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The Structure of *S. lividans* Acetoacetyl-CoA Synthetase Shows a Novel Interaction Between the C-terminal Extension and the N-terminal Domain

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Abstract

The AMP-forming acyl-CoA synthetase enzymes catalyze a two-step reaction that involves the initial formation of an acyl adenylate that reacts in a second partial reaction to form a thioester between the acyl substrate and CoA. These enzymes utilize a *Domain Alternation* catalytic mechanism, whereby a ~110 residue C-terminal domain rotates by 140° to form distinct catalytic conformations for the two partial reactions. The structure of an acetoacetyl-CoA synthetase (AacS) is presented that illustrates a novel aspect of this C-terminal domain. Specifically, several acetyl- and acetoacetyl-CoA synthetases contain a 30-residue extension on the C-terminus compared to other members of this family. Whereas residues from this extension are disordered in prior structures, the AacS structure shows that residues from this extension may interact with key catalytic residues from the N-terminal domain.

Keywords

Enzymes; Structural Biology; Adenylate-Forming Enzymes; ANL Superfamily; Lysine Acetylation

AMP forming acyl-CoA synthetases are members of the ANL Superfamily, a family of adenylate-forming enzymes that are involved in primary and secondary metabolism. This family contains additionally the NRPS adenylation domains as well as beetle luciferases.[1] These enzymes range in size from 50–70 kD and contain a large N-terminal domain of 400–550 residues and a smaller C-terminal domain that is approximately 120 residues in length. The enzymes catalyze two-step reactions that involve the initial adenylation of a carboxylate substrate to form an acyl adenylate. This initial reaction is followed by a second partial reaction, generally transfer of the acyl group from the adenylate to the pantetheine thiol of either CoA or a *holo*-acyl or peptidyl carrier protein. In contrast, the luciferase enzymes catalyze an oxidative decarboxylation to produce the photoactive product. The enzymes of

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this family exhibit several interesting features of their structural and biochemical mechanisms. First, the enzymes undergo a process known as domain alternation whereby the smaller C-terminal domain uses a 140° rotation to adopt two distinct catalytic conformations to carry out the two partial reactions. With this rotation, a catalytic lysine that is part of a conserved region that has been designated the A10 motif is transferred into the active site to assist the formation of the adenylate intermediate. Numerous biochemical and structural studies have confirmed this domain alternation for acyl-CoA synthetases[2, 3], NRPS adenylation domains[4, 5], and beetle luciferase enzymes.[6, 7]

A second interesting feature of the acyl-CoA synthetases concerns their regulation by post-translational acetylation of the A10 lysine within some family members. The features that dictate recognition of acyl-CoA synthetases by the protein acetyl transferase (PAT) enzymes are unknown and have been the target of structural and biochemical investigation. Acetyl-CoA synthetase from *Salmonella enterica*, for example, is regulated by acetylation of the catalytic lysine 609.[8] The crystal structure of the native and acetylated enzymes[9] demonstrate no large structural changes that result from the acetylation and the inhibition of the acetylated enzyme is thought to result specifically from the unavailability of the side chain amine of the lysine residue. Recent work[10, 11] has shown that the C-terminal domain of acetyl-CoA synthetase can dictate the PAT interaction. Specifically, *Streptomyces lividans* contains two closely related enzymes that differ in acetylation susceptibility. The *S. lividans* acetyl-CoA synthetase (*SlAcs*) is acetylated inefficiently by the *SlPatA*, while an acetoacetyl-CoA synthetase (*SlAacS*) is readily acetylated.

Similarly, the *SlPatA* enzyme is able to recognize and acetylate the *Acs* enzyme from *Salmonella enterica* (*SeAcs*).[10] Studies with chimeric enzymes between *SlAcs* and *SeAcs* narrowed down the difference in recognition by the *SlPatA* acetyltransferase to a stretch of 77 residues from the C-terminal domain. Because of conservation of residues between the two proteins, as few as 41 amino acid residues were responsible for the difference in *PatA* recognition.

