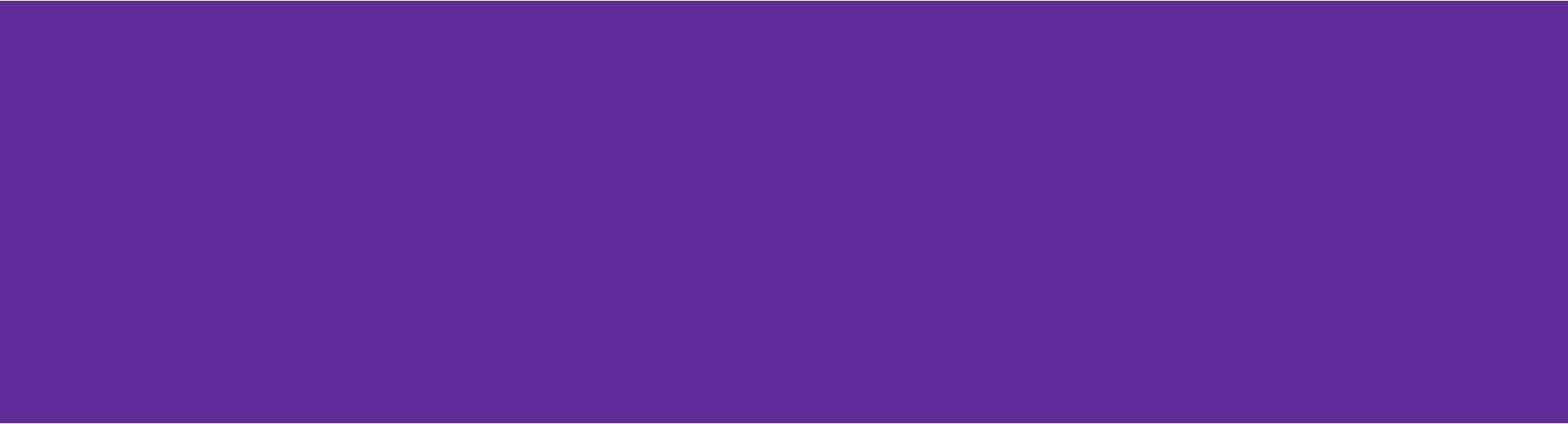


Phosphoproteome analysis

Hazal Koptagel, Julius Lautenbach, Maryia Ropat & Linnea Axelsson



Isoelectric point-based fractionation by HiRIEF coupled to LC-MS allows for in-depth quantitative analysis of the phosphoproteome

