

# Using/ Calling DrawAlignR

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The visualization tool can be called via two methods, either via installing the repository using devtools github package installer or by calling shiny::runGitHub.

## Calling tool via Installing DrawAlignR

```
#### Install devtools if not already installed.  
## install.packages("devtools")  
## Install DrawAlignR from Roestlab Github Repository  
devtools::install_github("Roestlab/DrawAlignR")  
## To run the tool  
DrawAlignR::runDrawAlignR()
```

OR

## Calling tool via Github repo

```
#### Install shiny  
## install.packages("shiny")  
shiny::runGitHub(repo = "Roestlab/DrawAlignR/", username = "Roestlab",  
  subdir = "inst/shiny-script")
```

# User Interface

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There are three major tabs in the left side pannel:

- **General Settings**
  - These are general settings for uploading chromatogram files (.mzML or .sqMass), library assay files (.ppq), OpenSwathWorkflow results file (.osw). The user can also set the working directory that contains sub-directroys for osw and mzml files.
  - The user selects the peptide and charge state to visualize.
  - The user selects how many plots to show for each chromatogram run file supplied.
  - The user can select the alignment option to perform an alignment for the selected peptide.
  - The user can visualize the reference plot, experiment plot and the experiment aligned plot.
- **Alignment Settings**
  - The user can change various alignment parameters
- **Plot Settings**
  - The user can change various plot visualization settings

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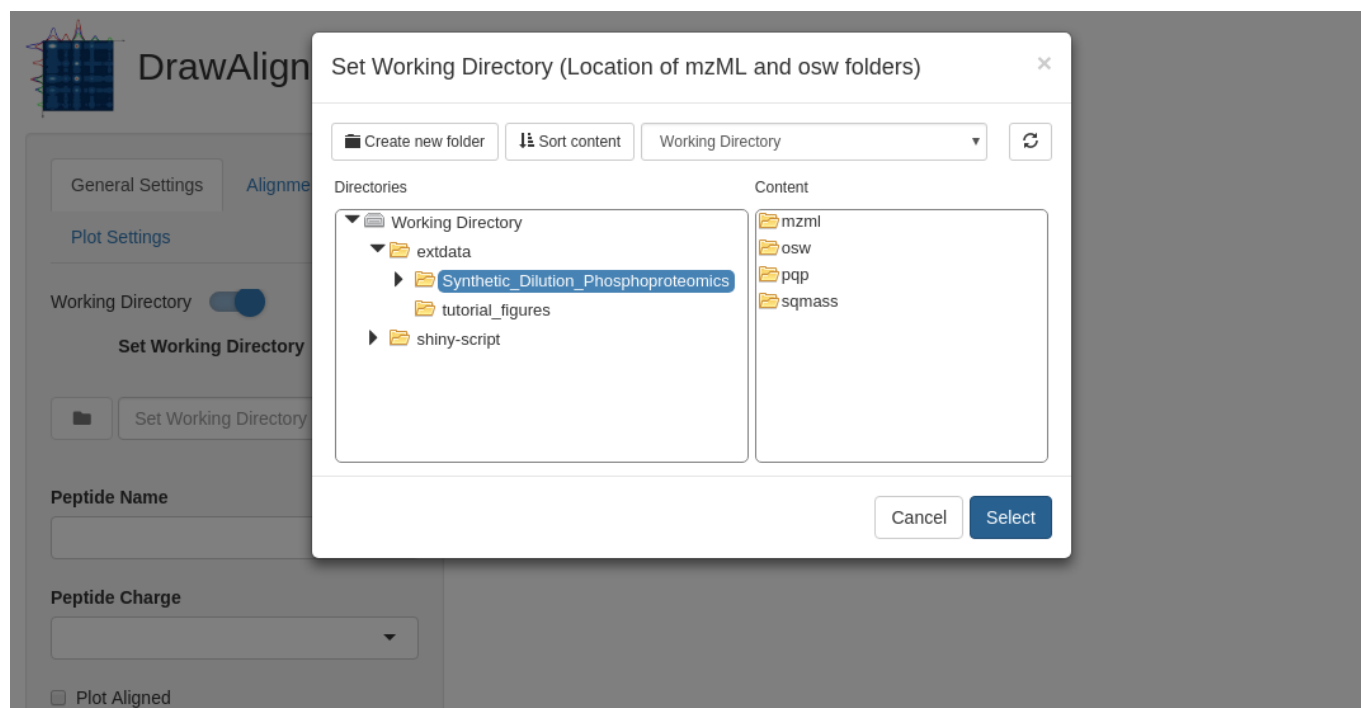
# Tutorial for Performing Alignment

## Set Working Directory

Use the Set Working Directory button to set the working directory that contains an mzml folder with .chrom.mzml files and an osw file with a merged.osw file, and optionally a library file in the .ppp format. Or you can directly enter the path to the working directory using the input textbox area. DrawAlignR will parse the working directory and look for `../osw`, `../mzml` ( or `../sqmass` ), and `../ppp` subfolders containing their corresponding file types. You can alternatively toggle the **Working Directory** button to enter chromatogram, library and osw files separately. **Note:** For alignment it is preferred if you enter a working directory.

An example Working Directory should be ideally structured and named as below:

```
/Project_Working_Directory
|__ /mzml
|   |__ /run0.chrom.mzML
|   |__ /run1.chrom.mzML
|__ /osw
|   |__ /merged.osw
|__ /ppp
|   |__ /assay_library.pqp
|__ /sqmass(Optional, either mzml or sqmass format is acceptable)
|   |__ /run1.chrom.sqMass
|   |__ /run1.chrom.sqMass
```



## Select a Peptide to visualize Chromatogram alignment

Choose which peptide you want to visualize using the Peptide dropdown list. The dropdown list is searchable, so you can easily search for a specific peptide to visualize. The list of peptides is extracted from either the input library file if available, or an osw file if available.

The screenshot displays the DrawAlignR web application interface. The browser address bar shows the URL `http://127.0.0.1:5681`. The application title is `/media/justincsing/ExtraDrive1/Documents2/Roest_Lab/Github/DrawAlignR/inst/shiny-script - Shiny`. The application logo is **DrawAlignR** Ver: 0.1.0.

