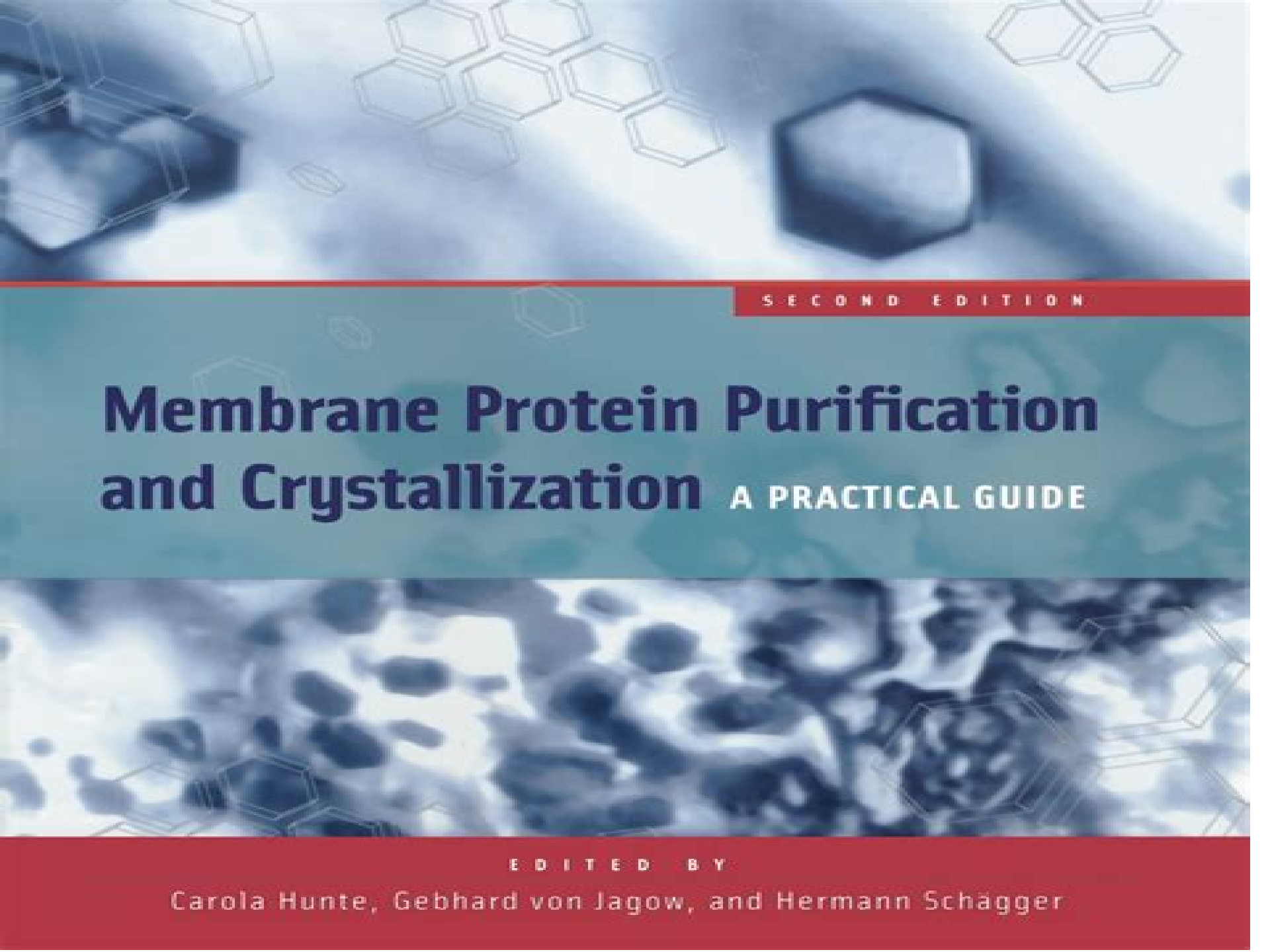




SECOND EDITION

Membrane Protein Purification and Crystallization

A PRACTICAL GUIDE

EDITED BY

Carola Hunte, Gebhard von Jagow, and Hermann Schagger

Membrane Protein Purification And Crystallization Second Edition A Practical Guide

