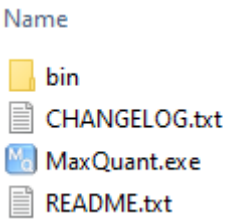
For this demo, we will analyze explore proteomics data and run a search using MaxQuant

Getting the data

1. MaxQuant can be downloaded from https://www.maxquant.org/ or alternatively https://drive.google.com/file/d/15mAi0UO8CLx0XYBZ_lD6PGjLWpUZGeFj/view?usp=sharing if you have problem
2. Mass spectrometry data (.raw files) and protein database (.fasta) can be downloaded from https://drive.google.com/drive/folders/1ddfY1TBVty0gueVhbex_IfGujuD_ikR1?usp=sharing
    a. The data came from https://zenodo.org/record/4274987 and are proteomics data of healthy human serum sample, digested with Trypsin and analyzed on a Q-Exactive Plus mass spectrometer
3. MZmine tool for visualizing mass spectra can be downloaded from https://github.com/mzmine/mzmine3/releases/tag/v3.2.8 (select the _portable file if you don't want to install it)
4. A proteomics paper where we can learn to spot key protocol details https://www.dropbox.com/s/bni0chcs1kp4nfj/3000788_Fall2022_L16_MS_paper_100622.pdf?dl=0

Setting up software

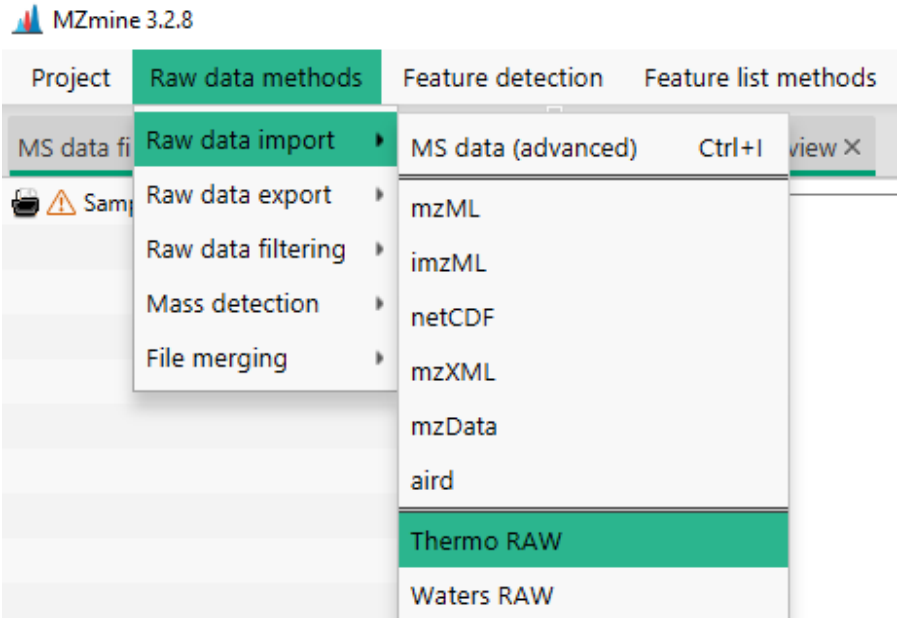
Just unzip the MaxQuant and MZmine (if you selected the_portable version). If there is an error about Microsoft .NET framework, please follow the link to download and install .NET.



