Supporting Information for

"Characterizing the Hydration Properties of Proton Binding Sites in the ATP Synthase C-rings of *Bacillus* Species"

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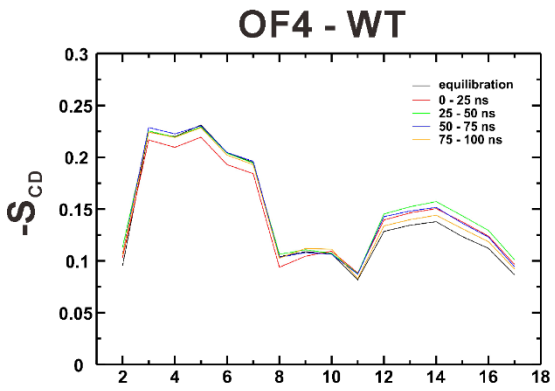
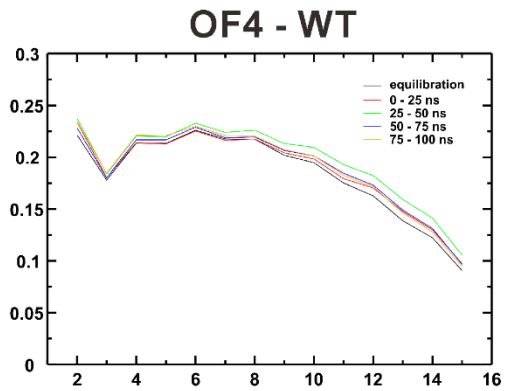
Table S1: Area per lipid and membrane thickness. Averages of the area per lipid of the POPC bilayer for all systems. The data is averaged over the entire 100 ns simulation time for each system. Errors represent standard deviations. Experimental data for the area per POPC lipid are 62.7 Å^2 and 64.3 Å^2 at 20 °C and 30 °C, respectively[1]. The POPC thickness was shown experimentally at 20 °C and 30 °C to be 39.8 Å and 39.1 Å, respectively[1].

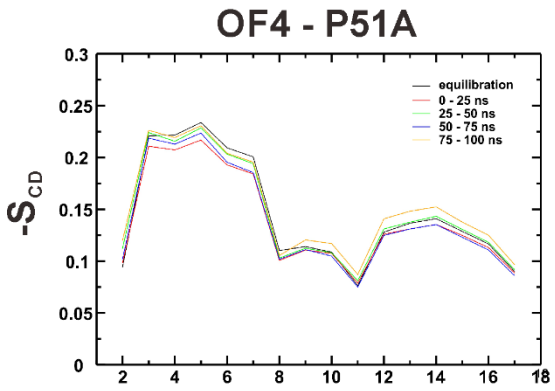
System	Area per lipid (Å^2)	Membrane thickness (Å)
OF4 – WT	64.2 ± 1.3	37.2 ± 1.4
OF4 – P51A	64.6 ± 1.3	37.8 ± 1.2
PS3 (water modelled) – 300 K	64.5 ± 0.9	39.1 ± 1.0
PS3 (no water modelled) – 300 K	64.4 ± 0.9	39.0 ± 0.8
PS3 (water modelled) – 338 K	68.2 ± 1.0	38.2 ± 0.7
PS3 (no water modelled) – 338 K	68.1 ± 1.1	38.3 ± 1.4

Table S2: Convergence analysis of the free energy calculations. The raw desolvation free energy values for a water molecule in the c-ring binding site is shown for different time blocks. The desolvation free energy in bulk solvent and entropic corrections are not incorporated in these values. All free energies are reported in kcal/mol. Errors were derived as the standard deviation over the three data sets.

