Supplementary information

Cryogenic temperature effects on membrane protein structure and dynamics

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Table S1 Number of molecules, ions, and atoms in the simulated protein-water systems.

System	**POPC molecules**	**TIP3 molecules**	**Na^+ ions**	**Cl^- ions**	**Protein atoms**	**Total atoms**
Membrane	266	40773	113	112	21488	179676
Water	0	98586	280	279	21488	317805

Table S2 Average values and standard deviations (SD) of simulated parameters for the three simulations of each temperature state of membrane-protein-water and protein-water systems.

Seed	**303 K - membrane Hot state**	**SD**	**85 K - membrane Cold state**	**SD**	**85 K - membrane Cooled state**	**SD**	**303 K - water Hot state**	**SD**	**85 K - water Cold state**	**SD**
Average root-mean-square deviation (nm)										
1	0.28	0.03	0.13	0.03	0.11	0.03	0.35	0.04	0.14	0.004
2	0.31	0.04	0.13	0.02	0.11	0.03	0.37	0.04	0.14	0.004
3	0.30	0.05	0.13	0.03	0.11	0.03	0.37	0.04	0.15	0.005
Average	**0.30**	**0.04**	**0.13**	**0.03**	**0.11**	**0.03**	**0.37**	**0.04**	**0.15**	**0.005**
Average radius of gyration (nm)										
1	4.03	0.02	3.80	0.03	3.98	0.05	3.93	0.03	3.84	0.003
2	4.01	0.02	3.81	0.02	3.96	0.05	3.91	0.02	3.83	0.003
3	3.99	0.03	3.81	0.03	3.97	0.05	3.93	0.03	3.83	0.004
Average	**4.01**	**0.03**	**3.81**	**0.03**	**3.97**	**0.05**	**3.92**	**0.03**	**3.83**	**0.003**
Average solvent accessible surface area (nm^2)										
1	605	8	529	3	589	4	587	6	534	2
2	610	7	529	3	581	3	582	6	536	2
3	613	9	529	3	598	4	586	7	536	2
Average	**609**	**8**	**529**	**3**	**589**	**4**	**585**	**6**	**535**	**2**
Average number of residues forming C83 α-helix										
1	13	2	15	1	12	2	16	1	15	1
2	14	2	15	1	15	0	14	1	14	1
3	12	2	13	1	14	0	14	1	16	1
Average	**13**	**2**	**14**	**1**	**14**	**1**	**15**	**1**	**15**	**1**
Average number of residues forming PS1 β-strands										
1	5	1	0	0	6	0	1	1	3	3
2	1	1	6	0	0	0	0	0	6	0
3	1	2	0	0	0	0	4	1	5	2
Average	**2**	**2**	**2**	**0**	**2**	**0**	**2**	**1**	**5**	**2**
Average distance between Cα-atoms of Asp257 and Asp385 (nm)										
1	1.15	0.03	1.02	0.01	1.16	0.02	1.66	0.06	1.11	0.01
2	1.15	0.05	1.05	0.01	1.12	0.02	1.40	0.08	1.07	0.01
3	1.14	0.03	1.05	0.02	1.15	0.02	1.30	0.04	1.07	0.01
Average	**1.15**	**0.04**	**1.04**	**0.01**	**1.14**	**0.02**	**1.46**	**0.06**	**1.08**	**0.01**
Average number of hydrogen bonds between C83 and PS1										
1	9.26	1.77	10.85	0.43	9.40	0.59	7.25	1.54	9.02	0.19
2	9.27	2.07	11.85	0.38	9.47	0.63	6.53	1.55	9.74	0.70
3	12.32	2.06	9.10	0.48	10.99	0.20	6.22	1.72	10.37	0.67
Average	**10.28**	**1.97**	**10.60**	**0.43**	**9.95**	**0.48**	**6.67**	**1.60**	**9.71**	**0.52**

Table S3 Correlation (R^2) between R_g and SASA of full γ-secretase-C83 complex.

Seed	**303 K - membrane Hot state**	**p-value**	**85 K - membrane Cold state**	**p-value**	**85 K - membrane Cooled state**	**p-value**	**303 K - water Hot state**	**p-value**	**85 K - water Cold state**	**p-value**
1	0.11	0.00	0.70	0.00	0.77	0.00	0.05	0.00	0.29	0.00
2	0.27	0.00	0.60	0.00	0.66	0.00	0.05	0.00	0.13	0.00
3	0.33	0.00	0.71	0.00	0.76	0.00	0.002	0.02	0.21	0.00

Table S4 Average R_g (nm) of each subunit of γ-secretase. In 6IYC, R_g for nicastrin is 3.0 nm, presinilin-1 is 2.10, APH-1A is 2.0, PEN-2 is 1.98 and C83 is 1.87 nm.

Seed	**303 K - membrane Hot state**	**SD**	**85 K - membrane Cold state**	**SD**	**85 K - membrane Cooled state**	**SD**	**303 K - water Hot state**	**SD**	**85 K - water Cold state**	**SD**
Nicastrin										
1	3.03	0.01	2.91	0.01	3.00	0.02	3.01	0.01	2.92	0.002
2	3.03	0.01	2.92	0.00	2.99	0.02	3.01	0.02	2.92	0.003
3	3.04	0.02	2.92	0.01	3.03	0.02	3.01	0.01	2.92	0.003
Average	**3.03**	**0.01**	**2.92**	**0.01**	**3.01**	**0.02**	**3.01**	**0.01**	**2.92**	**0.003**
Presinilin-1										
1	2.14	0.01	2.06	0.01	2.11	0.01	2.13	0.01	2.08	0.002
2	2.16	0.02	2.06	0.01	2.14	0.01	2.14	0.01	2.08	0.002
3	2.14	0.01	2.06	0.01	2.10	0.01	2.13	0.01	2.08	0.002
Average	**2.15**	**0.01**	**2.06**	**0.01**	**2.12**	**0.01**	**2.14**	**0.01**	**2.08**	**0.002**
APH-1A										
1	2.02	0.02	1.95	0.01	2.00	0.01	2.00	0.01	1.96	0.002
2	2.02	0.01	1.95	0.01	1.99	0.01	1.97	0.02	1.96	0.002
3	2.03	0.02	1.94	0.01	2.01	0.01	2.00	0.02	1.96	0.002
Average	**2.02**	**0.02**	**1.95**	**0.01**	**2.00**	**0.01**	**1.99**	**0.01**	**1.96**	**0.002**
PEN-2										
1	1.97	0.01	1.92	0.03	1.96	0.05	1.96	0.01	1.95	0.003
2	1.97	0.01	1.93	0.02	1.93	0.04	1.98	0.02	1.95	0.004
3	1.97	0.01	1.93	0.03	1.97	0.04	1.96	0.02	1.95	0.003
Average	**1.97**	**0.01**	**1.93**	**0.02**	**1.95**	**0.04**	**1.97**	**0.01**	**1.95**	**0.003**
C83										
1	1.96	0.02	1.88	0.03	1.94	0.04	1.89	0.02	1.89	0.004
2	1.91	0.02	1.87	0.02	1.92	0.04	1.87	0.03	1.90	0.004
3	1.93	0.02	1.86	0.03	1.95	0.03	1.94	0.03	1.89	0.004
Average	**1.93**	**0.02**	**1.87**	**0.02**	**1.94**	**0.04**	**1.90**	**0.03**	**1.89**	**0.004**

