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G Protein-Coupled Receptor Screening Assays

Methods and Protocols

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Preface

G Protein-Coupled Receptors (GPCRs) are one of the largest known families of membrane proteins, with around 1,000 GPCR-like sequences identified in the human genome. These highly specialized proteins play a key role in signal transduction, converting changes in extracellular information into changes in intracellular functions. The diversity of ligands that interact with GPCRs is vast and includes neurotransmitters, metals, odorants, taste ligands, biogenic amines, fatty acids, amino acids, peptides, proteins, steroids, and light. The involvement of GPCRs in many physiological or disease-related processes has made them one of the favorite targets for researchers in academia and in the pharmaceutical industry. This significance is emphasized by the fact that one third of the drugs currently available in the market interact in some way with a GPCR. Still, the fact that those drugs target only ~30 members of the family and that ligands and structure are unknown for around 100 "orphan" GPCRs makes it clear that their pharmacological potential remains largely untapped.

In the past 20 years, considerable efforts have been directed towards the development of GPCR screening assays in order to disclose GPCR acting compounds, elucidate signaling mechanisms, or evaluate compound's efficacy. The majority of the approaches target particular steps in the GPCR signaling cascade ranging from the ligand binding event to functional, cell-based assays where partners from both signaling and regulation mechanisms are screened. Furthermore, the combination of genetically engineered cells expressing a large variety of GPCRs with automatic fluid handling and read-outs has originated high-throughput (HTS) or high-content (HCS) platforms that can screen and assay millions of compounds in a parallel fashion. As these technologies are solidly introduced in drug discovery programs, new and exciting findings regarding GPCR signaling are being disclosed. It is now well recognized that GPCRs can signal independently from their associated G proteins, that in living cell the GPCR signaling is a product of a complex network of positive and negative feedbacks from multiple receptors and that some ligands stabilize different receptor conformations in such a way that different signaling pathways can be favored in detriment to others. As a result, new screening assays, where reconstituted cell lines are being replaced with more realistic cellular systems such as tissue or animal models and biosensor technology for noninvasive "in vivo" cell testing, are being implemented.

These new opportunities along with the widely recognized need for better and safer pharmaceutical drugs constitute the main motivation for editing this book. Acknowledging the principle that no screening assays are ideal, the book "GPCR Screening Assays" intends to provide the reader, both the beginner and the experienced researcher, with an updated overview of not only the established but also the innovative technologies that promise to advance GPCR drug research. The book targets all those involved in the discovery of GPCR-active drugs, whether they come from academia or industry, but also the novice who is being introduced to the subject.

The book is organized into two major sections: (1) Introduction and (2) GPCR screening assays. The topics presented and discussed in the introductory chapter of the first

section provide the necessary foundations for the understanding of GPCR action and the rationale behind the design of the available screening assays. In Part II, detailed protocols are provided for different screening approaches. The individual chapters were selected and laid down to provide a transversal overview of the different levels of GPCR signaling that are addressable in the different screening strategies and present practical examples of how current assay technologies are contributing to new paradigms in GPCR drug research.

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