Electronic Supplementary Material (ESI) for RSC Advances.

Molecular Mechanism of Skin Permeation Enhancing Effect of Ethanol: A Molecular Dynamics Study

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Supporting Information

S1. Interaction of ethanol with CER, CHOL, FFA and water at various ethanol concentration

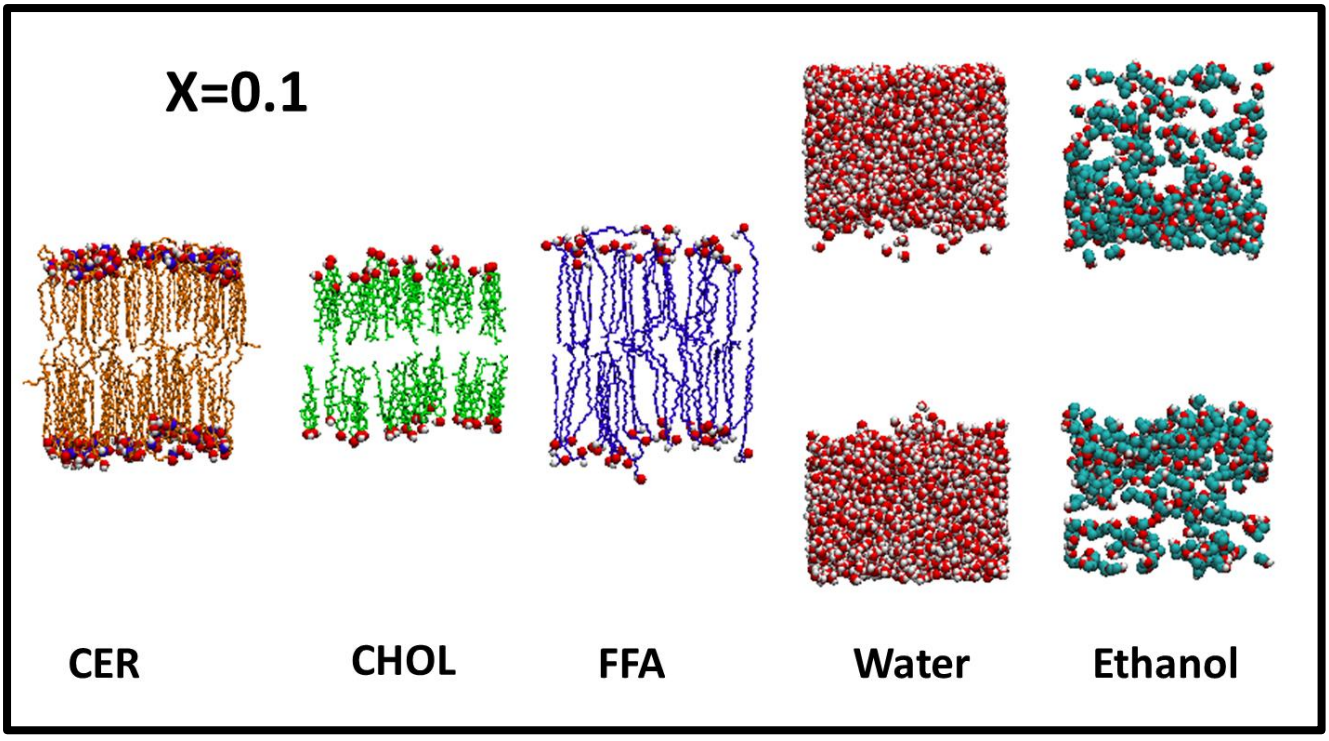

Figure S1. The snapshot of each component of the system at (x=0.1) in the end of 1.0 µs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software.

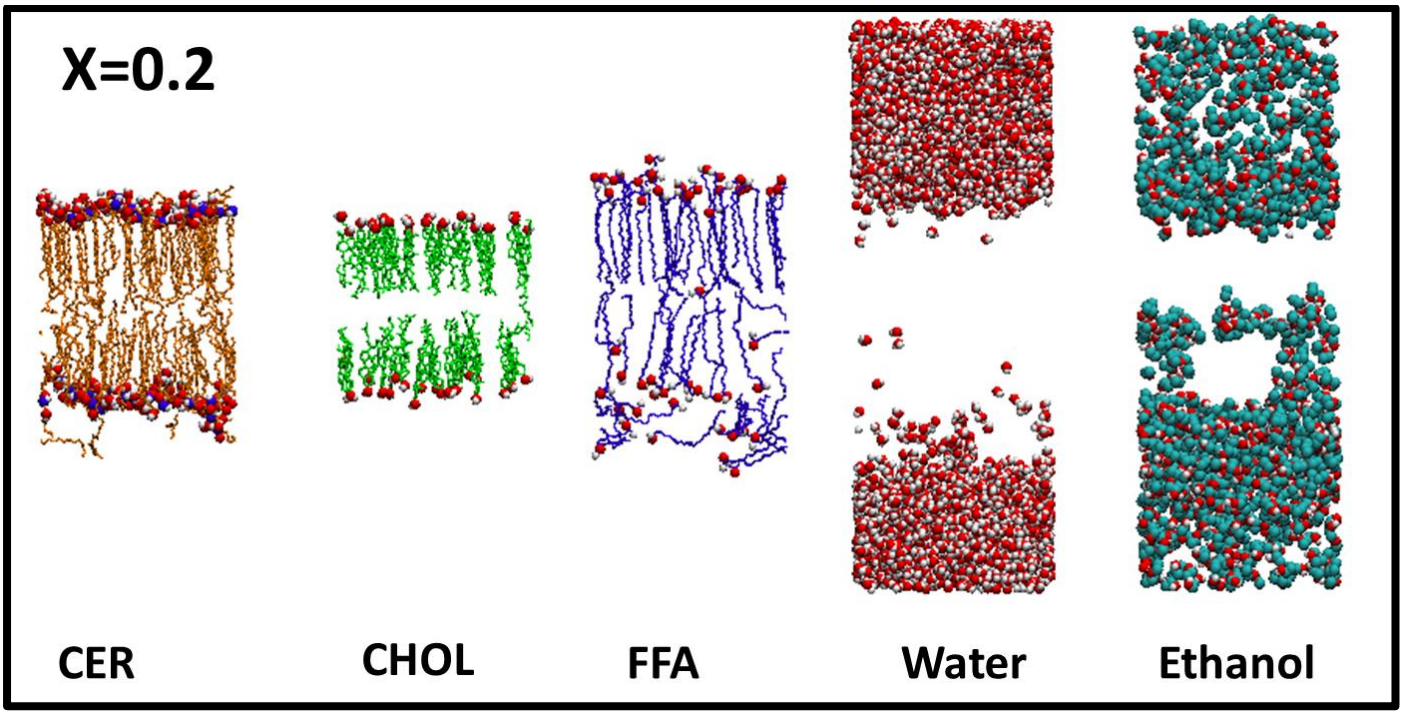

Figure S2. The snapshot of each component of the system at (x=0.2) in the end of 1.0 µs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software.

