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IN SILICO ANALYSIS OF TRANSLOCATOR-CHAPERONE INTERACTION FROM YSA-YSP TYPE III SECRETION SYSTEM OF YERSINIA ENTEROCOLITICA

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ABSTRACT: The interaction of Translocator YspC and chaperone SycB is an important event in regulation of *Yersinia* secretion apparatus- *Yersinia* secretion protein type III secretion system of *Yersinia enterocolitica.* The homology model of YspC depicted a Y-shaped structure comprising of alpha helices interspersed by coiled regions. There are three distinct regions in YspC forming the arms and the base of the Y-shaped structure. ConSurf analysis showed that maximum structural and functional conservation is in the concave core of SycB formed by the Tetratricopeptide Repeats (TPRs), whereas the N-terminal helix is moderately conserved. Molecular docking studies showed SycB exists in a dimeric state. The long N-terminal helix of one SycB molecule interacts with its counterpart in the other SycB molecule to form the SycB dimer. Similar docking studies also revealed SycB localizes within the fork formed by the arms of Y-shaped YspC. The first two TPRs of SycB forming the concave core are mainly responsible for its interaction with YspC. However, due to the bulky nature of YspC, stearically it becomes impossible for SycB to maintain its dimeric state and one of the SycB molecules gets displaced. Therefore, the dimeric state of chaperone SycB dissociates upon interaction with translocator YspC and it forms a 1:1 heterodimeric complex with YspC.

KEYWORDS: Homology model, Evolutionary conservation, Tetratricopeptide Repeats, Molecular docking, Translocator-chaperone interaction

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1. INTRODUCTION

Yersinia enterocolitica is a gram negative bacterium and an opportunistic pathogen. It causes many enteric diseases in immune-compromised individuals. *Yersinia enterocolitica* enters through food and water, and invades the gastro-intestinal tract. While in the intestine it causes intestinal phase of the infection. Then the bacterium crosses the intestinal barriers through M-cells overlying the Peyer's patches and spreads to the spleen, mesenteric lymph nodes and other visceral organs, and causes the systemic phase of the infection. *Yersinia* secretion component- *Yersinia* outer protein (Ysc-Yop) is a type III secretion system (T3SS) used by *Y. enterocolitica* to overcome the primary host immune responses and the effectors of Ysc-Yop T3SS causes systemic phase of the infection. It is encoded by the pYV plasmid of *Y. enterocolitica* [1, 2, 3, 4, 5, 6, 7]. Another T3SS required for the gastro-intestinal phase of the infection is *Yersinia* secretion apparatus- *Yersinia* secretion protein (Ysa-Ysp) T3SS. It is encoded by a ~200 Kb Pathogenecity Island present on the bacterial chromosome of *Yersinia enterocolitica* biovar 1B (serotypes O:4, O:8, O:13 and O:21) [8, 9]. This T3SS consists of structural proteins, regulators, effector proteins, translocators and chaperones. *syc-ysp* operon present within the 200 kb Pathogenecity Island encodes for proteins like SycB, YspB, YspC, YspD, YspA and AcpY. SycB, YspB, YspC and YspD show homology in sequence to SicA, SipB, SipC and SipD from *Salmonella* SPI-1 T3SS and IpgC, IpaB, IpaC, and IpaD from *shigella* Mxi-Spa T3SS. However, YspC could not complement IpaC in *Shigella flexneri*, neither it could act as a functional substitute of YopD [8, 9, 10, 11]. Ysa-Ysp T3SS in general and syc-ysp operon in particular has been comparatively much less explored though it contains some very important chaperone like SycB, and unique translocator like YspC. SycB plays the dual role of a chaperone and a regulator of T3SS. It is a class II chaperone of T3SS, which binds to the major translocator YspB and minor translocator YspC, sequesters them in the bacterial cytoplasm and releases them after activation of the T3SS. SycB also acts as a regulator of T3SS by interacting with the transcriptional activator YsaE and activating the transcription of *sycB, yspBCDA*. Activation signal of Ysa-Ysp T3SS is mediated by phospho-relay proteins YsrR, YsrS, YsrT, RcsB, where transcriptional regulators YsaE and SycB also play a major role [7, 11, 12, 13, 14, 15, 16]. A narrow conduit is required for translocation of toxic effector proteins from bacterial cell to the host cell cytoplasm. A needle like substructure projects out from basal body formed on the bacterial cell membrane. The tip of the needle is the translocation apparatus or translocon which interacts with the host cell membrane and mediates the delivery of effector toxins within the host cell cytoplasm. This translocon is formed by hydrophobic translocator proteins like YspB and YspC, which sits on the scaffold formed by hydrophilic translocator protein YspD. So, these proteins while forming the translocon are exposed to the extracellular milieu. Hence, they are potential therapeutic targets against the infection of *Yersinia*

