Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supporting Information

Cholesterol Driven Alteration of the Conformation and Dynamics of Phospholamban in Model Membranes

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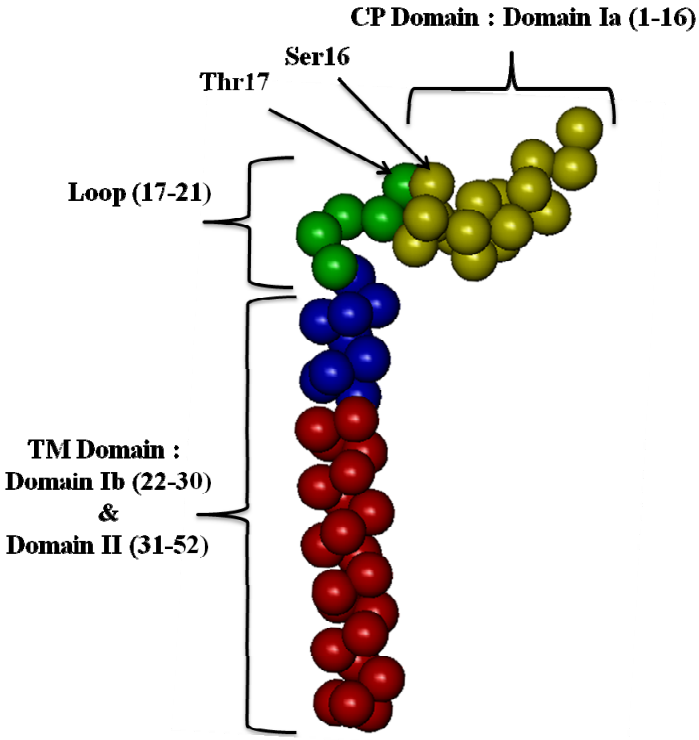

Fig. S1 The model of AFA-PLB (PDB code 1N7L), showing its four dynamic domains, highlighted as: domain Ia (residues 1-16) in yellow, loop (residues 17-21) in green, domain Ib (residues 22-30) in blue and domain II (residues 31-52) in red. PLB has three distinct structural domains; CP-helix and TM-helix connected by the loop region.