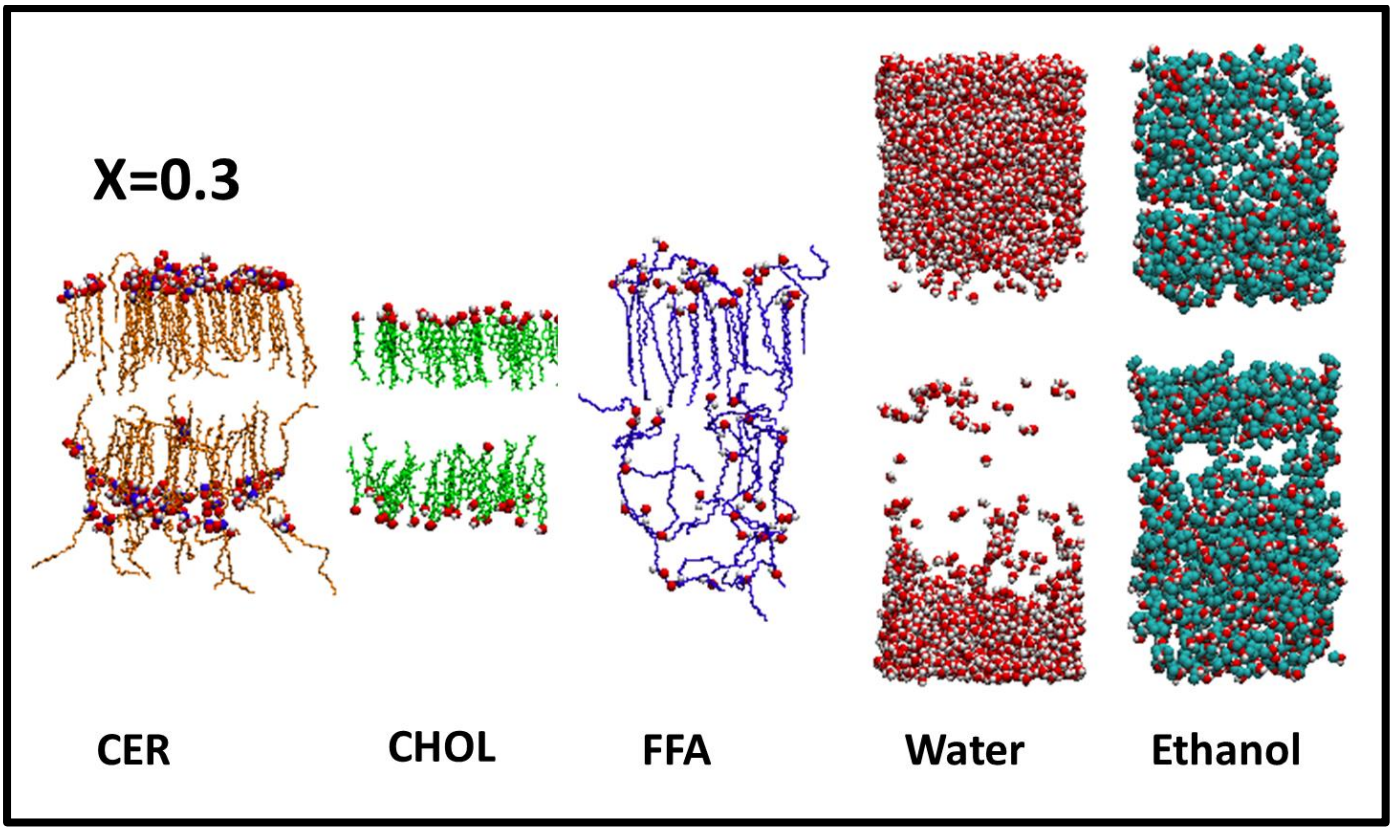

Figure S3. The snapshot of each component of the system at (x=0.3) in the end of 1.0 µs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software.

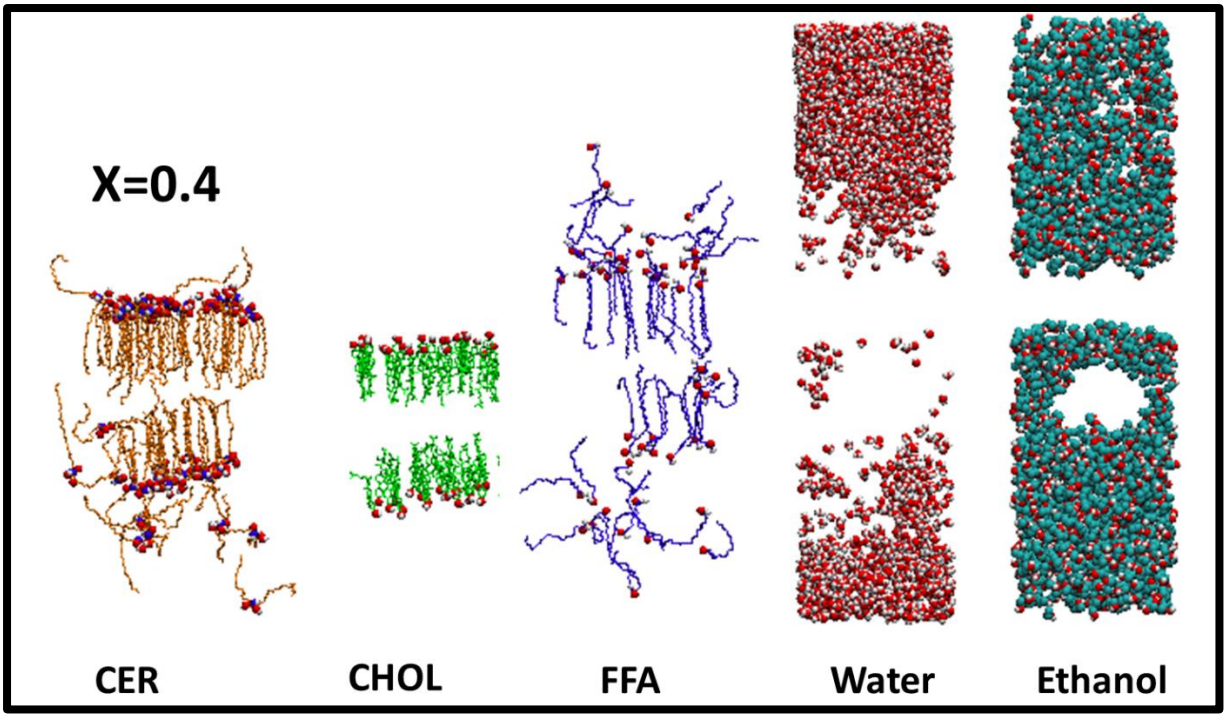

Figure S4. The snapshot of each component of the system at (x=0.4) in the end of 1.0 µs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software.

