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RESEARCH GROUP  
**BIOINFORMATICS**  
HAGENBERG



MS Amanda

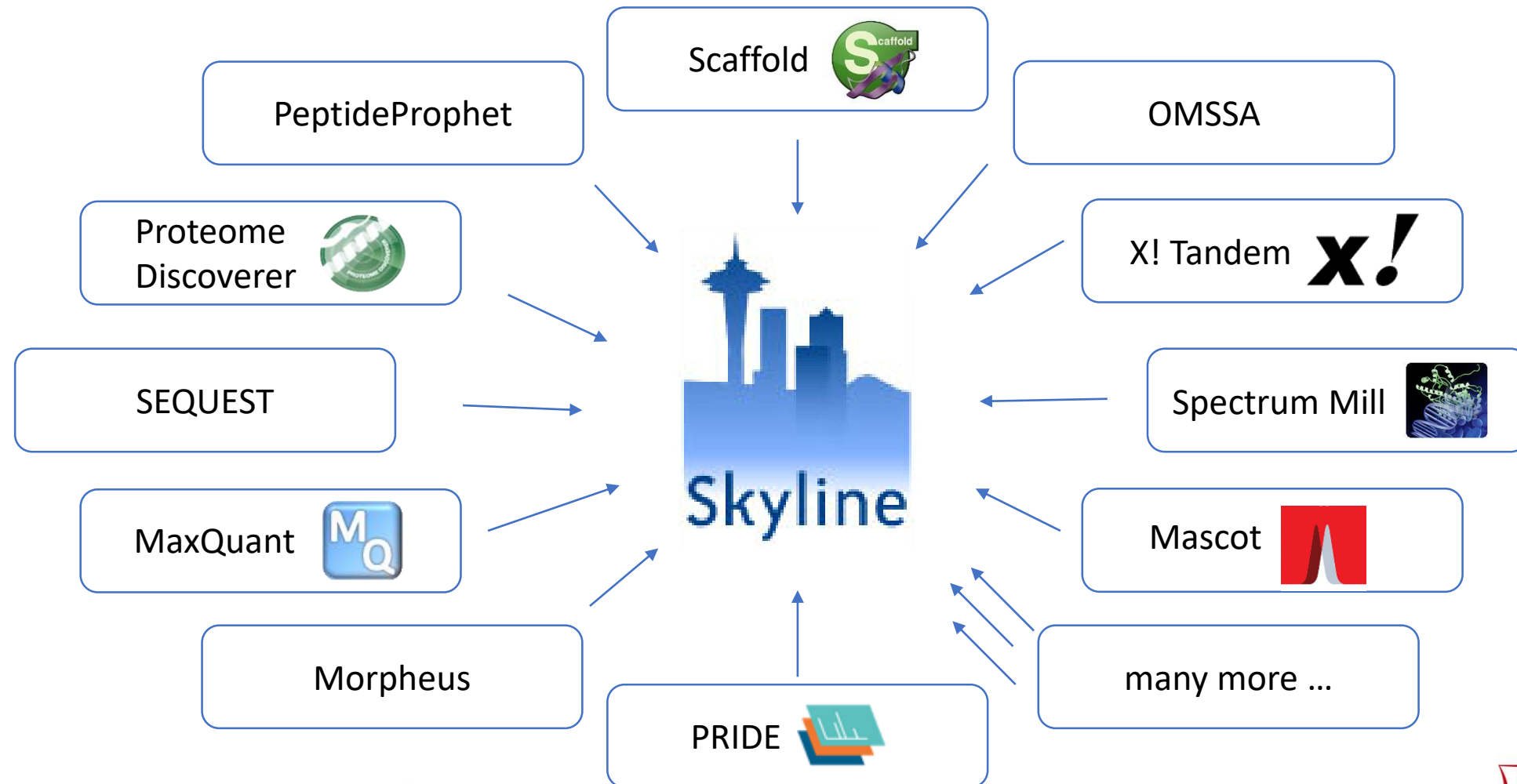


## MS Amanda goes West: Integrating a Search Engine into Skyline

Viktoria Dorfer, Marina Strobl, Nicholas Shulman, Matthew C. Chambers, Michael J. MacCoss, and Brendan MacLean  
Skyline User Meeting 2020

