Biochemical and Biophysical Research Communications 434 (2013) 65–69

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Crystal structure of the response regulator spr1814 from *Streptococcus pneumoniae* reveals unique interdomain contacts among NarL family proteins

Ae Kyung Park [a,1,2], Jin Ho Moon [b,1], Jae Soon Oh [a], Ki Seog Lee [c], Young Min Chi [a,*]

[a] Division of Biotechnology, College of Life Sciences, Korea University, Seoul 136-713, Republic of Korea
[b] Institute of Life Sciences and Natural Resources, Korea University, Seoul 136-713, Republic of Korea
[c] Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan 609-757, Republic of Korea

ARTICLE INFO

Article history:
Received 8 March 2013
Available online 30 March 2013

Keywords:
Two-component phosphotransfer pathways
Response regulators
NarL subfamily
Helix-turn-helix fold
Streptococcus pneumoniae

ABSTRACT

Spr1814 belongs to the NarL/FixJ subfamily of signal transduction response regulators (RR), and has been predicted to regulate the neighboring ABC transporter, which translocates antibiotic molecules in *Streptococcus pneumoniae*. Here, we report the crystal structure of full-length unphosphorylated spr1814 at 1.7 Å resolution. The asymmetric unit contains two spr1814 molecules, which display very different conformations. Through comparisons with other RRs structures, we concluded that one molecule adopts a general inactive conformation, whereas the other molecule adopts an intermediate conformation. The superposition of each molecule showed that rotational change of the effector domain occurred in intermediate conformational state, implying that domain rearrangement could occur upon phosphorylation.

1. Introduction

Two-component systems (TCSs) are signaling pathways which allow organisms to perceive and respond to a wide variety of environmental stimuli, including nutrient uptake, sporulation, chemotaxis, virulence, quorum sensing, and cell adhesion [1]. Typical TCSs consist of a membrane-bound signal-sensing protein histidine kinase (HK) and a cytosolic cognate response regulator (RR) protein [2]. In the presence of extracellular stimuli, HKs catalyze a transfer of the phosphate group to a receiver domain of cognate RR. Usually phosphorylated RRs function as transcription factors inducing the expression of target genes [3]. RR transcription factors can be classed into subfamilies based on their structural similarity within their DNA-binding effector domains. Spr1814, the RR studied here, belongs to the NarL/FixJ subfamily, which is characterized by a helix-turn-helix (HTH) DNA-binding domain, and accounts for approximately 19% of all response regulators [4–6].

Until now, there have only been three full-length structures of NarL/FixJ subfamily identified, and all of them were crystallized as unphosphorylated form [4,7,8]. Although they share a similar overall structure in terms of their receiver and effector domains, different contacts between domains have been observed. The most extensive interdomain interactions were found to occur in NarL and DosR in two very distinct manners, but few contacts were observed in StyR [4,7,8]. The structure of unphosphorylated full-length spr1814 also displays interdomain contacts, but its arrangement is totally different from above three structures. Spr1814 exhibits relatively moderate contacts, that only one helix from each domain participates in during domain contacts. Although structural information of RRs, which belong to the NarL/FixJ subfamily, has suggested that individual RRs exhibit unique domain organization, they are proposed to be activated by domain rearrangements disrupting the interdomain contacts [2,4]. In this respect, our previously reported structure of the N-terminal receiver domain of spr1814 emphasized the role of the linker region in phosphorylation-induced domain rearrangement [9]. A comparison of structures with the presence and absence of the phosphoryl analog beryllium fluoride (BeF_3^-) revealed that the linker region underwent a conformational change that could have an effect on the interaction between two domains upon phosphorylation. However, a detailed understanding of the regulation mechanism of RRs is limited due to the lack of structural information of full-length phosphorylated RRs.

In this report, we determined the crystal structure of full-length unphosphorylated spr1814 at 1.7 Å resolution. Spr1814 contains two molecules per asymmetric unit in solution. In many RRs, phosphorylation mediates dimerization of the receiver domains, which is thought to promote DNA binding and transcription activation [10,11]. In this respect, the dimer formation of the inactivated state of spr1814 may be the result of the high protein concentrations.

