Carbene Protein Footprinting

BMSS Biomacromolecular SIG Training

27 July 2020

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Overview

- Introduction and Background
- Protein and Probe
- Photochemical activation
- Proteolysis and MS analysis
- Data processing

Introduction and Background

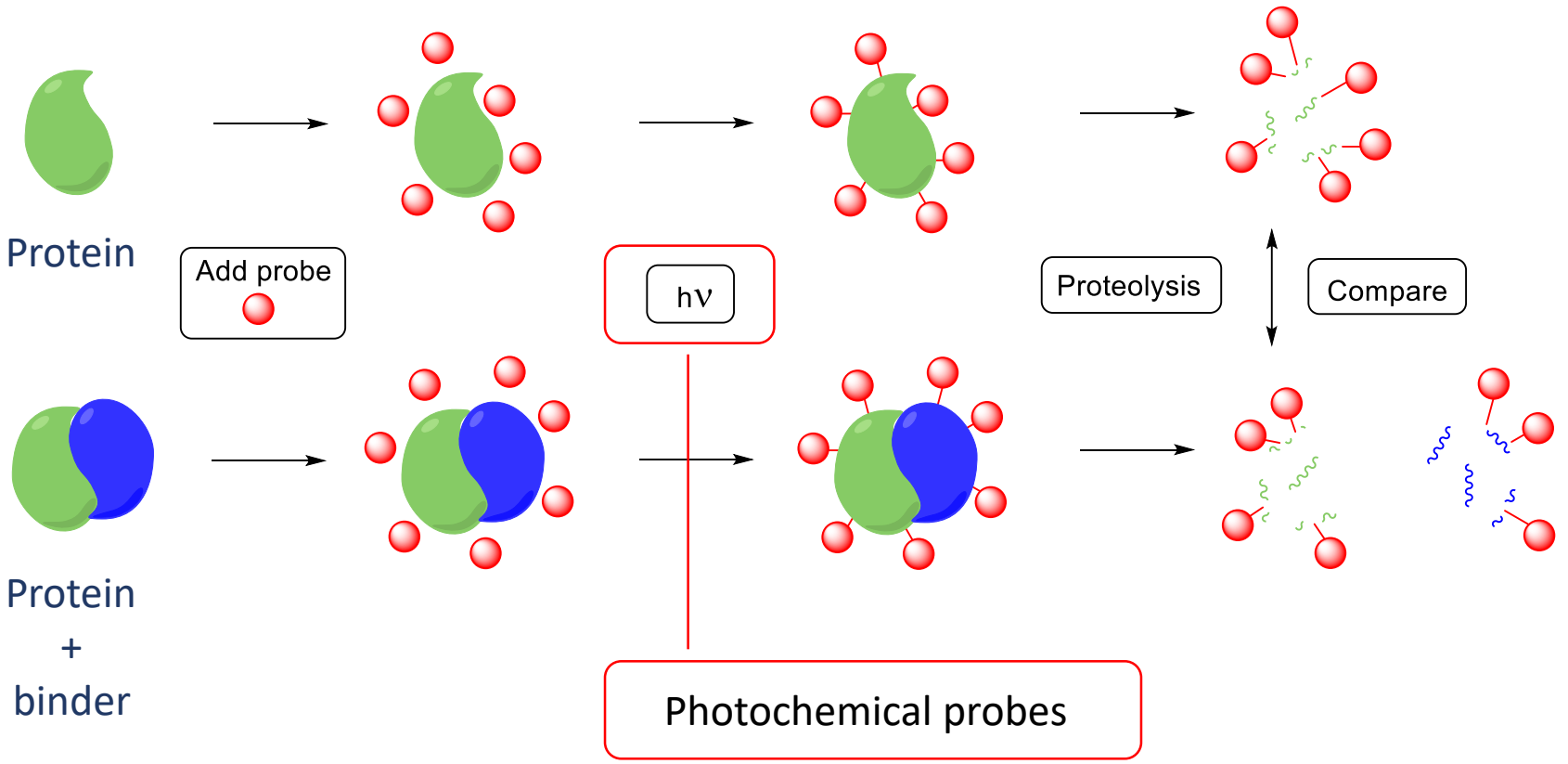
Concept

- Covalent labelling of a protein can be used to reveal solvent accessibility of the protein's surface

- Quantitative changes in labelling associated with masking by a binding partner allow binding sites on the protein to be identified

Introduction and Background

Concept



Introduction and Background

Probes

Radicals

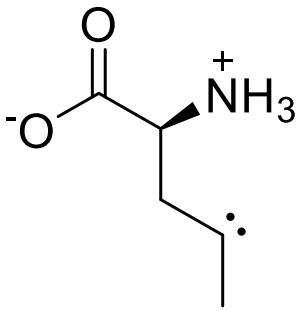
$\dot{O}H$ (e.g. from H_2O_2 and hν @248 nm in FPOP)

Gross *et al. JACS*, 18724 (2012)

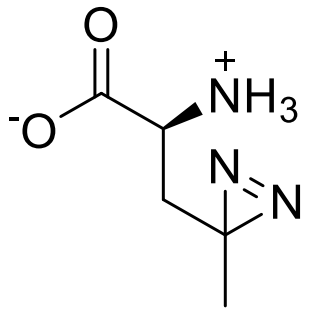
$\dot{C}F_3$ (e.g. from $\dot{O}H$ and CF_3SO_2Na)

Gross *et al. Angew. Chem.*, 14007 (2017)

