Deciphering the activation and recognition mechanisms of *Staphylococcus aureus* response regulator ArlR

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ABSTRACT

*Staphylococcus aureus* ArlRS is a key two-component regulatory system necessary for adhesion, biofilm formation, and virulence. The response regulator ArlR consists of a C-terminal DNA-binding effector domain and an N-terminal receiver domain that is phosphorylated by ArlS, the cognate transmembrane sensor histidine kinase. We demonstrate that the receiver domain of ArlR adopts the canonical α5β5 response regulator assembly, which dimerizes upon activation, using beryllium trifluoride as an aspartate phosphorylation mimic. Activated ArlR recognizes a 20-bp imperfect inverted repeat sequence in the ica operon, which is involved in intercellular adhesion polysaccharide production. Crystal structures of the inactive and activated forms reveal that activation induces a significant conformational change in the β4-α4 and β5-α5-connecting loops, in which the α4 and α5 helices constitute the homodimerization interface. Crystal structures of the DNA-binding ArlR effector domain indicate that it is able to dimerize via a non-canonical β1–β2 hairpin domain swapping, raising the possibility of a new mechanism for signal transduction from the receiver domain to effector domain. Taken together, the current study provides structural insights into the activation of ArlR and its recognition, adding to the diversity of response regulation mechanisms that may inspire novel antimicrobial strategies specifically targeting *Staphylococcus*.

INTRODUCTION

*Staphylococcus aureus* has been recognized as a major human pathogen, the leading cause of skin and soft tissue infections. It can also cause endocarditis and disseminated abscesses anywhere, as well as life-threatening systemic infections including septic shock, and toxic shock syndrome (1). *S. aureus* is categorized as an ESKAPE pathogen, with its incredible ability to acquire antibiotic resistance by uptake of mobile genetic elements (2, 3), and methicillin-resistant *S. aureus* (MRSA) has emerged as a particularly resistant and widespread healthcare threat. Besides its many antibiotic resistance mechanisms, the success of *Staphylococcus* as a human pathogen is highly dependent on the production of multiple virulence factors that contribute to evasion of the host immune system. Despite its pathogenic potential, most *S. aureus* colonizes the skin and mucosal surfaces of approximately 30% of the healthy human population (4). This widespread prevalence and pathogenicity of *S. aureus* derives from its ability to survive in a variety of conditions and to adapt to many different environments, including almost every organ of the human body (5).

Bacterial two-component regulatory systems constitute the dominant signal transduction mechanism for bacterial adaptation to a variety of environment stimuli using a feedback mechanism (6, 7). Two-component regulatory systems typically consist of a membrane sensor histidine kinase, which receives a specific environment stimuli, and its cognate response regulator. After receiving a phosphorylation signal from the histidine kinase, the response regulator modulates target gene expression, hence providing a feedback response to environmental stimuli (7, 8).

In *Staphylococcus aureus*, there are more than 16 two-component regulatory systems that allow adaptation to diverse microenvironments (9). *S. aureus* two-component reg-

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ulatory systems have been implicated in virulence factor production (10), resistance to antibacterial agents and nitrosative stress (11,12), cell wall stability (13), biofilm formation, and survival in blood (14). Two-component regulatory systems are thus essential for pathogenesis in Staphylococcus aureus and potential targets for antimicrobial therapy (15,16).

Recently, the Staphylococcus aureus two-component regulatory system ArlRS has been implicated in pathogenesis mechanisms including adhesion, autolysis, biofilm formation, virulence factor regulation, agglutination and capsule regulation (17–20). Gene expression modulation by bacterial response regulators and their activation by phosphorylation have been extensively characterized, with a large number of response regulator structures solved (6,21,22). Despite the accumulated knowledge on two-component regulatory systems (22,23), the mechanism and specificity of the two-component regulatory system family in response to different environmental stimuli is variable, and the mechanism of the ArlRS two-component regulatory system has remained elusive.

The response regulator ArlR consists of an N-terminal receiver domain, which receives a phosphorylation signal from its cognate histidine kinase, ArlS, and a C-terminal DNA-binding effector domain that modulates downstream target gene expression. Here we report the X-ray crystal structures and biophysical characterization of the ArlS receiver domain in the presence and absence of phosphorylation mimic beryllium trifluoride, as well as the DNA-binding effector domain. We have further identified a 20-bp inverted repeat target sequence of activated ArlR located within the intracellular adhesion gene cluster (ica operon), and bioinformatics searching for similar sequences has identified additional potential ArlR-targeting DNA sequences within the S. aureus genome.

MATERIALS AND METHODS

Cloning, expression and purification of ArlR

DNA fragments corresponding to full length ArlR (amino acids 1–219), receiver domain (amino acids 1–121), and effector DNA-binding domain (amino acids 122–219) (numbering according to Uniprot Q9KJN4), were amplified from S. aureus and ligated into a pET28a expression vector with an N-terminal 6His tag or with a thrombin-cleavable purification, and the activated dimer fractions were further used for crystallization. ArlR receiver domain in complex with BeF$_3$ and magnesium, 5.3 mM BeSO$_4$, 35 mM NaF and 7 mM MgCl$_2$ were supplemented during the purification, and the activated dimer fractions were further used for crystallization (25,26). ArlR receiver domain in complex with BeF$_3$ and magnesium were crystallized in 0.2 M sodium formate, 20% (w/v) polyethylene glycol 3350; crystals of ArlR receiver domain in complex with magnesium were grown with 0.2 M magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5 and 25% (w/v) polyethylene glycol 3350 in the reservoir; the free ArlR receiver domain was grown in 0.2 M calcium chloride, 0.1 M MES and 20% (w/v) polyethylene glycol 3350. Crystals were cryoprotected with the reservoir condition in addition to 15% glycerol and flash frozen in a 100 K nitrogen stream. ArlR DNA-binding domain with His tag was crystallized in a reservoir with 0.1 M Tris pH 8.5 and 2.0 M ammonium sulfate. The ArlR DNA-binding domain with His tag cleaved was observed in the condition of 3.2 M sodium chloride, 0.1 M sodium acetate trihydrate pH 4.6.
Datasets were collected at Beamline 18U1 and 19U1 at Shanghai Synchrotron Radiation Facility. All the X-ray diffraction data were processed and scaled with XDS (27). Data collection details and statistics are summarized in Table 1.

