Top Med Chem (2021) 36: 1–24
https://doi.org/10.1007/7355_2020_97

Published online: 6 May 2020

Function, Structure and Topology of Protein Kinases

Sandra Röhm, Andreas Krämer, and Stefan Knapp

Contents

Abstract Protein kinases represent one of the most successful target classes for the development of new medicines. Because of their key roles in cellular signalling, kinases are stringently regulated by a large diversity of mechanisms such as post-translational modifications, interacting domains and proteins and cellular localization. The high plasticity of protein kinases has been exploited for the development of new inhibitor types such as type-II and type-I½ inhibitors targeting inactive states of the kinase catalytic domain and allosteric inhibitors that target induced binding pockets either adjacent (type-III) or distantly located (type-IV) to the kinase ATP-binding site. Here we discuss structural elements of the kinase active site, key mechanisms of kinase regulation and how these mechanisms can be exploited for the development of selective kinase inhibitors.

Keywords Allosteric inhibitors, Kinase activation, Kinase regulation, Structure-based drug design

S. Röhm, A. Krämer, and S. Knapp (✉)
Johann Wolfgang Goethe-University, Institute for Pharmaceutical Chemistry, Frankfurt, Germany

Johann Wolfgang Goethe-University, Buchmann Institute for Life Sciences, Frankfurt, Germany
e-mail: knapp@pharmchem.uni-frankfurt.de

1 Introduction

The human kinome constitutes a large superfamily of essential enzymes with more than 500 family members [1]. Kinases can be grouped in two main classes based on their catalytic activity on serine/threonine and on tyrosine residues. Based on their primary sequence and conserved structural features, the human kinome has been classified into eight major kinase groups, namely, the AGC (protein kinase **A**, **G** and **C**), CaMK (calcium/calmodulin-dependent kinases), CMGC (**c**yclin-dependant kinases, **M**AP kinases, **g**lycogen synthase kinases, **c**asein kinases 2), TK (**t**yrosine **k**inases), STE (homologues of yeast **s**terile 7), CK1 (**c**asein **k**inases), TKL (**t**yrosine **k**inase-like) and the RCG (receptor guanylate **c**yclases) kinase families [1]. In addition, a large number of kinases share only weak sequence homology with any of these major groups and have been classified as "other" and atypical kinases. While the group of other kinases are typical protein kinases, the group of atypical kinases lack canonical sequence motifs of the kinase catalytic domain. Some of the atypical kinases have therefore been reassigned as non-kinase proteins, whereas there are also several recent additions to this group such as the FAM20 kinases [2–4]. Interestingly, around 10% of all human kinases are considered catalytically inactive and have been classified as pseudokinases. Pseudokinases share a typical kinase domain fold, but they lack at least one conserved structural motif which is considered important for catalytic activity [5, 6]. Pseudokinases have essential signalling function despite their lack of catalytic activity by acting as scaffolding proteins and allosteric regulators of catalytically active kinases.

Many protein kinases are deregulated in human disease which made protein kinases major drug targets. However, only few have been targeted to date offering huge opportunities for future drug development efforts [7, 8]. The high sequence homology within the kinase ATP-binding site which is the target of most kinase inhibitors poses however challenges on the development of selective inhibitors. On the other hand, design efforts for selective inhibitors are now facilitated by the large number of crystal structures that are now available covering about 40% of the kinase family. The protein data bank (PDB; http://www.rcsb.org/) and the KLIFS database (http://klifs.vu-compmedchem.nl/) [9] currently list 4,521 crystal structures covering 293 kinases. Current strategies for the development of selective inhibitors comprise now several structure-based strategies such as covalent targeting of unique cysteine residues with in the ATP-binding site [10, 11], allosteric inhibitors [12, 13] and conventional inhibitors with good shape complementarity [14]. Here we review structural features important for kinase catalytic function and regulation as well as strategies for structure-based kinase inhibitor design.

2 The Kinase Active State

The tertiary structure and overall folding of the kinase catalytic domain is highly conserved throughout the kinase family and harbours around 300 amino acids. The first crystal structure of PKA in complex with ATP and a pseudosubstrate in 1991 paved the way for a better understanding of the catalytic mechanism, cofactor binding and kinase regulation [15–17].

The canonical kinase catalytic domain fold consists of two domains, also called lobes, which are connected to each other via a flexible hinge region. Both lobes form a hydrophobic cleft which serves as binding site for ATP (Fig. 1a). The smaller N-terminal lobe (N-lobe) comprises five β-sheets (β1–β5) and one helix called αC, whereas the C-terminal lobe (C-lobe) is mainly alpha helical in structure (αD–αI).

Four main interactions were evident from the first PKA crystal structure [15–17]: (1) The adenosine ring of ATP is placed in the catalytic centre and forms hydrogen bonds with the hinge region amino acids. (2) A flexible loop region between the sheets β1 and β2 harbours a glycine-rich sequence motif GXGXφG, where φ refers to a hydrophobic residue which coordinates the ATP phosphates coining the name phosphate-binding loop. (3) The β3-sheet contains a conserved VIAK (Val-Ile-Ala-Lys) motif. The lysine adopts important structural features, by forming a salt bridge with ATP phosphate groups and to a conserved glutamate residue

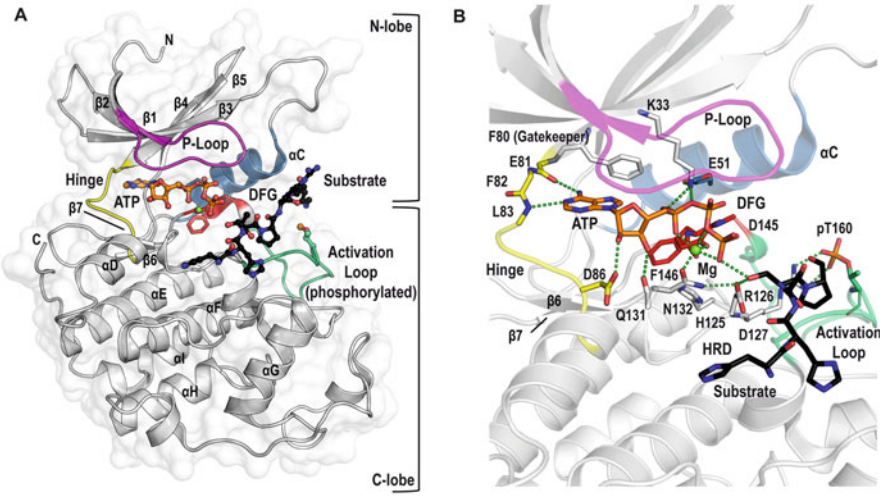

Fig. 1 Architecture of the kinase catalytic domain. (**a**) Conserved tertiary structure of a protein kinase exemplified by CDK2 (PDB: 1QMZ). Catalytic active state of CDK2 in complex with ATP and a peptide substrate. The P-loop is highlighted in pink, the αC-helix in blue, the hinge region in yellow, the activation loop in green, ATP in orange and the peptide substrate in black. The Mg^{2+} ion is shown as green sphere. The N- and C-termini are marked. (**b**) Details of the ATP-binding site. ATP and substrate are shown in stick representation. Colour schemes highlighting structural elements are the same as in panel (**a**). Key hydrogen bonds are shown as dotted lines

located in helix αC. Subsequent crystal structures showed that helix αC is flexible and can dislodge from the active site (αC out). The VIAK lysine/αC glutamate salt bridge is therefore a hallmark of the active kinase conformation. (4) A tripeptide motif DFG (Asp-Phe-Gly) is located between strands β8 and β9 in the C-lobe and marks the beginning of the activation segment (A-loop). The conserved Asp interacts with Mg^{2+} itself coordinating to the ATP phosphates [18].

Protein kinases phosphorylate their substrates on Ser/Thr or Tyr residues after binding of substrate sequences to the substrate binding site in an extended conformation. The substrate interaction site can be described as a shallow surface groove that is formed by side chains located in the four main C-terminal lobe helices (αD–αH) as well as the A-loop APE (Ala-Pro-Glu) motif. Usually the A-loop is 20–30 residues long, and it is usually unstructured in kinases that are regulated by A-loop phosphorylation [19]. The unstructured inactive A-loop conformation partially impedes substrate interactions, but does not necessarily affect substrate binding [20]. Phosphorylation of the A-loop locked this flexible motif in a defined conformation resulting in kinase activation as described below.