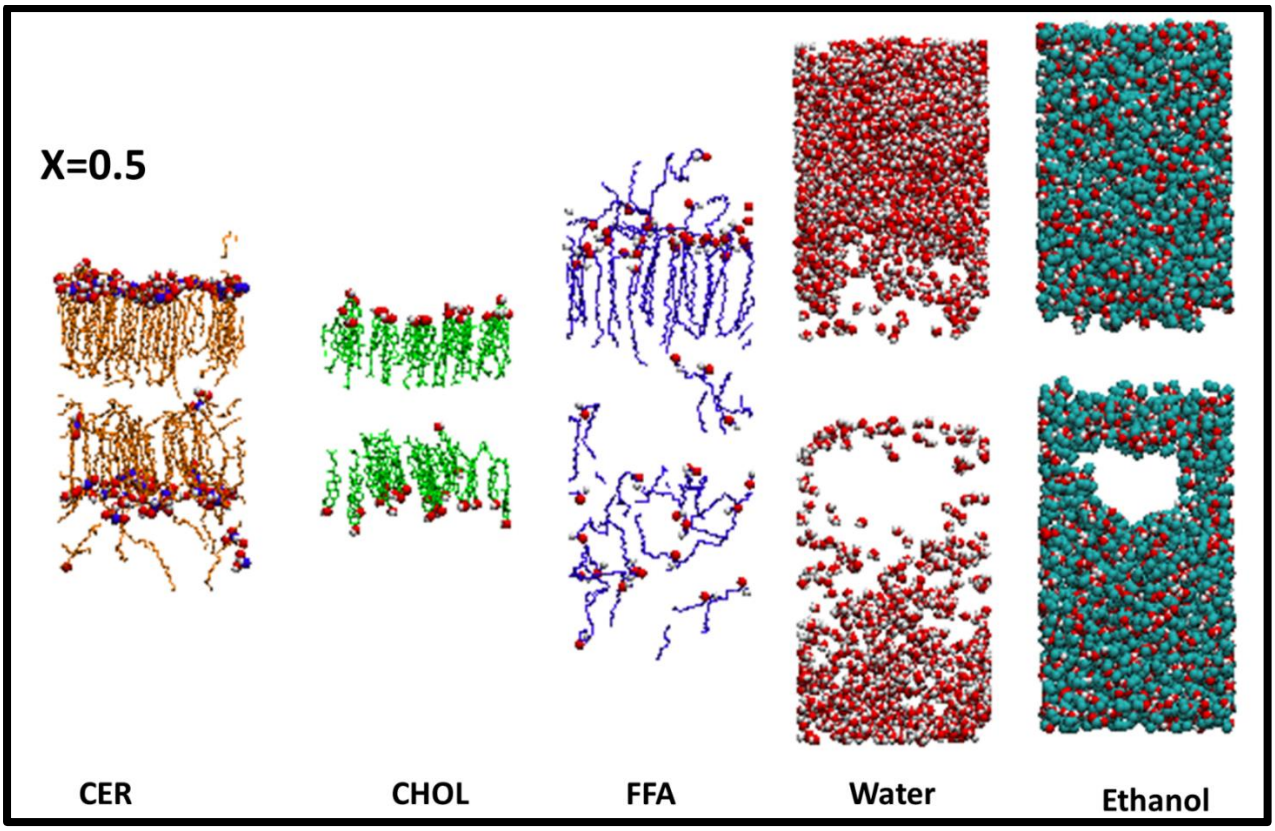

Figure S5. The snapshot of each component of the system at (x=0.5) in the end of 1.0 µs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software.

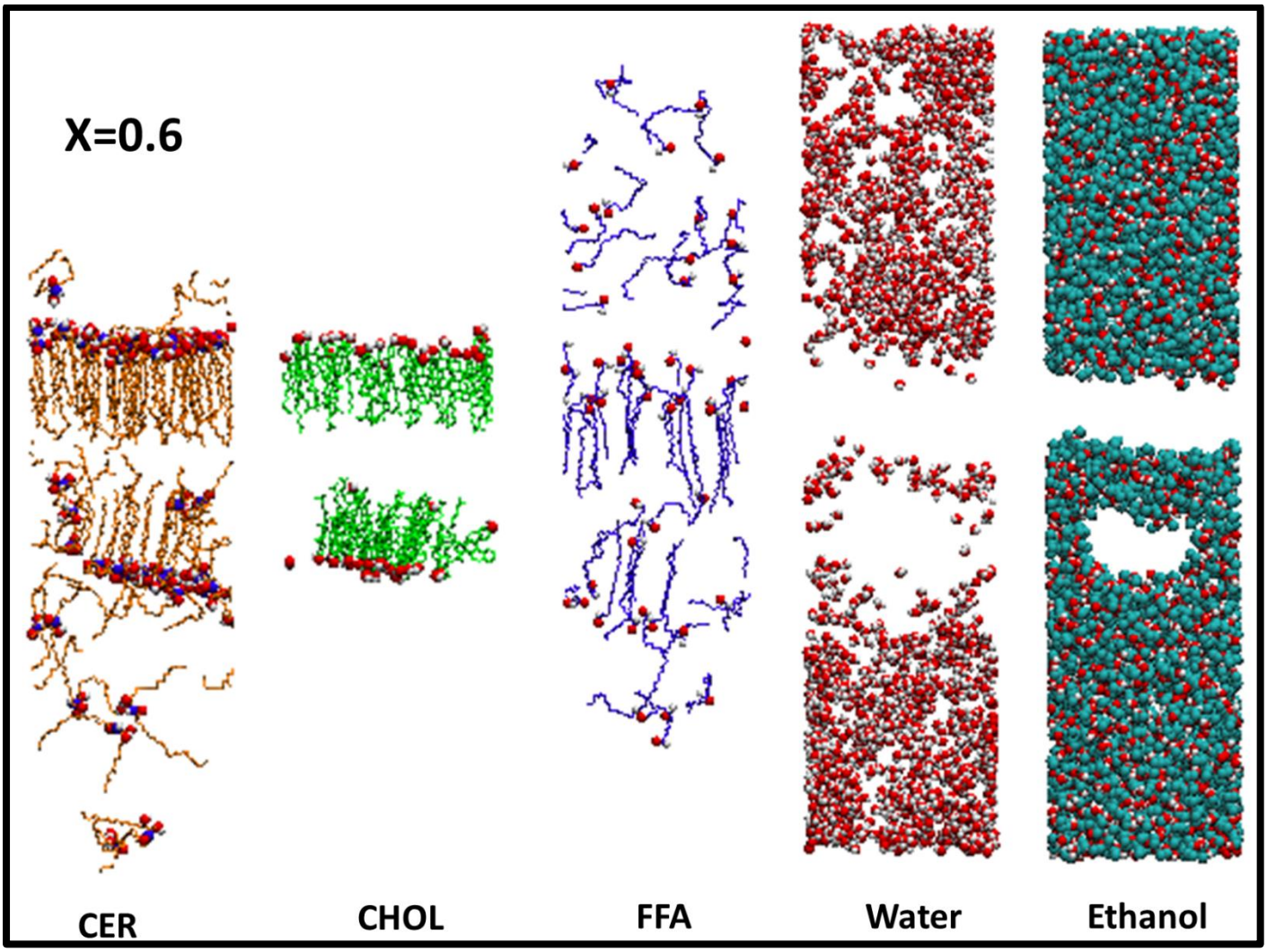

Figure S6. The snapshot of each component of the system at (x=0.6) in the end of 1.0 µs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software.