The C-terminal domain of members of this adenylate-forming superfamily starts with a two stranded antiparallel β -sheet that has been referred to as the A8 motif, one of 10 conserved sequence regions in this enzyme family.[1] This A8 loop is followed by a small domain composed of a central three stranded β -sheet that is surrounded by several α -helices. Interestingly, many short chain acyl-CoA synthetase enzymes are larger than other superfamily members because of a C-terminal extension. Following a common α -helix at the C-terminus that is shared by all family members, the structures of yeast[12] and bacterial[9] *Acs* contain an additional helix (Figure 1A). Inspection of sequence databases show that this extension is common to enzyme family members that produce CoA thioesters with short chain acids such as acetate, propionate, and acetoacetate. Unfortunately, the C-terminal extension is not fully resolved in any of the structures published to date.

To continue our investigation of the structural aspects of domain alternation of the ANL family of enzymes, we have determined the structure of the acetoacetyl-CoA synthetase from *S. lividans* (*SlAacS*). The structure in the adenylate-forming conformation and the loop joining the two C-terminal helices is visible. We present here this structure along with an

analysis of the C-terminal domain and the regions that have been identified from chimeric studies as potentially playing a role in recognition by the protein acetyl transferase.

Materials and Methods

Protein purification

The *aacS* gene encoding acetoacetyl-CoA synthetase gene from *S. lividans* TK24 (NCBI Accession #: AJ17050.1; E.C. 6.2.1.16) was cloned, expressed, and purified as described previously.[11] For structural studies, *SIAacS* was dialyzed into 10 mM Tris-HCl, pH 8.0 concentrated to 11.4 mg mL⁻¹ (~157 μ M).

Kinetic analysis of *SIAacS*

The kinetic parameters for acetoacetate, ATP, and CoA for *SIAacS* were measured using the NADH consumption assay which detects the oxidation of two mol of NADH to NAD⁺ for every 1 mol of AMP formed through the sequential catalytic activities of myokinase, pyruvate kinase, and lactate dehydrogenase.[13] For each determination of apparent kinetic constants, the non-varied substrates were kept constant at 1.25 mM. Variable substrate concentrations were tested for acetoacetate at 0.025 to 5 mM, for ATP at 0.015 to 2 mM, and for CoA from 10 μ M and 0.6 mM. The 100 μ L reactions contained 15 mM MgCl₂, 400 μ M NADH, 3 mM phosphoenol pyruvate, 1 U myokinase (EC 2.7.4.3), 1 U pyruvate kinase (EC 2.7.1.40), and 1 U lactate dehydrogenase (EC 1.1.1.27) in 50 mM HEPES-KOH (pH 7.5, 30° C). The reaction was initiated by the addition of *SIAacS* to a final concentration of 0.250 μ M. Activity was monitored by the decrease in absorbance at 340 nm over 5 min in a 96 well black wall, clear bottom plate (Costar) using a BioTek Synergy4 plate reader in kinetics mode with continuous shaking. Kinetic analysis was computed through Prism.

SIAacS Structure Determination

Initial crystallization hits were obtained from co-crystallization of *SIAacS* at 11 mg mL⁻¹ with 1.5x molar excess of buffered AMP and acetoacetate from a sparse matrix screen by hanging-drop vapor diffusion at 4°C. Initial crystals were observed in vapor diffusion experiments in which the cover slip was slightly ajar. This was found to be reproducible and optimized crystals grew in ~1 week with K₃Citrate (0.7–1.2M) and 50 mM BTP-HCl pH 7.0 at 14 °C with the cover slip left ajar. Hexagonal rod-shaped crystals were cryoprotected through sequential washes with reservoir solution with increasing concentrations of ethylene glycol (8, 16, and 24%, respectively) and vitrified in liquid N₂ prior to shipment to SSRL beam-line 7.1 for remote data collection on the ADSC Quantum 315r CCD using BluIce. [14]

Diffraction data were processed with HKL2000.[15] Molecular replacement was used for initial phases using phenix.AutoMR maximum-likelihood method with full length *S. enterica* Acetyl-CoA Synthetase (PDBID:1PG4) stripped of waters and cofactors/substrates as the search model. Phases derived from the molecular replacement solution were provided to the autobuild module of PHENIX with default settings.[16] The remaining model was manually built in COOT and refined in PHENIX using simulated annealing and individual

B-factor modeling. No TLS refinement was included. Model validation was carried out during the building process with MOLPROBITY [17] implemented in Phenix.[16]

The structure factors and coordinates have been deposited in the Protein Data Bank with accession code **4WD1**.

