Electronic Supplementary Material (ESI) for Molecular BioSystems.

† **Electronic supplementary information (ESI)**

Conformational states of HAMP domains interacting with sensory rhodopsin membrane systems: an integrated all-atom and coarse-grained molecular dynamics simulation approach

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Table S1. Structure validation report for HAMP1 homology model (84-159) in various web servers.

Servers		**HAMP1**	**2L7H**
PROCHECK	Most favored regions (%)	91.50	94.20
	Additionally allowed Regions (%)	7.00	5.80
	Generously allowed Regions (%)	1.50	0
	Disallowed regions (%)	0	0
	Overall G-factor	0.16	0.39
Verify3D	Averaged 3D-1D Score > 0.2	63.29	48.28
ERRAT	Overall Quality	87.14	100
Prove	Z-score mean	-0.73	0.42
ProSa	Z-score	-4.00	-3.68
ProQ	LGscore	1.63	0.58
	MaxSub score	0.28	0.09
	Bad backbone bonds (%)	0 (0/598)	0 (0/451)
MolProbity	Bad backbone angles (%)	0.12 (1 / 802)	0 (0/606)
	Cβ deviations >0.25Å (%)	0	0

Table S2. Binding energy calculation between HAMP1 domain in *Nps*rII/htrII heterodimer and homodimer complex systems using MM/PBSA.

Molecules			**Polar Contribution**			**Non-polar Contribution**	
	[1]ΔG_{bind}	**[2]ΔG_{coul}**	**[3]ΔG_{ps}**	**[4]ΔG_{polar}**	**[5]ΔG_{vdw}**	**[6]ΔG_{nps}**	**[7]$\Delta G_{nonpolar}$**
G-state (2:2)	-21.03 (5.84)	2523.32 (27.81)	-2194.03 (27.73)	329.29	-306.98 (3.84)	-43.33 (0.25)	-350.31
I-state (2:2)	-54.68 (4.60)	2216.70 (22.17)	-1981.42 (22.18)	235.28	-254.45 (2.79)	-35.51 (0.18)	-289.96
Homodimer (1:1)	-103.16 (6.17)	3095.44 (14.93)	-2830.80 (14.07)	264.64	-327.26 (3.87)	-40.54 (0.25)	-367.80

[1]Binding free energy, [2]Coulombic term, [3]Polar solvation terms, [4]Polar solvation energy, [5]van der Waals energy, [6]Nonpolar solvation energy, [7]Nonpolar solvation terms. Standard errors are presented in parenthesis, G- ground state and I- intermediate state.

Table S3. Binding free energy (kJ mol^{-1}) calculated by MM/PBSA from the *Np*srII-htrII (mutant) complex.

Molecules	Polar Contribution				Non-polar Contribution		
	[1]ΔG_{bind}	[2]ΔG_{coul}	[3]ΔG_{ps}	[4]ΔG_{polar}	[5]ΔG_{vdw}	[6]ΔG_{nps}	[7]$\Delta G_{nonpolar}$
Y199^{SrII}	-318.47 (13.30)	-1236.92 (17.90)	1431.66 (18.95)	168.04	-428.20 (13.00)	-58.30 (0.34)	-486.51
T189^{SrII}	-304.38 (13.22)	-1233.55 (17.68)	1463.18 (19.16)	229.63	-473.19 (13.00)	-60.81 (0.34)	-534.01
N74^{HtrII}	-333.08 (12.47)	-1290.67 (17.81)	1477.16 (18.96)	186.49	-458.02 (12.28)	-61.54 (0.34)	-519.57
E43^{HtrII}	-300.13(13.43)	-849.93 (16.88)	1065.99 (18.23)	216.06	-455.83 (13.27)	-60.37 (0.34)	-516.20
S154^{SrII}	-361.23 (12.32)	-1319.38(17.67)	1478.18 (19.06)	158.80	-458.93 (12.49)	-61.09 (0.34)	-520.02
D102^{HtrII}	-354.72 (12.61)	-1311.60 (17.98)	1476.43 (19.21)	164.83	-458.16 (12.52)	-61.38 (0.34)	-519.55
R162^{SrII}	-347.16 (14.05)	-423.99 (16.77)	589.74 (18.23)	165.75	-452.82 (13.38)	-60.09 (0.34)	-512.92
S62^{HtrII}	-338.97 (12.74)	-1288.13 (17.54)	1475.21 (18.99)	187.08	-464.52 (12.63)	-61.52 (0.34)	-526.05

[1]Binding free energy, [2]Coulombic term, [3]Polar solvation terms, [4]Polar solvation energy, [5]van der Waals energy, [6]Nonpolar solvation energy, [7]Nonpolar solvation terms. Standard errors are presented in parenthesis.