entercolitica, caused by the activation of Ysa-Ysp T3SS [7, 8, 9, 10, 11, 12]. Our previous study showed that SycB interacts with YspC and forms a 1:1 heterodimeric complex [17, 18]. In this Study, we would translate the biochemical observations into a working model in order to provide further insight into YspC-SycB interaction.

2. MATERIALS AND METHODS

Homology modeling

The homology model of YspC and SycB were generated by I-TASSER server. The sequence of YspC and SycB were obtained from National Centre for Biotechnology Information (NCBI) protein database. The Sequence of YspC and SycB were uploaded in FASTA format separately. Considering the C-score, TM-score and RMSD, the best possible models were selected [17, 19].

ConSurf Analysis of SycB

Homology model of SycB was loaded onto the ConSurf server. Multiple sequence alignment was built using MAFFT. The homologues were collected from UNIREF 90 database. Homolog search algorithm was CS-BLAST and the E-value was kept at 0.0002. Number of CSI-iterations was 5. Maximal % ID between sequences were 95, Minimal % ID for homologues were 20. Maximum number of homologues was 150. There are 22 CSI-BLAST hits. 21 of them are unique, including the query. The calculation was performed on the 21 unique sequences [20].

Molecular Docking

Molecular docking was performed using ZDOCK server (version 3.0.2). To study the dimerization of SycB molecule, the homology model of SycB was loaded as input protein 1 as well as the input protein 2. However, when we analyzed the interaction of YspC and SycB, The homology model of SycB was loaded as input protein 1 and that of YspC as input protein 2 [21].

Representation of the Models

The homology models and the models obtained by molecular docking were represented using Jmol and PyMOL Molecular Graphics System [22, 23]. Chimera was used for representation of the model of SycB along with conservation colour codes, as generated by ConSurf [24, 25].

3. RESULTS AND DISCUSSION

Homology Model of YspC shows an elongated Y-shaped structure with three distinct regions

YspC is a unique translocator protein in many respects. Unlike other minor hydrophobic translocators, it does not localize in the inclusion bodies, neither does it exists as a molten globule. It is highly soluble and very stable and possesses rigid tertiary structure. Till now, only the structures of small peptides or fragments of translocator proteins are available [18, 26, 27, 28]. Therefore, it would