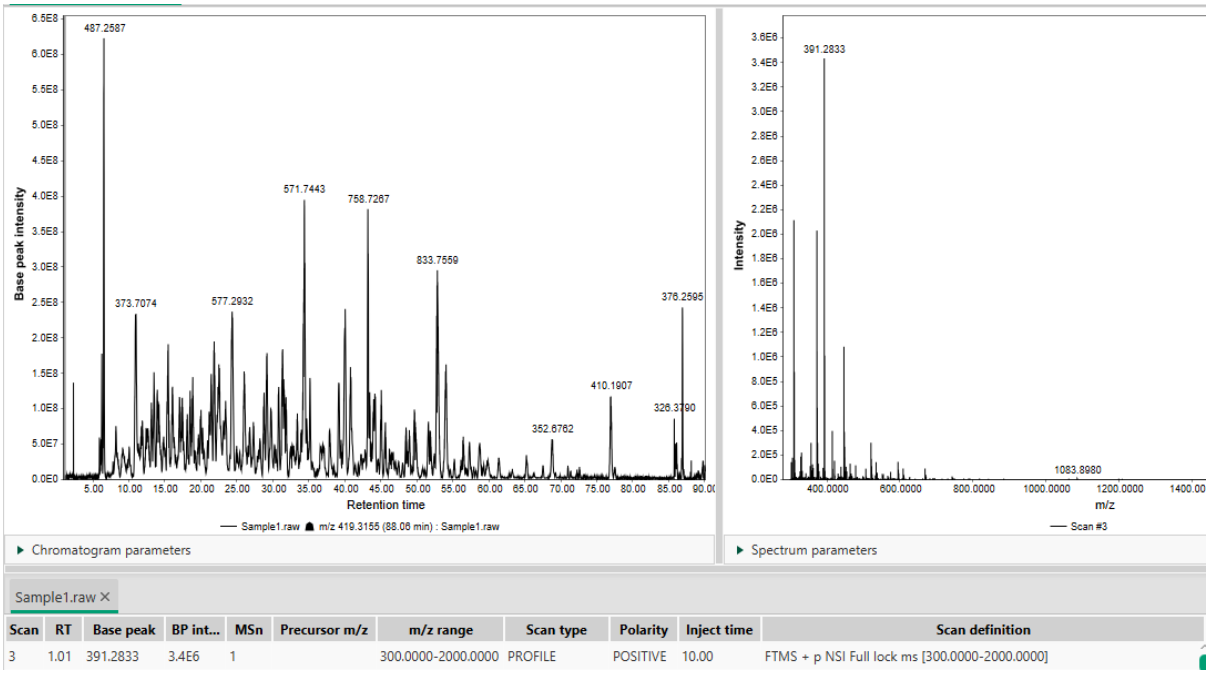
Running the demo

Section 1: MS data exploration

1. The .raw files came from Thermo Scientific's mass spectrometer machine. We can view the spectra in MZmine by specifying the import format as **Thermo RAW** as shown below



2. Once imported, you will be shown the **Chromatogram** (left panel). This show the most intense ions that eluded out from liquid chromatography into the mass spectrometer.

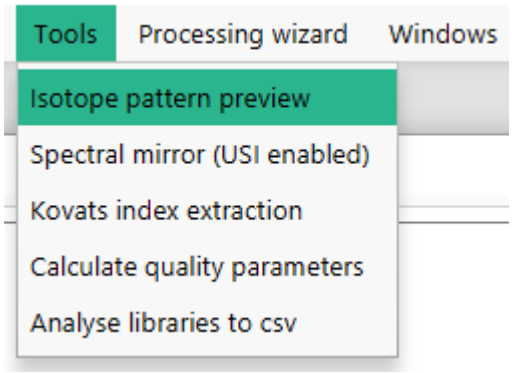


3. At the bottom, you will see the list of all spectra (sorted by scan number, or time). The **MSn** column tells us whether the spectrum is **MS1** (of intact peptides) or **MS2** (of fragmented peptides). **MS1** is like a survey of existing peptides while **MS2** provides signature ions that can be matched to protein/peptide databases.

