3D structure analysis of PAKs
A clue to the rational design for affinity reagents and blockers

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Abbreviations: PAK, p21-activated kinase; PDB, Protein Data Bank; ATP, adenosine triphosphate; AID, auto-inhibitory domain; IS, inhibitory switch; Di, dimerization segment; Ki, kinase inhibitory; CRIB, CDC42/RAC interactive binding; RMS, root mean square; SH2, src homology region 2; SH3, src homology region 3; CFP, cyan fluorescent protein

The p21-activated kinase (PAK) family plays a versatile role in cell signaling by forming a hub of interactions. PAKs bind the GTPases like RAC and CDC42. Their proline-rich motifs bind SH3 adaptor proteins such as PIX and NCK. PAKs display nuclear localization signal sites and a potential Integrin binding site. No fully complete structure of the PAKs has been published; partial 3D structures of the PAK family kinases include portions of the auto-inhibited PAK1, GTPase bound to small peptides from PAKs, and the kinase domains from PAK1 and PAK4–6 (with small ligands in a few cases). This review focuses on exploring the intermolecular interaction regions in these 3D structures and we offer insights on the missing regions in crystal structure of the auto-inhibited PAK1. Understanding and modulation of PAK intermolecular interactions can pave the way for PAK blockers and biosensors.

Introduction

The p21-activated kinase (PAK) family plays a diverse role in cell signaling and is implicated in cancer and other diseases. Because PAKs function as a hub of interactions with multiple partners, there is a strong interest in understanding the structure-function relation. Phylogentically, PAKs are categorized as group I, which consists of PAK1, PAK2 and PAK3, or group II, which consists of PAK4, PAK5 and PAK6. The last decade has seen substantial amount of progress in 3D structure determination of PAKs. However, no fully complete structure of a PAK has been published. Partial 3D structures of the PAK family kinases include portions of the auto-inhibited PAK1, GTPase bound to small peptides from PAKs and the kinase domains from PAK1 and PAK4–6 (with small ligands in some cases). This review focuses on the intermolecular interaction regions in these 3D structures

and we offer insights on the missing regions in crystal structure of the auto-inhibited PAK1. We pay special attention to the potential for modulation of the PAK system via binders.

PAKs have been extensively characterized and the regions involved in protein-protein interactions have been clearly mapped.[1,2] All PAKs consist of a catalytic kinase domain and a p21-binding domain (PBD). In PAK1, PAK2, PAK3 and PAK5, PBD was known to overlap with an auto-inhibitory domain (AID) until very recently, which showed that all group II PAKs also carry sequence-related AIDs.[3]

Catalytic (Kinase) Domain

Multiple structures of PAK catalytic kinase domain exist in the Protein Data Bank (PDB).[4] Each structure consists of two sub-domains, N-lobe and C-lobe, which are also typical for kinases from other non-PAK families. Structures of the activated form of kinase domain are known for PAK4 (PDB ID: 2BVA[5] and 2J0I[6]), PAK6 (PDB ID: 2C30[5]) and PAK1 (PDB ID: 3Q52[7]); these are all phosphorylated at an exposed activation loop. The backbone of those structures can be super-imposed within 1.6 Å RMS deviation. PAK1 kinase domain structures are also available for both non-phosphorylated form (PDB ID: 1YHW[8]) and a mimic of the phosphorylated form (T423E mutation, PDB ID: 1YHV[8]). The backbones of the phosphorylated, non-phosphorylated and phosphorylated mimic PAK1 kinase domain differ immeasurably (less than 0.2 Å RMS deviation). In particular, the activation loop conformations are very similar except that in the non-phosphorylated structure, the activation loop shows a higher B-factor. In all cases, backbone interaction between the residues A412 on the activation loop and I386 on the kinase domain is observed.

A remarkable disorder of the activation loop occurs in the auto-inhibited form of PAK1 (PDB ID: 1F3M[9]). In this case, the residue S144 on the kinase inhibitory tail, K$_i$, from the AID interacts with the residue I386. We speculate that this interaction outcompetes the interaction of residue A412 with residue I386, destabilizing the activation loop.

We evaluated one kinase domain structure each from group I and group II PAKs for the presence of ligand binding pockets

which could accommodate small molecules. Such sites are of interest for potential antagonists or inhibitors. PAK1 and PAK4 kinase domains were scanned using the Q-SiteFinder[10] method. This method calculates the non-bonded interaction energy between a methyl probe and the protein molecule. Surface regions with most favorable binding energy are considered putative binding sites. Both PAK1 and PAK4 kinase domains shared overall similar ligand binding sites locations (**Fig. 1**), but these had distinguishable differences in shape and size. Hence, a ligand designed to bind the kinase domain of any PAK potentially has a broad specificity toward both group I and group II PAKs. At the same time, the subtle differences in the sites across different PAKs can be exploited to have a molecule bind with higher affinity for one than the other. Recently a few potent PAK1-specific inhibitors (IC_{50} ~10 nM) were developed by Afraxis; the IC_{50} for PAK4–6 is poorer by 100-fold (~1 μM),[11] suggesting that the target ATP-binding pocket of these two distinct kinases substantially differ from each other in atomic detail. Our Q-SiteFinder ligand binding pocket models display an appreciable size and shape difference at this site between PAK1 and PAK4 (**Fig. 1**).