* Corresponding author. Fax: +82 82 2 921 3702.
E-mail address: ezeg@korea.ac.kr (Y.M. Chi).
[1] These authors contributed equally.
[2] Present address: Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.065

Therefore, unphosphorylated spr1814 will be considered as a monomeric protein in this work. Two monomers of spr1814 had different conformations and comparison between them revealed that there was a rotational change of the effector domain of one monomer (molecule B), resulting in a loosening of the interdomain contacts. The active-like conformation of phosphorylation site in the receiver domain triggers domain reorganization in molecule B, therefore, we postulate that molecule B is an intermediate state. From a structural perspective, the conformational changes of the molecule B have a meaning that it is the first structure in NarL/FixJ family, revealing the domain reorganization, which could occur upon phosphorylation.

2. Materials and methods

2.1. Recombinant protein expression, purification and crystallization

The heterologous expression of spr1814 (formerly known as DesR), purification and crystallization were performed as previously described [12]. Briefly, spr1814 was purified from the culture supernatant of transformed *Escherichia coli* BL21 (DE3). Following purification, the crystals of spr1814 belonging to the space group $P2_12_12_1$ were obtained by the hanging-drop vapor diffusion method at 22 °C; each drop was made up of a 1 μl protein solution (18 mg/ml) [20 mM Tris–HCl pH 7.9, 200 mM NaCl, 2 mM DTT, 5 mM $MgCl_2$, 10% glycerol] and a 1 μl reservoir solution [0.1 M sodium citrate tribasic dehydrate pH 5.0, 18% (*v*/*v*) Jeffamine ED-2001 pH 7.0], and was equilibrated over a 500 μl reservoir solution. For single-wavelength anomalous dispersion (SAD) phasing, the selenomethionine-substituted (SeMet-substituted) protein was cultured by growing the expression plasmid in *E. coli* B834 (DE3), using the M9 cell culture medium containing extra amino acids. Subsequent purification of SeMet-substituted spr1814 was identical to that described above for the native protein, except for the presence of 10 mM β-mercaptoethanol in all buffers used during the purification steps for protection from oxidizing of selenomethionyl protein. The crystals of SeMet-substitute spr1814 were grown under the same conditions of apo-crystal. Suitably sized crystals were obtained within 3 d and were used for X-ray diffraction.

2.2. Data collection and structure determination

For cryogenic experiments, SeMet-substituted crystals were soaked in mother liquor plus 25% (*v*/*v*) ethylene glycol and were flash-frozen in a liquid-nitrogen stream. The diffraction data set of SeMet-substituted crystal were collected on beamline 4A at the Pohang Light Source (Pohang, South Korea) using an ADSC Quantum 310 CCD detector. For the SeMet crystal, a total of 360 frames were collected with an oscillation range of 1°. Diffraction data were collected at a single wavelength corresponding to the peak (λ = 0.9798 Å) of a selenium *K*-edge absorption profile, and were indexed, integrated and scaled by using *HKL*2000 [13]. The data-collection statistics are summarized in Table 1.

The structure of spr1814 was determined by the single-wavelength anomalous dispersion (SAD) method using a SeMet-substituted crystal. The location of heavy atom positions, initial phase calculations, phase improvements through density modification, and initial maps, were calculated using the *PHENIX* program suite [14]. In SeMet-substituted structure, a total of 14 selenium sites in the asymmetric unit were determined, and the overall figure of merit (FOM) was improved to 0.47, and automatically built 286 residues of 398 residues. Model building and refinement were performed with *Coot* [15] and *REFMAC5* [16]. At this stage a higher-resolution native dataset was used for further refinement of the apo structure. After several cycles of model building, simulated annealing, positional refinement, and individual B-factor refinement, the final R_{factor} and R_{free} values of the apo structure were determined to be 19.8% and 24.1%, respectively. The final apo structure contains one protein dimer, 400 amino acids residues, and 244 water molecules. Structural validation by *MolProbity* [17] suggests that 98.7% of protein residues fall in the most favored region of the Ramachandran plot. The refinement statistics are summarized in Table 1. All structural figures were generated using *PyMOL* [18].

