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PDZ binding to the BAR domain of PICK1 is elucidated by coarse-grained molecular dynamics

Yi He¹, Adam Liwo^{1,2}, Harel Weinstein³, and Harold A. Scheraga^{1,*}

¹ Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853-1301, USA ² Faculty of Chemistry, University of Gdansk, Sobieskiego 18, 80-952 Gdansk, Poland ³ Department of Physiology and Biophysics, Weill Cornell Medical College, Cornell University, 1300 York Avenue, New York, New York 10065, USA

Abstract

A key regulator of AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor traffic, PICK1 is also known to interact with over 40 other proteins, including receptors, transporters, and ionic channels, and to be active mostly as a homodimer. The current lack of a complete PICK1 structure determined at atomic resolution hinders the elucidation of its functional mechanisms. Here, we identify interactions between the component PDZ and BAR domains of PICK1 by calculating possible binding sites for the PDZ domain of PICK1, PICK1-PDZ, to the homology-modeled crescent-shaped dimer of the PICK1-BAR domain using multiplexed replica-exchange molecular dynamics (MREMD) and canonical molecular dynamics (MD) simulations with the coarse-grained UNRES force field. The MREMD results show that the preferred binding site for the single PDZ domain is the concave cavity of the BAR dimer. A second possible binding site is near the N-terminus of the BAR domain that is linked directly to the PDZ domain. Subsequent short MD simulations, used to determine how the PICK1-PDZ domain moves to the preferred binding site on the BAR domain of PICK1, revealed that initial hydrophobic interactions drive the progress of the simulated binding. Thus, the concave face of the BAR dimer accommodates the PDZ domain first by weak hydrophobic interactions, and then the PDZ domain slides to the center of the concave face, where more favorable hydrophobic interactions take over.

Keywords

PICK1; binding; hydrophobic interactions; UNRES force field; molecular dynamics

Introduction

PICK1 (Protein Interacting with C Kinase 1)¹ is a multi-domain mammalian membrane protein. Its monomeric form contains one PDZ (post-synaptic density-95/discs large/zonula occludens-1)^{2, 3} and one BAR (Bin/Ampiphysin/Rvs)⁴ domain. Although PDZ and BAR domains are common protein-function domains, PICK1 is the only protein that contains both a PDZ and a BAR domain, and interacts with over 40 proteins in the cell, including receptors, transporters, and ionic channels.^{5–26} The proteins that interact with PICK1 are

*Corresponding author, phone: (607) 255 4034, fax: (607) 254 5400, has5@cornell.edu.

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involved in different functions ranging from protein phosphorylation, to neurotransmission, and cell adhesion. The interaction between PICK1 and AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors is essential for induction of synaptic long-term depression (LTD)^{27, 28} as well as for a new form of cerebellar synaptic plasticity termed calcium-permeable AMPA receptor plasticity. Recent findings also identify an important role for PICK1 in the neuroendocrine system both in insects and vertebrates.²⁹

PICK1 is believed to perform its biological function by regulating the trafficking of its binding partners^{30–32} or by using PKC α (protein kinase C alpha) to facilitate their phosphorylation.^{33–35} The interplay between the two main structural domains of PICK1, the PDZ and BAR domains, depends on the dynamics of the tertiary structure of the protein that involves the flexible connecting loops and termini, especially since the biological function of the protein appears to depend on its dimerization.

The structure of the PDZ domain of PICK1 was determined recently,³⁶ but the structure of the PICK1-BAR domain is still unknown; notably, however, PICK1-BAR has a high sequence similarity to the Arfaptin and Endophilin BAR domains whose structures are known^{37,38}. Besides this sequence similarity, the PICK1-BAR domain is known to dimerize³⁴ and is considered to adopt a banana-like shape whose concave surface carries positively charged regions, like the two structurally known proteins. The concave surface of the BAR dimer can act as a curvature sensor that preferentially interacts with curved lipid membranes containing negatively charged lipids.^{39–42} Based on this information, Han and Weinstein⁴³ used homology modeling and docking methods to build a model of the PICK1 dimer, shown in Fig. 1. The linkers (colored orange and purple) between the PDZ and BAR domains are shown only to complete the protein structure, but the structures of the flexible linker regions remain unknown.

