Supplementary Material

Insights into functions of the H channel of cytochrome *c* oxidase from atomistic molecular dynamics simulations

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Figure S1: Comparison between C1 and C2 simulations. Snapshots from C1-VI (brown) and C2-IV (green) are compared. When the C_α of both subunits I (shown as cartoons) are aligned, an RMSD of 2.2 Å is obtained, which is reduced to 1.1 Å when the loops are not considered. Positions of H channel residues are shown as sticks and are very similar (RMSD of 1.2 Å, or 0.8 Å if only the backbone is considered).

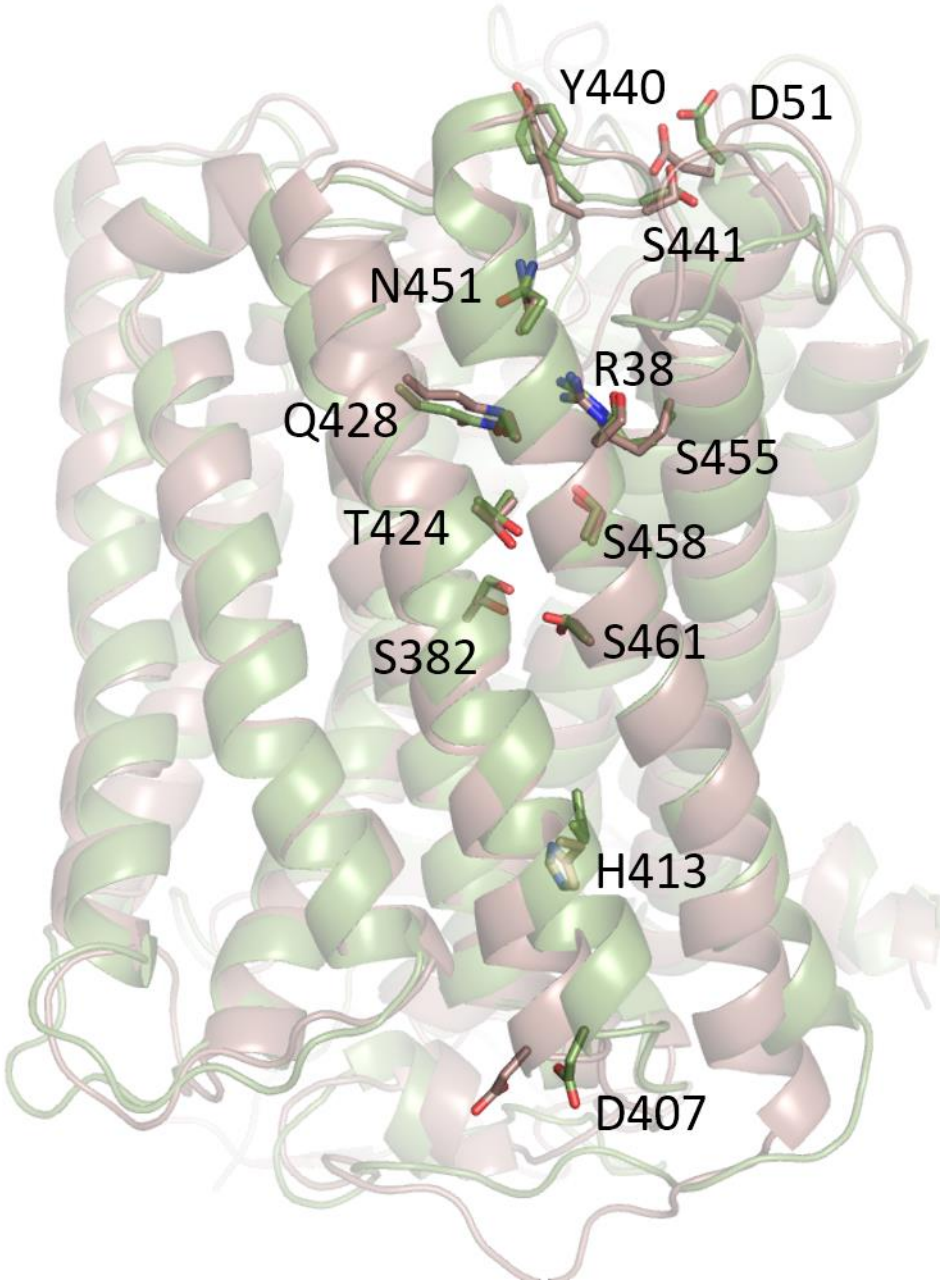