A hallmark of the active kinase conformation is the accurate spatial arrangement of the conserved catalytic domain motifs for efficient catalysis. The active conformation is therefore structurally well-defined as exemplified for CDK2 in Fig. 1b. Active protein kinases harbour a highly conserved Y/HRD (Tyr/His-Arg-Asp) motif between strand β6 and β7. While some variations of the Y/HRD motif exist in active kinases, the aspartate of this motif is strictly required for catalytic activity of the phosphoryl transfer reaction. The Tyr/His in Y/HRD typically forms hydrogen bond interactions with the DFG backbone linking these two key elements. A salt bridge network between Y/H in Y/HRD to the phosphate moiety of the activation loop phosphorylation site (pThr160 in CDK2) stabilizes the activation segment and further links this segment to the catalytic loop. The "R" in the HRD motif is not strictly conserved. The presence of this residue has been thought to be indicative of the requirement of A-loop phosphorylation (so-called RD kinases) which seems not always be the case [21]. However, in RD kinases the polar interactions of the HRD arginine with the A-loop phosphorylation site largely contribute to the conformation and stability of the A-loop. Finally, the Asp (D127 in CDK2) of the HRD motif acts as a catalytic base deprotonation the hydroxyl group of the peptide substrate Ser/Thr or Tyr residue promoting the nucleophilic attack onto the γ-phosphate of ATP. The Asp is further stabilized by a conserved hydrogen bond with an Asn from the activation loop (N132 in CDK2) [22].

Overall the kinase active state is characterized by a structured A-loop with a "DFG-in" conformation and an αC-helix in closed proximity to ATP site. A characteristic canonical salt bridge between the VIAK motif lysine and the conserved αC glutamate residue stabilizes the active form. Normally the N- and C-lobes adopt a closed conformation with a structured P-loop, although some of these structural features may not be present in crystal structures of active kinases.

Because of the structural diversity that is also often observed in active kinases, Taylor et al. established a better definition of the active state by analysis of hydrophobic spines that bridge both kinase lobes and interconnect all elements important for activity. The spines are present in all active kinases, and this structural feature seems therefore better suited defining the active state. Consequently, in inactive kinases the spines are broken leaving one of more structural motif disconnected from the active state position [23–26] (Fig. 2).

The "catalytic spine" (C-spine) is complemented by the ATP cofactor with an aromatic interaction of the adenine ring system bridging both kinase lobes. In PKA (*p*rotein *k*inase *A*), the C-spine comprises two bulky hydrophobic residues (Met231 and Leu227) located in helix αF in the C-lobe linking this helix via Met128 to αD and the sheet β7 (Leu172, Ile174). Leu173 in the β7 sheet extents the C-spine to the ATP-binding side and is complemented in the ATP-bound state by the adenine ring which connects the C-spine to the N-lobe where the C-spine is complemented by Val57 and Ala70.

The "regulatory spine" (R-spine) starts with Tyr164 in the C-terminal kinase lobe of PKA interacting with the DFG phenylalanine (Phe185) which forms the bridge to

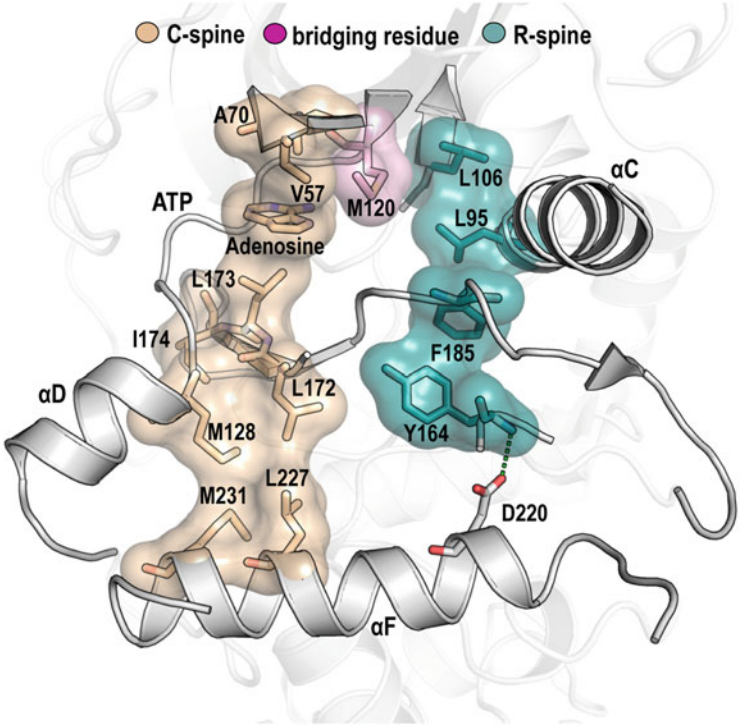

Fig. 2 Alignment of hydrophobic spines in the catalytic domain of active PKA (PDB:1ATP). C-spine is coloured in gold and R-spine in teal. The bridging residue between both spines in the N-terminal lobe, Met120, is highlighted in pink

the N-lobe. The R-spine is completed by the N-lobe residues L95 (alpha C) and Leu106 located in the β4-strand. The formation of the R-spine is indicative of an active conformation of αC and the DFG-motif and is therefore a structural hallmark of the kinase active state.

In agreement with the important structural role of the C-spine, mutation of Leu173 to alanine in PKA and the analogues residue in CDK2 results in kinase inactivation [27], and R-spine mutations have been detected oncogenic mutants resulting in kinase activation in particular in the so-called C-spine gatekeeper position that controls access to an extended ATP pocket in kinases with small amino acids in this position [28–30].

3 Mechanism of Kinase Activation

Activation of protein kinase is tightly controlled by a multitude of regulatory mechanisms. A key regulatory structural element is the activation segment, which is typically 20–40 residues long and consists of the DFG magnesium ion-binding motif, a short β-strand (β9), the actual activation loop and the P+1 loop. The activation segment shows considerable conformational diversity between two invariable anchor points at the N- and C-terminus of this segment: the DFG motif and the P+1 loop linking the activation segment to helix αEF [19].

For kinases requiring phosphorylation for activity, the unphosphorylated activation segment is usually unstructured or assumes an inactive conformation [31, 32]. Phosphorylation of the activation segment at a serine, threonine or tyrosine residue located typically about 11 residues N-terminal to the APE sequence motif stabilizes the A-loop. (Fig. 3). The role of further phosphorylation sites is however less clear. Phosphorylation at a secondary site is required for the activity of extracellular signal-regulated kinase 2 (ERK2) [33]. In contrast, introducing a second phosphorylation site in glycogen synthase kinase 3 (GSK3) increases the catalytic activity only moderately, and modulation of substrate selectivity has been suggested as a potential role of these phosphorylation events [34].

There are two mechanisms of activation segment phosphorylation – autoactivation and phosphorylation by a kinase acting upstream in a signal transduction pathway. Autoactivation is poorly understood as it requires that an inactive (unphosphorylated) kinase activates itself by *trans*-phosphorylation. Several models have been established including increasing local concentration by ligand-induced receptor dimerization [35, 36] or oligomerization in the cytoplasm as observed for CAMK2 [37–39]. Some intramolecular mechanisms have also been described. For instance, the -dual-specificity tyrosine phosphorylation-regulated kinase phosphorylates its own activation segment on a tyrosine residue. After this initial intramolecular phosphorylation event, this kinase trans-phosphorylates exclusively substrates on serine and threonine residues, whereas tyrosine autophosphorylation in GSK3 requires the presence of chaperonins [40, 41].

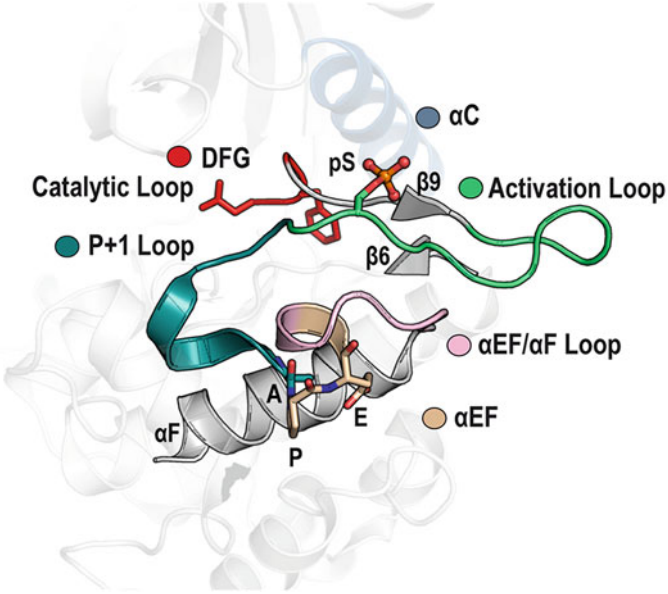

Fig. 3 Architecture of the kinase activation segment. Shown is the phosphorylated activation segment of PAK4 (PDB: 2CDZ). Highlighted are the activation segment structural elements including the DFG motif (red), the activation loop region (green) and the P+1 loop (dark green). The phosphorylation site is shown in ball and stick representation. The APE is indicated

An interesting model of autoactivation has been recently suggested. A number of kinases have been reported to form transient dimers in crystal structures. Intriguingly, these kinases all contain additional domains increasing local concentration and therefore the dimeric state of the inactive enzyme. In dimers of these kinases, the activation segment domain-exchanged, thereby forming an active kinase in a trans configuration, in which the phosphorylation sites are placed in the active site of the interacting protomer. It has been suggested that this conformation is important for autoactivation of kinases at non-consensus sites, offering an explanation of how kinases can active on sites [42–44] (Fig. 4a).

