Institution: University of Oxford

Unit of Assessment: 8 Chemistry

Title of case study: UOA08-02: A modified mass spectrometer provides a new tool for structural biology.

1. Summary of the impact

Carol Robinson's research at the University of Oxford in the mid-1990s led directly to her proposing a new type of mass spectrometer to enable more detailed analyses of larger molecular assemblies than previously possible. The design is marketed worldwide by Micromass UK Ltd (part of Waters Corporation), generating a new area of research within industry and academia in which intact protein complexes can be analysed by mass spectrometry and the chemistry of small molecules and drugs bound to them investigated, thus contributing to the search for novel pharmaceuticals. Since 2008, Waters' successful commercialisation of the new technology has led to sales worth many millions of dollars.

2. Underpinning research

Carol Robinson was employed at the University of Oxford's Department of Chemistry between 1991 - 2001 and 2009 - present, and at the University of Cambridge between 2001 - 2009. From 1993 onwards, Robinson has used electrospray-ionisation mass spectrometry (ESI-MS) to decipher protein structure and function. Research by Robinson and colleagues in 1994 investigated GroEL, a molecular chaperone that catalyses protein-folding, by directly monitoring its hydrogen exchange kinetics [1]. This work provided new insights into the GroEL mechanism, and supported the potential of ESI-MS for studying non-covalent protein complexes. Importantly, it showed that large protein assemblies (800 kDa) could be transferred *intact* into a vacuum even though they were not directly detected by the existing MS instruments. Quadrupole time-of-flight (Q-ToF) mass spectrometers (in which an ion's mass-to-charge ratio (*m/z*) is determined via a time measurement) could not directly detect the intact complexes because the high *m/z* ratio of large molecules was beyond the operating limits of commercial quadrupoles. Two specific instrument advances were required for the large complexes to be analysed; first, there needed to be improved transmission of high-mass molecules through Q-ToF mass spectrometers, and second there needed to be improved selectivity between ions with high *m/z* values.

In 1998, Robinson approached Micromass UK Ltd, who had launched their Q-ToF mass spectrometer in 1996, with a proposal to modify the instrument to enable more detailed investigation of the molecular system outlined in her 1994 paper. Proof-of-principle research using prototype and home-improvements on commercial instruments was performed, revealing that transmission of very large protein assemblies was possible, including GroEL (800 kDa) [2] , ribosomes (2.3 MDa) [3], and a virus (2.5 MDa). The virus analysis demonstrated that 180 protein subunits could remain intact during flight through the mass spectrometer and be dissociated to give monomeric units and assemblies, thus indicating that the packing of other viruses could be addressed using ESI-MS. The mass spectra of intact ribosomes revealed that not only the 70S particle but also the 30S and 50S complexes could be observed with charge-state resolution. These findings had huge consequences since, at the time, preservation of non-covalent interactions in such large complexes was completely unexpected. This key step towards obtaining mass spectra of intact protein complexes was achieved on both time-of-flight (ToF) and Q-ToF instruments by making adjustments to collisional cooling (via the differential pressure regimes) in the instrument, reducing the internal energy of the complexes and hence reducing their tendency to fragment. A patent for this method was filed in 2000 [4].

The second aspect of Robinson's instrumentation conceived at Oxford was to reduce the radio-frequency of the guiding field applied to the quadrupole. She was confident that this would optimise analysis of macromolecular complexes, and better exploit the ability of ESI-MS to preserve interactions between proteins and other molecules in the gas phase. The quadrupole frequency was reduced such that an *m/z* value of 32,000 could be isolated and transmitted. This was the first time that such a modification had been made to a Q-ToF instrument, and it was considered to be high-risk; it was not clear to Micromass, or others, that sufficient mass resolution would be

achieved with a lower frequency quadrupole. Initially, therefore, they produced only one mass spectrometer for Robinson's use, delivered to her Oxford laboratory in December 2000.

The first research results from the prototype instrument, obtained in Oxford in early 2001 and first reported in May 2001 at the 49th ASMS Conference on Mass Spectrometry and Allied Topics (Chicago, Illinois), showed unequivocally that intact macromolecular assemblies could be maintained and transmitted through the mass spectrometer, their masses measured with high accuracy using the new low-frequency quadrupole, and their overall topology examined. It was evident that with Robinson's innovative modification ESI-MS could now be used to analyse not just small proteins and modest non-covalent complexes but also macromolecular assemblies with masses as large as 1,000,000 Da.