Figure S2: Predominantly hydrophobic environment around H413. Sidechains of the residues around H413 are shown as sticks and water molecules are shown as red spheres. Top panel: Crystal structure (PDB 1V54). Middle panel: After 300 ns (setup C1-I) with H413 in its neutral form. Lower panel: After 150 ns (setup C1-VI) with H413 in its cationic (+1) form. Protonation of H413 attracts water molecules to the H channel (see main text). The pocket occupied by H413 and the surrounding water molecules is shown as a surface.

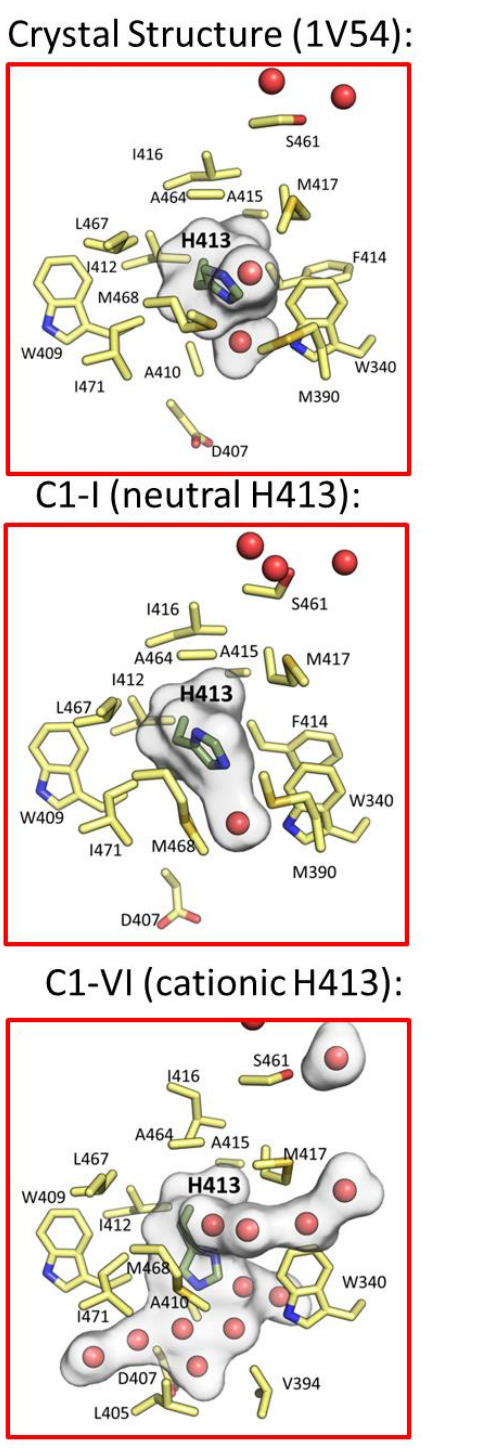

Figure S3. Number of water molecules (oxygen atoms) within 8 Å of His413 from simulations C3-II (five independent runs). All simulations started from a fully hydrated lower region of the H channel.