Table 1
Data collection and refinement statistics.

	Spr1814	
Data collection		
	Native	SAD (peak)
Space group	$P2_12_12_1$	$P2_12_12_1$
Wavelength (Å)	1.0000	0.9798
Cell dimension (Å)	a = 46.91, b = 71.38, c = 117.73	a = 46.81, b = 71.46, c = 117.63
Resolution range (Å)	50–1.7 (1.76–1.70)	50.0–2.6 (2.64–2.60)
Total reflections	340,515	100,448
Unique reflections	42,035	12,538
Completeness (%)	94.7 (67)	97.1 (90.3)
R_{merge}[a] (%)	6.5 (29.2)	8.5 (21.2)
Redundancy	8.1 (2.9)	8.0 (4.9)
$\langle I/\sigma(I)\rangle$	25 (1.9)	31.6 (6.3)
Refimenet		
Resolution range (Å)	50.5–1.7	
R/R_{free}[b] (%)	19.8/24.1	
No. of atoms protein/waters	400/244	
RMS deviation of		
Bond length (Å)	0.001	
Bond angle (°)	0.9	
In Ramachandran plot		
Most favored (%)	98.7	
Additionally allowed (%)	1.3	

Values in parentheses refer to the highest resolution shell.

[a] $R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| \sum_h \sum_i I(h)_i$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections, and $\sum_i$ is the sum over i measurement of reflection h.

[b] $R = \frac{\sum ||F_{obs}| - |F_{calc}||}{\sum |F_{obs}|}$, where R_{free} is calculated for a randomly chosen 5% of reflections, which were not used for structure refinement.

2.3. Protein data bank accession number

The coordinates of the structure together with the structure factors have been deposited in the Protein Data Bank (http://www.rcsb.org/pdb) with the accession code of 4HYE.

3. Results and discussion

3.1. Overall structure

The structure of full-length unphosphorylated spr1814 was determined by SAD phasing at resolution of 1.7 Å. Like most RRs [1], spr1814 is composed of two domains: the N-terminal receiver domain (residues 1–119) exhibiting the classic α/β fold observed in all RRs, and the C-terminal, effector domain (residues 141–199) with a helix-turn-helix DNA-binding fold (Fig. 1A). The site of phosphorylation is a conserved Asp residue (Asp53) located in the C-terminal edge of the β3 strand. Spr1814 is categorized as a member of the NarL/FixJ subfamily based on the homology of the C-terminal domain. The two domains are connected by a flexible linker region (residues 120–140) which is made up of an extended loop and short α helix. Spr1814 shares low sequence similarity with RRs, which were structurally identified in previous reports, however, the residues corresponding to phosphorylation site were well conserved. Although the overall structures of receiver and effector domains of spr1814 are highly similar to those of NarL/FixJ subfamily, there is one notable distinction. Spr1814 is comprised of two monomers that have very different conformations. The superposition of molecules A and B shows the slight change in the position of Cα atoms in the effector domains (r.m.s.d. of 0.62 Å). In contrast to this observation, large conformational changes are observed in the receiver domains, specifically two loop regions (β4-α4 and β5-α5). Consequently, there is an approximately 74° rotational change of the effector domain of molecule B with respect to molecule A (Fig. 1B) [19]. This movement is achieved by a flexible linker region, and this remarkable conformational change loosens the interdomain contacts of molecule B.

