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# A molecular modeling study of the interaction between SRP-receptor complex and peptide translocon

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ABSTRACT

The signal recognition particle (SRP) mediated protein translocation pathway is universal and highly conserved in all kingdoms of life. Significant progresses have been made to understand its molecular mechanism, yet many open questions remain. A structure model, showing how nascent peptide inserts into peptide translocon with the help of SRP protein Ffh and its receptor FtsY, is desired to facilitate our studies. In this work, we presented such a model derived by computational docking of the Ffh–FtsY complex onto the translocon. This model was compatible with most available experiments. It suggested that the Ffh–FtsY complex approached the translocon with its G domains and was locked up by the cytoplasmic loop of SecG and the C5/C6 loops of SecY. Several residues were expected to play important roles in regulating GTP hydrolysis. Additionally, a hypothesis on the yet ambiguous function of FtsY A domain was proposed. These interesting results invite experimental investigations.


The signal recognition particle (SRP) usually targets integral membrane proteins to the cellular protein translocation machinery during translation [1]. This pathway is highly conserved in all three kingdoms of life. First, SRP recognizes the signal sequence of a nascent membrane protein as it appears from the ribosome, and forms a cytosolic targeting complex. This complex is then recognized by the SRP receptor and directed to the protein translocation machinery, a complex collectively known as the translocon. The translocon is embedded either in the endoplasmic reticulum membrane (in eukarya) or the cytoplasmic membrane (in prokarya). Following the binding of ribosome and translocon, SRP and its receptor dissociate from the nascent chain in a GTP-dependent manner.

The bacterial SRP system, consisting of the SRP protein Ffh, 4.5S SRP RNA, SRP-receptor FtsY, and SecYEG translocon complex, represents the simplest and most conserved part of all SRP systems. Understanding how these components interact has attracted constant attentions. Significant progresses have been made. In 2004, the structure of Ffh–FtsY complex from *Thermus aquaticus* was solved, revealing a GTP-dependent symmetric heterodimer conformation [2]. Later, a cryo-electron microscopy (cryo-EM) structure of an opened *Escherichia coli* translocon was reported [3]. In 2006, the cryo-EM structure of *E. coli* Ffh and 4.5S RNA interacting with a nascent chain at the peptide exit of a ribosome was captured [4]. Assuming these bindings functionally relevant, it is tempting to assemble these pieces together to create a model showing how SRP and its receptor guide a nascent chain into the peptide translocon, facilitating further mechanistic studies on this conserved protein translocation system. In 2007, the *E. coli* FtsY structure was also solved [5], marking that all the monomer structures required for such an integrated model became available in *E. coli*. Knowing that FtsY interacts physically and functionally with the translocon [6], and with the above structures, the only missing part is the binding conformation between Ffh–FtsY complex and peptide translocon.

Protein–protein docking provides a potential means to fill this gap. In some cases, atomic-level prediction accuracy is within reach, as exemplified by the performances of several docking programs [7]. The ZDOCK software has a long-standing history in protein docking. It has produced high-accuracy predictions for multiple protein–protein complexes in many rounds of the CAPRI blind challenge [8]. ZDOCK uses fast Fourier transformation to search all possible binding modes between proteins. Ranks are given to putative conformations based on shape complementarity, desolvation energy, and electrostatics. Its official site claims that in 49% of the test cases, native structures can be identified in top 4 predictions.

In this study, we modeled the *E. coli* Ffh–FtsY complex referencing the *T. aquaticus* Ffh–FtsY complex [2]. Computational docking and structure optimizations were then used to predict the binding mode between *E. coli* Ffh–FtsY complex and translocon. Based on available complex structures containing other SRP components, these components were then assembled onto the predicted Ffh–FtsY-translocon complex. The resulting model was assessed and discussed in the sequel.

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## Materials and methods

The reported *E. coli* translocon complex in open conformation (2AKI) [3] is a backbone-only structure. We therefore prepared an all-atom model with Modeller 8v2. The *E. coli* Ffh–FtsY complex structure was constructed with *E. coli* Ffh (taken from 2J28) [4] and *E. coli* FtsY (2QY9) [5] referencing the *T. aquaticus* complex between them (1RJ9) [2]. Only the NG domains of Ffh–FtsY complex were used in subsequent docking. This is because that FtsY A domain did not have any available structure or suitable template, and conflicting reports existed for the arrangement of Ffh M domain [9,10]. As discussed below, FtsY A domain was not likely to involve in peptide insertion [11,12]. Its exclusion was therefore unlikely to affect docking accuracy. However, we did construct a reference structure with the Robetta ab-initio folding server [13], for estimation of its size and electrostatic properties. The final model was validated for its compatibility. Also, parallel ZDOCK runs were performed with different M domain arrangements. Results showed that no distinctive binding mode was missed because of M domain exclusion.