Crystal structure determination and refinement

The structure of ArlR receiver domain was solved by molecular replacement, implemented in Phaser program suite program (28), using BeF$_3^-$-activated DrrD receiver domain structure (PDB: 3NNN) as the search model (29). Further manual model building and refinement were carried out with COOT and PHENIX suite (30,31). The crystal structure of ArlR receiver domain, bound with magnesium and in complex with magnesium and BeF$_3^-$, were all solved in P1 space group but with different cell constants. The ArlR receiver domain structure without magnesium was solved to 1.55 Å with an $R_{work}$ of 0.1736 and $R_{free}$ factor of 0.2058 in the unit cell of $a = 99.7^\circ$, $b = 102.3^\circ$, $c = 86.5^\circ$ and $a = 38.4$, $b = 41.2$, $c = 42.9$. The model contains two ArlR receiver domain monomers and includes amino acid residues 2–120, 222 water molecules, 3 calcium ions, and 1 MES molecule. One of the ArlR receiver domain monomer active sites is occupied by 1 calcium ion, but the other is not, as we further describe in the results section.

The ArlR receiver domain structure bound to magnesium was solved to 1.59 Å and refined to a final $R_{work}$ of 0.1553 and $R_{free}$ factor of 0.1893 with cell constants of $a = 99.7^\circ$, $b = 102.3^\circ$, $c = 86.5^\circ$ and $a = 43.6$, $b = 45.8$, $c = 61.8$. The final structure contains four ArlR receiver domain monomers (amino acid residues 2–120), 524 water molecules, 4 magnesium ions, 1 SO$_4$ ion and 1 HEPES molecule.

The ArlR receiver domain in complex with magnesium and BeF$_3^-$ was determined to 1.59 Å with a final $R_{work}$ of 0.1587 and $R_{free}$ factor of 0.1889 with cell parameter of $a = 90.4^\circ$, $b = 105.5^\circ$, $c = 92^\circ$ and $a = 61.1$, $b = 61.7$, $c = 66.1$. The final model contains 8 ArlR receiver domain monomers (amino acid residues 2–120), 1159 water molecules, 8 magnesium ions and 8 BeF$_3^-$ ions.

The ArlR DNA-binding domain with His tag was solved to 1.85 Å with a final $R_{work}$ of 0.1960 and $R_{free}$ of 0.2250. The crystal pack in a cell parameter of $a = 90^\circ$, $b = 120^\circ$ and $a = b = 66.9$, $c = 153.1$ in $P6_422$ space group. The final model contains a single ArlR DNA-binding domain (amino acid residue 123–219) and 105 water molecules. The ArlR DNA-binding domain with His tag cleaved was solved to 2 Å with $R_{work}$ of 0.1995 and $R_{free}$ of 0.2425 in the same space group and cell parameter as the ArlR DNA-binding domain with 6 His tag (Supplementary Table S5), the structure factor data used for the final re-

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Table 1. X-ray data collection and refinement statistics

| Crystal  | ArlR receiver domain | ArlR receiver domain with Mg$^{2+}$ | ArlR receiver domain with Mg$^{2+}$ & BeF$_3^-$ | ArlR DNA-binding domain |
|----------|----------------------|-------------------------------------|-----------------------------------------------|------------------------|
| Spacegroup | P1 | P1 | P1 | $P6_422$ |
| $a, b, c$ (Å) | 38.4, 41.2, 42.9 | 43.6, 45.8, 61.8 | 61.1, 61.7, 66.1 | 66.9, 66.9, 153.1 |
| Resolution (Å) | 33.0–1.55 (1.60–1.55) | 31.7–1.59 (1.65–1.59) | 31.7–1.59 (1.65–1.59) | 32.67–1.85 (1.92–1.85) |
| Statistics for the highest resolution shell are shown in parentheses. |
Electrophoretic mobility shift assay
The LightShift Chemiluminescent EMSA Kit (Thermo Scientific) was used for EMSA experiments, with DNA labeled with biotin, for the detection of protein-DNA interactions. The sequence used for the EMSA was the ica operon between icaADBC and icaR, the 5′-end biotin-labeled. The EMSA reaction protocol was the same as described previously (33). Briefly, binding reactions were incubated in 10 μl volumes containing 40 fmol DNA and protein in the binding buffer (10 mM Tris, 50 mM KCl, 1 mM DTT, 5% glycerol, pH 7.5) for 30 min. The reaction mixtures were loaded onto a 5% native polyacrylamide gel and separated in 0.5× TBE at 100 V on ice, followed by western blotting for biotin.

Quantitative PCR
The ArlR knockout and site-directed mutagenesis strains were generated using the protocol described previously (34). For the quantitative PCR experiments, 500 ul of ArlR knockout and site-directed mutant strains from overnight cultures were grown in 50 ml of TSB (17 g/l tryptone, 3 g/l soya peptone, 2.5 g/l D-glucose, 5 g/l NaCl, 2.5 g/l K2HPO4, pH 7.3) at 37°C for 12 h. Total RNA was isolated from planktonic cells using a Qiagen RNeasy Mini kit. RNA purity was checked by the Abs260/Abs280 ratio of 2.0 to 2.1. First-strand cDNA synthesis from total RNA was performed with the High-Capacity cDNA Reverse Transcription Kit (Bio-Rad) using 300 ng of total RNA as the template following the manufacturer’s instructions. Expression of icaA was determined by two-step real-time PCR using the Power SYBR Green PCR Master Mix (Bio-Rad) and Real-Time PCR System (Bio-Rad). Primers were annealed at 60°C, and the 16S-RNA was used as reference to normalize all data. The specificity of all qRT-PCR primers (Supplementary Table S1) was verified using normal PCR. Fold changes in various icaA transcripts in ArlR knockout and site-directed mutant strains in relative to pLi50-ArlR were calculated using the 2−ΔΔCt formula (35).