Sample1.raw ×

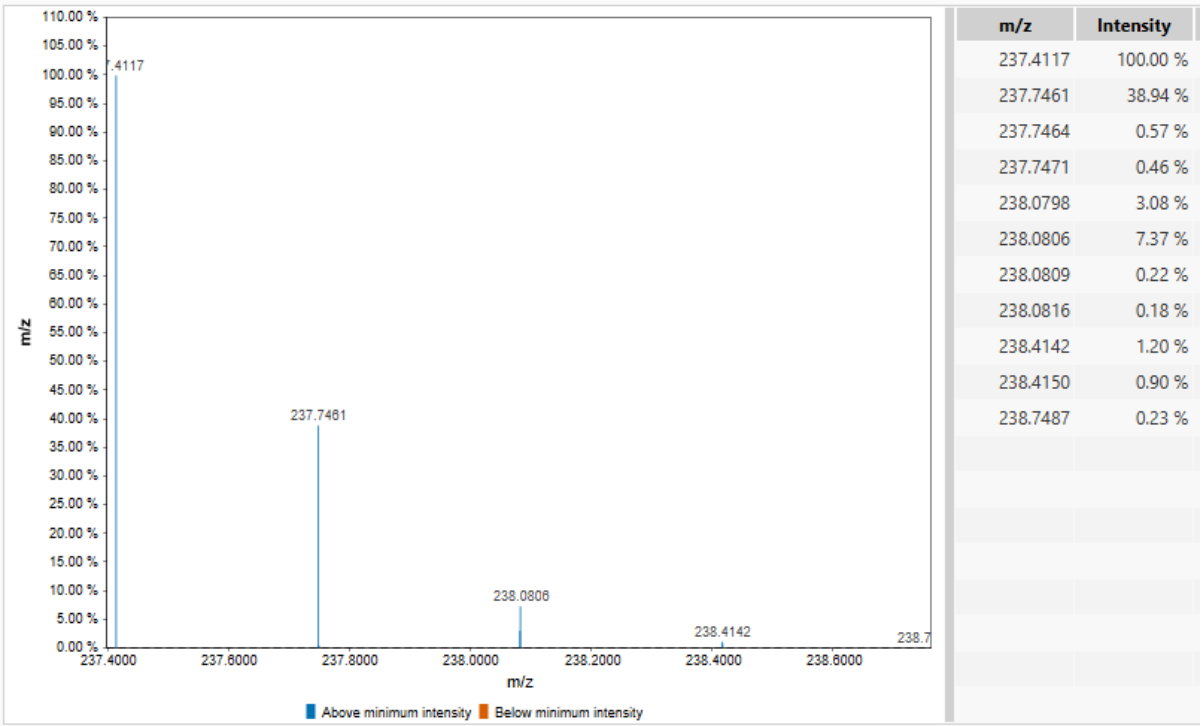
Scan	RT	Base peak	BP int...	MSn	Precursor m/z	m/z range	Scan type	Polarity	Inject time	Scan definition
1	1.00	391.2833	3.6E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
2	1.01	391.2833	4.0E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
3	1.01	391.2833	3.4E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
4	1.02	391.2832	3.8E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
5	1.02	310.0276	6.7E4	2	388.0055	80.6667-1210.0000	CENTROIDED	POSITIVE	80.00	FTMS + c NSI d Full ms2 388.0055@hcd25.00 [80.6667-1210.0000]
6	1.02	391.2834	3.9E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
7	1.03	391.2850	3.4E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
8	1.03	391.2833	3.7E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
9	1.04	391.2834	3.7E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
10	1.04	391.2834	3.9E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
11	1.05	391.2833	3.9E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
12	1.05	391.2834	3.6E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
13	1.06	391.2834	3.5E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
14	1.06	391.2833	3.9E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
15	1.07	391.2833	3.7E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
16	1.07	391.2835	5.2E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
17	1.08	391.2834	3.8E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]
18	1.08	97.0412	6.5E3	2	381.8745	53.3333-800.0000	CENTROIDED	POSITIVE	80.00	FTMS + c NSI d Full ms2 381.8745@hcd25.00 [53.3333-800.0000]
19	1.09	298.9213	2.4E4	2	376.8994	78.3333-1175.0000	CENTROIDED	POSITIVE	80.00	FTMS + c NSI d Full ms2 376.8994@hcd25.00 [78.3333-1175.0000]
20	1.09	391.2833	3.4E6	1		300.0000-2000.0000	PROFILE	POSITIVE	10.00	FTMS + p NSI Full lock ms [300.0000-2000.0000]

4. We can visualize isotopic envelope pattern using the tools shown below.



5. Given a chemical formula, $C_xH_yN_zO_w$, we can compute the expected abundance of all isotopes of this molecule based on the frequencies of naturally occurring C^{13}, C^{14}, N^{15}, and O^{18}.

