**SUPPORTING INFORMATION**

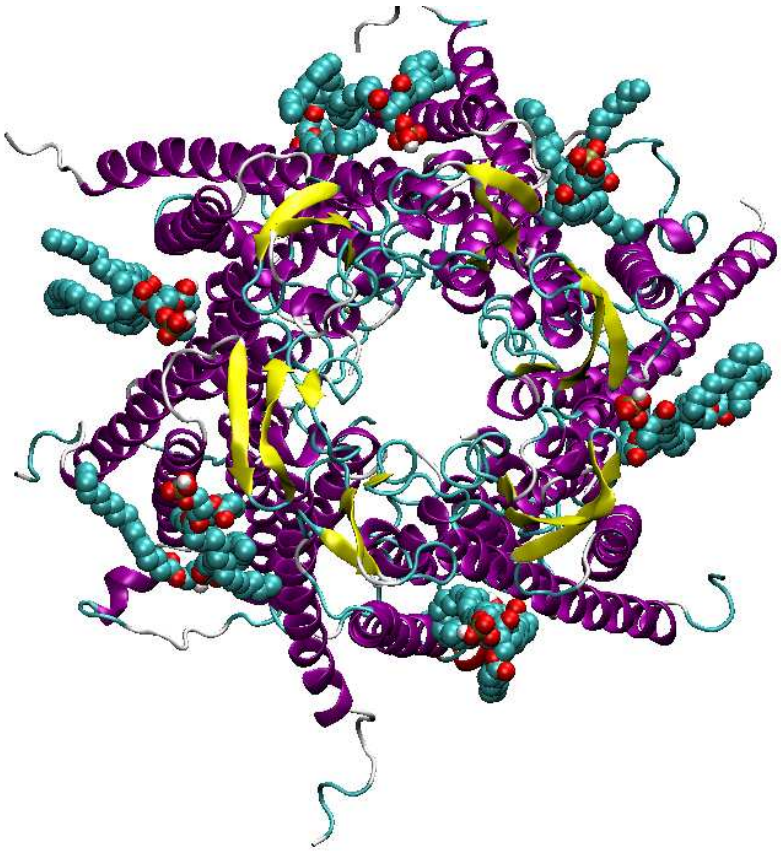
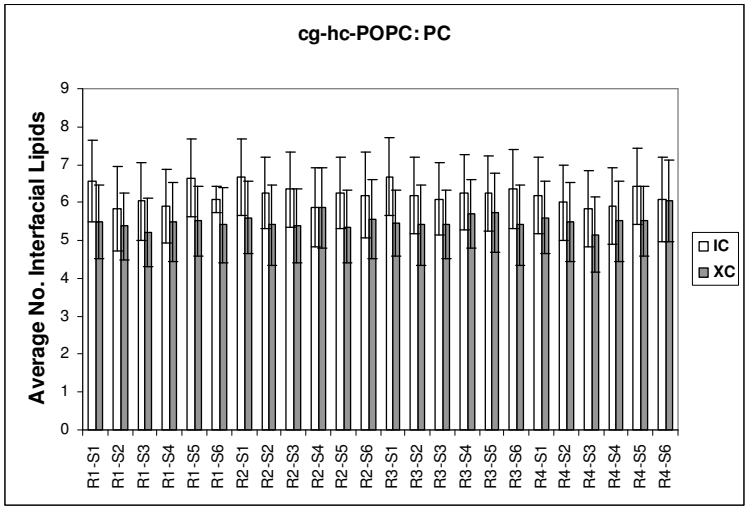
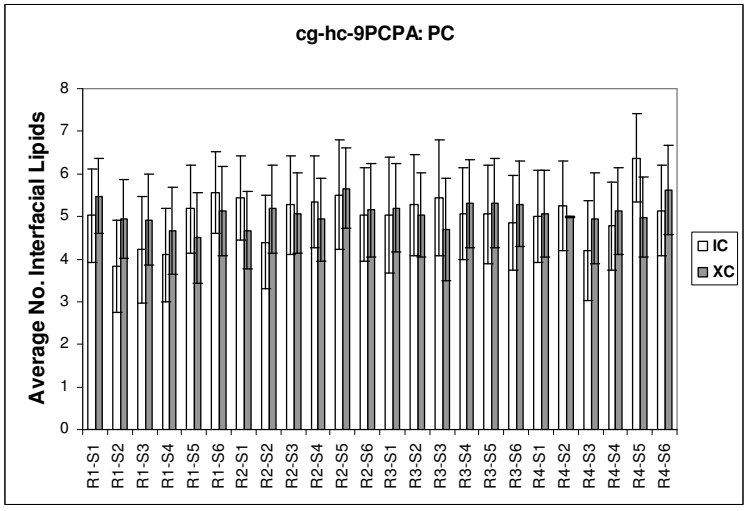


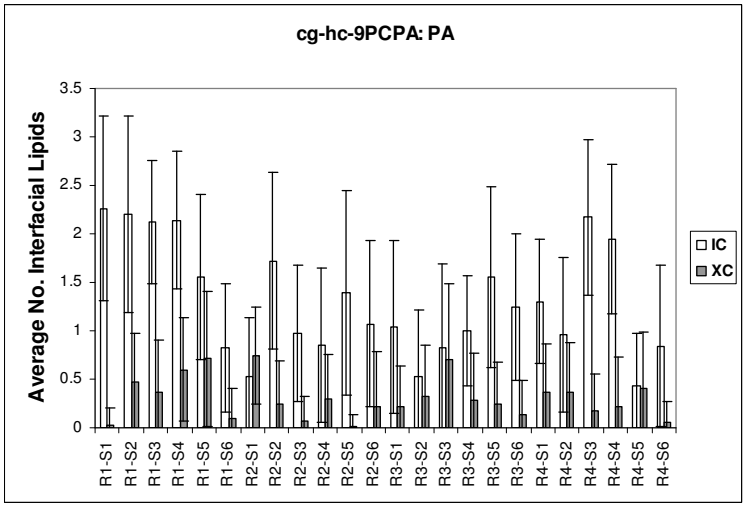
**Figure S1.** Graphical illustration of the initial structure of the atomistic mixed-lipid simulation **aa-hc-PCPA**, indicating POPA placement. The hemichannel is shown in ribbon representation. POPA lipids are shown as van der Waals' spheres.


cg-hc-POPC: PC
Average No. Interfacial Lipids
0
1
2
3
4
5
6
7
8
9
R1-S1
R1-S2
R1-S3
R1-S4
R1-S5
R1-S6
R2-S1
R2-S2
R2-S3
R2-S4
R2-S5
R2-S6
R3-S1
R3-S2
R3-S3
R3-S4
R3-S5
R3-S6
R4-S1
R4-S2
R4-S3
R4-S4
R4-S5
R4-S6
IC
XC

A)

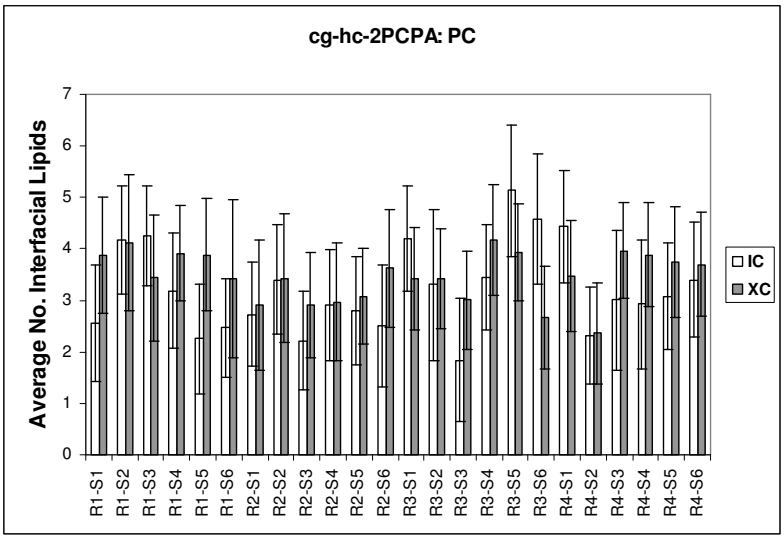

cg-hc-9PCPA: PC
Average No. Interfacial Lipids
0
1
2
3
4
5
6
7
8
R1-S1
R1-S2
R1-S3
R1-S4
R1-S5
R1-S6
R2-S1
R2-S2
R2-S3
R2-S4
R2-S5
R2-S6
R3-S1
R3-S2
R3-S3
R3-S4
R3-S5
R3-S6
R4-S1
R4-S2
R4-S3
R4-S4
R4-S5
R4-S6
IC
XC

