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Changes in quaternary structure in the signaling mechanisms of PAS domains

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Abstract

FixL from *Bradyrhizobium japonicum* is a PAS sensor protein in which two PAS domains covalently linked to a histidine kinase domain are responsible for regulating nitrogen fixation in an oxygen-dependent manner. The more C-terminal PAS domain, denoted *bj*FixLH, contains a heme cofactor that binds diatomic molecules such as carbon monoxide and oxygen and regulates the activity of the FixL histidine kinase as part of a two-component signaling system. We present the structures of ferric, deoxy, and carbon monoxide-bound *bj*FixLH in a new space group (P1) and at higher resolutions (1.5–1.8 Å) than those previously obtained. Interestingly, *bj*FixLH can form two different dimers (in P1 and R32 crystal forms) in the same crystallization solution, where the monomers in one dimer are rotated ~175° relative to the second. This suggests that PAS monomers are plastic and that two quite distinct quaternary structures are closely similar in free energy. We use screw rotation analysis to carry out a quantitative pairwise comparison of all PAS quaternary structures, which identifies five different relative orientations adopted by isolated PAS monomers. We conclude that PAS monomer arrangement is context-dependent and could differ depending on whether the PAS domains are isolated or are part of a full-length protein. Structurally homologous residues comprise a conserved dimer interface. Using network analysis, we find that the architecture of the PAS dimer interface is continuous rather than modular: the network of residues comprising the interface is strongly connected. A continuous dimer interface is consistent with the low dimer-monomer dissociation equilibrium constant. Finally, we quantitate quaternary structural changes induced by carbon monoxide binding to a *bj*FixLH dimer, in which monomers rotate by up to ~2 degrees relative to each other. We relate these changes to those in other dimeric PAS domains and discuss the role of quaternary structural changes in the signaling mechanisms of PAS sensor proteins.

The PAS (Per-ARNT-Sim) superfamily is composed of sensor and polymerization domains that receive and transmit environmental signals in order to regulate cellular behavior (1). Signals include light, oxygen, and redox potential. PAS domains regulate many different types of effector domains such as kinases, phosphodiesterases, and transcription factors in a signal-dependent manner (2). The modular nature of proteins containing PAS or other signaling domains (3) enables signaling networks to be diverse and poses an interesting question: how can a single type of sensor domain with a largely conserved tertiary structure regulate effector domains with very diverse structures and functions? We address this question by examining signal recognition, initiation, and propagation in the PAS sensor domain.

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Heme-PAS domains comprise a subset of the PAS superfamily distinguished by their iron protoporphyrin IX (heme b) cofactor, which binds oxygen and other diatomic molecules such as CO and nitric oxide (4). One example of a heme-PAS signaling protein is FixL, found in rhizobia and bacteria, which is responsible for regulating nitrogen fixation, anaerobic and microaerobic respiration, and hydrogen metabolism under hypoxic conditions (3). Rhizobial FixL proteins contain a histidine kinase effector domain, C-terminal to the heme-PAS domain, that is active and undergoes autophosphorylation in the absence of oxygen, and is inactive in the presence of oxygen (5). The phosphate is transferred from FixL to FixJ, the response regulator in this two component signaling system (6), and in a cascade of events, leads to the up-regulation of transcription from genes encoding proteins involved in nitrogen fixation (5-7). Regulation provides a safeguard against the prodigal use of energy to produce nitrogenase, an exquisitely oxygen-sensitive protein, in the presence of oxygen.

Crystallographic studies of ligand-bound and ligand-free, deoxy (ferrous) forms of the heme-PAS domains of FixL proteins from *Bradyrhizobium japonicum* (*bj*FixLH), *Sinorhizobium meliloti* (*sm*FixLH) and of the direct oxygen sensor from *Escherichia coli* (*ec*DOSH) have explored the mechanisms of signal recognition and initiation in heme-PAS domains (8-12). These structures suggest that ligand-dependent tertiary structural changes in FixLH are at least partly responsible for regulating effector domain activity. *Bj*FixLH undergoes ligand-dependent conformational changes in the FG-loop and the H_β and I_β-strands. The movement in the FG-loop has been described as the “FG-loop switch” and may be important for the signal-dependent regulation of FixL kinase activity (10-13). Quaternary structural changes may also play a role. *Bj*FixL forms a homodimer and in the absence of oxygen autophosphorylates at a histidine residue that is conserved among sensor histidine kinases (6,14,15). Key and colleagues suggested (10) that quaternary structural changes which reorient *bj*FixLH subunits relative to each other may facilitate *bj*FixL autophosphorylation. Quaternary structural changes may be a general mechanism by which PAS sensor proteins modulate the activity of their effector domains. For example, the blue light photosensor LOV domain of the dimeric PAS sensor protein, YtvA, in *Bacillus subtilis* (*bs*YtvA-LOV) undergoes small but significant quaternary structural changes as a result of light absorption (16).