Giuseppe Attardi, Anne Chomyn



Membrane Protein Purification And Crystallization Second Edition A Practical Guide:

Membrane Protein Purification and Crystallization Carola Hunte, Gebhard von Jagow, Hermann

Schagger, 2003-01-05 This second edition of *Membrane Protein Purification and Crystallization A Practical Guide* is written for bench scientists working in the fields of biochemistry biology and proteomic research This guide presents isolation and crystallization techniques in a concise form emphasizing the critical aspects unique to membrane proteins It explains the principles of the methods and provides protocols of general use permitting researchers and students new to this area to adapt these techniques to their particular needs This edition is not only an update but is comprised mainly of new contributions It is the first monograph compiling the essential approaches for membrane protein crystallization and emphasizes recent progress in production and purification of recombinant membrane proteins Provides general guidelines and strategies for isolation and crystallization of membrane proteins Gives detailed protocols that have wide application and low specialized equipment needs Emphasizes recent progress in production and purification of recombinant membrane proteins especially of histidine tagged and other affinity epitope tagged proteins Summarizes recent developments of Blue Native PAGE a high resolution separation technique which is independent of the use of recombinant techniques and is especially suited for proteomic analyses of membrane protein complexes Gives detailed protocols for membrane protein crystallization and describes the production and use of antibody fragments for high resolution crystallization Presents a comprehensive guide to 2D crystallization of membrane proteins *Methods and Results in Crystallization of Membrane Proteins* So Iwata, 2003 [Encyclopedia of Cell Biology](#), 2015-08-07 The *Encyclopedia of Cell Biology* Four Volume Set offers a broad overview of cell biology offering reputable foundational content for researchers and students across the biological and medical sciences This important work includes 285 articles from domain experts covering every aspect of cell biology with fully annotated figures abundant illustrations videos and references for further reading Each entry is built with a layered approach to the content providing basic information for those new to the area and more detailed material for the more experienced researcher With authored contributions by experts in the field the *Encyclopedia of Cell Biology* provides a fully cross referenced one stop resource for students researchers and teaching faculty across the biological and medical sciences Fully annotated color images and videos for full comprehension of concepts with layered content for readers from different levels of experience Includes information on cytokinesis cell biology cell mechanics cytoskeleton dynamics stem cells prokaryotic cell biology RNA biology aging cell growth cell Injury and more In depth linking to Academic Press Elsevier content and additional links to outside websites and resources for further reading A one stop resource for students researchers and teaching faculty across the biological and medical sciences [Molecular Biology in Medicinal Chemistry](#) Theodor Dingermann, Dieter Steinhilber, Gerd Folkers, 2006-03-06 This readily comprehensible book explains the identification of molecular targets via cellular assays reporter genes or transgenic models as well as surveying recent

