

# **PERCEPTRON** *v 1.0.0.0*

A Next Generation Top-Down Proteoform  
Identification and Characterization Platform

## **USER MANUAL**

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## 3 Introduction to PERCEPTRON

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This chapter introduces the user to the PERCEPTRON platform along with a description of its features.

### 3.1. About PERCEPTRON

PERCEPTRON is a freely available web-based proteoform identification pipeline for Top-Down Proteomics (TDP). Top-down proteomics is an emerging experimental protocol for analysis of intact proteoforms. PERCEPTRON search pipeline brings together algorithms for: (i) intact mass tuning, (ii) *de novo* peptide sequence tag extraction, (iii) *in silico* spectral comparison, (iv) identification of post-translational modifications as well as truncated proteins, and (v) a novel composite scoring scheme for candidate protein scoring. PERCEPTRON achieves high performance by leveraging NVIDIA GPU technology coupled with Microsoft ASP.NET and ANGULAR frameworks. The search results obtained include a list of proteins, their scores and details on the matching information. This information can be visualized as well as downloaded. Overall, PERCEPTRON is aimed at filling the crucial void of open-source and open-architecture protein identification software for TDP data, employing state-of-the-art algorithms.

### 3.2. Features

The salient features of the pipeline are summarized below:

- **Graphical User Interface (GUI)** - A set of rich and intuitive graphical user interface has been developed for setting up the search parameters as well as for integrating the main components of the engine.
- **Whole Protein Molecular Weight Estimation** - The protein identification begins with the tuning of precursor protein's monoisotopic MW (MS1) as guided by its fragmentation spectra (MS2). Relative abundances and mass/charge ( $m/z$ ) ratios are used to calculate the consensus MW which is then employed in the search and scoring process.
- **Peptide Sequence Tag Extractor** - Peptide sequence tag ladders (PST) are extracted from the spectra by enumerating successive peaks having MW differences equal to an amino acid and within the user specified mass tolerance. Protein database is then filtered for proteins reporting these PSTs. The length of PST ladders, cumulative mass off-sets and relative abundances are used in calculating the PST scores.
- ***In silico* fragmentation** – *In silico* fragments of candidate proteins are generated by the user selected fragmentation techniques. *In vitro* and *in silico* spectral comparisons are performed and scored.

- **Post-translational Modification (PTM) Search** - Support for predicting typical PTMs has been provided in the tool. Users can select and search variable and fixed PTMs of their choice along with blind-PTMs by simply selecting them from the GUI.
- **Multifactorial Composite Scoring System** - A multifactorial candidate protein scoring scheme incorporating the aforementioned algorithms has been developed. User customization of the parameters and weights in the scoring function is admitted via a GUI.
- **Single and Batch Search** – PERCEPTRON provides support for search in single as well as batch modes. Towards an automated processing of multiple spectral data files, a batch processing mode allows for the selection of multiple files from the folder by clicking the attach file button. The experimental spectra, search parameters and results are automatically stored in the project directory for further processing and visualization.

## 4 Hardware and Software

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### 4.1. Hardware

For in-house deployment of PERCEPTRON, the hardware requirement includes a graphical processing unit (GPU) that supports CUDA TOOLKIT 7.0.

### 4.2. Software

Compilation and software build of PERCEPTRON requires the following software:

- Windows Server 2012 R2
- Visual Studio 2013
- Angular 1.7.4
- Node.js 8.11.1
- SQL Server Management Studio 17.6
- CUDA TOOLKIT 7.0
- CUDAFy.NET.1.29.5576.13786
- Microsoft Windows Server v6.2
- .NET Framework 4.5

### 4.3. Testing

The freely available public deployment of PERCEPTRON is hosted on a Dell Power Edge R730, 2 x Intel Xeon E5-2620, 160 GB RAM (16GBx10) and an NVIDIA Tesla K40C (2880 Cores). Following Windows versions are compatible with PERCEPTRON:

- Windows 8.1
- Windows 7
- Win Server 2012 R2
- Win Server 2008 R2

## 5 Video Tutorials

Several video tutorials have been developed for employment of PERCEPTRON in protein search. These tutorials are available as a playlist at: [https://www.youtube.com/playlist?list=PLaNVq-kFOn0Z\\_7b-iL59M\\_CeV06JxEXmA](https://www.youtube.com/playlist?list=PLaNVq-kFOn0Z_7b-iL59M_CeV06JxEXmA)

## 6 Getting Started with PERCEPTRON

PERCEPTRON manual, samples and issues database is freely available (under the MIT open license) at (<https://perceptron.lums.edu.pk/>)

To initiate use of PERCEPTRON, the user has to register and log in by clicking on the link provided above.

