Supporting information for

All-Atom Molecular Dynamics Simulation of Photosystem II Embedded in Thylakoid Membrane

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Modeling of PSII with thylakoid membrane

A 3D-model of PSII protein embedded into a thylakoid membrane was built as close as the native state. As the preparation, 3D-models of lipids attaching two fatty acid chains at sn-1 and sn-2 positions are generated. Then, the thylakoid membrane in plants mainly contains four lipid classes (MGDG, DGDG, SQDG and PG)[1]. And each lipid has two fatty acid chains at sn-1 and sn-2 sites, which are two of six fatty acids (16:0, 16:3, 18:0, 18:1, 18:2 and 18:3(9,12,15))[2]. And for thylakoid membrane in cyanobacteria, 42 specific lipids having the fatty acids, 16:1, 18:3(6,9,12) and 18:4 were added and 186 lipid models were generated in total. The lipid composition of head group in the thylakoid membrane of T. vulcanus has been reported by Sakurai et al.[1b] However, no investigations of the positional distributions of fatty acid for T.valcanus have been reported. Therefore, we referred the distributions of Synechocystis sp. PCC6803[3], which belongs to the same family with T.vulcanus (Table S1). Using the lipid models, 3D-model of T.valcanus' thylakoid membrane, of which the size is 42×24×2 and is enough to cover the dimeric PSII and cofactors in the crystal structure, was built.

In the PSII crystal structure, the lack of coordinates of the subunits (Table S2) was assigned appropriately in the models. Then, the unknown ligands named UNK in the PDB file were assigned the ligands in 3BZ1 (Table S3)[4], which superimposed onto monomeric PSII structures. And unassigned UNK ligands were removed from the

dimeric PSII model. The dimeric PSII and cofactors were relaxed by the optimization in vacuum using AMBER11 software package. The dimeric PSII was embedded into the thylakoid membrane model. Then lipids having the short distance less than 3.6 Å with heavy atoms in PSII were removed. Finally, the thylakoid membrane contains 1259 lipids. The lipids were relaxed using 1 ns MD simulation in vacuum state with fixed PSII, cofactor and water molecules in the PSII crystal structure. Finally the initial structure of PSII with the thylakoid membrane was obtained.

Parameters of cofactors and lipids for MD simulation

The parameters of bond length, bond angle, torsion angle, improper torsion angle and van deer Waals interaction for the cofactors and lipids, exception of chlorophyll A, heme and OEC, were assigned the General Amber force field (GAFF)[5] using the antechamber software package[6]. The charges for Coulomb potential in each atom were obtained by the quantum chemistry (QM) calculation using density functional theory at B3LYP/6-31G(d) level with Gaussian09 software package[7]. After QM calculation, ESP charges were obtained using antechamber software. The parameter of chlorophyll A, the same sets reported by Ceccarelli *et al.*[8] was used. The parameter of heme in amber force field was used. The force constant of bond, angle and torsion angles in OEC were appropriately set at 1500 kcal/mol/Å^2, 500 kcal/mol/rad^2 and 100 kcal/mol to keep the OEC structure during the simulation. The equilibrium bond lengths, bond angles and torsion angles were set to the average of OECs in chain A and B. The atomic charges of Mn1, Mn2, Mn3, Mn4 in OEC were set to +3, +4, +4 and +3, respectively. The present crystal structure of OEC may take a damage from X-ray irradiation[9]. However, in our simulation of room temperature, the structural errors of X-ray (0.1 Å ~ 0.4 Å) are not so significantly large when we compare with the thermal fluctuation (0.5 Å ~ 1 Å) during the simulation. Therefore, the structure of Mn_4CaO_5 cluster was used as the crystal structure of an X-ray structure and kept their geometry during the simulation.