Carbenes



from

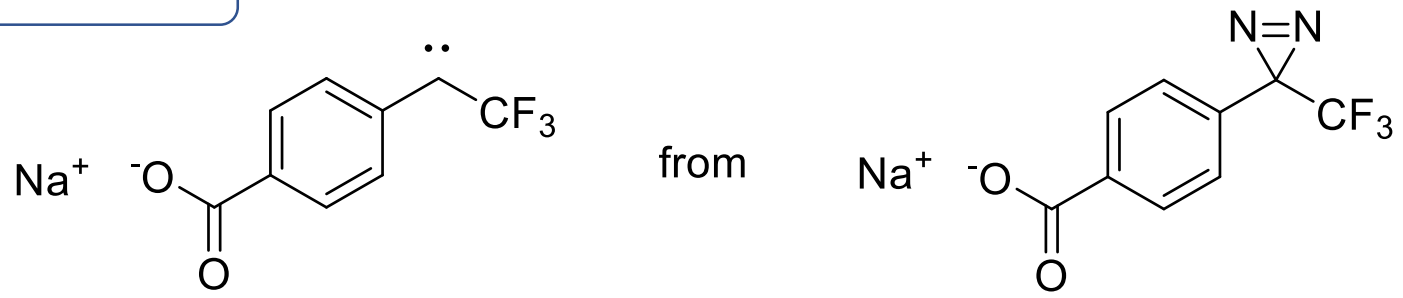


and hν @352 nm

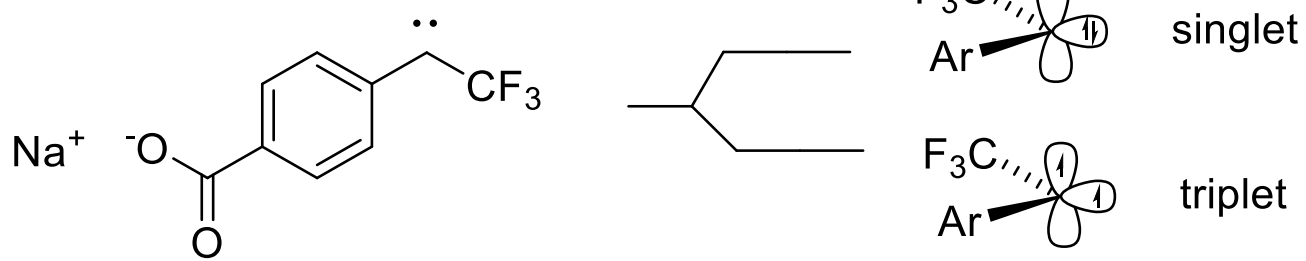
Schriemer *et al. Anal. Chem.*, 2913 (2011)

