

Methods in Molecular Biology™

VOLUME 228

Membrane Protein Protocols

*Expression, Purification,
and Characterization*

Edited by

Barry S. Selinsky



HUMANA PRESS

Membrane Protein Protocols Expression Purification And Characterization Methods In Molecular Biology

Paul G. Winyard, Derek A. Willoughby



Membrane Protein Protocols Expression Purification And Characterization Methods In Molecular Biology:

Membrane Protein Protocols Barry S. Selinsky, 2008-02-03 Knowledge of the three dimensional structure of a protein is absolutely required for the complete understanding of its function The spatial orientation of amino acids in the active site of an enzyme demonstrates how substrate specificity is defined and assists the medicinal chemist in the design of specific tight binding inhibitors The shape and contour of a protein surface hints at its interaction with other proteins and with its environment Structural analysis of multiprotein complexes helps to define the role and interaction of each individual component and can predict the consequences of protein mutation or conditions that promote dissociation and rearrangement of the complex Determining the three dimensional structure of a protein requires milligram quantities of pure material Such quantities are required to refine crystallization conditions for X ray analysis or to overcome the sensitivity limitations of NMR spectroscopy Historically structural determination of proteins was limited to those expressed naturally in large amounts or derived from a tissue or cell source inexpensive enough to warrant the use of large quantities of cells However with the advent of the techniques of modern gene expression many proteins that are constitutively expressed in minute amounts can become accessible to large scale purification and structural analysis

Cytokine Protocols Marc De Ley, 2008-02-03 A collection of biochemical cellular and molecular techniques for unraveling and quantifying the events occurring between the initial contact of a cytokine at the membrane receptor and the eventual activation of gene transcription The techniques used include the generation of transfectants the immunohistochemical detection of cytokines in tissue sections and optimized staining for cytoplasmic detection Highlights include RT PCR of small amounts of mRNA in situ hybridization biosensor analysis measurement of biological activities and standardization immunohistochemical and single cell detection and receptor isolation characterization and crystallization Enjoy a quick and smooth introduction to the key methods used in cytokine research Use readily reproducible techniques that ensure successful experimental results Employ antisense RNA RT PCR of small amounts of mRNA and in situ hybridization

Lentivirus Gene Engineering Protocols Maurizio Federico, 2008-02-03 Cell gene engineering is emerging as a field with outstanding impact not only in medicine biology but also and perhaps most importantly in agriculture and in all those food sciences involved in the fight against world hunger Lentivirus vector based technologies represent the last frontier in the development of powerful and reliable methods for both in vitro and in vivo gene transfer in eukaryotic animal cells Although the design of lentivirus vectors is closely reminiscent of those already successfully applied to the construction of oncoretroviral vectors some unique features e g the efficiency in transducing both postmitotic and stem cells render the use of lentivirus vectors invaluable It has been a great pleasure to edit *Lentivirus Gene Engineering Protocols* owing in part to the high level of enthusiasm that the authors demonstrated in contributing to this book The fact that so many outstanding scientists engaged in lentivirus vector research have provided articles renders it something more than a technical handbook In addition to detailed descriptions of the most innovative

methodologies the reader may find very informative ov views concerning both theoretical and practical aspects of the origin and the development of diverse lentivirus vector types This in my opinion rep sents a unique added value of this volume which should help our work resist the passage of time to which books such as this are particularly sensitive *Inflammation Protocols* Paul G. Winyard,Derek A. Willoughby,2008-02-03 Inflammation has been described as the basis of many pathologies of human disease When one considers the updated signs of inflammation they would be vasodilation cell migration and in the case of chronic inflam tion cell proliferation often with an underlying autoimmune basis Gen ally inflammation may be divided into acute chronic and autoimmune though the editors believe that most if not all chronic states are often the result of an autoimmune response to an endogenous antigen Thus a proper understanding of the inflammatory basis may provide clues to new therap tic targets not only in classical inflammatory diseases but atherosclerosis cancer and ischemic heart disease as well The lack of advances in classical inflammatory diseases such as rh matoid arthritis may in part arise from a failure to classify the disease into different forms That different forms exist is exemplified in patients with d fering responses to existing antiinflammatory drugs ranging from nonresponders to very positive responders for a particular nonsteroidal an inflammatory drug NSAID Though researchers have progressively unr eled the mechanisms the story is far from complete It should also be noted that the inflammatory response is part of the innate immune response or to use John Hunter s words in 1795 inflammation is a salutary response That may be applied in particular to the defensive response to invading micro ganisms **Functional Genomics** Michael J. Brownstein,Arkady Khodursky,2008-02-03 This collection of robust readily reproducible methods for microarray based studies includes expert guidance in the optimal data analysis and informatics On the methods side are proven techniques for monitoring subcellular RNA localization en masse for mapping chromosomes at the resolution of a single gene and for surveying the steady state genome wide distribution of DNA binding proteins in vivo For those workers dealing with massive data sets the book discusses the methodological aspects of data analysis and informatics in the design of microarray experiments the choice of test statistic and the assessment of observational significance data reduction and clustering **Protein Kinase C Protocols** Alexandra C. Newton,2008-02-03 Since the discovery that protein kinase C PKC transduces the ab dance of signals that result in phospholipid hydrolysis this enzyme has been at the forefront of research in signal transduction Protein Kinase C Protocols covers fundamental methods for studying the structure function regulation subcellular localization and macromolecular interactions of PKC Protein Kinase C Protocols is divided into 11 sections representing the major aspects of PKC regulation and function Part I contains an introduction and a historical perspective on the discovery of PKC by Drs Yasutomi Nishizuka and Ushio Kikkawa Part II describes methods to purify PKC Part III describes the standard methods for measuring PKC activity its enzymatic activity and its stimulus dependent translocation from the cytosol to the membrane Part IV describes methods for measuring the membrane interaction of PKC in vivo and in vitro Part V provides methodologies and techniques for measuring the ph

