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OxiTMT: elucidating the cysteine redoxome using differential chemical labelling

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Introduction

The local redox conditions that reign inside a cell have a determining effect on a number of biological processes. These conditions are often influenced by Reactive Oxygen Species (ROS). Members of the ROS family have been long known for their deleterious effects and linked to a number of pathologies [1], [2]. However, over the past decade, it has become increasingly evident that these species play a key regulating role on a cellular level [3].

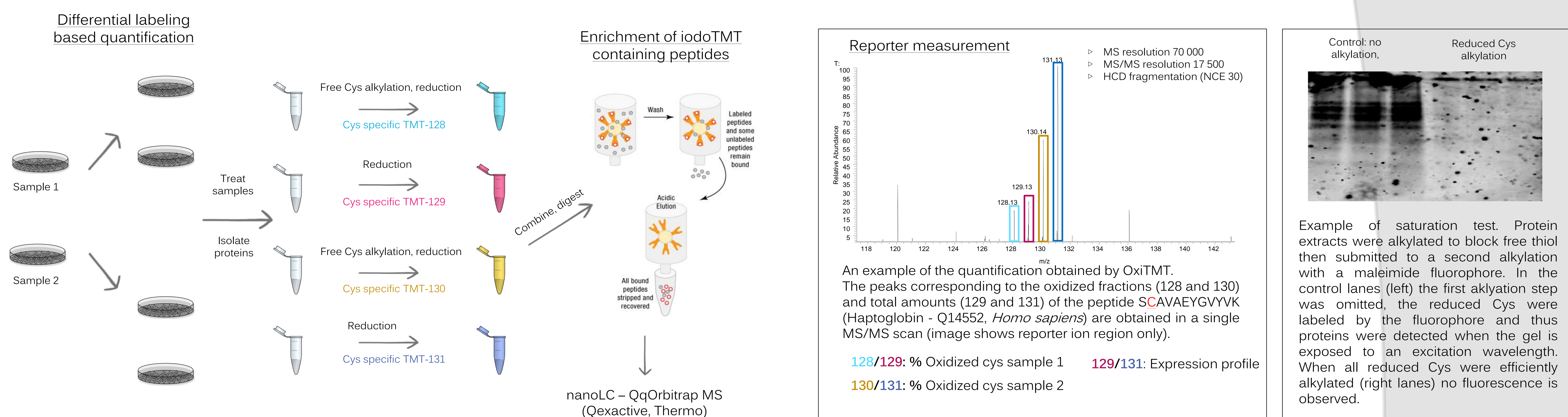
ROS have a number of targets that range from small molecules to DNA. On the proteome level, thiol groups of cysteinyl side chains constitute the major target. Posttranslational modifications (PTMs) on these residues include reversible and irreversible changes.

Problematics & Aims

A number of analytical techniques based on mass spectrometry (MS) have been developed to characterize the cysteine redoxome, often facing a number of technical challenges, mostly related to the lability and heterogeneity of the oxidized forms. Oxidized cysteines constitute a minority, meaning that an enrichment step is needed to increase the dynamic range. Furthermore, any PTM quantification method needs to take the parent protein's expression level into account.

While taking all these limitations into consideration, our group has developed OxiTMT, a cysteine specific chemical labeling based comparative method.

OxiTMT Procedure & Optimization



An example of the quantification obtained by OxiTMT.
The peaks corresponding to the oxidized fractions (128 and 130) and total amounts (129 and 131) of the peptide SCAVAEYGVYVK (Haptoglobin - Q14552, *Homo sapiens*) are obtained in a single MS/MS scan (image shows reporter ion region only).

128/129: % Oxidized cys sample 1

130/131: % Oxidized cys sample 2

129/131: Expression profile

Example of saturation test. Protein extracts were alkylated to block free thiol then submitted to a second alkylation with a maleimide fluorophore. In the control lanes (left) the first aklyation step was omitted, the reduced Cys were labeled by the fluorophore and thus proteins were detected when the gel is exposed to an excitation wavelength. When all reduced Cys were efficiently alkylated (right lanes) no fluorescence is observed.

