

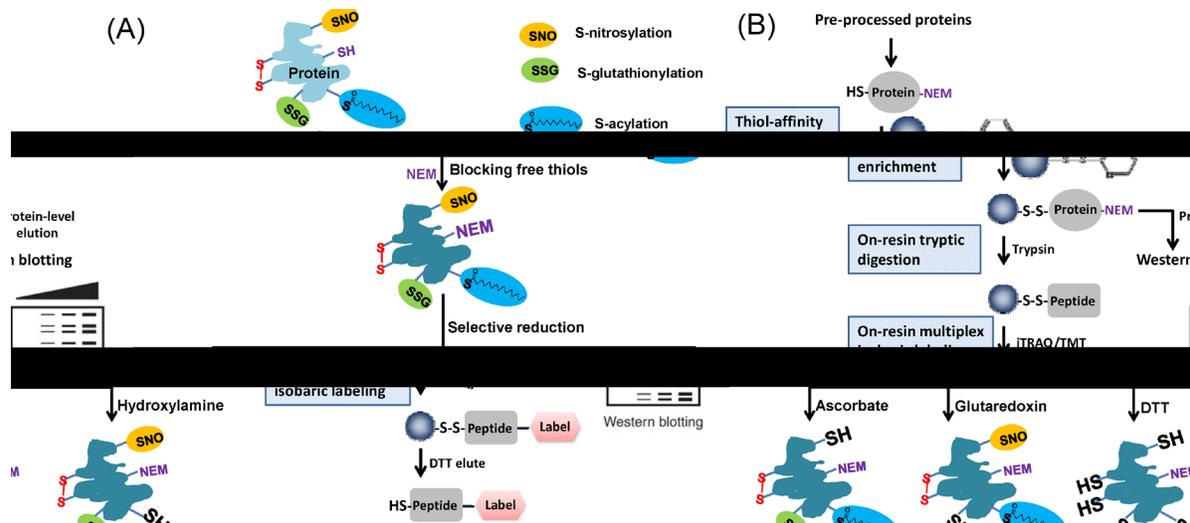
## 128. Quantitative Site-Specific Redox Proteomics on Protein Thiols and Broad Light/Dark Modulation of Thiol Oxidation in Cyanobacteria

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

*Quantitative redox proteomics:* 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 novel 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.<sup>3</sup>

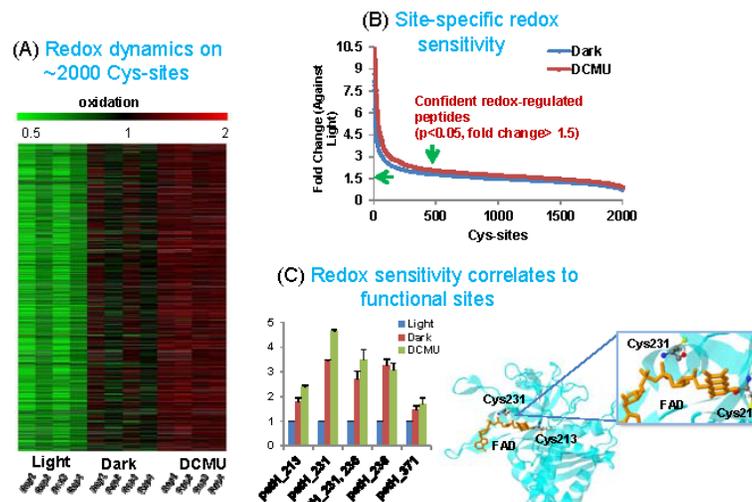


**Figure 1.** (A) Scheme of sample pre-processing for different reversible cysteine modifications. Different types of modifications are selectively reduced by different reagents. (B) Enrichment method for quantitative analysis of reversible cysteine modifications. (Guo, et al., Nat. Protoc. 2014)

Figure 1 illustrates the general principle of selective enrichment and quantification of site-specific

redox modifications. Briefly, thiol specific modifications can be reduced by specific reagents and the converted free thiols can be 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, SSG, and total thiol oxidations in multiple organisms.

Proteome-wide light/dark modulation of thiol-oxidation in cyanobacteria. By applying the 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, we observed redox dynamics for ~2,200 cysteine sites from 1,060 proteins under different conditions (light, dark, and in the presence of a photosystem II inhibitor DCMU) (Fig. 2A and 2B). The results revealed broad proteome-wide changes in thiol oxidation in many key biological processes, including photosynthesis, carbon fixation, and glycolysis. Moreover, the redox sensitivity data enabled prediction of potential functional cysteine sites for proteins of interest (Fig. 2C).



**Figure 2.** (A) Heatmap of the relative levels of oxidation of ~2000 identified Cys-sites under light, dark or DCMU conditions. (B) The redox sensitivity of individual Cys-sites. (C) The correlation of site-specific redox sensitivity with functional Cys sites.

Taken together, our results not only demonstrate the effectiveness of redox proteomics for profiling site-specific thiol modifications under physiological conditions, but also provide significant novel insights into the broad redox regulation of photosynthetic organisms.

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