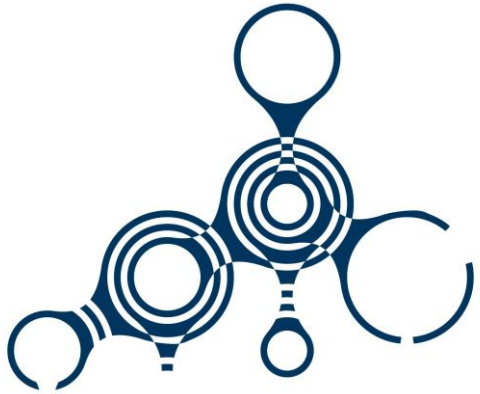
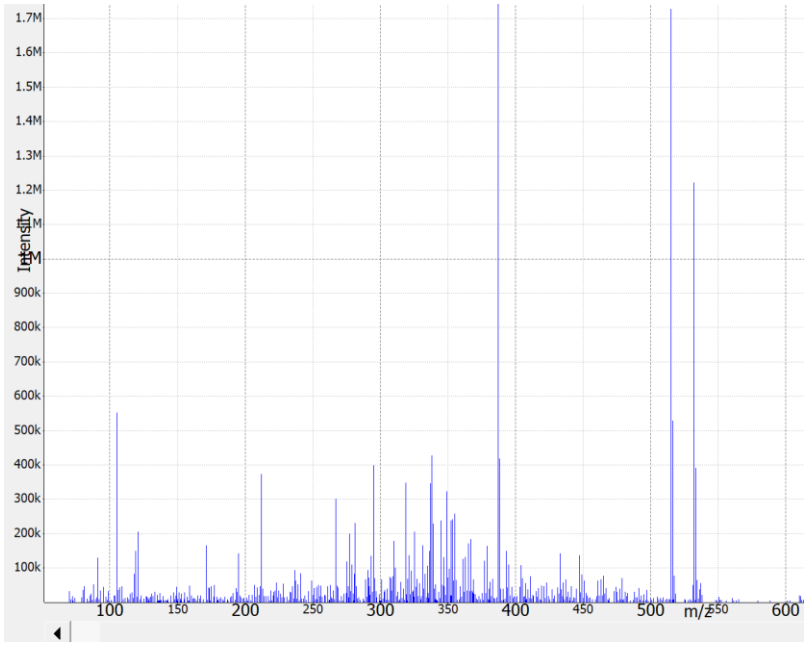


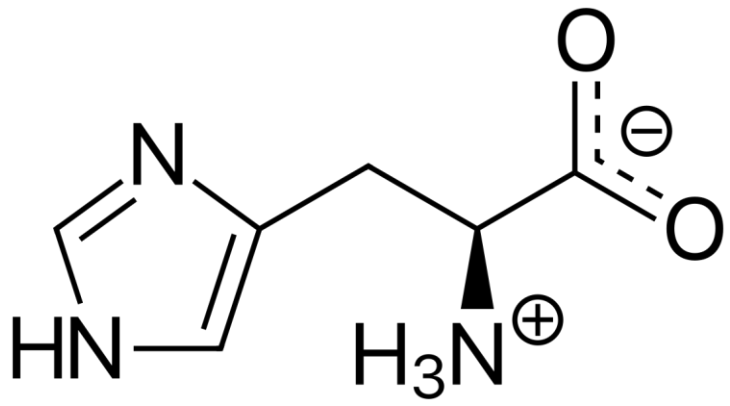
Rapid Development of Improved Data-Dependent Acquisition Strategies

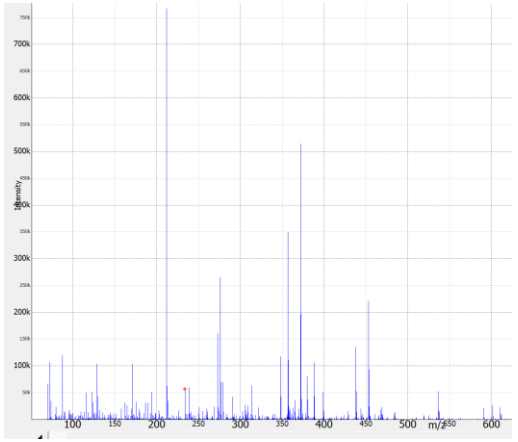
- ***Joe Wandy***, *Vinny Davies, Stefan Weidt, Justin J.J. van der Hooft, Alice Miller, Rónán Daly and Simon Rogers*