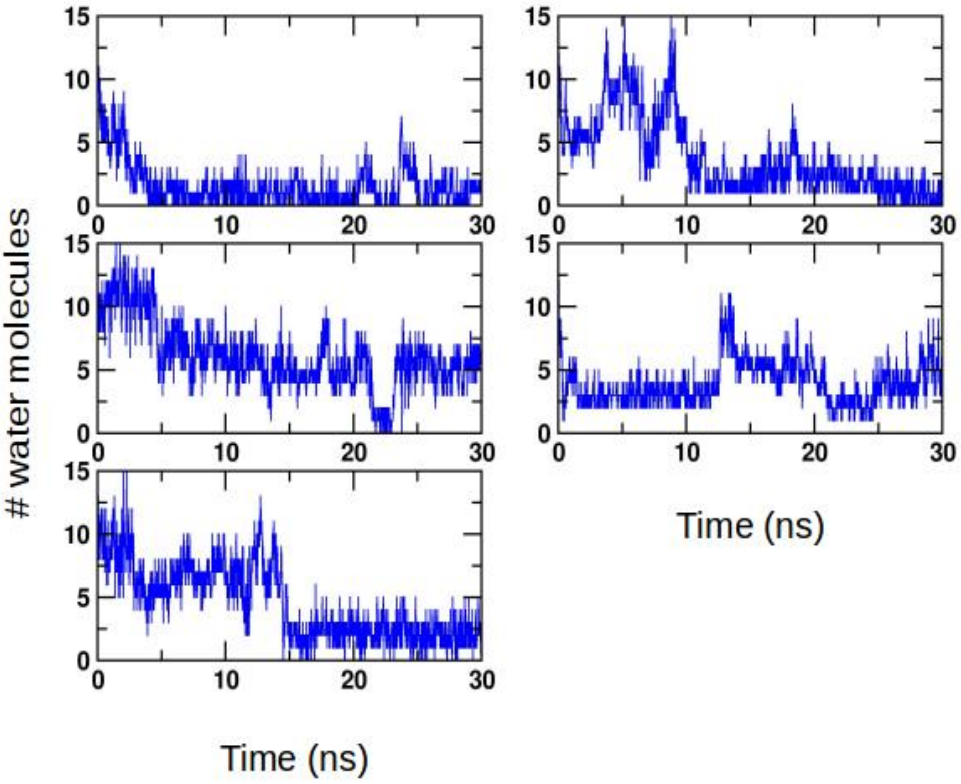

Figure S4. Number of water molecules (oxygen atoms) within 8 Å of His413 from simulations of C1-I (three independent runs). All simulations started from a fully hydrated lower region of the H channel. For clarity, plots display a running average of 20 snapshots.