3.2. Active sites

As mentioned above, receiver domain structures of molecule A and B in unphosphorylated full-length spr1814 are not identical. In detail, the phosphorylation site of spr1814 has two very different conformations (Fig. 2). In general, the distinct rotameric states of two highly conserved switch residues, Ser/Thr on β4 strand and Phe/Tyr on β5 strand, are the most distinguishable characteristic of the inactivated and activated states. In inactivated state, these residues adopt outward positions which are oriented away from the active site, on the other hand, in the active conformation, they are oriented toward the active site, adopting inward positions [3]. The corresponding residues from spr1814 are Thr81 and Phe83. The above two residues of molecule A adopt the outward position in a similar manner to other receiver domain structures of inactive conformation. On the other hand, in molecule B, the conserved switch residues adopt an inward position, which is regarded as an active-like state, and the effector domain is somewhat released

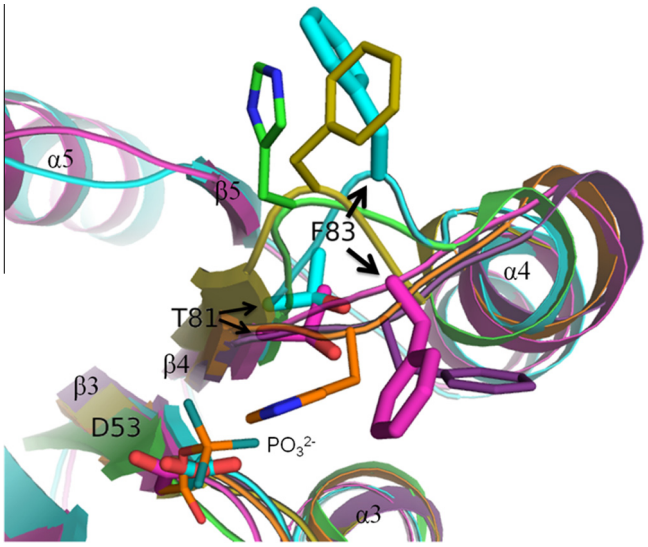

Fig. 2. Superposition of the phosphorylation site from molecule (A) and (B) of spr1814 (cyan and magenta, respectively) with inactive receiver domains of FixJ (green, PDB 1DBW) [24] and Spo0A (olive, PDB 1DZ3) [25] and active receiver domains of FixJ (orange, PDB 1D5W) [26] and Spo0A (purple, PDB 1QMP) [27]. The side chains are shown for position 'T81' and 'F83', and D53 residue of spr1814 and phosphorylated D54 of active FixJ. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

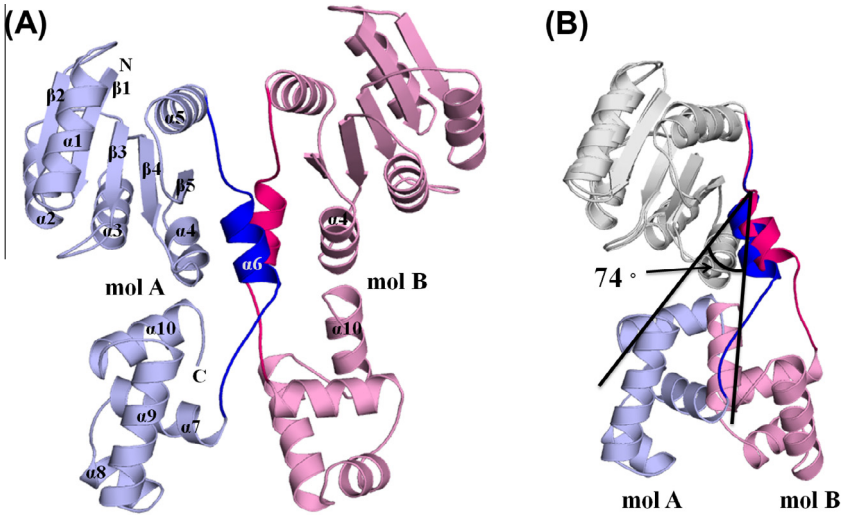

Fig. 1. X-ray crystal structure of full-length unphosphorylated spr1814. (A) Representation of spr1814. The two subunits are distinguished by light blue and light pink coloration, and the two linker regions are shown in blue and magenta. (B) Comparison of the effector domain between molecule A and B of spr1814. The only effector domain was colored for clarity and the same color scheme used for (A) was applied. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from the receiver domain in comparison with that of molecule A. In the previous reports, it has been suggested that phosphorylation triggers the release of the effector domain from the receiver domain in the NarL/FixJ subfamily [2,4,20]. Therefore, this has led us to speculate that molecule B exhibits an intermediate state between inactive and active conformations.

