# Introduction to Kinases, Cellular Signaling, and Kinase Inhibitors


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**Abstract** Protein kinases are essential regulators of cellular functions and responses to extracellular signals. Through phosphorylation of substrates, protein kinases control cell proliferation and survival. Proliferative disorders, such as cancer, are often observed to have excess protein kinase activity due to genetic mutations. Thus, the development of specific drugs to inhibit protein kinases in cancer cells has been a major goal of academic and pharmaceutical industry research during the last three decades. This chapter will provide a brief historical overview of groundbreaking discoveries describing the importance of protein kinases and the identification of clinically relevant kinase inhibitors. An outline of protein kinase classes, signaling pathways, and structural features will introduce current kinase inhibitor approaches and provide the rationale for identifying alternative approaches to block excess protein kinase activities that promote disease.

**Keywords** Kinase · Inhibitors · Disease · Drug discovery

## Historical Overview of Protein Kinases and Targeted Inhibition

Kinases, derived from the Greek word *kinein* meaning "to move," are ubiquitous enzymes that have become prominent therapeutic targets in the treatment of a variety of diseases. Kinase enzymatic activity is in every cell of every species and facilitates physiological responses to both intracellular and extracellular signals. Through the process of phosphorylation, kinases move or transfer cellular information that regulates a variety of other proteins essential for the survival of the organism. The presence and appreciation of phosphorylated proteins and their importance in biological processes began in the early 1900s. It was in Phoebus Levene's laboratory at

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the Rockefeller Institute for Medical Research where phosphorylated serine residues were identified on the proteins casein and phosvitin, which are abundant in milk and egg yolks, respectively [1]. Subsequently, in the 1940s, Gerty and Carl Cori's research at Washington University in St. Louis discovered active and inactive forms of the phosphorylase enzymes that transfer inorganic phosphate to acceptor molecules and are involved in the process of glycogen metabolism. Cori's research was awarded a Nobel Prize in Physiology or Medicine in 1947 and provided the foundation for understanding the process of reversible phosphorylation [2]. Following this award, the significance of the enzymatic activity of kinases and the process of phosphorylation received a significant boost from the pioneering work of George Burnett and Gene Kennedy at the University of Chicago. Their 1954 publication in the *Journal of Biological Chemistry* conclusively demonstrated that rat liver mitochondria extracts contained a protein enzymatic activity, which the authors referred to as a phosphokinase, that extracts a phosphate group from the energy molecule ATP and covalently links it to another protein [3]. These studies revealed a new area of biology that describes how cells use the mineral phosphorus, and its biological form phosphate, to convey information and regulate biological molecules via phosphorylation to accomplish specific cellular functions.

Subsequent discoveries by Edmond Fischer and Edwin Krebs in 1956, at the University of Washington, demonstrated that kinases regulate protein functions in response to extracellular signals and that kinase-mediated phosphorylation events are reversible [4]. The significance of the work by Dr. Fischer and Dr. Krebs, who had trained in the previously mentioned Cori laboratory, was recognized with the Nobel Prize in Physiology or Medicine in 1992. The balance between the activities of kinases that mediate phosphorylation and phosphatase enzymes that facilitate de-phosphorylation is essential for regulation and maintenance of most cellular functions. There are numerous genetic alterations, which will be highlighted in subsequent chapters, responsible for dysregulated kinase activity and the disruption of the balance between phosphorylation and dephosphorylation events. These dysregulated phosphorylation events alter the steady state, or homeostasis, of cellular functions and, ultimately, contribute to the pathology of a variety of diseases.

