**The extracted PRM peak intensity (XPI) manual**

1. **XPI program**
    a. The XPI program was developed to quantify parallel reaction monitoring (PRM) data of stable isotope labeled peptides. As a result, this software is currently optimized for Thermo instrument .RAW file data. The XPI program extracts the centroided peak intensity of each PRM target ion scan.
2. **Developers**
    a. Lang Ho Lee, Brett Pieper, Sasha A. Singh
    b. For issues or help please contact LHL (LLEE27@PARTNERS.ORG) or SAS (SASINGH@PARTNERS.ORG)
3. **Copyright**
    a. This software script is protected by the Copyright Act of 1976, 17 U.S.C. §§ 101-810, as amended. Rights reserved. Please contact The Brigham and Women's Hospital, Inc. for further information.
4. **License**
    a. GPL (http://www.gnu.org/licenses/)
5. **Update history**
    a. XPI-v.1.0 on May 31, 2016
6. **XPI program Installation**
    a. Download of XPI program
        i. Visit CICS homepage and download XPI at below link.
            1. http://cics.bwh.harvard.edu/software
    b. Python installation
        i. **We recommend Python 3.4.3 because XPILib was coded using Python 3.4.3**
        ii. See the link below to the Python website
        iii. https://www.python.org/downloads/release/python-343/
        iv. For Windows users
            1. You may need to add a python directory path to the Path environment variable.
    c. Required packages
        i. The XPI program requires several Python libraries. Follow the links and install libraries.
        ii. Pymzml
            1. Use >= 0.7.7 version.
            2. `$ python -m pip install pymzml`

3. http://pymzml.github.io/intro.html#download

iii. NumPy and SciPy
    1. `$ python -m pip install scipy`
    2. `$ python -m pip install numpy`
    3. http://www.scipy.org/install.html

iv. Statsmodels
    1. `$ python -m pip install statsmodels`
    2. http://statsmodels.sourceforge.net/devel/install.html
    3. For windows binaries
        a. http://statsmodels.sourceforge.net/binaries/

v. Matplotlib
    1. `$ python -m pip install matplotlib`
    2. http://matplotlib.org/users/installing.html

vi. Pyteomics
    1. `$ python -m pip install pyteomics`
    2. https://pythonhosted.org/pyteomics/installation.html
    3. `$ python -m pip install lxml`
    4. http://www.lfd.uci.edu/~gohlke/pythonlibs/#lxml

## 7. Execution example

a. This example already includes mzML files.

b. The XPI program consists of 4 Python scripts, XPILib, XPIQuant, XPIPeak and XPIViz, and provides one example dataset “testset”. The “testset” data consists of PRM data of apoA-I protein at different D0-Leu:D3-Leu mixing ratios (1:1 to 1:1,000).

c. In the test set, D0-Leu and D3-Leu are named as Light and Heavy, respectively.

d. Go to the directory where you unzipped the downloaded XPI and check XPI files.

```
BWH003067:ver.1.0 CICS$ ls -al
total 360
drwxr-xr-x   9 CICS  staff     306 May 31 11:48 .
drwxr-xr-x  14 CICS  staff     476 May 31 11:54 ..
-rw-r--r--@  1 CICS  staff    8196 May 31 11:48 .DS_Store
-rw-r--r--   1 CICS  staff  149157 May 30 17:12 XPILib.pyc
-rw-r--r--   1 CICS  staff    1364 May 27 15:57 XPIPeak.py
-rw-r--r--   1 CICS  staff    2505 May 27 15:11 XPIQuant.py
-rw-r--r--   1 CICS  staff    7084 May 30 16:48 XPIViz.py
-rw-r--r--@  1 CICS  staff     516 May 31 11:48 test.sh
drwxr-xr-x  13 CICS  staff     442 May 31 11:16 testset
```

e. Follow below steps

**Step 1. PRM peak extraction.** First, execute XPIQuant.py to extract PRM ion intensities of peptides listed in the inclusion list.

```
$ python XPIQuant.py ./testset/XPIQuant_config.txt
```

```
BWH003067:ver.1.0 CICS$ python XPIQuant.py ./testset/XPIQuant_config.txt
Reading Config & Transition
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
Copyright        : This software script is protected by the Copyright Act of 1976, 17 U.S.C. §§ 101-810, as ame
nded. Rights reserved.  Please contact The Brigham and Women's Hospital, Inc. for further information.
License          : GPL
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Light        L(0.0)
                 : Heavy        L(3.01883025)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Transition file is successfully generated
Found 21 transitions
Reading PRM Runs
Found 6 file(s) in ./testset
Reading ./testset/01_BP_HR-PRM_20min_APOA1_5%-20%_1000_Set1.mzML ... Done in 18.3783 seconds
Reading ./testset/01_BP_HR-PRM_20min_APOA1_5%-20%_100_Set1.mzML ... Done in 18.1164 seconds
Reading ./testset/01_BP_HR-PRM_20min_APOA1_5%-20%_1_Set1.mzML ... Done in 22.7192 seconds
Reading ./testset/01_BP_HR-PRM_20min_APOA1_5%-20%_500_Set1.mzML ... Done in 19.5160 seconds
Reading ./testset/01_BP_HR-PRM_20min_APOA1_5%-20%_50_Set1.mzML ... Done in 20.0820 seconds
Reading ./testset/01_BP_HR-PRM_20min_APOA1_5%-20%_5_Set1.mzML ... Done in 20.2564 seconds
Done in 119.0694 seconds
Finding target PRM ions
01_BP_HR-PRM_20min_APOA1_5%-20%_500_Set1.mzML... 90252/90252
01_BP_HR-PRM_20min_APOA1_5%-20%_1000_Set1.mzML... 88234/88234
01_BP_HR-PRM_20min_APOA1_5%-20%_100_Set1.mzML... 86396/86396
01_BP_HR-PRM_20min_APOA1_5%-20%_5_Set1.mzML... 92254/92254
01_BP_HR-PRM_20min_APOA1_5%-20%_50_Set1.mzML... 91441/91441
01_BP_HR-PRM_20min_APOA1_5%-20%_1_Set1.mzML... 108397/108397
Done in 39.0950 seconds
Writing all the detected PRM Ions ... Done in 0.5668 seconds
XPI output files were generated in ./testset
```

Results (“XPI_transition.txt” (Potential fragment ions that have labeled residues) and XPI_output_all.txt (Extraction of all the PRM ions)) will be generated in the data directory (for the test set, “testset” directory).