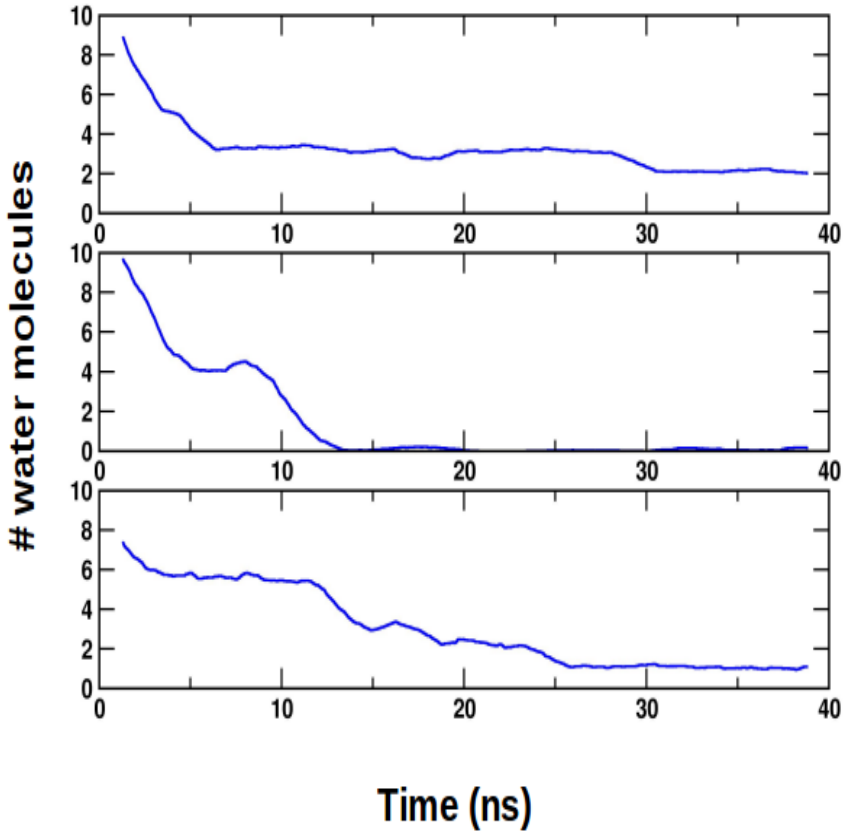

Figure S5. Free energy profiles for the hydration of lower region of H channel when His413 is neutral (top panel) or protonated (bottom panel). As reaction coordinate we have used $\langle r_{wat\text{-}H413} \rangle$, the average distance between the imidazole ring of His413 and the center-of-mass of the 10 water molecules closest to His413. The results for the protonated His413 (C1-VI simulations, red) show a single minimum at $\langle r_{wat\text{-}H413} \rangle$=5.5 Å, representing a stable water wire. Deprotonation of His413 (C1-I, blue) changes the free energy landscape, and the water wire is no longer stable. Minima are found instead between $\langle r_{wat\text{-}H413} \rangle$=9-11 Å, which are not compatible with a water wire connecting the bulk and the lower part of the H channel. Notice that the zero of the energy scale correspond to the most stable conformation for each of the systems.