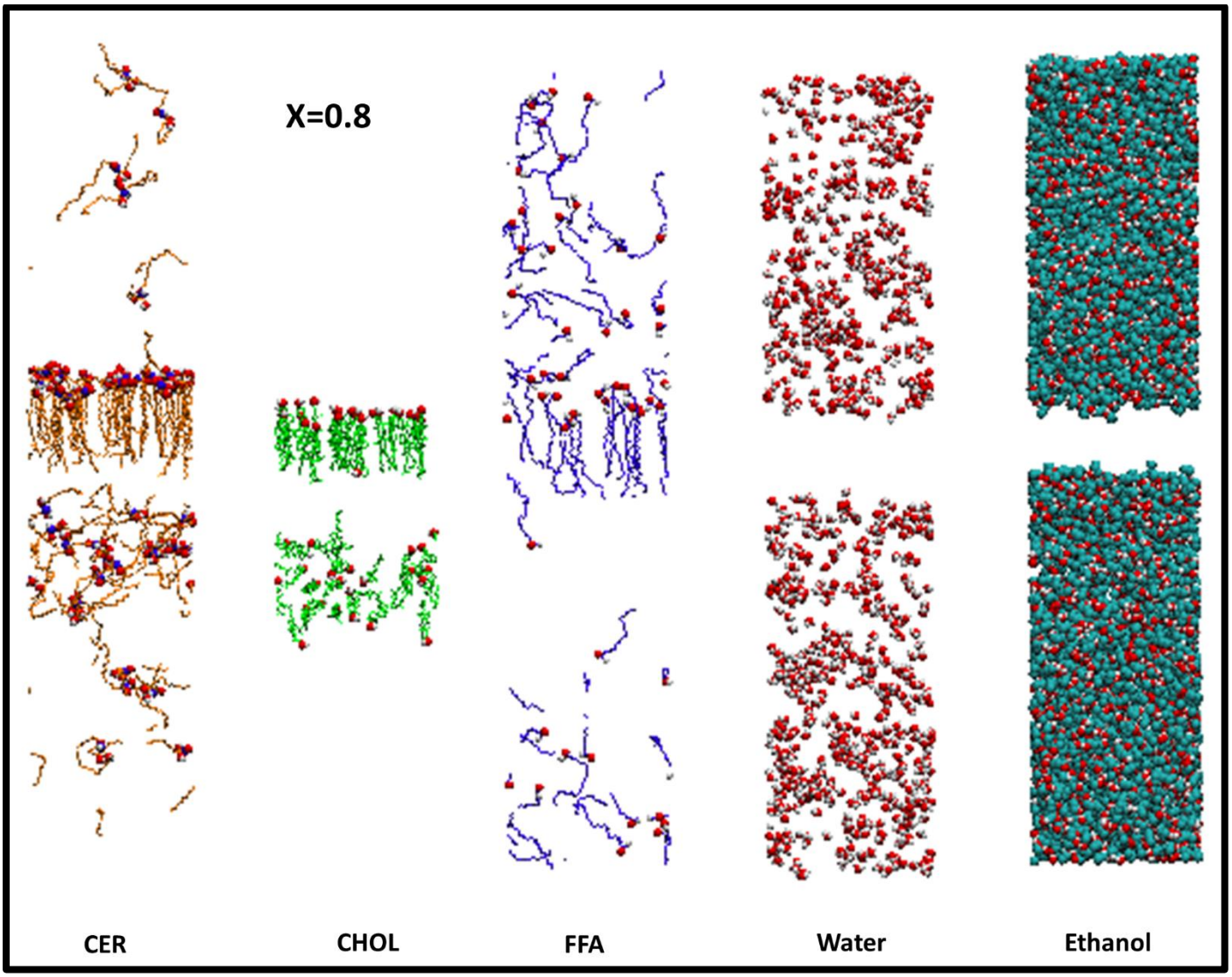

Figure S7. The snapshot of each component of the system at (x=0.8) in the end of 1.0 µs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software.

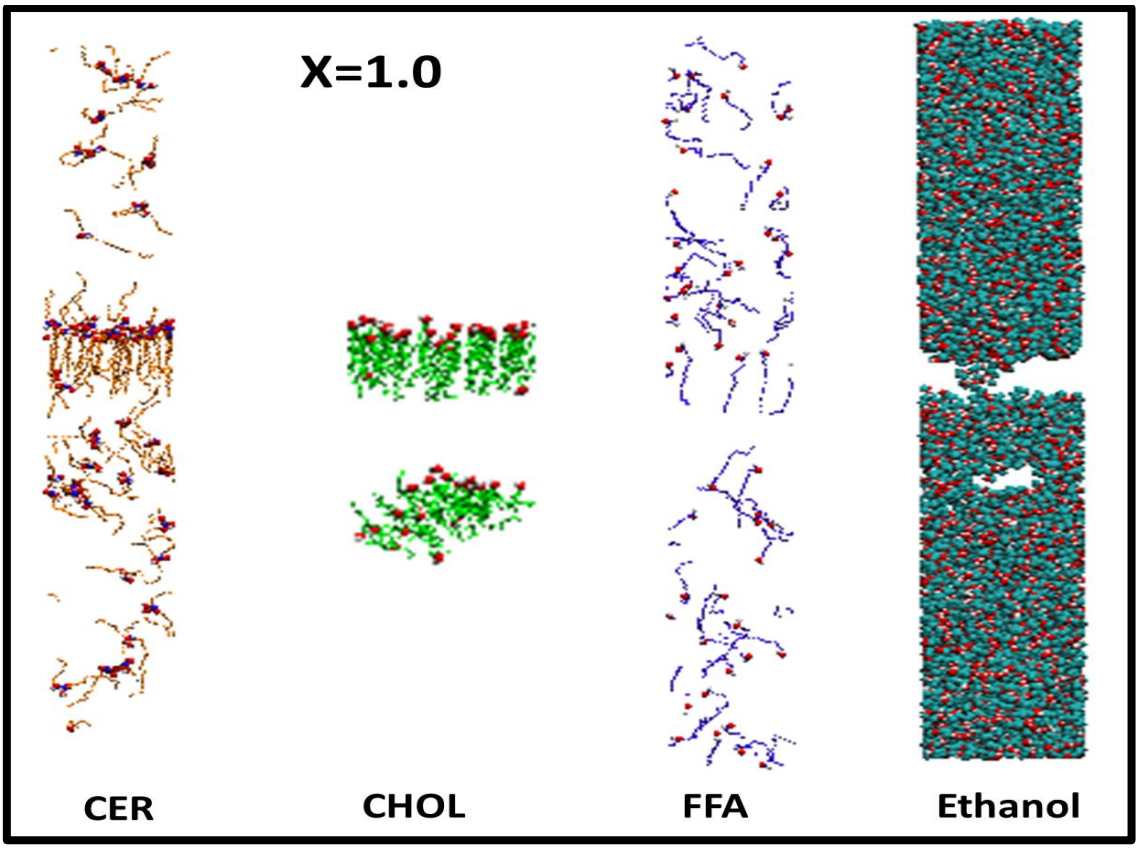

Figure S8. The snapshot of each component of the system at (x=1) in the end of 1.0 μs simulation run. The CER, CHOL and FFA are shown in orange, green and blue colour respectively. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software

S2. Interaction of FFA and CER with ethanol and various extraction mechanism

Movie S1: Extend conformation of the CER molecule. The CER, CHOL, FFA, ethanol and water are shown in orange, green, blue, red and cyan colour respectively and in point representation of VMD visualization software. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software. One of the CER having extended conformation is shown in vdw representation.

Movie S2: FFA extraction and its translocation back to the lipid layer. The CER, CHOL, FFA, ethanol and water are shown in orange, green, blue, red and cyan colour respectively and in point representation of VMD visualization software. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software. One of the FFA being extracted is shown in vdw representation.

Movie S3: CER extraction and its translocation back to the lipid layer. The CER, CHOL, FFA, ethanol and water are shown in orange, green, blue, red and cyan colour respectively and in point representation of VMD visualization software. The headgroup atoms of all three lipids are shown in vdw representation of VMD visualization software. One of the CER being extracted is shown in vdw representation.

S3. Time evolution of distance between head and tail group atoms of CER and FFA

The distance between the headgroup atom and tail group atom of both CER and FFA are calculated and tracked throughout the simulation time. Some of the patterns are shown in Fig S9.