Protein docking was performed with the ZDOCK software bundled with the InsightII package version 2005 (Accelrys™ Inc.). Fifty four thousand potential binding conformations were generated to ensure exhaustive sampling. Improbable ones, in which contacts were found at the extracellular surface of the translocon, were immediately discarded. Top 2000 conformations were clustered on the basis of pair-wise RMSD using a hierarchical clustering algorithm to identify rough binding modes. Structures within a 10 Å threshold were designated as one mode. It is reported that ribosome binds Ffh in cytosol and remains bonded until the end of a protein targeting cycle. Their binding mode was available in a cryo-EM structure [4], which was further supported by independent cross-linking results [14]. Referencing this conformation, only two binding modes permitted reasonable placement of a ribosome (i.e. entirely inside the membrane and its peptide exit faced the translocon). All individual conformations clustered into these two binding modes were optimized as below. One binding conformation was found best in energy and was taken as the putative binding mode.

In this work, modeled structures were optimized with molecular dynamics simulations as follows. Energy minimization was first performed with steepest descent method for 100 steps followed by conjugated gradient method for 300 steps, to release conflicting contacts. After the system was gradually heated from 10 to 300 K over 60 ps using the NVT ensemble, the equilibration dynamics of the entire system was performed for 100 ps. Finally, 1 ns MD simulation was conducted at 1 atm. A time step of 2 fs was used. Coordinates were saved every 1 ps. The average structure from 800 to 1000 ps was calculated and submitted for energy minimization with a conjugate gradient method for 5000 steps to obtain the final optimized model.

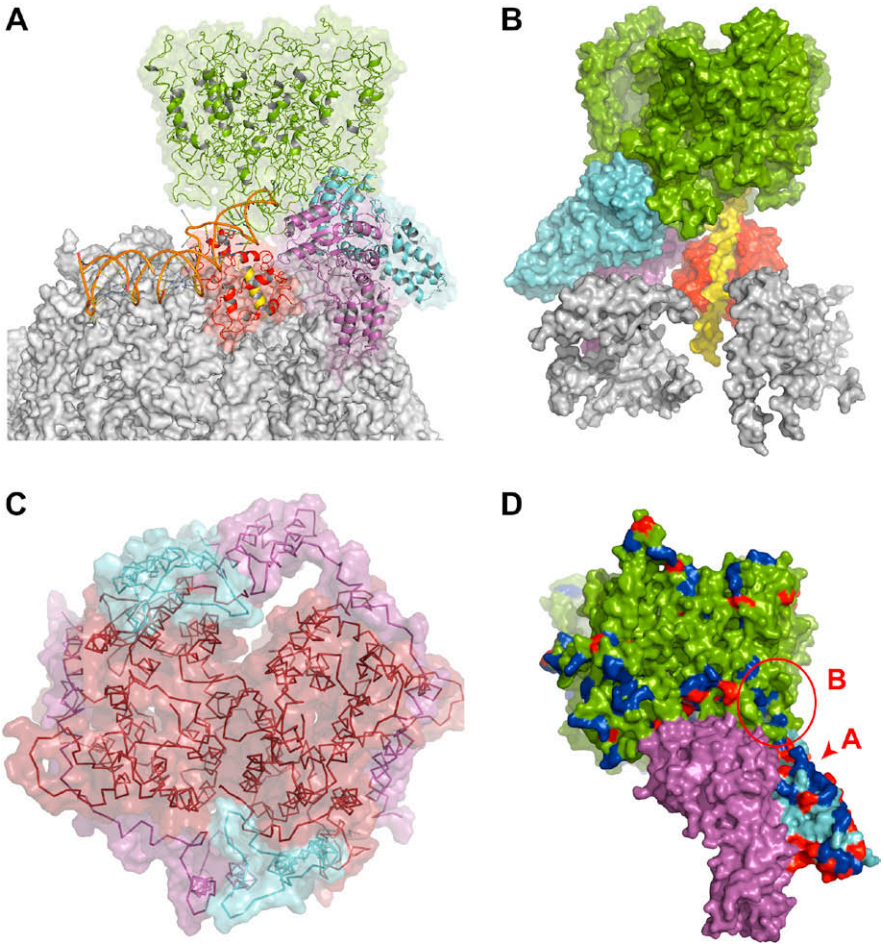


**Fig. 1.** Structure model of a nascent peptide being inserted into a peptide translocon with the help of SRP and its receptor. (A) Front view of the entire complex. The translocon was colored in green, Ffh NG domain in magenta, M domain in red, FtsY in cyan, signal peptide in yellow, and ribosome in grey. (B) Lateral view of the entire complex. Only the protein components of ribosome were shown to create a clearer view of the signal peptide orientation. (C) Structure of an opened translocon. SecY was colored in ruby, SecE in magenta and SecG in cyan. (D) Ffh–FtsY-translocon complex showing the putative membrane-associative patch of lysine and hydrophobic residues (site A) and the cavity on translocon (site B) that may interact with FtsY A domain. Positively (blue) and negatively (red) charged residues were marked on FtsY and translocon surface.