Project Goals: The main objectives of this early career research project are to develop novel proteomic approaches that will enable quantitative measurements of site-specific regulatory protein posttranslational modifications (PTMs) and targeted quantification approach for monitoring the dynamics of specific pathways. The ability to effectively and quantitatively characterize site-specific PTMs and pathways is essential for understanding the regulation of cellular signaling and protein functions, as well as for enabling a systems biology approach to study organisms as well as communities important for bioenergy or environmental applications. Our developments have been primarily focused on: (1) reversible redox modifications on protein thiols, and (2) ultrasensitive targeted quantification to enable measurements of low-abundance proteins or PTMs in a given pathway or network.

Quantitative redox proteomics: One of the major regulatory mechanisms is redox regulation where nearly all organisms sense subtle changes in their redox homeostasis due to environmental changes. Functional cysteinyl residues in proteins serve as “redox switches” through reversible oxidation, which is recognized as a fundamental mechanism of redox regulation in almost all organisms. We have developed a robust quantitative redox proteomics approach for measuring different types of reversible modifications on individual cysteine thiols to study redox regulation in metabolism or stress conditions of different organisms.1 The general principle of this approach involves the blocking of free thiols, selective reduction, enrichment, and quantification of site-specific redox modifications.

We have applied this redox proteomics approach to profiling the in vivo dynamics of thiol oxidation modulated by light/dark in Synechocystis sp. PCC 6803, an oxygenic photosynthetic prokaryote.2 Reversible protein thiol oxidation is an essential regulatory mechanism of photosynthesis, metabolism, and gene expression in photosynthetic organisms. The redox dynamics of ~2,100 Cys-sites from 1,060 proteins under light, dark, and 3-(3,4-ichlorophenyl)-1,1-dimethylurea (DCMU, a photosystem II inhibitor) conditions were quantified. In addition to relative quantification, the stoichiometry or percentage of oxidation (reversibly oxidized/total thiols) for ~1,350 Cys-sites was also quantified. The overall results revealed broad changes in thiol oxidation in many key biological processes, including photosynthetic electron transport, carbon fixation, and glycolysis. Moreover, the redox sensitivity along with the stoichiometric data enabled prediction of potential functional Cys-sites for proteins of interest. The functional significance of redox-sensitive Cys-sites in NADP-dependent glyceraldehyde- 3-phosphate dehydrogenase, peroxiredoxin (AhpC/TSA family protein Sll1621), and glucose 6-phosphate dehydrogenase was further confirmed with site-specific mutagenesis and biochemical studies. Together, our findings provide significant insights into the broad redox regulation of photosynthetic organisms. In our ongoing work, we have confirmed that S-Glutathionylation is a major form of the redox modification in Synechocystis 6803 and glutaredoxin enzymes play a key role in its regulation. Similarly, we have extended the approach to measure redox changes modulated by light/dark in a more complex microbial system, phototrophic mat-derived unicyanobacterial consortia.

Ultrasensitive targeted proteomics. In addition to global profiling, MS-based targeted proteomics using
selected reaction monitoring (SRM) offers unique advantages in its accuracy of multiplexed quantification, throughput, and sensitivity for enabling the measurements of the dynamics of proteins and PTMs belong to specific gene networks or pathways for both individual organisms and community samples. Traditionally, one of the challenges in targeted quantification is the insufficient sensitivity for low-abundance proteins. Recently, we achieved a significant advance in SRM sensitivity by developing the PRISM (high-Pressure and high-Resolution Separations coupled with Intelligent Selection and Multiplexing) technology, which offers ~200-fold enhancement in sensitivity. PRISM enables direct quantitation of extremely low-abundance proteins (<100 copies per cell) and PTMs without the need for affinity enrichment. This high sensitivity of PRISM-based targeted quantification makes it ideal for monitoring temporal dynamics of specific sets of proteins or protein isoforms in complex systems such as plants and soil microbial community samples. Preliminary data on quantification of plant enzyme isoforms will be presented.

References:

This work is supported by the DOE Early Career Research Award (to WJQ) under the Office of Biological and Environmental Research (BER) in the DOE Office of Science and by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences grant number DE-FG02-99ER20350 (to HBP). This work uses capabilities developed under the BER-supported Pan-omics project at PNNL and in the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the DOE. AYN has been supported by an NSF Graduate Research Fellowship.