Auto-Inhibited PAK1 Structure

PAK1 is an obligate asymmetric homodimer in the auto-inhibited state. One of the most striking partial structures of PAK1 (PDB ID: 1F3M) is a homodimer of two kinase domains each bound to an auto-inhibitory domain (AID).[9] Three critical interactions are responsible for the inhibition. First, the dimerization segment, Di (residues 81–87) forms an anti-parallel β-strand pair between the monomers. Second, the inhibitory switch domain, IS (residues 88–136), forms the majority of interactions with the kinase domain. The IS domain consists of three helices (Iα1, Iα2 and Iα3) of which Iα2 and Iα3 pack against the C-lobe of the kinase domain between helices αEF and αG. The total surface area buried by this interaction is approximately 1600 Å^2.[12] The IS domain, when expressed and purified alone, showed characteristics of unfolded protein,[9,12] suggesting that folding is mediated by the interaction with the kinase domain. The third interaction takes place between the kinase inhibitory tail, K_i (residues 137–149) and the kinase active site. This interaction is also responsible for displacing the activation loop into a disorder state.

Because structure 1F3M has missing regions, the chain connectivity of the disjoint segments is not evident. Later work showed that auto-inhibition in PAK1 homodimers is in *trans* where there is domain swap between the two monomers of PAK1.[13] Thus, the K_i from one monomer is inhibiting its partner's kinase active site.

Overall the PAK1 structure hints at three potential strategies for controlling the activity of the group I PAKs. Residues 75–90 correspond to the CDC42/Rac interactive-binding (or CRIB) motif where the activation of PAK1 is initiated upon binding of these GTPases. Thus, CRIB motif analogs should compete with the CDC42/RAC binding site and inhibit the PAK1 activation process. Similarly, an analog of K_i may bind to the active site of the kinase and keep it inhibited. PAK1 hyperactivity is implicated in cancerous cell-proliferation, thus such suppressors may offer therapeutic interest. Conversely, a competitor of IS domain for the binding spot on the kinase domain might slow down the activation or the auto-inhibition process. The approach has been
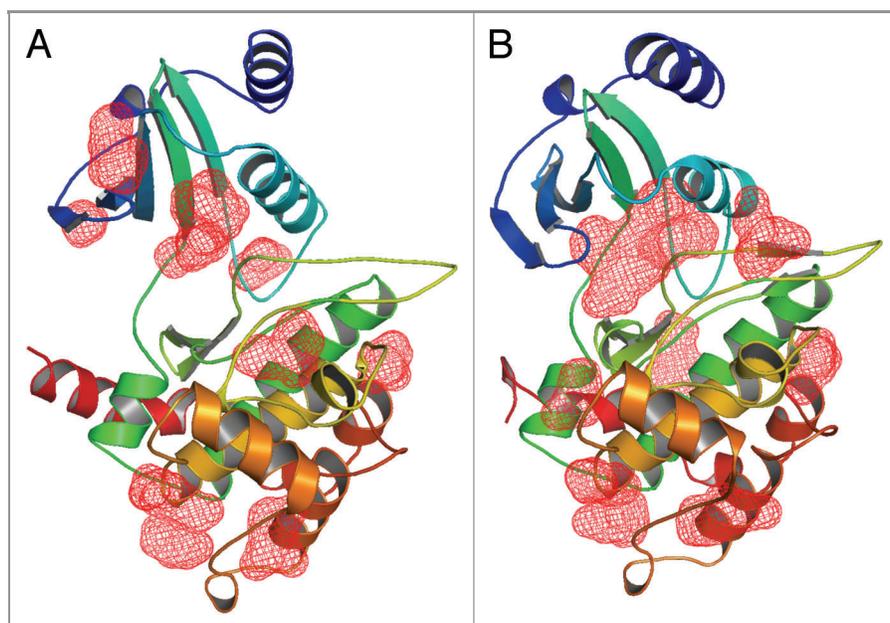

Figure 1. Exploring small molecule (ligand) binding sites in kinase domain from group I and group II PAKs. (A) PAK1 kinase domain (PDB ID: 3Q52[7]) and (B) PAK4 kinase domain (PDB ID: 2J0I[6]) were evaluated for ligand binding sites (shown as "red" mesh) using Q-SiteFinder method.[10] The figures were created using PyMOL.[44]

discussed in detail in a later section. Interestingly, group II PAKs also have an AID within the GTPase-binding domain[3] and may be susceptible to these strategies as well.