MD simulation

The AMBER11 package[10] with Cornell potential force field[11] and TIP3P water

model sets[12] were used in all the MD simulations. The PSII with the thylakoid membrane was solvated in water boxes—the sizes of which were 275 Å× 195 Å × 220 Å—consisting of more than 300000 TIP3P water molecules. The system are strongly negative charged and were neutralized by adding K+ ions. After neutralization, the PSII with the thylakoid membrane was optimized with respect to water molecules by using the conjugate gradient method in three steps: (i) relax the hydrogen atom constraints due to heavy atoms in the system; (ii) relax the water, ion, and hydrogen atom constraints due to heavy atoms in proteins and ligands; and (iii) relax all constraints in the system. The systems were then heated to 300 K for 30 ps under the constraint to PSII, cofactors, lipids and waters which contain in PDB file. After these optimization and heating procedures, 2 ns MD simulation with constrained to PSII in the canonical ensemble (NPT) at 300 K controlled by a Langevin thermostat were performed. The particle mesh Ewald (PME) method was used with a direct-space non-bonded cutoff of 10 Å. Bond lengths, including the lengths of bonds with hydrogen atoms, were constrained with SHAKE. The time step for all MD simulations was set at 1 fs. The harmonic force constant in the constraint for the atomic positions was set at 5.0 kcal/mol/Å^2 and gradually reduced 0.25 kcal/mol/Å^2 at 10 ps time interval. After 2 ns simulation, 8 ns MD simulation were performed and 1600 snapshots for analysis were extracted at 5 ps intervals.

Rms fluctuation analysis

Rms-fluctuations were calculated for mass center of residues, ligands and water molecules, to observe the mobility of the residues and ligands. First of all, the dimeric PSII and ligands in the trajectory were superimposed onto the initial structure. For superimposed structures, the rms-fluctuation, u, was calculated for the mass center of each residue and ligand. Then the average (μ) and the standard deviation (σ) of the rms-fluctuations were calculated from the residues in PSII protein. The deviation of rms-fluctuation, f, calculated as $f = (u - \mu)/\sigma$. The f value represents the mobility of the residues or ligands based on the mobility of the residues in dimeric PSII protein. To classify the mobility of the residues and ligand, the residues having $f < -1$ and $f > 1$ were regarded as the small- and large-movement residues, respectively.

Table S1 Lipid composition and positional distribution of fatty acid in thylakoid membrane

Lipids	Ratio (%)[1]	Position	Fatty acid (%)[2]							
			16:0	16:1	18:0	18:1	18:2	18:3[3]	18:3[4]	18:4
MGDG	43.5	sn-1	6.8	6.1		3.6	33	49	0.3	1.5
		sn-2	98		0.3	1.4	0.4	0.8		
DGDG	25.6	sn-1	7.9	6.4	0.3		26	57	0.8	3.1
		sn-2	95	1.2	1.5	2.3	0.3	0.1		
SQDG	24.8	sn-1	29	14		13	42	1.3	1.8	
		sn-2	94	0.2	2.7	2.8				
PG	6.1	sn-1	10	1.4		5.7	68		17	
		sn-2	95	0.4	3.6	1.0				

[1] The values of lipid composition (mol %) are taken from Sakurai et al. (2006).[1b] The values of positional distribution of fatty acids (mol %) are taken from Okazaki et al. (2006).[3] 18:3 (6,9,12). [4] 18:3(9,12,15)

Table S2 Modeling region of PSII protein

Chain	Region		Length	Chain	Region		Length
	Start	End			Start	End	
A	1	10	10	a	1	10	10
C	1	4	4				
				d	1	1	1
E	1	2	2	e	1	2	2
F	1	10	10	f	1	12	12
J	1	2	2				
M	35	36	2	m	35	36	2
T*	31	32	2	t*	31	32	2

* The coordinates of residues 31 and 32 were assigned from those at the equivalent position in chain T of 3BZ1.