Table S5 Average SASA (nm^2) of each subunit of γ-secretase. In 6IYC, the SASA of nicastrin is 309 nm^2, presinilin-1 is 179 nm^2, APH-1A is 143 nm^2, PEN-2 is 79 nm^2, and C83 is 37 nm^2.

Seed	**303 K - membrane Hot state**	**SD**	**85 K - membrane Cold state**	**SD**	**85 K - membrane Cooled state**	**SD**	**303 K - water Hot state**	**SD**	**85 K - water Cold state**	**SD**
Nicastrin										
1	319	4	285	1	312	2	319	4	287	1
2	323	4	284	1	310	2	317	4	288	1
3	326	4	286	1	322	2	315	4	289	1
Average	**322**	**4**	**285**	**1**	**315**	**2**	**317**	**4**	**288**	**1**
Presinilin-1										
1	188	3	167	1	182	1	181	3	171	1
2	191	3	168	1	183	1	183	3	170	1
3	184	3	168	1	173	1	185	3	170	1
Average	**188**	**3**	**168**	**1**	**180**	**1**	**183**	**3**	**171**	**1**
APH-1A										
1	145	2	138	1	140	1	145	3	136	1
2	146	2	136	1	141	1	144	4	137	1
3	147	3	138	1	145	1	150	3	137	1
Average	**146**	**2**	**137**	**1**	**142**	**1**	**147**	**3**	**137**	**1**
PEN-2										
1	79	1	78	1	79	1	80	1	76	1
2	78	1	78	1	76	1	79	1	77	1
3	80	1	78	1	79	1	79	1	77	1
Average	**79**	**1**	**78**	**1**	**78**	**1**	**79**	**1**	**77**	**1**
C83										
1	44	1	41	1	43	1	42	1	42	0
2	43	1	42	0	44	1	42	1	42	0
3	44	1	41	1	43	1	43	1	42	0
Average	**44**	**1**	**41**	**1**	**43**	**1**	**42**	**1**	**42**	**0**

Table S6 Correlation (R^2) between R_g and SASA of each subunit of γ-secretase.

Seed	303 K - membrane Hot state	p-value	85 K - membrane Cold state	p-value	85 K - membrane Cooled state	p-value	303 K - water Hot state	p-value	85 K - water Cold state	p-value
Nicastrin										
1	0.04	0.00	0.16	0.00	0.49	0.00	0.26	0.00	0.11	0.00
2	0.03	0.00	0.22	0.00	0.48	0.00	0.23	0.00	0.01	0.00
3	0.23	0.00	0.21	0.00	0.45	0.00	0.20	0.00	0.02	0.00
Average	**0.10**	**0.00**	**0.20**	**0.00**	**0.47**	**0.00**	**0.23**	**0.00**	**0.05**	**0.00**
Presinilin-1										
1	0.08	0.00	0.45	0.00	0.57	0.00	0.33	0.00	0.07	0.00
2	0.42	0.00	0.39	0.00	0.42	0.00	0.52	0.00	0.10	0.00
3	0.26	0.00	0.55	0.00	0.45	0.00	0.27	0.00	0.08	0.00
Average	**0.25**	**0.00**	**0.46**	**0.00**	**0.48**	**0.00**	**0.37**	**0.00**	**0.08**	**0.00**
APH-1A										
1	0.36	0.00	0.38	0.00	0.40	0.00	0.31	0.00	0.05	0.00
2	0.39	0.00	0.27	0.00	0.45	0.00	0.48	0.00	0.08	0.00
3	0.65	0.00	0.29	0.00	0.52	0.00	0.54	0.00	0.05	0.00
Average	**0.47**	**0.00**	**0.31**	**0.00**	**0.46**	**0.00**	**0.44**	**0.00**	**0.06**	**0.00**
PEN-2										
1	0.11	0.00	0.18	0.00	0.63	0.00	0.04	0.00	0.02	0.00
2	0.09	0.00	0.25	0.00	0.25	0.00	0.02	0.00	0.09	0.00
3	0.13	0.00	0.19	0.00	0.58	0.00	0.08	0.00	0.00	0.00
Average	**0.11**	**0.00**	**0.21**	**0.00**	**0.49**	**0.00**	**0.05**	**0.00**	**0.04**	**0.00**
C83										
1	0.07	0.00	0.34	0.00	0.44	0.00	0.01	0.00	0.03	0.00
2	0.16	0.00	0.24	0.00	0.44	0.00	0.04	0.00	0.02	0.00
3	0.02	0.00	0.47	0.00	0.56	0.00	0.00	0.75	0.02	0.00
Average	**0.08**	**0.00**	**0.35**	**0.00**	**0.48**	**0.00**	**0.02**	**0.25**	**0.02**	**0.00**

Table S7. Average radius of gyration and solvent accessible surface area of the complete γ-secretase-C99 complex in membrane-water system simulated at 303 K.