Small angle X-ray scattering
SAXS data collection was performed at the Shanghai Synchrotron Radiation Facility BL19U2 Beamline, carried out within a moment transfer range from 0.04 to 0.45 Å−1. ArlR DNA-binding domain, receiver domain, and full length with or without BeF3− and magnesium freshly purified from size exclusion chromatography were measured under batch mode at 293 K. For each sample, twenty frames were collected without measurable radiation damage detected. The molecular weight determination was carried out with the MoW2 server and Porod volume calculation (36). The ATSAS package was used for data interpretation, PRIMUS was used for the buffer subtraction and extrapolation, Guinier region was determined by Guinier approximation, the program GNOM was used to evaluate the radius of gyration Rg, forward scattering I0, maximum particle dimension Dmax, and the distance distribution function P(r) (37). The overall comparison of the crystal structure to SAXS experimental data was generated by CRYSOL (37,38). The small angle X-ray scattering data collection, analysis, and statistics information are summarized in Supplementary Table S2.

RESULTS
ArlR dimerizes in the presence of aspartate phosphorylation analogue BeF3− and Mg2+
To gain insight into the regulatory mechanism of the S. aureus response regulator ArlR, we successfully expressed and purified both full-length recombinant ArlR and its N-terminal receiver domain. Both constructs behaved as monomers in solution, as judged from their size exclusion chromatography profiles (Figure 1A). Within the response regulators, phosphorylation and dimerization are required for recognition of target DNA and downstream gene activation (22,23). Since BeF3− has been extensively used to mimic the phosphorylated aspartate group of the active state (25), full-length ArlR and its receiver domain were incubated with BeF3− and Mg2+. The addition of BeF3− and Mg2+ induced dimerization in both constructs, as indicated by size exclusion chromatography (Figure 1A, see Supplementary Table S2 for molecular weight estimation based on calibration curve).

We carried out small angle X-ray scattering (SAXS) experiments to further confirm ArlR dimerization induced by BeF3− (Supplementary Table S2). The Guinier region analysis and distance distribution function demonstrate that full-length BeF3−-ArlR exists in a larger assembly, as indicated by Dmax (Figure 1B and C). Further molecular weight calculation, generated from Porod volume using MoW2 software, is consistent with ArlR dimer formation in the presence of BeF3−. The SAXS data are consistent with the size exclusion chromatography results, strongly supporting that the aspartate phosphorylation analogue BeF3− induces dimerization of ArlR.

BeF3−-activated ArlR recognizes an inverted repeat in the ica operon
Staphylococcal biofilm formation depends on the production of sugar polymers by enzymes encoded by the icaADBC operon. ArlR was previously suggested to regulate biofilm formation in an ica-dependent manner, activating icaADBC operon expression in S. epidermidis (39). Recently, it has also been reported that ArlRS can modulate icaADBC expression at the transcriptional level using northern blotting in S. aureus (5). However, the recognition sequence for ArlR was not identified, possibly due to the low binding affinity of unphosphorylated ArlR. We have successfully used BeF3− to mimic phosphorylation of ArlR and generate stable activated dimeric ArlR. To further
validate the physiologic relevance of the phosphomimetic BeF$_3^-$-induced dimer, we studied its binding to the ica operon using electrophoretic mobility shift assay (EMSA). The ArlR wildtype monomeric protein binds the full promoter region between icaR and icaADBC with low affinity, as described previously (39). However, dimeric ArlR in complex with BeF$_3^-$ and Mg$^{2+}$ displayed significantly increased affinity, as indicated by EMSA (Figure 2A). We further applied DNase I footprinting assay to identify the DNA-binding motif of ArlR, using full-length ArlR in complex with BeF$_3^-$ and magnesium. The binding of activated ArlR protects a 20-bp AT-rich inverted repeat (Figure 2B): ATNGYANNTAANNTRCNAT, where N is any nucleotide and Y/R is a pyrimidine/purine, respectively (Figure 2B). The ArlR binding motif was located in the ica operon between the icaR and icaADBC loci, at around 50 bp upstream of the icaADBC start codon and 90 bp upstream from the icaR start codon on the other side (Supplementary Figure S1).

To further verify the identified ArlR-binding sequence within the ica operon, a short 24-bp DNA fragment including the 20-bp imperfect inverted repeat (with a 2-bp extension at both ends) was used for EMSA instead of the whole ica operon region. ArlR bound to the 24-bp fragment and caused a shift at an affinity comparable to that seen with the full ica operon region (Figure 2C). Mutation of position 4 or positions 1 and 6 in the identified sequence attenuated binding of activated ArlR (Figure 2D and E, respectively). Combined mutations of positions 1, 4 and 6 completely abolished the binding of activated ArlR to the DNA fragment (Figure 2F).

ArlR is reported to regulate over 114 genes involved in autolysis, cell division, growth, and pathogenesis (40), and multiple ArlR targeting sites have been speculated because of its broad regulation profile. We have performed bioinformatics searching on the whole S. aureus genome and identified more than 15 potential ArlR binding sites sharing sequence similarity with the identified imperfect inverted repeat ica operon DNA sequence (Supplementary Table S3). The consensus DNA sequence derived from whole genome searching indicate highly conserved A1,T2,G4,A6 and T9 positions, with positions 3 and 10 showing preferences for A or T, as shown using WebLogo (Figure 2G) (41). Many of the potential ArlR targeting sequence are located within or near the regulating regions of previously reported genes regulated by ArlR. Of particular interest, one of the top sequences identified was ATAGCATATTTATATGCAAT, which is within 40 bp of hypothetical protein SA0257 and probable ribokinase rbsK SA0258, genes shown to be downregulated in biofilm formation and close to other genes shown to be downregulated by ArlR (SA0252 and SA0253) (40,42).