Here we report the structures of met (ferric), deoxy and CO-bound forms of *bj*FixLH in a new space group, P1, and at a higher resolution (1.5-1.8 Å) than previously obtained (1.8-2.0 Å) (10). Four *bj*FixLH monomers in the unit cell are arranged as two dimers related by non-crystallographic two-fold symmetry. Since packing of protein molecules in the crystal lattice can influence conformational changes (17), we compare *bj*FixLH structures and signal-dependent conformational changes in the P1 space group with those previously observed in the C2 and R32 space groups (10). We quantitate the differences in quaternary structure of *bj*FixLH in the different space groups (P1AB, P1CD, C2AB and R32) and compare the quaternary structures of *bj*FixLH to those of other PAS dimers, using screw rotation analysis. To characterize the architecture of the PAS dimer interface we carry out network analysis on contact maps describing the interactions among dimer interface residues. Screw rotation and network analyses identify how PAS monomers pack together to form dimers, and the continuous or modular nature of the dimer interface.

Materials and Methods

Expression and Crystallization

*Bj*FixLH (residues 140-280) was purified as described (10,18). Crystals were grown in the oxidized, met form using the hanging drop vapor diffusion method with 3.75M NaCl, 1.5%PEI, 50mM CAPSO (pH 9) at a protein concentration of 60mg/mL. Deoxy-*bj*FixLH

and CO-*bj*FixLH crystals were made as previously described (10) by reducing the met form with mother liquor containing 200mM ascorbate, and then gently bubbling in either nitrogen or CO for 1 or 2 hours, respectively. All crystals were cryo-cooled by plunging into liquid nitrogen (100K). No additional cryoprotectants were found necessary.

Data collection, reduction and refinement

Monochromatic X-ray oscillation data were collected at 100K at the BioCARS 14 BM-C beamline at the Advanced Photon Source (Argonne National Laboratory, Argonne, IL). Data indexing, scaling and integration was done using HKL2000 (19). Molecular replacement was conducted using EPMR (20) with deoxy-*bj*FixLH [Protein Data Bank (PDB) entry 1XJ3] structure as a search model (10). Rigid body refinement, simulated annealing refinement, individual isotropic *B*-factor refinement, and conjugate-gradient minimization were performed using CNS (21) and REFMAC (21,22). Model building was conducted using X-fit/Xtalview (23) and Coot (24). Figures were created using MOLMOL (25), Pymol (26), and MegaPov.

Structure Analysis

The buried surface area and theoretical free energy of solvation were calculated using the computer program PISA (27). The number of inter- and intra-chain contacts was calculated using the CCP4 program NCONT (28) with a cut-off value of $<5\text{\AA}$ between two atoms of interacting residues. The specificity and chemical nature of these interactions was determined using the program Contacts of Structural Units (CSU) (29) and visualized using the computer program Pajek (30). To identify main chain tertiary structural differences, we superimposed individual monomers using $\text{C}\alpha$ least-squares fit analysis and then calculated the root-mean-square-deviation (rmsd) of $\text{C}\alpha$ atoms (31,32). Quaternary structural changes were determined using rigid-body screw rotation analysis, which can be described as a “fit-refit” method (32-35). Two monomers from the pair of dimers being compared were superimposed using $\text{C}\alpha$ least-squares fit analysis. The screw-rotation parameters were then obtained, namely the magnitude of rotation (ρ) and translation (d) associated with a second (rigid body) superposition between the previously non-superimposed monomers. Superposition and calculation of rotation and translation parameters were carried out using the CCP4 program LSQKAB (28,36).