Seed	Average radius of gyration (nm)	SD	Average solvent accessible surface area (nm^2)	SD
1.00	4.20	0.03	738	9
2.00	4.22	0.02	729	8
Average	**4.21**	**0.03**	**734**	**9**

Table S8 Number of clusters in the equilibrated trajectories.

Seed	**303 K – membrane Hot state**	**85 K – membrane Cold state**	**85 K – membrane Cooled state**	**303 K – water Hot state**	**85 K – water Cold state**
1	20	1	1	55	1
2	18	1	1	54	1
3	27	1	1	73	1
Average	**22**	**1**	**1**	**61**	**1**
SD	**4**	**0**	**0**	**9**	**0**

Table S9 Presence of the β-strands in PS1 subunit (one, both or none of the β1 and β2 strands) of the representative structure from trajectory clustering.

Seed	**303 K - membrane Hot state**	**85 K - membrane Cold state**	**85 K - membrane Cooled state**	**303 K - water Hot state**	**85 K - water Cold state**
1	Both	None	Both	None	None
2	None	Both	None	None	Both
3	None	None	None	Both	Both

Table S10 Comparison of the cooled state with hot and cold states of the protein-water-membrane system. 6IYC refers to the cryo-electron microscopy structure (PDB code 6IYC).

Cooled state properties

TIP3 density	**Lipid order**	**RMSD**	**R_g**	**SASA**	**RMSF**	**PCA**	**C83 Secondary structure**	**PS1 Asp-Asp distance**
Similar to hot state	Similar to hot state	Similar to cold state	Similar to 6IYC, slightly smaller than hot state	Similar to 6IYC. Somewhat smaller than hot state	Similar to cold state (slightly higher)	Similar to cold state although PC1 is slightly larger	Two states - one similar to 6IYC and one similar to hot state	Similar to 6IYC and hot state

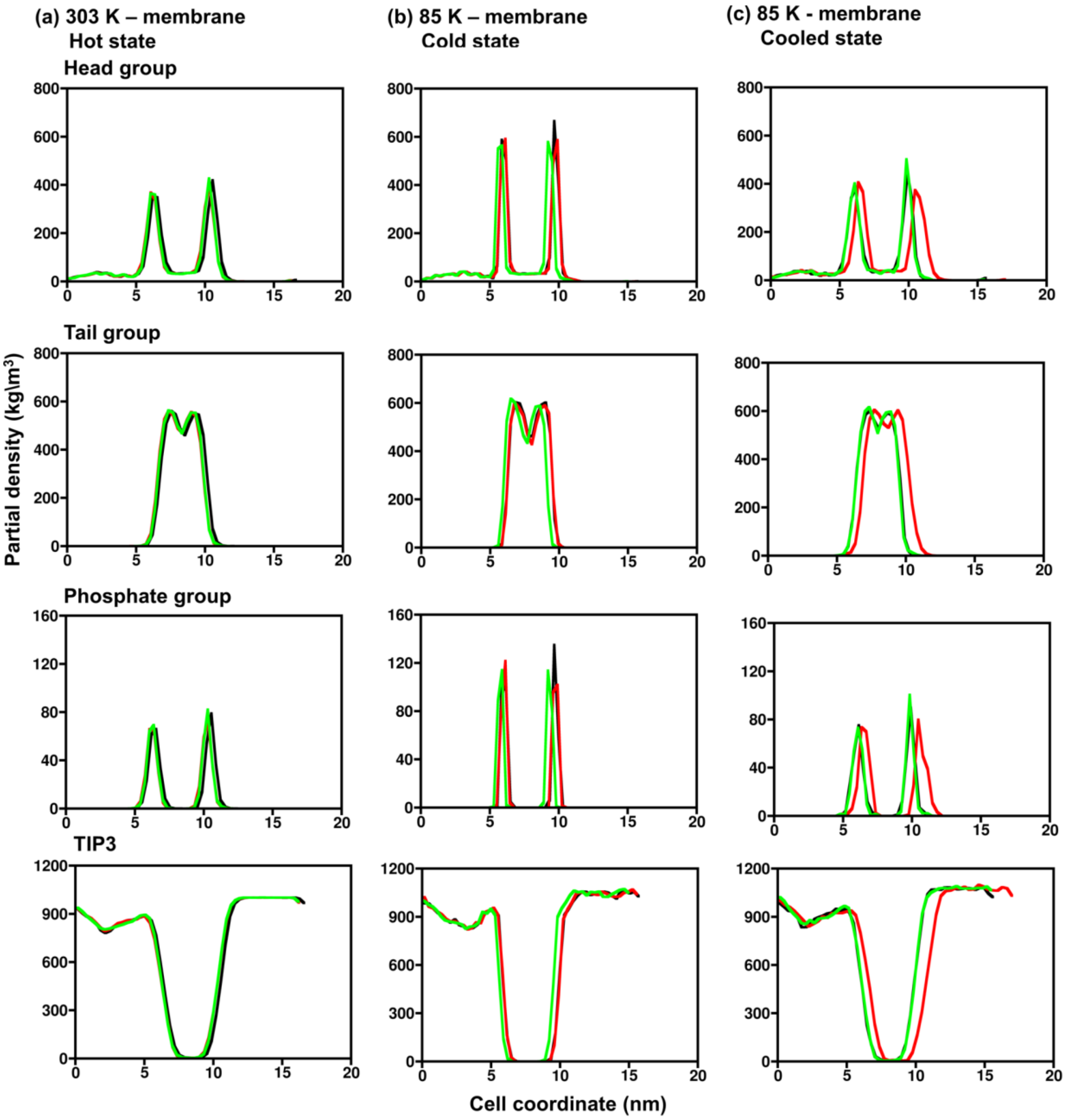

Figure S1. Partial densities of the POPC and TIP3P groups in membrane system simulations.

