



Mass Spectrometry Core Facility

The high demand to discover new drugs and to identify new therapeutic agents has led researchers to focus on cellular proteins. This has been greatly facilitated by significant advances of MS in the field of proteomics. MS now forms an integral part of proteomics and drug discovery processes and can also provide relevant information about structural biology. The Mass Spectrometry Core Facility provides the research community at IRB Barcelona with modern chromatographic and mass spectrometric tools for the identification and characterisation of proteins and other biological species.

The facility implements intact protein analysis (Top-down approach), which aims to provide the complete characterisation of proteins. In this approach, protein ions are introduced into the gas phase and subsequently fragmented in the mass spectrometer, thereby yielding the molecular mass of both the protein and the fragment ions. Top-down is successful for the analysis of targeted proteins of less than 100 kDa; however, no platform is available for extending this approach to whole proteome analysis and the approach still requires improvements for high quality fractionation.

The facility also works with the classical proteomic approach (Bottom-up), which consists of the MS analysis of peptides resulting from previous digestion of proteins with an enzyme for their identification, the determination of post-translational modifications (PTMs), and quantitation. 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.

Set up in September 2007, the Mass Spectrometry Core Facility provides service to 17 research groups from the five IRB Barcelona programmes and has established collaborations within the Institute and with external institutions. For the complete characterisation of intact proteins, in 2009 the facility has implemented the dynamic Top-down methodology. By Top-down, proteins are introduced into the gas phase and fragmented inside of the mass spectrometer by several techniques. In doing this, the goals are to have the intact protein mass, plus a sufficient number of informative fragment ions that can provide a complete description of the primary structure of the protein and reveal all its modifications, as well as any correlations between these modifications. To undertake this approach, we use a chromatographic device coupled to an Advion Triversa Nanomate, which in turn is coupled to an LTQ-FT mass spectrometer (Figure 1). NanoESI is performed by chip technology and full MS data is acquired on the LTQ-FT at a chromatographic scale. Liquid chromatography (LC) fractions are simultaneously collected and reanalysed off-line by static Top-down, performing the fragmentation of selected intact ions by CID (collision-induced dissociation), ECD (electron capture dissociation) or IRMPD (infrared multiphoton dissociation). ProSight software was purchased in May 2009 to analyse Top-down data. We are applying this approach to examine moderately sized proteins in non-pure mixtures. In this regard, Histone 1 in *Drosophila* is being studied in order to provide an accurate map of its PTMs, thereby contributing to the understanding of its functions. Moreover, proteins up to 80 kDa are being analysed by Top-down or Middle-down



Figure 1. Advion Triversa Nanomate coupled to the LTQ-FT mass spectrometer. Instrument configuration implemented for Top-down protein analysis.

(cleaved with CNBr) techniques to determine their binding sites to covalent inhibitors or to determine catalytic mechanisms in the case of enzymes.

In 2009 we have been using the technology implemented last year for the detection of intact non-covalent complexes in our Synapt mass spectrometer. β -amyloid protein oligomers (Figure 2), DNA complexes and protein bound to organometallic compounds, among others, have been analysed. These studies have been complemented with data from ion mobility (IM) experiments, performed in the same instrument. The capacity of the instrument to measure experimental cross sections implies that these experiments provide information about the structure of the macromolecule or the macromolecule-complex. The facility is working on the application of IM-MS methodology to study intact proteins in more complex samples, using a nanoLC device (purchased in April 2009) coupled to IM-MS. The purpose is to complement the Top-down analysis performed in the LTQ-FT in order to enhance the detection and structural characterisation of posttranslational modified proteins by using the potential offered by IM gas phase fractionation.

Throughout 2009, iTraQ (Isobaric Tag for relative and absolute quantitation) and free-label quantitation methodologies have been used, in collaboration with the PCB Proteomics Platform, to search for biomarkers of amyotrophic lateral sclerosis (ALS) in cerebrospinal fluid originating from familiar and sporadic ALS patients. Statistical studies have been performed in conjunction with the IRB Barcelona Biostatistics/Bioinformatics Unit to identify significant changes in the level of expression of identified proteins between SALS, FALS and controls.

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.



