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Research Article

MOLECULAR DOCKING AND DYNAMICS STUDIES ON THE PROTEIN-PROTEIN INTERACTIONS OF ELECTRICALLY ACTIVE PILIN NANOWIRES OF *GEOBACTER SULFURREDUCENS.*

D. Jeya Sundara Sharmila[1]*, J. Jino Blessy[1], K. S. Subramanian[1], K. Gunasekaran[1] and Sivakumar Uthandi[2]

[1] *Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India*
[2] *Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India*

Abstract: Molecular interactions are key aspects in biological recognitions applicable in nano/micro systems. Bacterial nanowires are pilus filament based structures that can conduct electrons. The transport of electron is proposed to be facilitated by filamentous fibers made up of polymeric assemblies of proteins called pilin. *Geobacter sulfurreducens* is capable of delivering electrons through extracellular electron transport (EET) by employing conductive nanowires, which are pilin proteins composed of type IV subunit PilA. Protein-protein interactions play an important role in the stabilization of the pilin nanowire assembly complex and it contains transmembrane (TM) domain. In current study, protein-protein docking and multiple molecular dynamic (MD) simulations were performed to understand the binding mode of pilin nanowires. The MD result explains the conformational behavior and folding of pilin nanowires in water environment in different time scale duration 20, 5, 5, 10 and 20ns (total of 60ns). Direct hydrogen bonds and water mediated hydrogen bonds that play a crucial role during the simulation were investigated. The conformational state, folding, end-to-end distance profile and hydrogen bonding behavior had indicated that the *Geobacter sulfurreducens* pilin nanowires have electrical conductivity properties.

Keywords: *Geobacter sulfurreducens;* bacterial nanowires; protein-protein interaction docking; molecular modeling simulation.

Note: Coloured Figures available on Journal Website in "Archives" Section

Introduction

Bacterial nanowires are extracellular filaments that can transport electrons relatively to a long distance. These nanowires could also act as terminal electron acceptors during respiration and are associated with metal-reducing bacteria to oxidize metals in the environment (Chang *et al.*, 2006). These micro organisms can extract and conduct electrons by directly involving in power process such as motility and indirectly involved in energy supply such as adenosine triphosphate (ATP). ATP is produced by the transfer of electron from donor molecules to acceptor molecules via reducing oxidation reaction. Many soluble electron acceptors like oxygen, nitrate and sulfate are freely available to intracellular enzymes in prokaryotes. Bacterial nanowires have electrically conductive pilus like appendages. However, the mechanism of extra cellular electron transport in biological nanowires remains unclear (El-Naggar *et al.*, 2008).

Corresponding Author: **D. Jeya Sundara Sharmila**
E-mail: djssharmila@gmail.com

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Nanowire produced by the bacterium *Geobacter sulfurreducens* is a filament based structure with thickness of 3-5nm and made up of multiple pilin type IV subunit PilA. In *G. sulfurreducens,* the first nanowire conductivity was observed and demonstrated using conducting-probe atomic force microscopy (Reguera *et al.*, 2005). In account, *G. sulfurreducens* biofilms conductive properties were measured using microbial fuel cells and the measurements showed the conductivity of 5 ms cm^{-1} which is similar to polyaniline, a synthetic organic metallic conductor (Reguera *et al.*, 2006). Sheared off nanowires of *G. sulfurreducens* are reported to show a conductivity of 4 μS cm^{-1} and to have a temperature related to that of organic metals. PilA itself has the capacity to transport electrons through π-π inter chain stackings of aromatic residues and confer metal like properties (Malvankar *et al.*, 2011). The ability of conduction based transport via nanowire to carryout extracellular electron transport enabled these bacteria to play important role in mineral and nutrient cycling, bioremediation of toxic metals and energy production via microbial fuel cell (MFC) (Vargas *et al.*, 2013).

In present work, three dimensional structure of *Geobacter sulfurreducens* pilin protein [PDB ID: 2M7G] was retrieved from protein databank (PDB) (Reardon and Mueller, 2013). The *Geobacter sulfurreducens* pilin protein was initially simulated using molecular dynamic (MD) simulation technique for 20 ns. Protein – Protein docking was done for the simulated *Geobacter sulfurreducens* pilin protein [System I] against *Geobacter sulfurreducens* pilin PDB protein 2M7G [System II] using GRAMM-X software (Tovchigrechko and Vakser, 2006). Multi simulations: First, the pilin-pilin nanowire docked complex was simulated for 5 ns simulation run and the minimum energy conformation generated during the simulation was extracted and subjected to further MD simulation for another 5ns. Second, the minimum energy conformation of pilin-pilin nanowire complex obtained during the last 5ns simulation was retrieved and further MD simulated for another 10ns duration. The minimum energy conformation of above simulation was again MD simulated for another 20ns duration resulting to a total of 60ns. The trajectories of multi-simulation *Geobacter sulfurreducens pilin-pilin nanowire* protein complex binding was observed for the determination of favorable conformations and their interactions responsible for the presence of electron conductivity.

Materials and Methods

Protein Preparation: The 3D structure [PDB ID: 2M7G] of electrically conductive bacterial nanowire of *Geobacter sulfurreducens* type IVa family of pilin protein (Reardon and Mueller, 2013) was downloaded from protein databank PDB. The pilin protein was prepared using protein preparation wizard of Maestro v9.2 (Maestro, 2009). The Impref minimization was carried out for the protein model using molecular mechanics force field optimized potentials for liquid simulations [OPLS] (Kaminski *et al.*, 2001) to optimize the model and to remove steric clashes. The restrained minimization gets completed when the root mean square deviation (RMSD) reaches 0.30 Å.

