

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

- 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.

This PC > Downloads				v 0 /
Name	^	Date modified	Туря	Size
v Today (1)				
isample_data-agilent-only		5/15/2023 10:07 AM	Compressed (zipped) Folder	568,408 K
				984
2010 01 34 have 64 500 1	d in the second s			File folder
2019_01_24_BOVCA_ECD_1.0				
2019_01_24_00VCA_ECD_1.2 2022-03-24_2x phospho SR	domain_GSK 1_500_MS2_1000]_more conc_10V CE_d0012.d		File folder
2019_01_24_b0vCA_ECD_1.c 2022-03-24_2x phospho SR 2022-11-03-NIST_Native_na	domain_GSK 1_500_MS2_1000 no_Extended_CID100V_Tuned	0_more conc_10V CE_d0012.d ICAECD1_LovmassCut_Pressure28_0	0038.d	File folder File folder
2022-03-24_2x phospho SR 2022-03-24_2x phospho SR 2022-11-03-NIST_Native_na 20210203_insulin_ECD_CE0_	domain_GSK 1_500_MS2_1000 no_Extended_CID100V_Tuned 10.d	0_more conc_10V CE_d0012.d ICAECD1_LovmassCut_Pressure28_0	0038.d	File folder File folder File folder

 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.

Characterize lable PTMs	Isstument: Aglent 654507 QTOF Sangle name: SARS.GaV.2 by Initian SR-ach Inher Istroduction method namogray Procusor: 688 miz (10*) Collision nenge; TVV	Open 12
Analyze doutliste bunded proteins	Isstument: Aglent 6545XT QTOF Sample name: Disulfive bonded Insulin beta chain Initialutation member UC Presunant (66 miz (64) Collision nonlig: UV	Open 18
Distinguish sobaric residues	Isatument Aginet 65450T QTOF Sample name REALMac/DEUISH/FEK Isttoduction method LC Precuetor 680 mit (3+) Collision neergy, 0V	Open (2
Characterize longer pepides and proteins (top-down)	testument Aglent 6445/C () TOF Sample neuro: Catolonia attybulan Istoluction method disci rikuson Precumo: 1.452 mtz (201-) Collision receipt; IVV	Open (C
Characterize ionger peptides and proteins (top-down)	Instrument Agient 6545XT 0/TOF Sample nome: Native NIST nARLC Introduction method: namospray Productor: nit	Open 6

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:

Сору	C.	
Symmetric Symbols	S	
Dot Size	•	
Dot Spacing	•	
Residue Font	•	
Residue Spacing	•	
Line Spacing	+	
Save image	•	Save as PNG image Save as TIFF image Save as BMP image Save as JPEG image Save as SVG (vector graphics)
		Image 200 DP1

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.

· ·	Display Monoisotopic	
	De-charge	
•••••	De-isotope	
	Enhancements	•

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:

k	on Candidates	Targets	lons	Isotopic Peaks	m/z Devi	ation Ef	ficiency	Available Measure	ments
N-term Residue N-term Position									
~	R						1		
	Matched		Name			lonScore		RankOrder	Spearman
0			w15 1+			13.94		89	0.8625
lumn			w15 2+			11.64		149	0.749
-	0		w15 3+			o		3017	0
Ţ	~		w15-H2	0 1+		10.94		206	0.7114
65	0		w15-H2	0 2+		3.3		1025	0.666
			w15-H4	02 1+		4.68		830	0.5776

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.

Target narrie	Sequence	Monoisstopic Weight	Min Charge	Max Charge
Substance P	RFKPQQFFGLM.(Amidated)	1346.7281	1	2
Thymosin	(Acety/SDKPOMAEEK/DKSKLKK	4960.4863	1	7
Ubiquitin	MQIPVKTLTGKTITLEVEPSOTIENV	8559.6172	1	6
Ubiquitin,C-clip	MQIPVKTLTGKTITLEVEPSOTIENV	8445.57428	1	11
Carbonic Anthydrase	(Anglothiko)GANIKATANAKATANARAGANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATAN NEBOSADKANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKATANAKAT	29006.6836	1	20
Myogisbin	GLEDGEWOQUNIVWSKYEALMGHOQUULUTISHEFETLESDKFCHLETALMKKEEDKCHGTWUTMLG GUERRICHHEALUFLAQHATGHOPIOLEPISDAIIWUHGRPGDFGADAQSAMTKAELFRDUAAKYEL GEQS	16940.9648	1	14
840 Phosphoprotein	SOGPOPPEPSIQEDASATORICOPSCELOPOPULAPQLOSUHQQGRAATASHHGQGAAATERSHISS; Phosphof/TAGTELDEGAEELSPTRIGERS(PhosphoJAPTNIIIAAQEVGRELRIMS(Phospho)DEFEGSEK CUPIPKSAGTATOARQEAQUTRICQSWWDRILGKIGSTPSQ	18044.4082	1	15

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.