**Step 2. Peak refinement.** Execute XPIPeak.py to refine PRM peaks

```
$ python XPIPeak.py ./testset/
```

```
BWH003067:ver.1.0 CICS$ python XPIPeak.py ./testset/
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
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nded. Rights reserved.  Please contact The Brigham and Women's Hospital, Inc. for further information.
License          : GPL
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Light        L(0.0)
                 : Heavy        L(3.01883025)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Processing peak data... Done!
Writing peak information and Picking effective peaks... Done!
Drawing plots..... 30/30
Drawing peak picking profile... Done!
```
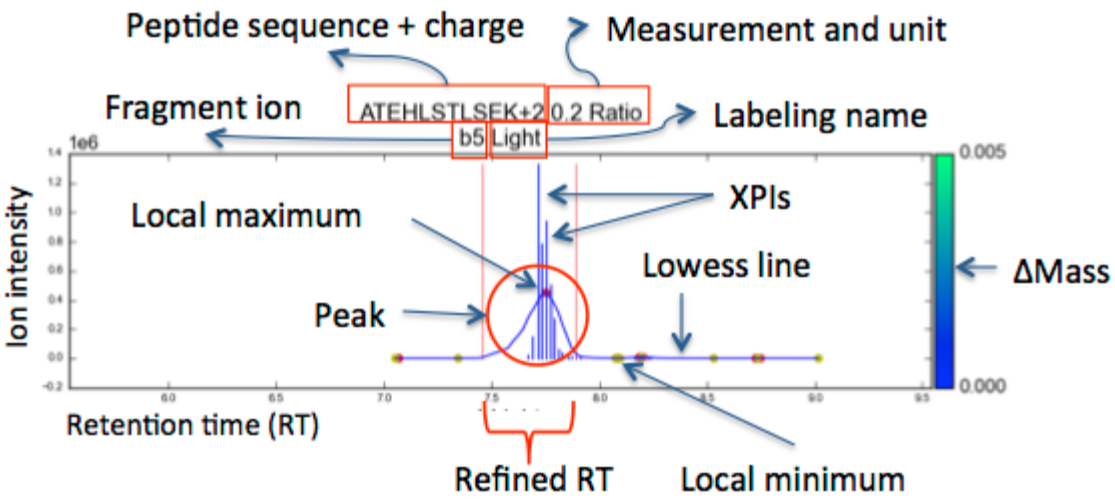

The XPIPeak produces peak selection plots as well as the peak refining result in the file, “XPI_output_peaks.txt”. The processing time for the PRM peak refinement depends on how many mzML files and peptides are being processed, but XPIPeak basically takes minutes to choose appropriate peaks. This can replace the manual peak selection that is often required and laborious for the XIC method.

The peaks in the graphics consist of XPIs (colored by ΔMass to the theoretical mass, green (large difference, ie. 005 Da) to deep blue (small difference ie. 001 Da)). Red lines are refined retention time (RT). Red and yellow dots are local maximum and minimum, respectively of the smoothed lowess line (blue line).



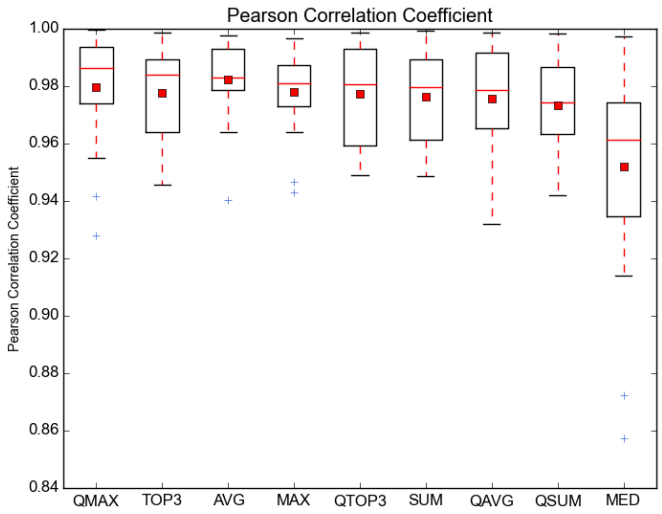

Peak plots will be saved in the "Peak_Picking" directory in the data directory. "XPI_output_peaks.txt" (the refined retention time information) and "XPI_output_4check.txt" (quantification data in various methods) will also be generated inthe data directory.

**Step 3. Quantification and PRM ion filtering.** Choose a quantification method and ion-filtering threshold. During this step, you can evaluate the candidacy of PRM ions for reliable quantification (more below).

```
$ python XPIViz.py ./testset/ fil
```

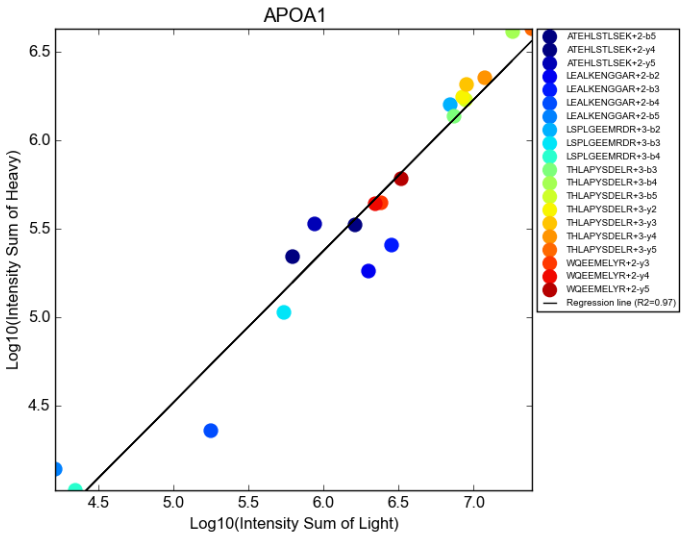
```
BWH003067:ver.1.0 CICS$ python XPIViz.py ./testset/ fil
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
Copyright        : This software script is protected by the Copyright Act of 1976, 17 U.S.C. §§ 101-810, as ame
nded. Rights reserved.  Please contact The Brigham and Women's Hospital, Inc. for further information.
License          : GPL
Selected quantification method: QMAX
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Heavy        L(3.01883025)
                 : Light        L(0.0)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Found 21 transitions
Drawing enrichment_filter plots..... 1/1
Drawing enrichment_filter_log plots..... 1/1
All the results are generated at ./testset/
```

The XPI program provides box plots of Pearson's r between the intended and the observed mixing ratio. For the test set, QMAX shows relatively higher Pearson's r so, we will use QMAX for the test set. QMAX is a maximum number in the second and third quartiles. If you want to follow traditional XIC quantification, SUM method is recommended. If you want to get more information about quantification methods, go to section 9.c.

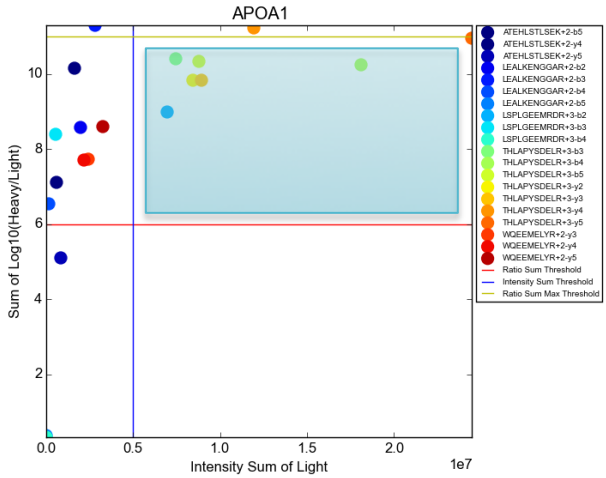



At this step, XPI program provide two more plots for the ion filtering:

1. A fragment ion scatter plot in log10 scale, standard label (section 8.c for more description) ion intensity (for the test set Light) vs. other labeled ion intensity (for the test set Heavy),



2. A fragment ion scatter plot, standard label ion intensity (for the test set Light) vs. ratio or enrichment (for the test set Heavy/Light). The ion-filter is based on this plot. The blue line is the reference ion intensity threshold (x=0.5E+07) and red line is ratio or enrichment threshold to filter out potential noise (y=6). The yellow line is ratio or enrichment threshold to filter out outliers (y=11). With three thresholds, we can limit fragment ions for further analyses.




All the plots will be saved in “Filtering” directory of the data directory.