System/window	0.2 – 2 ns	0.2 – 0.8 ns	0.8 – 1.4 ns	1.4 – 2 ns
OF4 – WT	9.6 ± 0.3	9.4 ± 0.4	9.7 ± 0.2	9.7 ± 0.3
OF4 – P51A	9.5 ± 0.3	9.6 ± 0.3	9.3 ± 0.4	9.7 ± 0.3
PS3 (1^{st} water) – 300 K	11.3 ± 0.3	11.3 ± 0.3	11.4 ± 0.6	11.0 ± 1.1
PS3 (2^{nd} water) – 300 K	4.6 ± 0.5	4.4 ± 0.3	4.6 ± 0.3	4.7 ± 0.5
PS3 (1^{st} water) – 338 K	10.7 ± 0.3	10.7 ± 0.1	10.7 ± 0.3	10.6 ±0.4
PS3 (2^{nd} water) – 338 K	4.6 ± 0.3	4.7 ± 0.2	4.6 ± 0.3	4.6 ± 0.5


OF4 - WT
-S_CD
equilibration
0 - 25 ns
25 - 50 ns
50 - 75 ns
75 - 100 ns


OF4 - WT
equilibration
0 - 25 ns
25 - 50 ns
50 - 75 ns
75 - 100 ns


OF4 - P51A
-S_CD
equilibration
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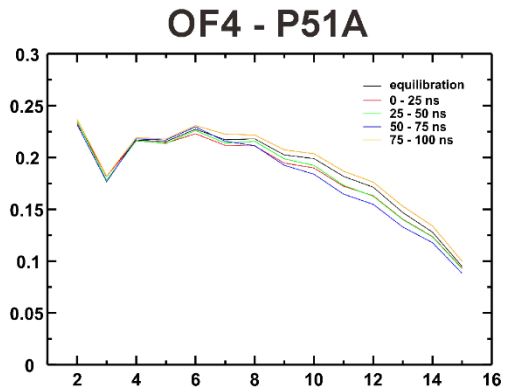
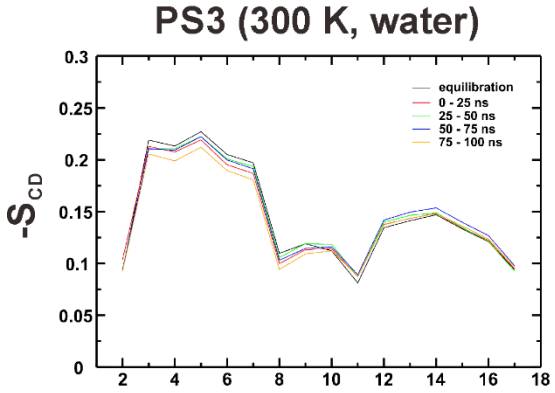
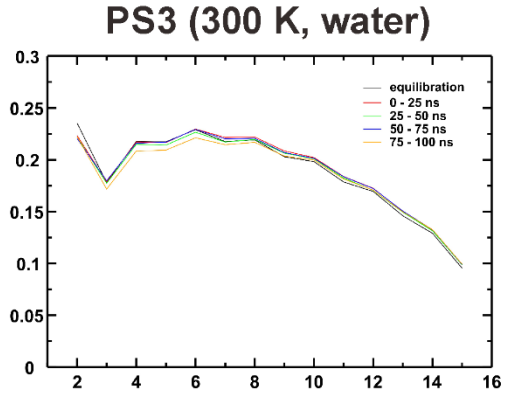
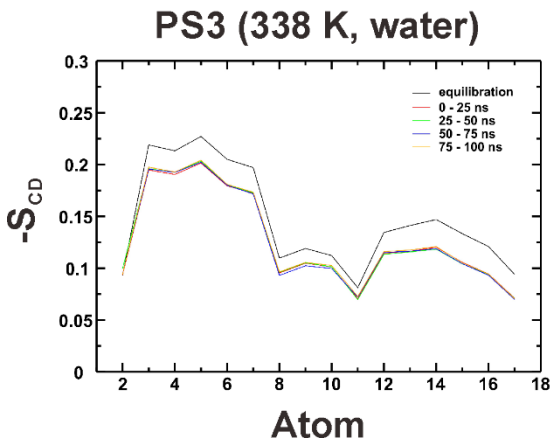
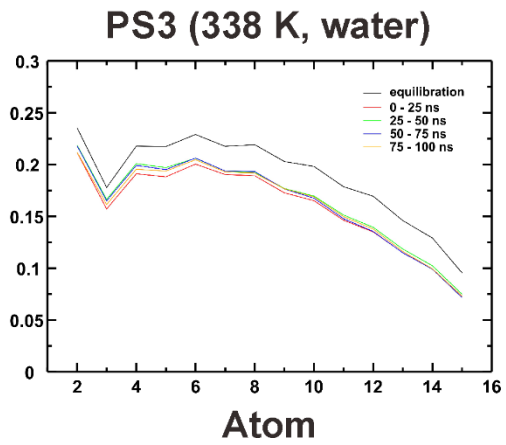
50 - 75 ns
75 - 100 ns


