ExDViewer Quick Start Guide

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Using This Quick Start Guide

The purpose of this Quick Start Guide is to make new ExDViewer users productive with their own samples as fast as possible. Using provided example data and results files, it covers the major functions of the ExDViewer software. The order of presentation is deliberately laid out to build on previous exercises. It is recommended that users install the software and follow along with the operations as they are described.

What Is ExDViewer Software?

ExDViewer is part of e-MSion's solution for the comprehensive analysis of proteins and peptides. We offer a device called the ExD cell that enables Electron Capture Dissociation (ECD) as an add-on to the Agilent 6545XT AdvanceBio and 6560 Ion Mobility LC/Q-TOF. ECD allows users to:

- Fragment large protein ions.
- Characterize labile post-translational modifications (PTMs), e.g., phosphorylation, glycosylation.
- Differentiate isobaric residues such as aspartate/isoaspartate, leucine/isoleucine.
- Obtain complementary sequence information to CID b/y-ions.
- Learn more about disulfide-bonded peptides and proteins.

ExDViewer enables rapid and easy processing of ECD, Electron Induced Dissociation (EID) and Collision Induced Dissociation (CID) data. It is available as an application installable on the hard disk of a Windows PC or can be run in a web browser with data and results stored on a server.

Minimum Configuration

Hardware

- Windows-type PC
- Intel Core i3 CPU or equivalent
- 4 GB Random Access Memory (RAM)
- 50 GB hard disk space (more if many large data files will be processed)

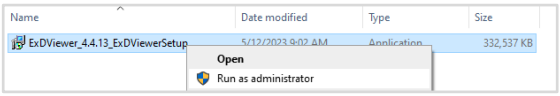
Software

- Microsoft Windows 10 or higher operating system
- For the web browser-based version, Google Chrome version 113 (or higher) or equivalent

Installing ExDViewer

1. Download the ExDViewer executable file.

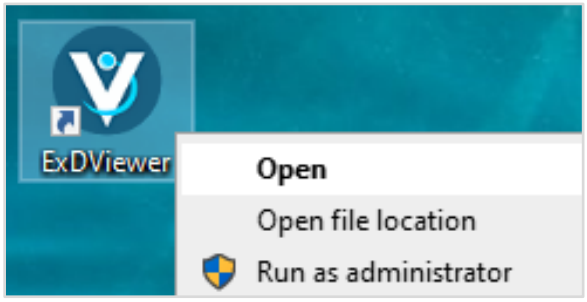
2. Right-click on the file and select **Run as administrator.**



3. Follow the prompts to select where the application will be installed and whether to create a Desktop icon.

Starting ExDViewer

Right-click on the ExDViewer icon and select **Open**.



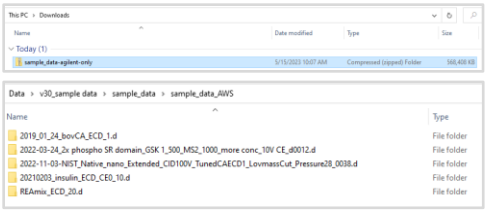
The ExDViewer software program will open.

Getting Started with ExDViewer

Download the Sample Data Files

1. Go to the **File > Download Sample Data** menu item. A file will begin to download.

2. Go to the Downloads folder in the Windows Explorer and extract the zipped file.



3. Move the data files to the normal place for LC/Q-TOF data files on the PC, e.g. \MassHunter\Data.

Loading Example Runs

exdview files are result files created by ExDViewer. They contain annotated mass spectra, sequence information and coverage (including ion types and PTMs assigned to positions), tables of results, and window layouts.

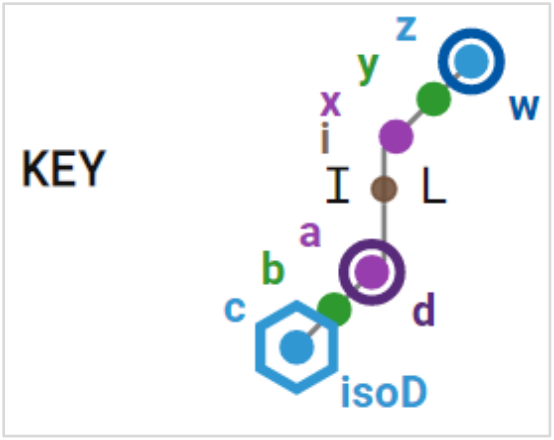
To load an example exdview file, go to the **File > Load Example Run** menu item and locate the example data file to **Distinguish isobaric residues** and click the **Open** button.