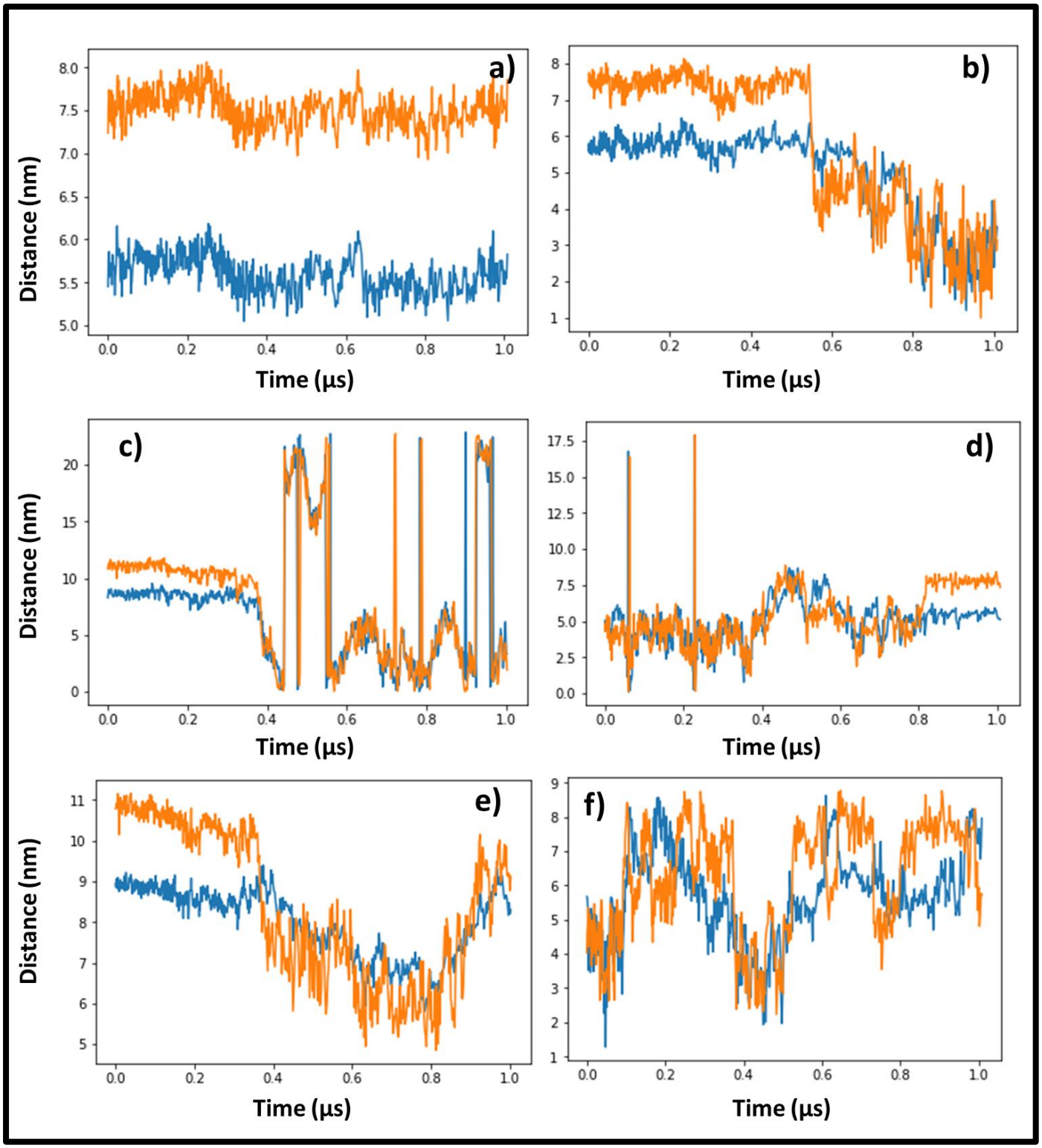

Figure S9. Time evolution of the distance between the headgroup atom and tail group atom of lipid molecule. The nitrogen and oxygen atom of CER and FFA and last carbon of FFA and CER tails are chosen to determine the distance between the headgroup and tail group atoms.

Based on patterns available in Fig S9, following events can be concluded

a. Lipid molecule stays inside the bilayer throughout the simulation time
b. The lipid molecule comes out (after 0.5 μs) from the lower leaflet of the bilayer and remain near to the headgroup and ethanol/water interface
c. The lipid has been extracted and reached in the bulk and crossed the periodic boundary of the box several times.
d. The lipid inside the bilayer only but going through several conformational changes
e. The lipid molecule come out of from the lower leaflet, remain near to the headgroup for some time and goes back into the bilayer interior but not with regular conformation
f. The lipid moves inside the bilayer interior and interact with ethanol/water at interface situated at both leaflets

We have manually checked time evolution of individual CER and FFA molecule in each system. Two numbers are reported here, i) number of lipids which comes out of the lipid bilayer and ii) total number of lipids which are extracted. In the former one, lipid which has shown pattern b, c, d and e are used and for latter one only event c is used.

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S4. Evolution of density of bilayer constituents along the bilayer normal

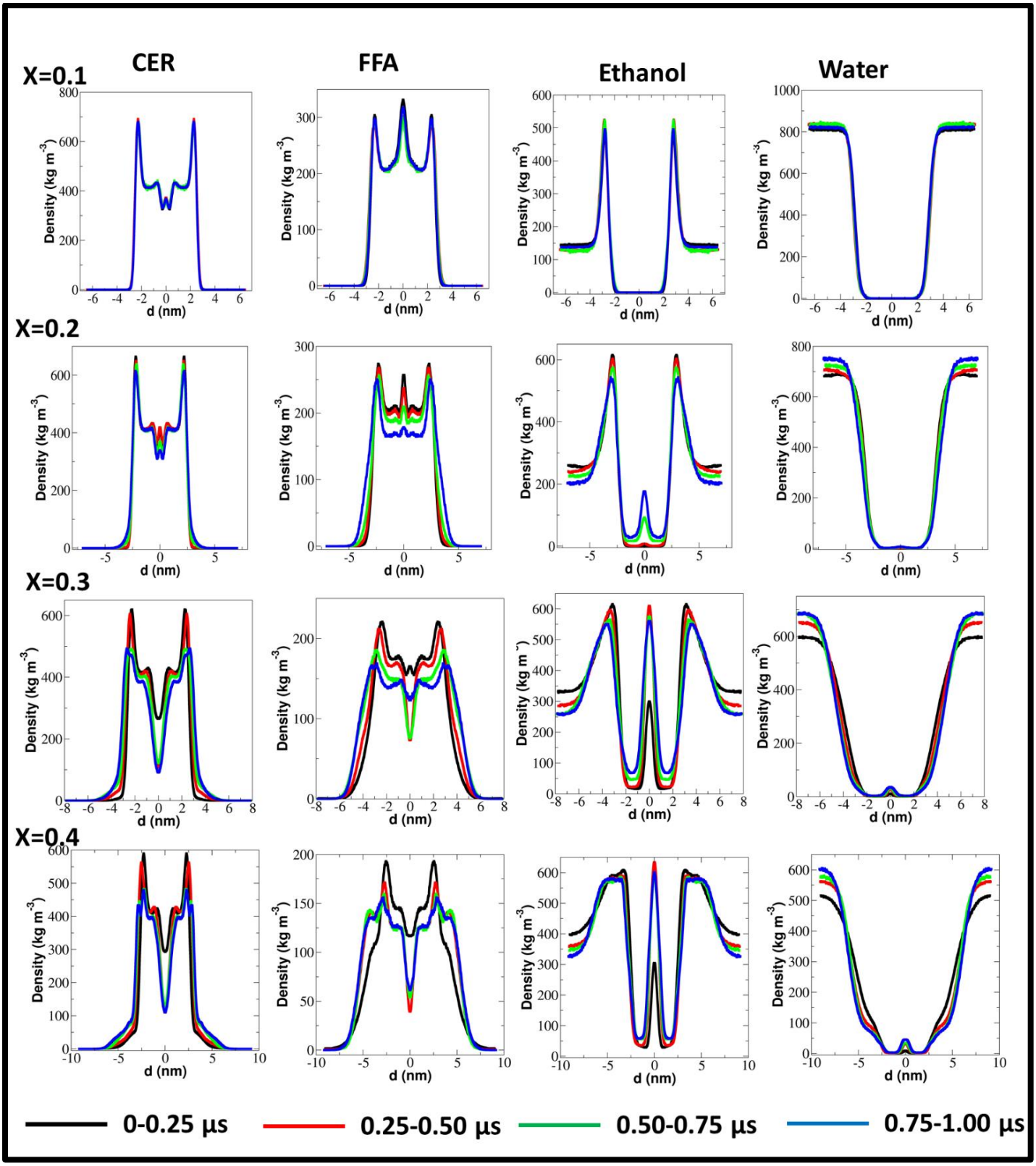

Figure S10. Density of a) ethanol b) water c) FFA and d) CER and along the bilayer normal calculated in time interval of every 0.25 µs at various ethanol concentration.

