Profiling the cysteine redox proteome: chemical *vs.* metabolic labeling

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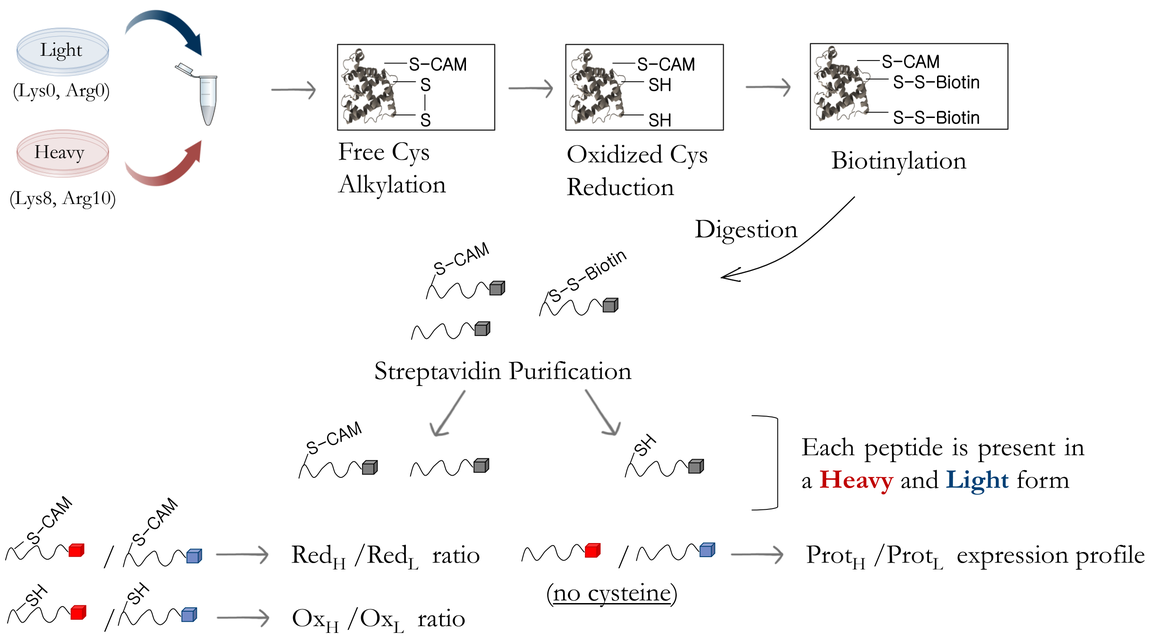
Introduction

The local redox conditions that reign inside a cell have a determining effect on a number of biological processes. These conditions are often set by Reactive Oxygen Species (ROS) and/or Reactive Nitrogen Species (RNS). Members of the ROS and RNS families have been long known for their deleterious effects and thus been linked to a number of pathologies ranging from cancer [1] to aging and neurodegenerative diseases [2]. However, over the past decade, it has become increasingly evident that these species play a key regulating role on a cellular level, hence establishing redox changes as physiological, rapid and specific cell signaling events [3].
ROS and RNS have a number of targets that range from small molecules to lipids, proteins and DNA. On the proteome level, thiol groups of cysteinyl side chains constitute the major target. Posttranslational modifications (PTMs) on these residues include reversible changes such as S-nitrosylation, sulfenylation and the formation of disulfide bridges. Cysteines can also undergo irreversible modifications leading to the formation sulfinic and sulfonic acids.