Results and Discussion

S/AacS Kinetics

We initially analyzed the kinetic parameters of *S/AacS* to allow enzymatic comparison with the *S/AacS* chimeric protein utilized in this study. The NADH consumption assay monitors the oxidation of NADH as AMP is released in the *S/AacS* reaction. Briefly, for ATP, *S/AacS* has a $k_{\text{cat}} = 6.43 \text{ s}^{-1}$, $k_{\text{cat}}/K_{\text{M}} = 3.24 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, and a specific activity of $5.36 \mu\text{mol min}^{-1} \text{ mg}^{-1}$ at 1.25 mM of ATP. For CoA, *S/AacS* has a $k_{\text{cat}} = 8.16 \text{ s}^{-1}$, $k_{\text{cat}}/K_{\text{M}} = 2.36 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, and a specific activity of $6.8 \mu\text{mol/min/mg}$ at 1.25 mM CoA. For acetoacetate, *S/AacS* has a $k_{\text{cat}} = 5.89 \text{ s}^{-1}$, $k_{\text{cat}}/K_{\text{M}} = 2.66 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, and a specific activity of $4.91 \mu\text{mol min}^{-1} \text{ mg}^{-1}$ at 1.25 mM acetoacetate.

The crystal structure of *S/AacS* Structure

The *S/AacS* structure was determined by molecular replacement, which identified one molecule in the asymmetric unit (consistent with the Matthews coefficient of 2.8) using full-length *S. enterica* Acetyl-CoA Synthetase **1PG4** [9] as a search model. Phases derived from the molecular replacement solution were used for autobuilding with PHENIX, which placed 436 residues out of 661 residues for full-length WT *S/AacS*. These residues were localized in the N-terminal domain; initial electron density for the C-terminal domain was present. The model was completed through iterative manual model-building and refinement. Refinement continued to a final crystallographic R_{factor} of 16.1 ($R_{\text{free}}=19.4$) (Table 1). The final model of *S/AacS* contains residues 9–661, 507 waters, and 22 ethylene glycol molecules. Residue Gln534 from the antiparallel two-stranded sheet of the A8 motif and residues 563–567 forming the loop connecting the two longest strands of the β -sheet in the C-terminal subdomain were not resolved in the electron density.

As discussed above, members of this enzyme family adopt two catalytic conformations that have been captured crystallographically. The *S/AacS* protein adopted the adenylate forming conformation (Figure 1B). The *S/AacS* structure is most similar to yeast acetyl-CoA synthetase (1RY2), which also adopted the adenylate-forming conformation [12]. The rms displacement calculated with LSQKAB of the CCP4 suite [18] is $1.95 \text{ \AA}$ over 520 structurally conserved residues of the full length protein. The DALI server [19] reports a value of $2.4 \text{ \AA}$ over 584 residues. The A10 motif was resolved in the density with the exception of the catalytic lysine side chain, which was omitted from the final model. The loop was positioned in a catalytically relevant pose even though no density for ligands was evident in the active site.

In addition to the two catalytic states, additional conformations have been observed crystallographically that likely play a role in allowing access and egress of substrates and products from the relatively buried active site. This conformational flexibility has influenced

the crystal structures of members of this family and frequently manifests as less well-ordered C-terminal domains. Frequently this results in higher B-factors and electron density of lower quality for the C-terminal domain. In the most dramatic cases, no electron density was observed for the entire ~110 residue C-terminal domain although it was present in the crystal and not proteolytically cleaved. Additionally, several groups have genetically removed the C-terminal domain to enable crystallization and structure determination. A list of structures of members of this family that exhibit poorer or absent electron density for the C-terminal domain is included in the Supplementary Table S1.

B-factor analysis reveals that the C-terminal domain position exhibits higher average B-factor values. Specifically, the average B-factors for the N-terminal subdomain, the C-terminal subdomain, and the entire protein were 18.8, 42.0, and 23.1 Å², respectively. The Supplementary Material includes additional validating information including a real space correlation plot (Figure S1), the PHENIX polygon histogram that compares the overall quality to structures of similar resolution (Figure S2), and electron density figures (Figure S3).