advances in the synthesis separation and analysis of drugs A special section is devoted to molecular genetics methods With its examination of these novel methods and generous practical advice this is essential reading for all pharmaceutical chemists molecular biologists and medical researchers using molecular methods to study drugs and their action Advances in Planar Lipid Bilayers and Liposomes Aleš Iglič, 2010-11-03 Advances in Planar Lipid Bilayers and Liposomes Volume 9 continues to include invited chapters on a broad range of topics covering both main arrangements of the reconstituted system namely planar lipid bilayers and spherical liposomes The invited authors present the latest results in this exciting multidisciplinary field of their own research group Many of the contributors working in both fields over many decades were in close collaboration with the late Prof H Ti Tien the founding editor of this book series There are also chapters written by some of the younger generation of scientists included in this series This volume keeps in mind the broader goal with both systems planar lipid bilayers and spherical liposomes which is the further development of this interdisciplinary field worldwide Incorporates contributions from newcomers and established and experienced researchers Explores the planar lipid bilayer systems and spherical liposomes from both theoretical and experimental perspectives Serves as an indispensable source of information for new scientists **Introduction to Experimental Biophysics** Jay L. Nadeau, 2017-10-10 Praise for the First Edition essential reading for any physical scientist who is interested in performing biological research Contemporary Physics an ambitious text Each chapter contains protocols and the conceptual reasoning behind them which is often useful to physicists performing biological experiments for the first time Physics Today This fully updated and expanded text is the best starting point for any student or researcher in the physical sciences to gain firm grounding in the techniques employed in molecular biophysics and quantitative biology It includes brand new chapters on gene expression techniques advanced techniques in biological light microscopy super resolution two photon and fluorescence lifetime imaging holography and gold nanoparticles used in medicine The author shares invaluable practical tips and insider s knowledge to simplify potentially confusing techniques The reader is guided through easy to follow examples carried out from start to finish with practical tips and insider s knowledge The emphasis is on building comfort with getting hands wet with basic methods and finally understanding when and how to apply or adapt them to address different questions Jay L Nadeau is a scientific researcher and head of the Biomedical Engineering in Advanced Applications of Quantum Oscillatory and Nanotechnological Systems BEAQONS lab at Caltech and was previously associate professor of biomedical engineering and physics at McGill University A Practical Guide to Membrane Protein Purification Gebhard von Jagow, 2013-10-22 A Practical Guide to Membrane Protein Purification is written especially for researchers who have some familiarity with separation of water soluble proteins but who may not be aware of the pitfalls they face with membrane proteins This guide presents techniques in a concise form emphasizing the aspects unique to membrane proteins The book explains the principles of the methods permitting researchers and students new to this area to adapt these techniques to their particular

needs The second volume in the series this book is an essential manual for investigations of structure and function of native membrane proteins as well as for purification of these proteins for immunization and protein sequencing Separation Detection and Characterization of Biological Macromolecules is a new series of laboratory guides Each volume focuses on a topic of central interest to scientists and students in biomedical and biological research Introductory chapters are followed by clear step by step protocols that present principles and practice These concise manuals are designed for optimal understanding of methods as well as for practical benchtop use Provides general guidelines and strategies for isolation of membrane proteins Describes detailed practical procedures that have been the widest applications and lowest specialized equipment needs Gives special emphasis to new native and denaturing electrophoresis techniques Explains modifications of techniques used for water soluble proteins

Proteomics Sample Preparation Jörg von Hagen, 2011-08-24 This long awaited first guide to sample preparation for proteomics studies overcomes a major bottleneck in this fast growing technique within the molecular life sciences By addressing the topic from three different angles sample method and aim of the study this practical reference has something for every proteomics researcher Following an introduction to the field the book looks at sample preparation for specific techniques and applications and finishes with a section on the preparation of sample types For each method described a summary of the pros and cons is given as well as step by step protocols adaptable to any specific proteome analysis task

Mitochondria Dario Leister, Johannes M. Herrmann, 2007-06-12 Mitochondrial Genomics and Proteomics Protocols offers a broad collection of methods for studying the molecular biology function and features of mitochondria In the past decade mitochondrial research has elucidated the important influence of mitochondrial processes on integral cell processes such as apoptosis and cellular aging This practical guide presents a wide spectrum of mitochondrial methods each written by specialists with solid experience and intended for implementation by novice and expert researchers alike Part I introduces major experimental model systems and discusses their specific advantages and limitations for functional analysis of mitochondria The concise overview of general properties of mitochondrial systems is supplemented by detailed protocols for cultivation of model organisms Parts II VI comprise a robust collection of protocols for studying different molecular aspects of mitochondrial functions including genetics and microbiology biochemistry physiology dynamics and morphology and functional genomics Emphasis is placed on new and emerging topics in mitochondrial study such as the examination of apoptotic effects fusion and fission of mitochondria and proteome and transcriptome analysis