Particularly, Glu55 shows remarkable conformational change in both molecules (Fig. 3). The previous studies have reported that the residue which is equivalent to the Glu55 in spr1814 is one of the important determinants in modulating autodephosphorylation rates by altering accessibility of the attacking water molecule to the phosphoryl group [21,22]. In spr1814, it is suggested that two different conformations of Glu55 in molecule A and B affects the interdomain interactions resulting in a rotational change of the effector domain of molecule B. The previous receiver domain structure of spr1814 complexed with phosphoryl analog (BeF_3^-) revealed that (BeF_3^-) interacts with side chains of Thr81 and Lys103, which are conserved to be residues among RRs [9]. In the receiver domain of molecule B of unphosphorylated full-length spr1814, instead of (BeF_3^-), Glu55 interacts with the above two residues to retain their active-like conformations. In detail, the OE2 atom of the Glu55 hydrogen bonded with the OG1 atom of the Thr81, and an additional salt-bridge between the OE1 atom of Glu55 and the NZ atom of Lys103 was also formed. On the other hand, Glu55 points toward the opposite direction in molecule A, and thus, it is impossible for it to interact with the above residues. Instead, Glu55 of molecule A interacts with Arg85 on β4-α4. Also, Arg85 of molecule A is sequentially connected to Glu188 on the α10 helix of the C-terminal domain by a salt-bridge. As shown in Fig. 3, the interaction connecting Glu55, Arg85, and Glu188 keeps the effector domain of molecule A in order to tether to the receiver domain. On the other hand, the lack of these interactions due to the differing conformation of Glu55 in molecule B results in the released of the effector domain from the receiver domain. Furthermore, there is an additional interaction which is only observed in molecule A, of tightening a domain interaction between the oxygen atom of Phe83, as a conserved switch residue, and NH1 atom of Arg135 in the linker region.

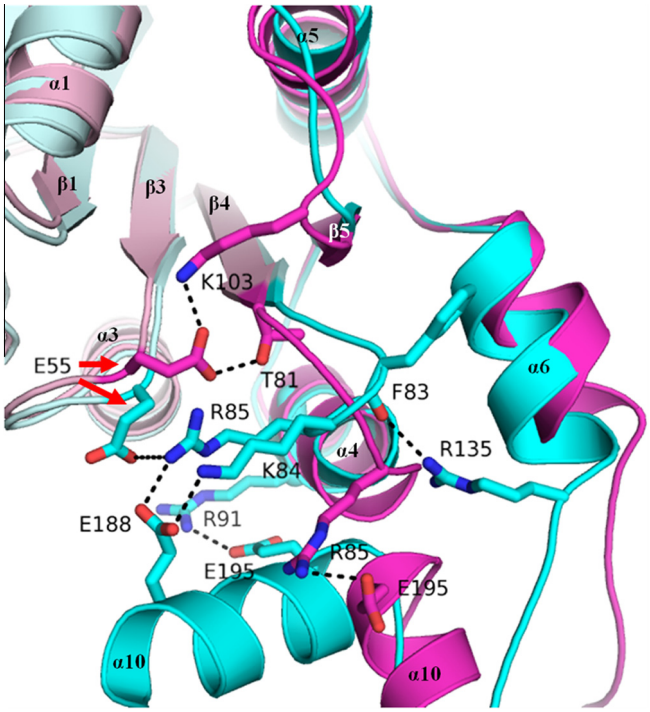

Fig. 3. Close-up view of the phosphorylation site and interdomain interface. The molecule A and B are colored as cyan and magenta, respectively. Note that E55 shows remarkable conformational change in both molecules. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Domain interfaces