**Crystal structures of activated and inactive ArlR receiver domain suggest its activation mechanism**

To further explore the activation mechanism of ArlR, we solved the crystal structure of activated ArlR receiver domain in complex with BeF$_3^-$ and Mg$^{2+}$ at 1.59 Å (Table 1). The ArlR receiver domain adopts a canonical αβ5 topology arranged as a central parallel β-sheet sandwiched by α1 and α5 on one side and α2, α3, and α4 on the other side.
**Figure 2.** BeF$_3^-$-activated ArlR recognizes an imperfect inverted repeat in the ica operon. (A) Purified ArlR in complex with BeF$_3^-$ and Mg$^{2+}$ is activated and binds the icaABCD operon, as indicated by EMSA. 0 µM, 1 µM and 5 µM ArlR with or without added BeF$_3^-$ and Mg$^{2+}$ is indicated. (B) The activated ArlR recognizes the AT-rich imperfect inverted repeat binding box (ATNGYANNTAANNTRCNAT) identified through DNase I footprinting. (C) EMSA experiment with the identified 24-bp ArlR-binding DNA sequence with concentration of 0 µM, 0.1 µM, 0.5 µM, 1 µM and 5 µM ArlR used in D, E, F panel as well. (D) EMSA experiment of activated ArlR with the identified ica operon DNA sequence with position 4 mutated. (E) EMSA experiment of activated ArlR with the identified recognition motif, with a buried surface area of 960 Å$^2$. A detailed interaction profile is shown in Supplementary Table S2. (G) Bioinformatics searching of the S. aureus genome for similar DNA sequences suggests a consensus sequence (Supplementary Table S3), shown here as generated by the WebLogo server.

(Figure 3A). Similar to other PhoB family members, there is a prominent dimerization interface comprised of α4-β5-α5, involving 21 hydrogen bonds and 22 salt bridges and burying a total surface area of 960 Å$^2$, as determined by PDBPISA (Figure 3A, B, Supplementary Table S4) (43). Positively charged residues R111, R113 and R118 from α5 interact with negatively charged α residues D97, D92 and D96 across the dimerization interface. Interestingly, Y98, which is involved in the typical response regulator Y–T coupling activation mechanism, forms a hydrogen bond with R111 across the dimer interface through its backbone. R118 also forms hydrogen bonds across the dimer interface with S72 in the loop between α3 and β4 (Figure 3B). A detailed list of dimer-forming interactions is provided in Supplementary Table S3. ArlR receiver domain has an overall sequence identity of 36% to the well-characterized OmpR/PhoB family response regulator, PhoP (Figure 3C). Most of the residues involved in dimerization, S72, D96, D97, Y98, R111 and R118, are conserved between ArlR and PhoP.

The highly conserved aspartate residue D52 at the C-terminal end of β3 is the phosphorylation site (Figure 3C). The aspartate phosphorylation analogue BeF$_3^-$ was found on the conserved D52 residue as expected. The phosphorylation of the aspartate residue in the response regulator domain regulates the activity of the effector DNA-binding domain. The atomic resolution crystal structure of ArlR receiver domain in complex with BeF$_3^-$ and Mg$^{2+}$ demonstrates that there are 7 residues from the β2-β5 strands and 2 water molecules involved in the complexation of BeF$_3^-$ and Mg$^{2+}$. BeF$_3^-$ binds the OD1 atom of D52, and the paired Mg$^{2+}$ ion coordinates with one fluoride atom from BeF$_3^-$. OD2 of D52, side chains of E8 and D9, the main chain carbonyl oxygen of M54 and two water molecules (Figure 4A). The other two fluoride atoms form hydrogen bonds with T79, A80, K101 and M54 (Figure 4A). These residues involved in BeF$_3^-$ and Mg$^{2+}$ binding are highly conserved in most response regulator family members (Figure 3C).

To assess the conformational changes between the active and inactive state of ArlR, we also solved the crystal structure of inactive ArlR receiver domain in the absence of BeF$_3^-$ and Mg$^{2+}$. Structural alignment of ArlR in the presence versus the absence of BeF$_3^-$ and Mg$^{2+}$ demonstrated an RMSD of 0.312 Å. Although inactive ArlR exists as monomer in solution, it crystallized as an active state-like dimer, similar to other members of this family (44, 45). This occurs because, within the PhoB family, helices α4 and α5 possess a fair degree of flexibility. Phosphorylation shifts the equilibrium towards an active conformation, but an active-like conformation is also present as a minor species in the unphosphorylated form, and this is what tends to crystallize. Thus, the dominant inactive conformation, which is
not conducive to dimerization, is not observed in the crystal structures. Nevertheless, it can be observed, by comparing the ArlR receiver domain in the absence and the presence of BeF$_3^-$/Mg$^{2+}$ that BeF$_3^-$/Mg$^{2+}$ binding pulls the loops connecting β4-α4 and connecting β5-α5 toward D52. Specifically, T79, which is located in β4-α4, and K101, located in β5-α5, directly complex BeF$_3^-$, causing conformational changes in their respective loops. In solution, it would be expected that these conformational changes be further transmitted to helices α4 and α5 to impact the dimerization surfaces in a phosphorylation-dependent manner. It was originally proposed in PhoP that the highly conserved threonine (T79 in PhoP) side chain on β5 is coupled to a conformational rearrangement of the adjacent aromatic Y98 residue located on β5, giving rise to a ‘Y–T’ coupling activation mechanism (26,45,46). However, this coupling was not observed in our ArlR crystal structures, suggesting that it may not be a feature universal to this family. Instead, what we observe is a conformational change in the β4-α4 and β5-α5-connecting loops, with the largest conformational changes to be found in K81, S82, D83 and Y86 in the β4-α4 connecting loop (Figure 4B).

