Integrated Software Platform for Analyzing Hydrogen-Deuterium Exchange and Oxidative Footprinting Data

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PROTEIN METRICS

NIGMS Washington University in St.Louis Mass Spec Resource

Introduction

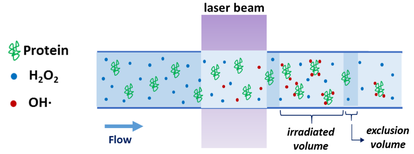
Mass spectrometry has become a powerful tool for studying high-order protein structure and dynamics by correlating changes in solvent accessibility with conformation or binding. Two popular methods are hydrogen-deuterium exchange (HDX) and covalent labeling – for example, oxidative footprinting by fast photochemical oxidation of proteins (FPOP). These methods are complementary:

- The time scale probed by oxidative footprinting is several orders of magnitude shorter than the time scale probed by HDX.
- FPOP labels side chains whereas HDX probes backbone secondary structure.
- FPOP labels reactive amino acid residues covalently, so it is compatible with almost any digestion and chromatography. HDX labels backbone more uniformly, but due to back-exchange requires low-pH digestion, low temperature, and fast chromatography.

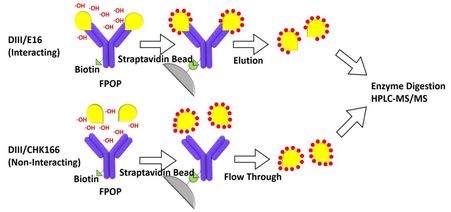
We describe here the first software platform that can provide integrated reports of both HDX and covalent labeling experiments. We demonstrate the software by analyzing the binding of West Nile Virus envelope protein domain III (WNV DIII) with neutralizing antibody E16 (Oliphant et al., 2005). WNV DIII catalyzes endosomal-membrane fusion and plays a key role in host infection.

Methods – FPOP

- WNV DIII was incubated with interacting antibody E16 for the bound-state sample. Non-interacting antibodies were used for the control sample.
- Scavenger and H_2O_2 were added just prior to FPOP.



- After irradiation, antibodies were removed by affinity-capture purification based on streptavidin-biotin interactions.



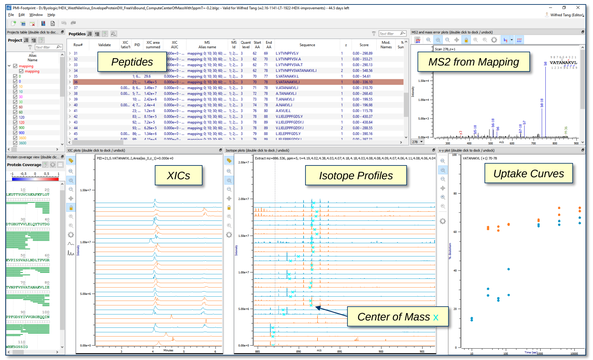
- Samples were reduced, alkylated, and digested with a range of enzymes.
- Samples were separated on a reversed-phase column by an UltiMate 3000 HPLC and analyzed by a Thermo Q Exactive Plus MS. Resolving power (at *m/z* 400) was 70,000 for MS^1 and 17,500 for MS/MS.

Methods – HDX

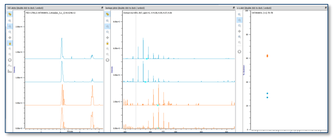
- WNV DIII with either E16 antibody or non-interacting antibodies was exposed to D_2O for 0 (control), 10, 30, 60, 120, 900, 3600, and 14400 seconds before quenching at low pH.
- Quenched proteins were immediately injected onto a pepsin column, and the resulting peptides were trapped and desalted on an Eclipse Zorbax XDB C8 cartridge (Agilent) at 200 µL/min before being eluted through an XSelect CSH C18 column (Waters) at 50 µL/min directly to the ESI source on the LTQ-FT hybrid MS (Thermo).
- Mapping employed high resolution MS^1 and low resolution MS^2 while timed deuteration LC runs used high resolution MS^1 only.

Software Platform

We adapted Byologic™ software to support HDX by adding center of mass calculation, uptake curves, and automatic selection of isotopes for XICs and scan times for isotope profiles. The same platform was previously adapted for FPOP (Huang et al., 2016).