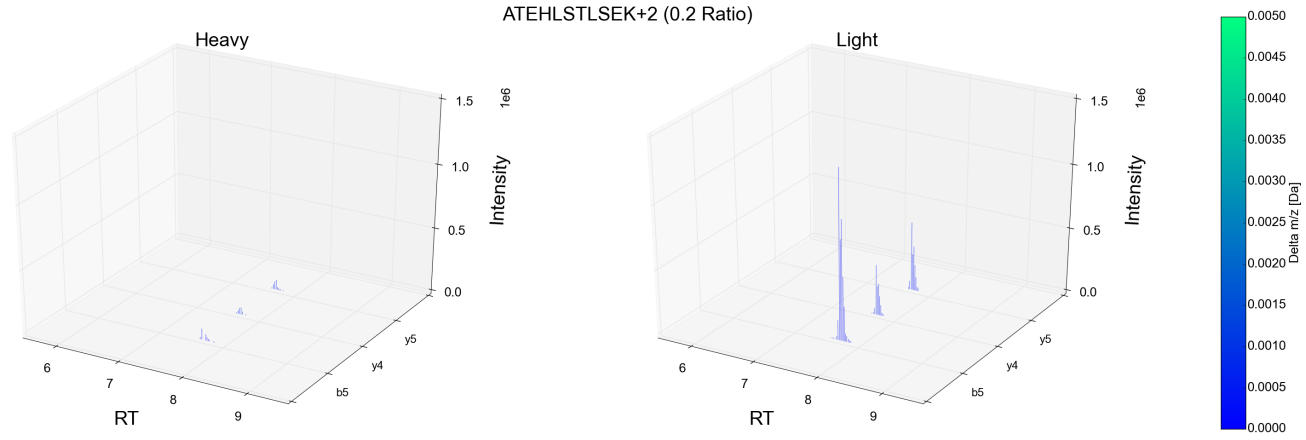
**Step 4. Visualization.** The XPI program provides visualization modules to draw several plots.

### 3D mass profiles

1. This plot shows the detected XPIs for a peptide.
2. Plots will be saved at “3D_Profile” directory of the data directory.
3. XPIs are colored by ΔMass to the theoretical mass, green (large difference) to deep blue (small difference)

```
$ python XPIViz.py ./testset/ 3dp
```

```
BWH003067:ver.1.0 CICS$ python XPIViz.py ./testset/ 3dp
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
Copyright        : This software script is protected by the Copyright Act of 1976, 17 U.S.C. §§ 101-810, as ame
nded. Rights reserved.  Please contact The Brigham and Women's Hospital, Inc. for further information.
License          : GPL
Selected quantification method: QMAX
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Light        L(0.0)
                 : Heavy        L(3.01883025)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Found 21 transitions
/Library/Frameworks/Python.framework/Versions/3.4/lib/python3.4/site-packages/matplotlib/tight_layout.py:225: U
serWarning: tight_layout : falling back to Agg renderer
  warnings.warn("tight_layout : falling back to Agg renderer")
Drawing 3D-plots..... 30/30
All the results are generated at ./testset/
```



**2D mass profiles**

4. This plot shows the detected XPIs for each fragment ions.
5. Plots will be saved at “3D_Profile” directory of the data directory.
6. XPIs are colored by ΔMass to the theoretical mass, green (large difference) to deep blue (small difference)

```
$ python XPIViz.py ./testset/ 2dp
```

```
BWH003067:ver.1.0 CICS$ python XPIViz.py ./testset/ 2dp
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
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License          : GPL
Selected quantification method: QMAX
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Heavy        L(3.01883025)
                 : Light        L(0.0)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Found 21 transitions
Drawing 2D-plots...... 126/126
All the results are generated at ./testset/
```

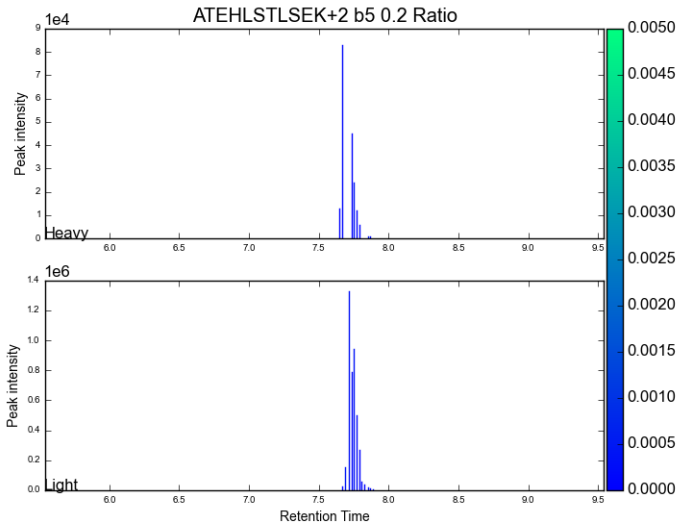

**Standard curve**

7. This scatter plot is to evaluate the linearity between the intended and the observed mixing ratio for proteins and fragment ions.
8. Red line is regression line and blue dots are the detected PRM ion ratio.
9. Results will be generated in “Standard_Curves” of the data directory.

```
$ python XPIViz.py ./testset/ stdc
```

```
BWH003067:ver.1.0 CICS$ python XPIViz.py ./testset/ stdc
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
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nded. Rights reserved.  Please contact The Brigham and Women's Hospital, Inc. for further information.
License          : GPL
Selected quantification method: QMAX
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Light        L(0.0)
                 : Heavy        L(3.01883025)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Found 21 transitions
Processing results..... 6/6
Completed making 6 standard curve figures
Standard curve for protin APOA1 is done (R2=0.9834)
Completed writing Standard_Curves_R2.csv
All the results are generated at ./testset/
```