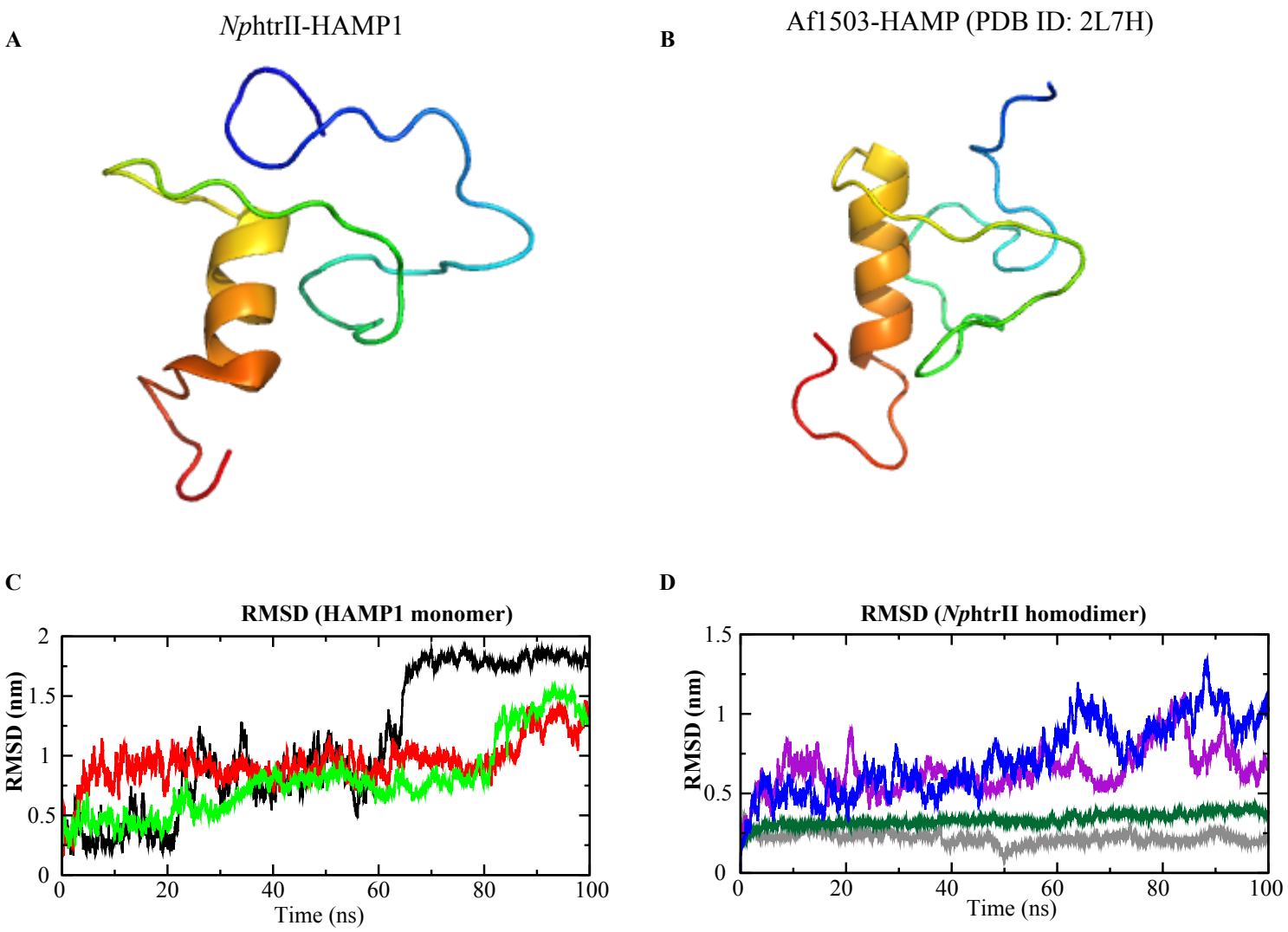

Fig. S1 Illustration of the simulated conformation of the HAMP monomer in (A) NphtrII HMAP model and (B) Af1503 solution NMR conformation after 100 ns MD simulation. Stability parameters for HAMP1 monomer homodimer during 100 ns time period. (C) Graphs show unstable RMSD values for HAMP1 monomer in different force fields, (D) RMSD graphs for HAMP1 dimer (84–136 and 23–159) in different temperature and salt environment. The black, red and green RMSD graphs are obtained for