Integrated software platform for analyzing hydrogen-deuterium exchange and oxidative footprinting data for solvent accessibility

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Introduction

Mass spectrometry (MS) has become a powerful tool for studying protein higher-order structure (HOS) and dynamics. Two MS-based techniques for following conformational changes are hydrogen-deuterium exchange (HDX) and hydroxyl radical protein footprinting (HRPF) using fast photochemical oxidation of proteins (FPOP) among other methods. Both HDX and hydroxyl radical footprinting measure solvent accessibility, but differ in their details, and hence give complementary information.

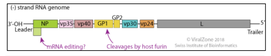
- HDX measures deuterium replacement of amide hydrogens along the protein backbone. Deuterium uptake rate depends upon solvent accessibility, as well as secondary structure stability.
- Hydroxyl radical footprinting measures oxidations of amino acid side chains. Oxidation rate depends upon solvent accessibility and reactivity of side chains (C, M, W, H, F are fast).
- Deuterium exchange typically uses exposure times of 1 to 10,000 seconds.
- Hydroxyl radical footprinting occurs in microseconds.
- HDX uses low pH to minimize back exchange, and typically uses pepsin digest and ~10-min liquid chromatography (LC) gradients.
- Hydroxyl radical footprinting can use tryptic or multiple digests and standard LC.

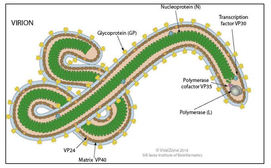
Here we report on a software platform in development for efficient analysis of both HDX and HRPF data.

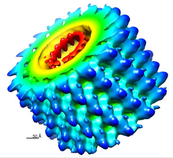
Filoviruses

Filoviruses such as Marburg and Ebola are negative-sense, single-stranded RNA viruses and important human pathogens that cause severe hemorrhagic fevers with mortality rates above 50%. As of June 2019, there is an active outbreak of Ebola in the Democratic Republic of Congo. Filoviral protein VP35 is a high priority therapeutic target due to its role in uncontrolled viral RNA replication. It is a non-enzymatic cofactor for viral RNA polymerase and a potent suppressor of innate antiviral signaling pathways in all filoviruses.

The Marburg viral genome codes for 7 proteins, including an RNA polymerase (L), glycoproteins (GP1 and GP2), and a polymerase cofactor (VP35).





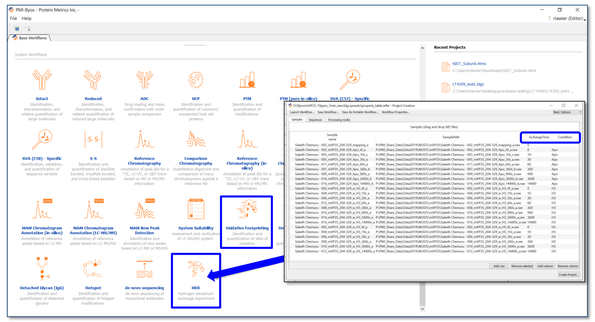


Cryo-EM reconstruction of virion cross-section, diameter ~80 nm

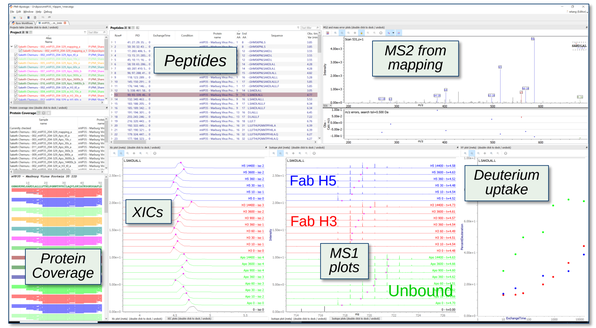
The primary animal reservoirs for filoviruses are African fruit bats.

Software Platform

Protein Metrics Byos™ platform includes workflows for severa MS-based biopharma assays. Byos accepts data from any MS instrument vendor and almost any type of mass spectrometer. Each workflow prompts the user for necessary inputs, for example, ExchangeTime and Condition for HDX.

