PERCEPTRON-XFMS: An Open-Source Web Server for Analysis of X-ray Footprinting with Mass Spectrometry Data

User Guidelines

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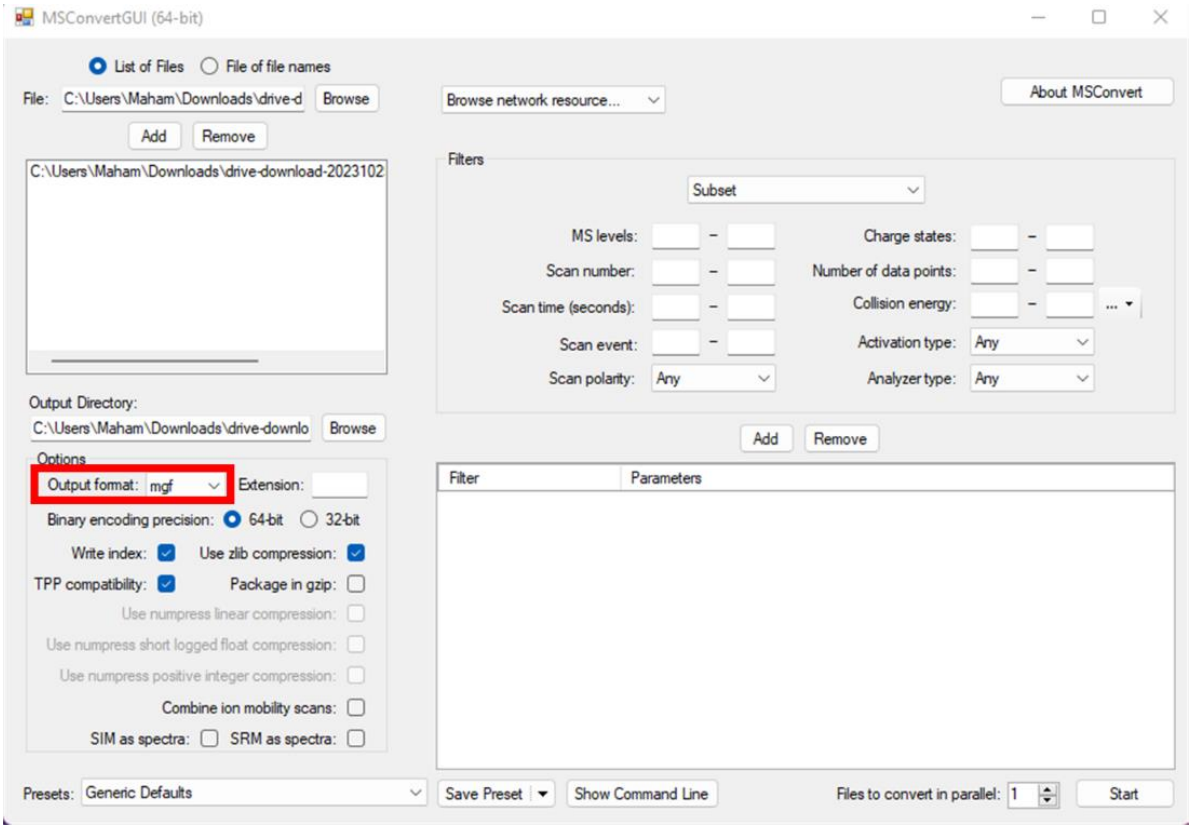
1. Step-by-Step Guidelines for using MASCOT

PERCEPTRON-XFMS is a highly interactive web server developed to assist in XFMS data analysis. The pipeline initiates with input of LC-MS/MS data along with parameter files. Upon receiving the dose-dependent LC-MS/MS data, the user must perform peptides identification process using MASCOT. The step-by-step process for CheY protein is listed below.

