

**P019** Identification of thioredoxin targets using a quantitative proteomics approach based on isotope-coded affinity tags **Hägglund, P.<sup>1</sup>, Bunkenborg, J.<sup>2</sup>, Maeda, K.<sup>1</sup> Finnie, C.<sup>1</sup> and Svensson, B.<sup>1</sup>**

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Thioredoxin is a protein disulfide reductase implicated in a wide range of cellular processes, e.g. metabolic control, protection against oxidative stress, transcriptional regulation, and cell division. However, the detailed target specificity is still not well understood. To further the understanding of the molecular recognition of thioredoxin, we have developed a novel quantitative proteomics approach for reliable identification of specific targets *in vitro* based on isotope-coded affinity tags (ICAT) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). By exploring the thiol-specific reactivity of the iodoacetamide-based ICAT reagent, specific target disulfides are identified and thioredoxin-mediated reduction is quantified by measuring ratios of peptides labeled with ICAT reagents containing “heavy” (<sup>13</sup>C) and “light” (<sup>12</sup>C) carbon isotopes. We applied the method to identify targets of a cytosolic h-type thioredoxin (HvTrxh1) in dissected barley embryo. Previously characterized target proteins such as peroxiredoxin and cyclophilin were identified as well as a range of novel putative targets including several ribosomal proteins.

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