

Article

Not peer-reviewed version

The Role of the Omega Subzone in Determining the Membership of a Protein in One of the Two Families of the LexA/Signal Peptidase-Like Superfamily

[Alexander I. Denesyuk](#)^{*}, Konstantin Denessiouk, [Mark S. Johnson](#), [Vladimir N. Uversky](#)^{*}

Posted Date: 28 January 2026

doi: 10.20944/preprints202601.2195.v1

Keywords: signal peptidase; LexA endopeptidase; 3D structure; catalytic core; Omega subzone; protein family; structural marker



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Article

The Role of the Omega Subzone in Determining the Membership of a Protein in One of the Two Families of the LexA/Signal Peptidase-Like Superfamily

Alexander I. Denesyuk ^{1,*}, Konstantin Denessiouk ¹, Mark S. Johnson ¹
and Vladimir N. Uversky ^{2,*}

¹ Structural Bioinformatics Laboratory, Biochemistry, InFLAMES Research Flagship Center, Faculty of Science and Engineering, Åbo Akademi University, Turku 20520, Finland

² Department of Molecular Medicine and USF Health Byrd Alzheimer's Research Institute, Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA

* Correspondence: alexandre.denesyuk@abo.fi (A.I.D.); vuversky@usf.edu (V.N.U.)

Abstract

LexA/signal peptidase-like superfamily proteins are serine proteases that use the Ser-Lys catalytic dyad to carry out their biological functions. Here, we investigate the two known families of LexA/signal peptidase-like superfamily proteins, the type I signal peptidase and LexA endopeptidase domain-like, and describe the structural catalytic cores that govern the catalytic residues in these proteins. We show that the structural catalytic core of these proteins is a combination of two subzones, the NucBaseOmega and Omega. While the NucBaseOmega subzone is a pattern observed in all proteins of the studied superfamily, the Omega subzone in the type I signal peptidase family differs from that of the LexA endopeptidase domain-like family. Thus, the amino acids and 3D characteristics of the Omega subzone are a structural marker of the proteins belonging to a specific family.

Keywords: signal peptidase; LexA endopeptidase; 3D structure; catalytic core; Omega subzone; protein family; structural marker

1. Introduction

Earlier, we described the structural catalytic core (SCC) of the β -lactamase/transpeptidase-like superfamily of proteins [1]. From the point of view of function, the superfamily of β -lactamases/transpeptidases included bacterial enzymes that provided multi-resistance to β -lactam antibiotics such as penicillins [2]. Most proteins in that superfamily carried out their biological functions through the employment of a Ser-Lys catalytic dyad (InterPro entry: IPR012338 [3]). The SCC in those proteins had been defined as a minimal conserved structural organization that has common structural features and also incorporates the most important catalytic, functional and supporting residues. In [1] we also showed that the SCC in β -lactamases included residues of the Omega subzone, which was a specific substructure introduced by us, but which included fragments of the already known Ω -loop and the antiparallel bridge joining its terminal residues. From earlier studies, the Ω -loop is known to play an important role in molecular recognition and protein function [4,5], and the unrelated antiparallel bridge is one of the seven known patterns observed in the secondary structure of proteins, where between residues in positions “i” and “j” there are two hydrogen bonds that are characteristic of the β -structure [6].

The β -lactamase/transpeptidase-like proteins, structure and composition of their catalytic fragments, which are part of the described SCC, did stem from variations of the catalytic Ser/His/Asp triads in the overall group of serine proteases, and from the variety of emerging unconventional catalytic triad/dyad combinations in these enzymes [7,8]. In the current study, besides the β -

lactamase/transpeptidase-like superfamily there is another unrelated superfamily, the LexA/signal peptidase-like superfamily (InterPro entry: IPR036286), whose enzymes also use the Ser-Lys catalytic dyad to carry out their function, and which originate from the same pool of unconventional serine proteases. In addition, there is a notable structural similarity between proteins of both superfamilies in the organization of their oxyanion holes [7]. Additionally, the functional relationship between proteins of the two superfamilies is also highlighted by two facts: that (1) β -lactam antibiotics induce the SOS response in *S. aureus*, which involves activation of the LexA protein [9], and (2) bacterial signal peptidases are also inhibited by some β -lactam compounds [10].

Here, we decided to look at the SCC of LexA/signal peptidases and see how it relates to the SCC of β -lactamases/transpeptidases in order to evaluate structural active-site homology between the two superfamilies.

2. Results and Discussion

Members of the LexA/signal peptidase-like superfamily have an unconventional catalytic Ser-Lys dyad instead of the classical catalytic Ser-His-Asp triad. No other structural amino acid motifs have been identified for this superfamily of proteins. Consequently, it is possible that their Structural Catalytic Cores (SCCs) predominantly use main-chain interactions for their local substructure.

2.1. Creating a Dataset of the LexA/Signal Peptidase-Like Superfamily Proteins

In the SCOP database [11], the LexA/signal peptidase-like superfamily includes two families: (1) the type I signal peptidase family with only one known protein, and (2) the LexA endopeptidase domain-like family with six different proteins. Additionally, the InterPro database [3] adds three other proteins to the type I signal peptidase family, totaling four known different proteins in that family; and it also adds two other proteins to the LexA endopeptidase domain-like family, totaling eight different proteins in that family. All in all, the resulting set includes 12 different proteins in the LexA/signal peptidase-like superfamily. Each of these 12 different proteins has one or several known 3D structures in the Protein Data Bank (PDB [12]), where one structure each can be chosen as a “protein representative”. These structures are summarized in Table 1. In addition to the protein representatives, one structure can be chosen as a “family representative” for the type I signal peptidase family, and one for the LexA endopeptidase domain-like family, respectively, based on resolution, the overall quality and presence in literature and research. These two structures are the signal peptidase I protein (LEP_ECOLI [13]; PDB ID: 1B12; R = 1.95 Å, [10]) for the Type I signal peptidase family (Table 1), and the UmuD’ mutagenesis protein (UMUD_ECOLI [13]; PDB ID: 1UMU; R = 2.50 Å, [14]) for the LexA endopeptidase domain-like family (Table 1). The signal peptidase I protein (PDB ID: 1B12) is a hydrolase, which cleaves the leader peptide from a variety of secreted proteins [15], and the UmuD’ mutagenesis protein (PDB ID: 1UMU) is involved in the global SOS response to DNA damage, where UmuD is self-cleaved to produce UmuD’ [14,16,17].

Table 1. Structural catalytic core (SCC) in 12 LexA/signal peptidase-like superfamily representative proteins.

N	PDB ID	R (Å)	Protein	Nuc hexapeptide	Omega subzone	Cross-sheet ladder	Ref.
Superfamily: LexA/signal peptidase-like							
Family: Type I signal peptidase							
1	1B12_A	1.95	LEP_ECOLI	85 QIPSGS 90	272 GDNRDNS AD 280	82 EPF 84; 101 ILVE 104;294 GRATA 298;130 IVVF 133;143 YIKR 146;270 MM 271; S 281	[10]
2	4WV1_A	1.90	LEP_STA AC	382 TIKGES 387	497 GDNREVS D 505	379 TPY 381;398 VAVN 401;519 GKVS– 522;415 VVVF 418;426 YVKR 429;495 VL 496; S 506	[18]

