

Methods in Molecular Biology™

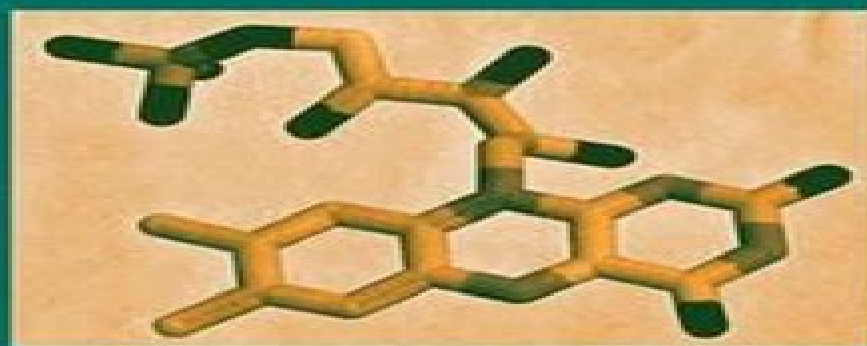
VOLUME 131

# Flavoprotein Protocols

*Edited by*

Stephen K. Chapman

Graeme A. Reid



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# Flavoprotein Protocols

**RM Cervero**



## **Flavoprotein Protocols:**

**Flavoprotein Protocols** Steven K. Chapman, Graeme A. Reid, 2008-02-03 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. *Mycotoxin*

*Protocols* Mary W. Trucksess, Albert E. Pohland, 2008-02-05 Mycotoxins produced by molds are common contaminants of many important crops including wheat, corn, rice and peanuts. Some mycotoxins are found in fruits and vegetables. These contaminants have a broad range of toxic effects including carcinogenicity, neurotoxicity and reproductive and developmental toxicity. The occurrence of mycotoxins in foods is an unavoidable worldwide problem. About 80 countries have imposed regulatory limits to minimize human and animal exposure to mycotoxins. Regulatory limits including international standards have tremendous economic impact and must be developed using science based risk assessments. The purpose of *Mycotoxin Protocols* is to provide the scientific and technological basis for analytical methods for use in obtaining the exposure data needed for risk assessments. *Mycotoxin Protocols* is divided into four sections which are interconnected. The first section Chapters 1-5 describe the general techniques for mycotoxin analysis with emphasis on the importance of method validation based on statistical parameters, sampling procedures for collecting a sample as representative as possible of a bulk lot, the isolation of mycotoxins for use as analytical standards or for toxicological studies, the evaluation of purity and preparation of standards and the detection and identification of impurities in isolated mycotoxins. Sections 2-4 Chapters 6-19 describe the most current chromatographic and immunochemical methods for studies on the major mycotoxins. **Immunotoxin**

**Methods and Protocols** Walter A. Hall, 2008-02-02 Immunotoxins represent a new class of human therapeutics that have widespread applications and a potential that has not yet been fully recognized since they were first conceived of by Paul Ehrlich in 1906. The majority of advances in the development and implementation of immunotoxins has occurred over the last