BACK TO BASICS

# Build a library from DDA data in Skyline

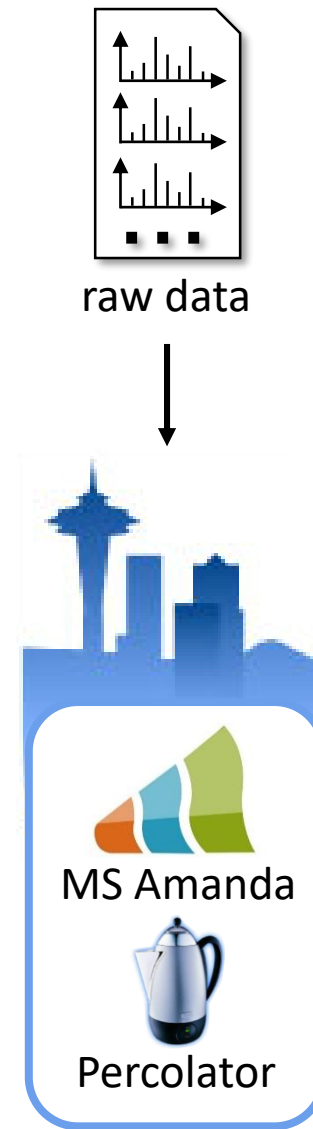
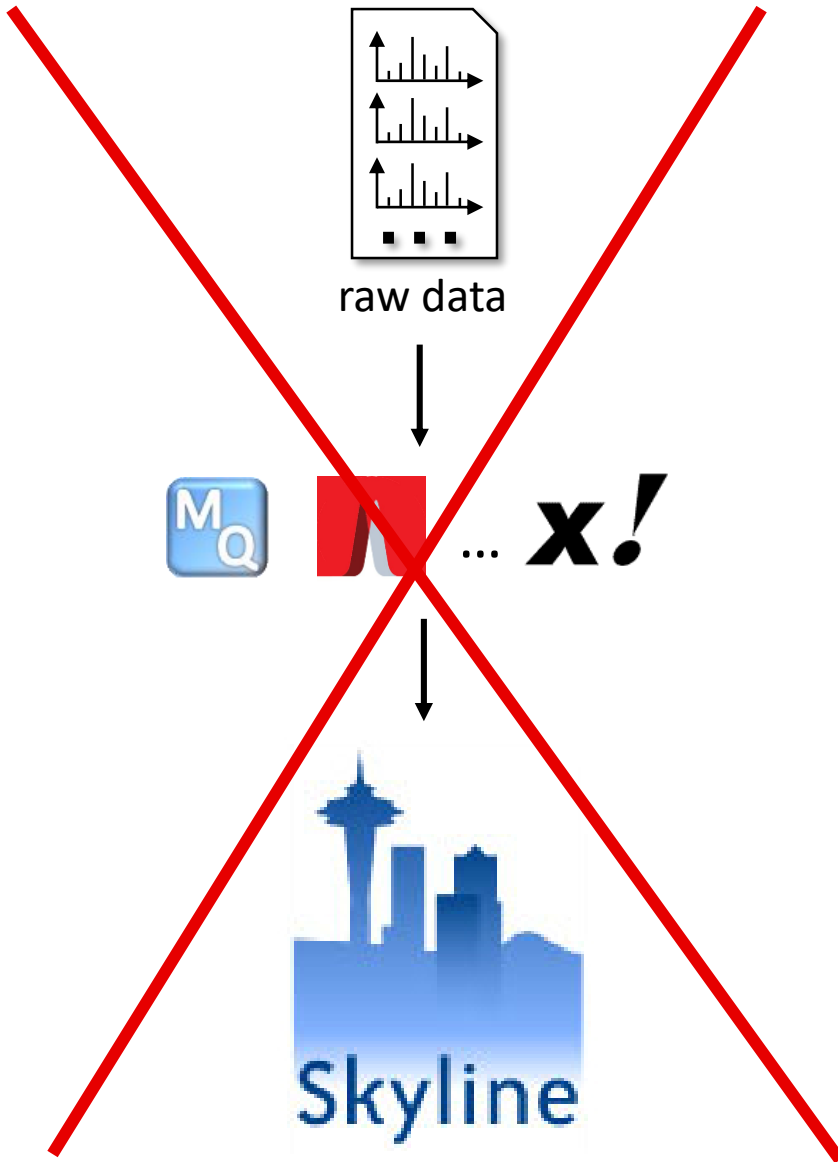