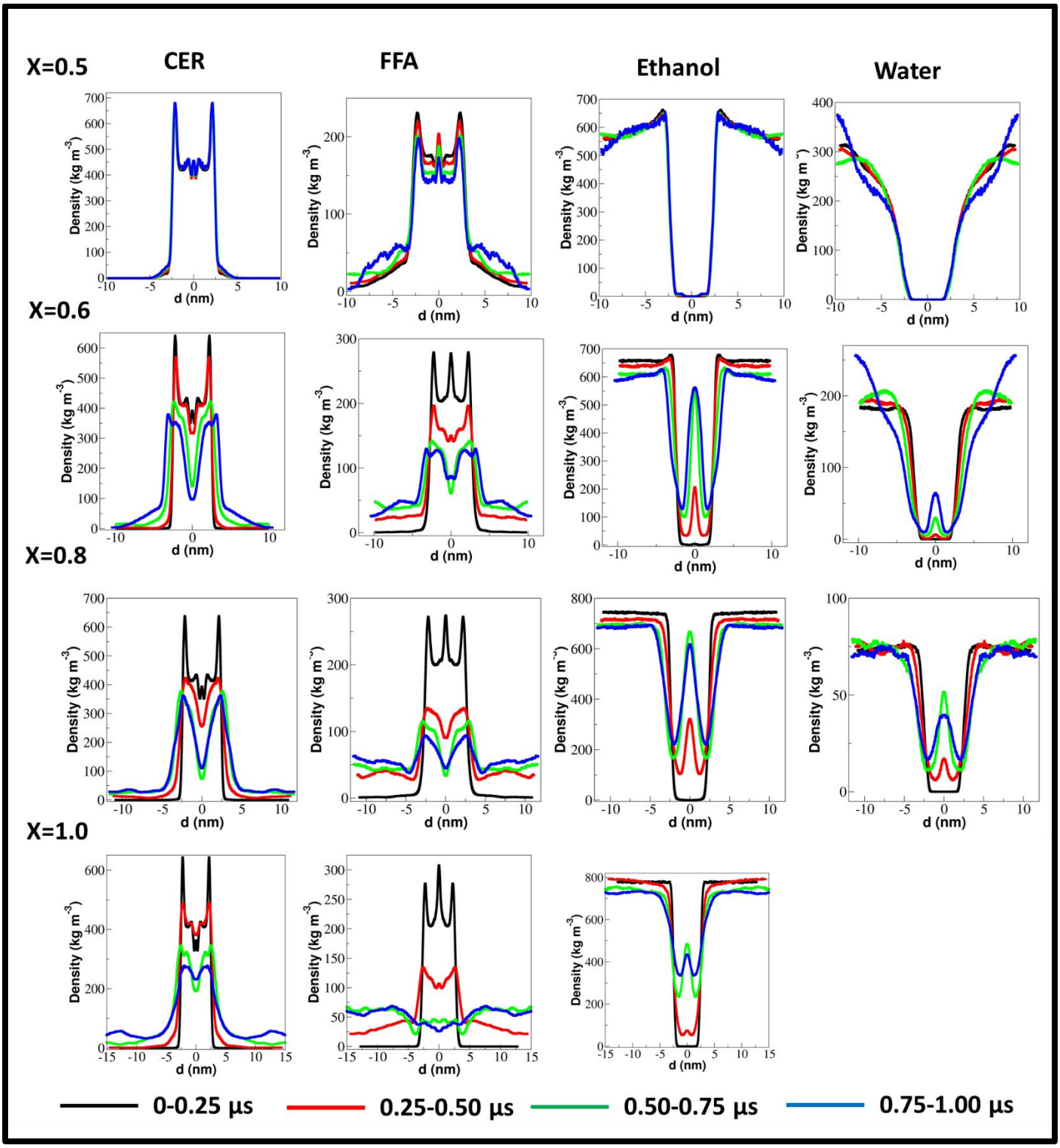

Figure S11. Density of a) ethanol b) water c) FFA and d) CER and along the bilayer normal calculated in time interval of every 0.25 µs at various ethanol concentration.

S5. Free energy profile of individual simulations at various ethanol concentrations

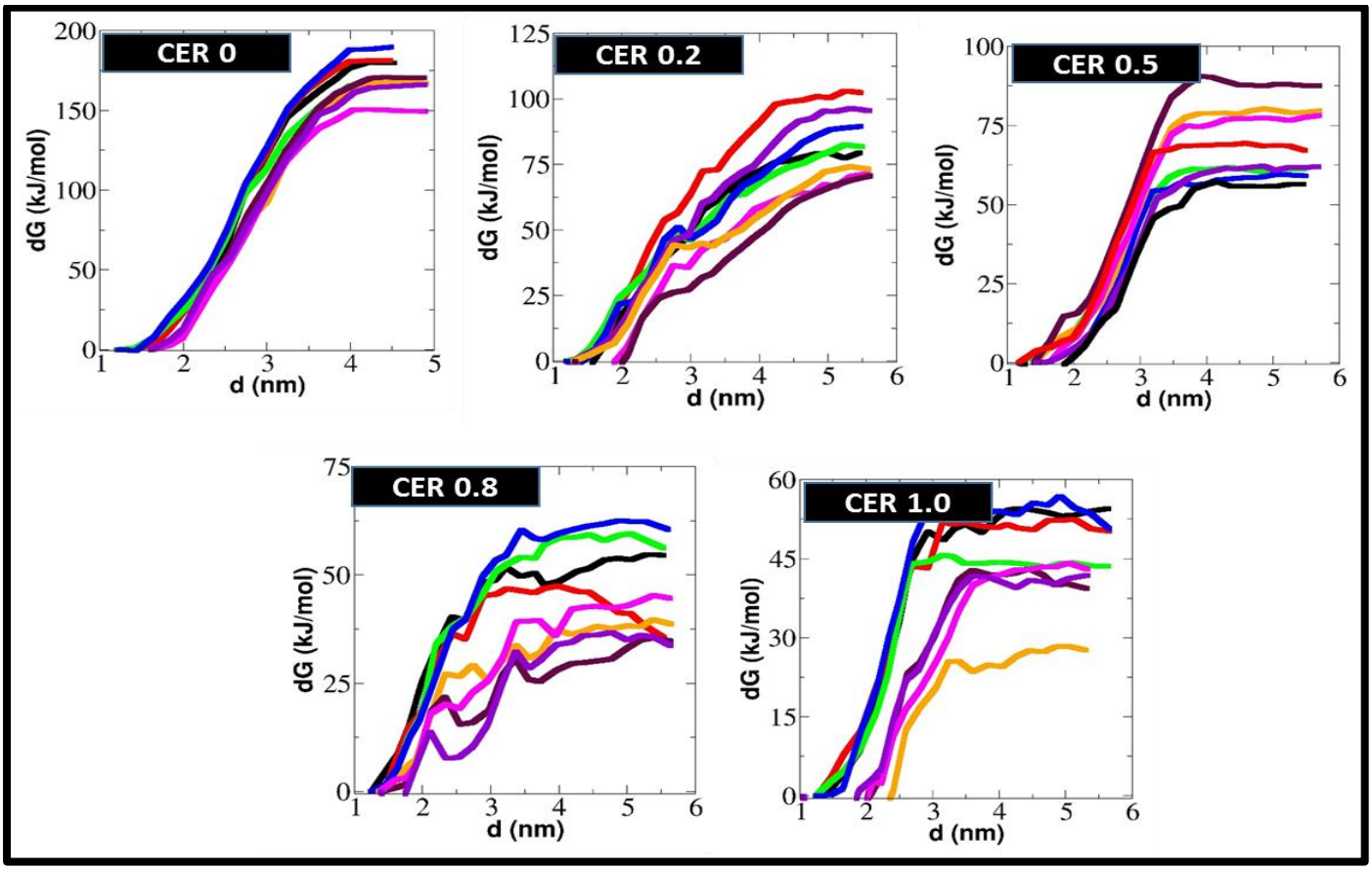

Figure S12. Free energy of extraction of CER from the skin lipid bilayer at various ethanol concentration. For each case in total 8 different simulations are performed and profile for each simulation is shown here. Average of these profiles are used for the calculation of average free energy of extraction. The profiles are similar for a given case but different in magnitude due to local environment.