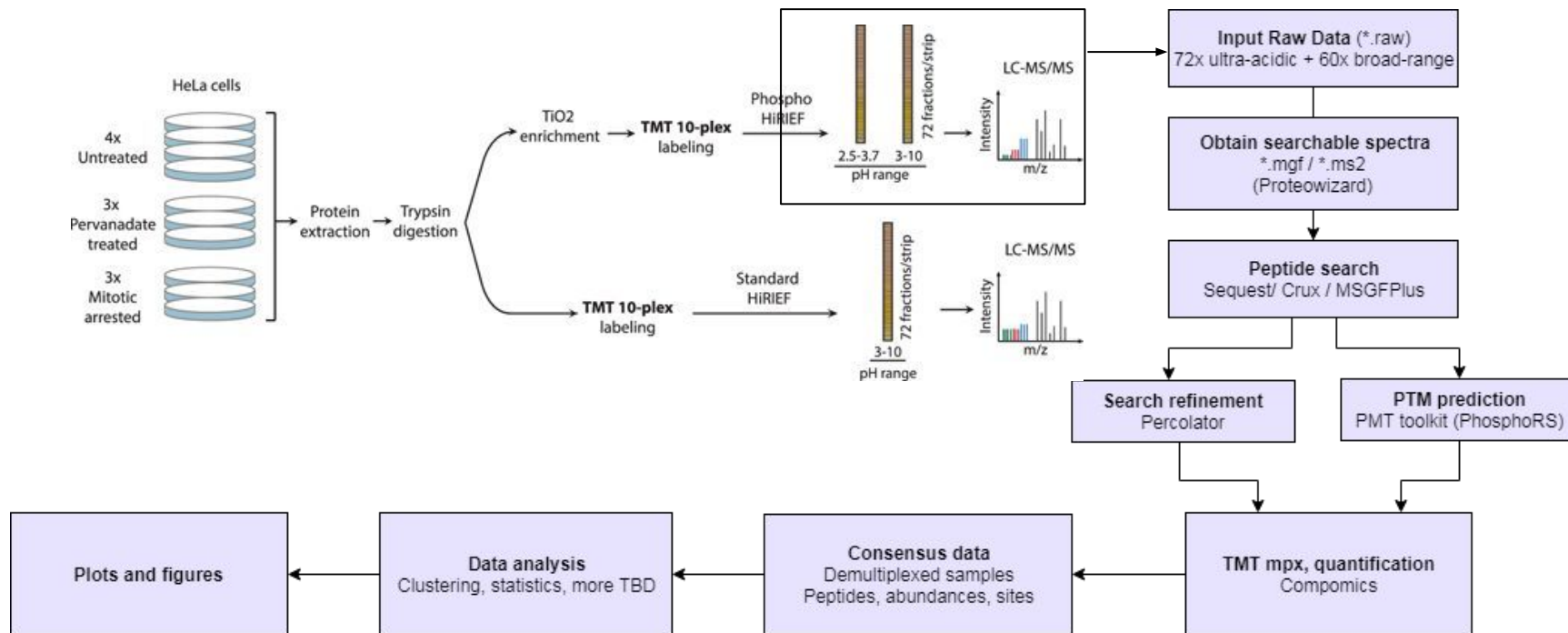
Elena Panizza, Rui M. M. Branca, Peter Olaviusson, Lukas M. Orre & Janne Lehtiö

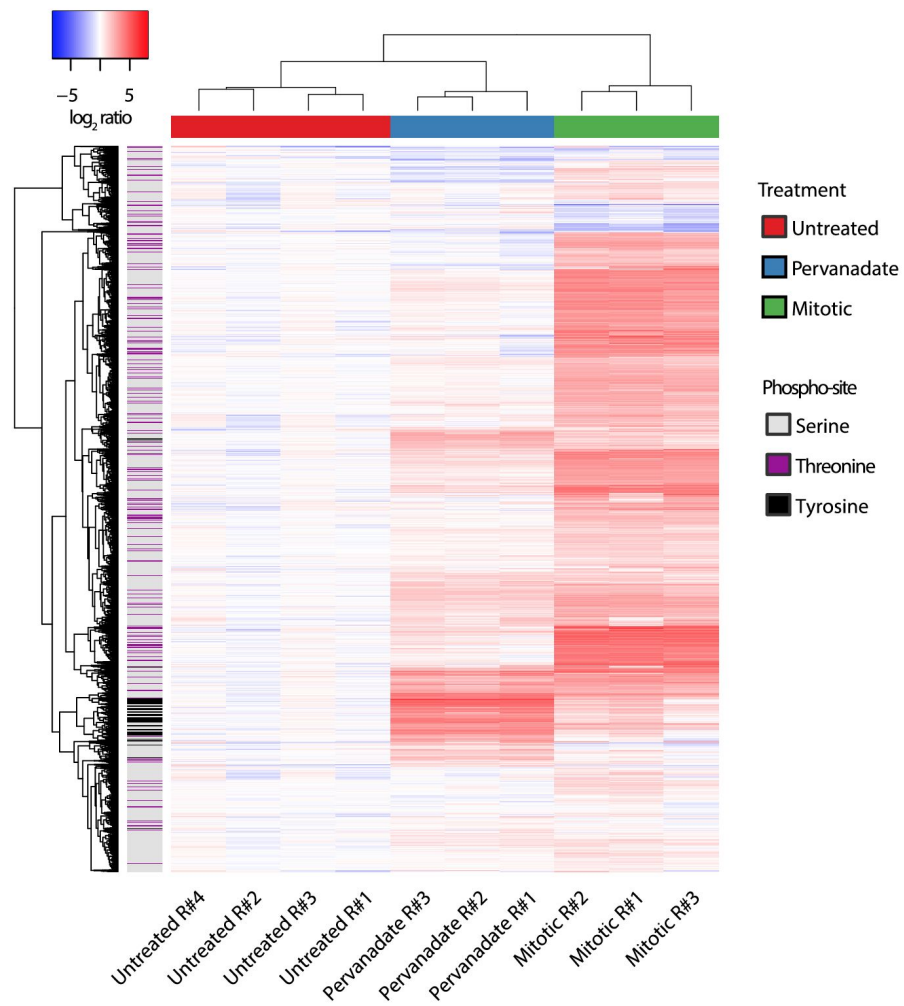
Titanium dioxide phospho-enrichment coupled with isobaric labeling by Tandem Mass Tags (TMT) and high-resolution isoelectric focusing (HiRIEF) before in-depth quantitative phosphoproteomics starting with a low sample quantity