Introduction and Background

Carbene Probe



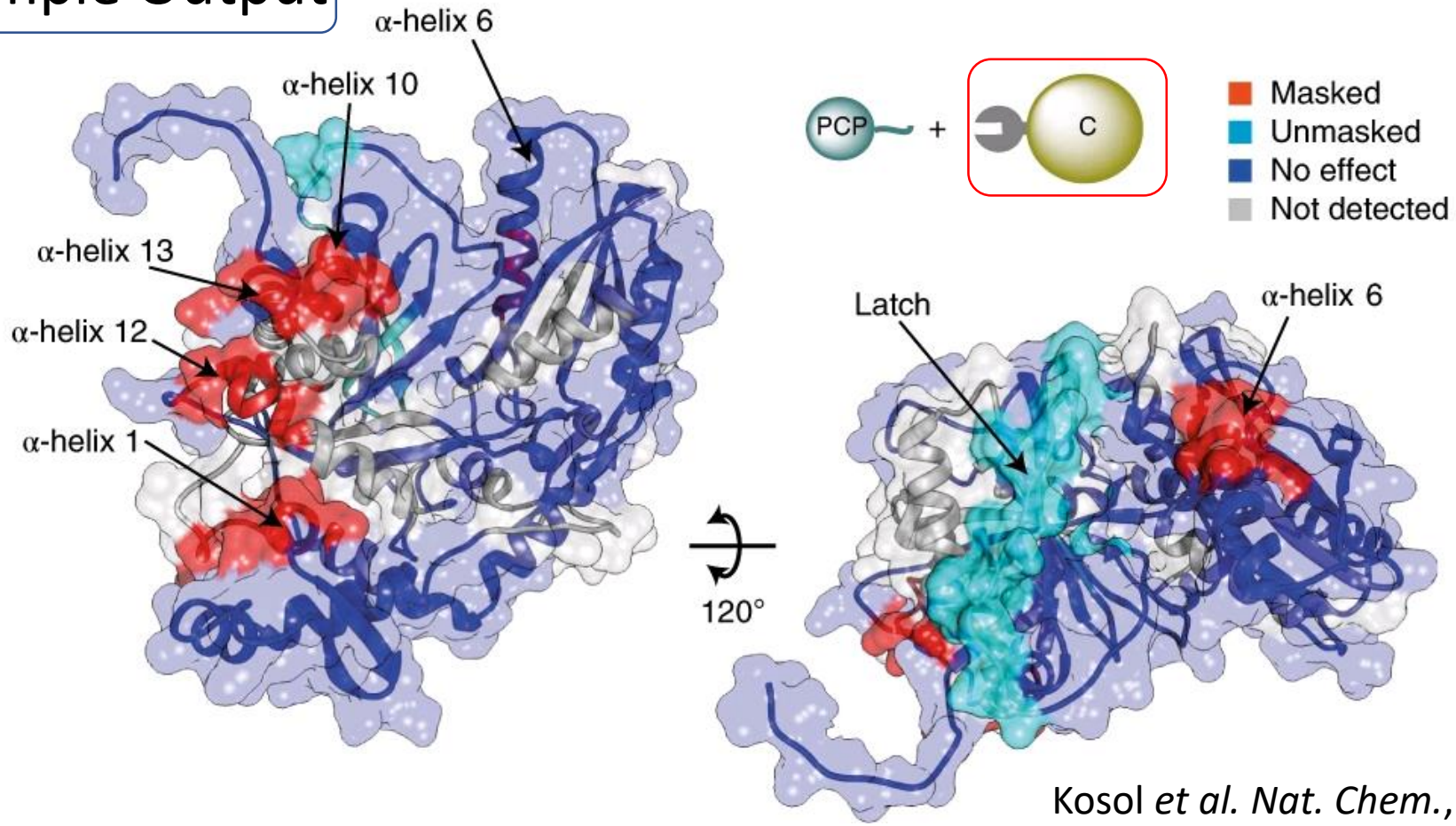
and hν @352 nm



Manzi *et al. Nat. Comm.*, 13288 (2016)

Introduction and Background

Example Output



Kosol *et al. Nat. Chem.*, 913 (2019)

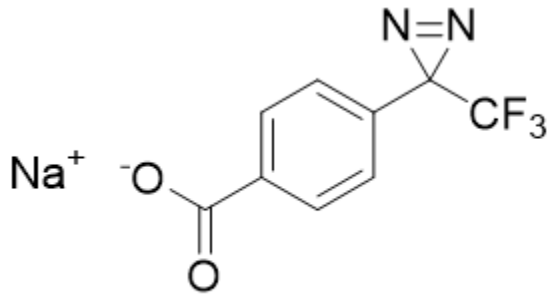
Protein and Probe

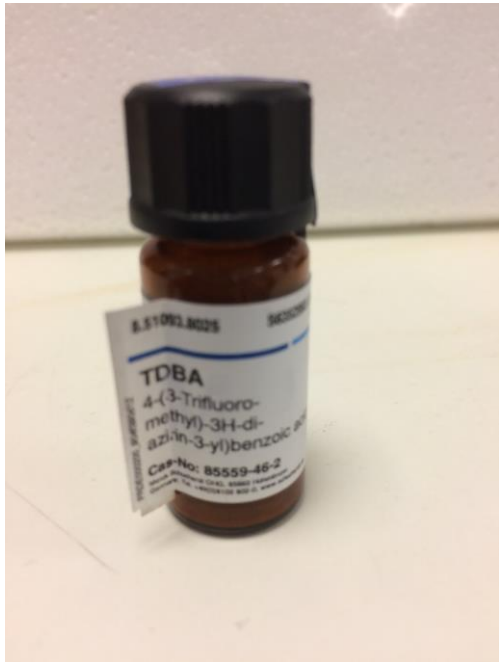
Protein

- Typical concentration 10-50 µM, typical volumes 20-100 µL
- Suitable buffers include Tris-HCl, PBS etc.
- No special considerations – just keep conditions constant across experiments

Protein and Probe

Probe





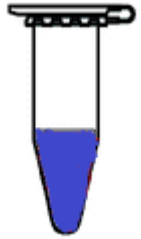
- Free acid (TDBA) is commercially available

  (Merck (Novabiochem) 25 mg - £ 180)

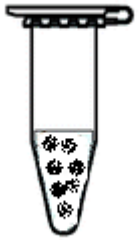
- To make sodium salt – add 1.1 mol. excess. of TDBA to NaOH solution of desired conc. (e.g. 40 mM) and centrifuge to remove undissolved free acid. Solution can be freeze dried, but is stable at 4 ^{o}C

Protein and Probe

Protein
(20-100 µM)



Protein + Binder
(20-100 µM)

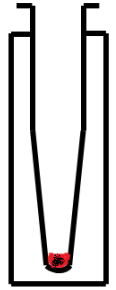


Protein
(10-50 µM)
+
Probe
(10-30 mM)

Probe
(20-60 mM)

Protein + Binder
(10-50 µM)
+
Probe
(10-30 mM)

