Quantitative Site-Specific Redox Proteomics and Broad Light/Dark Modulation of Thiol Oxidation in Cyanobacteria

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Overview

- 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 protein cysteine thiols, **(2)** proteolytic processing and protein N-terminal modifications[1], and **(3)** glycosylation[2]. All three classes of modifications are ubiquitous in both prokaryotic and eukaryotic cells and their importance for cellular regulation and signaling have been increasingly recognized.

Summary

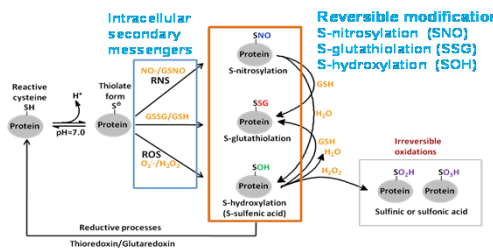
- Functional cysteinyl residues in proteins serve as "redox switches" through reversible oxidation, which is recognized as a fundamental mechanism of redox regulation in almost all organisms.
- We have developed a novel quantitative redox proteomics approach for measuring different types of reversible modifications on individual cysteine thiols to study redox regulation in metabolism or stress conditions of different organisms[3].
- Application of redox proteomics approaches to *Synechocystis sp. PCC* 6803, an oxygenic photosynthetic prokaryote, revealed broad *in vivo* dynamics of thiol oxidation modulated by light/dark cycles.
- Redox dynamics for ~2,200 cysteine sites from 1,060 proteins were observed under different conditions (light, dark, and in the presence of a photosystem II inhibitor DCMU).
- Redox sensitive proteins are involved in many key biological processes, including photosynthesis, carbon fixation, and glycolysis.
- The redox sensitivity data enabled prediction of potential functional cysteine sites for proteins of interest.
- Together, our results demonstrate the effectiveness of redox proteomics for profiling site-specific thiol modifications and provide novel insights into the broad redox regulation of photosynthetic organisms.

References

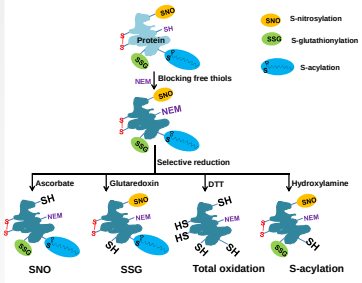
1. Kim, J. S., *et al.*, Resin-assisted enrichment of N-terminal peptides for characterizing proteolytic processing. ***Anal. Chem.*** **2013, 85 (14), 6826-32.**
2. Wang, L., *et al.*, Mapping N-linked glycosylation sites in the secretome and whole cells of Aspergillus niger using hydrazide chemistry and mass spectrometry. ***J. Proteome Res.*** **2012, 11 (1), 143-56.**
3. Guo, J., *et al.*, Resin-assisted enrichment of thiols as a general strategy for proteomic profiling of cysteine-based reversible modifications. ***Nat. Protoc.*** **2014, 9 (1), 64-75.**
4. Su, D., *et al.*, Proteomic identification and quantification of S-glutathionylation in mouse macrophages using resin-assisted enrichment and isobaric labeling. ***Free Radic. Biol. Med.*** **2014, in press.**

Quantitative redox proteomics

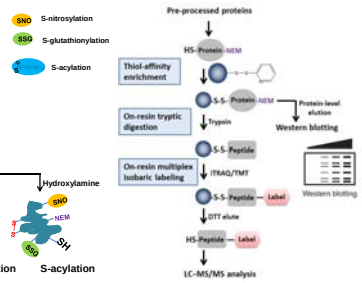
General mechanism of redox modifications



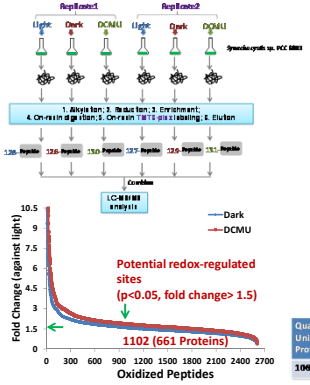
Chemistry principles

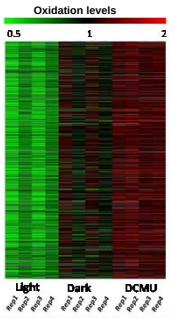


Enrichment and quantification



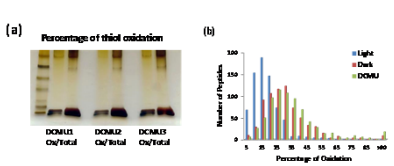
Broad light/dark modulation of thiol-oxidation in photosynthetic cyanobacteria (*Synechocystis sp. PCC* 6803)



