
Preface

The objective of *Receptor Binding Techniques, Second Edition* is to provide detailed practical methods for studying of receptors *in silico*, *in vitro*, and *in vivo*. The sequencing of the human genome has largely been completed. In addition to the established families of receptors, more than one hundred gene sequences have been predicted to encode novel G protein-coupled receptors (excluding olfactory receptors) together with a much smaller number of tyrosine kinase and nuclear receptors. Initially these are designated “orphan” receptors since their activating ligand has not been identified. Many sequences encoding orphan receptors have been artificially expressed in cell lines and are being paired with their cognate endogenous ligands by screening compounds from existing libraries or from tissue extracts. An unprecedented number of new receptor systems are emerging to be explored through ligand binding techniques. In addition, there is an expanding wealth of animal models such as disruption of genes in mice (knock-outs or knock-ins) in which to apply these techniques as a means to unravel the role of established or emerging orphan receptors.

In addition to curated databases of sequences encoding receptors, a large body of experimental data on ligand receptor interactions is available from public websites. These are dedicated to the major families together with sites concerned with downstream processes in intracellular, second messenger signaling. The efficient mining of these databases described in the first chapter is a logical first step in researching novel and established receptor systems. Chapter 2 provides detailed methodological information for the pairing of “orphan” receptors to identify their cognate ligand by fluorometric imaging plate reader assays that are amenable to high throughput screening. Molecular techniques can provide unequivocal evidence for the presence of mRNA encoding a specific receptor in a particular tissue. The quantitative measurement of mRNA in homogenates of tissue or cells using real-time polymerase chain reaction assays (Chapter 3) and determining the precise cellular or anatomical localization by *in situ* hybridization in tissue sections or Northern analysis in homogenates (Chapter 4) can provide initial clues for a role of de-orphanized receptors recently paired with their cognate ligands, narrowing the search for function.

The three principal radioligand binding assays used to characterize receptors (saturation, competition, and kinetic) are described in Chapter 5. Analysis by iterative curve-fitting programs provides key binding parameters: the equilibrium dissociation constant, receptor density, and Hill slope. A powerful

alternative to measure these parameters is the use of scintillation proximity assays (Chapter 6), which avoids the need to separate bound from free radioligand, particularly when rapid, high-throughput screening is required. Chapter 7 widens the scope of radiolabeled binding to encompass studying the distribution of enzymes, allosteric modulators of ion channels, and second messengers by either macro autoradiography or higher resolution micro autoradiography. A key protocol is provided for measuring unlabeled agonist-enhanced binding of [^{35}S]-GTP γ S, widely used in the mapping of G protein-coupled receptors that can also be exploited when radiolabeled analogs have not yet been developed for a particular transmitter molecule.

Immunocytochemistry (Chapter 8) exploiting selective antisera to map the distribution of receptor protein, compliments radioligand binding, particularly in permitting the precise identification of cell types expressing a specific receptor by dual labeling visualized by confocal microscopy. Applications include the characterization of mice following targeted disruption of gene-encoding receptors. The analysis of ligand receptor interactions in living cells at the subcellular level by confocal microscopy is the focus of Chapter 9. Protocols are given for visualizing intracellular trafficking in real time with fluorescent-labeled ligands or with the receptor itself labeled with green fluorescent protein. This chapter also describes techniques for measuring dimerization of receptors or interaction with other proteins by fluorescence resonance energy transfer.

Positron emission tomography (PET) is the only quantitative technique with sufficient sensitivity to detect the binding of radiolabeled ligand to receptors in living animals. The most widely used positron-emitting isotopes used to label ligands, ^{11}C and ^{18}F , have short half-lives and are difficult to detect by conventional film-based autoradiography. The penultimate chapter (10) outlines strategies for measuring binding parameters in vitro by phosphor imaging to evaluate the sensitivity and specificity of novel PET ligands for use in vivo. Following the development of tomographs specifically designed for rodents that can achieve remarkably high resolution, the final chapter (11) considers applying these ligands to functional imaging of receptors in vivo.

I am very grateful to the authors for their excellent contributions to this second edition of *Receptor Binding Techniques*, reflecting the success of the first edition edited by Mary Keen.

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