Phosphorylated proteins

- Protein phosphorylation is a fundamental regulatory mechanism in eukaryotic cells
- Deregulated protein phosphorylation is involved in the development of many diseases
- Identification of phosphorylated peptides is challenging due to their low abundance and poor ionization efficiency

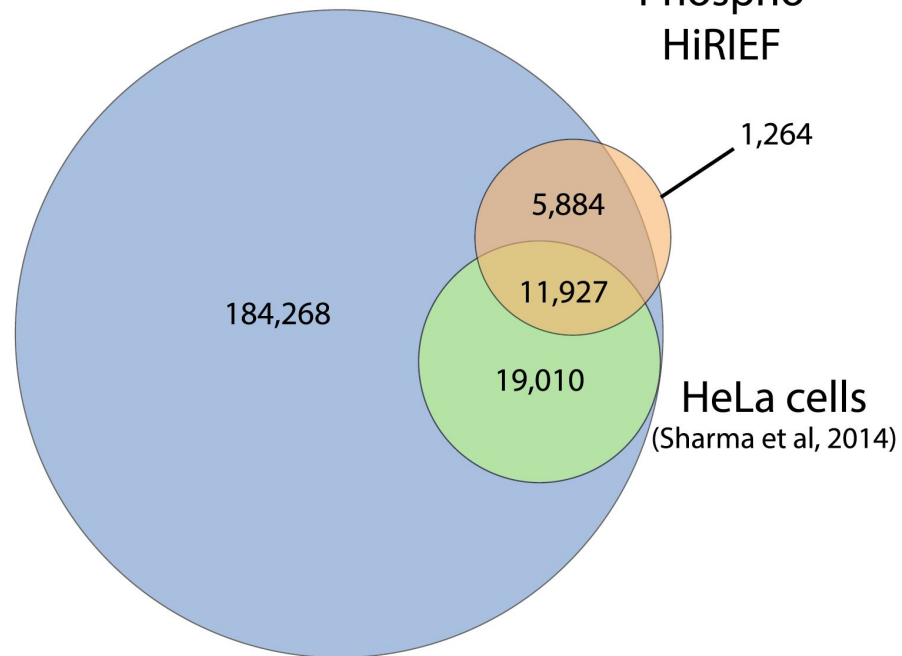
Analysis Workflow





PhosphoSitePlus

Phospho
HiRIEF



Linnea log

LinneaAx / [bb_2491](#)

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Fork 0

<> Code

Issues 0

Pull requests 0

Projects 0

Wiki

Insights

Settings

Project log

LinneaAx edited this page just now · 2 revisions

EditNew Page

Welcome to the bb_2491 wiki!

29/11

Downloaded proteowizard and tried to convert the first data file using default settings. Crashed within seconds, repeated times. Will try to download and run on my work computer.