**General Settings**

- Alignment Settings** (selected)
- Plot Settings**
- Working Directory**: `/media/justincsing/ExtraDrive1/Do` (with a "Set Working Directory" button)
- ☒ **Use mzml** ☐ **Use sqmass**
- Peptide Name**: A dropdown menu showing a list of peptides, including `ANS(UniMod:21)SPTTNIDHLK(UniMod:21)`, `ANSS(UniMod:21)PTTNIDHLK(UniMod:21)`, `ANSSPT(UniMod:21)TNIDHLK(UniMod:21)`, `ES(UniMod:21)TAEPDLSR(UniMod:267)`, `EST(UniMod:21)AEPDLSR(UniMod:267)`, `ES(UniMod:21)T(UniMod:21)AEPDLSR`, `ESTAEPDS(UniMod:21)LSR(UniMod:267)`, and `KDS(UniMod:21)NTNVLK(UniMod:250)`.
- ☐ **Plot Aligned**
- Select Reference Run for Alignment**: `chludwig_K150309_001b_SW_1_64`
- Experiment(s) to Align**: A list of experiments including `chludwig_K150309_002b_SW_1_32`, `chludwig_K150309_003b_SW_1_24`, `chludwig_K150309_004b_SW_1_16`, `chludwig_K150309_005b_SW_1_12`, `chludwig_K150309_006b_SW_1_8`, `chludwig_K150309_007b_SW_1_6`, `chludwig_K150309_008_SW_1_4`, `chludwig_K150309_009_SW_1_3`, `chludwig_K150309_010_SW_1_2`, `chludwig_K150309_011_SW_1_1point5`, `chludwig_K150309_012_SW_1_1`, and `chludwig_K150309_013_SW_0`.
- ☐ **Show Original Peak Annotation**

**Chromatogram Plots**

The right side of the interface shows three empty chromatogram plots, each with a title bar and a toolbar. The titles are:

- Run: 001b\_1\_64 | Precursor: | Peptide: | Charge: | m/z:
- Run: 002b\_1\_32 | Precursor: | Peptide: | Charge: | m/z:
- Run: 003b\_1\_24 | Precursor: | Peptide: | Charge: | m/z:

Each plot has a y-axis from 0 to 1 and an x-axis from 0 to 1.

# Select a Charge State for Selected Peptide

Choose which charge state to visualize for the selcted peptide.

General Settings

Alignment Settings

Plot Settings

Working Directory

Set Working Directory

/media/justincsing/ExtraDrive1/Do

Use mzml

Use sqmass

Peptide Name

EST(UniMod:21)AEPDSLRS(UniMod:267)

Peptide Charge

2

2:3

2

3

Run 9

Run 10

Run 11

Run 12

Run 13

Plot Aligned

Select Reference Run for Alignment

chludwig\_K150309\_001b\_SW\_1\_64

Experiment(s) to Align

chludwig\_K150309\_002b\_SW\_1\_32

chludwig\_K150309\_003b\_SW\_1\_24

chludwig\_K150309\_004b\_SW\_1\_16

chludwig\_K150309\_005b\_SW\_1\_12

chludwig\_K150309\_006b\_SW\_1\_8

chludwig\_K150309\_007b\_SW\_1\_6

chludwig\_K150309\_008\_SW\_1\_4

chludwig\_K150309\_009\_SW\_1\_3

chludwig\_K150309\_010\_SW\_1\_2

chludwig\_K150309\_011\_SW\_1\_1point5

chludwig\_K150309\_012\_SW\_1\_1

chludwig\_K150309\_013\_SW\_0

Show Original Peak Annotation

EST(UniMod:21)AEPDSLRS(UniMod:267)

Run: 001b\_1\_64 | m/z: 641.2653

Int

RT

Detecting

1068\_1+\_y6\_684.3551

1069\_1+\_y4\_472.2753

1070\_1+\_y7\_813.3976

1071\_1+\_y8\_884.4348

1072\_1+\_y5\_587.3023

1073\_1+\_y1\_185.1272

EST(UniMod:21)AEPDSLRS(UniMod:267)

Run: 002b\_1\_32 | m/z: 641.2653

Int

RT

Detecting

1068\_1+\_y6\_684.3551

1069\_1+\_y4\_472.2753

1070\_1+\_y7\_813.3976

1071\_1+\_y8\_884.4348

1072\_1+\_y5\_587.3023

1073\_1+\_y1\_185.1272

EST(UniMod:21)AEPDSLRS(UniMod:267)

Run: 003b\_1\_24 | m/z: 641.2653

Int

RT

Detecting

1068\_1+\_y6\_684.3551

1069\_1+\_y4\_472.2753

1070\_1+\_y7\_813.3976

1071\_1+\_y8\_884.4348

1072\_1+\_y5\_587.3023

1073\_1+\_y1\_185.1272

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# Select Reference Run

Choose which chromatogram file to use as the reference run. These are set through the searchable dropdown lists, which extracts the filenames from the supplied chromatogram files without the .chrom.mzml extension

General Settings

Alignment Settings

Plot Settings

Working Directory

Set Working Directory

/media/justincsing/ExtraDrive1/Do

☒ Use mzml

☐ Use sqmass

Peptide Name

EST(UniMod:21)AEPDSLRSR(UniMod:267)

Peptide Charge

2

Choose Runs to Display:

☒ Run 1

☒ Run 2

☒ Run 3

☒ Run 4

☒ Run 5

☒ Run 6

☒ Run 7

☒ Run 8

☒ Run 9

☒ Run 10

☒ Run 11

☒ Run 12

☒ Run 13

☐ Plot Aligned

Select Reference Run for Alignment

ch|

chludwig\_K150309\_013\_SW\_0

chludwig\_K150309\_008\_SW\_1\_4

chludwig\_K150309\_009\_SW\_1\_3

chludwig\_K150309\_010\_SW\_1\_2

chludwig\_K150309\_012\_SW\_1\_1

chludwig\_K150309\_006b\_SW\_1\_8

chludwig\_K150309\_007b\_SW\_1\_6

chludwig\_K150309\_001b\_SW\_1\_64

chludwig\_K150309\_007b\_SW\_1\_6

chludwig\_K150309\_008\_SW\_1\_4

chludwig\_K150309\_009\_SW\_1\_3

chludwig\_K150309\_010\_SW\_1\_2

chludwig\_K150309\_011\_SW\_1\_1point5

chludwig\_K150309\_012\_SW\_1\_1

chludwig\_K150309\_013\_SW\_0

☐ Show Original Peak Annotation

EST(UniMod:21)AEPDSLRSR(UniMod:267)

Run: 001b\_1\_64 | m/z: 641.2653

Detecting

1068\_1+\_y6\_684.3551

1069\_1+\_y4\_472.2753

1070\_1+\_y7\_813.3976

1071\_1+\_y8\_884.4348

1072\_1+\_y5\_587.3023

1073\_1+\_y1\_185.1272

EST(UniMod:21)AEPDSLRSR(UniMod:267)

Run: 002b\_1\_32 | m/z: 641.2653

Detecting

1068\_1+\_y6\_684.3551

1069\_1+\_y4\_472.2753

1070\_1+\_y7\_813.3976

1071\_1+\_y8\_884.4348

1072\_1+\_y5\_587.3023

1073\_1+\_y1\_185.1272

EST(UniMod:21)AEPDSLRSR(UniMod:267)

Run: 003b\_1\_24 | m/z: 641.2653

Detecting

1068\_1+\_y6\_684.3551

1069\_1+\_y4\_472.2753

1070\_1+\_y7\_813.3976

1071\_1+\_y8\_884.4348

1072\_1+\_y5\_587.3023

1073\_1+\_y1\_185.1272

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## Select Experiment Run(s)

Choose which chromatogram file to use as the experiment run. These are set through the searchable dropdown lists, which extracts the filenames from the supplied chromatogram files without the .chrom.mzml extension

Shiny interface for DrawAlignR (Ver: 0.1.0) showing settings and three chromatogram plots.

**General Settings**

- Working Directory: ☐ ☒ ?
- Set Working Directory:
- ☒ Use mzml ☐ Use sqmass
- Peptide Name:
- Peptide Charge:
- Choose Runs to Display:
  - ☒ Run 1 ☒ Run 2 ☒ Run 3 ☒ Run 4
  - ☒ Run 5 ☒ Run 6 ☒ Run 7 ☒ Run 8
  - ☒ Run 9 ☒ Run 10 ☒ Run 11
  - ☒ Run 12 ☒ Run 13
- ☐ Plot Aligned
- Select Reference Run for Alignment:
- Experiment(s) to Align:
  - chludwig\_K150309\_001b\_SW\_1\_64
  - chludwig\_K150309\_002b\_SW\_1\_32
  - chludwig\_K150309\_003b\_SW\_1\_24
  - chludwig\_K150309\_004b\_SW\_1\_16
  - chludwig\_K150309\_005b\_SW\_1\_12
  - chludwig\_K150309\_006b\_SW\_1\_8
  - chludwig\_K150309\_007b\_SW\_1\_6
  - chludwig\_K150309\_008\_SW\_1\_4
  - chludwig\_K150309\_009\_SW\_1\_3
  - chludwig\_K150309\_010\_SW\_1\_2
  - chludwig\_K150309\_011\_SW\_1\_1point5
  - chludwig\_K150309\_012\_SW\_1\_1 |
- ☐ Show Original Peak Annotation

**Chromatogram Plots (Int vs RT):**

EST(UniMod:21)AEPDSLRSR(UniMod:267) Run: 001b\_1\_64 | m/z: 641.2653

EST(UniMod:21)AEPDSLRSR(UniMod:267) Run: 002b\_1\_32 | m/z: 641.2653

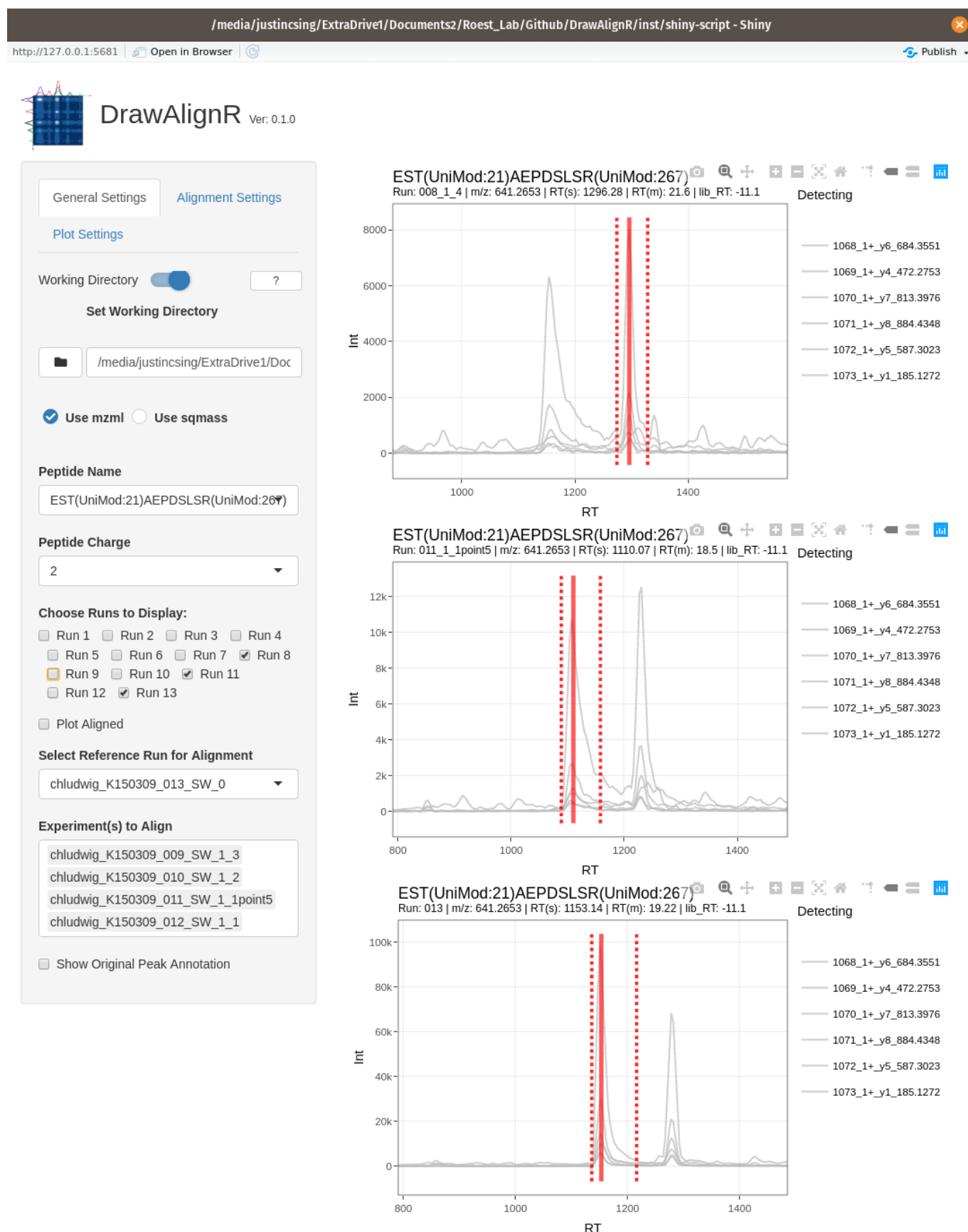
EST(UniMod:21)AEPDSLRSR(UniMod:267) Run: 003b\_1\_24 | m/z: 641.2653

