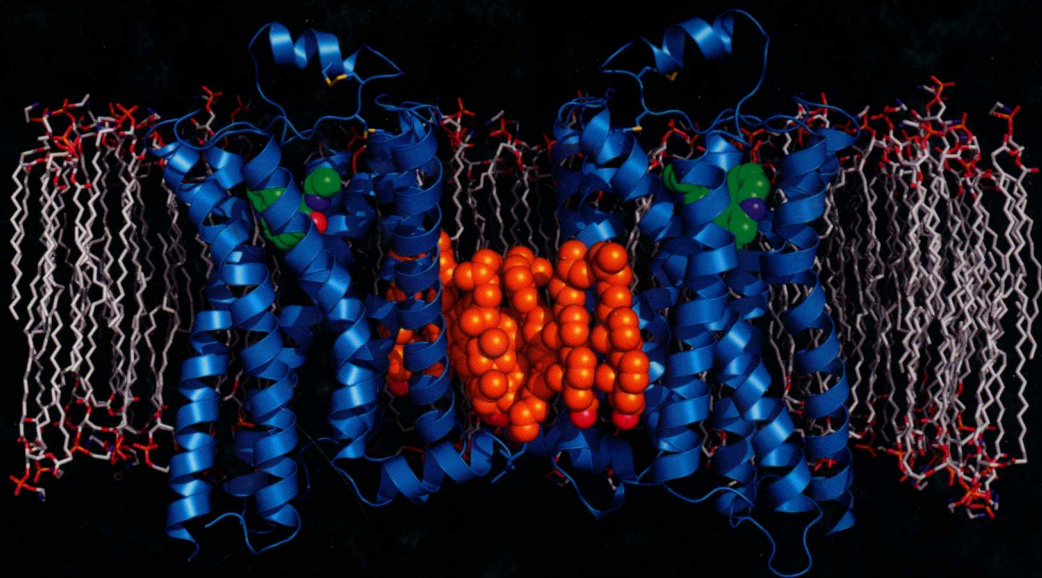


G Protein-Coupled Receptors

Structure, Signaling, and Physiology

edited by
Sandra Siehler and Graeme Milligan



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STRUCTURE, SIGNALING, AND PHYSIOLOGY

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Sandra Siehler

Novartis Institutes for BioMedical Research

Graeme Milligan

University of Glasgow



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G PROTEIN-COUPLED RECEPTORS

This text provides a comprehensive overview of recent discoveries and current understandings of G protein-coupled receptors (GPCRs). Recent advances include the first mammalian non-rhodopsin GPCR structures and reconstitution of purified GPCRs into membrane discs for defined studies, novel signaling features including oligomerization, and advances in understanding the complex ligand pharmacology and physiology of GPCRs in new assay technologies and drug targeting.

The first chapters of this book illustrate the history of GPCRs based on distinct species and genomic information. This is followed by discussion of the homo- and hetero-oligomerization features of GPCRs, including receptors for glutamate, GABA_B, dopamine, and chemokines. Several chapters are devoted to the key signaling features of GPCRs. The authors take time to detail the importance of the pathophysiological function and drug targeting of GPCRs, specifically β -adrenoceptors in cardiovascular and respiratory diseases, metabotropic glutamate receptors in CNS disorders, S1P receptors in the immune system, and Wnt/Frizzled receptors in osteoporosis.

This book will be invaluable to researchers and graduate students in academia and industry who are interested in the GPCR field.

Dr. Sandra Siehler is a Research Investigator at the Novartis Institutes for BioMedical Research in Basel, Switzerland. Dr. Siehler is a member of the American Society for Pharmacology and Experimental Therapeutics and the British Pharmacological Society.

Dr. Graeme Milligan is Professor of Molecular Pharmacology at the University of Glasgow. He is actively involved in numerous associations, such as the Biochemical Society and the British Pharmacological Society. Dr. Milligan was awarded the Ariens Award for Pharmacology from the Dutch Pharmacological Society in 2006.

Contributors

Rosa López Almagro, Ph.D.