3	4N31_A	2.20	R9TES9_S TRPY	43	IINTND	48	140 NDYREERL D 148	40 GVM 42; 59 VLYY 62; 162 GKIST 166; 73 VVVY 76; 83 KVGR 86; 138 IL 139; S 149	[19]
4	7P2P_A	4.90	SC11A_H UMAN	51 56	VVLSGS		115 GDNNA-- VD 121	48 PIV 50; 67 LFLT 70; 140 GRARG 144; 82 IVVF 85; 94 IVHR 97; 113 TK 114; D 122	[20]
Family: LexA endopeptidase domain-like									
5	1UM_U_A	2.50	UMUD_E COLI	55 60	KASGDS		109 PMNSA-- YSP 116	52 YFV 54; 72 LIVD 75; 129 GVVIH 133; 85 IVIA 88; 95 TVKK 98; 107 LI 108; I 117	[14]
6	3K2_Z_A	1.37	LEXA_T HEMA	114 119	KVKGES		169 PANRE-- MSS 176	111 FLL 113;131 VLVR 133;188 GKVVG 192;144 IVAA 147;154 TLAK 157;167 LR 168; M 177	[21]
7	6A2_Q_A	1.48	LEXA_M YCTU	155 160	KVIGDS		210 PHNPA-- FDP 217	152 FLL 154;172 VVVR 175;227 GKVVT 231;185 IVAA 188;195 TVKT 198;208 LM 209; I 218	[22]
8	8B0V_A	1.70	LEXA_PS EAI	120 125	RVRGMS		175 AENPE-- FAP 182	117 YLL 119;137 LAVH 140;196 GLSVG 200;150 VVVA 153;160 TVKR 163;173 LL 174; I 183	[23]
9	1JHF_A	1.80	LEXA_EC OLI	114 119	RVSGMS		169 PENSE-- FKP 176	111 FLL 113;131 LAVH 134;190 GLAVG 194;144 VVVA 147;154 TVKR 157;167 LL 168; I 177	[24]
10	2HN_F_A	1.80	Q7B004_E COLX	144 149	EVEGNS		205 PLNPQ-- YPM 212	141 FWL 143;166 ILVD 169;223 GKVIA 227;179 FCIA 182;190 TFAK 193;203 LQ 204; I 213	[25]
11	1F39_A	1.90	RPC1_LA MBD	144 149	EVEGNS		205 PLNPQ-- YPM 212	141 FWL 143;166 ILVD 169;223 GKVIA 227;179 FCIA 182;190 TFKK 193;203 LQ 204; I 213	[26]
12	2FJR_A	1.95	RPC1_BP 186	122 RSE--- 124	166 GG---- KVP 170		119 MAI 121;128 YFVD 131;182 GRVVG 186;140 LWLV 143;150 SIRE 153;164 VA 165; F 171	[27]	

2.2. The Type I Signal Peptidase Family: SCC in the Signal Peptidase I from E. coli

2.2.1. The NucBaseOmega Subzone Around the Ser-Lys Catalytic Dyad

The structure of LEP_ECOLI has a mainly β -sheet protein fold, consisting of two large antiparallel β -sheet domains, I and II [10]. The β -sheet of domain I consists of seven β -strands, β 1 through β 7, and incorporates the catalytic base, Lys₁₄₅, the catalytic nucleophile, Ser₉₀, and the oxyanion atom N/Ser₉₀, where the catalytic nucleophile lies in the loop following strand β 1 (Tyr₈₁-Gln₈₅), and the catalytic base lies within strand β 5 (Asp₁₄₂-Gly₁₄₉). The β -sheet of domain I twists significantly into a barrel-like structure, so that β -strands β 1 and β 5 approach each other and the catalytic base, Lys₁₄₅, and nucleophile, Ser₉₀, approach in space, which is required by protein function. Strands β 6 and β 7 turn away from the β 1- β 5 barrel-like structure, and the β 1 and β 5 strands are locked by two separate paths (two cross-sheet ladders, “a” and “b”) made of consecutive antiparallel bridges [6] (ladder steps), described in the Introduction. The cross-sheet ladder “a” starts with Glu₈₂ (β 1) and ends with Tyr₁₄₃ (β 5) (Figure 1A; Table S1), while the cross-sheet ladder “b” starts with Phe₈₄ (β 1) and ends with Arg₁₄₆ (β 5) (Figure 1B; Table S1). The steps of the cross-sheet ladders “a” and “b” are summarized in Table S2. The cross-sheet ladder “a” has four steps, which are all antiparallel bridges, I-IV (Figure 1A), according to criteria given in [6]. The cross-sheet ladder “b” also has four steps, I-IV (Figure 1B), but only two of those, I and IV, are typical antiparallel bridges according to criteria given in [6], while steps II and III are antiparallel bridge-like interactions, where antiparallel bridge-like interactions differ from antiparallel bridges by appearance of additional intermediate

elements, such as additional interactions or intermediate water molecules between contacting residues.

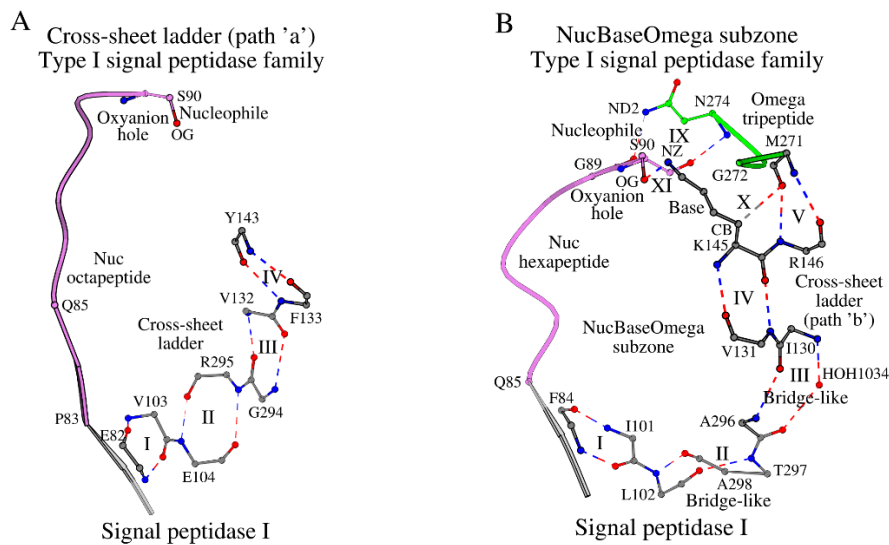


Figure 1. Plots **A** and **B** show paths “a” and “b” of the cross-sheet ladder in the type I signal peptidases, respectively. **A.** Four antiparallel bridges, which are marked with numbers I-IV, form path “a” of the cross-sheet ladder. The Nuc octapeptide is the peptide Pro₈₃-Ser₉₀ joining the catalytic nucleophile with the cross-sheet ladder via the β 1 β -strand. **B.** The NucBaseOmega subzone includes the Nuc hexapeptide (Gln₈₅-Ser₉₀), three antiparallel bridges (steps I, IV and V of the cross-sheet ladder), two antiparallel bridge-like interactions (steps II and III of the cross-sheet ladder), and the Omega tripeptide (Gly₂₇₂-Asn₂₇₄). Interactions marked as I through XI are described in Tables S2 and S3.

In addition to steps I-IV, the cross-sheet ladder “b” has a joint antiparallel bridge V, which connects strands β 5 and β 6 (Figure 1B). The summary of amino acid segments (21 residues), which incorporate all interacting amino acids from the cross-sheet ladders “a” and “b”, are shown in Tables 1 and S1 (“Cross-sheet ladder”). Met₂₇₁, which resides in strand β 6 in LEP_ECOLI and forms bridge V (Figure 1B), does also interact with the catalytic base Lys₁₄₅ via a weak hydrogen bond (X in Figure 1B; column X in Table S3), and borders the tripeptide Gly₂₇₂-Asn₂₇₄ (Omega tripeptide), which coordinates positioning of the Ser₉₀ nucleophile via a conventional hydrogen bond (IX in Figure 1B; column IX in TableS3). Finally, the Ser-Lys catalytic dyad forms a canonical functional interaction between these two residues (shown as XI in Figure 1B). As a result, the interlocked NucBaseOmega subzone around the Ser-Lys catalytic dyad is formed, which includes: (1) the β 1-loop-Nucleophile peptide (shown as Nuc octapeptide in Figure 1A or Nuc hexapeptide in Figure 1B); (2) the “Cross-sheet ladder (β 1- β 5)/Catalytic Base” local structure, joined by the antiparallel bridges; and (3) the Omega tripeptide. The choice of the Omega tripeptide and not the entire Omega subzone here is due to its functional role of coordinating the catalytic nucleophile residue. The NucBaseOmega subzone can be traced in all proteins of the Type I signal peptidase family.