Detecting

- 1068\_1+\_y6\_684.3551
- 1069\_1+\_y4\_472.2753
- 1070\_1+\_y7\_813.3976
- 1071\_1+\_y8\_884.4348
- 1072\_1+\_y5\_587.3023
- 1073\_1+\_y1\_185.1272

## Select Which Chromatogram Runs to Display

Choose which chromatogram runs to display by checking or unchecking the Run **n** checkbox.





## Select Plot Align checkbox to perform alignment

Check the Plot Aligned checkbox to perform the alignment of the two runs for the selected peptide You can also use the

- Show Original Peak Annotation to show where the original peak was annotated before alignment.

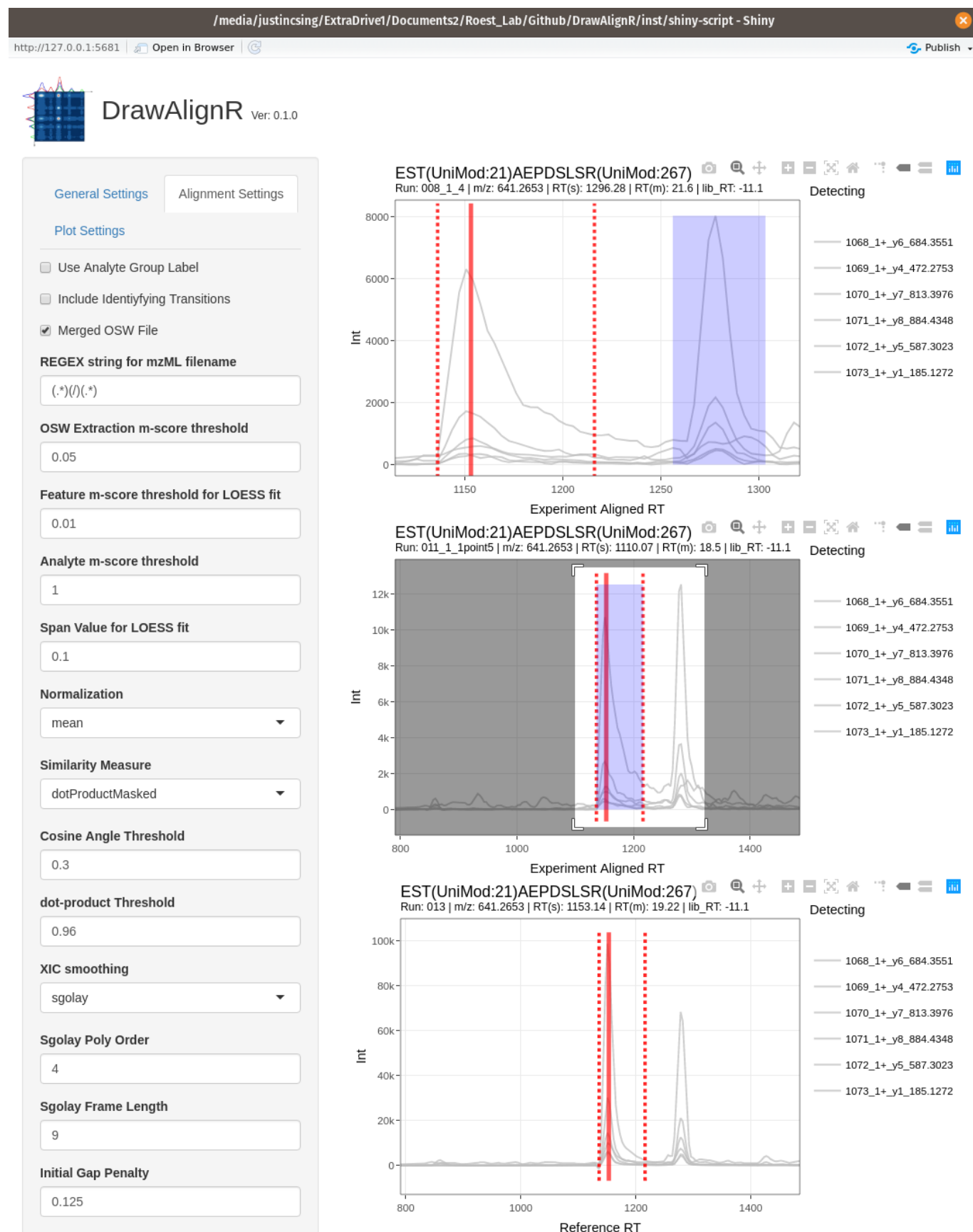


# Change Alignment Parameters

Select the Alignment settings tab to change various alignment settings

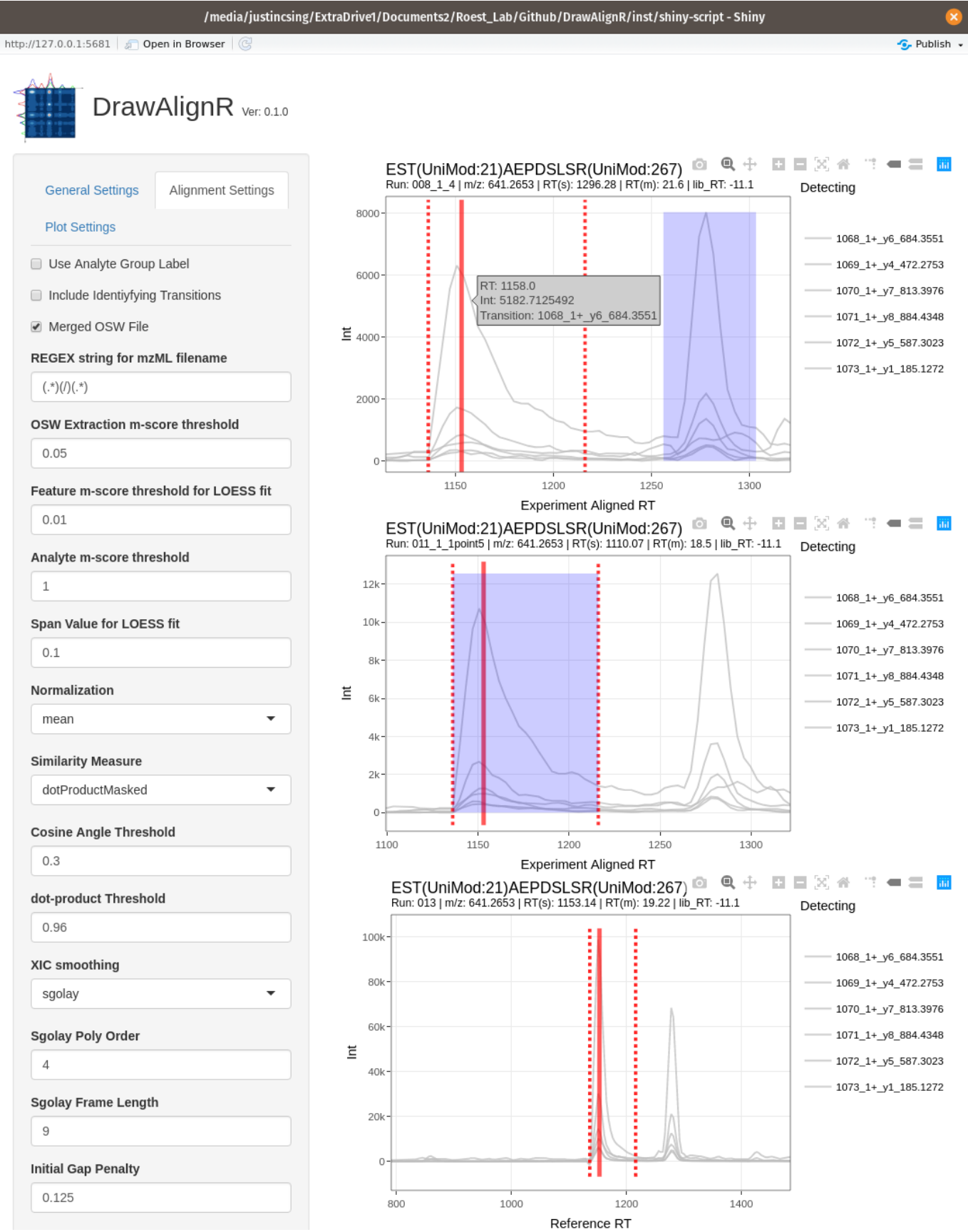


## Zoom into each chromatogram for further inspection



# Use the Hover-tooltip

You can hover over the chromatogram traces to see information such as Retention time and Intensity



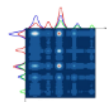
# Visualizing Chromatograms

The user can also just visualize the chromatograms alone without performing alignment to visually inspect each trace. If the user has an IPF dataset, they can visualize site-determining ions (unique identifying transitions) of the modified peptide.



## Precursor, Detecting, Identifying

The user can choose to display the precursor trace, or the 6 detecting traces, or the unique identifying traces. The precursor trace is displayed in **black**, the detecting traces are displayed in a **light gray** and the unique identifying transitions are **colored**