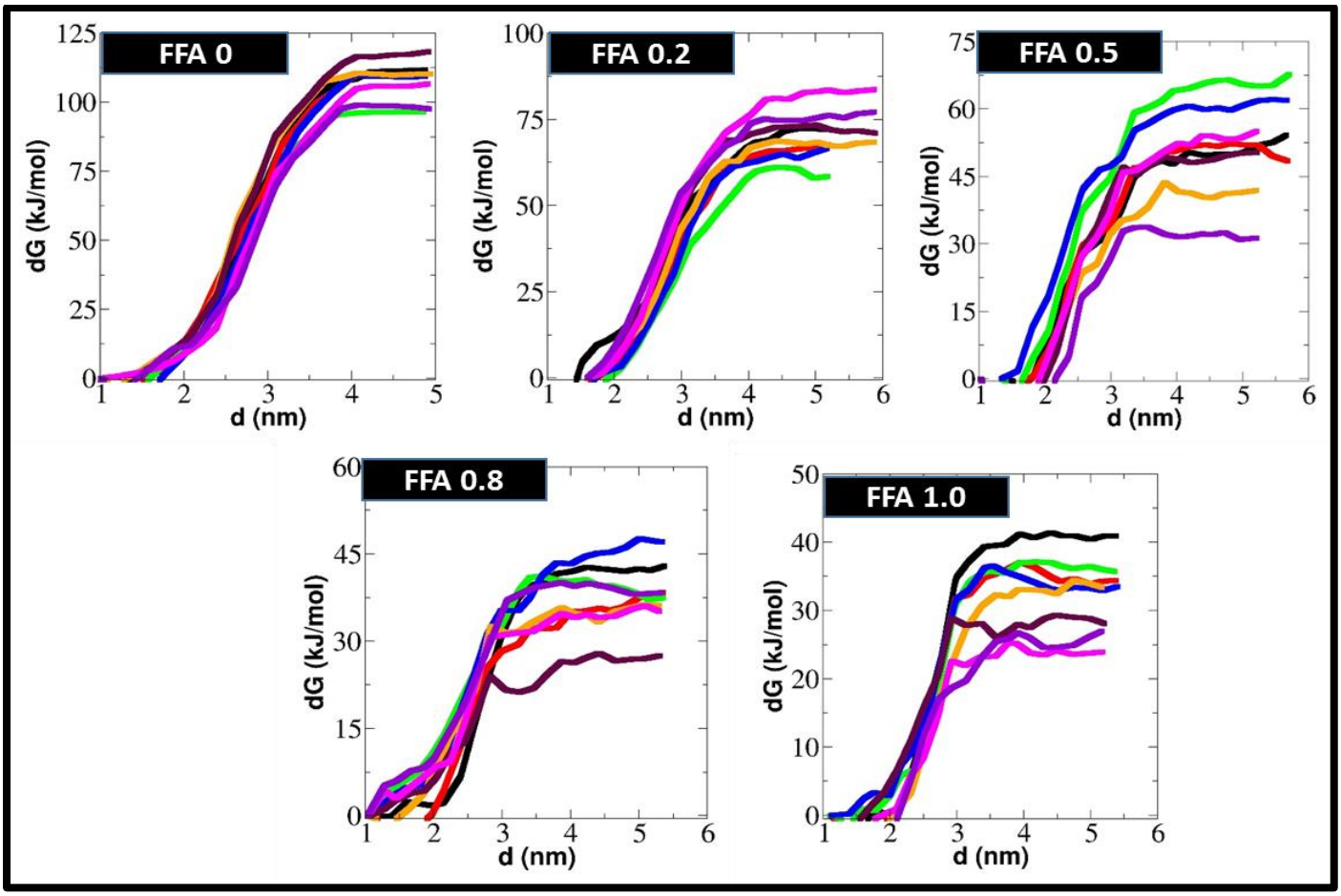

Figure S13. Free energy of extraction of FFA from the skin lipid bilayer at various ethanol concentration. For each case in total 8 different simulations are performed and profile for each simulation is shown here. Average of these profiles are used for the calculation of average free energy of extraction. The profiles are similar for a given case but different in magnitude due to local environment.

S6. Convergence of free energy profile and umbrella sampling simulations

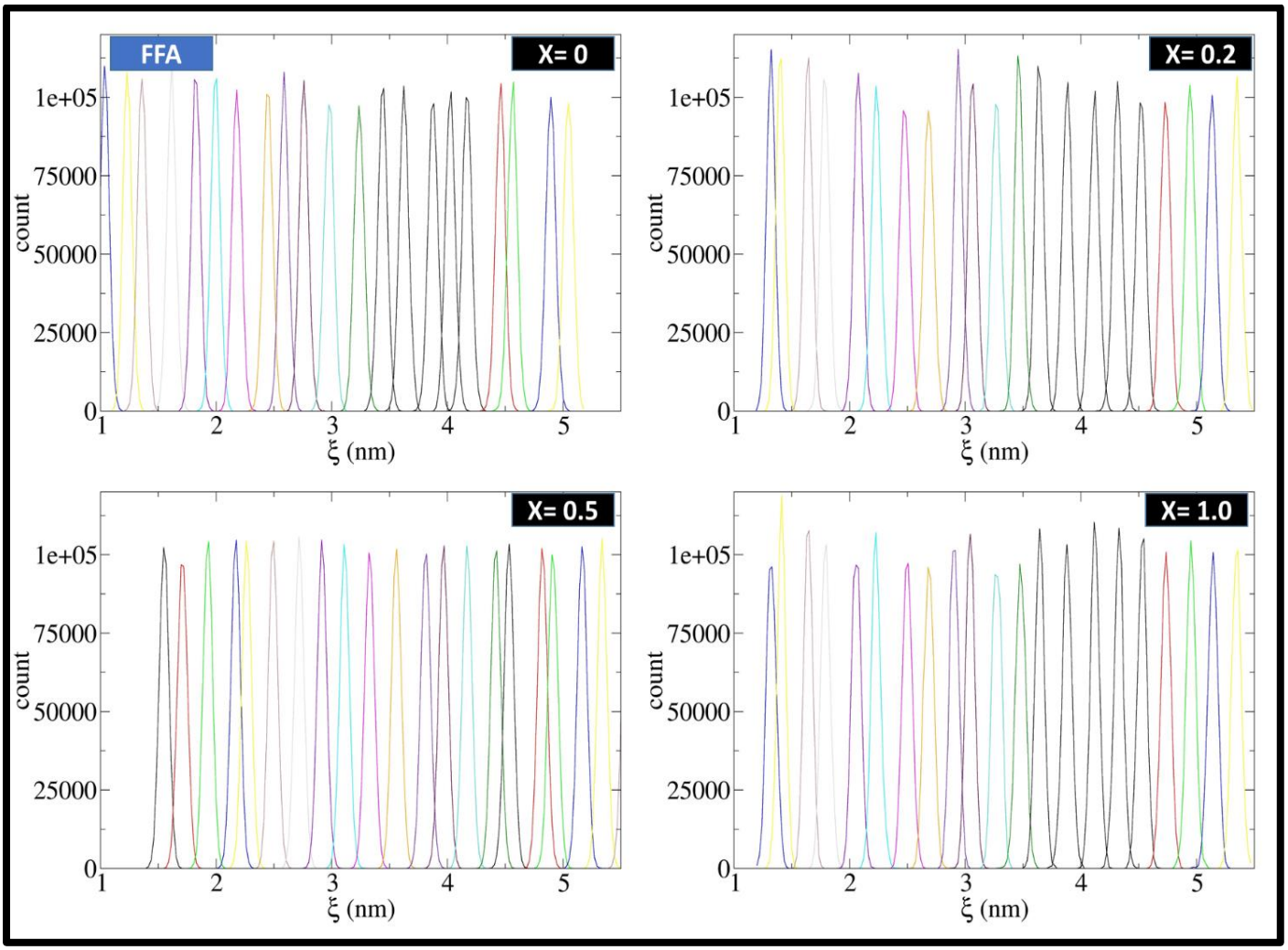

Figure S14. Overlap of simulation windows during umbrella sampling run (FFA extraction) at various ethanol concentration.