Molecular Docking and Multiple Molecular Dynamic (MD) Simulation: In this study, we used Desmond v3.0 software for multiple MD simulation in different time scale. First, we simulate the *Geobacter sulfurreducens* pilin protein subunit for 20ns simulation time at 12ps interval for recording of energy. The protein was solvated using periodic boundary conditions 10×10×10 Å orthorhombic box by adding TIP3P water model (Jorgensen *et al.*, 1983) using the force field OPLS_2005. The protein was neutralized by adding Na^+ and Cl^- counter ions to balance the net charge of the system. The system was relaxed and minimized using NPT ensemble (number of atom, pressure, and temperature) restraining non-hydrogen solute atom for 12ps and the temperature was maintained at 300 K and pressure at 1.01325 bar. The system contains **20719** atoms. Particle-mesh Ewald method was used to calculate long-range electrostatic interactions and 9 Å cut-off was set for van der waals (VDW) forces (Essmann *et al.*, 1995); Strahan *et al.*, 1998). Constraint on hydrogen bond geometry was satisfied using SHAKE algorithm. Second, simulated *Geobacter sulfurreducens* pilin protein subunit [System I] was docked against the PDB *Geobacter sulfurreducens* pilin subunit [System II] protein using the docking web server GRAMM-X. GRAMM-X web server worked based on Fast Fourier Transformation (FFT) GRAMM methodology and knowledge based scoring. In GRAMM methods, intermolecular energy potential approximating calculation is based on smoothed Lennard-Jones potential to set grid representation in the molecule. Based on knowledge based scoring function, refinement optimization and rescoring calculations are employed (Tovchigrechko and

Vakser, 2006). Third, the docked protein-protein complex was prepared using protein preparation wizard of Schrodinger suit and MD simulation was done for 5ns and at the recording energy interval of 12ps simulation run. The whole system contains 31743 atoms. The complex was simulated as mentioned in the above state condition. Fourth, the minimum energy conformation of the complex generated in previous 5ns simulation run, was retrieved from the frame no. 534 (2.563 ns) and prepared using protein preparation wizard and further simulation was done for 5ns and at 12ps recording interval for energy. The whole system contains 33592 atoms and the parameters used in this simulation are stated in above mentioned simulation. Fifth, the minimum energy conformation of the complex generated in previous 5ns simulation run, i.e., from the frame no. 465 (2.232 ns), was retrieved and prepared using protein preparation wizard & further simulation was done for 10ns at 12ps recording interval energy. The whole system contains 31042 atoms. The complex was simulated as before mentioned state condition. Sixth, the minimum energy conformation of the complex generated in previous 10ns simulation run, i.e., from the frame no. 2084 (7.642 ns), was retrieved and prepared using protein preparation wizard and further simulation was done for 20ns at recording interval energy of 12ps. The whole system contains **30518** atoms. The complex was simulated as before mentioned condition.

Results and Discussion

Molecular Docking and Multiple Molecular Dynamic (MD) Simulation Study

***Geobacter sulfurreducens* pilin protein MDs simulation:** MD simulation was carried out for *Geobacter sulfurreducens* pilin protein subunit for 20ns in aqueous environment using Desmond software. The conformational change and the folding of pilin protein were monitored in trajectory data obtained during 20000 ps. The trajectory data obtained during MD simulation for pilin protein was plotted for Energy (Figure 1) and Ramachandran plot (Figure 3). The energy plot explains that the energy of whole system was stable throughout the simulation. The minimum energy conformation was generated at global minimum potential energy of -66223kcal/mol at 18371ps. From the minimum energy conformer, Ramachandran plot was plotted and found that each residues of pilin protein was in the allowed region. The folding profile of pilin subunit protein during 20ns was shown in (Figure 3). The simulated *Geobacter sulfurreducens* pilin protein was considered as system I for further protein-protein docking and MD simulation studies.

Multiple Simulation Stage-1 5ns: Protein-protein docking was performed using GRAMM-X web server. *Geobacter sulfurreducens* pilin simulated protein (system I) and PDB protein of *Geobacter*