The model of the PICK1 dimer structure shown in Fig. 1 illustrates the main hypothesis for the regulation of the PICK1 protein by auto-inhibition/disinhibition. Thus, the occlusion of the concave face of the BAR dimer, which is considered to interact with membranes, by the interacting PDZ domains of PICK1, as indicated in Fig 1, has been proposed as the regulatory mechanism for BAR domain function, in which the auto-inhibited complex is activated by the dissociation of the PDZ domains from the BAR surface following their own interaction with the C-termini of specific membrane proteins⁴⁰ or proximity to the membrane⁴⁴.

Given the lack of detailed structural information about the complete PICK1 and its putative dimer, it becomes necessary to evaluate the proposed complex with computational modeling. Because PICK1 is the only protein that contains both a PDZ and a BAR domain, making it impossible to employ homology modeling to determine their modes of interaction, the structural and energetic feasibility of the putative auto-inhibited form of PICK1, and the manner in which the PICK1-PDZ domain finds its way to the binding sites on the crescent PICK1-BAR dimer, was explored here with restrained multiplexed replica-exchange molecular dynamics (MREMD) and canonical molecular dynamics (MD). The simulations to determine the binding mode of the PICK1-PDZ domain on the BAR dimer surface were started by initially placing the PICK1-PDZ domain somewhere near the crescent homology-modeled PICK1-BAR domains, and were carried out starting from different initial positions for the PICK1-PDZ domain near the PICK1-BAR domains. The physics-based united-residue (UNRES) force field for off-lattice protein structure simulations^{45–63} was used to facilitate the long computational explorations of the large PDZ/BAR complex system. We show here that the preferred binding site on the BAR domain is similar to that found by Han and Weinstein⁴³, and that hydrophobic interactions are the dominant stabilization forces,

with the large convex face of the BAR dimer attracting the PDZ domain which then slides to the central binding pocket where it forms the most favorable hydrophobic interactions.

The reliability of the novel application of the UNRES force field to compute the binding between different large domains within the PICK1 protein is tested here first with cognate simulations for the known structure of Sorting Nexin 9 (SN9, PDB id: 2RAI)⁶⁴, a protein that includes a PX (phox) domain and a BAR domain. As in PICK1, the SN9-BAR domain is also known to dimerize as shown in Fig. 2a. Compared to other coarse-grained approaches to model polypeptide chains, which are largely knowledge-based, UNRES is a physics-based force field, using a cluster-cumulant expansion of the effective free energy of a protein plus the surrounding solvent^{49, 65}. Recently, temperature dependence was included in UNRES⁶¹ to complete its free-energy function character, and the force field was reparameterized based on a global search of the parameter space.⁶² To treat large proteins containing over 500 amino-acid residues, the energy and force calculations were parallelized⁶³. The new force field has been shown to perform well in simulations of middle- to large-size proteins containing α , β and $\alpha+\beta$ structures⁶², and is shown here to produce excellent results for the known structure of SN9. Therefore, the same procedure was applied to study the model for the auto-inhibition of PICK1.

Results

The present version of the UNRES force field⁶² has already been tested on 13 proteins with α , β and $\alpha+\beta$ structures; however, all of them are single-domain proteins. Therefore, before the work on PICK1 was started, the UNRES force field was explored for its ability to pack the domains of the SN9 dual-BAR construct, which contains a PX domain and two BAR domains, in accordance with the structure determined from x-ray crystallography⁶⁴. The binding of the SN9-PX and SN9-BAR domains and the computed structure of the linker were determined by fixing the conformation and location of the BAR domains and varying the position of the PX domain.

Test MREMD simulations with SN9

Because the focus is the binding of the PX to the BAR domain in the SN9 dual-BAR monomer, the internal geometries of the PX and BAR domains were restrained; however, no restraints were applied to the 40-residue “linker region”^{66–69} between the PX and BAR domains. The initial structure for MREMD simulation is shown in Fig. 3. Each trajectory was run for a total of 8,000,000 steps. The set of conformations obtained in the last 80,000 steps of each trajectory was analyzed for the presence of native-like conformations.

The C^α rmsd distribution of the MREMD simulation results compared to the “native” structure of the SN9 dual-BAR monomer (shown in Fig. 2b and based on the X-ray structure illustrated in Fig. 2a) is shown in Fig. 4a. A maximum is centered at C^α -rmsd = 2.5 Å from the “native” structure. The resulting structures were also subjected to a cluster analysis (with the single-linkage method⁷⁰ and an rmsd cutoff = 1.5 Å), and the representative structure of the largest cluster is shown in Fig. 4b compared to the “native” structure shown in Fig. 4c. From these analysis results, the SN9 dual-BAR monomer exhibits a native-like binding pattern of the PX and BAR domains, except for some differences in the 40 residue linker region, showing that the UNRES-based calculations reproduce the correct binding between the PX and dual BAR domains when the distant PX domain is allowed to relax back to its preferred position near the BAR domain. This procedure was then applied to PICK1.

