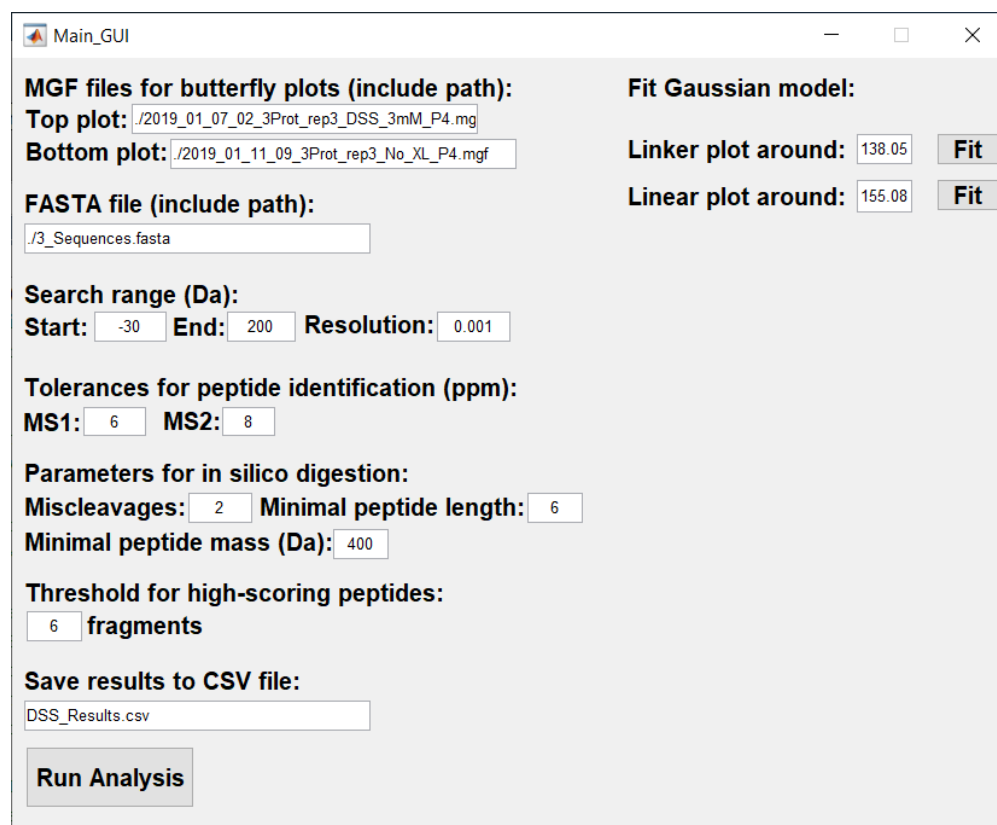


Requirements: This analysis package requires MATLAB 2015 (or newer) to be installed on your computer.

Installation: Unzip the folder. The folder contains all the required MATLAB scripts, and example files of DSS cross-linking data.

Running instruction:

1. Run MATLAB. Change your MATLAB working directory to the unzipped folder.
2. In the MATLAB Command Window write “run Main_GUI” and press enter. The following window will open:



The screenshot shows a MATLAB window titled "Main_GUI" with the following parameters and controls:

- MGF files for butterfly plots (include path):**
 - Top plot:
 - Bottom plot:
- FASTA file (include path):**
 -
- Search range (Da):**
 - Start: End: Resolution:
- Tolerances for peptide identification (ppm):**
 - MS1: MS2:
- Parameters for in silico digestion:**
 - Miscleavages: Minimal peptide length:
 - Minimal peptide mass (Da):
- Threshold for high-scoring peptides:**
 - fragments
- Save results to CSV file:**
 -
- Fit Gaussian model:**
 - Linker plot around:
 - Linear plot around:
-

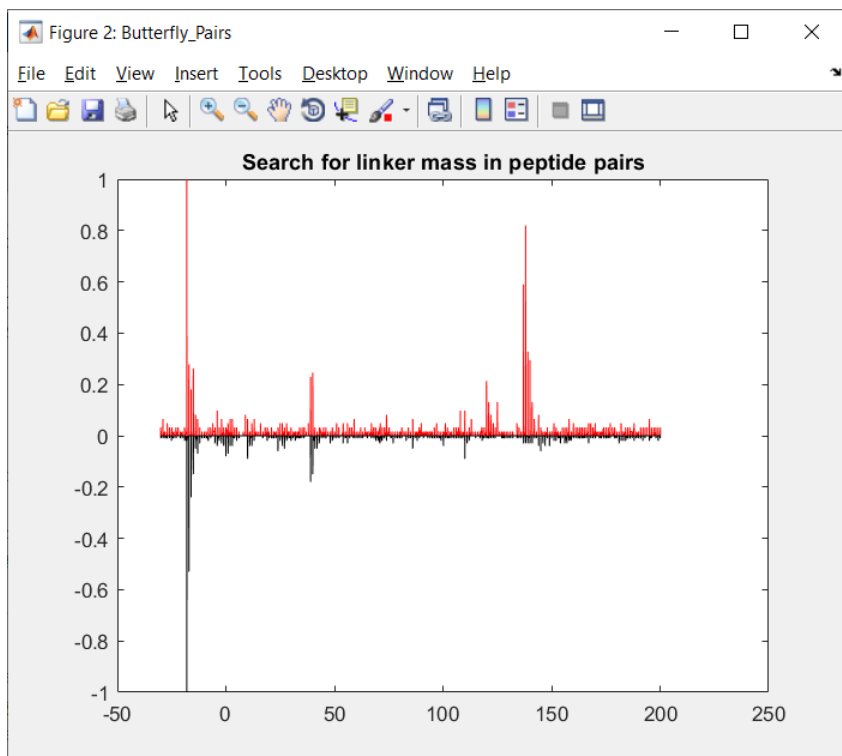
The values appearing in the window are those used for the analysis in the paper. They are a good starting point for a search.

3. Enter the name of the MGF file of the **cross-link** sample into the box “Top plot”. The corresponding example file is the default.
4. Enter the name of the MGF file of the sample that was **not** cross-linked into the box “Bottom plot”. The corresponding example file is the default.
5. Enter the name of the FASTA file with the relevant sequences into the box “FASTA file”. The corresponding example file is the default.
6. Set the search range. Wider range will increase the run time.

7. Press “Run Analysis”.

8. The following actions will follow: (i) The MGF files will be read and the spectra de-ionized. The results are stored in a file with the extension of “_fastload”. This may take a few minutes. Once the “_fastload” files are created, they are not computed again, so that successive runs are faster. (ii) The MS1 offset of each MGF file is estimated. (iii) The main analysis is started and its progress is shown in the MATLAB command window. The main analysis is run once for the Top plot (cross-link) and once for the Bottom plot (No XL). This may take a few minutes.

9. Once the analyses are done, the following window will appear showing the butterfly plot contrasting the XL (top red)/NoXL (bottom black) conditions. Here for example you can see the peaks of 138 and 120 Da exclusive for DSS. You can **zoom** in on any specific peak.



10. A similar window to the one above is opened, showing the modifications on linear peptides.

11. The data used to make the butterfly plots are also exported to a CSV file that you can open in Excel.

12. You can fit a Gaussian around any peak by using the “Fit” buttons on the right of the GUI (see below). The parameters of the Gaussian are printed in the command window.

