Role of lipids in endosomal/lysosomal cholesterol transport

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Contents

1 Simulations

- 100 lipids (50 per leaflet), 10% cholesterol and 30% DPPC/CERA/SM16/LBPA

system	NPT simulation [ns]	FEP simulation [ns]
90 POPC + 10 CHOL	500	100+100
60 POPC + 10 CHOL + 30 DPPC	500	101+100+100+100
60 POPC + 10 CHOL + 30 CERA	500	100+100
60 POPC + 10 CHOL + 30 SM16	500	101+100
60 POPC + 10 CHOL + 30 $LBPA_{18:1}$	500	103+100
60 POPC + 10 CHOL + 30 $LBPA_{16:0}$	500	100+100
60 POPC + 10 CHOL + 30 $LBPA_{14:0}$	500	100+100

Old test simulations, results not shown here

- Small membranes, 100 lipids (50 per leaflet), 10% cholesterol and 16% LBPA

system	NPT simulation [ns]	FEP simulation [ns]
74 POPC + 10 CHOL + 16 $LBPA_{RR}$	200	
74 POPC + 10 CHOL + 16 $LBPA_{SS}$	200	

- Big membranes, 200 lipids (100 per leaflet), 10% cholesterol and 15% CERA/SM16/LBPA

system	NPT simulation [ns]	FEP simulation [ns]
180 POPC + 20 CHOL	200	23
150 POPC + 20 CHOL + 30 CERA	200	20
150 POPC + 20 CHOL + 30 SM16	200	11
150 POPC + 20 CHOL + 30 $LBPA_{RR}$	200	17
150 POPC + 20 CHOL + 30 $LBPA_{SS}$	200	23

- Big membranes, 200 lipids (100 per leaflet), 10% cholesterol and 90% CERA/SM16

system	NPT simulation [ns]	FEP simulation [ns]
180 CERA + 20 CHOL	200	18
180 SM16 + 20 CHOL	200	20

- Big membranes, 200 lipids (100 per leaflet), 0.5 % cholesterol

system	NPT simulation [ns]	FEP simulation [ns]
199 POPC + 1 CHOL	200	17
169 POPC + 1 CHOL + 30 CERA	200	18
169 POPC + 1 CHOL + 30 SM16	200	24
169 POPC + 1 CHOL + 30 $LBPA_{RR}$	200	18
169 POPC + 1 CHOL + 30 $LBPA_{SS}$	200	24