OF4 - P51A
equilibration
0 - 25 ns
25 - 50 ns
50 - 75 ns
75 - 100 ns


PS3 (300 K, water)
-S_CD
equilibration
0 - 25 ns
25 - 50 ns
50 - 75 ns
75 - 100 ns


PS3 (300 K, water)
equilibration
0 - 25 ns
25 - 50 ns
50 - 75 ns
75 - 100 ns


PS3 (338 K, water)
-S_CD
equilibration
0 - 25 ns
25 - 50 ns
50 - 75 ns
75 - 100 ns
Atom


PS3 (338 K, water)
equilibration
0 - 25 ns
25 - 50 ns
50 - 75 ns
75 - 100 ns
Atom

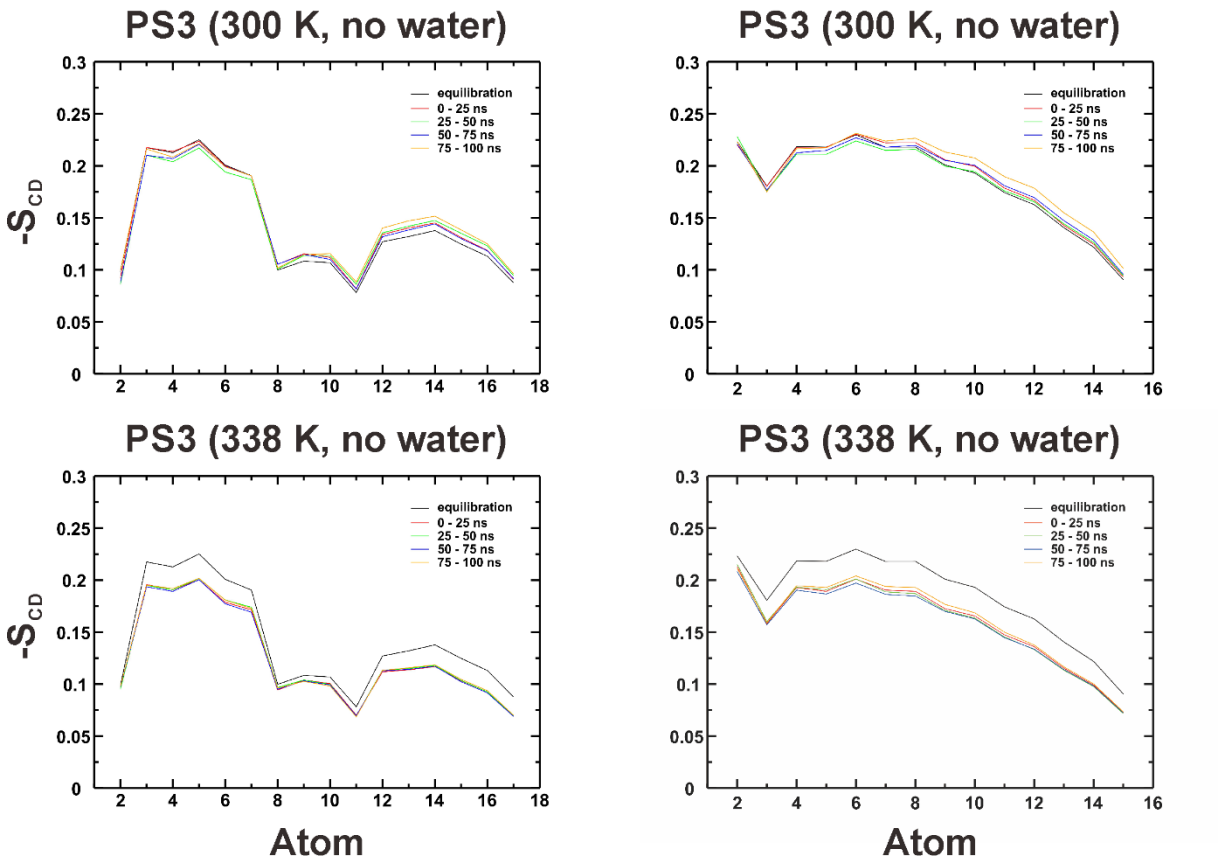


Figure S1: Time dependent order parameters of POPC lipids for all simulation systems. Order parameter profiles are shown for each of the lipid acyl tails on the left and right. The results were divided into the equilibration period and subsequent 25 ns blocks of the production runs.