B)

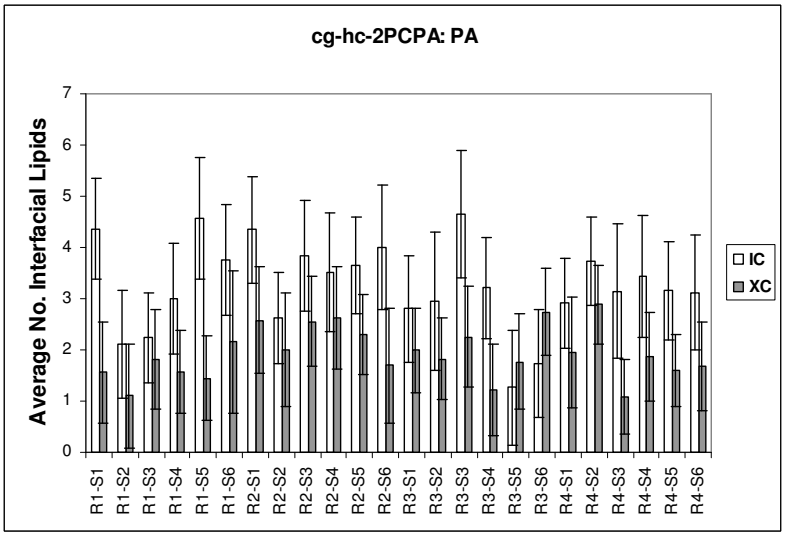

cg-hc-9PCPA: PA
Average No. Interfacial Lipids
0
0.5
1
1.5
2
2.5
3
3.5
R1-S1
R1-S2
R1-S3
R1-S4
R1-S5
R1-S6
R2-S1
R2-S2
R2-S3
R2-S4
R2-S5
R2-S6
R3-S1
R3-S2
R3-S3
R3-S4
R3-S5
R3-S6
R4-S1
R4-S2
R4-S3
R4-S4
R4-S5
R4-S6
IC
XC

C)

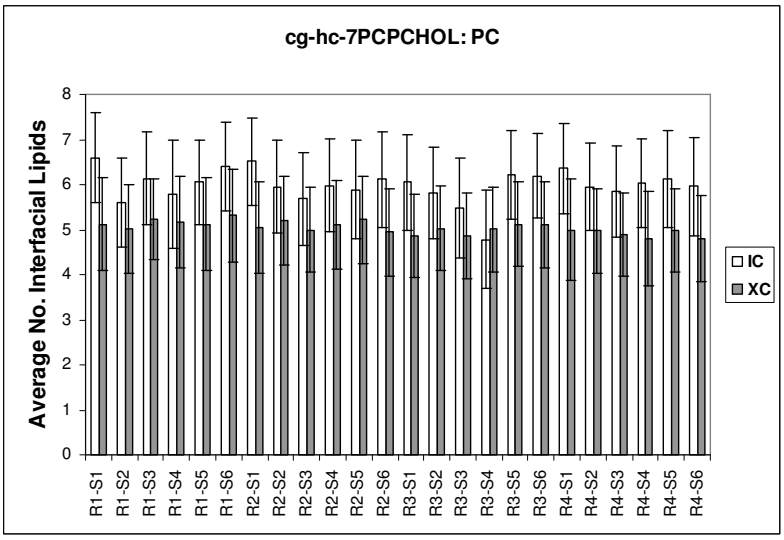
D)


cg-hc-2PCPA: PC
Average No. Interfacial Lipids
0
1
2
3
4
5
6
7
R1-S1
R1-S2
R1-S3
R1-S4
R1-S5
R1-S6
R2-S1
R2-S2
R2-S3
R2-S4
R2-S5
R2-S6
R3-S1
R3-S2
R3-S3
R3-S4
R3-S5
R3-S6
R4-S1
R4-S2
R4-S3
R4-S4
R4-S5
R4-S6
IC
XC

E)


cg-hc-2PCPA: PA
Average No. Interfacial Lipids
0
1
2
3
4
5
6
7
R1-S1
R1-S2
R1-S3
R1-S4
R1-S5
R1-S6
R2-S1
R2-S2
R2-S3
R2-S4
R2-S5
R2-S6
R3-S1
R3-S2
R3-S3
R3-S4
R3-S5
R3-S6
R4-S1
R4-S2
R4-S3
R4-S4
R4-S5
R4-S6
IC
XC

F)


cg-hc-7PCPCHOL: PC
Average No. Interfacial Lipids
0
1
2
3
4
5
6
7
8
R1-S1
R1-S2
R1-S3
R1-S4
R1-S5
R1-S6
R2-S1
R2-S2
R2-S3
R2-S4
R2-S5
R2-S6
R3-S1
R3-S2
R3-S3
R3-S4
R3-S5
R3-S6
R4-S1
R4-S2
R4-S3
R4-S4
R4-S5
R4-S6
IC
XC

G)