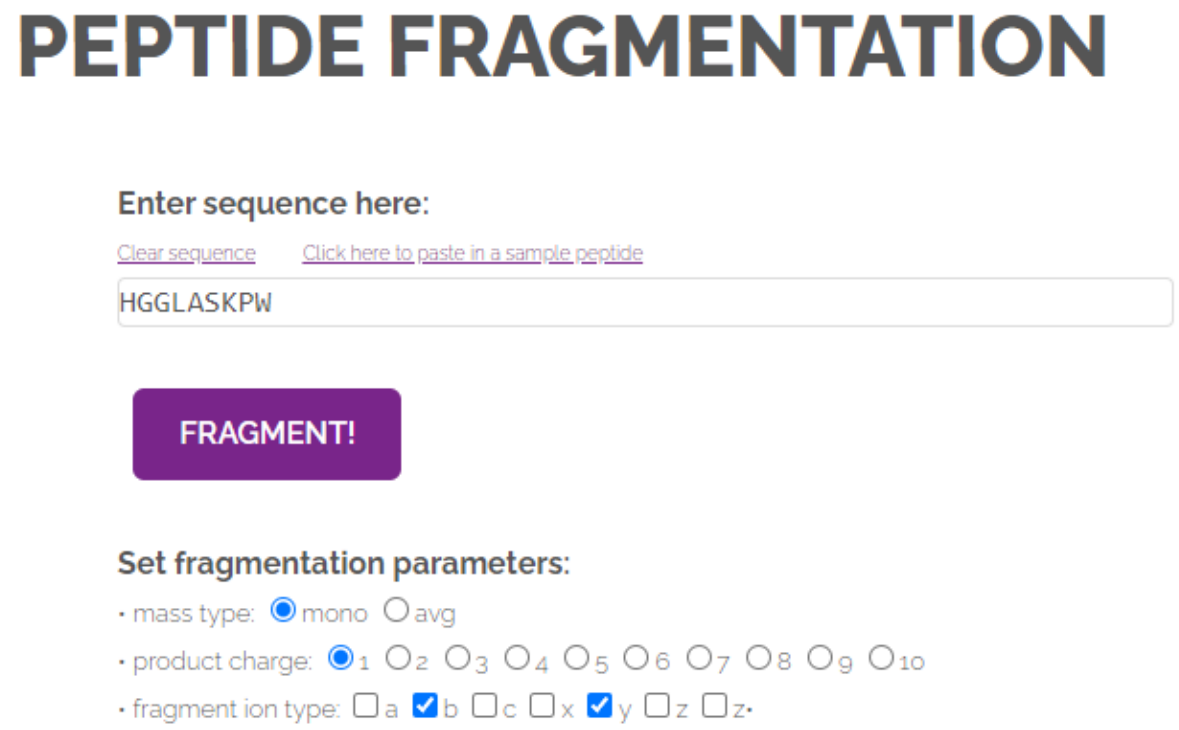
Please set the parameters



m/z	Intensity
237.4117	100.00 %
237.7461	38.94 %
237.7464	0.57 %
237.7471	0.46 %
238.0798	3.08 %
238.0806	7.37 %
238.0809	0.22 %
238.0816	0.18 %
238.4142	1.20 %
238.4150	0.90 %
238.7487	0.23 %

Chemical formula	C36H40O15
Minimum intensity	0.1 %
Merge width (m/z)	0.0001
Charge	3

6. To visualize the process of peptide fragmentation, we use an online tool https://proteomicsresource.washington.edu/cgi-bin/fragment.cgi. Let's input a peptide **HGGLASKPW**.

