Application of iTRAQ reagents to relatively quantify the reversible redox state of cysteines

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The dynamic nature of the proteome ensures that the cell is able to respond to environmental, genetic, biochemical and pathological perturbations. How the proteome responds to these stimuli is of considerable interest as it can relate to the cells stress response and can take the form of post-translational modifications and inter-protein interactions with subsequent effects on translation and transcription. Improvements in mass spectrometry has lead to the development of a number of techniques to quantify the abundance of proteins within any given sample, these include isotope coded affinity tags (ICAT), stable isotope labelling of amino acids in cell culture (SILAC) isobaric tags for relative and absolute quantification (iTRAQ). However, measuring the relative quantity of a protein between two samples does not tell us anything about the functional state of the protein itself, this is especially important in reference to redox proteins that contain thiol switches and are susceptible to activation or inactivation. We have used the redox sensitive yeast enzyme alcohol dehydrogenase, which has a decrease in the number of free thiols and loss of activity in response to oxidative stress. This protein contains two cysteine containing tryptic peptides that are amenable to MS analysis. We have applied iTRAQ technology to these peptides to relatively quantify the reversible redox state of cysteine peptides both in a control and test state, in a single analysis.