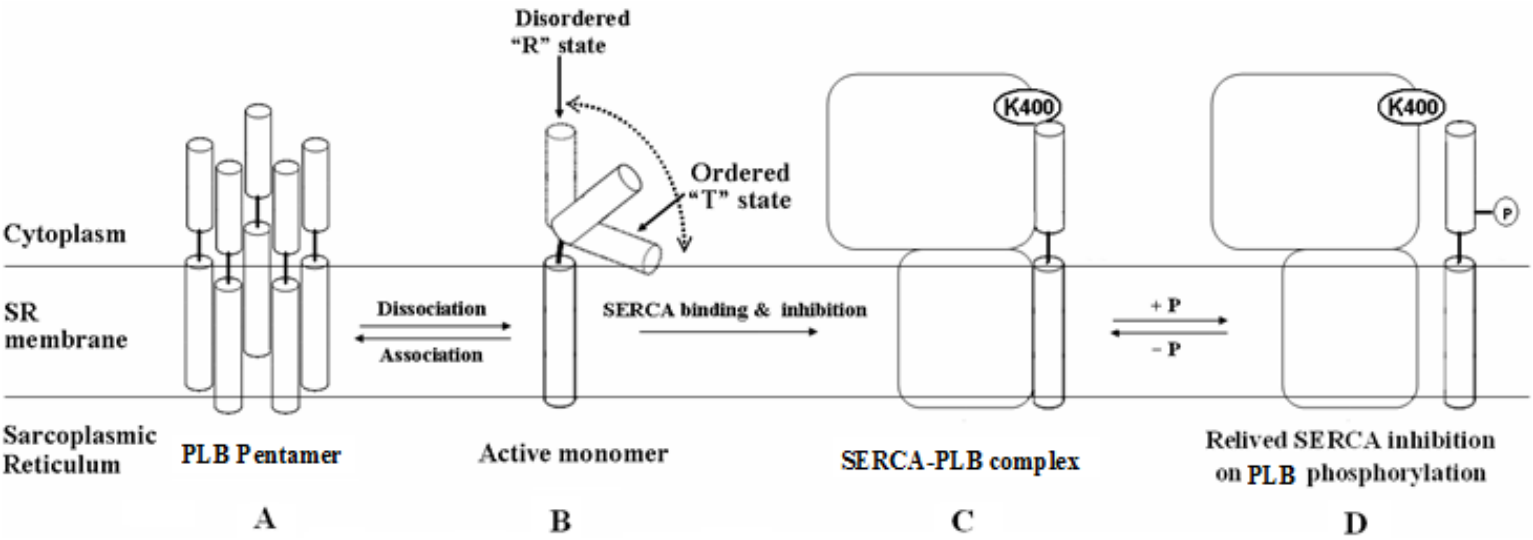

Fig. S2 A schematic representation showing the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) regulatory mechanism of phospholamban (PLB). (A)-(B) Dynamic equilibrium between the storage homopentamer and active monomer. (B) Equilibrium between the different conformational states of PLB, depending on the orientation of N-terminal cytoplasmic domain of PLB. (C) Binding and inhibition of SERCA by the extended conformer of PLB. (D) Phosphorylation cause dissociation of pPLB form SERCA-PLB inhibitory complex and SERCA retrieves its Ca^{2+} transport activity.

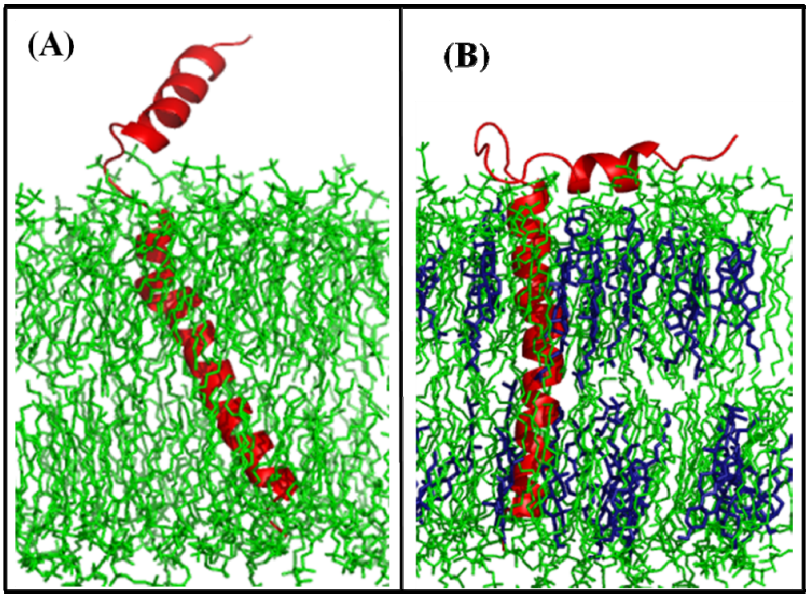

Fig. S3 Final snapshots of PLB (starting with extended conformer, Figure 1B of main text) (A) in pure POPC (cholesterol-free) bilayers and (B) in POPC bilayer containing 33.33 mol % of cholesterol. Phospholipids are shown as thin green lines, cholesterols as blue sticks and PLB as red cartoon.

Simulation with 50 mol% cholesterol (E50)

The cholesterol concentration reminiscent of biological membrane is typically about 10 – 30 mol%, although it may reach beyond the half of lipid concentration in specific membranes of higher vertebrates, e.g. it may reach 50 % in red blood cells and 70 % in the ocular lens.[1-4] Our additional simulation of PLB with 50 mol % of Chol together with our previous simulations, effectively cover the entire range Chol concentration: from 0 to 50 mol % in POPC bilayer and provide a more comparative and detailed study of effect of Chol on PLB conformational dynamics.