In 2008, while at Cambridge, Robinson found that by releasing a membrane-protein complex from a detergent micelle transported into the gas phase, she could record the mass spectrum of an intact membrane-protein complex, further supporting the initial proposal made at Oxford in 1998. Unexpectedly, these complexes also carried with them specifically bound lipid molecules. After Robinson returned to Oxford in 2009 she developed this research to show the consequences of lipid binding to membrane-embedded complexes [5]. This work is particularly important for the discovery of new drugs, since many drugs target membrane-bound proteins and lipid binding can either prevent the entry of drugs into the cell or adapt the cavity for drug recognition.

Robinson continues to pursue cutting-edge research into drug binding to a broad range of membrane-protein complexes. Research published in 2013 again used ESI-MS to study the binding in P-gp, a membrane-embedded pump which is responsible for clearing xenotoxins from the cell. Over-expression of P-gp in tumour cells impairs targeted drug delivery, since the pump recognises chemotherapeutics as toxins and exports them. Robinson and colleagues were able to probe the effect of a combination of drugs and lipids on the equilibrium in P-gp and detect independent and simultaneous binding, with important consequences for studying drug binding in general [6].

3. References to the research

Asterisked outputs denote best indicators of quality; University of Oxford authors are underlined.

1. Robinson CV, Gross M, Eyles SJ, Ewbank JJ, Mayhew M, Hartl FU, Dobson CM & Radford SE. Conformation of GroEL-bound α-lactalbumin probed by mass spectrometry. *Nature* 372, 646-651 (1994). DOI:10.1038/372646a0

2. * Rostom AA & Robinson CV. Detection of the intact GroEL chaperonin assembly by mass spectrometry. *J Am Chem Soc* 121, 4718-4719 (1999). DOI: 10.1021/ja990238r
   First analysis of GroeEl as an intact molecular assembly using the modified mass spectrometer.

3. * Rostom AA, Fucini P, Benjamin DR, Juenemann R, Nierhaus KH, Hartl FU, Dobson CM, Robinson CV. Detection and selective dissociation of intact ribosomes in a mass spectrometer. *PNAS* 97, 5185-5190 (2000). DOI: 10.1073/pnas.97.10.5185
   First mass spectrum of an intact ribosome using the modified mass spectrometer.

4. Improvements in, or relating to, microfluidic sample preparation and mass spectrometry. Patent application number WO2000050880 A3, filed 22nd February 2000. Inventors: Carol Robinson and Mark Tito. https://www.google.com/patents/WO2000050880A3

5. * Zhou M, Morgner N, Barrera NP, Politis A, Isaacson SC, Matak-Vinković D, Murata T, Bernal RA, Stock D, Robinson CV. Mass spectrometry of intact V-type ATPases reveals lipid binding and the effects of nucleotide binding. *Science* 344, 380-385 (2011). DOI: 10.1126/science.1210148
   First report of a membrane motor with structural consequences for lipid and nucleotide binding.

6. Marcoux J, Wang S, Politis A, Reading E, Ma J, Biggin P, Zhou M, Tao H, Zhang Q, Chang G, Morgner N & Robinson CV. Mass spectrometry reveals synergistic effects of nucleotides, lipids, and drugs binding to a multidrug resistance efflux pump. PNAS 110, 9704-9709 (2013). DOI: 10.1073/pnas.1303888110

4. Details of the impact

Robinson's novel modification of Micromass' original Q-ToF mass spectrometer, a proposal arising directly from her research needs, has generated a new area of mass spectrometry research in industry as well as academia, in which intact protein complexes can be studied and the chemistry of small molecules and drugs investigated. Using earlier designs of mass spectrometer optimised for proteomics and small-molecule drug analysis, it was not possible to maintain the non-covalent interactions in the gas phase since the pressure regimes and mass range of the instrumentation were not sufficient.

The prototype instrument described in Section 2 outperformed all anticipated specifications; the suite of initial papers that Robinson published (including references [2] and [3], and also reported in May 2001 at the ASMS Conference [7]) contained solid evidence that the new design could record the mass spectra of large intact molecules, and resulted in a high level of interest from other laboratories. This, in turn, created a demand for the modified Q-ToF mass spectrometer to be made commercially available. The Senior Director of Mass Spectrometry Research at Micromass UK Ltd, part of Waters Corporation, confirms that 'the first [commercially available] extended mass quadrupole units for the Q-ToF were developed in 2000, following the suggestions of Professor Robinson', and based on the prototype tested by Robinson at Oxford University [8]. Two model classes of mass spectrometers manufactured by Waters contain Robinson's modification: the Q-ToF and the SYNAPT. A rival company, now AB Sciex, also thought the new design was sufficiently commercially viable to invest in, and manufactured their own version, the QStar, in 2006. However, only a few QStar instruments have been sold, most likely because these were significantly more expensive than Micromass' instruments.