Annual Plant Reviews, Plant Proteomics Christine Finnie, 2008-04-15 The proteome comprises all protein species resulting from gene expression in a cell organelle tissue or organism By definition proteomics aims to identify and characterise the expression pattern cellular location activity regulation post translational modifications molecular interactions threedimensional structures and functions of each protein in a biological system In plant science the number of proteome studies is rapidly expanding after the completion of the Arabidopsis thaliana genome sequence and proteome

analyses of other important or emerging model systems and crop plants are in progress or are being initiated. Proteome analysis in plants is subject to the same obstacles and limitations as in other organisms but the nature of plant tissues with their rigid cell walls and complex variety of secondary metabolites means that extra challenges are involved that may not be faced when analysing other organisms. This volume aims to highlight the ways in which proteome analysis has been used to probe the complexities of plant biochemistry and physiology. It is aimed at researchers in plant biochemistry, genomics, transcriptomics and metabolomics who wish to gain an up-to-date insight into plant proteomes, the information plant proteomics can yield and the directions plant proteome research is taking.

Membrane Proteins - Engineering, Purification and Crystallization, 2015-04-30. Membrane Proteins: Engineering, Purification and Crystallization, a volume of *Methods in Enzymology*, encompasses chapters from the leading experts in the area of membrane protein biology. The chapters provide a brief overview of the topics covered and also outline step by step protocol for the interested audience. Illustrations and case example images are included wherever appropriate to help the readers understand the schematics and general experimental outlines. Volume of *Methods in Enzymology* contains a collection of a diverse array of topics in the area of membrane protein biology ranging from recombinant expression, isolation, functional characterization, biophysical studies and crystallization.

β -barrel Channel Proteins as Tools in Nanotechnology, Marco Fioroni, Tamara Dworeck, Francisco Rodriguez-Ropero, 2013-11-04. β -barrel outer membrane channel proteins (OMP) are useful as robust and flexible models or components in nanotechnology. Over the last decade, biotechnological techniques allowed to expand the natural characteristics of OMPs by modifying their geometry and properties. The present book is oriented towards a broad group of readers including graduate students and advanced researchers. It gives a general introduction to the field of OMP-based nano-component development as well as the state of the art of the involved research. On the example of the *E. coli* FhuA, the transformation of an OMP into a tailored nano-channel will be outlined. An exhaustive description of the scientific strategy including protein selection, analytical methods and *in silico* tools to support the planning of protein modifications for a targeted application, consideration on the production of a custom-made OMP and an overview on technological applications including membrane polymers, some technology will be provided.

Mitochondrial Biogenesis and Genetics, Giuseppe Attardi, Anne Chomyn, 1995. The critically acclaimed laboratory standard for forty years, *Methods in Enzymology* is one of the most highly respected publications in the field of biochemistry. Since 1955, each volume has been eagerly awaited, frequently consulted and praised by researchers and reviewers alike. More than 250 volumes have been published, all of them still in print, and much of the material is relevant even today. Truly an essential publication for researchers in all fields of life sciences.

Key Features: Structural and functional analysis of oxidative phosphorylation complexes; Import of proteins and RNA into mitochondria; Ion and metabolite transport systems in mitochondria; Biophysical methods for mitochondrial function analysis; Mitochondrial inheritance and turnover.