2 Results

2.1 Membrane area

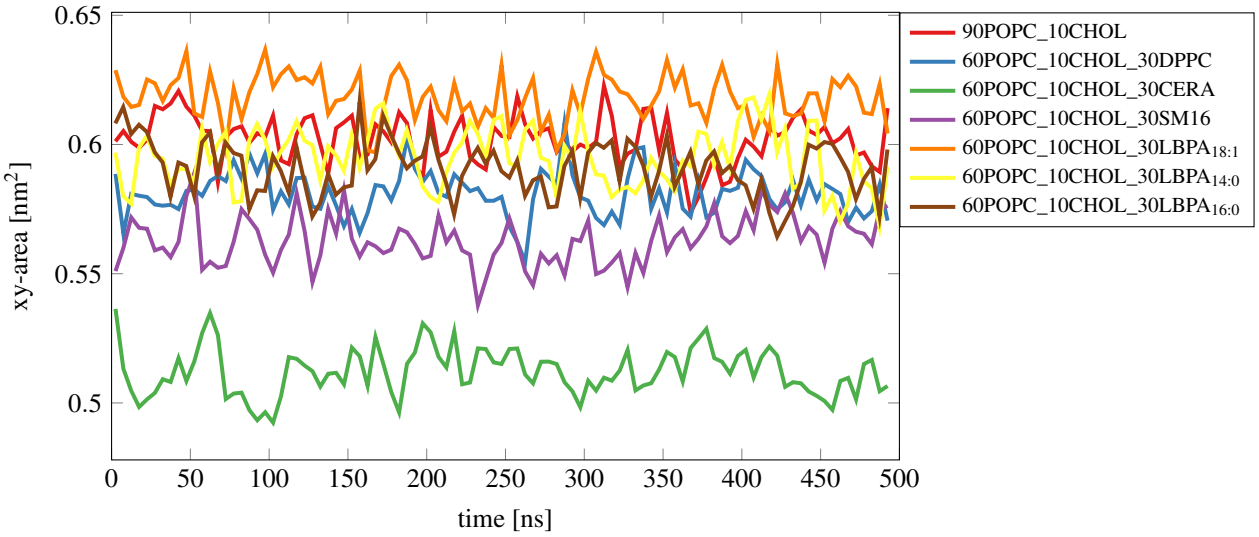


Figure 1: **Area per lipid.**

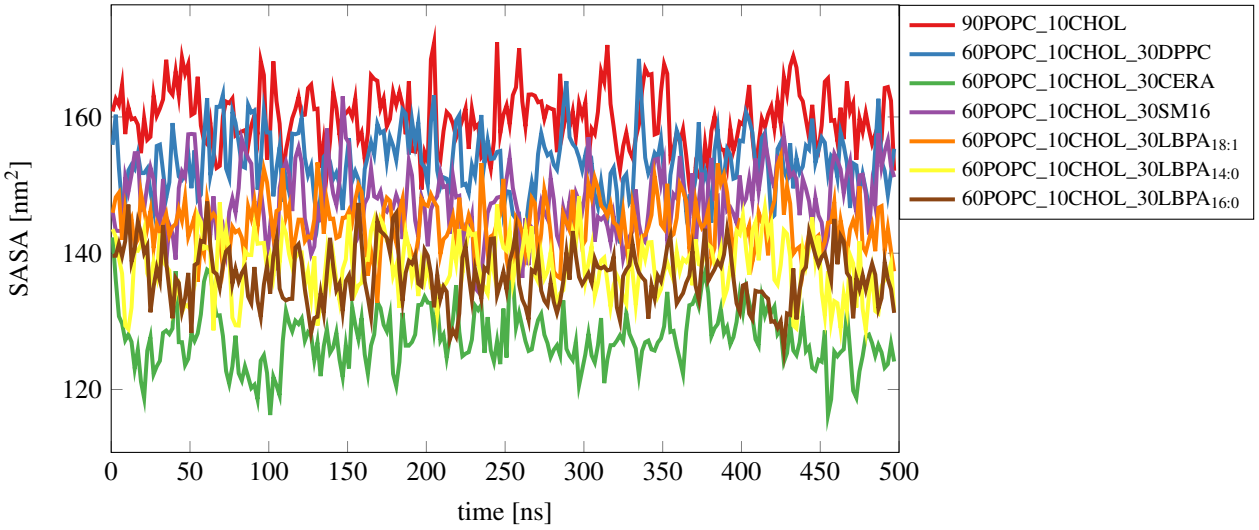


Figure 2: **Solvent accessible surface area (SASA) of membrane.**

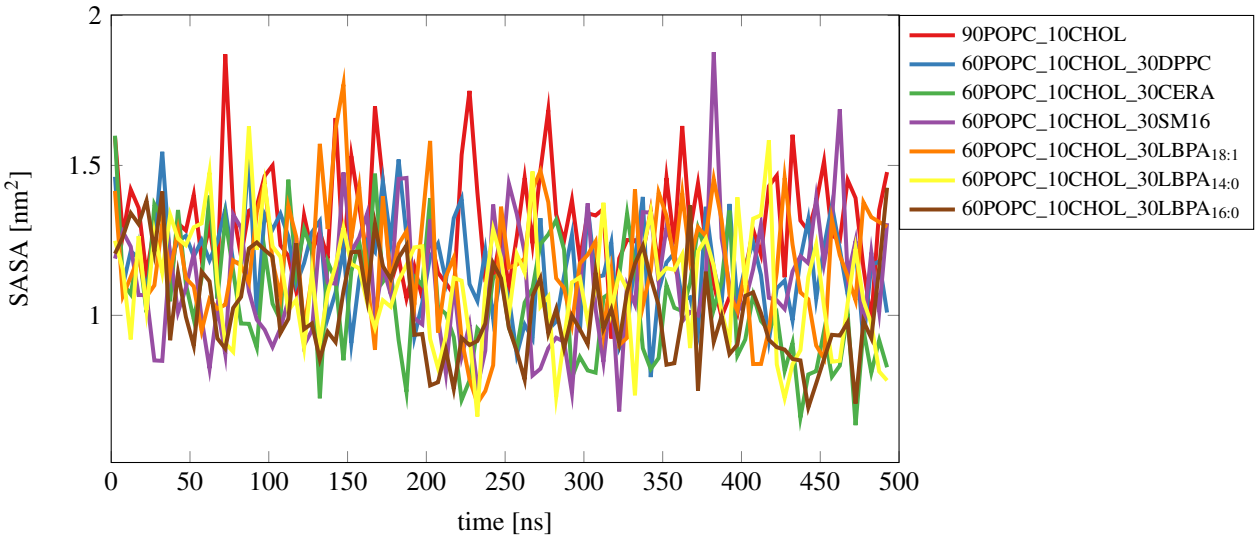


Figure 3: **Solvent accessible surface area (SASA) of cholesterol.**

2.2 Atom distance from bilayer center

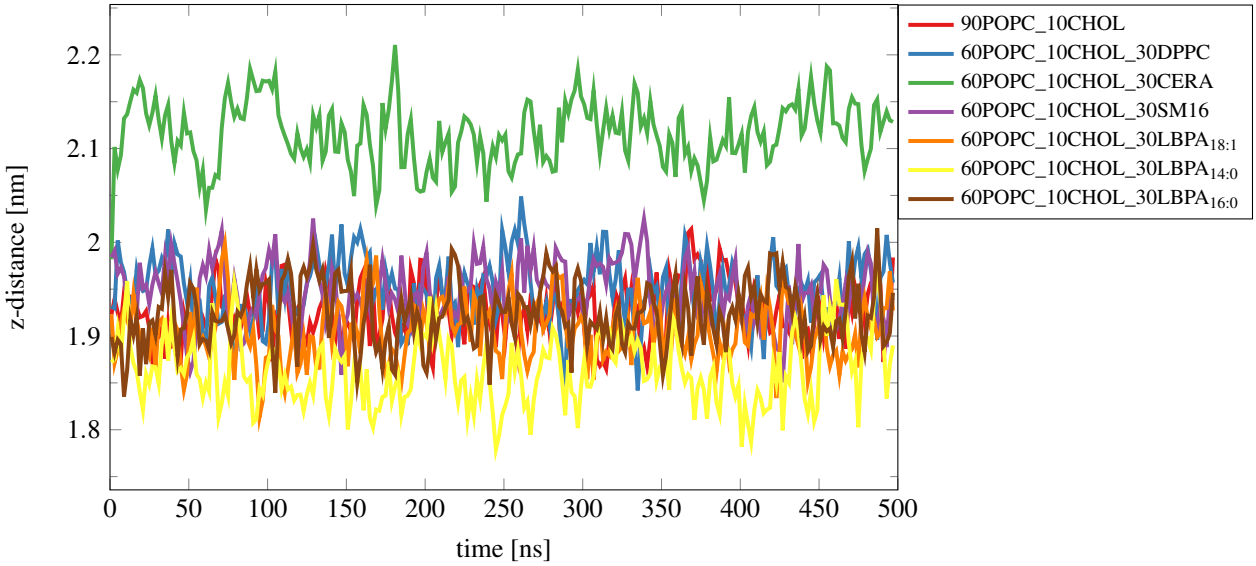


Figure 4: **Absolute distance of POPC Phosphate from bilayer center.**

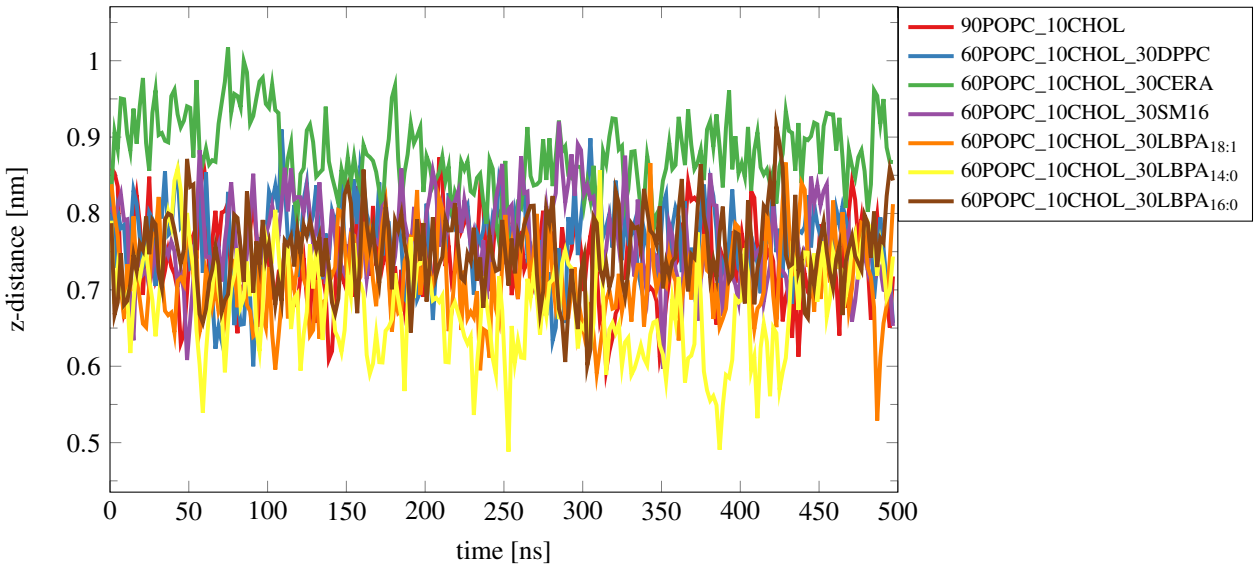


Figure 5: **Absolute distance of cholesterol COM from bilayer center.**

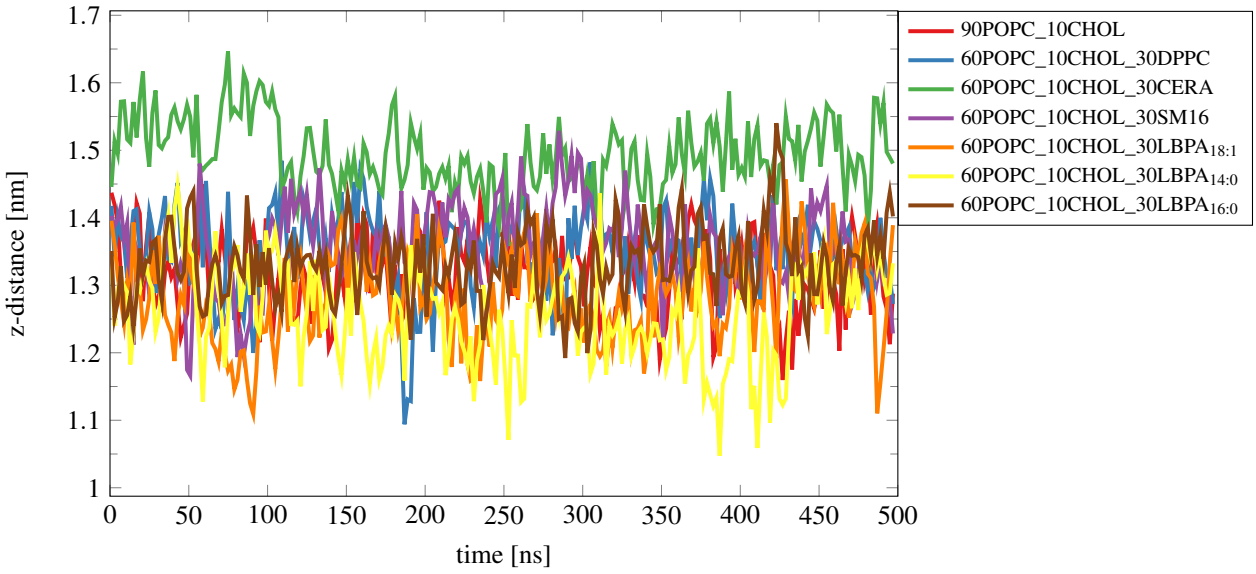


Figure 6: **Absolute distance of cholesterol C3 atom from bilayer center.**

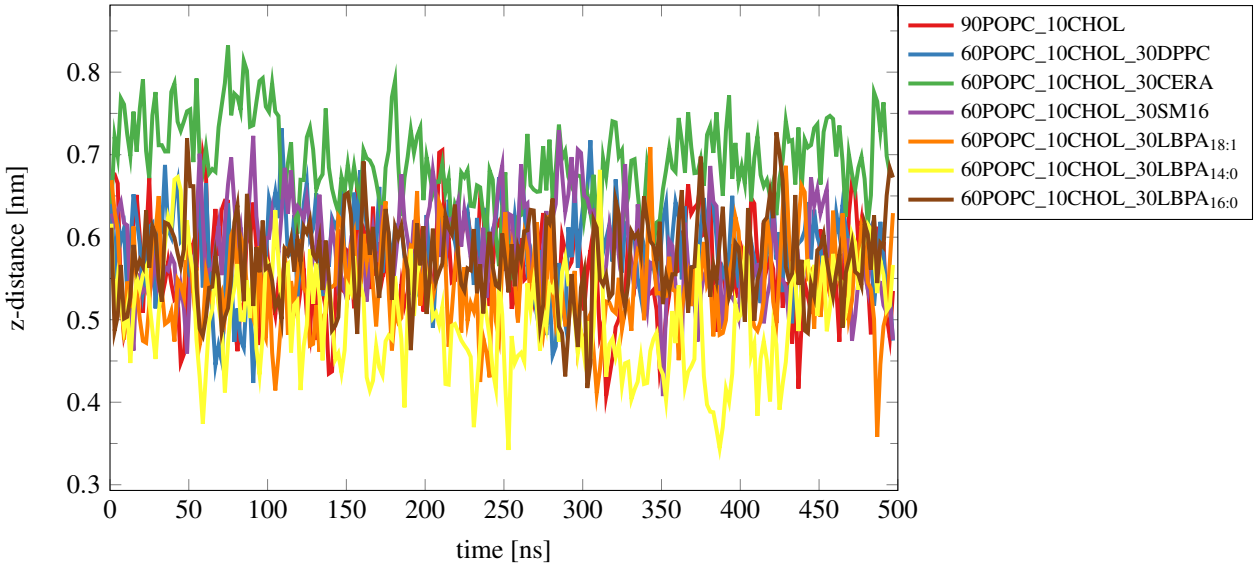


Figure 7: **Absolute distance of cholesterol C17 atom from bilayer center.**

2.3 Flip flops

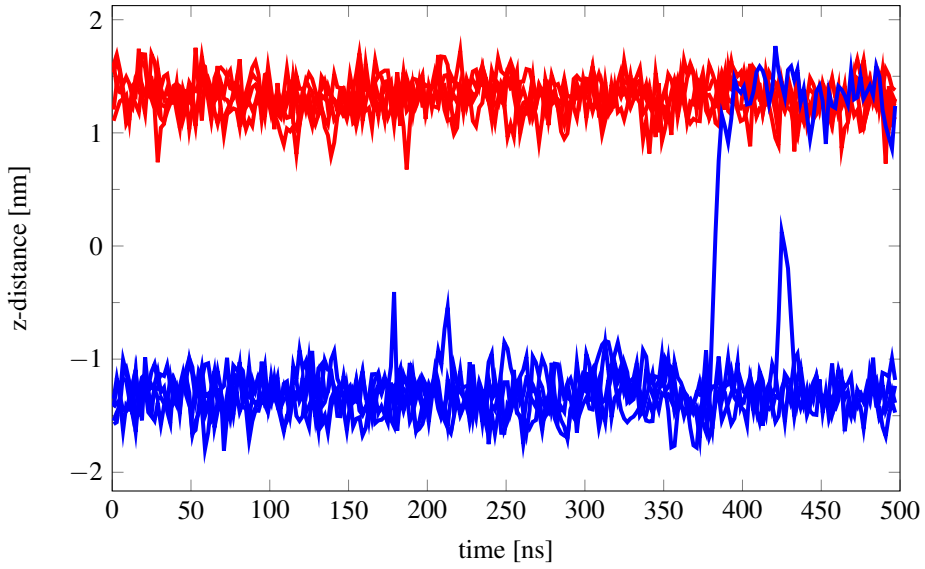


Figure 8: **Distances of all cholesterol C3 atoms from bilayer center. 90POPC_10CHOL**

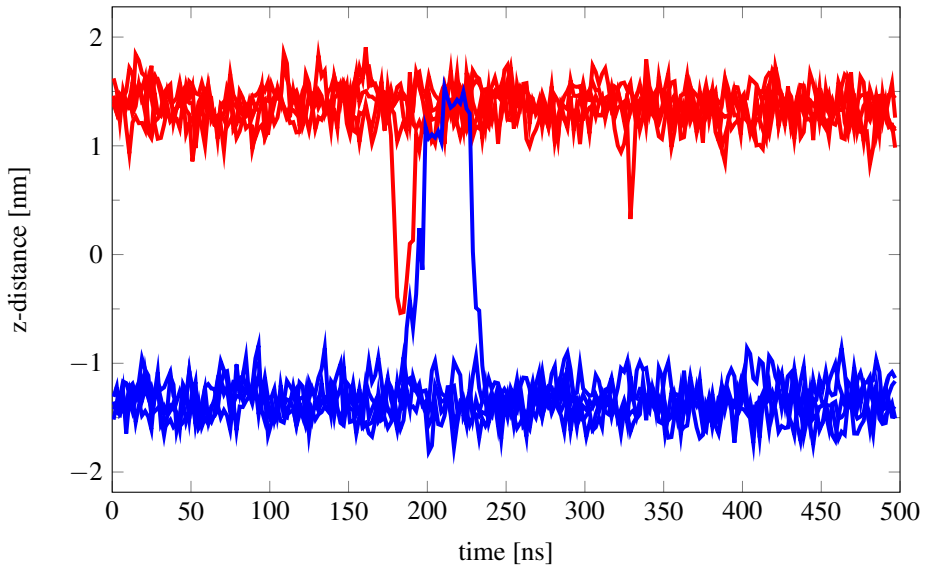


Figure 9: **Distances of all cholesterol C3 atoms from bilayer center. 60POPC_10CHOL_DPPC**

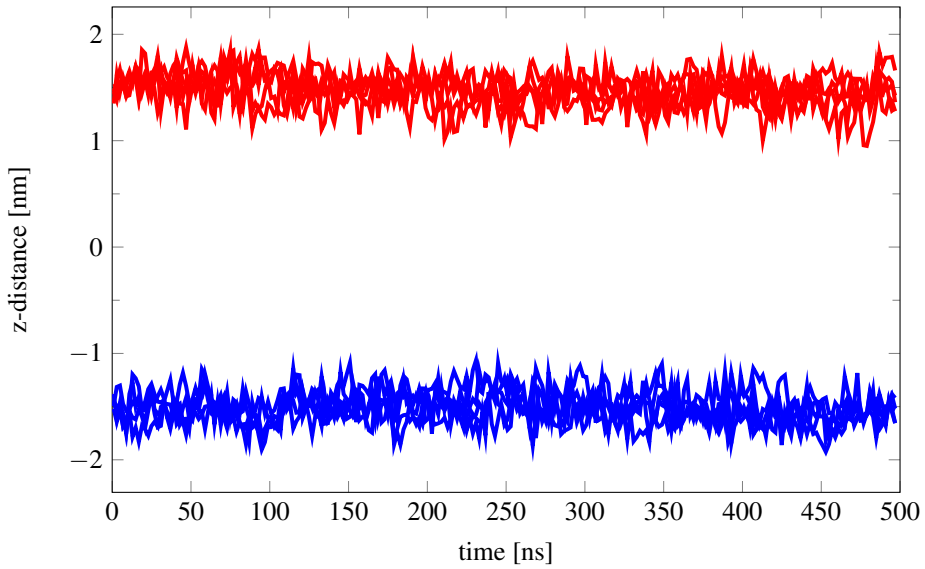


Figure 10: **Distances of all cholesterol C3 atoms from bilayer center. 60POPC_10CHOL_CERA**

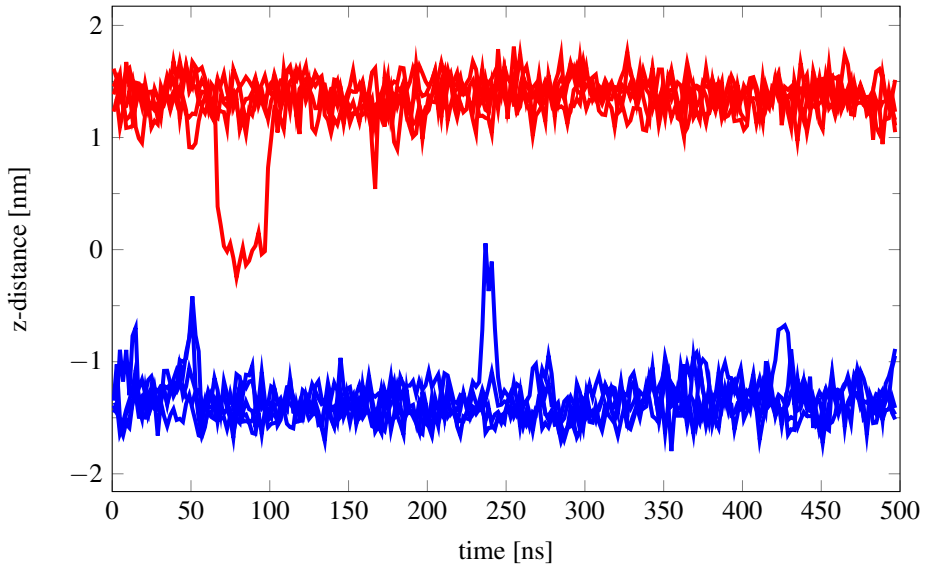


Figure 11: **Distances of all cholesterol C3 atoms from bilayer center. 60POPC_10CHOL_SM16**

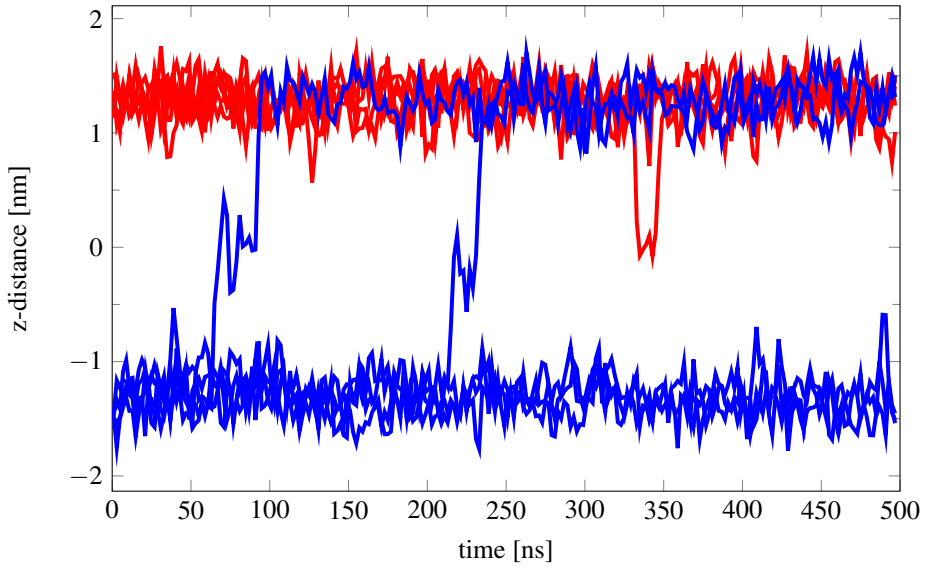


Figure 12: **Distances of all cholesterol C3 atoms from bilayer center. 60POPC_10CHOL_LBPA$_{18:1}$**

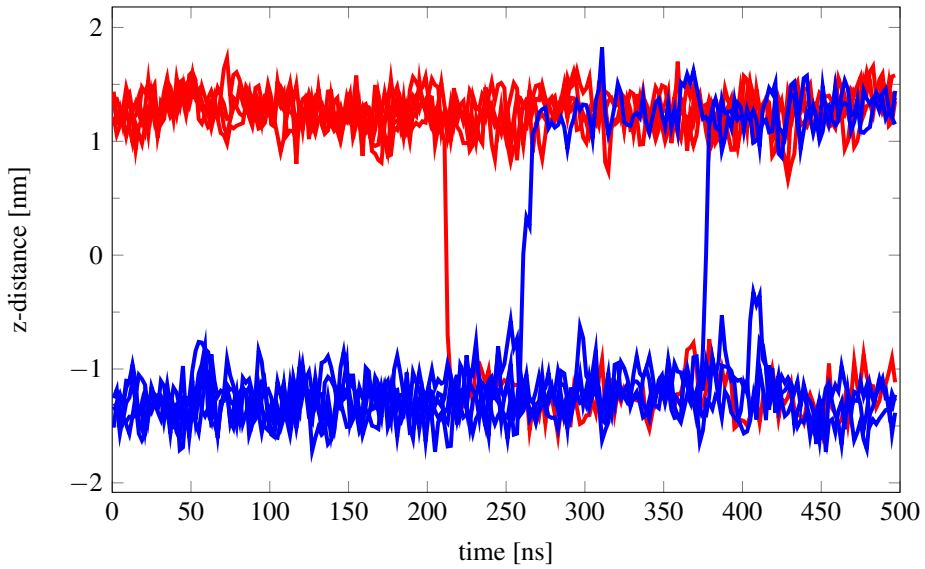


Figure 13: **Distances of all cholesterol C3 atoms from bilayer center. 60POPC_10CHOL_LBPA$_{14:0}$**

