1. Click “open file” button to select the MS data. MS data should be converted to MS1 format in advance. Multiple selection is allowed.
2. Click “Tool” menu strip and click “Selenoproteins”. In this form, target selenopeptides and modifications on amino acids can be set. In the two sheets, New row can be added by double clicking the blank next to the last row in the first column. Double click the blank to add or modify text, then single click other part of the sheet to confirm the change.
   a) Set modifications in the first sheet. The first column should be the 1-letter symbol of the amino acid (selenocysteine is represented by “U”). The second column should be the element difference of the modified amino acid and the original amino acid. The format should be “E1N1E2N2···EnNn”, where En is the element and Nn is the number of this element. For example, the acetylation on lysine should be set as “K” in the first column and “C2H2O1” in the second column.

Amino Acid	Static Modification
K	C2H2O1

   b) Set target selenopeptides in the second sheet. The first column should be the corresponding protein of the selenopeptide (format is not required), and the second column should be the peptide sequence. The third column should be the observed charge of this peptide in MS data. For example, if the peptide “VLLIENVASLUGTTVR” of GPX1 is observed with charge of +2 and +3, it should be set as the figure below.

Protein	Peptide	Charge
GPX1	VLLIENVASLUGTTVR	+2
GPX1	VLLIENVASLUGTTVR	+3

   c) Use “Save” button to save the configuration. Then it can be retrieved by clicking “Load” button to select the configuration file in future.
   d) Click “OK” to save the change and back to main form.
3. Click “Start” to start computation. The result can be found in the same directory of the MS data. It’s recommended to open the result file by Excel.