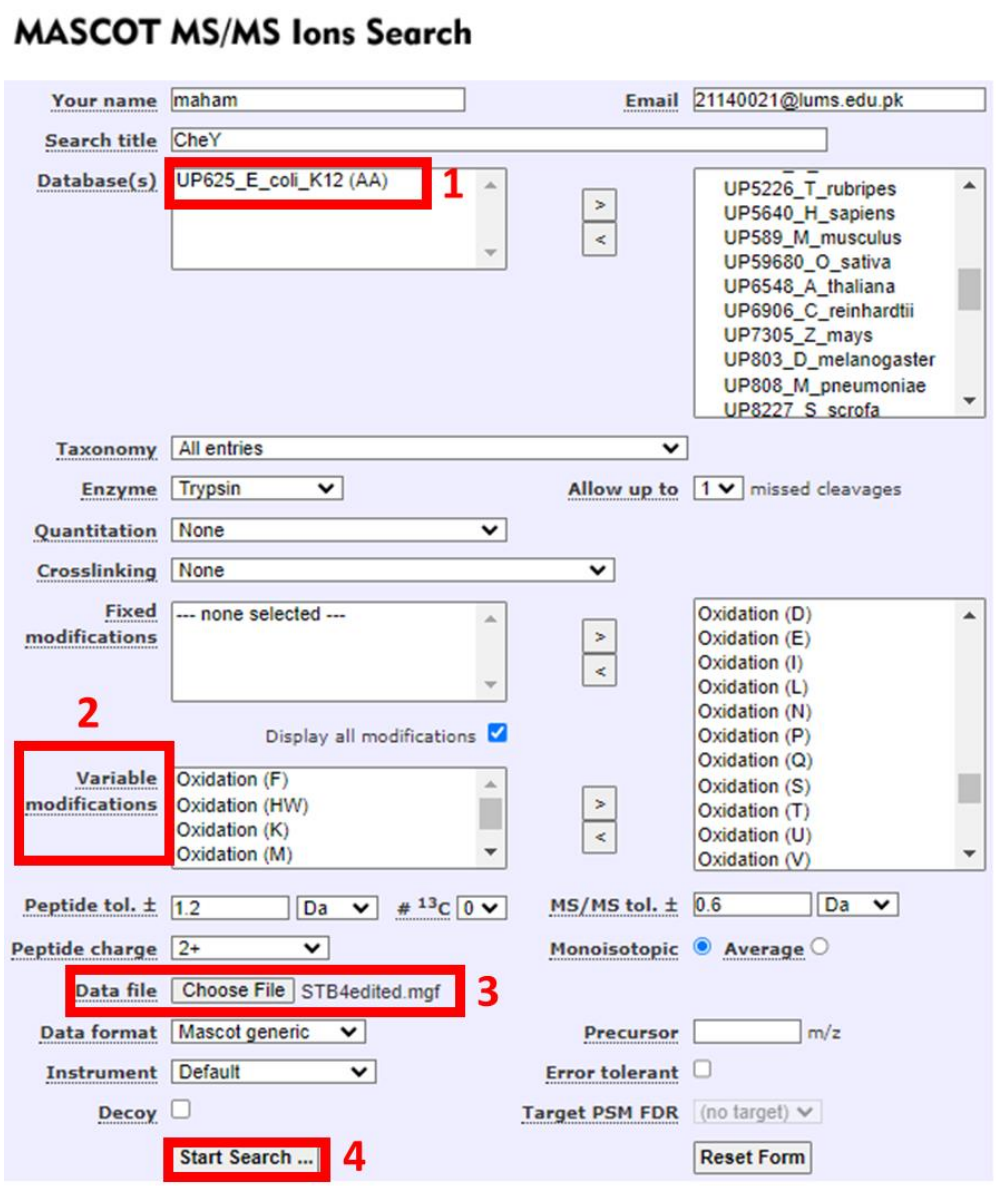
A. Mgf file for MASCOT

1. Open MSConvert GUI and browse the MS/MS file with the highest dosage.
2. Select the output format as '.mgf' and press the 'Start' button.



B. Generating Mascot Result File

1. Open Mascot MS/MS Ions search through the following link and select: UP625_E_Coli_K12 (AA) database
2. Select the desired variable modification.
3. Upload the '.mgf' file.
4. Start searching and download the results.



2. Preparation of Input Files

Create 2 separate folders for “MS/MS Input Files” containing (1) experimental data, and (2) “Additional Input Files” having the following parameters.