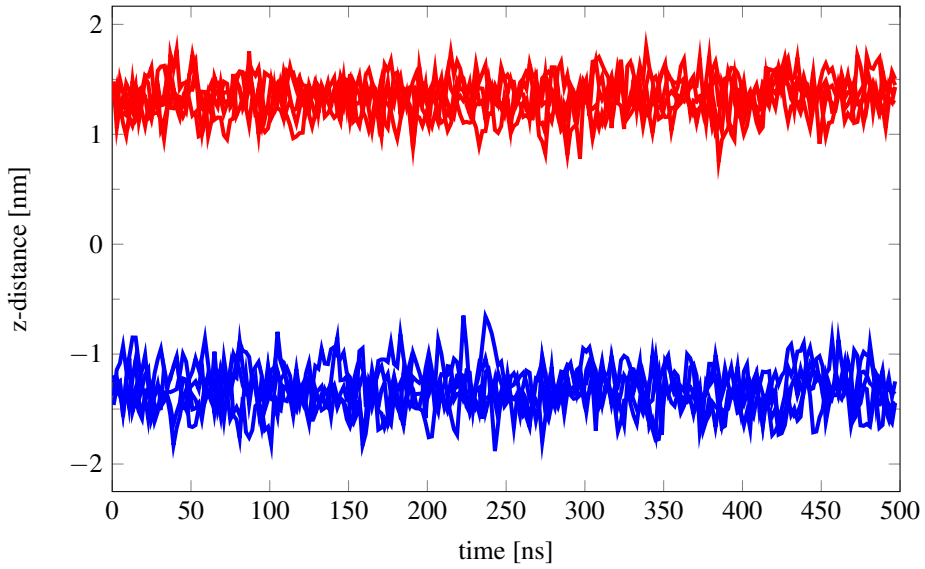
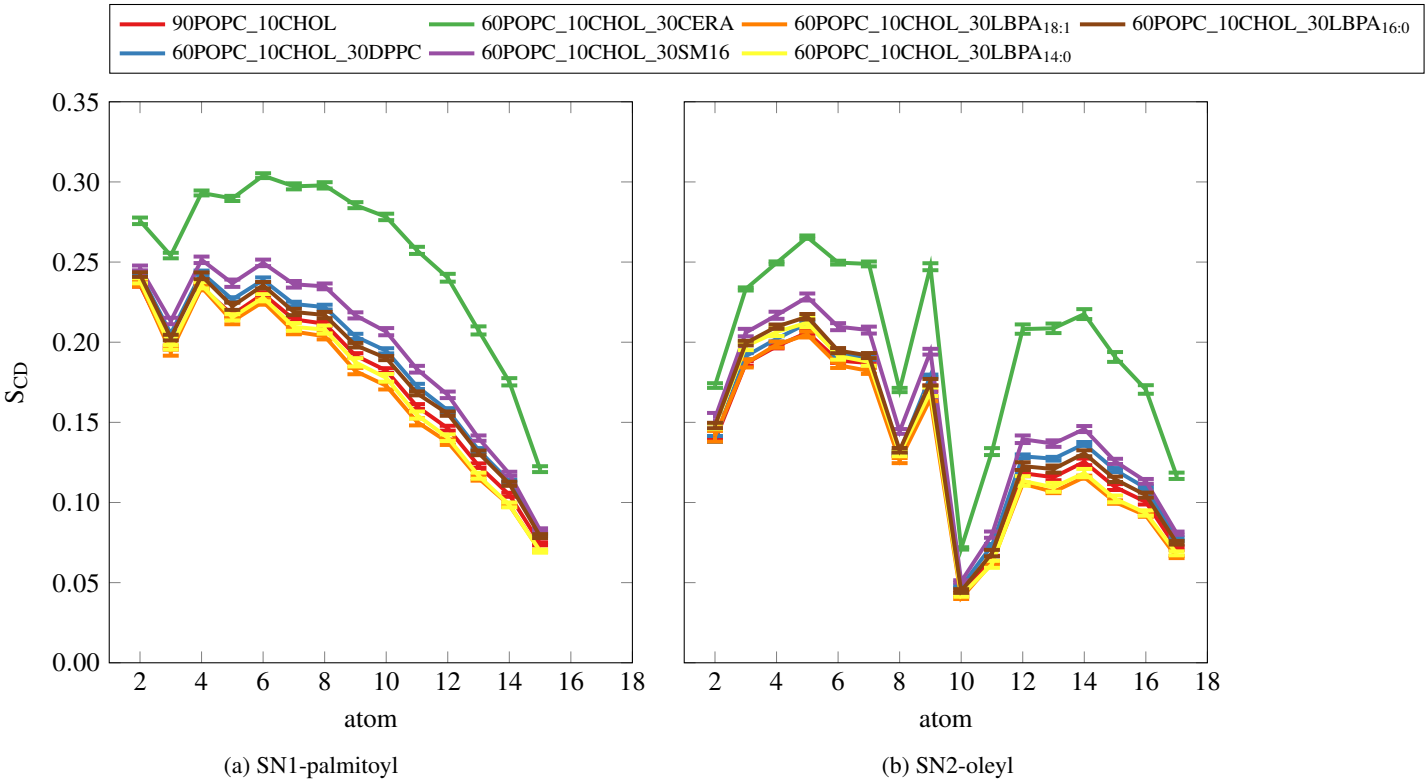


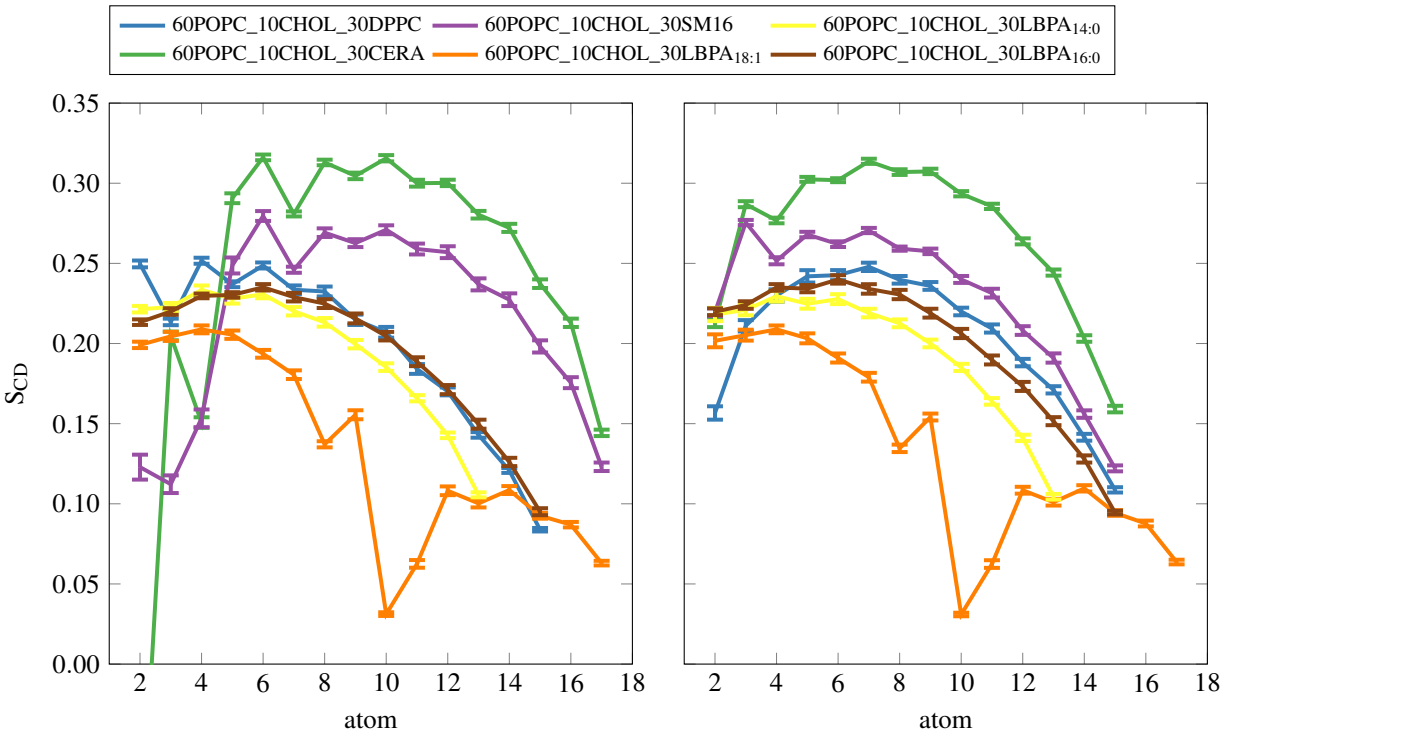
Figure 14: **Distances of all cholesterol C3 atoms from bilayer center. 60POPC_10CHOL_LBPA$_{16:0}$**

2.4 Order parameter



(a) SN1-palmitoyl (b) SN2-oleyl

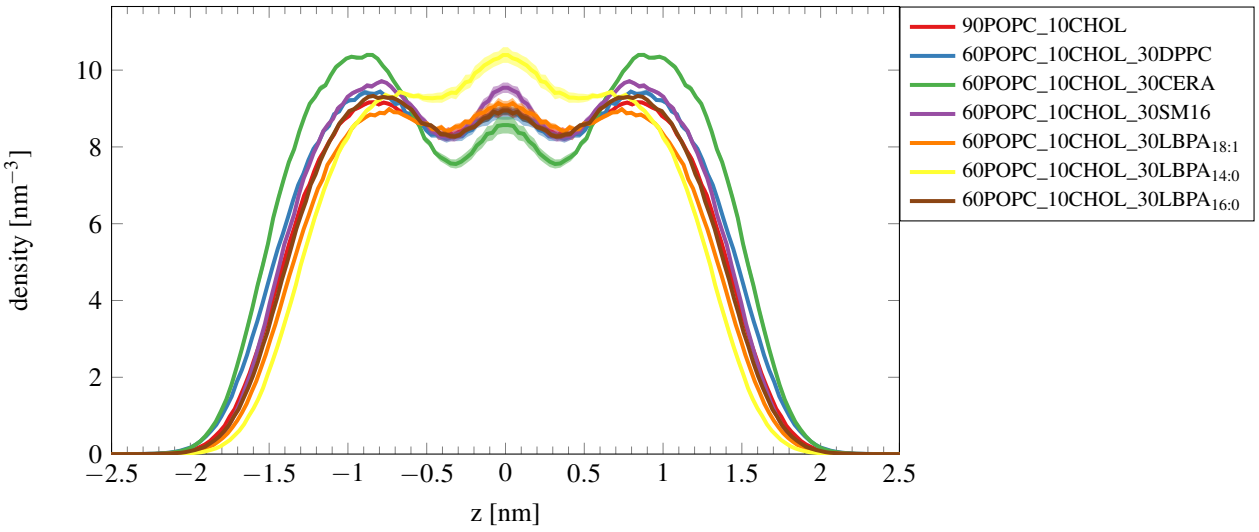
Figure 15: **POPC tail deuterium order parameters.**



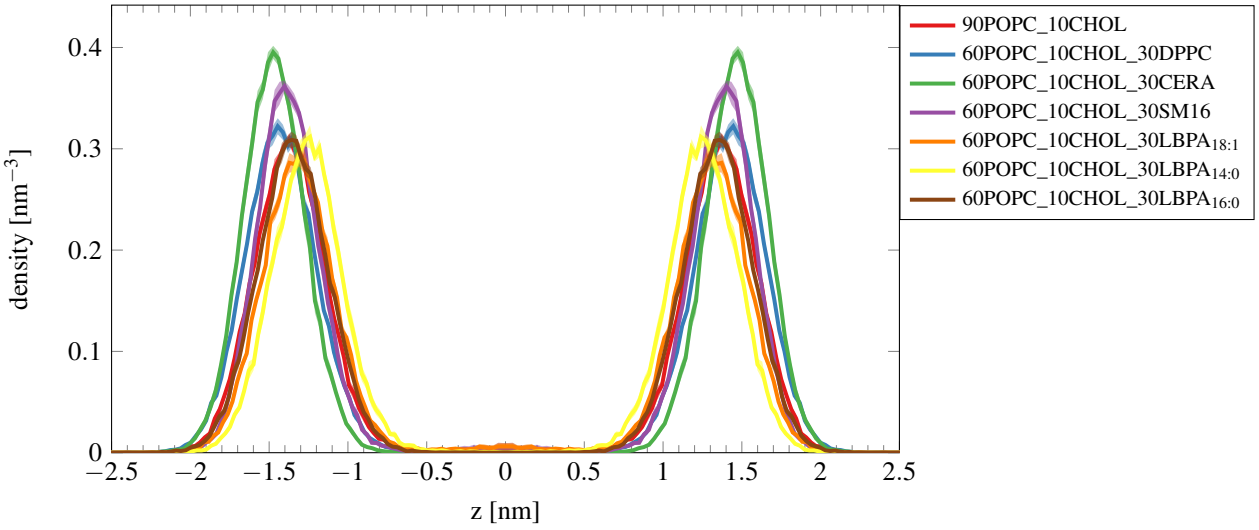
(a) DPPC SN1, CERA/SM16 sphingosine, LBPA tail 1 (b) DPPC SN2, CERA/SM16 palmitoyl, LBPA tail 2