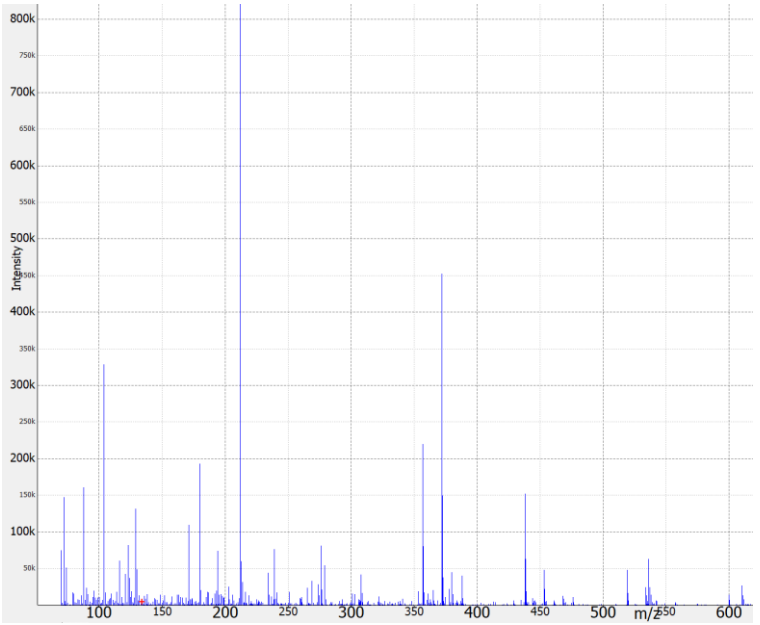
MassBank

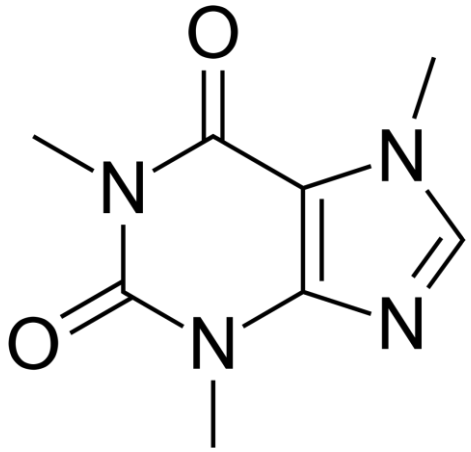


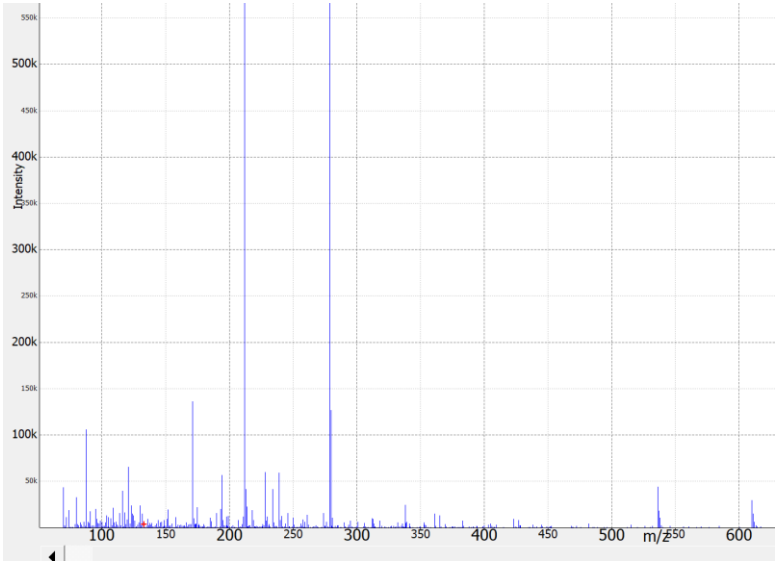
GNPS

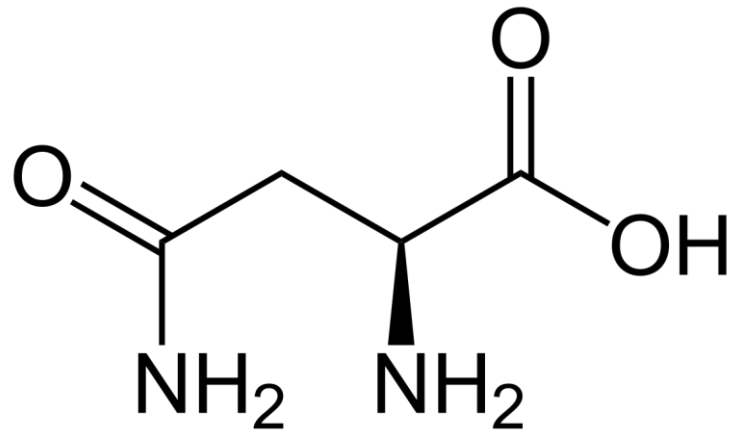

O
N
HN
H3N


Intensity
m/z


Intensity
m/z

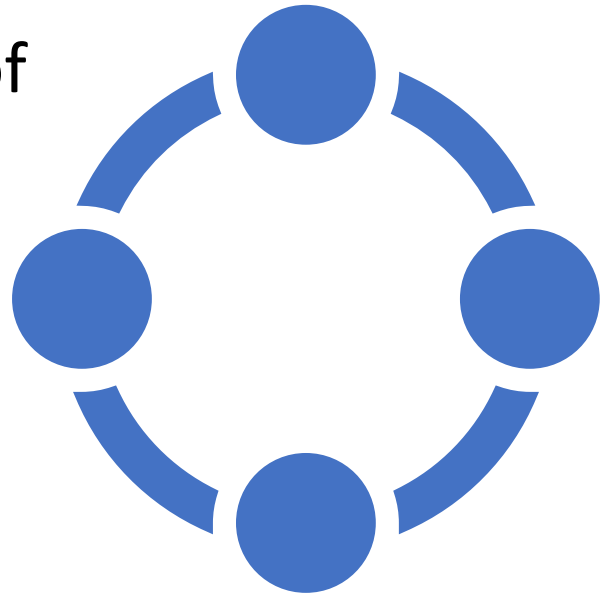

O
N
N
O
N
N


Intensity
m/z


O
O
OH
NH2
NH2

Data-dependent Acquisition (DDA)

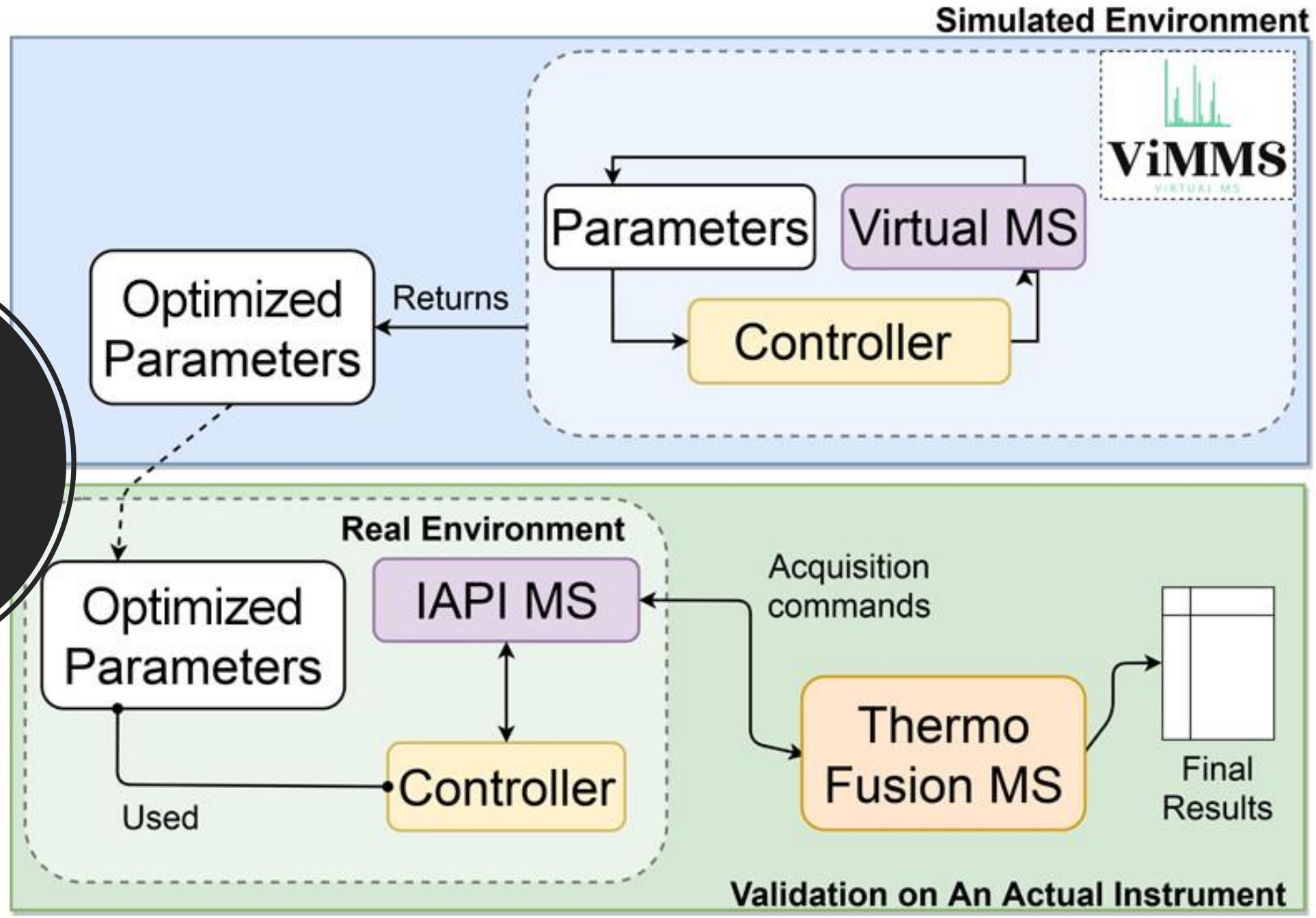
- In a duty cycle:
  - A survey (MS1) scan is performed.
  - A set of *N* ions (usually the most intense ones) is selected.
  - The chosen ions are isolated and fragmented in a series of MS2 scans.
- MS2 spectra produced from DDA are ready to use.
  - Each fragmentation spectrum is generated by fragmenting a small m/z isolation window to target particular chemical species.