Over the last several decades, a vast amount of research has discovered and described specific kinases and their functions in regulating specific physiological functions. These findings have revolutionized our understanding of the role of kinases in disease processes and the development of kinase-specific drug therapies. In most cases, the therapeutic objective is to inhibit constitutively active kinase activity found in proliferative disorders, like cancer. Despite a detailed understanding of kinase structures and functions, as well as the availability of potent and selective kinase inhibitors, the ability to achieve sustained patient responses to most of the current kinase inhibitors is limited. This raises the questions of how much is really known about the regulation of kinases in complex biological systems and their role in regulating disease. Furthermore, the multifaceted nature of diseases like cancer may limit the use of potent and selective kinase inhibitors that only target one aspect of the disease but allow compensatory kinase signals that protect cancer cell proliferation and survival. In contrast to the development of very selective

kinase inhibitors, there is growing interest in developing inhibitors that target multiple kinases simultaneously or polypharmacologic properties that are uniquely effective against several dysregulated kinases associated with specific diseases [5].

The first, and arguably most successful, program to develop specific small-molecule kinase inhibitors to treat a specific type of cancer was realized by Drs. Nicholas Lydon and Brian Druker in the 1990s with the drug imatinib for the treatment of chronic myelogenous leukemia (CML) [6]. It was well known that CML cells contained a genetic translocation resulting in the fusion of the breakpoint cluster region (BCR) gene on chromosome 22 with the Abelson tyrosine kinase (ABL) gene from chromosome 9. The resulting BCR-Abl fusion protein is constitutively active, and this mutant tyrosine kinase drives the proliferation of white blood cells in nearly every CML patient. Dr. Druker hypothesized that BCR-Abl is a viable drug target and that inhibition of BCR-Abl would improve the therapeutic outcomes of CML patients. Dr. Lydon, working on drug discovery programs at Ciba-Geigy (now part of Novartis), provided the compound STI571, also referred to as imatinib mesylate (brand name Gleevec® or Glivec®), which is an ATP competitive inhibitor of BCR-Abl and other tyrosine kinases. As Drs. Lydon and Druker pointed out, there was skepticism from other scientists and the pharmaceutical industry that specific kinases inhibitors could be developed and that targeted inhibition of a single kinase would be effective against cancer cells with multiple genetic defects [7]. However, the results of the first clinical trials in 1998 and 1999 testing imatinib in CML patients had remarkable outcomes with almost every patient showing improvement. Moreover, the patients had very few side effects and a 5-year follow-up showed patient survival approaching 90% compared to 50% for patients on traditional chemotherapies [7]. While the success of imatinib in treating CML patients could be partially attributed to its off-target effects on other tyrosine kinases, these groundbreaking trials provided the justification for the Food and Drug Administration (FDA)'s approval in 2001 for clinical use. Imatinib remains one of the top-grossing cancer drugs with over $1.5 billion in sales in 2018. Importantly, the introduction of tyrosine kinase inhibitors such as imatinib has significantly improved the survival of CML patients as compared to before these drugs were available [8]. With the therapeutic success of imatinib, scientists and the pharmaceutical industry were provided the framework to pursue the development of other kinase-selective inhibitors for treating disease.

Despite the clinical success of imatinib in treating CML, sustained treatment responses using inhibitors of kinases for other cancer types or diseases have been difficult to achieve. The current approaches used to inhibit protein kinases involved in disease consist of small-molecular-weight compounds (e.g., small molecules) or monoclonal antibodies. As of June 2019, there were approximately 50 small-molecule inhibitors of protein kinases approved by the FDA for clinical use. The number of FDA-approved small-molecule kinase inhibitors from 1999 to 2018 shows an increasing trend over the last decade (Fig. 1). An excellent comprehensive description of the pharmacological properties of FDA approved small-molecule kinase inhibitors is available [9]. In addition, there are more than 30 FDA-approved monoclonal antibodies developed to block the activity of mostly receptor and non-