Figure 16: **DPPC/CERA/SM16/LBPA tail deuterium order parameters.**

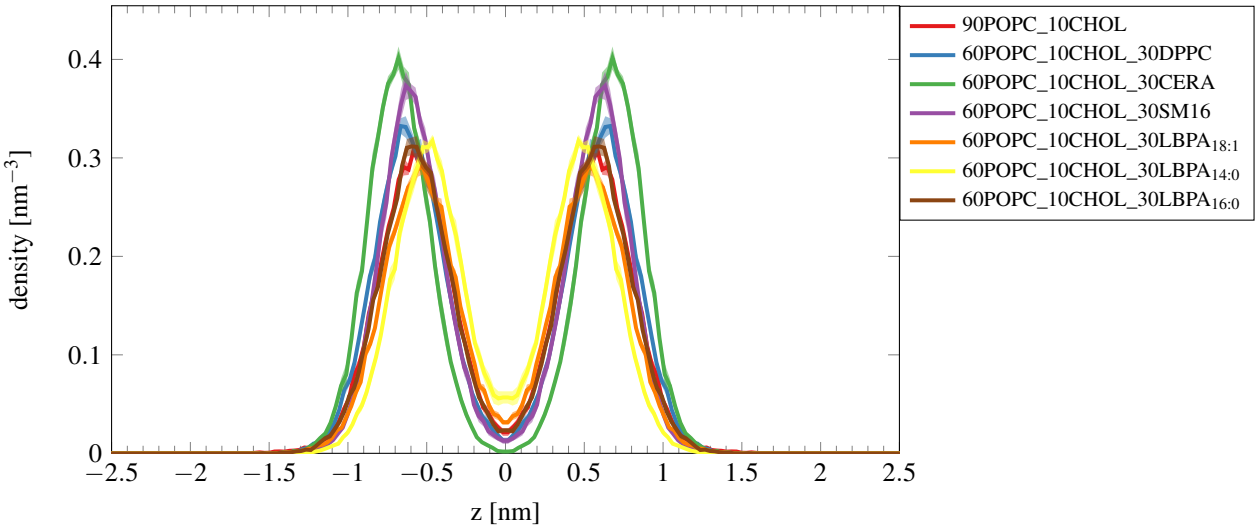
2.5 Cholesterol density



(a) All cholesterol atoms



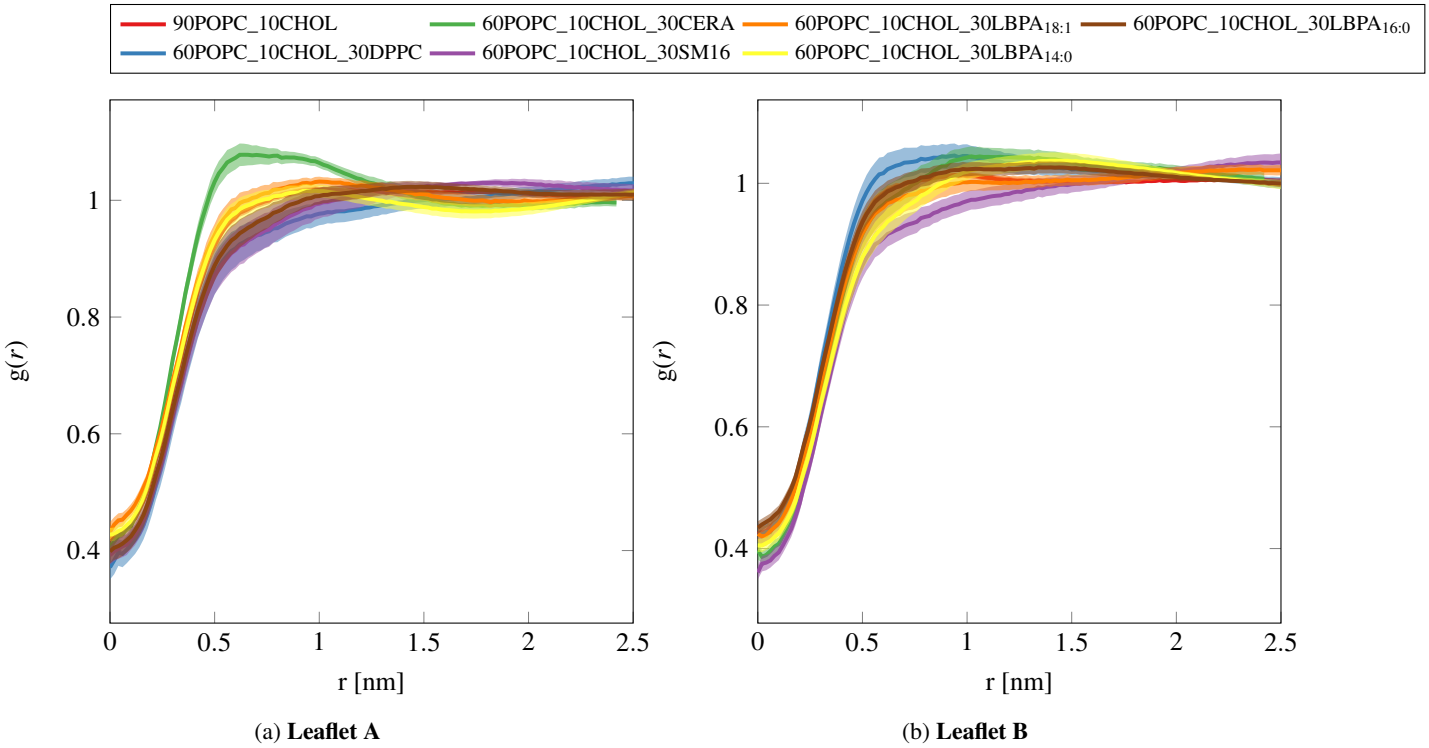
(b) Cholesterol C3-atoms.



(c) Cholesterol C17-atoms.

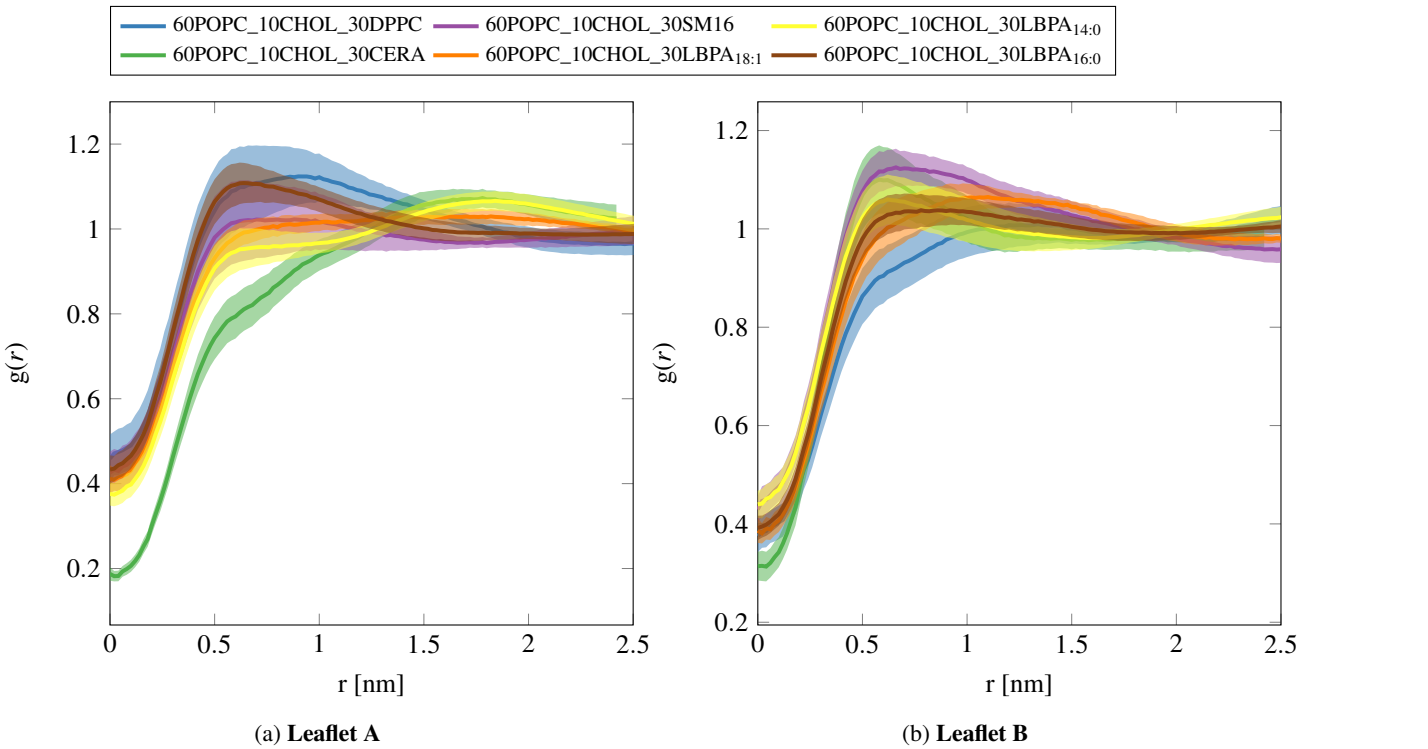
Figure 17: **Number density of cholesterol atoms through membrane.**

2.6 RDF of lipids around cholesterol



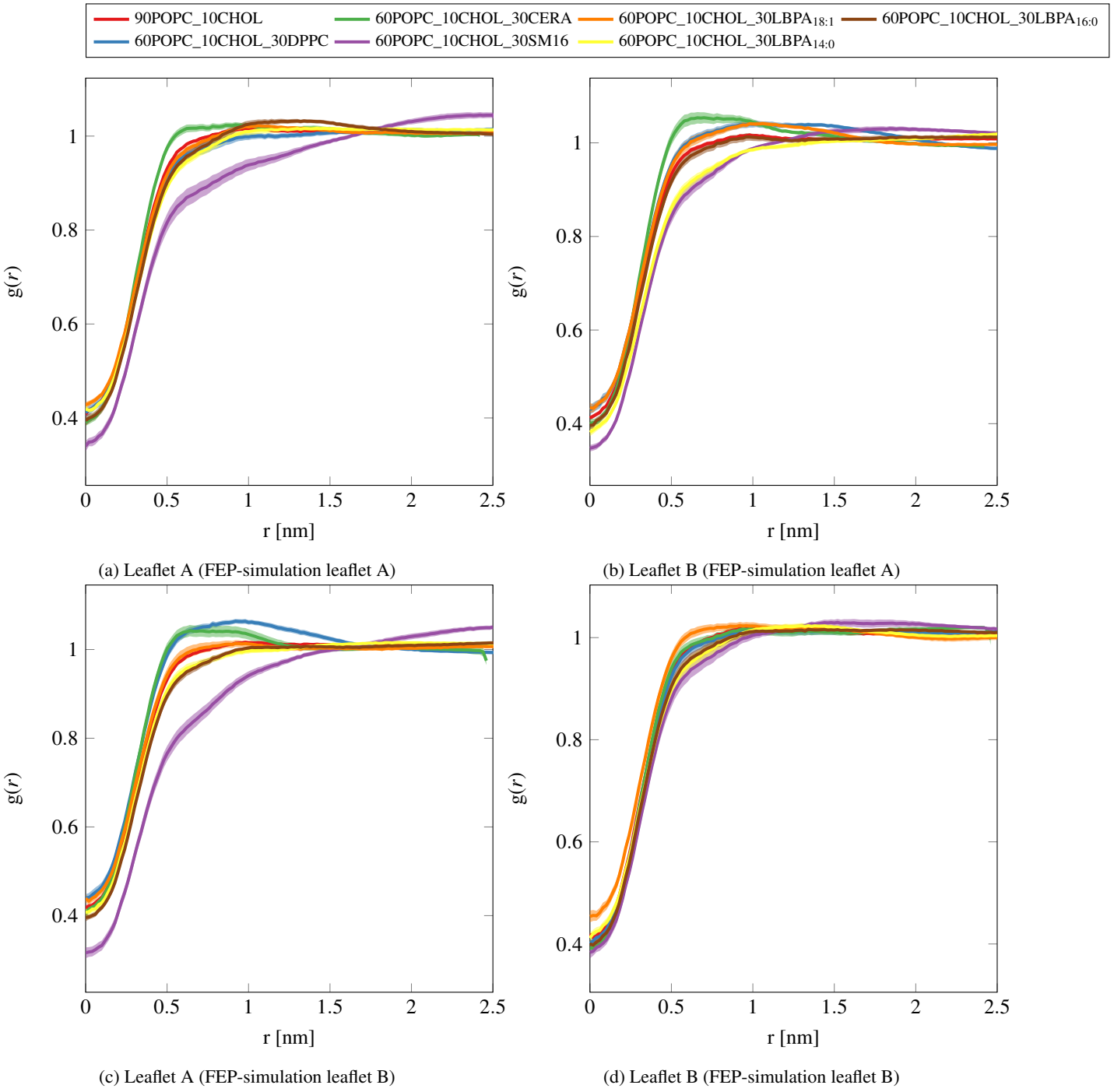
(a) **Leaflet A** (b) **Leaflet B**

Figure 18: **Radial distribution function (RDF) of POPC around cholesterol.**



(a) **Leaflet A** (b) **Leaflet B**

Figure 19: **Radial distribution function (RDF) of DPPC/CERA/SM16/LBPA around cholesterol.**



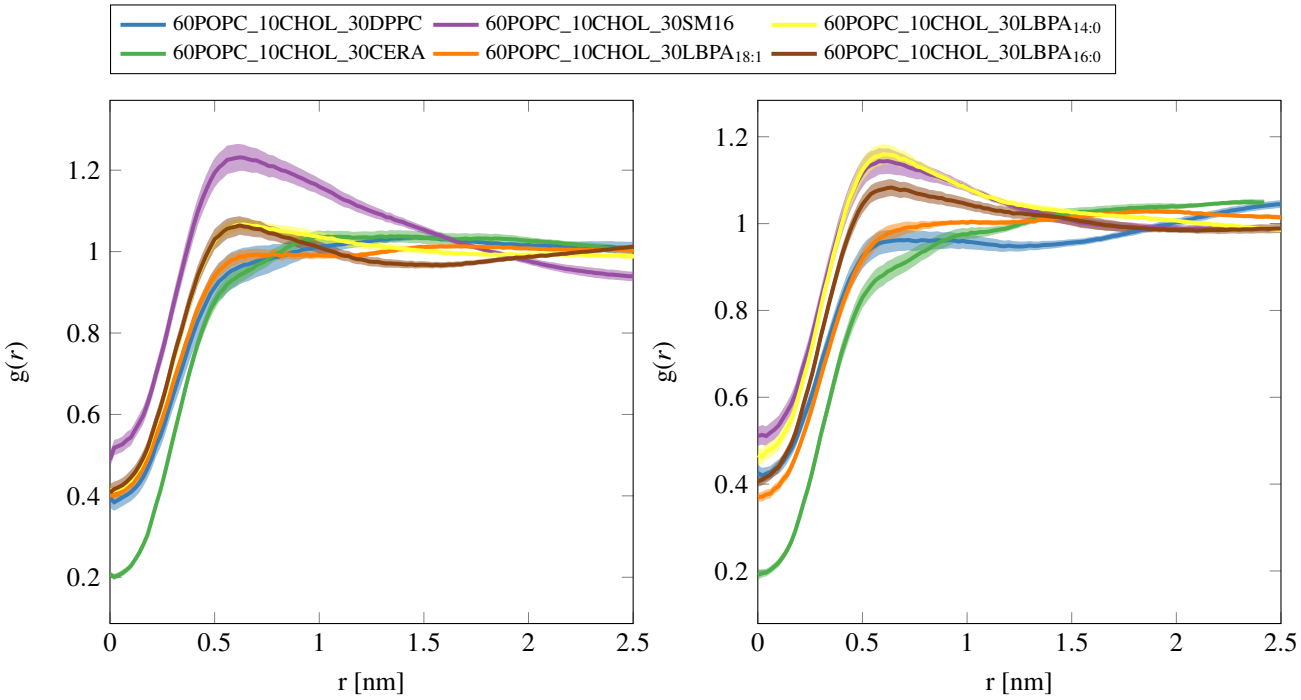
(a) Leaflet A (FEP-simulation leaflet A)