The results will load into ExDViewer:

Reviewing ExDViewer Results

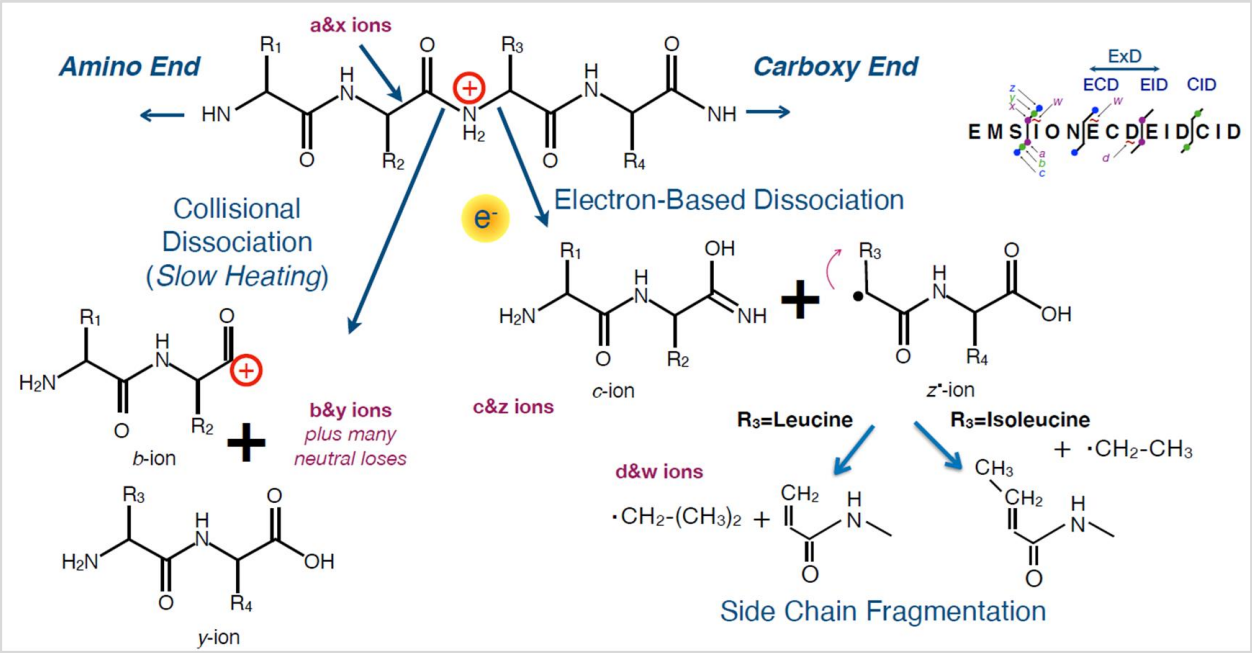
Sequence Coverage Map

REALLYisoD is a 16 residue synthetic peptide with isoaspartate (the blue D) in position 6 as well as four leucines (L) and one isoleucine (I). One of the challenges of top-down analysis is differentiating isobaric residues such as aspartate/isoaspartate and leucine/isoleucine



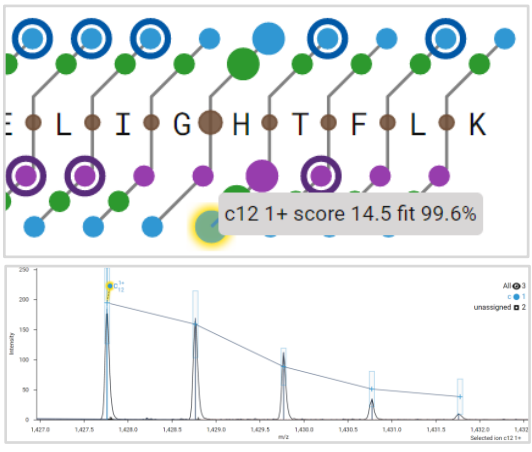
First, review the **KEY** which shows the representation of the different types of ions in the Sequence Coverage Map

These ion types are generated by ECD, EID and CID fragmentation mechanisms. ECD and EID are collectively referred to as ExD:

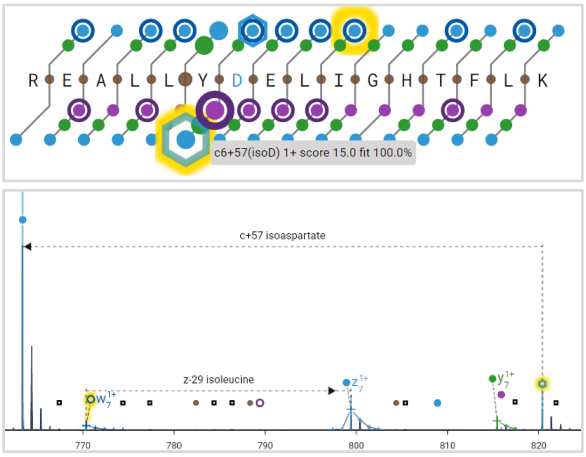


Most residues in the Sequence Coverage Map are in black text; however, those that have a PTM such as glycosylation or phosphorylation are in red. Isoaspartate is in blue text.

Clicking on a particular ion in the Sequence Coverage Map will zoom into it in the Spectrum view:



Leucine and isoleucine can be differentiated using w ions from side chain fragmentation while isoaspartate is indicated by an isotopic cluster 57 Daltons greater than the c ion:



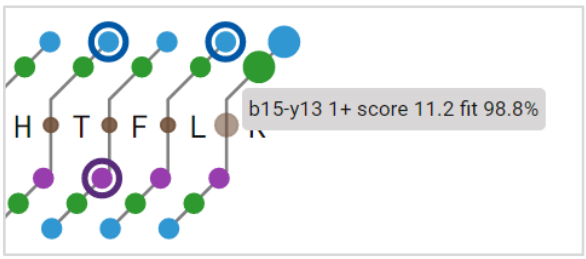
In the lower right corner, the Sequence Coverage Map displays the percentage of the total sequence that is found and annotated:

Sequence Coverage: 100% (without internal ions)

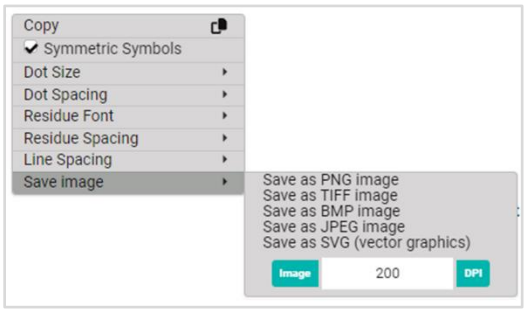
Internal ions are fragmented on both the amino and carboxyl end. ExDViewer detects

and annotates them but does not include them in the total sequence coverage.

Detection of internal ions can be configured in the Hybrid Deconvolution Settings dialog during the Hybrid Deconvolution workflow.



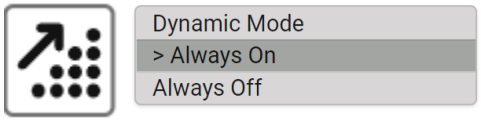
Right-clicking will bring up a menu to change the format of the Sequence Coverage Map or save an image as a publication-quality graphic:



Spectrum Window

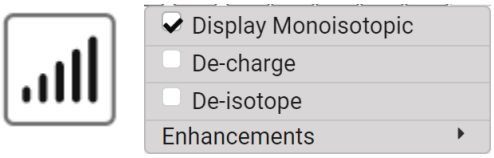
The Spectrum Window has several user interface (UI) elements for transforming or filtering. The Toolbar at the top enables zooming, panning as well as de-charging and de-isotoping the spectrum:

Toggle ion density view allows the ion dots to either be lined up neatly (Always On) or shown with all ions in their exact place (Always Off). Dynamic Mode determines the best positioning for the ion dots:



Toggle view of peak data has several options:

- Selecting De-charge consolidates information from different charge states of the same ion and transforms the x axis of the spectrum from *m/z* to Mass. This greatly simplifies the mass spectrum and aids in interpretation.
- In addition to de-charging, it is possible to display just the monoisotopic ion and de-isotope the isotopic clusters to collapse them to a single mass peak.



Toggle selection of visible ions will alternate between the currently selected ion and all other ions. **De-select all ions** will remove all selection of ions:

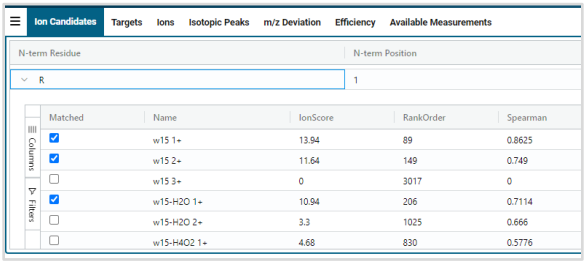
Hide selected ion(s) will make the currently selected ions disappear. **Unmatch selected ion(s)** will remove the match information for that ion:

The Legend on the right side has a summary of all ions found including unassigned ions (those matching an Averagine model). Selecting an ion (e.g., the blue z ion dot) will highlight all ions of that type in the Spectrum Window and Sequence Coverage Map.