I. **Folder 1 (MS/MS Input Files) -** This folder contains the LC-MS/MS mzXML experimental files.

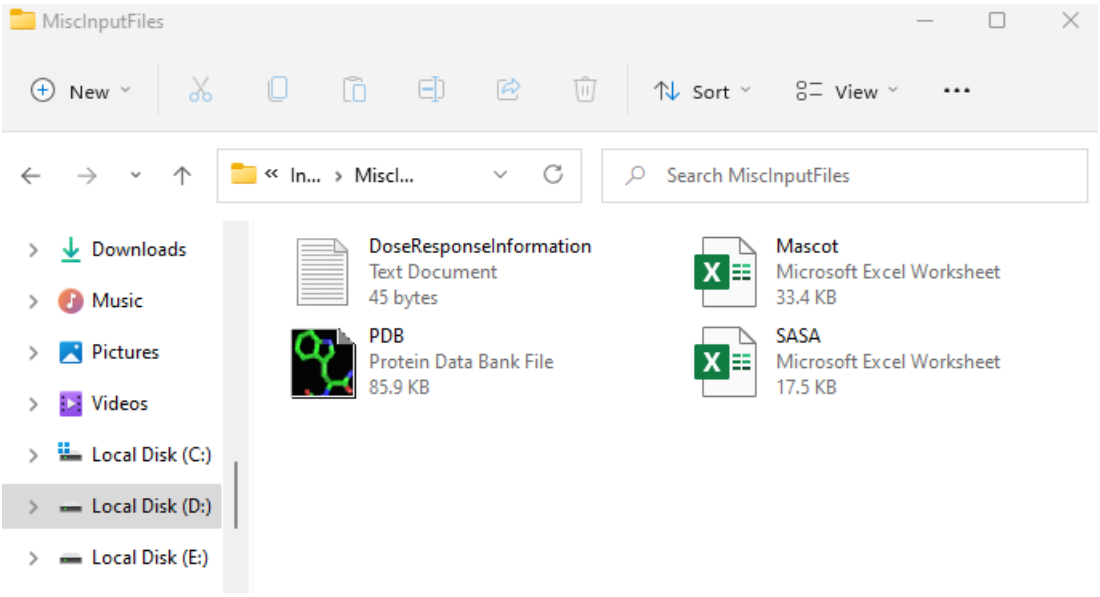
II. **Folder 2 (Additional Input Files) -** This folder contains the following files.

a) **Mascot.xlsx** – Mascot result file

b) **Protein_structure.pdb** – protein structure file

c) **SASA.xlsx** – Solvent accessible surface area (SASA) file generated by POPS server. The file includes detailed residue SASA information including Residue Name, Chain, Residue Number, Hydrophobic SASA (Phob/A^2), Hydrophilic SASA (Phil/A^2), Total SASA (Total/A^2), Relative SASA (Q(SASA)), Number of Overlaps (N(overlap)), and Surface Area (Surf/A^2). Currently, PERCEPTRON-XFMS plots the log(PF) value against the Total SASA by default.

d) **DoseResponseInformation.txt** – A text file that contains the X-ray doses used in the study.


MiscInputFiles
New
Sort
View
In... › Miscl...
Search MiscInputFiles
Downloads
Music
Pictures
Videos
Local Disk (C:)
Local Disk (D:)
Local Disk (E:)
DoseResponseInformation
Text Document
45 bytes
Mascot
Microsoft Excel Worksheet
33.4 KB
PDB
Protein Data Bank File
85.9 KB
SASA
Microsoft Excel Worksheet
17.5 KB

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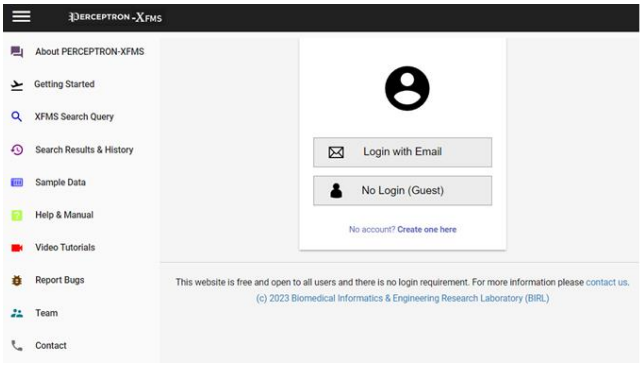
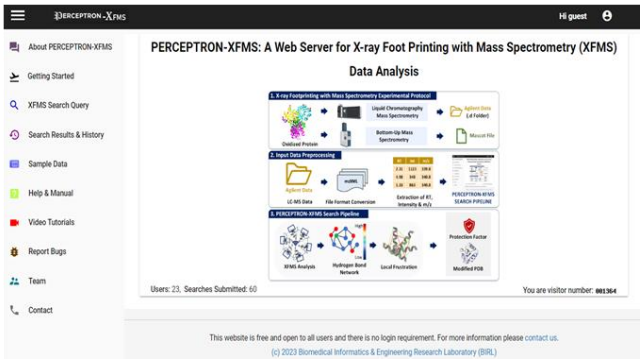
3. Step-by-Step Guidelines for Using Webserver

Step 1- Login PERCEPTRON-XFMS as a guest (without user account) or register for PERCETRON-XFMS user account to login using an existing email account.