Improving DDA strategies

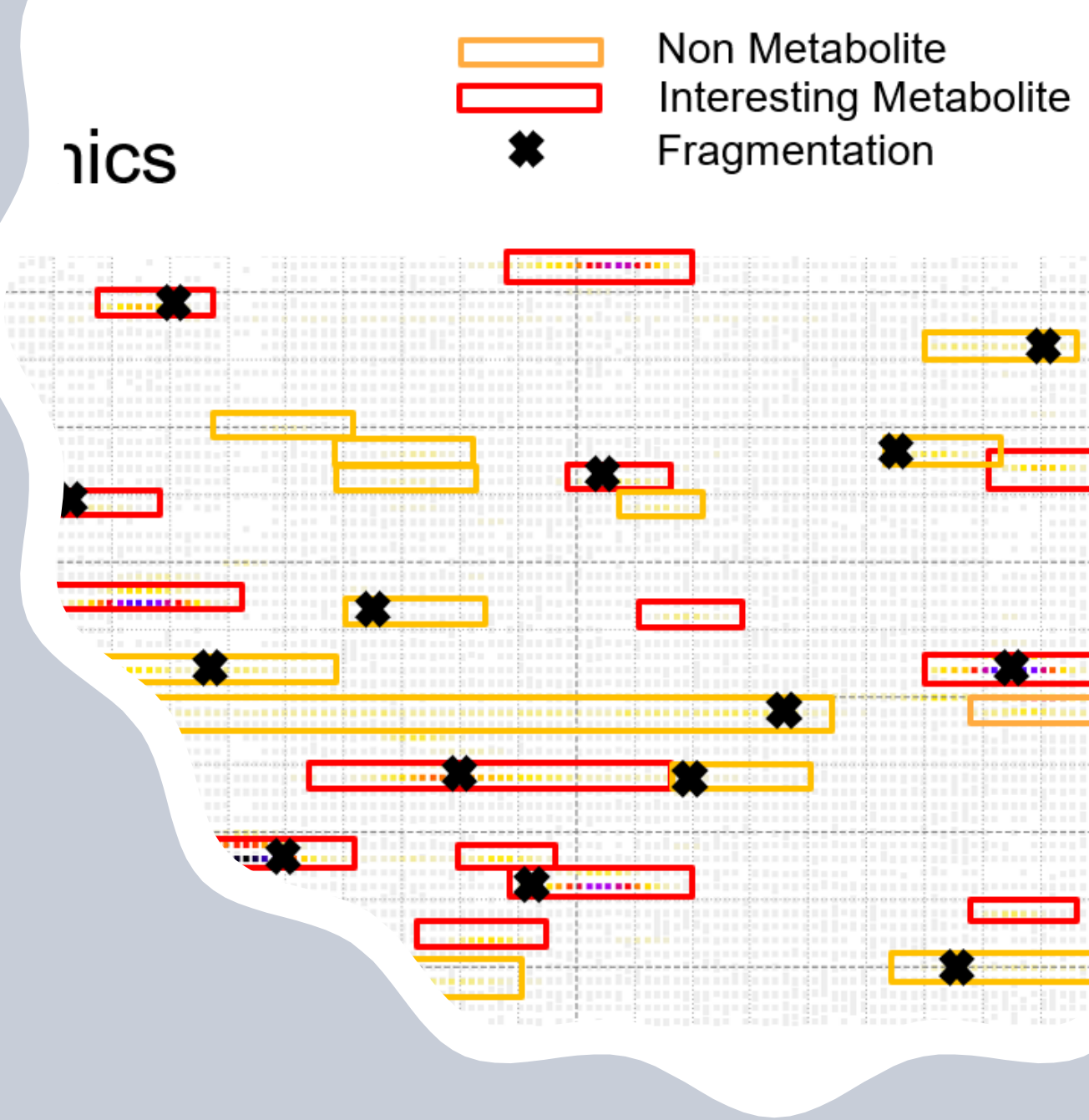
- One of the main criticisms of the performance of DDA (with respect to DIA) is its lower **coverage**, particularly for standard TopN method.
- Not much work has been done to improve DDA performance for single injections in metabolomics.
- Why?
  - Costly access to instruments
  - No common framework to develop fragmentation strategies