The Missing Regions in Auto-Inhibited PAK1 Structure

In order to crystallize the PAK1 protein, major deletions in the protein sequence proved necessary: residues 1–77 and 148–248 are omitted (deleted or disordered) in the crystal structure (PDB ID: 1F3M[9]). It is known that residues in the 1–77 segment of PAK1 are responsible for interacting with other (SH2/SH3) adaptor proteins like NCK[14,15] and Grb2.[16] These adapters localize the PAK to membrane bound Tyr-kinase receptors. The residue stretch 148–248 carries the PIX (SH3) binding motif of 18 amino acids (186–203).[17,18] S21 and T212 that belong to the missing regions in PAK1 crystal structure are phosphorylated by kinases such as Akt/PKB[19] and Cdc2/Cdk5[20,21]).

Earlier studies show these regions, PAK1–77 and PAK148–248, are largely disordered. Using several biophysical methods, a truncated PAK1 (residues 70 to C-terminal, called PAK70-C) showed a larger hydrodynamic radius and extended size than the theoretical dimer size.[9] It was concluded that the residue stretch 150–248 might form extended loop. Using the disEMBL[22] server, we predicted the structural propensity of the sequences of PAK1–77 and PAK148–248. disEMBL found similar sequences in the PDB that could have defined electron density but with high B-factors. Sequence propensity analysis predicts these sequences to be loops/coils (**Table 1**).

Thus, there is a reason to expect that these unstructured regions could be at least partially ordered. Moreover, if the two protein segments were disordered, the binding regions in PAK1–77 and PAK148–248 would undergo a huge entropy loss upon binding with the partner protein resulting in substantial decrease in binding affinity. This generally has an evolutionary disadvantage unless required for very fine control of signaling. Caution is needed while considering these regions totally

disordered and understanding the possible "collapsed" state of these regions can help design efficient blockers of PAK since both PAK1–77 and PAK148–248 are functionally very important segments of PAK1.

Proteins Bound to PAK Peptides

A substantial number of structures of proteins bound to PAK peptides are available in the PDB. The notable ones are β-PIX bound to peptides from PAK1 and PAK2 (PDB ID: 1ZSG[23] and 2DF6[24]), RAC bound to PAK1-CRIB and PAK4-CRIB (PDB ID: 2QME[25] and 2OV2[26]), CDC42 bound to a PAK1 peptide and PAK6-CRIB (PDB ID: 1E0A[27] and 2ODB[28]) and lastly dynein light chain, LC8, bound to a peptide from PAK1 (PDB ID: 3DVP[29]). All these interactions are part of PAK-mediated signaling. These interactions can potentially be exploited for PAK blockers, where an analog of PAK peptide will compete with it to bind to the partner protein; hence suppressing the PAK-mediated signaling in the transformed cells. Alternatively, binders of the PAK peptides can be created to compete with the native partner protein and inhibit the PAK-mediated signaling. In fact, Merlin, the tumor suppressor NF2 gene product, was shown to compete with RAC/CDC42 for binding to the CRIB domain of PAK1, directly inhibiting its kinase activity.[30,31] Similarly, the Proline-rich sequence that binds to SH3 domain of NCK was successfully utilized to prevent the localization of PAK to cell-cell junctions and PAK regulated permeability in endothelial cells and in athero-prone regions.[32,33]

Figure 2 shows the conformation of all of these peptides from their bound state structures. To visualize the allowed variability in these binding motifs, we have overlaid the peptides from different PAK sources if they bind to the same partner protein. The similarity of these is remarkable because, the PAK binding regions represented by the peptides usually undergo a dramatic conformational change upon binding to the partner proteins. In multiple cases, if they are disordered, they become ordered at the expense of intermolecular interaction energy.

Table 1. disEMBL prediction for ordered or disordered states of PAK1 residues 1–77 and PAK1 residues 148–248

	PAK1 residues 1–77 (PAK1–77)
Loops/coils (sequence propensity)	**MSNNGLDIQD KPPAPPMRNT STMIGAGSKD AGTLNHGSKP LPPNPEEKKK KDRFYRSILP GDKTNKKKEK ERPEISL**
Hotloops (B-factors)	**MSNNGLDIQD KPPAPPMR**nt stmigagskd **AGTLNHGSKP LPPNPEEKKK KDRFYRSILP GDKTNKKKEK ERPEISL**
Remark-465 (electron density in PDB)	msnngldiqd kppappmrnt stmigagskd agtlnhgskp lppnpeekkk kdrfyrsilp gdkt**NKKKEK ERPEISL**
	PAK1 residues 148–248 (PAK148–248)
Loops/coils (sequence propensity)	**KSAEDYNSSN ALNVKAVSET PAVPPVSEDE DDDDDDATPP PVIAPRPEHT KSVYTRSVIE PLPVTPTRDV ATSPISPTEN NTTPPDALTL NTEKQKKKPK M**
Hotloops (B-factors)	**KSAEDYNSSN ALNVKAVSET PAVPPVSEDE DDDDDDATPP PVIAPRPEHT KSVYTRSVIE PLPVTPTRDV ATSPISPTEN NTTPPDALTL NTEKQKKKPK M**
Remark-465 (electron density in PDB)	**KSAEDYNSS**n alnvkavse**T PAVPPVSEDE DDDDDD**atpp pviaprpeht ksvytrsvie plpvtptrdv atspispten nttppdaltl n**TEKQKKKPK M**