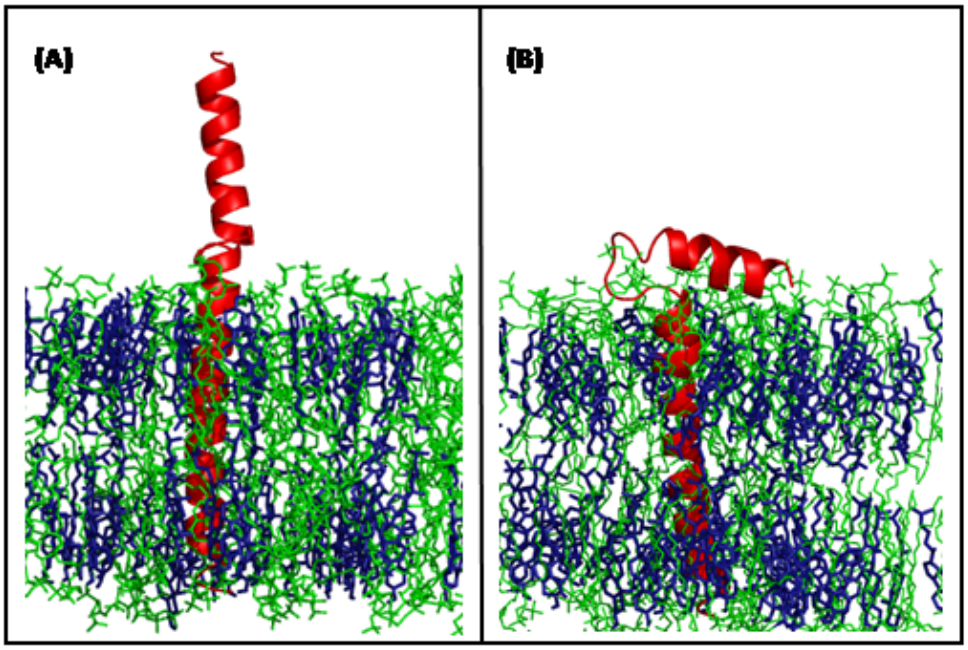

Fig. S4 (A) Initial (extended conformer as starting structure) and (B) final snapshots of PLB in POPC bilayer containing 50 mol % of cholesterol. Phospholipids are shown as thin green lines, cholesterols as blue sticks and PLB as red cartoon.

Secondary structure of phospholamban

Table S1 Average (over entire simulation time span) helicity of phospholamban and its domains

Simulations* of PLB in different POPC/cholesterol bilayers	PLB helix (%)	N-terminal helix (%)	C-terminal helix (%)
Chol 0 mol%	83.23 ± 3.36	79.56 ± 9.62	92.43 ± 1.63
Chol 11.11 mol%	77.92 ± 2.31	78.22 ± 5.36	87.9 ± 2.42
Chol 22.03 mol%	72.66 ± 3.69	52.08 ± 7.58	91.18 ± 2.62
Chol 33.33 mol%	71.14 ± 4.8	69.85 ± 8.98	86.62 ± 4.9

*Starting form "L-shaped" PLB conformer

Salt bridges of phospholamban

Table S2 Salt bridges observed in different PLB systems

Negatively charged residues	Positively charged residues	Probability of salt bridge formation (%)			
		L0	L11	L22	L33
Glu2	Lys3	5.62	20.33	9	4.7
Glu2	Arg9	5.3	3.8	9.21	3.5
Glu19	**Arg13**	-	17.85	29.46	57.06
Glu19	Arg14	11.77	-	-	-
Glu19	Arg25	2.44	1.8	3.56	2.11

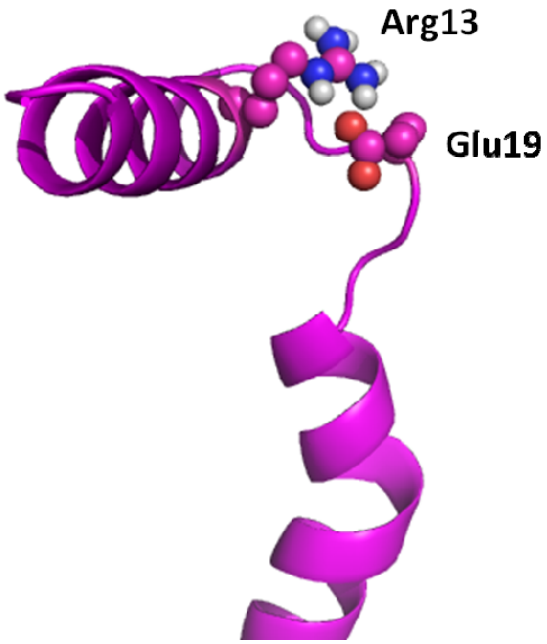

Fig. S5 Salt-bridge formed between Arg13 and Glu19 of PLB in L33 (containing 33.33 mol % of cholesterol) system.