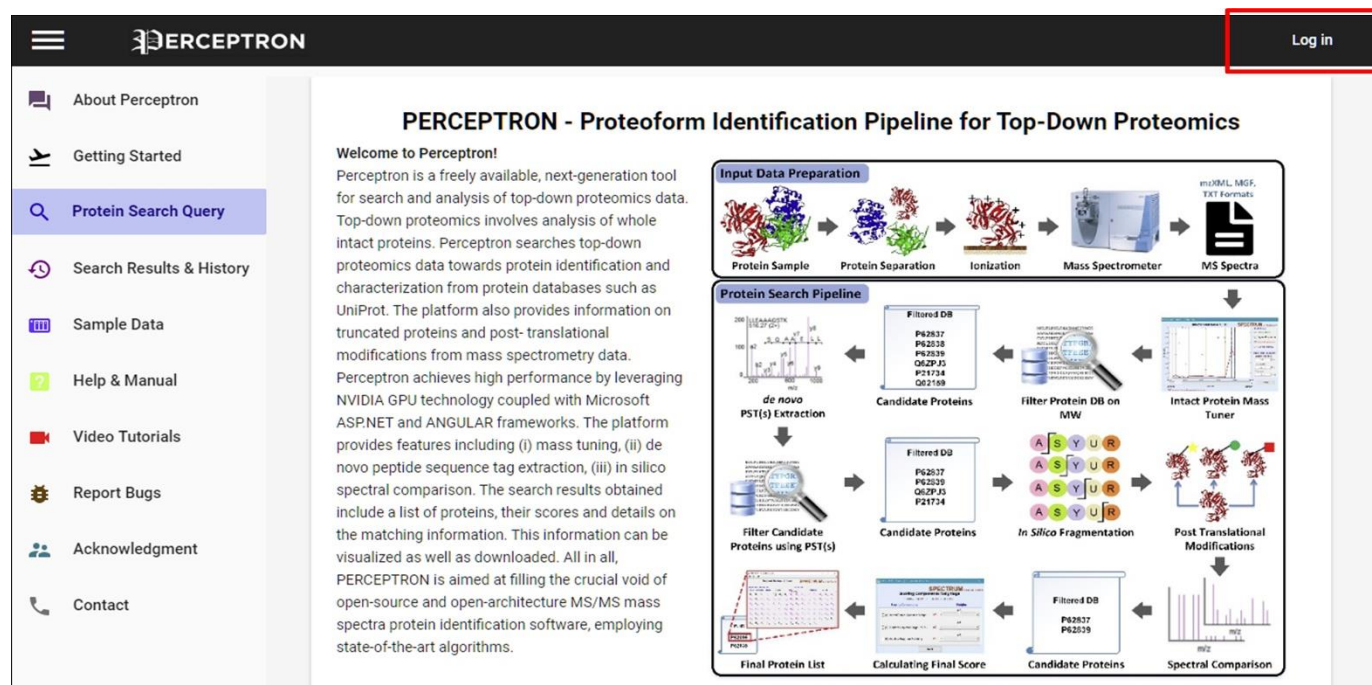
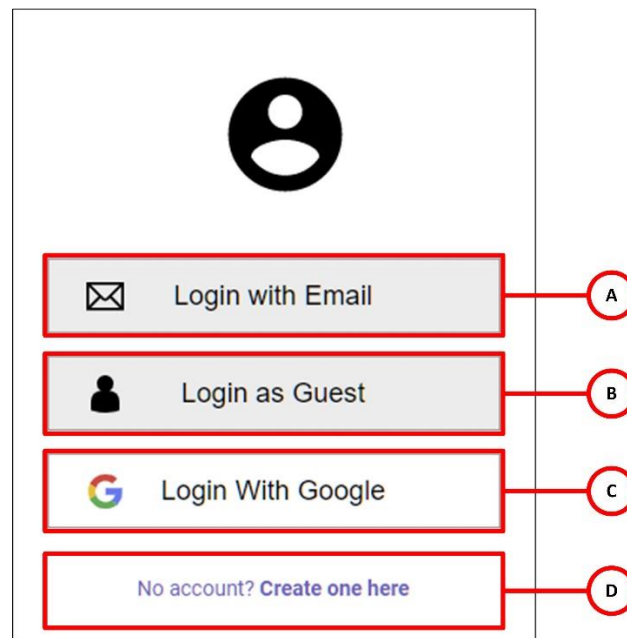


Figure 1. PERCEPTRON Homepage and Log in button

Click the 'log in' button on the top right. A window will appear. Enter user credentials to proceed.





*Figure 2. PERCEPTRON Login options*

- A. User can login with an existing email account
- B. Enables user to login as a guest, perform search in PERCEPTRON. Note that results will not be saved in this case.
- C. User can also use their Google account to login.
- D. Otherwise, users can create a new local account in order to login.

## 7 GUI description:

This chapter presents the interface overview for user facilitation.