Table S3 Assignment of UNL ligands from ligands in 3BZ1 crystal structure

3ARC		3BZ1			3ARC		3BZ1		
Chain	**No.**	**Name**	**Chain**	**No.**	**Chain**	**No.**	**Name**	**Chain**	**No.**
A	748	LMT	O	274	a	763		Removed	
A	763		removed		b	750		Removed	
B	750		removed		b	807	LMT	B	535
B	807	LMT	B	535	c	745	LHG	A	374*
C	745	LHG	A	374*	c	785		Removed	
D	722		removed		d	722		Removed	
D	723	SQD	D	361*	d	723	SQD	D	361*
E	800		removed		e	800		Removed	
I	732	LMT	I	230	i	732	LMT	I	230
I	733		removed		i	733		Removed	
I	749		removed		i	748	LMT	O	274
J	788		removed		i	749		Removed	
J	794	BCR	J	115	j	788		Removed	
K	798		removed		j	794	BCR	J	115
L	743		removed		k	798		Removed	
T	786	LMT	T	227	l	743		Removed	
T	802		removed		t	786	LMT	T	227
X	746	LMT	D	363	t	802		Removed	
Z	785		removed		x	746	LMT	D	363

* Modeling of the fatty acids, in which atoms are lack of coordinates.

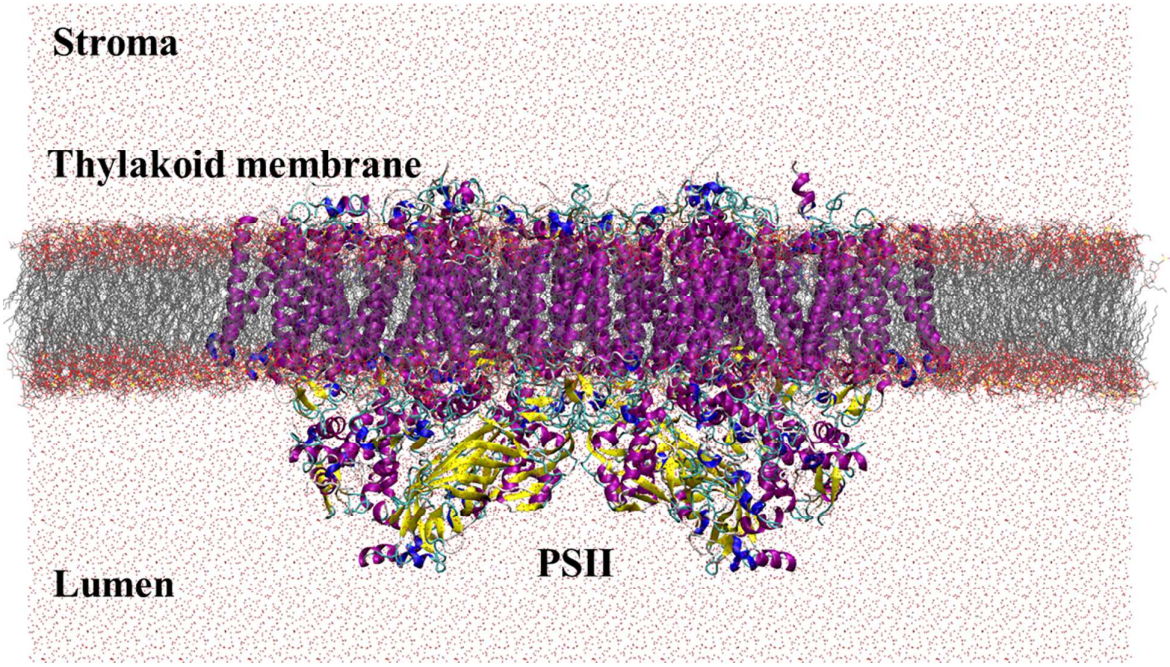

Figure S1. Initial structure of PSII complex embedded in thylakoid membrane. PSII, ligands and waters are shown by a New Cartoon, line model and CPK model, respectively. The C atoms are colored in gray and the other atoms are colored in the default colors of vmd[13].