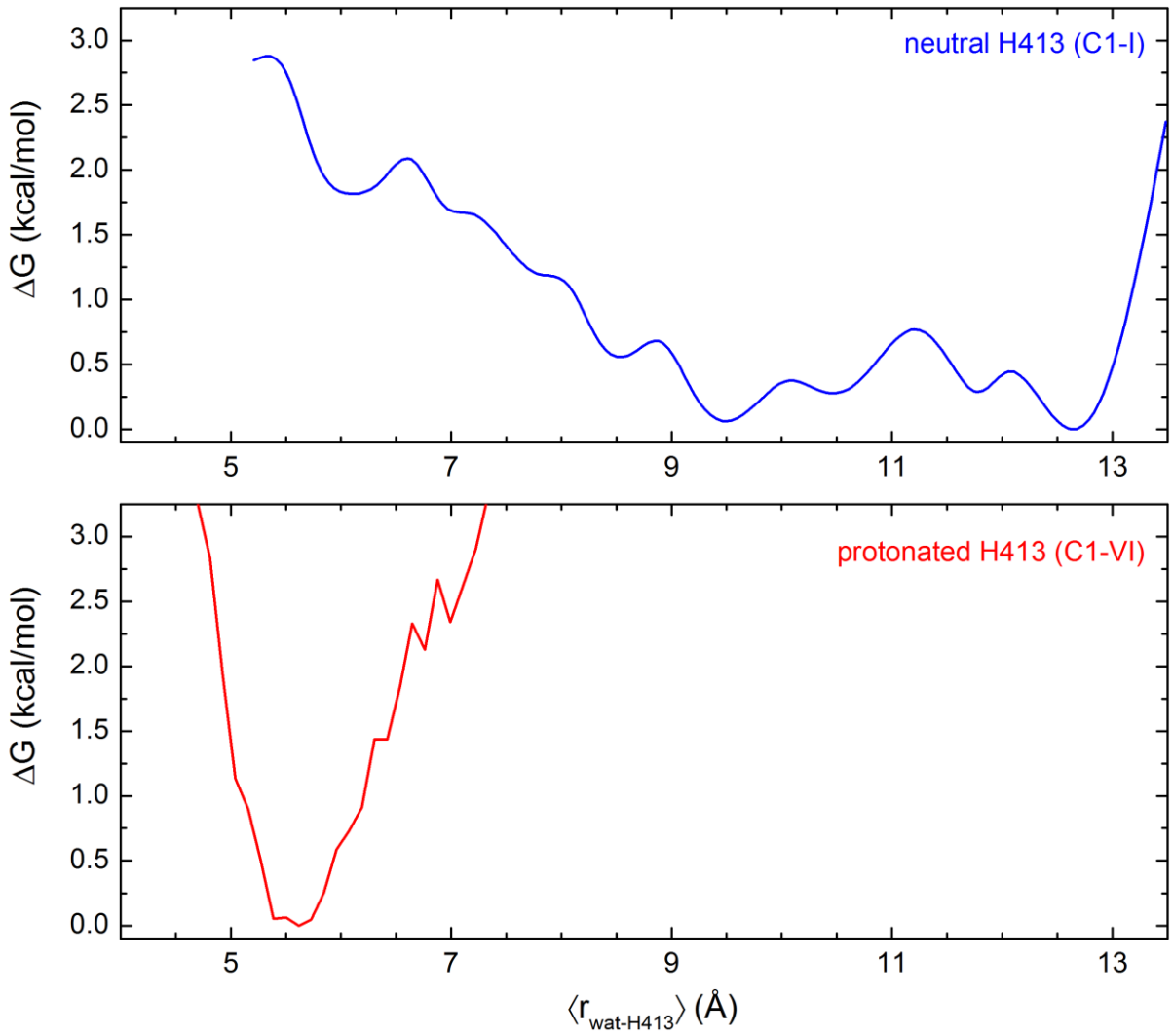

Figure S6: Snapshots at different time intervals display the fluctuation of electric dipole moment from simulations C2-I (red) and C2-II (blue). The amino acid side chains (yellow for C2-I, and brown for C2-II) are shown (see also Table 6 in main text). Backbone is displayed as white transparent ribbon, and low-spin heme is in licorice representation (green for C2-I and purple for C2-II).