Three different criteria were used by the server for the assignments. Upper case and bold amino acid symbol represents disordered state. Lower case amino acid symbol represents ordered state.
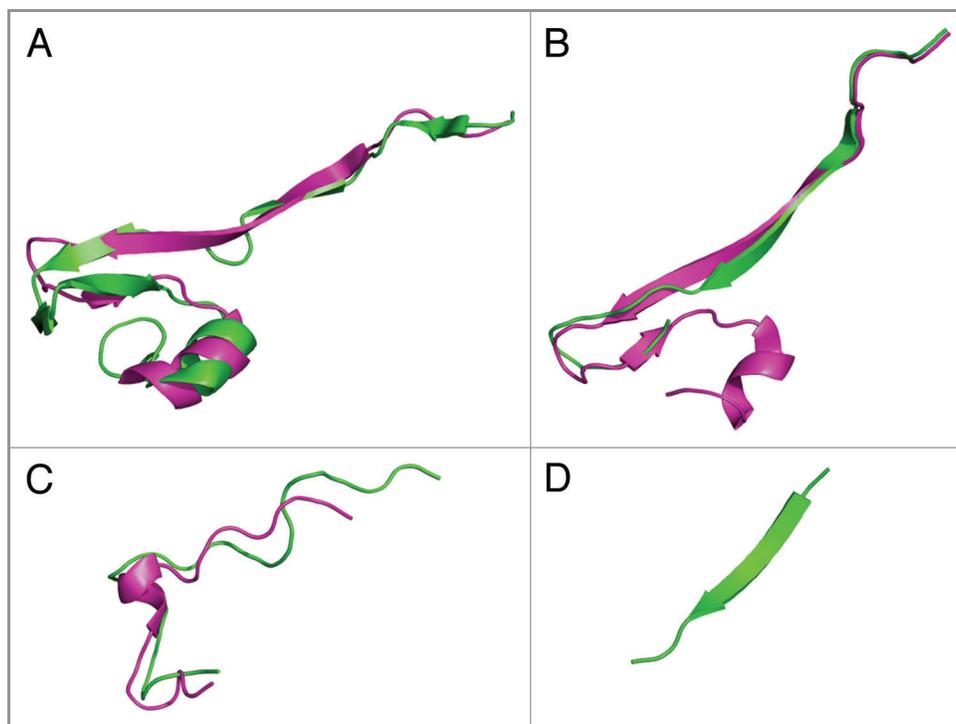
Figure 2. Conformational representation of PAK peptides known to interact with other proteins. (A) Aligned CRIB domains from PAK1 (green) and PAK6 (magenta) that interact with CDC42 (PDB ID: 1E0A[27] and 2ODB[28]). (B) Aligned CRIB domains from PAK1 (green) and PAK4 (magenta) that interact with Rac3 (PDB ID: 2QME[25] and 2OV2[26]). (C) Aligned β-PIX binding regions from PAK1 (green) and PAK2 (magenta) (PDB ID: 1ZSG[23] and 2DF6[24]). (D) LC8 interacting peptide from PAK1 (PDB ID: 3DVP[29]). Stabilized analogs of these peptides, which are pre-formed in the bound-state conformation, can block the interactions between PAKs and the partner proteins.

This suggests that a competitive inhibitory protein might be designed to be pre-formed in the optimal configuration and thus be energetically favored over the native sequences requiring re-arrangement. This will minimize the entropy loss upon binding and results in tighter binding affinity. A suitable way to design competing peptides as PAK blockers would be to use amino acids with high propensity for secondary structure of the peptide observed in the complexes of PAK peptides with the partner proteins (**Fig. 2**). Alternatively, the designed peptide can be rigidified by use of covalent (such as disulfide) or non-covalent interactions (such as hydrogen bonds or salt bridges).

PAK Kinase Domains Bound to Small Molecules

The 3D structures of PAK bound to small molecules (ligands) give an idea of the binding pockets and the kind of interactions that exist with native or non-native molecules. The ATP binding pocket of PAK1 is highly accommodating of alternative ligands of much larger size and differing shapes. One structure of PAK1 bound to a native ligand (ATP) and three structures to a non-native ligand (e.g., inhibitors) are known (PDB ID: 3Q53,[7] 3FXZ,[34] 3FY0[34] and 2HY8[35]). The backbone structures of these ligand-bound PAK1 kinase domains are globally identical (< 0.5 Å RMS deviation), but locally show structural deviations in the binding pocket (**Fig. 3**). In all non-native cases, the ligand occupies the same ATP binding pocket that lies between the N-lobe and the

C-lobe of the PAK1 kinase domain. Local ligand-dependent backbone displacements are concentrated in the β1 and β2 strands of the N-lobe (**Fig. 3B**). In one case (PDB ID: 3FXZ[34]), the ligand causes an opening of the binding pocket and displaces the A280 residue on β1–β2 hairpin by 2.38 Å away from the C-lobe. Conversely, the pocket contracts upon binding of the most potent PAK1 inhibitor ST-2001 (3-OH derivative of staurosporine with the IC_{50} around 1 nM[36-38]) and residue A280 on β1-β2 hairpin moves 3 Å toward the C-lobe (PDB ID: 2HY8[35]). This apparent flexibility of the N-lobe offers a broad range of options to design PAK1 blockers that are not necessarily ATP analogs, by providing an opportunity for enhanced selectivity of therapeutics based on size and electronic charge distribution.

