The DeconTools Framework: an Application Programming Interface Enabling Flexibility in Accurate Mass and Time Tag Workflows for Proteomics and Metabolomics

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Overview

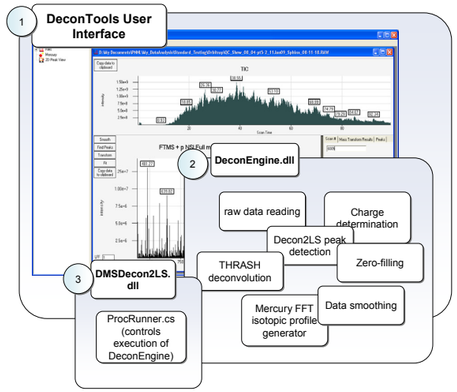
- A new .NET application programming interface (API) geared towards early MS data processing
- All processing tasks designed to be pluggable, allowing easy changes to overall workflow.
- Two workflows are demonstrated: standard accurate mass and time (AMT) tag data processing, and 2) a new targeted AMT tag workflow that begins with the target peptide and aims to score or quantify ($^{16}O/^{18}O$ or $^{14}N/^{15}N$ experiments) the peptide's MS signature.
- Demonstrated use of the new API by inserting a validation task and by substituting an alternative deconvolution task into the standard workflow.

Introduction

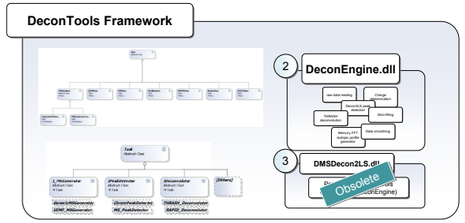
- Identifying peptides using the high throughput accurate mass and time (AMT) tag proteomics approach involves several data processing steps.
- At the beginning of the data analysis pipeline, the software tool Decon2LS[1] (pronounced "decon tools") extracts raw data, detects peaks, and finds peptide features (e.g., masses, elution times, etc.).
- Until now, Decon2LS users were limited to a single workflow and restricted to using only the algorithms made available by the DeconEngine, a C++ library.
- Addition of new deconvolution algorithms or alternative workflows required cumbersome changes that led to unmanageable 'spaghetti' code.
- Here, we wrap the previous DeconEngine in a new .NET framework and provide intuitive data objects and workflow control structures to facilitate early MS data processing.

Methods

Before: Decon2LS UI and Engine[1]



Now: DeconTools UI + DeconTools Framework



- Developed in C# .NET; open-source (available fall 2010)
- Framework organizes data structures and facilitates use of DeconEngine
- Adds new workflow control structures; abstraction of workflow processes ('Tasks')
- Allows new processing tasks to be easily added.
- Unit Testing: maintaining code integrity and demonstrating code usage.

Results

Inserting new modules into traditional workflows

1) Alternative Deconvolution using RAPID[2]

- **Question:** *Can we substitute our standard THRASH[1] deconvolutor with RAPID[2]?*
- **Approach:** Wrap RAPID with a class that implements from Task and insert into workflow.

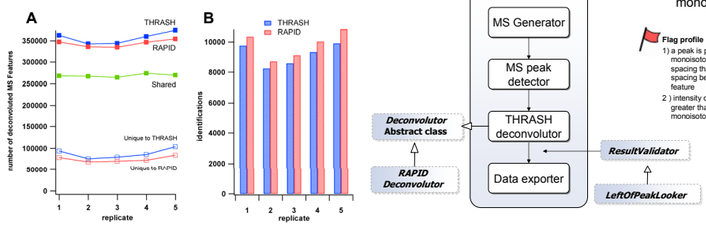


Figure 1. Comparison of THRASH and RAPID deisotoping of data from five quality-control Orbitrap *Shewanella oneidensis* datasets. **A)** Number of deisotoped features for each dataset, and **B)** AMT tags identified following processing through the AMT tag standard workflow.