Reinigung und Charakterisierung von mTMEM16A und der C-terminalen

Endodomäne des hP2X7-Rezeptors für die 2D-Kristallisation Frederik Rudolph, 2011-11-21 Strukturelle Charakterisierung der C-terminalen Endodomäne des hP2X7-Rezeptors Der P2X7-Rezeptor P2X7R gehört zur P2X-Rezeptorfamilie ATP-gesteuerter Kationenkanäle. Die sieben Isoformen P2X1-P2X7 teilen eine gemeinsame Topologie mit zwei Transmembranregionen, einer großen extrazellulären Schleife und intrazellulären N- und C-terminalen Domänen. Drei Untereinheiten einer Isoform assemblieren zu einem funktionellen homotrimeren Rezeptor. Aufgrund seiner Lokalisation in Zellen mit Beteiligung an Schmerzentstehung, Entzündungsprozessen und neurodegenerativen Erkrankungen ist der P2X7R ein interessanter Angriffspunkt für die Entwicklungen neuer Arzneimittel gegen verschiedene Krankheiten. Strukturell unterscheidet sich die 595 Aminosäuren umfassende P2X7R Untereinheit von anderen P2XR-Subtypen P2X1R-P2X6R durch eine um 120-200 Aminosäuren längere C-terminale Endodomäne. Funktionelle und biochemische Daten, die von unserer Arbeitsgruppe zusammen mit der Arbeitsgruppe von Prof. Markwardt in Halle publiziert wurden (Becker et al. 2008), lassen den Schluss zu, dass die C-terminale Endodomäne des P2X7-Rezeptors ein Gating-Modul darstellt, wobei eine trimere Anordnung des Moduls zur trimeren Struktur der P2X7-Rezeptoren passen würde. Modelle wie solche cytoplasmatische Domänen, das Öffnen von Ionenkanälen regulieren, wurden bereits für spannungsabhängige Kanäle durch Kombination funktioneller Daten mit Kristallographie-Strukturdaten abgeleitet. Die C-terminale Endodomäne des hP2X7-Rezeptors hP2X7 355-595 Protein wurde in dieser Arbeit in der methylothrophen Hefe *P. pastoris* überexprimiert und durch Ni²⁺-NTA-Affinitätschromatographie aufgereinigt. Nach Optimierung der Aufreinigungsbedingungen konnte 1,5 mg hP2X7 355-595 Protein in hoher Reinheit pro Liter Kulturmedium aufgereinigt werden. 2D-Kristallisationsversuche mit dem His-hP2X7 355-595 Protein wurden in Kooperation mit Prof. Schmidt-Krey an der Fakultät für Biologie am Georgia Institute of Technology in Atlanta im Rahmen eines einjährigen Aufenthalts durchgeführt. Durch die Optimierung der Kristallisationsparameter (Lipid-Protein-Verhältnis, LPR, Salz sowie Temperatur) wurde die Ausbildung von DMPC-Membranstrukturen günstig beeinflusst, und eine Abnahme von zuvor vorhandenen Proteinaggregaten beobachtet. Nach elektronenmikroskopischer Auswertung der Proben zeigte sich, dass es durch DMPC-Zugabe in einem Lipid-Protein-Verhältnis von 1 bis 5 zu einer Stabilisierung des His-hP2X7 355-595 Proteins kommt. Durch Zugabe von NaCl zum Dialysepuffer konnte die Ausbildung von DMPC-Membranen gefördert werden. Eine Rekonstitution des His-hP2X7 355-595 Proteins in die DMPC-Doppelschicht und eine Ausbildung von kristallinen Bereichen konnte allerdings mit keiner der Parameterveränderungen mit Sicherheit beobachtet werden. Die Optimierung weiterer Kristallisationsparameter ist daher Gegenstand aktueller Untersuchungen. Expression und Reinigung von TMEM16A für 2D-Kristallisationsexperimente Die TMEM16A-Anoctamin-Kanäle konnten 2008 unabhängig von drei Arbeitsgruppen als lang gesuchte Ca²⁺-aktivierte Chloridkanäle identifiziert werden. In unserer Arbeitsgruppe konnte mittels BN-PAGE-Analyse und Crosslinking-Experimenten erstmals gezeigt werden, dass das in *X. laevis*-Oozyten und HEK293-Zellen exprimierte TMEM16A-Protein stabil zu Homodimeren oligomerisiert und in homodimerer Form auch in der Plasmamembran dieser Zellen vorliegt.