the HAMP1 monomer in amber99sb-ildn, gromos96 53a6 and opls-aa/l force fields, respectively. The grey, green, pink and blue graphs represents the RMSD graphs for the NphtrII homodier in 323K/1.15M (aa84-136), 283K/0.15M (aa84-136), 323K/1.15M (aa23-159) and 283K/0.15M (aa23-159), respectively.

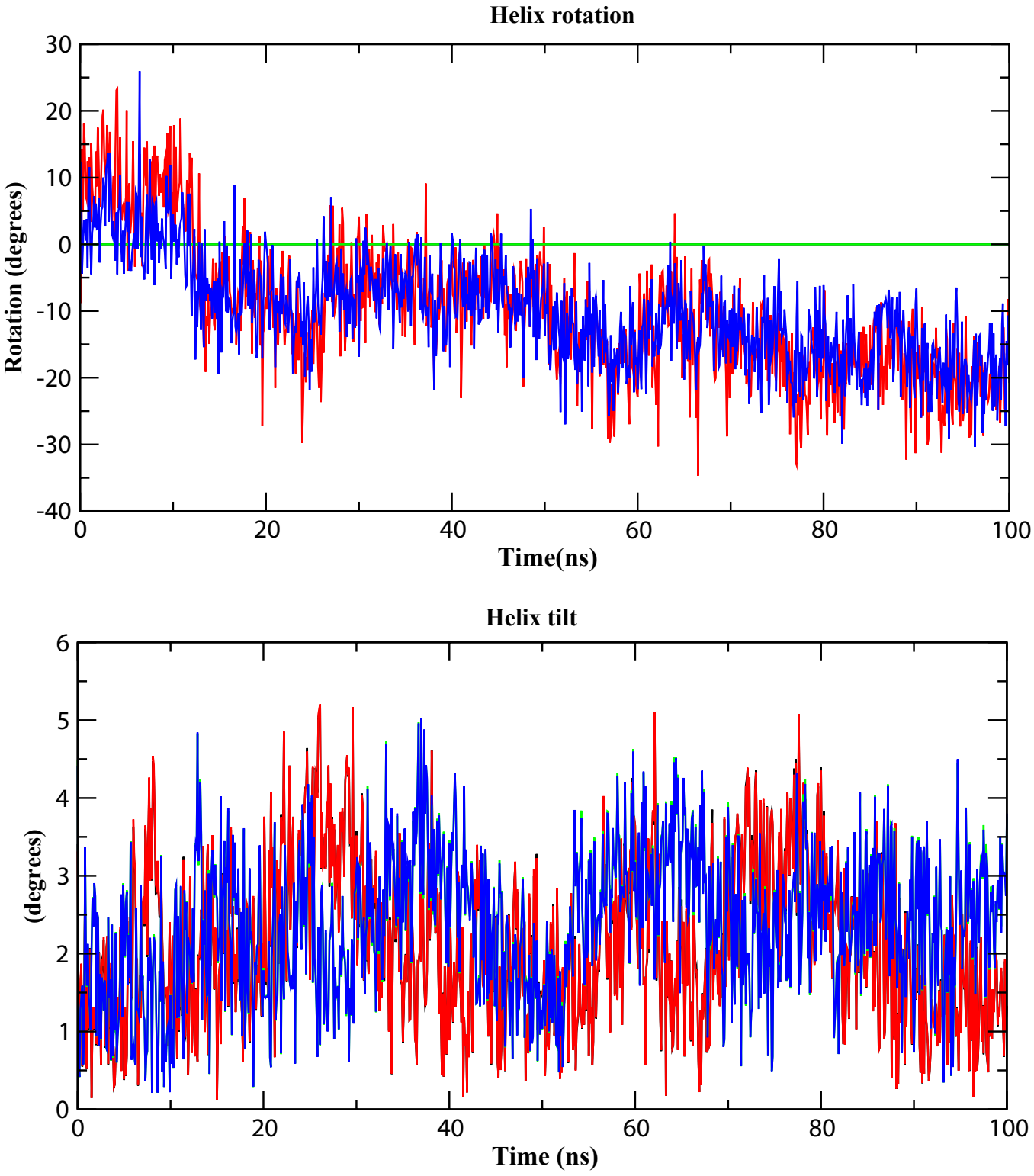

Fig. S2 *Np*htrII-TM2 helix rotation and tilt in the signaling state conformation. Blue and red graphs represents the TM2 helices of both *Np*htrII molecules in the 2:2 complex.