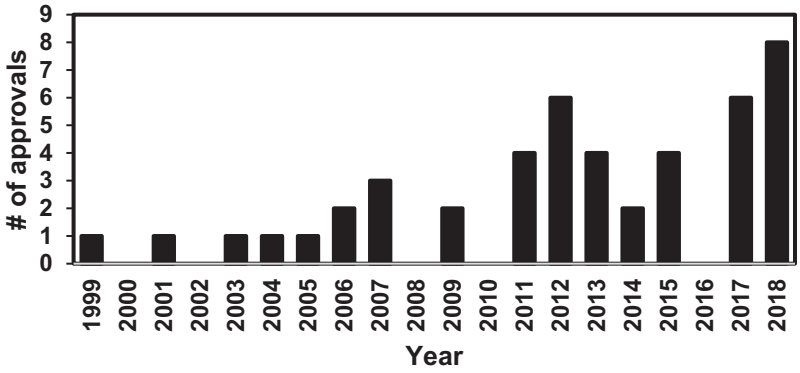


**Fig. 1** FDA-approved small-molecule kinase inhibitors (1999–2018)

receptor tyrosine kinases involved in disease. Several reviews outline the development of monoclonal antibodies along with their therapeutic potential and limitations [10, 11]. However, the development of kinase-targeted monoclonal antibodies will not be the focus of subsequent chapters.

A major goal of this book project is to convey that the current approaches to block kinases, with either small molecules or monoclonal antibodies, have mostly failed to produce effective or sustained clinical responses despite the significant evidence supporting kinases as key drivers of disease. As such, new approaches to block important kinase activities involved in disease need to be explored. It is reasonable to suggest that the lack of effective kinase inhibitors can be explained by an inadequate understanding of the biological and genetic determinants that drive the disease. As such, inhibition of a key kinase predicted to drive the pathology of a specific disease is not enough and additional biological targets need to be considered. While this is likely true for many conditions, it can also be argued that the kinase being targeted is appropriate; it is just that the approach used to target a specific kinase is ineffective at producing durable clinical responses. To set the stage for the discovery of new approaches to inhibit kinases, the first chapters will provide an overview of the kinase structure, kinase-signaling networks, and the current approaches to develop small-molecule inhibitors of kinases involved in disease. Therapeutic uses and limitations of the current kinase inhibitors, including the emergence of drug resistance will be highlighted.

Small-molecule kinase inhibitors are classified into six categories commonly referred to as type I–VI kinase inhibitors that are grouped largely based on the structural interactions between the inhibitor and target kinase [12]. Chapter 2 provides a concise summary of the basic features of the type I and II kinase inhibitors, which are currently the most common approach to target kinases, and act by preventing interactions with ATP when the enzyme is in an active or inactive state, respectively. Chapter 3 will summarize recent developments in the type III–VI kinase inhibitors. Type III kinase inhibitors target allosteric sites in the kinase domain but do not affect ATP binding whereas type IV kinase inhibitors target allosteric sites outside

the kinase domain and are generally designed to interfere with the interactions between kinases and other regulatory proteins or substrates. Type V kinase inhibitors are referred to as bivalent compounds that target both the ATP-binding site and unique allosteric sites outside the kinase domain. Finally, type VI kinase inhibitors form covalent interactions with cysteine and other amino acids in the ATP-binding site or other regions of the kinase. A recent review of approaches to target the extracellular signal-regulated kinases-1 and 2 (ERK1/2) provides an excellent visual description of the mechanism of action for type I–VI kinase inhibitors [13].

The second major goal of this book will be to highlight new approaches to target protein kinases, including the development of novel type IV small-molecule kinase inhibitors and kinase-targeted peptides that selectively inhibit specific kinase functions. Function-selective kinase inhibitors account for the diversity of protein substrates that are regulated by kinases involved in proliferative diseases, such as cancer, or inflammatory disorders. There are documented examples of how kinase-mediated regulation of substrates can drive a cellular response, but regulation of other substrates is involved in modulating that response through negative feedback mechanisms. Thus, kinases contribute to maintaining homeostasis in normal and diseased cellular responses through both positive and negative feedback regulation. Inhibition of kinase activity with the current type I and II inhibitors block all positive and negative enzyme activity whereas disruption of key kinase-substrate interactions has the potential to block undesirable kinase functions (e.g., protumorigenic) while maintaining desirable kinase functions (e.g., antitumorigenic negative feedback). Lack of durable responses of many kinase inhibitors used in the clinic may be attributed to inhibition of both positive and negative kinase functions. It will be interesting to see whether novel proteolysis targeting chimera (PROTAC) approaches that can be designed to selectively degrade kinases and other proteins involved in disease [14] will also have similar issues with efficacy.