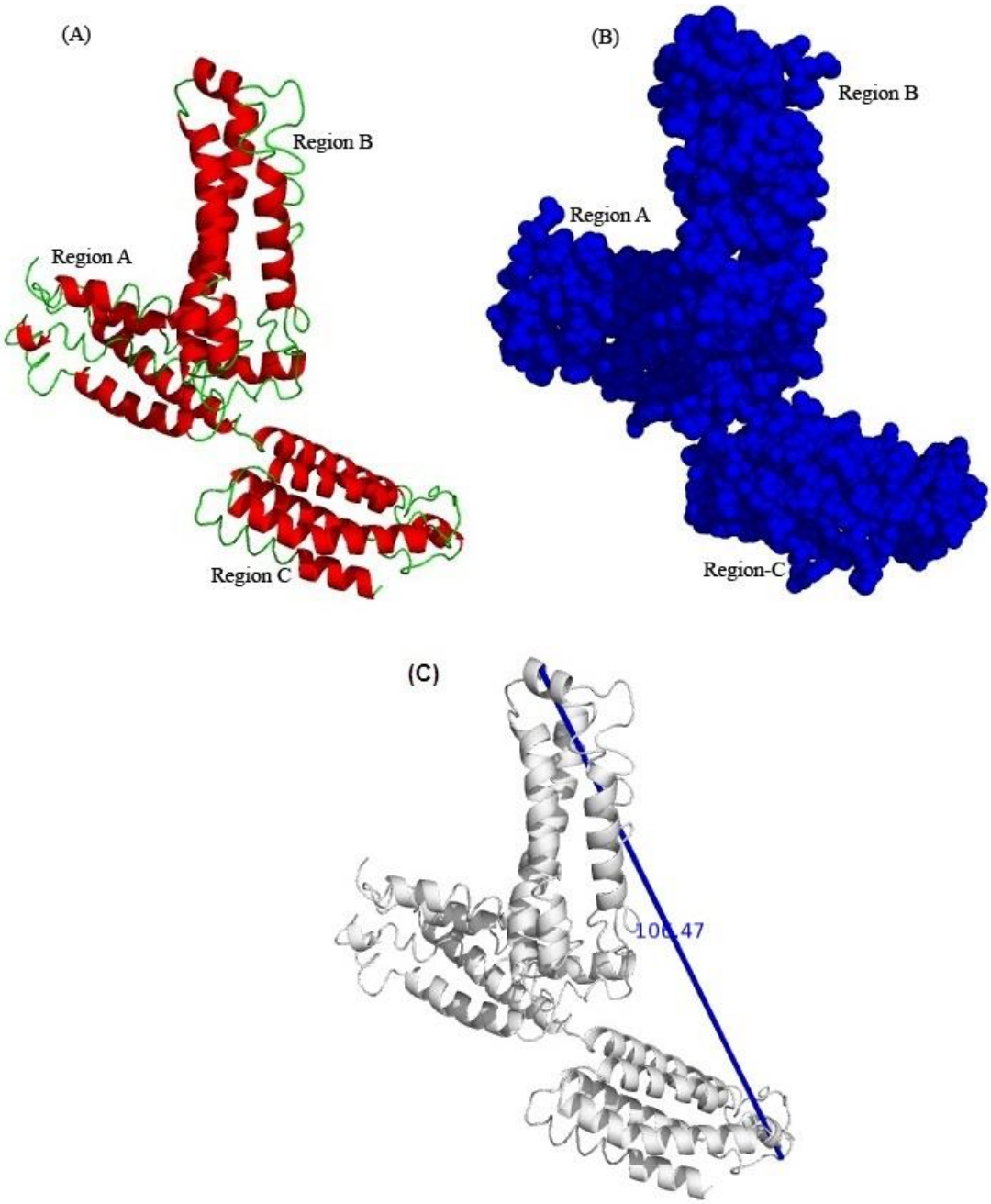

Figure 1: Homology Model of YspC shows a Y-shaped structure consisting of alpha helices interspersed by coiled regions (A) Cartoon Representation of homology model of YspC. The helices were shown in red and the coiled regions were shown in green colour. There are three distinct regions in YspC homology model. In region A, there are 7 helices forming a bundle like structure. Region B, also shows a bundle like structure formed by five alpha helices. In region C, there are six helices forming a perfect bundle like structure. (B) The atoms of the homology model were represented as spheres in deep blue colour. (C) Cartoon representation of the model of YspC showing its maximum length.

be interesting to know the spatial model of YspC. The homology model of YspC was obtained by using I-TASSER server [19]. In I-TASSER server the structure templates are identified by LOMETS (a meta-server threading approach) from the PDB library. LOMETS contains multiple threading programs, and each of these threading programs could generate more than ten thousand template alignments. The significance of the threading alignments was measured by the Z-score. The 10 best templates in order as selected from the LOMETS threading program for YspC were PDB Hits (5ic0A, 4hpqA, 4uxvA, 3zx6A, 1ciiA, 3zx6A, 4uxvA, 2ch7A, 4hpqC, 5ic0A). In general, the template with the highest Z-score is selected from each of the threading program. The C-score for YspC model is -1.19, which is well within the acceptable range and the estimated TM-score is 0.57$\pm$ 0.15, which is more than 0.5 indicating that the model is of correct topology. The homology model of YspC depicted a Y-shaped structure and contains short to long helices interspersed by loops. The model of YspC could be differentiated into three distinct regions forming the base and the two arms of Y. In each of these regions helices assemble like bundles joined by loop like structures. In region A, there are 7 helices forming a bundle like structure. Two of these helices are very short in length. Interestingly, these helices do not occur in a sequential manner. However, the folding of these helices is so unique that they form a bundle like structure together. Region B, also shows a bundle like structure formed by five alpha helices. In region C, there are six helices forming a perfect bundle like structure [Figure 1A & 1B]. The model of YspC is elongated and showed a maximum length of 10.6 nm, which depicts an extended conformation for a 48.3 KDa protein [Figure 1C]. This observation was corroborated by Size exclusion chromatography (SEC) data [18]. Thus, the structure of YspC could be visualized as a union of three different bundles joined by looped regions.

