Structural Basis for Asymmetric Association of the βPIX Coiled Coil and Shank PDZ

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βPIX (p21-activated kinase interacting exchange factor) and Shank/ProSAP protein form a complex acting as a protein scaffold that integrates signaling pathways and regulates postsynaptic structure. Complex formation is mediated by the C-terminal PDZ binding motif of βPIX and the Shank PDZ domain. The coiled-coil (CC) domain upstream of the PDZ binding motif allows multimerization of βPIX, which is important for its physiological functions. We have solved the crystal structure of the βPIX CC–Shank PDZ complex and determined the stoichiometry of complex formation. The βPIX CC forms a 76-Å-long parallel CC trimer. Despite the fact that the βPIX CC exposes three PDZ binding motifs in the C-termini, the βPIX trimer associates with a single Shank PDZ. One of the C-terminal ends of the CC forms an extensive β-sheet interaction with the Shank PDZ, while the other two ends are not involved in ligand binding and form random coils. The two C-terminal ends of βPIX have significantly lower affinity than the first PDZ binding motif due to the steric hindrance in the C-terminal tails, which results in binding of a single PDZ domain to the βPIX trimer. The structure shows canonical class I PDZ binding with a β-sheet interaction extending to position −6 of βPIX. The βB–βC loop of Shank PDZ undergoes a conformational change upon ligand binding to form the β-sheet interaction and to accommodate the bulky side chain of Trp −5. This structural study provides a clear picture of the molecular recognition of the PDZ ligand and the asymmetric association of βPIX CC and Shank PDZ.

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Introduction

PIX (p21-activated kinase interacting exchange factor) proteins belong to a group of guanine nucleotide exchange factors used by Rho GTPase family members Rac1 and Cdc42. Rho family proteins are known to regulate the actin cytoskeleton and to be involved in the formation of various types of focal adhesion structures.1 They have also been implicated in a variety of other biological processes, including activation of gene transcription, cell cycle progression, microtubule dynamics, and vesicular trafficking.2 During these processes, PIX interacts with numerous signaling proteins through its different domains and mediates the effects of various extracellular signals. PIXs are encoded by two genes, αPIX and βPIX, whose products include multiple splice variants (designated as β1PIX, β2PIX, and β3PIX).1 Both αPIX and βPIX (βPIX is used for β1PIX unless otherwise designated) display
similar domain structures, including an N-terminal Src homology 3 (SH3) domain and a central Dbl homology/pleckstrin homology domain. The central Dbl homology/pleckstrin homology module mediates the nucleotide exchange for Rac and Cdc42. The C-terminal portion of PIX interacts with the Spa2 homology domain of GITs (G-protein-coupled receptor kinase interacting proteins), and the PIX SH3 domain associates with a proline-rich sequence in PAK (p21-activated kinase), thereby forming a large GIT–PIX–PAK heteromolecular complex.\(^7\) αPIX is distinguished from most βPIX isoforms by the presence of a calponin homology domain.\(^4\) At their C-terminal ends, both αPIX and βPIX contain a coiled-coil (CC) domain, whereas the corresponding region of β2PIX is serine rich. The multimerization by its CC domain is essential for the peripheral localization of β1PIX and to form a large PIX–PAK heteromolecular complex.\(^5,6\) βPIX has a PDZ binding motif in its extreme C-terminus that promotes the synaptic localization of βPIX by associating with the PDZ domains of Shank and hScrib.\(^7,8\)

The Shank/ProSAP family of multidomain scaffolding proteins is involved in organizing synaptic protein complexes.\(^9\) Shank contains several protein interaction domains, including ankyrin repeats, an SH3 domain, a PDZ domain, a proline-rich region, and a sterile α-motif domain. The Shank PDZ interacts with the GKAP (guanylate-kinase-associated protein) family of synaptic scaffolding proteins, as well as with a number of membrane proteins, including glutamate and somatostatin receptors.\(^10\) The canonical PDZ domain contains ~90 amino acids and folds into a compact globular structure composed of a six-stranded β-sandwich flanked by two α-helices. It binds specifically to short peptides at the extreme C-terminus of target proteins and/or an internal sequence that adopts a β-finger structure.\(^11\) Through interactions with their target proteins, PDZ domains are often involved in organizing signal transduction complexes, clustering membrane receptors, and maintaining cell polarities.\(^12\)