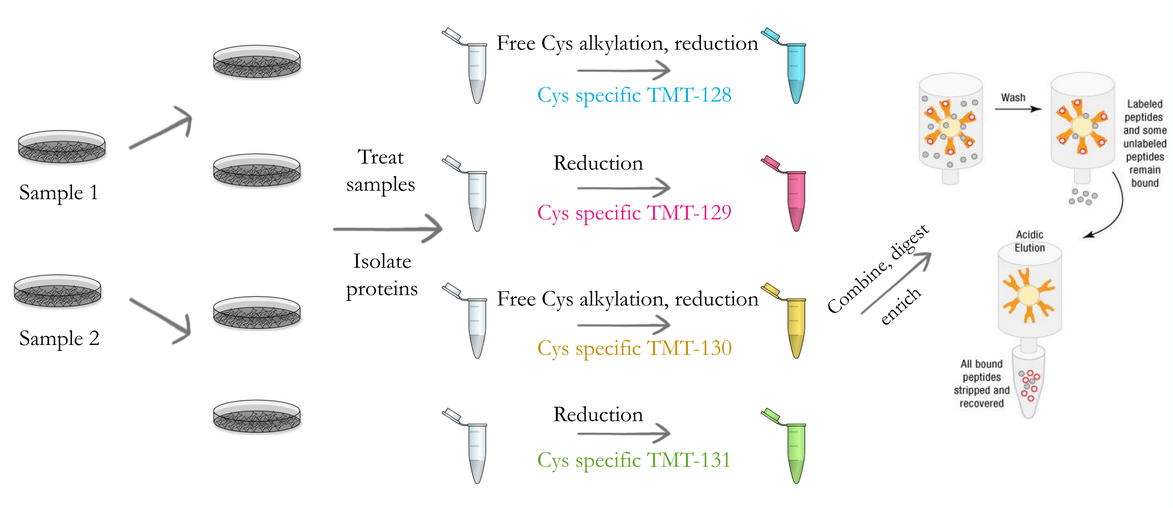
Problematics & Aims

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 on the proteome level meaning that an enrichment step is needed to increase the dynamic range to reach the least abundant forms. 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 OcSILAC, a comparative proteomics method based on metabolic labeling, and presented it at the 2013 EuPA meeting in Saint Malo (France). We're also developing a second method, OxiTMT, based on chemical labeling to complete our panel of redoxomic analytical tools.

OcSILAC : Procedure



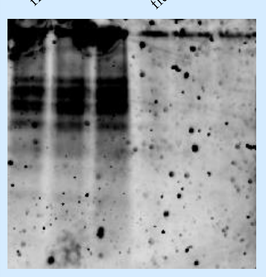
OxiTMT : Procedure



128/129: % Oxidized cys sample 1
130/131: % Oxidized cys sample 2

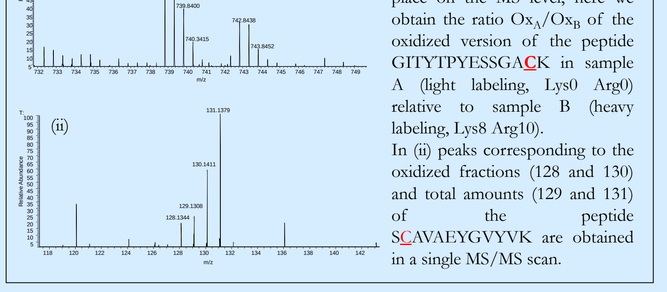
129/131: Expression profile

Optimization & Results



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 alkylation 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.

Both methods needed to be tested for thiol saturation. In both cases we proceeded by adding a maleimide activated fluorophore following the alkylation step, to ensure that the concentration of alkylant we used was enough to block all free thiols before reduction. When this is the case, no fluorescence should be detected. Tests carried out on HeLa extracts for both methods showed that we were working in saturating conditions in both cases. However, OxiTMT optimization tests on tissue showed that saturation conditions were less reliable and highly dependent on the quantity of extracted proteins which itself is dependent on the quantity and especially nature of the studied tissue.



An example of the quantification obtained by the two different methods: (i) OcSILAC and (ii) OxiTMT.
In (i) the quantification step takes place on the MS level, here we obtain the ratio Ox_A/Ox_B of the oxidized version of the peptide GITYTPYESSGACK in sample A (light labeling, Lys0 Arg0) relative to sample B (heavy labeling, Lys8 Arg10).
In (ii) peaks corresponding to the oxidized fractions (128 and 130) and total amounts (129 and 131) of the peptide SCAVAEYGVYVK are obtained in a single MS/MS scan.

Discussion

Data processing was done using MaxQuant for OcSILAC and PEAKS Studio for OxiTMT. While OxiTMT provided absolute quantification of the percentages of oxidized cys in a sample, OcSILAC provided relative data and percentages were accessible only when all three ratios provided by the method were available. However, OcSILAC presents the advantage of eliminating any sort of bias before any sample handling, whereas with OxiTMT we were faced with the problematic of data normalization. Furthermore, on the MS level, OxiTMT suffers from interference co-isolation, an issue faced with all isobaric labeling tandem mass quantification methods. Finally, OxiTMT presents the main advantage of being compatible with tissue studies, which remains the most important limitation of OcSILAC.

OcSILAC	OxiTMT
Limited bias due to sample handling	Access to percentages of oxidized/reduced cys
	Can be applied to tissue
Relative quantification	Samples mixed after labeling
Not compatible with tissue study	Pic co-isolation in MS

Conclusion

We have developed two quantitative methods based of two different principles of labeling to characterize the cysteine redoxome by mass spectrometry. The two methods, OcSILAC and OxiTMT present downsides related to the respective labeling technique used as well as some valuable advantages giving us access to a rich toolbox of redox proteomics analytical techniques.

References
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