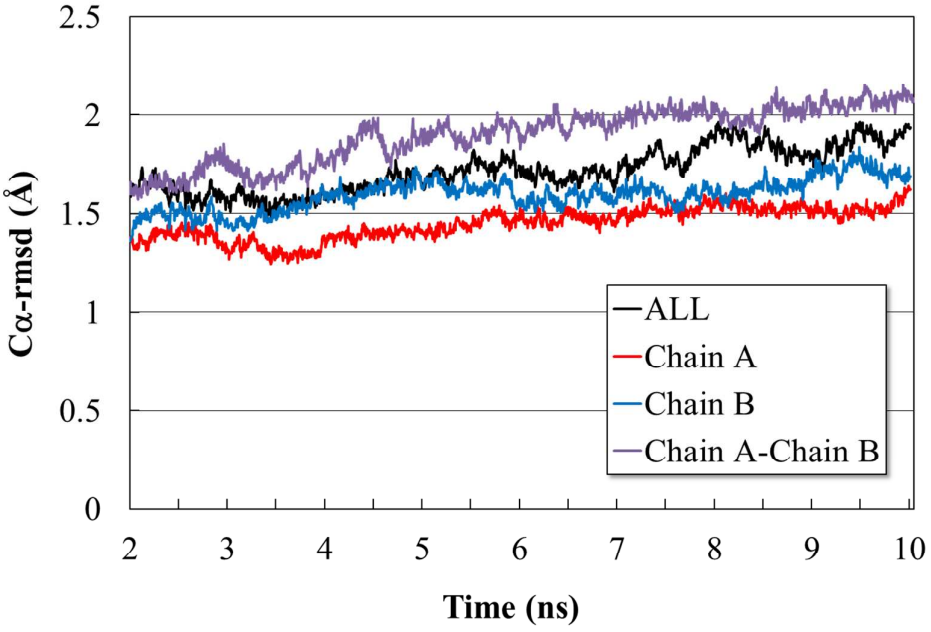

Figure S2. Plots of Cα-rmsd calculated for dimeric and monomeric PSII early in the simulation. The Cα rmsd values of ALL, Chain A and Chain B were calculated every 5 ps for initial structure. The Cα rmsd values between Chains A and B were also calculated every 5.

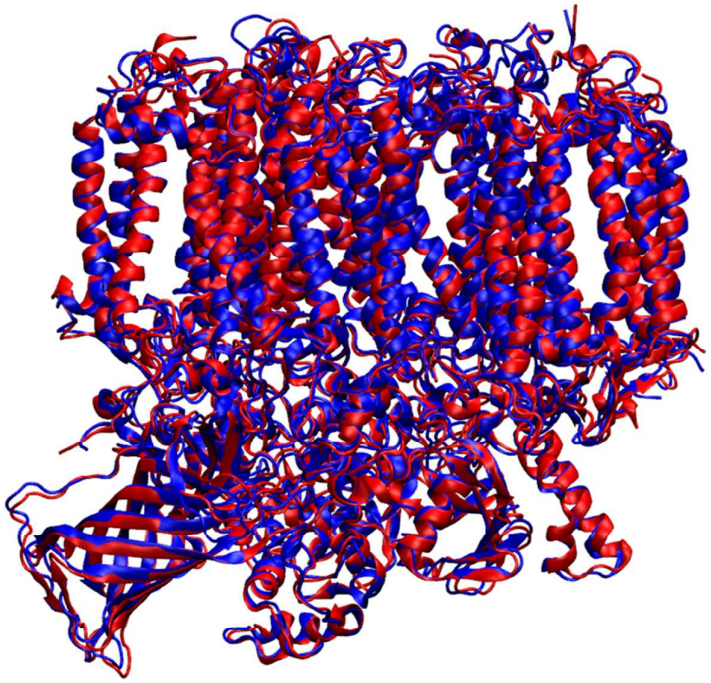

Figure S3. Superimposed structures of monomeric PSII structures. The structures were extracted from the MD trajectory at t=10ns. PSII structures are shown by a New Cartoon drawing. The chains A and B are colored in blue and red, respectively.