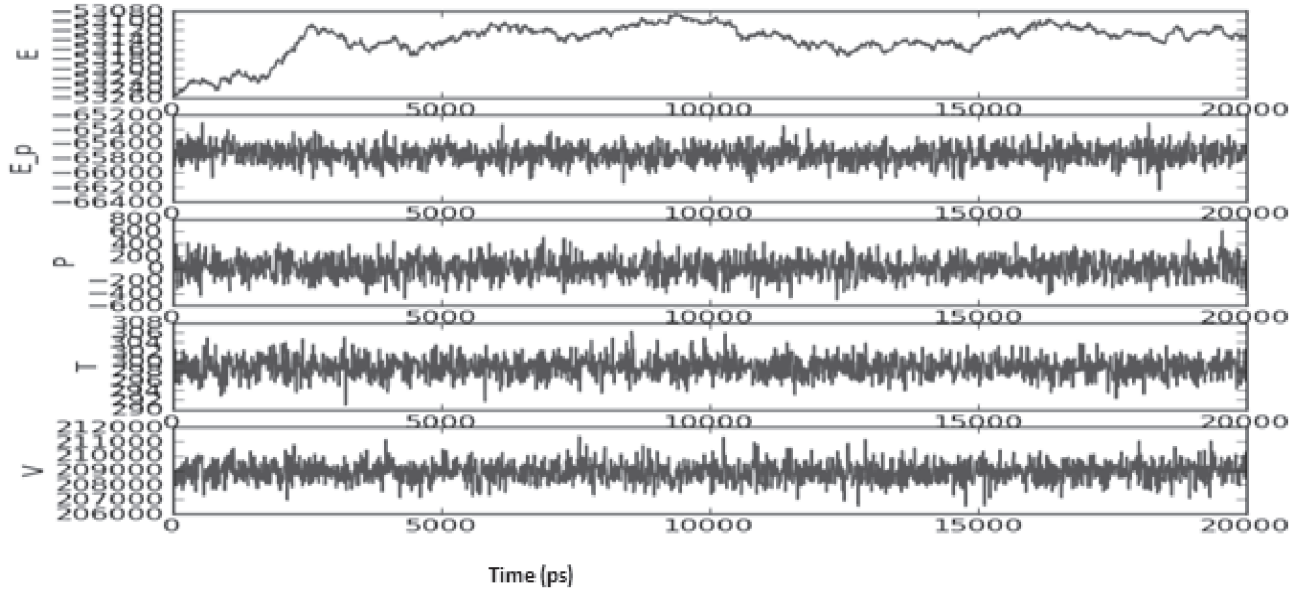

Figure 1: **Energy plot of *Geobacter sulfurreducens* pilin protein for 20ns MD simulation run (E: Total Energy in kcal/mol; E_P: Potential Energy in kcal/mol; P: Pressure in bar; T: Temperature in K; V: Volume in Å^3)**

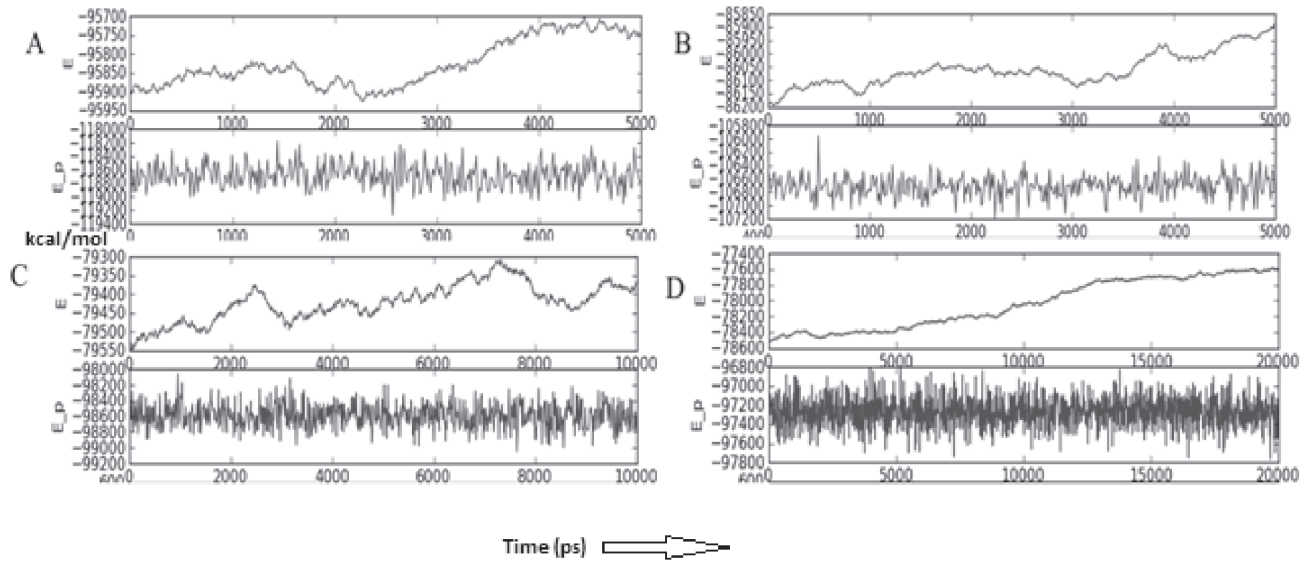

Figure 2: **Energy plot of *Geobacter sulfurreducens* pilin protein complex for multi-simulation of (A) First 5ns, (B) Second 5ns, (C) Third 10ns and (D) Final 20ns (E: Total Energy in kcal/mol; E_P: Potential Energy in kcal/mol)**

