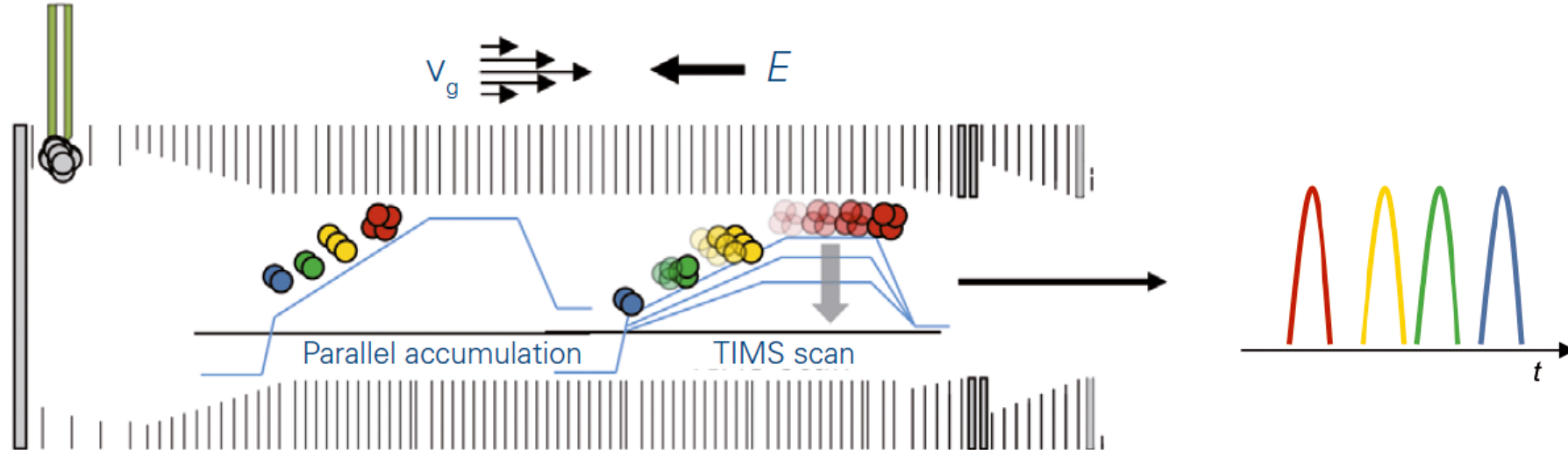
Meier, F., et al. 'DiaPASEF: Parallel Accumulation–Serial Fragmentation Combined with Data-Independent Acquisition'. *Nature Methods* (2020)

Backup

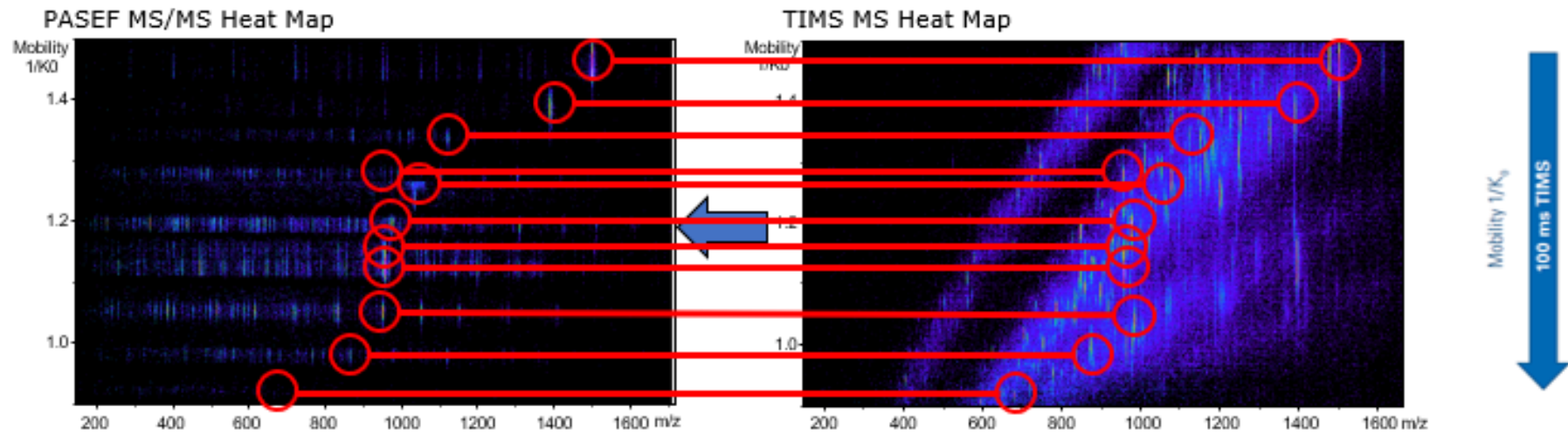
Many flavours of ion mobility separations



