

# METHODS IN MOLECULAR BIOLOGY™

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# Inositol Phosphates and Lipids

## Methods and Protocols

Edited by

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## Preface

This book seeks to cover a broad range of techniques encountered by those working within the field of phosphorylated inositols. This field started with the observation by the Hokins of the turnover of phosphatidylinositol, a minor membrane lipid, in response to agonists. However, it was more than 20 years before Michell proposed a hypothesis suggesting a functional link between this lipid turnover and intracellular calcium homeostasis that spurred a new wave of interest, cumulating with the description of  $IP_3$  as a second messenger by Berridge, Irvine, and their colleagues. This was followed by the later discovery of phosphatidylinositol 3 kinase by Cantley and co-workers. These significant advances were, however, just the beginning of a field that now encompasses every conceivable area of cell biology. The all-pervasiveness of these compounds in cellular regulation is largely facilitated by the unique structure of inositol which, when phosphorylated either in its native or lipid-derived forms, leads to the production of more than 30 unique isomeric forms with multiple functions. The tools of analysis necessarily reflect that complexity. The field is now divided into three major areas. These are the role of  $IP_3$  in handling intracellular calcium, the inositol lipids (and particularly the 3-phosphorylated lipids), and the higher phosphorylated inositol polyphosphates. Within the last 20 years, there have been two excellent methodological books on inositol compounds, edited by Irvine and Shears, respectively, each separated by approximately a decade. This new inositide methods book not only introduces the basic methodological tools to measure inositol lipids and phosphates but also reflects new approaches that have become available in the last 10 years, including RNA silencing and the use of fluorescently labeled PH domains to measure inositides in real time in live cells as well as new sensitive methods to measure mass of both phosphates and lipids. Inositol pyrophosphates are an important current area of inositol phosphate research, and this is reflected in a number of chapters within the book. After an overview of methodologies, we start with inositol phosphates, move on to the  $IP_3$  receptor and PLC, and then focus on the inositol lipids in the last section. Thus, the aim of this book is to compile many of the techniques that underscore phosphorylated inositol cell biology under one roof.

*Stockholm, Sweden*

*Christopher J. Barker*



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