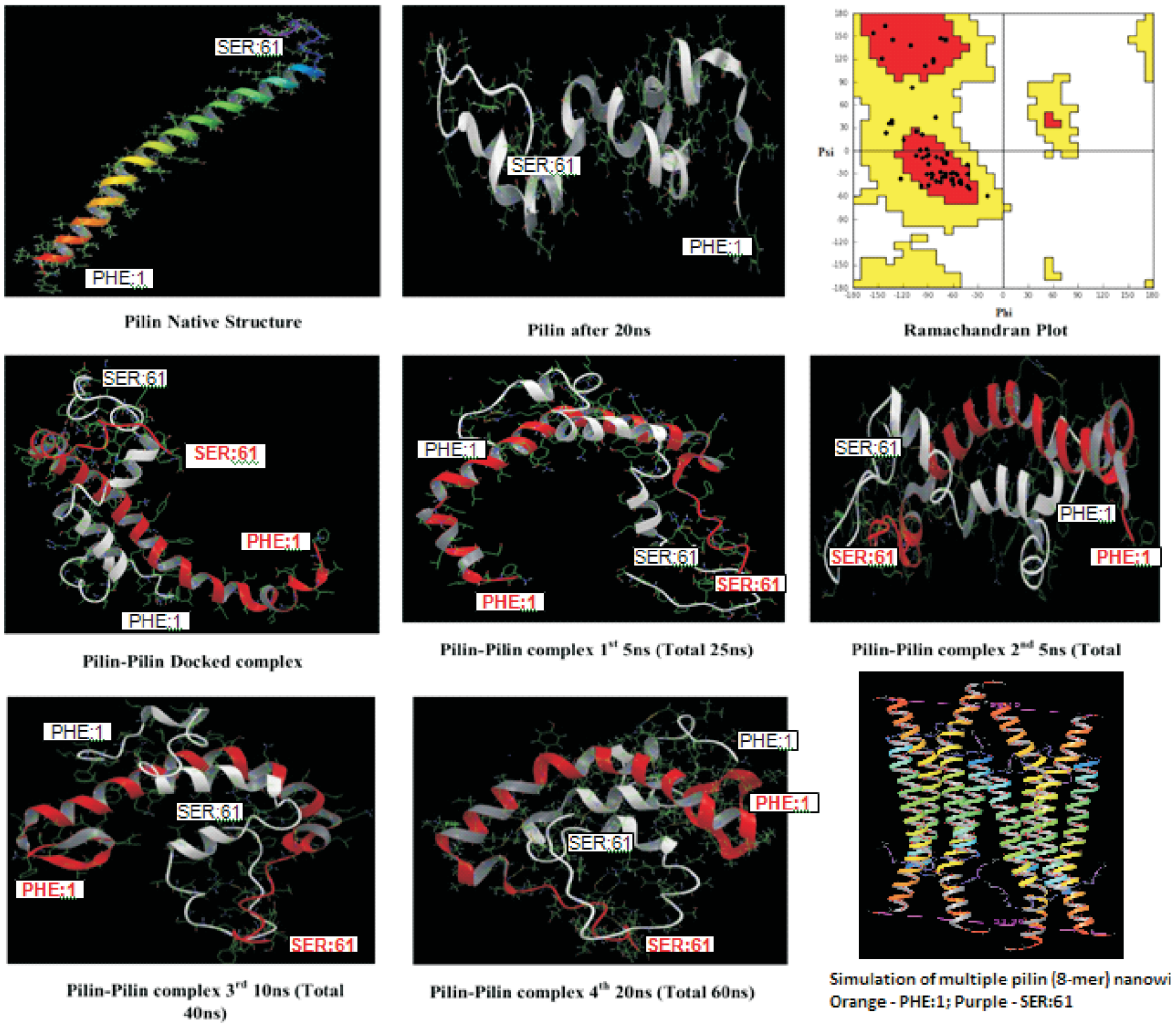

Figure 3: **Folding of *Geobacter sulfurreducens* pilin nanowire, Ramachandran plot for Pilin after initial 20ns and Pilin-pilin nanowire complex in different time scale (Total 60ns; A Chain-White; B Chain-Red)**

sulfurreducens pilin protein (system II) was docked to know their inter atomic interactions as well as the mode of binding. Multiple MD simulations were performed for protein-protein (system I and system II) docking complex using Desmond software for 1st 5ns followed by 2nd 5ns, 3rd 10ns and final 20ns subsequently to the total of 60ns including initial 20ns run. First, the pilin complex was simulated for 5ns. The energy plot and hydrogen bond interactions were analyzed for pilin complex. The energy plot explains the energy of the whole system was stable during the simulation period (Figure 2). The minimum energy conformation was generated for potential energy at -119260kcal/mol at 2563ps and at this point the complex was retrieved and used for further 5ns simulation. In the present study, direct intermolecular hydrogen bonding interactions and water mediating hydrogen bonds play a vital role for the stabilization of complex. In total, 8 direct hydrogen bonds (Table 1) and 7 water mediating hydrogen bonds (Table 2) were involved in the pilin-pilin nanowire nano wire interactions. In biological materials, hydrogen bond interaction is strong and it plays a vital role in organic electrical conductivity. In most of the polypeptides (proteins), non-covalent interaction is due to highly specific H-bonding. In proteins, the non-covalent H-bonding interactions mediate self-organization of molecular system as well as engage active part in many organic electrically conducting resources (G1owacki *et al.*, 2013). The amino acid residues of System I: ARG:41, THR:45, SER:37, GLU:60, SER:49, GLN:55, GLN:23 & THR:2 formed intermolecular hydrogen bond with the residues of System II: ASP:54, PRO:59, ASN:33, ARG:41, TYR:57, TYR:57, SER:25 & ALA:18 respectively (Table 1). The residue of System I: ASP:53, TYR:57, THR:45, ALA:50, PHE:51, SER:37 & ASP:39 formed water bridging (HOH) with residues of System II: ASP:54, ASP:54, ARG:41, ALA:52, ASP: 53, ARG:41 & ALA:36 respectively (Table 2). The aromatic residues TYR:57 and PHE:51 are found to be participating as effective electron agents in the intermolecular hydrogen bond interactions that may be responsible for their electron conduction phenomenon. Another earlier report also suggested that aromatic amino acid residues TYR and PHE of *Geobacter sulfurreducens* pilA are essential for pilin conductivity (Liu *et al.*, 2014). The favorable folding conformation of pilin-pilin nanowire complex for 5ns is shown in (Figure 3). In addition to the contribution from aromatic residues, the negatively charged residues ASP:53 and ASP:54 are found to be preferably aligned on the surface of pilus and are engaged in water mediated hydrogen bond interactions with another pilin that is detailed in Table 2.

Multiple Simulation Stage-2 5ns: In order to more examine the protein-protein interaction dynamics of the pilin dimers, the previously generated MD minimum energy conformation of pilin-pilin nanowire complex from the frame 534 at 2.563ns was further MD simulated for another 5ns. The H-bond interactions and water mediating H-bond interactions were studied during the second 5ns simulation run. The energy of the pilin-pilin nanowire complex was stable throughout the simulation and Energy (Figure 2) of the system was plotted for 5ns. The minimum energy conformation was obtained during the simulation in the frame number 465 at 2.232 ns of second 5ns simulation. The obtained conformation was further subjected to another 10ns simulation time. In pilin complex, nine direct intermolecular hydrogen bonding interactions and nine water mediating hydrogen bonds were involved in stable interaction during 5000ps simulation run. The amino acid residues of System I: ALA:50, ARG:41, ARG:41, SER:37, SER:37, GLU:60, GLU:60, ASN:33 & ARG28 formed H-bond with System II residues GLN:55, ASP:54, ASP:54, ASN:33, VAL:29, ARG:41, ARG:41, GLU:48 & GLU:5 respectively (Table 3). The residue of System I: GLU:48, GLU:48, LYS:30, ILE:4, ILE:4, SER:49, ASN:42, GLU:60 & ASP:39 formed water mediating hydrogen (HOH) with residues of System II: SER:38, ALA:46, TYR:27 , LEU:6, GLN:23, GLU:48, GLU:48, PRO:59 and ALA:36 respectively (Table 4). The negatively charged exterior positioned amino acids GLU:48, ASP:54 and GLU:60 along with aromatic TYR:27 are found to be participating in direct as well as water mediated intermolecular protein-protein hydrogen bond interaction. Another earlier X-ray micro diffraction report also explains the essential role of aromatic amino acids of *Geobacter sulfurreducens* to promote metal like conductivity and involved in long range electron transport (Malvankar *et al.*, 2015). The folding conformation of pilin-pilin nanowire complex for 2nd 5ns is shown in Figure 3.