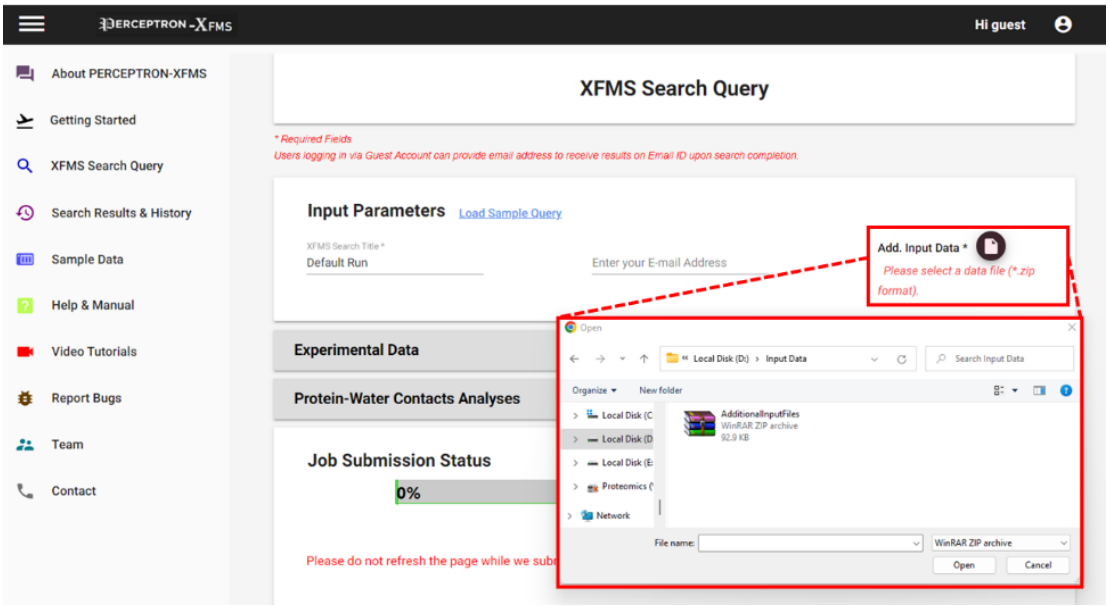




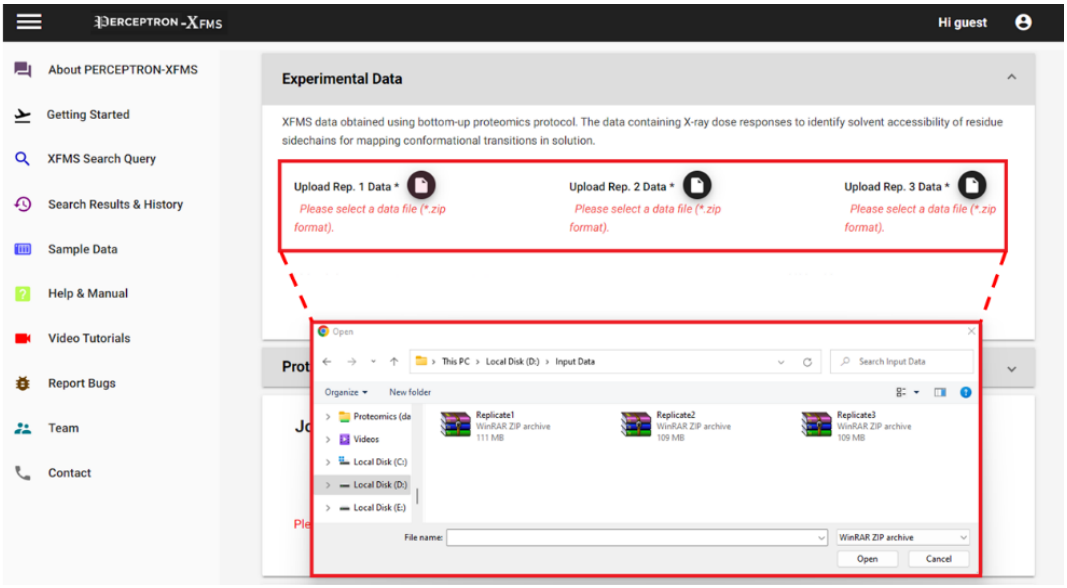
Step 2 - Input Parameters

Go to the 'XFMS Search Query' in the menu on the left and enter 'XFMS Search Title' to start data analysis.