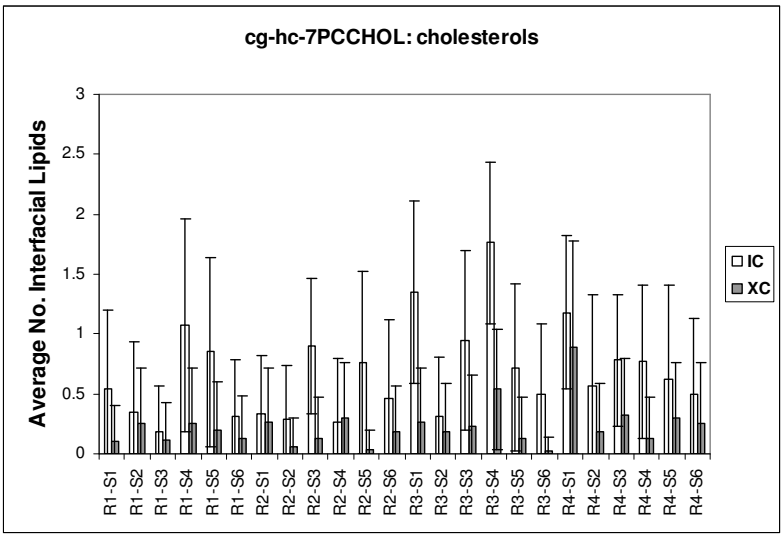
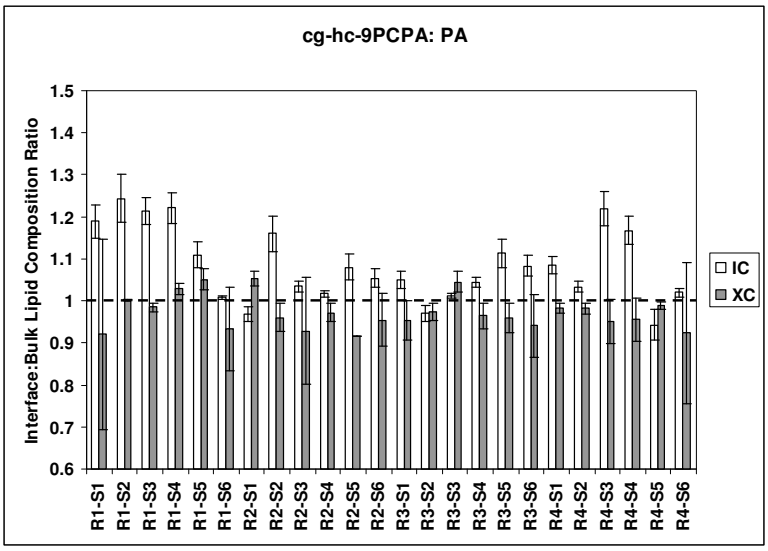
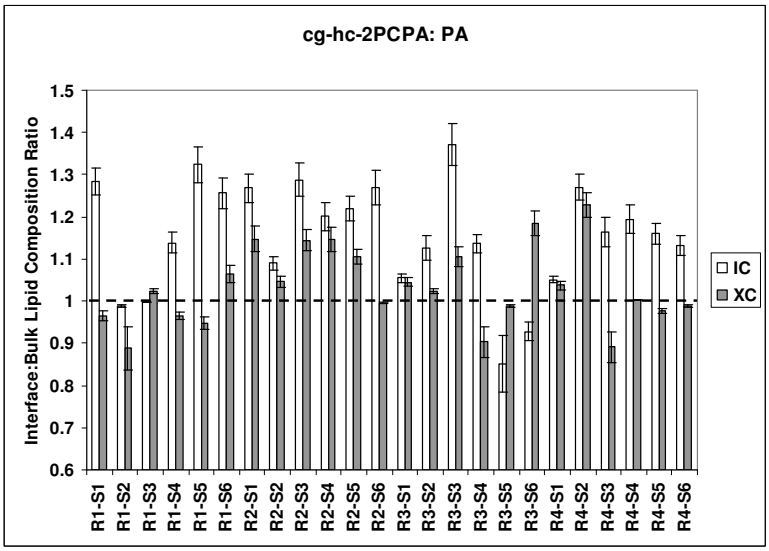


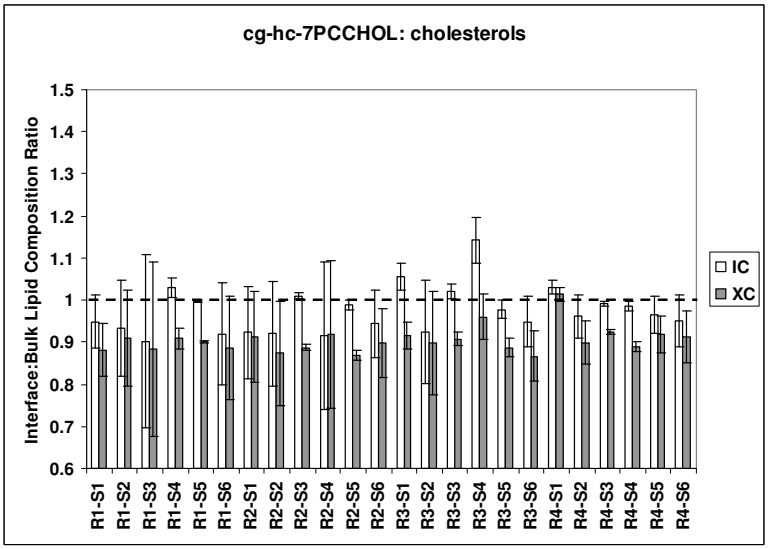

**Figure S2(A-G).** Bar charts of average numbers of extra- and intra-cellular interfacial lipids around each of the 6 subunits of the protein for all four replicate CG simulations of each system over the final 3200 ns. Each bar is labelled with simulation replicate and subunit ID (eg. R1S1 = replicate 1, subunit 1). Error bars indicate standard deviations calculated over each trajectory. .




A)

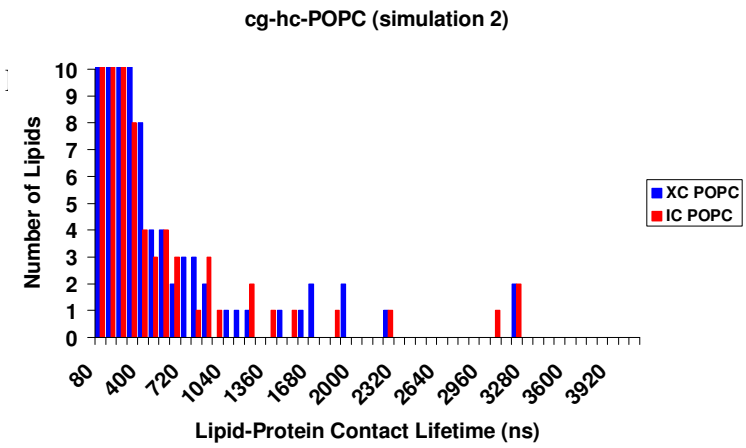


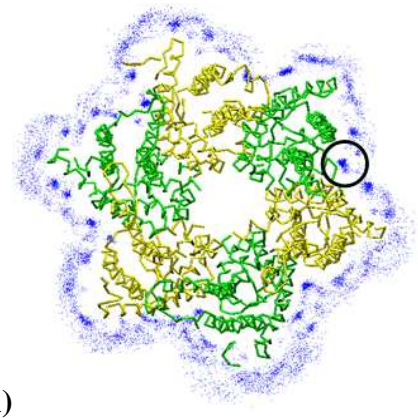

B)



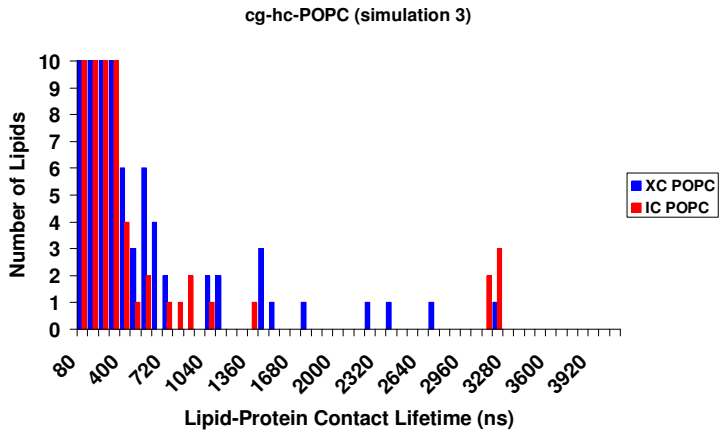

C)

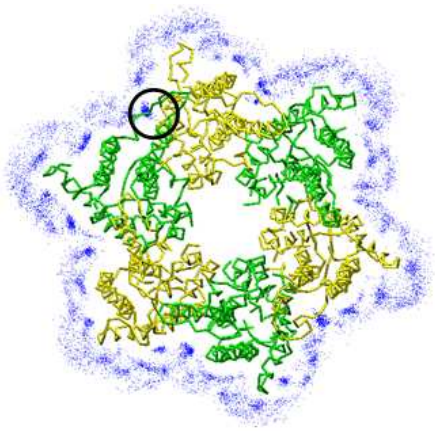
**Figure S3(A-C).** Bar charts of interface:bulk lipid composition ratio for POPA for each subunit and replicate simulation of **A) cg-hc-9PCPA** and **B) cg-hc-2PCPA**; and for cholesterol in **C) cg-hc-7PCCHOL**. Each bar is labelled as described in Fig. S2 caption.