Lipid-protein interactions

POPC-PLB hydrogen bonds

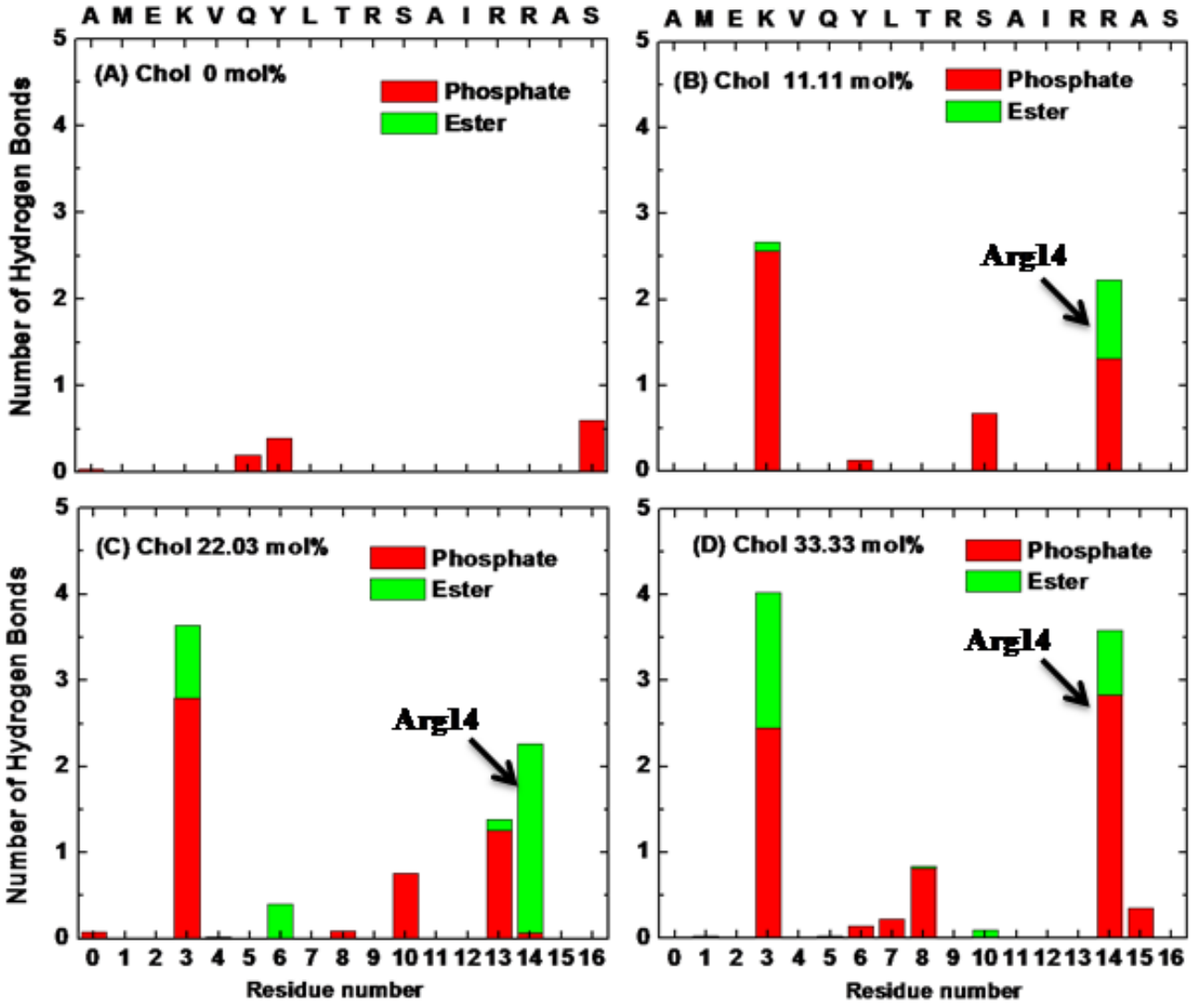

Fig. S6 Average number of hydrogen bonds formed between individual residues of CP-domain (residues 1-16) of PLB with POPC head group.