Shank recruits βPIX and βPIX-associated PAK to synaptic sites by its PDZ domain and regulates postsynaptic complex formation.\(^7\) Recent structural studies revealed that βPIX forms a parallel trimer by its CC domain.\(^13\) Still, there is limited knowledge about the precise mode of βPIX CC association with Shank PDZ. Here, we report the crystal structure of the complex between Shank PDZ and the C-terminal CC domain of βPIX. We examined how the complex adopts an unusual asymmetric architecture in which the βPIX CC trimer binds one Shank PDZ by hydrodynamic and thermodynamic analysis. The PDZ ligands used for structural studies have so far been limited to short peptides that mimic the C-terminal ends of target proteins. The current structure includes a PDZ ligand extending 60 residues upstream from the C-terminus and reveals how the binding motif associates with PDZ in conjunction with the upstream CC structure.

Results

Overall structure of the βPIX CC–Shank PDZ complex

The rat βPIX CC and Shank PDZ were coexpressed in Escherichia coli using a bicistronic expression vector (Fig. 1a). The crystal structure of the complex was determined using SeMet multiple anomalous dispersion (MAD) at 2.8-Å resolution. The resultant 2F\(_{o}\)–F\(_{c}\) electron density map clearly shows the bound βPIX C-terminal residues in a binding pocket (Fig. 1b). The refined structure consists of three copies of βPIX (residues 587–646) in a triple CC and a single Shank PDZ domain bound to one of the C-termini of βPIX chains (Fig. 1c). As expected from the predicted secondary structure, the βPIX CC domain, which spans 52 residues (residues 587–638), forms a single α-helix, and the three copies of the CC domain form a parallel CC structure (Fig. 1d and e).

The overall conformations of the three CC domains are similar, with a C\(^\alpha\) rmsd of 1.2–1.7 Å for residues 589–634. The CC is 76 Å in length and contains seven heptad repeats (Leu590, Leu597, Leu604, Met611, Glu618, Leu625, and Val632) (Fig. 1f). Its structure is formed by hydrophobic residues at positions a and e of the heptad repeats, which is characteristic of CC structures.\(^14\) Thirty-three basic residues (11 basic and 5 acidic residues per subunit) are located in the C-terminal half of the CC, which gives it an overall positive electrostatic potential (Fig. 1g). βPIX is well conserved among mammals, with the rat homolog showing 94% identity to the human one. The amino acid sequences of the rat βPIX CC region (βPIX CC) and the Shank PDZ used in this study are identical with the human homologs. In addition, the amino acid residues contributing to CC formation are strictly conserved among PIX family members and the eight variable residues are all located on the surface of the structure, suggesting that the human αPIX and β1PIX isoforms might form homo- or heterotrimers (Fig. 1f).

The βPIX CC structure is terminated by Pro639, which is immediately followed by a PDZ binding motif (AWDETNL). Unexpectedly, the C-terminal residues of each subunit have completely different conformations (Fig. 1h). The seven C-terminal residues of chain A, which interacts with the Shank PDZ, form an extended β-strand structure, while the C-terminal residues of chains B and C form random coils. The C-terminus of chain B is exposed to the solvent and folds back toward the base of the CC, while the C-terminus of chain C (residues 638–646) is disordered and not visible in the electron density map. This conformational difference in the C-terminal ends of the βPIX subunits suggests that the flexible PDZ binding motif is disordered in its unbound state and undergoes conformational change to form a β-strand upon PDZ binding.
Structural determinant for the binding of βPIX to the Shank PDZ

The Shank PDZ is a compact globular domain containing eight secondary structural elements. Six β-strands and two α-helices form a β-sandwich structure, and a 19-residue loop is inserted between βB and βC. The seven C-terminal residues of βPIX are positioned in a groove between strand βB and helix αB and are oriented as an additional strand anti-parallel with βB (Figs. 1c and 2a). The orientation of the C-terminal PDZ binding motif is parallel with the CC, and the PDZ domain is closely bound to the base of the CC structure (Fig. 1c). The three C-terminal residues (TNL) bind to the PDZ domain in a canonical class I PDZ interaction. Leu 0, the first residue, binds to the hydrophobic pocket, and Thr −2 makes a hydrogen bond with His735, which is a signature of the class I PDZ interaction [−(T/S)−X−L] (Fig. 2a). The side chain of Asn −1 is exposed to the solvent and does not interact with the PDZ domain. In addition, Glu −3 makes an electrostatic contact with the conserved Arg679, and the side chain of Trp −5 is positioned in the hydrophobic groove formed by Tyr701, Arg679, and Phe696 of the βB−βC loop (Fig. 2a and b). Finally, the side chains of Asp −4 and Asp −8 are oriented toward the basic patch formed by Lys682 and Arg736 at a distance of 3.6–4.5 Å, which appears to further stabilize the PDZ–ligand interaction.