Results

Structure of *bj*FixLH in the P1 space group at 1.5Å to 1.8Å resolution

The structures of *bj*FixLH in the deoxy (ferrous, Fe^{2+}), met (ferric, Fe^{3+}), and CO-bound states were determined in the P1 crystal form at resolutions ranging from 1.5 to 1.8Å (Figure 1). CO was used instead of oxygen, the physiological ligand of *bj*FixL, to study signal-induced conformational changes in three crystal forms (P1, C2, and R32) of *bj*FixLH. Currently only one crystal form of oxygen-bound *bj*FixLH has been determined (13). Both oxygen and CO form low-spin complexes with the ferrous heme in *bj*FixLH and inhibit *bj*FixL autophosphorylation (37). Oxygen and CO-binding also elicit largely similar tertiary conformational changes in *bj*FixLH, which suggests that oxygen and CO share a common signaling mechanism (10). Data collection and refinement statistics are presented in Table 1. Electron density could be identified and fitted for residues 153-257 in all four monomers A, B, C and D in the unit cell in each state, but not for the 11 N-terminal residues nor for the C-terminal residues 258-268, which form an α -helix in the R32 crystal form (10). All tertiary structures are closely similar (rmsd $< 1.0\text{\AA}$) to those found in other crystal forms and in other PAS domains. The four monomers are found as two dimers, P1AB and P1CD, related by a two-fold non-crystallographic symmetry axis. When either P1AB or P1CD is superimposed on the C2 dimer (C2AB), the two non-superimposed dimers are rotated and

displaced relative to each other. This reflects the different crystal packing arrangement in P1 compared to C2.

Comparison of PAS domain quaternary structures

BjFixLH dimer formation in the crystal may reflect the dimeric nature of full-length *bjFixL* sensor protein in solution (18). However, it is not known how PAS domains arrange themselves relative to each other within a signaling complex, nor what role quaternary structural changes play (if any) in the signaling mechanism of PAS sensor proteins. To address the first, we compared the dimeric structures of *bjFixLH* in three different crystal forms (P1, C2, and R32). *BjFixLH* forms non-crystallographic dimers in the P1 (P1AB and P1CD) and C2 (C2AB) crystal forms and a crystallographic dimer in the R32 crystal form.

The arrangement of monomers within a complex can be characterized by the position and type of residues comprising the interface, the buried surface area and/or the number of contacts made by interface residues, and by screw rotation parameters that describe any differences in quaternary structure. Each monomer in the P1 crystal form of *bjFixLH* contacts six neighbors and generates six candidate intra- and inter-unit cell dimeric assemblies. To assess which of the possible dimers is most stable and potentially of physiological relevance, we calculated the buried surface area and theoretical stability of all six (27). Only two dimers (P1AB and P1CD) contain more than 900 Å² of buried surface area (BSA) and have a theoretical solvation free energy for forming the dimer interface lower than -8kcal/mol, benchmarks for physiologically relevant assemblies (38,39). These two intra-unit cell dimers are shown in Figure 1. The buried surface area in P1AB and P1CD is approximately 1600 Å², which is similar to that in the C2 (1400 Å²) non-crystallographic and R32 (1200 Å²) crystallographic dimers. In both P1AB and P1CD the dimer interface is comprised of a central hydrophobic patch and flanking hydrogen bonding regions largely contributed by residues in the G β -, H β - and I β -strands.

We quantitated the differences in quaternary structure using screw rotation angle (ρ) and translation (d) parameters (Table 2a). When one monomer from each of the P1 and C2 dimers is superimposed (rmsd ~ 0.5 Å), the second (non-superimposed) monomer exhibits a small screw rotation of up to 3.5° and a translation of less than 1 Å. In contrast, a large screw rotation of ~175° is observed when comparing a monomer from either the P1 or C2 dimer with a monomer in the R32 form. The ability of *bjFixLH* to adopt two different quaternary structures (in the P1 and R32 crystal forms) in the same solvent reflects the plasticity of PAS domains, namely their ability to arrange themselves in different ways within a complex.

Do these observations of different quaternary structures in *bjFixLH* extend to other PAS domains that form dimers? To address this question we compared the dimers of *bjFixLH* (in the P1, C2, and R32 space groups) (10), YtvA-LOV from *Bacillus subtilis* (*bsYtvA*-LOV) (16), DOSH from *Escherichia coli* (*ecDOSH*) (8), NifL from *Azotobacter vinelandii* (*avNifL*) (40), FixLH from *Sinorhizobium meliloti* (*smFixLH*) (9), and LOV1 from *Chlamydomonas reinhardtii* (*crPhot*-LOV1) (41) according to their screw rotation parameters. The last two molecules each form crystallographic dimers. Each physiologically relevant dimer was identified using the same criteria used to identify dimers of *bjFixLH*. Five distinct monomer orientations (Table 2b) were identified by carrying out hierarchical clustering analysis on the pairwise comparisons of PAS dimer quaternary structure. Qualitative differences in PAS quaternary structure are presented in Figure 2, in which the left monomers are superimposed by a rigid body Ca least-squares fit superposition to chain B in P1AB, and the right monomers display a range of orientations. Several interesting results are evident from Table 2b. Structures containing ordered N-terminal helices (*smFixLH*, *ecDOSH*, *avNifL*) exhibit similar quaternary structures ($\rho = 16.7^\circ \pm 4.6^\circ$) and a small translation ($d = 1.6 \pm 0.8$ Å). Structures lacking an ordered N-terminal helix (*bjFixLH*,