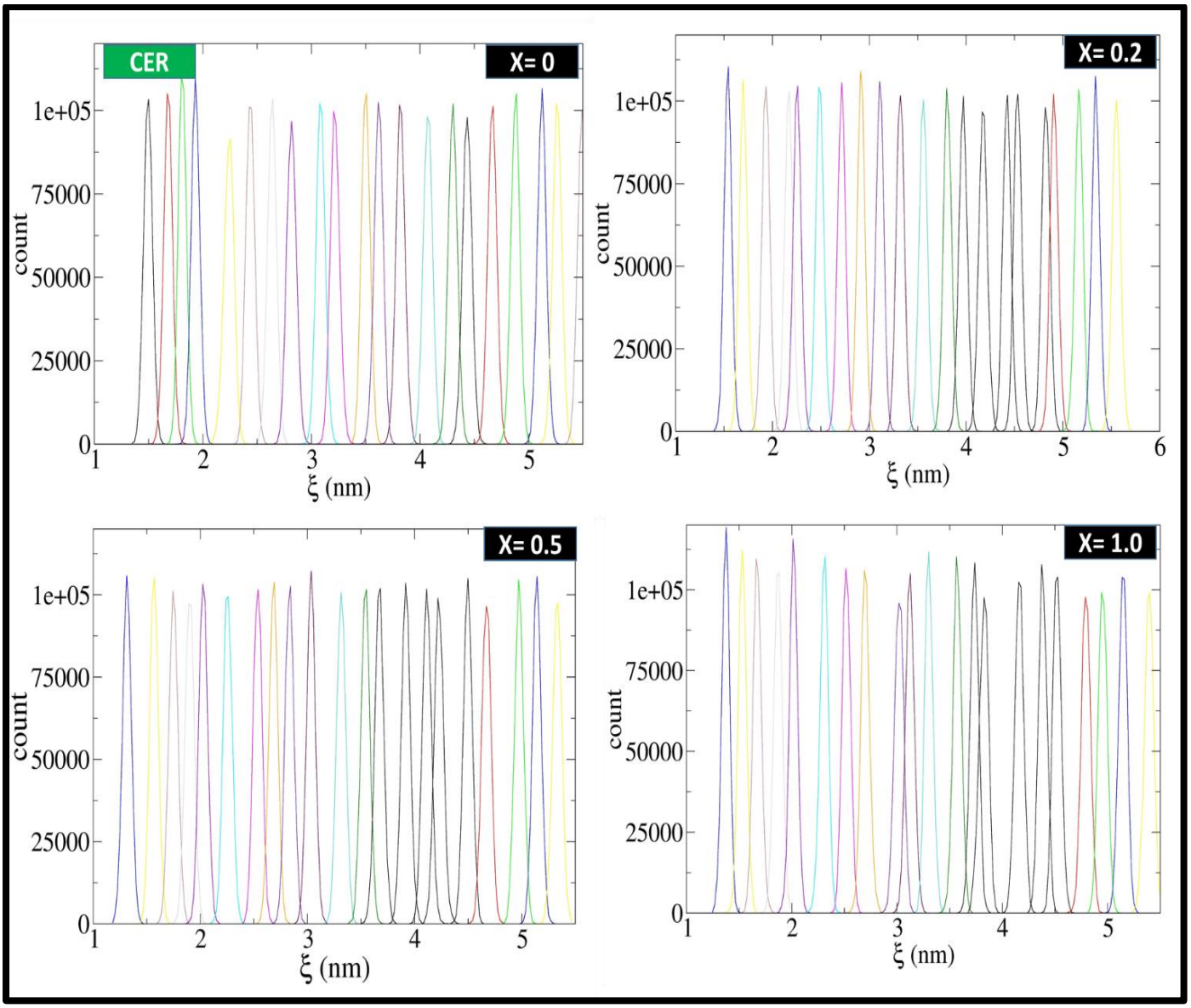

Figure S15. Overlap of simulation windows during umbrella sampling run (CER extraction) at various ethanol concentration.

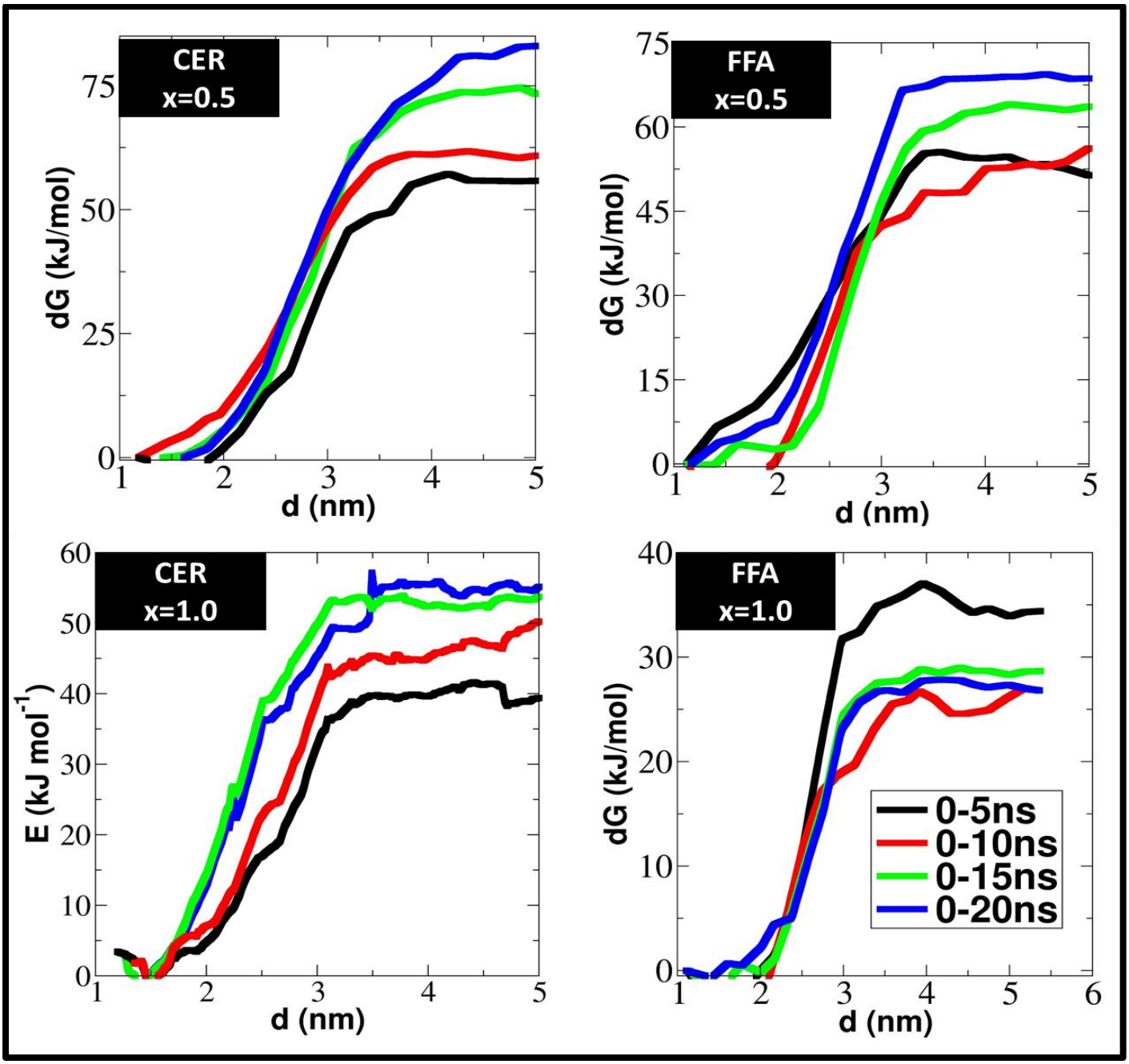

Figure S16. Convergence of free energy profile during 20 ns umbrella sampling run in various cases. The profiles are obtained at various time interval as shown in the index.