To examine the PDZ–ligand interaction in more detail, we carried out Ni-NTA (nitrilotriacetic acid)
pulldown of βPIX mutant and His-tagged Shank PDZ from cell lysate (Fig. 3). Mutation of Trp−5 of βPIX to Ala significantly reduced PDZ binding, but alanine mutation of Glu−3 or Asp−4 had little effect. Mutation of the conserved Arg679 in the Shank PDZ to Ala completely abolished the ligand binding. Arg679 interacts with residues −3 and −5 of βPIX: its guanidyl group makes an electrostatic contact with Glu−3, and the stalk of its side chain forms a wall of the hydrophobic pocket into which Trp−5 binds. This suggests that Glu−3 and Trp−5 act to increase ligand affinity and specificity by interacting with Arg679 in strand βB. Truncation of the βB–βC loop to 6 residues (ΔβB–βC loop) abolished the association with βPIX. This is consistent with the weak binding of the alanine mutant of Trp−5 in that the βB–βC loop provides a binding pocket for Trp−5. The deletion of 13 residues (587–599) at the N-terminus of the βPIX CC (construct 600–646) did not disrupt formation of the βPIX–Shank PDZ complex. To test whether the proximity of the CC domain and the PDZ binding motif is essential, we inserted a flexible loop with 15 residues between the CC domain and the PDZ binding motif (AWDETNL). The loop insertion (LI) mutants showed binding similar to the wild type, which confirms that the upstream of the 7 C-terminal residues is not directly involved in PDZ binding.

Conformational change in the Shank PDZ upon βPIX binding

Superposition of the βPIX–Shank PDZ complex and the apo PDZ domain [Protein Data Bank (PDB) ID 1IQO] reveals the ligand-induced conformational changes in the PDZ domain. The major change in the Shank PDZ structure occurs in the βB–βC loop (Fig. 4). This conformational change was not examined in a previous structural study of the Shank PDZ–GKAP peptide complex because the βB–βC loop was disordered by the lack of interaction with the short peptide. The long
βB–βC loop (residues 683–698) is highly flexible; in fact, it has the highest B-factors in the structure, and three residues (687–689) were not well defined in the electron density map. Upon βPIX binding, the N-terminal part of loop βB–βC (residues 680–682) moves toward helix αB to accommodate the bulky hydrophobic Trp–5 residue of βPIX. The reorientation of the backbones of residues 680–682 creates a β-sheet interaction with the C-terminal residues of βPIX at positions −4, −5, and −6 (Fig. 4). This conformational change eliminates the clashes of Trp–5 with residues 680–682. Lys682 undergoes the largest conformational change, during which the Cα atom is displaced by 11 Å. The relocated Lys682 makes an electrostatic contact with Asp–8 of βPIX, and the side chain of Phe696 rotates toward Trp–5 to form a hydrophobic groove. This conformational change in loop βB–βC appears to be essential for ligand binding in that it provides a hydrogen-bonding partner and a hydrophobic pocket for the −4, −5, and −6 positions of the PDZ binding motif of βPIX.

**Oligomeric association of the βPIX–Shank PDZ complex**

βPIX and Shank PDZ form a complex with a 3:1 molar ratio in the crystal. The structure of the βPIX CC determined in this study is consistent with the recent structural studies that demonstrated that the C-terminal domain of βPIX forms a parallel CC trimer in crystal and in solution.13 The Shank PDZ domain binds to only one of the three βPIX CC chains and makes few interactions with the other two chains of the βPIX trimer. Coexpression of wild-type βPIX CC and Shank PDZ in E. coli produced a complex of a 3:1 molar ratio with excess free PDZ protein (Fig. 5a and b). We confirmed that the isolated Shank PDZ is a monomer in solution as demonstrated by hydrodynamic analysis (Fig. 5c). The measured R11 value of free PDZ (2.1 nm) is consistent with the value derived from the structure of monomeric PDZ (2.0 nm). The experimental Stokes radius of the βPIX–Shank PDZ (3.2 nm) agreed with the calculated value (3.3 nm) from the structural coordinates of the 3:1 complex (Fig. 5c).