Although the crystallization experiments included acetoacetate and AMP, neither was present in the active site. Additional datasets from protein that was co-crystallized with AMP and acetoacetate with increasing concentration (up to 1.5 mM each) of co-crystallization reagents produced no evidence of ligand binding. Superposition of the ligands from related structures identify conserved and unusual features of the active site (Figure 1C). Asp508 is a universally conserved residue that interacts with the ribose hydroxyls of the nucleotide. Members of this family contain additionally a conserved A5 motif [1] that contains the stretch of amino acids with the motif $\phi(G/W)xTE$ motif, where ϕ represents an aromatic amino acid that stacks against the adenine ring of the nucleotide. The second residue of this motif is most commonly a glycine, which allows binding of larger substrates. In acetyl- and propionyl-CoA synthetases, this residue is a tryptophan that closes the acyl binding pocket for the small substrates. Finally, the glutamate of this motif binds a Mg²⁺ ion that coordinates the phosphates of ATP. *SlAacS* contains an unusual SGGTD motif in this position, and therefore does not have an aromatic residue to stack against the adenine ring. The aspartic acid should still be able to coordinate a Mg²⁺ ion however this has not been observed previously in any structurally characterized enzyme.

Members of this enzyme family contain an aromatic residue from the A4 motif that exhibits multiple side chain conformations dependent on the conformational state of the C-terminal domain.[1] In *SlAacS*, the A4 aromatic residue is Trp317. The side chain has been modeled into the density with X₁=-53°, however there is some negative difference within the side chain and positive difference density nearby. This density likely results from the side chain adopting multiple positions in the lattice. Attempts to model the side chain into partial occupancy conformations were not satisfactory and we have left the model in a single conformation that is most strongly supported by the electron density.

To identify the acetoacetate binding site, we examined the position of the propyl moiety from the propyl-AMP inhibitor that was co-crystallized with *SeAcs* (Figure 1C). The propyl group is surrounded by mostly hydrophobic residues including Met318 and Met319, which

adopts two side chain conformations, Gly422 of the A5 motif, and Thr392. This threonine residue provides the only hydrogen bonding moiety and potentially interacts with the β -carbonyl of the acetoacetate substrate. Examination of proteins annotated as acetoacetyl-CoA ligases demonstrates the conservation of this threonine residue.

The C-terminal Extension of Acyl-CoA Synthetases

The C-terminal domain undergoes a large conformational change in the catalytic mechanism of acyl-CoA synthetases.[1] As discussed above, the acetyl-CoA synthetases from *S. enterica* (*SeAcs*) and *Saccharomyces cerevisiae* (*ScAcs*) each contain a C-terminal extension that is longer by ~25–30 residues than other members of this family.[9, 12] This region contains a second α -helix that follows the common α -helix that terminates all other members of this family. Interdomain interactions were identified throughout the protein. In *SeAcs* and *ScAcs*, electron density for the loop that joins these two helices was not observed (Figure 1A). The model of *SlAacS* is the first structure to show complete density for this C-terminal extension, including the terminal helix and the residues that span these two helices.

Interestingly, biochemical evidence suggests that this C-terminal extension is important for catalytic activity. In particular, in an investigation of the acetylation susceptibility of the Acs enzymes from *S. enterica* and *S. lividans*, several chimeric enzymes were made that swapped homologous regions from these two enzymes [10]. A chimeric enzyme, designated chimeric *A1*, that replaced the entire C-terminus of *SlAcs* (residues 520 through 651) with the homologous C-terminal domain from *SeAcs* resulted in an inactive chimeric enzyme. This activity was fully restored in the *B3* chimeric if only residues 550–618 were substituted. (Note that the catalytically essential A10 lysine occurs at position Lys609 in *SlAcs* so in this *B3* chimeric the catalytic lysine is provided by the *SeAcs* sequence.) However, the *B4* chimeric containing a substitution of residues 550–627 reduced activity to ~30% and the *B5* chimeric replacing residues 550–638 reduced activity to less than 10%. This demonstrates that residues located between 618 and 638 (the difference between the active *B3* chimeric and the inactive *B5* chimeric enzymes) are important for activity. In fact, this is the region of the protein that spans the two C-terminal helices and is the region that is observed for the first time in the *SlAacS* structure.