Multiple Simulation Stage-3 10ns: With the intention of advance dynamics studying of the protein-protein interaction of the pilin dimers, the previously generated minimum energy conformation of pilin-pilin complex obtained

Table 1
Intermolecular hydrogen bonding between System I and System II complex for 5 ns simulation run.

S. No	*System I*	*System II*	*Distance Å*
1	ARG:41 & HH22	ASP:54 & OD1	1.817
2	THR:45 & HG1	PRO:59 & O	1.950
3	SER:37 & HG	ASN:33 & OD1	1.622
4	GLU:60 & OE1	ARG:41 & HH21	1.762
5	SER:49 & O	TYR:57 & HH	1.772
6	GLN:55 & HE21	TYR:57 & OH	2.118
7	GLN:23 & HE21	SER:25 & OG	2.115
8	THR:2 & H	ALA:18 & O	1.992

Table 2
Water mediated hydrogen bonds between System I and System II complex for 5 ns simulation run

S.No	*System I*	*Water Mediating Hydrogen Bonds*	*Distance Å*	*System II*	*Distance Å*
1	ASP:53 & H	HOH 3644	1.971	ASP:54 & OD2	1.876
2	TYR:57 & O	HOH 2958	1.662	ASP:54 & O	1.740
3	THR:45 & OG1	HOH 1370	1.841	ARG:41 & HE	1.890
4	ALA:50 & O	HOH 10002	1.745	ALA:52 & O	1.930
5	PHE:51 & O	HOH 1002	2.051	ASP: 53 & OD1	1.685
6	SER:37 & OG	HOH 10405	1.798	ARG:41 & HH11	1.909
7	ASP:39 & OD1	HOH 2720	1.665	ALA:36 & O	1.851

Table 3
Intermolecular hydrogen bonding between System I and System II complex for second 5 ns simulation run

S.No	*System I*	*System II*	*Distance Å*
1	ALA:50 & H	GLN:55 & O	2.494
2	ARG:41 & HH22	ASP:54 & OD1	1.723
3	ARG:41 & HH12	ASP:54 & OD2	1.709
4	SER:37 & HG	ASN:33 & OD1	2.338
5	SER:37 & HG	VAL:29 & O	2.433
6	GLU:60 & OE1	ARG:41 & HE	1.643
7	GLU:60 & OE2	ARG:41 & HH21	1.756
8	ASN:33 & HD21	GLU:48 & OE2	2.176
9	ARG:28 & HH22	GLU:5 & OE1	1.828

Table 4
Water mediated hydrogen bonds between System I and System II complex for second 5 ns simulation run

S.No	*System I*	*Water Mediating Hydrogen Bonds*	*Distance Å*	*System II*	*Distance Å*
1	GLU:48 & OE1	HOH:6304	1.753	SER:38 & O	1.834
2	GLU:48 & O	HOH:679	1.887	ALA:46 & O	2.332
3	LYS:30 & HZ2	HOH:10411	1.798	TYR:27 & OH	1.954
4	ILE:4 & O	HOH:2220	1.783	LEU:6 & O	1.761
5	ILE:4 & O	HOH:2220	1.783	GLN:23 & HE22	2.109
6	SER:49 & H	HOH:3437	1.879	GLU:48 & OE2	1.534
7	ASN:42 & OD1	HOH:9241	1.858	GLU:48 & OE2	1.750
8	GLU:60 & OE1	HOH:531	1.805	PRO:59 & O	2.064
9	ASP:39 & OD1	HOH:9842	1.726	ALA:36 & O	2.168

during the 2nd MD simulation trajectory in the frame 465 at 2.232 ns was further simulated for 10 ns. Totally 31042 atoms were present in the system. The Energy of the system (Figure 2), H-bond interaction and water mediating H-bond interactions were analyzed to know the stable nature of the complex. H-bond interaction and water mediating H-bond interactions plays an important role during the simulation. In an earlier report, Chojnacki (1970) explained the essential role of H-bonding in electrical conductivity and its dependence on the direction of hydrogen bonds in biological systems. H-bonds in biological system were responsible for higher electrical conductivity especially in solid state and bioelectronic systems (Chojnacki, 1970). In this study, totally eight direct hydrogen bonds and ten water mediating hydrogen bonds were formed and involve in strong interaction. The amino acid residues of System I: GLN:55, GLU:60, GLU:60, SER:25, GLU:48, ALA:50, ASP:54 & ASP:54 formed hydrogen bond with amino acid residues of System II: GLN:55, ARG:41, ARG:41, GLU:5, SER:34, ARG:41 & ARG:41 respectively (Table 5). The residue of System I: ASP:39, ALA:36, PRO:58, LYS:44, THR:45, PHE:1, ILE:4, GLU:48, ILE:15 & ASP:54 formed water mediating hydrogen (HOH) with residues of System II: ASN:42, ASP:39, GLU:60, GLU:48, GLU:48, ILE:19, LEU:6, THR:45, ALA:18 & ASP:53 respectively (Table 6). After carefully analyzing the energy plot, the minimum energy conformation obtained in the 2084th frame in the trajectory at 7.642 ns was further simulated for 20ns. At the closing stage of 3rd MD simulation of 10ns (Total 40ns) pilin-pilin nanowire complex, closer dimer folding of pilin-pilin nanowire complex was found than the previous folding conformation (Figure 3). The negatively charged residues situated on the surface of MD simulated pilin GLU:48, ASP:53, ASP:54 and GLU:60 are maintaining the direct and water mediated hydrogen bond interactions (Table 5 & 6) in the protein-protein binding of pilin-pilin nanowire complex even through 40ns simulation. The presence of negatively charged amino acids existing in the outer areas of the pilus with maximum exposure to the solvent are reported earlier by Feliciano *et al* (2016). However, the present paper documents the systematic analysis of direct and water mediated hydrogen bond profiles between the pilin-pilin nanowire complex using multiple MD simulation at different time scale.