cg-hc-POPC (simulation 2)
Number of Lipids
0
1
2
3
4
5
6
7
8
9
10
80
400
720
1040
1360
1680
2000
2320
2640
2960
3280
3600
3920
Lipid-Protein Contact Lifetime (ns)
XC POPC
IC POPC

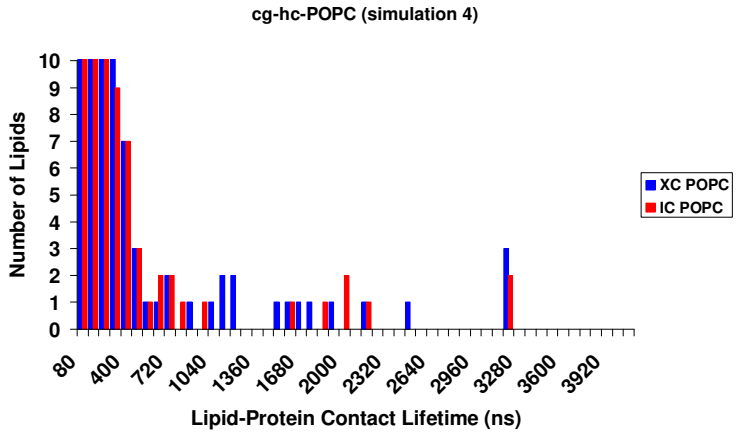


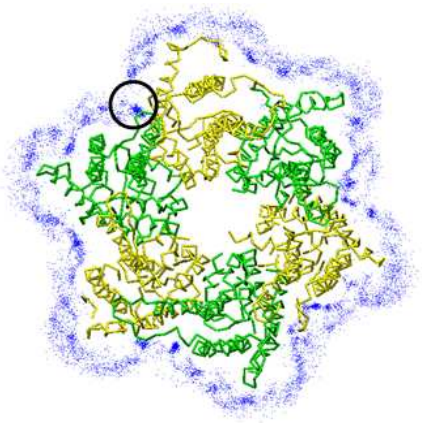
**A)**


cg-hc-POPC (simulation 3)
Number of Lipids
0
1
2
3
4
5
6
7
8
9
10
80
400
720
1040
1360
1680
2000
2320
2640
2960
3280
3600
3920
Lipid-Protein Contact Lifetime (ns)
XC POPC
IC POPC



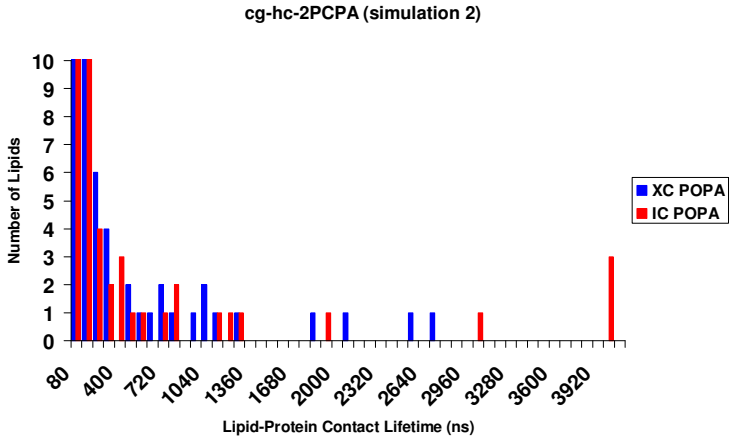
**B)**

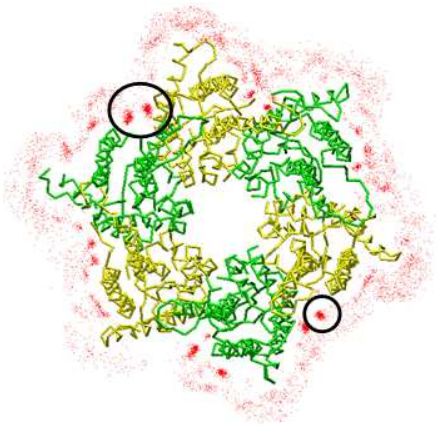


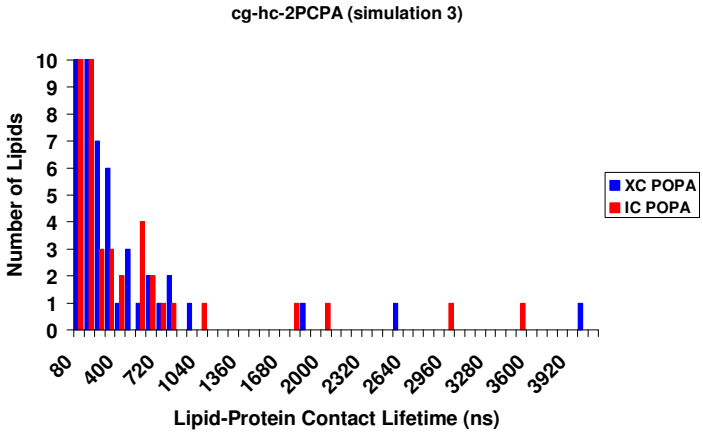
**C)**

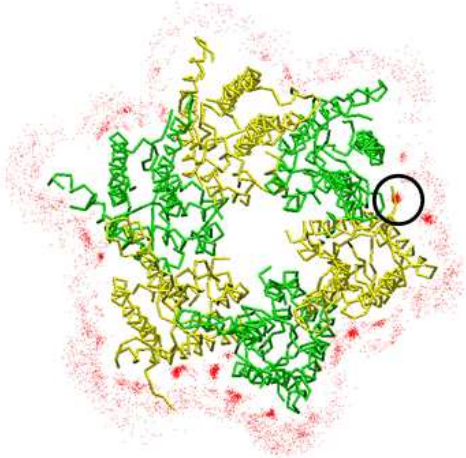
**Figure S4:** Histograms of lipid-protein contact lifetimes calculated over the entire course of each simulation trajectory for the **cg-hc-POPC** replicate simulations 2 (A) to 4 (C) The y-axes are truncated at 10 to ensure visibility of the bars for high lifetime lipids. IC dot plots with longest lifetime lipids circled are shown beneath each histogram.


cg-hc-2PCPA (simulation 2)
Number of Lipids
Lipid-Protein Contact Lifetime (ns)
XC POPA
IC POPA
0
1
2
3
4
5
6
7
8
9
10
80
400
720
1040
1360
1680
2000
2320
2640
2960
3280
3600
3920

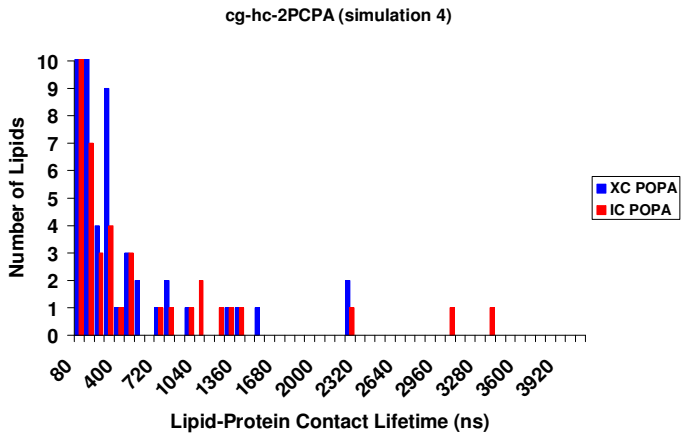


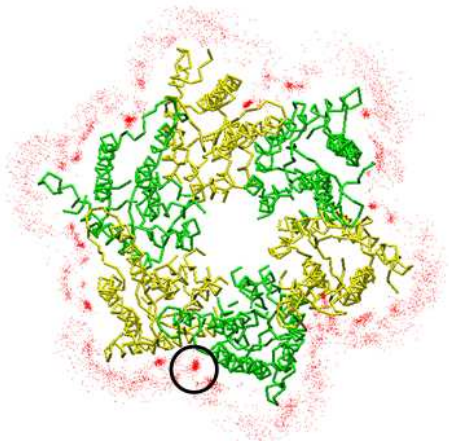
**A)**


cg-hc-2PCPA (simulation 3)
Number of Lipids
Lipid-Protein Contact Lifetime (ns)
XC POPA
IC POPA
0
1
2
3
4
5
6
7
8
9
10
80
400
720
1040
1360
1680
2000
2320
2640
2960
3280
3600
3920