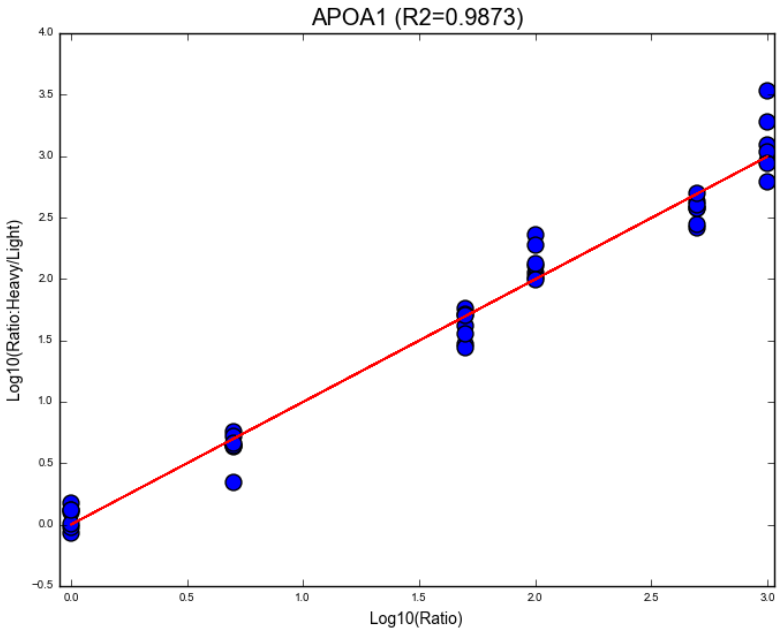

**Peptide plots**

10. The XPI program provides two plots for peptides, the filling plot and the line graph.
11. Results will be generated at “Scatter_Plots_Peptide” of the data directory.

```
$ python XPIViz.py ./testset/ pep
```

```
BWH003067:ver.1.0 CICS$ python XPIViz.py ./testset/ pep
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
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nded. Rights reserved.  Please contact The Brigham and Women's Hospital, Inc. for further information.
License          : GPL
Selected quantification method: QMAX
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Light        L(0.0)
                 : Heavy        L(3.01883025)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Found 21 transitions
Drawing scatter plots of peptides..... 2/2
Drawing filling plots of peptides..... 2/2
All the results are generated at ./testset/
```
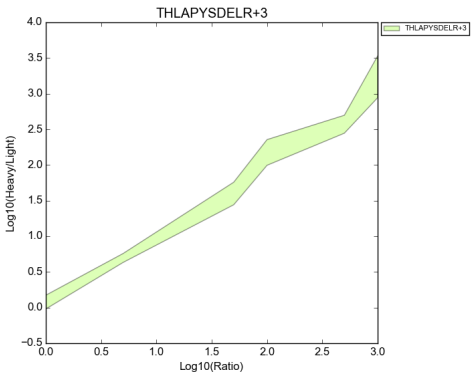
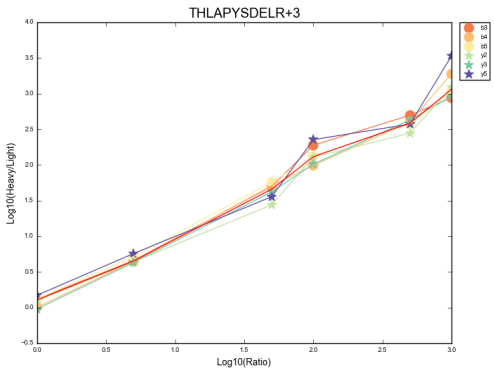

The filling plot and the line graph



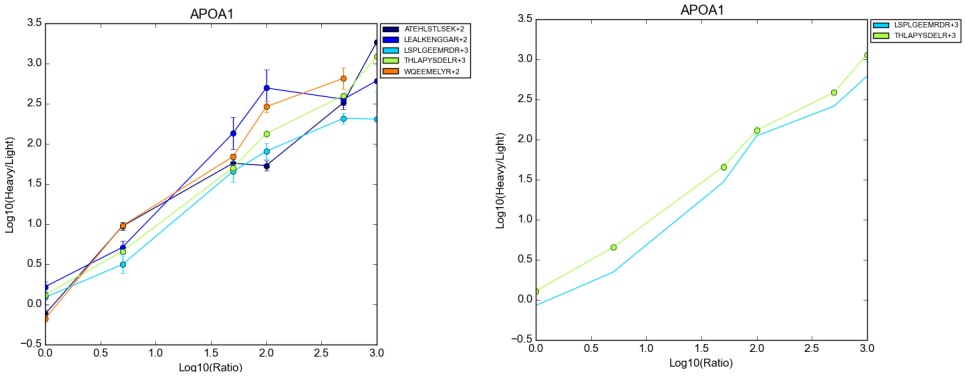
**Protein plots**

12. The XPI program provides three plots for proteins, the error plot, the filling plot and the scatter plot.
13. Results will be generated at “Scatter_Plots_Protein” of the data directory.

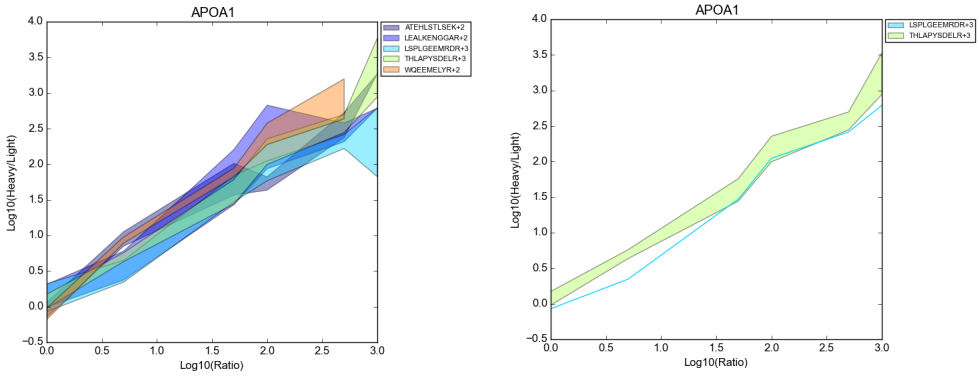
```
$ python XPIViz.py ./testset/ prot
```

```
BWH003067:ver.1.0 CICS$ python XPIViz.py ./testset/ prot
eXtraction of Prm ion Intensities (XPI) ver.1.0 (2016-05-31)
Credits          : Lang Ho Lee, Brett Pieper, Sasha Singh
Maintainer       : Lang Ho Lee (llee27@partners.org)
Copyright        : This software script is protected by the Copyright Act of 1976, 17 U.S.C. §§ 101-810, as ame
nded. Rights reserved.  Please contact The Brigham and Women's Hospital, Inc. for further information.
License          : GPL
Selected quantification method: QMAX
No modification found
Data directory   : ./testset
Inclusion list   : ./testset/Inclusion_list.txt
Labeling         : Heavy        L(3.01883025)
                 : Light        L(0.0)
MS2 window       : 0.005
Max fragment     : 5
Ratio            : Heavy/Light
Enrichment       : False
TIC Normalize    : False
Skip Inclusion   : False
Background subtraction: False
Max L sites      : all
Found 21 transitions
Drawing time_series plots of proteins..... 1/1
Drawing filling plots of proteins..... 1/1
Drawing error_bar plots of proteins..... 1/1
All the results are generated at ./testset/
```

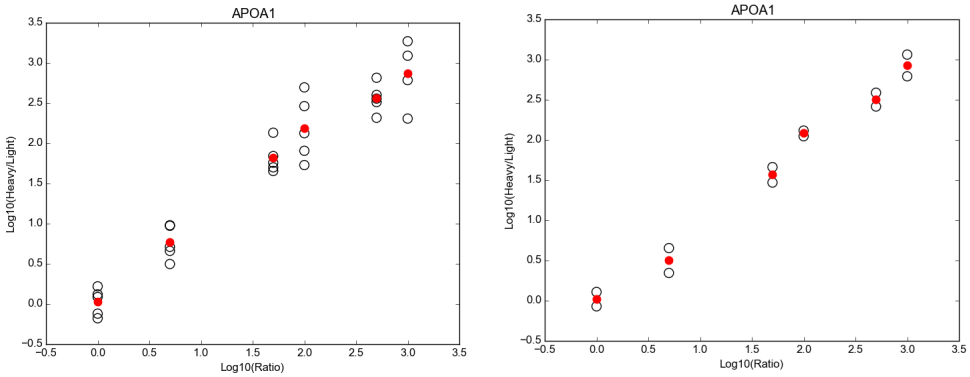
The error plot (before and after the ion-filtering at the step 3)



The filling plot (before and after the ion-filtering at the step 3)




The scatter plot (before and after the ion-filtering at the step 3)



