**Doctorat PSL (ED388, spécialité Chimie analytique, protéomique)**

# Study of redoxome and associated post-translational changes: application to oxidative stress of endoplasmic reticulum

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## Description of the PhD project

When translocated into the endoplasmic reticulum (ER), polypeptides are glycosylated and folded in their native form. For many of them, folding is stabilized by disulfide bonds in a process called oxidative protein folding. The formation of these disulfides is catalyzed by ER oxidase Ero1, a FAD enzyme that reduces $O_2$ to $H_2O_2$ and in turn oxidizes into catalytic disulfide (1). The poor folding of proteins causes stress in the ER, triggering a signaling cascade called Unfolded Protein Response (UPR) to restore cell homeostasis. Indeed, if this stress is not resolved, it can lead to cell apoptosis. The elements influencing the fate of cells during ER stress have been the subject of intense investigations, as they have relevant implications for many human pathologies, particularly in cancer and diabetes (2).

In addition to glycosylation, oxidation plays a central role in cellular processes. Its physiological role and influence in the processes related to cancer or aging are little understood. While oxidative stress can disrupt biological functions, oxidation-reduction (redox) reactions in a cell are often very finely regulated. A growing number of comments indicate that Cys oxidation should be considered as a post-translational modification involved in protein regulation. Methionine residues are also highly reactive and have been described as being involved in the crosstalk effects of protein oxidation and glycosylation. The redox protein status often has an impact on its catalytic activity, conformation, or interactions with metals.

Our group is currently involved in a research project (ANR Erred2) to study redox metabolism's impact on ER pathophysiology. We have already developed an analytical pipeline to quantify redox cysteine events at the proteome scale (5). These approaches should provide a snapshot as close as possible to the redox state. However, the analysis of proteins involved in ER stress signaling is still limited by their low abundance. It is thereby important to establish the identity and functions of two of the still poorly characterized components of ER metabolism in order to identify (i) the pathways providing reducing equivalents of thiols and (ii) the sources of $H_2O_2$ produced during stress and their functional link with the oxidation pathway of thiols and with UPR. Specific tools need to be designed to monitor stress indicators. Today, many bio-analysis tools are used to monitor the stress of ER (3). These are mainly based on measuring the levels of certain key proteins by immunodetection assays and analysis of

associated transcripts. Besides, the signaling cascade involving phosphorylation is also monitored by specific antibodies

The main limitation of these techniques is that they are a heterogeneous set of indirect detection tools that need antibodies and primers. A first attempt to monitor low-abundance stress protein indicator levels in ER has recently been carried out (4), showing potential improvements that mass spectrometry can make in this area. However, profiling of post-translational changes involved in the UPR is still lacking.

This PhD project aims to implement a targeted method of mass spectrometry to monitor in a single analysis many indicators of ER stress, including protein abundance tracking and post-translational changes such as cysteine oxidation and serine/threonine phosphorylation. This will be done by combining capillary chromatography, protein chemistry, and high-resolution mass spectrometry methods at various stages.

Several technological aspects of the project:

- Working on confined microfluidic systems to overcome redox phenomena induced by the presence of surrounding oxidative species.
- Developing an optimal dedicated nano-chromatography system by manufacturing dedicated home-made columns (75 m or less internal diameter) by eliminating dead volumes.
- Developing and optimizing MS/MS nanoLC coupling with or without ion mobility separation to best cover the complex sample and detect minor species with their heterogeneity.
- Characterizing the analysis methods dedicated to the study of oxidation, glycosylations, and phosphorylations of proteins of interest.

The group will strongly support this central project, which will benefit from the unique infrastructures for proteomics (ESPCI facility) and microfluidics (IPGG facility).

## Keywords

microfluidics, mass spectrometry, redoxomics, glycoproteomics

**References**

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# Unité de recherche

Spectrométrie de masse biologique et protéomique, ESPCI Paris, Université PSL, 10 rue Vauquelin 75005 Paris (France)

## Description of the research Unit

Our research group was established in 2009. It provides proteomic solutions and makes constant technological developments. We have been particularly involved in sensitive multidimensional protein quantification strategies, differential analysis, including SILAC, and characterization of post-translational changes by mass spectrometry. In particular, we recently combined analytical chemistry, biochemistry, and protein chemistry to study protein oxidation and glycosylation. We have recently developed a specific strategy to identify the proteins that will be implemented as part of this project. The project will beneficiate from the hosted fully equipped proteomics technological platform, and the microfluidic devices will be produced on the IPGG Institute's microfabrication platform. We want to develop a home-made dedicated nano separation device.

## Name of the supervisor

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## 3i Aspects of the proposal

**Intersectionality** The project will include a multidisciplinary strategy using mass spectrometry, microfluidics, microfabrication, proteomics, and bioinformatics. It is of the utmost importance for industrial application as there is a significant need to study post-translational modifications, either for quality control of production and storage or for diagnosis and clinical management.

**Interdisciplinarity** The project is an interdisciplinary project at the interface between protein chemistry, physical chemistry, microfluidics, and biology. There needs to be a strong involvement of the candidate who will have to be trained in various disciplines, from nanochromatography to microfabrication and interface design, as well as biology and proteomics.

**International Mobility** If successful, the redoxomic strategy will be applied to projects in collaboration with Italian groups on thyroid cancer. More generally, there is no strategy available to address redox and protein glycosylation simultaneously, and once validated, the protocol will be transferred to the ESPCI proteomics platform (national platform, with more than 100 users/year)

## The Candidate

This doctoral project is at the interface of analytical chemistry and biology.

Expected Profile of the candidate: Master M2 with a dominant in analytical chemistry. A (theoretical) knowledge of mass spectrometry and miniaturized separation techniques is expected. He must have validated a full M2 master's degree or an engineering degree.

The candidate must have a good background in biochemistry and analytical chemistry. Previous experiments in separative techniques, mass spectrometry, or protein chemistry are welcome. Finally, the analysis of the results uses data processing tools in bioinformatics that may require the development of specific tools

**Call for applications:** from March 01 to May 31, 2021

**Interviews:** Fifo, videoconferences or presential

**Application:** Please apply by email by sending a file containing a detailed CV, cover letter for the project, copy, and transcript of the last two diplomas, letters of recommendation, or certification for possible prior experiences.

- At the time of the call for applications deadline, applicants must be in possession of or finalizing their Master's degree or equivalent/postgraduate degree.
- At the time of recruitment, applicants must be in possession of their Master's degree or equivalent/postgraduate degree, which would formally entitle them to embark on a doctorate.
- Candidates in possession of their Master's degree at the time of the call for applications must be within the first four years (full-time equivalent research experience) of their research career (excluding career breaks). Career breaks referred to periods when the candidate was not active in the research, regardless of employment status (sick leave, maternity leave, etc.). Short stays such as holidays and/or compulsory national service are not taken into account.
- Applicants must be available to start the program on time (no later than September 2021)
- Citizens of any nationality may apply.