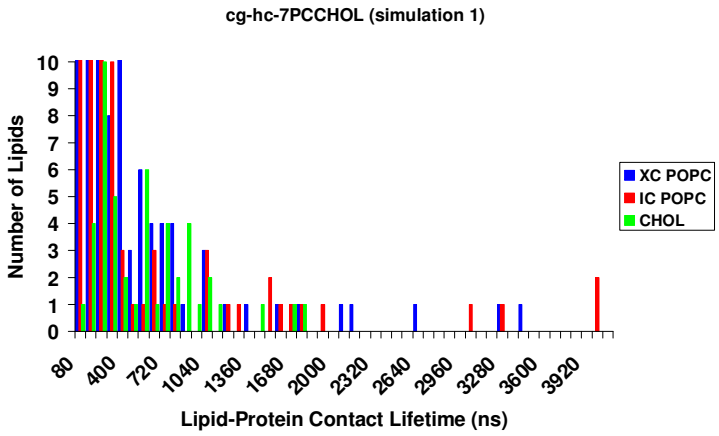
**B)**



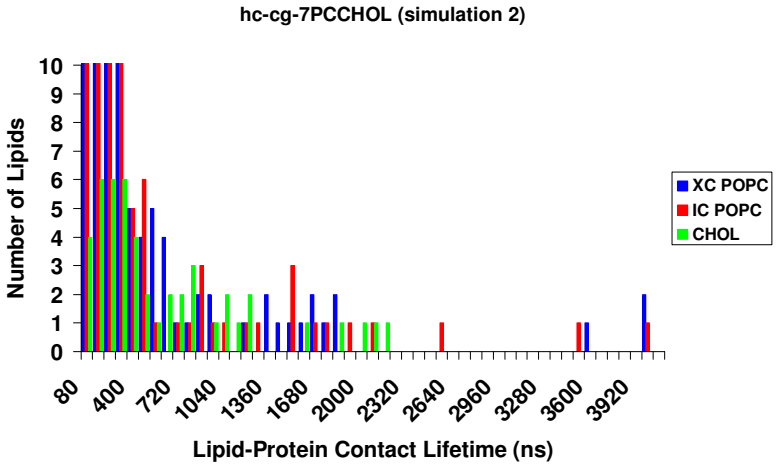



**C)**

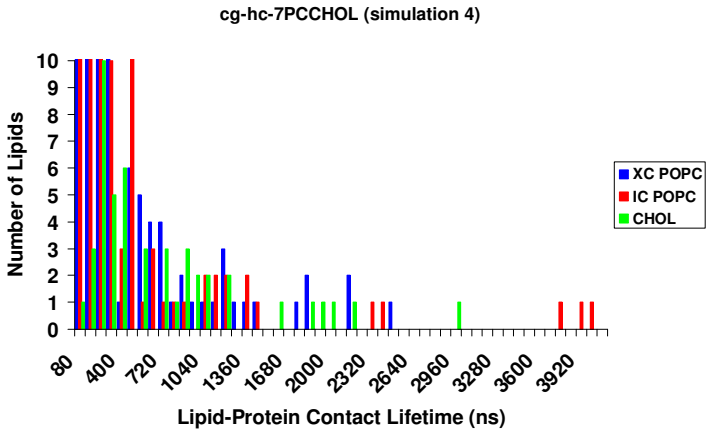
**Figure S5:** Histograms of lipid-protein contact lifetimes calculated over the entire course of each simulation trajectory for the **cg-hc-2PCPA** replicate simulations 2 (A) to 4 (C). IC dot plots with longest lifetime POPA circled are shown beneath each histogram.




A)

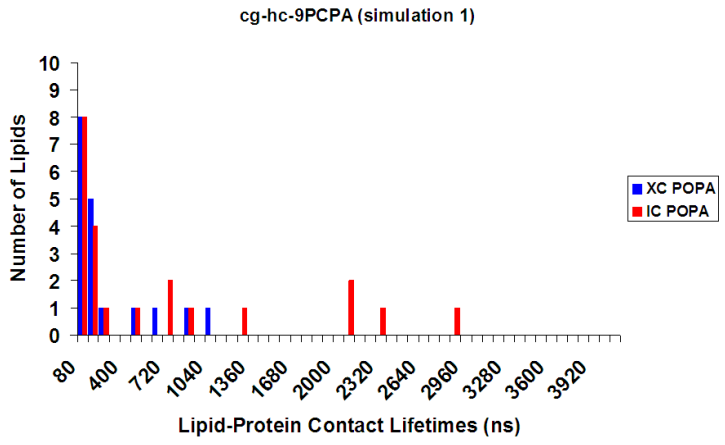



B)

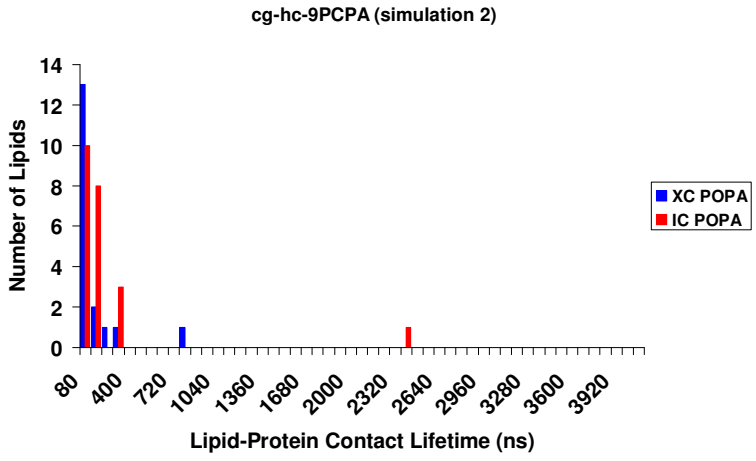



C)

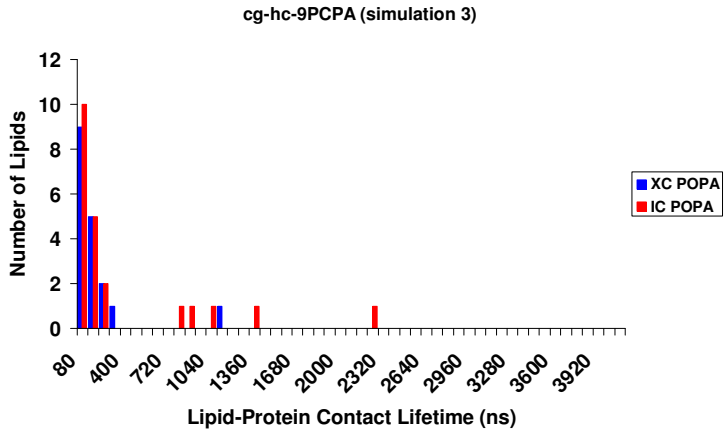
**Figure S6:** Histograms of lipid-protein contact lifetimes calculated over the entire course of each simulation trajectory for the **cg-hc-7PCCHOL** replicate simulations 1 (A), 2 (B) and 4 (C).