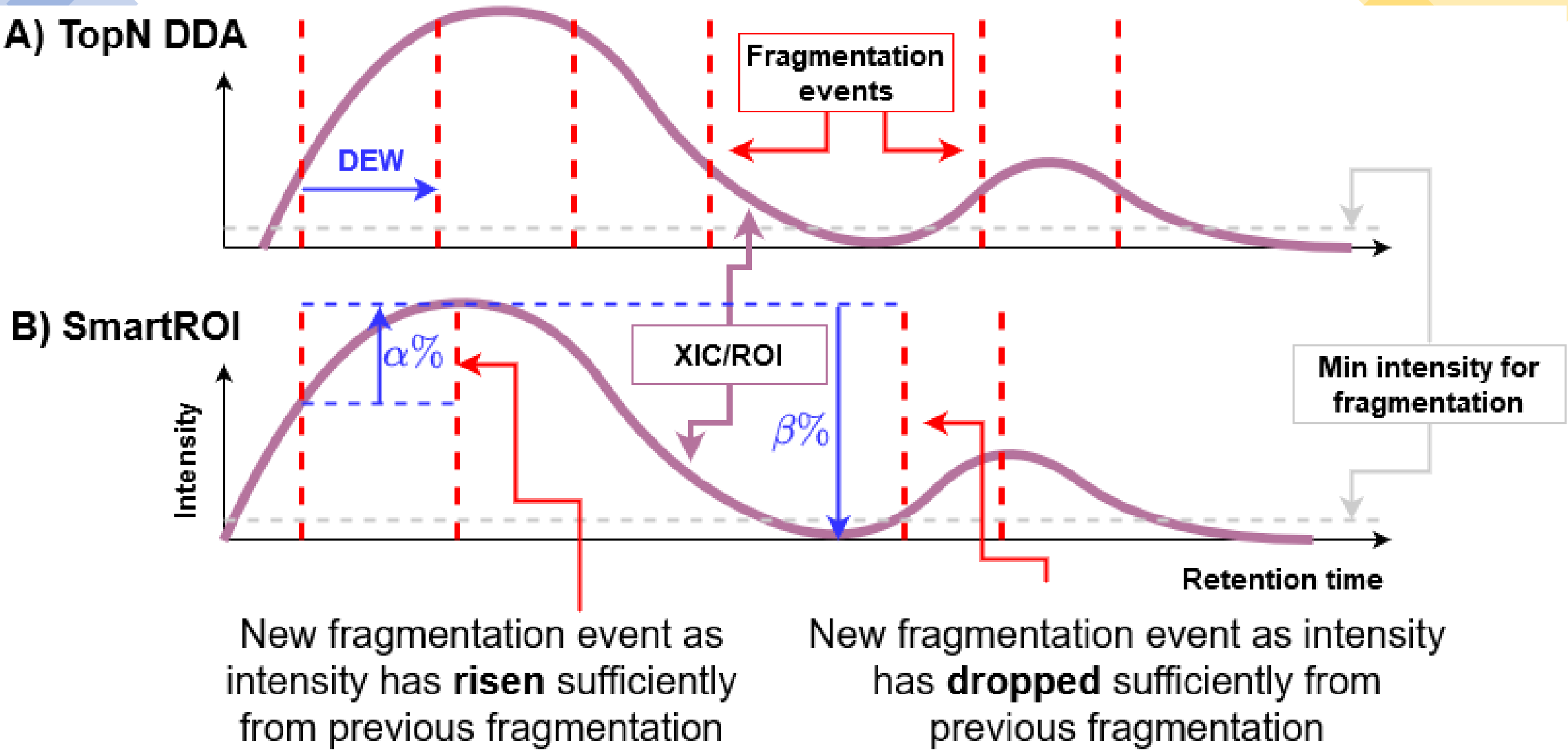
A framework to develop, optimize and test new strategies



SmartROI: Real-time Tracking of ROIs

- Many MS2 scans produced by TopN target ions that were not subsequently picked as peaks.
- Creation of ROIs is the first step in many peak-picking methods,
- Fragmentation events outside ROIs are almost certainly wasted.
- SmartROI keeps track of regions of interest (ROIs) in real time and only fragments peaks within ROIs.