Simulations of PICK1

(1) Simulated binding of the PDZ to the BAR domains of PICK1 by MREMD—

Simulations of PICK1 were started from the initial structures illustrated in Fig. 5. As shown there, two positions were chosen for placing the PDZ domain with respect to the BAR domains, one on the top of the concave face of the BAR domains as shown in Fig. 5a, and another on the opposite side of the BAR domains as shown in Fig. 5b. A total of 10,000,000 MD steps were run with MREMD for both initial structures. The set of conformations obtained in the last 80,000 steps of each trajectory were analyzed for possible native-like conformations.

To identify the most likely complexes between the interacting domains we parsed the resulting complexes with the single-linkage method⁷⁰ of clustering and a C α rmsd cut-off of 2.0 Å. The cluster distributions from simulations, starting with the structures of Fig. 5a and Fig. 5b, respectively, are shown in Fig. 6. Because we focused on predicting the binding of the PICK1-PDZ to the PICK1-BAR domains, the linker region was excluded from the rmsd cluster analysis calculations. Representative conformations of the top three most populated clusters obtained in MREMD simulations, starting from the initial structures shown in Fig. 5a and 5b, respectively, are shown in Fig. 7a–c and Fig. 7d–f, respectively.

Remarkably, the representative structures of the largest two clusters in both MREMD simulations are nearly the same in spite of the large difference in starting point. In the most populated clusters (Fig. 7a and Fig. 7d), the PICK1-PDZ domain is bound to the center of the BAR dimer, and the position in which PDZ binds to the PICK1-BAR domain is almost the same as in the homology-modeled structure⁴³ (Fig. 1). However, the energy-based analysis also yields a binding mode of PICK1-PDZ to PICK1-BAR that is different from that predicted by Han and Weinstein⁴³. In the second populated clusters (Fig. 7b and Fig. 7e), the PICK1-PDZ domain is bound closer to the N-terminus of the BAR domain that is connected directly to the PDZ domain. The third populated cluster in each MREMD simulation of PICK1 (Fig. 7c and Fig. 7f), exhibits a different binding pattern in the two independent MREMD simulations, starting from the structures shown in Fig. 5a and Fig. 5b, respectively. In Fig. 6a, there are three clusters, numbers 8, 13 and 17, each containing 24 structures. Because the PDZ domain in clusters 8 and 17 binds to a similar position as shown in Fig. 7b, the representative structure of cluster 13 was treated as the third largest cluster starting from the initial structure of Fig. 5a. Thus, the third cluster (Fig. 7c) obtained in simulations started from the initial structure illustrated in Fig. 5a is similar in the relative PDZ-BAR position to the largest one (Fig. 7a), but with the PDZ domain rotated by 180°. Both the binding position and binding pattern of the structure shown in Fig. 7c are similar to those reported by Han and Weinstein⁴³ (note, however, that this structure did not appear in the MREMD simulation starting from the structure shown in Fig. 5b, which yielded a position closer to the far edge of the crescent).

(2) Linker region of PICK1—The cluster analysis of the 40-residue linker segment of the PICK1 dual-BAR construct did not reveal any large linker clusters at the 3 Å rmsd cut-off; even the increase of the cut-off to 4 Å resulted in only two very small clusters. These results indicate that the linker region is flexible and does not adopt any dominant characteristic conformations.

(3) Exploring the pathway of PDZ binding to the BAR dimer with MD simulations—

To determine how the PDZ domain moves from a position remote from the convex face of the BAR dimer to the binding position, we carried out a series of short canonical MD simulations (a total of 64 trajectories) starting from the initial structure shown in Fig. 5b at T = 350 K. No simulation was attempted with the initial structure shown in Fig.

5a because the PDZ domain prefers to bind to the concave face in both the MREMD simulation of the dual-BAR construct of PICK1 and the homology modeled structure of PICK1⁴³, and the initial position of the PDZ domain of the PICK1 structure in Fig. 5a may have a bias toward the concave cavity of the BAR domains of PICK1. Each trajectory was run for 3,000,000 steps (14.7 ps). Figure 8 shows the two observed pathways, drawn with PYMOL⁷¹ and marked 1 and 2 in the Figure, that were found to lead to the binding of the PDZ domain to the center of the concave surface of the BAR-domain dimer.