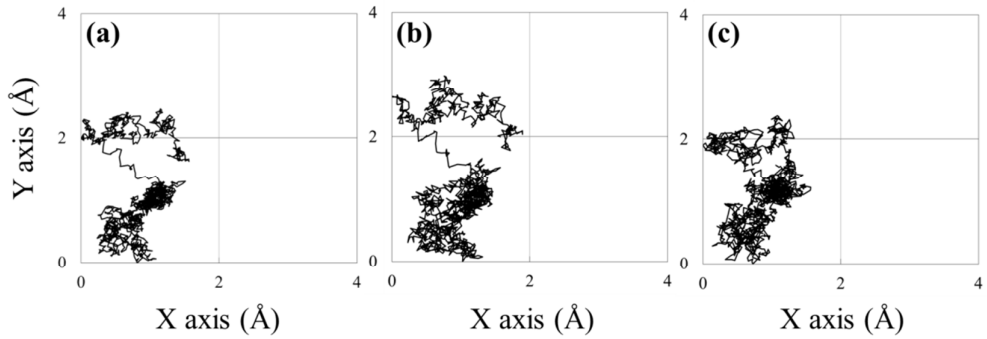

Figure S4. Trajectories of moving PSII, (a) dimeric PSII, (b) chain A (chain A-Z) and (c) chain B (chain a-z), projected the mass center of dimeric and individual PSIIs onto XY-plain.

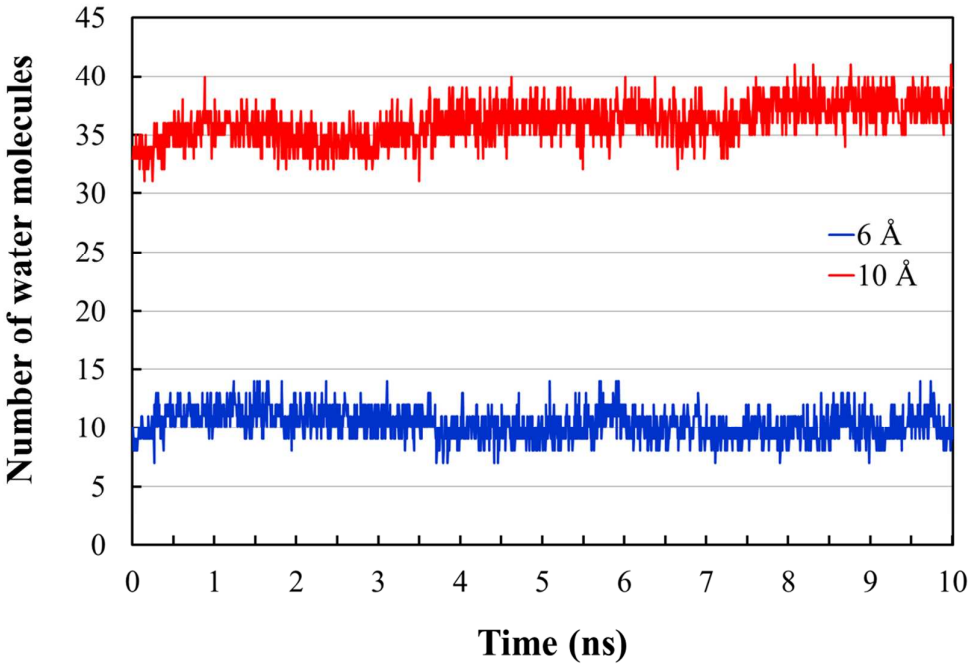

Figure S5. Plot of the number of water molecules within 6 Å and 10 Å from OEC colored in blue and red, respectively.