A comparison of SmartROI fragmentation strategy to conventional Top-N strategy. Keeping track of an ROI in real-time allows for fewer, more targeted MS2 events

WeightedDEW

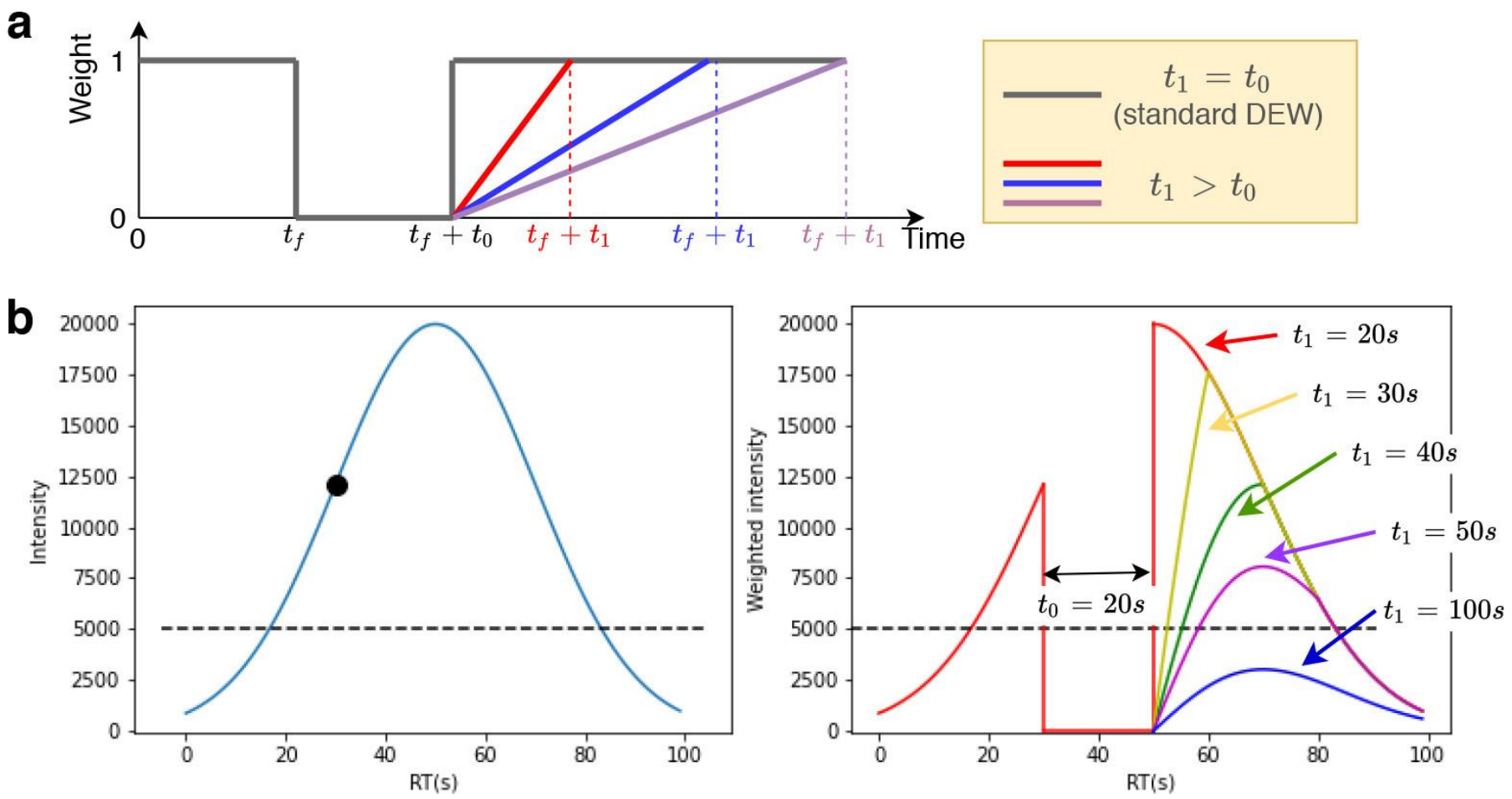
Fragmenting with Weighted Dynamic Exclusion Scheme

- TopN DDA uses the intensity of the ion in the survey scan for fragmentation prioritization.
- Standard dynamic exclusion window (DEW): peaks are excluded from repeated fragmentation as long as their m/z and RT values are still within the dynamic exclusion window of previously fragmented ions.
- This is a binary window:
  - 0: excluded
  - 1: included