Evolutionary conserved residues are mainly located in the Concave core of SycB

ConSurf is a web based server which determines the structurally and functionally conserved regions in a protein, thereby predicting the pathway of evolution of a protein. The conserved residues were indicated in the homology model of SycB and represented by Chimera [20, 24, 25]. The extent of conservation could be determined by adjoining conservation scale. ConSurf analysis clearly depicted that certain regions in the concave core of SycB are highly conserved evolutionarily. On the Contrary, the N-terminal helix is only moderately conserved. The Tetratricopeptide Repeats (TPRs) forming the concave core, are maximally conserved structurally and functionally. Amongst the TPRs, the second TPR showed maximal evolutionarily conserved regions. Whereas the first and the third TPR also contains some conserved residues [Figure 2A & 2B]. Therefore, it could be predicted that the conservation within the chaperone might play a role in the Chaperone-translocator interaction.

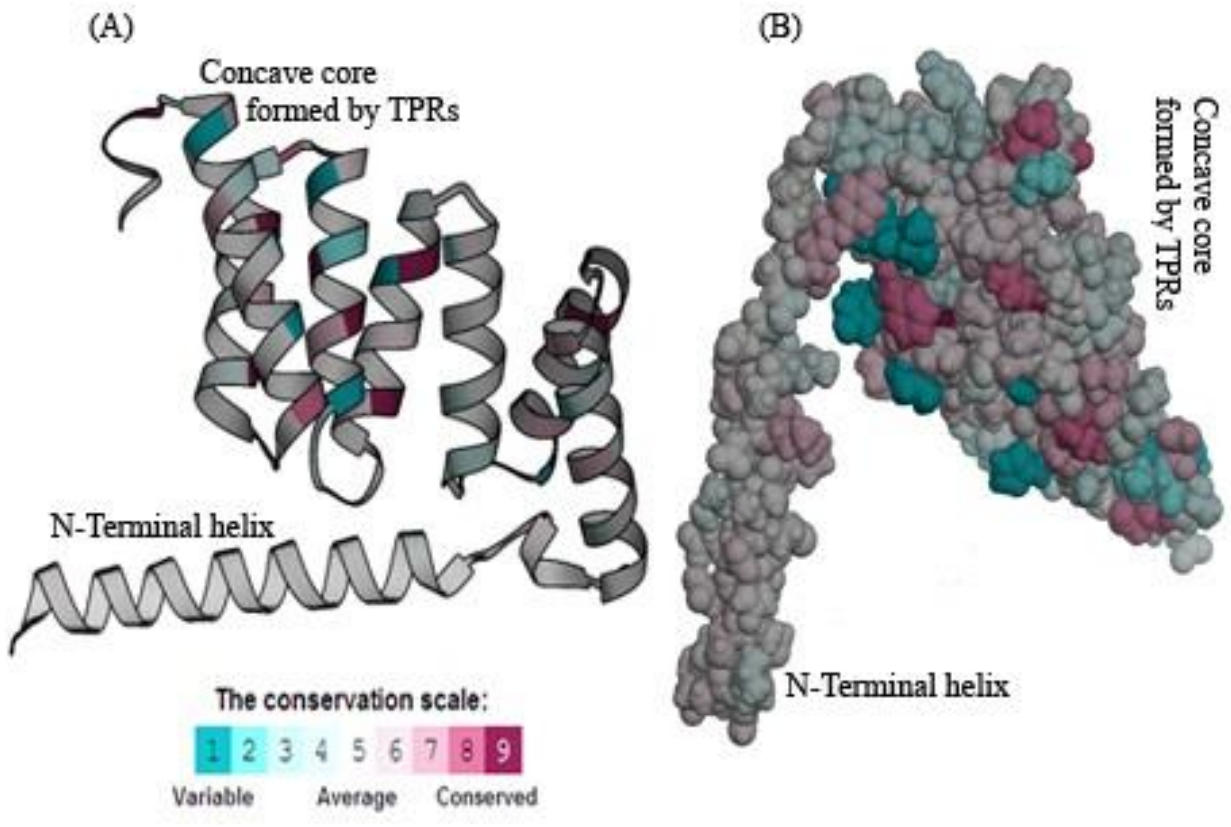

Figure 2: Homology model of SycB showing evolutionarily conserved residues as predicted by ConSurf. (A) Cartoon representation of homology model of SycB (B) The atoms of homology model of SycB were represented as spheres. Both the figures showing the extent of conservation of SycB molecule based on the adjoining colour graded conservation scale.

