

# METHODS IN MOLECULAR BIOLOGY

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# Flavins and Flavoproteins

## Methods and Protocols

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## **Dedication**

We would like to dedicate this volume to our long-term collaborator Kenichi Hitomi, who died unexpectedly and much too young on December 30, 2013. Kenichi was a highly enthusiastic flavin enzymologist who made seminal contributions, both at the Scripps Research Institute and at Osaka University, to understanding various light-active flavoproteins such as photolyases and cryptochromes. We mourn his untimely death and keep his friendship and scientific expertise in our hearts.



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## Preface

After the first discovery of the flavin cofactor in the early 1930s, it was soon recognized that riboflavin and its derivatives are essential and ubiquitously encountered organic cofactors in biology. Since these pioneering years, a number of important protein classes harboring flavin cofactors have been isolated and their specific functions elucidated based on the method spectrum that was available at the respective time. In 1999, Chapman and Reid presented a comprehensive volume in the “Methods in Molecular Biology” series devoted solely to flavoproteins. The focus of this issue was on general characterizations of flavins and flavoproteins, using optical, vibrational, and magnetic-resonance spectroscopies, as well as on computational methods. Moreover, protocols for cofactor reconstitution, the handling of flavoproteins, and protein modification were provided. Since then, despite being only about 15 years later, tremendous progress was made on improving these techniques and methods. Increased spectral and temporal resolutions, in combination with sensitivity enhancements have been accomplished for most spectroscopic techniques. These are of course essential and beneficial for in-depth investigations of flavoproteins, as well as of free flavins in isotropic and anisotropic media. With methods for determining primary to quaternary protein structures being nowadays more or less routinely available and extremely successful, precise spectroscopic data can now be correlated to gain structure–function relationships at a molecular level. This is aided by the continuously increasing computational power and methodology. Moreover, novel roadmaps for cofactor synthesis have been developed with the aim of incorporating stable isotopes at virtually any desired position in the flavin and/or chemically altering the isoalloxazine moiety. On the other hand, completely new classes of flavoproteins with yet-to-be-fully unraveled functions have been discovered in the last one and a half decades, such as the light-activated flavoproteins that are involved in light signaling and DNA repair, or the redox-sensing flavoprotein apoptosis-inducing factor, that is involved in initiating a caspase-independent pathway of apoptosis.

With the present volume, we intend to encounter the above-mentioned developments and are proud to present an update to “Flavoprotein Protocols.” Different from the other issues of this series, we not only included “conventional” protocols but also invited distinguished scientists to provide protocols in a review style to exemplify the variety, the power, and the success of modern techniques and methods in application to flavoproteins.

Part I of this volume covers general properties, syntheses, and applications of free flavins as well as its analogs, and flavoproteins. Specifically, Chapter 1 by Ana Edwards opens up the field of flavins and flavoproteins by introducing the structure and general properties of flavins. In Chapter 2, the detailed biosynthesis of flavins and its derivatives is described by Markus Fischer and coworkers. Matthias Mack and coworkers have provided an overview on the synthesis and the application of natural flavin analogs (Chapter 3). Many spectroscopic techniques rely on isotope-labeled flavins for assignment and/or resolution enhancement. Therefore, a comprehensive strategy for isotope labeling of flavins is presented in Chapter 4 under the aegis of Adelbert Bacher and Markus Fischer. Two classes of flavoproteins, namely flavin-containing electron-transfer dehydrogenases and oxygenases,

are presented in Chapters 5 and 6 by the groups of Willem van Berkel and Miagros Medina, respectively. This part closes with a contribution by Katja Becker and coworkers on applications of flavins to medicine (Chapter 7).

Part II covers characterizations of flavins and flavoproteins using modern experimental techniques as well as theoretical methods. The reader should keep in mind that this volume is designed as an upgrade with respect to the first volume of the “Flavoprotein Protocols.” Therefore, a novice to flavoproteins is strongly encouraged to first study the book from 1999 to get introduced into the very basics of flavoprotein handling.

In the present volume, Chapter 8, written by the group of Nigel Scrutton, covers a practical protocol for the use of kinetic isotope effects as probes to determine the enzymatic activity of flavoproteins. Aleksandra Bury and Klaas Hellingwerf provided an article that deals with the *in vivo* characterization of redox states exemplified by flavin-containing photoreceptors, an upgrade to the general protocol for potentiometric measurement of oxidation–reduction potentials of flavins. A global overview on recent progress in computational spectroscopy with emphasis to the dynamics of photoactive flavoproteins was contributed by Tatiana Domratcheva and coworkers (Chapter 10).

Chapters 11–17 focus on individual spectroscopic techniques. These chapters are arranged in terms of their respective excitation energies. Hence, this part starts with three chapters dealing with magnetic resonance spectroscopies: In Chapters 11 and 12, Franz Müller and Anne-Francis Miller cover the field of modern NMR spectroscopy of flavins and flavoproteins, both in liquids as well as in solids. Additionally, Chapter 11 provides a comprehensive database of chemical shifts of various nuclei in flavin derivatives, flavoproteins, and chemically modified flavins. Chapter 13 by the group of Robert Bittl reviews a number of timely electron paramagnetic resonance experiments that have been successfully used to investigate and characterize flavoprotein radicals and flavin-based radical pairs. The adjacent region of electromagnetic radiation is the infrared region, from which information on molecular vibrations can be obtained. Two techniques, Fourier transform infrared spectroscopy and resonance Raman spectroscopy are introduced in detail by Hideki Kandori and Teizo Kitagawa with their coworkers, respectively.

Last but not least, two advanced optical methods and their applications to flavins and flavoproteins are described in Chapters 16 and 17. First, Tilo Mathes, Ivo van Stokkum, and John Kennis introduce the setup of ultrafast spectroscopy and the analysis of spectra obtained from this method using global analyses. And second, Robert Stanley and coworkers demonstrate how information on excited states of flavins can be obtained via Stark spectroscopy.

The editors are indebted to all contributors for their efforts and persistent verve in preparing and editing their chapters. We also wish to thank J and J Walker for giving us the opportunity to coordinate this volume and for their endless patience. At last, we hope that this volume will convince many readers that the field of flavoproteins is timeless and still evolving, and that the modern protocols presented in this volume can help to tackle the countless questions that need to be answered to more fully comprehend the vast diversity and specificity of flavin-governed biological processes.

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