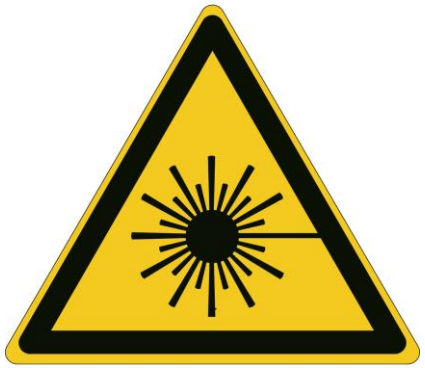
Photochemical Activation



5 µL

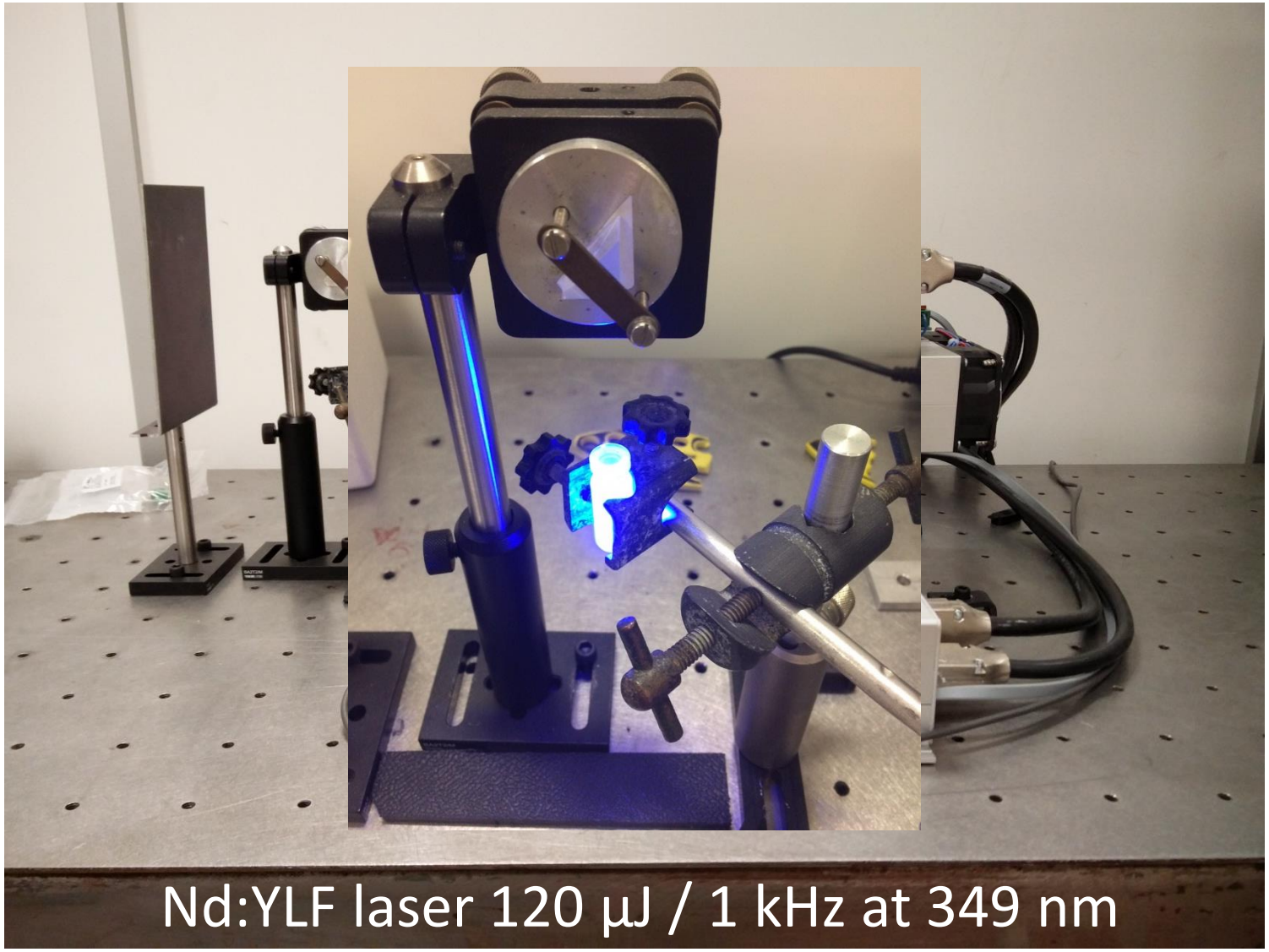
Flash freeze

Liquid nitrogen

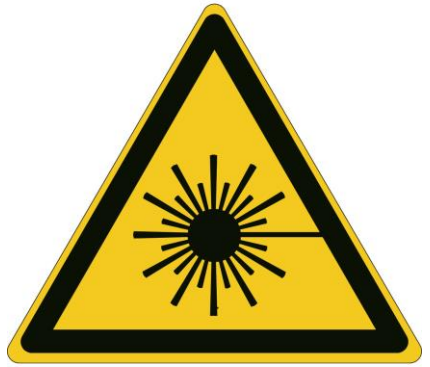


Laser irradiation

Photochemical Activation



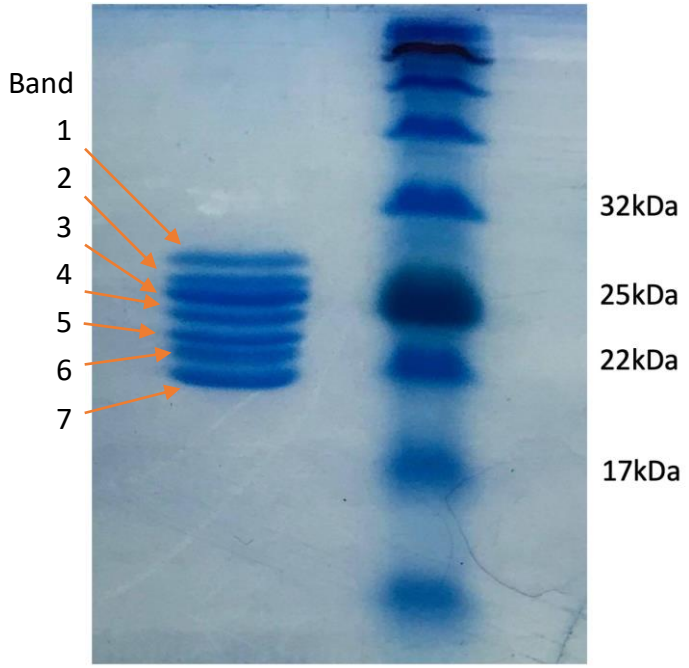
Nd:YLF laser 120 µJ / 1 kHz at 349 nm

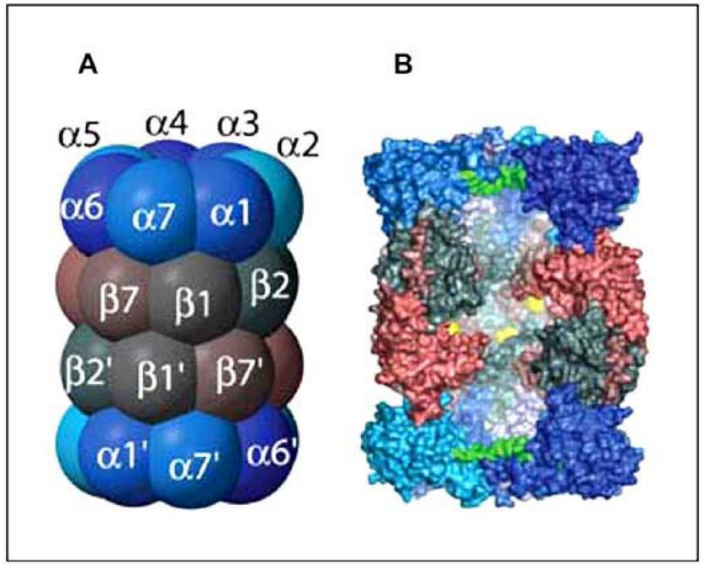


For normal operation the beam should be fully enclosed

Proteolysis and MS analysis

- Covalent labelling is irreversible
- Protein sample can be separated by SDS-PAGE





20S proteasome

Proteolysis and MS analysis

- Protein digestion can be in gel or in solution
- A range of enzymes can be used:

Trypsin
AspN
GluC
Pepsin
Chymotrypsin
Elastase

Run digests (in silico and actual) on the protein(s) before labelling to guide enzyme choice