Add Target	Delete Selected	Show Target Editor Rules
Target name	e Seque	nce
target name	TARGE	TSEQUENCE

To add PTMs to target sequences, ExDViewer uses a derivative of <u>Unimod</u> 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:

EAD Phosphophotan SSRC1014FFFFEGDASK17BCL025F1ECD/OPH_ARDL020HH002GAAATGH00GAAAET65H5 18044.062 EAD Phosphophotan SSRC104FEGFSEGDASK17BCL02HH002FK155 18044.062

To exit the Target Editor, click the \mathbf{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.

•	•	•	•
inout	Scan Selection	Peak Picking	Hybrid Deconvolution Settings
			Previous Next Rut Now Cor
Stream spectrum from instrument			
Enter spectrum manually			
iput File:*			
Characterist Databangle, SAX (20210-00, RD	1271e0_6C0_01.4	.4 .mm, mobili, .mgt, tot	
plent. Therma, and Watters vendor formats are pupp	of the states with spen-acycle and he of based formate.		
e-charged Output File:			
			milit, militte, m
arget:			
Realities		*	
Average Spectrum 💫 MS1 🔹 N	62		
Automatic Binning RT tolerance ()	ecords) 5.0 Precursor m/z tolerance (Da) 0.05		
IS2 m/z tolerance (ppm) 20.0			
Relabel MS1 as MS2			
Use centroids and noise threshold from	input file		
Run baseline filter			
Apply preset settings [Hybrid Deconvol	ution settings are now locked		

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

ReallyIsoD	~
Single Target	
Substance P	
Thymosin	
Ubiquitin	
Ubiquitin_C-clip	
Carbonic Anhydrase	
Myoglobin	
BAD Phosphoprotein	
SOD	
ReallyIsoD	
NISTmAB LC	
NISTmAB LC Native	
NISTmAB Intact	
Insulin a	
Multiple Target	
From MZID file	
Multiple, User Specified	

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.

		•		
Input		Scan Selection	Posit Picking	Hybrid Deconvolution Settings
				Previous Next Bun Now Can
ak Picking				
s done to control noise in at recease that threshol three advanced settings	complex spectra without disca ling	ting too many peaks in sparse spectra. If you increase noise t	restolds for certroiding leve, consider setting	Thilses to believe in Hybrid Deconvolution to
and the second s	125.4	Deurphin		
ignel_bo_miles	0.1	Minimal agratito noise ratio for a peak to be access IOD toatties	shit extensions	
cjevala.		Dut of MS levels for which the peak picking is applied. If empty, a	Ito mode is enabled, will peaks which aven't picked yet will get picked. Other	er scars are copied to the output without charges
njeveli port,PMHH	No	List of MS kinks for which the peak picking is applied. If empty, a Add metadata for THINH (as foatQataking named TWHM or T	vio mode is enabled, will peeks which aren't picked yet will get picked. Other ANN(.gpm: depending on person 'report,/NINN(.unit') for each picket pe	er som are copied to the output without charges. Al.
njevelik port,PMHR port,PMHR_print	false relative	List of MS levels for which the peak picking is applied. Hempty, a Add meradate for failfuld (as footSeaking, named FWHM or P Unit of FBHMC Efter atoxicte in the unit of input, e.g. m/c for a	vice mode is enabled, will peaks which even't picked yet will get picked. Othe WHX_genr, depending on person 'report,788556_unt1) for each picked pea pectra, or relative as gent (only encodes for guestra, est chromotogram).	er som av copiel to the output without therges. Al
njevela port/Wella. port/Wella.por plytevlij?ter	false matter below ware threshold	List of MS leaves for which the peak poling is applied. Hamply, a Add metadate for PMIHID (as footDataking named TWHM) or P Unit of PMIHID Ether associate in the unit of input, e.g. wort for a Removes peaks that reak lower in intersity than a sufficient numb	uto mode to enabled, sill peaks which event polied yet will get ploket. Other MMM_gover, depending on person report, PMMM_unit's for each polied per sector, or relative as gove (only annibile for questor, set chromotogover), et all other predis within the tenk littler vinition. May speed up run times for	er som av opped to the output without therges as, or hyp-essivition data.
njevela port/Wellizeri port/Wellizeri plyteric/Her rk/Herzeridor/willia	Nax realize before rose threshold 027	List of MS levels for which the peak policing is applied. If energy, a Rod mendana for 700 MIA (as foat/backway named TWMR or fo Unit of F80 MS. Ether attacke is the unit of riput, ag, wort the decrement peaks that in which laver is intending them sufficient numb Rapics of the wendow used for well (Review, min);	zo mode is enabled all pasis which event points yet will ge points DM WMI gam: depending on parts import, 2003/01 for each point pe- partie, or interive as gen (only another for gents, not chronotogome), er of other peaks within the serie liber weeks. May speed up on investing	e som av oppd to the output without charges at. Ir high-resolution date.
njeveli port,PMHR port,PMHR,witt ply, ankj,Pher rik,Pher, andror, jedna rik,Pher, predicid	Nata centre betro rose trackol 021 5	Los of KE leave far which the peak-poling is septied. If enging a Add exectance for Fillinki gas facultariances, research TVMH or P Unit al Fillinki. Ether attack and is the and of paper, any the lea- ferences peaks that noise in intervals there an all-leave number factors of the modes used for each fillering, a mile Medican and below mithing again. We remember the filling	ue make enabled, all passe reints wert polind yet of app polint. DNA Margane, Repending on person "report, 7881%, wert for and polind para estate, or relative as giver long's avoidable for sources, and showen appending er of other peels within the senii liber vanism. Nay speed up run times for roughd.	e com are copied to the output without changes al.
ngawik sport/WHI port/WHIgan polyweighte stightegonitorywika ragineghaniais ngen	Nation Interface Decision record thread-bold 0.027 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.	List of US leaves for which the peak points is applied. If empty and Add reactions for PUMIA as forest-backway reared TWWM or P Units of TMMA (Elever assistants in the and integra, or you'll the Removes peaks that each leave in intercely them a sufficient work features of the incidence with the peak of the removed from the the window leaves in the servers.	de make sendest af gezo with wert points per uit gezond. Ob Mill gent, Begending on pean report, PBING with for each points pe and an an without ang get of particular by peak or act socio-stagenous, et of other peaks within the seniil filter window. May speed up namines for codgat.	er som are copied to the output vithout charges al.