Dimerization of the catalytic domain plays also a role for a number of diverse kinases, but in contrast to the symmetric dimers reported for kinases autophosphorylating at non-consensus sites, these kinases form asymmetric dimers or heterodimers. The receptor tyrosine kinase EGF1R (epidermal growth factor-1 receptor), for instance, forms homo- or heterodimers with its closely related family members HER2, HER3 and HER4 including the inactive pseudokinase HER3 [45, 46]. This activation model represents a refined mechanism of the canonical ligand-induced receptor activation which has major implications for our understanding of the mode of action of selective therapeutic antibodies and kinase drugs [47–49]. Ligand binding to the extracellular domain of EGFR induces large structural rearrangements and dimerization which orients the catalytic domains

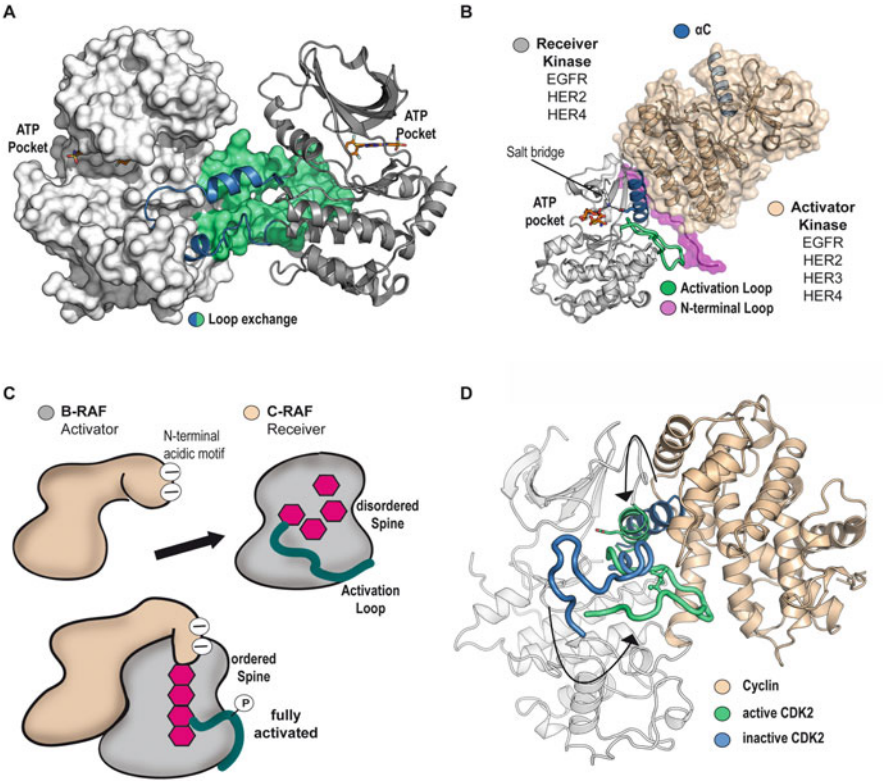

Fig. 4 Activation models of kinases. (**a**) Activation by activation loop exchange allowing phosphorylation on non-consensus sites. (**b**) Asymmetric activation of EGFR receptor kinases. (**c**) Activation of B/C-RAF by heterodimerization. (**d**) Activation of CDK2 by cyclin interaction. Shown is inactive CDK2 (PDB: 1HCK) superimposed onto active CDK2/cyclin A (PDB: 1JST)

on the cytoplasmic side in an asymmetric dimeric assembly in which the C-lobe of one protomer, called *the activator*, stabilizes the second catalytic domain, called *the receiver*, in an active state. This allosteric activation is achieved by a tight contact of the C-lobe of the activator catalytic domain with the N-lobe of the receiver kinase domain in a conformation that is reminiscent of the interaction of activating cyclins with cyclin-dependent kinases (Fig. 4d) [50–53]. This model explains how also catalytically inactive kinases such as HER3 participate in EGFR signalling by acting as activators in heterodimeric receptors [54]. Recent extension of this model also suggested multimeric assemblies [55] (Fig. 4b).

Dimerization is also a key regulatory event in the activation of mitogen-activated protein kinase (MAPK) signalling. The RAS-RAF-MEK-ERK cascade represents a key MAPK signalling pathway controlling cellular proliferation and survival [56] which is often deregulated in cancer [57, 58]. Similar to EGFR several isoforms exist on each level of this pathway. For instance, the serine/threonine-specific

protein kinase RAF (rapidly accelerated fibrosarcoma) comprises three isoforms, A-RAF, B-RAF and C-RAF, which despite their homology differ in the mechanism of their activation. The highly studied family member B-RAF dimerizes in a typical receiver/acceptor asymmetric dimer. Interestingly, kinase inhibitors that bind to the active state of B-RAF stabilize the activator kinase resulting in paradoxical activation of MAPK signalling [59, 60]. The activation of the pathway is also thought to be mediated by C-RAF though B-RAF/C-RAF heterodimerization [61–63]. Kinase inactive mutants of B-RAF, but not C-RAF, can therefore still activate the MAPK cascade by acting as allosteric B-RAF activators [64]. The structural reasons explaining the inability of C-RAF kinase dead mutants activating MAPK signalling have recently been elucidated [65]. Full activity of RAF requires phosphorylation at the activation segment [66, 67] at two sites as well as at the N-terminal acidic (NtA) motif [68, 69]. In B-RAF the NtA motif is acidic (sequence SSDD) and constitutively phosphorylated [69]. In contrast C-RAF and A-RAF lack the two acidic aspartate residues requiring phosphorylation by upstream kinases on their SSYY and SGYY NtA motifs [70]. Hu et al. revealed that NtA motif phosphorylation is only required for the "activator" but not the "receiver" kinase offering a rational why B-RAF can activate C-RAF but not vice versa [65]. Importantly, the oncogenic mutant B-RAF(V600E) does not require dimerization for activity explaining the efficacy of B-RAF inhibitors supressing MAPK signalling in tumours harbouring this mutant but not in wild-type tissue where activation is observed. The paradoxical activation of MAPK signalling in wild-type tissue has been associated with the development of both benign and malignant cutaneous manifestations, ranging from seborrheic dermatitis-like rashes to eruptive keratoacanthomas and squamous cell carcinomas [71]. These examples demonstrate how the complex activation mechanisms of protein kinases may lead to unexpected adverse clinical manifestations. A schematic of the B-RAF/C-RAF activation model is shown in Fig. 4c.

3.1 Kinase Activation by Interacting Domains and Proteins

The dimerization models of kinase activation highlight the importance of protein interactions stabilizing the kinase active state and suggest that also other proteins and domains may act as kinase activators or inhibitors. Indeed, a large number of interactions regulating kinase activity have been described which include flanking domains, for instance, the SH2 which plays a role stabilizing the inactive [72, 73] as well as active state [74, 75], and these interactions might be exploited therapeutically [76].

One of the best studied examples of kinase regulation by an interacting protein is the cyclin-dependent kinases (CDKs) which are stringently regulated by their interaction partners the cyclins. CDKs are master regulators of the cell cycle, and dysfunction of CDK regulation is a major driver of tumourigenesis and attractive drug targets [77–79]. CDKs typically require activation segment as well as binding of a cyclin for full activity [80–82]. In addition, ATP and peptide binding contribute

to the active state [83]. The main role of the cyclin interaction is to push αC towards the active site, while phosphorylation stabilizes an active conformation of the activation segment by interaction with the HRD arginine residue as described above (Fig. 4d).

In analogy to the role of the pseudokinase HER3 in EGFR activation, other catalytically inactive (pseudo) kinases have been shown to activate catalytically competent kinases in trans. Examples include the activation of JAK family members by N-terminal pseudokinase domains [84] as well as the activation of LKB1 (liver kinase B1) by the pseudokinase "STE20-related adaptor protein" (STRADα or STRADβ) [85]. STRAD forms a heterotrimeric complex with LKB1 and the scaffolding protein MO25 which dramatically enhances the activity of LKB1, a kinase that does not require activation segment phosphorylation [86]. Despite the lack of catalytic activity, STRAD is still capable of binding ATP with high affinity. The structure of the trimeric complex revealed that MO25 and ATP binding stabilizes an active-like state of STRAD in which MO25 stabilizes active conformation of αC in a similar way as reported for CDK2. This interaction was also observed in dimeric complexes of MO25 with active kinases [87]. The active-like conformation of STRAD is required for LKB1 activation which is achieved by tight contacts of STRAD with the LKB1 substrate binding site [88, 89].

4 Canonical Type-I and Type-II Inhibitor Binding Mode

The plasticity of the kinase catalytic domain that is essential for kinase regulation also offers an opportunity of targeting structurally diverse states. The most explored design strategies are inhibitors targeting the active state (type-I inhibitors), as well as inhibitors that target the so-called DFG-out conformation (type-II inhibitors), an inactive conformation of the DFG motif that leads to an additional large pocket. Since the active state is most stable, the largest fraction of known structural models available in the protein data bank represent the type-I binding mode. Type-I inhibitors are ATP mimetics, thus similar to the adenosine ring of ATP, they form 1-3 hydrogen bonds with the main chain backbone of the kinase hinge region. A large number of typically heterocyclic aromatic mono- to tricyclic ring systems have been explored as ATP mimetic type-I scaffolds. As the active state is highly conserved, selectivity of many type-I inhibitors is low. However, shape complementarity and sequence variations can be used for the development of inhibitors with high or restricted selectivity (Fig. 5a).