The oligomeric species with a 3:3 or 3:2 molar ratio were not detected in wild-type constructs during purification. This observation raises a key question regarding the asymmetric association of a βPIX trimer and a Shank PDZ since the CC of βPIX has three identical C-termini for PDZ binding. The PDZ binding motifs begin right after the C-terminal ends of CC domains and all three C-termini are in close proximity to one another. This configuration might interfere with the binding of additional PDZ molecules to the 3:1 complex by steric hindrance. To prove this hypothesis, we inserted a flexible loop with 15 residues before the PDZ binding motif to provide spatial separation of the three binding motifs. The insertion mutants were then analyzed by size-exclusion chromatography and SDS-PAGE to measure the ratio of βPIX and PDZ molecules in the complexes (Fig. 5a and b). The insertion mutant showed a significant decrease of retention volume compared with the wild-type complex in size-exclusion chromatography. The shift of the peaks can be best explained by the incorporation of additional PDZ molecules to the complex. The densitometric analysis of Coomassie-stained protein bands corresponding to each peak demonstrates that the insertion mutants have a molar ratio close to 3:3. In order to confirm that the single PDZ association to βPIX trimer is caused by steric hindrance, we measured the binding affinity of Shank PDZ to βPIX trimer by isothermal titration calorimetry (Fig. 5d). The binding curve could not be fit to a single-binding-site model. Instead, the curve was best fit to a two-binding-site model with a strong binding site (Kd = 2.6 μM) and a weak binding site (Kd = 16.3 μM). We could not measure the accurate stoichiometry because the protein solubility could not be reached to measure the full curve of strong and weak binding sites. This result can be comprehended such that the second binding sites are influenced by the PDZ binding to the first site by steric hindrance. Therefore, the spatial separation by the insertion of a linker would fully expose the three C-terminal ends for independent PDZ bindings. As expected, insertion of a 15-residue loop between the CC and the PDZ binding motif showed a single binding curve (Kd = 9 μM) with a stoichiometry of 3 (Fig. 5e), suggesting that the three C-terminal ends of mutant βPIX are fully exposed for PDZ binding and form a 3:3 complex with Shank PDZ. These data confirm that the crowding of the C-terminal PDZ binding motifs by oligomerization of the CC domain leads to reduced binding of the second and third binding motifs by steric hindrance, which explains the formation of the asymmetric 3:1 βPIX–Shank PDZ complex.
Discussion

The mode of βPIX CC–Shank PDZ binding is consistent with the canonical class I PDZ interaction. The side chains of Leu 0, Thr −2, Glu −3, and Trp −5 directly interact with the Shank PDZ, and the affinity is enhanced by auxiliary interactions with the residues upstream of the core motif (TNL) of βPIX. Recognition of the residue at position −5 is mediated by the βB−βC loop and Arg679. The Shank PDZ accompanies a conformational change in the βB−βC loop upon βPIX binding. Notably, the βB−βC loop varies greatly with respect to sequence, length, and main-chain conformation among different PDZ domains, which suggests that the βB−βC loop may serve as an additional determinant of ligand specificity and affinity. Previously, we observed in the Shank PDZ accompanies a conformational change in the βB−βC loop upon βPIX binding. Notably, the βB−βC loop varies greatly with respect to sequence, length, and main-chain conformation among different PDZ domains, which suggests that the βB−βC loop may serve as an additional determinant of ligand specificity and affinity. Previously, we proposed a model of a dimeric Shank PDZ–βPIX complex based on the artifactual PDZ dimer observed in the crystal lattice. In this study, we confirmed that Shank PDZ is a monomer in solution by dynamic light scattering analysis. We found that the βPIX CC domain is a CC trimer and that βPIX and Shank PDZ form a 3:1 complex. The trimeric CC structure is consistent with a recent structural study on βPIX CC domain with an rmsd of 0.8 Å. The full-length Shank has multiple domains on either side of the N- and C-termini of the PDZ domain, and the βPIX trimer has three C-terminal PDZ binding motifs close to one another. Therefore, the βPIX trimer in vivo would associate with one Shank molecule due to the reduced affinities of the second and third PDZ binding motifs by steric hindrance. The PDZ binding motif is present only in the βPIX isoform; therefore, the hetero-oligomers of βPIX isoforms would have an asymmetric association with PDZ, similar to the βPIX–Shank PDZ complex seen in this crystal structure. Since the binding of one Shank molecule is sufficient to recruit the βPIX, the 3:1 association of βPIX CC and Shank PDZ would be physiologically relevant. Asymmetric association of βPIX is not limited to Shank protein. βPIX and GIT1 are known to form homo-oligomers by their CC domains, and they also form an asymmetric heteropentameric complex consisting of a PIX trimer and a GIT1 dimer. GIT and PIX proteins are tightly associated as a multimeric complex capable of linking important signaling molecules, including PAKs. βPIX and GITs colocalize at focal complexes, the cell periphery, and cytoplasmic complexes. Oligomerization by the CC domains appears to be essential for specific subcellular localizations since mutations that disrupt PIX oligomerization cause it to be diffusely distributed in the cytoplasm. We did not detect membrane binding of the C-terminal CC region of βPIX using liposomes composed of phosphatidylcholine, phosphatidylethanolamine, and phospholipids in vitro (data not shown). The localization of βPIX seems to be mediated by the C-terminal PDZ binding motif downstream of the CC region because Shank associates with βPIX via its PDZ domain and recruits βPIX and PAK to synaptic sites. The PDZ domain of hScrib scaffolding protein also directly binds to βPIX, which is required to anchor βPIX at the cell cortex. It thus appears that hScrib and Shank act as membrane anchors for βPIX, which can then recruit GIT and other associated proteins. In summary, our structural studies provide a clear picture of the asymmetric association of βPIX and Shank PDZ, which is the basis for βPIX recruitment to synaptic sites.