Proteolysis and MS analysis

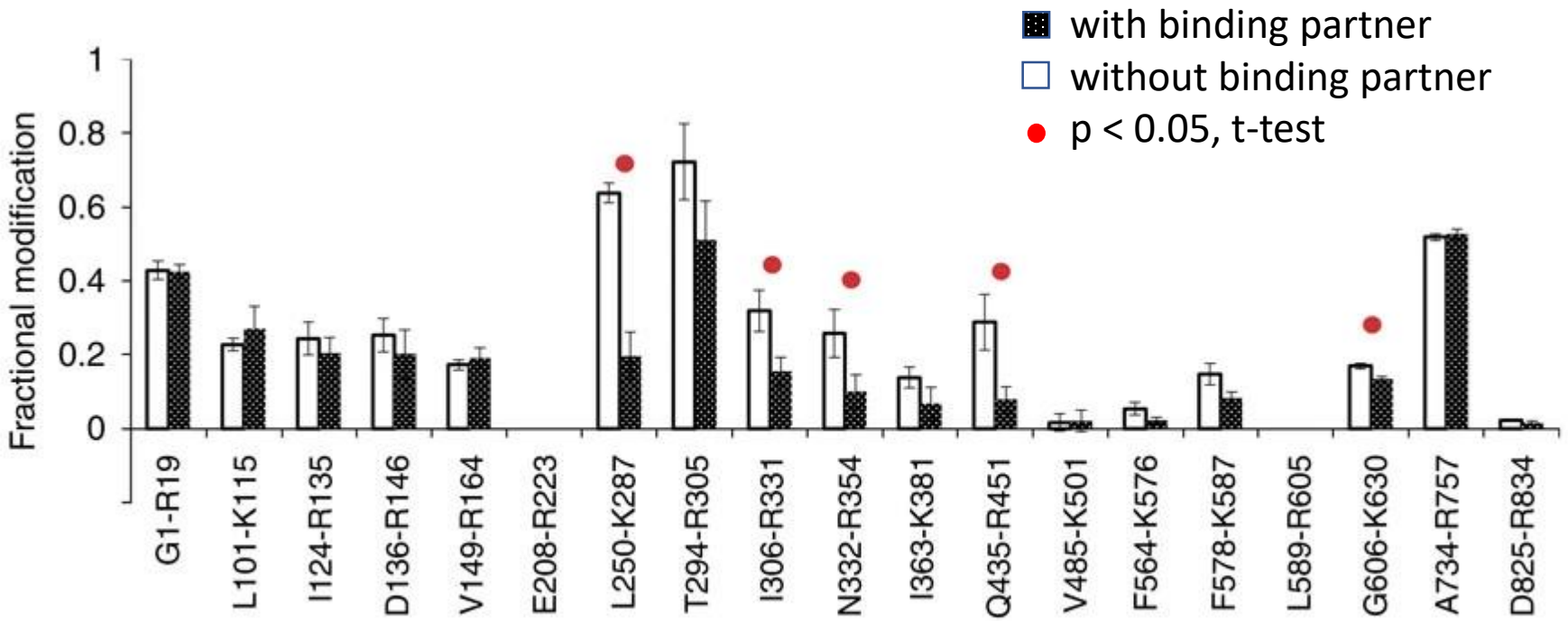
- Nano or cap LC-ESI-MS preferred for digest analysis
- FT-MS or TOF-MS (HR and accurate mass) beneficial
- MS/MS (CID or ECD/ETD) confirms ID of unlabelled peptides; and can be used to map labelling at the sub-peptide level
- No special requirements for MS analysis beyond those typically adopted for peptide analysis/bottom-up proteomics

Data Processing

- Output of experiment – ~10 LC-MS files containing data for modified and unmodified peptides (usually in 2 *groups*)
- Data can be analysed *manually* -
  - generate EICs for each labelled and unlabelled peptide
  - integrate peaks - PA
  - determine fractional modification $f_m = \frac{PA_{mod}}{PA_{mod}+PA_{unmod}}$
  - Plot f_m for each peptide and compare between *groups*