cg-hc-9PCPA (simulation 1)
Number of Lipids
0
1
2
3
4
5
6
7
8
9
10
80
400
720
1040
1360
1680
2000
2320
2640
2960
3280
3600
3920
Lipid-Protein Contact Lifetimes (ns)
XC POPA
IC POPA

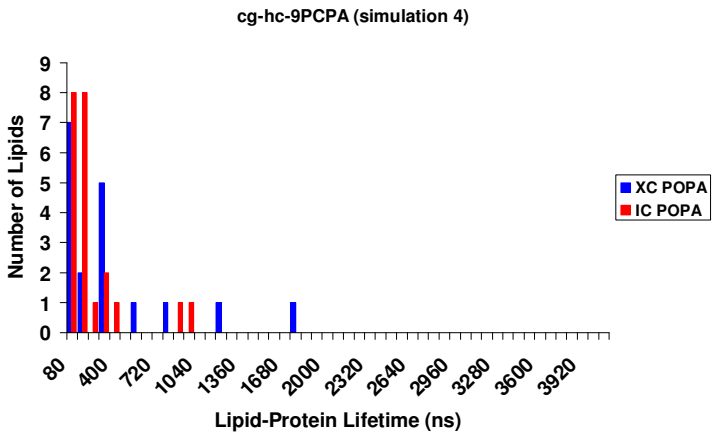
A)


cg-hc-9PCPA (simulation 2)
Number of Lipids
0
2
4
6
8
10
12
14
80
400
720
1040
1360
1680
2000
2320
2640
2960
3280
3600
3920
Lipid-Protein Contact Lifetime (ns)
XC POPA
IC POPA

B)

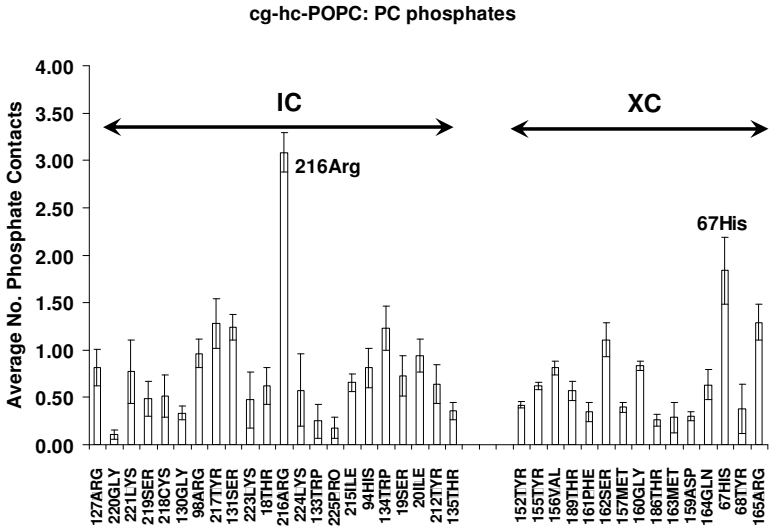

cg-hc-9PCPA (simulation 3)
Number of Lipids
0
2
4
6
8
10
12
80
400
720
1040
1360
1680
2000
2320
2640
2960
3280
3600
3920
Lipid-Protein Contact Lifetime (ns)
XC POPA
IC POPA

C)

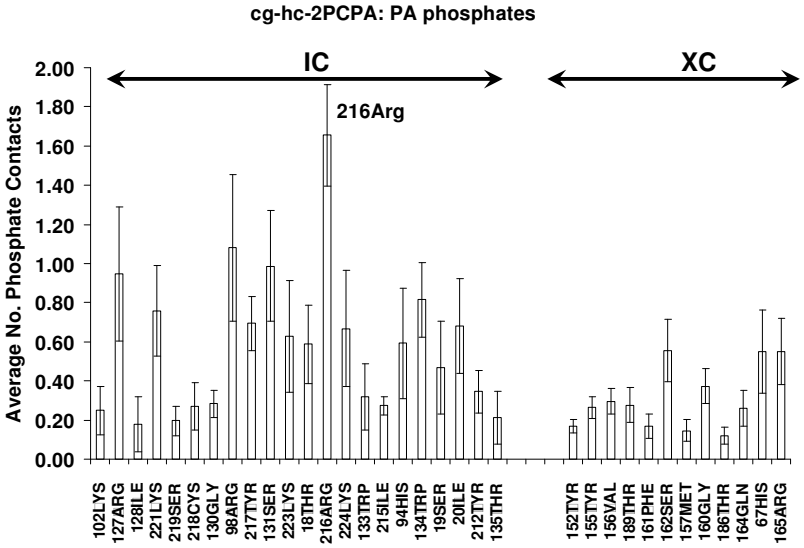



**D)**

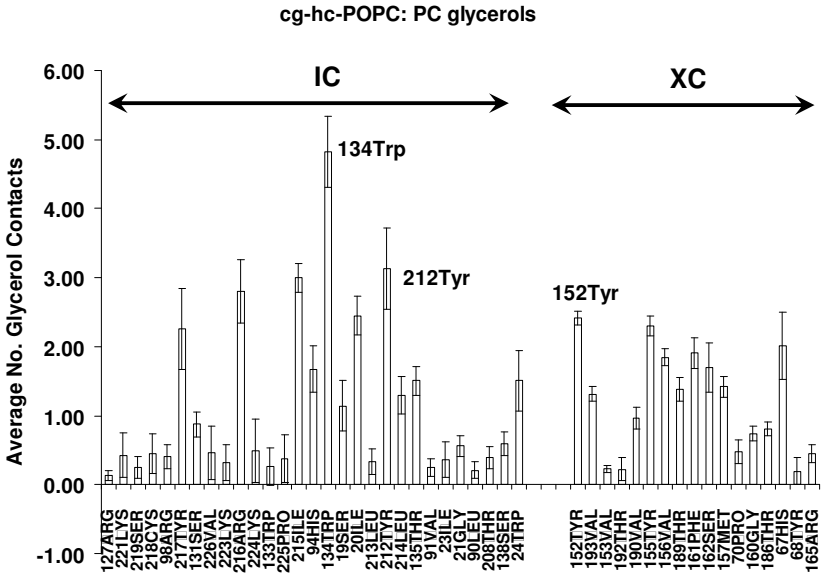
**Figure S7:** Histograms of lipid-protein contact lifetimes calculated over the entire course of each simulation trajectory for the **cg-hc-9PCPA** replicate simulations 1 (A) to 4 (D).


cg-hc-POPC: PC phosphates
Average No. Phosphate Contacts
IC
XC
216Arg
67His
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
127ARG
220GLY
221LYS
219SER
218CYS
130GLY
98ARG
217TYR
131SER
223LYS
18THR
216ARG
224LYS
133TRP
225PRO
215ILE
94HIS
134TRP
19SER
20ILE
212TYR
135THR
152TYR
155TYR
156VAL
189THR
161PHE
162SER
157MET
160GLY
186THR
163MET
159ASP
164GLN
67HIS
68TYR
165ARG

A)


cg-hc-2PCPA: PA phosphates
Average No. Phosphate Contacts
IC
XC
216Arg
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
102LYS
127ARG
128ILE
221LYS
219SER
218CYS
130GLY
98ARG
217TYR
131SER
223LYS
18THR
216ARG
224LYS
133TRP
215ILE
94HIS
134TRP
19SER
20ILE
212TYR
135THR
152TYR
155TYR
156VAL
189THR
161PHE
162SER
157MET
160GLY
186THR
164GLN
67HIS
165ARG

B)


cg-hc-POPC: PC glycerols
Average No. Glycerol Contacts
IC
XC
134Trp
212Tyr
152Tyr
6.00
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
127ARG
221LYS
219SER
218CYS
98ARG
217TYR
131SER
226VAL
223LYS
216ARG
224LYS
133TRP
225PRO
215ILE
94HIS
134TRP
19SER
20ILE
213LEU
212TYR
214LEU
135THR
91VAL
23ILE
21GLY
90LEU
208THR
138SER
24TRP
152TYR
193VAL
153VAL
192THR
190VAL
155TYR
156VAL
189THR
161PHE
162SER
157MET
70PRO
160GLY
186THR
67HIS
68TYR
165ARG