Research and Development Center
Almirall
Barcelona, Spain

J. Kurt Chuprun, Ph.D.

Center for Translational Medicine
Thomas Jefferson University
Philadelphia, PA

Michele Ciccarelli, MD

Center for Translational Medicine
Thomas Jefferson University
Philadelphia, PA

Laetitia Comps-Agrar, Ph.D.

Department of Molecular
Pharmacology
Institut de Génomique Fonctionnelle
Montpellier, France

John F. Cryan, Ph.D.

Senior Lecturer
School of Pharmacy
Department of Pharmacology
and Therapeutics
University College Cork
Cork, Ireland

Yehia Daaka, Ph.D.

Department of Urology
UF Prostate Disease Center
University of Florida
College of Medicine
Gainesville, FL

Etienne Doumazane, Ph.D.

Department of Molecular
Pharmacology
Institut de Génomique
Fonctionnelle
Montpellier, France

Thierry Durroux, Ph.D.

Department of Molecular
Pharmacology
Institut de Génomique
Fonctionnelle
Montpellier, France

Karin F. K. Ejendal, Ph.D.

Postdoctoral Research Associate
Department of Medicinal Chemistry
and Molecular Pharmacology
School of Pharmacy and
Pharmaceutical Sciences
Purdue University
West Lafayette, IN

Susan R. George

Professor
Department of Pharmacology
and Toxicology
University of Toronto
Toronto, Ontario, Canada

Nuria Godessart, Ph.D.

Head of Autoimmunity Department
Almirall Laboratories
Llobregat, Spain

J. Silvio Gutkind, Ph.D.

Oral & Pharyngeal Cancer Branch
National Institute of Dental and
Craniofacial Research
National Institutes of Health
Bethesda, MD

Ahmed Hasbi, Ph.D.

Postdoctoral Fellow
Department of Pharmacology
and Toxicology
University of Toronto
Toronto, Ontario, Canada

Ralf Heilker, Ph.D.

Boehringer Ingelheim Pharma
GmbH & Co. KG
Department of Lead Discovery
Biberach, Germany

Peter Hein, MD, Ph.D.

Postdoctoral Researcher
Department of Molecular and Cellular
Pharmacology and Psychiatry
University of California at San
Francisco
San Francisco, CA

Daniel Hoyer, Ph.D.

Neuropsychiatry
Neuroscience Research
Novartis Institutes for BioMedical
Research
Basel, Switzerland

Terry Kenakin, Ph.D.

Department of Biological Reagents
and Assay Development
Molecular Discovery
GlaxoSmithKline Research and
Development
Research Triangle Park, NC

Brian K. Kobilka, MD

Professor
Department of Molecular and Cellular
Physiology
Stanford University
Stanford, CA

Walter J. Koch, Ph.D.

Center for Translational Medicine
Thomas Jefferson University
Philadelphia, PA

Adam J. Kuszak

Department of Pharmacology
University of Michigan
Ann Arbor, MI

Carlos Martínez-A., Ph.D.

Professor
Department of Immunology and
Oncology
Centro Nacional de Biotecnología
Madrid, Spain

Damien Maurel, Ph.D.

Scientist
Ecole Polytechnique Fédérale de
Lausanne
Lausanne, Switzerland

Mario Mellado, Ph.D.

Research Scientist
Department of Immunology
and Oncology
Centro Nacional de
Biotecnología
Madrid, Spain

Graeme Milligan, Ph.D.

Professor
Neuroscience and Molecular
Pharmacology
University of Glasgow
Scotland

Carine Monnier, Ph.D.

Department of Molecular
Pharmacology
Institut de Génomique Fonctionnelle
Montpellier, France

Zhongzhen Nie, Ph.D.

Department of Urology
UF Prostate Disease Center
University of Florida
College of Medicine
Gainesville, FL

Richard M. O'Connor

School of Pharmacy
Department of Pharmacology
and Therapeutics
University College Cork
Cork, Ireland

Brian F. O'Dowd

Professor
Department of Pharmacology and
Toxicology
University of Toronto
Toronto, Ontario, Canada

Stefan Offermanns, MD

Director
Department of Pharmacology
Max-Planck Institute for Heart and
Lung Research
Hessen, Germany

Jean Phillippe Pin, Ph.D.

