Sensing and Measuring of redox modifications in proteins

Lavrentis Dimitrios Galanopoulos, David J. Clarke

EaStChem, School of Chemistry, University of Edinburgh, Edinburgh, Scotland, EH9 3JJ

L.Galanopoulos@ed.ac.uk

First Year Summary

The first year of the PhD consisted of a training period to acquire research skills and knowledge on sensing principles and applications in industry.

Furthermore, practical projects were conducted to develop specific skills, like 3D printing, prototyping, software development and oral skills.

Protein Redox Regulation – Redox Switches

Sulfur occupies a unique position in biology because of its ability to adopt a broad range of oxidation states (-2 to +6) and distinct chemical forms (chemotypes). This oxidative diversity, along with the inherent reactivity of the thiol and thiolate modifications, drive to the amino acid cysteine being susceptible to a diverse range of redox reactions which can lead to an array of protein covalent modifications. There is overwhelming evidence for the notion that these reversible oxidative post-translational adjustments (oxPTMs) can potentially act as binary 'switches' - regulating protein function; and, as such, constitute an essential regulative mechanism widespread throughout the proteome. In this project, we explore mass spectrometry techniques for mapping oxPTMs and examine their applicability at the proteomic scale.

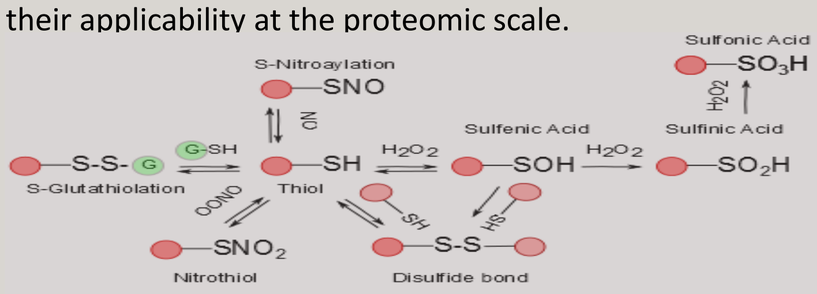


Figure 1: Oxidative thiol modifications.

Aims and Objectives

Here we propose to develop an automated platform for efficient and fast mapping oxPTMs of proteins. This system will be hyphenated with FT-ICR mass spectrometry to achieve accurate intact protein mass characterisation and middle down fragmentation MS techniques to analyse specific oxPTMs.

A short systematic study of redox modification of protein will be performed using a carefully selected well established set of proteins. The whole procedure will be optimized by using automated robotic fluidic handling. During this project, new procedures will be settled to multiplex the current workflows allowing the rapid determination of oxPTMs for various proteins, in a parallel manner, by developing a software.

Selected References

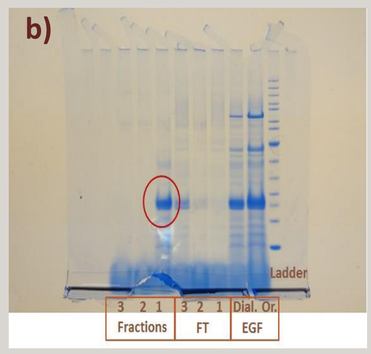
1. J. Scotcher, B. Bythell and A. Marshall, *Analytical Chemistry*,2013, **85**, 9164-9172.
2. S. Thurlow, D. Kilgour, D. Campopiano, C. Mackay, P. Langridge-Smith, D. Clarke and C. Campbell, *Analytical Chemistry*, 2016, **88**, 2727-2733.
3. J. Scotcher, D. Clarke, C. Mackay, T. Hupp, P. Sadler and P. Langridge-Smith, *Chemical Science*, 2013, **4**, 1257.
4. Conte, M., Carroll, K., The chemistry of thiol oxidation and detection. *In Oxidative Stress and Redox Regulation*, ed. Jakob, U. and Reichmann, D., Springer, Netherlands, 2nd edn, 2013, vol. 1,ch. 4, pp 1-42.,