The design of function-selective kinase inhibitors is based on a large body of information describing the structural features that determine specific protein-protein interactions (PPIs) and the biological consequences of those interactions. Chapter 4 will provide examples of PPIs focusing on specific kinase interactions with substrate proteins. These studies have helped in the design of new approaches to target key PPIs involved in disease. In addition, Chap. 4 will overview the emergence of acquired drug resistance to current kinase inhibitors used in the clinic, which presents a major barrier to sustained and durable patient responses. It is hypothesized that function-selective kinase inhibitors will prevent or mitigate the emergence of drug resistance observed with current kinase inhibitors that block all enzyme activity.

The final chapters will describe specific examples of theoretical and experimental approaches to develop kinase inhibitors that act outside of the ATP/catalytic site and inhibit specific kinase functions. Chapter 5 describes how computational models can facilitate the rationale design and analysis of new compounds that target specific PPIs and, in particular, those that involve kinase interactions with specific protein substrates. Chapter 6 provides a comprehensive description of known substrate-docking sites on the extracellular signal-regulated kinases (ERK1/2) and

opportunities to develop type IV inhibitors that target these sites for treating cancer. Chapter 7 will describe the synthesis of novel peptide sequences that lock into secondary structures that recognize specific kinase sites involved in substrate recognition. Finally, Chap. 8 expands on the use of peptides that selectively modify kinase functions and outlines the challenges that need to be overcome before these agents can be used in the clinic. In conclusion, the evidence presented in these chapters will provide support for the discovery and development of novel kinase inhibitors that selectively block some, but not all, enzymatic functions. The development of new approaches aimed at partial inhibition of kinase functions involved in disease is predicted to lead to more effective and sustained therapeutic responses.

## Overview of Protein Kinase Signaling Pathways

Protein kinases are essential regulators of cellular functions and responses to external signals. Protein kinases accomplish their regulatory role mostly, but not always, by catalyzing the transfer of phosphate from ATP onto substrates. Of the more than 500 distinct genes that encode for human protein kinases [15], it is estimated that ~80% fall into the category of serine or threonine kinases while the remaining 20% consist of tyrosine or histidine kinases [16]. However, it is estimated that roughly 90% of all phosphorylation events in human cells occur on serine residues while approximately 10% occurs on threonine residues, and less than 1% of phosphorylation events occur on tyrosine residues [16]. Given that most of the kinase inhibitors used in the clinic today block the actions of tyrosine kinases, these numbers suggest there are tremendous opportunities for the discovery of new kinase inhibitors.

Protein kinases are classified into AGC, CAMK, CMGC, CK1, STE, TK, and TKL subgroups based on their phylogenetic tree [15]. The AGC protein kinase group consists of approximately 60 serine/threonine kinases related to protein kinases A, G, and C. One feature key to the regulation of AGC kinase activity is a hydrophobic region in the C-terminal that interacts with a pocket in the catalytic region. This interaction site was named the PIF, 3-phosphoinositide-dependent protein kinase–1 (PDK1)-interacting fragment, unique to the ACG family [17]. The CAMK group is the abbreviation for around 80 serine/threonine kinases related to the calcium-calmodulin–dependent protein kinases. The identification of a unique calcium/calmodulin (CaM)-binding domain is a regulatory feature found in about half of kinases in the CAMK family [18].

