MARTA VILASECA

Mass Spectrometry Core Facility

Mass Spectrometry (MS) plays a pivotal role in several scientific disciplines. Today it is an integral part of proteomics and drug discovery processes and also provides relevant information about structural biology. The MS Core Facility provides the IRB Barcelona research community with modern chromatographic and spectrometric tools for the identification and characterisation of a broad range of biological species. One of the main objectives of the Facility is to implement intact protein analysis (top-down approach) for their complete characterisation. In this approach, protein ions are introduced into the gas phase by electrospray and are subsequently fragmented in the mass spectrometer, thereby yielding the molecular mass of both the protein and the fragment ions. The top-down approach is successful for targeted studies of single proteins of less than 100 kDa; however, extending the approach to the analysis of the whole proteome still requires improvements in proteome fractionation. The Facility is working on the development of bottom-up proteomic techniques for protein quantitation and for the determination of post-translational modifications. Moreover, the novel ion mobility-MS coupling methodology is being used to study the macromolecular structure and conformation of proteins and nucleic acids. Along the same line, non-covalent protein-protein and protein-ligand interactions can be directly detected and studied, thereby providing clues as to the mechanisms of action of these proteins in biological processes.

General activities over the year

The MS Core Facility was established in 2007 with the preparation of the lab site and the arrival of instruments at the end of the year. During 2008 all instrumentation was set up and the Facility grew from 2 to 3 members of staff with the incorporation of a second research officer. By June the Facility was providing service to groups in 4 out of the 5 Research Programmes at IRB Barcelona. The Facility also supported research groups at the University of Barcelona and the Autonomous University of Barcelona.

In 2008 we implemented the technology necessary to detect intact non-covalent protein-protein, protein-ligand and DNA complexes of moderate size by infusion. The detection of non-covalent ions is done by applying gradual changes in vacuum between the source and the analyser (ion-cooling). For macromolecular structure and conformation studies, we use ion mobility technology coupled to MS. The calibration of drift time data to collision cross sections is done with protein standards of known cross sections.

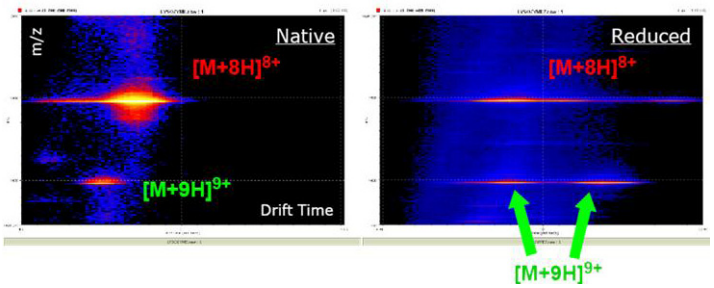

Figure 1. *Example that shows the capacity of Synapt HDMS. This instrument recognises different conformers of a particular charge state. In the mobilogram m/z is represented on the y axis and drift time on the x axis. Note the clear difference between 2 conformers for charge 9 of Hen egg lyzozyme once the native form is reduced.*

Acting Facility Manager Marta Vilaseca **Research Officers** Claudio Diema, Núria Omeñaca

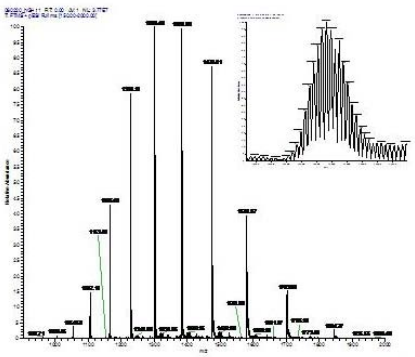

Figure 2. *MS spectra of hGH on LTQ-FT.*

The Facility has set up instrumentation for bottom-up proteomic applications and has begun to implement the techniques for protein quantitation with this approach. Applications of these novel methods are being applied for the identification of biological markers in body fluids.

For the complete characterisation of intact proteins, the above mentioned top-down approach is one of the targeted analyses of the Facility. Using Fourier Transform Mass Spectrometry, accurate data on intact proteins and their fragments ions, formed by Electron Capture Dissociation (ECD) and collision activated dissociation (CAD), facilitate the characterisation of coding polymorphisms (cSNPs), alternative splice events, and post-translational modifications (PTMs) by providing information on 100% of the primary protein structure. This is a challenging approach which we have begun to explore for moderately sized proteins in pure samples as a first step before addressing more complex mixtures.

Services for IRB Barcelona researchers

The services offered include MS, MS/MS and MSn analysis using atmospheric pressure ionization techniques (electrospray and APcI) coupled to LC, nanoLC or infusion inlets. The Facility also provides consultancy services and analytical method development for specific applications, as well as mass spectra data processing. Samples are analysed either directly by the service or by researchers (previously trained by Facility members) who can use mass spectrometers through an open-access system.

Equipment and specialised applications

1-LTQ FT Ultra (ThermoFisher Scientific). Hybrid Mass Spectrometer consisting of a linear Ion Trap, combined with a Fourier Transform Ion Cyclotron Cell. This instrument has exceptional applications for the identification and structural characterisation of small molecules. It permits both bottom-up (proteomics) and top-down approaches for the analysis of intact proteins, including post-transductional modifications (Figure 2).

2-Synapt High Definition MS System (Waters-Micromass). Hybrid QTOF instrument with an incorporated Triwave Cell. This instrument allows tandem MS to be combined with ion mobility, thus permitting the analysis of samples differentiated not only by their mass to charge ratio (like standard analysers) but also by their size and shape. The instrument is used to analyse the macromolecular structure and conformation of intact proteins and to study non-covalent interactions (Figure 3).

3-LCT-Premier XE (Waters-Micromass). Orthogonal acceleration time-of-fight mass spectrometer ideal for analysing high molecular weight compounds. This instrument has been modified to achieve inert conditions inside the ionisation source, to allow amide H/D exchange experiments for the study of the dynamic and structural properties of proteins and their complexes.

Figure 3. *Synapt HDMS Mass Spectrometer.*

The Facility also has 2 LC devices: a) Acquity UPLC (Waters; attached to the LCT-Premier), and b) a quaternary micro LC pump with a micro-autosampler (Thermo) attached to the LTQ-FT and an infusion inlet, Advion Triversa Nanomate, based on chip infusion.

SCIENTIFIC OUTPUT

Collaborations

Cerebrospinal fluid proteomic analysis of patients with Amyotrophic Lateral Sclerosis (ALS)
Jacques Borg, Jean Monet University (Saint-Étienne, France), Joan Guinovart, IRB Barcelona (Barcelona, Spain) and Eliandre de Oliveira, PCB Proteomic Platform (Barcelona, Spain)

DNA non-covalent complexes studies by ion mobility mass spectrometry
Modesto Orozco, IRB Barcelona (Barcelona, Spain) and Ramon Eritja, IRB Barcelona (Barcelona, Spain)

H/D exchange determined by ESI to study molecular recycling in Aβ(1-42) amyloid fibrils
Natàlia Carulla, IRB Barcelona (Barcelona, Spain)

Top-down mass spectrometry
Ernest Giralt, IRB Barcelona (Barcelona, Spain), Michaela Scigelova and Vlad Zabrouski, ThermoFisher Scientific (Bremen, Germany)