Browse for the zipped folder containing the additional input data files



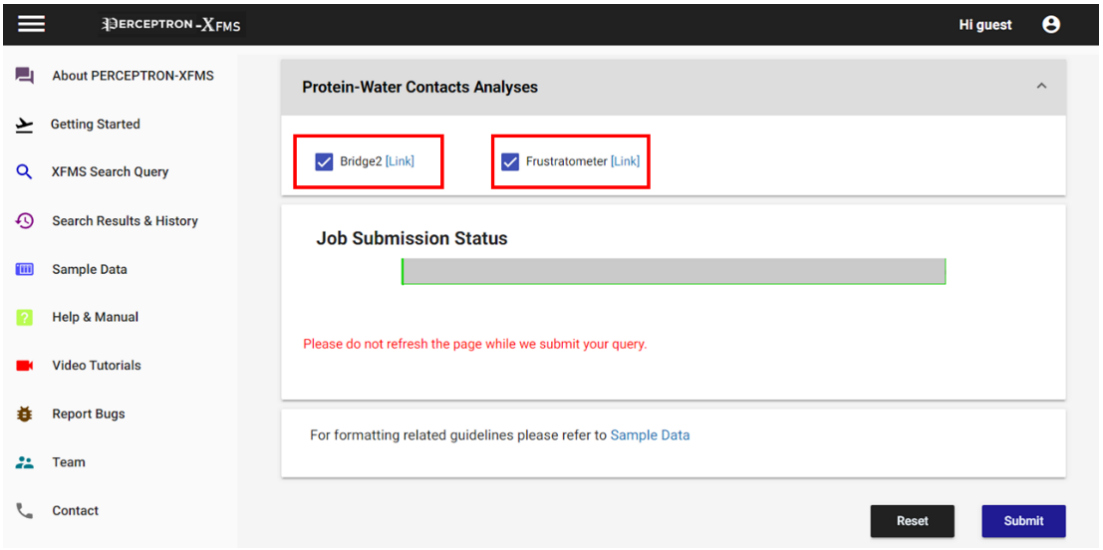
Step 3 - Upload the LC-MS/MS experimental data. PERCEPTRON-XFMS requires experimental mzXML-formatted data to be arranged as three separate replicates for each X-ray dose. Specifically, the user must provide three replicates per dose, with each replicate stored as an individual mzXML file. The total number of mzXML files required is n × 3, where n is the number of doses used in the experiment. Names of files must strictly follow the same naming structure as the DoseResponseInfo file i.e., “Dose0.mzXML” etc.



Step 4 – Protein Contact Analyses (Advanced Users)

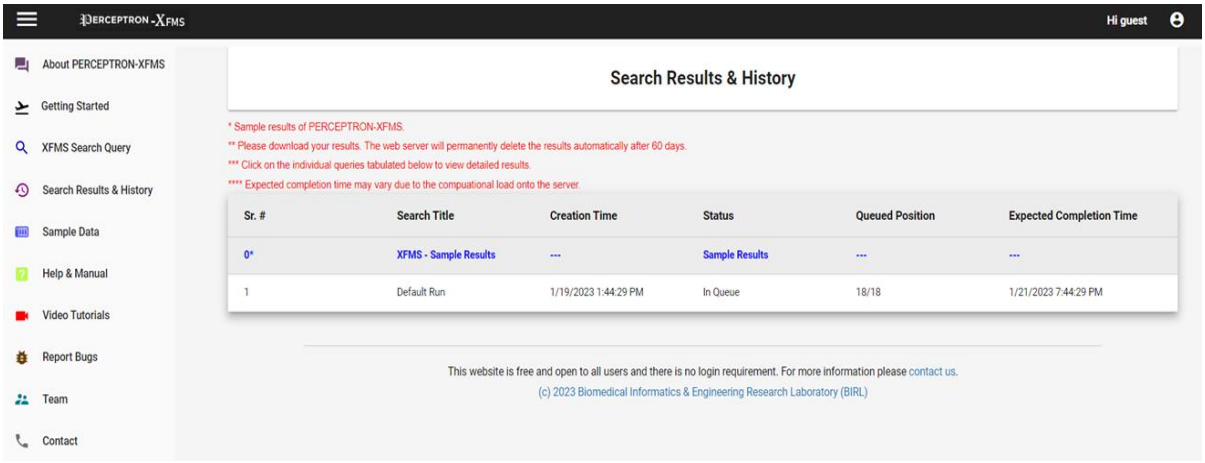
To analyze protein-water hydrogen bond graphs click the ‘Bridge Algorithm’ box.