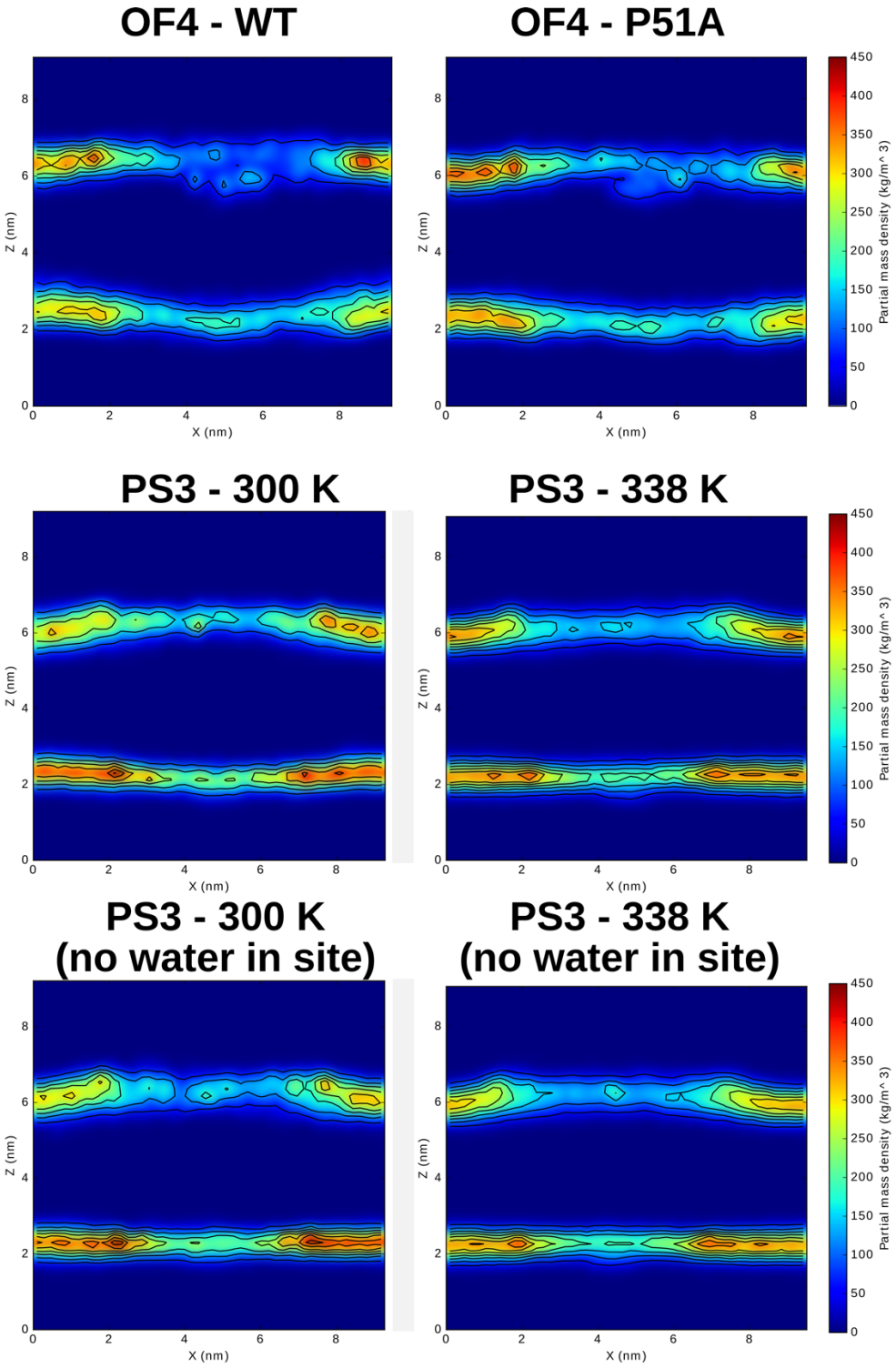


Figure S2. Partial densities of the POPC membrane for each system. The figure shows the side view of the density of the phosphate groups averaged over the entire simulation time.

