



DEER of Spin-Labeled Stearic Acids in Model Phospholipid Membranes Reveals Alternative Cluster Formation in Two Opposing Leaflets

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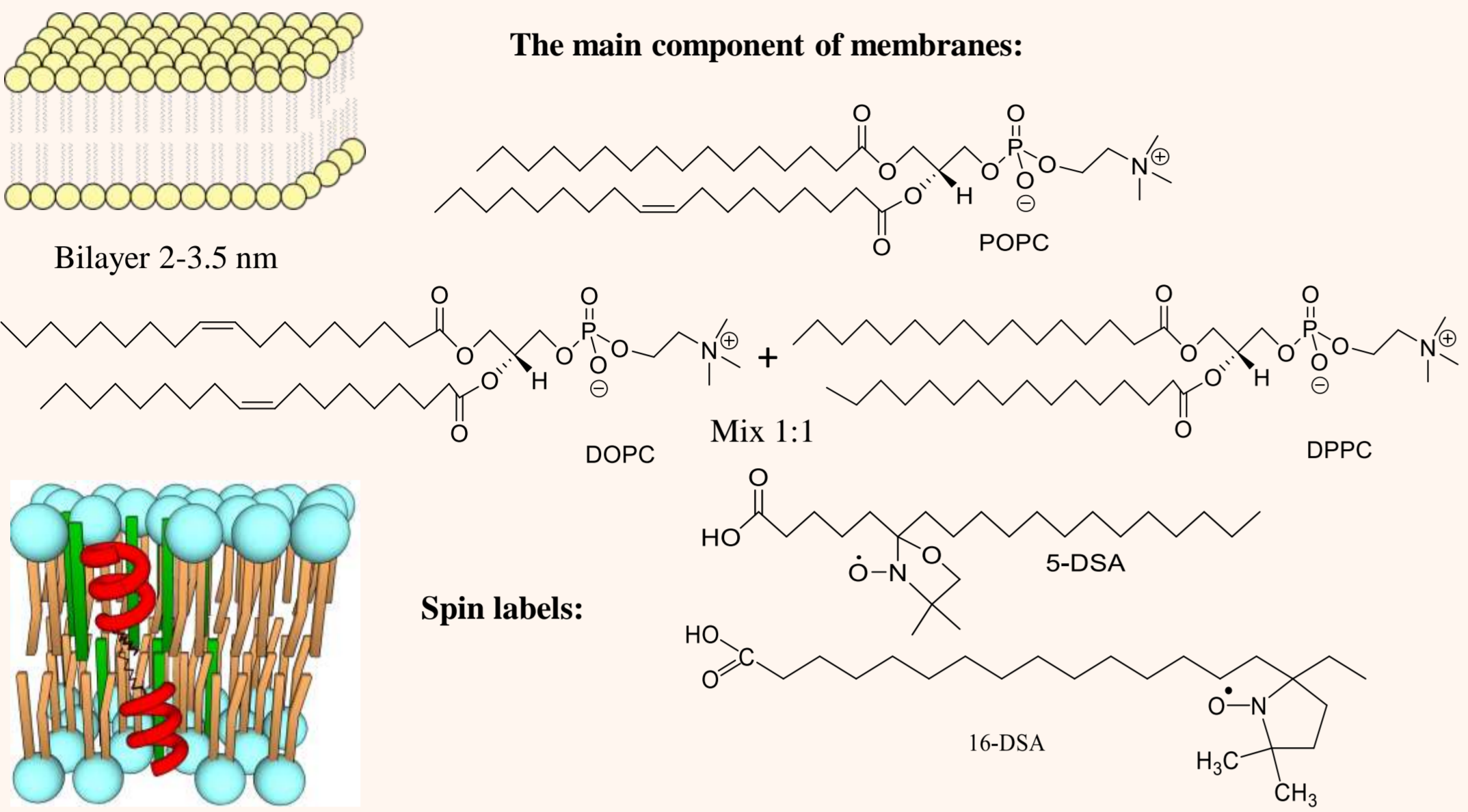
Introduction

Free fatty acids are essential components of biological membranes that are important for their functioning: they increase the membrane fluidity, serve as energy supply and a source of structural components, and participate in lipid metabolism and other biological processes.