For instance, unique sequence features flanking the hinge, such as rare amino acids in hydrophobic regions and variable structural elements, have been shown to increase selectivity of type-I inhibitors. Noteworthy is the gatekeeper residue, which controls the access to the hydrophobic back cavity. Small gatekeeper residues such as threonine provide access to a larger back cavity and are present in only about 5% of all kinases. Type-I inhibitors targeting this hydrophobic site have therefore favourable selectivity profiles by excluding ATP sites with bulkier gatekeeper

residues. Mutating this residue to glycine, which is not present in any human kinases, has been exploited for the development of kinase-specific ATP analogues for functional studies [28].

Combination of two rare sequence variations led to exclusively selective inhibitors. For instance, the p38 inhibitor skepinone-L is a potent and selective type-I inhibitor exploiting the presence of a small gatekeeper residue and an unusual glycine residue located in the kinase hinge region [90–92].

Canonical type-II inhibitors are ATP competitive and target an inactive state of the kinase catalytic domain which is created by the "outward" flip of the DFG motif. This binding mode gained popularity in drug design after it has been found that the first approved kinase inhibitor Gleevec induces and stabilizes this conformation in its main targets ABL and KIT kinase [93, 94]. All type-II inhibitors protrude into the so-called deep pocket which is made accessible in this inactive state. Type-II inhibitors are therefore slightly elongated small molecules when compared to type-I inhibitors. However, the type-II binding mode needs to be confirmed experimentally as a large diversity of binding modes have been observed for putative type-II inhibitors including canonical type-I interactions. In the DFG-out state, the aspartate of the DFG rotates ~180° and moves ~5 Å away from the ATP-binding site, inactivating the kinase. However, the canonical DFG-out state represents only one of many possible conformations of the DFG motif, and a large number of intermediate states have been described.

Initial expectations that type-II inhibitors would be more selective since they include the dynamic properties of the DFG-out movement were however not confirmed by more comprehensive studies, demonstrating that the DFG-out conformation can be induced by many kinases including also CDKs that are additionally constrained by interaction with cyclins [95–98]. A number of studies

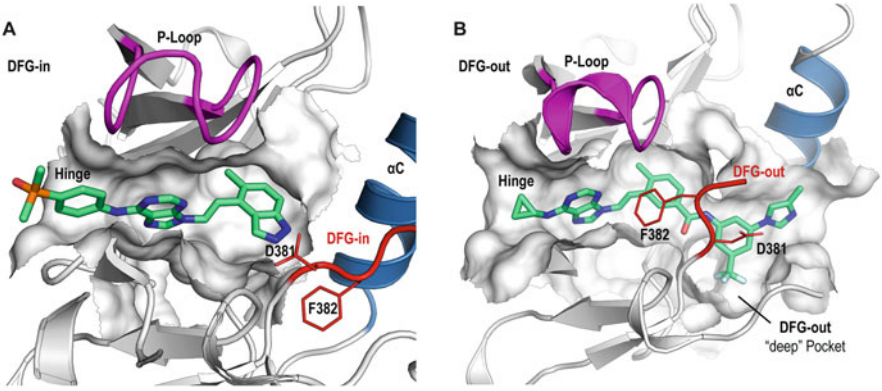

Fig. 5 Comparison of the ATP-binding site in the DFG-in and DFG-out conformation of mouse ABL kinase. Shown is the active state (**a**) (PDB: 3KF4) and the canonical DFG-out conformation in (**b**) (PDB: 3KFA). The ATP pocket is shown as a solid surface to demonstrate the structural differences within the pocket. The DFG motif (red) in panels. Inhibitors are shown as stick representation

demonstrated that type-II inhibitors have slow off-rates resulting in prolonged target residence times. However, comparing larger data sets, slow off-rates have also been reported for canonical type-I inhibitors suggesting that a diversity of structural mechanisms contribute to target residency [22, 99, 100].

The type-II pharmacophore shares a common heterocyclic hinge-binding head group which is connected with typically an amide, urea or another hydrophilic linker with a hydrophobic deep pocket binding moiety. Besides the important hinge-binding donor and acceptor interactions, contacts with the highly conserved aspartate backbone from the DFG motif and glutamate present in the αC-helix are common [13, 101].

4.1 Noncanonical Binding Modes

The dynamic nature of the kinase catalytic domain gives rise to many noncanonical binding modes that target additional pockets or that constitute intermediate states between classical type-I and type-II binding modes discussed above. One variant is the type-I½ binding mode, an ATP competitive binding mode recognizing an either active or an intermediate conformation of the DFG motif which is not fully in the canonical out conformation. Often also an αC-out conformation is observed in type-I½ structures resulting in distorted or interrupted R-spines [102]. An interesting type-I½ inhibitor is, for instance, a derivative of the p38 type-I inhibitor skepinone-L, which interacts with the hinge region inducing the Gly110 backbone flip while inserting into the R-Spine with a thiophene moiety (Fig. 6a) [103]. Similar to some type-II inhibitors, the induced structural changes result in prolonged target residency and slow off-rate kinetics.

Flexible structure elements such as the αC-helix, P-loop or the A-loop can adopt several types of inactive conformations in addition to the DFG-in and DFG-out state. Besides canonical type-I, type-II and type-I½ binding, different ways of trapping inactive, high-energy conformations of a given kinase, creating less solvent exposed and more buried cavities, can be found in diverse studies. Lapatinib targets a DFG-in conformation inducing large, inactivating conformational rearrangements unique to the kinase domain in epidermal growth factor receptor (EGF1R) explaining the exceptional selectivity of this drug [104]. The DFG-in inhibitor GSK2606414 targets a unique binding pocket created by an inactive activation segment conformation in the protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) again resulting in exclusive selectivity [105, 106].

P-loop folded conformations have been found for a set of kinases which harbour aromatic amino acids such as Tyr and Phe at the tip of this loop region [107]. In the active state, these aromatic residues orient their side chains away from the ATP site supporting interaction with the ATP cofactor. In contrast, crystal structures of inhibitor complexes showed that these aromatic side chains can interact with the inhibitor resulting in distortion of the P-loop conformation and capture the P-loop inside the ATP-binding active site. Inhibitors inducing these

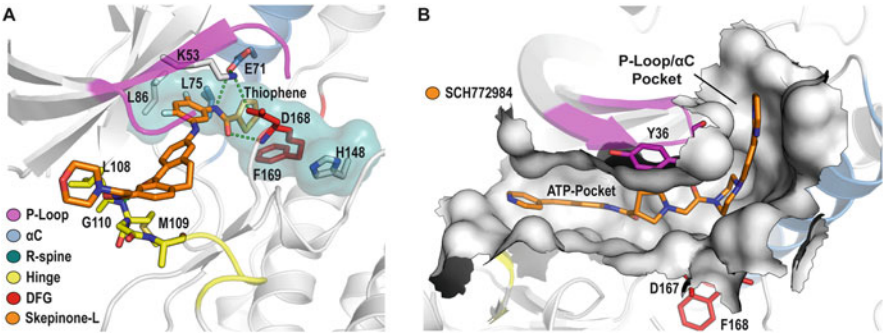

Fig. 6 Examples of noncanonical binding modes. (**a**) A derivative of the p38 inhibitor skepinone-L assuming a type-I1/2½ inhibitor binding mode by inserting a thiophene moiety into the R-spine (PDB: 5TBE). (**b**) The ERK1/2 inhibitor SCH772984 targets a pocket induced by a folded P-loop and αC out (PDB: 4QTA)

folded P-loop conformations are usually characterized with significantly narrower selectivity profiles [107].

A folded P-loop allowed also access to a little conserved binding pocket located between the P-loop and αC-helix exemplified by the ERK1/2 inhibitor SCH772984. This unique binding pocket is additional enlarged by an out movement of αC (Fig. 6b). Also in this case, targeting of this unique pocked resulted in high inhibitor selectivity [108].

The MET inhibitor SGX523 binds to a DFG-in conformation with excellent shape complementarity with the ATP-binding site and targets an additional binding pocket created by an unusual conformation of the activation segment [109]. Aromatic stacking interactions of the ligand with the conserved Tyr^{1248} residue relocated the A-loop with around a 14 Å conformational change inside the phosphate-binding region of ATP, thus inactivating the catalytic function of the kinase in a highly specific mode. Overall inhibitors with noncanonical binding modes have demonstrated to deliver highly potent and selective compounds, which mostly benefit over canonical type-I and type-II inhibitors. However, as most of the compounds were found serendipitous, it stays a challenging task to find lead structures for the design of noncanonical inhibitors addressing a kinase of interest [14].