The interactions between the receiver domain, the linker region, and the effector domain of spr1814 show a relatively moderate interface in comparison with that of NarL and DosR. While the buried surface area (BSA) of NarL and DosR are 900 $Å^2$ and 2500 $Å^2$, respectively [8,23], the BSA of molecule A and B of spr1814 are 634 $Å^2$ and 465 $Å^2$, respectively. The interdomain interactions of spr1814 are mediated mainly by salt-bridges in both molecules (Fig. 3). As mentioned previously, Arg85 in molecule A forms a salt bridge with Glu188 on α10 helix. Despite the different conformations, Arg85 of molecule B contributes to the domain interactions by forming a salt bridge with Glu195, which resides in the α10 helix. The salt-bridge between Arg85 and Glu195 is the only intra-side-chain interdomain interactions found in molecule B. On the other hand, additional interactions were observed in molecule A. The Glu188, which interacts with Arg85, also forms a salt-bridge with Lys84 on β4-α4. Moreover, a further salt-bridge is formed between Arg91 on α4 helix and Glu195 on α10 helix. Taken together, although the interdomain interactions between α4 and α10 helices are maintained, regardless of their conformational states, the tight interactions between the two domains that were observed in molecule A, were loosened in molecule B, which is regarded as an intermediate state. This finding suggests that domain movement, which causes the loosening of effector domain from receiver domain, could occur upon phosphorylation, and it could be achieved by the conformational change of the flexible linker region.

Acknowledgments

We thank the staff at beamline 4A of Pohang Light Source, South Korea for assistance during data collection. This work was supported by a Korea Research Foundation Grant (KRF-2007-359-C00026) funded by the Korean Government (MOEHRD).

References

[1] A.M. Stock, V.L. Robinson, P.N. Goudreau, Two-component signal transduction, Annu. Rev. Biochem. 69 (2000) 183–215.
[2] J.A. Hoch, Two-component and phosphorelay signal transduction, Curr. Opin. Microbiol. 3 (2000) 165–170.
[3] R. Gao, A.M. Stock, Biological insights from structures of two-component protein, Annu. Rev. Microbiol. 63 (2009) 133–154.
[4] I. Baikalov, I. Schröder, M. Kaczor-Grzeskowiak, K. Grzeskowiak, R.P. Gunsalus, R.E. Dickerson, Structure of the *Escherichia coli* response regulator NarL, Biochemistry 35 (1996) 11053–11061.
[5] K. Kurashima-Ito, Y. Kasai, K. Hosono, K. Tamura, S. Oue, M. Isogai, Y. Ito, H. Nakamura, Y. Shiro, Solution structure of the C-terminal transcriptional activator domain of FixJ from *Sinorhizobium meliloti* and its recognition of the fixK promoter, Biochemistry 44 (2005) 14835–14844.
[6] M.Y. Galperin, Structural classification of bacterial response regulators: diversity of output domains and domain combinations, J. Bacteriol. 188 (2006) 4169–4182.
[7] M. Milani, L. Leoni, G. Rampioni, E. Zennaro, P. Ascenzi, M. Bolognesi, An active-like structure in the unphosphorylated StyR response regulator suggests a phosphorylation-dependent allosteric activation mechanism, Structure 13 (2005) 1289–1297.
[8] G. Wisedchaisri, M. Wu, D.R. Sherman, W.G. Hol, Crystal structures of the response regulator DosR from *Mycobacterium tuberculosis* suggest a helix rearrangement mechanism for phosphorylation activation, J. Mol. Biol. 378 (2008) 227–242.
[9] A.K. Park, J.H. Moon, K.S. Lee, Y.M. Chi, Crystal structure of receiver domain of putative NarL family response regulator spr1814 from *Streptococcus pneumoniae* in the absence and presence of the phosphoryl analog beryllofluoride, Biochem. Biophys. Res. Commun. 421 (2012) 403–407.