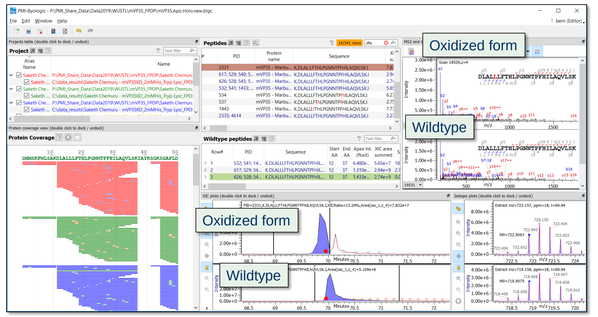


The workflow processes the data and produces a project file (SQLite) that can be opened with an interactive viewer for expert curation of results. The interactive viewer presents data in a format appropriate to the experiment. For HDX, the viewer shows an annotated MS2 spectrum from the mapping phase on undeuterated peptides, along with stacked XIC and MS1 plots of deuterated peptides, all ordered by exposure time. A red ✕ in the MS1 plot shows the center of mass; the average number of deuteriums (difference in center of mass between undeuterated and deuterated peptides) is shown in the uptake plot on logarithmic time axis. The picture below shows a peptide with sequence SAKDLAL that is protected in by both antibodies H3 and H5.



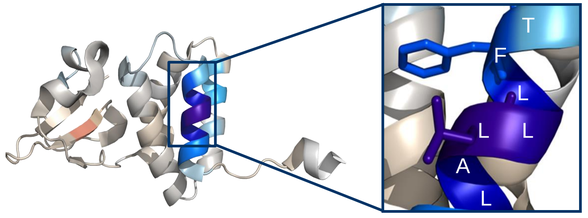
Hydroxyl Radical Protein Footprinting

Hydroxyl radical protein footprinting can also be run in Byos. For HRPG, the interactive viewer shows stacked XIC, MS1, and MS2 plots of oxidized and unoxidized ("unmodified") peptides for easy comparison and, if necessary, adjustment of XIC time limits, for example, to exclude in-source oxidation as shown below. Byos also exports graphical reports in user-customizable formats, including bar charts, spectrum plots, and so forth.



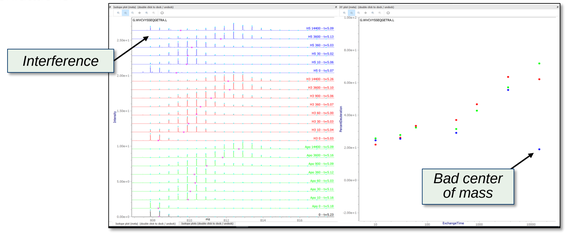
Epitope Mapping Results

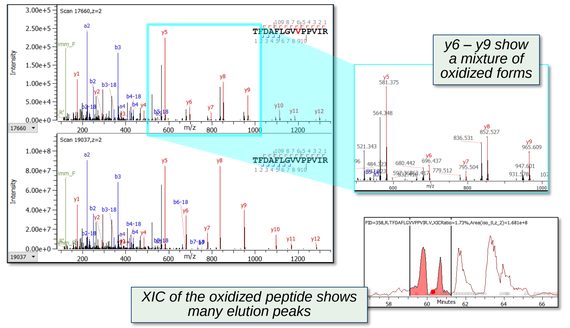
Differential deuterium uptake of mpVP35 protein in complex with novel Fab H3 mapped to a 3D ribbon diagram (PDB 4GHL) of mpVP35 protein. The inset shows the two residues L215 and F218 (sequence …SAKDLALLLF…) most important for binding. This part of the protein also shows significant differences in oxidation upon binding as measured by FPOP — in fact, the critical binding residues were localized by FPOP. Even relatively unreactive residues such as L can give enough oxidation to measure protection.



Curation

Byos reduces but does not eliminate manual curation required for HDX and HRPF workflows. For HDX, curation involves correcting or removing peptide interferences. For HRPF, curation involves excluding in-source oxidation (as shown at the left) and, if sub-peptide resolution is the goal, localization of oxidations.





Conclusions and Acknowledgments

In summary:

- HDX and HRPF have proved effective for epitope mapping and often give complementary information.
- Byos for higher order structure is still a work in progress, but the new workflows can already improve the efficiency of data analysis for labeling experiments, including HDX, HRPF, and reagent-based labeling.

Acknowledgments

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