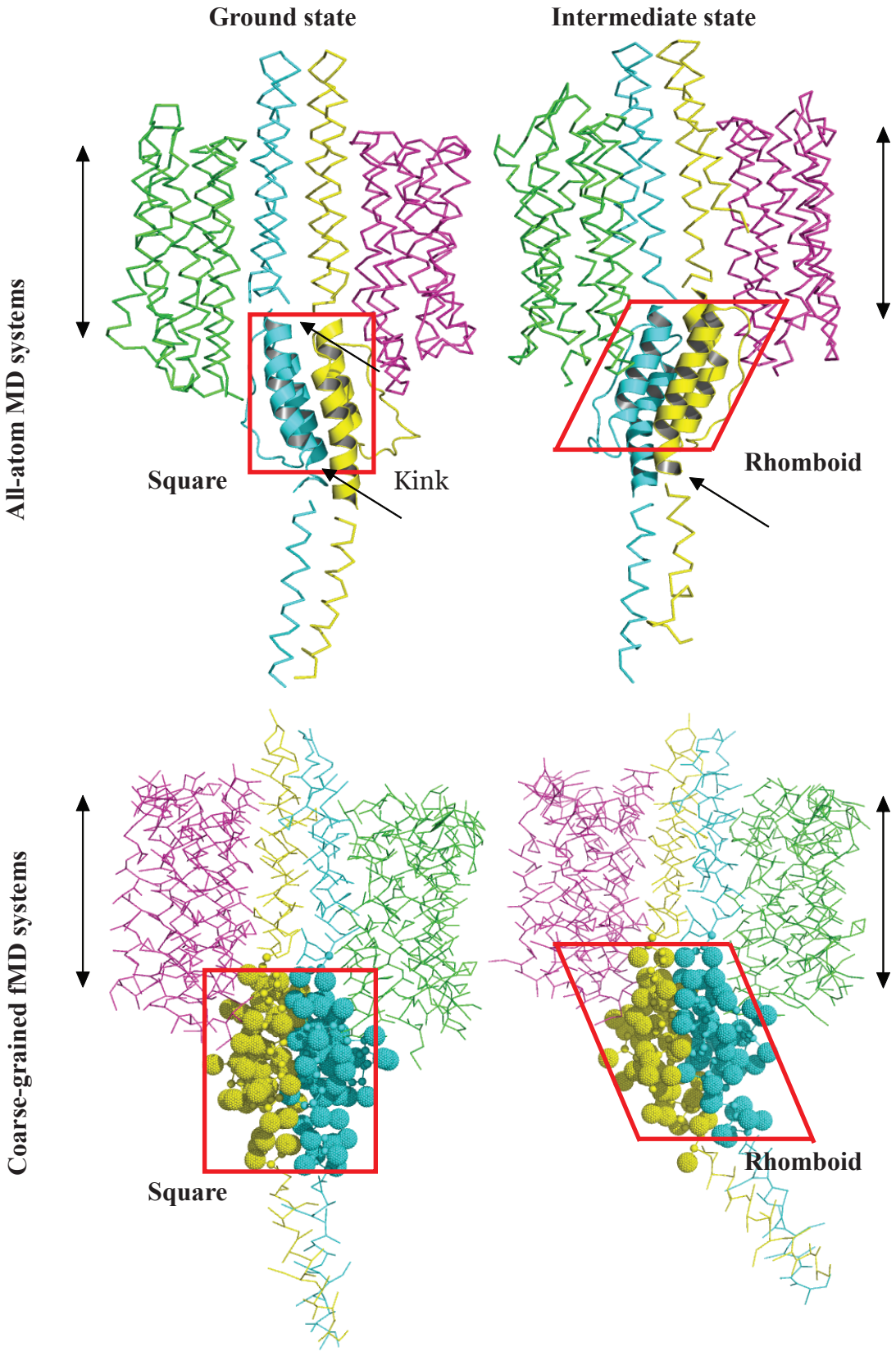

Fig. S3 HAMP1 conformational states and helical arrangements in all-atom and CG-MD simulation. The rectangular and rhomboid like arrangements in HAMP four-bundle helices in the ground and intermediate state, respectively, are shown from the final MD snapshots. Cartoon and VWD representation of HAMP helices are shown in VMD for all-atom and CG-MD complexes, respectively. Other protein domains are shown as lines. Arrows show small helical kinks at serine and threonine residues. The membrane position is indicated by the double sided black arrows.

