
Preface

A multitude of fundamental cellular processes are driven by a class of ~90 proteins known as kinases, including cell division, DNA replication and repair, cell death, and differentiation. Kinases typically exist in a steady-state inhibited conformation which upon activation initiates a series of downstream signaling cascades, known as signaling networks. Aberrations in the expression and regulation of kinases are major causes of human disease, including cancer, diabetes, atherosclerosis, and infectious disease, among others. It is therefore unsurprising that kinases represent the largest class of proteins for which drugs have been developed. In some instances, these drugs have revolutionized therapy outcomes resulting in significant improvements in patient benefit and quality of life. Given the key roles that kinases play in health and disease, high-quality tools and innovative methods for in-depth characterization of these proteins and their associated signaling networks are necessary to overcome technological bottlenecks that limit our ability to gain a deeper mechanistic understanding of kinase biology.

Over the past two decades, advances in the development of experimental methods to systematically interrogate kinase signaling networks have led to fundamental insights into the inner workings of the cell and their interactions with the extracellular microenvironment. More recently, computational strategies to model and distil large experimental datasets have become essential in the quest to first deconstruct the kinase signaling “parts list” and subsequently rebuild *in silico* signaling networks for integrative and predictive cell biology. In this *Methods in Molecular Biology* volume in kinase signaling networks, we describe cutting-edge techniques in three distinct and complementary areas of contemporary kinase biology research.

In the first section “Synthetic Biology, Chemical Biology, and Screening Approaches,” we include chapters describing a range of diverse methodologies encompassing emerging and interdisciplinary approaches to characterize kinases, their signaling, and biological functions. Synthetic biology is described broadly as the designing and constructing of biological modules, biological systems, and biological machines for useful purposes. Using next-generation optogenetic tools, Goglia et al. outline the use of light inputs to exquisitely control the regulation of the mitogen-activated kinase (MAPK) pathway and imaging MAPK responses using live-cell microscopy. In a complementary approach, Ray et al. illustrate a strategy that utilizes the RapRTAP methodology to control kinase activity using small molecules. Resolving kinase signaling in heterogeneous cellular populations remains a significant challenge due to the limited number of single cell analysis approaches currently available. Pargett et al. demonstrate the power of fluorescent biosensors to parse

out population level effects in cellular signaling by measuring subcellular localization of extracellular signal-regulated kinase (ERK) activity at single cell resolution. One of the major challenges of kinase analysis is the lack of tools to measure kinase activity in the endogenous context without ectopic expression of a genetically encoded version of the protein. Beck et al. discuss the use of kinase activity chemosensors based on sulfonamido-oxine (Sox) fluorophores to quantify the enzymatic activity of endogenous kinases in tissue homogenates. While as a field we have developed a range of tools to measure kinase signaling, there are virtually no methods to synthesize phosphorylated proteins for interrogating kinase signaling. Barber and Rinehart describe their efforts in engineering recombinant phosphoproteins that incorporate phosphoserine residues to understand the role of phosphorylation in regulating kinase structure and function.

Chemical biology involves the use of chemical techniques and compounds to study and manipulate biological systems. Included in this section are a number of chapters that describe robust chemical assays to characterize the biochemical properties of kinases. Register et al. have developed a series of assays to characterize the allosteric effects of ATP competitive inhibitors of the Src-family kinases (SFKs) on distal domains of kinases, while Lucet and Murphy describe a unique fluorescence-based thermal shift assay that is capable of characterizing the interaction between the enigmatic class of pseudokinases and their ligands. The next chapter by Golkowski et al. highlights the use of affinity enrichment and proteomics to perform kinome-wide binding studies of small molecular inhibitors. To conclude the chemical biology part of this section, Stewart and Banerji illustrate the use of bead-based Luminex technology to measure small molecule inhibitor effects on kinase signaling networks in a high-throughput and reproducible fashion. With the democratization of genome- and kinome-wide screening strategies in routine laboratory research, this section also includes several chapters describing screening-based approaches to interrogate kinase biology and signaling networks. These include the use of RNAi screens for the analysis of kinase signaling networks and drug resistance (Stockwell and Mitnacht, Singleton et al.), as well as the identification of driver kinases from next-generation cancer genome sequencing data (Leonidou et al.).

The second section of the book delves into the use of protein mass spectrometry and metabolic approaches to analyze kinase signaling networks. Proteomic and phosphoproteomic analysis by mass spectrometry is one of the key technologies currently employed for in-depth characterization of kinase signaling, and chapters in this section cover label-free analysis methodology (Wilkes and Cutillas), analysis of bidirectional signaling between different cell types (Tape and Jorgensen), characterizing the phosphorylated complement of the cellular adhesome (Robertson et al.), defining phosphotyrosine signaling in cancer cell lines (Broncel and Huang), and targeted analysis of phosphopeptides using multiple reaction monitoring mass spectrometry (Payne and Huang). Additional developments in this area include tools to measure protein phosphorylation in specific cellular compartments such as mitochondria (Renvoise et al.), the use of mass spectrometry to define immune cell signaling (Koppenol-Raab and Nita-Lazar), methods to tackle the challenging problem of defining absolute kinase phosphorylation stoichiometry (Chen et al.), developing assays to identify kinase substrates based on kinase assay-linked phosphoproteomics (Hsu et al.), combining subcellular fractionation and quantitative proteomics to map global spatial protein networks (Mardakheh), and tools for kinome-wide analysis of signaling networks using mass spectrometry (Beck et al.).

In addition to protein mass spectrometry, next-generation analytical tools such as mass cytometry have revolutionized our ability to characterize kinase signaling networks at the

single cell level. Bandyopadhyay et al. describe the use of the CyTOF mass cytometer to acquire multidimensional signaling data in cellular systems. Another complementary approach to study the physiological and pathological effects of kinases is the use of metabolic analysis. Chung describes the use of magnetic resonance spectroscopy technique to determine metabolic changes in response to kinase inhibitor therapy, while Poulogiannis illustrates the utility of targeted mass spectrometry to characterize the metabolic networks associated with oncogenic signaling.

The final section of the book is focused on computational analysis of kinase signaling networks and aims to provide a broad description of contemporary computational approaches to model experimental data and generate new insights into kinase biology. Fey et al. provide an in-depth chapter that describes the development and use of mechanistic models for predictive tyrosine kinase signaling. Along the same theme, Reis et al. illustrate the combined use of experimental and computation to develop and optimize kinetic models of the MAPK signaling pathway. An interesting area of kinase research is the use of large-scale phosphoproteomic data to study the evolution of protein phosphorylation and signaling modules. Tan provides a review on the databases and computational tools for conservation analysis of novel phosphorylation sites across multiple model organisms to establish evolutionary relationships. The ability to predict tumor cell responses to kinase inhibitors remains a significant challenge in the field, and both Berlow and Pal and Tang describe novel computational tools based on large-scale analysis of kinase inhibitor profiles to infer cellular networks and predict potent drug combinations in the context of cancer. The section concludes with a contribution by Kreeger who describes the use of partial least squares regression as a tool for data reduction and multivariate analysis of kinase signaling and phenotypic outcomes.

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