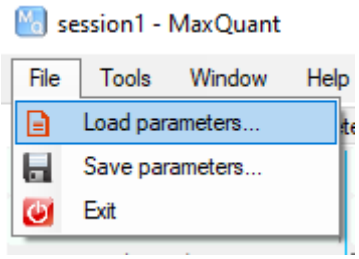


7. The tool will show all b- and y-ions that could be produced from this peptide. The default setting is to show the monoisotopic masses (without extra neutron) and to the show the masses at charge state of +1.
    a. b-1 ion (from the N-terminus) consists of only a Histidine. Histidine's molecular weight is 137.06. The weight shown is Histidine with an addition of a proton = 138.066.
        i. b-ions at +1 charge = 1 + total amino acid molecular weight
    b. y-1 ion (from the C-terminus) consists of only a Tryptophan. Tryptophan 's molecular weight is 186.08. The weight shown is Tryptophan with an addition of H_2O + proton = 205.097.
        i. y-ions at +1 charge = 19 + total amino acid molecular weight
    c. The mass difference between adjacent ions is equal to amino acid mass
        i. Glycine's weight is 57.02 = 195.088 – 138.066 = 252.109 – 195.088

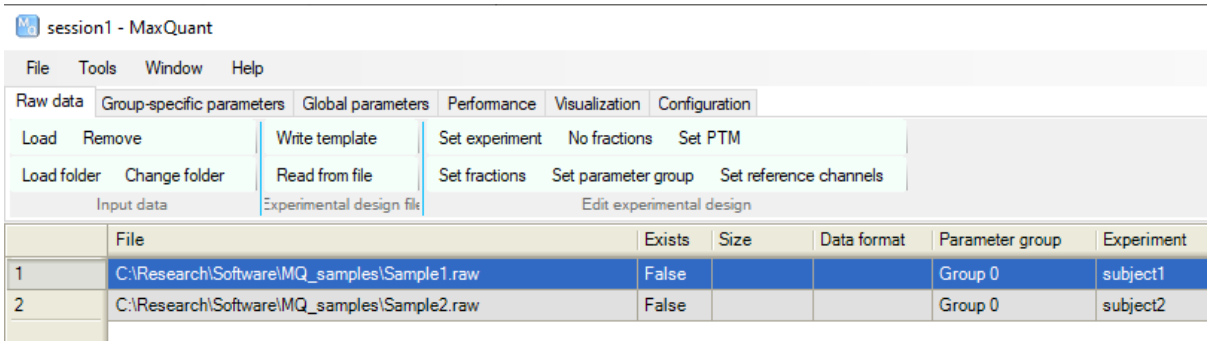
b^+		AA		y^+
138.066188	1	H	9	
195.087652	2	G	8	815.441015
252.109116	3	G	7	758.419551
365.193180	4	L	6	701.398087
436.230294	5	A	5	588.314023
523.262322	6	S	4	517.276910
651.357285	7	K	3	430.244881
748.410049	8	P	2	302.149918
	9	W	1	205.097154

Section 2: Protein/peptide search with MaxQuant

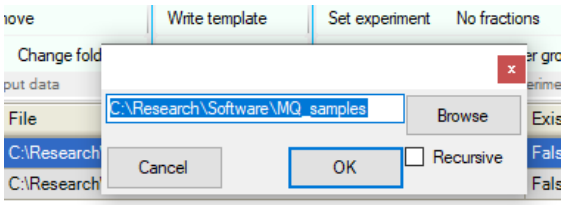
1. We will use MaxQuant to perform the analysis. First, import the parameter file mqpar.xml into the software. This contain all settings that are specific to the data being analyzed.