bsYtvA LOV, *crPhot-LOV1*) exhibit three distinct quaternary structures ($\rho \sim 50^\circ$, 140° or 170°) accompanied by a similarly small translation ($d = 0.9 \pm 0.8 \text{ \AA}$). This is opposed to when a structure containing an ordered N-terminal helix is compared to one lacking this helix, where a large translational component (range 1.6-12.9 $\text{\AA}$, with a mean of $7.2 \pm 3.9 \text{ \AA}$) is observed. The identification of only five distinct monomer orientations is surprising given that 21 pairwise comparisons were carried out to evaluate the quaternary structure of seven PAS dimers. The sources of constraints on monomer orientation are discussed below.

Conserved PAS dimer interface

Those residues in the P1AB, P1CD, C2, and R32 dimers that have more than 50% of their surface area buried at the dimer interface are indicated in the sequence alignment of PAS domains in Figure 3. From the alignment it is apparent that homologous residues in *bjFixLH*, *smFixLH*, *ecDOSH*, *bsYtvA-LOV*, *avNifL* and *crPhot-LOV1* form the dimer interface in each protein despite in some cases very different quaternary structures. All are located on β -strands. Thus, a conserved set of residues forms the PAS domain intra-dimer interface regardless of crystal form, quaternary structure or type of PAS domain. This is consistent with the observation by Erbel and colleagues (42) that the β -sheet is important for intra- and inter-molecular interactions and versatile in its ability to accommodate both homo- and hetero-dimerization among PAS monomers (42).

To visualize the three-dimensional relationship among interface residues and the degree to which each residue contributes to the dimer interface, we determined the number of intra- and inter-unit cell crystal contacts made by each residue in the P1, C2 and R32 forms of *bjFixLH*, *smFixLH*, *ecDOSH*, *bsYtvA*, *avNifL* and *crPhot-LOV1*, and mapped these contacts on to their tertiary structures (Figure 2). The conserved dimer interface residues are labeled in each structure.

Continuous or modular dimer interface?

Is the network of residues comprising the PAS dimer interface continuous or can it be partitioned into clusters of residues, as described by Reichmann and colleagues (43) for proteins such as CheY/CheA and barnase/barstar? Clusters may contain as few as three residues. The boundaries between clusters are identified based on the premise that residues within a cluster form many close connections (hydrophobic, aromatic, electrostatic and hydrogen bonding) whereas relatively fewer connections are made between residues from different clusters (43).

To determine whether a PAS dimer interface is continuous or modular with ≥ 2 clusters, we created contact maps (Figure 4; see *Materials and Methods*) for P1AB, P1CD, C2AB and R32 dimers containing those interface residues with $>50\%$ buried surface area at the dimer interface. Only specific interactions between two side chains or a side chain and main chain with contact atom distances $\leq 5 \text{ \AA}$ were included. Nodes represent residues and edges represent interactions between residues. The results of a hierarchical clustering analysis of each contact map revealed that the *bjFixLH* dimer interface contains only one large cluster i.e. a continuous patch at the dimer interface. This result was maintained when the threshold for the distance between interacting atoms was lowered from $5 \text{ \AA}$ to $3.5 \text{ \AA}$.

We extended this analysis to *smFixLH*, *ecDOSH*, *bsYtvA-LOV*, *avNifL* and *crPhot-LOV1* and observe the same result: in all molecules the PAS dimer interface contains a continuous patch. A large, well-developed cluster has been correlated with tight binding between monomers (43). The high affinity between PAS monomers (for example, $K_d < 500 \text{ nM}$ for *bsYtvA* LOV) (16) is consistent with the observation that PAS domain interfaces are comprised of a complex and continuous network of residues.