Pathway 1: PDZ binds initially to the N-terminus of the BAR domain (Fig. 8b), and there are no direct hydrophobic interactions between the PDZ and the BAR domain (i.e., all nonpolar residues of the PDZ domains are farther than 8.0 Å from those of the BAR domain). Rather, the stabilization comes from hydrophobic interactions between the linker region and the PDZ/BAR domains; note that the surface of PDZ that eventually interacts directly with the BAR dimer seen in the final model (Fig. 8f) of the complex (identified in red in the surface view), is pointing away from the BAR domain at this stage. Since the linker region is very flexible, the structure of Fig. 8b is not stable. The PDZ domain soon moves away from this metastable state towards the concave surface of the BAR-dimer domain (Fig. 8c) where it forms more hydrophobic contacts (Cys28/Leu140 and Val68/Phe400). Figures 8d to 8f show the subsequent repositioning of the PDZ domain towards the central binding pocket on the concave surface of the BAR dimer.

Pathway 2: In the second binding mode (path 2), the system bypasses the initial binding to the N-terminal part of the BAR domain seen in Fig. 8b and 8c, and the PDZ domain reaches the concave surface of the BAR-domain dimer directly (Fig. 8d); then, from the structure shown in Fig. 8d, the pathway goes through the position in Fig. 8e to that shown in Fig. 8f.

The characteristics of the two types of binding trajectories identified above for the interaction between the PICK1-PDZ domain and the PICK1-BAR-domain dimer suggest that the central binding pocket ultimately stabilizes the PDZ domain in Fig. 8f through favorable hydrophobic interactions involving residue pairs Leu16/Phe386, Ile21/Leu158, Val34/Leu158 and Val68/Phe161. The pathways, however, show that the interactions of the linker are significant at several putative intermediate positions of the PDZ domain. The specific role of the linker in the structural dynamics of PICK1 is as yet unclear, however, and must be elucidated in the context of neighboring lipids because, in some analogous systems containing BAR domains (e.g., endophilin) the linker region has been suggested to play an active role in the interactions with the cell membrane and thereby affect the BAR domain interactions.⁷²

Interactions that drive the binding of PDZ to the BAR dimer

Our results for the mode of stabilization of PDZ in the concave region of the BAR dimer suggest a key role for hydrophobic interactions. Notably, the cognate BAR dimers of SN9 and Arfaptin^{37, 64} also have large nonpolar patches in this concave region corresponding to the PDZ binding locus identified here. To learn more about the role of hydrophobic interactions in the binding process, the number of contacts between the nonpolar residues of the PDZ domain and those of the BAR domain were monitored during the simulation. Two residues were considered to be in hydrophobic contact if the distance between their side chains was < 7.0 Å (for GLY, the distance between C^α atoms was used). Residues were assigned as nonpolar following the classification in our previous work⁷³. The variation of the number of hydrophobic contacts with snapshot number is plotted in Fig. 9 where it can be seen that the number of hydrophobic contacts increases as the simulation progresses and reaches the highest values after approximately 230 snapshots (11.2 ps). The increase in

number of hydrophobic contacts corresponds to the binding of the PDZ domain to the hydrophobic pocket of the BAR dimer.

A detailed set of hydrophobic interactions, corresponding to the structures in Fig. 8, is shown in Table 1. From this Table, it can be seen that the number of hydrophobic interactions increases as binding proceeds. By comparing the hydrophobic contact pairs during the whole binding process, shown in Table 1, it can be seen that Leu16 and Val68 in the PDZ domain each form more than one contact with the BAR domain. Therefore, these two residues are important in driving the binding of the PDZ domain to the BAR domains. Because the PDZ domain moves a long way to the final binding position on the BAR domain, many nonpolar residues in the BAR domain contribute to this process. Also (shown only partially in Table 1f), residues Leu16, Ile21, Gly22, Val34 and Val68 in the PDZ domain and residues Leu158, Phe161, Tyr162, Phe386 of the PICK1 dual-BAR construct (corresponding to Phe161 on chain B) and His393 (corresponding to His168 on chain B) in the BAR domain are possible important residues that stabilize the PDZ and BAR domain binding. The BAR-domain binding site for the PDZ domain is the most hydrophobic region of the BAR of PICK1; it is also in the BAR domains of SN9 and Arfaptin.

