Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

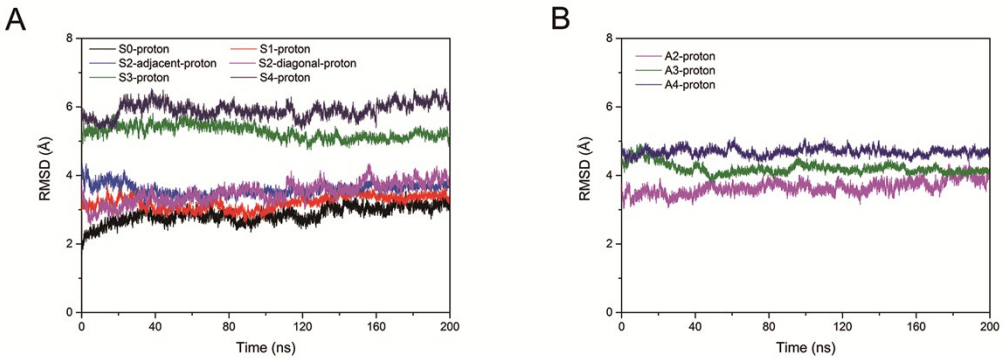

Fig. S1. The backbone RMSD with respect to the starting structure at different protonation states of H19 tetrad. (A) The backbone RMSD in wild systems. The curves in S0-proton (colored black), in S1-proton (colored red), in S2-adjacent-proton (colored blue), in S2-diagonal-proton (colored magenta), in S3-proton (colored olive) and in S4-proton (colored royal blue). (B) The backbone RMSD in mutant systems. The curves in A2-proton (colored magenta), in A3-proton (colored olive) and in A4-proton (colored royal blue).

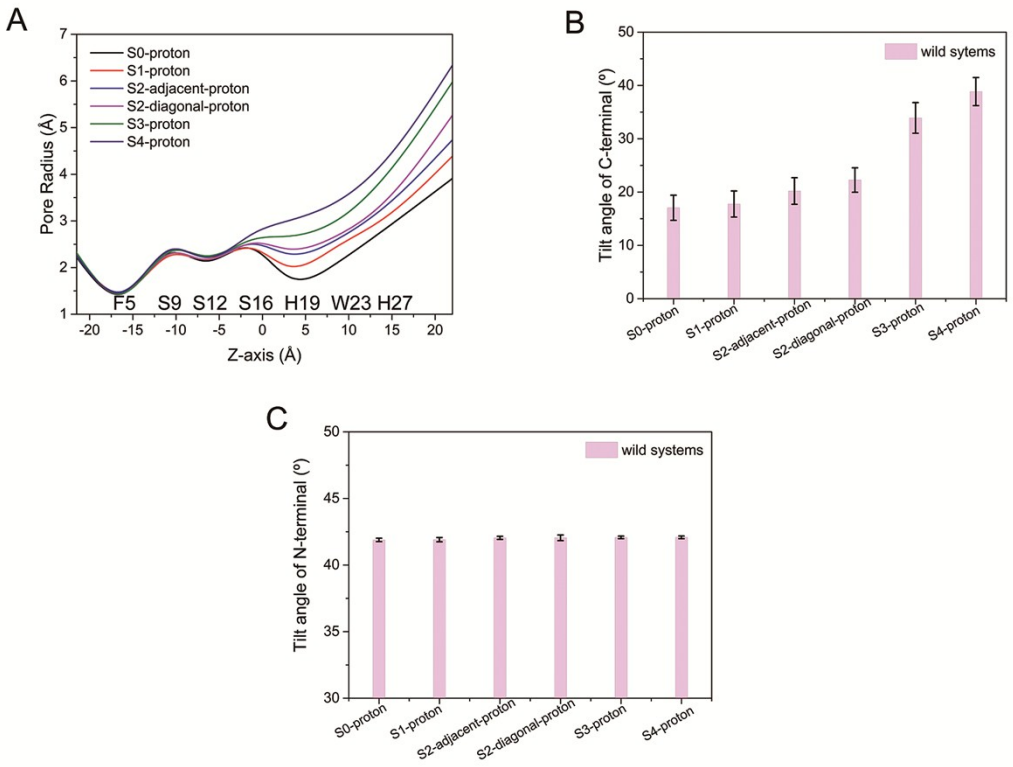

Fig. S2. (A) Pore radius along the channel in wild systems. Pore radius were averaged over the simulation time. (B) Tilt angle of the C-terminal portion (residues 17-33) and (C) N-terminal portion (residues 1-16) of helices relative to the channel axis in wild systems. Angles were averaged over all four helices and simulation time. Error bars indicate the standard deviations (colored black).