**Divalent cation binding of ArlR**

A divalent cation is essential for the addition or removal of the phosphoryl group at D52 in the receiver domain. Most response regulator receiver domains prefer Mg$^{2+}$, but other divalent ions such as Mn$^{2+}$ and trivalent ions have been reported to promote reactions with even tighter binding, although without physiological validation (47,48). Crystals of apo ArlR receiver domain were produced in the presence of 0.2 M CaCl$_2$ in the reservoir. Interestingly, the resulting structures show that, within a dimer of ArlR receiver domains, one monomer was found to have a Ca$^{2+}$ ion bound, while the other monomer was free of cation. The calcium ion occupied the expected cation binding site liganded to four residues: D52, D9, E8 via a single water molecule, and the carbonyl group of M54. The binding of calcium caused a rotational switch of D52 resulting in conformational changes in K101 and M54 (Figure 5A). To investigate the divalent ion binding further, we crystallized the ArlR receiver domain in the presence of 10 mM Mg$^{2+}$ without BeF$_3^-$. Mg$^{2+}$ bound in a similar manner, but with six-ligand coordination (compared to the Ca$^{2+}$-bound form, two additional water molecules are seen complexing the Mg$^{2+}$ ion), in which the coordination with a BeF$_3^-$ fluoride atom was replaced by a water molecule (Figure 5B). Compared to the metal ion-free state of ArlR receiver domain, the Mg$^{2+}$-bound and Ca$^{2+}$-bound ArlR receiver domain were both structurally very similar, having RMSD values of 0.3 Å (Figure 5C). Notably, however, the Mg$^{2+}$-bound ArlR receiver domain more resembled the activated form even in the absence of BeF$_3^-$, whereas the Ca$^{2+}$-bound more resembled the form with no metal ion bound (Figure 5D).

**Crystal structure of the ArlR DNA-binding domain reveals domain swapping of the β1–β2 hairpin**

Based on sequence homology, the ArlR DNA-binding domain was classified as OmpR/PhoB-type, having a winged helix DNA-binding domain. The ArlR DNA-binding domain has an overall sequence identity of around 40% to the well-characterized PhoB, with most of the conserved residues in the DNA-binding helix-turn-helix region (Supplementary Figure S2). Here, we solve the crystal structure of the ArlR DNA-binding effector domain. The ArlR effector domain consists of four N-terminal β strands (β1–β4), followed by a three-helix-bundle (α1–α3) and a C-terminal β hairpin (β5–β6). However, compared to all other known
To validate the ArlR DNA-binding domain structure, we performed small angle X-ray scattering in solution. The Kratky plot is informative of the globularity and flexibility of a protein in solution. The curvature of the ArlR DNA-binding domain SAXS data was consistent with a globular domain with a partially unfolded region (Figure 6C). The Porod volume and SAXS MoW2 software indicated that the ArlR DNA-binding domain exists as a monomer in solution, consistent with the size exclusion chromatography data (Supplementary Table S2). However, the SAXS data are not consistent with a fully folded single compact domain, with a high $X^2$ value of 10.7 when the experimental data is fit against the curve predicted for the PhoB DNA-binding domain (Figure 6D). Inclusion of an additional N-terminal 6-His-tag on the PhoB model slightly improved the $X^2$ value to 9.4, indicating that the flexible His-tag on our ArlR construct could not account for the SAXS curve. Instead, the structure of a monomeric ArlR DNA-binding domain, with a β1-β2 hairpin extended away from the rest of the domain, demonstrated a compatible fit with the small angle x-ray scattering experiment data, with a $X^2$ value of 1.9. The fit of the experimental ArlR DBD SAXS data to a β1-β2-truncated ArlR DBD model yields a $X^2$ value over 100, as generated by FoXS (49). The hypothetical SAXS curve generated from the β1-β2-truncated ArlR DBD model is suggestive of a compact shape, similar to PhoB DBD, but demonstrated an apparent smaller size with $R_g$ of 12.6 Å as compared to the full ArlR DBD of 18.7 Å, both of which are inconsistent with the experimental SAXS data for ArlR DBD. Taken together, the SAXS experiment supports dissociation of the β1-β2 strands in the ArlR DBD (Figure 6D). It is likely that ArlR DBD exists in an ensemble of states that includes partially folded monomer (unfolding of the N-terminal β1-β2 strands), giving rise to the observed SAXS data.

It is interesting to observe that the N-terminal β sheet of the ArlR DNA-binding domain is unstable with a propensity to domain swap (Figure 6A). A β1-β2-β3-β4 structural sequence alignment of multiple PhoB/OmpR family DNA-binding domain generated from PROMALS3D indicates conserved secondary structure elements with relatively low sequence identity (Figure 6E) (50). In the well-characterized PhoB/OmpR family DNA-binding domain structure, the 4 residues between the β2 and β3 strand comprise a tight turn. PhoB and YycF have been shown to possess Type I β turns. However, the 4-residue sequence of ArlR at this position, KNAF, has a relatively low propensity for forming such a β turn structure, with lower turn propensities of each amino acid type at every position of the turn compared to PhoB: K (0.53), N (0.74), A (0.63), F (1.03) for ArlR (overall score of 0.25) versus P (1.28), T (0.82), S (1.49), H (1.13) for PhoB (overall score of 1.77) (51). Thus, it is possible that the main factor destabilizing the intramonomeric β-sheet in ArlR versus PhoB is the preference of the sequence between strands β2 and β3 in ArlR to form a structure other than a tight turn.

The program H-predictor calculates theoretical unfolding temperatures ($\Delta E/\Delta L$) at every position in a protein by treating each position as a putative hinge region for domain swapping, and then calculating the predicted change in enthalpy ($\Delta E$) divided by the change in average minimal path
(ΔL) (52). H-predictor analysis of ArlR unfolding temperatures (ΔE/ΔL) revealed that the 134KNAF137 sequence has a global minimum ΔE/ΔL value, which supports the notion that the KNAF sequence is a potential hinge region for domain swapping (Supplementary Figure S3).

To further support the domain swapping of the β1–β2 hairpin observed in the ArlR DNA-binding domain, we engineered double cysteine point mutations, A136C and F137C with its distance favorable for cysteine disulfide bridge engineering. These residues form intermolecular contacts in the domain-swapped dimer (Figure 6A). It is known that following purification of ArlR directly from an affinity column, there exists a small population of active ArlR able to bind to DNA without addition of BeF$_3^-$, which has been explained as being due to the response regulator accepting phosphoryl groups from intermediary metabolites such as acetyl phosphate and carbamoyl phosphate acting as phosphor-donors (39,53). Consistent with this low basal level of ArlR activation and dimer formation, the A136C-F137C double mutant form of ArlR full-length protein is observed to form a small population of disulfide-linked dimer, and the addition of DTT eliminated this dimer population (Figure 6F).