8. **Configuration file for XPIQuant.py (XPIQuant_config.txt)**
    a. All the items should be tab-delimited.
    b. Data directory
        i. The directory path of mzML files and configuration files
    c. Inclusion list
        i. The file path of inclusion list that was used for PRM data generation
        ii. Format
            1. Mass [m/z]
                a. m/z for precursor isolation (will be used for the scan number match)
            2. CS [z]
                a. Charge of the peptide
            3. Start [min]
                a. Starting retention time for the precursor ion isolation
            4. End [min]
                a. Ending retention time for the precursor ion isolation
            5. Sequence
                a. Peptide sequence
            6. Protein
                a. Protein name
        iii. Should be tab-delimited text file
        iv. Example

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Mass [m/z] | CS [z] | Start [min] | End [min] | Sequence | Protein |
| 2 | 434.5543314 | 3 | 11.53 | 15.53 | THLAPYSDELR | APOA1 |
| 3 | 434.8872047 | 3 | 11.64 | 15.64 | LSPLGEEMRDR | APOA1 |
| 4 | 579.3172941 | 2 | 5 | 9 | LEALKENGGAR | APOA1 |
| 5 | 608.3144171 | 2 | 5.54 | 9.54 | ATEHLSTLSEK | APOA1 |
| 6 | 642.2898879 | 2 | 20.3 | 24.3 | WQEEMELYR | APOA1 |
| 7 | | | | | | |

    d. Skip Inclusion
        i. If it is True, skip parsing inclusion list and use existing “XPI_transition.txt” file.
        ii. If you modified “XPI_transition.txt”, set this to True.
    e. MS2 window

i. Maximum mass difference allowed for XPI identification
ii. ΔMass = |theoretical mass – observed mass|

f. Labeling
   i. Labeling information.
   ii. Format
      1. “Labeling name”, “Labeled residue”:”Exact mass shift”
      2. e.g.) For deuterated leucine labeling
         Labeling Light, L:0
         Labeling Heavy, L:3.01883025

g. Enrichment
   i. If it is True, XPI program will calculate Enrichment (e.g. Heavy/(Heavy+Light)).
   ii. If it is False, XPI program will calculate simple ratio described above (e.g. Heavy/Light).

h. Ratio
   i. A ratio formula you want to compute.
   ii. Labeling name should be same to what stated in “Labeling” section.
   iii. “Labeling name 1”/“Labeling name 2” or “Labeling name 2”/“Labeling name 1”.
   iv. e.g.)
      If you named labeling at “Labeling” as Light (for unlabeled ions) and Heavy (for labeled ions), the XPI program will calculate Heavy/Light when “Enrichment” = False and Heavy/(Heavy+Light) when “Enrichment” = True.

i. Max L sites
   i. Maximum number of labeling sites.
   ii. If it is 1, the XPI program will consider only one labeled residue and ignore others for mass shift calculation caused by labeling.
   iii. Set 'all' if you want to consider all the possible mass shifts.

j. Background
   i. If it is True, background signal will be subtracted from the XPI intensity (See section 6. Step 2).
      1. The background signal threshold is the median intensity of XPIs in a MS/MS scan.
      2. An XPI whose ion intensity is less than the background signal threshold will be considered as noise.
      3. XPI program removes noises during the XPI extraction.
   ii. If it is False, XPI program will accept PRM intensity itself.

k. TIC Normalize
    i. Normalize XPI intensity by dividing by TIC (Total Ion Current).
    ii. If it is True, XPI intensity will be divided by TIC,
    iii. If it is False, XPI will accept XPI intensity as it is.
l. Modification
    i. If there is modified amino acid, use this option.
    ii. Format
        1. “Residue name used in peptide sequence”:”Mono isotopic mass”
        2. e.g.) Modification m:131.04048491299
m. Max fragment
    i. Maximum fragment length for quantification
    ii. For example, if it is 5, XPI will generate b1 to b5 and y1 to y5 ions.

## 9. Configuration file for XPIPeak.py (XPIPeak_config.txt)

a. XPIPeak narrows down the retention time to identify the correct peaks to calculate ratio or enrichment. First, XPIPeak selects commonly found peaks defined by the location (RT and scan no. of the M0). XPIPeak considers the number of commonly found peaks within the RT window and the rank of peak intensity. Next, XPIPeak applies the refined retention time, defined by the standard label (Section 8.c, ie., Light in the test set), to the labeled isotope (ie., Heavy in the test set).

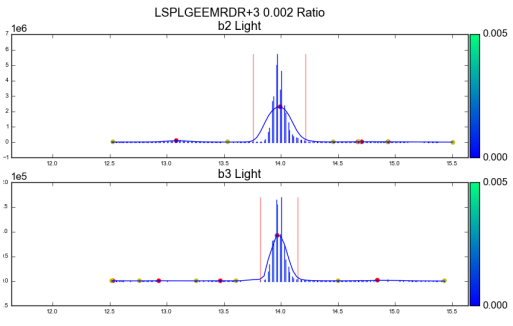
b. LOWESS fraction
   i. XPI uses LOWESS for the curve smoothing.
   ii. To get more information about LOWESS implemented to XPI, see the below website
      1. http://statsmodels.sourceforge.net/devel/generated/statsmodels.nonparametric.smoothers_lowess.lowess.html
   iii. Between 0 and 1. The fraction of the PRM peak used when estimating each PRM ion intensities.

c. LOWESS weight
   i. The number of residual-based reweightings to perform.

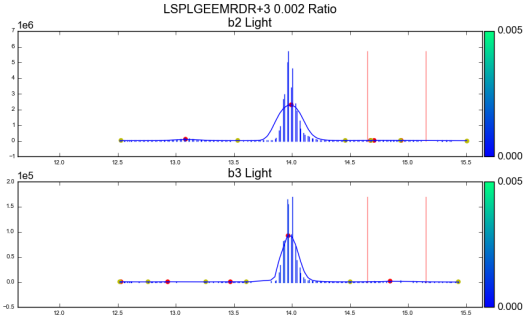
d. Standard label
   i. Unlabeled ion's label name (ie., Light or M0)
   ii. Standard label should be detected stronger than other labels.
   iii. Label name should be same to what was used in XPIQuant configuration file (Section **7.f**) and XPIViz configuration file (Section **7.f**).

e. RT window
   i. Retention time window to find commonly detected peaks in standard label (ie. Light – M0 ions for all target peptide fragments). XPIPeak compares local minimum (Section 6.Step2) retention time (peak RT) to find commonly found peaks. The commonly found peaks are defined by retention time so XPI considers peaks (Section 6.Step2) within the RT window.

RT window = 0.05

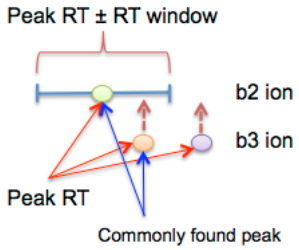



RT window = 0



ii. Above example (left panel) shows that XPI successfully selected b2 and b3 ions (within red lines). However, right panel misses correct peaks because peak retention times of b2 and b3 ions are not within the RT window = 0.

iii. If RT window = 5, XPIPeak will consider the peak RT – 5 and the peak RT + 5.