(b) Leaflet B (FEP-simulation leaflet A)

(c) Leaflet A (FEP-simulation leaflet B)

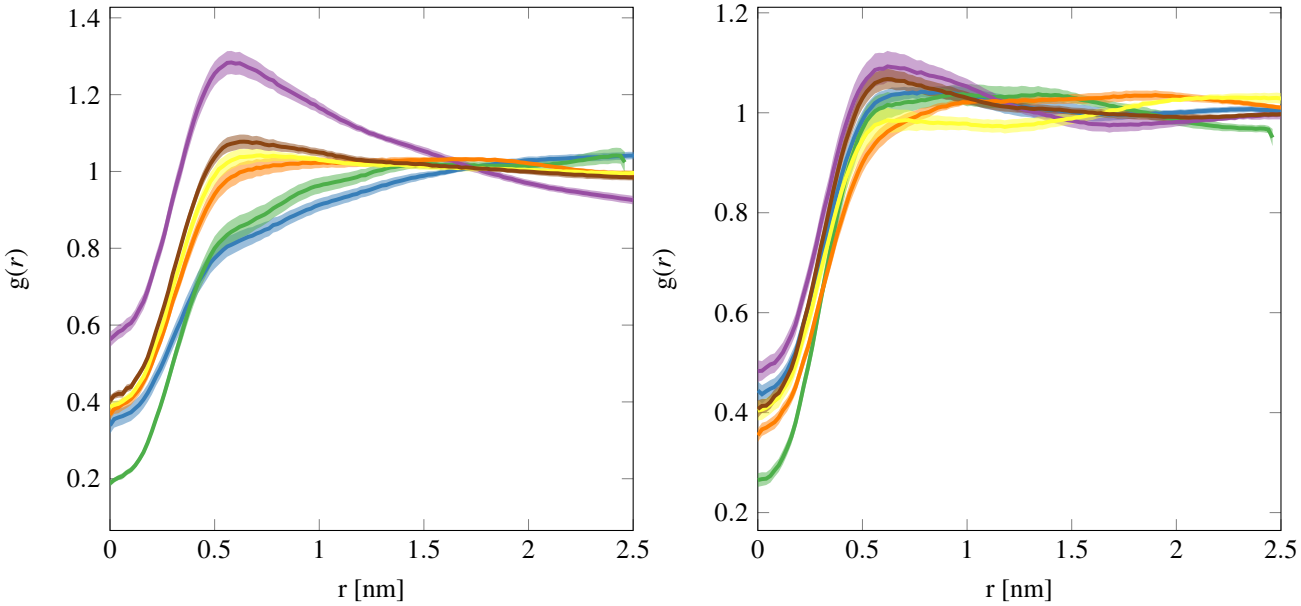
(d) Leaflet B (FEP-simulation leaflet B)

Figure 20: **Radial distribution function (RDF) of POPC around cholesterol. Calculated from FEP simulations (lambda0).**



(a) Leaflet A (FEP-simulation leaflet A)

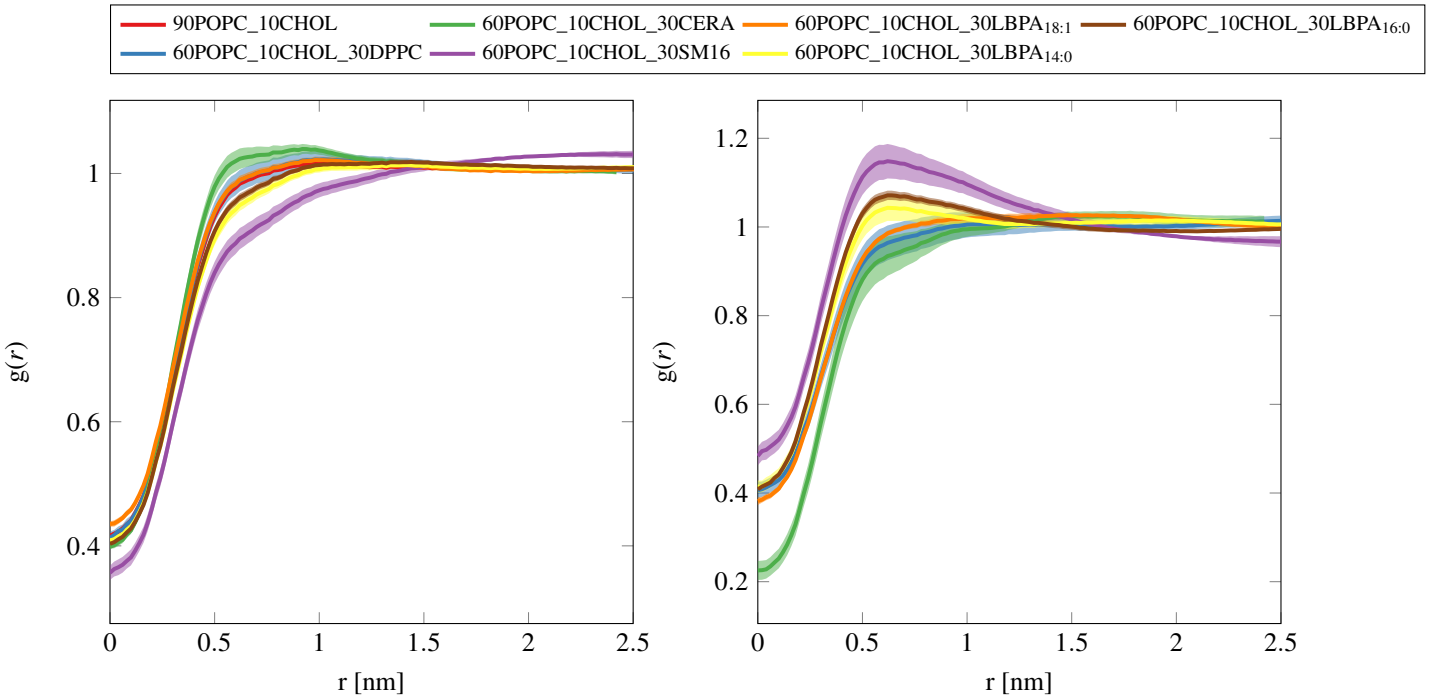
(b) Leaflet B (FEP-simulation leaflet A)



(c) Leaflet A (FEP-simulation leaflet B)

(d) Leaflet B (FEP-simulation leaflet B)

Figure 21: **Radial distribution function (RDF) of DPPC/CERA/SM16/LBPA around cholesterol. Calculated from FEP simulations (lambda0).**



(a) POPC

(b) DPPC/CERA/SM16/LBPA

Figure 22: **Radial distribution function (RDF) of lipids around cholesterol. RDFs from NPT and FEP simulations averaged.**