# Amazing, but...

- ... you need to
  - respecify several parameters
    - modifications
    - mass thresholds
    - the FASTA file used
    - ...
  - remember the correct result file

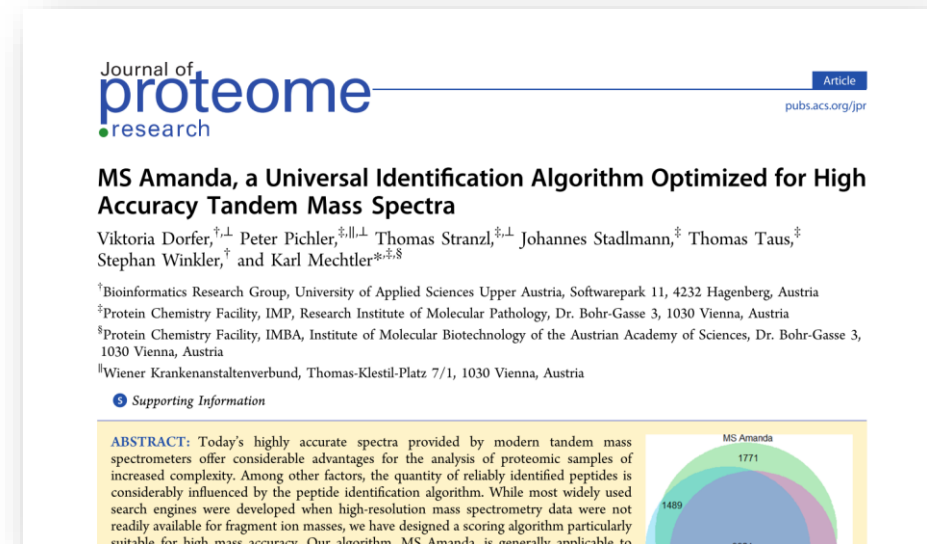