Data Processing

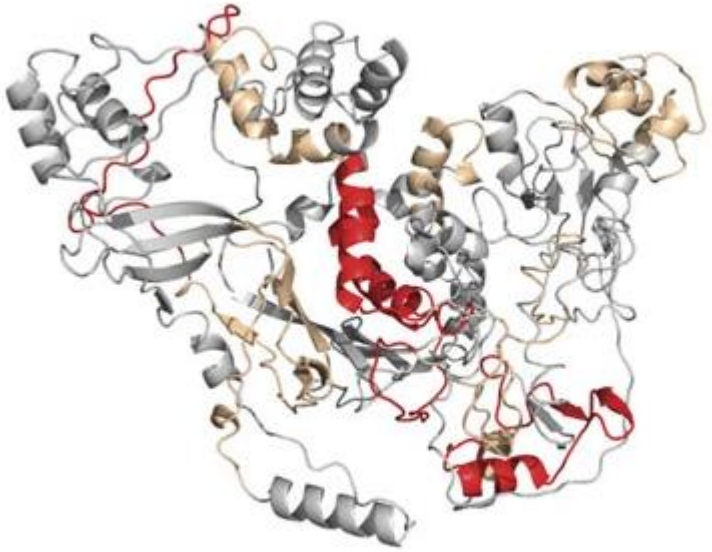
- Differences between *groups* report areas of masking and unmasking



Manzi *et al. Nat. Comm.*, 13288 (2016)

Data Processing

- Differences can then be plotted on protein structures (X-ray, NMR, cryoEM or homology models)

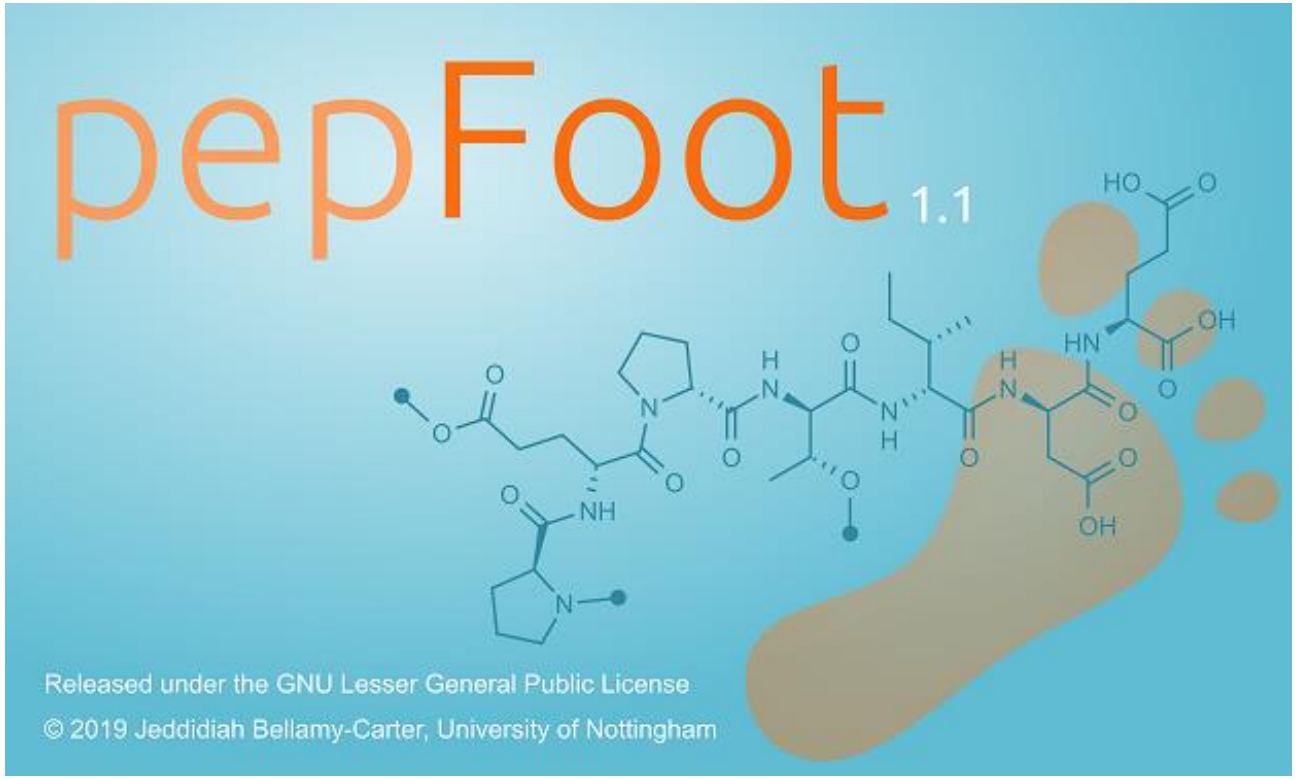


Significant masking by binding partner

Manzi *et al. Nat. Comm.*, 13288 (2016)