Director
Department of Molecular
Pharmacology
Institut de Génomique Fonctionnelle
Montpellier, France

Laurent Prézeau, Ph.D.

Department of Molecular
Pharmacology
Institut de Génomique Fonctionnelle
Montpellier, France

Julie A. Przybyla

Department of Medicinal Chemistry
and Molecular Pharmacology
School of Pharmacy and
Pharmaceutical Sciences
Purdue University
West Lafayette, IN

Sören G. F. Rasmussen, Ph.D.

Postdoctoral Scholar
Department of Molecular and
Cellular Physiology
Stanford University
Stanford, CA

Georges Rawadi, Ph.D.

Business Development & Alliance
Manager
Galapagos
Romainville, France

Marie-Laure Rives, Ph.D.

Postdoctoral Research Fellow
Columbia University
New York, NY

José Miguel Rodríguez-Frade, Ph.D.

Research Scientist
Department of Immunology and
Oncology
Centro Nacional de
Biotecnología
Madrid, Spain

Philippe Rondard, Ph.D.

Department of Molecular
Pharmacology
Institut de Génomique
Fonctionnelle
Montpellier, France

Andreas Russ, Ph.D.

Department of Biochemistry
University of Oxford
Oxford, United Kingdom

Torsten Schöneberg, Ph.D.

Molecular Biochemistry
Institute of Biochemistry
University of Leipzig
Leipzig, Germany

Kristin Schröck, Ph.D.

Molecular Biochemistry
Institute of Biochemistry
University of Leipzig
Leipzig, Germany

Sandra Siehler, Ph.D.

Research Investigator II
Center for Proteomic Chemistry
Novartis Institutes for Biomedical
Research
Basel, Switzerland

Claudia Stäubert, Ph.D.

Molecular Biochemistry
Institute of Biochemistry
University of Leipzig
Leipzig, Germany

Roger K. Sunahara, Ph.D.

Associate Professor
Department of Pharmacology
University of Michigan
Ann Arbor, MI

Gema Tarrasón, Ph.D.

Research and Development Center
Almirall
Barcelona, Spain

Erin Trinquet, Ph.D.

Cisbio Bioassays
Parc technologique Marcel Boiteux
Bagnols/Cèze, France

José Vázquez-Prado, Ph.D.

Professor
Department of Pharmacology
Center for Research and Advanced
Studies
National Polytechnic Institute
Mexico

Ivan Toma Vranesic, Ph.D.

Neuropsychiatry
Neuroscience Research
Novartis Institutes for BioMedical
Research
Basel, Switzerland

Val J. Watts, Ph.D.

Department of Medicinal Chemistry
and Molecular Pharmacology
School of Pharmacy and
Pharmaceutical Sciences
Purdue University
West Lafayette, IN

Michael Wolff, Ph.D.

Department of Lead Discovery
Boehringer Ingelheim Pharma GmbH
& Co. KG
Biberach, Germany

Xiao Jie Yao

Research Associate
Department of Molecular and
Cellular Physiology
Stanford University
Stanford, CA

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Introduction

Sandra Siehler and Graeme Milligan

This book provides a comprehensive overview of recent discoveries and the current understanding in the G protein-coupled receptor (GPCR) field.

A plethora of distinct GPCRs exist on the cell surface of every cell type and generate signals inside cells to regulate key physiological events. The human genome contains between 720 and 800 GPCRs with specific tissue and subcellular expression profiles. Chapter 1 of this volume illustrates the evolutionary history of GPCRs based on genomic information available from distinct species and ancient genomic information. Many GPCRs are involved in olfactory/sensory mechanisms. Three hundred sixty-seven non-sensory human GPCRs are known or predicted to be activated by native ligands; endogenous ligands for 224 human GPCRs are described currently, but remain to be identified for 143 orphan receptors. Three hundred sixty-seven ligand-activated non-sensory GPCRs consist of 284 class A (rhodopsin-like) receptors, 50 class B (secretin-like) receptors, 17 class C (metabotropic receptor-like) receptors, and 11 belong to the atypical class of frizzled-/smoothened receptors. Polymorphisms (e.g., of β adrenoceptors, see Chapter 15) and alternative splicing (e.g., of metabotropic glutamate receptors, see Chapter 16) further increase the variety of GPCR proteins. Posttranslational modifications such as N-linked glycosylation or carboxyterminal palmitoylation can influence their function.

