MAASTRICHT . THE NETHERLANDS

IMSC 2022

ABSTRACT BOOK

27 AUGUST - 2 SEPTEMBER 2022

Plenary Talks

Sunday 28 August 2022: 17:00 – 17:30

PROTEOMES IN 3D

Abstract ID: **848**

Presenting author: Paola Picotti, ETH Zurich

Introduction

Proteomics has been broadly applied to detect changes in protein levels in response to perturbations and derive information on altered pathways. Beyond protein expression changes, however, biological processes are also regulated by molecular events such intermolecular interactions, chemical modification, aggregation and protein conformational changes. These events do not affect protein levels and therefore escape detection in classical proteomic screens. Protein structures integrate all these different types of regulatory molecular events. In my talk I will demonstrate that global, in situ analyses of protein structures allow detecting various types of protein functional changes simultaneously, yielding a more detailed and nuanced picture than measurement of abundance changes alone.

Methods

Our group developed the limited proteolysis-coupled mass spectrometry (LiP-MS) approach to monitor protein structural changes directly within complex biological extracts and on a proteome-wide scale. It is based on the application of unspecific proteases to native proteome extracts for a short time, followed by complete trypsin digestion under denaturing conditions and mass spectrometric analysis. Comparison of structure-specific proteolytic fingerprints from different conditions identifies structurally altered proteins and allows pinpointing structurally altered regions. Differential proteolytic patterns are represented along the protein sequence in the format of structural barocdes.

Preliminary data (results)

In my talk, I will demonstrate that a global protein structural readout based on LiP-MS detects various types of functional alterations in situ, such as enzyme activity changes, phosphorylation, protein aggregation, and protein complex formation in microrganisms undergoing nutrient adaptation and responding to acute stress. The structural resolution of the approach pinpoints individual regulated functional sites such as binding and active sites, thus supporting the generation of mechanistic hypotheses and linking holistic and reductionist approaches. I will show how the structural readout allows detecting novel regulatory interactions due to metabolite-protein interactions. Further, LiP-MS can be used to identify drug targets o and binding sites in an unbiased manner and directly from cell and tissue extracts. Last, I will show how the approach supports the identification of a novel type of protein biomarker based on altered protein structures (structural biomarkers) and present an application to Parkinson’s disease. I will discuss the power and limitations of this new concept.

Please explain why your abstract is innovative for mass spectrometry?

This structural MS readout substantially increases the coverage of classical proteomics screens and supports the direct generation of mechanistic hypotheses. It paves the way for in situ structural systems biology.

CAN WE PREDICT THE PREFERENCE FOR ADDUCT FORMATION IN ELECTROSPRAY?

Abstract ID: **495**

Presenting author: Stepan Stepanovic, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva, Quai Ernest Ansermet 24, 1211 Geneva, Switzerland

Introduction

When electrospray ionization method (ESI) is utilized, the obtained spectra are often complicated by adduct formation with metal or small organic ions. Annotation methods, such as the multi-layered approach, are very powerful, but they do not offer deeper insight into the microscopic mechanisms. In order to tackle these phenomena, we have developed a simple theoretical approach that can be used to predict the preference of ion adducts formation, associated with several protonation/deprotonation equilibria that also play an important role. The strategy consists of using explicit solvation of reactive sites and density functional theory (DFT) as method of choice. It is simple and bypasses complicated ab-initio molecular dynamics calculations, necessary to treat the system dynamics as well as bond making-bond breaking properly.

Methods

All DFT calculations were performed with the Amsterdam Modelling Suite (AMS2021) program package. Initial structures were refined with global minimization techniques available in AMS, namely Basin Hopping and systematic variation of dihedral angles, using XTB semiempirical methodology. The obtained low energy structures were later reoptimized with PBE DFT method, using full electron TZ2P Slater type orbitals basis, Grimme G4 dispersion correction and implicit (as well as partial explicit) solvation, until the maximum gradient component was less than $5 \cdot 10^{-4}$ a.u (default is 10^{-3}). The nature of stationary points is confirmed by calculating analytical Hessians.