[10] S. Da Re, J. Schumacher, P. Rousseau, J. Fourment, C. Ebel, D. Kahn, Phosphorylation-induced dimerization of the FixJ receiver domain, Mol. Microbiol. 34 (1999) 504–511.
[11] R.K. Lewis, D.J. Scott, J.A. Brannigan, J.C. Ladds, M.A. Cervin, G.B. Spiegelman, J.G. Hoggett, I. Barák, A.J. Wilkinson, Dimer formation and transcription activation in the sporulation response regulator Spo0A, J. Mol. Biol. 316 (2002) 235–245.
[12] A.K. Park, S.M. Bong, J.H. Moon, Y.M. Chi, Crystallization and preliminary X-ray crystallographic studies of DesR, a thermosensing response regulator in a two-component signalling system from *Streptococcus pneumonia*, Acta. Crystallogr. Sect. F 65 (2009) 727–729.
[13] Z. Otwenowsky, W. Minor, Processing of X-ray diffraction data collected in oscillation mode, Methods Enzymol. 276 (1997) 307–326.
[14] P.H. Zwart, P.V. Afonine, R.W. Grosse-Kunstleve, L.W. Hung, T.R. Ioerger, A.J. McCoy, E. McKee, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter, L.C. Storoni, T.C. Terwilliger, P.D. Adams, Automated structure solution with the PHENIX suite, Methods Mol. Biol. 426 (2008) 419–435.
[15] P. Emsley, K. Cowtan, *Coot*: model-building tools for molecular graphics, Acta. Crystallogr. Sect. D 60 (2004) 2126–2132.
[16] G.N. Murshudov, P. Skubák, A.A. Lebedev, N.S. Pannu, R.A. Steiner, R.A. Nicholls, M.D. Winn, F. Long, A.A. Vagin, *REFMAC5* for the refinement of macromolecular crystal structures, Acta. Crystallogr. Sect. D 67 (2011) 355–367.
[17] V.B. Chen, W.B. Arendall 3rd, J.J. Headd, D.A. Keedy, R.M. Immormino, G.J. Kapral, L.W. Murray, J.S. Richardson, D.C. Richardson, *MolProbity*: all-atom structure validation for macromolecular crystallography, Acta. Crystallogr. Sect. D 66 (2010) 12–21.
[18] W.L. DeLano, The PyMOL User's Manual, DeLano Scientific, San Carlos, CA, USA, 2002.
[19] G.P. Poornam, A. Matsumoto, H. Ishida, S. Hayward, A method for the analysis of domain movements in large biomolecular complexes, Proteins 76 (2009) 210–212.
[20] C. Birck, M. Malfois, D. Svergun, J. Samama, Insights into signal transduction revealed by the low resolution structure of the FixJ response regulator, J. Mol. Biol. 321 (2002) 447–457.
[21] S.A. Thomas, J.A. Brewster, R.B. Bourret, Two variable active site residues modulate response regulator phosphoryl group stability, Mol. Microbiol. 69 (2008) 453–465.
[22] Y. Pazy, A.C. Wollish, S.A. Thomas, P.J. Miller, E.J. Collins, R.B. Bourret, R.E. Silversmith, Matching biochemical reaction kinetics to the timescale of life: structural determinants that influence the autodephosphorylation rate of response regulator proteins, J. Mol. Biol. 392 (2009) 1205–1220.
[23] V.L. Robinson, T. Wu, A.M. Stock, Structural analysis of the domain interface in DrrB, a response regulator of the OmpR/PhoB subfamily, J. Bacteriol. 185 (2003) 4186–4194.
[24] P. Gouet, B. Fabry, V. Guillet, C. Birck, L. Mourey, D. Kahn, J.P. Samama, Structural transitions in the FixJ receiver domain, Structure 7 (1999) 1517–1526.
[25] R.J. Lewis, K. Muchová, J.A. Brannigan, I. Barák, G. Leonard, A.J. Wilkinson, Domain swapping in the sporulation response regulator Spo0A, J. Mol. Biol. 297 (2000) 757–770.
[26] C. Birck, L. Mourey, P. Gouet, B. Fabry, J. Schumacher, P. Rousseau, D. Kahn, J.P. Samama, Conformational changes induced by phosphorylation of the FixJ receiver domain, Structure 7 (1999) 1505–1515.
[27] R.J. Lewis, J.A. Brannigan, K. Muchová, I. Barák, A.J. Wilkinson, Phosphorylated aspartate in the structure of a response regulator protein, J. Mol. Biol. 294 (1999) 9–15.