DrawAlignR Ver: 0.1.0

General Settings
Alignment Settings

Plot Settings

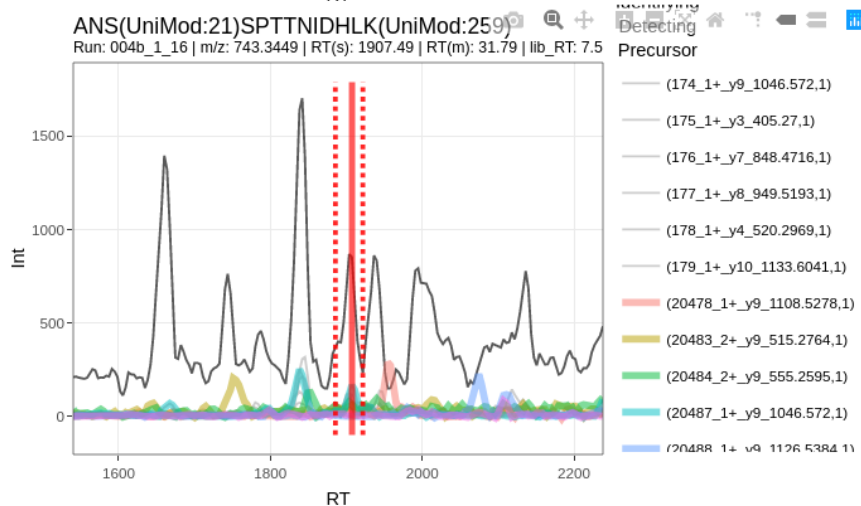
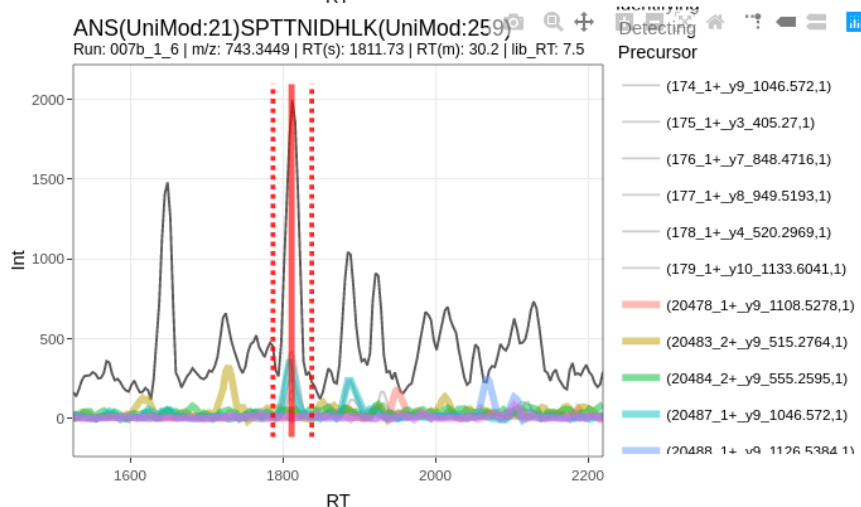
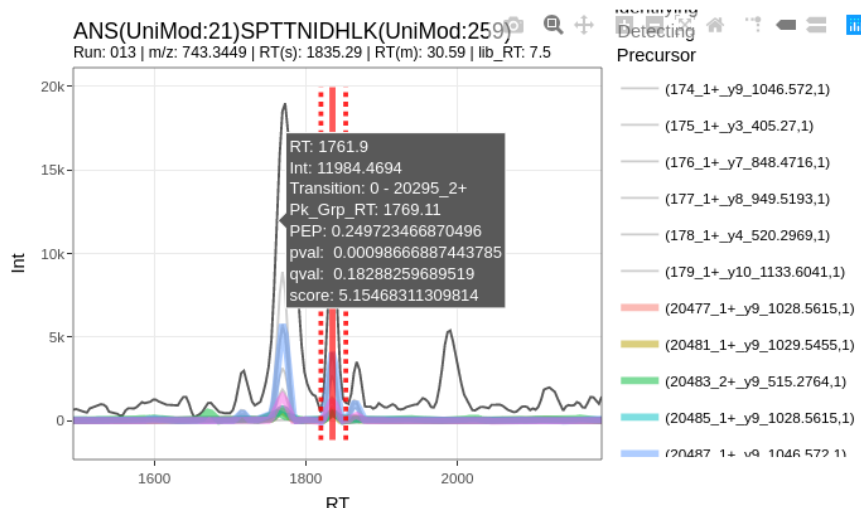
☒ Plot Precursor Trace  
☒ Plot Detecting Traces  
☒ Plot Unique Identifying Traces

Show n Identifying Traces

identifying y-ions

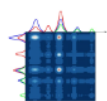
identifying b-ions

☒ Show Transition Scores (hover tooltip)  
☐ Show All Peak-Groups



## Displaying Transition Scores

The user can hover of the traces to display the transition scores such as the transitions posterior error probability, q-value and score.



DrawAlignR Ver: 0.1.0

General Settings
Alignment Settings

Plot Settings

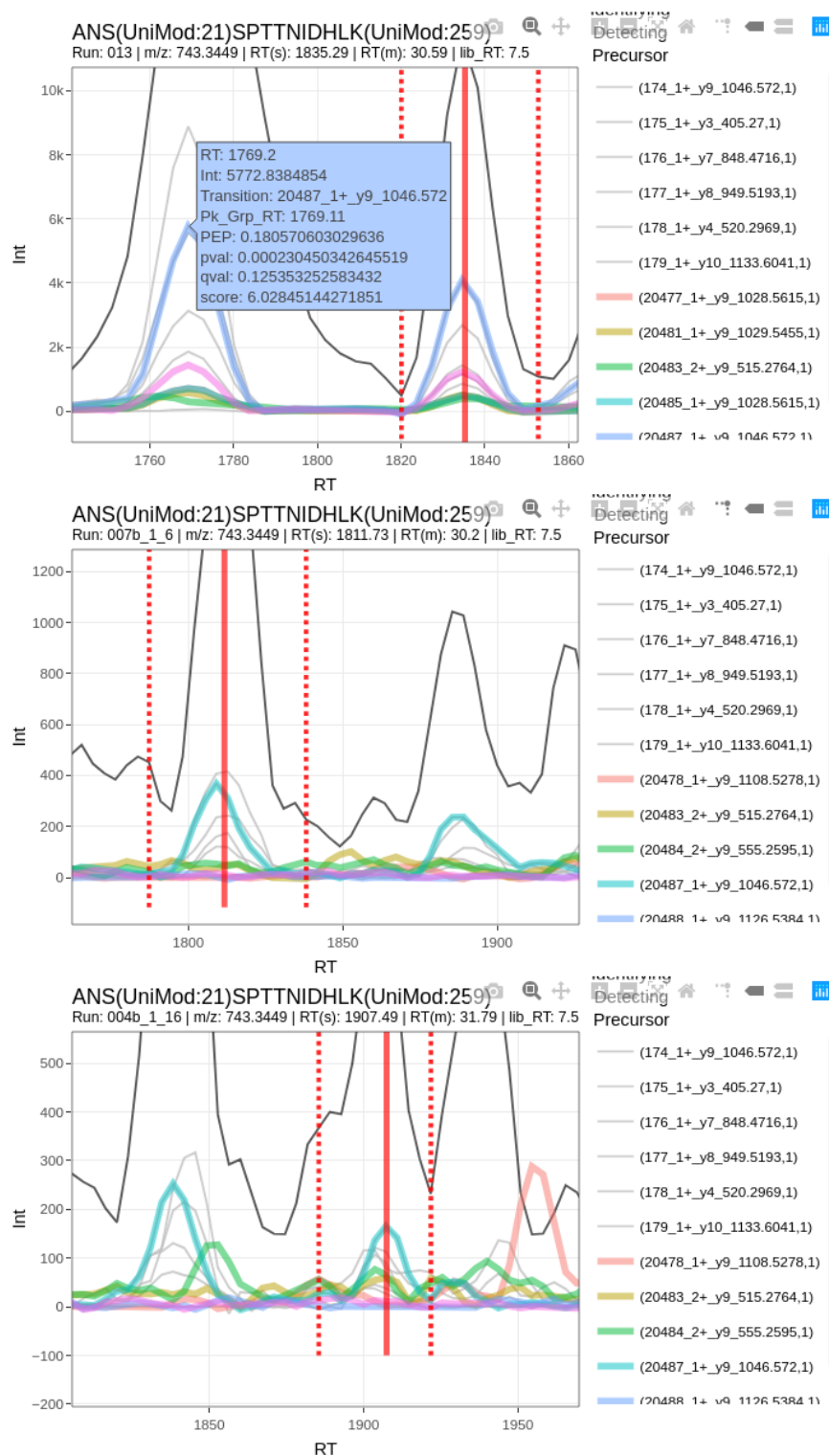
☒ Plot Precursor Trace  
☒ Plot Detecting Traces  
☒ Plot Unique Identifying Traces