Cholesterol-PLB hydrogen bonds

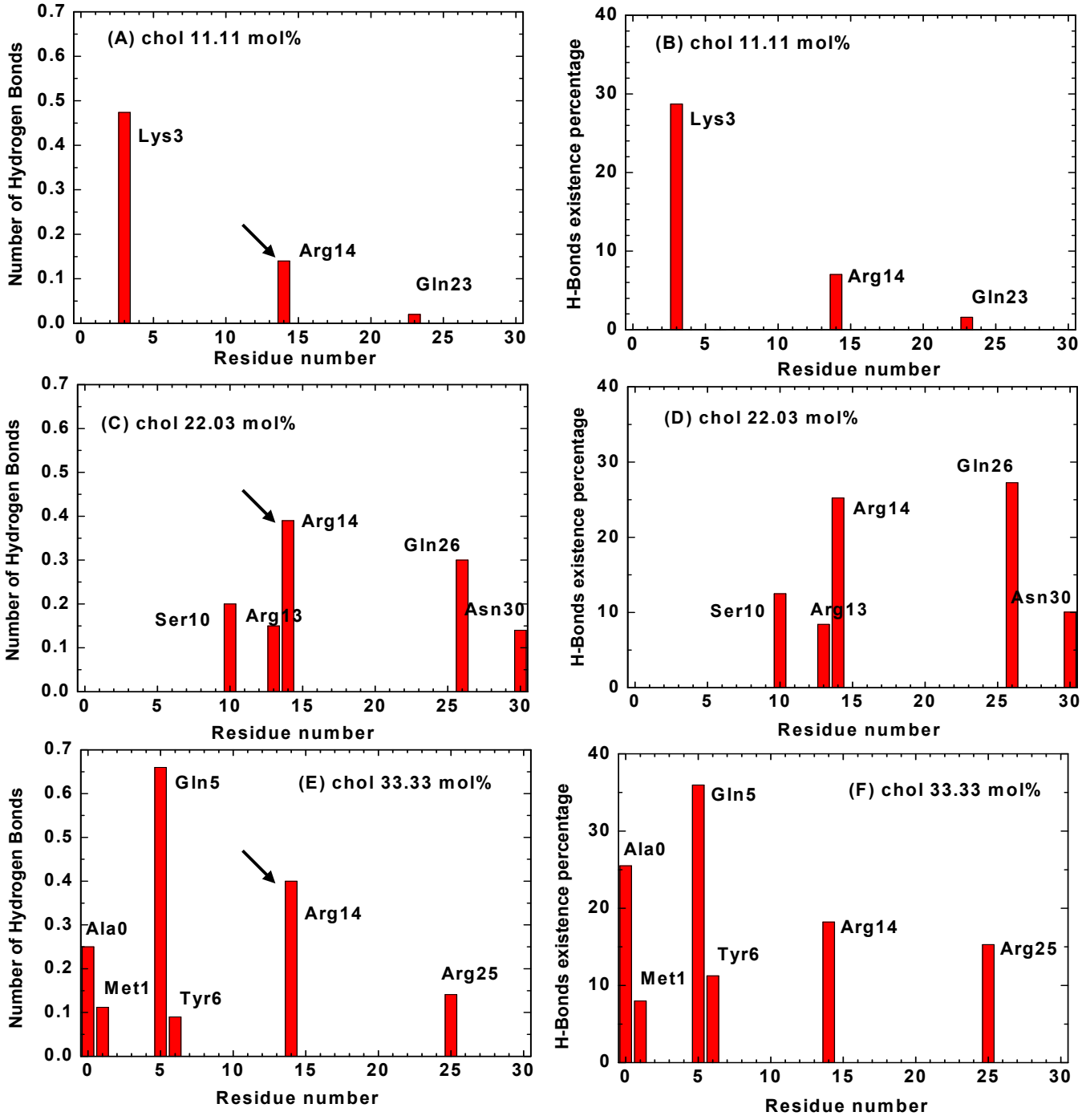

Fig. S7 Average number of hydrogen bonds (A,C,E) formed between PLB (residues 1-30) with hydroxyl group of cholesterol, along with their existence percentage (B,D,F).

Table S3 The average interaction energy between N-terminal helix of PLB and membrane

Cholesterol concentration (mol %)	Interaction energy (KJ/mol)
0	-649.14±31.53
11.11	-874.19±39.01
22.03	-988.08±32.85
33.33	-1102.69±33.26
50	-1198.58±56.82

Stronger interaction with increasing cholesterol concentration

Table S4 Lateral Diffusion Coefficients of Local POPC (within 10Å of PLB heavy atoms)

Systems	Cholesterol concentration (mol %)	Lateral Diffusion Coefficients (10^{-8} cm^2/sec)
L11	11.11	4.011± 0.32
L22	22.03	2.94 ± 0.13
L33	33.33	2.25 ± 0.21

The lateral diffusion coefficients of the local POPC molecules are smaller as compared to the overall lateral diffusion coefficient values of all POPC present in the system (Fig. 9, inset plot, main text).

References

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