Developing a specific multiplex analytical strategy for redox proteomics

Shakir Shakir[1], Giovanni Chiappetta[1], Alise Ponsero[2], Michel B. Toledano[2], Joëlle Vinh[1]

1 ESPCI-ParisTech, Spectrométrie de Masse Biologique et Protéomique, CNRS USR 3149, 10 rue Vauquelin 75231 Paris, France
2 Laboratoire Stress Oxydants et Cancer, DSV, IBITECS, CEA-Saclay, Gif-sur-Yvette, France

Contact: shakir.shakir@espci.fr

The accumulation of reactive oxygen species (ROS) inside cells is a condition leading to oxidative stress, a phenomenon tied to a number of biological processes ranging from normal processes like aging to pathological processes such as cancer and neurodegenerative diseases. At the proteome level, the presence of an increased amount of ROS leads to a number of posttranslational modifications (PTMs); notably the oxidation of cysteine residues leads to the formation of sulfenic acid (-S-OH), S-nitro groups (S-NO) and disulfides bridges, the latter known as oxidative folding. These general conclusions have led to the development of analytical techniques in proteomics aiming to exhaustively characterize and quantify PTMs on cysteine residues. Redox proteomics remain a technical challenge due to the labile nature of thiol-redox reactions. Furthermore, compared to other PTMs, the number of modified residues per protein can be high. The low abundance of oxidized proteins, combined to the intrinsic heterogeneity of the oxidized forms is another source of complexity.

OcSILAC is a shotgun proteomics approach based on the differential labeling of thiols, coupled to a SILAC strategy. The concept of this approach was presented at the last EUPA meeting in Saint Malo. In brief, two cell cultures, representing two experimental conditions, are grown in a medium supplemented with either normal or heavy lysine ($^{13}C_6$ $^{15}N_2$) and arginine ($^{13}C_6$ $^{15}N_4$). The protein extracts are mixed and the oxidized cysteine residues are selectively labeled with a thiol specific reagent. Following a tryptic digestion, the labeled peptides are enriched and analyzed by LC-MS/MS (QExactive, Thermo Scientific) to identify/localize the oxidized cysteines and quantify the redox differences between the two cultures. OcSILAC represents a discovery step; it has been successfully applied to a thioredoxin reductase-1 knockout yeast model.

We here present in detail all the post-analysis treatment needed in order to quantify the change in protein expression profiles with a high confidence level. Furthermore, cysteine containing peptides receive a special treatment allowing the localization and the quantification of the change in redox state. This value is normalized by the change in expression profile. The emphasis is put on the high precision and resolution needed to reach the confidence levels that allow us to precisely characterize and quantify the redox state of a protein down to the cysteine level. This is achieved thanks to the FTMS instrument used in this study.