+ Add a custom footer

Pages 2

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Clone this wiki locally

<https://github.com/LinneaAx>

Julius log

LautenbachMJ / bb2491_project

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⌚ Issues 0

🔗 Pull requests 0

📁 Projects 0

📖 Wiki

📊 Insights

⚙ Settings

Julius_Individual_Diary_2018 11 28

Edit

New Page

LautenbachMJ edited this page 5 minutes ago · 1 revision

- first group meeting
- discussion how to do the project and how to understand the tasks
- start of a project plan
- downloading of raw data, attempts of preprocessing it (FAILED)

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▼ Pages 4

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[Julius_Individual_Diary_2018 11 29](#)

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https://github.com/Lauter

📄

https://github.com/LautenbachMJ/bb2491_project/wiki

Hazal log

https://github.com/koptagel/bb2490/wiki/Hazal_Individual_Diary

Hazal_Individual_Diary

koptagel edited this page 3 hours ago · 3 revisions

- 28-29 Nov: I met with the group to prepare Seminar 1's presentation.
- 29 Nov: I looked at the supplementary data of the article. I started preparing clustering / visualisation scripts.

▼ Pages **2**

[Home](#)

[Hazal_Individual_Diary](#)

Maryia log

<https://maropbb2491.blogspot.com/>

Experiments

torsdag 29 november 2018

Week 1 of Data analysis project

Dear diary!

This week I have accomplished the following:

1. Extract the list of software used in related publication
2. Read up on *.raw / *.ms2 / *.mgf formats and software capabilities
Conclusion: *.ms2 conversion -> Crux search is the most flexible option
3. Found the software that used to be PhosphoRS: <http://ms.imp.ac.at/index.php?action=pmt>
4. Spent too much time lining up figures for the flowchart

Upplagd av mrop kl. 09:37

Inga kommentarer:



Om mig



Visa hela min profil



Bloggarkiv

▼ 2018 (1)

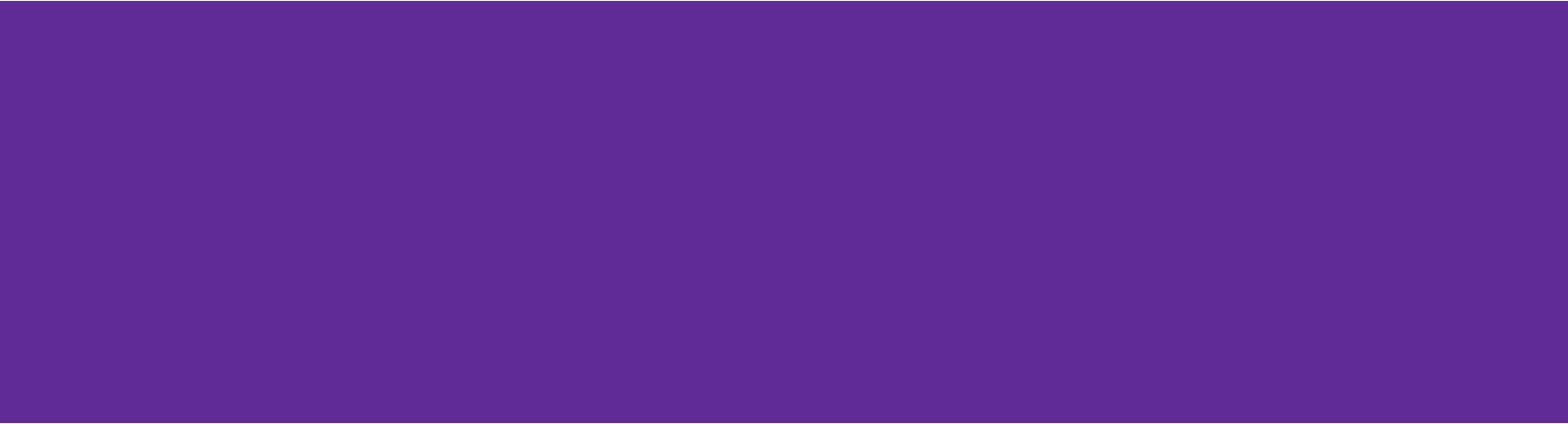
▼ november (1)