2.2.2. The Omega Subzone in the Type I Signal Peptidase Family Proteins

The cross-sheet ladder of the NucBaseOmega subzone in type I signal peptidases involves amino acids from β -strands β 1- β 6, which are linked to each other via nine antiparallel bridge and bridge-like interactions: four interactions in ladder “a”, four interactions in ladder “b”, and one interaction V between Arg₁₄₆ and Met₂₇₁ (Figures 1A and 1B). However, the adjacent Met₂₇₀ from strand β 6 forms a reinforcing antiparallel bridge-like interaction to Ser₂₈₁ (shown as VI in Figure 2A), which can also be observed in all Type I signal peptidases, and thus Ser₂₈₁ and its respective residues in the other Type I signal peptidases were also added to the total cross-sheet ladder connection (Tables 1, S1 and

S2). Existence of interaction VI indicates existence of a separate, locked structural Omega subzone (Figure 2A). The conformation of the Omega subzone is stabilized by several hydrogen bonds (shown as bridges VI, VII and VIII in Figure 2A; and in columns VI, VII and VIII in Tables S2 and S3). Here, residue Asn₂₇₄ not only participates in the interaction with the catalytic nucleophile (Figure 1B), but also, in the interactions with Asn₂₇₇ (Figure 2A), fixing the conformation of the Omega subzone. As discussed below, this part of the Omega subzone participates in ligand binding by Type I signal peptidases.

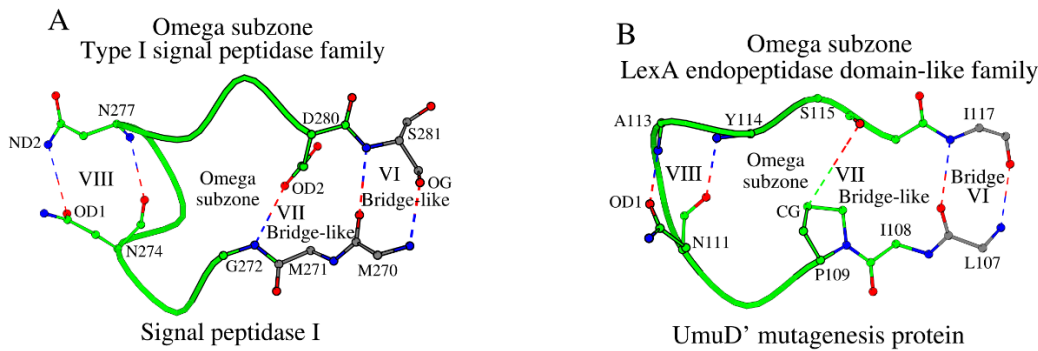


Figure 2. Plots **A** and **B** show Omega subzone in the type I signal peptidase family and the LexA endopeptidase domain-like family, respectively. **A.** The representative structure is the signal peptidase I. **B.** The UmuD' mutagenesis protein. Interactions marked as VI, VII and VIII are described in Tables S2 and S3.

2.2.3. The Structural Catalytic Core (SCC) of the Type I Signal Peptidases

The Structural Catalytic Core (SCC) of the Type I signal peptidases incorporates the NucBaseOmega and Omega subzones (Figure 3). Figure 3 shows how residues of the cross-sheet ladder function as a spine, supported by the β -sheet, for the key functional loops and residues. Visually, the SCC in Figure 3 resembles letter “B”, and thus, for simplicity, we will further refer to this type of SCC as the B-type SCC or B-SCC.

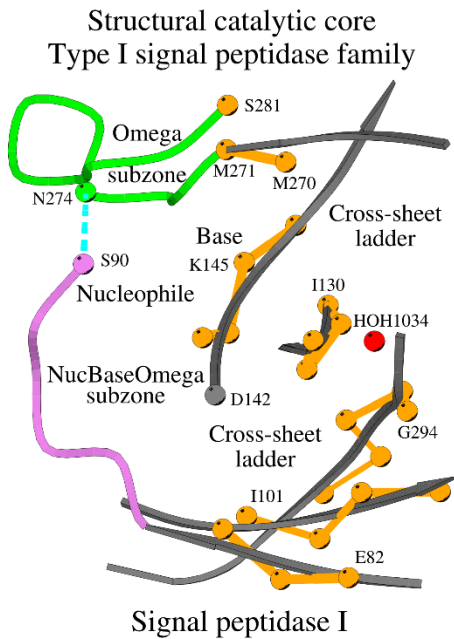


Figure 3. Overview of the B-type Structural Catalytic Core or B-SCC of the type I signal peptidase-like family. The Nuc-peptide is shown in purple, residues of the cross-sheet ladder in orange, and the Omega subzone in

green. The dotted line shows the contact between the nucleophile and the conserved asparagine of the Omega subzone. Several amino acids are marked for reference purposes.

2.3. The Type I Signal Peptidase Family: SCC in the Other Proteins

Table 1 contains four representative proteins of the type I signal peptidase family, all having the B-type SCC. Below, we will compare the B-SCC in signal peptidase I from *E. coli* and in the other three representative structures of the family.

2.3.1. SCC in the Signal Peptidase IB from *S. Aureus*

Comparison of B-SCCs in LEP_ECOLI and LEP_STAAC [18] showed only three minor differences. The first difference lies in the structure of the cross-sheet ladder, where the Gly₂₉₄-Ala₂₉₈ in LEP_ECOLI is one amino acid shorter with Gly₅₁₉-Ser₅₂₂ in LEP_STAAC (Tables 1 and S1). Instead, an additional water molecule HOH₈₁₄ is present in LEP_STAAC in the structure of the bridge II (see column II in Table S2). The second difference is replacement of Asn₂₇₇ (LEP_ECOLI) with Val₅₀₂ (LEP_STAAC) in the turn segment of the Omega subzone, which may lead to higher flexibility of the Omega tripeptide and the overall Omega subzone (see interaction VIII in Figure 2A, and in Tables 1, S1 and S3). Finally, the last difference is the presence of a water molecule-mediator HOH₈₅₇ between residues of the catalytic dyad (as opposed to the direct interaction (see XI in Figure 1B and in Table S3).

2.3.2. SCC in the Signal Peptidase-Like Protein from *S. Pyogenes*

This signal peptidase-like protein (R9TES9_STRPY) lacks typical catalytic Ser and Lys residues characteristic of type I signal peptidases (Tables 1 and S1). They are replaced by Asp₄₈ and Gly₈₅ residues, respectively, which play no part in function [19]. Additionally, there are minor changes in composition of the Omega subzone: Asn₂₇₄ (LEP_ECOLI) → Tyr₁₄₂ (R9TES9_STRPY) and Asn₂₇₇ (LEP_ECOLI) → Glu₁₄₅ (R9TES9_STRPY). All these changes do not affect the relative positioning of the Nuc peptide, the Omega subzone, and the cross-sheet ladder, i.e. they have no effect on the structural arrangement of the B-SCC, and thus it remains conserved between LEP_ECOLI and R9TES9_STRPY (Table S3).

2.3.3. SCC in the Human Signal Peptidase Complex Catalytic Subunit SEC11A

The last representative structure of the type I signal peptidase family of proteins is the human signal peptidase complex catalytic subunit (SC11A_HUMAN) determined by the electron microscopy method and having a relatively poor resolution of R = 4.90 Å [20]. SC11A_HUMAN exists in structures of two paralogs: paralog A (PDB ID: 7P2P) and paralog C (PDB ID: 7P2Q). The two structures are indistinguishable at 5Å resolution, and paralog A was chosen for further analysis, because of a more stable conformation within the complex [20].

The catalytic dyads of the human (SC11A_HUMAN) and bacterial (LEP_ECOLI) signal peptidases differ in the type of amino acid used as the catalytic base: histidine *versus* lysine, respectively (Table 1). Also, there is a reduction in length of the Omega subzone. Regardless these changes, and also taking into account the poor resolution of 5Å, we can safely say that all the geometric parameters between the bacterial and human signal peptidases' B-SCCs are conserved (Tables S1–S3).

2.4. The Role of SCC in Peptide Ligand Binding in Type I Signal Peptidases

In the signal peptidase I from *E. coli* (LEP_ECOLI), the C-terminal segment of the Omega subzone participates in ligand binding, where Ser₂₇₈ and Ala₂₇₉ form hydrogen bonds with the ligand atoms, shown as XVIII in Figure 4A and described in column XVIII in Table S4. The catalytic base, nucleophile and the oxyanion hole interact with the central ligand atoms O8, OG and O10 as a part of protein function (Figure 4A). On the other side of the peptide ligand, in the C-terminal part, the

O17 and O19 atoms are sandwiched between the Nuc-peptide and the cross-sheet ladder, and form interactions XIII, XIV, XVI and XVII (Figure 4A and Table S4).