Multiple Simulation Stage-4 final 20ns: In an attempt to learn the extended multiple dynamics of protein-protein interaction of the pilin dimmers at varying time scale, the minimum energy conformation generated in 2084th frame of trajectory at 7.642 ns during previous 10 ns simulation run was further MD simulated for 20 ns. The system was composed of 30518 atoms. Energy trajectory of the system (Figure 2) was analyzed to study the energy profile, H-bond interaction and water mediating H-bond interactions to understand the steady nature of the pilin-pilin nanowire interaction. Totally, twenty amino acid residues were involved in intermolecular hydrogen bonding and eleven residues were involved in water bridging. The amino acid residues of System I: ARG:28, ARG:28, SER:25, SER:37, SER:38, ARG:41, GLU:48, ARG:41, ALA:50, SER:61, ARG:41, ALA:50, ASP:54, ASP:54, GLN:55, SER:37, GLN:23, LYS:30, VAL:9 & ALA:52 formed intermolecular hydrogen bonds with residues of System II: GLU:5, GLU:5, GLN:23, ASN:33, GLU:60, GLU:60, ASN:33, ASN:33, SER:34, LYS:30, GLU:60, SER:34, ARG:41, ARG:41, GLN:55, ASN:33, ASP:39, ASP:39, ASN:42 & GLN:55 (Table 7). The residue of System I: SER:38, ALA:18, PHE:1, LEU:47, ASN:39, ASP:54, TYR:57, ARG:28, GLN:55, ARG:41 & ALA:46 formed water mediating hydrogen (HOH) with residues of System II: ARG:41, ILE:15, LEU:3, GLU:48, ASN:42, GLN:54, ASP:53, TYR:32, LYS:44, SER:38 & LEU:43 respectively (Table 8). Over-all, first 21 amino acids of pilin subunit exhibit poor participation in Protein-Protein interaction through direct hydrogen bonds to recognize another pilin, as they were already buried in bacterial inner membrane prior to fiber formation as discussed in a previous study of electrically conductive bacterial nanowires of *Geobacter sulfurreducens* (Reardon and Mueller, 2013). During the 4th & final 20ns (Total 60ns) pilin-pilin nanowire complex simulation, a more firm folding of pilin-pilin nanowire complex was established compared to previous folding conformation (Figure 3). The negatively charged amino acid residues GLU:48, ASP:53, ASP:54, and GLU:60 involve in solvent mediated H-bonds and their neighbor aromatic residue TYR:57 is found to play a part as efficient electron charge transporter in the intermolecular hydrogen bond interactions. The positively charged amino acids ARG:28, ARG:41 and LYS:30 were found to interact with negatively charged counterparts GLU:5, GLU:60 and ASP:39 respectively in the pilin-pilin nanowire complex in addition to polar residue SER:61 interacting with LYS:30. The extensive dynamic

Table 5
Intermolecular hydrogen bonding between System I and System II complex for third 10 ns simulation run

S. No	*System I*	*System II*	*Distance Å*
1	GLN:55 & O	GLN:55 & HE21	1.707
2	GLU:60 & OE1	ARG:41 & HE	1.565
3	GLU:60 & OE2	ARG:41 & HH21	1.676
4	SER:25 & HG	GLU:5 & O	2.088
5	GLU:48 & OE1	SER:34 & HG	1.634
6	ALA:50 & H	SER:34 & O	1.862
7	ASP:54 & OD2	ARG:41 & HH12	1.664
8	ASP:54 & OD1	ARG:41 & HH22	1.988

Table 6
Water mediated hydrogen bonds between System I and System II complex for third 10ns simulation run

S.No	*System I*	*Water Mediating Hydrogen Bonds*	*Distance Å*	*System II*	*Distance Å*
1	ASP:39 & O	HOH:9045	2.037	ASN:42 & HD21	2.415
2	ALA:36 & O	HOH:6983	2.040	ASP:39 & OD1	1.991
3	PRO:58 & O	HOH:7548	1.862	GLU:60 & O	2.002
4	LYS:44 & O	HOH:9002	2.150	GLU:48 & OE2	1.834
5	THR:45 & O	HOH:4723	2.411	GLU:48 & O	1.735
6	PHE:1 & H1	HOH:2023	1.837	ILE:19 & O	2.135
7	ILE:4 & O	HOH:3269	1.811	LEU:6 & O	1.804
8	GLU:48 & O	HOH:9093	2.092	THR:45 & HG1	2.495
9	ILE:15 & O	HOH:934	1.944	ALA:18 & H	1.996
10	ASP:54 & OD2	HOH:8836	1.928	ASP:53 & OD2	1.881

Table 7
Intermolecular hydrogen bonding between System I and System II complex for final 20 ns simulation run