Molecular docking study depicts the dimeric spatial model of SycB

The model of SycB could be segregated into two distinct domains. The N-terminal of SycB comprises of a long helix followed by a short flexible helix. And there is a concave core formed by TPRs. The N-terminal helix is joined to the concave core by a short flexible helix. The TPRs are formed by two antiparallel helices of 34 amino acid residues. There are three TPRs forming the concave core or two TPRs and one TPR-like region according to the TPR prediction server and conserved domain database [Figure 3A] [17, 29, 30]. Molecular docking studies using ZDOCK showed that SycB exists as dimer [21]. The dimeric state of SycB was stabilized by interaction

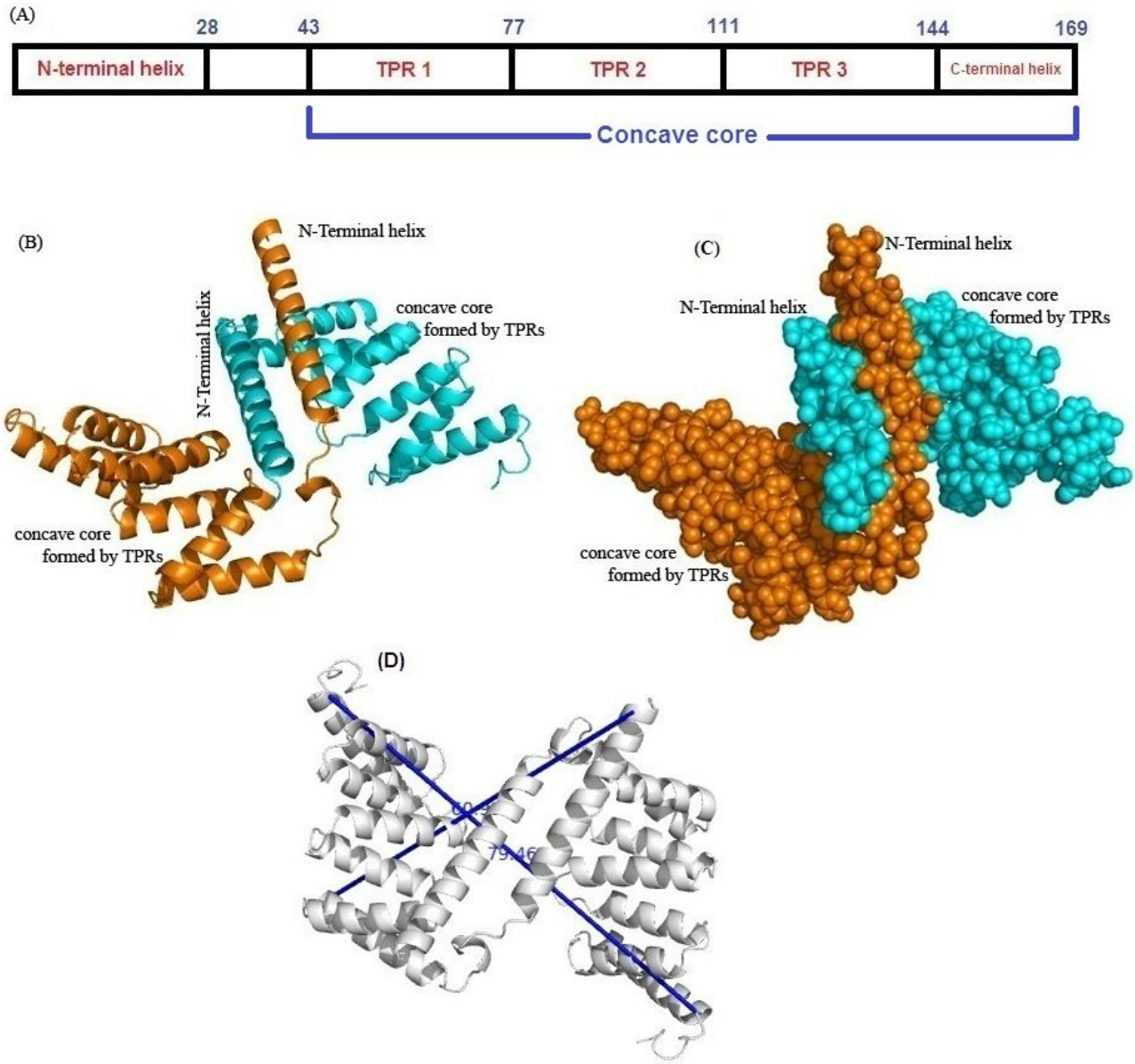

Figure 3: Molecular docking studies reveal that the N-terminal helix is mainly responsible for the dimerization of SycB. (A) Schematic representation of various regions within SycB. (B) Cartoon representation of the SycB dimer. (C) The atoms of SycB were represented as spheres in the SycB dimer. In both the figures one of the SycB molecule was represented in orange and the other SycB molecule in Cyan colour. (D) Cartoon representation of the SycB dimer showing its dimensions.

mediated mainly by the long N-terminal helix. The short-helix joining the long N-terminal helix to the first helix of the Tetraticopeptide repeat (TPR) provides the additional flexibility to the long N-terminal helix, thereby, allowing it to interact with its counterpart and form the dimeric model of

SycB [Figure 3B & 3C]. This model was further corroborated by the fact that size exclusion chromatography and chemical crosslinking experiments clearly revealed the dimeric nature of SycB. Also, deletion of the N-terminal helix disrupts the stable dimeric state of SycB [17]. The Model of SycB dimer is almost globular in conformation, with diameter varying from 6.1 nm to 7.9 nm, which is almost similar to the