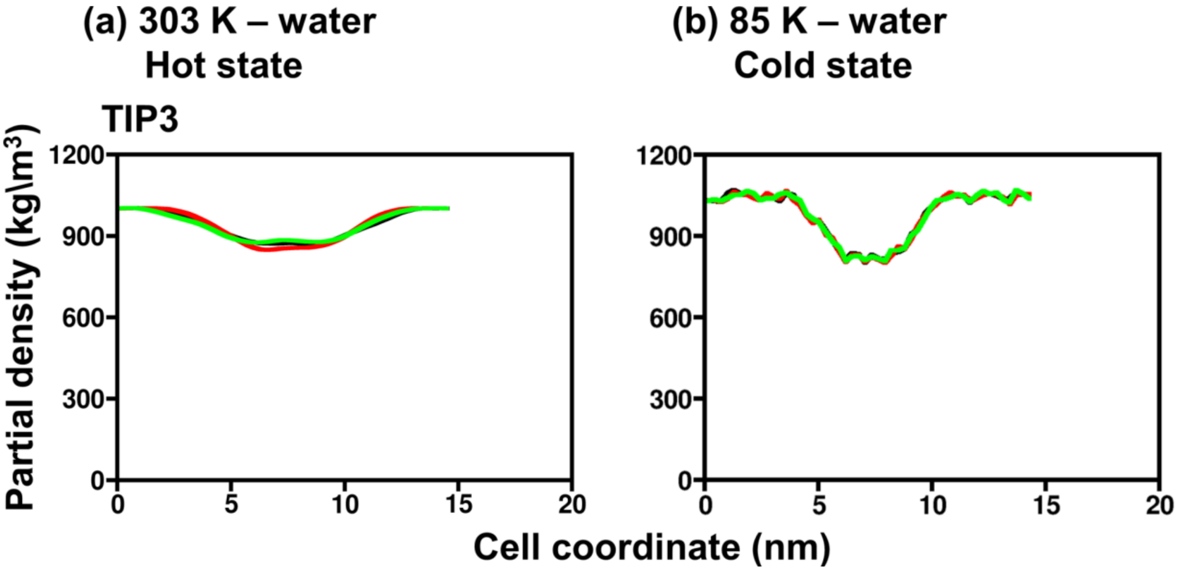

Fig. S2 Partial densities of TIP3P in water system simulations.

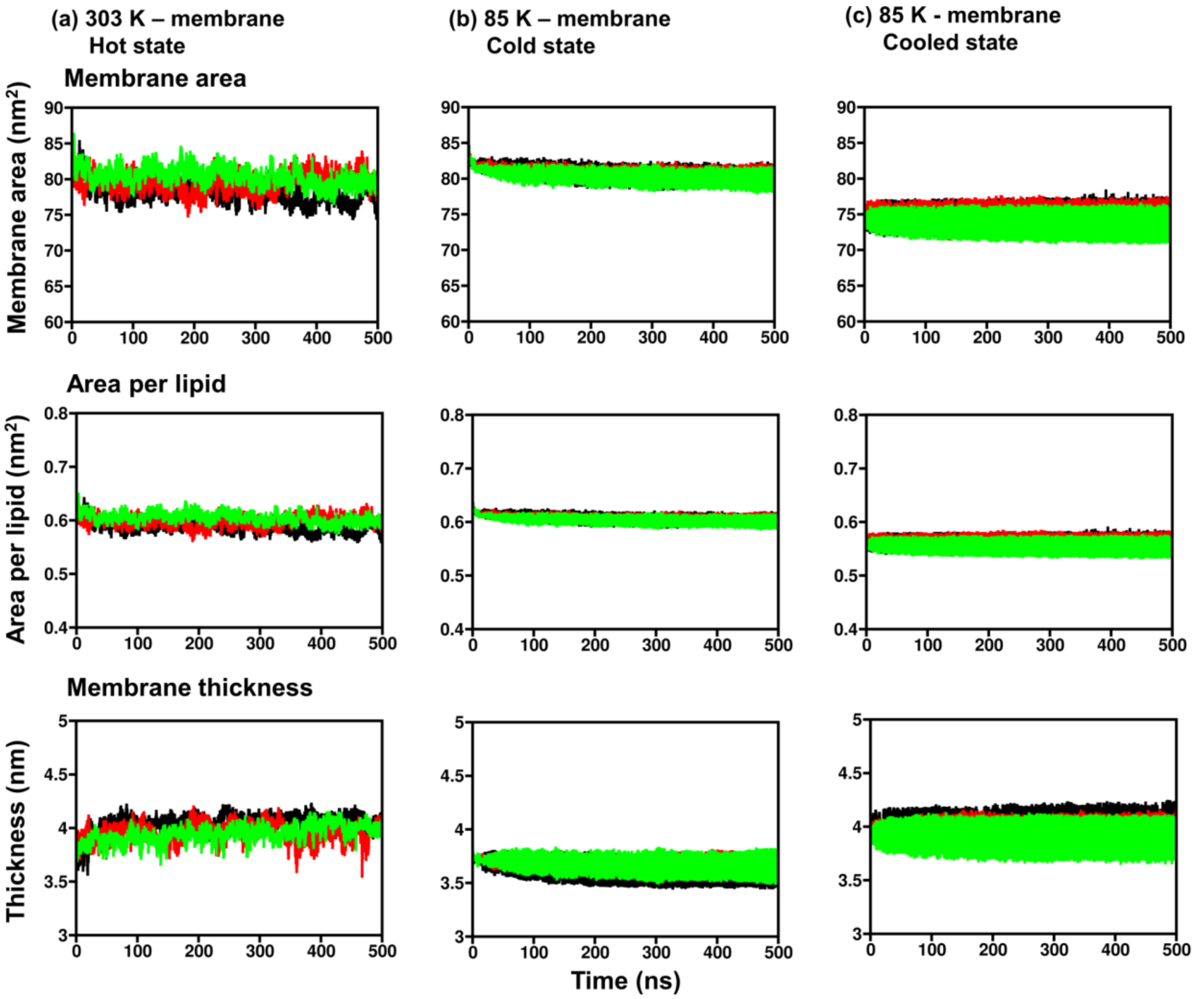

Figure S3. Membrane properties (membrane area, area per lipid and thickness) calculated for the three states. **(a)** Hot state of protein in membrane. **(b)** Cold state in membrane. **(c)** Cooled state in membrane.