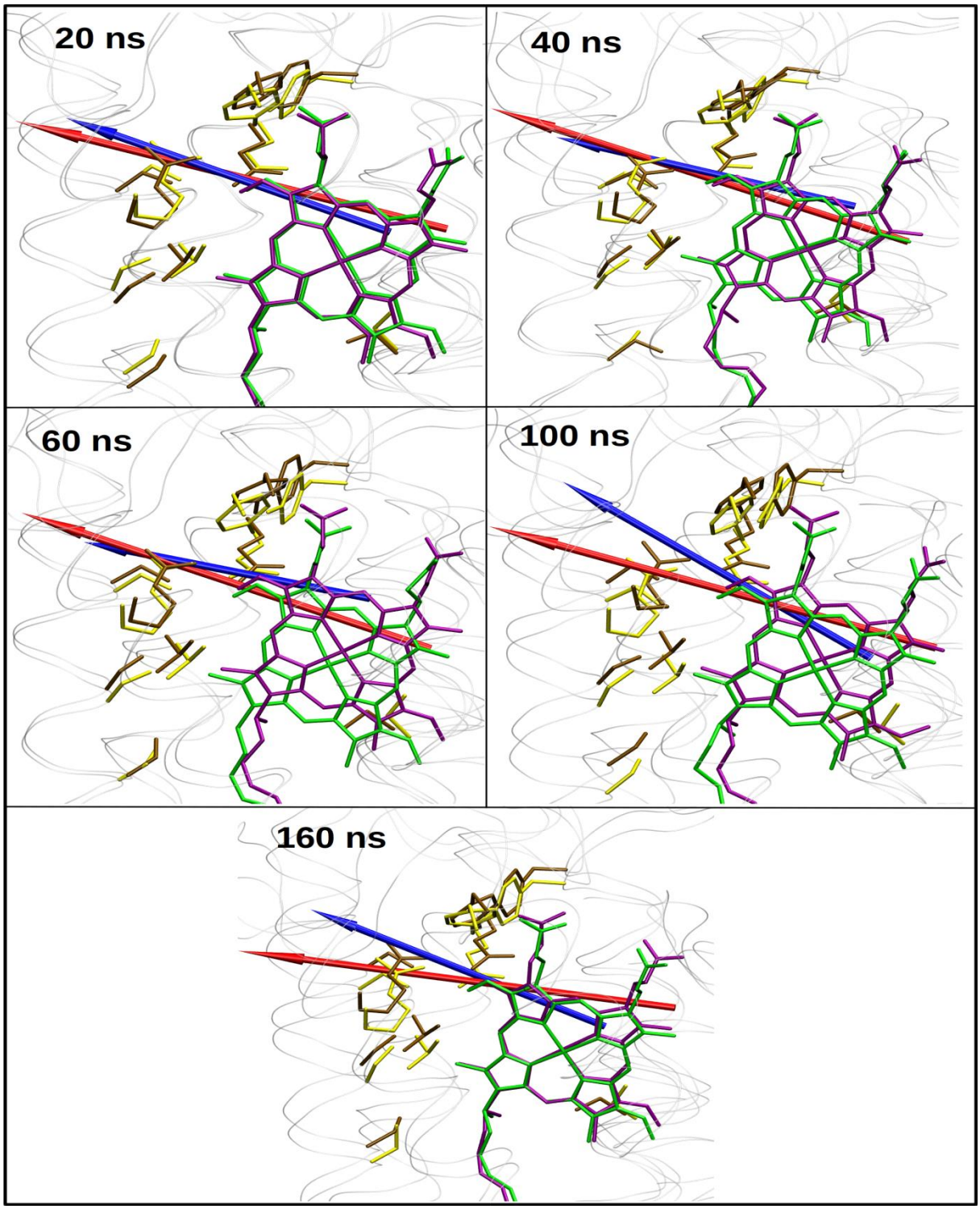

Figure S7: Root mean square deviation (RMSD) in Å *vs* simulation time (in ns) for C1 simulations. The structural alignment to zeroth simulation frame and RMSD calculations were performed using Cα atoms.