4.2 Allosteric Kinase Inhibitors

Two types of allosteric inhibitors have been described: type-III and type-IV inhibitors. Type-III inhibitors interact with the allosteric back-pocket adjacent to the ATP-binding catalytic region, not participating in any hinge-binding interaction. They are considered as steady-state ATP uncompetitive or noncompetitive as

they can bind simultaneous with ATP to an active DFG-in conformation of a kinase disrupting catalytic function by distorting the kinase fold. Some type-III inhibitors also bind in an ATP competitive manner and stabilize the inactive DFG-out state as demonstrated by allosteric inhibitors targeting p38, FAK or IGF1R kinases [110–112]. The *N*-phenylsulfonamide LIMK2 inhibitor published by Goodwin et al. is the first example addressing tyrosin-like kinases (TKL) in an allosteric way. The high potency and the exceptional selectivity of the compound have been achieved by an DFG-out/αC-out binding mode which has been confirmed by a *co*-crystal structure [113].

The most prominent examples of allosteric type-III inhibitors are clinically approved MEK1/2 inhibitors trametinib, cobimetinib, binimetinib [114–116] and many similar drug candidates that are currently in the clinical development pipeline. Trametinib shows exceptionally high efficiency, potency and selectivity, and it was the first allosteric kinase inhibitor approved by the FDA for the treatment of adult B-RafV600E and V600K-mutated metastatic melanoma [117]. The compound binds to the allosteric back-pocket adjacent to the ATP-binding site making hydrogen bounds to the conserved β3-lysine and hydrophobic contacts to the β5-strand, the activation loop and the αC-helix. Upon binding the αC-helix gets displaced leading to inhibition of the kinase activity. The activation loop further adopts a closed conformation prohibiting substrate binding [118, 119]. Stimulated by the success of the first-generation MEK inhibitors, many diverse allosteric MEK1/2 inhibitors occupying this pocket have been reported and are now tested clinically in diverse cancer indications such as non-small cell lung cancer (NSCLC), leukaemia and thyroid and colon cancer [120]. About 30 MEK kinase structures in complex with small-molecule allosteric inhibitors and ATP have been co-crystallized providing inside in important structural aspects of inhibitor binding. Figure 7a exemplifies the allosteric pocket of refametinib in complex with ATP [121].

A potent and isoform selective type-III inhibitor has been published recently by Bagal et al. targeting tropomyosin receptor kinases (TrkA) [122]. As confirmed by structural studies, the inhibitor interacts with an allosteric pocket adjacent to the ATP-binding site accessible in the DFG-out conformation of this kinase. Hydrophobic interactions and hydrogen bonds stabilize the inhibitor also taking advantage of the structurally diverse TrkA juxtamembrane domain. As the targeted pocket in the juxtamembrane domain is unique to TrKA, the compound gains selectivity over the closely related TrK family members TrKB and TrKC [123, 124]. The compound has demonstrated to be efficient in preclinical pain models [122].

Type-IV inhibitors bind reversibly to induced binding pocket that are in contrast to type-III inhibitors distantly located from the ATP-binding site. The ability of type-IV inhibitors inducing structural changes in the catalytic domain results often in inhibition, but not all induced or stable pockets targeted in kinases also abrogate catalytic activity [125]. For instance, a unique binding mode has been observed for GNF-2 with binds to the myristate pocket located at the C-terminal lobe of ABL (Fig. 7b) [126]. GNF-2 and its derivative have an interesting

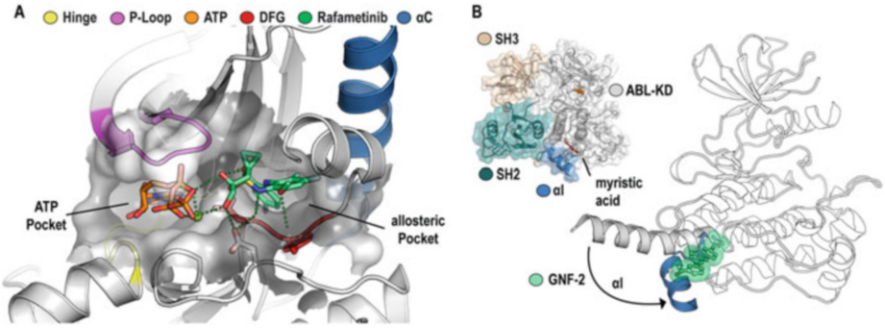

Fig. 7 Examples of binding modes of type-III and type-IV inhibitors. (**a**) Complex of refametinib (green) with MEK1. The inhibitor binds adjacent to the ATP site making hydrophilic interactions with ATP (orange) (PDB: 3E8N). (**b**) Induced changes by the type-IV inhibitor GNF-2. Structural changes of helix I are indicated (PDB: 3K5V and 3KF4). The insert shows the closed autoinhibited conformation of the ABL catalytic domain interacting with the flanking SH2 and SH3 domains. The location of the type-IV binding site is indicated (PDB: 1OPL)

mode of action: the inactive form of ABL is stabilized in an autoinhibited closed conformation, where a myristoylated N-terminal residue binds to the allosteric myristate cleft of the kinase domain (Fig. 7b) [127]. In the BCR-ABL fusion protein, which drives tumourigenesis in a subtype of chronic myelogenous leukaemia (CML) and acute lymphoblastic leukaemia (ALL), the autoinhibitory function of the kinase is lost due to the fusion with BCR, resulting in constitutive activation [128]. GNF-2 mimics the myristate residue disrupting the kinase catalytic function by inducing this inactive state [129]. Structural comparisons showed that the overall conformation is very similar to the native autoinhibition state of ABL, as the N-terminal αI-helix is rotated ~90° inwards upon binding of GNF-2 and assembles the SH2 and SH3 domain [126, 130, 131]. In the active state, this αI-helix normally adopts an extended conformation, which was exemplified by the use of allosteric agonists [132]. Interestingly, GNF-2 binding can take place simultaneously with inhibitors targeting the ATP-binding pocket, such as the ATP competitive inhibitors nilotinib, erlotinib or imatinib. Numerous reports in oncology have therefore highlighted the benefits of a combinatorial drug therapy using ATP competitive inhibitors together with type-IV inhibitors as this treatment strategy reduces the risk of drug-inactivating resistance mutations [126]. A promising novel allosteric type-IV BCR-ABL inhibitor asciminib (ABL001) has now entered clinical testing. The compound binds similar to GNF-2 to the myristate pocket of BCR-ABL, therefore interrupting the catalytic function of this constitutively active kinase [133, 134].

Besides the myristate pocket, also other allosteric pockets in the kinase domain have been targeted by allosteric inhibitors. Non-ATP competitive allosteric inhibitors of checkpoint kinase 1 (CHK1) such as the thioquinazolinones target an allosteric site adjacent to the αD-helix. As this pocket normally serves as a substrate recognition site of CHK1-activating kinases, the kinase cannot

longer be activated [135, 136]. The phosphoinositide-dependent protein kinase-1 (PDK1) inhibitors RS1 and RS2 target a hydrophobic motif called PDK1-interacting fragment (PIF) pocket, exclusively found at the N-terminal lobe of PDK1 [137]. Interaction of these inhibitors allosterically inactivates the catalytic function of PDK1.

AKT1 kinase is another interesting target for the design of allosteric inhibitors by offering an alternative mode of inhibition, as all previous described ATP-competitive inhibitors failed in clinical trials so far [132]. The complex domain architecture of AKT provides the possibility to target the kinase allosterically, as demonstrated by the highly selective AKT1 antagonist MK-2206 [138–140]. The compound binds via hydrogen bond and π-π stacking interactions at the interface between the AKT kinase domain and the pleckstrin homology (PH) domain locking the kinase in an inactive conformation preventing membrane association. In this closed so-called PH-in state, the ATP-binding pocket is no longer accessible. In addition, activation of AKT1 is prevented by abrogating recruitment to the plasma membrane. Other described allosteric AKT inhibitors address, for instance, the PH domain preventing structural rearrangements for activation [141–144].

The activation of cyclin-dependent kinases (CDKs) is reliant on their regulatory proteins of the cyclin family, and CDKs are key regulators of cell cycle control [145, 146]. CDKs have been extensively studied as drug targets, and their role in cancer and inflammatory diseases and three CDK4/6 inhibitors has been approved for treatment of cancer [147]. For CDK2 it has been shown that the kinase activity can be interrupted by targeting an allosteric pocket formed between the αC-helix and the β3-, β4- and β5-strand of the N-lobe [148]. The simultaneous binding of two 8-anilino-1-naphthalene sulfonate (ANS) inhibitors changes the orientation of the β-strands, moving the αC-helix outwards and prohibit the binding of the cyclins [149, 150]. However, these inhibitors are at a very early stage of development and have still not been optimized for in vivo use.

