# Biomolecular mass spectrometry

James Ault, Helen Beeston, Paul Devine, Henry Fisher, Aneika Leney, George Preston, Caroline Pritchard, Charlotte Scarff, Dale Shepherd, Tom Watkinson, Lucy Woods, Lydia Young and Alison Ashcroft.

## Introduction

The main focus of our research is the development and application of mass spectrometric techniques to investigate the tertiary and quaternary structures of biomolecules. We use non-covalent electrospray ionisation-mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS) to determine the mass, conformational properties, stoichiometry, stability, and binding characteristics of proteins and protein complexes. We are also pioneers of ion mobility spectrometry-mass spectrometry (IMS-MS), which offers a unique opportunity to separate co-populated biomolecular entities on the basis of their physical shape and to measure their mass and cross-sectional area (Ω) in a single, rapid (≤ 2 mins) experiment.
Our major areas of research focus on protein folding, function and self-aggregation, protein-ligand interactions, biomolecular complex assembly, and oligonucleotide structure.

## Results

Our recent method development work on membrane proteins has shown that ESI-MS and ESI-IMS-MS can be used to study these hydrophobic proteins in a native-like state. The procedure involves the use of amphipols (amphipathic polymers), which are a mild alternative to detergent micelles, to solubilise membrane proteins and to protect and preserve their structure from solution through to the gas phase. The bacterial β-barrel outer-membrane proteins PagP (20.2 kDa) and OmpT (33.5 kDa), whose interactions with amphipols had not been studied previously, were selected for this study (Figure 1).

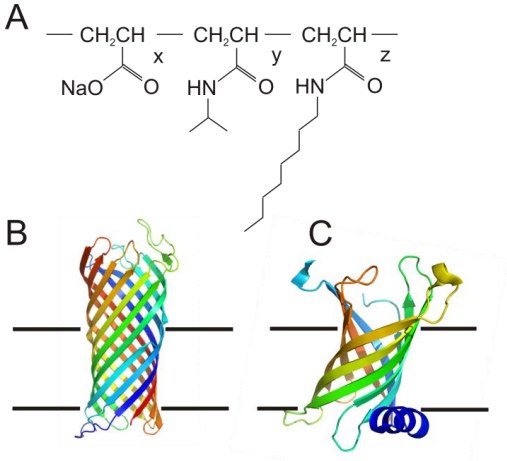


**Figure 1: (A)** Amphipol A8-35 (9-10 kDa; x = 29-34%, y = 25-28%, z = 39-44%). Crystal structures of the membrane proteins **(B)** OmpT (PDB:1I78) and **(C)** PagP (PDB:1THQ)

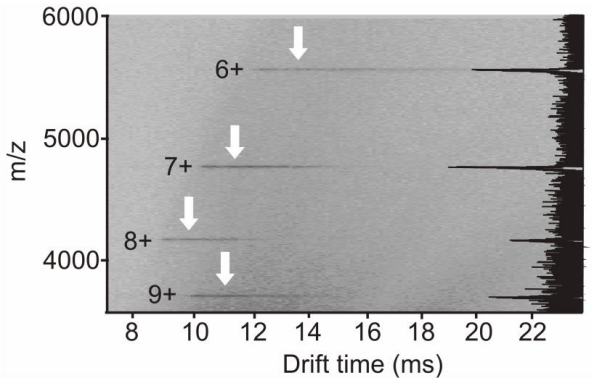


**Figure 2:** ESI-IMS-MS driftscope plot of the OmpT:amphipol complex. The charge states of the ions are labelled and highlighted with white arrows. The summed m/z spectrum for the complex is displayed on the right-hand side.

The PagP and OmpT membrane protein:amphipol complexes were analysed using ESI-IMS-MS. In these analyses, the proteins were separated from the amphipol in the gas phase, thus enabling measurement of the molecular mass of each protein, together with its cross-sectional area. Data for OmpT are shown in Figure 2. The cross-sectional area of OmpT was measured as 2705 $Å^2$ (ave. 6+, 7+ and 8+ ions), which is consistent with the cross-sectional area calculated from the crystal structure (1I78; 2718 $Å^2$).

Amphipols thus offer a simple method of trapping membrane proteins in detergent-free aqueous solutions and preserving them in a native-like conformation, from which ESI-MS analyses can be performed on µMolar amounts of material.

**Publications**

Arscott, S., Descatoire, C., Buchaillot, L. & Ashcroft, A. (2012) A snapshot of electrified nanodroplets undergoing coulomb fission. *Appl. Phys. Lett.* **100:** 074103.

Hodkinson, J., Radford, S. & Ashcroft, A. (2012) The role of conformational flexibility in beta(2)-microglobulin amyloid fibril formation at neutral pH. *Rapid Commun. Mass Spectrom.* **26:** 1783-1792.

Kang, L., Moriarty, G., Woods, L., Ashcroft, A., Radford, S. & Baum, J. (2012) N-terminal acetylation of alpha-synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer. *Protein Sci.* **21:** 911-917.

Leney, A., McMorran, L., Radford, S. & Ashcroft, A. (2012) Amphipathic polymers enable the study of functional membrane proteins in the gas phase. *Anal. Chem.* **84:** 9841-9847.

Morrissey, B., Leney, A. , Toste Rego, A., Phan, G., Allen, W. , Verger, D., Waksman, G., Ashcroft, A. & Radford, S. (2012) The role of chaperone-subunit usher domain interactions in the mechanism of bacterial pilus biogenesis revealed by ESI-MS. *Mol. Cell Proteomics* **11:** M111.015289.

Ndlovu, H., Ashcroft, A., Radford, S. & Harris, S. (2012) Effect of sequence mutation on the mechanical response of amyloid fibrils probed by steered molecular dynamics simulation. *Biophys. J.* **102:**587-596.

Phillips, H., Chernikov, A., Fletcher, N., Ashcroft, A., Ault, J., Filby, M. & Wilson, A. (2012) The use of electrospray mass spectrometry to determine speciation in a dynamic combinatorial library for anion recognition. *Chem. Eur. J.* **18:** 13733-13742.

Preston, G., Radford, S., Ashcroft, A. & Wilson, A. (2012) Covalent cross-linking within supramolecular peptide structures. *Anal. Chem.* **84:** 6790-6797.

**Funding**

This work was funded by the BBSRC, the EPSRC, the Wellcome Trust, Waters UK Ltd., LGC, AZ, GSK, UCB and Medimmune. We thank the BMSS for student travel grants.

**Collaborators**

***Leeds:*** S. Radford, N. Stonehouse, S. Harris, A. Wilson, P. Henderson and P. Stockley.
***External:*** S. Arscott (CNRS, Lille, France), G. O'Connor (LGC, UK), M. Morris & K. Giles (Waters UK Ltd.).