

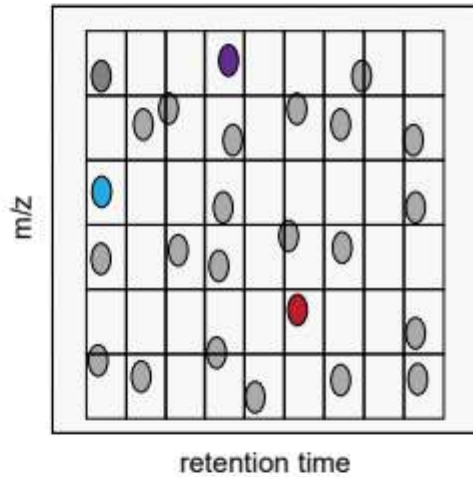
Webinar #26

Spectrum-centric analysis of DIA datasets combining FragPipe, DIANN and Skyline

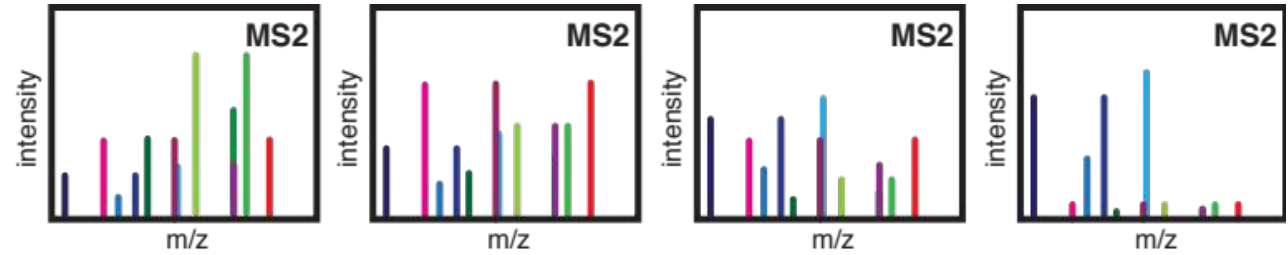
Eduard Sabidó
CRG/UPF Proteomics Unit

In Data-independent acquisition (DIA) we generate MS1 and MS2 data for all the space independently of peptide signals

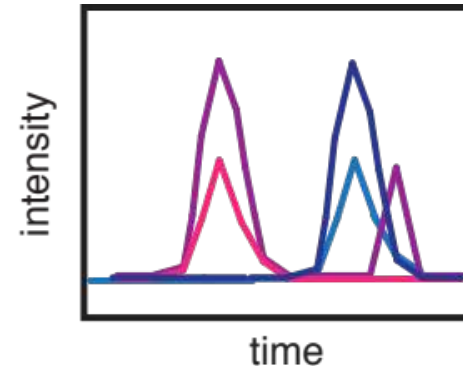
DIA



Deterministic
Not targeted



MS2 extracted ion chromatograms

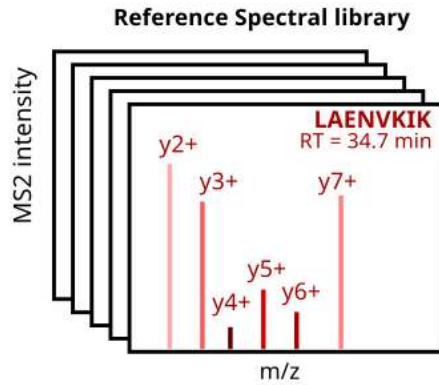


Targeted data extraction

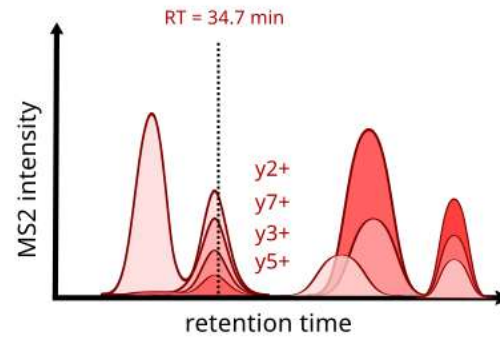
Peptide-centric DIA data analysis

Scoring peptide identification based on prior knowledge

Prior knowledge



Experimental data



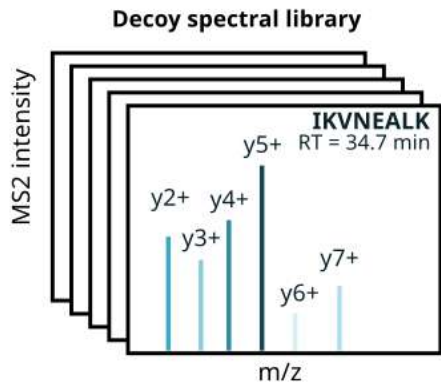
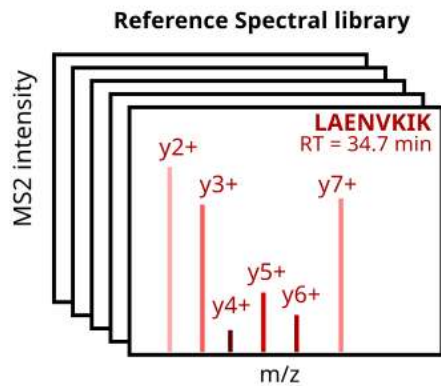
Scoring system