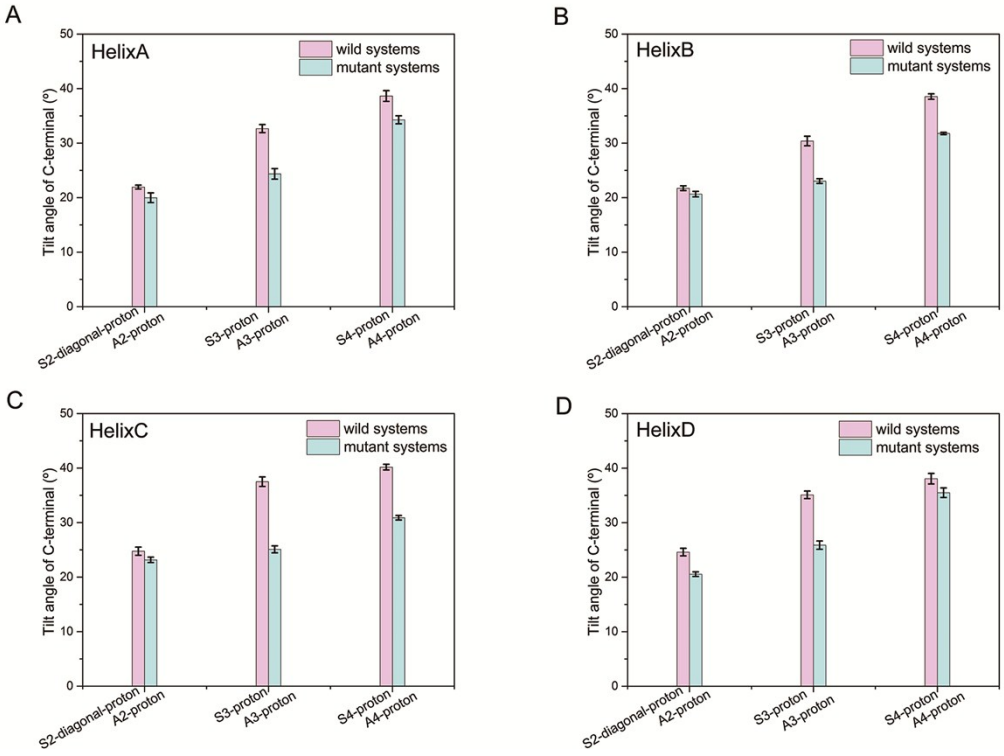

Fig. S3. Tilt angle of the C-terminal portion (residues 17-33) of helices relative to the channel axis in wild systems (light pink) and mutant systems (light cyan). Each panel includes the data for one helix as labeled. Error bars indicate the standard deviations (coloured black).