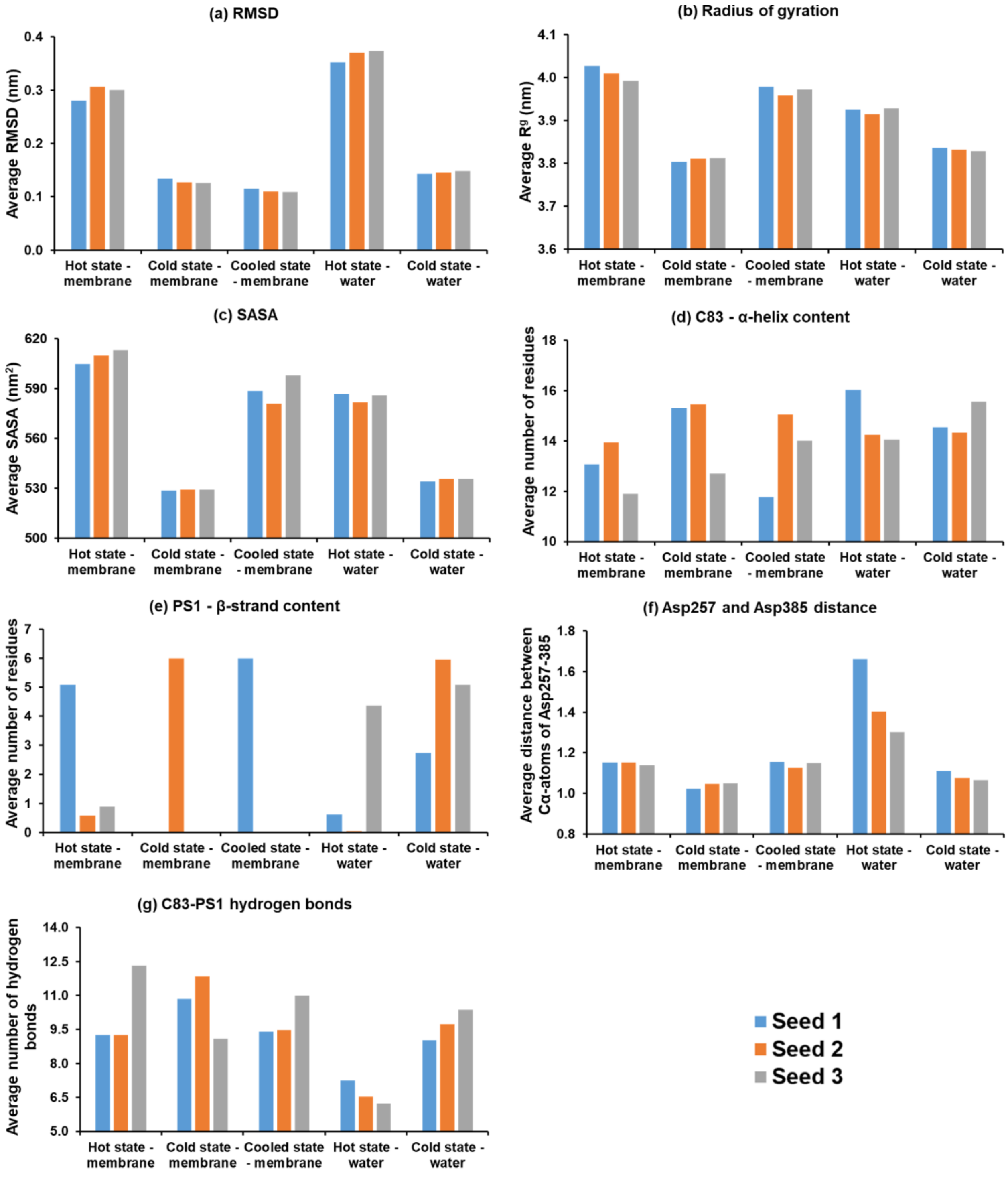

Figure S4. Average values of the simulation parameters over the three simulations of each temperature state of membrane-protein-water and protein-water systems.