# Solution



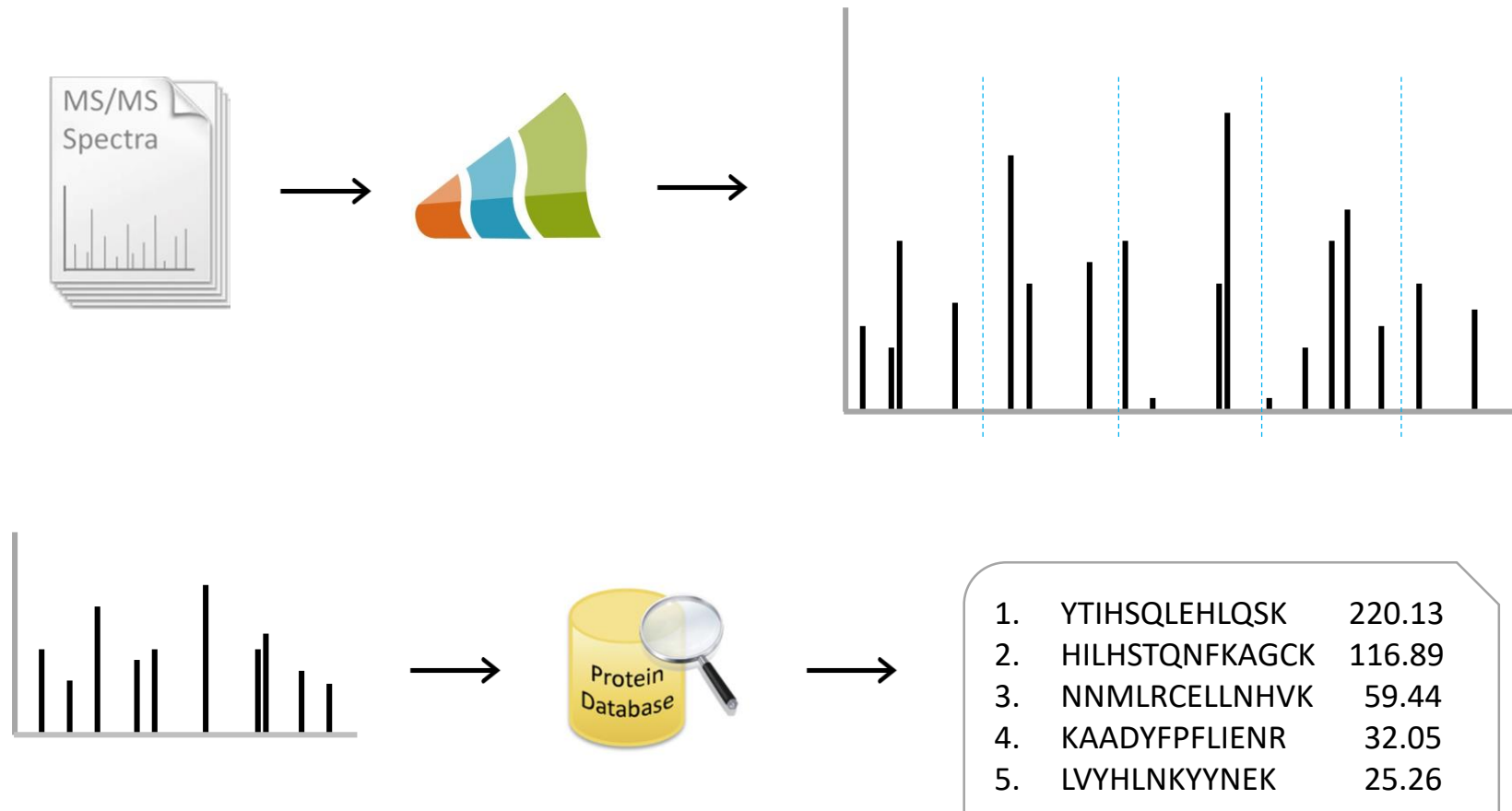
# MS Amanda

- Search engine for high resolution data sets
- Probability Score
- Intensity of explained peaks is considered
- Availability:
  - Free of charge
  - <https://ms.imp.ac.at/?goto=msamanda>

