MASS SPECTROMETRY OF PROTEIN INTERACTIONS

Edited by

KEVIN M. DOWNARD
The University of Sydney
Sydney, Australia
MASS SPECTROMETRY
OF PROTEIN
INTERACTIONS
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PREFACE

THROUGH THE LOOKING GLASS — PROTEIN INTERACTIONS AS VIEWED BY MASS SPECTROMETRY

Mass spectrometry has come a long way from its role in the discovery of isotopes for many of the chemical elements. In just a few decades, difficulties with introducing large, highly polar molecules such as proteins into a mass spectrometer have been overcome and the mass spectrometer, in its many guises, stands as a central technology for the analysis and sequencing of proteins. Perhaps even more astounding, given its construct, is the increasing role that mass spectrometry now plays in the study of protein and other macromolecular interactions.

There are a large number of experimental approaches at hand with which to examine some facet of protein interactions. Although mass spectrometry is not yet routinely practiced by all researchers for this application, it nonetheless can provide a unique window into the nature and stability of these interactions. Developments on a number of fronts from the direct detection of protein complexes and assemblies, to the use of hydrogen isotopic exchange and other chemical labeling approaches with mass spectrometry, to the application of ion mobility mass spectrometry, and the preservation of protein complexes on activated surfaces, have all advanced the study of protein interactions by mass spectrometry. Importantly, the salient features of mass spectrometric analysis—namely, the ability to detect molecules at low sample levels, to do so in complex mixtures without their purification, and to perform the analysis rapidly—are all transposed to these studies.
The use of mass spectrometry to investigate protein interactions using any one individual approach or a combination of approaches is beginning to move from the domain of specialist research laboratories involved in their development to protein scientists and biologists in general. Over a decade on from the earliest observations, an appropriate juncture has been reached at which to review the progress made thus far and report on the latest discoveries and applications as well as new and ongoing challenges. At the time of preparation, there is no book available that covers these developments in a single authoritative volume. This book aims to bring together a series of chapters covering the many avenues with which to study protein interactions by mass spectrometry, each written by international authorities, and in some cases pioneers of the approaches.

In teaching students of the wonders and wherefores of mass spectrometry, I have likened the mass spectrometer to a well-trained dog. Largely obedient, quick to perform, precise in execution, the mass spectrometer eagerly, expeditiously, and expertly can analyze and sequence proteins. But as owners, or should I say custodians, we in the mass spectrometry research community would like our “dog” to jump a little higher, run a little faster, and not without a little satisfaction outperform other “animals” (read analytical technologies).

I am reminded of the words of Lewis Carroll from *Alice’s Adventures in Wonderland*.

*Will you walk a little faster? said a whiting to a snail,*  
*There’s a porpoise close behind us and he’s treading on my tail.*  
*See how eagerly the lobsters and the turtles all advance!*  
*They are waiting on the shingle—will you come and join the dance?*  
*Will you, won’t you, will you, won’t you, will you join the dance?*

The contents of this book allows one to peer through the looking glass to view the present state-of-play, presents the latest achievements and challenges, and leaves the reader to wonder about what might be possible in the years ahead. On behalf of the contributing authors, I invite you, the reader, to come and join the dance.

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DIRECT CHARACTERIZATION OF PROTEIN COMPLEXES BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY AND ION MOBILITY ANALYSIS

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1.1 Introduction
1.1.1 Historical Perspective of ESI-MS for Measuring Protein Complexes
1.1.2 Types of Interactions that Are Probed by ESI-MS

1.2 Critical Aspects of the Experimental Procedure
1.2.1 Instrumental Parameters
   1.2.1.1 Electrospray Ionization Source
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1.3 Solution Phase Equilibria and Gas Phase Dissociation
1.3.1 Measuring Solution Dissociation Constants
1.3.2 Tandem Mass Spectrometry of Protein Complexes
1.1 INTRODUCTION

Beyond its primary, secondary, and tertiary structures, the quaternary structure of a protein can be defined as its interactions and associations with other proteins, macromolecules, and ligands that conspire to define its biological function. Thus, the structural determination of protein complexes can play an important role in the fundamental understanding of biochemical pathways. Traditionally, researchers have a variety of tools at their disposal to probe and measure such interactions. These tools include ultracentrifugation, light scattering, yeast two-hybrid, surface plasmon resonance, affinity chromatography, and native gel electrophoresis, and the methods that provide an “image” of the protein complex, such as cryoelectron microscopy, nuclear magnetic resonance (NMR) spectroscopy, and X-ray crystallography. Each of these methods has its advantages and disadvantages, and each provides a defined level of information detail, from low-resolution assembly size information (e.g., dynamic light scattering) to high-resolution structure from NMR and X-ray.

Mass spectrometry (MS) is becoming a tool for probing noncovalently bound protein–ligand associations. Its popularity is increasing for several reasons, including the impressive results from a number of researchers worldwide, including Carol Robinson [1] and Albert Heck [2], who have demonstrated the capabilities of MS to measure protein complexes as large as the 2 MDa ribosome [3]. In addition, the general field of proteomics has featured prominently and has encouraged more biochemical scientists to apply mass spectrometry into their research strategies. Perhaps the greatest incentive for the increasing interest in mass spectrometry is the improvements in the technology; sensitivity, resolving power, and mass accuracy have been improving steadily, and the availability of more MS systems tailored to specific requirements (e.g., laboratory space, budget) is increased. Although most of the improvements have targeted peptide-centric analysis for protein sequencing and identification, these improved features have benefited also the analysis of intact proteins and protein complexes.

As demonstrated by the pioneering work of John Fenn, who was awarded the Nobel Prize in Chemistry in 2002 for his development of electrospray