20 years The reasons for this use of immunotoxins in basic science and clinical research are the powerful concurrent advances in genetic engineering and receptor physiology Recombinant technology has allowed investigators to produce sufficient quantities of a homogeneous compound that allows clinical trials to be performed The identification of specific receptors on malignant cell types has enabled scientists to generate immunotoxins that have had positive results in clinical trials As more cellular targets are identified in coming years additional trials will be conducted in different disease states affecting still larger patient populations Modulation of the immune system to decrease the humoral response to immunotoxins may improve their overall efficacy As increasingly more effective compounds are generated it will be necessary to decrease the local and systemic toxicity associated with these agents and methods for doing so are presently being developed The work presented in *Immunotoxin Methods and Protocols* focuses on three specific areas of immunotoxin investigation that are being conducted by experts throughout the world The first section describes the construction and development of a variety of immunotoxins *Eicosanoid Protocols* Elias A Lianos, 2008-02-03 [Connexin Methods and Protocols](#) Roberto Bruzzone, Christian Giaume, 2008-02-05 Direct cell-cell communication is a common property of multicellular organisms that is achieved through membrane channels which are organized in gap junctions The protein subunits of these intercellular channels the connexins form a multigene family that has been investigated in great detail in recent years It has now become clear that in different tissues connexins speak several languages that control specific cellular functions This progress has been made possible by the availability of new molecular tools and the improvement of basic techniques for the study of membrane channels as well as by the use of genetic approaches to study protein function in vivo More important connexins have gained visibility because mutations in some connexin genes have been found to be linked to human genetic disorders *Connexin Methods and Protocols* presents in detail a collection of techniques currently used to study the cellular and molecular biology of connexins and their physiological properties The field of gap junctions and connexin research has always been characterized by a multidisciplinary approach combining morphology biochemistry biophysics and cellular and molecular biology This book provides a series of cutting edge protocols and includes a large spectrum of practical methods that are available to investigate the function of connexin channels *Connexin Methods and Protocols* is divided into three main parts [Chromatin Protocols](#) Peter B. Becker, 2008-02-03 More than 40 years after the discovery of the nucleosome as the fundamental unit of chromatin the multifaceted problem of how variations in chromatin structure affect the activity of the eukaryotic genome has not been solved However during the past few years research on chromatin structure and function has gained considerable momentum and impressive progress has been made at the level of concept development as well as filling in crucial detail The structure of the nucleosome has been visualized at unprecedented resolution Powerful multisubunit enzymes have been identified that alter histone-DNA interactions in ways that expose regulatory sequences to factors initiating and regulating such nuclear processes as transcription Though the importance of

posttranslational modifications of histones notably their acetylation has long been known the finding that a number of bona fide regulators increase transcription by acetylating nucleosomes has lent new support to the old idea that the process of gene regulation is intimately related to the nature of the chromatin environment A wealth of nonhistone proteins contribute to a continuum of structures with distinct biochemical properties and varying degrees of DNA condensation Perhaps the most important conclusion from a large number of studies is a fresh appreciation of the dynamic nature of chromatin structure the built in flexibility providing the basis for regulation

*Bioinformatics Methods and Protocols* Stephen Misener, Stephen A. Krawetz, 2008-02-02 Computers have become an essential component of modern biology They help to manage the vast and increasing amount of biological data and continue to play an integral role in the discovery of new biological relationships This in silico approach to biology has helped to reshape the modern biological sciences With the biological revolution now among us it is imperative that each scientist develop and hone today's bioinformatics skills if only at a rudimentary level

*Bioinformatics Methods and Protocols* was conceived as part of the *Methods in Molecular Biology* series to meet this challenge and to provide the experienced user with useful tips and an up to date overview of current developments It builds upon the foundation that was provided in the two volume set published in 1994 entitled *Computer Analysis of Sequence Data* We divided *Bioinformatics Methods and Protocols* into five parts including a thorough survey of the basic sequence analysis software packages that are available at most institutions as well as the design and implementation of an essential introductory *Bioinformatics* course In addition we included sections describing specialized noncommercial software databases and other resources available as part of the World Wide Web and a stimulating discussion of some of the computational challenges biologists now face and likely future solutions

*Neurotrophin Protocols* Robert A. Rush, 2008-02-03 The past decade has seen an extraordinary growth in research interest in neurotrophic factors and the study of the neurotrophin family has led this activity Nevertheless this area of research has often struggled as a result of techniques that were either inadequate or just emerging from other research fields and disciplines *Neurotrophin Protocols* has brought together many leaders in the neurotrophin field who detail their special expertise in a wide variety of techniques Though most procedures are valid across many different fields of research some of those described here have been developed to address particular issues within the neurotrophic factor field The protocols cover a broad range of biochemical histological and biological techniques that are often required by the modern laboratory However all have been written with sufficient detail to allow any laboratory to achieve proficiency without need of reference to other texts *Neurotrophin Protocols* is divided into four sections dealing with protein RNA recombinant and in vivo techniques Protein techniques have in general been less successfully employed than those dealing with RNA or DNA However procedures that achieve localization and quantification of the neurotrophins are now being used more extensively Their inclusion here should assist further studies at the protein level Transgenic cell lines and animals are commonplace in the scientific research literature but their inclusion in several chapters in this book provide