Week 1 of Data analysis project

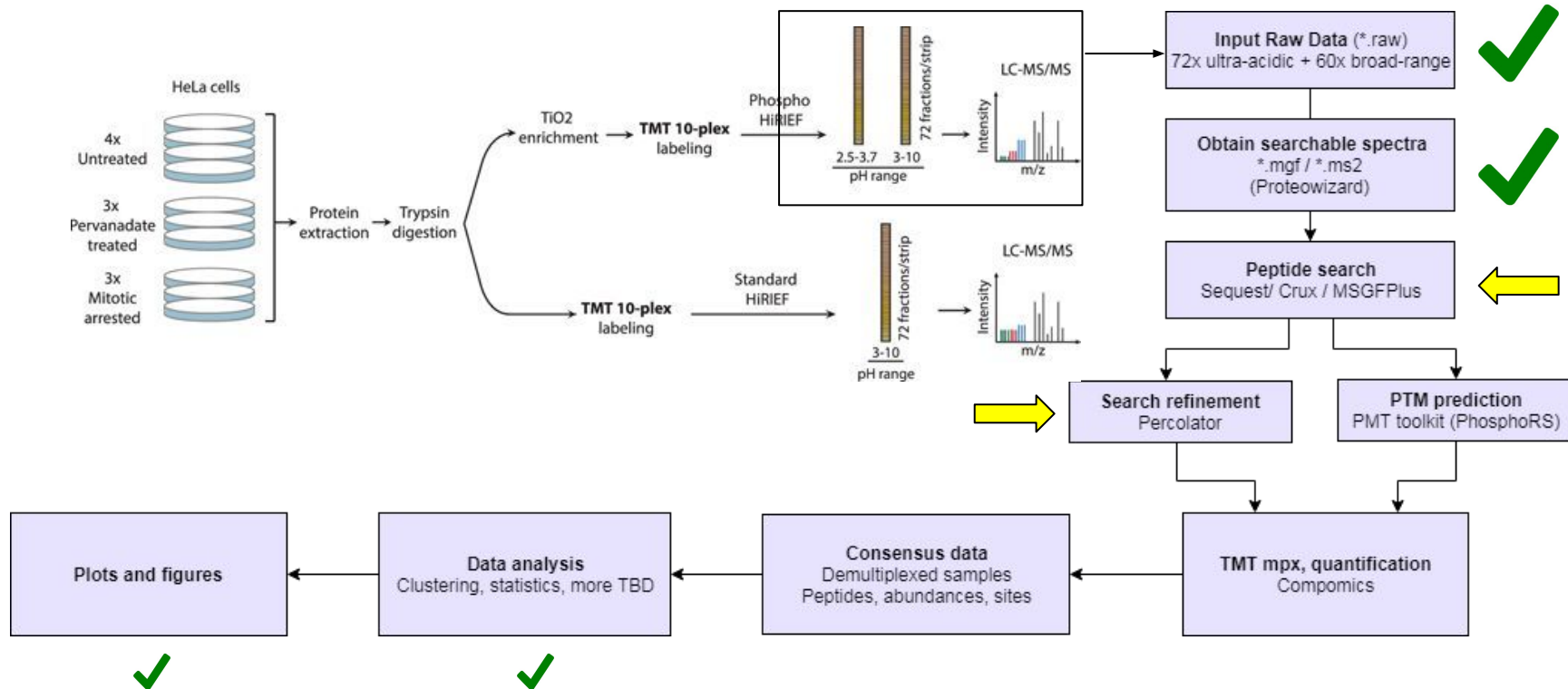


Phosphoproteome analysis

Hazal Koptagel, Julius Lautenbach, Maryia Ropat & Linnea Axelsson



Project Workflow



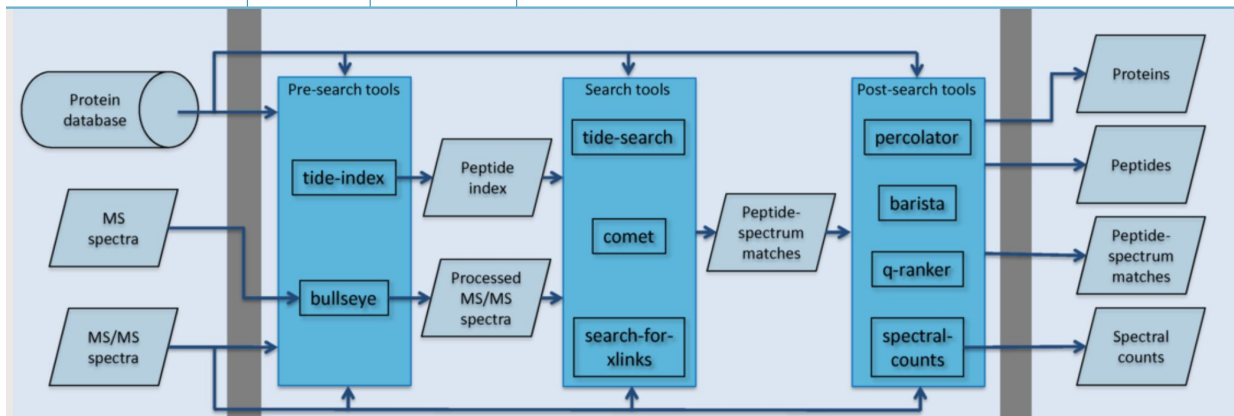
First steps

- preprocessing the raw data into .ms2 format (using Proteowizard - msconvert)
 - focus on the phosphoproteomics runs/files (132 files ~ 32GB)
- running crux/percolator
 - Semi-supervised learning for peptide identification from shotgun proteomics datasets
 - fix-modifications for carbamidomethylation on cysteine (by default)
 - mass modifications for lysine and N-terminus (TMT 10-plex)
- searchGUI with comet
 - good interface
 - possibility to follow Lab4 pipeline
 - output is not compatible percolator

crux



Home Download File Formats Contact



The Crux mass spectrometry analysis toolkit is an open source project that aims to provide users with a cross-platform suite of analysis tools for interpreting protein mass spectrometry data. The toolkit includes several search engines for both standard and cross-linked database search, as well as a variety of pre- and post-processing engines for assigning high-resolution precursor masses to spectra, assigning statistical confidence estimates to spectra, peptides and proteins, and performing