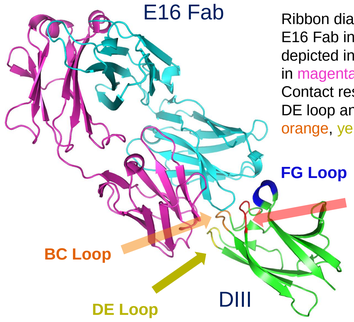


- The screenshot shows bound and unbound West Nile Virus DIII.
- The viewed peptide (VATANAKVL + H^+) is in the DE loop.
- 7 deuterium exposure times for bound, 6 for unbound, 2 replicates each. (XIC and Isotope plots include control (0 sec exposure) and mapping LC runs.)
- XICs and Isotope profiles sorted by deuterium time from 0 (bottom) to 4 hours (top).
- Selections may be filtered for peptide sequence, exposure time, position in protein, etc. Shown at right are XICs, Isotope Profiles, and Uptake limited to samples with 30 sec exposure.



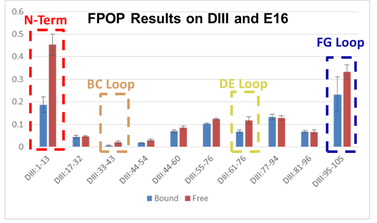
Results

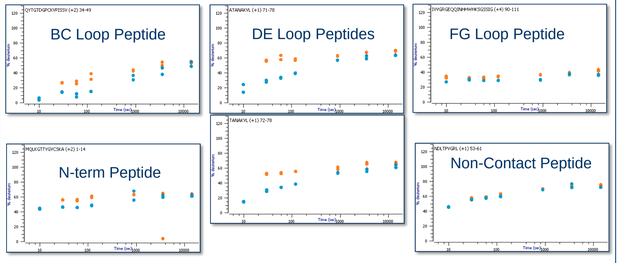
- To infect hosts, WNV undergoes endosomal-membrane fusion catalyzed by rearrangement of the receptor-binding domain III (DIII).
- E16 is a monoclonal antibody that inhibits infection by blocking this rearrangement.
- E16 binds DIII at 16 residues on four discontinuous segments (Nybakken et al., 2005).



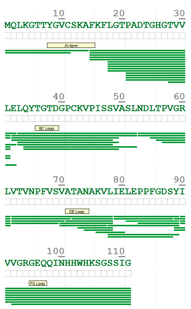
Ribbon diagram of crystal structure for E16 Fab in complex with DIII, with DIII depicted in green, the antibody light chain in magenta, and the heavy chain in cyan. Contact residues on N-terminus, BC loop, DE loop and FG loop are shown in red, orange, yellow and blue respectively.

- FPOP and HDX results were obtained previously by Huang et al., 2015. Here we reanalyze the HDX data with the new software.
- HDX uptake curves below show good agreement with FPOP results. N-terminal, BC, FG, and especially DE each showed protection in the bound state. Non-interacting sequences showed no protection.

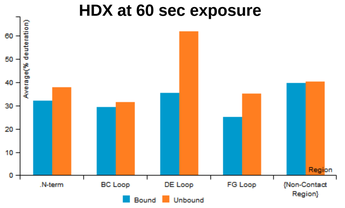




- Pivot tables and graphs make it fast and convenient to generate customized reports.



- Reports can include FPOP, HDX, and other higher-order structure experiments.
- Protein annotations can be integrated seamlessly.



Discussion and Conclusions

The Byologic software platform was initially developed for sequence variant analysis, but has since been adapted for stress studies, biosimilarity, oxidative footprinting, and now hydrogen-deuterium exchange. The advantages of a unified platform include code reuse, faster learning time, and integrated reports. Here we comment on some specific aspects of HDX processing.

- XICs and Isotope Profiles can help the user diagnose problems with deuterium uptake measurements, such as interference from other peptides or isotope profiles from scans that miss the elution peak.
- The most challenging automation task in HDX is selection of isotope profile time. We are continuing to reduce error rate, and also to provide convenient tools for user validation / correction.
- Improved automation should allow deuterium uptake to be tracked on more peptides, extending HDX to larger proteins and complexes.

References and Acknowledgments

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(Huang et al., 2016) Rapid Mapping Of Antigen-Antibody Binding Sites Utilizing Oxidative Footprinting And New Software, ASMS Conference 2016.

We gratefully acknowledge STTR grant R41 GM121133 from the NIH NIGMS for software development. The Washington University in St. Louis Mass Spec Center is supported by center grant P41 GM103422 and instrument grant 1S10OD016298-01A1.