Fallah et al 2010 Molecular Cellular Proteomics Ziel der fortf hrenden Arbeit war es das TMEM16A Protein in Milligramm Mengen zu exprimieren und zu reinigen um es f r strukturelle Untersuchungen mittels der 2D Kristallisation verf gbar zu machen Eine wichtige Frage war die Identifizierung von Detergenzien die TMEM16A schonend solubilisieren und gleichzeitig f r die Kristallisation geeignet sind Durch ein umfassendes Screening konnte das nicht ionische Detergenz C8E4 sowie das Detergenziengemisch CHAPS und Triton X 100 als Detergenzien identifiziert werden in denen sich TMEM16A als stabiles Homodimer verh lt In anderen untersuchten nicht ionischer und zwitterionischen Detergenzien lag TMEM16A im nicht denaturiertem Zustand in unterschiedlichen prozentualen Verh ltnissen als Monomer und Dimer vor Durch Kultivierung in einem Spinner System im Literma stab und affinit tschromatografischer Aufreinigung gelang es rekombinantes TMEM16A im Milligramm Mengen in einer Qualit t zu isolieren wie sie f r die 2D Kristallisation ben tigt wird Als weiteres Expressionssystem neben HEK293 Zellen erwiesen sich Sf158 Insektenzellen als geeignet Milligramm Mengen an homodimeren TMEM16A Protein zu produzieren Im Vergleich zu den in HEK293 Zellen exprimierten TMEM16A wies das Protein in Insektenzellen ein anderes Glykosylierungsmuster auf Erste 2D Kristallisationsexperimente mit dem aufgereinigten TMEM16A werden zurzeit im Labor von Prof Schmidt Krey am Georgia Institute of Technology durchgef hrt

Analytical Techniques in Biosciences Chukwuebuka Egbuna, Kingsley C. Patrick-Iwuanyanwu, Muhammad Ajmal Shah, Jonathan C. Ifemeje, Azhar Rasul, 2021-10-21 Analytical Techniques in Biosciences From Basics to Applications presents comprehensive and up to date information on the various analytical techniques obtainable in bioscience research laboratories across the world This book contains chapters that discuss the basic bioanalytical protocols and sample preparation guidelines Commonly encountered analytical techniques their working principles and applications were presented Techniques considered in this book include centrifugation techniques electrophoretic techniques chromatography titrimetry spectrometry and hyphenated techniques Subsequent chapters emphasize molecular weight determination and electroanalytical techniques biosensors and enzyme assay protocols Other chapters detail microbial techniques statistical methods computational modeling and immunology and immunochemistry The book draws from experts from key institutions around the globe who have simplified the chapters in a way that will be useful to early stage researchers as well as advanced scientists It is also carefully structured and integrated sequentially to aid flow consistency and continuity This is a must have reference for graduate students and researchers in the field of biosciences Presents basic analytical protocols and sample preparation guidelines Details the various analytical techniques including centrifugation spectrometry chromatography and titrimetry Describes advanced techniques such as hyphenated techniques electroanalytical techniques and the application of biosensors in biomedical research Presents biostatistical tools and methods and basic computational models in biosciences

Mitochondrial Function, Part A, 2009-04-03 The first of two new volumes covering mitochondria Mitochondrial Function Part A presents modern methods that have been developed to examine mitochondrial electron transport chain

complexes iron sulfur proteins and reactive oxygen species These new techniques provide investigators with sensitive original approaches to the study of disease states associated with mitochondrial malfunction The critically acclaimed laboratory standard for 40 years *Methods in Enzymology* is one of the most highly respected publications in the field of biochemistry Since 1955 each volume has been eagerly awaited frequently consulted and praised by researchers and reviewers alike With more than 400 volumes published each *Methods in Enzymology* volume presents material that is relevant in today's labs truly an essential publication for researchers in all fields of life sciences New methods focusing on the examination of normal and abnormal mitochondrial function are presented in an easy to follow format by the researchers who developed them Along with companion volume covering mitochondrial diseases and defects provides a comprehensive overview of modern techniques in the study of mitochondrial malfunction Provides a one stop shop for tried and tested essential techniques eliminating the need to wade through untested or unreliable methods