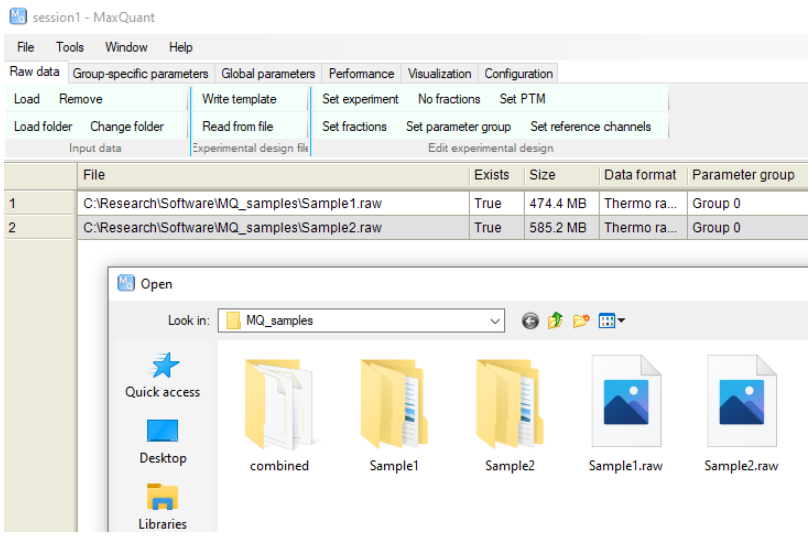
2. This will also import the location of .raw files and the experimental design (experiment name and group ID, etc.). You may notice that the **Exists** column says **False**. This is because the path to the .raw file that I used is likely different from where you put the files.



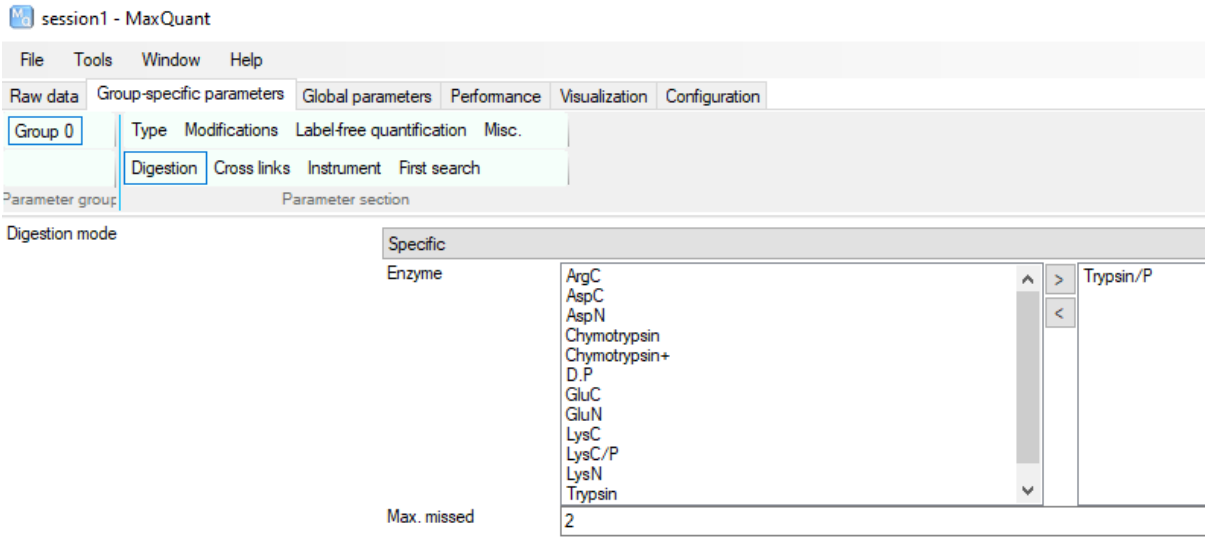
3. Click the **Change folder** button to point MaxQuant to the folder where you placed .raw files.