2) The ResultValidator

- **Question:** *What percentage of MS features extracted by THRASH and RAPID had monoisotopic peaks wrongly selected?*
- **Approach:** Add a 'ResultValidator' module to the workflow, that flags results if intense peaks are present to the left of the identified monoisotopic peak (see information below)

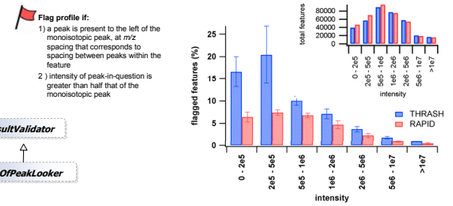
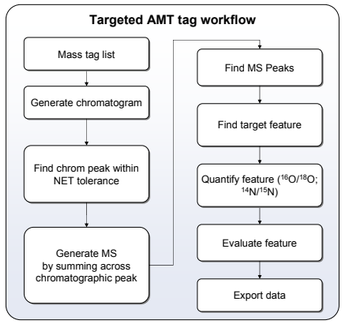


Figure 2: Occurrence of flagged isotopic profiles for quality control *S. oneidensis* datasets (n=5) run on an Orbitrap instrument, using THRASH[1] and RAPID[2] to deconvolute, binned by intensity. Inset shows the average total features detected for each bin.

New data processing workflows: a targeted AMT tag approach to quantification

- **Challenge:** Extracting unlabeled/labeled ratios from $^{16}O/^{18}O$ or $^{14}N/^{15}N$ data using traditional (THRASH-based) workflows can be error-prone, and can lead to missing data
- **Approach:** Use a targeted data processing workflow to direct the deconvolution of individual AMT tags
- **New additions to the DeconTools framework**
  1. Algorithms for rapidly generating selected ion chromatograms
  2. New data objects to support targeted workflow
  3. Simplified deconvolution algorithm
  4. Quantification algorithms



Scenario: Five mouse plasma $^{16}O/^{18}O$ datasets were processed through the typical analysis pipeline. While datasets averaged 1259 identifications, only 736 were shared across datasets (see Table 1.)

Can a targeted data analysis approach help recover missing $^{16}O/^{18}O$ values?

Table 1. AMT tag identifications across datasets

Replicate	Identifications
1	1231
2	1230
3	1255
4	1334
5	1246
Average	1259.2
Union	1708
Shared	736

Example of a case of missing $^{16}O/^{18}O$ ratio recovery

Mouse Complement C3; AMT Tag 106916571; MonoMW 1945.05194; Peptide EADVSLTAFVLIALQEAR

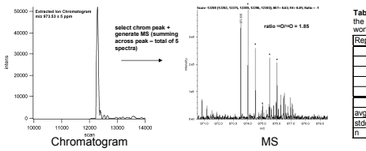


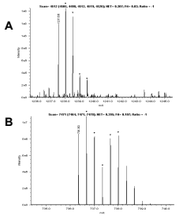
Table 2. $^{16}O/^{18}O$ ratios following the traditional and targeted workflow for AMT Tag 106916571

Replicate	traditional	targeted
1	-	1.62
2	1.62	1.85
3	-	1.56
4	-	2.00
5	-	1.98
avg	1.62	1.80
stdev	-	0.20
n	1	5

- For scenario to the left, the traditional workflow was successful in one out of five replicates, while a targeted approach retrieved $^{16}O/^{18}O$ ratios for all datasets
- Enables use of more peptides for calculating $^{16}O/^{18}O$ ratios at the protein level

Summary of larger scale analysis

- To retrieve missing $^{16}O/^{18}O$ ratios, all 1708 identified unique AMT tags (Table 1) were reprocessed through the targeted workflow
- Targeted excelled in cases:
  - where the +4 Da (^{18}O-) profile is extremely low or non-existent **(A)**
  - with larger MW peptides, in which the ^{16}O and ^{18}O profiles tend to overlap and confuse traditional deisotoping **(B)**
- Future work will examine AMT tags that are not found by the traditional approach in *any* dataset, but may have been missed because of low intensity profiles or overlapping ^{16}O and ^{18}O profiles



Conclusions and future directions

- DeconTools Framework API enabled use of RAPID, an alternative to Decon2LS' standard THRASH-based algorithm, without extensive modification to the codebase.
- Addition of automated result validation tasks is now possible and expected to provide downstream data processing tools with 'cleaner' MS features.
- Targeted AMT tag workflows are enabled by the framework. Application of these workflows to the extraction of quantification expression ratios will be further examined.

Acknowledgements

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References

1. Jaitly, N.; Mayampurath, A.; Littlefield, K.; Adkins, J. N.; Anderson, G. A.; Smith, R. D., Decon2LS: An open-source software package for automated processing and visualization of high resolution mass spectrometry data. *Bmc Bioinformatics 10* (2009).
2. Park, K.; Yoon, J. Y.; Lee, S.; Paek, E.; Park, H.; Jung, H. J.; Lee, S. W., Isotopic peak intensity ratio based algorithm for determination of isotopic clusters and monoisotopic masses of polypeptides from high-resolution mass spectrometric data. *Analytical Chemistry 80*:7294-7303 (2008).

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