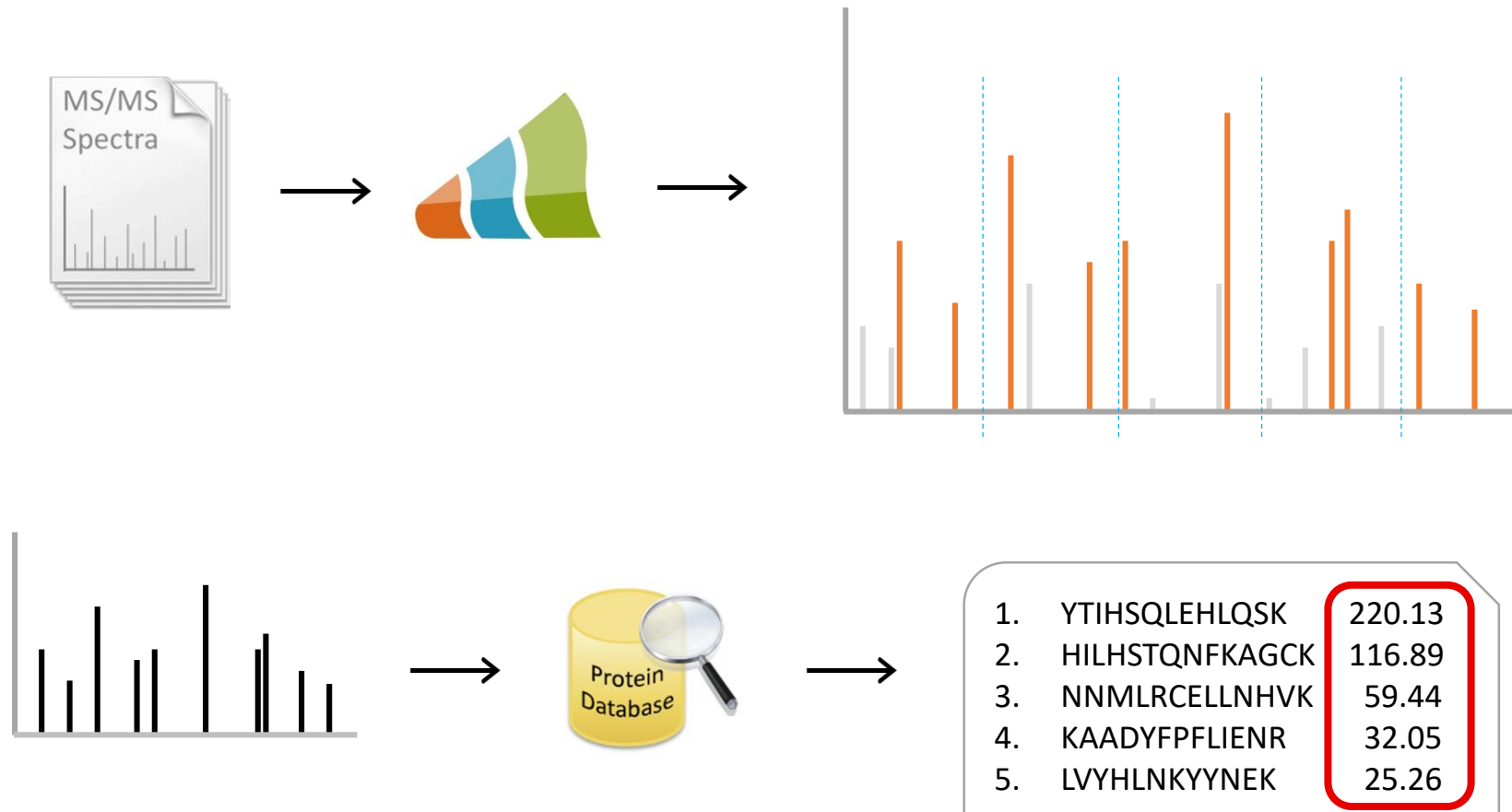


Dorfer et al., 2014, JPR

# MS Amanda Score



# MS Amanda Score

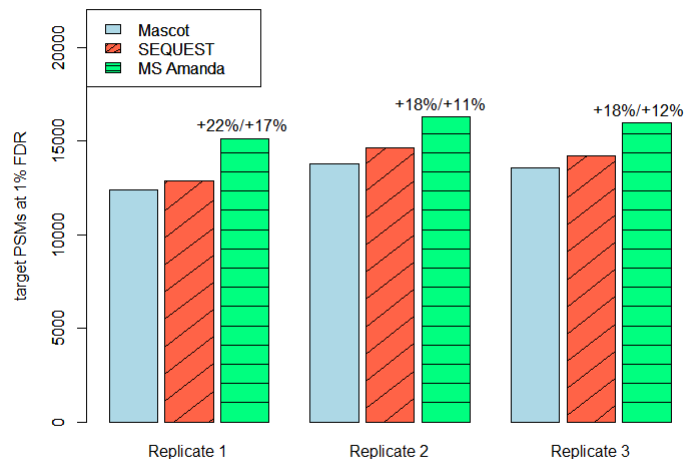




# MS Amanda Score

1. YTIHSQLEHLQSK	220.13
2. HILHSTQNFKAGCK	116.89
3. NNMLRCELLNHVK	59.44
4. KAADYFPFLIENR	32.05
5. LVYHLNKYYNE	25.26

$$AmandaScore = \max_{m \in [1..10]} \left( -10 \times \log \left( \frac{P(s, pep, m)}{eif(s, pep, m)} \right) \right)$$



Determine optimal peak picking depth

Enhance readability of score - the bigger the better!