Effect of quaternary structure on signal-induced tertiary structural changes

The observation that *bjFixLH* can adopt two distinct quaternary structures (P1 and R32) in the same crystallization solution raises an important question: to what extent do very different quaternary structures modify the signal-induced tertiary structural changes? CO-induced tertiary structural changes have been well characterized in the C2 and R32 crystal forms (10,44). Inspection of difference Fourier maps and calculation of C α -rmsd values for superimposed monomers (in the deoxy and CO-bound states) in the P1 and R32 crystal forms indicate that *bjFixLH* undergoes largely similar CO-induced tertiary structural changes regardless of its quaternary structure (with the exception of the dimer interface residue Thr250 discussed below). In both the P1 and R32 forms, very few tertiary structural changes are observed for main chain atoms. The largest tertiary structural changes occur in the side chains of residues known as the hydrophobic triad (L215, L236, I238) that surround the heme active site, and residues located in the β -sheet on the distal side of the heme and the FG-loop. The conformational changes in P1AB are similar in pattern but lesser in magnitude than those observed in P1CD. Most of these conformational changes are also observed for the third crystal form of *bjFixLH*, C2, despite quite different crystallization conditions (10).

Thr250 undergoes CO-induced conformational changes in dimers in the P1 but not C2 or R32 crystal forms. We attribute this difference between crystal forms to different contacts made between Thr250 and other dimer interface residues (Figure 4). A second water molecule is also incorporated into the dimer interface in both P1AB and P1CD upon CO binding. The additional water molecule makes hydrogen bonds to the main chain amide and the side chain hydroxyl group of Thr250 in chain B of the P1AB dimer and Thr250 in chain C of the P1CD dimer. Conformational changes by Thr250 do not appear to be necessary for evoking CO-induced quaternary structural changes since R32 monomers rotate 1.5° relative to each other upon CO-binding to the heme in the absence of tertiary structural changes at Thr250.

Signal-induced quaternary structural changes

To determine quaternary structural changes associated with ligand binding, we compared the structures of each dimer (P1AB, P1CD) in the P1 space group from two crystals, one in the CO-bound state and the other in the deoxy state. To provide a negative control, we also compared the structures of the P1AB and P1CD dimers derived from two deoxy crystals from which diffraction data were independently collected and refined to yield two deoxy structures. The magnitude of subunit rotation and translation in P1AB and P1CD dimers following CO binding are compared in Table 2c with those for *bsYtvA* LOV. In the negative control monomers apparently rotate less than 0.7°, but monomers in the P1AB and P1CD dimers rotate by up to 1.3° and 1.9°, respectively. A similar rotation was observed in the R32 form (1.5°), but not in the C2 form, perhaps due to differences in crystallization solvent, crystal contacts and interactions at the dimer interface. Signal-induced quaternary structural changes were also observed in the PAS domain dimer, *bsYtvA*, whose monomers rotate 2.2° following exposure to light (16). Although similar in their magnitude of quaternary structural change, the different monomer arrangements in *bjFixLH* and *bsYtvA*-LOV suggests that there are several ways in which PAS dimers could undergo quaternary structural changes as a result of responding to a signal.

Discussion

PAS domain quaternary structure

The quaternary structures of several PAS domain dimers have previously been determined (8-10,16,40,41). By quantitating differences in PAS quaternary structure using screw

rotation analysis, we identify five discrete monomer orientations (Table 2b) that are adopted by PAS domains. Most strikingly, the presence of ordered N-terminal helices seems to predispose a dimer to a particular orientation in which the monomers adopt a parallel arrangement. Different monomer orientations may arise from differences in crystallization conditions. However, recent studies suggest that the N-terminal flanking region (termed the Ncap) stabilize PAS dimers (9,45-47). The signal transduction histidine kinase (STHK) H-NOXA from *Nostoc punctiforme* (npSTHK H-NOXA), which has a PAS-like core fold, undergoes a change in monomer orientation as a result of deleting seven N-terminal residues flanking its core (48). In this case the N-terminal helices appear to be critical for maintaining a parallel orientation. Deletion of the N-terminal flanking region of a PAS domain from *Rhodobacter sphaeroides* PpsR, a transcription factor, disrupts its repressor activity but not its oligomerization state (49). Together these data suggest that the Ncap may be of general importance in establishing the quaternary structure and function of PAS domains.

The presence of N-terminal domains and in particular dimerization domains, may also affect the arrangement of PAS domains in PAS sensor proteins. Recently the dimeric structure of a PAS histidine kinase from *Thermotoga maritima* (ThkA) was solved in complex with its response regulator (TrrA) (50). In this structure the PAS domains are more than 100Å apart and do not interact with each other. However, the ThkA construct lacks ~400 N-terminal residues comprising predicted coiled-coil and GAF domains, which can greatly facilitate protein dimerization (51,52). Additional experiments are necessary to determine whether this arrangement of PAS monomers is physiologically relevant or arises from the particular, shorter construct used.