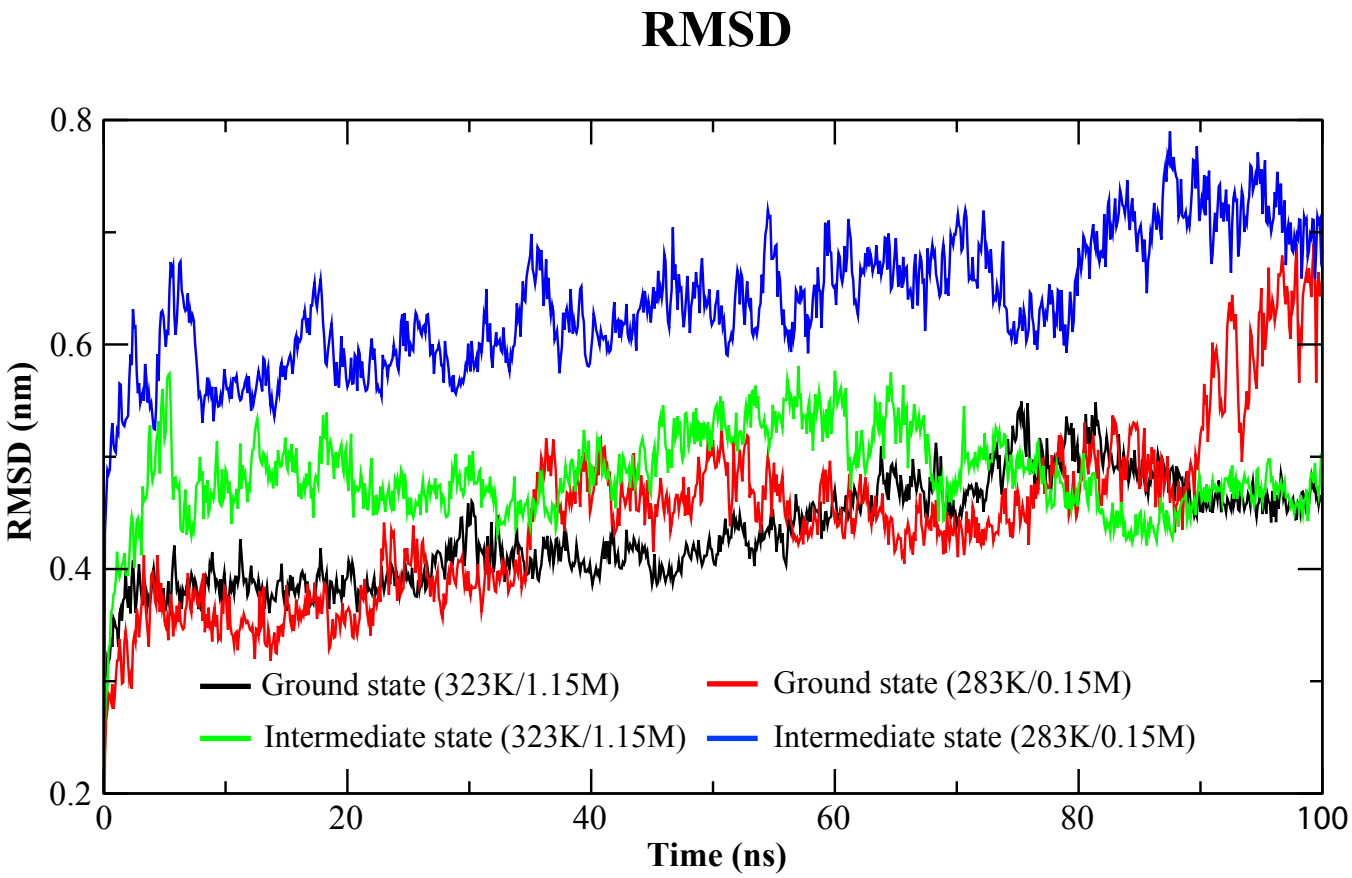

Fig. S4 Root mean square deviation interpretation of Cα-atoms in NpsrII-htrII complex (2:2) in ground and intermediate states derived from 100 ns MD simulation at different environmental conditions. The nonphysiological condition complex shows a relatively high and disorder backbone conformation.