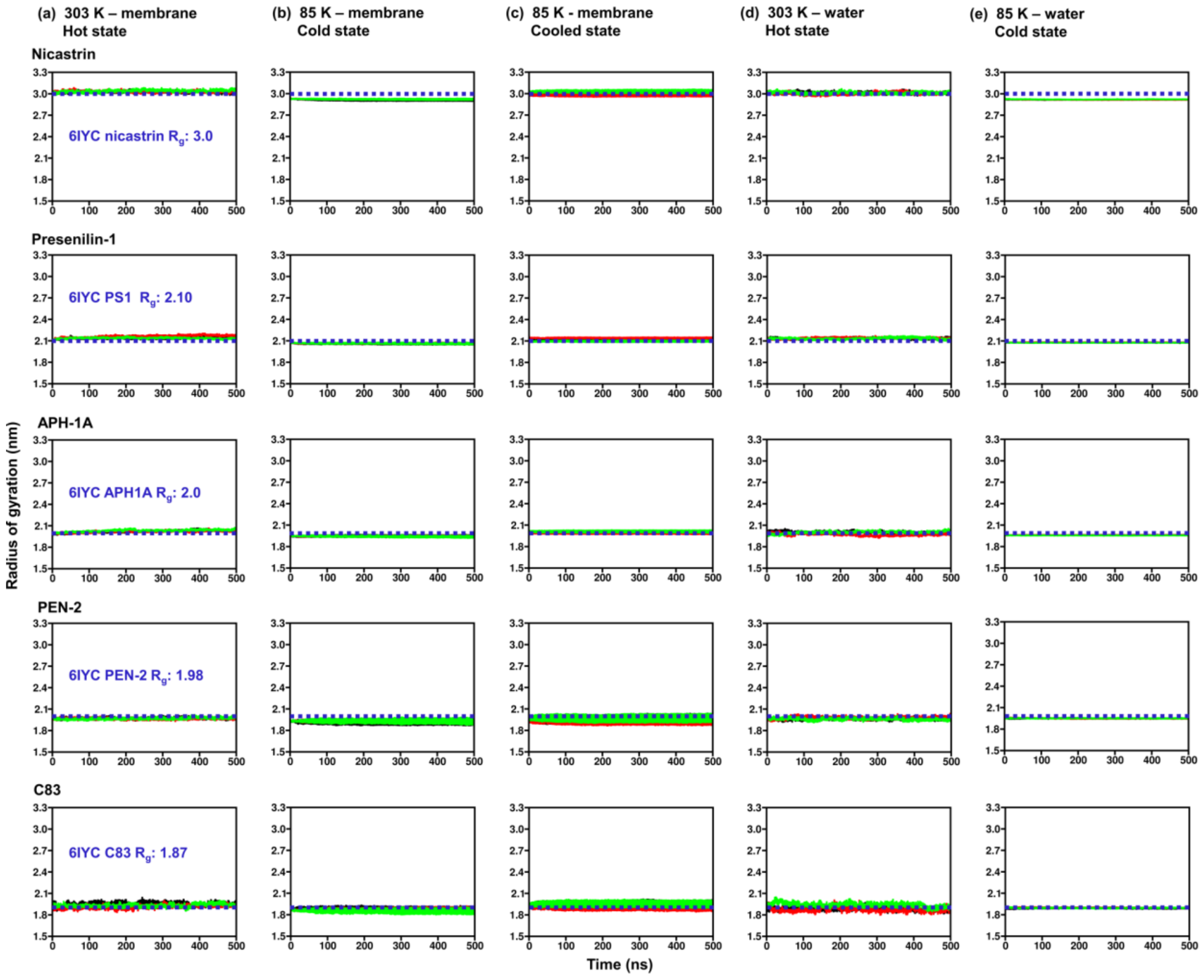

Figure S5. R_g of each subunit of γ-secretase complex.

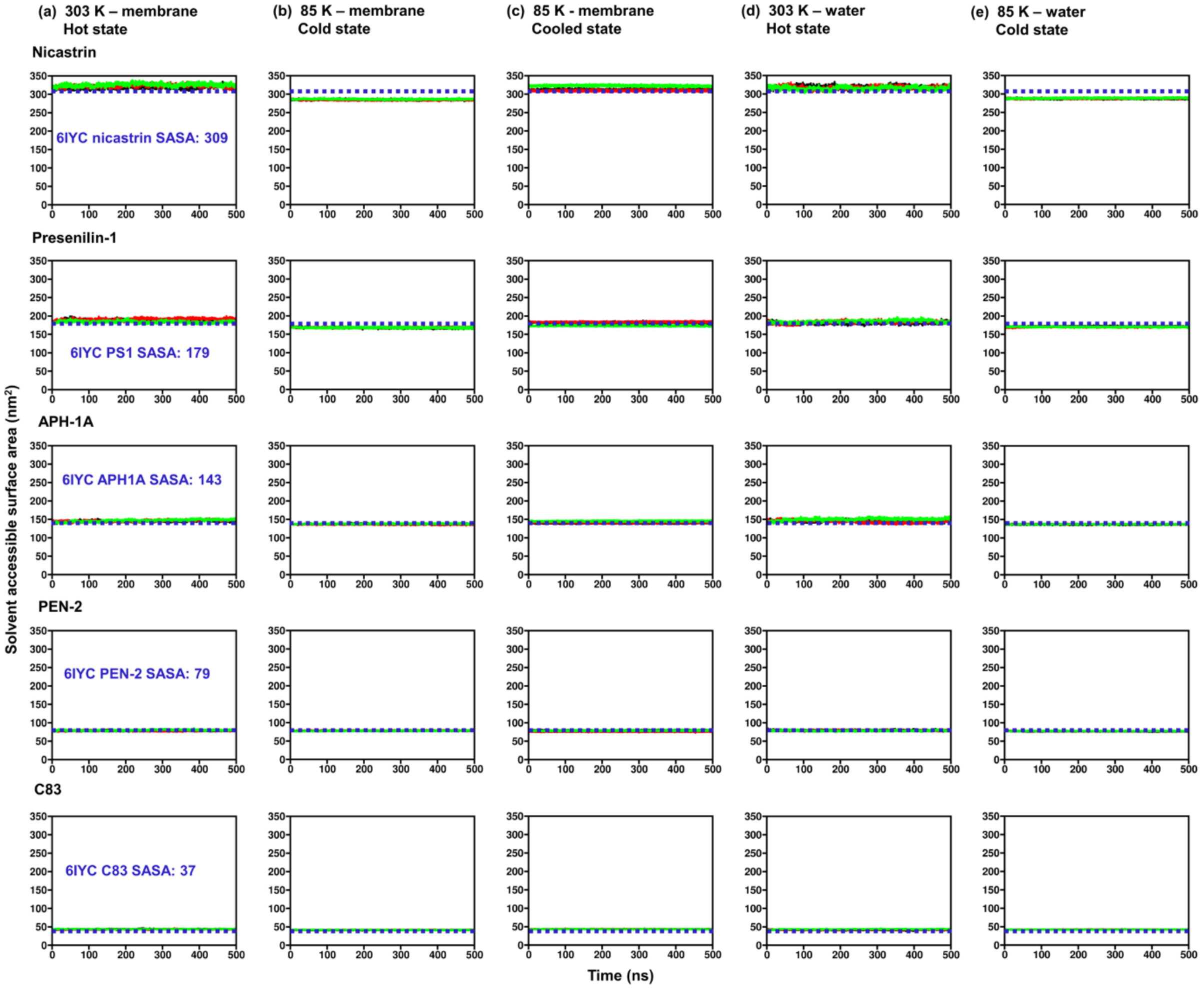

Figure S6. SASA of each subunit of γ-secretase complex.

