AN INVESTIGATION OF THE YIDC-MEDIATED MEMBRANE INSERTION OF A PF3 COAT PROTEIN USING MD SIMULATIONS

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SCANME

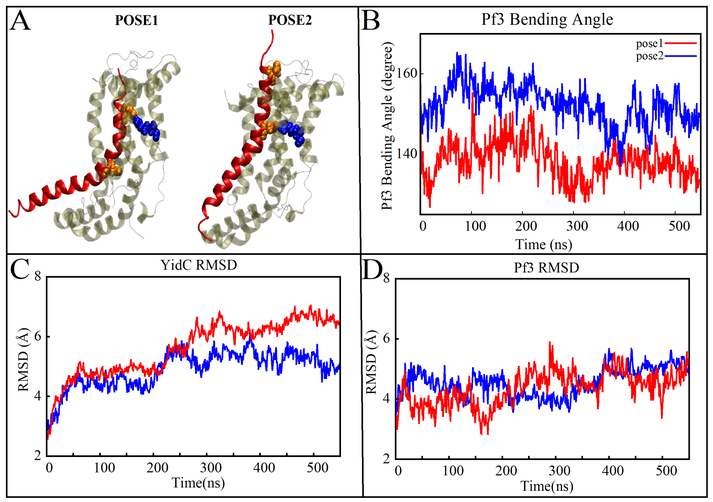
Arkansas High Performance Computing Center

BLUE WATERS

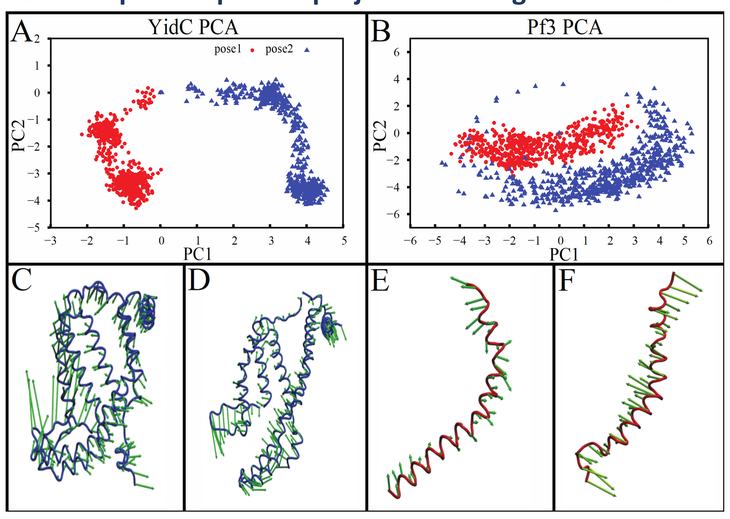
INTRODUCTION

33% of all proteins are membrane proteins, which must be inserted and embedded into the plasma layer cotranslationally. The YidC/Oxa1/Alb3 family of membrane proteins mediates the proper folding and insertion of incoming peptides and proteins in the membrane. YidC is involved in the insertion and placement of membrane proteins in microbes. They exist in all domains of life and are essential for the viability of cells. They are adaptable proteins and can work along with the Sec pathway to embed peptides into the membrane via the Signal Recognition Particle (SRP), or independently of the Sec pathway by with the ribosome. YidC completes its function either independently as a membrane insertase or as a chaperone in the SecYEG complex. Initially, the Sec independent pathway was thought to happen without the contribution of an insertase. Nonetheless, research about this has demonstrated that YidC is fundamental for the addition of the little phage coat proteins, for example, the Pf3 coat and M13 pro coat protein in a Sec-independent manner. Along these lines, our primary focus is on the mechanism of single-traversing substrate insertion in the membrane bilayer via the Sec-independent pathway. Using MD simulations, we have investigated the conformational dynamics of YidC, specifically the local and global conformational changes involved in the insertion of the Pf3 coat protein.