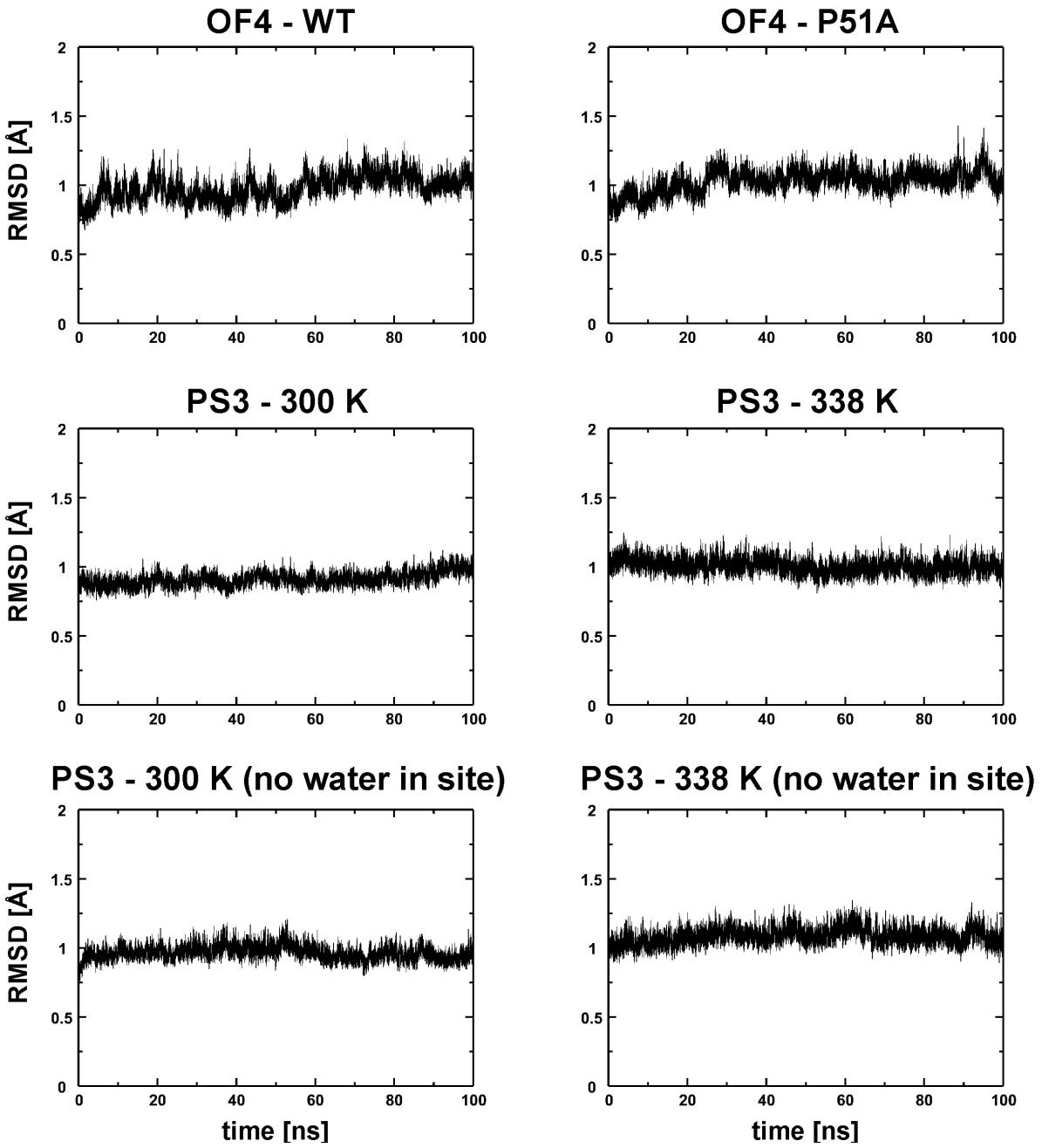


Figure S3: Stability of the c-ring during the simulations. The RMSD of the backbone of the entire c-ring is shown for each system for the production runs (equilibration period not shown), calculated with respect to the initial minimized structure.