Data Processing

Semi-automated data analysis

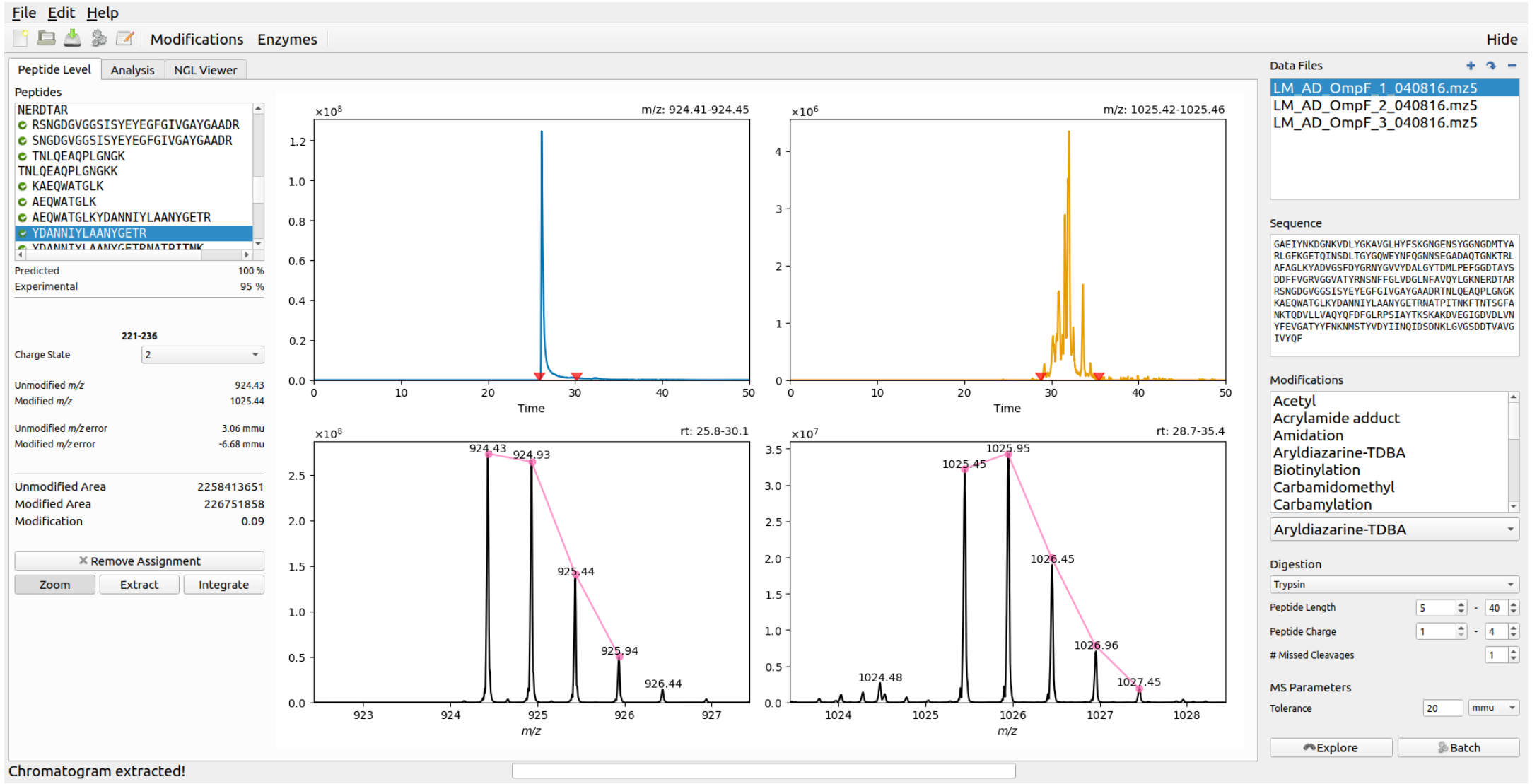


- Batch processing
- Data visualisation

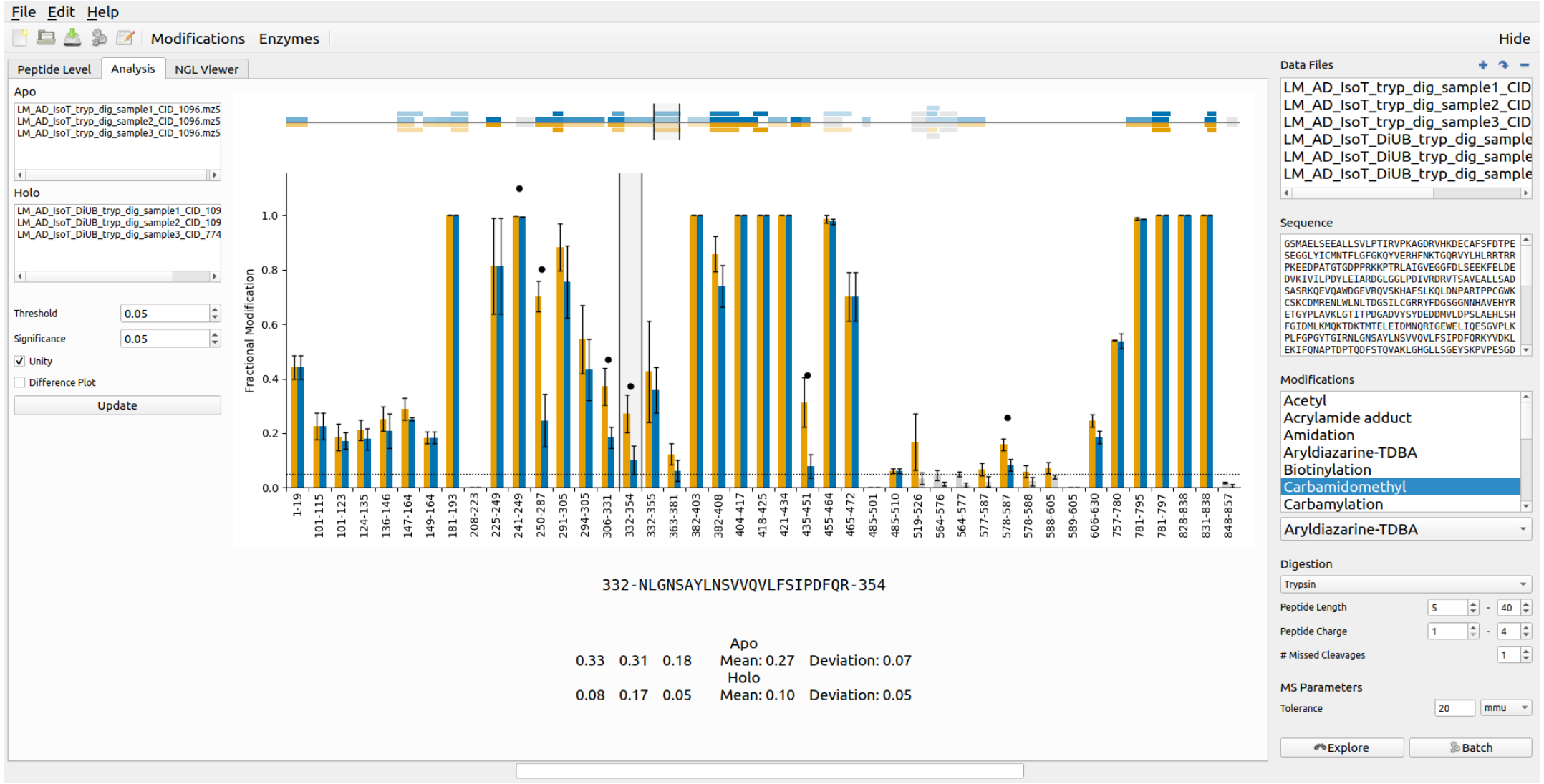
github.com/jbellamycarter/pepfoot