2.7 CHOL - contacts (0.6nm)

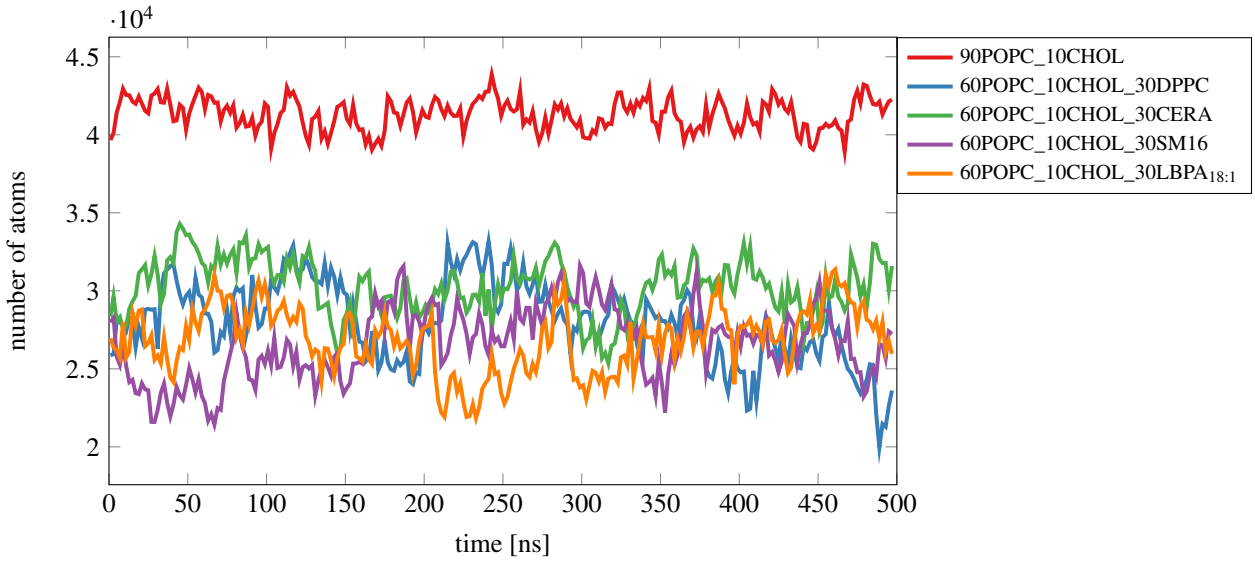


Figure 23: **Number of CHOL-POPC contacts (0.6 nm).**

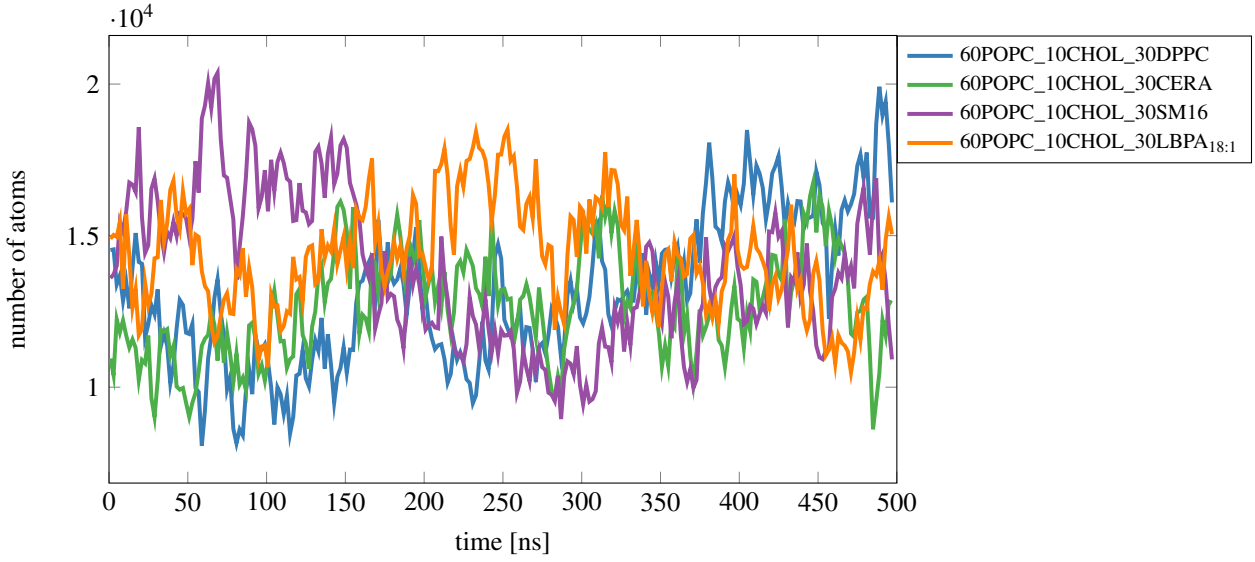


Figure 24: **Number of CHOL-DPPC/CERA/SM16/LBPA contacts (0.6 nm).**

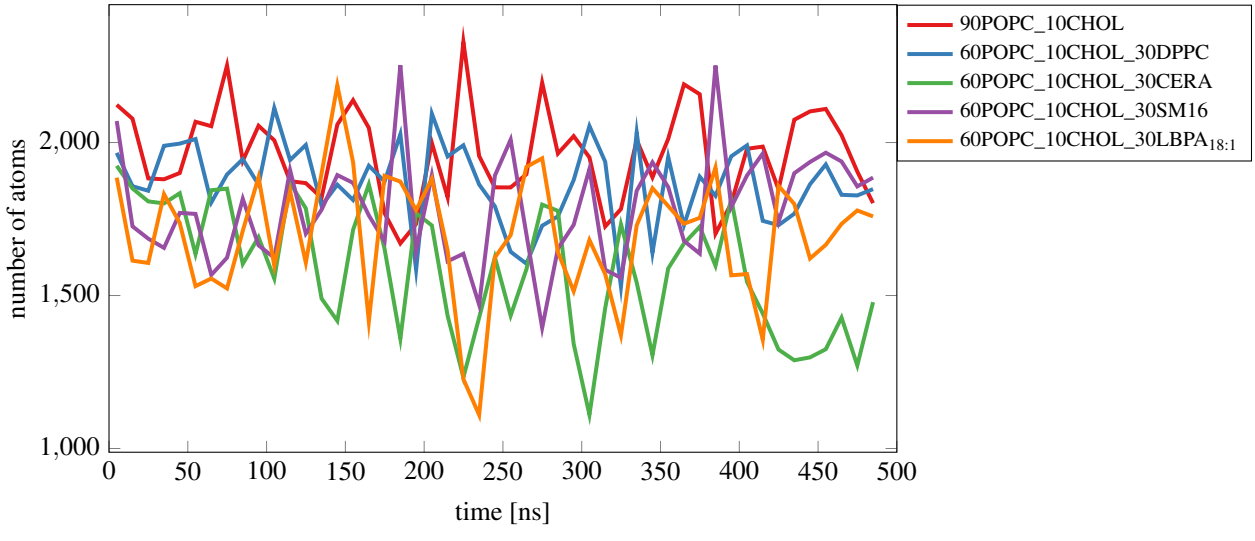


Figure 25: **Number of CHOL-Water contacts (0.6 nm).**

2.8 Hydrogen bonds

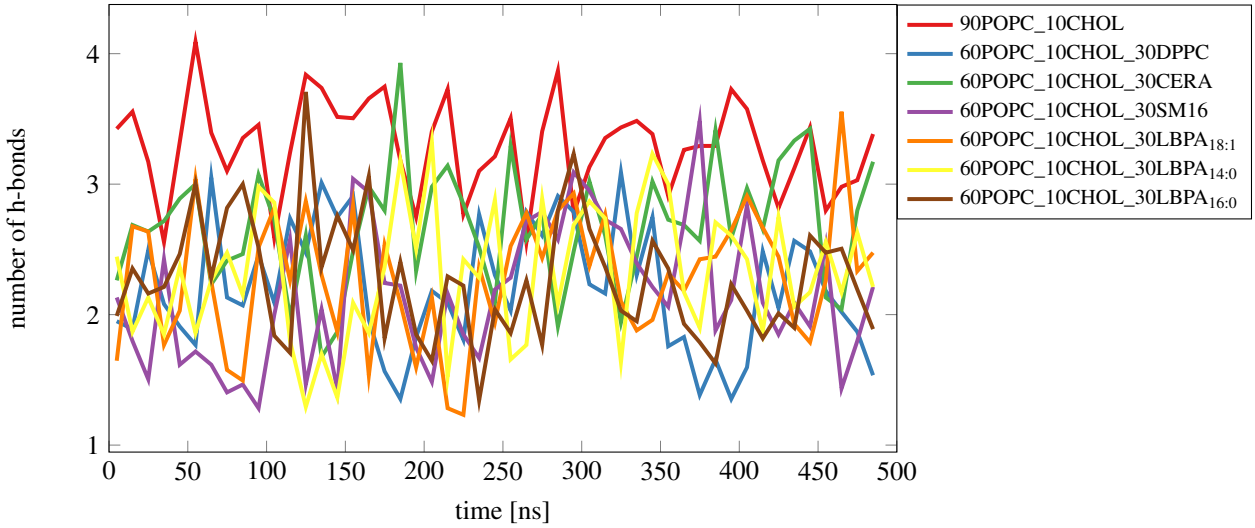


Figure 26: **Number of CHOL-POPC hydrogen bonds.**

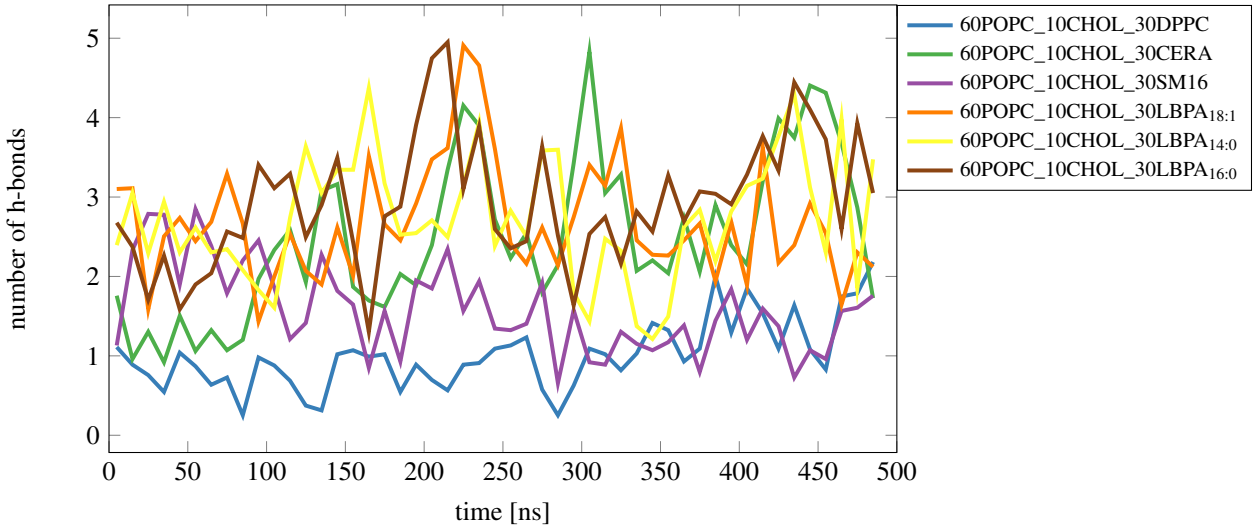


Figure 27: **Number of CHOL-DPPC/CERA/SM16/LBPA hydrogen bonds.**

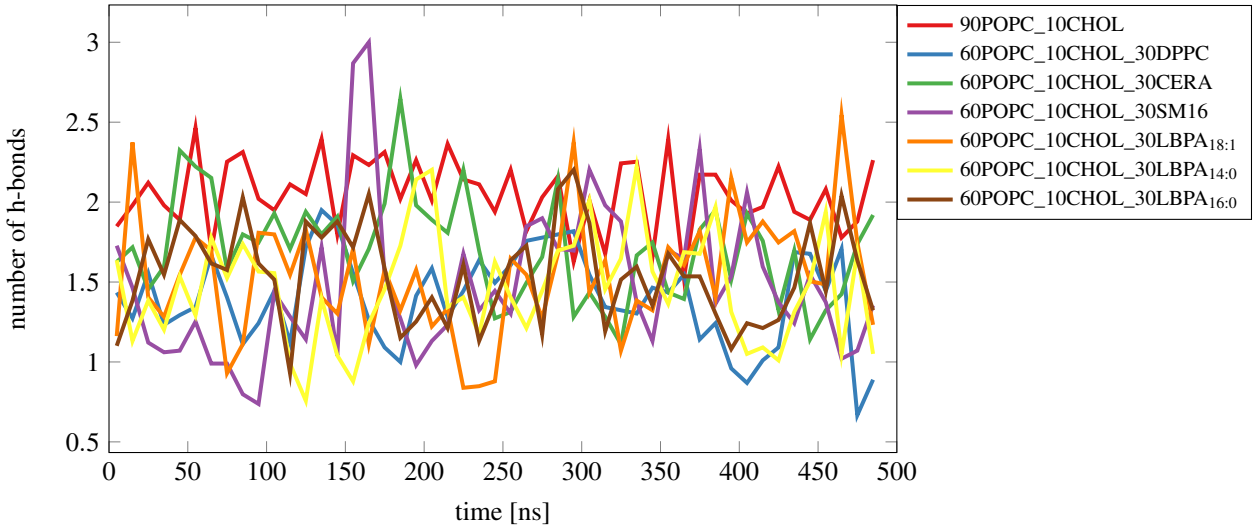


Figure 28: **Number of CHOL-POPC hydrogen bonds defined by only distance (angle ignored).**

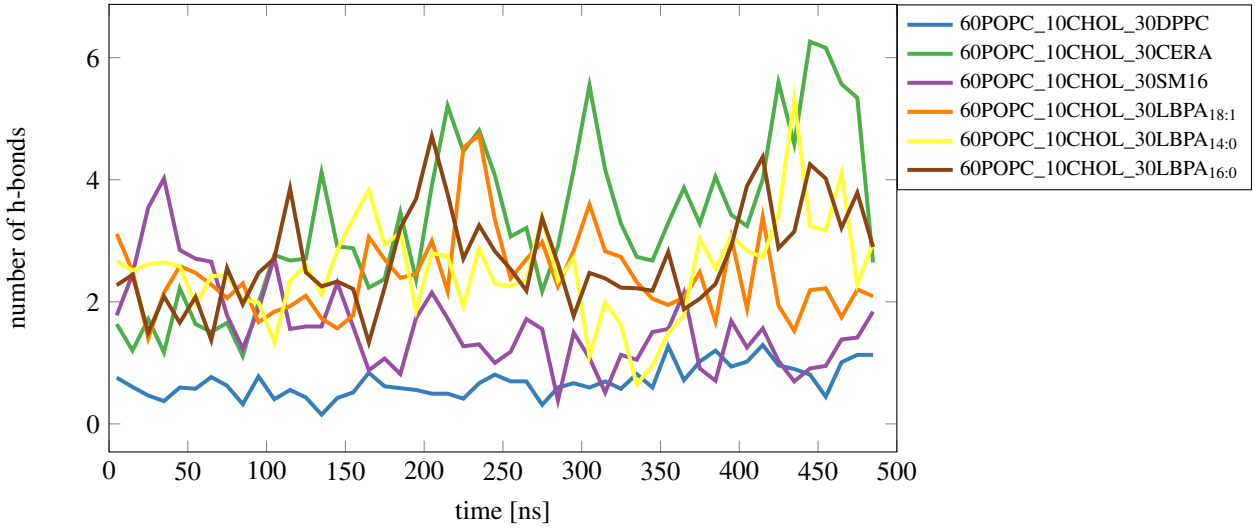


Figure 29: **Number of CHOL-DPPC/CERA/SM16/LBPA hydrogen bonds defined by only distance (angle ignored).**