GPCRs are integral membrane proteins containing an extracellular amino terminus of widely varying length, seven transmembrane α -helical stretches, and an intracellular carboxy terminus. The molecular understanding of GPCRs developed with the cloning of the β_2 adrenoceptor in 1986 and appreciation that it was related to the photon receptor rhodopsin. The majority of signaling events originate at the inner face of the plasma membrane and involve transactivation of one or more members of the four G protein families (G_s , G_i/o , $G_q/11$, $G_{12/13}$), which link GPCRs to effector cascades. Chapter 7 explains functions of mammalian G proteins elucidated using subunit- and tissue-specific gene targeting. Besides effector cascades involving G proteins, non-G protein-mediated signaling has been described for various GPCRs. Moreover, the activity of G proteins can be regulated by non-GPCR proteins such as receptor tyrosine kinases. The activity of GPCRs is further modulated by cellular signals in an auto- and trans-regulatory fashion. GPCRs form intra- and juxtamembrane signaling complexes

comprising not only G proteins, but also other GPCRs, ion channels, membrane and cytosolic kinases and other enzymes, G protein-modulatory proteins, and interact with elements of the cell cytoskeleton. Chapters 3–6 describe homo- and hetero-oligomerization features of GPCRs including receptors for glutamate, GABA_B, dopamine, and chemokines. Dopamine receptors can hetero-dimerize not only with other subtypes in the same receptor family, but also with less-related GPCR members and ion channels such as NMDA or GABA_A receptors. For class C receptors, which contain a large extracellular domain, oligomerization is mandatory for receptor function. For other GPCRs, oligomerization may result in altered and/or novel ligand pharmacology. Methods applied to measure GPCR complexes and oligomer signaling comprise GPCR-Gα protein fusion constructs containing either a mutated receptor or Gα mutant, and time-resolved fluorescence resonance energy transfer (TR-FRET).

Downstream of the cellular plasma membrane, the complexity of intracellular communication controlled by GPCRs increases dramatically. Ligand-activated GPCRs often internalize, which mostly causes desensitization of signaling events, although both prolonged signaling and even signaling initiated following receptor internalization have been described. Receptor hetero-oligomers can co-internalize, and activation and internalization of one partner can therefore silence the other interaction partner. Chapters 8–11 describe key signaling features of GPCRs better understood because of significant recent advancements. These include understanding of kinetics of receptor activation and signaling events studied using FRET and bioluminescent RET (BRET). Multiple related proteins control GPCR-mediated cell signaling processes. For example four RhoGTPase nucleotide exchange factors (Rho-GEFs) link G_{12/13} to pathways controlling, for example, contractile complexes of the cytoskeleton, whereas nine mammalian adenylyl cyclases (ACs) are regulated by GPCRs in a receptor- and tissue-specific manner. These enzymes are integral membrane proteins directly regulated by G_s and G_{i/o} proteins, although G_{q/11}-coupled GPCRs also influence AC activities via calcium and protein kinase C, and G_{12/13} proteins were recently found to regulate AC activity as well. Arrestins are known to bind to agonist-stimulated phosphorylated GPCRs and promote endocytosis. Novel functions of arrestins include interactions with non-GPCR receptors or direct interaction with signaling proteins including, for example, the ERK MAP kinases. Modern assay technologies to assess GPCR signaling and ligand pharmacology are described in Chapter 12. Multiplexing subcellular readouts using high content screening allows the simultaneous capture of multiple signals, in both temporal and spatial fashion. The pharmacological complexity of orthosteric and allosteric GPCR ligands in the context of both receptor-G protein complexes and activation state models, is illustrated in Chapters 13 and 14. Functional selectivity of GPCR ligands due to receptor allosterism toward intracellular effector pathways contributes to the complex pharmacological nature.

Dysregulated ligand concentration, GPCR protein level, coupling, and/or signaling are implicated in and often causative for many pathophysiological conditions including central nervous system (CNS) disorders, cardiovascular and