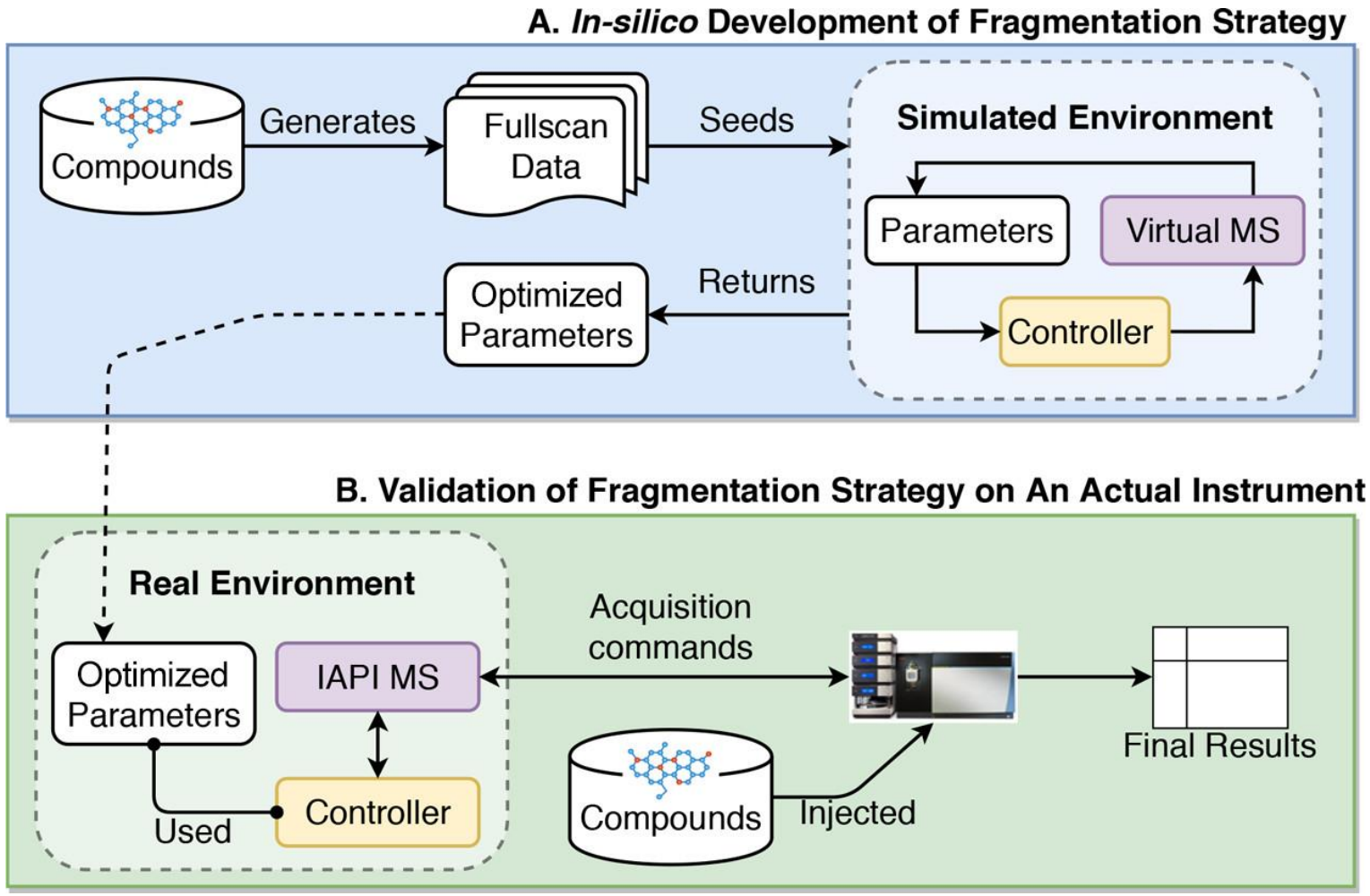
$$w = \begin{cases} 0 & \text{if } t_f < t < t_f + t_0 \\ \frac{t-(t_f+t_0)}{t_1-t_0} & \text{if } t_f + t_0 \leq t \leq t_f + t_1 \\ 1 & \text{otherwise,} \end{cases}$$

How about non-binary weights?

Non-linear weights in WeightedDEW



Validation on real instruments



Validation on real instruments

- Validate using beer and serum samples.
- For each beer and serum extract, we ran six injections:
  - one full-scan (for evaluating coverage and efficiency),
  - one TopN (using the controller optimized as part of the development of ViMMS(10))
  - four injections for the new fragmentation strategies.
- IAPI bridge was used to let the SmartROI and WeightedDEW controllers communicate with Thermo Orbitrap instruments.