### 7.1 Window 1: PERCEPTRON Tool for Top-down Proteomics

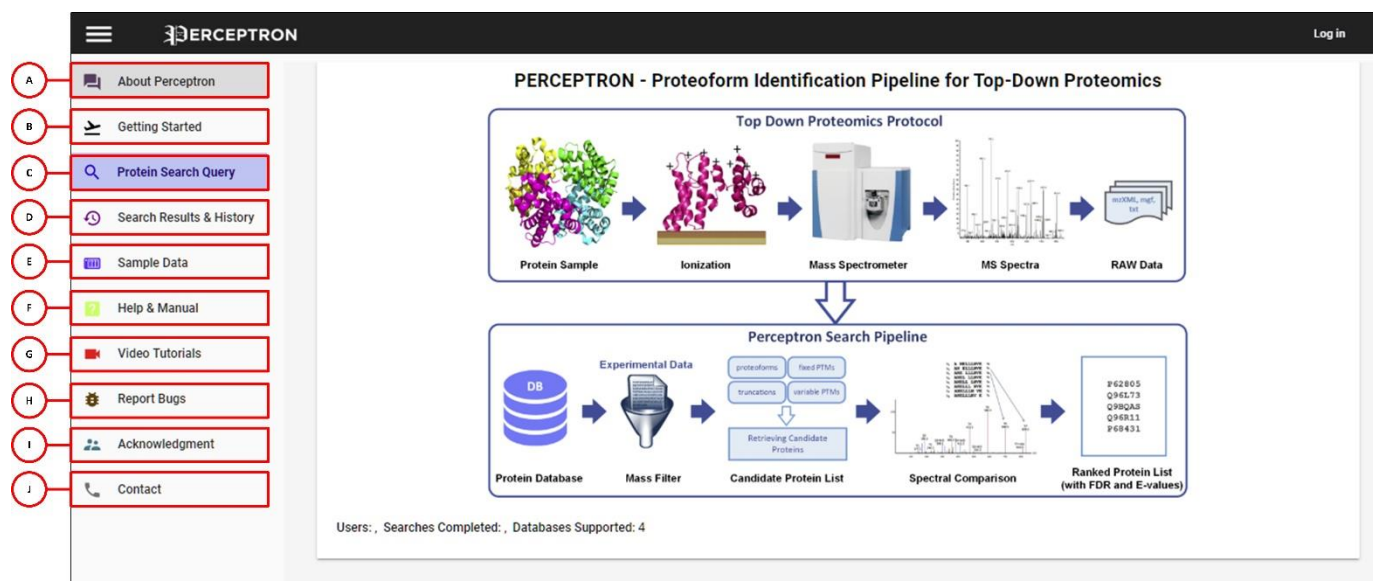


Figure 3. PERCEPTRON - Overview of User Interface

- A. About PERCEPTRON: A next-generation top-down proteoform search and identification platform
- B. Getting Started: Quick guide to proteoform search and identification using PERCEPTRON
- C. Protein Search Query: Job submission - Search top-down proteomics data files to identify and characterize proteoforms
- D. Search Results & History: View search results and history
- E. Sample Data: Sample top-down mass spectrometry data for use with PERCEPTRON
- F. Help & Manual: Get assistance with using PERCEPTRON and download manual
- G. Video Tutorials: View step-by-step video tutorials demonstrating usage of PERCEPTRON
- H. Report Bugs: Report problems and issues here
- I. Acknowledgement: PERCEPTRON project team members
- J. Contact: Contact us for further information

In order to start protein search, click on the 'Protein Search Query' tab and the following window will appear:

## 7.2 Window 2: Protein Search Query

### Basic Parameters:

The screenshot shows the 'Submit Protein Identification Query' form in the PERCEPTRON application. The form is titled 'Submit Protein Identification Query' and is divided into two main sections: 'Basic Parameters' and 'Set Experimental Parameters'. The 'Basic Parameters' section includes the following fields:

- Protein Search Title** (A): A text input field for the search title.
- Intact Protein Mass** (B): A text input field for the intact protein mass.
- Upload Mass-Spectrometry Data File(s)** (D): A file upload button with a '0 Files Selected' indicator.
- Select Database** (C): A dropdown menu for selecting the protein database.
- Enter your E-mail Address \*** (E): A text input field for the user's email address.
- Recieve Top** (F): A dropdown menu for selecting the number of top results to receive.

The 'Set Experimental Parameters' section includes expandable sections for:

- Set De Novo Sequencing Parameters**
- Set Protein Modifications Parameters**
- Set Scoring Components Weight**

At the bottom right of the form are two buttons: 'Reset' and 'Submit'. A sidebar on the left contains navigation links: 'About Perception', 'Getting Started', 'Protein Search Query', 'Search Results & History', 'Sample Data', 'Help & Manual', 'Video Tutorials', 'Report Bugs', 'Acknowledgment', and 'Contact'.