Do C-terminal flanking regions also play a role in establishing the quaternary structure of PAS domains? Different orientations of *bjFixLH* monomers are observed where in one case (R32) the C-terminal flanking helices are ordered and in the second case (P1AB, P1CD) they are disordered. The C-terminal helices in the R32 *bjFixLH* dimer interact with the helices from neighboring monomers. Although this specific orientation of its helices is likely to be a crystallization artifact, it demonstrates that interactions involving C-terminal helices could affect PAS domain quaternary structure.

Additional properties such as the presence of PAS domain effector domains and the composition of the dimer interface residues are likely to constrain PAS monomer orientation. In many cases PAS domains function as protein-interaction modules (53). However, PAS domain oligomerization may also be dictated by the effector domain to which they are linked. For example, full-length *ecDOS*, containing phosphodiesterase and heme-PAS domains, results in *ecDOS* tetramerization whereas the heme-PAS domains alone can only form dimers (54). We conclude that the plasticity of PAS monomers makes their rearrangement particularly sensitive to their context. Therefore, PAS domain quaternary structure could differ depending on whether the PAS domains are isolated or are part of a full-length protein.

Maintaining the proper orientation of PAS monomers in the presence and absence of a signal appears to be critical for PAS sensor protein function. A single point mutation in a PAS domain from the sensor histidine kinase KinA from *Bacillus subtilis* dimer interface residue may disrupt monomer orientation. This in turn can lead to functional defects (49,55). Signal-induced changes in quaternary structure are also observed in the PAS domain of a sensor histidine kinase from *Vibrio harveyi*, LuxQ (56).

Dimer interface

PAS domain dimers contain a conserved dimer interface that is comprised of five structurally homologous residues located throughout the β -sheet in the A β -, B β -, H β - and I β -

strands (Figure 3) which interact with the β -sheet or N-terminal helix of another PAS domain. The conservation of the interface is surprising given the low sequence identity of the residues that comprise it. In most cases the PAS dimer interface is comprised of hydrophobic residues that interact with residues from the opposing monomer in a specific “knobs-in-holes” fashion. In these cases monomer orientation is sterically constrained. Notably, more than one solution to the “knobs-in-holes” problem is possible, as observed for the two crystal forms of *bjFixLH* (P1 and R32). Charged residues have also been found at a PAS dimer interface and can establish quaternary structure via electrostatic interactions. A PAS heterodimer involving PAS-B monomers from the human hypoxia-inducible factor (HIF)-2 α and the aryl hydrocarbon nuclear translocator (ARNT) appears to be formed in part via electrostatic interactions between dimer interface residues of opposing monomers (57).

Network analysis (43) of the PAS dimer interface reveals that the interface is continuous, based on a single surface patch, rather than modular, based as two or more discrete surface patches. Thus, a single module establishes monomer affinity and quaternary structure, and prevents the retention of quaternary structure when residue(s) within that module are mutated (43). Indeed, mutating a single residue at the KinA PAS dimer interface leads to large changes (>10-fold) in monomer affinity (55). The effect of deleting the N-terminus on the quaternary structure of PAS domains (9,45,46) is consistent with the predictions made using network analysis: disrupting part of a module leads to changes in quaternary structure (43).

Crystal lattice effects have minimal impact on CO-induced conformational changes in *bjFixLH*

Crystal lattice effects are important to consider when using crystallography to study static protein structures and signal-induced conformational changes. While we cannot entirely rule out the effect of crystal packing, lattice effects on CO-induced conformational changes in *bjFixLH* appear to be quantitatively minor. Recently, it was shown that CO recombination kinetics of *bjFixLH* are closely similar in the R32 crystal form compared to solution (18,44). This demonstrates that *bjFixLH* can readily convert between deoxy and CO states in the crystal and that intermolecular forces in the lattice that may affect the free energy barrier between the deoxy and CO-bound states have at most a minor effect. We directly compare the nature of the CO-induced conformational changes in *bjFixLH* crystallized in different crystal forms (P1, R32, C2). We observe similar CO-induced conformational changes in each crystal form, despite very different intermolecular interactions and constraints within the three forms. This provides additional evidence for minimal crystal lattice effects on the integrity of the CO-bound structure of *bjFixLH*.

Role of quaternary structural changes in the signaling mechanism of PAS sensor proteins

Previous studies have invoked the β -sheet in the signaling mechanism of PAS sensor proteins (42,57,58). Cusanovich and colleagues suggest (58) that a signaling event initiated within a PAS domain is transmitted from the ligand binding site to the dimer interface via distortion of the β -sheet. Our results are consistent with this hypothesis. Many PAS sensor proteins including FixL exist and function as oligomers (53,54). While it is not necessary that an oligomer undergo quaternary structural changes to sense and respond to a signaling event, the quaternary structure of proteins often mediates allosteric communication. Quaternary structural changes originating in PAS domains could propagate to the linker region between the PAS and effector domain and ultimately to the effector domain itself. Signal-induced quaternary structural changes accommodate the ability of PAS sensor domains to regulate a wide variety of effector domains, since in such a model PAS and effector domains are not required to interact with each other in a structure-specific manner.