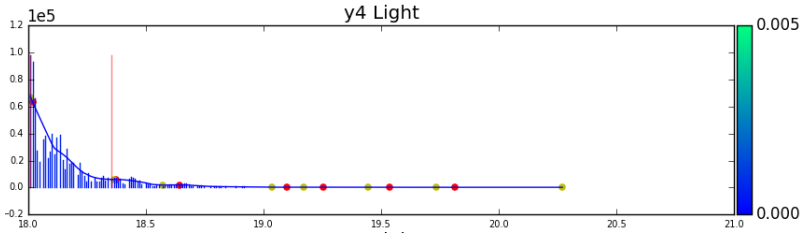

iv. If RT window is large, XPI could include noise peaks.

f. Borderline limit

i. If there are partial peaks located at either borderline RT, XPI recognizes the peak if it has more than the "Borderline limit" number of extracted PRM peak intensities.

ii. The required number of ion intensities to detect incomplete borderline peaks

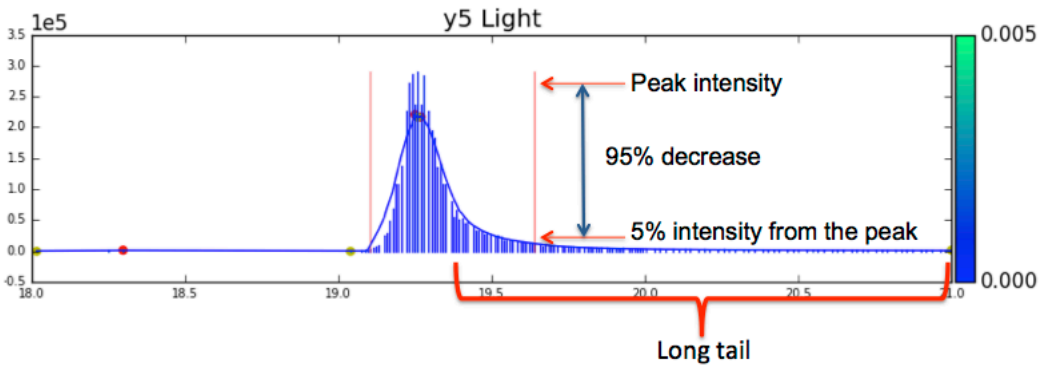
iii. Below peak detection plot shows that XPIPeak detected the partially detected peak (between red lines) using this option.




g. Long tail limit

i. If a peak has a long tail, XPIPeak cuts its tail by the ratio to its top intensity. For example, if Long tail limit = 0.05 (5% intensity to highest intensity of the peak), XPIPeak will set the RT limit to where XPI whose intensity is less than 5% to the highest intensity of the peak.

ii. Peaks spanned longer than 0.5 min. will be considered for long tail removal.

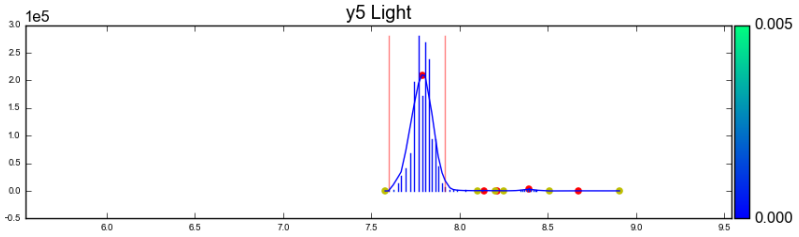


h. Show all peaks

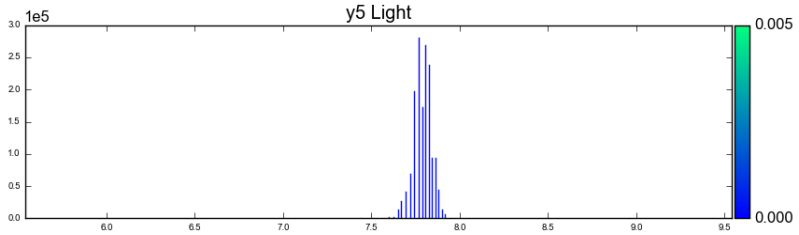
   i. If it is True, Peak profile will show LOWESS line and local minima and maxima as well as non-specific peaks

   ii. If it is False, XPI will show the filtered peaks only.

Show all peaks = True




Show all peaks = False

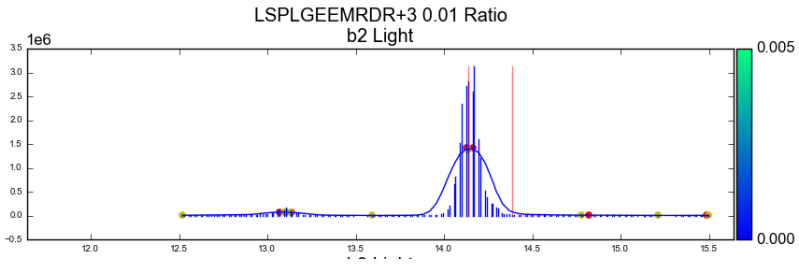



i. Merge close peaks

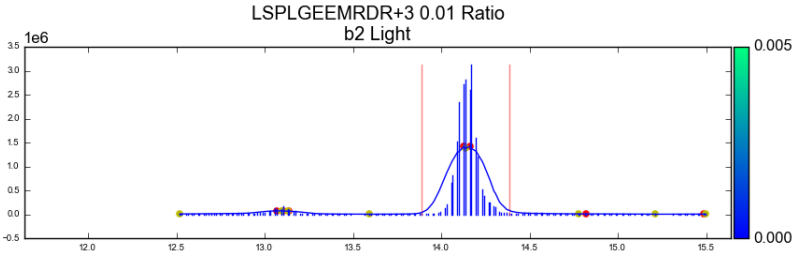
   i. If it is True, XPI will merge peaks closely located within RT window (Section 8.e).

   ii. If it is False, XPI will not merge peaks

Merge close peaks = False




Merge close peaks = True



**10. Configuration file for XPIViz.py (XPIViz_config.txt)**

a. XPIViz_config.txt should be tab-delimited plain text file.

b. Data

   i. “Data” is to get sample information

   ii. Format

      1. RAW data (file name)

         a. mzML file path that should be same to file names in “PRM_Run_Name” column of “XPI_output_4check.txt”.

      2. Measure

         a. This should be number, for example hours in time series data (0, 0.5, 2, 4 and 6) or mixing ratio (1, 0.2, 0.02 and 0.01).

         b. This number will be used to calculate Pearson’s r and plot drawing.

      3. Unit

         a. This is for x-axis label for plot drawing.

         b. If numbers in “Measure” is time, you can put “hour” or “minute”. If it is mixing ratio, you can put “Ratio”.

      4. Ratio:Label_1/Label_2

         a. “Ratio:” + the ratio formula that was used in “XPIQuant_config.txt”.

         b. e.g.)

            If your “Ratio” formula = Heavy/Light and “Enrichment” = False at “XPIQuant_config.txt” (Section 7.g and 7.h), your Ratio label will be “Ratio:Heavy/Light”

            If your “Ratio” formula = Heavy/(Heavy+Light) and “Enrichment” = True at “XPIQuant_config.txt” (Section 7.g and 7.h), your Ratio label will be “Ratio:Heavy/(Heavy+Light)”

c. Pick Method

   i. Intensity-picking method for quantification

   ii. If you want to follow the traditional XIC, use SUM method

   iii. Choose one of these

   iv. MAX

      1. One maximum PRM peak within the retention time window

   v. TOP3

      1. Sum of top 3 PRM peaks within the retention time window

   vi. SUM

1. Sum of all the PRM peaks within the retention time window

vii. AVG
1. Average of the PRM peaks within the retention time window

viii. MED
1. Median of the PRM peaks within the retention time window

ix. QMAX
1. One maximum PRM peak within the retention time window after removing out 1st and 4th quartile.