As we outlined above, epithermal growth factor receptor (EGFR) kinases are activated by asymmetric dimerization where the C-terminal lobe of the activator kinase activates the receiver kinase domain. EGFR can form either homo- or heterodimeric structures that are reminiscent on the interactions observed in cyclin-dependent activation of CDKs [53, 151]. The recently published inhibitor EAI045 binds to an allosteric pocked formed by displacement of the αC-helix interfering with this activating interaction of the EGFR kinase domain. The excellent selectivity of this allosteric inhibitor allows mutant-selective targeting of EGFR, sparing EGFR wild-type. In combination with the therapeutic antibody cetuximab, EAI045 potently inhibits the mutants EGFR(L858R/T790M) and EGFR(L858R/T790M/C797S) efficiently overcoming resistance of these mutants to current ATP competitive inhibitors [152]. Further allosteric EGFR inhibitors are in development and open new opportunities for the treatment of diverse cancer types such as non-small cell lung cancer (NSCLC) [153, 154].

The first potent and selective I kappa-B kinase β (IKKß) allosteric inhibitor which prevents IKKß activation has been publish by Liu et al. in 2018

[155]. The inhibitor 3,4-dichloro-2-ethoxy-*N*-(2,2,6,6-tetramethylpiperidin-4-yl) benzenesulfonamide targets the inactive form of IKKβ by binding between the kinase domain and ubiquitin-like domain abrogating activation of IKK as suggested by molecular dynamic simulations. The compound potently inhibited IκBβ/α phosphorylation and NF-κB activation in cells and opens the opportunity for the design of novel type-IV inhibitors for this key signalling kinase.

Even though the field of allosteric inhibitor development is in its infancy for most kinase targets, it has now been established that type-III and type-IV inhibitors offer several unique advantages when compared to conventional ATP competitive compounds. In particular, allosteric inhibitors are often exclusively selective for kinase isoforms or even mutants by exploring target specific mechanism of activation. They abrogate therefore not simply catalytic activity but also modulate scaffolding function of the kinase and its interaction with regulatory domains or proteins. Type-III and type-IV inhibitors open therefore opportunities for the development of inhibitors with new mode of action utilizing new and unexplored chemical scaffolds. However, despite these advantages, the design of novel lead structures remains a challenging task as no general strategies have been developed for their identification and optimization. Hence, allosteric inhibitors are often found serendipitously in high-throughput screens and by structural studies [13]. We predict, however, that type-III and type-IV inhibitors will play a major role in the future in kinase drug discovery.

5 Conclusions and Outlook

With 48 kinase targeting drugs that are currently approved, protein kinases have developed into one of the most promising areas of drug discovery. Recent approvals increasingly target kinases that play a role in a number of diverse diseases with small patient populations, such as rare oncogenic rearrangements and mutants which have been validated as dominant drivers of tumour development and growth. The current clinical inhibitors utilize predominantly conventional ATP mimetic scaffolds for the development of canonical type-I, type-I½ and type-II inhibitors. However, also new type of inhibitors such as covalent inhibitors [11] and allosteric inhibitors [13] have now entered clinical development pipelines and have been approved for clinical use.

Allosteric inhibitors are particularly attractive for the development of inhibitors with exclusive selectivity for closely related isoforms and in some cases even for oncogenic mutants. These recent developments may open other therapeutic areas for kinase inhibitor development that have not been successfully targeted by kinase inhibitors because of selectivity issues or the rigid hydrophobic nature of conventional ATP mimetic scaffolds that make it difficult to develop kinase inhibitors, for instance, for neurological applications or long-term systemic use. In addition, allosteric inhibitors also modulate scaffolding function of kinases and how they interact with regulatory proteins. These properties could be used

for the development of functional modulators of pseudokinases that have been linked to the development of many diseases but which have not been targeted so far. Efficient development of allosteric inhibitors would require an even better understanding of the molecular mechanisms of kinase regulation and the dynamic properties of these enzymes. The fraction of the kinome that has been successfully targeted is still quite small despite the large number of disease associations that have been identified for kinases that have not been extensively studied as targets for the development of drugs [8]. It is therefore likely that many new kinases will be explored for the development of new medicines in the future.

Compliance with Ethical Standards

Conflict of interest: The authors have no conflict of interest.

Funding: The authors are grateful for support by the SGC, a registered charity (number 1097737) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada, Innovative Medicines Initiative (EU/EFPIA), Janssen, Merck KGaA Darmstadt Germany, MSD, Novartis Pharma AG, Ontario Ministry of Economic Development and Innovation, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and Wellcome [106169/ZZ14/Z].

Ethical Approval: This manuscript is a review of previously published accounts, as such no animal or human studies were performed.

References

1. Manning G et al (2002) The protein kinase complement of the human genome. Science 298(5600):1912–1934
2. Ishikawa HO et al (2012) The Raine syndrome protein FAM20C is a Golgi kinase that phosphorylates bio-mineralization proteins. PLoS One 7(8):e42988
3. Cui J et al (2015) A secretory kinase complex regulates extracellular protein phosphorylation. elife 4:e06120
4. Zhang H et al (2018) Structure and evolution of the Fam20 kinases. Nat Commun 9(1):1218
5. Taylor SS et al (2013) Pseudokinases from a structural perspective. Biochem Soc Trans 41(4):981–986
6. Boudeau J et al (2006) Emerging roles of pseudokinases. Trends Cell Biol 16(9):443–452
7. Knapp S, Sundstrom M (2014) Recently targeted kinases and their inhibitors-the path to clinical trials. Curr Opin Pharmacol 17:58–63
8. Fedorov O, Muller S, Knapp S (2010) The (un)targeted cancer kinome. Nat Chem Biol 6(3):166–169
9. van Linden OP et al (2014) KLIFS: a knowledge-based structural database to navigate kinase-ligand interaction space. J Med Chem 57(2):249–277
10. Liu Q et al (2013) Developing irreversible inhibitors of the protein kinase cysteinome. Chem Biol 20(2):146–159
11. Chaikuad A et al (2018) The Cysteinome of protein kinases as a target in drug development. Angew Chem Int Ed Engl 57(16):4372–4385
12. Mobitz H, Jahnke W, Cowan-Jacob SW (2017) Expanding the opportunities for modulating kinase targets with allosteric approaches. Curr Top Med Chem 17(1):59–70
13. Cowan-Jacob SW, Jahnke W, Knapp S (2014) Novel approaches for targeting kinases: allosteric inhibition, allosteric activation and pseudokinases. Future Med Chem 6(5):541–561

14. Muller S et al (2015) The ins and outs of selective kinase inhibitor development. Nat Chem Biol 11(11):818–821
15. Zheng J et al (1993) 2.2 a refined crystal structure of the catalytic subunit of cAMP-dependent protein kinase complexed with MnATP and a peptide inhibitor. Acta Crystallogr D Biol Crystallogr 49(Pt 3):362–365
16. Knighton DR et al (1991) Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. Science 253(5018):407–414
17. Taylor SS et al (1992) Structural framework for the protein kinase family. Annu Rev Cell Biol 8:429–462
18. Fabbro D, Cowan-Jacob SW, Moebitz H (2015) Ten things you should know about protein kinases: IUPHAR review 14. Br J Pharmacol 172(11):2675–2700
19. Nolen B, Taylor S, Ghosh G (2004) Regulation of protein kinases; controlling activity through activation segment conformation. Mol Cell 15(5):661–675
20. Adams JA (2003) Activation loop phosphorylation and catalysis in protein kinases: is there functional evidence for the autoinhibitor model? Biochemistry 42(3):601–607
21. Johnson LN, Lewis RJ (2001) Structural basis for control by phosphorylation. Chem Rev 101(8):2209–2242
22. Roskoski Jr R (2015) A historical overview of protein kinases and their targeted small molecule inhibitors. Pharmacol Res 100:1–23
23. Kornev AP et al (2006) Surface comparison of active and inactive protein kinases identifies a conserved activation mechanism. Proc Natl Acad Sci U S A 103(47):17783–17788
24. Kornev AP, Taylor SS (2015) Dynamics-driven allostery in protein kinases. Trends Biochem Sci 40(11):628–647
25. Ten Eyck LF, Taylor SS, Kornev AP (2008) Conserved spatial patterns across the protein kinase family. Biochim Biophys Acta 1784(1):238–243
26. Taylor SS, Kornev AP (2011) Protein kinases: evolution of dynamic regulatory proteins. Trends Biochem Sci 36(2):65–77
27. Yang J et al (2009) Contribution of non-catalytic core residues to activity and regulation in protein kinase A. J Biol Chem 284(10):6241–6248
28. Bishop AC et al (2000) A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature 407(6802):395–401
29. Yun CH et al (2008) The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 105(6):2070–2075
30. Azam M et al (2008) Activation of tyrosine kinases by mutation of the gatekeeper threonine. Nat Struct Mol Biol 15(10):1109–1118
31. Sicheri F, Moarefi I, Kuriyan J (1997) Crystal structure of the Src family tyrosine kinase Hck. Nature 385(6617):602–609
32. Meng W et al (2002) Structure of mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 suggests a bifunctional switch that couples kinase activation with nuclear export. J Biol Chem 277(40):37401–37405
33. Prowse CN, Lew J (2001) Mechanism of activation of ERK2 by dual phosphorylation. J Biol Chem 276(1):99–103
34. Dajani R et al (2003) Structural basis for recruitment of glycogen synthase kinase 3beta to the axin-APC scaffold complex. EMBO J 22(3):494–501
35. Weiss A, Schlessinger J (1998) Switching signals on or off by receptor dimerization. Cell 94(3):277–280
36. Mellado M et al (2001) Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. Annu Rev Immunol 19:397–421
37. Rellos P et al (2010) Structure of the CaMKIIdelta/calmodulin complex reveals the molecular mechanism of CaMKII kinase activation. PLoS Biol 8(7):e1000426
38. Rosenberg OS et al (2006) Oligomerization states of the association domain and the holoenyzme of Ca2+/CaM kinase II. FEBS J 273(4):682–694
39. Rosenberg OS et al (2005) Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme. Cell 123(5):849–860