C)

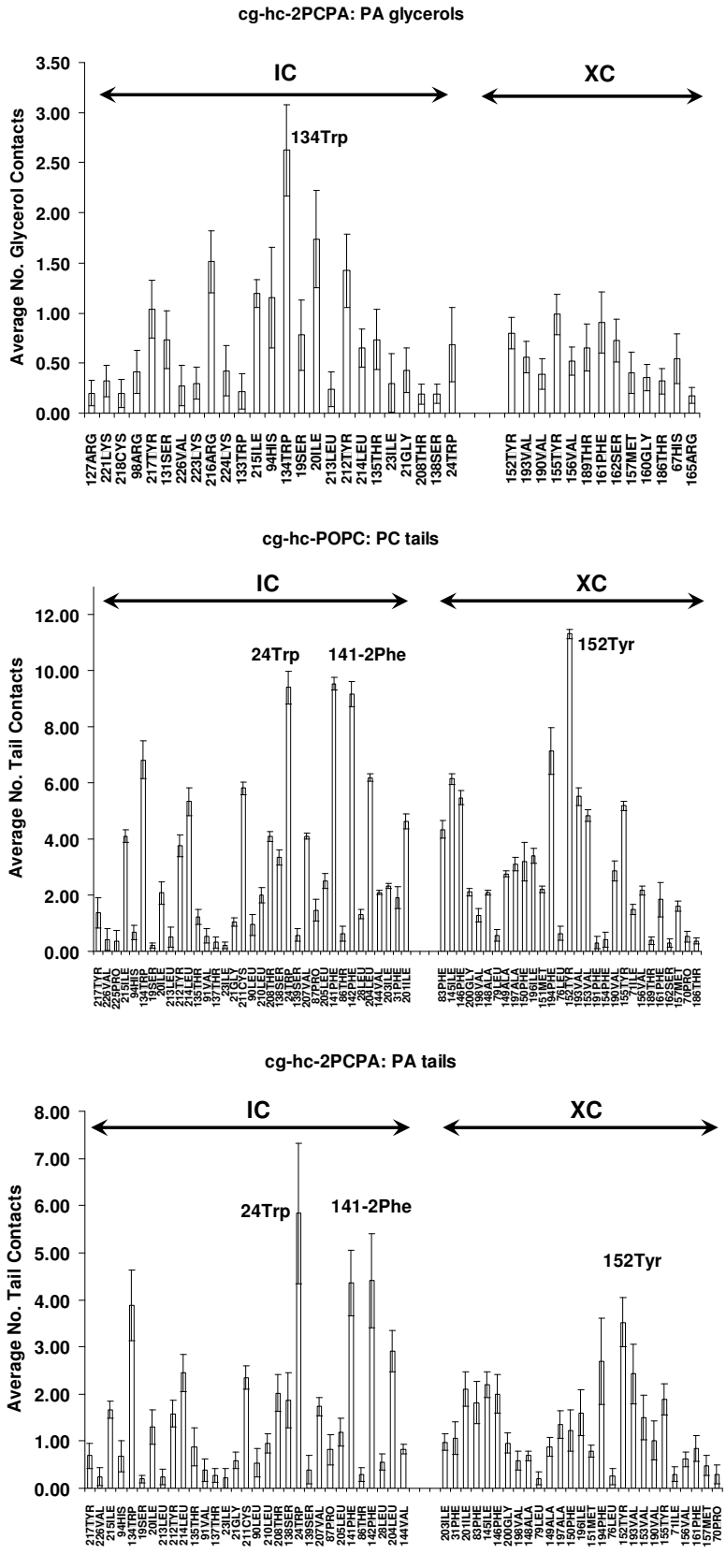
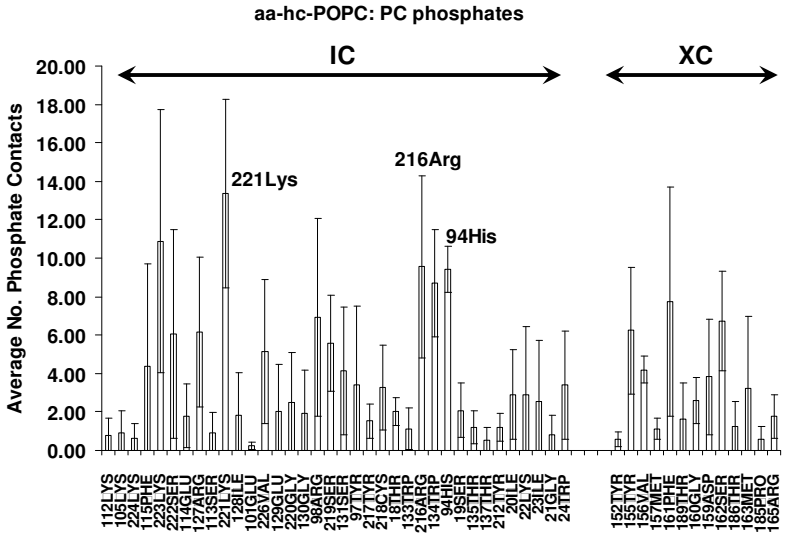
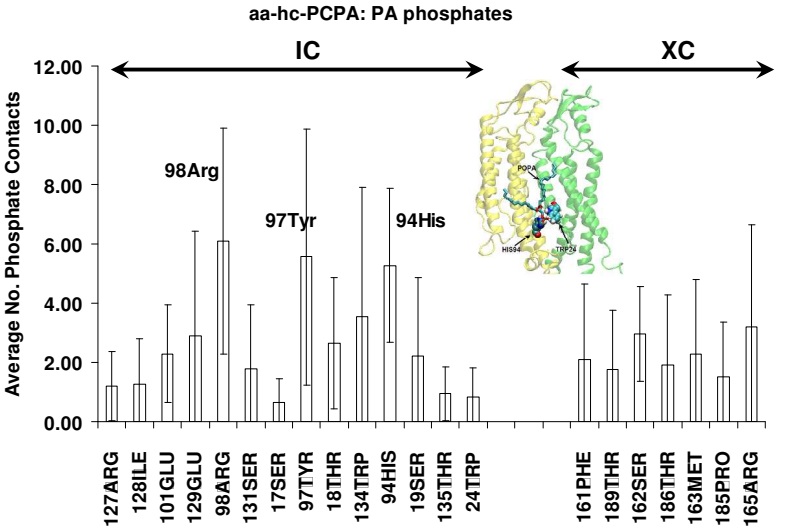