label free quantification. Crux comes pre-compiled for the Linux, Windows and MacOS operating systems. It is implemented as a single program that offers a wide variety of commands. If you use Crux in your research, please cite:

Christopher Y. Park, Aaron A. Klammer, Lukas Käll, Michael J. MacCoss and William Stafford Noble. "[Rapid and accurate peptide identification from tandem mass spectra.](#)" *Journal of Proteome Research*. 7(7):3022-3027, 2008.

For a more up-to-date description of Crux, please read:

Sean McIlwain, Kaipo Tamura, Attila Kertesz-Farkas, Charles E. Grant, Benjamin Diamant, Barbara Frewen, J. Jeffrey Howbert, Michael R. Hoopmann, Lukas Käll, Jimmy K. Eng, Michael J. MacCoss and William Stafford Noble. "[Crux: rapid open source protein tandem mass spectrometry analysis.](#)" *Journal of Proteome Research*. 13(10):4488-4491, 2014.

- includes several search engines (standard & cross-linked database search)
- pre- and post-processing engines for assigning high-resolution precursor masses to spectra
- estimation of statistical confidence for a spectra, peptides and proteins
- label free quantification

Next steps

- Decide which search engine to use
- Crux/percolator
 - variable modifications of S, T or Y for phosphorylations
- Apply search to all files