Quantitative Proteomic Profiling of Site-specific Redox Modifications, Proteolytic Processing and N-glycosylation

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Project Goals: One of the primary objectives of this early career research project is to develop novel proteomic approaches to enable quantitative measurements of site-specific regulatory protein posttranslational modifications (PTMs) in subcellular compartments. The ability to effectively and quantitatively characterize site-specific PTMs is essential for understanding the regulation of cellular signaling and protein functions at the post-translational level, and for enabling a systems biology approach to study organisms important for bioenergy or environmental applications. Our efforts have been primarily focused on three important classes of PTMs: (1) reversible redox modifications on cysteinyl thiols, (2) proteolytic processing and protein N-terminal modification, and (3) glycosylation. All three classes of modifications are ubiquitous in both prokaryotic and eukaryotic cells and their important roles in cellular regulation and signaling have been increasingly recognized.

Reversible redox modifications of protein thiols: 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 are developing approaches to enable parallel identification and quantification of several types of reversible thiol modifications such as S-nitrosylation (SNO) in response to different cellular reactive oxygen species. The strategy for achieving site-specific proteomic identification is illustrated in **Fig. 1A.** Briefly, thiol specific modifications can be reduced by specific reagents and the converted free thiols are subsequently captured and enriched by a thiol-specific resin and their dynamics can be quantified by isobaric labeling and LC-MS/MS. We have applied this approach to profile SNO in *Synechococcus* sp. PCC 7002. We identified 226 cysteine residues sensitive to SNO modification (**Fig. 1B**) and many of the sites were identified in proteins localized to the thylakoid, indicative of the role of redox

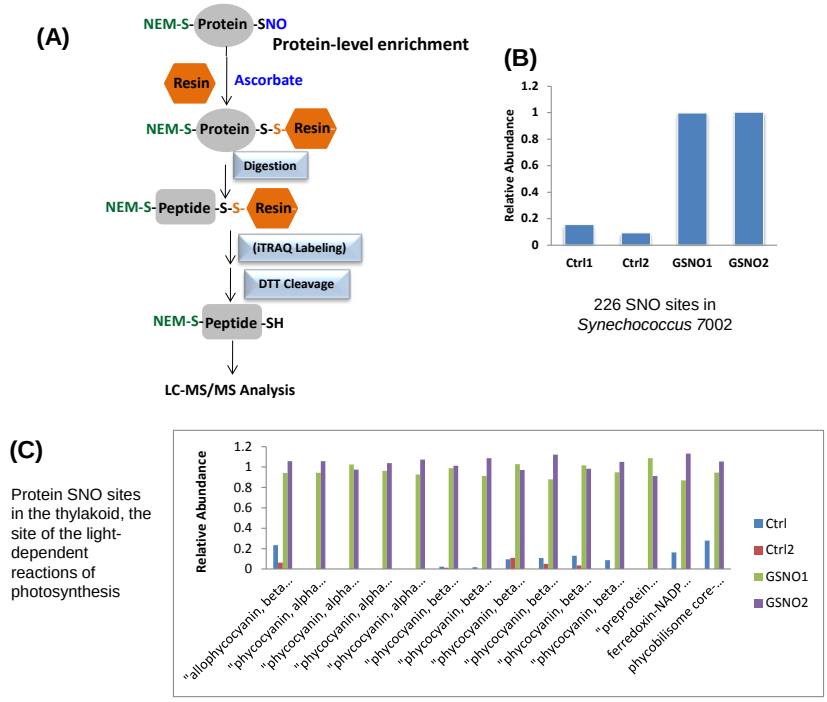

Figure 1. (A) The strategy for identification and quantification of S-nitrosylation (SNO); (B) SNO sites identified in 7002; (C) SNO sites identified in the thylakoid of 7002.

regulation in photosynthesis. Similarly, the application of this approach to a fungal organism, *Aspergillus niger*, also resulted in the identification of ~700 SNO-modified sites with many of the proteins being metabolic enzymes.

Proteolytic processing and N-terminal modifications: Proteolytic processing and *in vivo* N-terminal modifications are another common mechanism of regulation of protein functions. We have developed two complementary approaches to target specific protein N-termini and enrich N-terminal peptides for proteomic identifications. Applying these approaches to a whole cell lysate of *Aspergillus niger*, we were able to identity more than 2000 N-terminal sites with 228 *in vivo* modifications from 858 genes (**Fig. 2**). The number of N-terminal sites identified per gene reveals the complexity of the proteome due to proteolytic processing, which may reveal many novel functional proteins. Quantitative profiling will be performed to identify functional products.

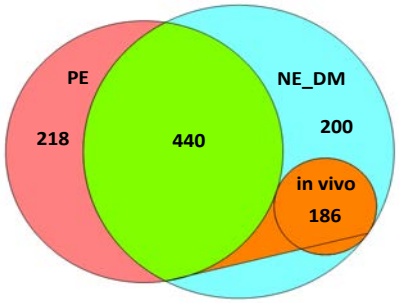

Figure 2. Number of genes identified with N-terminal sites. PE, positive enrichment; NE, Negative enrichment; *in vivo*, proteins with N-terminal sites modified *in vivo*.

Protein glycosylation: N- and O-linked glycosylation are known to play an essential role in cellular functions and secretory pathways. We have adapted and optimized of a hydrazide chemistry based enrichment approach for profiling N-glycosites. We have performed an extensive mapping of N-glycosylated sites in *A. niger* by applying this approach using hydrazide-modified magnetic beads. The optimized protocol was applied to profile N-glycosylated sites from both the secretome and whole cell lysates of *A. niger*. A total of 847 N-glycosylated sites from 330 N-glycoproteins (156 proteins from the secretome and 279 proteins from whole cells)[1] were confidently identified by LC-MS/MS. The identified N-glycoproteins in the whole cell lysate were primarily localized in the plasma membrane, endoplasmic reticulum, Golgi apparatus, lysosome, and storage vacuoles (**Fig. 3**), supporting the important role of N-glycosylation in the secretory pathways. The extensive coverage of N-glycosylated sites and the observation of partial glycan occupancy on specific sites in a number of enzymes provide important initial information for functional studies of N-linked glycosylation and as a prelude to their biotechnological applications in *A. niger*.

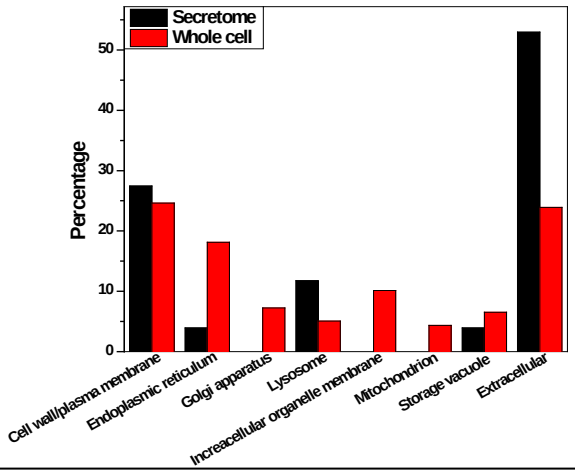

Figure 3. N-glycoproteins identified in cellular compartments based on gene ontology information.

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References:

[1] Wang, L., Aryal, U. K., Dai, Z., Mason, A. C., *et al.*, Mapping N-Linked Glycosylation Sites in the Secretome and Whole Cells of Aspergillus niger Using Hydrazide Chemistry and Mass Spectrometry. *J. Proteome Res.* 2011.