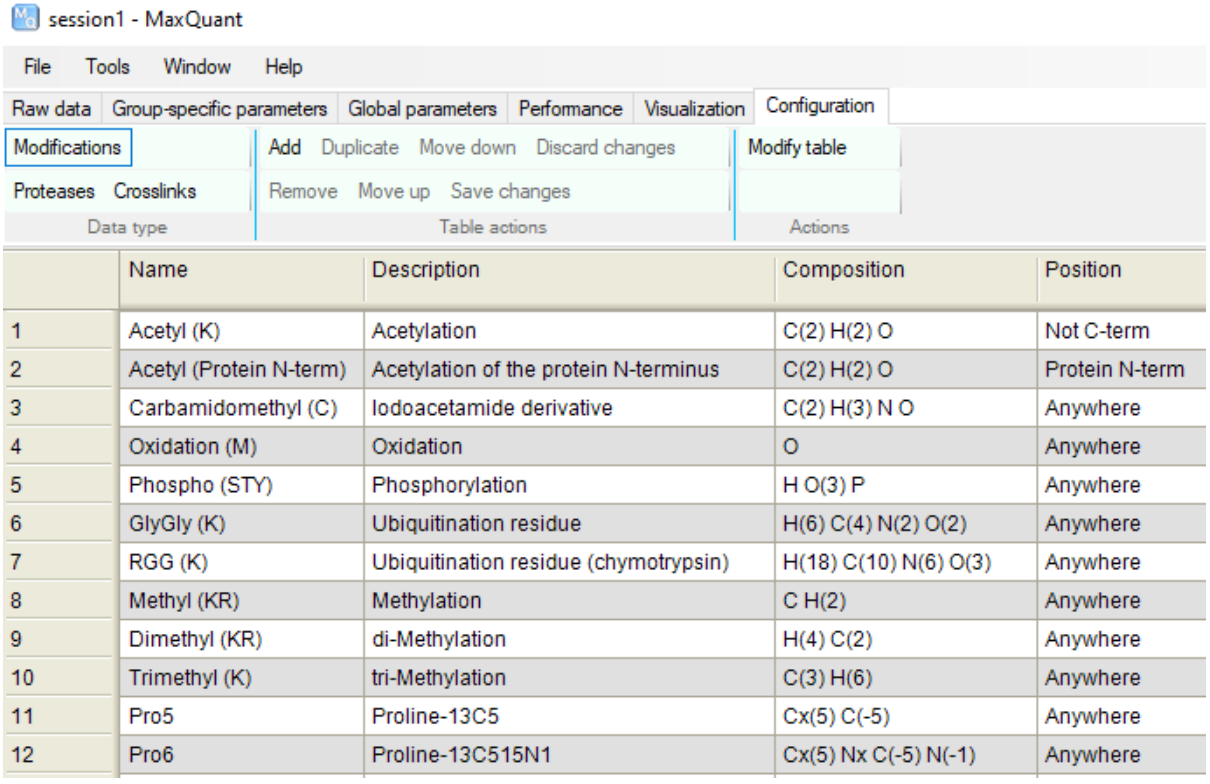
4. Alternatively, you can load individual .raw file or a folder containing multiple .raw files using the **Load** and **Load folder** buttons.



5. Next, let us explore the vast settings in MaxQuant (we will do this in class). Proteomics analysis is highly specific to the experimental conditions and mass spectrometer setups. So, if you want to re-analyze public data, you need to understand the entire protocol.



6. We can also add new post translational modification or chemical modification to be included in the protein/peptide search. This functionality is in the **Configuration** tab.



	Name	Description	Composition	Position
1	Acetyl (K)	Acetylation	C(2) H(2) O	Not C-term
2	Acetyl (Protein N-term)	Acetylation of the protein N-terminus	C(2) H(2) O	Protein N-term
3	Carbamidomethyl (C)	Iodoacetamide derivative	C(2) H(3) N O	Anywhere
4	Oxidation (M)	Oxidation	O	Anywhere
5	Phospho (STY)	Phosphorylation	H O(3) P	Anywhere
6	GlyGly (K)	Ubiquitination residue	H(6) C(4) N(2) O(2)	Anywhere
7	RGG (K)	Ubiquitination residue (chymotrypsin)	H(18) C(10) N(6) O(3)	Anywhere
8	Methyl (KR)	Methylation	C H(2)	Anywhere
9	Dimethyl (KR)	di-Methylation	H(4) C(2)	Anywhere
10	Trimethyl (K)	tri-Methylation	C(3) H(6)	Anywhere
11	Pro5	Proline-13C5	Cx(5) C(-5)	Anywhere
12	Pro6	Proline-13C515N1	Cx(5) Nx C(-5) N(-1)	Anywhere