In the signal peptidase IB from *S. aureus* (LEP_STAAC), the C-terminal residues of the Omega subzone, similar to LEP_ECOLI, are also used for ligand binding, antiparallel tripeptides Val₅₀₂-Lys₅₀₄ (from the protein Omega subzone) and Pro_{212_D}-K_{214_D} (from the ligand peptide) form hydrogen bonds with each other and interaction XVIII is present (Figure 4B; Table S4). Also, similar to LEP_ECOLI, the NZ atom of the catalytic base, Lys₄₂₈, interacts with atom O/Pro_{212_D} of the ligand making interaction XV (Figure 4B; Table S4), and the “oxyanion hole” main-chain NH atom of Ser₃₈₇ makes a hydrogen bond to the carbonyl oxygen atom of Ala_{211_D}, which is the C-terminal alanine of the conserved Ala-Xaa-Ala motif, after which (except there is a following proline) the enzyme cleaves the substrate [18]. As also seen in LEP_ECOLI, the N-terminal part of the peptide substrate (Pro₂₀₇-Lys₂₁₀ in LEP_STAAC) is sandwiched between the Nuc-peptide (Thr₃₇₉-Lys₃₈₄ in LEP_STAAC) and the cross-sheet ladder (Asp₄₂₅-Arg₄₂₉ in LEP_STAAC) forming interactions XIII, XIV, XVI and XVII (Figure 4B; Table S4). Moreover, in this sandwich, the Thr₃₈₂-Lys₃₈₄ tripeptide from the Nuc-peptide, the Pro₂₀₇-Lys₂₁₀ tetrapeptide from the ligand and the Asp₄₂₅-Val₄₂₇ tripeptide from the cross-sheet ladder have the conformation of a three-stranded parallel β -sheet that interacts with each other by means of the Acceptor-Donor-Acceptor (ADA) and Donor-Acceptor-Donor (DAD) interaction patterns, as seen in ligand binding of many unrelated protein complexes [28]. Because of this motif feature, we will refer to the Asp₄₂₅-Val₄₂₇ tripeptide, which interacts with the peptide ligand by the stretched β -sheet type ADA/DAD interactions XVI and XVII and precedes in sequence the catalytic base (Lys₄₂₈ in LEP_STAAC), as the DAD_{Base} motif tripeptide.

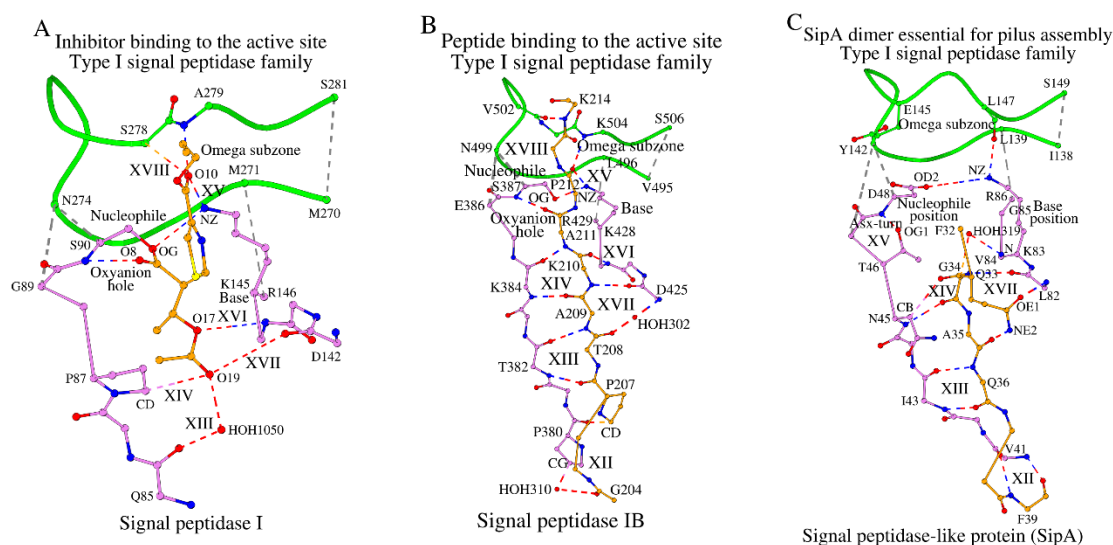


Figure 4. Three different types of sandwich-like interactions between proteins of the type I signal peptidase-like family and their peptide ligands. A) show signal peptidase I, B) show signal peptidase IB and C) show SipA protein. Ligands are shown in yellow, and the Omega zone in green. Interactions marked as XII through XVIII are described in Table S4.

In the signal peptidase homologue SipA from *S. pyogenes* (R9TES9_STRPY), binding of the peptide ligand is functionally different, because SipA has lost the catalytic dyad typical for signal peptidases and functions in polymerization of pilin subunits [19]. If we compare binding of ligand peptides in LEP_ECOLI (Figure 4A), LEP_STAAC (Figure 4B) and R9TES9_STRPY (Figure 4C), we can see several fundamental differences between R9TES9_STRPY and the canonical type I signal peptidases. Firstly, because of the functional differences between R9TES9_STRPY and the canonical type I signal peptidases, the Omega subzone in R9TES9_STRPY is not involved in ligand binding (Figure 4C). Then, unlike in LEP_STAAC (Figure 4B), the peptide ligand is sandwiched between the

Nuc-peptide segment Phe³⁹-Asp⁴⁸ and the Base-peptide segment Leu⁸²-Arg⁸⁶ in an antiparallel manner, where Ile⁴³-Asn⁴⁵ of the Nuc-peptide interacts with the Gly³⁴-Gln³⁶ of the bound ligand in an antiparallel β -strand conformation (interactions XIII and XIV in Figure 4C; and columns XIII and XIV in Table S4). Finally, in the signal peptidases I and IB, the DAD_{Base} motif tripeptide sandwiches the ligand chain with the Nuc-peptide from the other side, while in SipA the DAD_{Base} motif interacts with the side chain group of a single amino acid, Gln³³. Also, in R9TES9_STRPY the absence of the catalytic function is reinforced with blocking the oxyanion hole, N/Asp⁴⁸, by the presence of a Asx-turn [29]. Plus, the structural absence of a lysine at the catalytic base position 85 is partially compensated by a lysine at position 83 (Figure 4C), which forms interactions with the residue at the catalytic nucleophile position Asp⁴⁸, NZ/Lys⁸³-OD2/Asp⁴⁸, and with the Omega subzone, NZ/Lys⁸³-O/Leu¹⁴⁷.

2.5. SCCs in the Type I Signal Peptidase Family: Conclusions

In summary, the structural comparison of the SCCs of the four type I signal peptidase family representative proteins, the signal peptidase I from *E. coli* (LEP_ECOLI), the signal peptidase IB from *S. aureus* (LEP_STAAC), the signal peptidase-like SipA from *S. pyogenes* (R9TES9_STRPY), the and human signal peptidase complex catalytic subunit SEC11A (SC11A_HUMAN), we can conclude that bacterial and human signal peptidase SCCs are structurally similar, even though some differences remain, such as (1) missing residues and respective presence of additional water molecules in some steps of the cross-sheet ladder in LEP_STAAC, and (2) reduction in length of the Omega subzone in SC11A_HUMAN.

We can also conclude that in LEP_ECOLI and LEP_STAAC, binding of peptide ligands with the NucBaseOmega and Omega subzones is similar, while in R9TES9_STRPY the Omega subzone does not participate in peptide ligand binding, even though interactions between the peptide ligand and the NucBaseOmega subzone are similar in all three peptidases.

Finally, we can conclude that the three peptidases use the structural DAD_{Base} motif tripeptide for binding the peptide ligand in a similar donor-acceptor-donor way. This tripeptide borders the position of the catalytic base and has the overall schematics of "N_(base residue -1) – O_(base residue -3) – N_(base residue -3)", where "N" and "O" designate main-chain nitrogen and oxygen atoms of the residues preceding the catalytic base by one and by three, respectively.