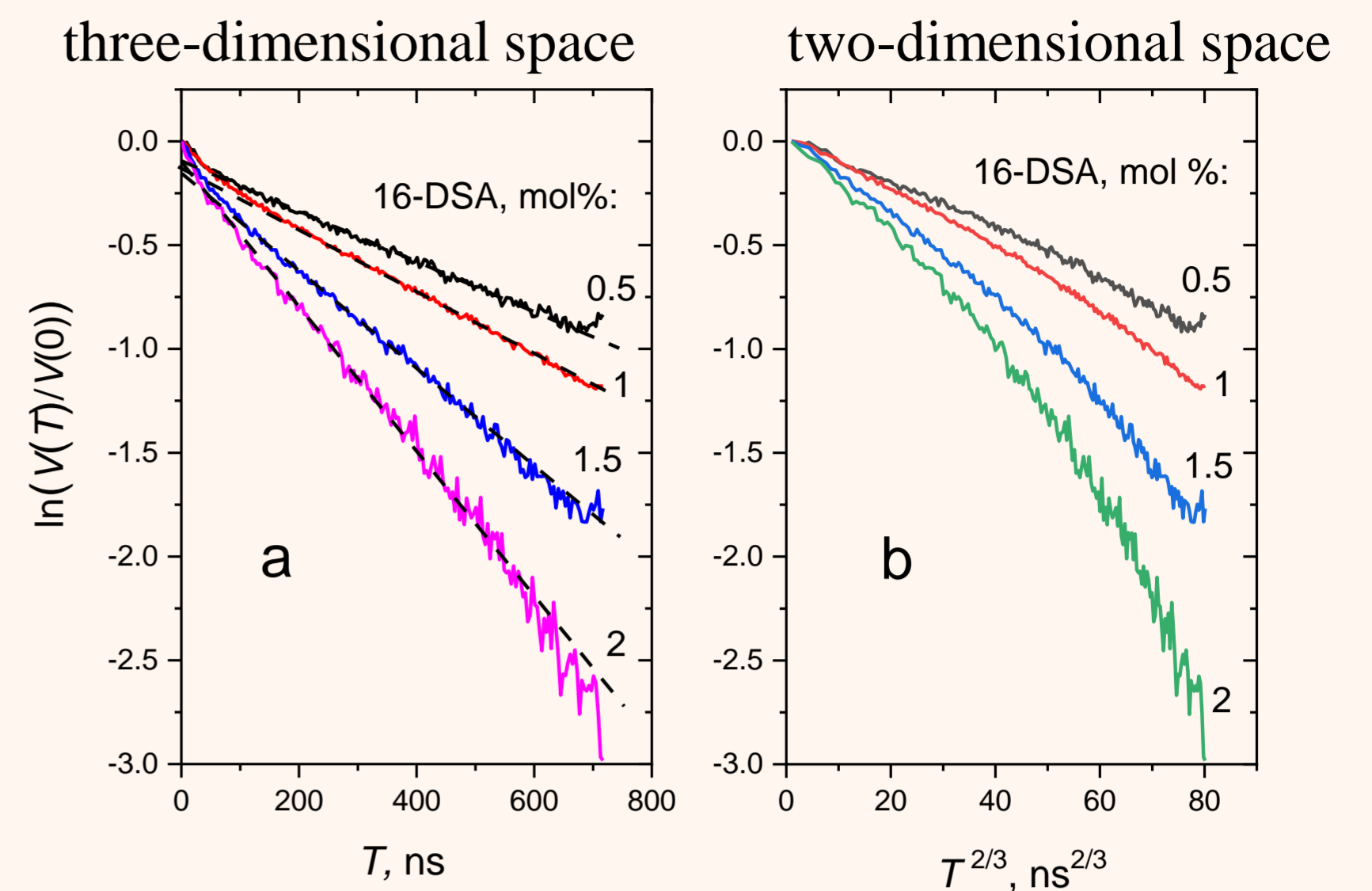
The main approach used was a pulsed version of EPR - double electron-electron resonance spectroscopy (DEER, also known as PELDOR). PELDOR spectroscopy is based on an electron spin echo phenomenon; it is used to determine the nanoscale distances between spin labels and study spatial distribution of spin labels. Here, PELDOR is applied to study the clustering of spin-labeled stearic acids in model synthetic membranes.

Model membranes

The main component of membranes:



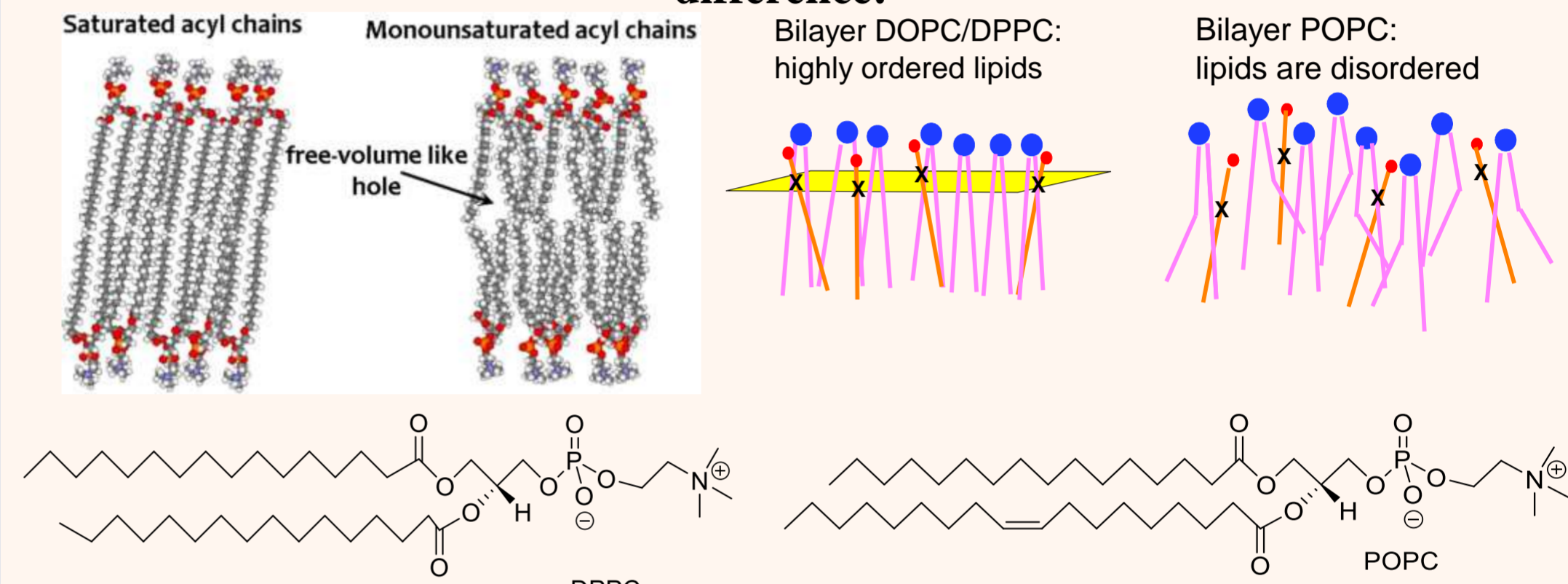
Bilayer POPC



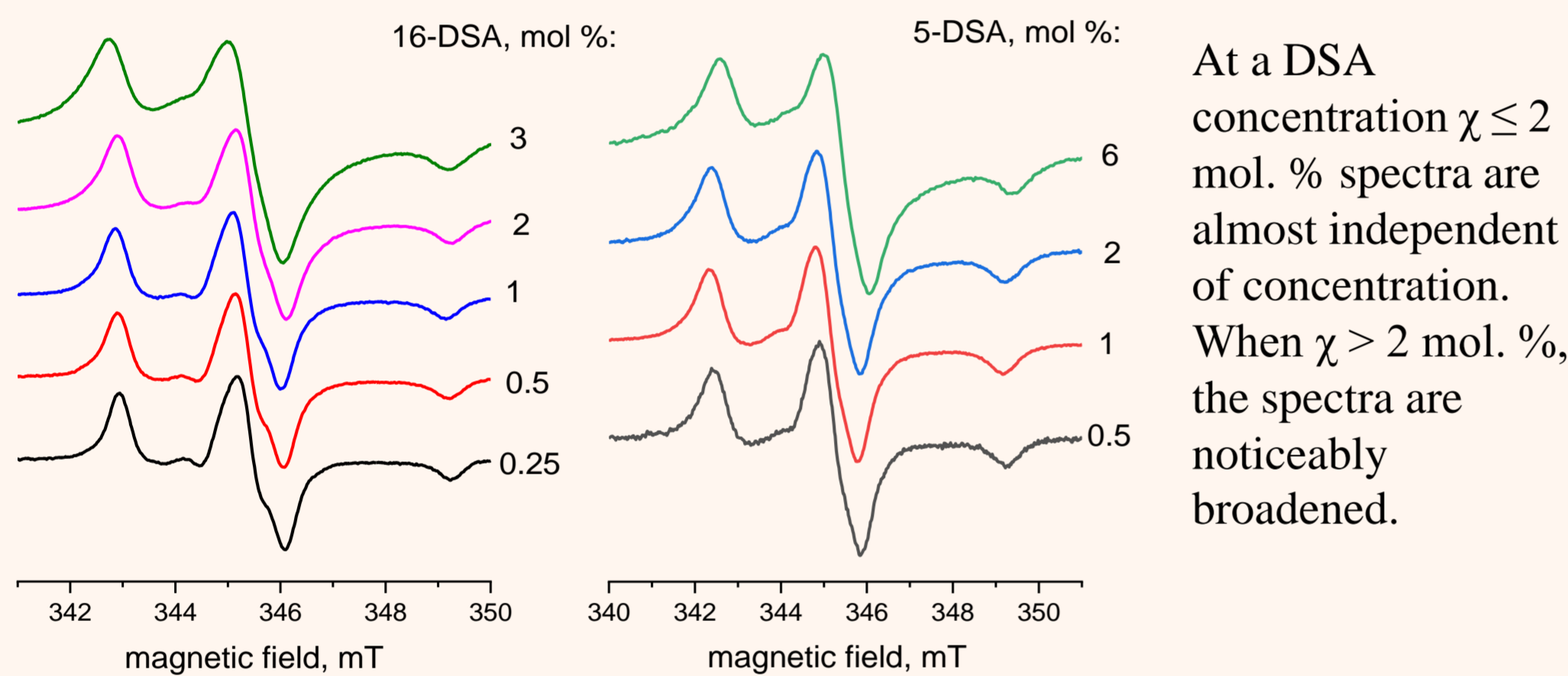
A three-dimensional spatial distribution is observed

