Overview of facility
The mass spectrometry (MS) facility has a Platform II electrospray ionisation (ESI) quadrupole instrument with on-line HPLC and CE, a Q-Tof orthogonal acceleration quadrupole-time-of-flight tandem instrument with nano-ESI, a TSQ 7000 tandem quadrupole instrument with ESI, and a surface enhanced laser desorption ionisation/matrix assisted laser desorption ionisation (SELDI/MALDI) mass spectrometer. There is also a MALDI instrument specifically for high-throughput proteomics screening in the department. The facility runs an analytical service as well as being actively involved in several research areas within the Astbury Centre for Structural Molecular Biology and the Faculty of Biological Sciences, in addition to other groups within the University and external collaborators.

Research
The research involves the development of MS techniques to aid the structural elucidation of biomolecules and can be categorised into four main areas:

i). Protein folding (see Fig. 1). Protein folding is an intriguing area of biochemistry and protein mis-folding is thought to be a contributing factor to several diseases. Working with Prof. S.E. Radford's group, ESI-MS is being used to monitor β2-microglobulin conformations using charge state distribution analysis, enzymatic digestion and H/D exchange to gain insights into folding intermediates and fibril formation.

ii). Protein-ligand non-covalent interactions and macromolecular assembly. In collaboration with Profs. P.G. Stockley and S.E. Radford and Dr N.J. Stonehouse, ESI-MS is being used to investigate non-covalently bound macromolecular structures. Such studies include protein-peptide, protein-protein, and protein-RNA complexes. The latter are important in virus assembly, an area we are investigating with respect to the MS2 and Qβ systems. Protein-protein macromolecular complexes are critical species in fibrillogenesis and are under investigation as an integral part of our β2-microglobulin folding studies.

iii). Reaction monitoring. A recent project includes measuring the uptake of ATP by the muscle protein myosin in collaboration with Profs. H. White (Eastern Virginia Medical School, USA) and J. Trinick.

iv). Structural elucidation and proteomics. Tandem MS (MS/MS) sequencing of proteins and peptides is an important bioanalytical technique. This is being used in collaboration with Prof. P.J.F. Henderson and Dr R.B. Herbert for the characterisation of labelled membrane proteins. The characterisation of Alzheimer's disease-related peptides is being carried out with Dr C. Exley (Keele University).

A proteomics project is in progress with Dr A. Grierson (University of Sheffield) to identify post-translational modifications of kinesin by the generation of mass maps and MS/MS sequence tags from 2D-gel digests.

Other structural analysis projects include the characterisation of lipopolysaccharides (Dr D. Devine, Oral Biology) and oligosaccharides (Prof. S.W. Homans).
Fig. 1 shows the ESI-MS analysis of the apical domain protein (ApEL) of the chaperonin GroEL. The apical domain is acknowledged to be the site where polypeptides bind during folding to their native state. The m/z spectrum of the apical domain (a) shows three conformations: the folded ($n = 8^+ \text{ to } 11^+$), the partially folded ($n = 12^+ \text{ to } 17^+$) and the unfolded ($n = 20^+ \text{ to } 28^+$) states. Non-covalent attachment of a peptide (ML) can be detected on the folded (b.i) and partially folded (b.ii) ApEL, but not on the unfolded conformation (b.iii).

Publications


**Group Members**
Antoni Borysic (with Prof. S.E. Radford),
Simona Francese (with Dr N. J. Stonehouse & Prof. P.G. Stockley),
Michelle Morgan (with Dr D. Devine)
Andrew Smith (with Profs. S.E. Radford & P.G.Stockley).

**Funding**
Financial support from the University of Leeds, the Wellcome Trust, BBSRC, the European Commission, Micromass UK Ltd and AstraZeneca is gratefully acknowledged.