2.9 POPC Diffusion

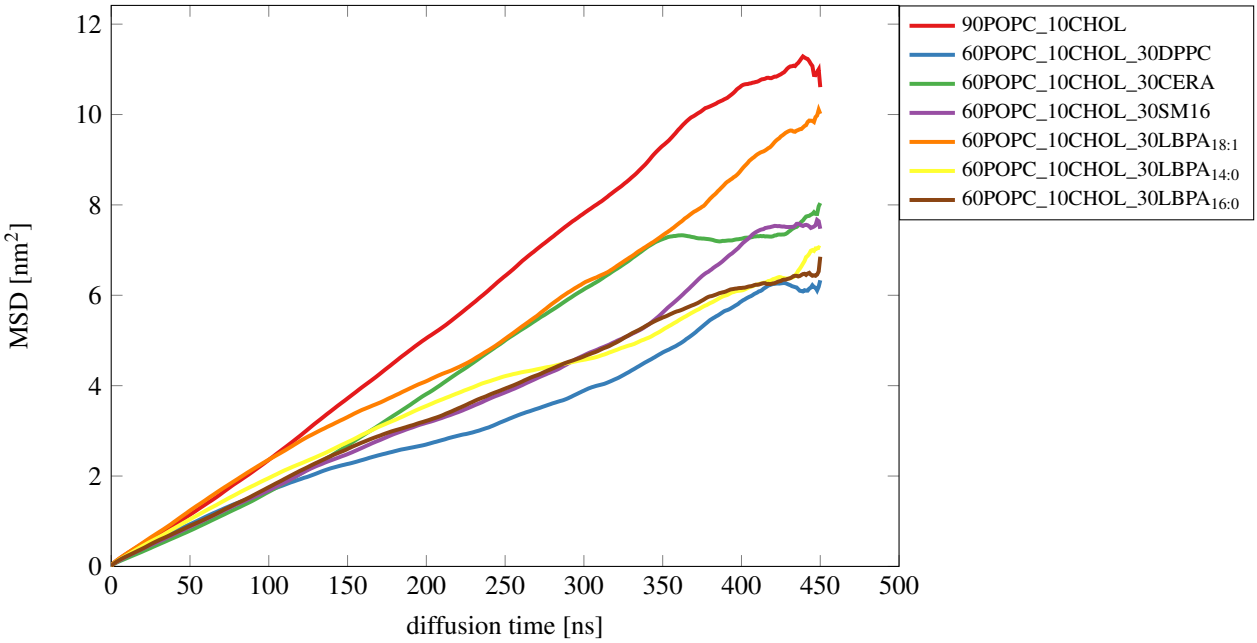


Figure 30: **MSD of POPC in xy-plane. Leaflet A.**

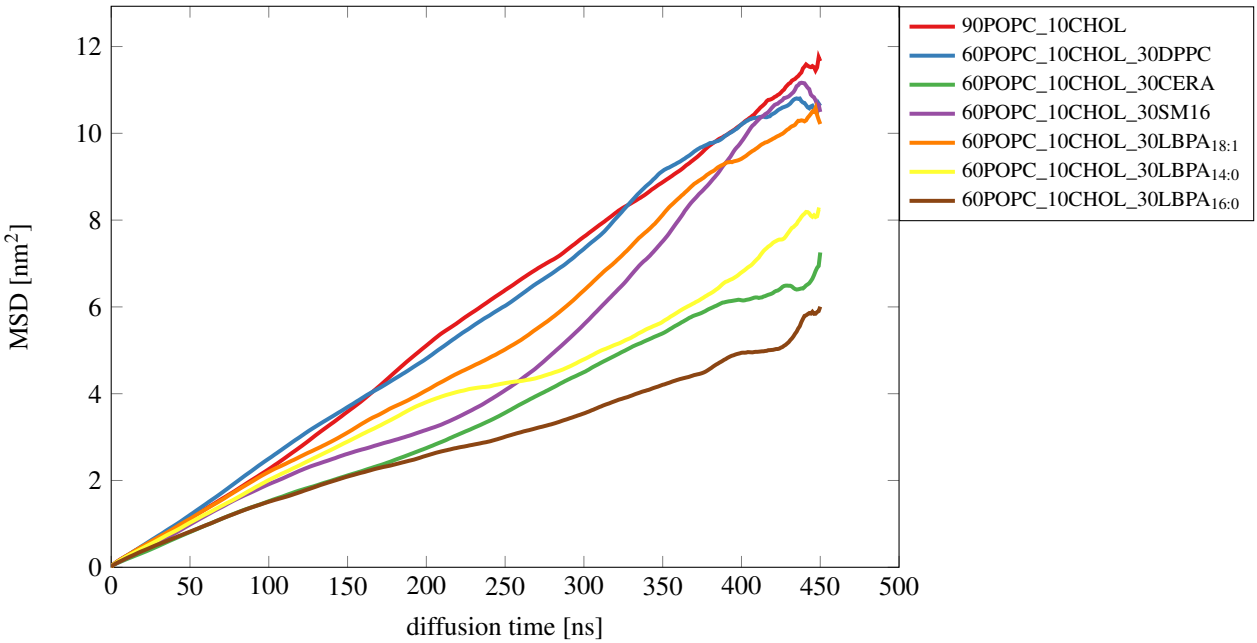


Figure 31: **MSD of POPC in xy-plane. Leaflet B.**

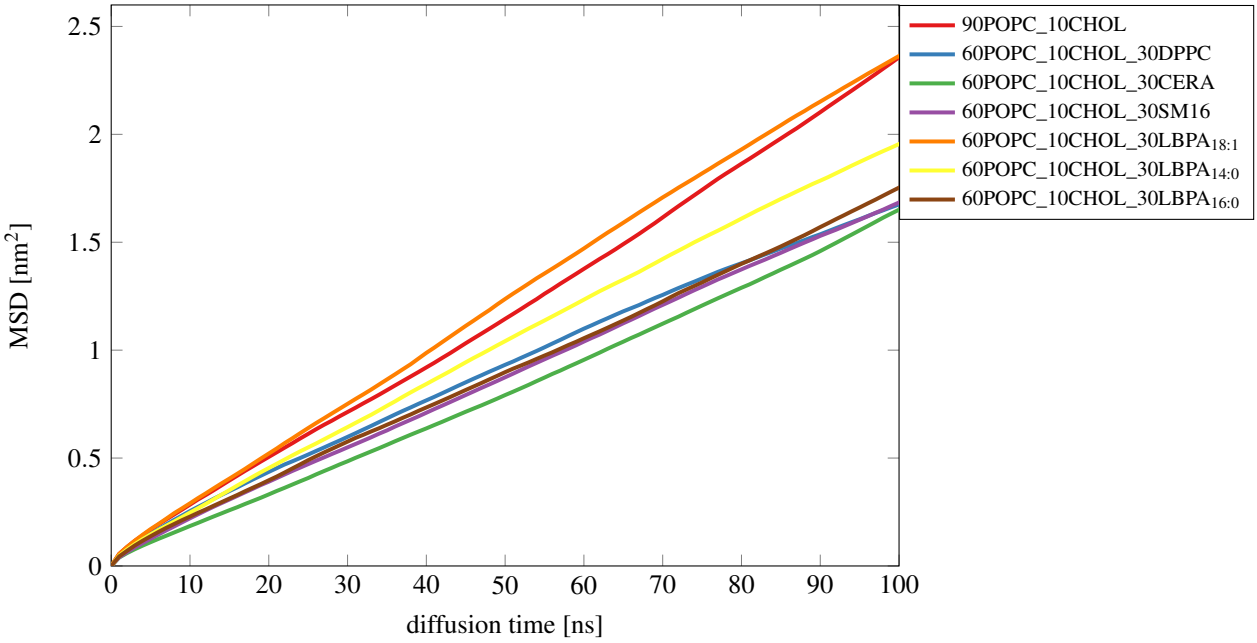


Figure 32: **MSD of POPC in xy-plane. 100ns diffusion. Leaflet A.**

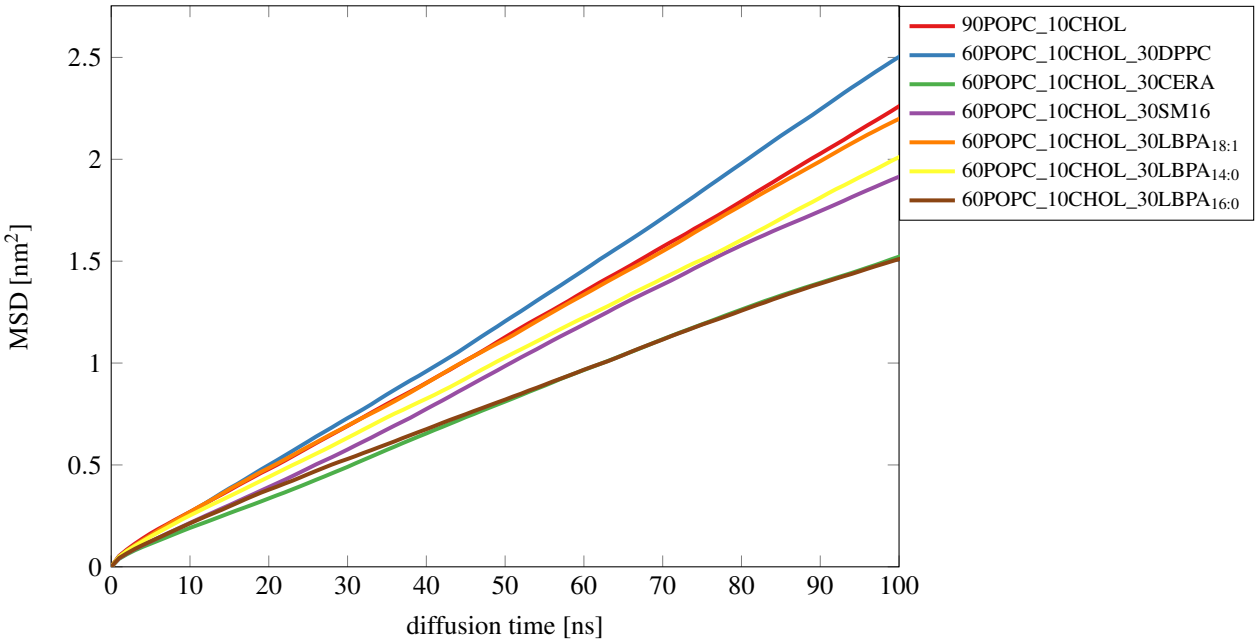


Figure 33: **MSD of POPC in xy-plane. 100ns diffusion. Leaflet B.**

2.10 CHOL diffusion

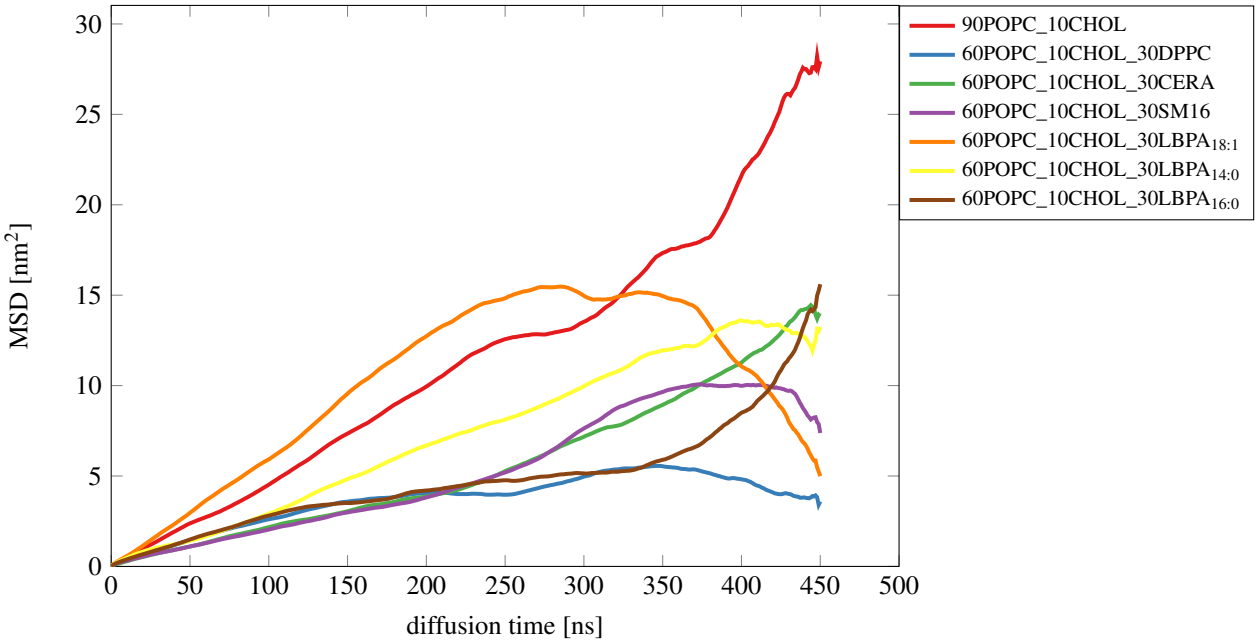


Figure 34: **MSD of cholesterol in xy-plane. Leaflet A.**

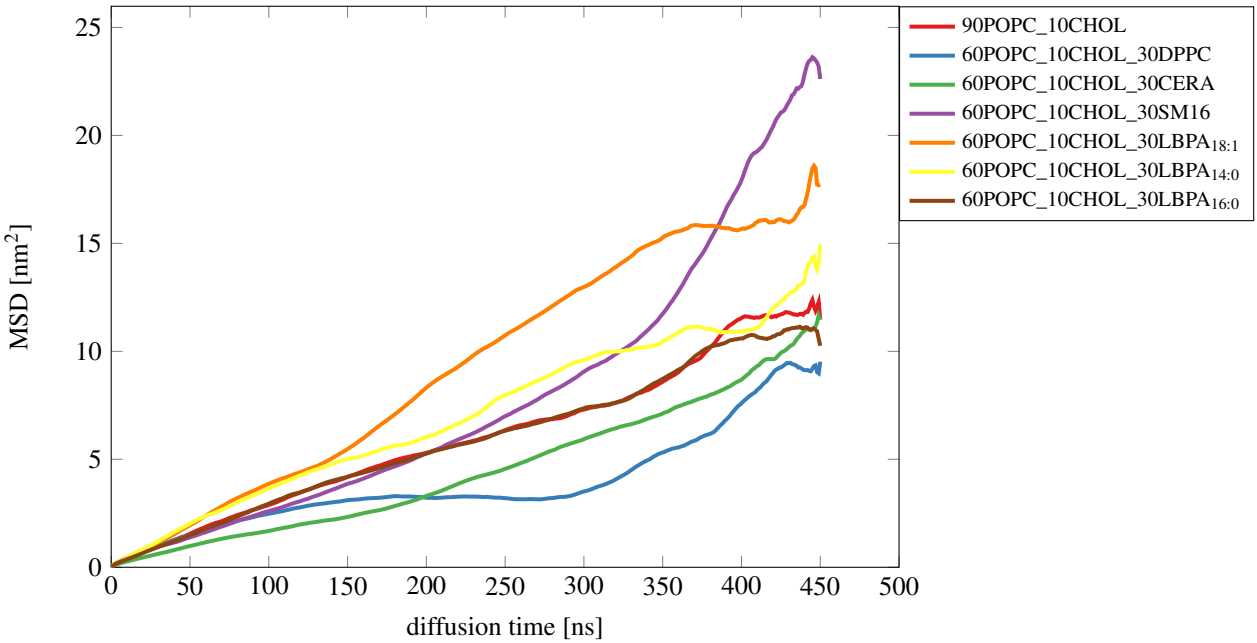


Figure 35: **MSD of cholesterol in xy-plane. Leaflet B.**

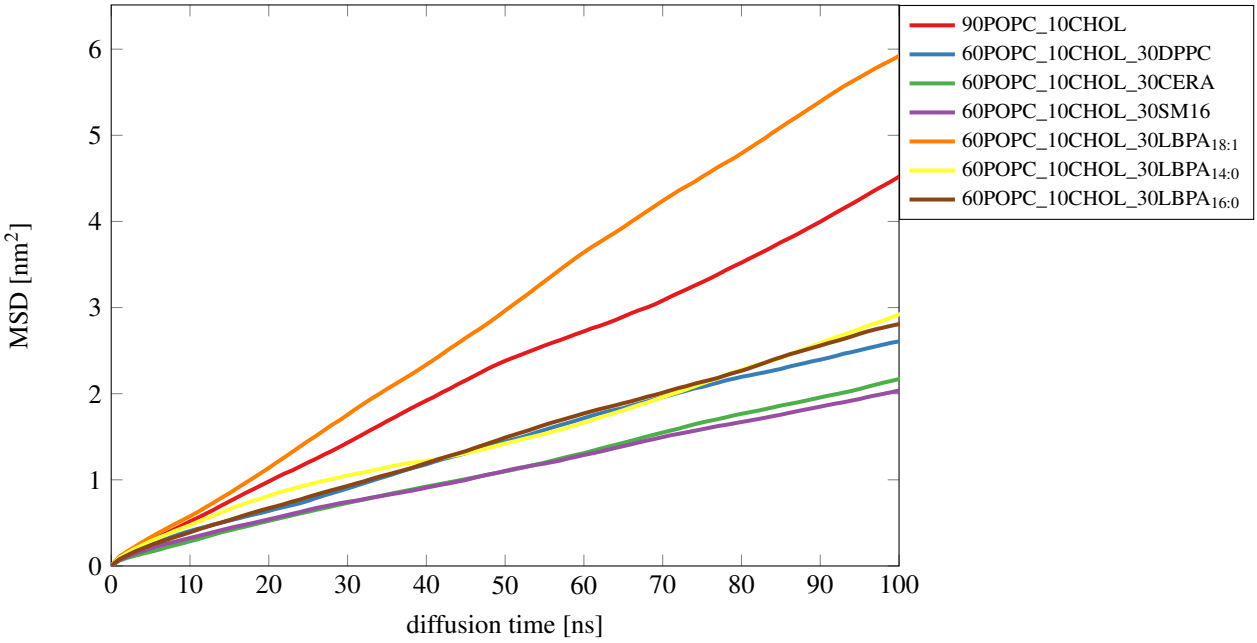


Figure 36: **MSD of cholesterol in xy-plane. 100ns diffusion. Leaflet A.**

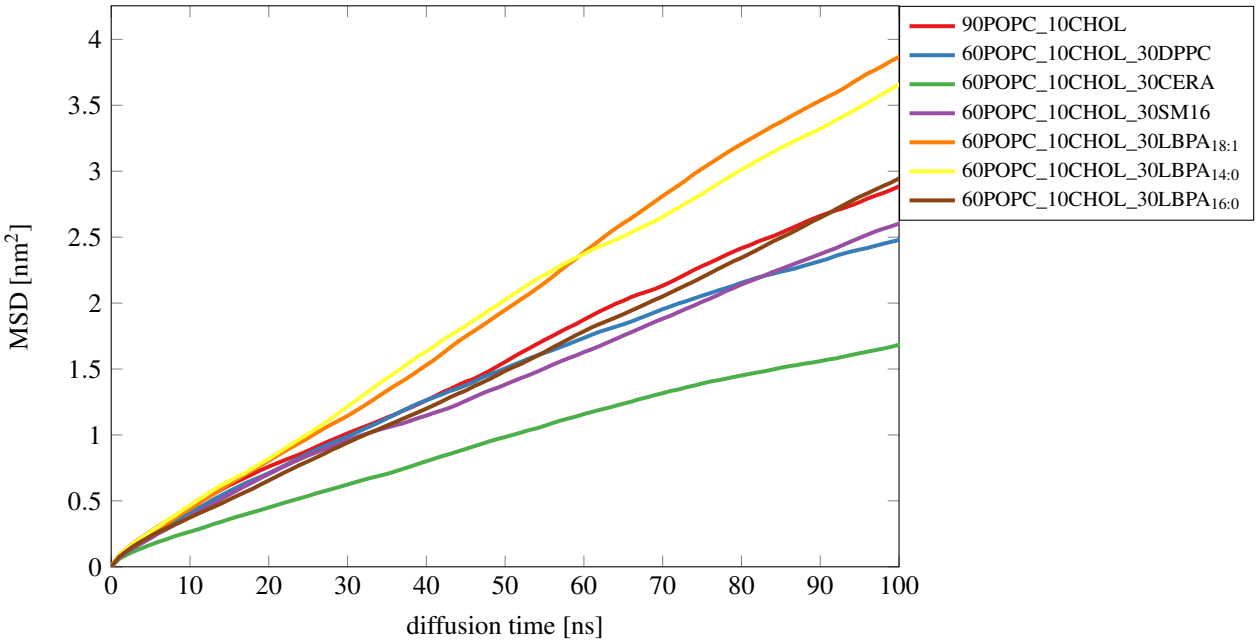


Figure 37: **MSD of cholesterol in xy-plane. 100ns diffusion. Leaflet B.**

2.11 Free energy calculations