The small angle X-ray scattering data for the full length ArlR in complex with BeF$_3^-$ and Mg2+ agrees with the rigid model generated from the receiver domain and domain swapped DNA-binding domain, with the missing N-terminal tag residues modeled as spheres with CORAL and SASREF program in the ATSAS package (Figure 6G) (37).

**Functional characterization of ArlR mutants involved in its activation and recognition**

We applied a series of point mutations to the receiver domain and DNA-binding domain to determine the functional relevance of these residues in vivo by monitoring the impact of mutations on icaA transcriptional level. The key mutations include D96A/D97A, Y98A, R111A, R113A, R117A/R118A, all designed to disrupt ArlR dimerization as confirmed by the size exclusion chromatography profiles of the recombinant mutants ArlR purification (Supplementary Figure S4). All of these mutants also demonstrated a significant decrease in icaA transcription, confirming that our structural models are consistent with those of other response regulators (33). Mutations in conserved residues in the DNA-binding domain, including R196A, Y216A and the minor groove anchoring residue R212A, were designed to disrupt recognition of target DNA, and all of these significantly reduced the transcriptional level of icaA (Figure 7). Interestingly, the deletion of the β1–β2 hairpin linking the receiver domain and the effector domain in ArlR also demonstrates a strong disruption of icaA transcription (Figure 7).

**DISCUSSION**

ArlR is classified as a member of the bacterial OmpR/PhoB response regulator family. Although its secondary structure elements and basic two-domain organization resemble that of the other family members, ArlR has a number of features that are unique and have not previously been observed in this family. First of all, the ArlR effector domain binds to target DNA sequences in the form of inverted repeats, whereas PhoB and most other homologs have been shown to bind to direct repeats. Generally speaking, however, winged helix-turn-helix DNA-binding domain response regulators have been reported to recognize either direct repeat or inverted repeat target sequences (54–58). DNA-binding domains that bind to inverted repeats tend to form symmetric dimers, we observe in the X-ray crystal structure of the domain-swapped DBD dimer, which also characterized in Mycobacterium tuberculosis of RegX3 (59).

The ArlR DNA-binding domain dimer is connected by a short KNAF sequence that is key to the domain swapping mechanism. This sequence is not favorable for the tight turn observed in other homologs, and it adopts a somewhat extended conformation that acts as a short connecting linker between monomers of the domain swapped dimer. The DNA-binding a3 helices that suggested to bind to the DNA major groove are perfectly spaced in the domain swapped dimer to bind to adjacent major grooves, corresponding to one helical B-DNA step or 10–11 nucleotides. Thus, dimerization of the receiver domain would potentially drive domain-swapped dimer formation of the DNA-binding domain with the domain orientation needed to bind the inverted repeat target sequence. However, in full-length ArlR, domain orientations would be constrained by the very short 4-residue PQKD linker between the receiver domain and DNA-binding domain. The inter-domain linkers from each monomer are brought into close proximity by dimerization of the receiver domain (see Figure 3A), and this would require a 60- to 90-degree twist of both DNA-binding domains along the A136-F137 connector sequence (see Figure 6A), necessitating a rearrangement of the A136-F137 dimeric interface. It is unknown whether this rearrangement would be more favorable in the context
of a domain-swapped dimer or a non-swapped dimer that would place fewer constraints on the A136–F137 interface. The twist rotation necessary to bring the N-termini of the DBD domains into close proximity (see Figure 6A) also positions the α3 helices into the right orientation (facing the same way) to bind a DNA inverted repeat, suggesting that this is the actual geometry behind dimerization-driven DNA recognition, further evidence such as solution NMR structure of ArlR full length would validate our hypothesis.

ArlR activation of the receiver domain engages a significant conformational change in the β4–α4 and β5–α5-connecting loops, and possible isomerization of the neighboring aromatic Y85 from α4, which would lead to a reorientation of helices α4 and α5 to promote dimerization. However, the classical Y–T coupling mechanism is not observed in ArlR, as Y98 remains in the same conformation in all crystal forms. ArlR may be part of a non-Y–T coupling activation mechanism subfamily of response regulators, similar to SdrG, for which it was proposed that a reorganization of several aromatic residues around a conserved motif is implicated in activation rather than Y–T coupling (60).

ArlR is a master regulator reported to globally regulate over hundred genes expression involved in autolysis, cell division, growth, and pathogenesis (40). However the direct targets of ArlR have not been identified, possibly due to the difficulty associated with preparing activated ArlR. The ArlR binding motif we have identified in the ica operon is the only ArlR binding site identified thus far in S. aureus, and it is distinct from the proposed binding motif involved in multidrug resistance transporter NorA modulation (61). This strongly supports the role of ArlR in regulating biofilm formation in an ica-dependent manner (62,63). The successful identification of an ArlR binding site within the ica operon has also suggested a consensus motif for binding that will enable the identification of other ArlR-modulated genes. This will help to characterize the ArlR regulation network and to elucidate its multi-faceted role in biofilm formation and pathogenesis.

In conclusion, we demonstrate that ArlR functions in a manner similar to other two-domain response regulators involving a distinct activation mechanism, with phosphorylation driving dimerization of the effector domain. The ArlRS two-component regulatory system plays a unique role in promoting the pathogenicity of S. aureus, making it an interesting potential target in the development of new antimicrobial strategies.

DATA AVAILABILITY
Structure factors and atomic coordinates have been deposited in the Protein Data Bank under accession codes: 6I51 (ArlR receiver domain in complex with BeF3− and Mg2+), 6I52 (ArlR receiver domain in complex with Mg2+), 6I53 (ArlR receiver domain) and 6I54 (ArlR DNA-binding domain).