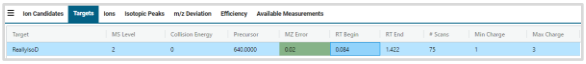
Tables

The Tables provide different views on the data including matched and unmatched ions, targets used, raw peaks, *m/z* deviation across the mass range, and fragmentation efficiency.

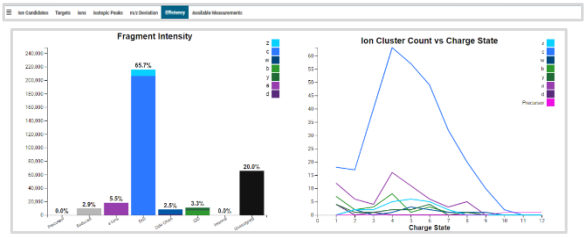
The Ion Candidates table lists theoretical fragment ions for the target sequence for each residue position and shows whether they are matched or unmatched in the Spectrum Window. The user may select or de-select whether an ion is Matched:



The Targets table shows information about the target sequence and spectra analyzed:

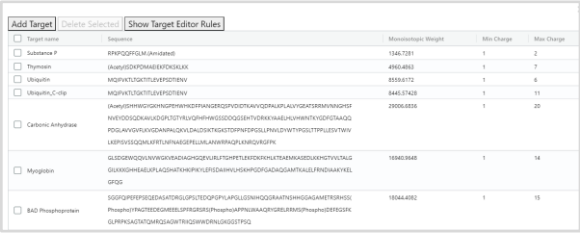


The Efficiency tab displays the intensity of ion types detected during the analysis including precursor, reduced precursor, ECD, EID, CID, internal, and unassigned ions. The count of ion clusters by charge state is graphed, which can assist in determining optimal conditions.



Setting up Target Sequences with Target Editor

The Target Editor enables the user to add, modify and curate target sequences used in the Hybrid Deconvolution workflow. To access it, select the **File > Target Editor** menu item.



To add a target sequence, select the **Add Target** button above. A new row will be created at the top with the dummy sequence name "target name." Fill this field in with your sequence name. Double-click in the **Sequence** field to enter your new amino acid sequence. The monoisotopic weight will automatically be calculated.

To edit a sequence, double-click in its **Target name** and/or **Sequence** field. When you are finished, press Enter to save the new information.

To add PTMs to target sequences, ExDViewer uses a derivative of Unimod syntax. To access a summary, select the **Show Target Editor Rules** button above. For example, a serine (S) residue that has a phosphorylation PTM will be annotated with (Phospho) as below:

To exit the Target Editor, click the **X** in the upper right corner.

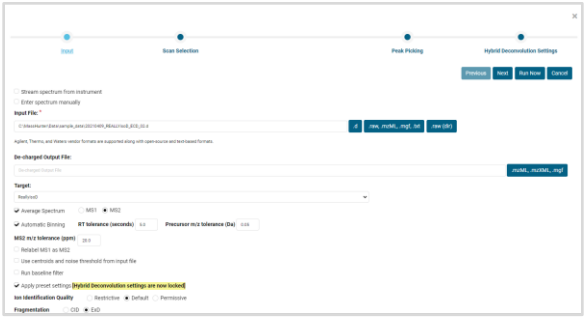
Running the Hybrid Deconvolution Workflow

The Hybrid Deconvolution workflow makes top-down analysis of a protein or peptide fast and easy. It answers the question "Does this spectrum match this protein or peptide?" The default set of preferences can be used to analyze a wide variety of proteins and peptides.

To begin the Hybrid Deconvolution workflow select the **Workflow > Hybrid Deconvolution** menu item.

Hybrid Deconvolution Step 1 – Input Dialog

In the Input Dialog select the data file **20210409_REALLYisoD_ECD_02.d** among the sample data that you downloaded earlier in the **Input Data** field. The Input Data can either be a data file or spectra. Note that Agilent MassHunter *.d, Thermo, Waters, mzML and MGF formats are supported.