Material and Methods

Protein expression and purification

DNA encoding the rat Shank PDZ (residues 653–763) was subcloned into the BamHI and HindIII sites of a pETDuet-1 vector modified to contain an N-terminal hexahistidine tag separated from the protein by a thrombin protease recognition site. The DNA encoding βPIX (residues 586–646) was subcloned into the second multiple cloning site of the modified pET-Duet vector using NdeI and XhoI (GenBank accession number NM_00113521.1 for βPIX; GenBank accession number NM_031751 for Shank). The βPIX–Shank PDZ complex was expressed in E. coli BL21(DE3) at 30 °C. The cell lysate was applied to a Ni-NTA affinity column. After washing the column with lysis buffer (200 mM NaCl and 50 mM NaH2PO4, pH 7.5), we eluted the complex using buffer (250 mM imidazole, 200 mM NaCl, and 50 mM NaH2PO4, pH 7.5). The eluate was concentrated to 15 mg/ml, and the histidine tag in the Shank PDZ was removed with thrombin protease. The protein complex was further purified by a Superdex 200 size-exclusion column (Pharmacia) equilibrated with buffer containing 20 mM Tris–

![Fig. 5. Oligomeric states of the βPIX–Shank PDZ complex. (a) Size-exclusion chromatography profiles of the βPIX–Shank PDZ constructs after Ni-NTA affinity purification. Red and green indicate wild type and the LI (loop insertion) mutant, respectively. The coexpression of two proteins in E. coli yielded higher levels of His-tagged Shank PDZ than βPIX CC, resulting in a mixture of the complex and the excess free Shank PDZ. In each profile, the first peak corresponds to the βPIX–Shank PDZ complex and the second peak corresponds to free Shank PDZ. (b) The SDS-PAGE of each peak from the size-exclusion chromatography and the results of the densitometric analysis are shown. The molar ratio of βPIX CC and Shank PDZ was measured by analyzing the amount of each protein from a scanned image using the program LabWorks. (c) Hydrodynamic analysis of the βPIX–Shank PDZ complex. The table shows the experimental and calculated Rs values. The experimental Stokes radii Rs were measured by dynamic light scattering. The calculated Rs values were derived from the crystal structures of the Shank PDZ and the βPIX–Shank PDZ complex. The R1/3 value of βPIX–Shank PDZ complex could not be measured due to the partial dissociation of PDZ from the complex upon isolation. The calculated Rs values were derived from a structural model of the 3:3 complex. The lower panels show the correlation curves of the dynamic light scattering data. (e) Isothermal titration calorimetry of Shank PDZ into mutant (linker insertion) βPIX CC solution.](en)
HCl, pH 8.0, and 100 mM NaCl. The fractions containing the recombinant protein were concentrated to 15 mg/ml. The SeMet-labeled protein was expressed in *E. coli* strain B834(DE3) in M9 minimal medium supplemented with selenomethionine and was purified using the same protocol used for the native protein complex.