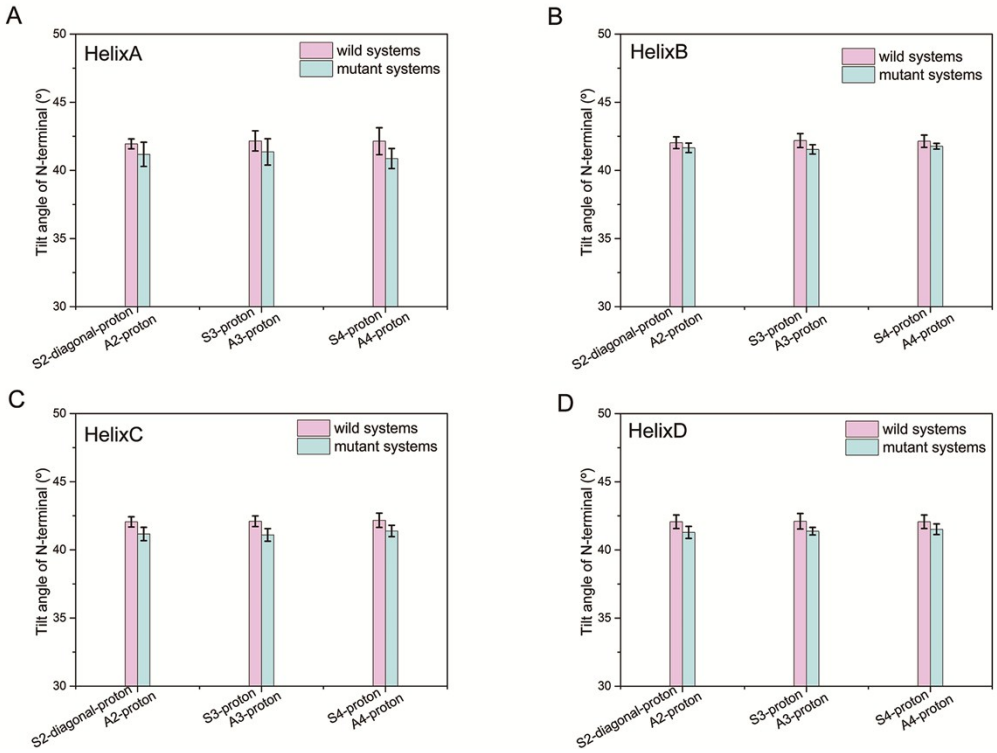

Fig. S4. Tilt angle of the N-terminal portion (residues 1-16) of helices relative to the channel axis in wild systems (light pink) and mutant systems (light cyan). Each panel includes the data for one helix as labeled. Error bars indicate the standard deviations (coloured black).

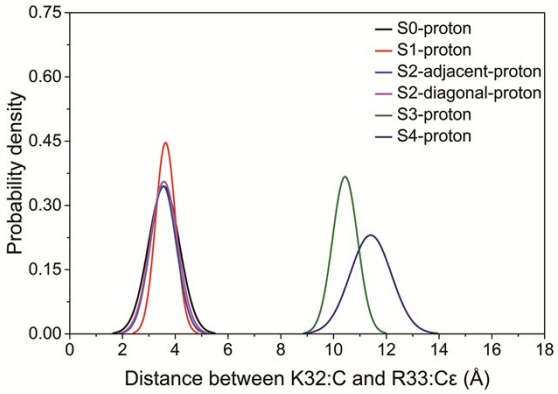

Fig.S5. Probability distribution of the average distance between the C atom of K32 of one helix and the Cε atom of R33 of its neighboring helix in wild systems. Distance was averaged over all four helices and simulation time.

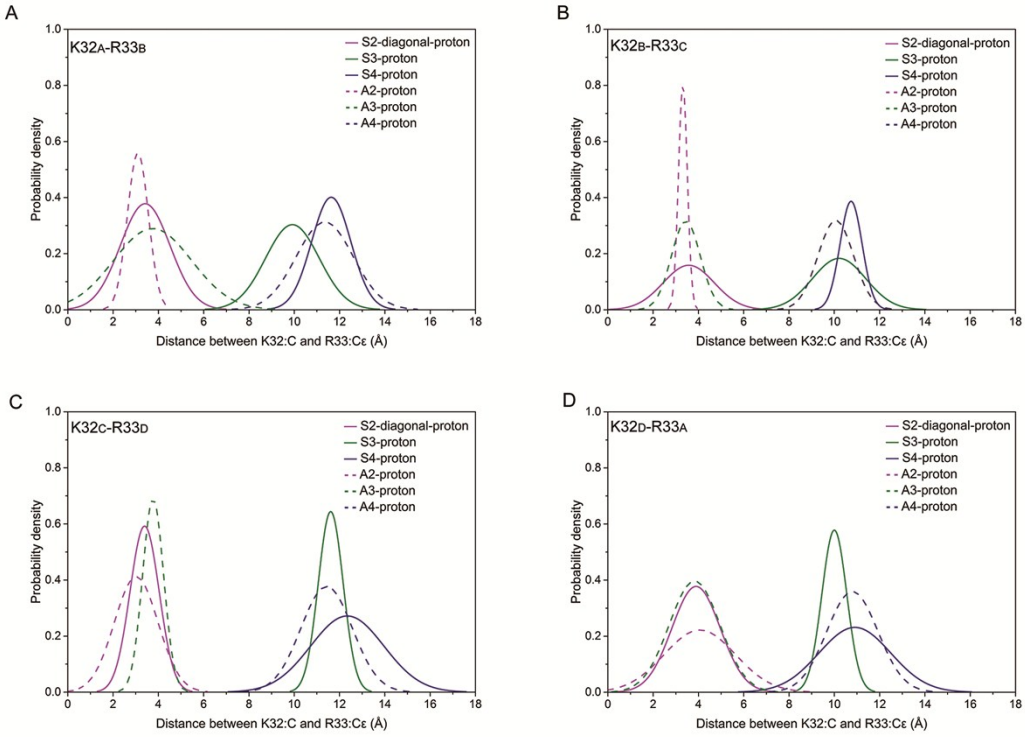

Fig. S6. Probability distribution of the distance between the C atom of K32 of one helix and the Cε atom of R33 of its neighbouring helix in wild systems (solid lines) and mutant systems (dotted lines). Each panel is for one K32-R33 pair.