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Abbreviations

PAS	Per-ARNT-Sim
CO	carbon monoxide
PEI	polyethylenimine
CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid

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Figure 1. Ribbon diagram of the structure of deoxy bjFixLH in the P1 crystal form
P1AB and P1CD dimers are shown in yellow and blue, respectively. Secondary structure elements are labeled on the P1B monomer.

Figure 2. Intra- and inter-unit cell contacts between *bjFixLH* dimers

The number of contacts made by residues in each monomer of (A) P1AB, (B) P1CD, (C) C2AB, (D) R32, (E) *ecDOSH*, (F) *smFixLH*, (G) *bsYtvA-LOV*, (H) *avNifL*, and (I) *crPhot-LOV1* are colored according to the color scale bar. Conserved dimer interface residues are labeled by residue type and number.

Figure 3. Structure-based sequence alignment of PAS domains

PAS sensor domain sequences were aligned using Dali (59) then manually adjusted to best align secondary structure elements. Residues that have greater than 50% of their surface area buried (as calculated using PISA) at the dimer interface are denoted in red. Residues in italics indicate those for which the structure is unknown. Purple bars and asterisks indicate residues in the conserved dimer interface. Non-crystallographic dimers: *bjFixLH* (P1AB, P1CD, and C2), *ecDOSH*, *bsYtvA-LOV* and *avNifL*. Crystallographic dimers: *bjFixLH* (R32), *smFixLH*, and *crPhot-LOV1*.

Figure 4. Contact map for the intra-dimer interface of bjFixLH dimers

The α -atoms for residues making specific contacts with residues within the same or neighboring monomer were mapped onto their respective ribbon structures and are shown as spheres. α -atoms from chain A of P1AB, C2AB, and R32 and from chain C of P1CD are shown as blue spheres. α -atoms from chain B of P1AB, C2AB, and R32 and from chain D of P1CD are shown as yellow spheres. Contacts are shown between two side-chain atoms (*solid line*), between a side-chain and main chain atom (*dottedline*) and between two residues involving both side chain-side chain and side chain-main chain interactions (*thick solid line*). Conserved dimer interface residues are underlined.

Table 1Data Collection and Refinement Statistics for *bj*FixLH in Space Group P1

Diffraction data			
Ligand state	met	deoxy	CO
Unit cell dimensions			
a, b, c (Å)	48.8, 50.0, 59.0	48.6, 49.8, 58.8	48.9, 50.3, 59.0
α , β , γ (deg.)	73.7, 70.4, 72.3	73.2, 71.1, 71.7	73.4, 70.3, 72.1
Resolution (Å) (last shell)	50.0-1.40 (1.45-1.40)	50.0-1.80 (1.86-1.80)	50.0-1.61 (1.67-1.61)
Unique reflections	91144	44159	57814
R _{merge} (%) (last shell)	3.4 (42.3)	4.6 (42.4)	3.5 (32.4)
Redundancy (last shell)	1.9 (1.9)	3.8 (3.7)	1.9 (1.8)
Completeness (%) (last shell)	94.6 (94.5)	96.1 (95.7)	90.7 (93.7)
I/ σ I (last shell)	25.3 (2.1)	38.0 (3.2)	29.5 (3.7)
Refinement			
Resolution (Å)	50.0-1.50	50.0-1.81	50.0-1.61
R work, R free (%)	18.2, 21.1	19.7, 24.7	24.9, 20.6
RMSD from ideal bond lengths (Å)	0.010	0.019	0.015
RMSD from ideal bond angles (deg.)	1.435	1.985	1.643
Ramachandran distribution			
Most favored (%)	92.4	91.0	91.3
Allowed (%)	7.6	9.0	8.7
Generously allowed (%)	0	0	0
Outside allowed (%)	0	0	0
PDB entry	2VV6	2VV7	2VV8

