



Proteomics beyond the state of the art

NEPAF is a state of the art proteomics facility with particular strengths in the application of LC-MS and LC-MS/MS methods to the identification, quantification and characterisation of proteins. We have five staff members who share more than 55 years of experience in protein chemistry, bioanalytical chemistry, clinical chemistry and/or proteomics.

We have the following equipment and software that is accessible to our collaborators:

- Bruker Ultraflex II MALDI TOF/TOF (particularly useful for the identification of proteins from 2DE gels)
- Applied Biosystems 4800 MALDI TOF/TOF (also suitable for Maldi imaging)
- Bruker HCT Ultra Proteome Discovery system (ETD) with Dionex Ultimate 3000 nano-HPLC
- Applied Biosystems Qtrap 4000 with Dionex Ultimate 3000 nano-HPLC
- ThermoScience Orbitrap XL (ETD) with Dionex Ultimate 3000 nano-HPLC
- Bruker maXis with Dionex Ultimate 3000 nano-HPLC
- Standalone Dionex Ultimate 3000 nano-HPLC system with micro fraction collector, capable of performing 2D-LC analyses;
- Zeptosens reverse protein array platform;
- GE BioCore X100
- GE Typhoon Trio+ gel scanner
- GE Akta explorer FPLC
- ProGenesis SameSpots and ProGenesis LCMS software; OpenMS software, in house Matrixscience Mascot Server.

The high-resolution LC-MS systems (Bruker maXis and Thermo Orbitrap) can both be deployed in detailed comparative protein expression studies following *in solution* proteolytic digests. Analysis of the resulting three-dimensional datasets using state of the art peak recognition and alignment software (Progenesis LCMS, OpenMS or SuperHirn) would generate the data that could subsequently be analysed using the statistical tools provided within these packages in combination with purpose-written (using R) supervised polyvariate statistical approaches.

Electron transfer dissociation (available on the HCT Ultra iontrap and ThermoScience Orbitrap) is a novel method for the generation of MS/MS spectra, that is particularly well suited for the analysis of peptides carrying post-translational modifications, including protein glycosylation and phosphorylation.

Proteins from two-dimensional gels can be identified following in-gel digest using either a Bruker ultraflex II MALDI-TOF/TOF or (in case of contaminated or complex samples) using a Bruker HCT Ultra (ETD) / Dionex Ultimate3000 LCMSMS system.

A complementary approach to using mass spectrometry based proteomics for the exploration of signal transduction pathways is provided by the immunochemical based reversed phase protein array Zeptosens platform. 32 samples can be analysed in duplicate quantitatively at atto-mol sensitivities on a chip, provided that antibodies against phosphorylated and non-phosphorylated proteins in the signal transduction cascades are available. This platform has the potential to screen hundreds of samples per week, once immunochemical assays have been developed and set up.

NEPAF can perform protein–protein interaction studies using a BiaCore X100 surface plasmon resonance system.

In addition NEPAF has a range of gel electrophoresis platforms from the leading supplier in the fields (GE Healthcare, BioRad, Fluorotechnics) for 1D and 2D gel electrophoresis, DiGE studies and high resolution scanning on a GE typhoon trio+ scanner.

North East Proteome Analysis Facility

Devonshire Building,
Newcastle University,
Newcastle upon Tyne,
United Kingdom.
NE1 7RU

Tel: +44 (0) 191 246 4804

Fax: +44 (0) 191 211 2596

e-mail: info@nepaf.com

Web: www.nepaf.com