Preliminary data (results)

There are many complicated factors that influence the intensity of a specific ion signal in the mass spectrum. With ESI ionization method, these factors include a delicate interplay between a condensed phase ionization process and subsequent solvent expulsion.

We have chosen a succinic acid as a model system for our model development, since it gives solely the ion adducts, has a complicated potential energy surface (with many close lying cyclic and acyclic conformers) and the most important signals involve carboxylic group deprotonation. Experimental spectra of succinic acid with the ion mixture (conc. of all salts is 2M) is shown on Figure 1. It can be seen that the most intensive peaks are from M^{2+} ion adducts and that only somewhat intense M^{+} adduct is with Li^{+} ion. Protonated succinic acid is not observed.

In order to obtain comparable results for both protonation, deprotonation and ion binding, we have treated all important interaction centers with two explicit water molecules. The model is depicted on Figure 1. Simple energetics of the entire process show that M^{2+} ions bind more favorably to succinic acid than M^{+} ions and that acid protonation is the least favorable option. Thus, it is experimentally demonstrated and theoretically confirmed that M^{2+} ion adducts should no longer be neglected.

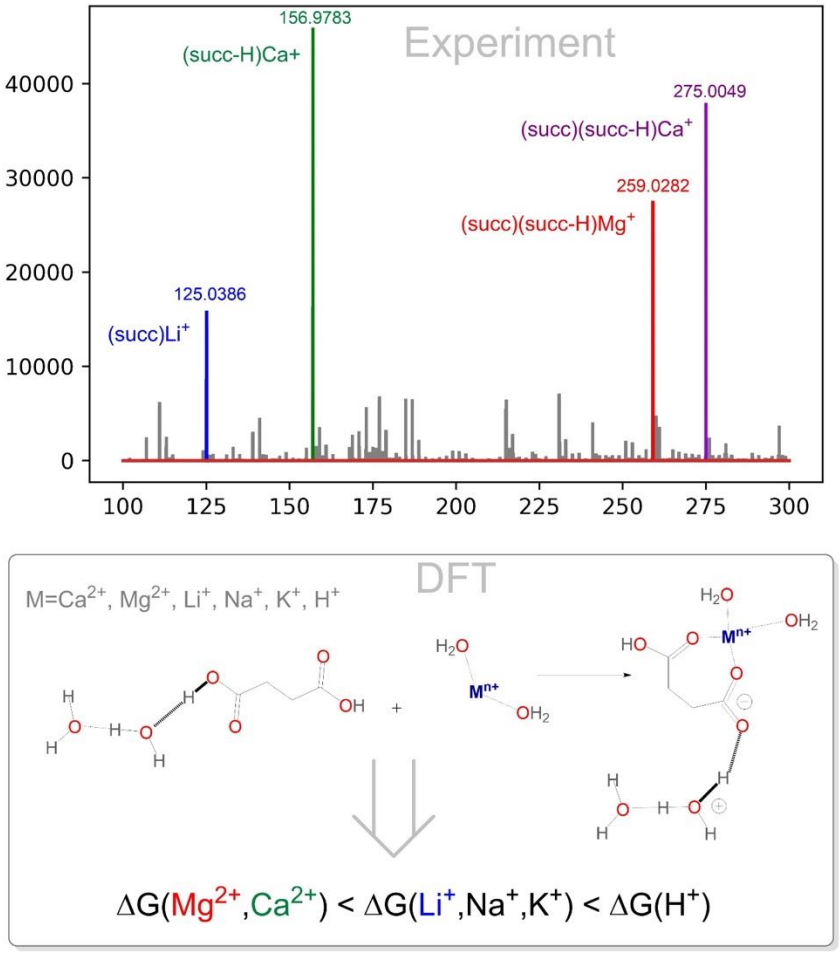
We have also applied our model to methylated uric acid derivatives, and obtained the preference for protonation instead of adduct formation, which is in excellent accordance with experimental results.

Please explain why your abstract is innovative for mass spectrometry?

The relatively simple methodology, to predict preference for ions adducts vs. protonation is presented.

Co-authors:

Gérard Hopfgartner, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva, Quai Ernest Ansermet 24, 1211 Geneva, Switzerland



Experimental mass spectra and theoretical model for succinic acid-ions mixture