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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REFERENCES

1. Tong, S.Y.C., Davis, J.S., Eichenberger, E., Holland, T.L. and Fowler, V.G. (2013) Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin. Microbiol. Rev., 28, 603–661.
2. Pendleton, J.N., Gorman, S.P., Gilmore, B.F., Pendleton, J.N. and Gilmore, B.F. (2013) Clinical relevance of the ESKEAPE pathogens. Expert Rev. Anti. Infect. Ther., 11, 297–308.
3. Malachowa, N. and DeLeo, F.R. (2010) Mobile genetic elements of Staphylococcus aureus. Cell. Mol. Life Sci., 67, 3057–3071.
4. Wertheim, H.F.L., Mellés, D.C., Vos, M.C., Van Leeuwen, W., Van Belkum, A., Verbrugh, H.A. and Nouwen, J.J. (2005) The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect. Dis., 5, 751–762.
5. Burgui, S., Gil, C., Solano, C., Lasa, I. and Valle, J. (2018) A systematic evaluation of the two-component systems network reveals that ArlRS is a key regulator of catheter colonization by Staphylococcus aureus. Front. Microbiol., 9, 1–11.
6. Stock, A.M., Robinson, V.L. and Goudreau, P.N. (2000) Two-Component signal transduction. Annu. Rev. Biochem., 69, 183–215.
7. Groisman, E.A. (2016) Feedback control of two-component regulatory systems. Annu. Rev. Microbiol., 70, 103–124.
8. Capra, E.J. and Laub, M.T. (2012) The evolution of two-component signal transduction systems. Annu. Rev. Microbiol., 66, 325–347.
9. Orlet, P., Whitworth, D.E., Sontella, C., Achouak, W. and Barakat, M. (2015) P2CS: updates of the prokaryotic two-component systems database. Nucleic Acids Res., 43, D536–D541.
10. Liu, Q., Yeo, W.S. and Bae, T. (2016) The SaRS two-component system of Staphylococcus aureus. Genes (Basel), 7, 1–20.
11. Kawada-Matsuo, M., Yoshida, Y., Nakamura, N. and Komatsuzawa, H. (2011) Role of two-component systems in the resistance of Staphylococcus aureus to antibacterial agents. Virulence, 2, 427–430.
12. Kinkel, T.L., Roux, C.M., Dunman, P.M. and Fang, F.C. (2013) The Staphylococcus aureus SrrAB two-component system promotes resistance to nitrosative stress and hypoxia. MBio, 4, 1–9.
13. Kolar, S.L., Nagarajan, V., Ozsminia, A., Rivera, F.E., Miller, H.K., Davenport, J.E., Riordan, J.T., Potempa, J., Barber, D.S., Koziel, J. et al. (2011) NsaRS is a cell-envelope-stress-sensing two-component system of Staphylococcus aureus. Microbiology, 157, 2206–2219.
14. Hall, J.W., Yang, J., Guo, H. and Ji, Y. (2015) The AirlRS two-component system contributes to Staphylococcus aureus survival in human blood and transcriptionally regulates sspABC operon. Front. Microbiol., 6, 1–12.
15. Watanabe, T., Okada, A., Gotoh, Y. and Utsumi, R. (2008) Inhibitors targeting two-component signal transduction. Adv. Exp. Med. Biol., 631, 229–236.
16. Gotoh, Y., Eguchi, Y., Watanabe, T., Okamoto, S., Doi, A. and Utsumi, R. (2010) Two-component signal transduction as potential drug targets in pathogenic bacteria. Curr. Opin. Microbiol., 13, 232–239.
23. Gao, R., Mack, T.R. and Stock, A.M. (2007) Bacterial response
21. Wen, Y., Ouyang, Z., Devreese, B., He, W., Shao, Y., Lu, W. and
19. Walker, J.N., Crosby, H.A., Spaulding, A.R., Salgado-Pabón, W.,
Malone, C.L., Rosenthal, C.B., Schleifer, P.M., Boyd, J.M. and
Horswill, A.R. (2013) The Staphylococcus aureus ArlRS Two-Component system is a novel regulator of agglutination and pathogenesis. *PLoS Pathog.*, 9, 1–17.

20. Luong, T.T. and Lee, C.Y. (2006) The arl locus positively regulates Staphylococcus aureus type S capsule via an mraA-dependent pathway. *Microbiology*, 152, 3123–3131.

26. Li, Y.C., Chang, C.K., Chang, C.F., Cheng, Y.H., Fang, P.J., Yu, T.,
Wen, Y., Ouyang, Z., Devreese, B., Higgins, P.G., Wu, Y., Wang, J.,
Wang, W., and Zheng, F. (2017) Crystal structure of master biofilm regulator CsgD regulatory domain reveals an atypical receiver domain. *Protein Sci.*, 26, 2073–2082.

24. Gao, R., Mack, T.R. and Stock, A.M. (2007) Bacterial response regulators: versatile regulatory strategies from common domains. *Trends Biochem. Sci.*, 32, 225–234.

29. Barbieri, C.M., Mack, T.R., Robinson, V.L., Miller, M.T. and Stock, A.M. (2010) Regulation of response regulator autophosphorylation through interdomain contacts. *J. Biol. Chem.*, 285, 32325–32335.

34. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCT method. *Methods*, 25, 402–408.

36. Fischer, H., De Oliveira Neto, M., Napolitano, H.B., Polikarpov, I. and Craievich, A.F. (2010) Determination of the molecular weight of proteins in solution from a single small-angle X-ray scattering measurement on a relative scale. *J. Appl. Crystallogr.*, 43, 101–109.

37. Petoukhov, M.V., Franke, D., Shkumatov, A.V., Tria, G.,
Kikhney, A.G., Gajda, M., Gorba, C., Mertens, H.D.T., Konarev, P.V. and Svergun, D.I. (2012) New developments in the ATSAS program for small-angle scattering data analysis. *J. Appl. Crystallogr.*, 45, 342–350.

38. Svergun, D., Barberato, C. and Koch, M.H.J. (1995) CRYSOL - a Program to Evaluate X-ray Solution Scattering of Biological Macromolecules from Atomic Coordinates. *J. Appl. Crystallogr.*, 28, 768–773.

39. Wu, Y., Wang, J., Xu, T., Liu, J., Yu, W., Lou, Q., Zhu, T., He, N., Qu, D., Ben, H. et al. (2012) The Two-Component signal transduction system arls regulates staphylococcus epidermidis biofilm formation in an ica-Dependent Manner. *PLoS One*, 7, e40041.