some novel uses that are not readily available elsewhere      Transforming Growth Factor-Beta Protocols Philip H. Howe, 2008-02-05 Philip H Howe and a group of well versed experimentalists present the first major volume a collection of indispensable classic and cutting edge TGF assays Described in great detail to ensure robust and successful results these readily reproducible techniques range from the growth inhibition assay for TGF to methods for monitoring its interactions with the mediating proteins Extensive notes discuss potential pitfalls and provide tips on how to avoid failures and throughout emphasis is given to detailing those technical steps critical for experimental success that are often omitted in the primary literature Concise and highly practical Transforming Growth Factor Beta Protocols provides today's molecular and cell biologists both expert and novice with time tested methods for the identification and analysis of the signal transduction pathways by which TGF induces and modulates physiological behavior      **Transcription Factor Protocols** Martin J. Tymms, 2008-02-03 The effort to sequence the human genome is now moving toward a conclusion As all of the protein coding sequences are described an increasing emphasis will be placed on understanding gene function and regulation One important aspect of this analysis is the study of how transcription factors relate transcriptional initiation by RNA polymerase II which is responsible for transcribing nuclear genes encoding messenger RNAs The initiation of Class II transcription is dependent upon transcription factors binding to DNA elements that include the core or basal promoter elements proximal promoter elements and distal enhancer elements General initiation factors are involved in positioning RNA polymerase II on the core promoter but the complex interaction of these proteins and transcriptional activators binding to DNA elements outside the core promoter regulate the rate of transcriptional initiation This initiation process appears to be a crucial step in the modulation of mRNA levels in response to developmental and environmental signals Transcription Factor Protocols provides step by step procedures for key techniques that have been developed to study DNA sequences and the protein factors that regulate the transcription of protein encoding genes This volume is aimed at providing researchers in the field with the well detailed protocols that have been the hallmark of previous volumes of the Methods in Molecular TM Biology series      *DNA Topoisomerase Protocols* Neil Osheroff, Mary-Ann Bjornsti, 2008-02-05 Beginning with the Escherichia coli protein or bacterial DNA topoisomerase I an ever increasing number of enzymes have been identified that catalyze changes in the linkage of DNA strands DNA topoisomerases are ubiquitous in nature and have been shown to play critical roles in most processes involving DNA including DNA replication transcription and recombination These enzymes further constitute the cellular targets of a number of clinically important antibacterial and anticancer agents Thus further studies of DNA topology and DNA topoisomerases are critical to advance our understanding of the basic biological processes required for cell cycle progression cell division genomic stability and development In addition these studies will continue to provide critical insights into the cytotoxic action of drugs that target DNA topoisomerases Such mechanistic studies have already played an important role in the development and clinical application of antimicrobial and chemotherapeutic agents The two volumes of DNA

Topoisomerase Protocols are designed to help new and established researchers investigate all aspects of DNA topology and the function of these enzymes. The chapters are written by prominent investigators in the field and provide detailed background information and step by step experimental protocols. The topics covered in Part I DNA Topology and Enzymes range from detailed methods to analyze various aspects of DNA structure from linking number knotting unknotting site specific recombination and decatenation to the overexpression and purification of bacterial and eukaryotic DNA topoisomerases from a variety of cell systems and tissues.

**Neuropeptide Y Protocols** Ambikaipakan Balasubramaniam, 2008-02-05 The observation that neuropeptide Y (NPY) is the most abundant peptide present in the mammalian nervous system and the finding that it elicits the most powerful orexigenic signal have led to active investigations of the properties of the NPY family of hormones including peptide YY (PYY) and pancreatic polypeptide (PP). Nearly two decades of research have led to the identification of several NPY receptor subtypes and the development of useful receptor selective ligands. Moreover, these investigations have implicated NPY in the pathophysiology of a number of diseases including feeding disorders, seizures, memory loss, anxiety, depression, and heart failure. Vigorous efforts are therefore continuing not only to understand the biochemical aspects of NPY actions but also toward developing NPY based treatments for a variety of disorders. To facilitate these efforts, it was decided to produce the first handbook on NPY research techniques as part of the Methods in Molecular Biology Series. In compiling **Neuropeptide Y Protocols**, I have gathered contributions on techniques considered critical for the advancement of the NPY field from experts in various disciplines. Each chapter starts with a brief introduction with Materials and Methods sections following. The latter sections are presented in an easy to follow step by step format. The last section of the chapter Notes highlights pitfalls and the maneuvers employed to overcome them. This information not usually disseminated in standard research publications may prove extremely useful for investigators employing these techniques in NPY research.