Click on the ‘Frustratometer Algorithm’ box for additional analysis of protein residue energetics.

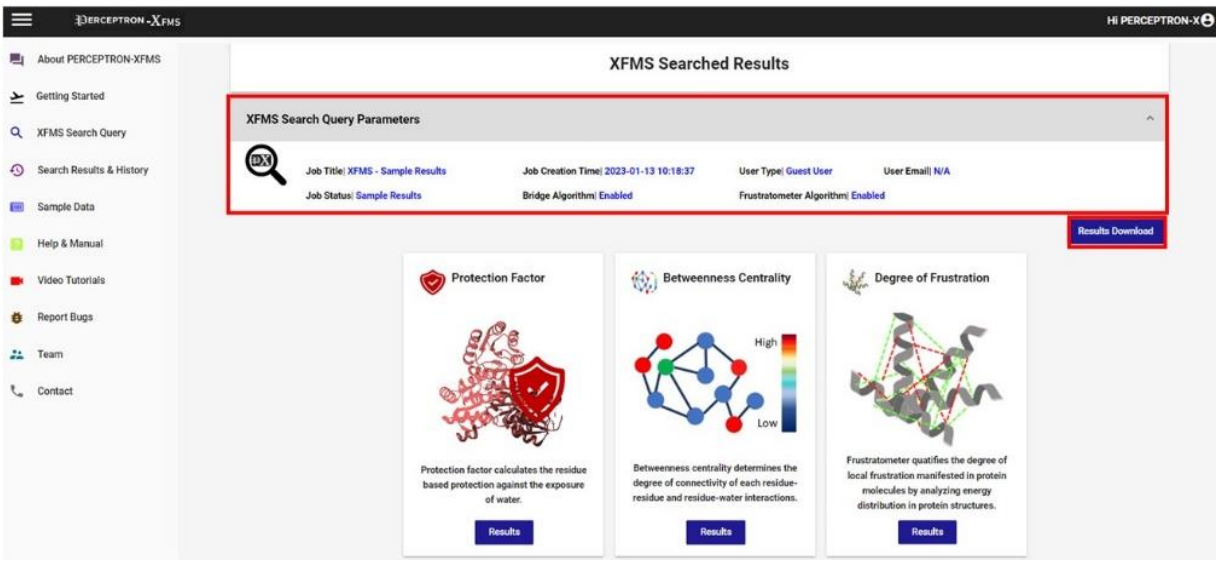


Submit the Query Search by clicking on the 'Submit' button.

Step 5- Click on 'Search Results & History' to see the search results. Click on your "Search Title" row.



Step 6 – Download results by clicking **'Results Download'** button option provided on top right. The results will be downloaded as a *.zip file.



A. Downloadable Result Files

Downloaded result (*.zip) file contains multiple following subfolders and files.

i. Several subfolders correspond to different peptides in the protein and each folder contains:
   a) Subfolders containing rate of oxidation for individual replicate.
   b) Dose-response plots of the rate of oxidation.
   c) **Mean_R1_R2_R3.xls** file contains the mean values of (1 - Rate of oxidation) for each modified residue reported in that peptide.

ii. **PeptideInfo.xls** file which includes information regarding the peptide sequence and their corresponding peptide folder.

iii. **PF_SASA_tab.xls** - contains a table with log protection factor of each modified residue.

iv. **SASAmain.png** - a graphical representation of the protection factor data with SASA values on the x-axis and log (PF) on the y-axis.

v. **Modified.pdb -** a pdb file incorporated with log (PF) values.

vi. **ResultsBridge.xlsx -** an Excel file that contains centrality scores of the residues using the Bridge algorithm.

vii. **FrustratometeR-Results** include images that quantify the degree of local frustration in each protein. Users also get tables with the calculated Frustration indexes and scripts to locally explore and manipulate the structures.