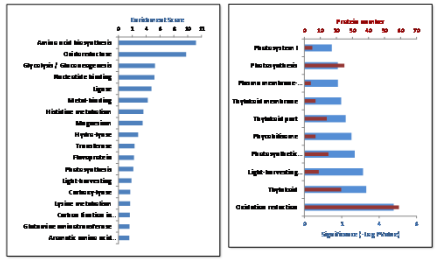


Quantifiable Unique Proteins	Quantifiable Unique Peptides	Protein coverage	Specificity (Cys/TotalCys)
1060	2067	33%	97 %

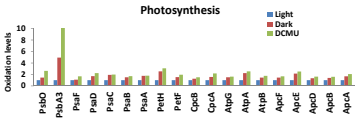
Stoichiometry of Cys oxidation



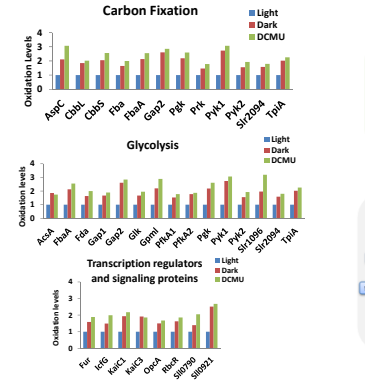
Functional categories

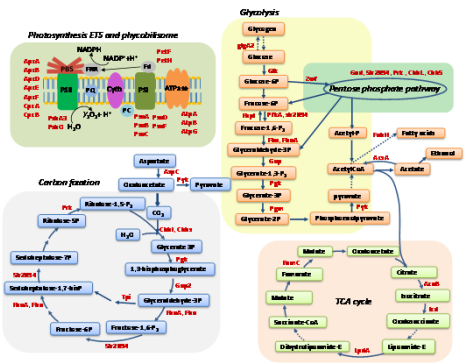


Oxidation of selected proteins

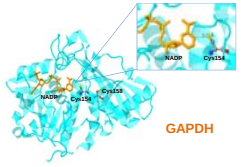


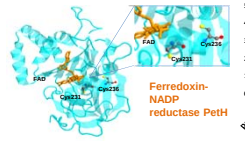
Redox-sensitive proteins in important biological processes





Correlation of site-specific redox sensitivity with functionality





Gene ID	Accession	Protein name	Cys-sites	Levels of oxidation in ratio* Dark/ Light	Levels of oxidation in ratio* DCMU/L ight
sll0080	P54899	N-acetyl-gamma-glutamyl-phosphate reductase (argC)	151	10.9	22.7
			151, 154#	3.3	42.8
			154#	3.9	5.0
			73	2.8	3.5
sll0427	P10549	Photosystem II manganese-stabilizing polypeptide (psbO)	48@	1.4	2.8
			73	1.5	2.4
sll1342	P80505	NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenase (gap2)	154#	3.4	4.1
			154#, 158	2.6	2.8
			158	2.6	2.7
			292	1.8	1.7
slr0394	P74421	Phosphoglycerate kinase (pgk)	216	2.2	2.3
			314@	3.0	4.5
			94	1.8	1.8
			94, 97	1.3	1.3
			97	1.8	1.8
slr0506	Q59987	Light-dependent NADPH-protochlorophyllide oxidoreductase (por)	37@	2.3	2.7
			33	1.3	1.5
			226@	3.7	7.5
slr0527	Q55468	Transcription regulator ExsB homolog (queC)	190@	1.8	2.7
slr1463	P28371	Elongation factor EF-G (fusA)	105@	2.2	2.6
			388	1.3	1.2
			676	2.0	2.2
			68	2.2	2.7
slr1643	Q55318	Ferredoxin-NADP+ oxidoreductase (petH)	213	1.8	2.4
			231@	3.4	4.6
			231@, 236	2.7	3.5
			236	3.2	3.1
			371	1.5	1.7

Active sites; @ functional sites based site-mutagenesis.

Acknowledgements

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