

Proteomics Core Unit



Keith Ashman

Unit Head

Keith Ashman was born in Newport, South Wales (UK), in 1956. He studied microbiology at the University of Wales Aberystwyth and obtained an MSc from the same University for studies on the protein chemistry of bacterial ribosomal proteins, carried out at the *Max-Planck-Institut für Molekulare Genetik* in Berlin (Germany).

In 1985 he was awarded an EMBL predoctoral fellowship to study at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany). His PhD dissertation at the University of Wales focused on "the chemical synthesis and expression of a gene coding for human tumour necrosis factor".

In 1989 he moved to Australia where he worked on the isolation of antigens for the development of vaccines against multi-cellular parasites. He returned to EMBL in 1995 to join the group of M. Mann and began to work in the mass spectrometry and proteomics fields. He then moved to Canada in the year 2000 to join the Samuel Lunenfeld Research Institute of Mount Sinai Hospital as Director of Mass Spectrometry.

In 2003 he was invited to join MDS Sciex as a Senior Research Scientist to develop mass spectrometry-based proteomic applications on new instrument platforms. In 2005 he returned to Australia as Director of Mass Spectrometry at the Australian Proteome Analysis Facility (APAF) in Sydney.

Since June 2008 he has been Head of the CNIO's Proteomics Core Unit.

Summary

The field of proteomics is progressing beyond protein identification into multi-plexed protein quantitation through quantitative mass spectrometry-based techniques such as stable isotopic labelling (SILAC, iTRAQ) and selective reaction monitoring (SRM) (see Figure). We can now investigate the changes in concentration of many hundreds of proteins in a single experiment. The CNIO Proteomics Core Unit is developing and implementing these technologies in order to better understand the molecular processes involved in cancer. The Unit also provides recombinant protein expression methodologies to CNIO researchers.

Main Objectives

- Implement and apply state-of-the-art proteomic technologies
- Establish protein post-translational modification (PTM) analysis techniques
- Offer advice on the experimental design of proteomic experiments
- Provide recombinant protein expression
- Develop quantitative protein assays





Staff scientist: Jorge L. Martínez. **Post-doctoral fellow:** M. Ángeles Abengózar. **Technicians:** Fernando García, Rut González, Nuria Ibarz, M. Isabel Ruppen and M. Pilar Ximénez de Embún.

Highlights

During 2010 the Proteomics Core Unit has collaborated with groups belonging to most of the CNIO Research Programmes including Clinical Research, Structural Biology and Biocomputing, BBVA Foundation – CNIO Cancer Cell Biology, Molecular Pathology and Molecular Oncology. We have recruited two new staff members which has strengthened the Unit considerably.

We installed a new mass spectrometer and its associated nano HPLC system. The LTQ Velos Orbitrap system facilitates extensive discovery proteomics experiments on complex protein mixtures. The high resolution (up to 100,000) and high scanning speed of the Velos ion trap, as well as the diverse scan modes of this instrument, will enable us to penetrate the various proteomes more deeply.

Offgel peptide separation has been implemented to widen the range of techniques for peptide isolation. The 5500 qTrap hybrid triple quadrupole linear ion trap has been used to establish absolute protein quantitation methods for the analysis of clinical samples in collaboration with the CNIO's Clinical Research Programme.

The Unit also provides state-of-the-art technology for heterologous recombinant protein expression in different hosts such as *Escherichia coli*, insect cell-baculovirus and mammalian cells. In collaboration with the CNIO Crystallography Group

we implemented the multigene baculovirus-based protein expression system – MultiBac – for protein complex production.

The Unit is also taking part in a project to provide new ways of producing human antibodies *in vitro* using phage display technology. This project focuses on the development and validation of recombinant antibody fragments (scFv) directed against several proteins involved in angiogenesis – such as ephrin B2 – and their potential application as anti-tumour agents in cancer therapy.

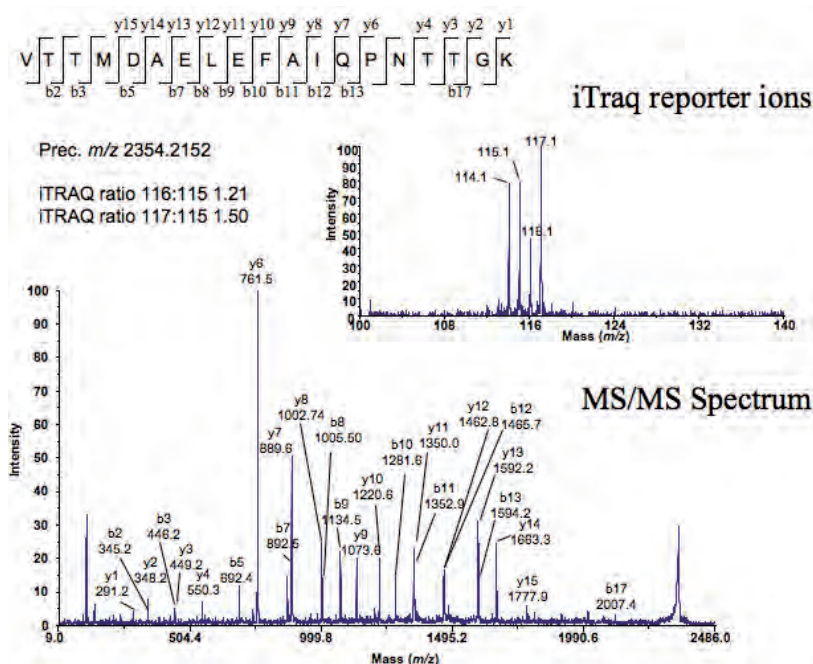


Figure: Typical MS/MS spectra used to identify proteins as well as the iTRAQ reporter ions used for quantitation.

Publications

Ruppen I, Grau L, Orenes-Piñero E, Ashman K, Gil M, Algaba F, Bellmunt J, Sánchez-Carbayo M (2010). Differential Protein Expression Profiling by iTRAQ-2DLC-MS/MS of human bladder cancer EJ138 cells transfected with the metastasis suppressor KiSS-1 gene. *Mol Cell Proteomics* 9, 2276-2291.

Luque-García JL, Martínez-Torrecedrada JL, Epifano C, Cañamero M, Babel I, Casal JI (2010). Differential protein expression on the cell surface of colorectal cancer cells associated to tumor metastasis. *Proteomics* 10, 940-952.

Patent

Martinez JL, Abengozar MA (2010). Antibody against ephrin B2 and its uses (OEPM-21/09/2010). Spanish Patent Application P201031402.