Foreword

Mass spectrometry (MS) is one of the core analytical techniques for the identification and confirmation of molecules. The origins of the technique can be traced back to the pioneering work of Nobel prize-winners J. J. Thomson and F. W. Aston at the University of Cambridge. Since then MS has undergone over one hundred years of continuous development and has won three Nobel prizes for key developments along the way. The analysis of labile, thermally unstable analytes (for example peptides, proteins, saccharides and complex natural products) has always been the driving force for the development of new MS approaches and methods. Moreover, the ability to detect and quantify metabolites from natural or physiological sources, or even *in situ* in plant material or animal tissue, has opened up the whole world of synthetic biology to MS analysis.

My own first experiences of MS were at Warwick University where, for my final year research project, I studied the analysis and sequencing of simple peptides by fast-atom bombardment (FAB)-MS. This technique was severely limited by molecular weight (less than 1500 Da) due to the competition between energy dissipation along the analyte molecule *vs.* bond fission. Additionally, not all peptides wanted to 'play ball' and just simply refused to fragment. When it worked, this methodology was, of course, a lot faster and more sensitive than non-MS approaches, but it was very unreliable and as a result didn't exactly 'set the world on fire'! After completing my degree, I returned to my home town of Cambridge and spent a couple of years working at the Mass Spectrometry service in the Department of Chemistry at Cambridge University, spending most of my time analysing heavy metal catalysts for groups of the ilk of Professor The Lord Lewis. Most of the analyses were performed by FAB-MS along with the allied technique of liquid secondary ion mass spectrometry (LSIMS). At the same time, I was often asked to analyse some of the more intractable samples from the natural product groups of

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James Staunton, Dudley Williams and Ian Fleming. This was very much my formative period and during this time I learned a considerable amount about both the theory and applications of MS, and the seeds of my future career were very much being sown. The biggest lesson I learned was that it was all too easy to give up on an analysis and blame the sample for poor quality spectra. However, I was a fast learner and also a bit of a perfectionist (comes with the job I think), and I soon learned that sample preparation was often as much to blame. This is an important lesson, especially when you are working for such distinguished chemists as Lewis, Staunton, Williams and Fleming! In particular, with FAB-MS, the quality of the resulting spectra was often very much due to the formation of the matrix/analyte drop on the probe tip. Often the drop would 'skin over' and no spectra could be obtained. Sometimes you would have to repeat the analysis five or six times to get a spectrum. We never really understood the skin formation process, but it was most probably down to the way the matrix dried, sometimes forming a crust that was impenetrable to the xenon beam. On a good day, I would go home happy if I had obtained more than ten good quality spectra.

After this period at Cambridge I returned to Warwick University to work with Peter Derrick towards my MSc by research. He had just obtained one of the first commercial matrix-assisted laser desorption/ionisation (MALDI) instruments using a short time-of-flight analyser. My project was to test the utility of this instrument and technique for the analysis of saccharides. Again, this project taught me about the importance of sample preparation. With MALDI you have the complexity of matrix choice, matrix/analyte ratios and spot preparation. In this time period (mid 1990s) little was really known about how MALDI worked and sample preparation was like a 'black art'. To the uninitiated, MALDI-MS can still seem like magic, but the extent of knowledge is now considerable, and there are matrices and successful sample preparation methodologies for almost all sample types. However, a universal matrix and sample preparation methodology remains elusive.

After completing my MSc, I stayed on for a short period at Warwick and helped with the establishment of the high-field Fourier-transform ion cyclotron resonance (FT-ICR) facility. This was a real eye opener for me and was a major step-change for the analysis of natural systems by MS in the UK. This instrument used electrospray ionisation (ESI) and, coupled with the high-resolution abilities of the instrument, intact biological compounds could be analysed rapidly with relative ease. FT-ICR-MS also brought an unsurpassed ability to perform controlled tandem and sequential MS as well as gas-phase chemistry experiments. After six months, I returned to the University of Cambridge to study for a BBSRC funded PhD with James Staunton on natural product MS using their newly acquired 4.7 Tesla FT-ICR-MS instrument. Although the ESI source on this instrument was fairly crude by today's standards, it allowed for a whole realm of analyses not previously available with older 'soft' ionisation techniques such as FAB, LSIMS or plasma desorption.

My PhD project was to try to develop a routine methodology for the analysis of target natural products being developed as potential new antibiotics

generated through a combination of genetic engineering of the bacteria, which produced the synthase enzymes, and biochemical techniques to manipulate the tailoring of enzymes at the protein level, which were in turn responsible for oxidation, reduction and glycosylation of the final (non-)natural products. This work produced a lot of very similar structural variants and co-metabolites and the group needed a fast, sensitive and reliable method of confirming structure to then feed back to the biochemists that their chemistry was (or was not) working. This required me to develop a high-resolution sequential MS protocol and a parallel understanding of molecular fragmentation. To cut a long story short, with my methodology, I was not only able to confirm that loading modules of the enzyme were functioning correctly, but also that the various tailoring enzymes were producing the correct final molecules from a five-minute experiment using accurate-mass, high-resolution ESI–FT-ICR-MS/MS.

After completion of my PhD, I stayed at Cambridge for a four-year post-doc, working with James Staunton and Steven Ley. During this time, I worked on increasing the understanding of natural product fragmentation in ESI-MS/MS and the exploitation of this increased understanding of structural elucidation. One of the more bizarre memories I have is of Dr Lopes (now Professor) climbing on a stepladder with a heat gun to heat the glass inlet. In the inlet was solid D_4-methanol at vacuum. We needed to generate a low vapour pressure of gaseous D_4-methanol to bleed into the FT-ICR cell to try to prove an unusual gas-phase substitution reaction. Needless to say, the experiment worked and it led to a paper in *Chemical Communications*. It was an unusual experiment, and a great lasting memory of a very productive and fun four years of academic research.

In 2003, I moved to my current position as manager of the School of Chemistry MS facility at the University of Bristol. During my time here, the facility has undergone substantial investment resulting in expansion from two aging (and failing) instruments to more than ten, including state-of-the-art Orbitrap, nanospray IMS-MS/MS and high-resolution MALDI-TOF-MS/MS instruments. The facility is used to carry out cutting edge MS-based research in natural products, proteomics, metabolomics (both biological and environmental) and petroleomics, as well as studies of protein structure and function. The facility also supports cutting edge research across a whole range of chemistries, including studies of synthetic life, superconductors, clean fuels, environmentally friendly catalysts, self-assembly nanomaterials and metallopolymers. The facility also hosts the departmental MS service, which primarily supports the synthetic and materials chemists in the School. The resulting samples range from simple low-mass organic compounds to metal catalysts, synthetic polymers, non-natural peptides and dendrimeric cage systems designed for drug transport. This diverse range of analytes brings with it its own challenges for the facility, not least of which is sample preparation. Somehow, I also find time to conduct my own research into natural product ionisation and fragmentation, and increasing our understanding of gas-phase fragmentation. It is my long-term hope that such knowledge can

be applied to ever increasingly complex samples such as blood plasma and crude oil. At the same time, I have also developed a totally new methodology for the MALDI analysis of small molecules using colloidal graphite as the matrix.

This book brings together an international array of renowned scientists active in a diverse range of MS research areas from multi-omics (proteomics, metabolomics, lipidomics) to enzyme action and natural product discovery. As a result, this book should serve as a comprehensive review of current MS applications in analytical synthetic biology including enzymology, biomedical research, natural products and drug discovery.

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