Evaluation metrics

Coverage: the number of picked peaks that contain a fragmentation event.

Efficiency: the ratio of the number of picked peaks that are fragmented to the number of MS2 scans.

Coverage Results

- **Coverage**: the number of picked peaks that contain a fragmentation event.

	Beer (4592 peaks)		Serum (3032 peaks)	
Method	**Iteration 1**	**Iteration 2**	**Iteration 1**	**Iteration 2**
TopN	1046		656	
WeightedDEW	1859	1768	1105	1226
SmartROI	1660	1546	991	1015
Optimal (using TopN scan timings)	2955		1542	

Efficiency Results

- **Efficiency**: the number of picked peaks that are fragmented divided by the number of MS2 scans.

		Beer (4592 peaks)				Serum (3032 peaks)			
Iter	**method**	**total**	**MS1**	**MS2**	**Eff**	**total**	**MS1**	**MS2**	**Eff**
	TopN	6404	583	5821	0.18	6317	575	5742	0.11
1	WeightedDEW	6282	572	5710	0.33	6235	567	5668	0.19
	SmartROI	4948	1050	3898	0.43	4271	1268	3003	0.33
2	WeightedDEW	6294	573	5721	0.31	6205	566	5639	0.22
	SmartROI	5078	1032	4046	0.38	4572	1237	3335	0.30

Conclusions

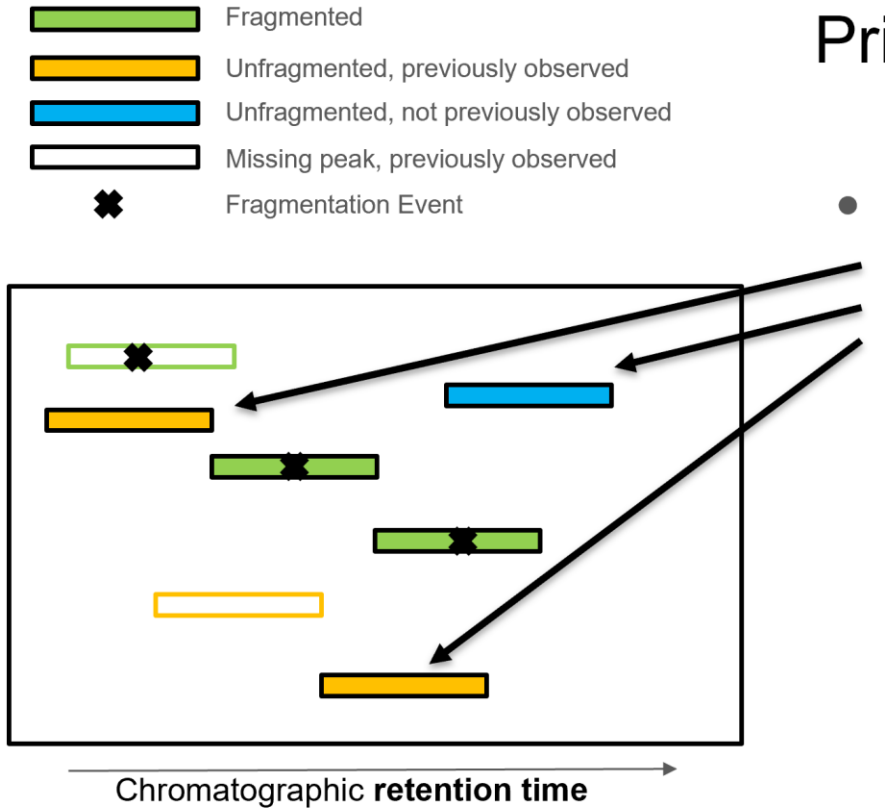
- Developing new acquisition methods typically requires extensive experimentation on the MS: **expensive** & **time consuming**.
- We introduce a framework to develop new methods by extending the capability of ViMMS
- Easily run fragmentation strategies (controllers) in the simulator and also on real MS equipment with minimal changes.

Conclusions

- We presented two new DDA strategies that outperform conventional TopN strategy
- SmartROI uses an ROI detection algorithm to only fragment molecules within ROIs and are likely to be picked as peaks.
- WeightedDEW generalizes the dynamic exclusion window approach to a real-valued weighting scheme.
- An optimal limit of DDA performance for a particular mixture can be computed via a bipartite graph matching scheme.
- Still far from reaching theoretical optima!

Future Works

- Predict which ROI contains a peak or not.
- Multi-sample methods
- Correcting RT drift in real-time



Priorities

- We want to prioritise peaks that haven't been fragmented before

Acknowledgements

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- Simon Rogers
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