

## PTMProphet – Tutorial version 0.4

For this tutorial we will be using a subset of data from Ferries et al. (2017, JPR, 16, 3448)

(<https://www.ncbi.nlm.nih.gov/pubmed/28741359>), the HCD Orbitrap data files (raw files labeled HCDOT).

Although not necessary for this tutorial, the complete dataset could be downloaded from ProteomeXchange

<http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD007058>

The data were already converted to mzML using msconvert and searched using Comet and X!Tandem. 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](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
  - Download and install TPP 5.2.0 or greater from here: <https://sourceforge.net/projects/sashimi/files/latest/download>
2. 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)
3. 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/ptm/Ferries2017.zip>
  - Click (Fetch Dataset)
  - Monitor the job by clicking [Refresh] until download and unzip is complete
4. Verify that the folder C:\TPP\data\tutorials\Ferries2017 contains the correct folders and files
- Click [Files] tab
  - In lower right, click on “tutorials” directory and “Ferries2017” directory below that
  - Verify that you see 1 file “Reference.csv” in the center and the following directories in the lower right:  
com\_HCDOT  
com\_tan\_HCDOT  
data  
dbase  
tan\_HCDOT
5. Run PTM Prophet on the example dataset
- Click [TPP Tools][Analyze PTMs]
  - Click on (Add Files), and navigate to **tutorials/Ferries2017/com\_tan\_HCDOT**
  - This directory contains the combined results of running iProphet on the Comet and X!Tandem search + PeptideProphet analyses: **interact.ipro.pep.xml**.
  - Checkmark interact.ipro.pep.xml and click (Select)
  - Under [Output file and location] set [Write output to file:] to:  
**interact.ptm.ipro.pep.xml**
  - Under [PTMProphet Parameters] set the following:
    - i. [Specify modifications] (you can use the pull-down triangles to assist you):  
[Residue(s)]: **STY**      [Mass shift]: **79.9663**  
[Residue(s)]: **MW**      [Mass shift]: **15.995**
    - ii. [Minimum PSM Probability for Computation]: **0.9**

- Click on (Run PTMProphet)
  - Click on [Refresh] to monitor progress.
  - Ensure that the job did not end with an error
6. Open the results and filter them
- Under the [OUTPUT FILES] section of the page, click on the [PepXML] link
  - This opens a new tab the output results, but notice many probability 0 results
  - Click the [Filtering Options] tab set the filters:
    - a. [iProphet Probability]:[min]: **0.9**
    - b. Click [Update Page] at bottom of green space (might need to make window larger)
    - c. After refresh, click on [Filtering Options] tab again to make it go away

7. Examine the results

The PTMProphet localization results are provided in the column called **PTM\_PEPTIDE**. The site probabilities for the two types of modifications localized are displayed as bars over each potential modification site. Modifications are assigned to the site with the highest probability, breaking ties by assigning the modification to the first from the n-terminus unoccupied site of the highest probability. Hovering the mouse over the gray and colored bars reveals the site probabilities. Additional PTMProphet calculations are provided in columns: **mBPr\_STY** (mean of best probabilities of phospho mods), **info\_STY** (per phosphor mod informational content), **lmods\_STY** (number of localized phospho mods), **mBPr\_MW** (mean of best probabilities of oxidation mods), **info\_MW** (per oxidation mod informational content), **lmods\_MW** (number of localized oxidation mods).

8. Open the original iProphet results (prior to PTMProphet)

- Switch back to the previous web browser tab containing the Petunia interface
- Click [Files]
- If not already there, navigate to **tutorials/Ferries2017/com\_tan\_HCDOT**
- Click on the PepXML link next to the file **interact.ipro.pep.xml** (NOT the new one with “ptm” in the file name)
- You now have three browser tabs: Petunia, PepXML Viewer with just ipro, and PepXML Viewer with ptm analysis

9. Apply the same filters to both PepXML Viewer tabs

- In the first PepXMLViewer tab apply these filters and sorting options as follows:

### [Filtering Options] tab

- a. [Required peptide text (regex allowed)]:  
***S167.00SSFREMENQPHK|SSI67.00SSFREMENQPHK|SSSI67.00FREMENQPHK***
- b. Next to that, [include modification (subscript) text]: **checked**
- c. [iProphet Probability]:[min]: **0.9**

### [Display Options] tab

- d. [Sorting]: by spectrum in ascending order
  - e. [Hilight peptide text (regex)]: ***S167.00***
  - f. Next to that, [include modification (subscript) text]: **checked**
  - g. Click [Update Page]
  - h. After the update, click on [Display Options] tab to make it disappear
- **Now apply the same settings to the PepXML Viewer in the other browser tab**

10. Compare the original search engine result and the PTMProphet result and the answer sheet (which synthesized peptides were placed in each sample pool) for the filtered peptides

- Fill in the table on the last page of this document with the results in the two PepXML Viewer tabs
- How often did the initial search match the correct site?
- How often did a \*confident\* PTMProphet result match the correct site?