1. co-elution
2. peak shape
3. number of fragments
4. Fragment relative intensity
5. mass accuracy
6. retention time

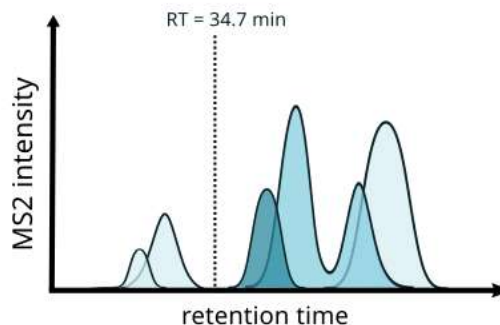
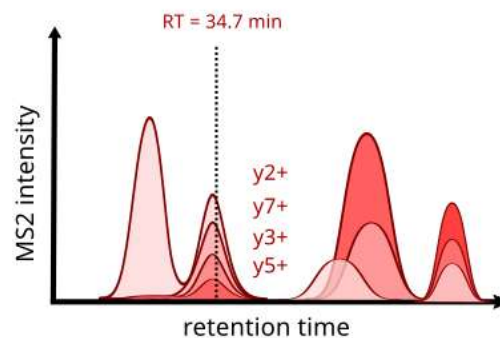
Peptide-centric DIA data analysis

Scoring peptide identification based on prior knowledge

Prior knowledge

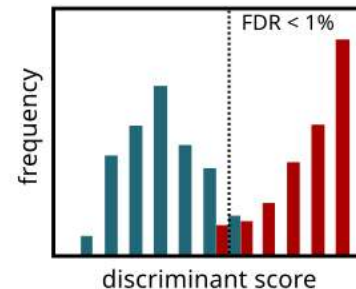


Experimental data



Scoring system

1. co-elution
2. peak shape
3. number of fragments
4. Fragment relative intensity
5. mass accuracy
6. retention time



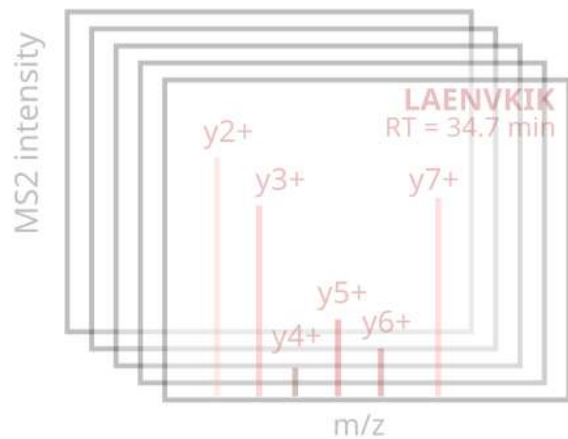
Spectrum-centric DIA data analysis



<https://diaumpire.nesvilab.org>

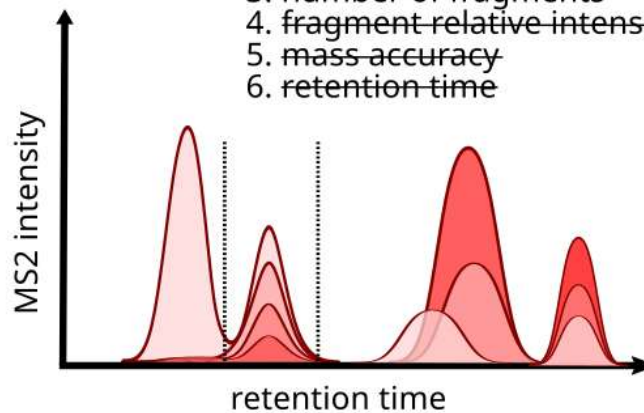
~~Prior knowledge~~

Reference Spectral library



Experimental data

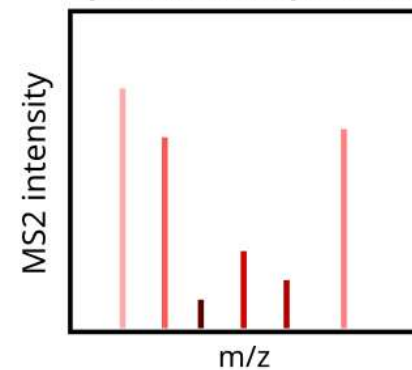
1. co-elution
2. peak shape
3. number of fragments
4. fragment relative intensity
5. mass accuracy
6. retention time