2.6. SCC in the LexA Endopeptidase Domain-Like Family Proteins

Similar analysis of the SCC in the UmuD' mutagenesis protein (UMUD_ECOLI) showed that there are no significant differences in construction of the NucBaseOmega subzone in UmuD' and the signal peptidase I proteins (Tables 1, S1 and S2). Existing differences between the SCC of the two families lie in the structure of their Omega subzones (Figures 2A vs 2B, Table S3), where in UmuD' it is one residue shorter, a single Tyr¹¹⁴ instead of a Asn²⁷⁷-Ser²⁷⁸ dipeptide (Table 1). Such sequence-difference shortening induces a Asx-motif [30] within interactions VIII in UmuD' (Figure 2B). Additionally, within the same Omega subzones, in UmuD' an internal strong hydrogen bond VII connecting their N- and C-ends is lost, and Pro¹⁰⁹ makes a weaker interaction (Figures 2A vs 2B; Table S3). Due to presence of the side-chain atoms of Pro¹⁰⁹ between the N- and C-ends of the Omega subzone, the distance between them increases by 1.1 Å in UmuD' compared to the signal peptidase I, which affects its interactions with the NucBaseOmega subzone and the catalytic nucleophile.

2.7. SCC in a Non-Canonical Repressor Protein CI

Of the other seven representative structures of the LexA endopeptidase domain-like family proteins, six have SCCs similar to the UmuD' mutagenesis protein and only the repressor CI from *Eganvirus ev186* (RPC1_BP186; PDB ID: 2FJR [27]) is different (Table 1). The RPC1_BP186 does not form a canonical active site due to the absence of the C-terminal half of the Nuc-peptide and the catalytic base. The size of the Omega subzone is also significantly decreased from eight residues to

five. However, the structure of the cross-sheet ladder in this protein is similar to that of all other LexA endopeptidases.

2.8. Conformation of SCC During Self-Cleavage of LexA Endopeptidase Domain-Like Family Proteins

Self-cleavage of the UmuD to produce UmuD' requires protein RecA in vivo. The 3D structure of UmuD in complex with a segment of RecA has been determined by electron microscopy (PDB ID: 8GMT, R = 3.31 Å) [31]. In 8GMT, the UmuD/RecA complex shows the catalytic domain of UmuD, which later participates in dimerization of UmuD' (Figure 5A). RecA affects self-cleavage of UmuD at the Cys₂₄-Gly₂₅ peptide bond. Figure 5A shows the self-cleavage segment, Pro₁₆-Ser₂₈, bound to the rest of the protein. To prevent self-cleavage, the catalytic base, Lys₉₇, was mutated to Ala₉₇. The conformation of SCC during self-cleavage of LexA endopeptidase domain-like proteins (Figure 5A) is similar to that of Type I signal peptidases (Figure 4B), but there are clear differences. Before the point of protein cleavage, fragments Gly₂₀₄-Pro₂₁₂ (in signal peptidase IB) and Pro₁₆-Cys₂₄ (in UmuD) interact the respective Nuc-peptides in identical manner. However, after the point of protein cleavage the conformation differs, where amino acids of the signal peptidase IB do cross contact the Omega subzone, while structurally similar amino acids of UmuD do not (Figure 4B vs. 5A). In UmuD, the polypeptide chain turns around and the DAD_{Base} (Phe₉₄-Phe₉₆ in UmuD) interactions XVI and XVII while exist, but bind substrate amino acids before (interaction XVI) and after (interaction XVII) the point of self-cleavage, unlike in signal peptidase IB, where the DAD_{Base} motif is entirely bound to the substrate peptide prior to the point of peptide cleavage (Table S4).

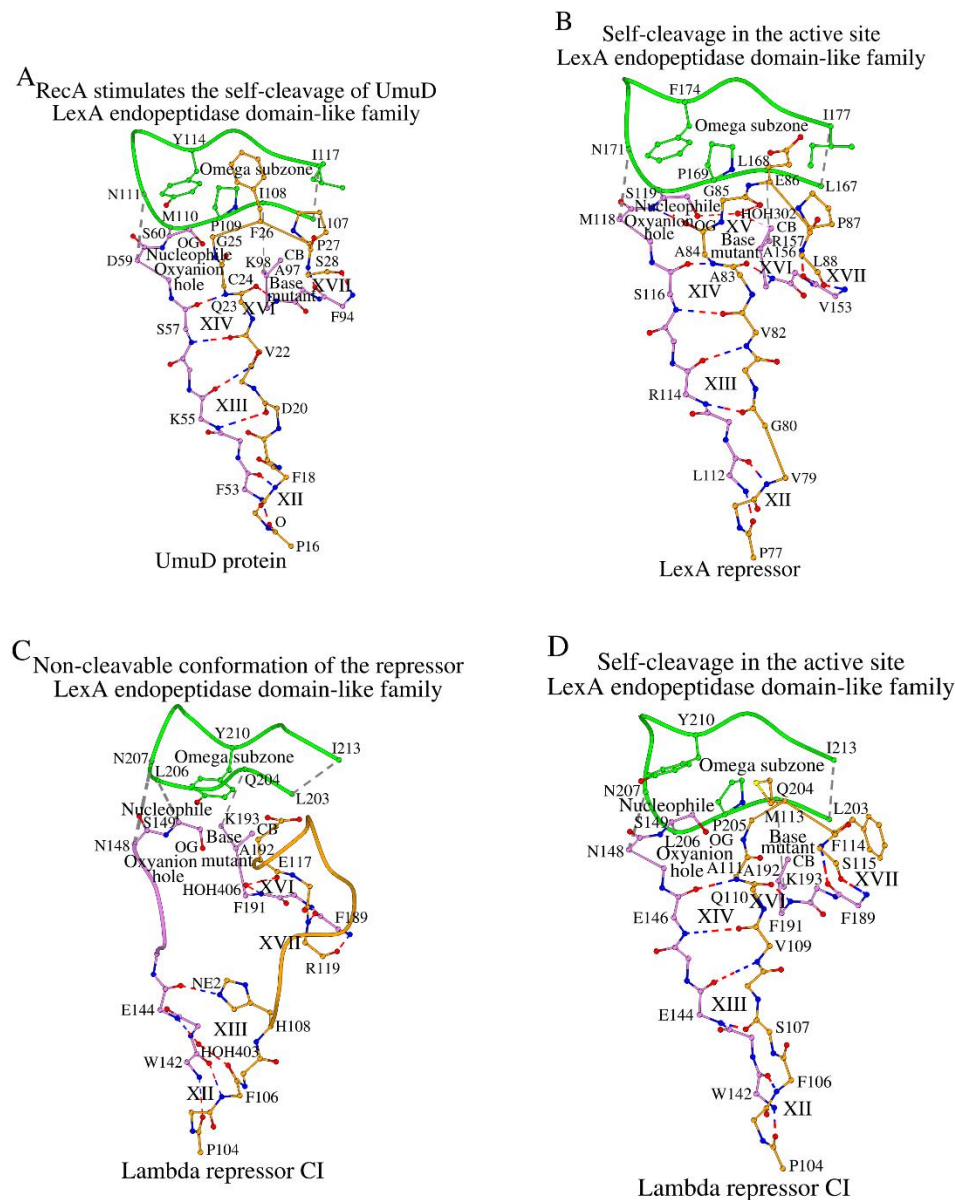


Figure 5. Four different types of interactions between proteins of the LexA endopeptidase domain-like family proteins with the target polypeptide chains. A) show the UmuD protein, B) show LexA repressor, C) and D) show non-cleavable and self-cleaved variants of the lambda repressor CI, respectively. Interactions marked as XII through XVIII are described in Table S4.

2.9. Conformation of SCC During Self-Cleavage of LexA Repressors

With the same conformation of the SCC as above, we see in two LexA repressors, the LexA repressor from *P. aeruginosa* (LEXA_PSEAI, PDB ID: 8S7G) [23], and the LexA repressor from *E. coli* (LEXA_ECOLI), in which there are three sets of coordinates with PDB IDs: 1JHE [24], 8GMS [31] and 8TRG [32] (Table S4). Similar results are obtained for all four 3D structures (Figure 5B).

2.10. Conformation of SCC in the Non-Activated (Unable to Self-Cleave) and RecA-Activated (Able to Self-Cleave) Forms of Lambda Repressor CI

For the lambda repressor CI, there are also two sets of coordinates: the non-activated (PDB ID: 2HNF) [25] and RecA-activated 3D structures (PDB ID: 8GMU) [31] (Figures 5C and 5D, respectively). The non-activated protein pattern does not show the typical parallel contact of the Nuc-peptide with the segment to be cleaved (Figure 5C vs 5A). Interactions structurally equivalent to XII and XIII do

exist, which are followed by interactions XVI and XVII of the DAD_{Base} motif (N/Phe₁₈₉-O/Phe₁₈₉-N/Phe₁₉₁) with the tripeptide Glu₁₁₇-Arg₁₁₉ (Table S4). However, after activation by protein RecA, the conformation of the lambda repressor CI changes into the standard conformation as seen for the UmuD and LexA proteins shown above (Figure 5D, Table S4).