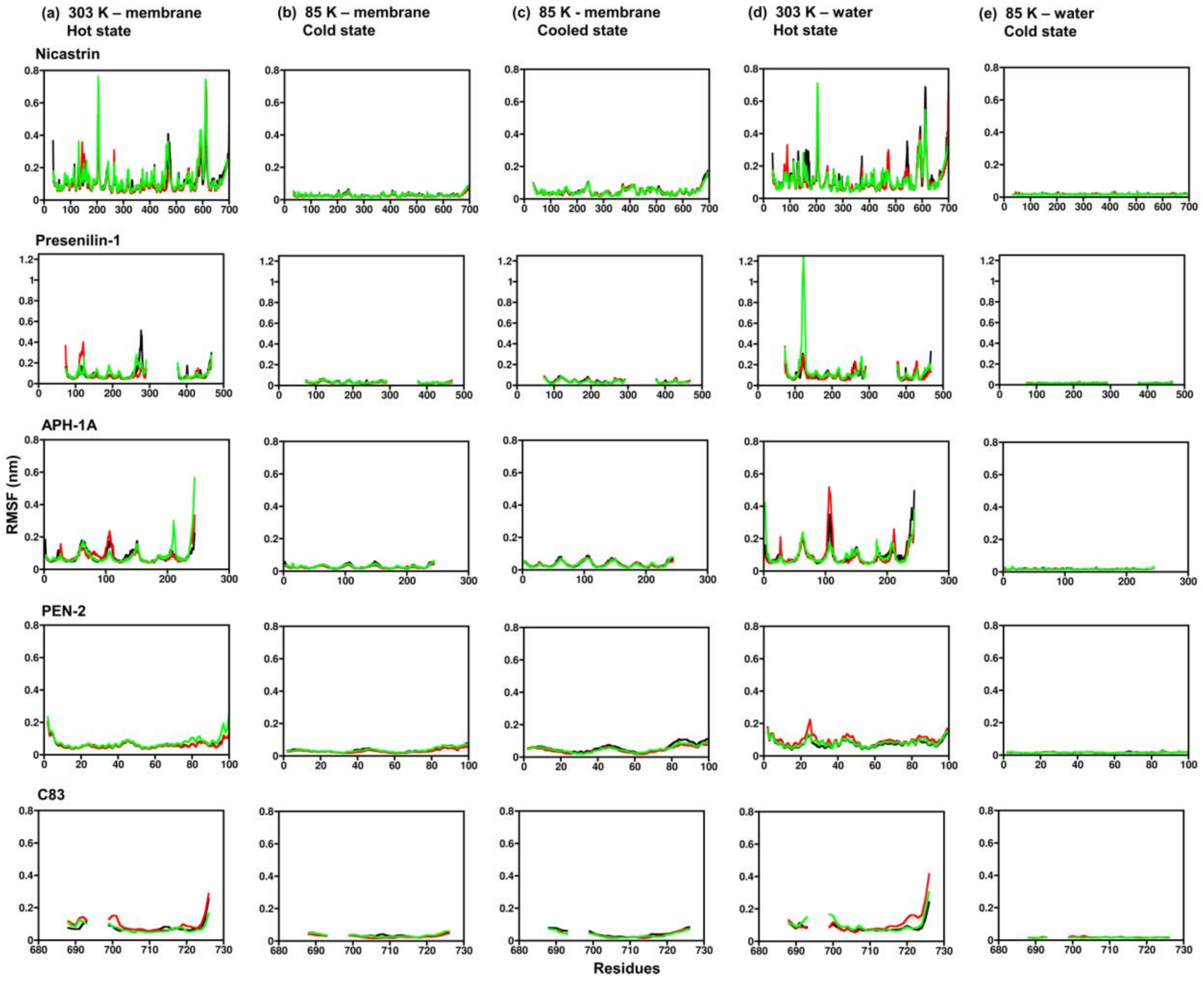

Figure S7. RMSF plots of the five chains of γ-secretase (nicastrin, presenilin-1, APH-1A, PEN-2 and C83) in the five states. **(a)** Hot state of protein in membrane. **(b)** Cold state in membrane. **(c)** Cooled state in membrane. **(d)** Hot state in water. **(e)** Cold state in water.