Structures of group II PAKs with small molecule ligands include PAK4 (PDB ID: 2X4Z[39] and 2CDZ[5]) and PAK5 (PDB ID: 2F57[5]). These structures also show ligands bound to the ATP binding pocket.

Allostery is an important event during PAK activity and interrupters of the protein motion could also work as PAK blockers. A potentially novel technique of designing PAK blockers is suggested by structure of PAK4 kinase domain bound to an 11-residues peptide, RRRRRSWYFDG (PDB ID: 2Q0N[40]). This peptide forms a β-strand mediated interactions with the activation loop (residues 474–477). The position of this incorporated strand appears to form a steric obstruction to the allosteric movement of the N-lobe with respect to the C-lobe.
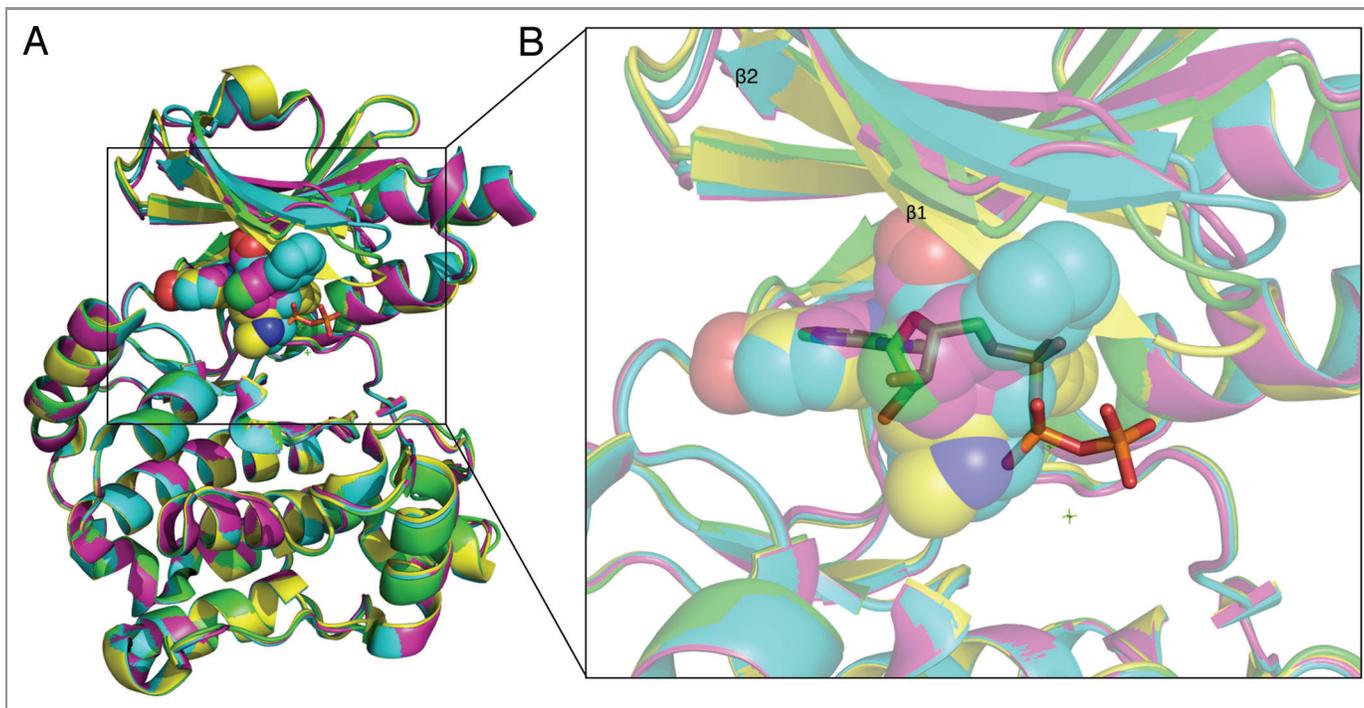
Figure 3. Native (ATP) and non-native (inhibitors) molecules bound in the active site of PAK1 kinase domain. (A) Structural alignment of PDB IDs 3Q53[7] (green), 3FXZ[34] (cyan), 3FY0[34] (magenta) and 2HY8[35] (yellow). (B) Magnified active site showing ATP (green sticks) and inhibitors (spheres in cyan, magenta and yellow). Depending on the molecule bound in the active site, strands β1, β2 and the loop connecting them show movements either away or toward the C-lobe of the kinase domain.