There are roughly 62 members of the serine/threonine kinase CMGC group that include the cyclin-dependent kinases (CDK), mitogen-activated protein kinases (MAPK), glycogen synthase kinases (GSK), and CDC-like kinases (CLK). The CK1 group, or casein kinase-1 family, is a small group of 12 serine/threonine kinases that are the most structurally distinct group of eukaryotic protein kinases. A unique aspect of the CK1 proteins is a variable C-terminal region that does not directly affect ATP catalysis but is important for regulating intracellular location and kinase functions [19, 20]. The STE kinase group, named for yeast sterile genes

involved in mating signals, comprises approximately 46 serine/threonine kinases that act primarily as upstream activators of the mitogen-activated protein kinase (MAPK) proteins and include the MAP2K, MAP3K, and MAP4K proteins. The MAP2K subfamily is unique in that it is a dual specificity kinase and can phosphorylate MAPK proteins on threonine (T) and tyrosine (Y) residues within a conserved TXY motif (X is any amino acid) that regulates kinase activation. The last kinase groups consist of approximately 90 receptor and nonreceptor tyrosine kinase (TK) proteins and another 43 tyrosine kinase-like (TLK) proteins. While the TLK group shares amino acid sequence similarity to TK proteins, these proteins function as serine/threonine kinases.

The process of phosphorylation adds a negative charge to a biological molecule, which alters the molecule's structure and ultimate function in the cell. The functions of biological molecules such as proteins, nucleic acids, carbohydrates, and lipids are all regulated by phosphorylation events. Phosphorylation is a highly regulated and reversible process. Cellular functions depend on the balance between the addition of phosphates by kinases to achieve a specific cellular response and the removal of the phosphate by protein phosphatases when that cellular response is no longer needed. A consequence of disrupted balance between protein phosphorylation and dephosphorylation often results in elevated protein phosphorylation, which contributes to the development and progression of many types of cancer and inflammation-related disorders. Excess protein phosphorylation is a consequence of genetic mutations or altered expression of kinases and phosphatases. Dysregulated and constitutively active protein kinases are the primary culprits that disrupt the balance between phosphorylation and dephosphorylation. As such, inhibition of dysregulated kinase activity is a major goal in the development of safe and effective therapies for cancer, inflammatory disorders, and many other diseases.

There are currently more than 200 kinases that have been linked to various disease states and most involve proliferative disorders such as cancer [21, 22]. However, dysregulated kinase activity is also recognized to contribute to cardiovascular, metabolic, and neurodegenerative diseases [23–28]. The causes of kinase dysregulation and its role in driving disease have been studied extensively and can be narrowed down to three genetic changes. These genetic alterations consist of point mutations that change single amino acids, gene amplification, and the fusion of two different genes, all of which result in kinases with elevated or constitutive activity [21]. Further analysis of over 1000 putative cancer causing or "driver" genes, which are essential for the cells proliferative advantage, identified 91 of these genes to be protein kinases [29]. Interestingly, a remarkable 40% of the protein kinase drivers were tyrosine kinases, which is consistent with the higher emphasis on the development of tyrosine kinase inhibitors. However, despite the prevalence of clinically available tyrosine kinase inhibitors, less than half of these kinase drivers have been targeted with therapeutic agents [29].

Protein kinases serve an important function in regulating cellular responses to extracellular signals. Figure 2 shows a simplified overview of major kinase signaling pathways that respond to extracellular signals, have been found to be dysregulated in disease, and are the targets of kinase inhibitors. Extracellular cytokines and