Table 2aQuaternary structure of *bj*FixLH dimers in different crystal forms

Dimer 1	Dimer 2	ρ	d
P1AB (1)	P1AB (2)	0.2	0.0
P1AB (1)	P1CD (1)	3.5	0.3
P1AB (1)	P1CD (2)	2.9	0.4
P1AB (1)	C2AB	2.4	0.1
P1AB (1)	R32	174.9	1.1
P1AB (2)	P1CD (1)	3.4	0.1
P1AB (2)	P1CD (2)	2.7	0.2
P1AB (2)	C2AB	2.4	0.2
P1AB (2)	R32	175.1	1.0
P1CD (1)	P1CD (2)	0.7	0.1
P1CD (1)	C2AB	2.3	0.2
P1CD (1)	R32	178.1	0.7
P1CD (2)	C2AB	2.1	0.1
P1CD (2)	R32	177.7	0.7
C2AB	R32	174.9	1.1

The monomer orientation of *bj*FixLH dimers in the deoxy state was compared using screw rotation analysis. Screw rotation parameters (rotation (ρ) and translation (d); degrees, Å) are shown for each pairwise comparison of dimers (Dimer 1, Dimer 2). Data for two separate crystals (denoted 1, 2) of deoxy *bj*FixLH in the P1 crystal form are shown.

Table 2b

Quaternary structures of PAS domain dimers fall into five distinct groups

Dimer 1	Dimer 2	ρ	d	Mean ρ , d ($\pm$ std. dev)
P1AB	R32	174.9	1.1	168.8 ± 7.7 , 5.4 ± 4.2
P1AB	* <i>smFixLH</i>	170.0	8.1	
P1AB	* <i>ecDOSH</i>	161.0	7.7	
P1AB	* <i>avNifL</i>	177.4	9.5	
<i>bsYtvA-LOV</i>	<i>crPhot-LOV1</i>	160.9	0.7	136.4 ± 7.5 , 6.4 ± 5.9
P1AB	<i>bsYtvA-LOV</i>	143.1	0.1	
R32	<i>crPhot-LOV1</i>	134.7	0.0	
* <i>smFixLH</i>	<i>crPhot-LOV1</i>	130.2	10.1	
<i>crPhot-LOV1</i>	* <i>ecDOSH</i>	128.7	9.5	76.4 ± 3.2 , 4.2 ± 1.7
<i>crPhot-LOV1</i>	* <i>avNifL</i>	145.4	12.3	
* <i>ecDOSH</i>	R32	78.7	3.0	
* <i>ecDOSH</i>	<i>bsYtvA-LOV</i>	74.2	5.4	
P1AB	<i>crPhot-LOV1</i>	50.8	1.9	53.0 ± 6.3 , 4.1 ± 4.4
R32	<i>bsYtvA-LOV</i>	47.7	1.7	
R32	* <i>smFixLH</i>	61.5	2.0	
R32	* <i>avNifL</i>	60.1	12.9	
<i>bsYtvA-LOV</i>	* <i>smFixLH</i>	46.9	4.5	16.7 ± 4.6 , 1.6 ± 0.8
<i>bsYtvA-LOV</i>	* <i>avNifL</i>	50.7	1.6	
* <i>smFixLH</i>	* <i>ecDOSH</i>	11.4	0.9	
* <i>smFixLH</i>	* <i>avNifL</i>	19.7	1.5	
* <i>ecDOSH</i>	* <i>avNifL</i>	19.0	2.4	

The monomer orientation of PAS domain dimers was compared using screw rotation analysis. Screw rotation parameters (rotation (ρ) and translation (d); degrees, Å) are shown for each pairwise comparison of dimers (Dimer 1, Dimer 2). Only P1AB and R32 crystal forms of *bjFixLH* are shown since P1AB, P1CD and C2AB have very similar quaternary structures ($\rho = 2.8 \pm 0.6^\circ$, $d = 0.2 \pm 0.1$ Å). The mean rotation and translation parameters are shown for the five different PAS domain orientations identified using hierarchical clustering analysis. Asterisks denote PAS domains that contain ordered N-terminal helices.

Table 2c

Signal-induced quaternary structural changes by PAS domains

Dimer 1	Dimer 2	ρ	d
P1AB (deoxy)	P1AB (CO)	1.3	0.1
P1CD (deoxy)	P1CD (CO)	1.9	0.0
C2AB (deoxy)	C2AB (CO)	0.7	0.0
R32 (deoxy)	R32 (CO)	1.5	0.2
<i>bsYtvA</i> -LOV (dark)	<i>bsYtvA</i> -LOV (light)	2.2	0.1

The monomer orientation of *bjFixLH* (in deoxy and CO-bound states) and *bsYtvA*-LOV (in dark and light states) was compared using screw rotation analysis. Screw rotation parameters (rotation (ρ) and translation (d); degrees, Å) are shown for each pairwise comparison of dimers (Dimer 1, Dimer 2).