A superposition of the C-terminal domains of *SeAcs*, *ScAcs*, and *SlAacS* shows indeed that the final C-terminal helices do adopt approximately the same overall position. The *SlAacS* structure shows that the protein chain between these helices adopts a mostly random coil that is interrupted in the middle by a single turn of a helix (Figure 2A). Interestingly, the *SlAacS* structures shows that the tip of this loop interacts with the N-terminal domain. Specifically, two residues from this loop, Asn637 and Ser640, form part of a highly structured network of direct and solvent-mediated hydrogen bonds between the N- and C-terminal domains (Figure 2B). One region from the N-terminal domain that interacts is the so-called P-loop, a glycine-, serine-, and threonine-rich region that interacts with the phosphates of ATP.[1] This P-loop adopts multiple conformations in the different crystal structures and may play an important role in the release of PP_i and trigger the conformational change. Specifically, the main chain carbonyls of Ser272, Gly274, and Gly277 form direct or water-mediated hydrogen bonds with Asn637 and Ser640. Asn637

also interacts directly with Arg183 and Asp187, while the carbonyl of Gly639 and the carbonyl and side chain oxygens of Ser640 interact with Ser184, Asp187, and Arg188.

The sequence of loop from the C-terminal domain that is observed crystallographically in *SlAacS* differs between the *Acs* enzymes and the acetoacetyl-CoA synthetase protein and there are likely to be differences in how this region interacts with the N-terminal domain in the *Acs* enzymes. While the sequence is more conserved between *SeAcs* and *SlAcs*, there are differences that are likely the cause of the drop in the activity observed in the study of the chimeric enzymes.[10]

Because of the importance of the C-terminal extension, we also asked whether the conformation of the C-terminal extension might be influenced by contacts made with neighboring molecules in the crystal lattice. First, we note that the conformation of the C-terminal domain has been observed in many structures previously, including the yeast acetyl-CoA synthetase and 4-chlorobenzoyl-CoA ligase structures (Figure S4). Second, the C-terminal domain projects into a cavity in the crystal lattice (Figure S5) suggesting it is free to adopt a preferred conformation and is not unduly influenced by neighboring protein chains.

However, there are interactions that occur between the C-terminal extension and a neighboring protein molecule in the lattice. Specifically, a salt bridge between the side chain of Asp633 and Arg308 from a neighboring molecule. A second hydrogen bond exists here between the side chain of Lys634 and the main chain carbonyl of Glu353. The third interaction was a hydrogen bond from the main chain amide of Leu644 to the side chain of Asp106 from a neighboring chain. Finally, Arg655 near the C-terminus hydrogen bonds to the main chain of the carbonyl oxygen from Pro119 of a neighboring molecule. The interface between the symmetry-related molecules is fairly well solvated with at least 12 water molecules and an ethylene glycol molecule located between the two protein chains, suggesting the conformation of this loop is not driven by the contacts between symmetry related protein molecules. Nonetheless, the impact of crystal contacts cannot be unequivocally ruled out unless a different crystal form is identified that also shows the new interactions between the N- and C-terminal domains, or until further biochemical experiments test this hypothesis. These experiments are on-going.

Contribution of the C-terminal Domain to PAT recognition

The motivation for the generation of the chimeric acetyl-CoA synthetases by Tucker and Escalante-Semerena[10] was to gain a better understanding of the factors that dictate recognition of the A10 catalytic lysine by the *SlPAT* acetyl transferase. Indeed, along with a better understanding of the role of the C-terminal extension to the domain alternation catalytic mechanism, that was a motivating factor for a structural investigation of the *SlAacS* protein. The same chimeric constructs between *SeAcs* and *SlAcs* that were examined for catalytic activity were also investigated for their susceptibility to protein acetylation at the Lys609 position. As opposed to the results with activity, which decreased sequentially from chimeric *B3* to *B4* to *B5*, the degree of acetylation also increased significantly from 30% in *B3* to >80% in *B4* to ~100% in *B5*, where the acetylation of the *SeAcs* was used as a standard to indicate 100% activity. This demonstrated that inclusion of the 20 residues that

follow Lys609 from the *SeAcs* enzyme that serves as a substrate for *SIPAT* increased the ability of the acetyl transferase to recognize the chimeric proteins. Further investigation of even smaller substitutions may further identify the exact recognition site between the *Acs* and *PAT* enzymes and will provide insight into the role of the C-terminal extension in this mechanism of post-translational regulation.