2.11. SCC of the Standalone Protein 186 Repressor CI

Section 2.7 mentioned the non-canonical SCC of the 186 repressor CI. However, this protein has a canonical cross-sheet ladder that begins with a tripeptide Met₁₁₉-Ala₁₂₀-Ile₁₂₁ (Tables 1, S1 and S2). Moreover, similar to the complexes described here, fragment Ala₁₂₀-Glu₁₂₄ sandwiches the pentapeptide Glu₈₉-Leu₉₃ by means of interactions structurally equivalent to XII, XIII and XIV (Figure 6; columns XII-XIV in Table S4). Due to the absence of the C-terminal half of the Nuc-peptide, the next interaction in the SCC is only XVII, where Glu₉₆ (equivalent to Ser₂₈ in UmuD) contacts with the N-terminal part of the DAD_{Base} motif (Figure 6; column XVII in Table S4). In an unrelated manner, but similar to SipA (Figure 4C) which does not have peptidase activity, in 186 Repressor CI there is interaction between the amino acid at the position of the catalytic base (Arg₁₅₂ in 186 Repressor CI and Lys₈₃ in SipA) and the main-chain oxygen atom (O/Val₁₆₉ in 186 Repressor CI and O/Leu₁₄₇ in SipA) of the Omega subzone. It is assumed that the molecular function of this protein is the lysogenic regulation in bacteriophage 186 [27].

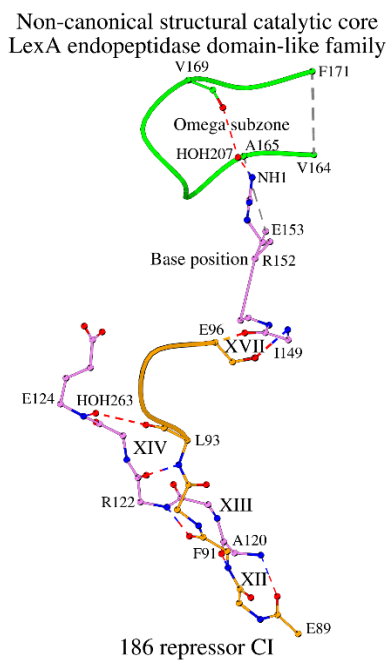


Figure 6. In the absence of a canonical active site due to the lack of both the C-terminal end of the Nuc-peptide and the catalytic base, the non-canonical SCC of the LexA endopeptidase domain-like protein 186 repressor CI interacts with the target polypeptide (shown orange color) in a unique way.

2.12. The LexA/Signal Peptidase-Like Superfamily: Existence of Two Protein Families with Different Molecular Function Correlates with Differences in Their Omega Subzones

The different direction of peptides after the point of cleavage in the families of type I signal peptidases and LexA endopeptidases can be explained by differences in the structure of their Omega subzones. These differences include: (1) different size of the Omega subzone; (2) appearance of a conservative proline (Pro₁₀₉ in UMUD_ECOLI) at a specific place of the N-terminal segment of the Omega subzone, and of an aromatic residue (Tyr₁₁₄ in UMUD_ECOLI) at the C-terminal segment of the Omega subzone in LexA endopeptidases; and (3) existence of a residue with a large side-chain group in the second amino acid position after the cleavage point in the same family (Phe₂₆ in

UMUD_ECOLI). Interactions of the DAD_{Base} motif support the change in polypeptide chain direction due to these differences.

2.13. Participation of the Cross-Sheet Ladder Step in Peptidase Dimerization

In the above study there were two structures of dimers without bound inhibitors, where the dimeric forms structurally demonstrated how a possible inter-chain function, such as self-cleavage or inter-chain inhibition of catalytic activity could take place. These structures are protein SipA from the family of Type I signal peptidases (R9TES9_STRPY; PDB ID: 4N31; Figure 4C) and (2) protein UmuD from the family of LexA endopeptidases (UMUD_ECOLI; PDB ID: 8GMT; Figure 5A).

Unlike these two structures, the signal dimer of the peptidase I from *E. coli* (LEP_ECOLI; PDB ID: 1B12; chains A and D) has a separate inhibitor in its active site. In LEP_ECOLI, the interface between chains A and D contains interacting glycine residues, which are located at position 320 at the C-terminus of the molecule: CA/Gly_{320_A/D}-O/Gly_{320_D/A} = 3.6/3.6 (2.6/2.7) 149°/140° (Figure 7). At the same time, Gly₃₂₀ forms a bridge with the Ile₂₉₉ of β -strand 3 of the same chain (see column “Dimer interface” in Table S3). In the structure with PDB ID: 1B12, structural coordinates for the Lys₃₀₅-Thr₃₁₂ fragment are missing, but it can be found in the structure with PDB ID: 3IIQ of the same protein [33]. With this fragment, the dimer interface is also involved in Nuc-peptide stabilization and ligand binding with interactions Glu₃₀₇-Pro₈₇ and OE2/Glu₃₀₇-O14/JZA₃₁₄, respectively. Finally, Ile₂₉₉, which forms a bridge with the interface Gly₃₂₀, lies next to the cross-sheet ladder step Gly₂₉₄-Ala₂₉₈. Ten out of twelve representative structures show the identical mode of dimer formation (Table S3).

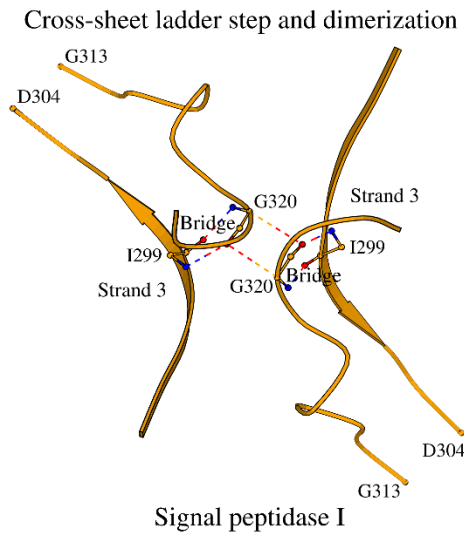


Figure 7. Dimer interface at step 3 of the cross-sheet ladder in type I signal peptidases.

2.14. Comparison of the NucBaseOmega Subzones Between the LexA/Signal Peptidase-Like Superfamily and the β -Lactamase/Transpeptidase-Like Superfamily

Recently a similarly derived SCC was introduced for the β -lactamase/transpeptidase-like superfamily [1]. As mentioned in the Introduction, there is a certain similarity in “function via a Ser-Lys catalytic dyad” between the β -lactamase/transpeptidases described in [1] and the LexA/signal peptidases described here. It is curious to see how their functional similarity translates into structural similarity between their SCCs. In addition to the catalytic residues and their respective subzones, formation of the Omega subzone affects SCCs in the two superfamilies. Figures 8A and 8B illustrate the NucBaseOmega subzones of the two superfamilies, using examples of the signal peptidase I and the β -lactamase CTX-M-14, respectively.

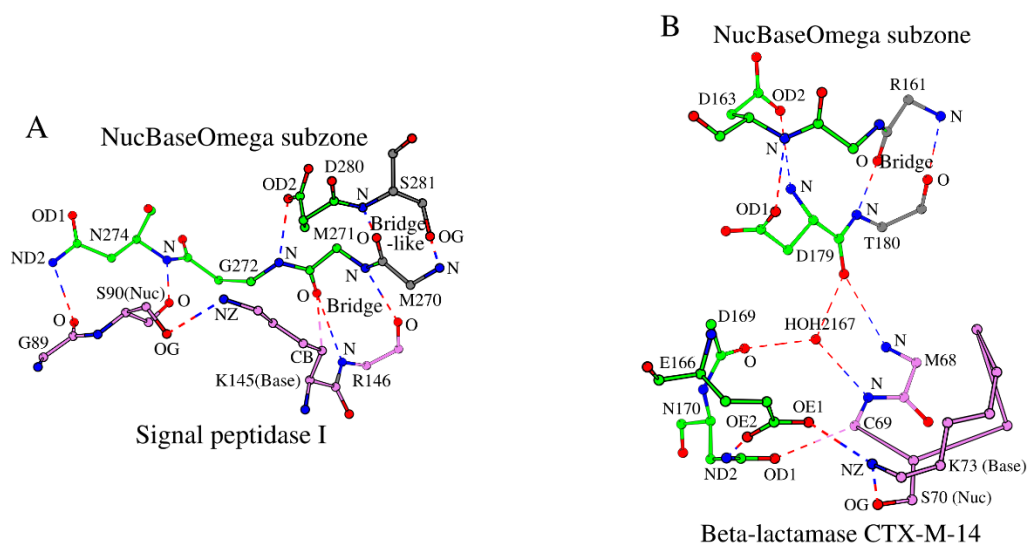


Figure 8. Comparison of the NucBaseOmega subzones in two different superfamilies of peptidases. **A.** The LexA/signal peptidase-like superfamily with the example of signal peptidase I. **B.** The β -lactamase/transpeptidase-like superfamily with the example of β -lactamase CTX-M-14.