Show n Identifying Traces

identifying y-ions

identifying b-ions

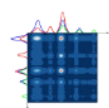
☒ Show Transition Scores (hover tooltip)  
☐ Show All Peak-Groups





## Displaying all Peak-Group Ranks

The user can choose to display the other potential peak-group ranks found by OpenSWATH



DrawAlignR Ver: 0.1.0

General Settings
Alignment Settings

Plot Settings

☒ Plot Precursor Trace

☒ Plot Detecting Traces

☒ Plot Unique Identifying Traces

Show n Identifying Traces

6

identifying y-ions

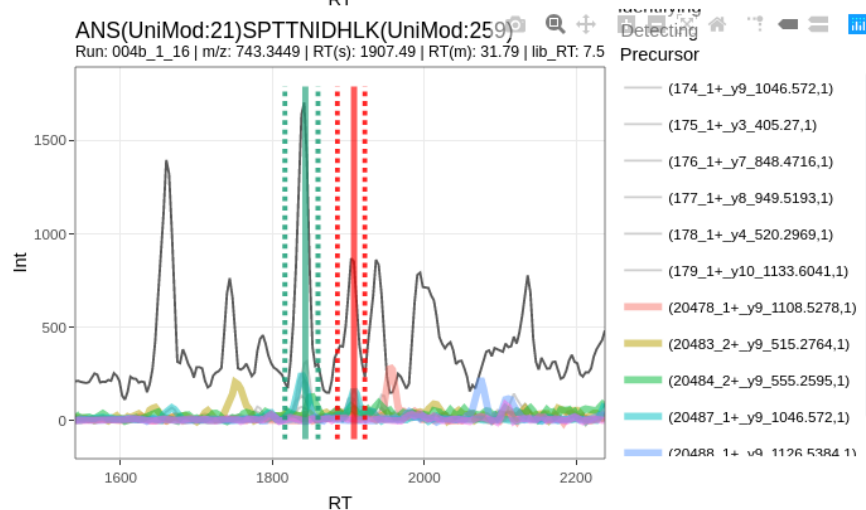
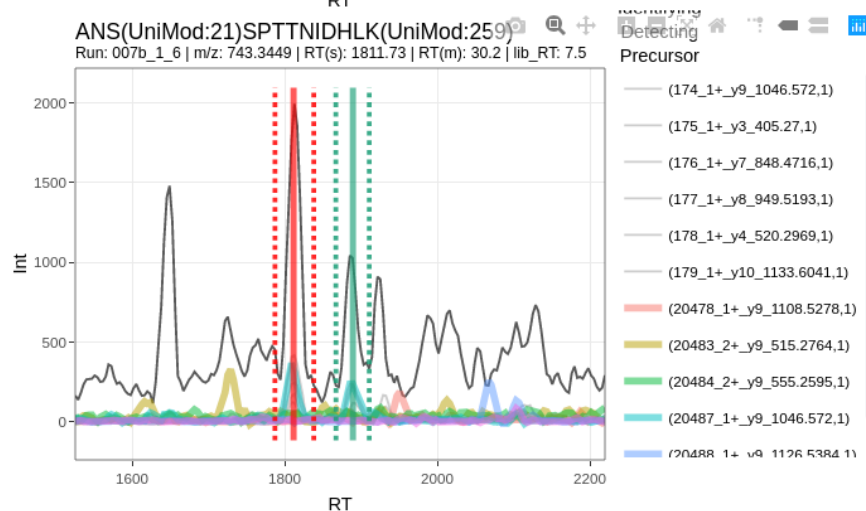
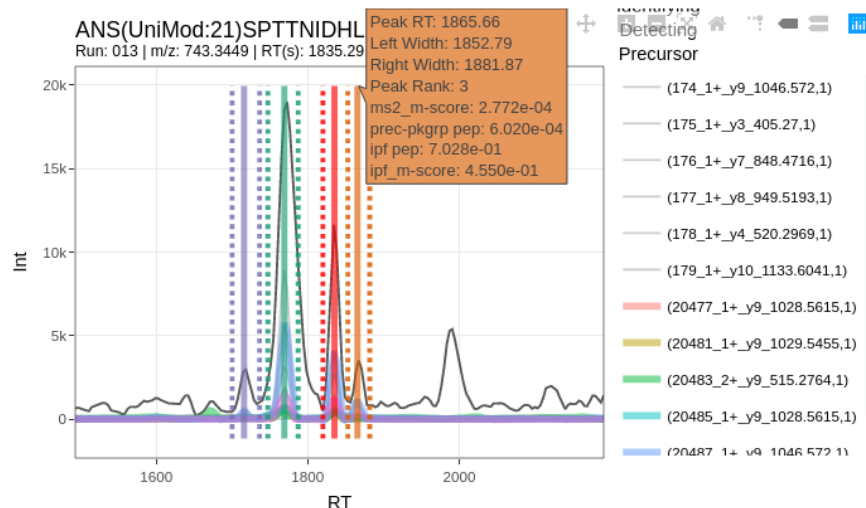
9,10