available SEC and dynamic light scattering data [Figure 3D] [17]. The Molecular weight of SycB dimer is almost same as that of YspC, but the significant difference in the dimensions of the two proteins reveal that YspC possess an elongated structure whereas SycB dimer is somewhat globular in nature.

YspC interacts with the TPRs of SycB and stearically disrupts the dimeric state of SycB

Our study of interaction between YspC and SycB by ZDOCK molecular docking server showed YspC interacts with SycB, using the TPR regions of SycB [17, 21]. However, the main interacting residues of SycB are confined to the first two TPR regions. IpgC, a class II chaperone and a close homologue of SycB, consists of pockets where the IpaB peptide localizes [27]. These pockets are formed by the residues mainly located in the first two TPR regions. Also PcrH (another class II chaperone) interacts with minor translocator PopD using the residues present within its concave cleft [26]. Region A and Region B of YspC is mainly responsible for its interaction with SycB. Region A and Region B forms a fork like structure in the Y-shaped YspC molecule. SycB localizes almost completely within the fork [Figure 4A & 4B]. The maximum length of YspC-SycB complex is 10.8 nm, which is almost similar to the size of YspC (10.6 nm), as SycB localizes almost completely within YspC [Figure 4C]. This is also evident from the SEC profile of YspC and YspC-SycB where both the proteins eluted corresponding to the same molecular mass [17, 18]. Since YspC is a bulky molecule, stearically it becomes impossible for SycB to maintain its dimeric state as the other SycB molecule is displaced once YspC interacts with SycB (Figure 3B & 3C, Figure 4A & 4B). Forsters resonance energy transfer analysis and further design of deletion mutants clearly revealed that the two TPR regions of SycB, were mainly responsible for its interaction with YspC, and YspC disrupts the dimeric state of SycB [17, 18].