phorylation state of PKC including a protocol for measuring the activity of PKC's upstream kinase PDK 1. Novel methods for identifying substrates are described in Part VI. Part VII presents protocols for expressing and analyzing the membrane targeting domains of PKC. Part VIII provides a compilation of methods used to identify binding partners for PKC. Part IX describes pharmacological probes used to study PKC. The book ends with a presentation of genetic approaches to study PKC. Part X and a discussion of approaches used to study PKC in disease. Part XI

Handbook of Assay

Development in Drug Discovery Lisa K. Minor, 2006-01-20. The Handbook of Assay Development in Drug Discovery describes all the tools currently available for performing various assay techniques. Featuring troubleshooting advice for common problems from experienced assay developers, the vendor community, and scientists in the pharmaceutical industry, the book presents descriptions of methods, laboratory guidelines, and protocols used to perform such methods, specific examples of each assay system, and troubleshooting tools. Designed as a guide to running an assay from start to finish, this is an ideal bench-top companion for scientists involved in drug discovery, screening, lead profiling, therapeutic target evaluation, and assay development and implementation.

Membrane Proteins: Production for Structural Analysis Isabelle Mus-Veteau, 2014-06-20. This book updates the latest development in production, stabilization, and structural analysis techniques of membrane proteins. This field has made significant advances since the elucidation of the first 3-D structure of a recombinant G Protein-Coupled Receptor (GPCR), rhodopsin, with the structure of several more GPCRs having been solved in the past five years. In fact, the 2012 Nobel Prize in Chemistry was awarded for groundbreaking discoveries on the inner workings of GPCRs. This book is essential reading for all researchers, biochemists, and crystallographers working with membrane proteins who are interested by the structural characterization of their favorite protein and who wish to follow the expression, migration, modifications, and recycling of a membrane protein.

p53 Protocols Sumitra Deb, Swati Palit Deb, 2008-02-02. Since the discovery of p53 as a tumor suppressor, numerous methods have evolved to reveal the unique structural features and biochemical functions of this protein. Several unique properties of p53 posed a challenge to understanding its normal function in the initial phase of its research. The low levels of p53 in normal cells, its stabilization under situations of genotoxic stress, induction of growth arrest and apoptosis with stabilization of the protein, obstructed the visibility of its normal, unmutated function. The property of p53 that can sense a promoter and transactivate or inhibit is still not well understood. It is still not known whether it is the absence of the protein that causes tumorigenesis or if its mutants have a dominant role in inducing cancer. p53 Protocols comprises eighteen chapters for the study of the diverse properties of p53 and related proteins. The methods included are invaluable for delineating the function of other proteins that may function as tumor suppressors or growth suppressors. The chapters are not presented in any schematic order for the importance and diversity of the functions of p53 make it impossible to organize them suitably. We have made a sincere effort to collect the methods most useful to those investigators working on tumor suppressors or growth suppressors. The purpose of p53

Protocols is not only to provide investigators with methods to analyze similar biochemical functions but also to familiarize them with the associated problems that arose during the course of investigations

Capillary Electrophoresis of Carbohydrates Pierre Thibault, Susumu Honda, 2008-02-03 A collection of cutting edge techniques for using capillary electrophoresis CE to analyze complex carbohydrates These readily reproducible protocols provide methods for sample preparation analysis of mono and oligosaccharides glycoproteins and glycoconjugates A useful appendix describes the structures of the most commonly encountered carbohydrate residues and oligosaccharides from mammalian and bacterial origins Each protocol contains detailed information on reagents apparatus notes comments and tips on procedures

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