PREFACE

The field of protein kinase inhibitors has exploded in the past 20 years. This pace of discovery has been driven by the diversity of biological processes controlled by protein kinases and the ability to develop small-molecule inhibitors capable of potently inhibiting kinases. This volume of *Methods in Enzymology* begins with a framework for understanding how protein kinases carry out the regulated transfer of phosphate to substrate proteins (Chapter 1). Protein kinase inhibitors were first viewed simply as ATP-competitive agents which occlude ATP binding and thereby block catalysis. As a result of the great number of structural studies, we now appreciate that certain inhibitors can bind to kinases in a conformation-specific manner or even outside the ATP pocket (Chapter 2). Following these mechanistic and structural chapters, we move to the discovery of inhibitors. There have been relatively few transformations in drug discovery since the implementation of structure-based design. One particularly noteworthy advance has been the appreciation of ligand efficiency and the implementation of this guiding principle at the earliest stages of kinase inhibitor discovery, termed fragment-based inhibitor discovery (Chapter 3). A particularly important clinical aspect of kinase inhibitors has been the need to inhibit oncogenic kinases potently such that the pathways in which they reside are inhibited at the level of 80% or greater (Vemurafenib clinical experience). The challenge of achieving such potent and durable inhibition while maintaining target specificity is a challenge the entire field faces. Covalent inhibitors which target nonconserved cysteines solve the potency and selectivity challenge simultaneously (Chapter 4). The main liability of covalent inhibitors, that of irreversible binding to off-targets, has been creatively addressed by the development of covalent-reversible inhibitors (Chapter 4). Although the core catalytic residues of kinases are conserved across the 538 family members, there are many residues in the active site which tolerate mutations resulting in drug resistance. The clinical emergence of these mutations and their creation and use *in vitro* to understand the mechanism of action of drugs is described in Chapter 5. The importance of identifying conformation-specific inhibitors and alternatives to traditional ATP site blockers has led to new tailored fluorescent screens (Chapter 6) and phenotypic screens (Chapter 7) for kinase inhibitors. Methods for discovering kinase inhibitors have improved greatly in the last 20 years, yet the kinase

family is enormous with 538 human kinases. Genetics offers a means to inactivate each family member individually, but the adaptability of kinase networks often masks the effect of knocking out or knocking down a specific kinase. In Chapter 8, the use of chemical genetics to develop an inhibitor of each protein kinase is described which combines the advantages of genetics and pharmacology in a widely used system across multiple organisms.

The field of kinase inhibitors is vast. Many important advances and techniques that were not covered in this volume will be covered in future editions.

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