Conventional database search

FRD controlled

pseudo-MS2 spectrum

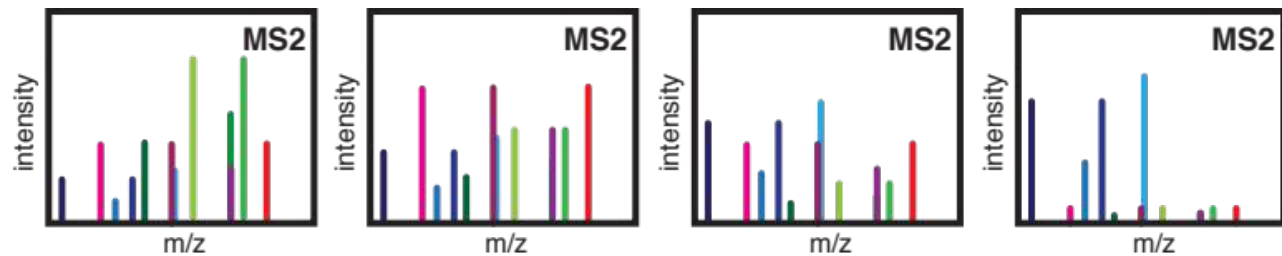


Spectrum-centric DIA data analysis



<https://fragpipe.nesvilab.org/>

Nesvizhskii's group

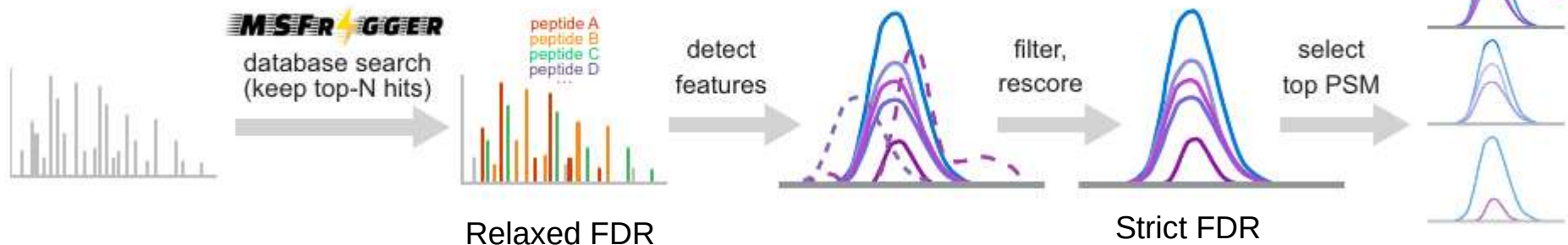
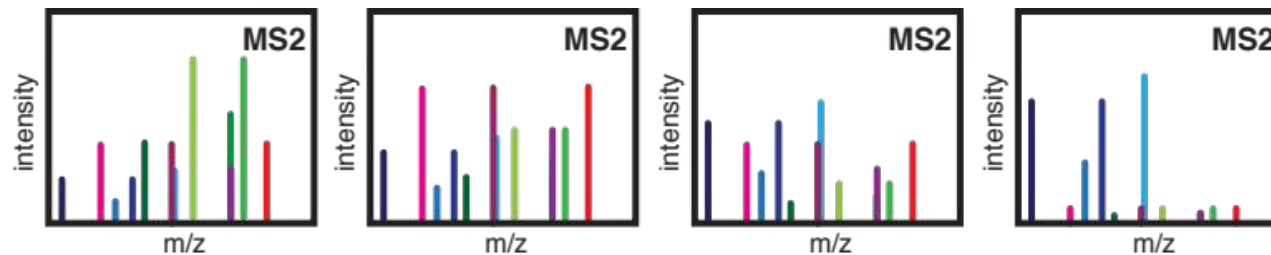