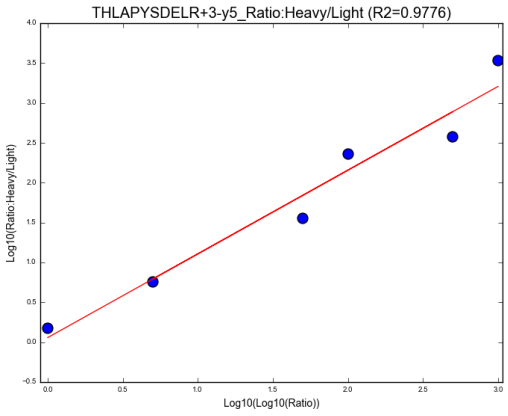
x. QSUM
1. Sum of all the PRM peaks within the retention time window after removing out 1st and 4th quartile.

xi. QTOP3
1. Sum of top 3 PRM peaks within the retention time window after removing out 1st and 4th quartile.

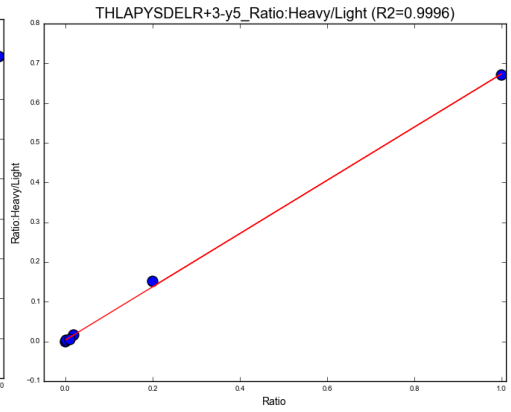
xii. QAVG
1. Average of the PRM peaks within the retention time window after removing out 1st and 4th quartile.

d. Log10
   i. For standard curve and protein or peptide scatter plots
   ii. If it is True, get log10 of both y-axis (the observed ratio or enrichment) and x-axis (the measured time or the intended mixing ratio)
   iii. If it is False, XPI will not get log10.

Log10 = True



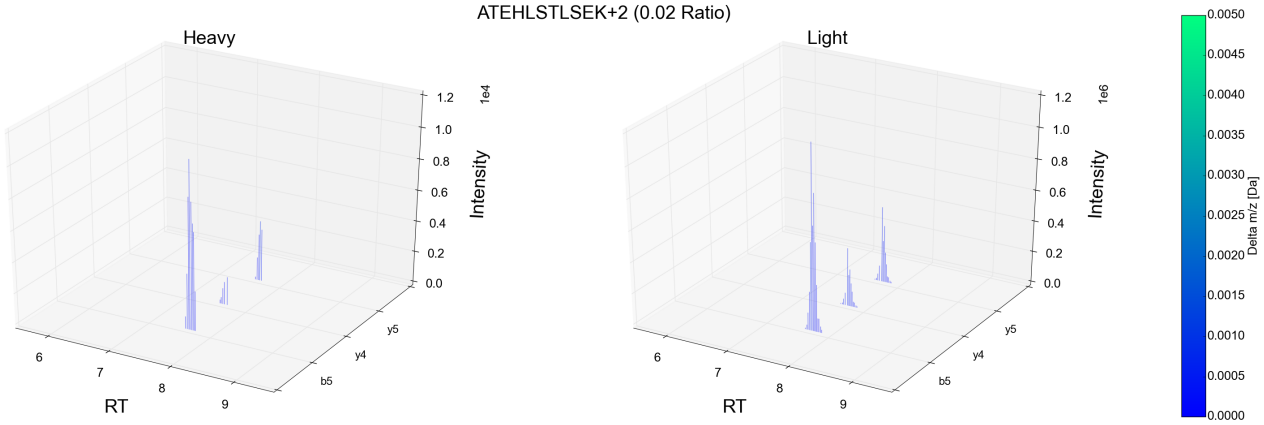

Log10 = False



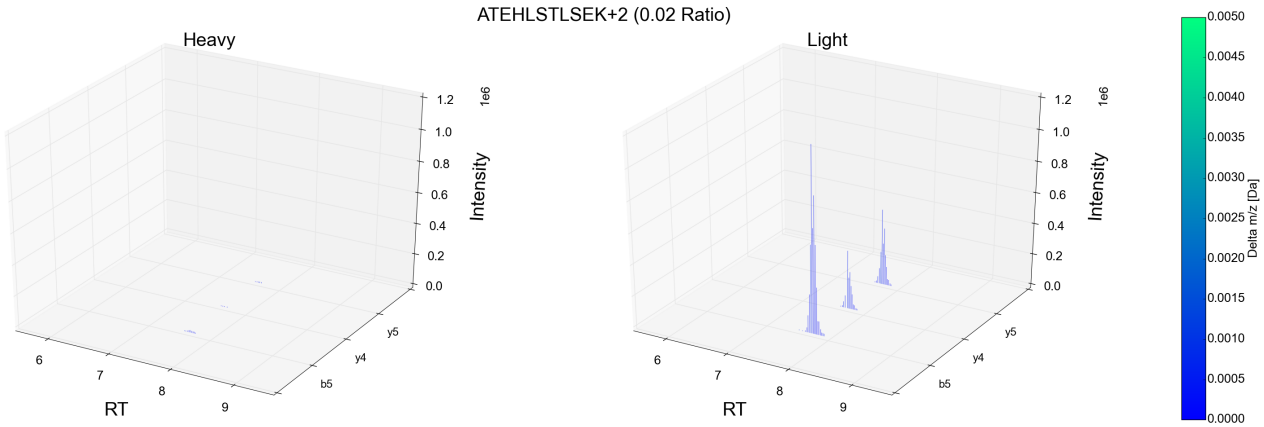

e. Make Zs same
   i. Only for 3D mass profile plot

ii. If it is True, XPI will draw 3D mass profile with same scale z-axis (peak intensity) in different labels

Make Zs same = False



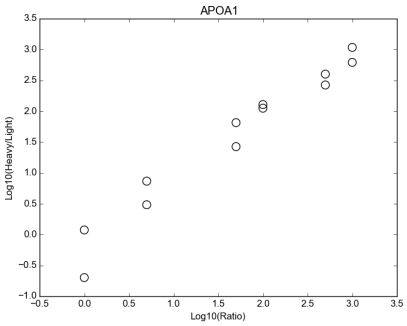

Make Zs same = True



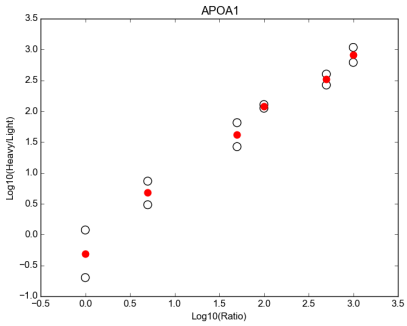

f. Median trend line

i. For protein scatter plot, if it is True, median trend line will be shown.