1. Yidc Undergoes Major Conformational Changes In Sec-independent Insertion Process.

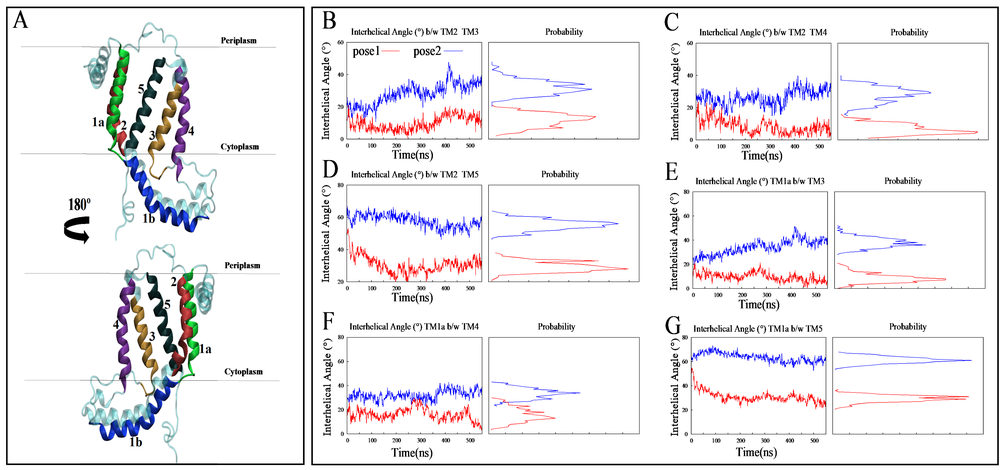


2. Principal component projections along PCs 1 and 2

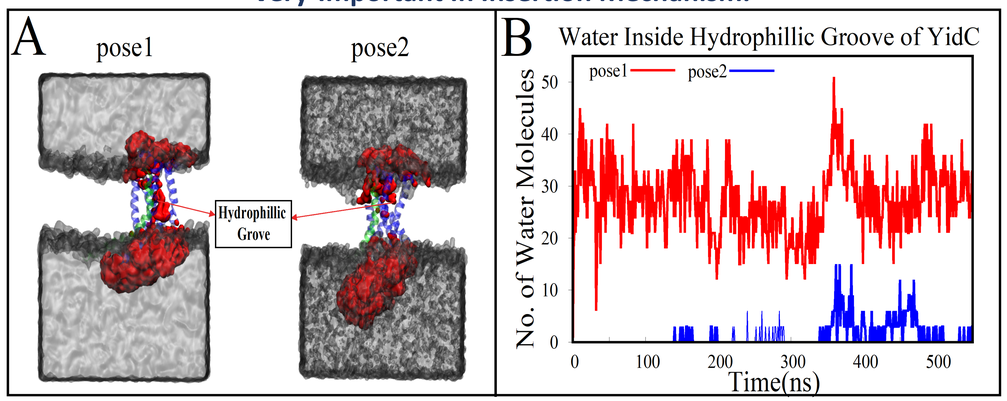


RESULTS

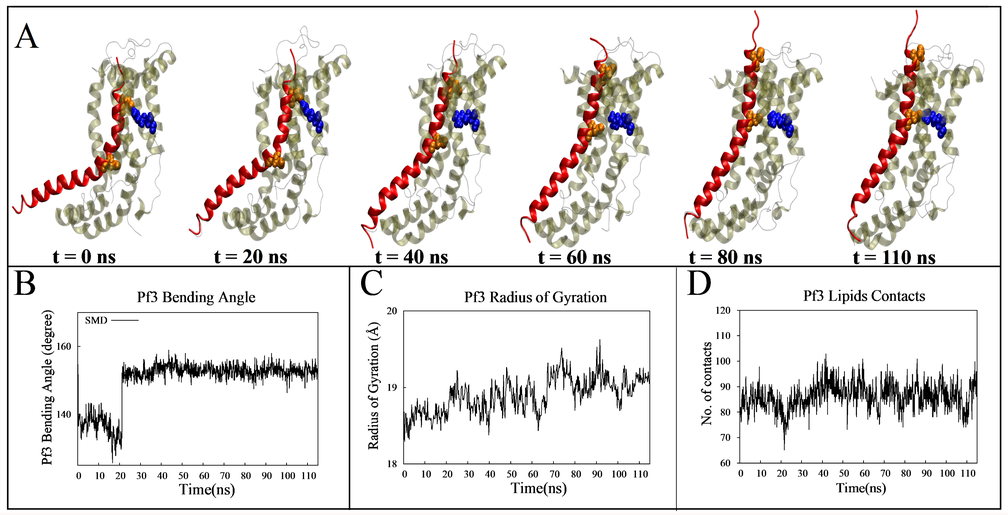
3. Widening Of The Transmembrane Domain Is Essential For The Insertion Of Signal Protein In The Insertion Process.



4. Hydration And Dehydration Of Hydrophilic Grove Of Yidc Is Very-Important In Insertion Mechanism.



5. Characterizing the insertion process using targeted MD simulations



METHODS

Initial atomic models were generated from crystal structures of YidC (PDB ID:3WO7). Missing atoms, and loops were constructed using MODELLER a modeling program used for building 3D structures of protein-based on spatial restraints. The solvated system contained ~142000 atoms and was neutralized by adding Na and Cl ions into the system until the concentration of salt was ~150mM. The system was solvated with TIP3P water, then energetically minimized and equilibrated for 600ns under constant pressure and temperature of 1 atm and 310 K respectively with a timestep of 1 fs. For simulations, the CHARMM36 forcefield was used. For minimization and system production, simulations were performed using NAMD 2.13. Docking models of Pf3 coat were generated using MOE software and equilibrated for 500ns in the POPE membrane along with the YidC. In the non-equilibrium simulations, we inserted the Pf3 coat protein into the hydrophilic grove of the YidC in a 100ns simulation using a distance collective variable followed by a relaxation of 15ns using the final frame of targeted MD simulation.

CONCLUSION

Based on our results, YidC must undergo a major conformational changes during the secY-independent insertion process. The incoming Pf3 coat protein would first come into contact with the cytoplasmic loops and then penetrate the hydrophilic groove, forming a salt bridge with R72. The YidC loops on the cytoplasmic side of the bilayer are critical for moving Pf3 into YidC's hydrophilic groove. These cytoplasmic loops make contact with the Pf3 coat at first. The negatively charged D7 residue of Pf3 interacts with the positively charged R72 of YidC to form a stable salt bridge. The formation of this salt bridge is crucial in the insertion process to stabilize Pf3 in YidC's TM groove. The Pf3 coat protein then travels towards the periplasmic side of the membrane, helped by the water slide force. The interactions with the membrane also aid in the passage of the protein towards the periplasmic side, which is also supported by the salt bridge between D18 of Pf3 and R72 of YidC; this combination stabilizes the position of Pf3 in the membrane. The protein then moves into the membrane through the water-filled cleft. Finally, after Pf3 completely enters YidC's hydrophilic groove, it will form contacts with the lipid tails, which will be aided by hydration of the groove, forcing the Pf3 coat into the bilayer.

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