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.

			•	
input	Scan Se	lection	Peak Picking	Hybrid Deconvolution Settings
				Previous Next Run Now Can
brid Deconvolution Set	tings			
tings that control matching of	observed profile and centroid data to	both known and unassigned ions. Known isotopic peak distribution	is are generated from the target AA sequence wit	h PTMs, and are matched preferentially over
osigned isotopic peak distribu	itions generated from a precomputed	AA-centric averagine model. Entries in yellow are set based on pre-	et selections.	
Apply preset settings <mark>(Hybrid C</mark>	econvolution settings are now locked			
three advanced settings				
lana	Value	Description		
noid, mapping, mass, and	10m	Mass withfor determining mapping between wold IDs and scan numbers		
noid, mapping, mass, tolerance	20.0	Mass tolevance for determining mapping between mold IDs and scan numb	les .	
ninjscow	15	Minimum ionScore to include a peak match, ionScore is roughly calculated	as the product of the spearman correlation of profile dat	times the correlation score of the centroid data.
min_score_by_type		Where minyacces defines the default accring threshold, this defines thresh for example, 'cu35_cu35_is15,#a15' would set the scoring thresholds to '	side for individual ion types. 'I denotes internal ions. W d D3 for c and s ions, and 1.3 for internal ideavage ions and	enotes unassigned matches (unidentified ions). unassigned ions.
ion_types	akolnyz	for types to include. To include internal ions, specify ion_types_internal		
iar, y pec, escare	akodagai	Ion types to include for re-scoring when subtracting signal from overlappin overlapping ions. IT and W are both accepted here, denoting internal ions.	g peaks. This can extend processing time for complex spe and unassigned lone, respectively	ctra yet is more sensitive at uncovering
ion_typec_internal	cabycyba	Specify prefix and suffix ion types of internal fragments to consider (separa One having a 'c' type at the C-terminus and a 'y' type at the N-terminus, th replicated as a general ion type in the 'lon, types' parameter.	ted by a dash). For example, 'c-y,b-d' denotes two interna e other containing 'b' and 'd' types at the corresponding t	I on types for every possible internal cleanage. armini, All types specified here must also be
ion_types_ms_lock		A subset of ion_types to use as lock meases for correction of mitrialues in	pofile and centroid data.	
min_scare_mz_lock	2	Minimum ionScore for ion matches used for correction of m/z values in pr	file and certroid data.	
ion, types, h	abodayar	Ion types to allow + 1/-1 hydrogen transfer readifications.		

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:

Input	Scan Selection	Peak Picking	Deconvolution Settings
			Next Bun Now Cano
Stream spectrum from instrument			
Enter spectrum manually			
nput File:*			
C Maximum Data seruk presidentes y BAL	Fe6_ECE_E2.4	d raw, mohil, regt, bit .raw (dr)	
igilet, Themis, and Mateix wedge formats are support	ed along with open-source and text-based formats.		
be-charged output Pile:			
			mann, manne, ma
Average Spectrum ● MS1 ○ MS	2		
Automatic Binning RT talerance (se	conds) 5.0 Precursor m/z tolerance (Da) 0.05		
Relabel MS1 as MS2			
Use centroids and noise threshold from it	rput file		
Pun baseline filter			
Apply deconvolution presets Deconvolution	ion Settings are now locked		
Ion Identification Quality O Restriction	e 🖲 Default 🕓 Permissive		
Iterative Matching 💦 Single-pass 🛞	Multi-pass (for overlapping ions)		
			tevious Next Run Now Cano

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 <u>https://viewer.e-</u> <u>msion.com/</u> 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.