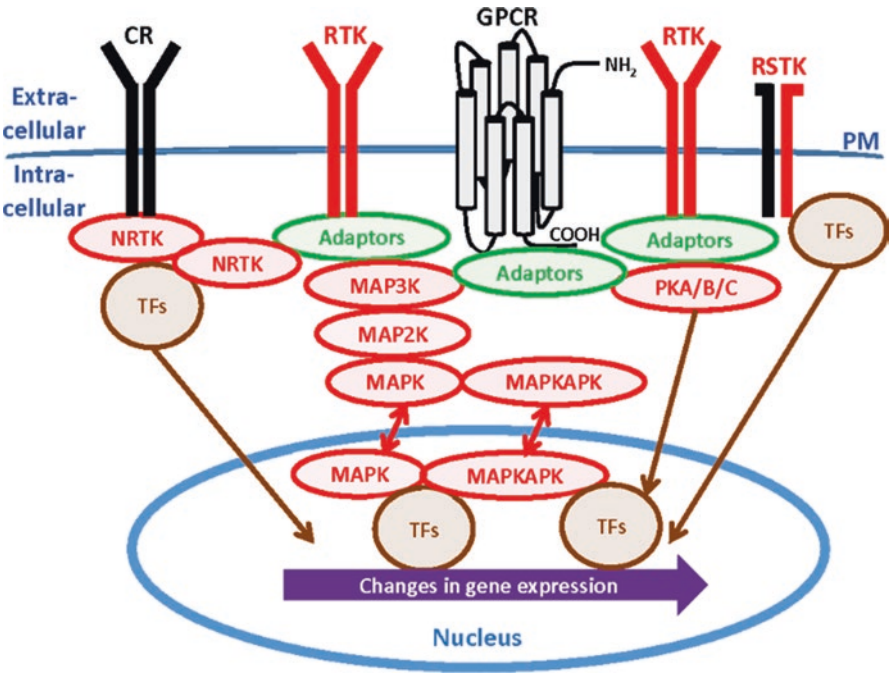


**Fig. 2** Receptor-mediated kinase signaling networks. Kinases (red) target transcription factors (brown) to mediate changes in gene expression and cellular responses to extracellular signals. Adaptors (green) include G-proteins and associated proteins that couple receptors to kinase cascades. Key: *CR* cytokine receptor, *RTK* receptor tyrosine kinase, *NRTK* nonreceptor tyrosine kinase, *RSTK* receptor serine/threonine kinase, *PM* plasma membrane, *TFs* transcription factors, *MAP3K/MAP2K/MAPK* mitogen-activated protein kinase cascade, *MAPKAPK* MAP kinase-activating protein kinase, *PKA/B/C* protein kinase A, B, or C

growth factors regulate cellular responses by interacting with plasma membrane–bound receptor tyrosine kinases (RTK), G-protein coupled receptors (GPCR), cytokine receptors (CR), and receptor serine/threonine kinases (RSTK). Engagement of the extracellular ligands induces receptor conformational changes that result in the dimerization and activation of monomeric receptor proteins and the recruitment of intracellular nonreceptor tyrosine kinases (NRTK) and other adapter proteins. The recruitment of intracellular adaptor proteins to the activated receptors led to the activation of kinase cascades and regulation of specific transcription factors and gene expression. However, kinases can regulate many other nongenomic processes by phosphorylating cytoplasmic substrates that affect the size or shape of the cell and its ability to migrate and interact with other cells. To add to the complexity, there are several examples of protein kinases regulating other proteins and biological outcomes through catalytic-independent functions [30]. In most of the cases where there is no phosphate transfer, the physical interaction between a kinase and a particular protein is sufficient to modulate the protein's function and a subsequent biological outcome.

The mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) signaling cascades are classic examples of RTK and GPCR-mediated signaling pathways that regulate cellular functions [31–36]. In the case of receptor serine/threonine kinases (RSTK), transcription factor activation is coupled directly to the ligand-activated receptor. The transforming growth factor-β (TGF-β) family is an example of secreted extracellular proteins that activate receptors with primarily serine/threonine kinase activity to modulate the cellular responses in many physiological systems [37]. Membrane-bound receptors, directly or through adaptor proteins, activate intracellular kinases, which in turn regulate a variety of substrates including transcription factors to alter gene expression and cellular responses. Dimerization of RTK monomers following ligand engagement facilitates inherent kinase activity of these receptors. In contrast, the ligand-activated cytokine receptors lack kinase activity and engage associated nonreceptor tyrosine kinases to initiate downstream signaling. In the case of the receptor serine-threonine kinases, only one of the monomers has kinase activity that directly phosphorylates transcription factors after receptor dimerization following ligand engagement. Several comprehensive reviews of these receptor-mediated kinase-signaling pathways are available [38, 39]. In addition, a more detailed description of specific kinase-signaling networks will be presented in later chapters.