Spectrum-centric DIA data analysis



<https://fragpipe.nesvilab.org/>

Nesvizhskii's group



Spectrum-centric DIA data analysis



FragPipe 23.1

Nesvizhskii's group

<https://fragpipe.nesvilab.org/>



DIANN 2.2

Demichev's group

<https://github.com/vdemichev/DiaNN>



Skyline 25.1

MacCoss Lab

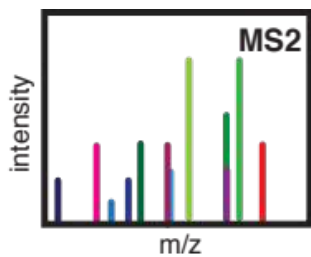
<https://skyline.ms>

Spectrum-centric DIA data analysis



Peptide candidates

DEPFIDEK
LLIMNSTFR
LAENVKIK

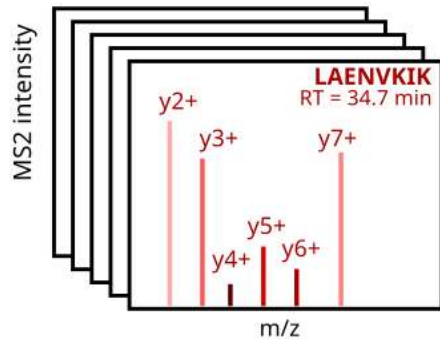