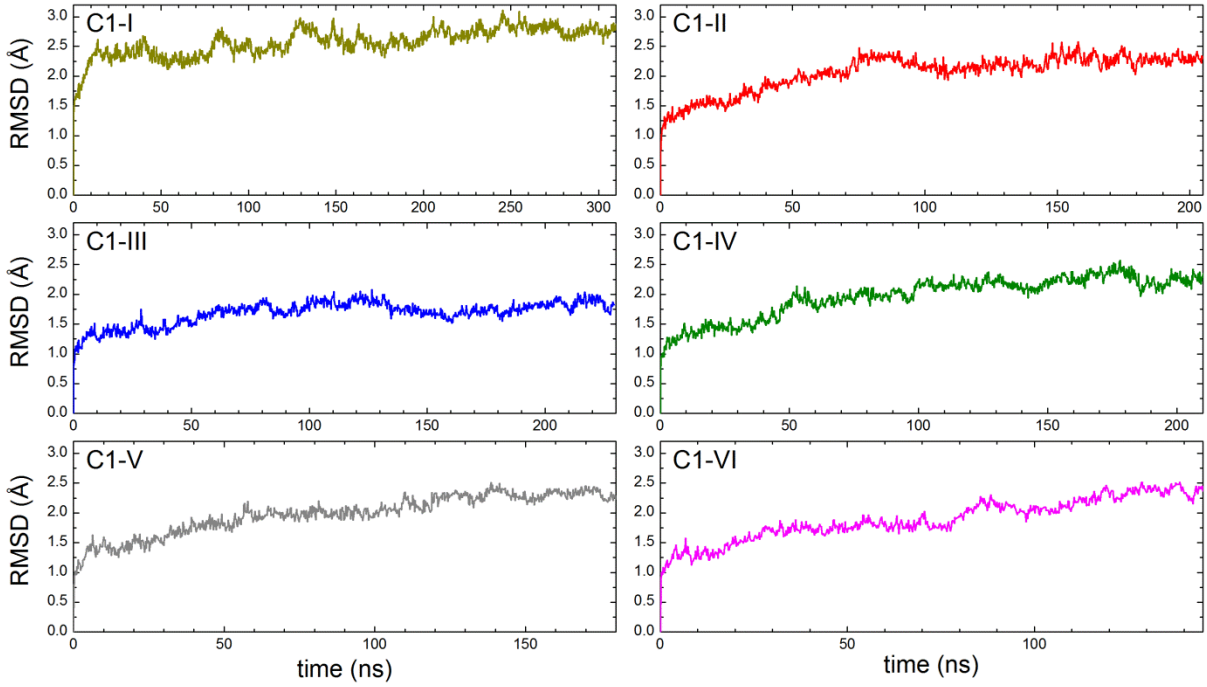

Figure S8: RMSD (in Å) *vs* simulation time (in ns) for C2 simulations. The structural alignment to zeroth simulation frame and RMSD calculation were performed using Cα atoms. Only the first replicas of setups C2 I, II, VII and VIII were used for RMSD calculations.

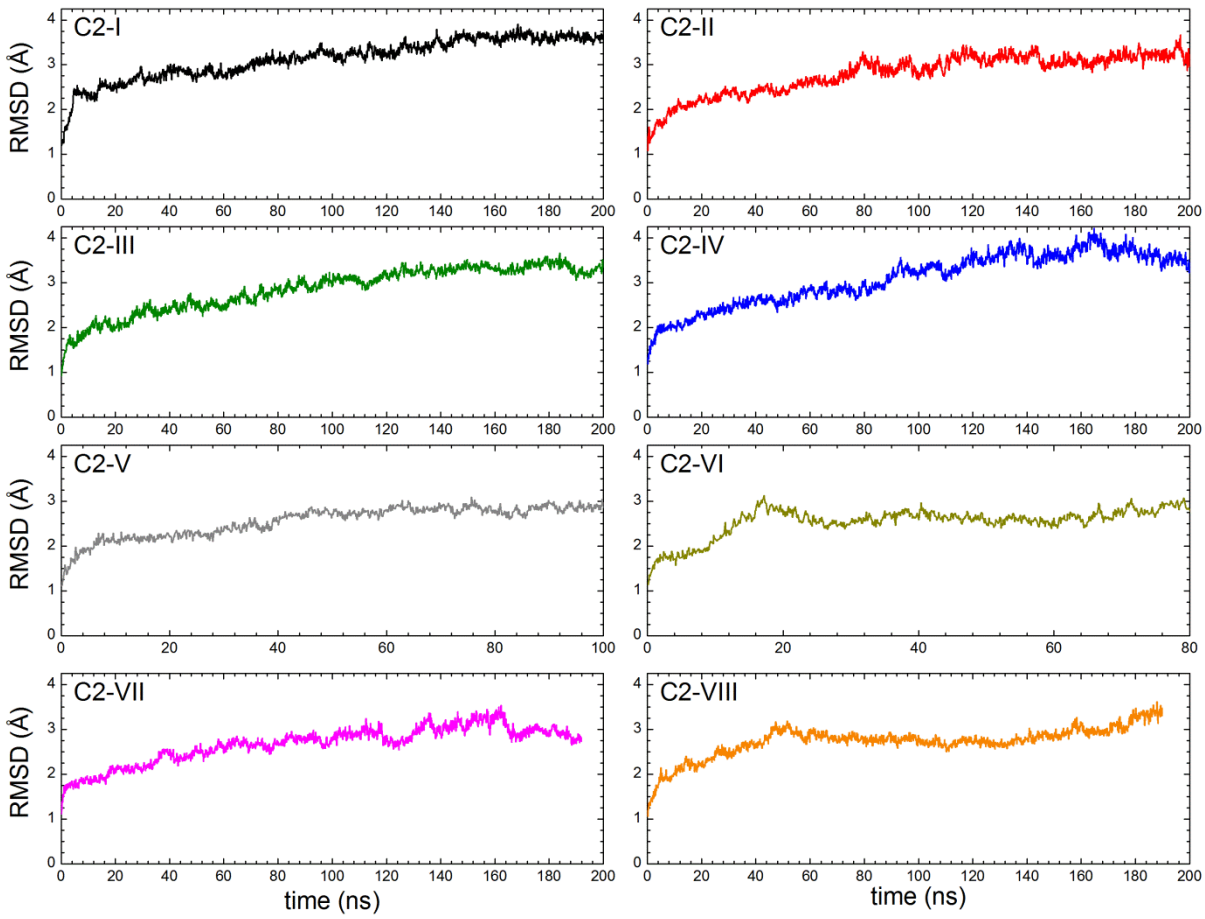

Table S1: Average number of water molecules in the upper region of the H channel in C1 simulations.