Calculate probability that match could have happened by chance

Include/consider explained intensity

# MS Amanda in Skyline

Import Peptide Search

### Spectral Library

Build     Use existing     Perform DDA Search

Cut-off score:  
0,95

Files to search:

HeLa\_1ug\_isow2\_1hgradient\_datasetA\_rep1.raw  
HeLa\_1ug\_isow2\_1hgradient\_datasetA\_rep2.raw

Add Files...  
Remove Files

iRT standard peptides:  
None

Include ambiguous matches

Workflow

DDA with MS1 filtering  
 DIA  
 PRM

Finish    Next >    Cancel

Import Peptide Search

### Adjust Search Settings

MS Amanda search settings:

MS1 tolerance: 10    Unit: ppm  
MS2 tolerance: 20    Unit: ppm  
Fragment ions: b, y  
Fixed modifications:  
 Carbamidomethyl (C) = C[57]  
 Oxidation (M) = M[16]

Additional Settings

< Back    Next >    Cancel

Import Peptide Search

### DDA Search

Search progress:

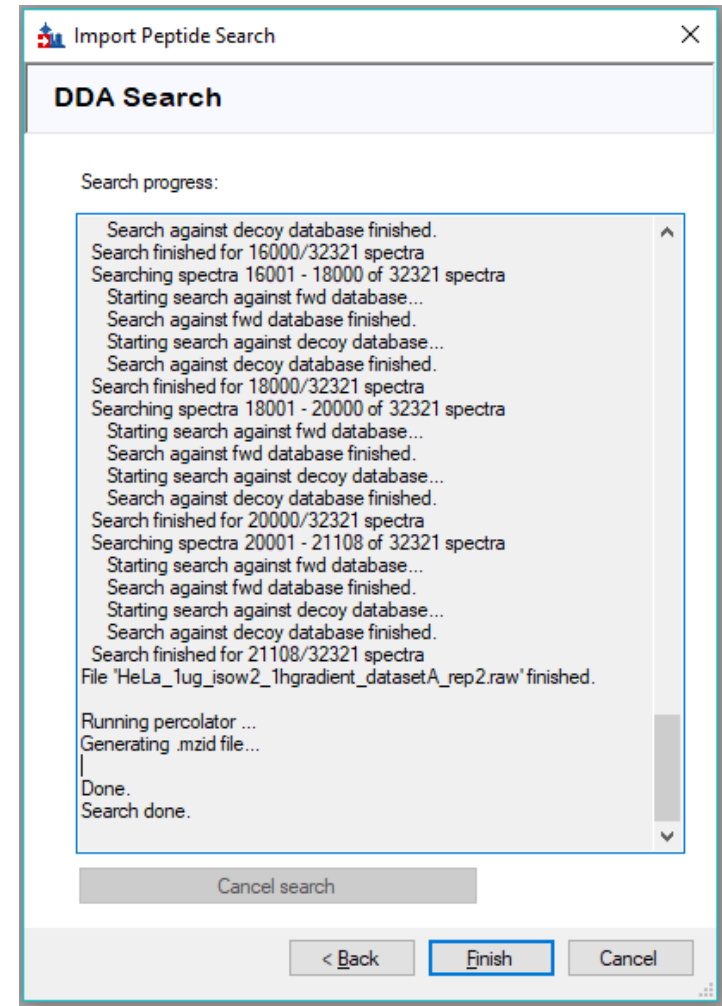
```
Starting search...
Input files contain 31523 ms2 spectra
Searching file 'HeLa_1ug_isow2_1hgradient_datasetA_rep1.raw'...
Searching spectra 1 - 2000 of 31523 spectra
Starting search against fwd database...
Search against fwd database finished.
Starting search against decoy database...
Search against decoy database finished.
Search finished for 2000/31523 spectra
Searching spectra 2001 - 4000 of 31523 spectra
Starting search against fwd database...
```