Figure 4. PERCEPTRON - Overview of Basic Parameters

- In order to start protein search, user must enter the 'Protein Search Title' (for example: "MyProject")
- Enter intact protein mass (MS1)
- Select protein database
- Browse and upload experimental data (.mzXML/ .MGF/ .txt) for Single mode; Peak-list files for Batch mode
- Enter the email address at which the link for downloading results will be sent. Note that users are notified about the availability of search results upon completion of the job.
- Provide the number of candidate protein hits to be received in results

## Set Experimental Parameters:

The screenshot shows the PERCEPTRON web interface. The sidebar on the left contains links: About Perceptron, Getting Started, Protein Search Query (highlighted), Search Results & History, Sample Data, Help & Manual, Video Tutorials, Report Bugs, Acknowledgment, and Contact. The main content area is titled 'Submit Protein Identification Query'. It includes a 'Basic Parameters' section with fields for Protein Search Title, Intact Protein Mass (Da), Upload Mass-Spectrometry Data File(s) (0 Files Selected), Select Database, Enter your Email Address \*, Recieve Top, and Results. Below this is the 'Set Experimental Parameters' section, which is expanded. It contains several input fields and checkboxes: Mass Mode (radio buttons for MH+ and M(Neutral)), Intact Mass Tolerance (Da), Peptide Tolerance (ppm), Select Fragmentation Type (dropdown), Filter Database using MS1 (checkbox), Handle Truncated Proteoforms (checkbox), Tune Intact Protein Mass (checkbox), Neutral Mass Loss (Da), and Select Corresponding Special Ions (dropdown). At the bottom of the main content area are sections for 'Set De Novo Sequencing Parameters', 'Set Protein Modifications Parameters', and 'Set Scoring Components Weight'. A 'Reset' button and a 'Submit' button are at the bottom right. Red circles A through I are placed around the interface, with lines pointing to specific parameters: A points to the Mass Mode radio buttons, B points to the Intact Mass Tolerance field, C points to the Peptide Tolerance field, D points to the Select Fragmentation Type dropdown, E points to the Filter Database using MS1 checkbox, F points to the Handle Truncated Proteoforms checkbox, G points to the Neutral Mass Loss field, H points to the Select Corresponding Special Ions dropdown, and I points to the Tune Intact Protein Mass checkbox.

Figure 5. PERCEPTRON - Overview of Experimental Parameters

- A. Select Mass Mode. MS data can only be provided in either m/z form with  $z = 1$  or neutral masses
- B. Set the tolerance value for Protein Mass and select its unit
- C. Select the tolerance value for Peptide and select its unit
- D. Select the 'Fragmentation type' from drop down menu
- E. User can filter database by checking the option 'Filter Database using MS1'
- F. Check 'Handle Truncated Proteoforms' to allow search for truncated proteoforms
- G. Provide the value of Neutral loss, if any
- H. Choose the corresponding special ions for the type of fragmentation selected (i.e. a', b', y', z'', a\*, b\*, y\*, z' ions)
- I. Check the option 'Tune Intact Protein Mass' to allow for tuning of MS1 using MS2 data

## Set De Novo Sequencing Parameters:

The screenshot shows the PERCEPTRON web interface. The left sidebar contains navigation links: About Perceptron, Getting Started, Protein Search Query (selected), Search Results & History, Sample Data, Help & Manual, Video Tutorials, Report Bugs, Acknowledgment, and Contact. The main content area is titled 'Submit Protein Identification Query'. It includes a 'Basic Parameters' section with fields for 'Protein Search Title', 'Intact Protein Mass', 'Da', 'Upload Mass-Spectrometry Data File(s)', '0 Files Selected', 'Select Database', 'Enter your E-mail Address \*', 'Recieve Top', and 'Results'. Below this is the 'Set Experimental Parameters' section, which is expanded to show 'Set De Novo Sequencing Parameters'. This section contains five parameters: 'Enable PST Filtering' (checkbox, labeled A), 'Tolerance For Each Hop' (dropdown menu, labeled B), 'Minimum Tag Length' (dropdown menu, labeled C), 'Maximum Tag Length' (dropdown menu, labeled D), and 'Set the overall mass error tolerance for the whole PST' (text input field, labeled E). Below these are 'Set Protein Modifications Parameters' and 'Set Scoring Components Weight' sections. At the bottom right are 'Reset' and 'Submit' buttons.