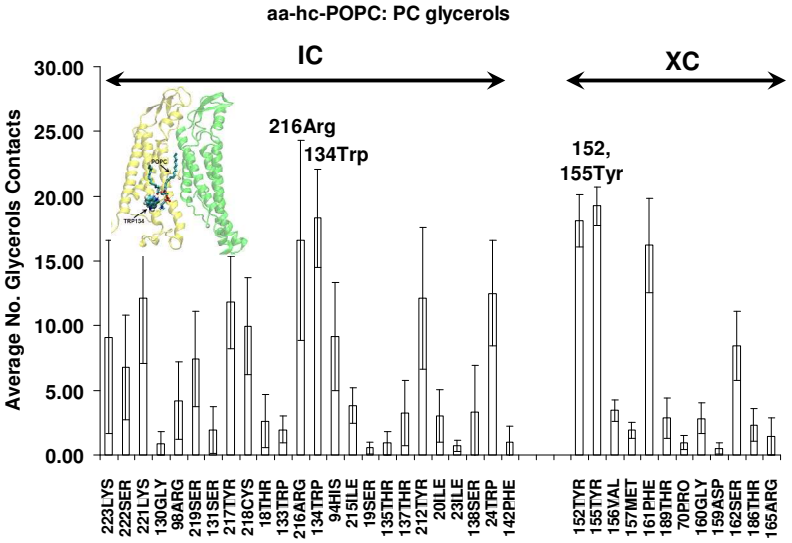

**Figure S8.** CG average numbers of contacts between lipid functional groups and Cx26 residues, for **A)** PC phosphates from simulation **cg-hc-POPC; B)** PA phosphates from simulation **cg-hc-2PCPA; C)** PC glycerols from **cg-hc-POPC; D)** PA glycerols from **cg-hc-2PCPA; E)** PC tails from **cg-hc-POPC**; and **F)** PA tails from **cg-hc-2PCPA**. Averages and standard deviations (error bars) for these charts are taken over the trajectory-averaged values (final 3200 ns) for six subunits in four replicate simulations.


aa-hc-POPC: PC phosphates
IC
XC
Average No. Phosphate Contacts
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
221Lys
216Arg
94His
112LYS
105LYS
224LYS
115PHE
223LYS
222SER
114GLU
127ARG
131SER
128ILE
101GLU
226VAL
129GLU
220GLY
130GLY
98ARG
219SER
131SER
97TYR
217TYR
218CYS
18THR
133TRP
216ARG
134TRP
94HIS
19SER
135THR
137THR
212TYR
20ILE
22LYS
23ILE
21GLY
24TRP
152TYR
155TYR
156VAL
157MET
161PHE
189THR
160GLY
159ASP
162SER
186THR
163MET
185PRO
165ARG

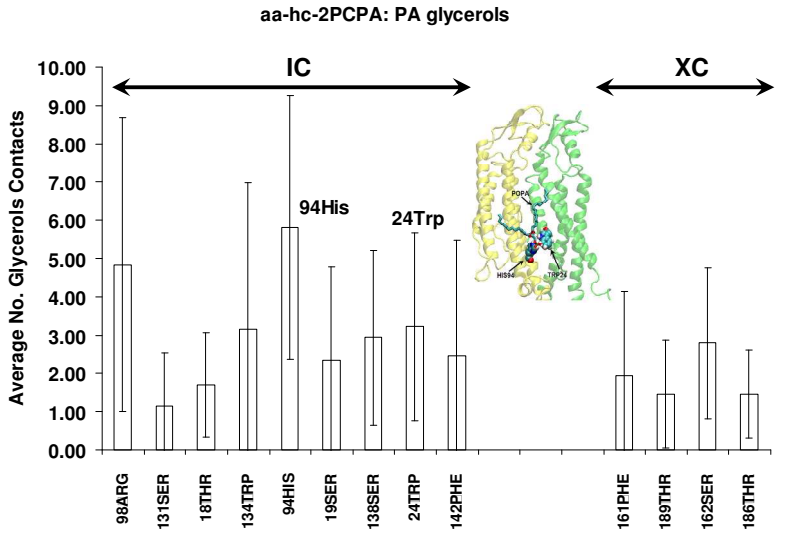
A)


aa-hc-PCPA: PA phosphates
IC
XC
Average No. Phosphate Contacts
12.00
10.00
8.00
6.00
4.00
2.00
0.00
98Arg
97Tyr
94His
127ARG
128ILE
101GLU
129GLU
98ARG
131SER
17SER
97TYR
18THR
134TRP
94HIS
19SER
135THR
24TRP
161PHE
189THR
162SER
186THR
163MET
185PRO
165ARG

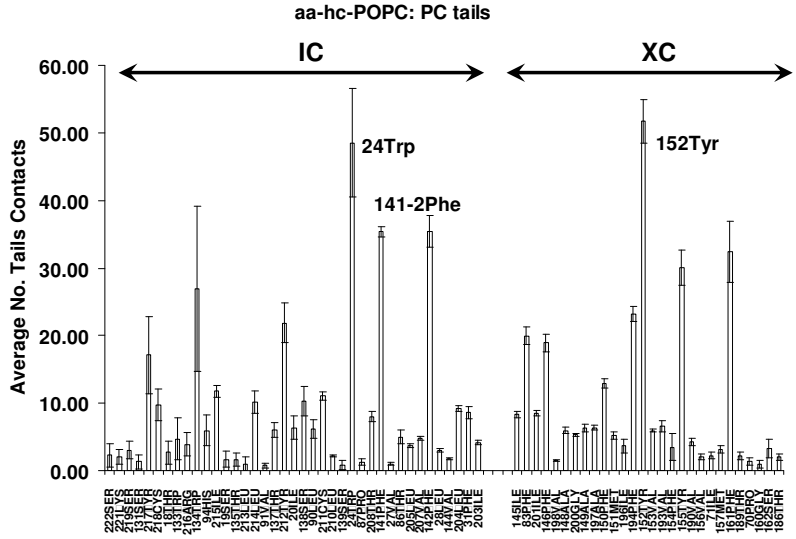
B)


aa-hc-POPC: PC glycerols
IC
XC
Average No. Glycerols Contacts
30.00
25.00
20.00
15.00
10.00
5.00
0.00
216Arg
134Trp
152,
155Tyr
223LYS
222SER
221LYS
130GLY
98ARG
219SER
131SER
217TYR
218CYS
18THR
133TRP
216ARG
134TRP
94HIS
215ILE
19SER
135THR
137THR
212TYR
20ILE
23ILE
138SER
24TRP
142PHE
152TYR
155TYR
156VAL
157MET
161PHE
189THR
70PRO
160GLY
159ASP
162SER
186THR
165ARG

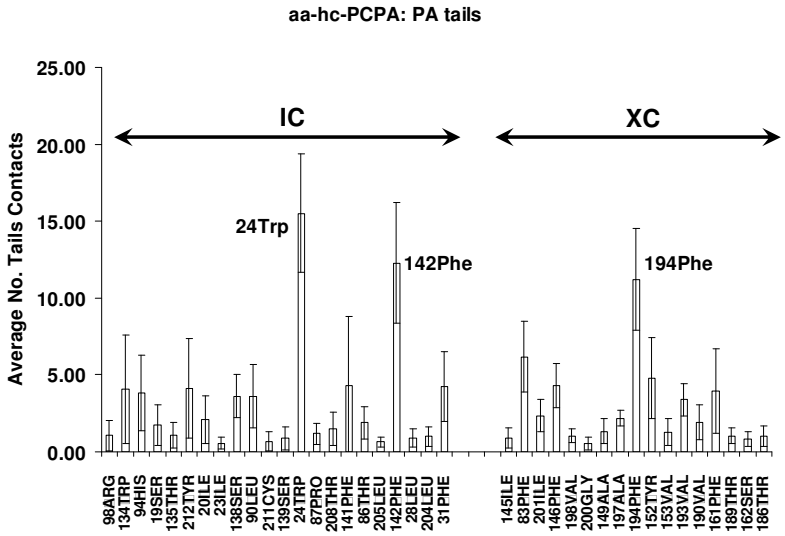
C)




D)




E)




F)

**Figure S9.** AA average numbers of contacts between lipid functional groups and Cx26 residues, for **A)** PC phosphates from simulation **aa-hc-POPC; B)** PA phosphates from simulation **aa-hc-PCPA; C)** PC glycerols from **aa-hc-POPC; D)** PA glycerols from **aa-hc-PCPA; E)** PC tails from **aa-hc-POPC**; and **F)** PA tails from **aa-hc-PCPA**. Averages and standard deviations (error bars) for these charts are taken over the trajectory-averaged values (final 6 ns) for six subunits.