M S F R G G L R

Rescoring
Percolator

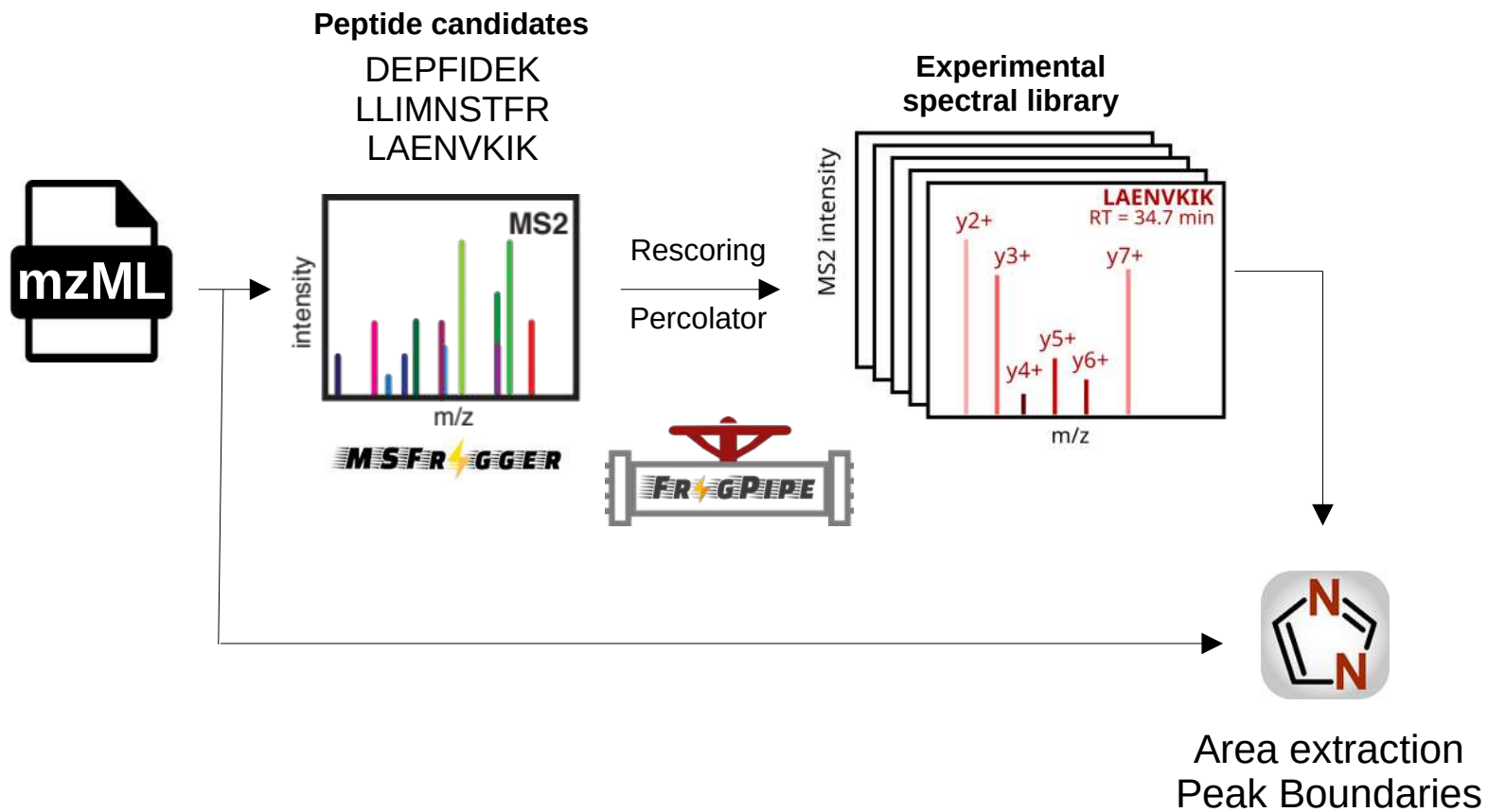


Experimental
spectral library

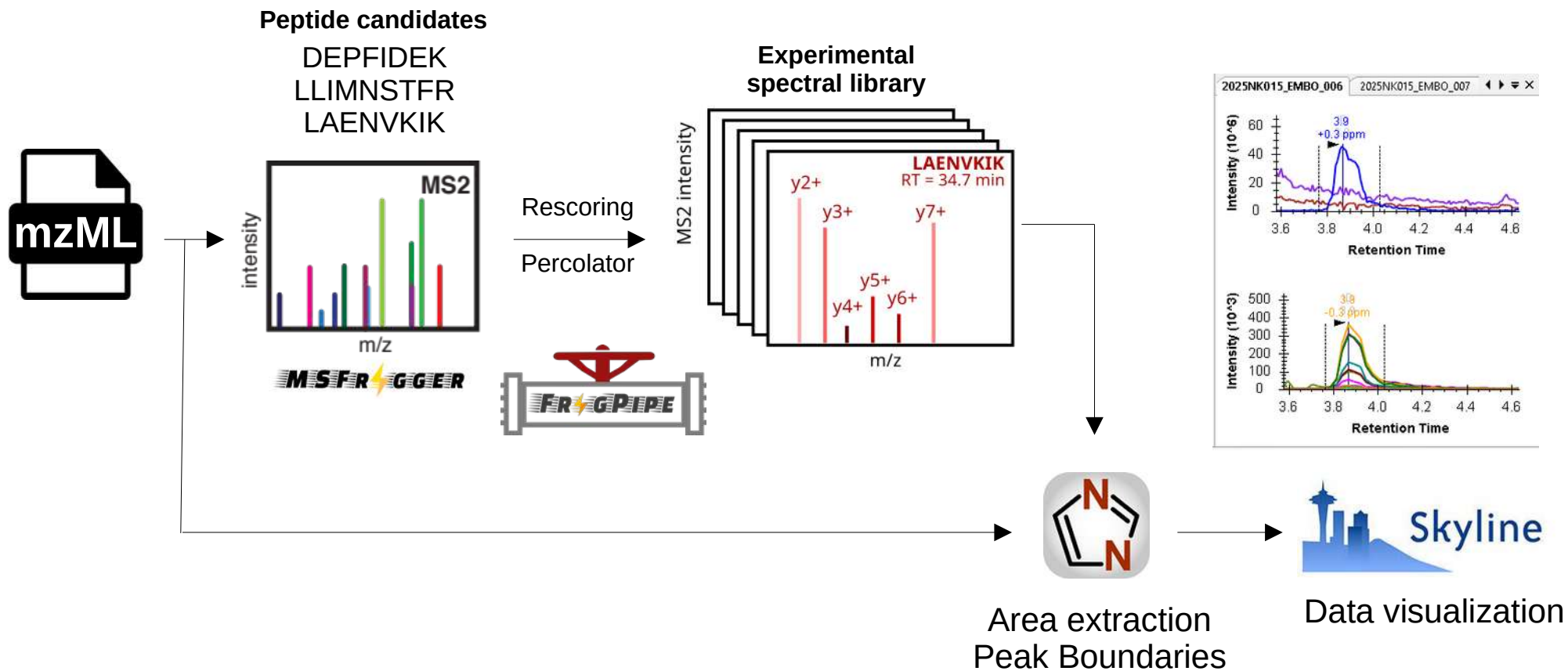


m/z

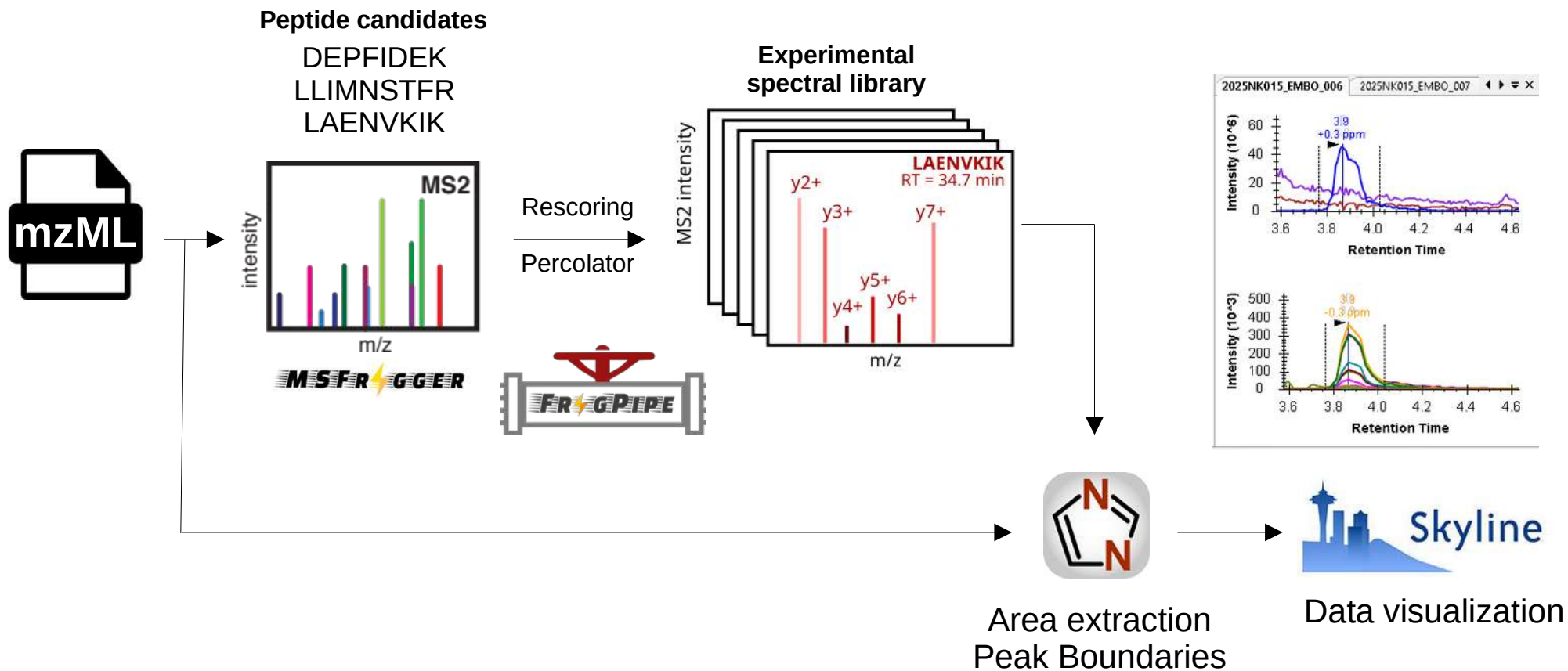
Spectrum-centric DIA data analysis



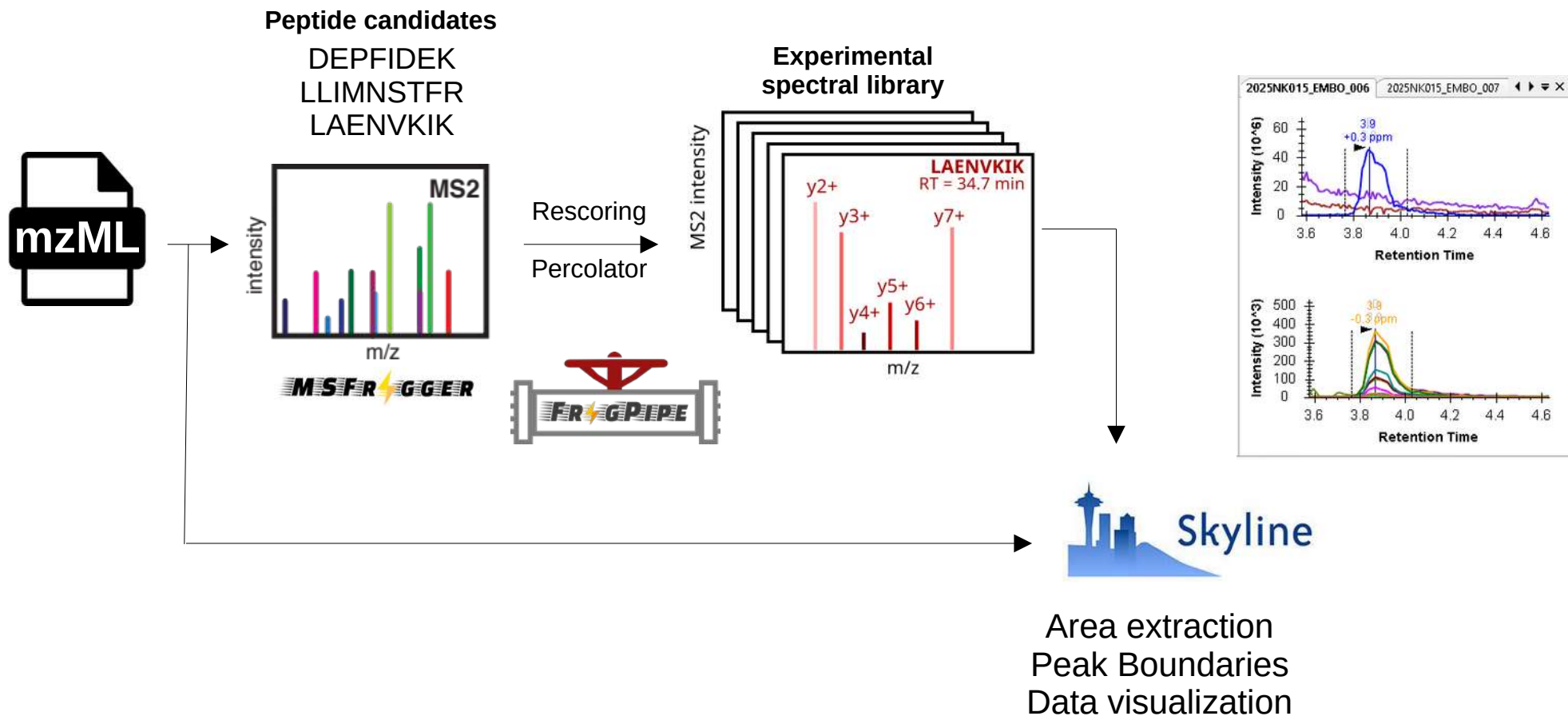
Spectrum-centric DIA data analysis



Spectrum-centric DIA data analysis



Spectrum-centric DIA data analysis



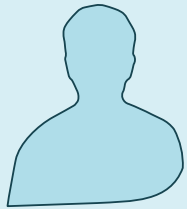
Biological experimental background



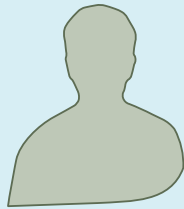
Deep plasma proteomics profiling of healthy humans at two different stages of adulthood

Eva Borràs^{1,2}, Federica Anastasi^{1,3,4}, Olga Pastor^{1,2}, Marc Suárez-Calvet^{3,4,5}, Eduard Sabidó^{1,2}

1. Experimental design



VS



Young
Age 18-25

Old
Aged 70-84

n= 30
Plasma

n= 30
Plasma

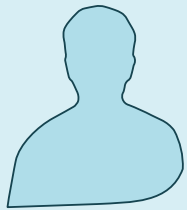
Biological experimental background



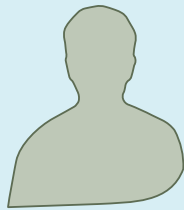
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1. Experimental design