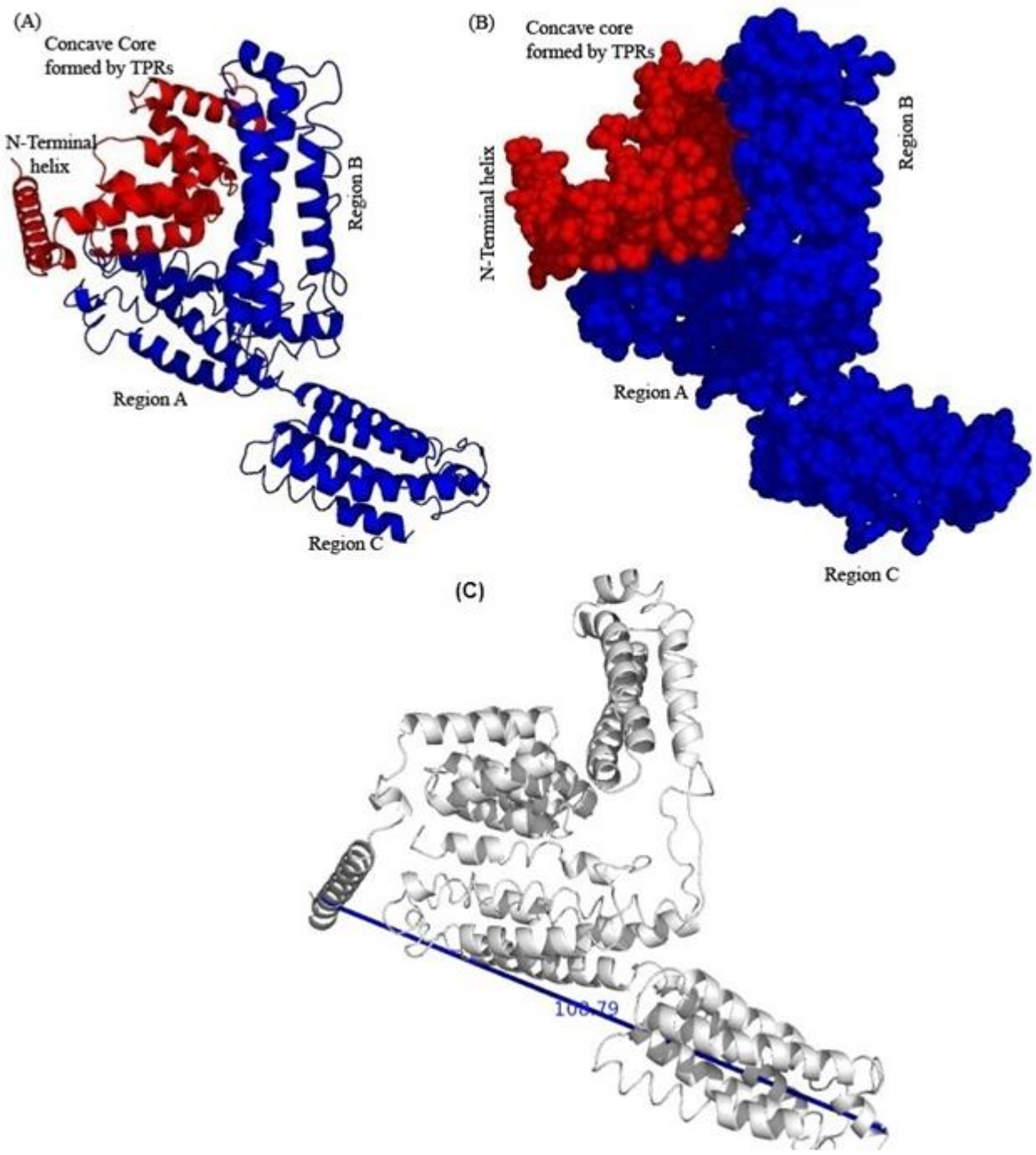

Figure 4: Molecular docking showed SycB localizes within the fork formed by the arms of Y-shaped YspC, by an interaction mediated by the TPRs of SycB (A) Cartoon representation of the model of YspC-SycB interaction. (B) In the YspC-SycB interaction model, the atoms of the YspC-SycB molecules were represented as spheres. The YspC molecule was represented in deep blue colour and SycB molecule was represented in red colour. (C) Cartoon representation of the model of YspC-SycB showing its maximum length.

4. CONCLUSION

Little information is available regarding the spatial model of Translocator-chaperone interaction. Some three dimensional experimental structures are available for small peptides of translocators interacting with chaperone [26, 27].Therefore, the model of YspC-SycB interaction would be significant. Here, a dimeric chaperone alters its physiological state upon interaction with translocator and forms a 1:1 heterodimeric complex. In the present case, the domains responsible for dimerization of the chaperone and chaperone-translocator interaction are separate. Still the dimeric state of the chaperone is disrupted once it interacts with the translocator. This model shows sequestration of the translocator within the bacterial cell cytoplasm, which is an important component of regulation of T3SS. For the first time, this model depicts the interaction of a full length minor translocator protein with its chaperone, which could be further used to analyze and dissect translocator-chaperone interaction.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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