
Preface

Since the initial discovery of the G protein-coupled receptor system that regulates cyclicAMP production, the G protein field has rapidly expanded. Cell surface receptors that couple to heterotrimeric G proteins, the G protein-coupled receptors (GPCRs), number in the hundreds and bind to a wide diversity of ligands including, biogenic amines (e.g., adrenaline), lipid derivatives (e.g., lysophosphatidic acid), peptides (e.g., opioid peptides), proteins (e.g., thyroid-stimulating hormone), and odorants to name a few. The GPCR system is found throughout biology in such simple organisms as yeast and in such more complex organisms as *Dictyostelium discoideum* (slime mold), *Caenorhabditis elegans* (nematode worm), and of course in humans. GPCRs and their associated G protein systems are the subject of intense academic research and because of their involvement in a human biology and disease, the pharmaceutical industry has large research initiatives dedicated to the study of GPCRs. By some estimates, more than 50% of the pharmaceuticals on the market are targeted at GPCRs.

The G protein/G protein-coupled receptor system consists of a receptor (GPCR), a heterotrimeric G protein consisting of α , β , and γ subunits, and an effector. G protein effector molecules, such as enzymes or ion channels, respond to activation by the G protein to generate second messengers or changes in membrane potential that lead to alterations in cell physiology. Superimposed on this classical G protein framework are the recently discovered regulators of G protein signaling (RGS proteins) that provide an additional level of G protein regulation, but whose physiological functions remain undefined. The heterotrimeric G proteins themselves form a diverse family of subunits with more than 20 α subunits, 6 β subunits and 13 γ subunits. There are many effectors, each of which may be represented by multiple isoforms. Analysis of such a complex system requires methods that can tackle each of these molecules and the interactions between them in biochemical and cell biological contexts. The variety of strategies that are employed range from studies with purified components to studies in cell biological and whole animal systems. In this volume we have gathered together many of the methodologies that are used to study mechanisms of G protein and G protein coupled receptor function and the roles of G protein subunits in cell biology and disease. Many of the chapters cover topics that use a variety of G protein methodologies, so within single chapters multiple specific methodologies are described in detail. In some cases not only is the method itself described, but the principles that underlie the experimental design are also out-

lined in ways that are not described in other publications. And of course in each chapter there is a Notes section dealing with critical details that are often not included in formal publications owing to space limitations and other issues.

G Protein Signaling: Methods and Protocols has been divided into seven parts somewhat arbitrarily, but based on the general types of systems being used. The first part deals with purification of G proteins and effector enzymes from heterologous expressions systems. To study G proteins and their interactions with receptors and targets it is often necessary to purify the proteins prior to analysis. The second part of the volume has protocols for assays of the interactions between these purified G proteins and effector enzymes. Alternate strategies outlined in Part III are for the study of G protein interactions with effectors in intact cells, either with the endogenous components or with expressed components. Each of these approaches has advantages and disadvantages with regard to data interpretation and analysis. The fourth part is concerned with various assays of G protein coupled receptor structure, function, and localization. The fifth part has protocols for studying the physiological roles for endogenous G proteins, either in cell culture systems or in whole animals, using approaches that inhibit these endogenous systems. Regulators of G protein signaling (RGS) proteins are a novel class of proteins that regulate the activity of heterotrimeric G proteins. Part VI describes methods for studying lipid and phosphate modifications of these proteins. Finally, green fluorescent proteins and their derivatives have been used to study the localization and interactions of many proteins in cells. In Part VII, the specific application of such green fluorescent proteins to G protein signaling systems are described.

The methods outlined in *G Protein Signaling: Methods and Protocols* should be of interest to scientists studying the physiological roles of G protein systems, the signal transduction systems associated with these G protein systems, as well as the molecular nature of the G proteins themselves. The volume contains complementary biochemical, molecular biological, and cell biological approaches to addressing specific questions so that they can be studied from multiple perspectives and adopted by different types of laboratories.

Alan V. Smrcka



<http://www.springer.com/978-1-58829-137-0>

G Protein Signaling

Methods and Protocols

Smrcka, A.V. (Ed.)

2004, XII, 250 p., Hardcover

ISBN: 978-1-58829-137-0

A product of Humana Press