40. Liang, X., Zheng, L., Landwehr, C., Lunsford, D. and Holmes, D. (2005) Global regulation of gene expression by ArlRS, a Two-Component signal transduction regulatory system of staphylococcus aureus. *J. Bacteriol.*, 187, 5486–5492.

41. Crooks, G., Hon, G., Chandonia, J. and Brenner, S. (2004) WebLogo: a sequence logo generator. *Genome Res.*, 14, 1188–1190.

42. Beeken, K.E., Dunman, P.M., Mcaulese, F., Macapagal, D., Murphy, E., Projan, S.J., Bleivins, J.S. and Smeltzer, M.S. (2004) Global Gene Expression in Staphylococcus aureus Biofilms. *J. Bacteriol.*, 186, 4665–4684.

43. Krissinel, E. and Henrick, K. (2007) Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.*, 372, 747–774.

44. Toro-Roman, A., Wu, T. and Stock, A.M. (2005) A common dimerization interface in bacterial response regulators KdpE and TorK. *Protein Sci.*, 14, 3077–3088.

45. Bachhawat, P. and Stock, A.M. (2007) Crystal structures of the receiver domain of the response regulator PhoP from Escherichia coli in the absence and presence of the phosphoryl analog beryllium fluoride. *J. Bacteriol.*, 189, 5987–5995.

46. Wang, S. (2012) Bacterial Two-Component Systems: Structures and Signaling Mechanisms. In: Cai, H (ed). *Protein Phosphorylation in Human Health*. InTech.

47. Needham, J. V., Chen, T.Y. and Falke, J.J. (1993) Novel ion specificity of a carboxylate cluster Mg (II) Binding Site: Strong charge selectivity and weak size selectivity. *Biochemistry*, 32, 3363–3367.

48. Febs, S., Lukat, G.S., Stock, A.M., and Stock, J.B. (1990) Divalent metal ion binding to the CheY Protein and its significance to phosphotransfer in bacterial chemotaxis. *Biochemistry*, 29, 5436–5442.

49. Schneidman-duShony, D., Hammel, M., Tainer, J.A., Sali, A., Angle, S. and Saxs, S.X. (2016) FoXS, FoXSDock and Multi-FoXS: Single-state and multi-state structural modeling of proteins and their complexes based on SAXS profiles. *Nucleic Acids Res.*, 44, 424–429.

50. Pei, J., Kim, B.H. and Grishin, N.V. (2008) PROMALS3D: A tool for multiple protein sequence and structure alignments. *Nucleic Acids Res.*, 36, 2295–2300.

51. Shen, Y. and Bax, A. (2012) Identification of helix capping and β-turn motifs from NMR chemical shifts. *J. Biomol. NMR*, 52, 211–232.

52. Ding, F., Prutzman, K.C., Campbell, S.L. and Dokholyan, N.V. (2006) Topological determinants of protein domain swapping. *Structure*, 14, 5–14.

53. Lukat, G.S., McClear, W.R., Stock, A.M. and Stock, J.B. (2006) Phosphorylation of bacterial response regulator proteins by low molecular weight phospho-donors. *Proc. Natl. Acad. Sci. U.S.A.*, 89, 718–722.

54. Edayathumangalam, R., Wu, R., Garcia, R., Wang, Y., Wang, W., Kreinberg, C.A., Bach, A., Liao, J., Stone, T.A., Terwilliger, T.C. et al. (2013) Crystal structure of Bacillus subtilis GabR, an autorepressor and transcriptional activator of gabT. *Proc. Natl. Acad. Sci. U.S.A.*, 110, 17820–17825.

55. Ryan, P.L., Friendship, R.M. and Raeside, J.I. (1986) Impaired estrogen production by Leydig cells of the naturally retained testis in unilaterally orchidectomized boars and stallions. *J. Androl.*, 7, 100–104.

56. He, X., Wang, L. and Wang, S. (2016) Structural basis of DNA sequence recognition by the response regulator PhoP in Mycobacterium tuberculosis. *Sci. Rep.*, 6, 1–11.

57. Martinez-Hackert, E. and Stock, A.M. (1997) The DNA-binding domain of OmpR: Crystal structure of a winged helix transcription factor. *Structure*, 5, 109–124.

58. Maris, A.E., Sawaya, M.R., Kazcor-Grzeskowiak, M., Jarvis, M.R., Pearson, S.M.D., Kopka, M.L., Schröder, I., Gunsalus, R.P. and Dickerson, R.E. (2002) Dimerization allows dna target site recognition by the nrl response regulator. *Nat. Struct. Biol.*, 9, 771–778.
59. King-scott,J., Nowak,E., Mylonas,E., Panjikar,S., Roessle,M., Svergun,D.I. and Tucker,P.A. (2007) The structure of a Full-length response regulator from mycobacterium tuberculosis in a stabilized Three-dimensional Domain-swapped, Activated State *. J. Biol. Chem., 282, 37717–37729.

60. Campagne,S., Dintner,S., Gottschlich,L., Thibault,M., Bortfeld-Miller,M., Kaczmarczyk,A., Francez-Charlot,A., Allain,F.H.T. and Vorholt,J.A. (2016) Role of the PFXFATG[G/Y] Motif in the activation of SdrG, a Response regulator involved in the alphaproteobacterial general stress response. Structure, 24, 1237–1247.

61. Fournier,B., Aras,R. and Hooper,D.C. (2000) Expression of the multidrug resistance transporter nora from staphylococcus aureus is modified by a Two-Component regulatory system. J. Bacteriol., 182, 664–671.

62. Toledo-arana,A., Merino,N., Vergara-irigaray,M., De,M. and Penade,R. (2005) Staphylococcus aureus develops an alternative, ica-Independent biofilm in the absence of the arlRS Two-Component System †. J. Bacteriol., 187, 5318–5329.

63. Wu,Y., Liu,J., Jiang,J., Hu,I., Xu,T., Wang,J. and Qu,D. (2014) Microbial Pathogenesis Role of the two-component regulatory system arlRS in ica operon and aap positive but non-bio fi lm-forming Staphylococcus epidermidis isolates from hospitalized patients. Microb. Pathog., 76, 89–98.