Conclusions

We present here the structure of the first acetoacetyl-CoA synthetase crystal structure, a previously uncharacterized member of the ANL superfamily of adenylating enzymes. The structure identifies that the active site shares many common features with other members of this family. However, the *SlAacS* enzyme also contains unusual residues that vary some otherwise well-conserved aspects of the active site. In particular, a common hydrophobic residue in the A5 motif is replaced by a serine residue. More interesting, the structure provides a view of the C-terminal extension that is shared in bacterial and eukaryotic acetyl-CoA synthetases. The presence of an additional helix has been observed however the loop joining this helix to the C-terminus of the shorter family members have never been observed. The structure of *SlAacS* not only shows the orientation of this loop but additionally demonstrates that it interacts with the N-terminal domain in the adenylate-forming conformation. Surprisingly, this loop interacts with the P-loop, a region of the N-terminal domain that interacts with the phosphates of the ATP substrate. This suggests that upon completion of the adenylation partial reaction, the release of PPi may communicate through this interaction to the C-terminal domain to trigger the conformational change necessary to catalyze the second partial reaction. Why acetyl, propionyl, and acetoacetyl-CoA synthetases contain this extension is unknown and is the subject of ongoing studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1. Structure of the *SlAacS* enzyme

A. Sequence alignment of the C-terminal residues from the Acs enzymes from *S. lividans*, *S. enterica*, and *S. cerevisiae*, as well as the *S. lividans* AacS enzyme. The yellow region highlights the hinge region that starts the C-terminal domain. The blue box highlights the A10 motif (PXXXXGK) that contains the catalytic A10 lysine. Green highlighting shows regions that were disordered in the three structurally characterized enzymes. The positions of the two C-terminal helices in *SeAcs* are shown. B. Ribbon diagram of the *SlAacS* enzyme. The C-terminal domain is shown in green. The A8 motif that follows the hinge is shown in pink. The position of the A10 lysine is shown with a red sphere. C. The propyl-AMP ligand from *SeAcs* (1PG4) was manually docked into the active site of *SlAacS* to illustrate the active site binding residues. Note that the side chain of Met319 is present in two conformations.

Figure 2. The C-terminal Domain of *SIAcS*

A. Ribbon diagrams for the C-terminal domains of *SeAcs* (cyan), *ScAcs* (red) and *SIAcS* (green). The hinge residue at the start of the C-terminal domain is shown. Dashed lines represent regions that were disordered in the crystal structures including the loop that spans the two C-terminal helices. B. Stereo illustration of the interactions between the C-terminal extension in *SIAcS* and the N-terminal domain. Residues Asn637 through Ser640 interact with the N-terminal helix from residues Arg183 through Arg188 and the P-loop at positions

Ser272 through Gly277. Note that the side chains of Ser272, Ser273, Thr275, and Thr276 are not shown for clarity as the only interactions occur with main chain carbonyl atoms.

Table 1**Diffraction and Refinement Statistics**

	<i>SI/AacS</i>
PDB Code	4WD1
Beamline	SSRL 7.1
Wavelength	0.9794
Space group	P3 ₁ 21
Unit cell a, b, c (Å)	104.4, 104.4, 133.9
Molecules per asymmetric unit	1
Resolution range (Å) ^a	50.0-1.9
No. observations	470190
No. unique reflections	66,573
Multiplicity	7.1
Completeness ^a (%)	99.2 (85.1)
$\langle I/\sigma \rangle^a$	16.4 (4.2)
R _{merge} ^a (%)	5.6 (24.6)
Structure Refinement	
R _{factor} ^a (%)	16.1 (19.3)
R _{free} ^a (%)	19.4 (23.3)
No. protein/water atoms	5001/507
RMSD bond distances (Å)	0.007
RMSD bond angles	1.00
Wilson B-factor (Å ²)	19.9
Average B-Factor (Å ²)	
Protein (all, main, side)	23.1, 22.0, 24.4
Ethylene Glycol, Ca ²⁺	30.9
Solvent	30.5
Ramachandran analysis (%)	
Favored	98
Allowed	2
Outliers	0
Molprobability ClashScore	4.2 (98 th percentile)

^aValues in parentheses are for the highest resolution shell (2.0 – 1.9 Å for diffraction data and 1.93-1.90 Å for refinement)