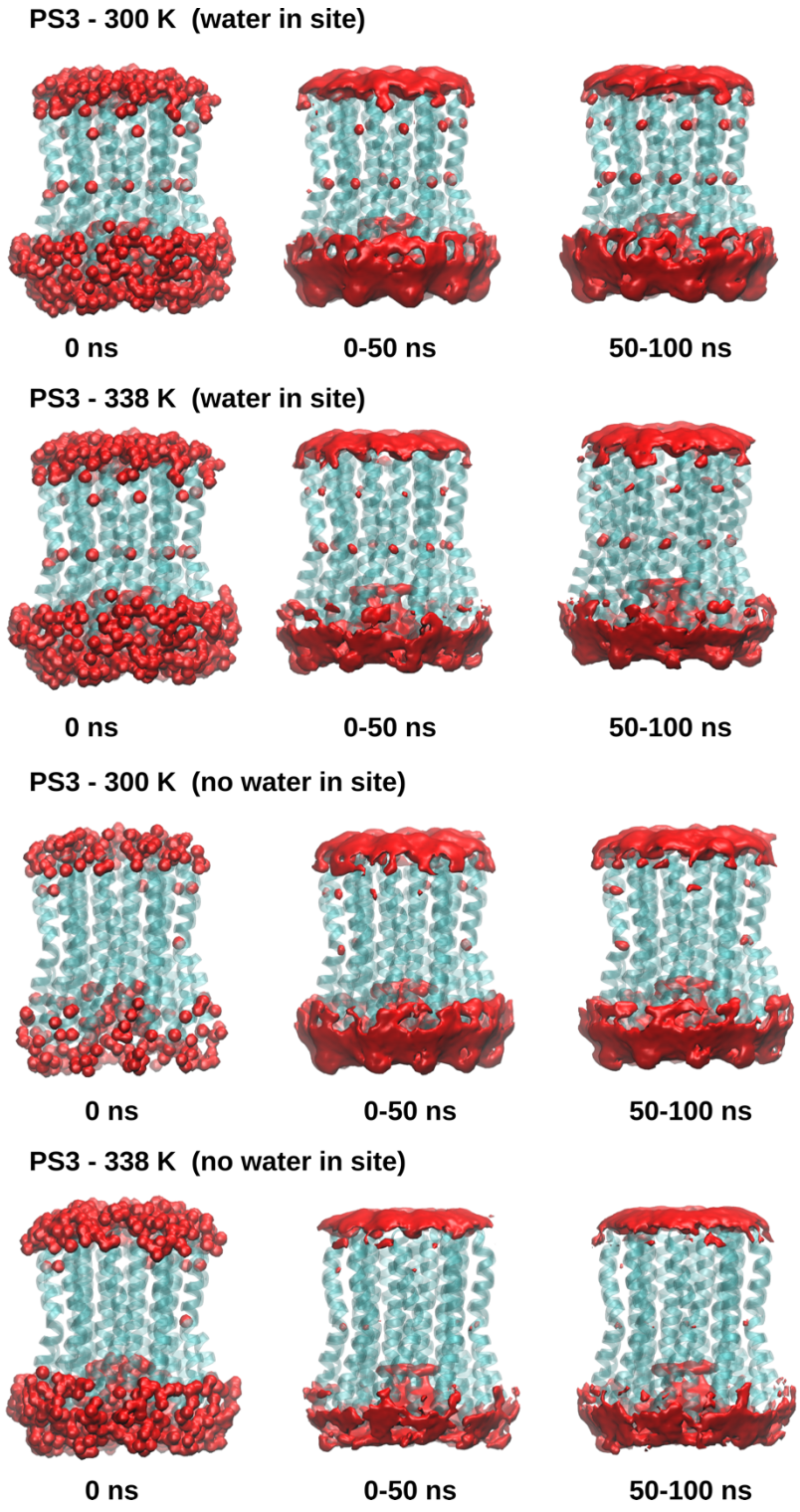


Figure S4. Time averaged water density map around the c-ring. The time averaged water density (red surface representation) is shown within 0.3 nm of the protein (cyan cartoon representation).

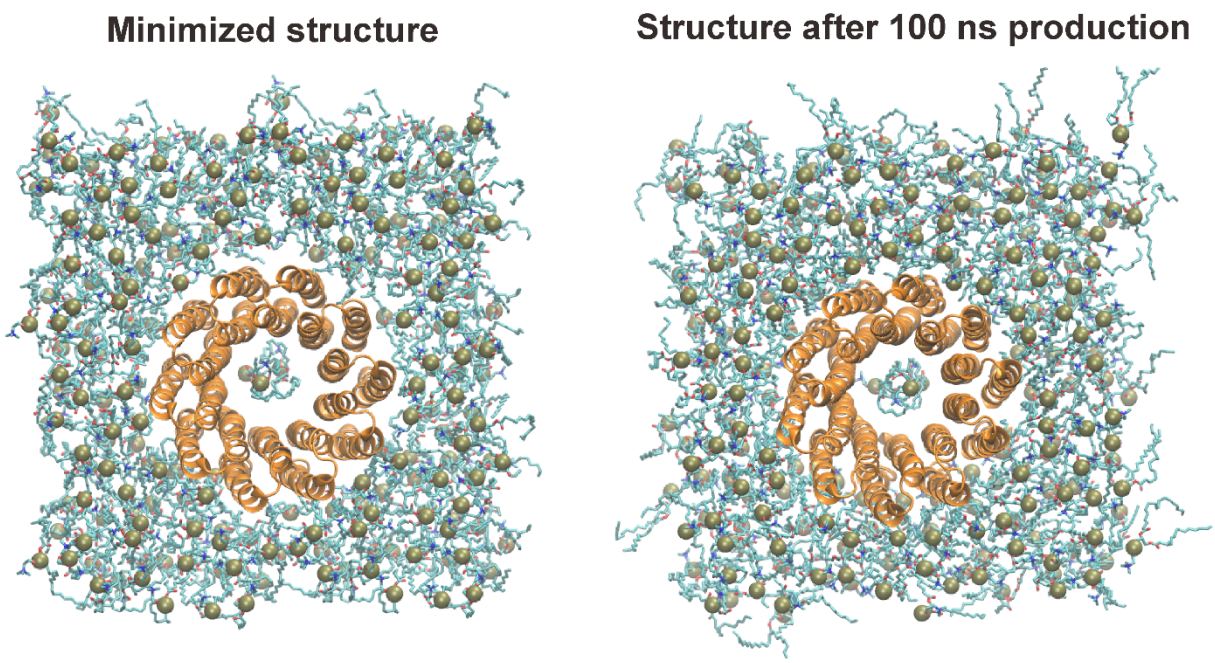


Figure S5: Top-down snapshots of c-ring embedded in membrane at different times in simulation. Snapshots are shown for the membrane embedded c-ring (*Bacillus* PS3) after energy minimization and 100 ns of production simulation. The protein is shown in orange cartoons format. POPC lipids are depicted in wireframe format with phosphate groups highlighted as tan-colored van der Waal's spheres.