The NucBaseOmega subzones of the two superfamilies differ significantly from each other, and they also differ in their folds, making the two superfamilies different locally and globally. Among similarities, one can generally pinpoint the presence of Asp, Asn and Glu residues in the region for both superfamilies. In particular, in both superfamilies, an asparagine of the Omega subzone, Asn₂₇₄ and Asn₁₇₀ in Figures 8A and 8B, interacts with the catalytic nucleophile and the residue preceding it.

3. Materials and Methods

The Protein Data Bank (PDB, <http://www.rcsb.org/>; 12 March 2025 [12]), Classification of protein families InterPro (<https://www.ebi.ac.uk/interpro/entry/InterPro/IPR036286/>; 12 March 2025 [3]) and the Structural Classification of Proteins database (SCOP, <https://www.ebi.ac.uk/pdbe/scop/>; 12 March 2025 [11]) were used to retrieve 19 representative structures of proteins from the LexA/signal peptidase-like superfamily (SCOP ID: 3000103). Probable quaternary structures were obtained using the PDBePISA server (<https://www.ebi.ac.uk/pdbe/pisa/>; 12 March 2025 [34]). Detailed structural information from the above set of PDB files is given in Section 2.1.

Structure visualization and structural analysis of interactions between amino acids in proteins (hydrogen bonds, hydrophobic, other types of weak interactions) were conducted using Maestro (Schrödinger Release 2023-1: Schrödinger, LLC, New York, NY, USA, 2021; <http://www.schrodinger.com/>; 12 March 2025) and software [35] to determine interatomic contacts, i.e., of ligand–protein contacts (LPCs) and contacts of structural units (CSUs).

Weak hydrogen bonds were identified based on geometrical criteria [36]. Figures were drawn with MOLSCRIPT [37] and PyMOL molecular graphics system (<https://pymol.org/>; 12 March 2025).

4. Conclusions

Structural studies of the catalytic site of 12 representative proteins that are part of the LexA/signal peptidase-like superfamily have revealed that the B-type Structural Catalytic Cores (B-SCCs) of these proteins consist of a combination of two conserved subzones: the NucBaseOmega subzone and the Omega subzone. The NucBaseOmega subzone incorporates (a) the Nuc-peptide, which is a hexapeptide, ending with the catalytic nucleophile; (b) the cross-sheet ladder, which is a set of bridge-like pair interactions, called steps, across the central β -sheet, and ending with the catalytic base; and (c) the N-terminal elements of the Omega subzone. The Omega subzone is a

standalone conserved substructure, which exists in two variants: (1) the Omega subzone of the type I signal peptidases; and (2) the Omega subzone of LexA endopeptidases. The latter variant is shorter than the prior, but a conservative proline and aromatic residue pair appear in it, which leads to a noticeable change in the local geometry between the two variants of Omega subzone.

Structural studies of the ligand binding and the self-cleavage process showed that proteins of the two families of the LexA/signal peptidase-like superfamily form complexes of two different types depending on the direction of the target polypeptide chain, which in turn is determined by differences in the structure of the C-terminal part of Omega subzone. The Nuc-peptide sandwiches the cleaved peptide in all enzymes such that the cleavage site is “correctly” placed near the catalytic nucleophile and the oxyanion hole. The catalytic base peptide (Xaa_{base-3}-Xaa_{base-2}-Xaa_{base-1}-LyS_{base}-Xaa_{base+1}) forms a donor-acceptor-donor DAD_{Base} motif (N/Xaa_{base-3}-O/Xaa_{base-3}-N/Xaa_{base-1}) from the other side of the Nuc-peptide/substrate sandwich prior and after the point of cleavage. The conserved asparagine of the Omega subzone and the main-chain oxygen of its N-terminal residue interact with the catalytic nucleophile and catalytic base in all structures.

Finally, the cross-sheet ladder is a key scaffold for the SCC supporting the Nuc-peptide (at strand β1), the dimerization interface (at strand β3), the catalytic base (at strand β5) and the Omega subzone (at strand β6).

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1. Two patterns of the cross-sheet ladders in 12 LexA/signal peptidase-like superfamily representative proteins. Table S2. Conserved geometric parameters (distance and angle) in two patterns of the cross-sheet ladder contacts in 12 LexA/signal peptidase-like superfamily representative proteins. Table S3. Conserved geometric parameters (distance and angle) of the Omega and NucBaseOmega subzones in 12 LexA/signal peptidase-like superfamily representative proteins. Table S4. Conserved geometric parameters (distance and angle) of interactions between SCC and an inhibitor or the target polypeptide chain in 9 LexA/signal peptidase-like superfamily representative proteins.

Author Contributions: A.I.D.: Study design, Formal analysis, Methodology, Visualization, Writing – Original Draft, Writing – Review & Editing; K.D.: Formal analysis, Methodology, Visualization, Writing – Original Draft, Writing – Review & Editing; M.S.J.: Formal analysis, Methodology, Writing – Original Draft; V.N.U.: Study design, Formal analysis, Methodology, Visualization, Investigation, Writing – Original Draft, Writing – Review & Editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting reported results can be found in the article and Supplementary Materials.

Acknowledgments: We thank the Biocenter Finland Bioinformatics Network (Jukka Lehtonen) and CSC IT Center for Science for computational support for the project. The Structural Bioinformatics Laboratory is part of the Solution for Health strategic area of Åbo Akademi University and within the InFLAMES Flagship program on inflammation and infection, Åbo Akademi University and the University of Turku, funded by the Academy of Finland.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Denesyuk, A.I.; Denessiouk, K.; Johnson, M.S.; Uversky, V.N. Alpha and Omega Classification of β-Lactamase/Transpeptidase-like Superfamily Proteins Based on the Comparison of Their Structural Catalytic Cores. *Molecules* **2025**, *30*, 2019. doi: 10.3390/molecules30092019

