

# METHODS IN MOLECULAR BIOLOGY™

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# Olfactory Receptors

## Methods and Protocols

Edited by

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*Cover illustration Caption:* The cover figure depicts a computational model of the human olfactory receptor hOR17-209 with the model of an isopentylacetate odorant ligand docked in the protein binding region. The OR-odorant system is embedded in a simulated plasma membrane. Water molecules surround the plasma membrane. Created by Peter C. Lai and Chiquito J. Crasto.

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## **Dedication**

For  
Hemant  
Jim  
Ed  
Jin-an  
Gordon



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## Preface

... *Some natures are so sensitive to certain smells; and it would even be a very fine question to study both in its pathological and physiological relation ...*

from *Madame Bovary*  
–Gustave Flaubert

A paper by Linda Buck and Richard Axel in the journal, *Cell*, in 1999, informed us of the discovery of an olfactory receptor gene. This tremendous discovery was rewarded by the Nobel Prize for Medicine in 2004. The discovery of ORs spurred research in identifying olfactory receptors as well as other chemosensory receptors such as taste and pheromone receptors, for several species.

When mammalian genomes were published, olfactory receptors were identified as constituting the largest families in these genomes. It appears that the higher the order of the species (whose olfactory repertoire has been mined and assembled from its genome), arguably, the lower is the acuity of olfactory function. This is evidenced by the relative number of nonfunctioning genes in the gene families, the percentage of these being highest in humans. Such a large gene repertoire should then conceal within it a gold mine of information as to the evolution of olfaction from lower order to higher order species. It also hints to the variability of the sequence (or structure)–function dichotomy, which allows fewer (despite its being a super-family) genes to discriminate several thousand odors singly or as complex mixtures.

Despite the best efforts of several and despite landmark discoveries and experimental ingenuity, challenges in the pursuit of research related to ORs exist. These hinder the functional characterization of olfactory receptors. Deorphanizing ORs—identifying odorants that will elicit an excitatory response from an OR—is difficult. This is because of the costs involved in testing odorants for an OR's response, a predictive notion for matching ORs and odorants being unavailable. This difficulty is exacerbated because of the promiscuous nature of OR–odor interactions. Protein expression, as is typically attempted in heterologous systems, has also proved difficult. Olfactory receptor repertoires consisting of several hundred genes have to be cataloged and made available to the community, informing of aspects related to the genomics, proteomics, and functional assessments. ORs are membrane-bound proteins, for which there currently exists no experimentally determined structure. Their protein structures have to be determined using *ab initio* or semi-empirical methods.

The chapters contained in this volume, titled *Olfactory Receptors: Methods and Protocols*, were chosen for the depiction of research that has resulted in significant contributions to addressing the issues mentioned in the above paragraph. Indeed, the parts within this volume (i.e. knowledge dissemination of ORs, theoretical assessments of OR structure and function, development and use of expression systems and experimental functional analysis) are designed to meet the above. The primary content of each chapter is written in the format of a laboratory manual, in a style that is readable to scientists as well as aspiring scientists.

This is in keeping with the general format for all volumes in the series. The chapters also provide a support-basis for methodological development for those involved in OR, GPCR, as well as membrane protein research.

ORs belong to the family of GPCRs, whose ubiquitous functionality has long been recognized and is being increasingly appreciated. At the time of completion of the development of this volume came a momentous announcement. In the words of the Nobel Committee: “*Robert Lefkowitz and Brian Kobilka are awarded the 2012 Nobel Prize in Chemistry for groundbreaking discoveries that reveal the inner workings of an important family of such receptors: G-protein-coupled receptors.*” ([http://www.nobelprize.org/nobel\\_prizes/chemistry/laureates/2012/](http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2012/)). ORs, because of the size of the gene family, the large variability in sequence (sustained by a uniformly similar three-dimensional structure), and the consequences to OR function, become, in several respects, a good testing ground for GPCR function.

Consequences of odor perception articulated by the prescient Flaubert notwithstanding, there is evidence that olfactory dysfunction can point to neurological disorders. Odor perception is perhaps more complex than specific odor recognition. It is possible that every individual possesses a unique olfactory fingerprint that is an agglomeration of a personal olfactory repertoire, a unique combination of neuronal processes and the health of the olfactory processing regions. ORs are responsible for the first step—odorant capture—that catalyze the cascade of events that results in olfaction. Understanding the mechanism of OR function will go a long way towards contributing to our knowledge of olfaction.

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To all the authors who have contributed to this volume, especially the corresponding authors: Eleonora Alfinito, Ricardo Araneda, Kaveh Baghaei, Yehuda Beh-Shahar, Richard Benton, Jerome Golebiowski, Kara Hoover, Dietmar Krautwurst, Charles Luetje, Joel Mainland, Bettina Malnic, Valery Matarazzo, Yoshihito Niimura, Tsviya Olender, Micherl Renou, Guenhaël Sanz, and Shuguang Zhang. This volume has been in development for some time and some of you have had to be very patient.

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To my dad, who passed away around the time I was thinking of working on a volume on olfactory receptors, to my mother, my brother, and my sister who continue to inspire and sustain me.

And when all's said and done and the dust's settled, I am fortunate to come home to my Budu, my Munder and my Avver.



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