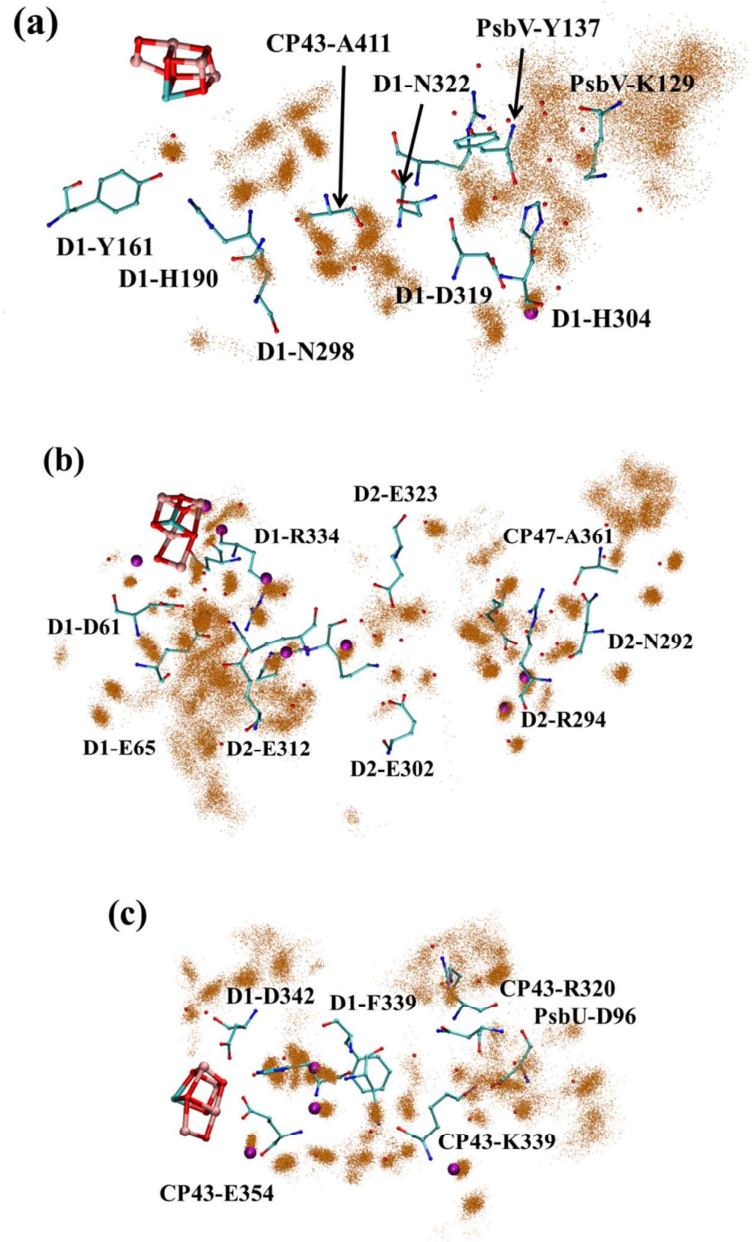

Figure S6. Pathways for water, oxygen and proton transfer marked (a) Path1, (b) Path2 and (c) Path3. Water molecules with small fluctuation are colored in purple. And the water distribution, which was generated by all the waters having the distance within 5 Å from the residues consisting of the channels in all the trajectories, is colored in orange. The viewpoints of these figures are different from those of Figure 4.

Video S1. Animation the simulation of PSII embedding into thylakoid membrane.

Video S2. Animation the simulation with leaving water molecules from the pathways. The water within 15 Å in the snapshot at 2 ns were shown as vdW model colored in orange.

Video S3. Animation the simulation with entering water molecules to the pathways. The water within 15 Å in the snapshot at 10 ns were shown as vdW model colored in orange.

PDB formatted file S1. Initial structure of PSII embedding into thylakoid membrane.

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