Research Group Members

Acting Core Facility Manager:

Marta Vilaseca

Senior Research Officer:

Claudio Diema

Research Officer:

Nuria Omeñaca



Equipment and specialised applications LTQ FT Ultra (Thermo Scientific)

Hybrid Mass Spectrometer consisting of a linear Ion Trap, combined with a Fourier Transform Ion Cyclotron Cell. It is also provided with ECD and IRMPD for complementary protein fragmentation in the mass spectrometer. This instrument is used to perform Top-down MS protein analysis and Bottom-up applica-

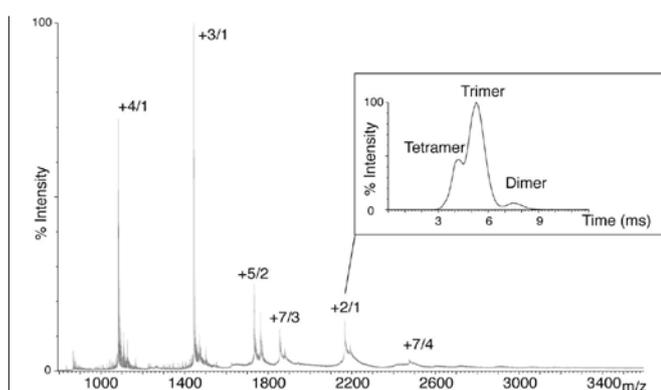


Figure 2. IM gas phase separation allows distinction between the dimer, trimer and tetramer of AB40. Work performed in collaboration with researchers Rosa Pujol, Ernest Giralt and Natàlia Carulla.

tions. In its normal configuration, this instrument works coupled to an LC device (Surveyor LC (Thermo) and a chip-based nanoESI interface (Advion Triversa Nanomate).

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, but also by their shape and size. The instrument is used to analyse the macromolecular structure and conformation of intact proteins and to study non-covalent interactions. When working in its normal configuration, it is attached to a chip-based nanoESI interface (Advion Triversa Nanomate), thus combining the analysis of samples by infusion mode and by nanoLC coupling (NanoAcquity UPLC, Waters; purchased in 2009).

LCT-Premier XE (Waters-Micromass)

Orthogonal acceleration time-of-flight mass spectrometer ideal for the analysis of high molecular weight compounds. This instrument has been modified to achieve inert conditions inside the ionisation source, in order to allow amide H/D exchange experiments for the study of the dynamic and structural properties of proteins and their complexes. In another set-up configuration, it is used to analyse small molecules for their structural characterisation and provides accuracies of less than 3ppm. For LC-purifications, it is normally attached to an LC device (UPLC Acquity, Waters).

Scientific output

Collaborations

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

Evaluation of CSF immunodepletion and fractionation strategies for MS-based biomarker discovery
Jacques Borg, Jean Monet University (Saint-Étienne, France); Àlex Campos, Eliandre de Oliveira, PCB Proteomic Platform (Barcelona, Spain); Joan Guinovart, IRB Barcelona (Barcelona, Spain) and David Rossell, IRB Barcelona (Barcelona, Spain)

Evaluation of Top-down and Middle-down MS strategies for the location of specific inhibitors binding sites to prolyl oligopeptidase
Ernest Giralt and Teresa Tarragó, IRB Barcelona (Barcelona, Spain); Michaela Scigelova and Vlad Zabousov, Thermo Fisher Scientific (Bremen, Germany)

Experimental cross section determination by Ion Mobility Mass Spectrometry of DNA non-covalent complexes
Ramon Eritja and Modesto Orozco, IRB Barcelona (Barcelona, Spain)

Study of the catalytic mechanism of glycosyltransferases: method development for the monitoring of the transference of glycosidic groups by LC-MS
Joan Carles Ferrer, University of Barcelona (Barcelona, Spain)

Top-down analysis of glucose 6-phosphate dehydrogenase: MS and MS/MS approaches to study its modification with methylglyoxal
Javier Luque, University of Barcelona (Barcelona, Spain); Eduardo Silva, Pontificia Universidad Católica de Chile (Santiago de Chile, Chile)

Top-down MS development for the analysis of PTMs in Drosophila melanogaster Histone 1
Ferran Azorin and Carles Bonet, IRB Barcelona (Barcelona, Spain)