Figure 6. PERCEPTRON - Overview of De Novo Sequencing Parameters

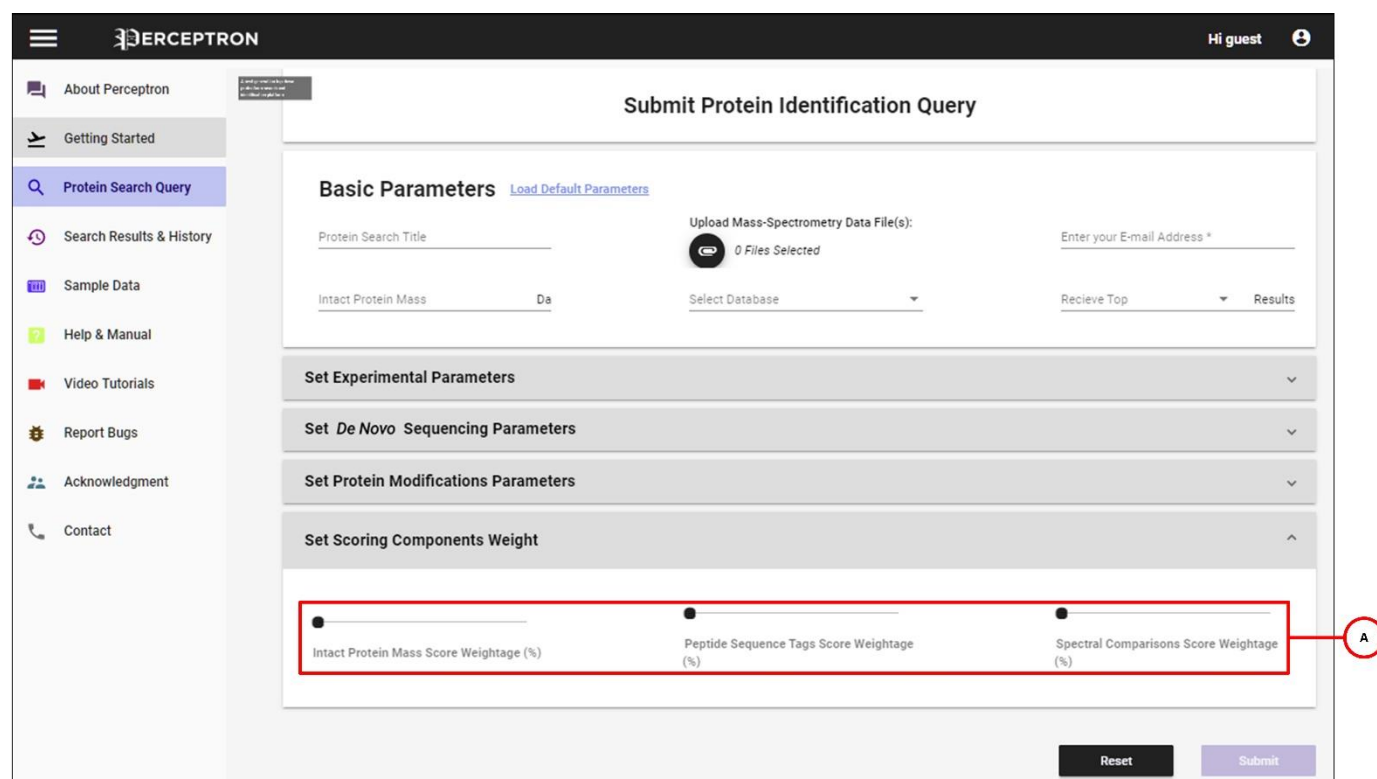
- A. Check 'Enable PST Filtering' to filter PSTs
- B. Set the 'Tolerance for each Hop'
- C. Tags will be filtered above the minimum length of PST selected from the drop down menu by the user
- D. Tags will be filtered below the maximum length of PST selected from the drop down menu by the user
- E. Overall mass error tolerance shows error margin for the whole PST

## Set Protein Modifications Parameters:

Figure 7. PERCEPTRON - Overview of Protein Modifications Parameters

- A. Allows the user to select specified terminal modifications. PERCEPTRON handles four cases: 1) None – No modification, 2) NME – N terminal methionine excision, 3) NME\_ACETYLTATION – N terminal acetylation with initiator methionine removed, and 4) M\_ACETYLTATION – N terminal methionine acetylation
- B. Select whether to perform Blind-PTM search and unknown modifications
- C. Set the tolerance value for Post Translational Modification (PTM) site selection
- D. Allows the user to select instrument specific modification on Methionine
- E. User can opt for required Variable 'Post translation Modifications' from the list of modifications
- F. Allows the user to select instrument specific modification on Cysteine
- G. Similarly, various 'Fixed Modifications' are also selected from the list

## Set Scoring Components Weight:



The screenshot displays the PERCEPTRON web application interface. On the left is a sidebar with navigation links: About Perceptron, Getting Started, Protein Search Query (highlighted), Search Results & History, Sample Data, Help & Manual, Video Tutorials, Report Bugs, Acknowledgment, and Contact. The main header shows the PERCEPTRON logo and a user status of 'Hi guest'. The main content area is titled 'Submit Protein Identification Query'. It contains several sections: 'Basic Parameters' with fields for 'Protein Search Title', 'Intact Protein Mass' (Da), 'Upload Mass-Spectrometry Data File(s)' (0 Files Selected), 'Select Database', 'Enter your E-mail Address \*', 'Recieve Top' (dropdown), and 'Results'; 'Set Experimental Parameters'; 'Set De Novo Sequencing Parameters'; 'Set Protein Modifications Parameters'; and 'Set Scoring Components Weight'. The 'Set Scoring Components Weight' section is highlighted with a red box and labeled 'A'. It contains three sliders for adjusting weights: 'Intact Protein Mass Score Weightage (%)', 'Peptide Sequence Tags Score Weightage (%)', and 'Spectral Comparisons Score Weightage (%)'. At the bottom right are 'Reset' and 'Submit' buttons.

