

Preface

This book describes a number of techniques relating to research on G protein-coupled receptors (GPCRs), written by a number of leading international authorities. In line with the rest of the essential methods series, each chapter contains an overview of the method and this is followed by a series of detailed protocols, providing a bench-side guide.

The literature on GPCRs is vast and the pace of investigation shows no sign of slackening. Given that these receptors are not only the largest protein family in the human genome but are also the single biggest target for therapeutic agents, this level of interest is not surprising. For any editor, this poses problems; the range of techniques that can be applied to GPCRs is vast and continues to grow, so it is impossible to cover them all in a single volume. In the case of this book, the techniques that are covered have been selected simply because we think that they will prove to be useful tools in future research and will contribute to increasing our understanding of GPCRs. In addition, they are of interest to the editors. Doubtless, readers wishing to find descriptions of additional methods will be able to find these elsewhere in the GPCR literature. Some of the techniques covered in this volume are very well known, such as mutagenesis or measurement of second messengers. We have included these because they are so fundamental to GPCR research and so might be of interest to the newcomer to the field. Some of the other techniques presented might not be so widely employed, but they have proved their worth in numerous laboratories.

Chapters 1 and 2, by Leach *et al.* and Gregory *et al.* respectively, review radioligand binding techniques (including detection of allosteric modulators) and measurements of a range of second messengers activated by GPCRs. Measurement of GTP γ S binding as an index of receptor activation is dealt with by Kara and Strange in Chapter 3. Quantitative GPCR imaging is considered in the context of receptor trafficking by James *et al.* in Chapter 4. In Chapter 5, Darby *et al.* describe methods for overexpressing GPCRs, concentrating on *Pichia pastoris* as a host. In Chapter 6, Jaeger *et al.* consider how bioluminescence resonance energy transfer can be used to look at GPCR complexes, whereas Krasel and Hoffmann describe in Chapter 7 how fluorescence resonance energy transfer can be used to measure receptor conformation. Chapters 8 and 12, by Li *et al.* and Liapakis and Javitich respectively, review the utility of engineered cysteines for artificial intramolecular disulfide bonds and cysteine scanning accessibility mutagenesis respectively. The new technique of fluorescence correlation spectroscopy

is described by Briddon *et al.* in Chapter 9. Mundell *et al.* (Chapter 11) and Kong *et al.* (Chapter 10) consider receptor regulation; how this can be quantified and how GPCR phosphorylation can be measured. Simms reviews techniques for receptor modelling in Chapter 13 and the Appendix (by Conner *et al.*) considers methods for mutagenesis.

We hope this volume will be useful to investigators in GPCR research.

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