Whilst initial sales of the modified mass spectrometers were confined to academia, the research demonstrating the ability of mass spectrometry to preserve protein complexes and to study the effects of drug binding (including [4] above) has led to purchases by industry, notably by pharmaceutical companies. The benefits for such customers within the pharmaceutical industry are illustrated by the three examples below.

The biotechnology and pharmaceutical company Amgen bought their second SYNAPT instrument in 2008 as a tool for analysing drug-like molecules. A recent Amgen white paper confirms that the instrument's increased quadrupole mass range allows for *'mass selection, transmission and ultimately activation and fragmentation of large protein complexes. This fragmentation allows for protein subunit complex stoichiometry determination, which has proven incredibly valuable if the stoichiometry of the protein complex is unknown, or if the complex is heterogeneous'* [9]. Amgen has used ion mobility-mass spectrometry to analyze polyethylene glycol and PEGylated polypeptides, IgG2 monoclonal antibodies and most recently the detection of large multimeric charge-reduced protein complexes [10]. Novo Nordisk, a global healthcare company with particular expertise in diabetes care, has purchased a SYNAPT and used it to carry out a joint collaborative study to monitor insulin aggregation [11]. A senior Novo Nordisk scientist confirms that the mass spectrometer modifications suggested by Robinson have given them *'insight into the structure and function of protein pharmaceuticals that would otherwise be difficult or impossible to obtain'* [12]. In addition, GSK is working with Pentraxin, a spin-out company from University College London, to develop small molecules that stabilise transthyretin, a blood protein linked to a rare but fatal disease called amyloidosis. This research programme has also used the modified spectrometers to perform analysis of the macromolecules and determine the efficacy of potential inhibitors in regulating the assembly and disassembly of transthyretin, directly building on Robinson's demonstration that it was possible to use the new instrumentation to study interactions within protein assemblies, particularly in relation to the effect of drugs.

Many of these experiments are not possible on standard mass spectrometers, since the required accuracy, resolution and pressure regimes cannot be achieved. Thus, Robinson's modified ESI-MS design has led to significant improvements in potential drug identification and analysis, since multimeric drug targets can be observed intact and the effect of potential inhibitors can consequently be revealed in an entirely new environment.

To give an indication of the context for sales of the new mass spectrometers, in 2012 Waters achieved net sales of over $ 1.8 billion, an increase of 17% since 2008 [13]. 45% of these sales were of instrument systems including its mass spectrometer range. Overall, Q-ToF and SYNAPT instruments containing the modification proposed by Robinson constitute about half of all Q-ToF instruments sold by the Waters Corporation [8]. There has thus been a significant impact on the commercial success of a leading multinational instrument manufacturer, worth many millions of dollars.

5. Sources to corroborate the impact

7. Conference paper, 'Structure and Subunit Dynamics of Small Heat Shock Proteins studied by Nanoflow ESI-MS', 49th ASMS Conference on Mass Spectrometry and Allied Topics, May 2001 (held on file). *Corroborates the use at Oxford University of the custom-built mass spectrometer provided by Micromass to record mass spectra of large intact molecules.*

8. Letter from the Senior Director of Mass Spectrometry Research at Micromass/Waters (held on file); *corroborates Robinson's suggestion of the extended-mass* QToF *instrument, and the impacts on Micromass/Waters' commercial success.*

9. Amgen White Paper, 'Advantages of the High-Mass QTOF for intact protein detection' (held on file), *confirming the advantages of the modified mass spectrometer for the characterisation of biopharmaceutical products.*

10. Campuzano IDG, Schnier PD. Coupling electrospray corona discharge, charge reduction and ion-mobility mass spectrometry: From peptides to large macromolecular protein complexes. International Journal for Ion Mobility Spectrometry, 16, 51-60 (2013). DOI: 10.1007/s12127-013-0120-x
Paper published by Amgen, in which the analyses were conducted using the modified mass spectrometer.

11. Rune Salbo et al. Traveling-wave ion mobility mass spectrometry of protein complexes: accurate calibrated collision cross-sections of human insulin oligomers. Rapid Communications in Mass Spectrometry 26, 1181–1193 (2012). DOI: 10.1002/rcm.6211
Paper co-published by Novo Nordisk; confirms in the Methods Section the use of Waters SYNAPT instruments to analyse insulin molecules.

12. Statement from a Senior Research Scientist at Novo Nordisk (held on file), *corroborating the ways in which the modified mass spectrometer design has enabled insights in the development of protein pharmaceuticals.*

13. http://www.waters.com/waters/nav.htm?cid=134619461&locale=en_US
Link to list of Waters Annual Reports; the 2012 Annual Report confirms the sales figures, and 2011 Annual Report *confirms the increase in sales growth on page 18*.