Proteomics data analysis

MS/MS data were analysed for protein identifications using MaxQuant 1.6.14 [[1](#_ENREF_1)] with the in-built Andromeda search engine [[2](#_ENREF_2)]. The raw files were searched against the UniProt [[3](#_ENREF_3)] mouse uniport proteome UP000000589, last modified on June 28, 2020 supplemented with the sequence of the BACE1-BirA construct. The mass tolerance was set to 4.5 ppm for precursor ions and trypsin was set as proteolytic enzyme with two missed cleavage allowed. Carbamidomethyl on cysteine, was set as fixed modifications. Oxidation of methionine, Acetylation of Protein N-term, Biotinilation, Deamidation of Asparagine and Glutamine, Dioxidation of Methionine and Tryptophan, and N-terminal Glutamate to Pyroglutamate Conversion were set as variable modification. The false-discovery rate for protein and peptide level identifications was set at 1%, using a target-decoy based strategy. The minimum peptide length was set to seven amino acids and protein quantification was performed on unique plus razor peptides [[4](#_ENREF_4)]. “Reverse Hits”, “Only identified by site” and “Potential contaminant” identifications were filtered out. Only protein groups with at least two unique peptide sequences and Andromeda protein score greater than 1 were selected for further quantification. For the differential protein expression analysis, the iBAQ values were analysed with the ProtRank package [[5](#_ENREF_5)] by comparing the three control samples versus the three 24 hours and 48 hours treatment samples. The analysis pipeline was implemented in python using the SciPy packages (<https://www.scipy.org/>) [[6](#_ENREF_6)] and Jupyter notebook (https://jupyter.org/).

Data Availability

The raw mass spectrometry files are deposited in the PRIDE database [[7](#_ENREF_7)], with accession id XXXXXX.

The analysis pipeline is available in GitHub (<https://github.com/mtinti/BACE1_pulldown>) and it is archived in Zenodo (https://zenodo.org/badge/latestdoi/283470327). The analysis pipeline can be easily reproduced using the mybinder app at (<https://mybinder.org/v2/gh/mtinti/BACE1_pulldown/master>).

The code to run the webserver for exploring the BACE1 interactome is available in GitHub (<https://github.com/mtinti/jennifer_bace1>) and it is archived in Zenodo (https://zenodo.org/badge/latestdoi/290785628).

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4. Cox, J., et al., *A practical guide to the MaxQuant computational platform for SILAC-based quantitative proteomics.* Nat Protoc, 2009. **4**(5): p. 698-705.

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