

Methods in Molecular Biology™

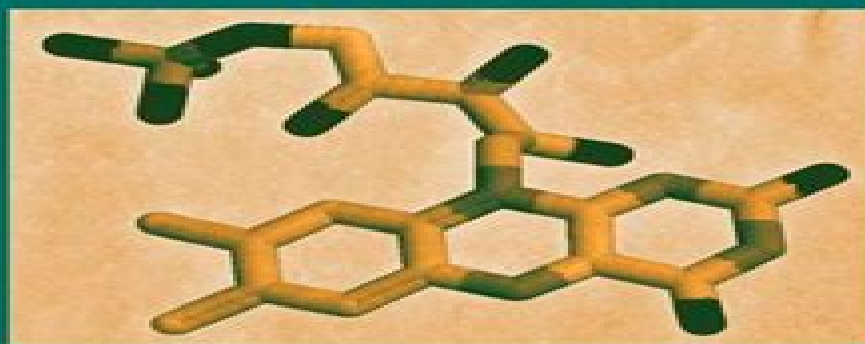
VOLUME 131

Flavoprotein Protocols

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HUMANA PRESS

Flavoprotein Protocols

Ambikaipakan Balasubramaniam



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

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

Eicosanoid Protocols Elias A. Lianos, 2008-02-03 **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. *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. **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

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 result of growth 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

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 regulate 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

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 well 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

Protein Kinase Protocols Alastair D. Reith, 2008-02-02 As key components of many cell signaling pathways protein kinases are implicated in a broad variety of diseases including cancers and neurodegenerative conditions and offer considerable potential as tractable targets for therapeutic intervention In Protein Kinase Protocols a panel of highly skilled laboratory investigators describe both basic and more sophisticated methods for the analysis of kinase mediated signaling cascades with emphasis on the identification of proteins according to their interactive relationships and the analysis of their functional properties Described in step by step detail these readily reproducible techniques offer novices quick access to a complicated field and provide more experienced investigators many novel time saving ploys Emphasis is given to the critical technical steps that are often omitted from methods published in the primary literature There are also tips on potential pitfalls and copious notes on how to adjust the protocols to work in related systems Broad in its range of techniques and thoroughly detailed to help ensure experimental success Protein Kinase Protocols offers both novice and experienced investigators powerful tools for understanding the functional roles of specific protein kinases within signaling cascades and for identification and evaluation of novel therapeutic targets

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.

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, developmental 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.

Calpain Methods and Protocols John S. Elce, 2008-02-05 The purpose of **Calpain Methods and Protocols** is quite straightforward: it is to present the actual experimental methods used in many different laboratories for the study of calpain. It will provide the vital experimental detail and the discussion of possible pitfalls for which the standard journals no longer provide space. This will make it as easy as possible for investigators interested in calpain to adopt established methods without repeating old mistakes and to adapt and apply these methods in novel approaches to the many outstanding calpain questions. These questions range from purely biochemical problems of protein structure and enzyme regulation at the molecular level through large areas of cell biology to applied and clinical aspects of calpain function in human disease. Within this panoply of topics, a wide range of investigators will find many fascinating and as yet unanswered questions about calpain. **Calpain Methods and Protocols** will provide instant access to

many essential techniques while saving them the time and effort involved in developing a new method. In addition to questions relating to the normal physiological roles of the calpains there is considerable evidence that inappropriate calpain activity may have pathological effects in many tissues for example following ischemia. This provides a major stimulus for the development of specific calpain inhibitors for therapeutic purposes and for the development of methods to evaluate such inhibitors.

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.

Adrenergic Receptor Protocols Curtis A. Machida, 2008-02-03 Adrenergic receptors are important modulators in the sympathetic control of various metabolic processes in the central and peripheral nervous systems. These receptors are localized at multiple sites throughout the central nervous system (CNS) and serve as important regulators of CNS mediated behavior and neural functions including mood memory neuroendocrine control and stimulation of autonomic function. **Adrenergic Receptor Protocols** consists of 35 chapters dealing with various aspects of adrenergic receptor analyses including the use of genetic RNA protein expression transactivator second messenger immunocytochemical electrophysiological transgenic and in situ hybridization approaches. This volume details the use of various methods to examine the adrenergic receptor system using aspects of the genetic flow of information as a guide. DNA RNA transactivator protein expression second messenger analyses cellular analyses transgenic whole animal approaches. **Adrenergic Receptor Protocols** displays step by step methods for successful replication of experimental procedures and would be useful for both experienced investigators and newcomers in the field including those beginning graduate study or undergoing postdoctoral training. The Notes section contained in each chapter provides valuable troubleshooting guides to help develop working protocols for your laboratory. With **Adrenergic Receptor Protocols** it has been my intent to develop a comprehensive collection of modern molecular methods for analyzing adrenergic receptors. I would like to thank the many chapter authors for their contributions.

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

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

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

Chaperonin Protocols Christine Schneider, 2008-02-05 **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

Uncover the mysteries within is enigmatic creation, Embark on a Mystery with **Flavoprotein Protocols** . This downloadable ebook, shrouded in suspense, is available in a PDF format (*). Dive into a world of uncertainty and anticipation. Download now to unravel the secrets hidden within the pages.

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