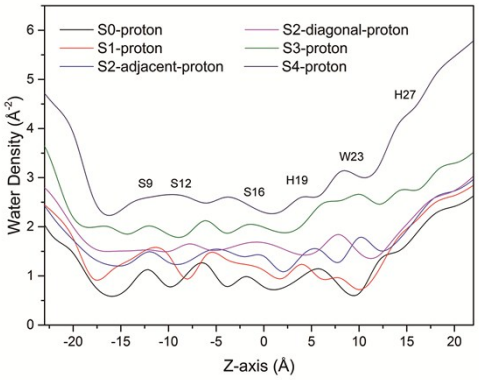

Fig.S7. Water density profiles across the channel axis in wild systems. The residues are those lining the pore: S9, S12, S16, H19, W23 and H27.

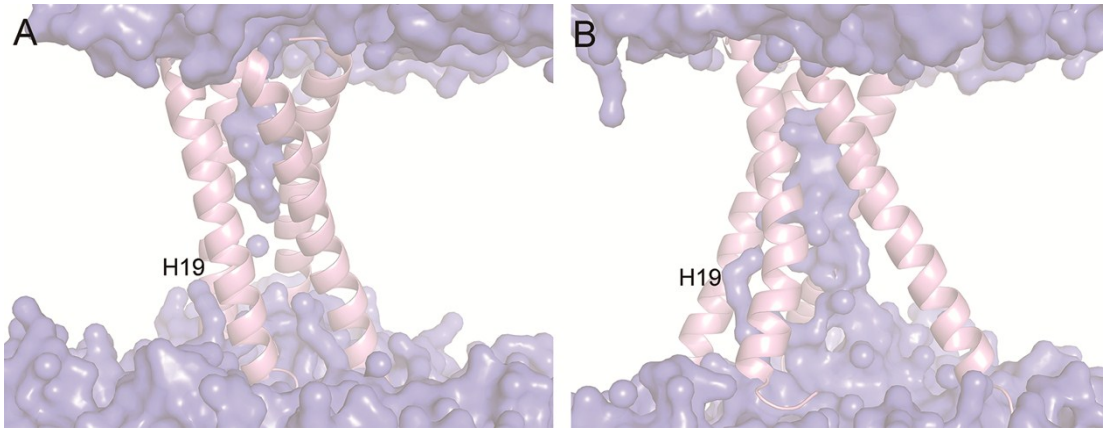

Fig.S8 (A) The distribution of water density for closed state is shown as blue shading. (B) The distribution of water density for open state calculated from the simulation is shown as blue shading.