Results

Oxidative stress essay

- Comparison of *E. coli* cells treated with 1 mM H_2O_2 for 30 minutes with untreated cells.
- Total cysteine and oxidized cysteine fraction in both cultures labeled as illustrated above
- Simultaneous LC-MS analysis. Data analysis performed using MaxQuant, data mining performed on Perseus.

- Enrichment step could be more efficient
- 1229 iodoTMT labeled cysteines identified in total associated to 580 proteins.
- 30 modulated proteins
- 18 significantly changed cysteine redox states
- Unexpectedly, all of the significantly changed states showed a decrease of abundance of the oxidized fraction.

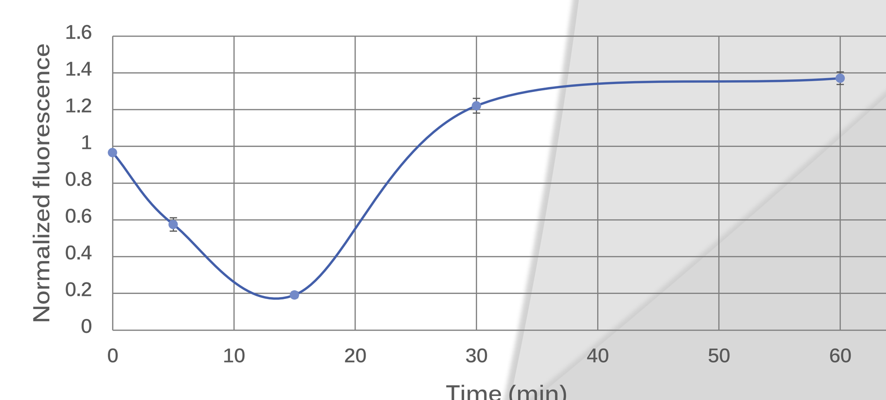
Protein	Expression level	Significance(p-value)
Peptide methionine sulfoxide reductase MsrB	1.68	2.7E-02
Thioredoxin reductase	1.66	3.5E-02
Thioredoxin-1	1.64	5.0E-02
Glutaredoxin-2	1.84	2.4E-03
Glutaredoxin-3	2.10	1.8E-05
Thiol-disulfide interchange protein DsbC	1.67	3.4E-02
Thioredoxin-2	1.84	2.3E-03

Selection of proteins with significantly changed expression levels in H_2O_2 treated cells. All these proteins are involved in maintaining the redox homeostasis.

Discussion

- Data show that the significant redox changes correspond to a decrease in the percentage of the oxidized fraction.
- Upon inspecting the significantly reduced cysteine residues, we find the active site (Cys64 - Cys67) of thioredoxin-2
- Thioredoxin-2 also found to be up regulated: this indicates an increase in the expression of the protein, mostly in its reduced form to counteract the effects of the H_2O_2 treatment.

- Burst of exogenous H_2O_2 leads to the activation of the antioxidant response that includes the thioredoxin pathway and the glutathione pathway, thus establishing a more reducing environment. Similar results, have already been reported [4].
- This observation was also tested by submitting *E. Coli* cells to a kinetic study following a 1 mM H_2O_2 exposure. The quantity of free thiols was estimated by Dylight 550 sulfhydryl labeling. The estimated free thiol quantity was corrected by the total protein amount in order to draw the kinetic plot (right).



The results show that at t = 30 min, the amount of free thiols is more important than in the basal condition (t = 0), thus confirming an adaptive response to H_2O_2 treatment.

Conclusion

Here, we report a novel method, OxiTMT, for the simultaneous quantification of protein expression levels and oxidized cysteine residues. OxiTMT was confirmed on an *E. Coli* model treated with H_2O_2. Results showed the method to be adequate for the analysis of cysteine PTMs. The OxiTMT concept allowed the generation of redox data that could be corrected by the protein expression profile. This information is crucial and must be integrated to all PTM studies, regardless of the concerned amino acid. Reporter ion PTM quantification still faces however some challenges though; our latest essays show that reporter ions relating to the least abundant species (often PTMs) are much more affected by signal to noise ratio compared to the more abundant reporter ions (total protein amount).

References
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