A Prominent Surface Exposure Around αEF and αG Helices in PAK1 (also in Other PAKs) upon Activation

The PAK1 surface is full of protein binding "hotspot" regions. The hydrophobic cleft formed by the helices αEF and αG is important because it is large and gets exposed only when the PAK1 is activated. Upon binding of the GTPases, CDC42 or RAC, to the CRIB region, the IS domain undergoes conformational changes and dislodges from the kinase domain.[9] The IS domain buries approximately 1,600 Å^2 of solvent accessible surface area at the interface[12] (~800 Å^2 on the kinase domain side) and offers a huge interest in designing affinity reagents to bind to that cleft. An affinity reagent designed to bind the αEF/αG cleft can be developed into a biosensor for sensing the endogenous active PAK1 and can also work as disrupters of auto-inhibition that can enhance PAK1-mediated apoptosis of cells.

The cleft is structurally conserved in all PAKs but has differences at the sequence level, which can be exploited for specificity. Earlier a binder derived from PAK1 IS domain showed a weaker binding interaction with the PAK5 kinase domain than the PAK1 kinase domain.[41]

A recent study by Wang et al.[7] supported by an earlier study by Pirruccello et al.[42] have shown that the cleft between αEF and αG are important for autophosphorylation of PAKs to attain full activation. Dimerization mediated by intermolecular contacts from αEF and αG helices were observed in both studies, though asymmetric in the

recent study and symmetric in the previous one, and could represent distinct intermediates during the course of *trans*-autophosphorylation event. In such a case, an affinity reagent targeted to αEF/αG cleft can also form an inhibitor of PAK activation.

PAK activation and deactivation (auto-inhibition) processes are multi-step processes. With increasing theoretical and experimental evidences of conformational sampling in proteins,[43] the expectation is that there would be a measurable population of every conformation of PAK and these conformations would be in a dynamic equilibrium with one another. An affinity reagent would only shift the pre-existing equilibrium toward one conformation in preference to another and would be effective in slowing down both activation and auto-inhibition processes in PAKs.

In order to achieve a preferential blocking of activation or auto-inhibition in PAK1, a fine detail can be added to the design strategy where an affinity reagent "senses" the presence of phosphate group on T423 on the structured activation loop in the vicinity of targeted αEF/αG cleft. An affinity reagent that "likes" the phosphorylated T423 (post *trans*-autophosphorylation state) will have improved affinity for αEF/αG cleft and would be a blocker of the auto-inhibition process of PAK1. Conversely, if the affinity reagent binds very tightly to αEF/αG cleft in the absence of structured activation loop (pre *trans*-autophosphorylation state) but is "repelled" by the phosphorylated T423 would be more effective in blocking the activation process of PAK1.
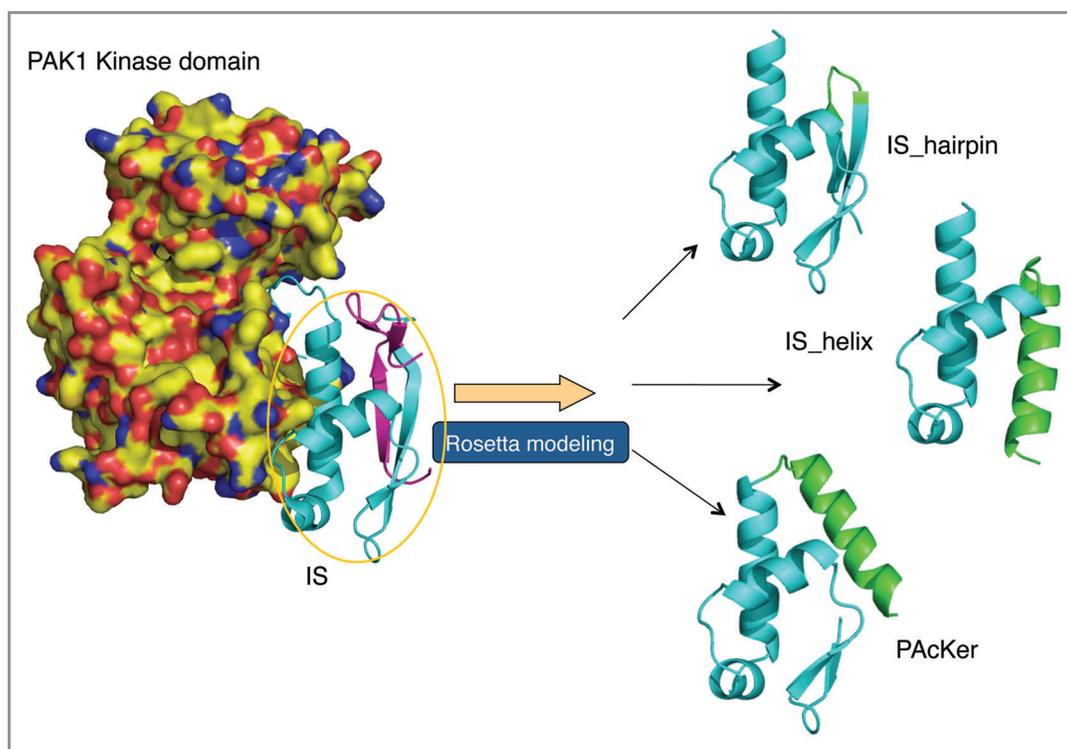
Figure 4. Redesigning the auto-inhibitory domain for biosensor applications. The designs were based on the IS domain (cyan), which interacts with the C-lobe of the kinase domain (yellow). Using Rosetta modeling, the IS domain of AID was redesigned for stability, solubility and improved binding affinity. New structural elements added in the redesign of IS domain are shown in green. This figure is reprinted from Jha et al.[41] with permission from Elsevier.