Figure 8. PERCEPTRON - Overview of Scoring Component Weight

- A. Set the respective weights of the Scoring Components from by shifting the slider left or right accordingly

## 7.3 Window 3: Summary and Detailed Results View

**Summary Results View**

Filter

Protein Rank	Protein Name	Protein ID	Molecular Weight	Terminal Modification	No. of Modification(s)	Protein Score
1	Protein 0	Q5TBE3	11359.7861	no	2	0.3159
2	Protein 1	P62805	11473.4114	no	2	0.0340
3	Protein 2	Q99525	11115.1934	no	2	0.0274
4	Protein 3	A6NFX4	11363.5022	no	2	0.0240
5	Protein 4	Q9C005	11355.8325	no	2	0.0227
6	Protein 5	Q9P1G2	11483.6480	no	2	0.0204
7	Protein 6	Q5T752	11334.5254	no	2	0.0183
8	Protein 7	Q9BTM9	11486.0357	no	2	0.0141
9	Protein 8	Q5T292	11431.1326	no	2	0.0136
10	Protein 9	P09341	11407.2531	no	2	0.0129
11	Protein 10	P19875	11494.3653	no	2	0.0129

Items per page: 5 0 of 0 < >

\* Click on the table row for corresponding detailed results.

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Figure 9. Summary Results window showing candidate proteins

Proteins found after the search are reported along with their protein ID, molecular weight and score. Users can click on any protein to see the ‘Detailed Result View’ of the protein. Click on the ‘protein ID’ to go to the detailed UniProt view of the protein.

**Detailed Result View**

User Search Parameters

**General Results**

Protein Rank:	2	Protein ID:	<a href="#">P62805</a>	Protein Name:	P62805
Protein Score:	0.034039	Molecular Weight:	11473.411430	# Matched Fragments:	12
Terminal Modification:	N/A	Truncation:	No	# Modifications:	3

\* Click on the Protein ID to access its information.

**Protein Search Time (in Seconds)**

Total:	00:00:00.0470323	Molecular Weight Module:	N/A	Peptide Sequence Tag Module:	00:00:00.0111953
Spectral Comparison Module:	00:00:00.0000004	Post-Translational Modification Module:	00:00:00.0039530	Truncation Module:	00:00:00.0470323

1. MSGRGKGK 2. LGKGAKRHR 3. KVLRDNIQI 4. TKPAIRRLR 5. RGVKRISGL 6. IYEETRGVLK 7. VFLENVIRDA 8. VTYTEHAKRK 9. TVTAMDVVYA 10. LKRGRTLYG 11. FGG

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Figure 10. Detailed Results window showing candidate proteins



## 8 Search

### 8.1 File Formats

PERCEPTRON provides support for plain text files (data in columns containing mass to charge ratios ( $m/z$ ) and relative intensities), eXtensible Markup Language (XML) files with  $m/z$  and relative abundances (mzXML)<sup>1</sup>, Mass Spectrometry Markup Language (mzML)<sup>2,3</sup> and Mascot Generic Format (MGF)<sup>4</sup> data formats in both single and batch file processing modes.

#### 8.1.1 Raw to mzXML File Format Conversion

User can convert raw data files to mzXML file format by using MS-Convert<sup>5</sup>.