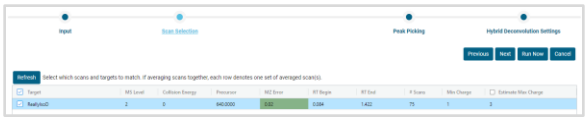


In the **Target** field use the dropdown to select the **ReallyIsoD** target sequence. All target sequences available in the Target Editor will show here.

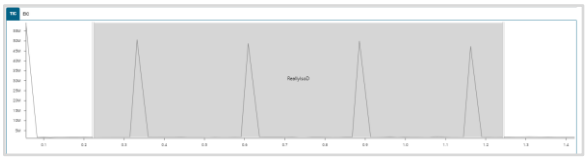
Leave the rest of the settings as their default values. Press the **Next** button.

Hybrid Deconvolution Step 2 – Scan Selection Dialog

In the Scan Selection dialog select the checkbox next to the target sequence **REALLYisoD**. The **MZ Error** for the spectra vs that target sequence is green, which indicates that it is within specification.



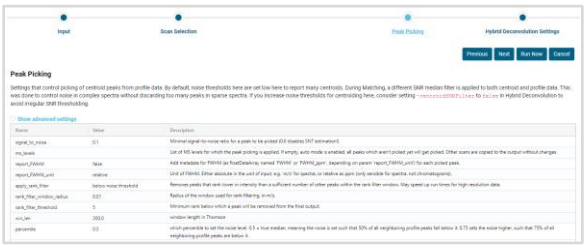
Below, the Chromatogram window is interactive; you can zoom in or out and sliders at each end allow you to select the range of scans for analysis.



Press the **Next** button.

Hybrid Deconvolution Step 3 – Peak Picking Dialog

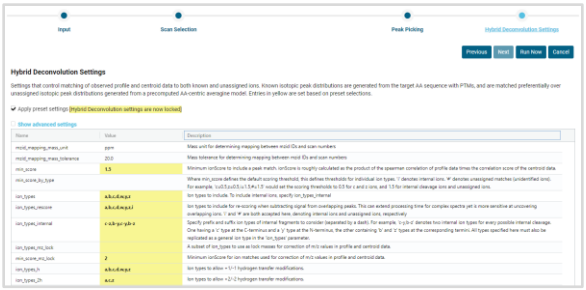
Most applications can use default values for peak picking. After initial analysis, it is possible to return to this dialog if adjustments are needed for settings such as signal to noise ratio.



Press the **Next** button.

Hybrid Deconvolution Step 4 – Hybrid Deconvolution Settings Dialog

Like the Peak Picking dialog box, most applications can use default match settings values. The values in yellow are locked to preset matching settings.



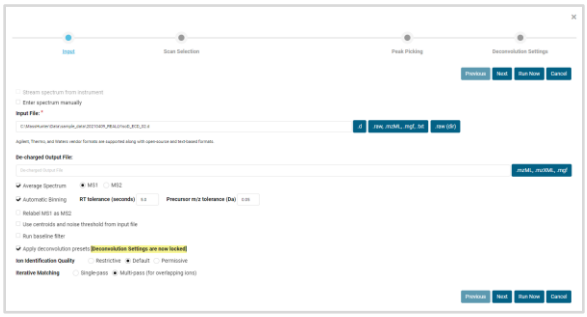
When finished, press the **Run Now** button to begin the analysis.

Running the Untargeted Deconvolution Workflow

The Untargeted Deconvolution workflow finds all ion fragments in a spectrum or set of spectra that match an Averagine model for amino acid residues. The ions are labeled as unassigned and annotated in the

spectra. The unassigned peptide ions can then be used for de novo sequencing.

To begin the Untargeted Deconvolution workflow, select the **Workflow > Untargeted Deconvolution** menu item. The Input dialog box will appear:



Similar to the Hybrid Deconvolution workflow, choose an input file and settings. It is possible to create a De-charged Output file in mzML, mzXML or MGF format for moving results to another software program.

Untargeted Deconvolution can also be used as a form of batch deconvolution by processing every spectrum in the data file.

The next three steps after the Input Dialog are a subset of the Hybrid Deconvolution workflow with the same settings and options.

Running the Browser-based ExDViewer

The browser-based version of ExDViewer is available at the URL https://viewer.e-msion.com/ The browser-based version has the same menu items as the desktop. In addition, it has a graphical navigation UI for viewing results (i.e. exdview files) and running the Hybrid Deconvolution workflow.