Computational Design of Affinity Reagents for Activated PAK1

In recent works, Jha et al. showed two different approaches to design affinity reagents that recognize the activated form of PAK1 specifically.[12,41]

In the first approach, the IS domain was redesigned for improved properties as an affinity reagent. The IS domain, if expressed alone, is not very soluble and also lacks any tertiary structure.[9,41] The binding affinity of the IS domain for the kinase domain is around ~4 μM. Since it is clear that the folded structure of the IS domain, shown in the crystal structure (PDB ID: 1F3M[9]), must be binding-mediated, one would anticipate that a pre-folded affinity reagent with an optimal orientation of the binding interface will have improved binding affinity.

Jha et al.[41] tested three different strategies to improve the structural property of the IS domain (**Fig. 4**). In each case, the goal was to improve the interactions in the core of the domain. The first strategy was inspired from the crystal structure of the auto-inhibited PAK1 (PDB ID: 1F3M[9]). Using computational methodology, where iterative rounds of sequence and structure optimizations of the region of interest were carried, the authors extended the dimerization segment to form a β-hairpin followed by a β-strand. The new β-strand in the computational model was intended to mimic the dimerization segment (Di) from the second auto-inhibitory domain as observed in the crystal structure of auto-inhibited PAK1. In the second strategy, Jha et al. created a

new helical stretch that connected the N-terminus of Iα1 helix. The new helical stretch packed against rest of the IS domain to form a small globular structure. Experimental evaluation showed the new sequence folded as a helix and had improved solubility. In the final strategy, a helical stretch was created at the C-terminus of Iα3. While core interactions were also created as the modeled helix packed against Iα3, the N- and C-termini of the model were brought in a close proximity to be able to get inserted in a loop of a fluorescent protein. The new sequence, PAcKer, when inserted in a cyan fluorescent protein (CFP) showed 10-fold improvement in binding affinity (~400 nM) for the PAK1 kinase domain. The same construct did not bind to the full-length PAK1, confirming that the designed affinity reagent can distinguish between PAK1 kinase domain (model for activated PAK1) and auto-inhibited full-length PAK1 and has a potential to be converted into a biosensor to detect spatio-temporal dynamics of the activated PAK1 in living cells. In vitro experiments also confirmed that CFP-PAcKer bound to the PAK1 kinase domain with a preference over PAK5 kinase domain, hence confirming that even though the overall structure of group I and group II PAKs remain very similar, there are differences at the atomic level and that can be exploited to create affinity reagents with specificity.