S. No	*System I*	*System II*	*Distance Å*
1	ARG:28 & HH22	GLU:5 & OE1	1.650
2	ARG:28 & HH12	GLU:5 & OE2	1.692
3	SER:25 & OG	GLN:23 & HE21	1.931
4	SER:37 & HG	ASN:33 & OD1	1.849
5	SER:38 & HG	GLU:60 & OE1	1.838
6	ARG:41 & HH21	GLU:60 & OE1	1.695
7	GLU:48 & OE2	ASN:33 & HD21	1.630
8	ARG:41 & HH11	ASN:33 & O	1.803
9	ALA:50 & H	SER:34 & OG	2.432
10	SER:61 & OG	LYS:30 & HZ1	1.830
11	ARG:41 & HE	GLU:60 & OE2	1.770
12	ALA:50 & H	SER:34 & OG	2.432
13	ASP:54 & OD1	ARG:41 & HH12	1.785
14	ASP:54 & OD2	ARG:41 & HH22	1.791
15	GLN:55 & HE21	GLN:55 & O	1.795
16	SER:37 & HG	ASN:33 & OD1	1.849
17	GLN:23 & HE22	ASP:39 & OD2	1.597
18	LYS:30 & HZ2	ASP:39 & OD2	1.653
19	VAL:9 & H	ASN:42 & OD1	2.368
20	ALA:52 & H	GLN:55 & O	2.489

Table 8
Water mediated hydrogen bonds between System I and System II complex for final 20ns simulation run

S.No	*System I*	*Water Mediating Hydrogen Bonds*	*Distance Å*	*System II*	*Distance Å*
1	SER:38 & OG	HOH: 2901	1.961	ARG:41& HH11	1.938
2	ALA:18 & H	HOH:134	2.005	ILE:15 & O	1.820
3	PHE:1 & H1	HOH:496	1.772	LEU:3 & O	2.485
4	LEU:47 & O	HOH:5940	1.700	GLU:48 & OE1	1.841
5	ASN:39 & OD2	HOH:1749	1.907	ASN:42 & HD21	1.815
6	ASP:54 & OD1	HOH:6147	1.806	GLN:54 & OE1	1.903
7	TYR:57 & OH	HOH:4118	2.199	ASP:53 & OD1	1.641
8	ARG:28 & HE	HOH:7897	1.648	TYR:32 & OH	1.929
9	GLN:55 & OE1	HOH:6086	2.102	LYS:44 & HZ2	1.662
10	ARG:41 & HH11	HOH:2901	1.938	SER:38 & OG	1.916
11	ALA:46 & H	HOH:3769	1.876	LEU:43 & O	2.232

analysis of direct and water mediated hydrogen bonds between the different residues of pilin-pilin nanowire complex through multiple MD simulation at different time scale disclose the electron charge movement that may be responsible for the electron conduction phenomenon.

Distance Analysis: The end-to-end distance analysis for *Geobacter sulfurreducens* pilin nanowire and pilin-pilin nanowire complex was done for the following terminals: nitrogen (N) of PHE:1 and carbon alpha (Cα) of SER:61 for different time scale 20ns, 5ns, 5ns, 10ns and 20ns (Total 60ns). (i) for System I (A Chain) terminals SER:61 (Cα) to PHE:1 (N) (ii) System II (B Chain) terminals SER:61 (Cα) to PHE:1 (N) (iii) System I (A Chain) SER:61 (Cα) to System II (B Chain) SER:61 (Cα) (iv) System I (A Chain) PHE:1 (N) to System II (B Chain) PHE:1 (N) (v) System I (A Chain) PHE:1 (N) to System II (B Chain) SER:61 (Cα) (vi) System I (A Chain) SER:61 (Cá) to System II (B Chain) PHE:1 (N) (Figure 4). In the end-to-end distance trajectory, System I (A Chain) PHE:1 (N) to System II (B Chain) PHE:1 (N) [yellow line] distance maintains a minimum variation throughout the multi-simulation process. System I (A Chain) terminals SER:61 (Cα) to PHE:1 (N) [blue line], System I (A Chain) SER:61 (Cα) to System II (B Chain) PHE:1 (N) [pink line] and System I (A Chain) PHE:1 (N) to System II (B Chain) SER:61 [green line] maintains maximum fluctuations during the multi-simulation steps which reveals the total dynamics of the molecules. During single pilin initial 20ns, System I (A Chain) terminals SER:61 (Cα) to PHE:1 (N) was compared to the rest of the pilin-pilin nanowire complex System I (A Chain) terminals SER:61 (Cα) to PHE:1 (N), distance fluctuation starts from 40 Å and rising up to 59 Å till 3000ps, and after that minimum distance is maintained in the range of 9 Å till the end of the simulation 20000ps. This was compared to rest of the pilin-pilin nanowire complex System I (A Chain) terminals SER:61 (Cα) to PHE:1 (N) in which each of the pilin-pilin nanowire complex simulation, the distance transition maintains between the range of 40 Å till end of the simulation and for 4th 20ns pilin-pilin nanowire complex transition occurs 30 Å and maintained till the end of the trajectory. The above observations clearly show the closer packing and folding of pilin nanowires mediated by direct intermolecular hydrogen bonds and water bridges.

