

SRM workshop

Quantitative Proteomics II

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This workshop demonstrates how one can use computational tools for visualizing and analyzing SRM data. First, we use Skyline to get initial familiarity with the data and perform manual analysis. Then, Anubis is used to perform automated analysis of biological samples, which can be highly useful for large studies with hundreds of samples and peptides.

Exercises, Skyline:

Install Skyline from <https://proteome.gs.washington.edu/software/skyline/>
Skyline is unfortunately only available on Windows, so if you do not have windows accessible, pair up with some windows user for the Skyline based exercises.

Data import and initial orientation

1. Open **Skyline**, and create a blank project
2. Click **File -> Import -> Transition list...**, and select the file
`151201_SRM-workshop_transition_list.csv`
3. Browse through the protein tree on the left-hand side
4. Save the project by some suitable name.
5. Click **File -> Import -> Results...**, click **OK**, and select
`101104_JT_spiketides_part1.mzML`
`101104_JT_spiketides_part3.mzML`
`101112_JT_p14_21.mzML`
`101112_JT_p15_09.mzML`
`101112_JT_p16_13.mzML`
`101112_JT_th4_08.mzML`
`101112_JT_th5_10.mzML`
`101112_JT_th6_12.mzML`
6. Tell Skyline to not remove any common prefix, and wait for the files to load.
7. Click on a few peptides on the left and look at some data
8. By default, Skyline automatically zooms in on the best detected peak.
Disable this by **right-click** on the chromatogram, and selecting

Auto-zoom X-axis -> None.

9. Click on some more peptides, and try some data different data-files using the top tabs.

Q: Can you find some chromatogram where the peak selected by Skyline is wrong? If so, drag along the chromatogram to select the correct peak.

Simple analysis

1. Click **View -> Arrange Graphs -> Tiled**, and re-enable the **Auto-zoom X-axis -> Best Peak**.

2. Browse around among the peptides

Q: Can you identify peptides that were successfully measured?

Q: Are these any failed peptides assays?

3. Click **View -> Peak Areas -> Replicate Comparison**.

Right-click the graph and select **Log scale**. Browse some peptides.

Q: What is the difference between the 2 **spiketides** samples and the other 6?

Export of results

1. Click **File -> Export -> Report...**

2. Click **Edit list...**, and click **Add....** Check the following boxes:

Proteins/Protein Name

Proteins/Peptides/Peptide Modified Sequence

Proteins/Peptides/Precursors/Precursor Charge

Proteins/Peptides/Precursors/Precursor Mz

Proteins/Peptides/Precursors/Precursor Results/Best Retention Time

Proteins/Peptides/Precursors/Precursor Results/Min Start Time

Proteins/Peptides/Precursors/Precursor Results/Max End Time

Proteins/Peptides/Precursors/Precursor Results/Total Area

Proteins/Peptides/Precursors/Precursor Results/Max Height

Proteins/Peptides/Peptide Results/Result File/File Name

Proteins/Peptides/Peptide Results/Result File/Acquired Time

3. Give the report the name **Precursor Report** and click **OK**

4. Click **OK** to exit the **Edit Reports** dialogue

5. Select the **Precursor Report** you just created in the **Export Report** dialogue, click **OK** and select a good name.

6. Open the newly created file in Excel or similar.

Q: How many rows were exported? How many did you expect?

Exercises, Anubis:

For these exercises you will need to have Java 1.6+ installed.

Note that the names of programs (.jar files) have been shortened for easier readability. Everything you need will be in the exercise folder, so if something does not work - check what files you have and figure out what's wrong =).

Creating the Reference file

1. Open the `ReferenceFileCreator.jar` by double-clicking.
2. In the drop-down menu, change from `unsched` to `sched`.
3. Click `Load transition list` and select
`151201_SRM-workshop_transition_list.csv`
4. Control that the loaded table seems correct
5. Select all rows (ctrl+A), click `Get mzML for selected peptides`,
and select the file `101104_JT_spiketides_part1.mzML`
Q: What changed in the table?
6. Select uncovered rows by clicking the `Select zero coverage` button.
Click `Get mzML for selected peptides` again, but this time select
the other spiketides mzML file.
7. Browse through all rows and check that the chosen peak (rectangle) in
the chromatograms contains the correct peak.
Q: Is this true for all peptide ions? If no, which ones are wrong?
Q: Are all peptide ions covered?
8. If you found errors in the peak-picking in 8) fix them by
 - a. Checking the `mouse zoom` check box
 - b. Find the correct peak. Drag to zoom, right-click to un-zoom
 - c. Uncheck `mouse zoom`.
 - d. Select the correct peak in the chromatogram by dragging
 - e. Click `set reference`.
 - f. Update the chromatogram by zooming around again to see
that the rectangle is now in the correct spot
9. Click `Save` and select an appropriate name. Ensure that the status-text
in the bottom of the program states that the reference file was
successfully saved before exiting.

Running Anubis

1. Start Anubis by double-clicking `Anubis.jar`.

2. Click **Reference file** and select the file you just created.
3. Fill in an appropriate output name.
4. Click **Choose mzML files** and select
 - 101112_JT_p14_21.mzML
 - 101112_JT_p15_09.mzML
 - 101112_JT_p16_13.mzML
 - 101112_JT_th4_08.mzML
 - 101112_JT_th5_10.mzML
 - 101112_JT_th6_12.mzML
5. Click **Analyze** and wait for Anubis to finish.
 - Q: how many output files did Anubis create?
6. Open the resulting file ending with **'.res.csv'** in Excel or similar
 - Q: were all peptides successfully quantified?
 - Q: what peptide had the largest average intensity?
 - Q: what sample had the smallest average intensity?

Viewing Anubis result as a heatmap using ResultHeatMapper

1. Open a command line interface, and navigate to the exercise folder
2. Type `java -jar ResultHeatMapper.jar ANUBIS_OUT.res.xml` to open the ResultHeatMapper program.
3. Click around on heatmap squares to see what happens.
 - Q: What do the options in the top left corner do?
 - Q: Can you draw any interesting conclusions on these samples?
4. Exit the ResultHeatMapper. We will open it again soon, don't worry.
5. Investigate the files `proteins.xml` and `samples.xml` in a text editor.
 - Q: What data does `proteins.xml` contain?
 - Q: What's in `samples.xml` then?
6. Use `proteins.xml` and `samples.xml` to improve your heatmap by running the following command on the command line:


```
java -jar ResultHeatMapper.jar
      --rep-group=samples.xml
      --prot-group=proteins.xml
      ANUBIS_OUT.res.xml
```

 - Q: How is the generated heatmap different from before?
 - Q: Can you draw any interesting conclusions now?