Median trend line = False




Median trend line = True

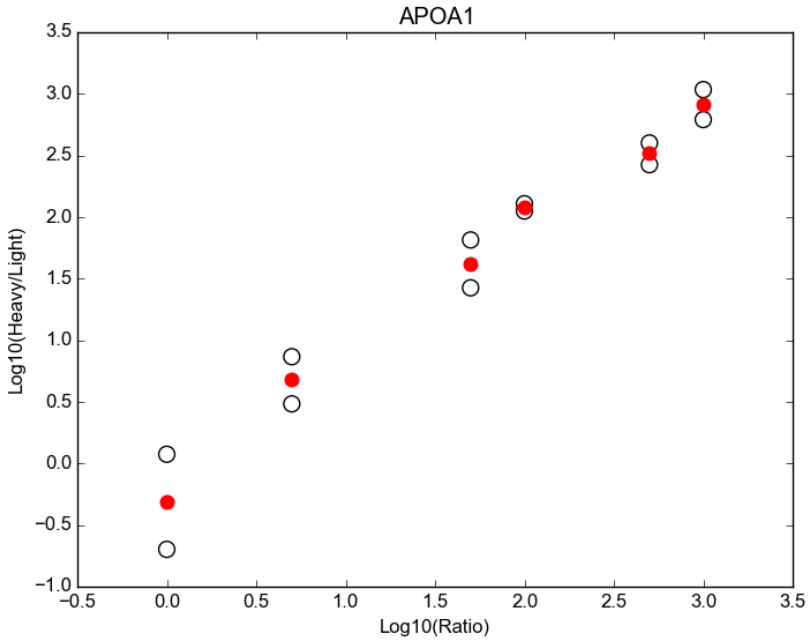


g. Percent ratio

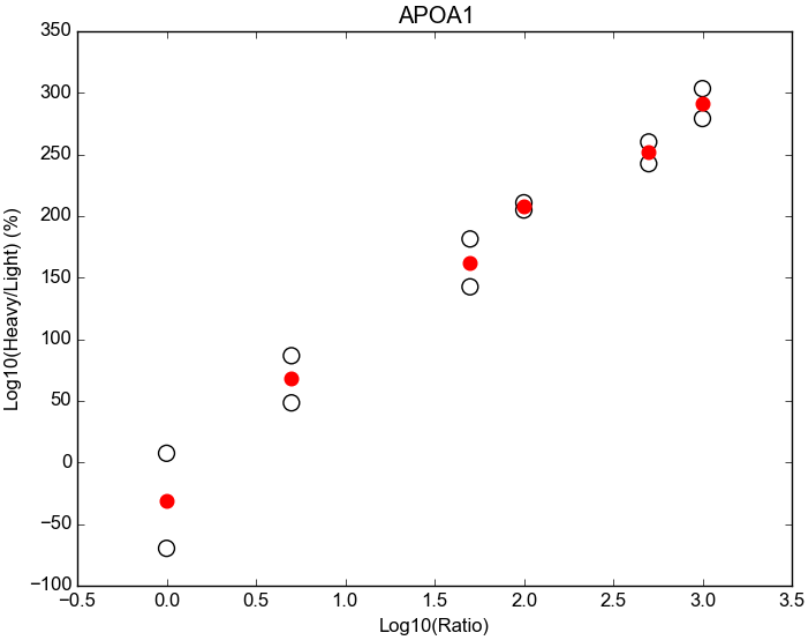
i. This option is for calculation of percentage enrichment, so if you want to calculate % enrichment, set this to “True”.

ii. If it is True, XPI will multiply 100 to enrichment or ratio.

Percent ratio = False




Percent ratio = True

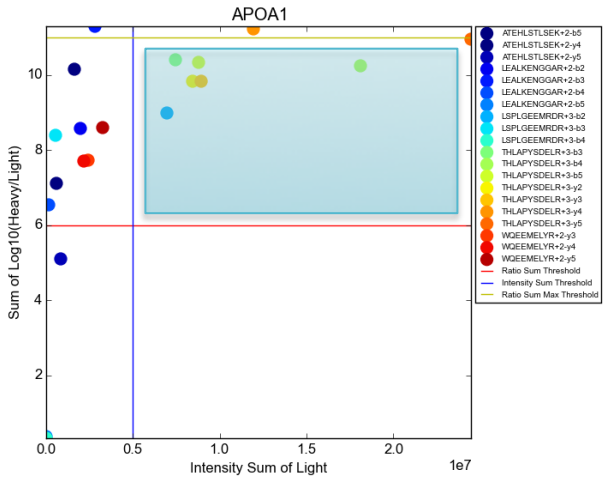


h. DPI
    i. Set the quality of XPI visualizations.
    ii. Recommend setting DPI to 100 for fast processing, but if you need publication quality, set DPI to 300.

i. Standard label
    i. Reference label that is usually more intense than other labels.
    ii. Label name should be same to what was used in “XPIQuant_config.txt” (Section 7.e).

j. Filtering (See also, Section 6.Step3)
    i. The thresholds for the ion filtering by enrichment or ratio level and m0 ion intensity.
    ii. Min_Ratio_Sum
        1. If it is bigger than 0, XPI will filter out fragment ions whose summed enrichment or ratio is less than Min_Ratio_Sum.
    iii. Max_Ratio_Sum
        1. If it is bigger than 0, XPI will filter out fragment ions whose summed enrichment or ratio is bigger than Max_Ratio_Sum.
        2. If Max_Ratio_Sum = 0, XPI doesn’t exclude any ions by Max_Ratio_Sum threshold.
    iv. Min_Intensity_Sum
        1. If it is bigger than 0, XPI will filter out fragment ions whose summed standard label ion intensity is less than Min_Intensity_Sum.
    v. If you set “Percent Ratio” as True, Min_Ratio_sum and Max_Ratio_sum should be multiplied by 100 to values in the intensity_vs_ratio plot.
    vi. Format (tab-delimited)

| | Filtering | Protein_Name | Min_Ratio_Sum | Max_Ratio_Sum | Min_Intensity_Sum |
|---|---|---|---|---|---|
| vii. e.g.) | Filtering | APOA1 | 6 | 11 | 5000000 |

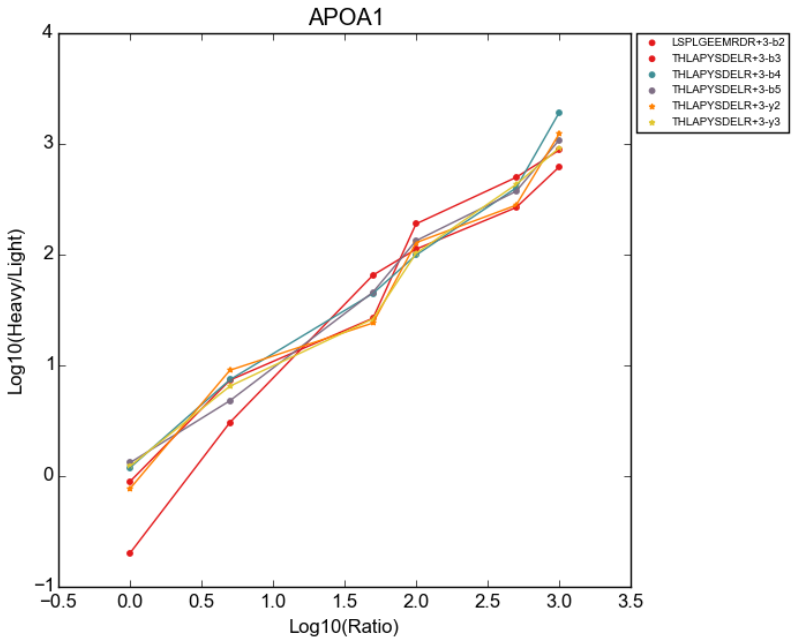


k. Protein Color

i. If it is True, protein scatter plot will be colored by fragment ions.

ii. If it is False, protein scatter plot will be black circles (median of fragment ions of a peptide). Red circle is average of peptides.

Protein Color = True




Protein Color = False