The above multi-simulation study explains the stable nature of interaction within the pilin-pilin nanowire complex. The pilin-pilin nanowire complex had folded finely during simulation and involved in intermolecular and water mediating H-bond interaction. The energy of the pilin and pilin-pilin nanowire complex was evaluated in different time scale 20ns, 5 ns, 5 ns, 10 ns and 20ns and found it was in stable nature. The amino acid residues of System I: ARG:41, SER:25, SER:37, GLN:55, GLN:23, GLU:60, ARG:28, GLU:48, ALA:50, ASP:54 are found to be involved in regular inter molecular hydrogen bond interaction with System II residues in during the trajectory of multiple MD simulation of various time durations such as 5ns, 5ns, 10ns and 20ns respectively. The water mediating hydrogen bonds play a vital role during the multi-simulation process. The end-to-end terminals distance calculation for nitrogen (N) of PHE:1 and carbon alpha (Cα) of SER:61 was evaluated for different

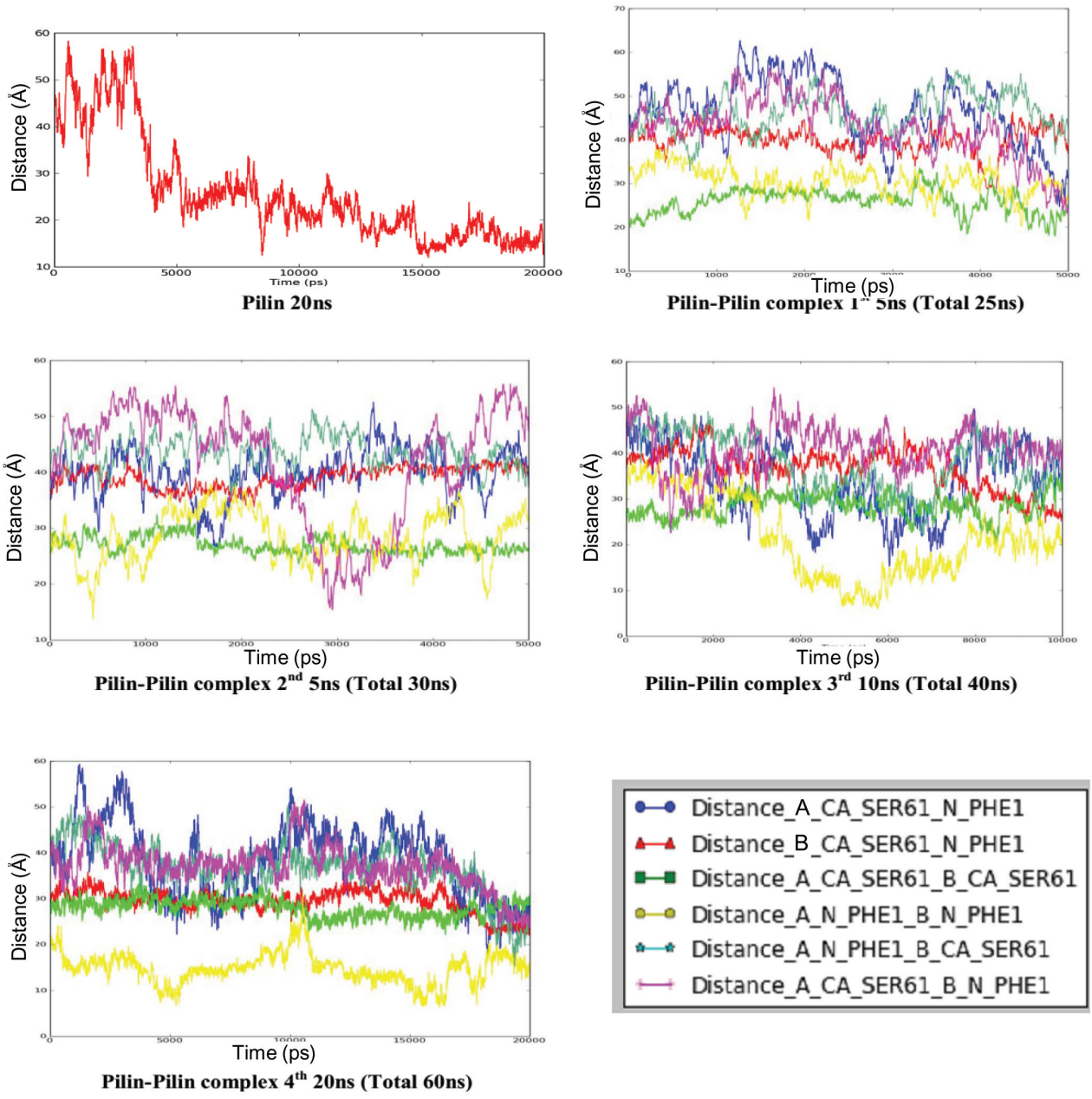

Figure 4: **End-to-end distance analysis was plot in different time scale for Pilin 20ns, Pilin-pilin nanowire complex 1st 5ns (Total 25ns), Pilin-pilin nanowire complex 2nd 5ns (Total 30ns), Pilin-pilin nanowire complex 3rd 10ns (Total 40ns), Pilin-pilin nanowire complex 4th 20ns (Total 60ns)**

possible folding and conformational preference achieved by the protein-protein interaction in the pilin-pilin nanowire complex. The distance of the terminals was analyzed and plotted to correlate with the energy of the pilin and pilin-pilin nanowire complex during the simulation.

Conclusion

The favorable conformations of protein-protein interaction obtained in the present multiple MD simulation of pilin and pilin-pilin nanowire complex (Total 60ns) clearly explains different energy profile with well distinguished hydrogen bond interactions. In the initial stage of simulation, the pilin monomer was unfold and linear. After simulation, pilin protein as well as the pilin-pilin nanowire complex were delicately folded with better inter molecular interaction. The water mediating hydrogen bonds also play a major role during the simulation. The conformation state of the pilin protein and the complex was studied through

Ramachandran plot in which each residue was in the allowed region. The end-to-end distance analysis was done for the six combinations of N-Cá terminus of the pilin complex. Therefore the multiple MD simulation approach is useful and may be successfully employed for the prediction of *Geobacter sulfurreducens* pilin nanowire structures through conformational investigation. These structural insights into protein-protein interaction of piln-pilin nanowire complex might be useful for designing and developing novel biological nanomaterials for electronic application as organic electrically conducting resources.

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Abbreviations

MD, molecular dynamics; H-bond, hydrogen bond; EET, *extracellular electron transport; pilA, pilin A*

Conflict of Interest

The authors do not have any conflict of interest with the contents of this manuscript.

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