

219. Pan-Omics: Activity-Based Proteomics

Aaron T. Wright¹* (email: aaron.wright@pnnl.gov), Natalie C. Sadler¹, Paul D. Piehowski¹, Stephen J. Callister¹, Richard D. Smith¹

¹Pacific Northwest National Laboratory, Biological Sciences Division, Richland, Washington, 99352

Project Goals: As part of the Pan-Omics Program we seek to enable a more complete assessment of the functional proteome of microbes and microbial communities by developing and deploying novel strategies in activity-based proteomics (ABP), which uses synthetic chemistry to create chemical probes that can measure enzyme functions, protein-substrate interactions, and protein redox regulation directly in living systems. Our research program has centered on increasing the technical capabilities of this technology by creating a multimodal probe suite for in situ studies, with accessibility to several analysis methods, and to correlate post-translational modifications to protein function, in conjunction with the use of advanced mass spectrometry-based platforms. Additionally, we are employing multiplexed probe studies to concomitantly address multiple functional proteins, or to analyze an active metabolic pathway more fully. Together, these ABP studies help link protein function, regulation, and interactions to the dynamic cellular physiology of microbial systems.

Activity-based proteomics addresses the fact that enzymatic function does not necessarily correlate with protein abundance, as cellular localization, allosteric and cofactor interactions, regulatory dynamics, and other details that mitigate function are generally not known. Activity-based probes can be used to monitor functional enzyme activity, redox regulation, and protein-substrate interactions on a proteome-wide basis. ABP not only provides a method to directly evaluate enzyme and substrate binding, and links between the proteome and metabolome, but when coupled to mass spectrometry-based top-down characterization methods can also provide quantitative information on specific proteoforms and PTMs that instigate activities (Figure 1). ABP can also provide information on the expression and functional status of proteins both in vitro and in living systems using chemical probes.

ABP involves the labeling of specific protein targets through the use of probes containing a click chemistry moiety (e.g., azide or alkyne), and effective enrichment of labeled proteins through click chemistry and affinity methods. ABP probes consist of three moieties: (i) a reactive group that forms an irreversible covalent bond with a target protein, (ii) a binding group that biases the probes toward a protein or protein family and may also impart cell permeability via active transport or diffusion, and (iii) a “clickable” reporter tag for rapid and sensitive measurement of labeled enzymes. Each of these three groups can be varied to create suites of probes.

When synthesizing new probes we attempt to keep the size small, thereby minimizing undesirable impacts on reactivity with the target proteins and maximizing cell permeability. Live cell or community labeling of active proteins enables the activity of a target to be monitored while maintaining the native cellular physiology, and prevents complications from the release of endogenous enzyme inhibitors following cell disruption. In addition to live studies we are increasingly multiplexing chemical probes to observe the complexity of myriad simultaneously functioning enzyme activities (Figure 2). Finally, since ABP captured proteins can be eluted at both the intact protein level or at the peptide level (i.e. following enzymatic digestion), both top-down and bottom-up MS-based approaches can be applied for a more comprehensive characterization of proteoforms (often revealing the specific sites of modifications that drive activity).

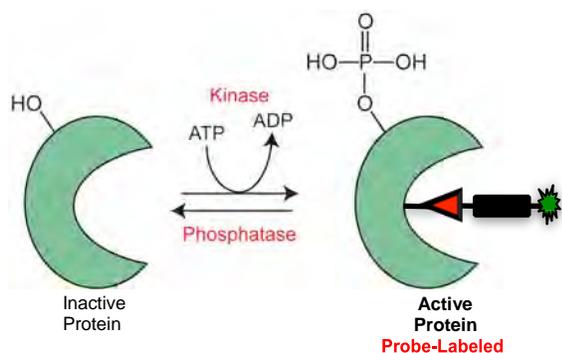


Figure 1. Intact ABP enables more effective characterization of active proteoforms.

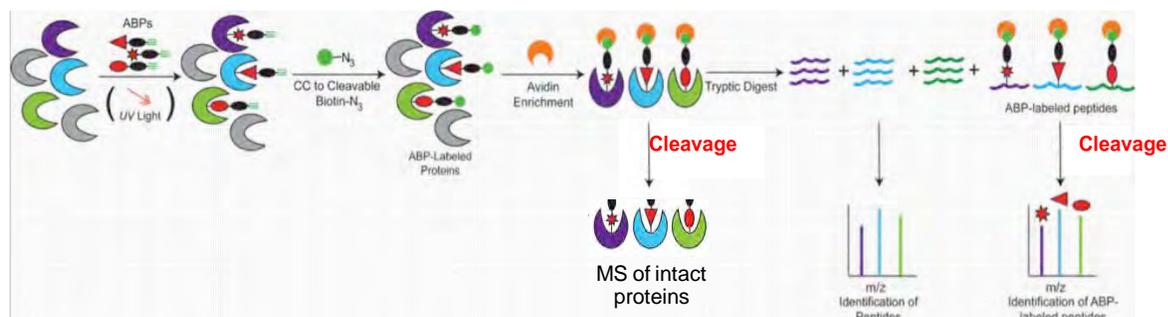


Figure 2. Multiplexed ABP approach for mass spectrometry-based intact and bottom-up proteoform characterization. A probe is added to a living cell or lysate, and only reacts with the active proteoform of the target enzyme. Click chemistry is used to attach biotin or other cleavable (e.g., by chemical reduction, light, or enzymatically) enrichment moiety, followed by enzyme capture and subsequent processing for MS analyses.

This research under the Pan-omics Program at PNNL was supported by the Genomic Science Program (GSP), Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Foundational Scientific Focus Area. The work conducted by the U.S. Department of Energy Joint Genome Institute was supported by the Office of Science of DOE under Contract No. DE-AC02-05CH11231. A portion of this work was performed in the William R. Wiley Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by OBER and located at PNNL.