Heterologous Expression of Membrane Proteins Isabelle Mus-Veteau, 2022-06-30 This detailed volume explores protocols for the production of membrane proteins in a panel of heterologous organisms for structural studies Beginning with techniques using *E. coli* as a host for the overproduction and purification of membrane proteins the book continues with chapters covering mammalian membrane protein production in yeast insect cells mammalian cells as well as using virus like particles and acellular systems Additionally new detergents and alternatives to detergents allowing membrane protein purification for structural analyses are described The book closes with a chapter exploring the use of microscale thermophoresis MST to evaluate the binding activity of heterologously expressed proteins directly in crude membrane extracts Written for the highly successful *Methods in Molecular Biology* series chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Authoritative and up to date *Heterologous Expression of Membrane Proteins Methods and Protocols* Third Edition serves as an ideal guide for scientists aiming to produce and purify functional recombinant membrane proteins for structural studies

Handbook of Ion Channels Jie Zheng, Matthew C. Trudeau, 2015-02-25 The New Benchmark for Understanding the Latest Developments of Ion Channels Ion channels control the electrical properties of neurons and cardiac cells mediate the detection and response to sensory stimuli and regulate the response to physical stimuli They can often interact with the cellular environment due to their location at the surface of cells

Membrane Protein Crystallization, 2009-05-29 This volume of *Current Topics in Membranes* focuses on Membrane Protein Crystallization beginning with a review of past successes and general trends then further discussing challenges of membranes protein crystallization cell free production of membrane proteins and novel lipids for membrane protein crystallization This publication also includes tools to enhance membrane protein crystallization technique advancements and crystallization strategies used for photosystem I and its complexes establishing Membrane Protein Crystallization as a needed practical reference for researchers

Membrane Proteins in

Aqueous Solutions Jean-Luc Popot, 2018-06-08 This book is the first to be entirely devoted to the challenging art of handling membrane proteins out of their natural environment a key process in biological and pharmaceutical research but one plagued with difficulties and pitfalls Written by one of the foremost experts in the field *Membrane Proteins in Aqueous Solutions* is accessible to any member of a membrane biology laboratory After presenting the structure functions dynamics synthesis natural environment and lipid interactions of membrane proteins the author discusses the principles of extracting them with detergents the mechanisms of detergent induced destabilization countermeasures and recent progress in developing detergents with weaker denaturing properties Non conventional alternatives to detergents including bicelles nanodiscs amphipathic peptides fluorinated surfactants and amphipols are described and their relative advantages and drawbacks are compared The synthesis and solution properties of the various types of amphipols are presented as well as the formation and properties of membrane protein amphipol complexes and the transfer of amphipol trapped proteins to detergents nanodiscs lipidic mesophases or living cells The final chapters of the book deal with applications membrane protein in vitro folding and cell free expression solution studies NMR crystallography electron microscopy mass spectrometry amphipol mediated immobilization of membrane proteins and biomedical applications Important features of the book include introductory sections describing foundations as well as the state of the art for each of the biophysical techniques discussed and topical tables which organize a widely dispersed literature Boxes and annexes throughout the book explain technical aspects and twelve detailed experimental protocols ranging from in vitro folding of membrane proteins to single particle electron cryomicroscopy have been contributed by and commented on by experienced users *Membrane Proteins in Aqueous Solutions* offers a concise accessible introduction to membrane protein biochemistry and biophysics as well as comprehensive coverage of the properties and uses of conventional and non conventional surfactants It will be useful both in basic and applied research laboratories and as a teaching aid for students instructors researchers and professionals within the field

Decoding **Membrane Protein Purification And Crystallization Second Edition A Practical Guide**: Revealing the Captivating Potential of Verbal Expression

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