identifying b-ions

3

☒ Show Transition Scores (hover tooltip)

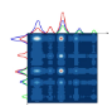
☒ Show All Peak-Groups





## Unselecting a Few Transitions to Display

The user can click on the legend to hide transitions they don't want to display



DrawAlignR Ver: 0.1.0

General Settings
Alignment Settings

Plot Settings

☒ Plot Precursor Trace  
☒ Plot Detecting Traces  
☒ Plot Unique Identifying Traces

Show n Identifying Traces

6

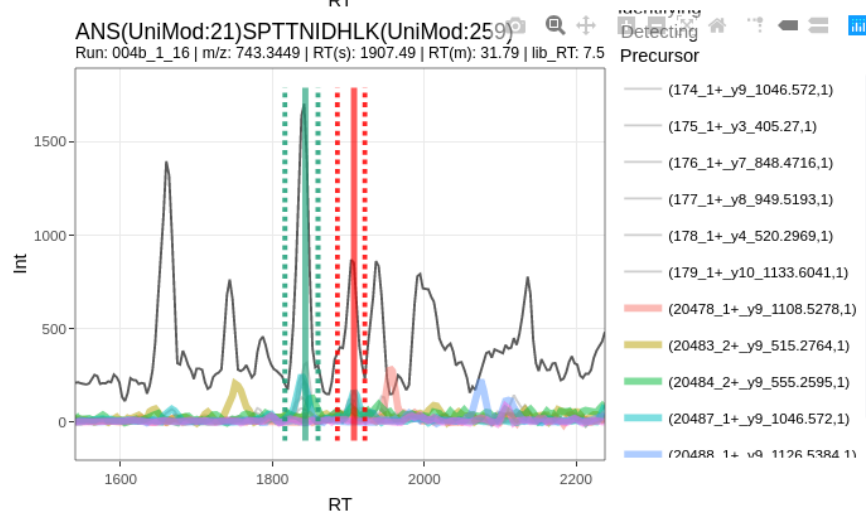
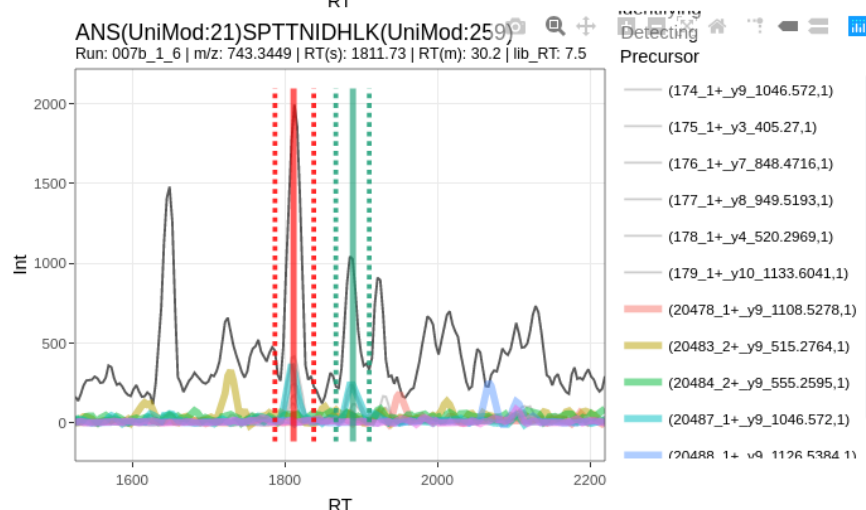
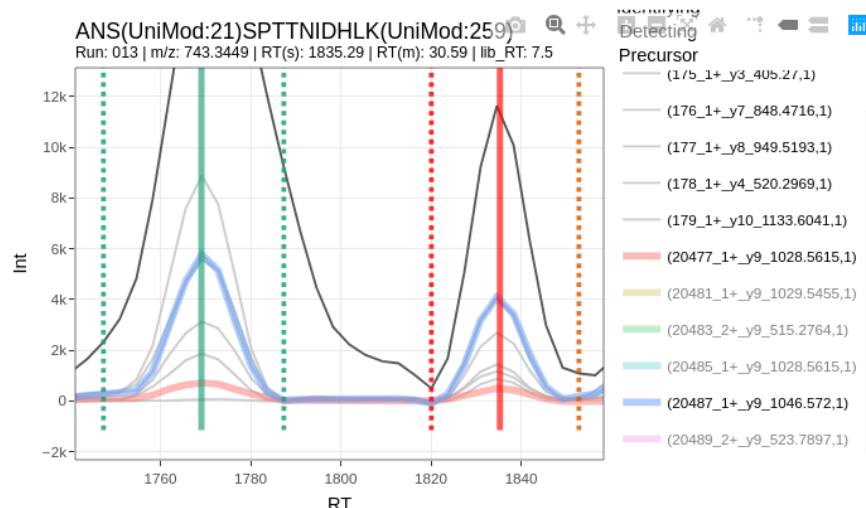
identifying y-ions

9,10

identifying b-ions

3

☒ Show Transition Scores (hover tooltip)  
☒ Show All Peak-Groups



The user can choose to display a single transition by double clicking on the transition legend they wish to display