Discussion

The initial test of the UNRES-based simulations demonstrated that the protocol that we applied, based on the use of the UNRES potential, is able to reproduce native binding in SN9 in which the structure of the PX domain bound to the BAR dimer is known. Applying the same protocol to the analysis of domain interactions in PICK1, MREMD simulations with UNRES identified two possible binding modes of the PDZ domain to the BAR-domain dimer. The first is located in the center of the concave cavity of the crescent-shaped BAR dimer, whereas the second is near the N-terminus of the PICK1-BAR domain that connects directly to the PICK1-PDZ domain. Hydrophobic interactions were found to be the dominant stabilization forces, with the large convex face of the BAR dimer serving as a “net” to “catch” the PDZ domain, which then slides to the central binding pocket where it forms the most favorable hydrophobic interactions. The preferred binding site on the BAR domain is similar to that found by Han and Weinstein⁴³, based on the Zdock⁷⁴, PatchDock⁷⁵ and Rosetta Dock⁷⁶ homology-modeling protocols. Experimental observations^{34, 40} suggested that the PICK1-PDZ domain inhibits the activity of the PICK1-BAR domain, and this auto-inhibition can be released by PICK1-PDZ ligand binding. Our simulation results clearly show that the PICK1-PDZ domain prefers to bind to the concave region of the PICK1 BAR-dimer which makes this site inaccessible to the interaction with membrane surfaces required for the common BAR dimer function.

The form of the final structure of the modified PICK1 is similar to that observed in Rac and Arfaptin binding³⁷. This observation suggests that occupation of the concave region of the BAR dimer is a very likely basis for auto-inhibition of the BAR domain in experimental observations, consistent with the earlier model⁴³. The other modes of binding obtained from the present work may arise from the fact that Han and Weinstein considered the PICK1 dimer that includes two PICK1-PDZ domains and studied the binding of a PDZ dimer to the BAR-domain dimer, whereas here we considered the binding of only a single PDZ domain. We note, however, that the alternative binding position of PDZ interacting with the BAR dimer identified here is near the N-terminus of the BAR domain, a region that has been shown to be essential for perturbing the membrane so as to facilitate the sensing and binding of curved membrane sections by BAR dimers (see ^{42, 77}). Therefore, this alternative position is also consistent with an inhibition of BAR-membrane interaction. Experimental evidence suggested that the auto-inhibition was released by PICK1-PDZ binding of ligands⁴⁴, and/or proximity to the membrane ⁴⁴

We plan to extend the present study to the full PICK1 dimer (containing two PDZ domains) and evaluate the changes produced by PDZ-ligand binding as soon as the fine-grained version of UNRES to treat multichain proteins becomes available.

Materials and Methods

The UNRES Force Field

In the UNRES model^{45–63}, a polypeptide chain is represented by a sequence of α -carbon atoms connected by virtual bonds with attached side chains. Two interaction sites are used to represent each amino acid, one located in the middle between two consecutive C^α atoms and the other one at the center of mass of the corresponding side chain. The C^α atoms serve only to define the geometry. UNRES is a physics-based force field which has been carefully derived as a restricted free energy (RFE) function of an all-atom polypeptide chain plus the surrounding water.⁴⁵ The all-atom energy is averaged over the degrees of freedom that are lost when passing from the all-atom to the simplified representation. The RFE is further decomposed into factors representing the interactions within and between a given number of united interaction sites. Expansion of these factors into a Kubo⁶⁵ generalized cumulant series made it possible to obtain approximate analytical expressions for the respective terms, including the multibody terms, which are derived in other force fields from structural databases or on a heuristic basis.⁷⁸ The details of the theoretical basis have been described previously⁴⁹. The energy function of the virtual-bond chain is expressed by eq. (1).

$$\begin{aligned}
 U(X, T) = & w_{sc} \sum_{i < j} U_{sc_i sc_j}(X) \\
 & + w_{scp} \sum_{i \neq j} U_{sc_i p_j}(X) \\
 & + w_{pp}^{VDW} \sum_{i < j-1} U_{p_i p_j}^{VDW}(X) \\
 & + w_{pp}^{el} f_2(T) \sum_{i < j-1} U_{p_i p_j}^{el}(X) \\
 & + w_{tor} f_2(T) \sum_i U_{tor}(\gamma_i) \\
 & + w_{tord} f_3(T) \sum_i U_{tord}(\gamma_i, \gamma_{i+1}) \\
 & + w_b \sum_i U_b(\theta_i) \\
 & + w_{rot} \sum_i U_{rot}(\alpha_{sc_i}, \beta_{sc_i}) \\
 & + w_{corr}^{(3)} f_3(T) U_{corr}^{(3)}(X) \\
 & + w_{corr}^{(4)} f_4(T) U_{corr}^{(4)}(X) \\
 & + w_{corr}^{(5)} f_5(T) U_{corr}^{(5)}(X) \\
 & + w_{corr}^{(6)} f_6(T) U_{corr}^{(6)}(X) \\
 & + w_{turn}^{(3)} f_3(T) U_{turn}^{(3)}(X) \\
 & + w_{turn}^{(4)} f_4(T) U_{turn}^{(4)}(X) \\
 & + w_{turn}^{(6)} f_6(T) U_{turn}^{(6)}(X) \\
 & + w_{bond} \sum_i U_{bond}(d_i)
 \end{aligned} \tag{1}$$