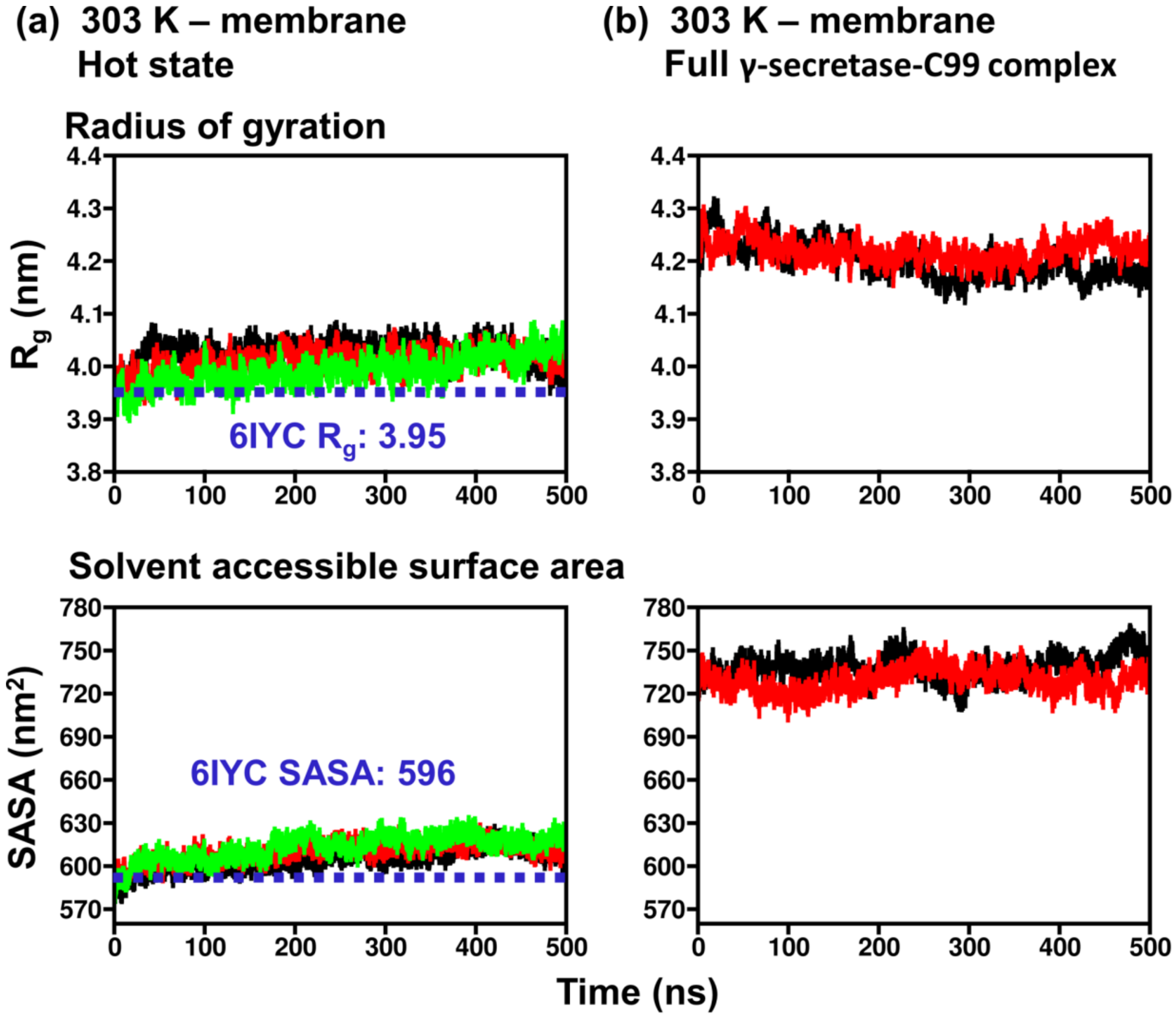

Figure S8. Comparison of R_g and SASA of the 6IYC-membrane-water system ("hot state", three simulations) with complete γ-secretase-C99 complex in membrane-water system (two simulations) at 303 K.

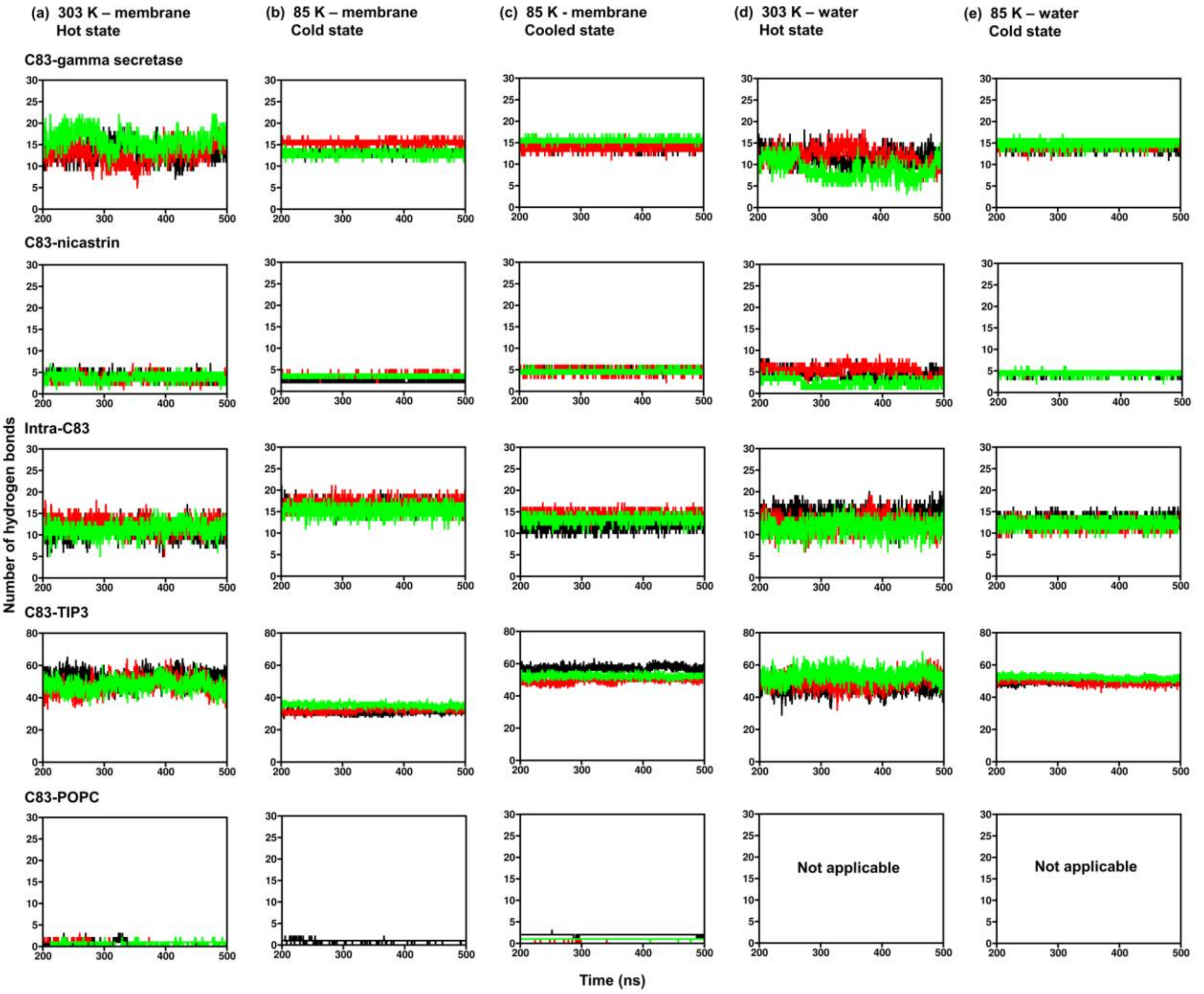

Figure S9. C83 interactions with other subunits including full protein γ-secretase, PS1, nicastrin, intra-C83, TIP3 and POPC. **(a)** Hot state of protein in membrane. **(b)** Cold state in membrane. **(c)** Cooled state in membrane. **(d)** Hot state in water. **(e)** Cold state in water.