VS



Young
Age 18-25

Old
Aged 70-84

n= 30
Plasma

n= 30
Plasma

2. Sample preparation

Enrich Plasma **Neat Plasma**

ENRICH-iST
Preomics Kit



n=60

iST-BCT
Preomics Kit



n=60

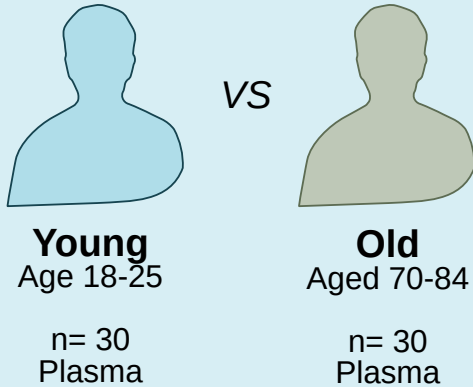
Biological experimentl background



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1. Experimental design



2. Sample preparation

Enrich Plasma *Neat Plasma*

ENRICH-iST
Preomics Kit



iST-BCT
Preomics Kit



3. Data Acquisition

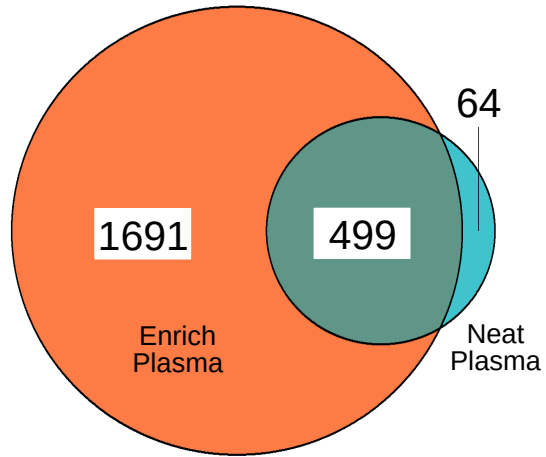
Data-Independent Acquisition
2-Da Windows
Orbitrap Astral