where X represents the set of coordinates of a conformation of the coarse-grained chain and T is the absolute temperature. The terms $U_{sciscj}(X)$ are the potentials of mean force of the interaction of isolated side chains in water (with the contribution from the solvent already included). The terms $U_{scipj}(X)$ are the excluded-volume potentials of interactions between side chains and peptide groups. The terms $U_{pi pj}^{VDW}(X)$ are the Lennard-Jones potentials and $U_{pi pj}^{el}(X)$ are the averaged electrostatic-interaction potentials between peptide groups. The terms $U_{tor}(\gamma_i)$ and $U_{tord}(\gamma_i, \gamma_{i+1})$ are the virtual-bond torsional and double-torsional potentials, respectively. The terms $U_b(\theta_i)$, $U_{rot}(\alpha_{scj}, \beta_{scj})$, and $U_{bond}(d_i)$ denote the energy of virtual-bond angle bending, side-chain rotamers, and virtual-bond stretching, respectively. The terms $U_{corr}^{(m)}$ represent the correlation or multibody contribution from the backbone-local and backbone-electrostatic interactions. The terms $U_{turn}^{(m)}$ denote the correlation contribution involving m consecutive peptide groups. The energy-term weights corresponding to second- and higher-order generalized cumulants are multiplied by the appropriate scaling factor, $f_n(T)$, defined in eq. (2), where n is the order of a cumulant-based term⁴⁹.

$$f_n(T) = \frac{\ln[\exp(1) + \exp(1)]}{\ln \left\{ \exp \left[\left(\frac{T}{T_0} \right)^{n-1} \right] + \exp \left[- \left(\frac{T}{T_0} \right)^{n-1} \right] \right\}} \quad (2)$$

T_0 is the arbitrary reference temperature (we set $T_0 = 300$ K), and T is the current temperature. The force field used in this work was developed by global search of the energy-term weights⁶².

Generating initial structures

(1) Sorting Nexin 9—To take advantage of massively-parallel computations to run simulations in real time, we used the recently developed fine-grained UNRES code⁶³ that has not yet been extended to treat multi-chain proteins. Preliminary UNRES/MD simulations of the SN9 monomer, extracted as chain A from the X-ray structure⁶⁴ of the dimer (data not shown) demonstrated that, in this monomer, PX binds to the side of the BAR domain (bottom side of the yellow-BAR domain in Fig. 2a) that normally contacts the BAR domain of chain B of the native dimer. Therefore, two BAR domains bound as in the native dimer were used to compute the binding between the PX and BAR domains. In order to make use of the (currently) single-chain UNRES code in this simulation, a single chain was constructed containing two BAR domains and one PX domain, based on the experimental structure of SN9, as shown in Fig. 2b. To this end, the PX domain from chain B of the dimer (colored light grey in Fig. 2a) was removed, and then the linker which connects the BAR and PX domains of chain B (colored purple in Fig. 2a) in the native dimer, was used to connect the BAR domain of chain B to that of chain A (colored yellow in Fig. 2a) to form a single chain. This artificial construct, which contains two monomeric BAR domains and one PX domain, will hereafter be referred to as a dual-BAR monomer of SN9, shown in Fig. 2b. Although the two BAR domains were artificially connected, their arrangement was maintained as in the experimental structure, so that the dual-BAR “monomer” of SN9 is very similar to the SN9 dimer except that it lacks one of the PX domains. Comparison of Fig. 2a and Fig. 2b shows that a second PX domain (colored light grey) is too distant from the PX-BAR complex to affect the interaction, making the dual-BAR monomer of SN9 suitable for the test simulations.