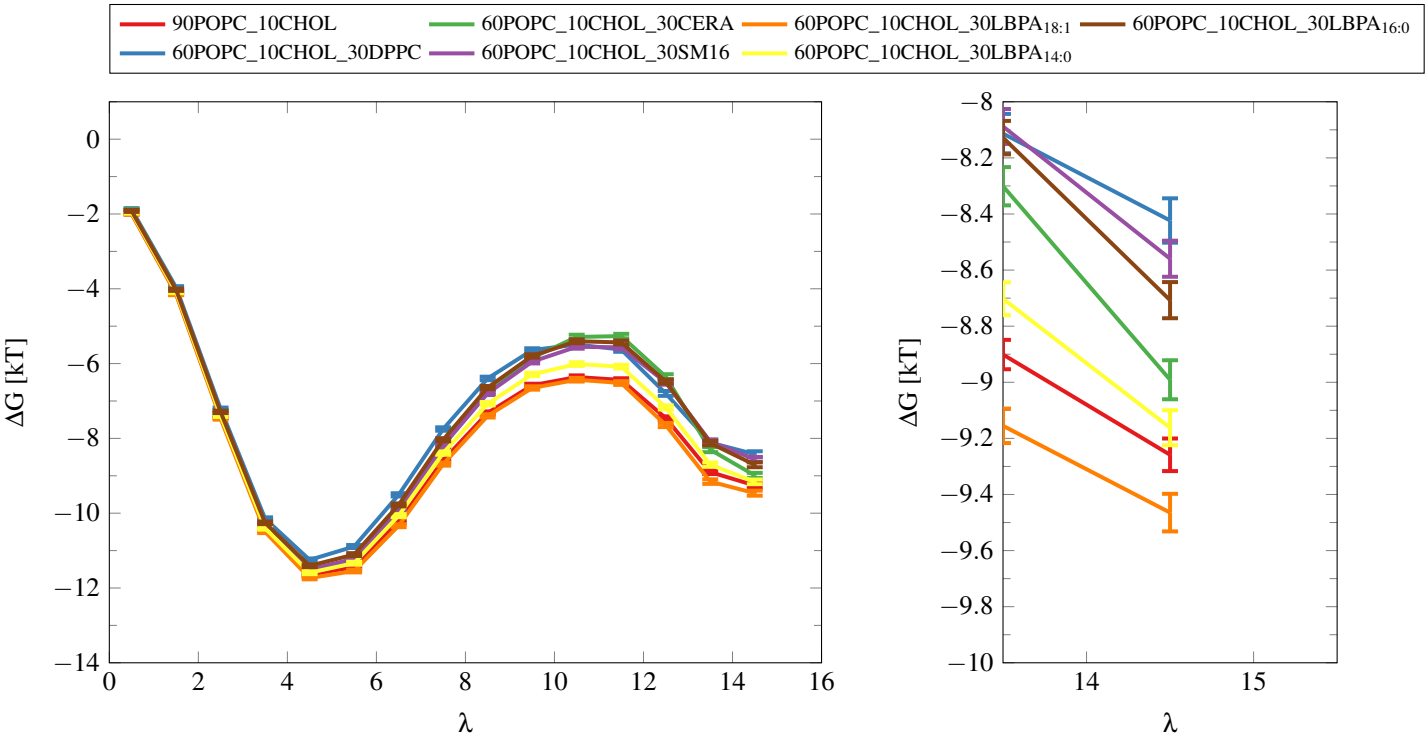


Figure 38: **Free energy integral. Leaflet A.** Length of simulations was 100 ns. The different inital structures were created by using different cholesterol molecules and different time frames of NPT-simulation. The plot is simply cumulative sum of ΔGs between lambdas (from bar.xvg file). The error estimate is sum of squares error (SSE) of bootsrap errors.

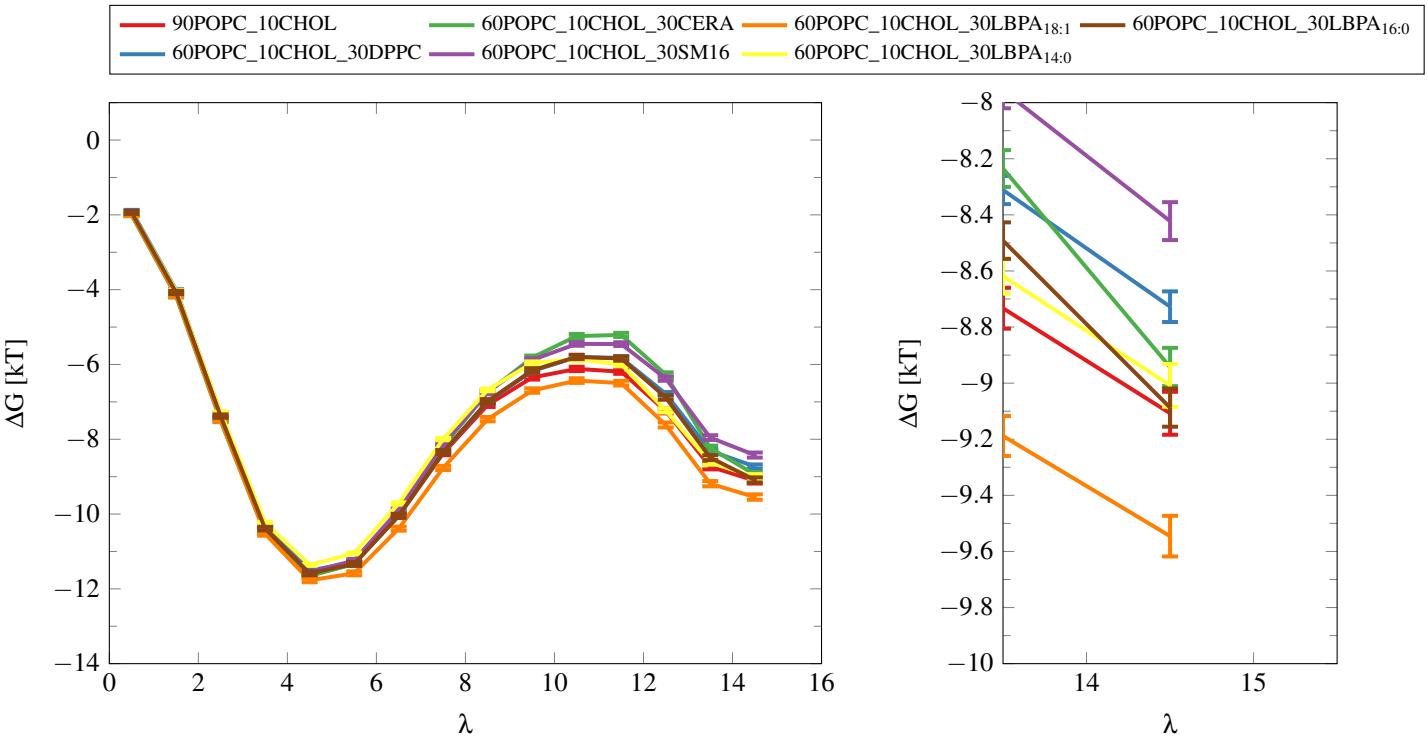


Figure 39: **Free energy integral. Leaflet B.** Same as figure above, but leaflet B was used for calculation. Simulation length was 100 ns. A flip-flop occurred in $\text{LBPA}_{14:0}$ system, and messed up the results.

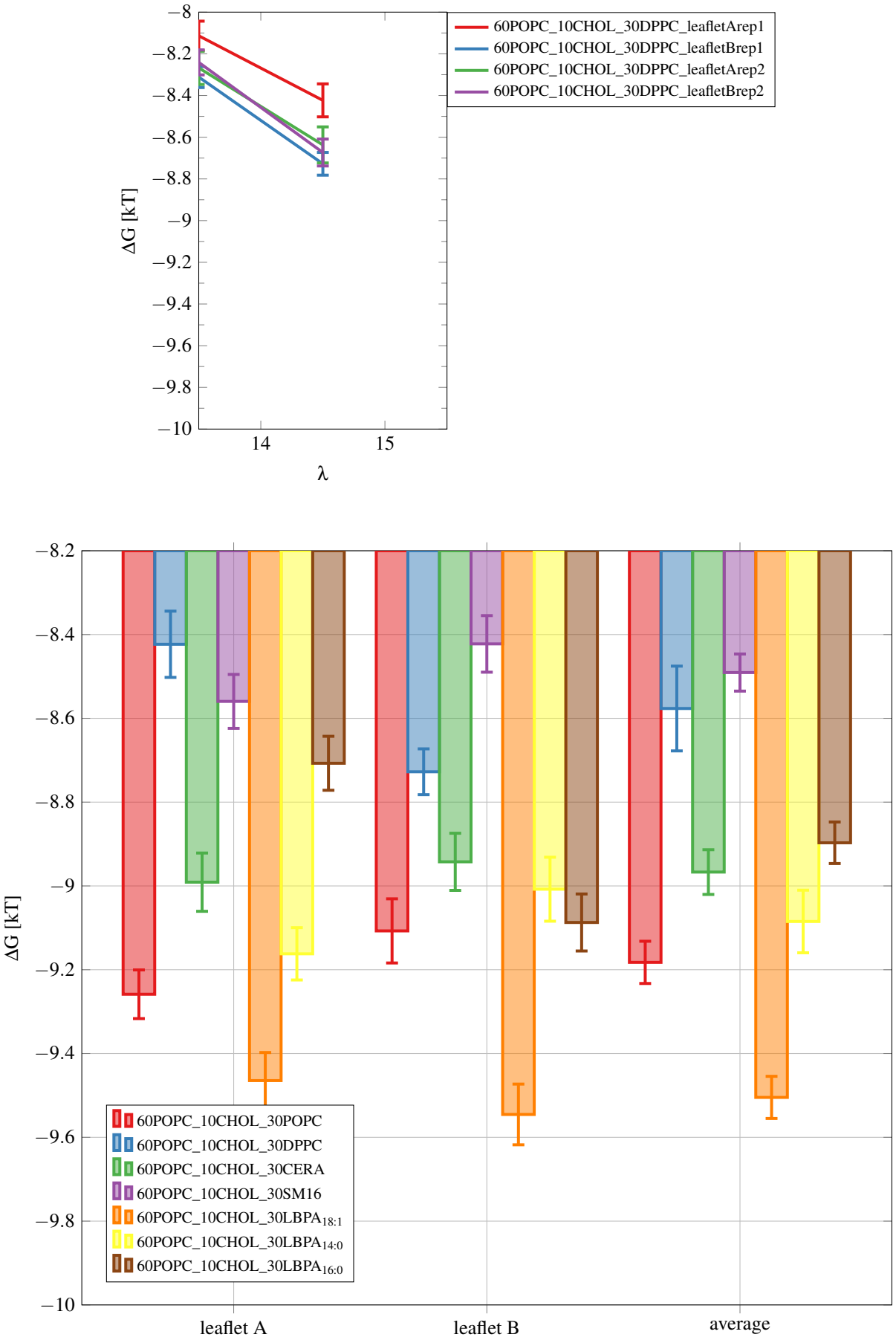


Figure 40: **ΔG of two leaflets compared.** Error estimate from using bootstrap with `nbmin=6` and `nbmax=10`

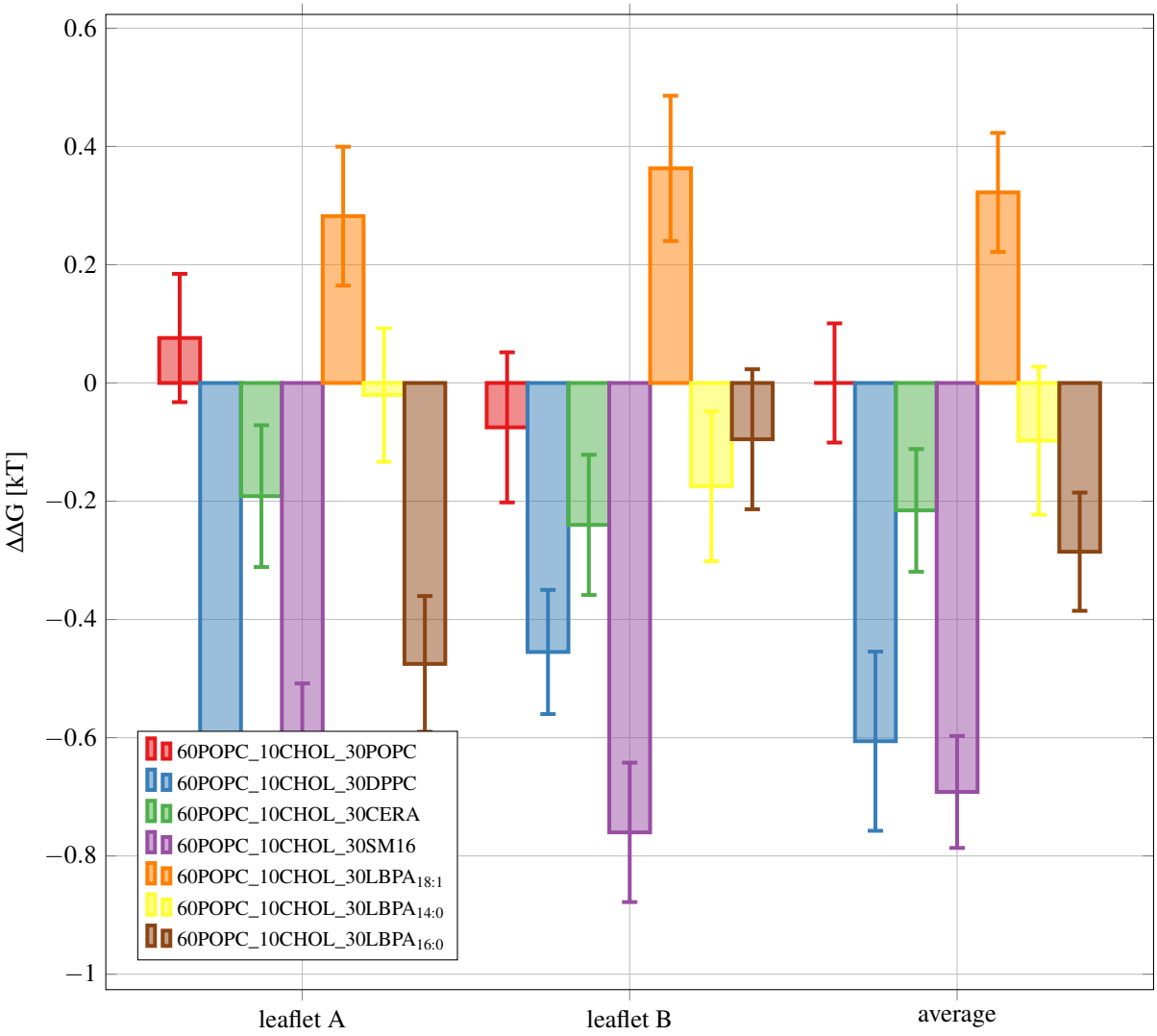


Figure 41: **Difference of ΔG compared to pure POPC+CHOL system (90POPC_10CHOL).**