**Amino Acid Analysis Protocols** Catherine Cooper, 2008-02-05 A collection of classic and cutting edge techniques of high utility in answering specific biological questions about amino acids. Common methods include those based on HPLC or gas chromatography separation and analysis after precolumn derivatization. New techniques based on capillary electrophoresis separation, high performance anion exchange chromatography and mass spectrometry are also presented. Each method is described in step by step detail to ensure successful experimental results and emphasizes sample preparation, particularly the collection and storage of bodily fluids. Up to date and highly practical **Amino Acid Analysis Protocols** offers analytical and clinical chemists as well as a broad range of biological and biomedical investigators a rich compendium of laboratory tools for the productive analysis of both common and uncommon amino acids.

**Nuclease Methods and Protocols** Catherine H. Schein, 2008-02-03 Nucleases, enzymes that restructure or degrade nucleic acid polymers, are vital to the control of every area of metabolism. They range from housekeeping enzymes with broad substrate ranges to extremely specific tools. 1. Many types of nucleases are used in lab protocols and their commercial and clinical uses are expanding. The

purpose of Nuclease Methods and Protocols is to introduce the reader to some we characterized protein nucleases and the methods used to determine their activity structure interaction with other molecules and physiological role Each chapter begins with a mini review on a specific nuclease or a nuclease related theme Although many chapters cover several topics they were arbitrarily divided into five parts Part I Characterizing Nuclease Activity includes protocols and assays to determine general processive distributive or specific mechanisms Methods to assay nuclease products identify cloned nucleases and determine their physiological role are also included here Part II Inhibitors and Activators of Nucleases summarizes assays for measuring the effects of other proteins and small molecules Many of these inhibitors have clinical relevance Part III Relating Nuclease Structure and Function provides an overview of methods to determine or model the 3 D structure of nucleases and their complexes with substrates and inhibitors A 3 D structure can greatly aid the rational design of nucleases and inhibitors for specific purposes Part IV Nucleases in the Clinic summarizes assays and protocols suitable for use with tissues and for nuclease based therapeutics

**Gene Knockout Protocols** Martin J. Tymms, Ismail Kola, 2008-02-03

As the major task of sequencing the human genome is near completion and full complement of human genes are catalogued attention will be focused on the ultimate goal to understand the normal biological functions of these genes and how alterations lead to disease states In this task there is a severe limitation in working with human material but the mouse has been adopted as the favored animal model because of the available genetic resources and the highly conserved gene conservation linkage organization In just of ten years since the first gene targeting experiments were performed in embryonic stem ES cells and mutations transmitted through the mouse germline more than a thousand mouse strains have been created These achievements have been made possible by pioneering work that showed that ES cells derived from preimplantation mouse embryos could be cultured for prolonged periods without differentiation in culture and that homologous recombination between targeting constructs and endogenous DNA occurred at a frequency sufficient for recombinants to be isolated In the next few years the mouse genome will be systematically altered and the techniques for achieving manipulations are constantly being streamlined and improved

**Cytoskeleton Methods and Protocols** Ray H. Gavin, 2008-02-03

Over the past two decades experimental studies have solidified the interpretation of the cytoskeleton as a highly dynamic network of microtubules actin microfilaments intermediate filaments and myosin filaments Rather than a network of disparate fibers these polymers are often interconnected and display synergy which is the combined action of two or more cytoskeletal polymers to achieve a specific cellular structure or function Cross communication among cytoskeletal polymers is thought to be achieved through cytoskeletal polymer accessory proteins and molecular motors that bind two or more cytoskeletal polymers Development of the modern concept of the cytoskeleton is a direct outgrowth of advances in experimental tools and reagents that are available to cell and molecular biologists Technological advances and refinements in cell imaging have made it possible to selectively image a single cytoskeletal polymer and monitor its dynamics through the use of fluorescence



probes in vitro and in vivo Two decades ago cytoskeletal research was limited to a few perturbation reagents that included colchicine and cytochalasin Today the perturbation arsenal has expanded to a highly selective group of reagents that includes Taxol nocodazole benomyl latrunculin jasplakinolide and such endogenous proteins as gelsolin These reagents enable the investigator to selectively perturb or destroy a cytoskeletal polymer while leaving other cytoskeletal polymers intact Site specific monoclonal antibodies that target a specific cytoskeletal polymer have proven to be highly selective affinity tools for cytoskeletal research