For the test simulations, the PX domain was separated from the BAR domain as far as the length of the covalent linker between it and the BAR domain of the chain would allow, and was placed above the BAR domains of the dual-BAR monomer of SN9 as shown in Fig. 3.

It should be noted that, in SN9, the linker between the PX and BAR domain consists of only 13 residues (167–179) compared to the 40-residue linker between the PDZ and BAR domains in PICK1. Therefore, in order to test the binding simulation power of UNRES for domains connected by a 40-residue linker, the linker region of SN9 was extended by sequestering an additional 12 residues (155–166) from the SN9-PX domain and 15 residues (180–194) from the BAR domain, and assigned them to the linker region as shown in Fig. 6. In this way, an initial structure of the SN9 dual-BAR construct with a 40-residue linker (colored orange) was built as shown in Fig. 3.

(2) PICK1—The same protocol as above was applied to PICK1. Since the experimental structure of the PICK1 dimer is unavailable, the homology-modeled structure of the PICK1-BAR dimer⁴³ was used. As was done for SN9, the PDZ domain (colored grey in Fig. 10a) was removed, the linker (colored purple) was then connected to the C-terminus of chain A of the homology-modeled structure of PICK1, and the BAR domains were kept in the same arrangement as in the homology-modeled structure (Fig. 10b). This artificial construct thus contains two monomeric BAR domains and a PDZ domain, and will hereafter be referred to as a dual-BAR monomer of PICK1, shown in Fig. 10c. The 40-residue linker of PICK1 is long enough to provide sufficient mobility to pull the PDZ domain away from the two artificially connected BAR domains. Two initial structures were therefore generated for PICK1 as shown in Fig. 5. In these two initial structures, the PICK1-PDZ domain is located on opposite sides, away from the PICK1-BAR domains. The initial shape of the 40-residue linker (colored orange in Fig. 5) between the PDZ and the BAR dimer was built with Modeller^{66–69}.

Simulation Details

During the simulations, C $^{\alpha}$ -distance harmonic restraints were applied to the structures of the SN9-PX, PICK1-PDZ and dual-BAR domains with a force constant of 50.0 kcal/(mol $\times$ Å²). However, there were no restraints between domains, and the linker between the two domains was free to move and change conformation.

(1) MREMD simulation details—To sample the conformational space and location of the binding sites for both proteins, MREMD simulations were carried out for both SN9 and PICK1. All MREMD simulations were run at 16 temperatures of T = 210, 230, 250, 270, 280, 290, 300, 310, 320, 340, 360, 380, 400, 420, 440 and 460 K. In our previous work⁶¹, we found that this temperature range enables a protein to explore the conformational space efficiently. Four trajectories per temperature were run (a total of 64 trajectories), and replica exchange was carried out after every 20,000 MD steps.

(2) MD simulation details—Canonical Molecular Dynamics (MD) simulations were also carried out for PICK1 to explore how the PDZ domain moves from a remote starting position to the most favorable binding sites on the BAR domains of PICK1. In previous work,⁶² when the force field was parameterized, a folding temperature of T = 300K was found for this force field for the tryptophan cage (trp-cage) and the tryptophan zipper 2 (trpzip2). Since PICK1 is much larger than these two proteins, it presumably has a higher folding transition temperature. On the other hand, in order to accelerate the simulation, T = 350K (the presumed folding temperature) was used for canonical MD simulation of the PICK1 dual-BAR construct shown in Fig. 5b. The Berendsen thermostat, implemented in UNRES/MD in our previous work,⁵⁷ was used to maintain constant temperature; the coupling constant was $\tau = 0.489$ fs, which was used in our previous work to provide a compromise between the extent of fluctuations of the potential and kinetic energy. The time increment for integrating the equations of motion was $\delta t = 4.89$ fs.

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Figure 1. Homology-modeled structure of the PICK1 dimer from Han and Weinstein.⁴³ Chain A consists of the PDZ domain (blue), the linker (orange), the BAR domain (yellow). Chain B consists of the PDZ domain (grey), the linker (purple), the BAR domain (green).

Figure 2.

(a) Experimental structure of the SN9 dimer determined by X-ray diffraction⁶⁴. Chain A consists of the PX domain (blue), the linker (orange), the BAR domain (yellow). Chain B consists of the PX domain (grey), the linker (purple), the BAR domain (green). (b) The artificial structure, termed dual-BAR construct of SN9, built for UNRES simulation based on the experimental structure. In the dual-BAR construct of SN9, the PX domain (grey) was removed from the BAR domain of chain B, and the linker sequence of chain B was used to connect the BAR domains of chains A and B; the connection is marked by a small black ellipse (Fig. 2b).