Bellamy-Carter and Oldham. *J. Proteome Res.*, 2925 (2019)

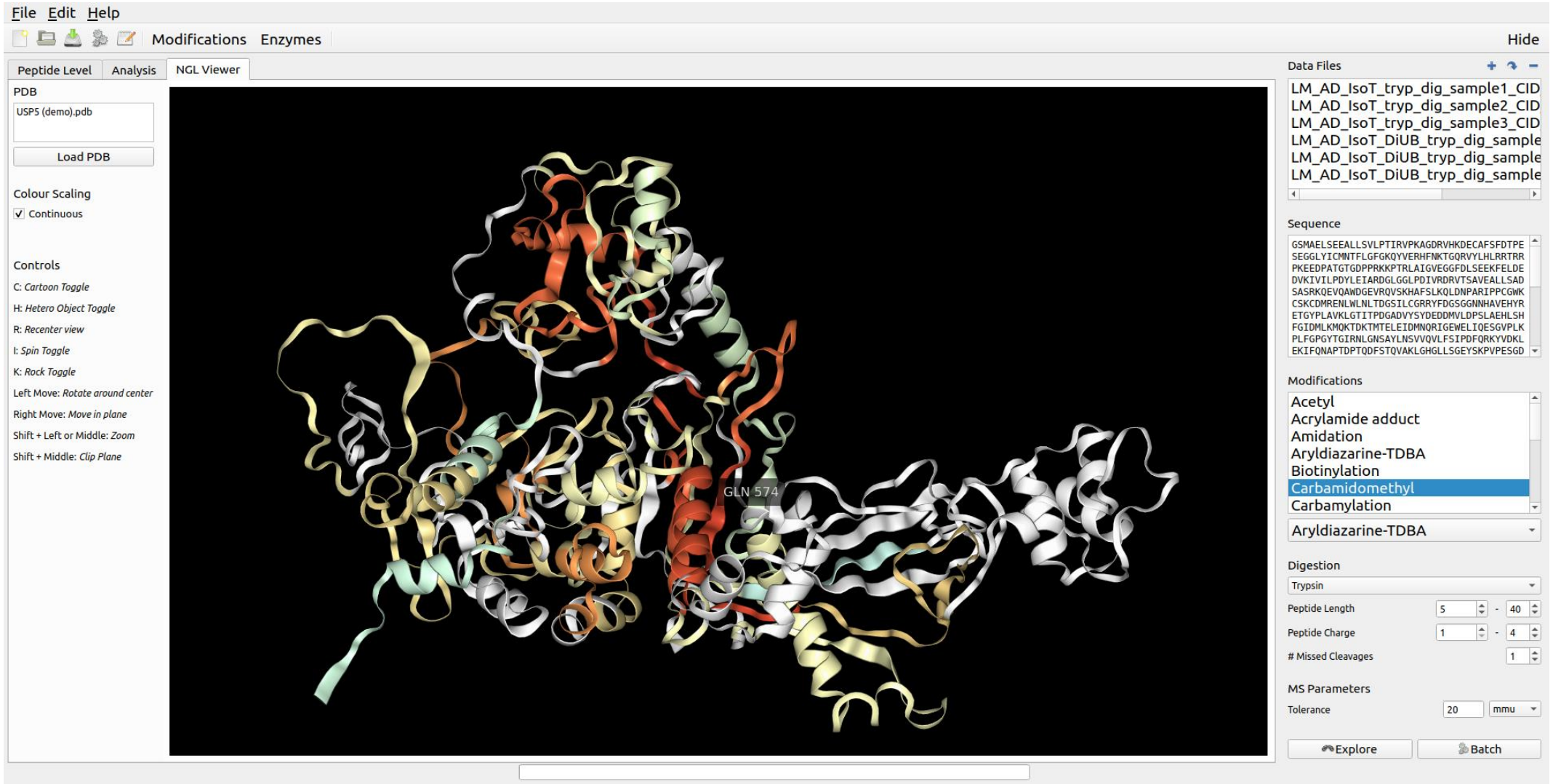
Data Processing



Data Processing



Data Processing



Summary

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