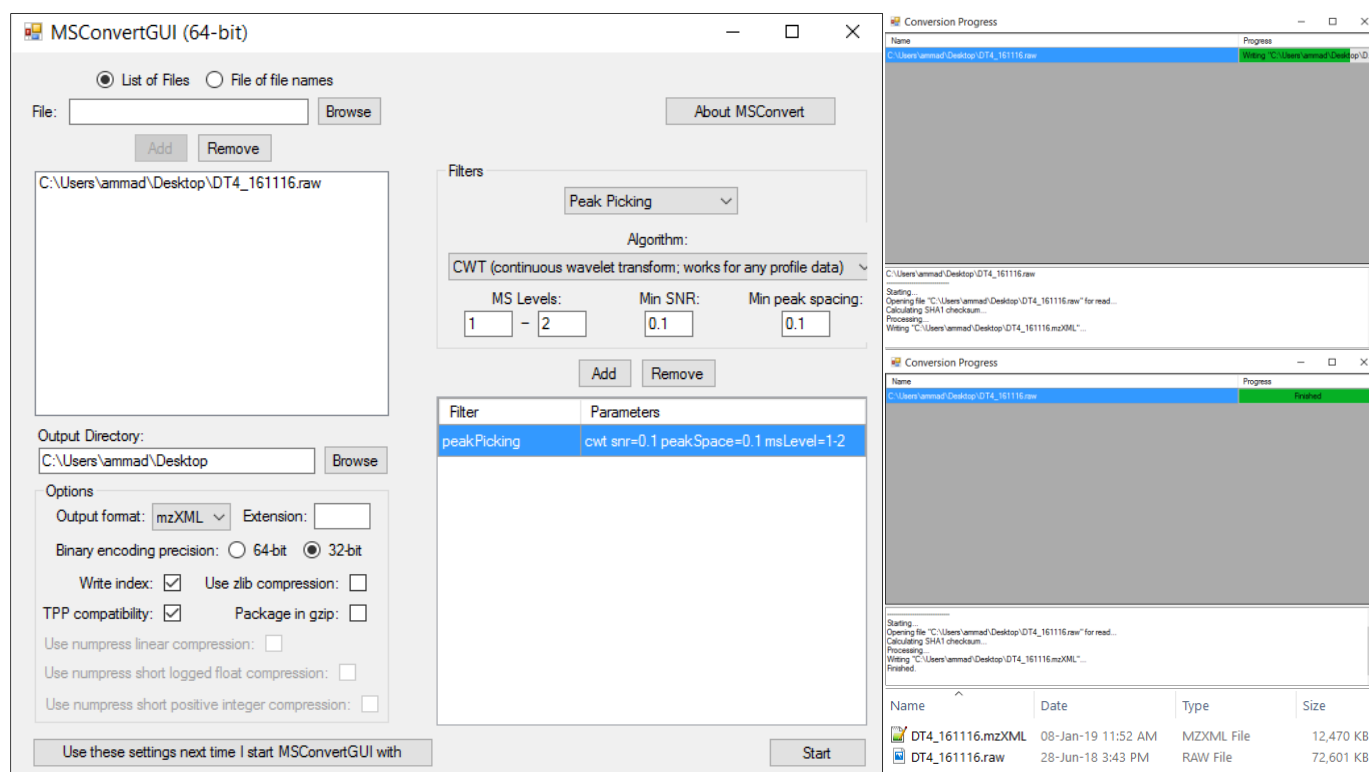


Figure 11. Conversion of raw to mzXML

#### 8.1.2 Raw to mzML File Format Conversion

Raw data files can be converted to mzML file format by using MS-Convert<sup>5</sup>.

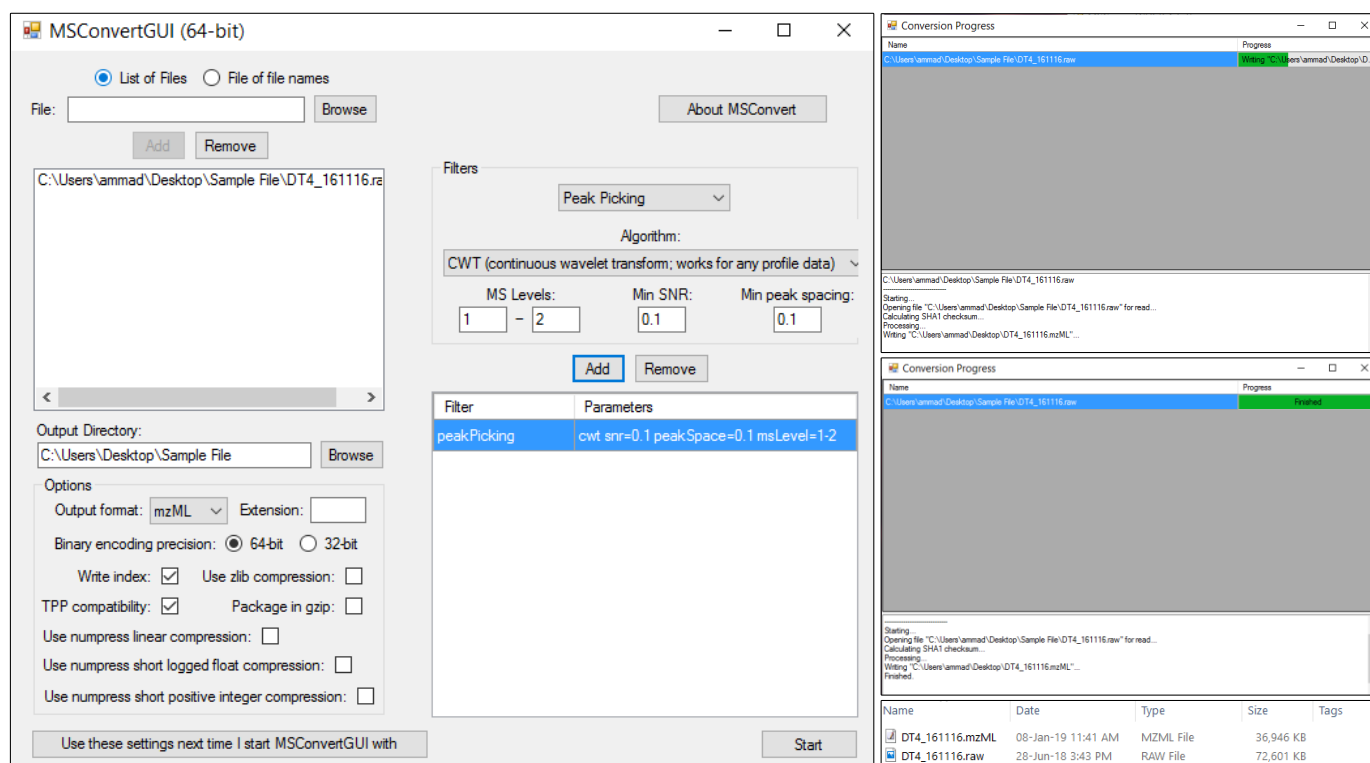


Figure 12. Conversion of raw to mzML

## 8.1.3 MzXML to MGF File Format Conversion

User can convert mzXML files to MGF using MS-Decov<sup>6</sup>.