Figure 3. Structure of the SN9 dual-BAR construct with the PX domain (colored blue) pulled away from the BAR domains. A 40-residue linker, colored orange, was produced artificially and built with Modeller^{66–69}. The structure shown here was the initial conformation for the subsequent UNRES optimization.

Figure 4.

(a) Distribution of C α rmsd from the native structure of the dual-BAR construct of SN9. (b) Lowest C α rmsd structure of the conformations of the dominant cluster of the SN9 dual-BAR construct obtained in UNRES/MREMD simulations. (c) The artificial structure, termed dual-BAR construct of SN9, built for UNRES simulation based on the experimental structure.

Figure 5. Initial structures with the PDZ domain pulled away from the BAR domains, selected for subsequent UNRES simulations of the PICK1 dual-BAR construct (built with Modeller^{66–69}).

Figure 6. Number of conformations of the PICK1 dual-BAR construct in clusters corresponding to the results of simulations started from (a) the structure shown in Fig. 5a, and (b) the one in Fig. 5b. The cluster number (x-axis) has no relationship to the unknown C^α rmsd.

Figure 7. Representative structures for the three top clusters from the UNRES/MREMD simulations with different initial starting structures of Fig. 5a(a–c) and Fig. 5b(d–f), respectively.

Figure 8.

Snapshots corresponding to two types of trajectories for binding the PICK1-PDZ domain to the dual-BAR domain, starting from the initial structure in which the PICK1-PDZ domain faces the convex surface of the PICK1-BAR dimer as shown in Fig. 5b. The initial structure of the PICK1 dual-BAR construct is colored light grey and shown in cartoon representation as a background to compare with the snapshots from the folding trajectory. The PDZ domain is shown as a surface view and is colored blue. The 40-residue linker region is shown as colored purple. The interaction surfaces (i.e., the interface in the final complex) of both the PICK1-PDZ and PICK1-BAR domains are shown as a surface view and colored red. (These structures are drawn with PYMOL⁷¹). The two different binding pathways are marked by arrows with 1 or 2 (or both if the conformations are similar in the two pathways).

Figure 9. Number of hydrophobic contacts between the PDZ and BAR domains of PICK1 during simulated binding.

Figure 10.

The process of generating the structure of the PICK1 dual-BAR construct from the PICK1 dimer structure predicted by Han and Weinstein⁴³. (a) The grey PDZ domain (in the black circle) was removed from its position in Fig. 1. (b) The purple linker region of chain B was artificially joined to the C-terminus of the BAR domain of chain A (colored yellow) with Modeller^{66–69}. The two small black circles with a black arrow between them indicate the two positions of the A and B chains, respectively, that are superposed to join the A and B chains as shown in panel (c).

Table 1

Hydrophobic contact map of structures shown in Fig. 8.

Figure number	Number of HBC ^a	Contact pairs ^b	
a	0	NONE	
b	0	NONE	
c	6	Gly24 / Gly404 Cys28 / Leu140 Val68 / Ile403	Gly24 / Val405 Val68 / Phe400 Val68 / Gly404
d	11	Leu16 / His393 Leu16 / Gly397 Val34 / Tyr147 Phe37 / Gly397 Val68 / Leu157 Val68 / Phe161	Leu16 / Phe396 Tyr27 / Leu146 Val36 / Phe400 Phe37 / Phe400 Val68 / Leu158
e	14	Leu16 / Gly397 Gly22 / Tyr147 Gly23 / Tyr147 Tyr32 / Tyr147 Val34 / Tyr147 Phe37 / Phe400 Val68 / Leu158	Leu16 / Phe400 Gly22 / Met150 Gly23 / Met150 Tyr32 / Met150 Val34 / Met150 Val68 / Leu157 Val68 / Phe161
f	16	Leu16 / Phe386 Gly18 / His393 Ile21 / Leu158 Gly22 / Leu158 Gly24 / Phe161 Val34 / Leu158 Phe37 / Phe396 Val68 / Leu164	Leu16 / His393 Ile19 / His393 Ile21 / Tyr162 Gly23 / Leu158 Tyr27 / His153 Phe37 / His393 Val68 / Phe161 Ile74 / Phe386

^a HBC means Hydrophobic Contacts.^b In the contact pairs, the residues on the left side of the forward slash are in the PDZ domain, and the residues on the right side of the forward slash are in the BAR domain.