2. Oliva, M.; Dideberg, O.; Field, M.J. Understanding the acylation mechanisms of active-site serine penicillin-recognizing proteins: a molecular dynamics simulation study. *Proteins* 2003, 53, 88-100. doi: 10.1002/prot.10450
3. Blum, M.; Andreeva, A.; Florentino, L.C.; Chuguransky, S.R.; Grego, T.; Hobbs, E.; Pinto, B.L.; Orr, A.; Paysan-Lafosse, T.; Ponamareva, I.; et al. InterPro: the protein sequence classification resource in 2025. *Nucleic Acids Res.* 2025, 53, D444-D456. doi: 10.1093/nar/gkae1082
4. Leszczynski, J.F.; Rose, G.D. Loops in globular proteins: a novel category of secondary structure. *Science* 1986, 234, 849-855. doi: 10.1126/science.3775366
5. Fetrow, J.S. Omega loops: nonregular secondary structures significant in protein function and stability. *FASEB J.* 1995, 9, 708-717. doi: 10.1096/fasebj.9.9.7601335
6. Kabsch, W.; Sander, C. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* 1983, 22, 2577-2637. doi: 10.1002/bip.360221211
7. Ekici, O.D.; Paetzel, M.; Dalbey, R.E. Unconventional serine proteases: variations on the catalytic Ser/His/Asp triad configuration. *Protein Sci.* 2008, 17, 2023-2037. doi: 10.1110/ps.035436.108
8. Buller, A.R.; Townsend, C.A. Intrinsic evolutionary constraints on protease structure, enzyme acylation, and the identity of the catalytic triad. *Proc. Natl. Acad. Sci. USA* 2013, 110, E653-E661. doi: 10.1073/pnas.1221050110
9. Maiques, E.; Ubeda, C.; Campoy, S.; Salvador, N.; Lasa, I.; Novick, R.P.; Barbé, J.; Penadés, J.R. Beta-lactam antibiotics induce the SOS response and horizontal transfer of virulence factors in *Staphylococcus aureus*. *J. Bacteriol.* 2006, 188, 2726-2729. doi: 10.1128/JB.188.7.2726-2729.2006
10. Paetzel, M.; Dalbey, R.E.; Strynadka, N.C. Crystal structure of a bacterial signal peptidase in complex with a beta-lactam inhibitor. *Nature* 1998, 396, 186-190. doi: 10.1038/24196
11. Andreeva, A.; Kulesha, E.; Gough, J.; Murzin, A.G. The SCOP database in 2020: expanded classification of representative family and superfamily domains of known protein structures. *Nucleic Acids Res.* 2020, 48, D376-D382. doi: 10.1093/nar/gkz1064
12. Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. *Nucleic Acids Res.* 2000, 28, 235-242. doi: 10.1093/nar/28.1.235
13. UniProt Consortium. UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Res.* 2025, 53, D609-D617. doi: 10.1093/nar/gkae1010
14. Peat, T.S.; Frank, E.G.; McDonald, J.P.; Levine, A.S.; Woodgate, R.; Hendrickson, W.A. Structure of the UmuD' protein and its regulation in response to DNA damage. *Nature* 1996, 380, 727-730. doi: 10.1038/380727a0
15. Tuteja, R. Type I signal peptidase: an overview. *Arch. Biochem. Biophys.* 2005, 441, 107-111. doi: 10.1016/j.abb.2005.07.013
16. Little, J.W. Mechanism of specific LexA cleavage: autodigestion and the role of RecA coprotease. *Biochimie* 1991, 73, 411-421. doi: 10.1016/0300-9084(91)90108-d
17. Butala, M.; Zgur-Bertok, D.; Busby, S.J. The bacterial LexA transcriptional repressor. *Cell Mol. Life Sci.* 2009, 66, 82-93. doi: 10.1007/s00018-008-8378-6
18. Ting, Y.T.; Harris, P.W.; Batot, G.; Brimble, M.A.; Baker, E.N.; Young, P.G. Peptide binding to a bacterial signal peptidase visualized by peptide tethering and carrier-driven crystallization. *IUCrJ* 2016, 3, 10-19. doi: 10.1107/S2052252515019971
19. Young, P.G.; Proft, T.; Harris, P.W.; Brimble, M.A.; Baker, E.N. Structure and activity of *Streptococcus pyogenes* SipA: a signal peptidase-like protein essential for pilus polymerisation. *PLoS One* 2014, 9, e99135. doi: 10.1371/journal.pone.0099135
20. Liaci, A.M.; Steigenberger, B.; Telles de Souza, P.C.; Tamara, S.; Gröllers-Mulderij, M.; Ogrissek, P.; Marrink, S.J.; Scheltema, R.A.; Förster, F. Structure of the human signal peptidase complex reveals the determinants for signal peptide cleavage. *Mol. Cell* 2021, 81, 3934-3948.e11. doi: 10.1016/j.molcel.2021.07.031
21. Zhang, A.P.P.; Rice P.A. Crystal structure of a LexA protein from *Thermotoga maritima*. 2010. doi: 10.2210/pdb3K2Z/pdb.

22. Chandran, A.V.; Srikalaivani, R.; Paul, A.; Vijayan, M. Biochemical characterization of Mycobacterium tuberculosis LexA and structural studies of its C-terminal segment. *Acta Crystallogr. D Struct. Biol.* 2019, 75, 41-55. doi: 10.1107/S2059798318016066
23. Vascon, F.; De Felice, S.; Gasparotto, M.; Huber, S.T.; Catalano, C.; Chinellato, M.; Mezzetti, R.; Grinzato, A.; Filippini, F.; Maso, L.; et al. Snapshots of Pseudomonas aeruginosa SOS response reveal structural requisites for LexA autoproteolysis. *IScience* 2025, 28, 111726. doi: 10.1016/j.isci.2024.111726
24. Luo, Y.; Pfuetzner, R.A.; Mosimann, S.; Paetzel, M.; Frey, E.A.; Cherney, M.; Kim, B.; Little, J.W.; Strynadka, N.C. Crystal structure of LexA: a conformational switch for regulation of self-cleavage. *Cell* 2001, 106, 585-594. doi: 10.1016/s0092-8674(01)00479-2
25. Ndjonka, D.; Bell, C.E. Structure of a hyper-cleavable monomeric fragment of phage lambda repressor containing the cleavage site region. *J. Mol. Biol.* 2006, 362, 479-489. doi: 10.1016/j.jmb.2006.07.026.
26. Bell, C.E.; Frescura, P.; Hochschild, A.; Lewis, M. Crystal structure of the lambda repressor C-terminal domain provides a model for cooperative operator binding. *Cell* 2000, 101, 801-811. doi: 10.1016/s0092-8674(00)80891-0
27. Pinkett, H.W.; Shearwin, K.E.; Stayrook, S.; Dodd, I.B.; Burr, T.; Hochschild, A.; Egan, J.B.; Lewis, M. The structural basis of cooperative regulation at an alternate genetic switch. *Mol. Cell* 2006, 21, 605-615. doi: 10.1016/j.molcel.2006.01.019
28. Denessiouk, K.A.; Johnson, M.S. "Acceptor-donor-acceptor" motifs recognize the Watson-Crick, Hoogsteen and Sugar "donor-acceptor-donor" edges of adenine and adenosine-containing ligands. *J. Mol. Biol.* 2003, 333, 1025-1043. doi: 10.1016/j.jmb.2003.09.017
29. Duddy, W.J.; Nissink, J.W.; Allen, F.H.; Milner-White, E.J. Mimicry by Asx- and ST-turns of the four main types of beta-turn in proteins. *Protein Sci.* 2004, 13, 3051-3055. doi: 10.1110/ps.04920904
30. Wan, W.Y.; Milner-White, E.J. A natural grouping of motifs with an aspartate or asparagine residue forming two hydrogen bonds to residues ahead in sequence: their occurrence at alpha-helical N termini and in other situations. *J. Mol. Biol.* 1999, 286, 1633-1649. doi: 10.1006/jmbi.1999.2552
31. Gao, B.; Liang, L.; Su, L.; Wen, A.; Zhou, C.; Feng, Y. Structural basis for regulation of SOS response in bacteria. *Proc. Natl. Acad. Sci. USA* 2023, 120, e2217493120. doi: 10.1073/pnas.2217493120
32. Cory, M.B.; Li, A.; Hurley, C.M.; Carman, P.J.; Pumroy, R.A.; Hostetler, Z.M.; Perez, R.M.; Venkatesh, Y.; Li, X.; Gupta, K.; et al. The LexA-RecA* structure reveals a cryptic lock-and-key mechanism for SOS activation. *Nat. Struct. Mol. Biol.* 2024, 31, 1522-1531. doi: 10.1038/s41594-024-01317-3
33. Luo, C.; Roussel, P.; Dreier, J.; Page, M.G.; Paetzel, M. Crystallographic analysis of bacterial signal peptidase in ternary complex with arylomycin A2 and a beta-sultam inhibitor. *Biochemistry* 2009, 48, 8976-8984. doi: 10.1021/bi9009538
34. Krissinel, E.; Henrick K. Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* 2007, 372, 774-797. doi: 10.1016/j.jmb.2007.05.022
35. Sobolev, V.; Sorokine, A.; Prilusky, J.; Abola, E.E.; Edelman, M. Automated analysis of interatomic contacts in proteins. *Bioinformatics* 1999, 15, 327-332. doi: 10.1093/bioinformatics/15.4.327
36. Derewenda, Z.S.; Derewenda, U.; Kobos, P.M. (His)C epsilon-H...O=C < hydrogen bond in the active sites of serine hydrolases. *J. Mol. Biol.* 1994, 241, 83-93. doi: 10.1006/jmbi.1994.1475
37. Kraulis, P.J. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Cryst.* 1991, 24, 946-950. doi: 10.1107/S0021889891004399

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.