**DISCO – Tutorial version 0.1**

For this tutorial we will be using some peak picked Orbitrap Elite DIA data

The data were already converted to mzML using msconvert. The search results were processed individually by PeptideProphet and combined using iProphet. This tutorial starts by running PTMProphet on the iProphet combined pepXML results.

1. Ensure that you are connected and have the latest Trans-Proteomic Pipeline (TPP)

* This tutorial is written with details for TPP on a Microsoft Windows operating system. If you have a different kind of operating system, this tutorial should still work, but the details of the installation and some file path locations will be somewhat different
* Ensure that you are Internet connected for this tutorial because you will download files
* This tutorial requires that you have TPP 5.2.0 or later installed on your system.
* If you have a pre-5.2.0 version of TPP already installed on your system, uninstall the older version first by clicking [Start] [Trans-Proteomics Pipeline] [Uninstall TPP]
* If TPP 5.2.0 or greater is not installed on your system, install that first by downloading and following the instructions at <http://tools.proteomecenter.org/wiki/index.php?title=TPP:5.2_Installation>. Note that you will need to restart your computer or manually start the Apache web server service

1. Launch the TPP web browser Graphical User Interface

* [Start] [Trans-Proteomic Pipeline] [TPP Web Interface] or open a web browser to: <http://localhost:10401/tpp>
* Login with the username ‘guest’ with password ‘guest’ (or alternate account if you have one)

1. In the web browser GUI, download the tutorial data:

* Click the [Files] tab at the top
* In the bottom right, create new directory: tutorials
* Click [TPP Tools] [fetch datasets]
* Click on [Show version information and available features]
* If there is a newer version, click on [Update to the latest version of fetchDataset]. Version 0.8.1 or greater is required
* Click [TPP Tools] [fetch datasets] again
* Click (Add Files)
* Checkmark the “tutorials” directory and click (Select)
* Paste the following URL into the [Dataset Identifier or URL] box:

http://www.tppms.org/tools/disco/DISCO.zip

* Click (Fetch Dataset)
* Monitor the job by clicking [Refresh] until download and unzip is complete

1. Run DISCO on the example dataset

* Click [TPP Tools]:[DIA:Extract MS2 Fragments]
* Click on (Add Files), and navigate to ***tutorials/DISCO***
* This directory contains the mzML data: ***011618-DIA-02.mzML***.
* Checkmark 011618-DIA-02.mzML and click (Select)
* Under [Choose DIA Windows File] select: ***c:/TPP/data/tutorials/DISCO/DIA\_85fixed.txt***
* Under [Choose Hardklor params] select: ***c:/TPP/data/tutorials/DISCO/Hardklor.conf***
* Under [Disco Options]
  1. [Scan Range]: [Start]: ***50000***
  2. [Scan Range]: [End]: ***51000***
  3. If you know you have multiple core, you can specify number of threads to number of cores
* Click on (Run Disco*)*
* Click on [Refresh] to monitor progress. (About 2 minutes on a modern computer)
* Ensure that the job did not end with an error

1. Run Comet on the DISCO generated data file
   * Open [TPP Tools]:[Comet Search]
   * Remove 011618-DIA-02.mzML as a selected file (checkmark file on right and click [Remove])
   * In “choose from list”, select the 011618-DIA-02\_ds.mzML file (note the **\_ds**)
   * Remove the Hardklor.conf from the Comet parameters file (checkmark and click [Remove])
   * Click [Add Files], navigate into “com” subfolder and then select the comet.params file and click [Select]
   * Next Choose a sequence database. Go up to DISCO folder. Then into dbase folder. Checkmark PA\_THISP\_Level1\_2018-05-01\_targetdecoy\_iRT.fasta and click [Select]
   * Click on (Run Comet)
   * Click on [Refresh] to monitor progress. (About 2 minutes on a modern computer)
   * Ensure that the job did not end with an error
2. Run the Prophets on the Comet results
   * Open [TPP Tools]:[Analyze Peptides]
   * Remove any selected input files if present by checkmarking them and clicking [Remove]
   * Navigate to the DISCO/comet folder
   * Checkmark the 011618-DIA-02\_ds.pep.xml file and click [Select]
   * Checkmark “Use accurate mass binning, using PPM”
   * Under iProphet options, select “RUN iProphet” and then also below that ProteinProphet
   * Click on (Run XInteract)
   * Click on [Refresh] to monitor progress. (About 1 minute on a modern computer)
   * Ensure that the job did not end with an error
3. Explore the results
   * Click on the [PepXML] link next to interact.pep.xml (first output file)
   * Click on a probability and examine the models. Do they seem reasonable?
   * What is the probability threshold, number correct and incorrect at 1% FDR?
   * Close the model viewer and PepXML Viewer tabs
   * Click on the [PepXML] link next to interact.ipro.pep.xml (second output file)
   * Click on a probability and examine the models. Do they seem reasonable?
   * What is the iProphet probability threshold, number correct and incorrect at 1% FDR?
   * Close models and filter the PepXML Viewer with an iProphet probability of 0.817
   * Do a CTRL+F to find YVTIIDAPGHR and click on the spectrum [9/20]
4. Run some StPeter (MS2-based) quantitation
   * Open [TPP Tools]:[Quantify Label-Free (MS2)]
   * Choose from list drop-down interact.ipro.prot.xml file
   * Change tolerance to 0.05
   * Set the total protein to 1 microgram
   * Click (Run StPeter) (this should finish super fast with such a small dataset)
   * Click on the [ProtXML] link next to interact.ipro.prot.xml
   * See the new quantitation results (may need to scroll to the right)
   * Click [File & Info] tab
   * In the bottom left drop down list, choose StPeter\_Quant and then click [Export Values]
   * Click on the new PloTPP link that comes up
   * In the drop-down at the top, choose interact.ipro.prot-StPeter\_Quant.data
   * You should see a histogram of the StPeter results, not normalized
   * If we had multiple samples here (we don’t in this tutorial), you could do relative quantitation