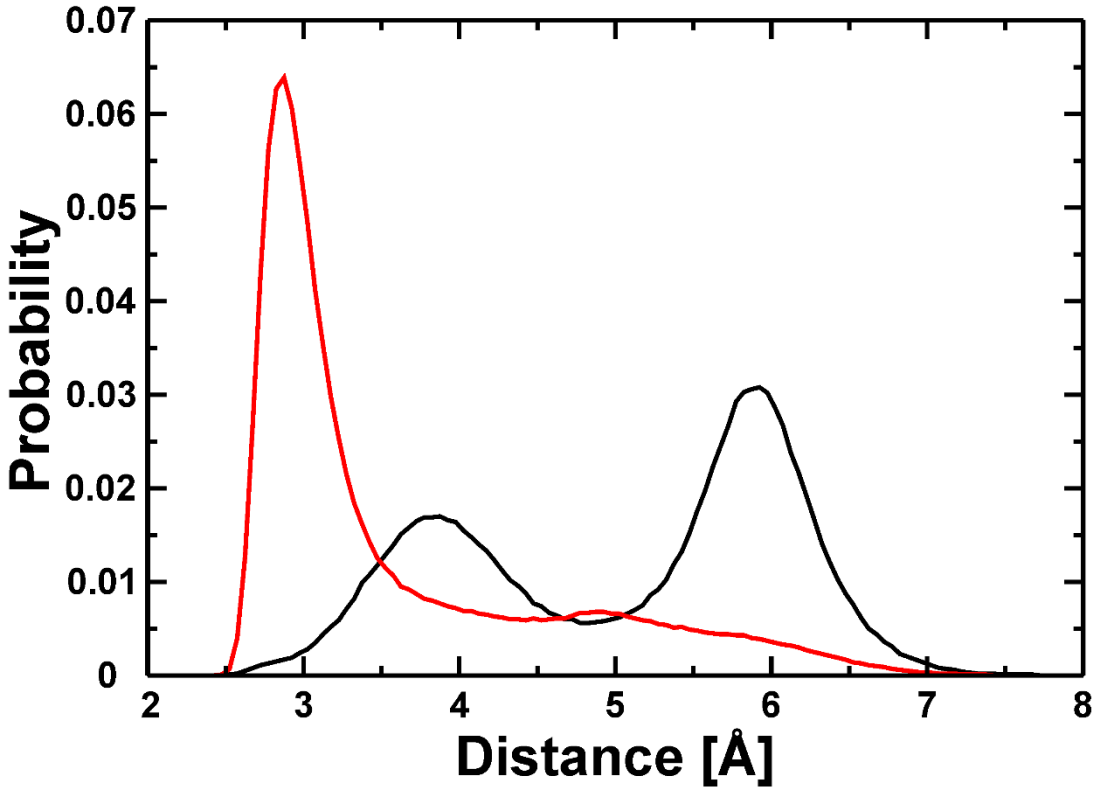


Figure S6: Distance distributions for key interactions at the ion binding site of the *Bacillus* PS3 system at 300 K. The distribution of distances between E56:Oε2 and L54:O is shown (black) for the case where water was not initially modelled in the binding site; sites which became occupied spontaneously by a water molecule during the simulations are not taken into account. For comparison, the distribution of distances between E56:Oε2 and water:O is shown (red) for the system where the water molecule was initially modelled (all sites taken into account).

References

(1) Kučerka, N.; Nieh, M. P.; Katsaras, J. Fluid Phase Lipid Areas and Bilayer Thicknesses of Commonly Used Phosphatidylcholines as a Function of Temperature. *Biochim. Biophys. Acta - Biomembr.* **2011**, *1808*, 2761–2771.