40. Lochhead PA et al (2006) A chaperone-dependent GSK3beta transitional intermediate mediates activation-loop autophosphorylation. Mol Cell 24(4):627–633
41. Lochhead PA et al (2005) Activation-loop autophosphorylation is mediated by a novel transitional intermediate form of DYRKs. Cell 121(6):925–936
42. Oliver AW, Knapp S, Pearl LH (2007) Activation segment exchange: a common mechanism of kinase autophosphorylation? Trends Biochem Sci 32(8):351–356
43. Oliver AW et al (2006) Trans-activation of the DNA-damage signalling protein kinase Chk2 by T-loop exchange. EMBO J 25(13):3179–3190
44. Pike AC et al (2008) Activation segment dimerization: a mechanism for kinase autophosphorylation of non-consensus sites. EMBO J 27(4):704–714
45. Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2(2):127–137
46. Citri A, Skaria KB, Yarden Y (2003) The deaf and the dumb: the biology of ErbB-2 and ErbB-3. Exp Cell Res 284(1):54–65
47. Yarden Y, Schlessinger J (1987) Self-phosphorylation of epidermal growth factor receptor: evidence for a model of intermolecular allosteric activation. Biochemistry 26(5):1434–1442
48. Lemmon MA, Schlessinger J, Ferguson KM (2014) The EGFR family: not so prototypical receptor tyrosine kinases. Cold Spring Harb Perspect Biol 6(4):a020768
49. Schlessinger J (2002) Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell 110(6):669–672
50. Jura N et al (2009) Mechanism for activation of the EGF receptor catalytic domain by the juxtamembrane segment. Cell 137(7):1293–1307
51. Jura N et al (2009) Structural analysis of the catalytically inactive kinase domain of the human EGF receptor 3. Proc Natl Acad Sci U S A 106(51):21608–21613
52. Jura N et al (2011) Catalytic control in the EGF receptor and its connection to general kinase regulatory mechanisms. Mol Cell 42(1):9–22
53. Zhang X et al (2006) An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. Cell 125(6):1137–1149
54. Endres NF et al (2013) Conformational coupling across the plasma membrane in activation of the EGF receptor. Cell 152(3):543–556
55. Huang Y et al (2016) Molecular basis for multimerization in the activation of the epidermal growth factor receptor. elife 5:e14107
56. Wellbrock C, Karasarides M, Marais R (2004) The RAF proteins take centre stage. Nat Rev Mol Cell Biol 5(11):875–885
57. Brose MS et al (2002) BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 62(23):6997–7000
58. Davies H et al (2002) Mutations of the BRAF gene in human cancer. Nature 417(6892):949–954
59. Farrar MA, Alberol-Ila J, Perlmutter RM (1996) Activation of the Raf-1 kinase cascade by coumermycin-induced dimerization. Nature 383(6596):178–181
60. Hatzivassiliou G et al (2010) RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature 464(7287):431–435
61. Weber CK et al (2001) Active Ras induces heterodimerization of cRaf and BRaf. Cancer Res 61(9):3595–3598
62. Heidorn SJ et al (2010) Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell 140(2):209–221
63. Poulikakos PI et al (2010) RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. Nature 464(7287):427–430
64. Garnett MJ et al (2005) Wild-type and mutant B-RAF activate C-RAF through distinct mechanisms involving heterodimerization. Mol Cell 20(6):963–969
65. Hu J et al (2013) Allosteric activation of functionally asymmetric RAF kinase dimers. Cell 154(5):1036–1046

66. Chong H, Lee J, Guan KL (2001) Positive and negative regulation of Raf kinase activity and function by phosphorylation. EMBO J 20(14):3716–3727
67. Zhang BH, Guan KL (2000) Activation of B-Raf kinase requires phosphorylation of the conserved residues Thr598 and Ser601. EMBO J 19(20):5429–5439
68. Diaz B et al (1997) Phosphorylation of Raf-1 serine 338-serine 339 is an essential regulatory event for Ras-dependent activation and biological signaling. Mol Cell Biol 17(8):4509–4516
69. Mason CS et al (1999) Serine and tyrosine phosphorylations cooperate in Raf-1, but not B-Raf activation. EMBO J 18(8):2137–2148
70. Morrison DK, Cutler RE (1997) The complexity of Raf-1 regulation. Curr Opin Cell Biol 9(2):174–179
71. Owen JL, Lopez IE, Desai SR (2015) Cutaneous manifestations of vemurafenib therapy for metastatic melanoma. J Drugs Dermatol 14(5):509–510
72. Young MA et al (2001) Dynamic coupling between the SH2 and SH3 domains of c-Src and Hck underlies their inactivation by C-terminal tyrosine phosphorylation. Cell 105(1):115–126
73. Hantschel O et al (2003) A myristoyl/phosphotyrosine switch regulates c-Abl. Cell 112(6):845–857
74. Filippakopoulos P et al (2008) Structural coupling of SH2-kinase domains links Fes and Abl substrate recognition and kinase activation. Cell 134(5):793–803
75. Lorenz S et al (2015) Crystal structure of an SH2-kinase construct of c-Abl and effect of the SH2 domain on kinase activity. Biochem J 468(2):283–291
76. Grebien F et al (2011) Targeting the SH2-kinase interface in Bcr-Abl inhibits leukemogenesis. Cell 147(2):306–319
77. Malumbres M, Barbacid M (2009) Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 9(3):153–166
78. Asghar U et al (2015) The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov 14(2):130–146
79. Whittaker SR et al (2017) Inhibitors of cyclin-dependent kinases as cancer therapeutics. Pharmacol Ther 173:83–105
80. De Bondt HL et al (1993) Crystal structure of cyclin-dependent kinase 2. Nature 363(6430):595–602
81. Russo AA, Jeffrey PD, Pavletich NP (1996) Structural basis of cyclin-dependent kinase activation by phosphorylation. Nat Struct Biol 3(8):696–700
82. Echalier A, Endicott JA, Noble ME (2010) Recent developments in cyclin-dependent kinase biochemical and structural studies. Biochim Biophys Acta 1804(3):511–519
83. Brown NR et al (1999) The structural basis for specificity of substrate and recruitment peptides for cyclin-dependent kinases. Nat Cell Biol 1(7):438–443
84. Babon JJ et al (2014) The molecular regulation of Janus kinase (JAK) activation. Biochem J 462(1):1–13
85. Boudeau J et al (2004) Analysis of the LKB1-STRAD-MO25 complex. J Cell Sci 117(Pt 26):6365–6375
86. Baas AF et al (2003) Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. EMBO J 22(12):3062–3072
87. Mehellou Y et al (2013) Structural insights into the activation of MST3 by MO25. Biochem Biophys Res Commun 431(3):604–609
88. Zeqiraj E et al (2009) Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation. Science 326(5960):1707–1711
89. Zeqiraj E et al (2009) ATP and MO25alpha regulate the conformational state of the STRADalpha pseudokinase and activation of the LKB1 tumour suppressor. PLoS Biol 7(6):e1000126
90. Koeberle SC et al (2011) Skepinone-L is a selective p38 mitogen-activated protein kinase inhibitor. Nat Chem Biol 8(2):141–143