**Mutagenesis**

The mutations were carried out with a QuikChange Site-Directed Mutagenesis Kit (Stratagene) using the modified pETDuet vector containing the *β*PIX CC and Shank PDZ genes as a template. The open-reading frames of all mutant genes were confirmed by DNA sequencing.

**Hydrodynamic analysis**

The experimental Stokes radii $R_H$ of the proteins were measured using a Wyatt quasi-elastic light scattering instrument with ASTRA V software. The calculated $R_H$ values of the *β*PIX–Shank PDZ complex and the free PDZ were derived from crystal structures using the program Hydropro.21

**Isothermal titration calorimetry**

The individual Shank PDZ, *β*PIX, and *β*PIX LI mutant were expressed in *E. coli* using the pGEX-4T-Shank PDZ, pHIS-*β*PIX, and pHIS-*β*PIX LI vectors, respectively. Shank PDZ was purified by glutathione affinity and size-exclusion chromatography. *β*PIX constructs with an N-terminal hexahistidine tag followed by a TEV cleavage site were purified by Ni$^{2+}$ affinity and size-exclusion chromatography. The N-terminal affinity tags were removed by protease cleavage during purification steps. Shank PDZ (2 mM, injectant), wild-type *β*PIX (75 μM, placed in the sample cell), and the linker insertion mutant of *β*PIX (75 μM, placed in the sample cell) were dissolved in 20 mM Tris–HCl, pH 8.0, and 40 mM NaCl. Titrations (25 injections of 12 μl of Shank PDZ) were performed at 25 °C using a VP-ITC Microcalorimeter (MicroCal), and data were analyzed using Origin software (Origin Lab).

**Crystallization**

Single crystals of the *β*PIX–Shank PDZ complex were grown at 21 °C in 2-μl hanging drops containing equal volumes of protein solution (15 mg/ml) and mother liquor [100 mM Mes–NaOH, pH 6.5, 15% (v/v) ethanol, and 10% (v/v) ethylene glycol]. The crystals grew to a maximum size of 0.1 mm × 0.2 mm × 1.0 mm over 1 week. The crystals were cryoprotected in reservoir solution supplemented with 20% ethylene glycol and flash frozen under N$_2$ gas at 95 K.

**Crystallographic analysis**

Native data were collected at 2.8-Å resolution from a single frozen crystal using an ADSC Quantum Q210 CCD detector at beamline 4A of the Pohang Accelerator Laboratory (South Korea). All data were processed and scaled using HKL2000 (HKL Research). In addition, MAD data sets were collected using SeMet-labeled crystals with an ADSC Quantum 315 CCD detector at beamline BL5 of...
the Photon Factory (Japan). MAD phasing was carried out using the program SOLVE at 3.1-Å resolution (Table 1). SeMet crystals displayed significant radiation decay and weak anomalous signals. Therefore, the wedge data collection was essential to obtain good MAD phasing. Of 14 sites in the complex, 2 Se sites were found. The phases were further improved using SOLVE, and it showed clear electron densities for βPIX and Shank PDZ. Automatic model building was carried out using RELEVE, with which about 50% of the structure was modeled. The structure of the Shank PDZ (PDB ID 1IQO) was fit into the partially built model. The remainder of the model was then built manually into a density-modified map using the program O, and the structure was refined using CNS and REFMAC with TLS refinement. The map using the program O, and the structure was refined model was then built manually into a density-modified map, which was fit into the partially built model. The remainder of the model was then built manually into a density-modified map using the program O, and the structure was refined using CNS and REFMAC with TLS refinement. The model constructed from the MAD data was used as a starting model for the 2.8-Å native data. After rigid-body refinement and cycles of simulated annealing, a readily interpretable map was obtained, and the structure was further built and refined. The eight C-terminal residues (638–646) of one of the three βPIX subunits were disordered and were not modeled in the structure. The distribution of GIT1. The Shank family of postsynaptic density proteins interacts with and promotes synaptic accumulation of the beta PIX guanine nucleotide exchange factor for Rac1 and Cdc42. J. Biol. Chem. 278, 19220–19229.