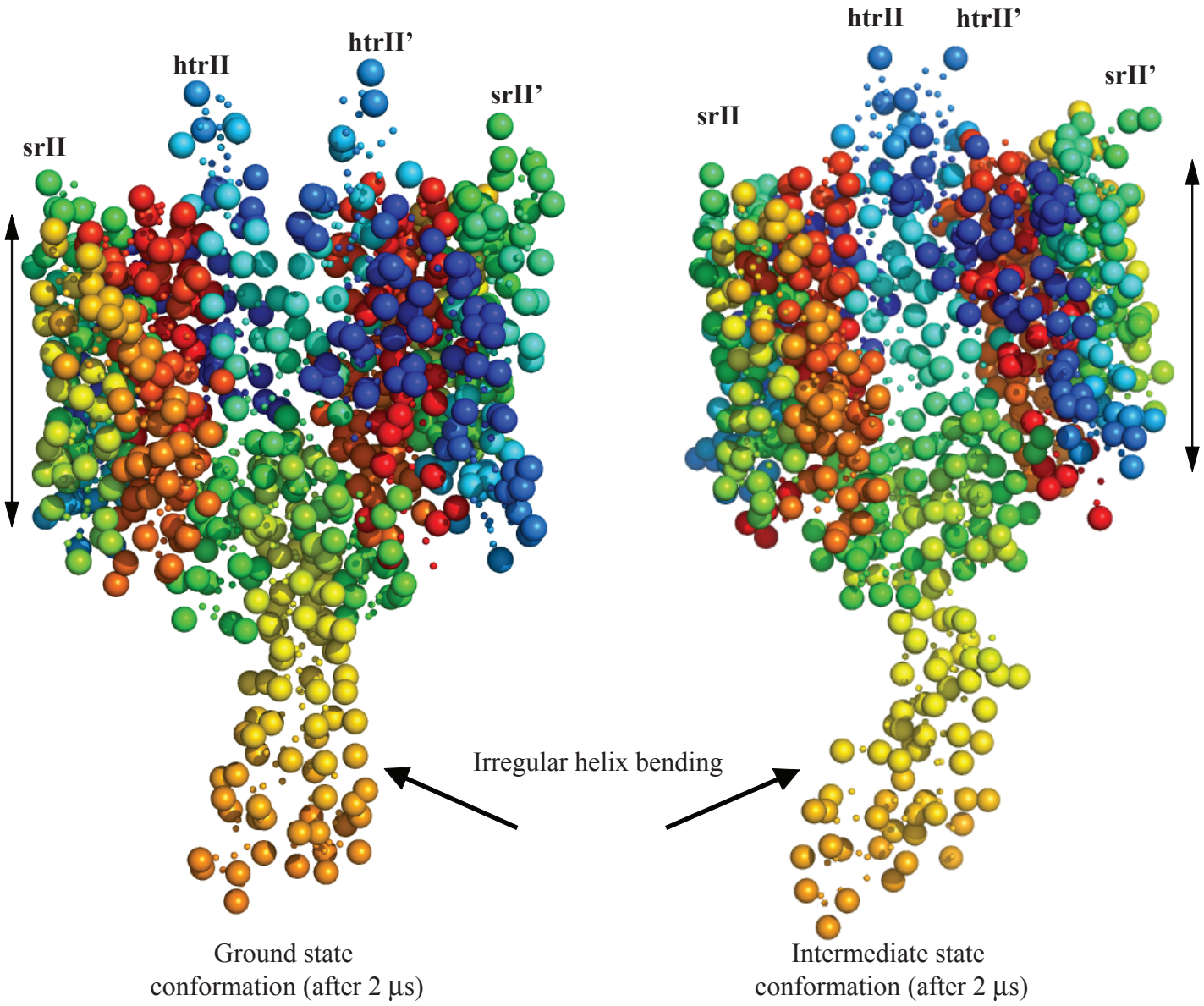

Figure S5. Coarse-grained models of srII-htrII (2:2) complex in nonphysiological environments. The snapshots are taken after 2 µs MD simulation and are shown in PyMOL. The protein molecule is shown as spheres and colored in rainbow spectrum. The large helical shifts and kinks are shown with arrows. The membrane position is indicated by the double sided black arrows.