Why did the PAcKer show improvement in binding affinity for the PAK1 kinase domain? In the crystal structure of auto-inhibited PAK1 (PDB ID: 1F3M[9]), two important interactions that anchor the N- and C-termini of the IS domain are observed. The N-termini anchoring occurs via the Di that undergoes a
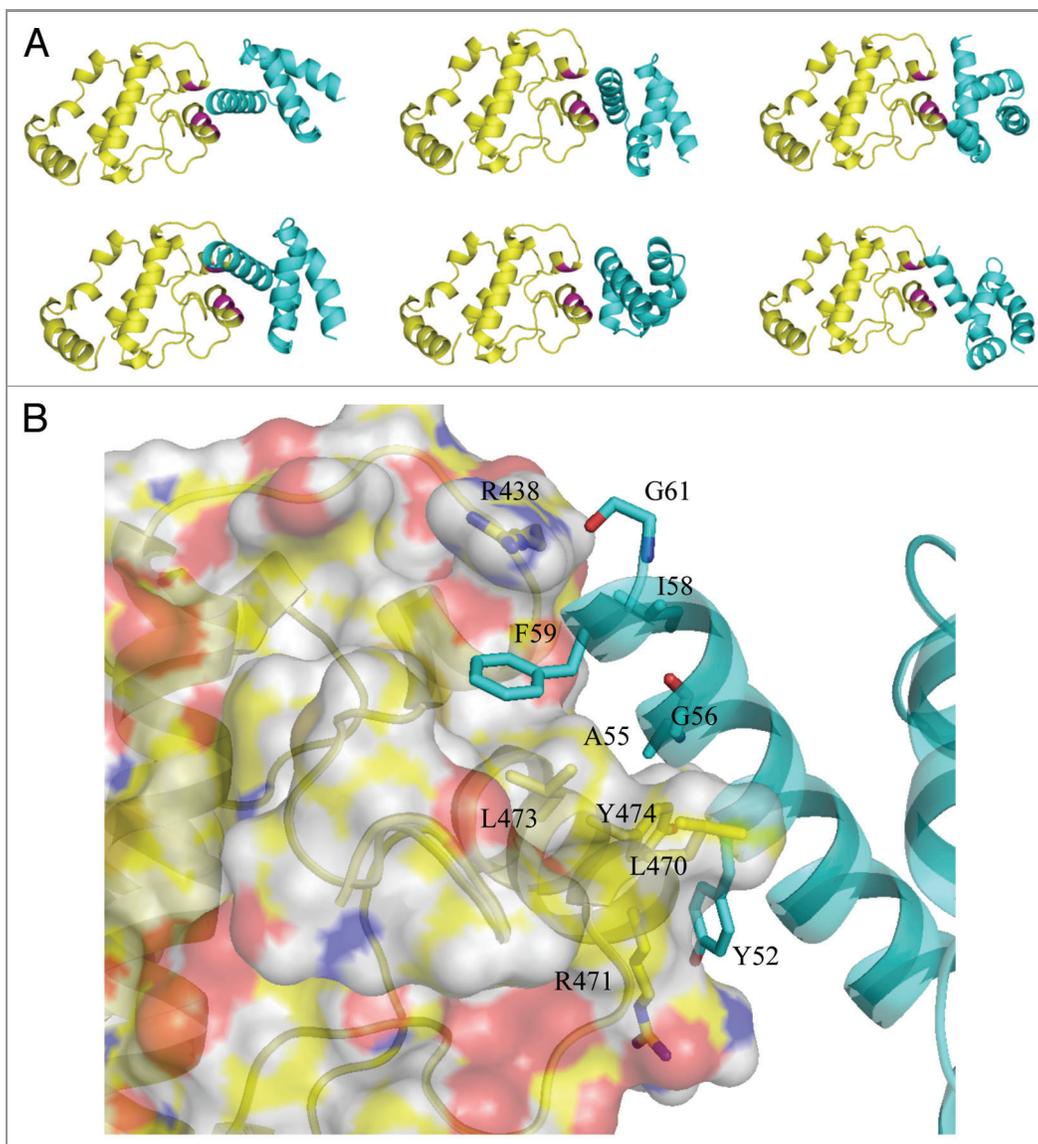
Figure 5. A de novo method for designing a novel affinity reagent for PAK1. (A) A scaffold protein (cyan) is randomly docked at the selected 'hotspot' site between helices αEF and αG of PAK1 kinase domain (yellow). This step is required for searching through conformational space for adequate shape complementarity. A truncated kinase domain is shown here for clarity. (B) An energetically 'good' docked conformation is designed on the scaffold side for maximal interaction with the kinase domain. A representative design, Spider Roll is shown here with the residues involved in protein-protein interaction shown as sticks. This figure is reprinted from Jha et al.[12] with permission from Elsevier.

β-strand swap with another AID to form a two-strand β-sheet. The C-terminus anchor is achieved by the interaction of kinase inhibitory tail (K_i) with I386 and the residues around it in the active site of the kinase domain. With the N- and C-termini anchored, the binding event of IS domain and kinase domain will undergo less entropy cost. PAcKer inserted into CFP, such that the N- and C-termini are anchored by the rigid structure of CFP is a reminiscence of the actual interactions that are observed in the auto-inhibited PAK1.

For the second approach to design an affinity reagent for the activated PAK1, Jha et al. took a de novo route where they started with a structural homolog of IS domain, called HYP, as a scaffold protein.[12] The authors of this work created a computational

protocol, DDMI (**d**ock **d**esign **m**inimize **i**nterface) under Rosetta macromolecular modeling suite, to design residues on HYP, such that it can interact with the helices αEF and αG of the PAK1 kinase domain. Using DDMI, the scaffold protein HYP was first docked at different orientations in the kinase cleft formed by αEF and αG helices (**Fig. 5A**), then using iterative rounds of sequence and structure optimization, the residues on HYP were optimized for good packing and hydrogen bonding interactions. The final selection of the model was made based on binding energy density (predicted binding energy/buried surface area), and the number of unsatisfied polar groups at the interface. A representative design called Spider Roll is shown here (**Fig. 5B**). Experimental evaluations showed Spider Roll bound to PAK1 kinase domain

with a binding affinity of 100 μM and did not show binding to full-length auto-inhibited PAK1. The authors speculated that small interface size (< 1,000 Å^2) in case of Spider Roll, was contributing factor for poor binding affinity for the kinase domain. As mentioned earlier, IS domain buries ~1,600 Å^2 of solvent accessible surface area at the interface with the kinase domain.[12]

Conclusion

The PAK family of proteins is a critical nexus for a variety of pathological conditions including cancers and thus provides opportunities for design of affinity reagents and specific blockers. While a full structure of PAK is not known, the backbone structure of the kinase domain across the family is extremely conserved overall. The deviations at the local level are more prominent. Understanding the similarity in the structure but exploiting the differences at restricted regions can help design affinity reagents or blockers with desired broad or narrow specificities. Using existing 3D knowledge of the PAKs, researchers can rationally start designing small molecules, peptides or small protein domains that can recognize a specific residue stretch, patch or pocket on these kinases, which can then alter the rate of switch between "closed" and "open" states and help manipulate their activities.

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