91. Fischer S et al (2013) Dibenzosuberones as p38 mitogen-activated protein kinase inhibitors with low ATP competitiveness and outstanding whole blood activity. J Med Chem 56(1):241–253
92. Martz KE et al (2012) Targeting the hinge glycine flip and the activation loop: novel approach to potent p38alpha inhibitors. J Med Chem 55(17):7862–7874
93. Schindler T et al (2000) Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. Science 289(5486):1938–1942
94. Mol CD et al (2004) Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. J Biol Chem 279(30):31655–31663
95. Karaman MW et al (2008) A quantitative analysis of kinase inhibitor selectivity. Nat Biotechnol 26(1):127–132
96. Goldstein DM, Gray NS, Zarrinkar PP (2008) High-throughput kinase profiling as a platform for drug discovery. Nat Rev Drug Discov 7(5):391–397
97. Zhao Z et al (2014) Exploration of type II binding mode: a privileged approach for kinase inhibitor focused drug discovery? ACS Chem Biol 9(6):1230–1241
98. Alexander LT et al (2015) Type II inhibitors targeting CDK2. ACS Chem Biol 10(9):2116–2125
99. Georgi V et al (2018) Binding kinetics survey of the drugged Kinome. J Am Chem Soc 140(46):15774–15782
100. Heroven C et al (2018) Halogen-aromatic pi interactions modulate inhibitor residence times. Angew Chem Int Ed Engl 57(24):7220–7224
101. Liu Y, Gray NS (2006) Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol 2(7):358–364
102. Zuccotto F et al (2010) Through the "gatekeeper door": exploiting the active kinase conformation. J Med Chem 53(7):2681–2694
103. Wentsch HK et al (2017) Optimized target residence time: type I1/2 inhibitors for p38alpha MAP kinase with improved binding kinetics through direct interaction with the R-spine. Angew Chem Int Ed Engl 56(19):5363–5367
104. Wood ER et al (2004) A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer Res 64(18):6652–6659
105. Axten JM et al (2012) Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-p yrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). J Med Chem 55(16):7193–7207
106. Wang H et al (2010) Structural determinants of PERK inhibitor potency and selectivity. Chem Biol Drug Des 76(6):480–495
107. Guimaraes CR et al (2011) Understanding the impact of the P-loop conformation on kinase selectivity. J Chem Inf Model 51(6):1199–1204
108. Chaikuad A et al (2014) A unique inhibitor binding site in ERK1/2 is associated with slow binding kinetics. Nat Chem Biol 10(10):853–860
109. Buchanan SG et al (2009) SGX523 is an exquisitely selective, ATP-competitive inhibitor of the MET receptor tyrosine kinase with antitumor activity in vivo. Mol Cancer Ther 8(12):3181–3190
110. Over B et al (2013) Natural-product-derived fragments for fragment-based ligand discovery. Nat Chem 5(1):21–28
111. Tomita N et al (2013) Structure-based discovery of cellular-active allosteric inhibitors of FAK. Bioorg Med Chem Lett 23(6):1779–1785
112. Heinrich T et al (2010) Allosteric IGF-1R inhibitors. ACS Med Chem Lett 1(5):199–203
113. Goodwin NC et al (2015) Discovery of a type III inhibitor of LIM kinase 2 that binds in a DFG-out conformation. ACS Med Chem Lett 6(1):53–57

114. Odogwu L et al (2018) FDA approval summary: Dabrafenib and Trametinib for the treatment of metastatic non-small cell lung cancers Harboring BRAF V600E mutations. Oncologist 23(6):740–745
115. Garnock-Jones KP (2015) Cobimetinib: First Global Approval. Drugs 75(15):1823–1830
116. Martin-Liberal J (2018) Encorafenib plus binimetinib: an embarrassment of riches. Lancet Oncol 19(10):1263–1264
117. Wu P, Clausen MH, Nielsen TE (2015) Allosteric small-molecule kinase inhibitors. Pharmacol Ther 156:59–68
118. Hashemzadeh S, Ramezani F, Rafii-Tabar H (2018) Study of molecular mechanism of the interaction between MEK1/2 and Trametinib with docking and molecular dynamic simulation. Interdiscip Sci 11(1):115–124
119. Roskoski Jr R (2017) Allosteric MEK1/2 inhibitors including cobimetanib and trametinib in the treatment of cutaneous melanomas. Pharmacol Res 117:20–31
120. Shang J et al (2016) Allosteric modulators of MEK1: drug design and discovery. Chem Biol Drug Des 88(4):485–497
121. Iverson C et al (2009) RDEA119/BAY 869766: a potent, selective, allosteric inhibitor of MEK1/2 for the treatment of cancer. Cancer Res 69(17):6839–6847
122. Bagal SK et al (2018) Discovery of allosteric, potent, subtype selective, and peripherally restricted TrkA kinase inhibitors. J Med Chem 62(1):247–265
123. Furuya N et al (2017) The juxtamembrane region of TrkA kinase is critical for inhibitor selectivity. Bioorg Med Chem Lett 27(5):1233–1236
124. Freeman SN, Mainwaring WI, Furr BJ (1989) A possible explanation for the peripheral selectivity of a novel non-steroidal pure antiandrogen, Casodex (ICI 176,334). Br J Cancer 60(5):664–668
125. Lamba V, Ghosh I (2012) New directions in targeting protein kinases: focusing upon true allosteric and bivalent inhibitors. Curr Pharm Des 18(20):2936–2945
126. Zhang J et al (2010) Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. Nature 463(7280):501–506
127. Nagar B et al (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. Cell 112(6):859–871
128. Pendergast AM, Witte ON (1987) Role of the ABL oncogene tyrosine kinase activity in human leukaemia. Baillieres Clin Haematol 1(4):1001–1020
129. Adrian FJ et al (2006) Allosteric inhibitors of Bcr-abl-dependent cell proliferation. Nat Chem Biol 2(2):95–102
130. Schneider R et al (2012) Direct binding assay for the detection of type IV allosteric inhibitors of Abl. J Am Chem Soc 134(22):9138–9141
131. Nagar B et al (2006) Organization of the SH3-SH2 unit in active and inactive forms of the c-Abl tyrosine kinase. Mol Cell 21(6):787–798
132. Fang Z, Grutter C, Rauh D (2013) Strategies for the selective regulation of kinases with allosteric modulators: exploiting exclusive structural features. ACS Chem Biol 8(1):58–70
133. Eadie LN et al (2018) The new allosteric inhibitor asciminib is susceptible to resistance mediated by ABCB1 and ABCG2 overexpression in vitro. Oncotarget 9(17):13423–13437
134. Schoepfer J et al (2018) Discovery of Asciminib (ABL001), an allosteric inhibitor of the tyrosine kinase activity of BCR-ABL1. J Med Chem 61(18):8120–8135
135. Converso A et al (2009) Development of thioquinazolinones, allosteric Chk1 kinase inhibitors. Bioorg Med Chem Lett 19(4):1240–1244
136. Vanderpool D et al (2009) Characterization of the CHK1 allosteric inhibitor binding site. Biochemistry 48(41):9823–9830
137. Rettenmaier TJ et al (2014) A small-molecule mimic of a peptide docking motif inhibits the protein kinase PDK1. Proc Natl Acad Sci U S A 111(52):18590–18595
138. Hartnett JC et al (2008) Optimization of 2,3,5-trisubstituted pyridine derivatives as potent allosteric Akt1 and Akt2 inhibitors. Bioorg Med Chem Lett 18(6):2194–2197

139. Siu T et al (2008) The design and synthesis of potent and cell-active allosteric dual Akt 1 and 2 inhibitors devoid of hERG activity. Bioorg Med Chem Lett 18(14):4191–4194
140. Bjune K et al (2018) MK-2206, an allosteric inhibitor of AKT, stimulates LDLR expression and LDL uptake: a potential hypocholesterolemic agent. Atherosclerosis 276:28–38
141. Huang BX et al (2017) Identification of 4-phenylquinolin-2(1H)-one as a specific allosteric inhibitor of Akt. Sci Rep 7(1):11673
142. Ahad AM et al (2011) Development of sulfonamide AKT PH domain inhibitors. Bioorg Med Chem 19(6):2046–2054
143. Kim D et al (2010) A small molecule inhibits Akt through direct binding to Akt and preventing Akt membrane translocation. J Biol Chem 285(11):8383–8394
144. Ranieri C et al (2018) In vitro efficacy of ARQ 092, an allosteric AKT inhibitor, on primary fibroblast cells derived from patients with PIK3CA-related overgrowth spectrum (PROS). Neurogenetics 19(2):77–91
145. Jeffrey PD et al (1995) Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. Nature 376(6538):313–320
146. Roskoski Jr R (2018) Cyclin-dependent protein serine/threonine kinase inhibitors as anticancer drugs. Pharmacol Res 139:471–488
147. Thill M, Schmidt M (2018) Management of adverse events during cyclin-dependent kinase 4/6 (CDK4/6) inhibitor-based treatment in breast cancer. Ther Adv Med Oncol 10:1758835918793326
148. Christodoulou MS et al (2017) Probing an allosteric pocket of CDK2 with small molecules. ChemMedChem 12(1):33–41
149. Mariaule G, Belmont P (2014) Cyclin-dependent kinase inhibitors as marketed anticancer drugs: where are we now? A short survey. Molecules 19(9):14366–14382
150. Betzi S et al (2011) Discovery of a potential allosteric ligand binding site in CDK2. ACS Chem Biol 6(5):492–501
151. Zhao P et al (2018) Crystal structure of EGFR T790M/C797S/V948R in complex with EAI045. Biochem Biophys Res Commun 502(3):332–337
152. Jia Y et al (2016) Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature 534(7605):129–132
153. Caporuscio F et al (2018) Identification of small-molecule EGFR allosteric inhibitors by high-throughput docking. Future Med Chem 10(13):1545–1553
154. Carlino L et al (2018) Structure-activity relationships of Hexahydrocyclopenta[c]quinoline derivatives as allosteric inhibitors of CDK2 and EGFR. ChemMedChem 13(24):2627–2634
155. Liu H et al (2018) A novel allosteric inhibitor that prevents IKKbeta activation. MedChemComm 9(2):239–243