Gradient 30 SPD
50-cm μ PAC Column

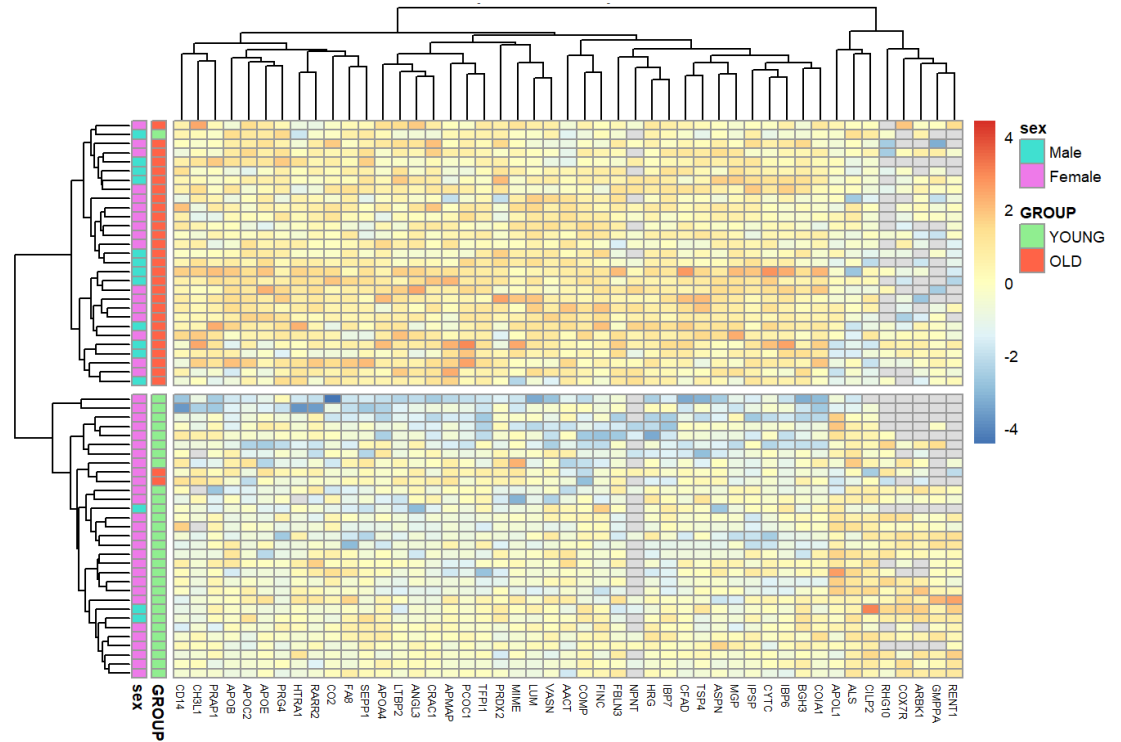


Results overview

More low-abundant proteins are identified in the ENRICH plasma dataset

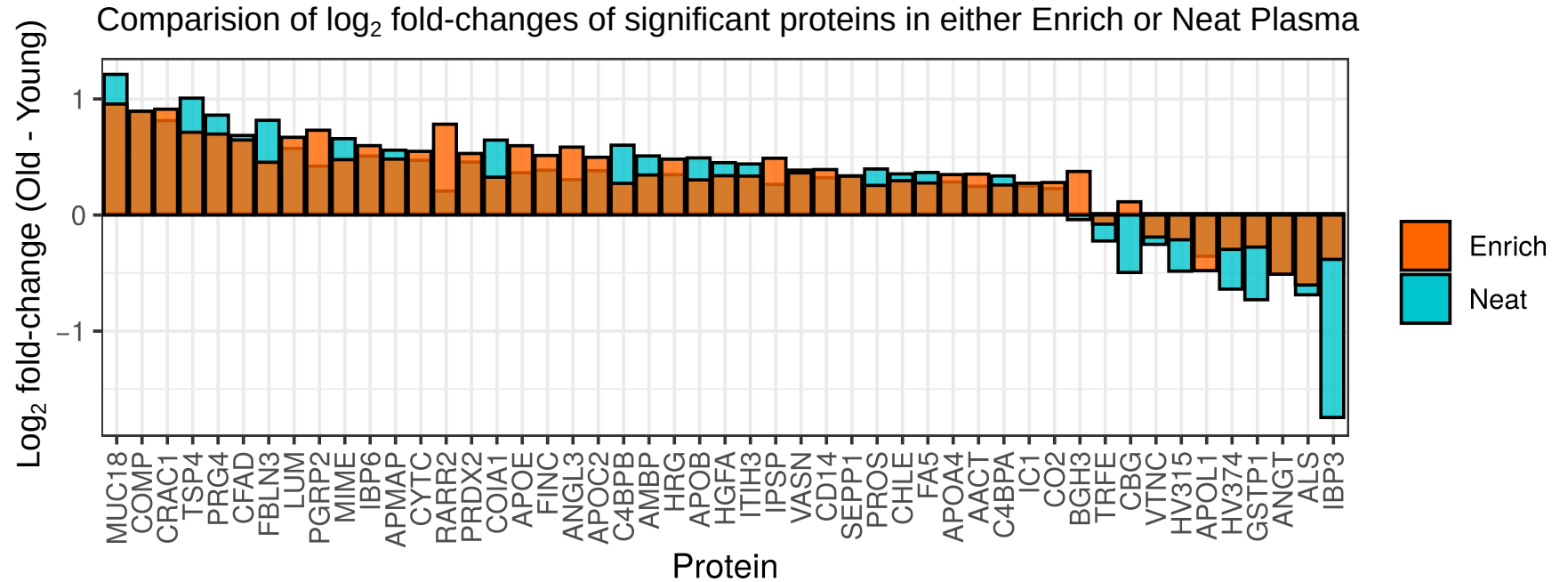


Heatmap of ENRICH plasma significantly changing proteins (adj.pvalue < 0.05)

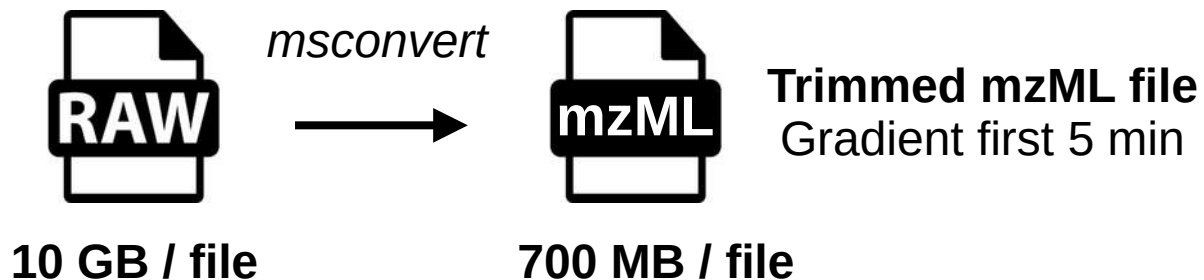
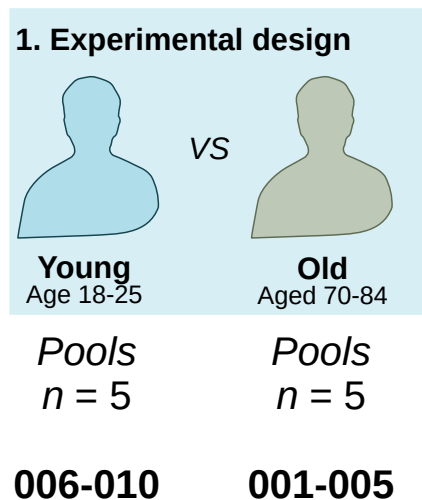


Results overview

The enrichment procedure does not alter the observed protein changes



The dataset to be analyzed



```
msconvert.exe --32 --filter "scanTime [0,300]" --filter "peakPicking vendor msLevel=1-"
```


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