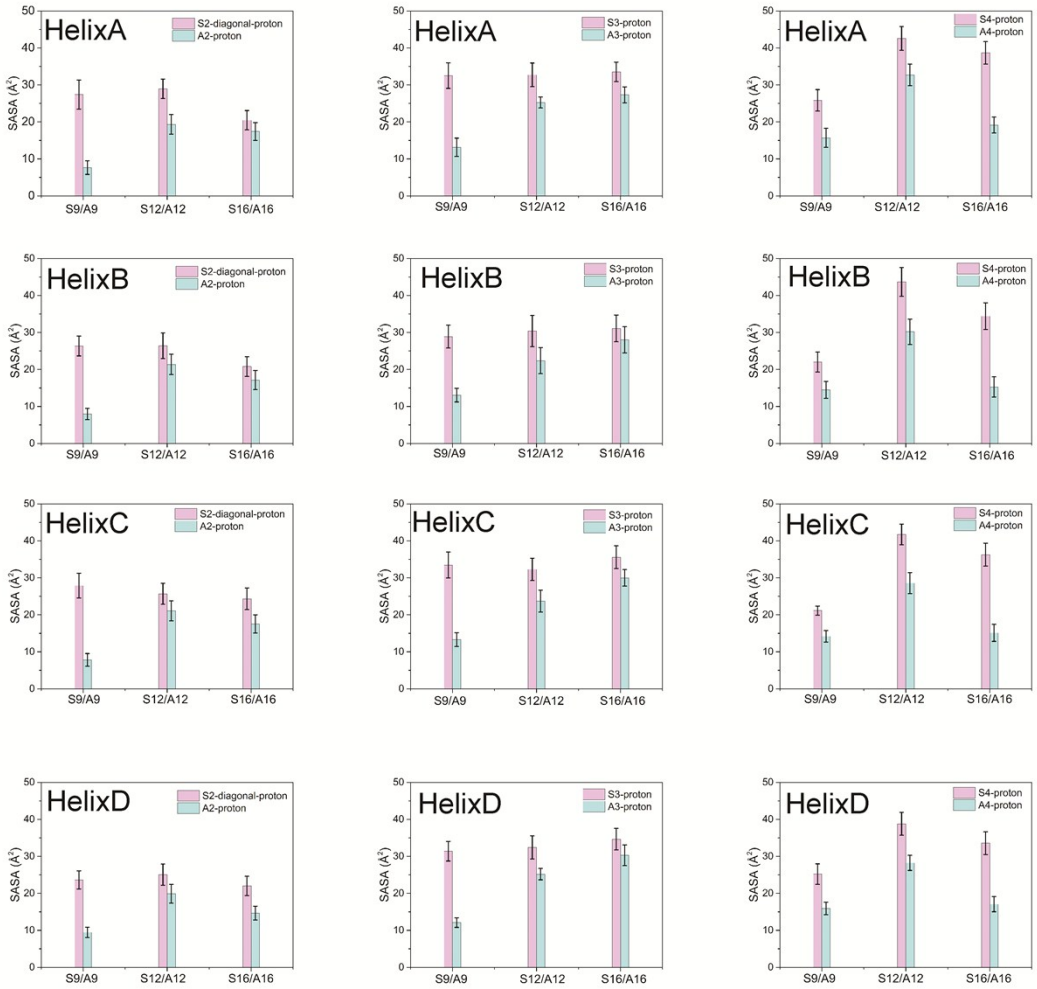

Fig.S9. The Solvent Accessible Surface Areas for residues S9/A9, S12/A12 and S16/A16 in wild systems (light pink) and mutant systems (light cyan). Each panel includes the data for one helix as labeled.

Table S1. Occupancy of the internal hydrogen bonds formed in protonated H19. A hydrogen bond is defined using a cutoff of 3.5 Å. NO stands for absent hydrogen bonds.

Protonation states of H19	Occupation (%)			
	HelixA	HelixB	HelixC	HelixD
S2-diagonal-proton	37.41	NO	37.66	NO
S3-proton	95.01	81.05	91.77	NO
S4-ptoton	99.50	99.50	98.25	95.76
A2-proton	NO	NO	NO	NO
A3-proton	NO	NO	NO	NO
A4-proton	NO	NO	NO	NO

Table S2. Occupancy of the hydrogen bonds between S16/A16 and protonated H19. A hydrogen bond is defined using a cutoff of 3.5 Å. NO stands for absent hydrogen bonds.

Protonation states of H19	Occupation (%)			
	HelixA	HelixB	HelixC	HelixD
S2-diagonal-proton	34.66	NO	32.42	NO
S3-proton	68.08	61.60	63.34	NO
S4-ptoton	95.01	86.53	89.78	91.27
A2-proton	NO	NO	NO	NO
A3-proton	NO	NO	NO	NO
A4-proton	NO	NO	NO	NO

Table S3. Occupancy of the interhelical hydrogen bonds between K32 of one helix and R33 of its neighboring helix. A hydrogen bond is defined using a cutoff of 4 Å for the distance between the C atom of K32 and Cε atom of R33. NO stands for occupancy below 20%.

Protonation states of H19	Occupation (%)			
	HelixA	HelixB	HelixC	HelixD
S0-proton	80.05	79.30	77.56	74.56
S1-proton	77.31	66.33	73.07	68.58
S2-adjacent-proton	59.60	56.87	56.11	61.60
S2-diagonal-proton	52.62	59.39	54.36	55.61
S3-proton	NO	NO	NO	NO
S4-proton	NO	NO	NO	NO
A2-proton	68.83	62.34	64.59	67.61
A3-proton	56.61	54.88	53.87	52.62
A4-proton	NO	NO	NO	NO