EGF5-7

A novel uncharacterized protein (EGF5-7) was studied. The protein was purified and afterwards the number of free cysteines was determined. This was achieved by treating 1 mM solution of protein with 8 mM TCEP and then 20 mM NEM. As follows from Figure 2a, the obtained mass spectra was identical to the previous one indicating that the cysteines are not freely available.

a)

AGIPDNKCDLSCPSNKVCVIENGKQTCKC
SERFVLENGVCICANDYKMEDGINCIAKNK
CKRKEYENICTNPNEMCAYNEETDIVKCEC
KEHYYRSSRGECILNDYCKDINCKENEECS
IVNFKPECVCKENLKKNNKGECIYE



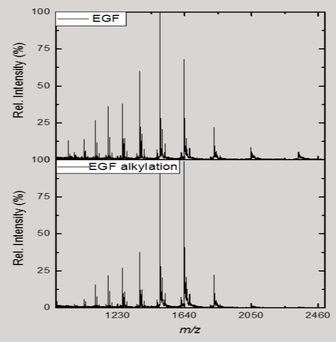


Figure 2: a) Primary sequence of EGF5-7. **b)** Purification of EGF5-7 domain **c)** Initial (1) and after TCEP and NEM addition (2) mass spectra of the protein.

Human neutrophil peptide 1 (HNP1), 2 (HNP2), 3 (HNP3)

Human neutrophil peptide 1 (HNP1), 2 (HNP2) and 3 (HNP3) were chosen as model peptides because each of them has three disulfide bonds. These disulfide bonds have been extensively defined, making these peptides a suitable choice for validation of the developed method.

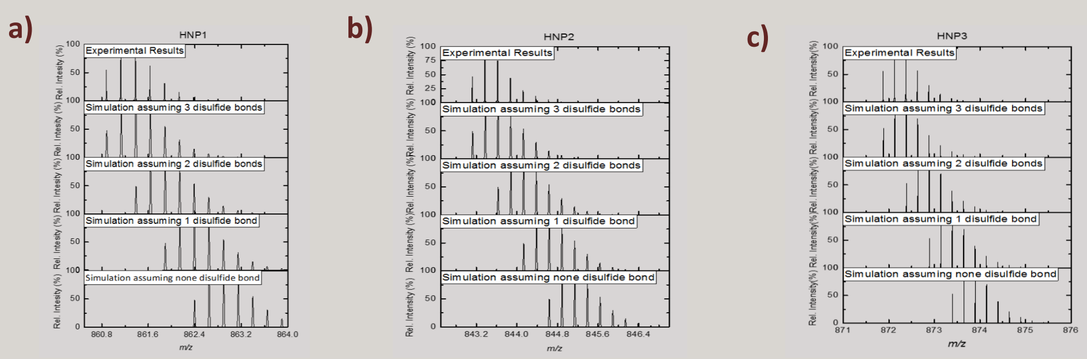


Figure 3: The MS spectra of HNP1 **(a)**, HNP2 **(b)** and HNP3 **(c)** and the comparisons of the experimental results with the simulations assuming 3, 2, 1 and none disulfide bonds.

Fragmentation	Dig.Peptides	Meas. Mass	Theor. Mass	Error	Comments
ECD	ACYR YGTCIYQGR	1667.73003	1667.7619	19.167	1 disulfide bond
	DCYCR YGTCIYQGR	1715.64534	1715.6852	23.233	1 disulfide bond
CID	ACYR YGTCIYQGR	1671.68467	1671.6953	6.402	1 disulfide bond

Table 1: ECD and CID of HNP1.

Research Plan

Major milestones	Deadline
1. Literature review work	Dec-17
2. Establishment of a platform for automated redox titrations	Aug-18
3. Establishments of software for automated data analysis using genetic algorithms	Feb-19
4. Modifying the software based on additional experiments	Aug-19
5. Establishments of oxPTMs of unknown proteins	Feb-20
6. Thesis writing	Aug-20