Cancel search

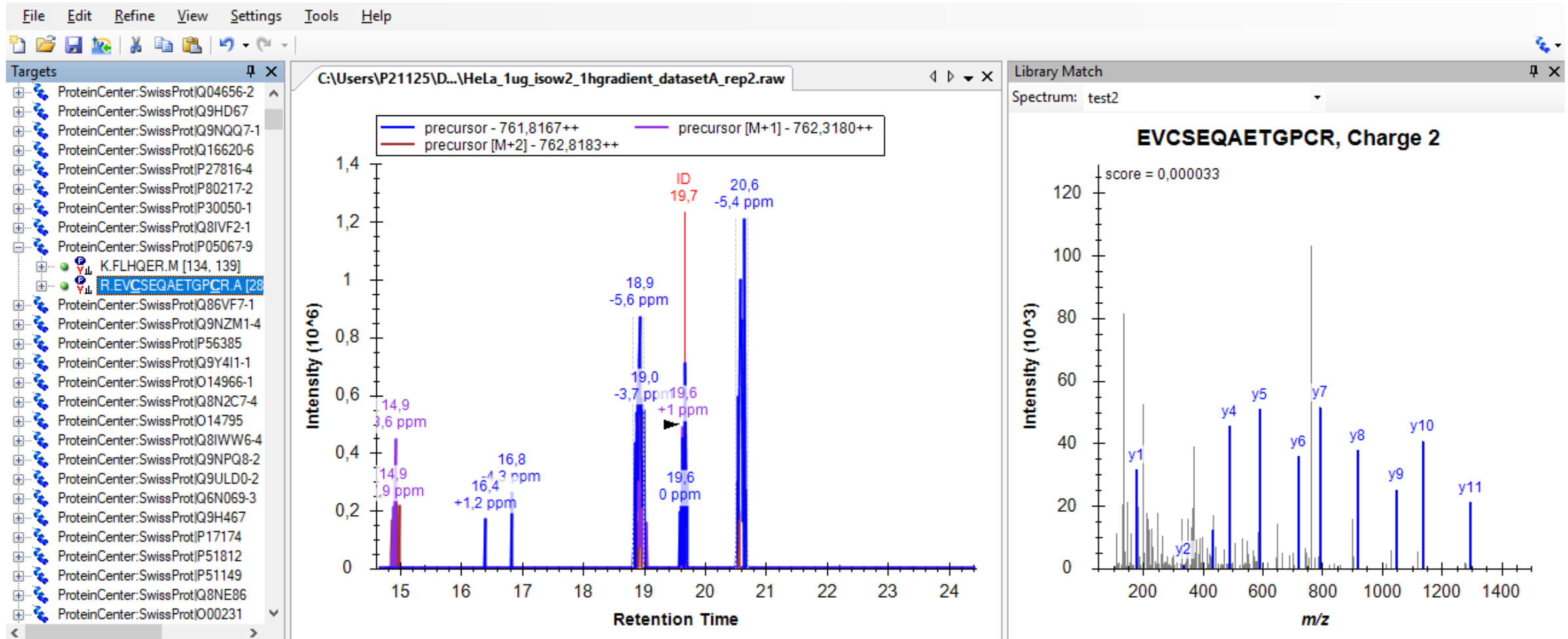
< Back    Finish    Cancel

# MS Amanda in Skyline

- Automatically performs a target/decoy search
- Runs percolator
- Generates .mzID
- Q-values are used as cut-off score



# MS Amanda in Skyline



# Availability and Future Perspectives

- Availability:

- Branch of ProteoWizard repository on Github
- [https://github.com/ProteoWizard/pwiz/tree/amanda to skyline](https://github.com/ProteoWizard/pwiz/tree/amanda%20to%20skyline)
- Planned to be fully integrated into Skyline



- Future Perspective:

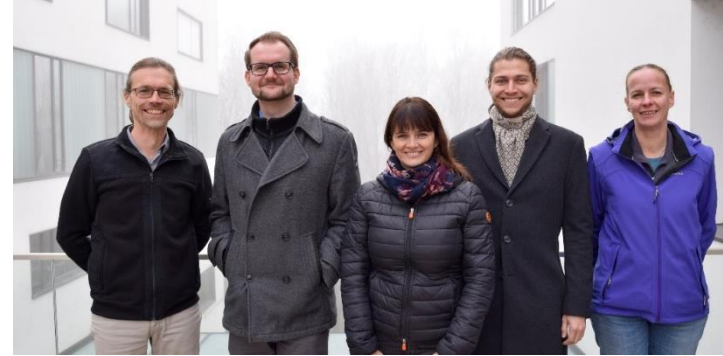
- Analyse DIA data without DDA library
- Use MS Amanda to search DIA data
- Extract often chimeric spectra using DIA Umpire



# Acknowledgements



MacCoss Group



Bioinformatics Research Group



Mechtler Group at IMP