**Plant Hormone Protocols** Gregory A. Tucker, Jeremy A. Roberts, 2008-02-04 Established investigators from around the world describe in step by step detail their best techniques for the study of plant hormones and their regulatory activities These state of the art methods include contemporary approaches to identifying the biosynthetic pathways of plant hormones monitoring their levels characterizing the receptors with which they interact and analyzing the signaling systems by which they exert their effects Comprehensive and fully detailed for reproducible laboratory success Plant Hormone Protocols offers plant biologists an indispensable compendium of today's most powerful methods and strategies to studying plant hormones their regulation and their activities

Developmental Biology Protocols Rocky S. Tuan, Cecilia W. Lo, 2008-02-05 Developmental biology is one of the most exciting and fast growing fields today In part this is so because the subject matter deals with the innately fascinating biological events changes in form structure and function of the organism The other reason for much of the excitement in developmental biology is that the field has truly become the unifying melting pot of biology and provides a framework that integrates anatomy physiology genetics biochemistry and cellular and molecular biology as well as evolutionary biology No longer is the study of embryonic development merely embryology In fact development biology has produced important paradigms for both basic and clinical biomedical sciences alike Although modern developmental biology has its roots in experimental embryology and the even more classical chemical embryology the recent explosive and remarkable advances in developmental biology are critically linked to the advent of the cellular and molecular biology revolution The impressive arsenal of experimental and analytical tools derived from cell and molecular biology which promise to continue to expand together with the exponentially developing sophistication in functional imaging and information technologies guarantee that the study of the developing embryo will contribute one of the most captivating areas of biological research in the next millennium

**Adipose Tissue Protocols** Gérard Ailhaud, 2008-02-03 Adipose tissue is recognized to be exquisitely sensitive to hormone action and is also now recognized as a secretory and endocrine organ required for reproduction and good health Adipocytes are smart cells able within the tissue to communicate with surrounding cells but also with various organs particularly via leptin acting on the central nervous system Brown adipose tissue BAT and white adipose tissue WAT are known to be distinct tissues whereas the heterogeneity of WAT depots is well established Unfortunately excess WAT leads to obesity which is the most common health problem in industrialized countries Therefore from both a scientific and a technical point of view the time has come to create

a survey of adipose tissues and their neglected adipocytes In Adipose Tissue Protocols I have attempted to gather together chapters from all areas of adipose tissue research from in vivo to in vitro studies and to provide methods covering a wide variety of techniques including the choice of adipose tissue depot and of morphological techniques for the study of BAT and WAT the isolation subcellular fractionation and transfection of adipocytes where the low density of these cells must be taken into account assays of nutrient and ion fluxes and the metabolic aspects of nutrient uptake assays of lipid related enzymes biopsies and quantification of lipid related mRNAs cultures of adipose precursor cells from WAT and BAT of various species including human tissue measurements of adipose secretory products and assessment of WAT metabolism in vivo

T Cell Protocols Kelly P. Kearse, 2008-02-02 The purpose of T Cell Protocols Development and Activation is to c lect a series of protocols particularly those that have been developed within the past few years to help investigators master new techniques or improve existing ones for the study of T cell Biology Invariably in putting together a book like this it is difficult to decide which methods to include and which to leave out To this end methods were selected from a variety of disciplines including cellular immunology b chemistry and molecular biology to try to provide something of interest for everyone who works on T cell development and activation I would like to mention that my primary reason for agreeing to put this book together is that when I was a graduate student I purchased a copy of Selected Methods in Cellular Immunology by Mishell and Shigii which proved a tremendous help in learning the basics of one and two dimensional gel te niques and other methods The cover has long since fallen off but it still remains one of my most valued reference books for the laboratory It is my hope that T Cell Protocols Development and Activation will prove similarly useful to current and future scientists wishing to learn new methods for expl ing the development and activation of T cells

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