Dual TIMS design on timsTOF Pro improves duty cycle



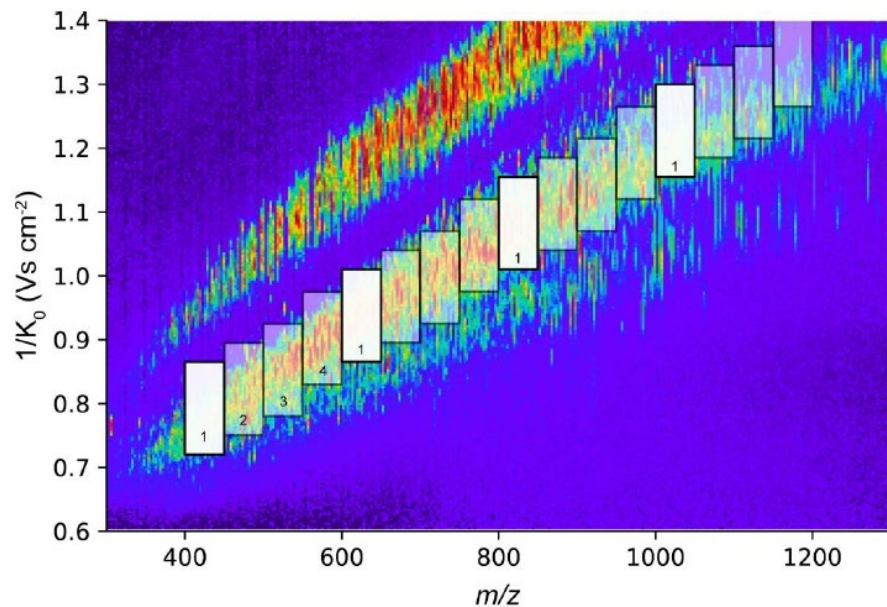
Parallel Accumulation – Serial Fragmentation



Meier, F, et al. *Journal of Proteome Research* (2015)

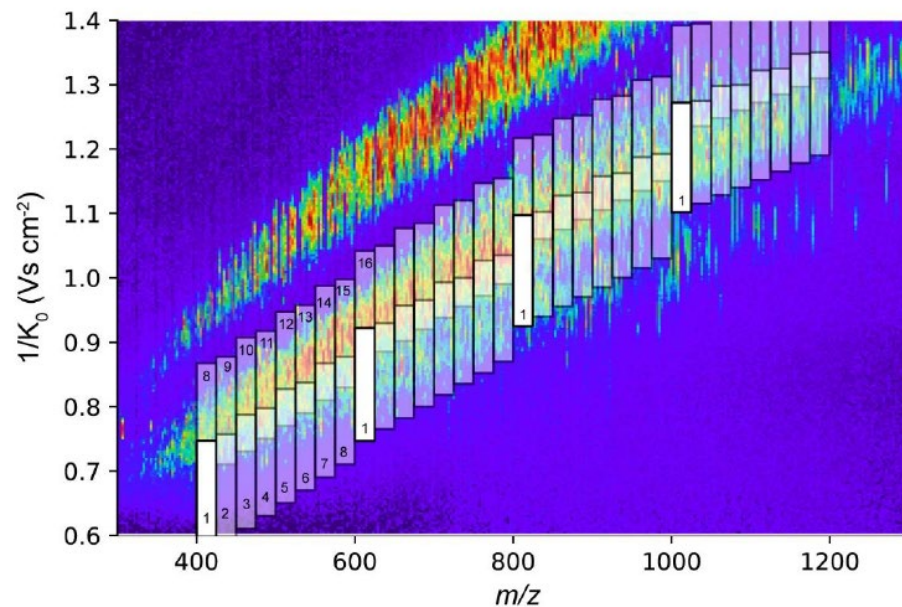
Meier, F., et al. *Molecular & Cellular Proteomics* (2018)

Balance between ion utilization efficiency, selectivity, and precursor coverage



high sensitivity diaPASEF

- 16 x 50 m/z windows
- 4 IM windows / 100 ms IM cycle
- 4 IM cycles to cover precursors ~ 0.4 sec
- Repeat 4x for ~ 1.7 sec duty cycle



Standard diaPASEF

- 32 x 25 m/z windows
- with IM overlap 64 windows
- 4 IM windows / 100 ms IM cycle
- 16 (8 x 2) IM cycles / ~ 1.7 sec duty cycle