11. Export the results to Excel

- Switch to the browser tab of the PepXML Viewer with the PTMProphet results
- Click [Other Actions]
- Click [Export Spreadsheet]
- After the page refresh, click on the hyperlink next to “**exported spreadsheet to**”
- Switch to the browser tab with the Petunia interface
- Navigate to **tutorials/Ferries2017/com\_tan\_HCDOT** or if already there, do a browser refresh
- Click on the [view] link next to interact.ptm.ipro.pep.xls and open with Excel
- Excel might complain about the format. Click [Yes] to open it anyway

12. Study the relationship between PeptideProphet (spectrum-level) Probability (column B) and PTM confidence metrics.

- Click column B
- CTRL + Click column D
- [Insert] and choose a scatter plot
- Right click on a data point and [Add Trendline...]
- Under the trendline options, select to display the r-squared
- What are the r-squared values of the relationships between PeptideProphet probability (column B) and PTMProphet localization metrics (columns D, E and F)?

13. Export the results to an mzIdentML file, using the TPP tool tpp2mzid.

- In Petunia, open the interface to tpp2mzid: go to [TPP Tools][Export MzIdentML]
  - Click on [Add Files], and go to [Current Directory]:  
**c:/TPP/data/tutorials/Ferries2017/com\_tan\_HCDOT**
  - Checkmark the PTMProphet result file: **interact.ptm.ipro.pep.xml** and click (Select)
  - Click on (Convert to mzIdentML)

Additional Notes:

- The Ferries et al. 2017 reference sheet for the whole dataset can be viewed by opening the following file in Excel or text editor: **tutorials/Ferries2017/Reference.csv**

spectrum	ModPosition	CometRight?	PTMProphetRight?	PTMProphetProbAtCorrectSite
plate08_A08.00027.00027.2	2			
plate08_A08.00030.00030.3	2			
plate08_A08.00053.00053.3	2			
plate08_A08.00090.00090.3	2			
plate08_A09.00017.00017.3	3			
plate08_A09.00020.00020.2	3			
plate08_A09.00026.00026.3	3			
plate08_A09.00040.00040.3	3			
plate08_A09.00057.00057.3	3			
plate08_A09.00068.00068.3	3			
plate08_A10.00029.00029.3	4			
plate08_A10.00032.00032.2	4			
plate08_A10.00038.00038.3	4			
plate08_A10.00064.00064.3	4			
plate08_A10.00069.00069.3	4			
plate08_A10.00082.00082.3	4			
plate08_A10.00120.00120.3	4			
plate08_A10.00130.00130.3	4			
plate08_A11.00033.00033.2	5			
plate08_A11.00049.00049.3	5			
plate08_A11.00076.00076.3	5			
plate08_A11.00085.00085.3	5			
plate08_A11.00103.00103.3	5			
plate08_A11.00138.00138.3	5			

spectrum	Synthesized ModPosition	SearchRight?	PTMProphetRight?	PTMProphetConfident?
<b>SF_200217_pPeptideLibrary_pool1_HCDOT_rep1.02448.02448.3</b>	3			

## PTMProphet -- Tutorial

SF_200217_pPeptideLibrary_pool1_HCDOT_rep1.02450.02450.2	3			
SF_200217_pPeptideLibrary_pool1_HCDOT_rep2.02436.02436.3	3			
SF_200217_pPeptideLibrary_pool2_HCDOT_rep1.02447.02447.3	2			
SF_200217_pPeptideLibrary_pool2_HCDOT_rep1.02480.02480.2	2			
SF_200217_pPeptideLibrary_pool2_HCDOT_rep2.02476.02476.3	2			
SF_200217_pPeptideLibrary_pool2_HCDOT_rep2.02487.02487.2	2			
SF_200217_pPeptideLibrary_pool5_HCDOT_rep1.02477.02477.3	1			
SF_200217_pPeptideLibrary_pool5_HCDOT_rep1.02507.02507.2	1			
SF_200217_pPeptideLibrary_pool5_HCDOT_rep2.02473.02473.3	1			
SF_200217_pPeptideLibrary_pool5_HCDOT_rep2.02504.02504.2	1			