Simulation system	Average number of water molecules*
C1-I	14 ± 3
C1-II	11 ± 1
C1-III	12 ± 1
C1-IV	12 ± 1
C1-V	12 ± 2
C1-VI	11 ± 2

* Average number of water molecules were calculated over entire simulation trajectories by counting water oxygens within 5 Å of residues 54 371 440 and 441 (highlighted in blue in Fig. 1B).

Table S2: Average number of water molecules in the middle region of the H channel in C1 simulations.

Simulation system	Average number of water molecules*
C1-I	8 ± 1
C1-II	7 ± 1
C1-III	7 ± 1
C1-IV	9 ± 1
C1-V	9 ± 2
C1-VI	8 ± 1

* Average number of water molecules were calculated over entire simulation trajectories by counting water oxygens within 5 Å of residues 38 424 454 and 458 (highlighted in cyan in Fig 1B).

Table S3: Average number of water molecules in the lower region of the H channel in C1 simulations.

Simulation system	Average number of water molecules*
C1-I	3 ± 1
C1-II	4 ± 1
C1-III	4 ± 1
C1-IV	5 ± 1
C1-V	4 ± 1
C1-VI	9 ± 1

* Average number of water molecules were calculated over entire simulation trajectories by counting water oxygens within 5 Å of residues 461 and 413 (highlighted in green in Fig. 1B).

Table S4: Average number of water molecules in the upper region of the H channel in C2 simulations.

Simulation system	Average number of water molecules*
C2-I (replica 1/2)	29 ± 6 / 24 ± 4
C2-II (replica 1/2)	17 ± 3 / 16 ± 2
C2-III	22 ± 5
C2-IV	31 ± 8
C2-V	26 ± 4
C2-VI	21 ± 1
C2-VII (replica 1/2)	27 ± 7 / 31 ± 5
C2-VIII (replica 1/2)	25 ± 6 / 18 ± 3

* Average number of water molecules were calculated over entire simulation trajectories by counting water oxygens within 5 Å of residues 54 371 440 and 441 (highlighted in blue in Fig. 1B).

Table S5: Average number of water molecules in the middle region of the H channel in C2 simulations.

Simulation system	Average number of water molecules*
C2-I (replica 1/2)	3 ± 1 / 3 ± 1
C2-II (replica 1/2)	3 ± 1 / 2 ± 1
C2-III	5 ± 2
C2-IV	4 ± 1
C2-V	5 ± 3
C2-VI	3 ± 0.3
C2-VII (replica 1/2)	3 ± 2 / 3 ± 1
C2-VIII (replica 1/2)	2 ± 1 / 4 ± 2

* Average number of water molecules were calculated over entire simulation trajectories by counting water oxygens within 5 Å of residues 38 424 454 and 458 (highlighted in cyan in Fig. 1B).

Table S6: Average number of water molecules in the lower region of the H channel in C2 simulations.

Simulation system	Average number of water molecules*
C2-I (replica 1/2)	1 ± 0.5 / 1 ± 1
C2-II (replica 1/2)	3 ± 1 / 2 ± 1
C2-III	2 ± 1
C2-IV	8 ± 1
C2-V	8 ± 1
C2-VI	2 ± 0.2
C2-VII (replica 1/2)	3 ± 1 / 2 ± 1
C2-VIII (replica 1/2)	3 ± 1 / 2 ± 1

* Average number of water molecules were calculated over entire simulation trajectories by counting water oxygens within 5 Å of residues 461 and 413 (highlighted in green in Fig. 1B).