## Overview of Protein Kinase Structural Features

The typical eukaryote protein kinase has a conserved bilobed 3-D structure consisting of amino- (N) and carboxy-(C) terminal lobes that are coordinated in their movement in relation to each other depending on kinase activity. Most protein kinases contain a conserved ATP-binding site, substrate interaction sites in the C-terminal lobe, and activation sites as shown in the example of protein kinase A (Fig. 3). The N-terminal lobe consists mainly of beta sheets while the C-terminal lobe contains alpha-helices. At the base of the N-terminal lobe sits the ATP-binding and catalytic site that serves the function of removing the terminal phosphate ($PO_4^{3-}$) from magnesium-ATP (MgATP) and catalyzing its transfer onto the hydroxyl ($OH^-$) group of a serine, threonine, or tyrosine residue located on the substrate protein. The $Mg^{2+}$ helps stabilize and position the negatively charged phosphate on ATP for transfer onto the substrate. Additional coordination of ATP involves a conserved glycine rich loop and lysine residue in the N-terminal lobe. Substrate proteins interact with specific residues in the C-terminal lobe and along a cleft formed between the N- and C-terminal lobes. However, the specific kinase residues involved in recognizing most protein substrates are not known. An overview of some of the known structural features and residues involved in kinase recognition of protein substrates will be the topic of Chap. 4. This information will be important for the identification of type IV kinase inhibitors that disrupt key kinase functions.

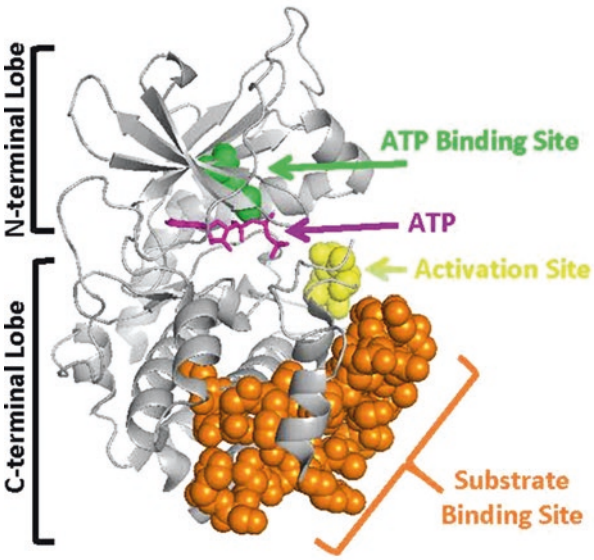


**Fig. 3** General overview of protein kinase structure. A model of protein kinase A (pdb: 3FJQ) highlights the N-terminal lobe containing the ATP-binding site (conserved lysine, K73, in green) and ATP (purple). The C-terminal lobe contains the activation sites (T196 and T198 in yellow) and substrate-binding site (residues 230–260 in brown)

Human protein kinases are dynamic structures, and multiple regions distal to the catalytic site have been implicated in coordinating activity. For a more detailed analysis of the structural features involved in protein kinase regulation, the reader is directed to several intriguing studies and comprehensive reviews. For example, McClendon et al. have presented compelling molecular modeling data showing that kinases also have unique local regions, consisting of 40–60 amino acid segments that undergo unique dynamic changes that provide allosteric regulation and additional control over kinase activity and function [40]. Wang and Cole provide an excellent review of the catalytic mechanisms of protein kinases and the transfer of a phosphoryl group from ATP onto substrates [41]. This review highlights the work of many scientists who have made significant contributions to our understanding of kinase structure and catalytic mechanisms. Although protein kinases share many conserved structural features that define the core region involved in phosphoryl transfer onto protein substrates, there are regions outside of the kinase core that facilitate catalytic activity, kinase complexes, and signaling events. Gógl et al. describe the presence of intrinsically disordered regions (IDRs) in protein kinases that help fine tune kinase catalytic activity and assembly of kinases in multiprotein signaling complexes [42]. Expanding knowledge of the regulatory features of protein kinases will provide opportunities to develop new approaches to modulate protein kinase functions in disease.

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