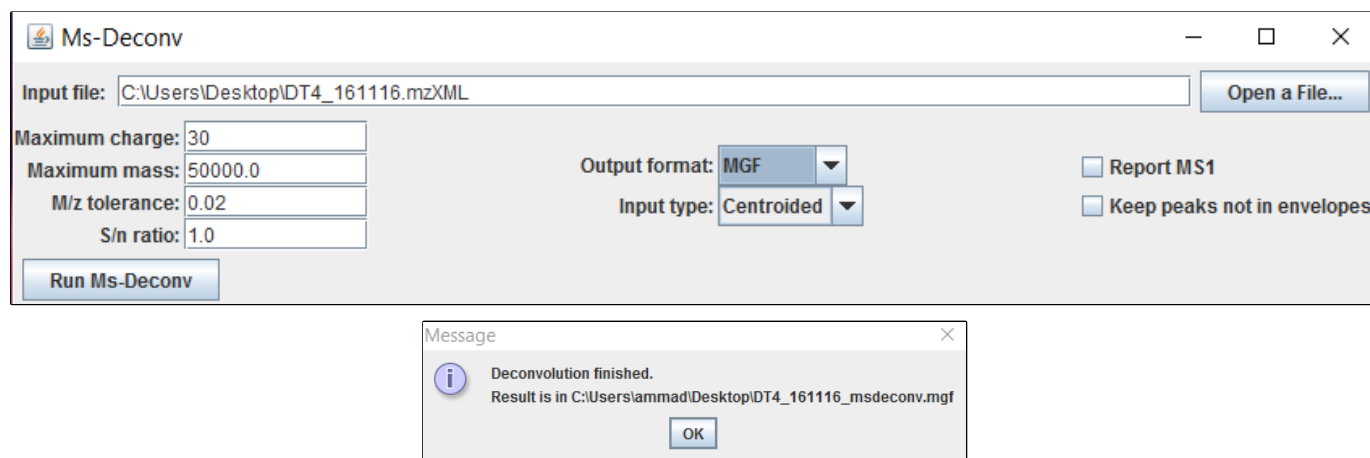


Figure 13. Conversion of mzXML to MGF

## 8.1.4 MGF to Flat Text File Format Conversion

PERCEPTRON converts MGF files to flat text (peak list) using built-in custom file reader.

## 8.2 Parameters

PERCEPTRON can employ from the following set of search parameters including: (i) Default Parameters, and (ii) Selected parameters.

### How to load default Parameters?

To submit the job using default parameters, select ‘Load Default Parameters’ option in front of Basic Parameters.

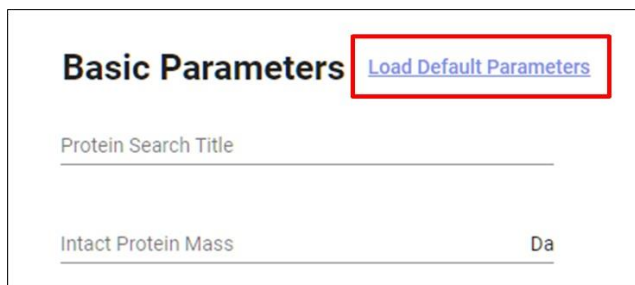
A screenshot of a web form titled "Basic Parameters". In the top right corner of the form, there is a blue text link "Load Default Parameters" which is enclosed in a red rectangular box. Below the title, there are two input fields: "Protein Search Title" and "Intact Protein Mass" (with a "Da" unit label to its right).

Figure 14. Load Default Parameters

## 8.3 Databases

SwissProt database is included in PERCEPTRON by default. User can take any protein sequence from other databases (such as Uniprot) in .fasta format.

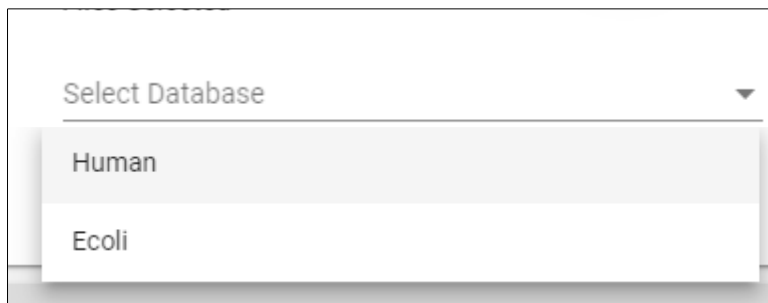
A screenshot of a "Select Database" dropdown menu. The menu is open, showing two options: "Human" and "Ecoli". The "Human" option is highlighted with a light gray background.

Figure 15. Selecting Protein Database

## 8.4 Modes

The search modes are auto-selected based on the number of files that the user inputs. If one file is given as input, PERCEPTRON runs single search mode whereas if multiple files are given as input, the mode switches to batch mode.

- (i) Single Search Mode
- (ii) Batch Mode

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Batch mode takes more processing time as it deals with larger data. The experimental spectra, search parameters and results are automatically stored in the project directory for further processing and visualization.

## 9 References

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