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## Preface

As a scientist with an interest in proteins you will, at some time in your career, isolate an enzyme that turns out to be yellow—or perhaps you already have. Alternatively, you may identify a polypeptide sequence that is related to known flavin-containing proteins. This may, or may not, be your first encounter with flavoproteins. However, even if you are an old hand in the field, you may not have exploited the full range of experimental approaches applicable to the study of flavoproteins. We hope that *Flavoprotein Protocols* will encourage you to do so. In this volume we have sought to bring together a range of experimental methods of value to researchers with an interest in flavoproteins, whether or not these researchers have experience in this area.

A broad range of techniques, from the everyday to the more specialized, is described by scientists who are experts in their fields and who have extensive practical experience with flavoproteins. The wide range of approaches, from wet chemistry to dry computation, has, as a consequence, demanded a range of formats. Where appropriate (particularly for analytical methods) the protocol described is laid out in easy-to-follow steps. In other cases (e.g., the more advanced spectroscopies and computational methods) it is far more apt to describe the general approach and relevance of the methods. We hope this wide-ranging approach will sow the seeds of many future collaborations between laboratories and further our knowledge and understanding of how flavoproteins work.

The most common methods are described in the earlier chapters. Anyone who isolates a flavoprotein will almost certainly use UV-visible spectroscopy as the first method of analysis. This simple, yet immensely informative, first approach is concisely described by Macheroux in Chapter 1. Now you have your flavoprotein—does it contain FAD or FMN and how much flavin is present? These questions can be answered by following the methods described by Aliverti et al. in Chapter 2. The electronic structure of flavins makes them amenable to fluorescence-based studies. The fluorimetric methods described in Chapter 3 by Munro and Noble can give useful information on how the protein environment influences the properties of the flavin molecule.

Most flavoproteins are redox active and one of the most important pieces of information relating to their redox activity is the reduction potential of the flavin. In many cases, but not all, it is possible to determine the potentials for

both the oxidized-semiquinone and semiquinone-reduced couples. The way to do this is explained clearly by Mayhew in Chapter 4.

A large proportion of flavoproteins, though certainly not all, are enzymes. Chapter 5, by van Berkel et al., describes spectroscopically based methods that allow a detailed analysis of the reaction kinetics of flavoenzymes. Both steady-state and stopped-flow methods are covered, enabling multiple steps in each reaction cycle to be studied. This chapter is complemented by Chapter 6, in which Ingledew describes freeze–quench methods for analyzing reaction intermediates of flavoenzymes, for example, by electron paramagnetic resonance (EPR) spectroscopy. The EPR method is itself described in Chapter 7 by Murataliev. EPR is ideal for observations of the flavin semiquinone, which is stabilized in some proteins and exists as a transient intermediate in the reactions of others.

Circular dichroism is a well-established method for looking at structural aspects of proteins. This technique is of particular use in the study of flavoproteins since the CD spectrum in the region where the flavin absorbs gives valuable information on the environment of the cofactor. Applying the CD technique to flavoproteins is outlined in Chapter 8 by Price et al. Vibrational spectroscopy, particularly surface-enhanced resonance Raman scattering (SERRS), is also of considerable use in the identification of structural and electronic factors associated with the catalytic mechanisms of many flavoenzymes. A protocol for the use of SERRS in such studies is covered in some detail by Macdonald in Chapter 9. Nuclear magnetic resonance, NMR, is a widely used method in the study of many types of protein, but in Chapter 10 Vervoort provides a guide to its particular usefulness in the study of flavin-containing proteins, in which it can provide extensive and detailed information on the flavin environment.

In most flavoproteins the cofactor is noncovalently bound to the protein and can be dissociated. In Chapter 11 Lederer et al. succinctly describe methods for the removal and re-incorporation of flavin. This method is complemented by Chapter 12 in which Edmondson and Ghisla review the use of labeled and chemically altered flavin analogs that can be used to probe the structure and function of the flavin-active site. Reconstituted proteins produced using a combination of procedures from Chapters 11 and 12 can then be analyzed by the spectroscopic and other methods described in the preceding chapters. In some proteins the flavin is covalently attached to an amino acid side-chain, generally altering its functional properties. It is very important to determine the chemical nature of this attachment in order to understand its effect on the

properties of the cofactor. In Chapter 13 Scrutton clearly describes methods to determine both the existence and the nature of such a covalent link.

Naturally occurring flavoproteins can be rather complex, and Dutton and colleagues have pioneered new and exciting synthetic approaches to develop simpler models for studying redox–protein function. Chapter 14 describes several methods for constructing such models by attaching flavins to synthetic peptides.

Computational methods are now impinging on almost every aspect of biochemistry, and the study of flavoproteins is no exception. In Chapter 15 Rietjens et al. describe some of the computational methods now available for detailed biochemical analysis of the reactions catalyzed by flavoenzymes.

Finally, Chapter 16 touches on a most important aspect of flavins and flavoenzymes, i.e., their potential for use in medicine. Becker et al. describe a range of diagnostic and therapeutic applications for flavins and flavoproteins that go far beyond the use of riboflavin as a vitamin supplement.

In bringing together the wealth of experience shared by the authors of the individual chapters, we hope that we have provided, in *Flavoprotein Protocols*, a wide-ranging and useful set of methods that can be used by biochemists whether or not they have experience with flavoproteins. It is very unlikely that anyone will use the full range of techniques described here, but by bringing them together in a single volume we hope that you can select those that can be most fruitfully applied to your particular experimental system.

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