Distribution of fatty acids in bilayers

"2D" for DOPC/DPPC bilayer and "3D" for POPC. Explanation of this difference:

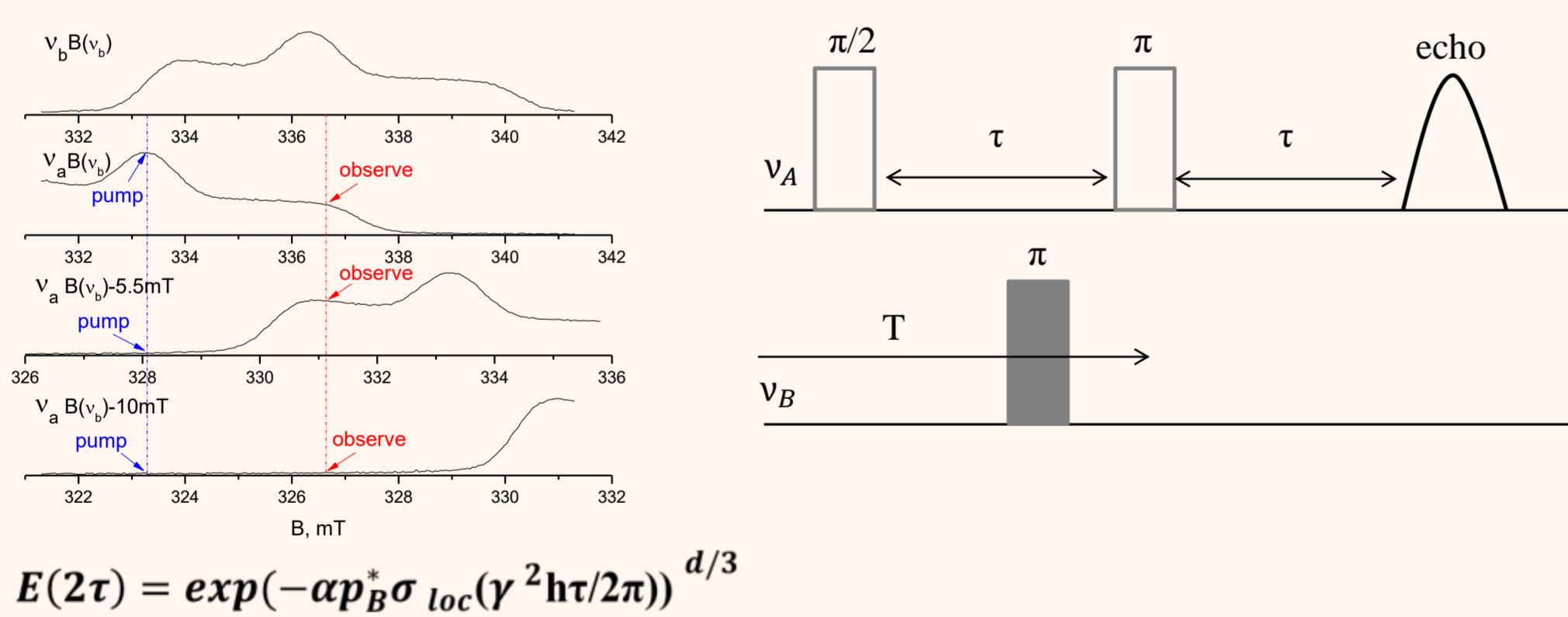


EPR spectra at 200 K for 16-DSA and 5-DSA at different concentrations in the DOPC/DPPC bilayer

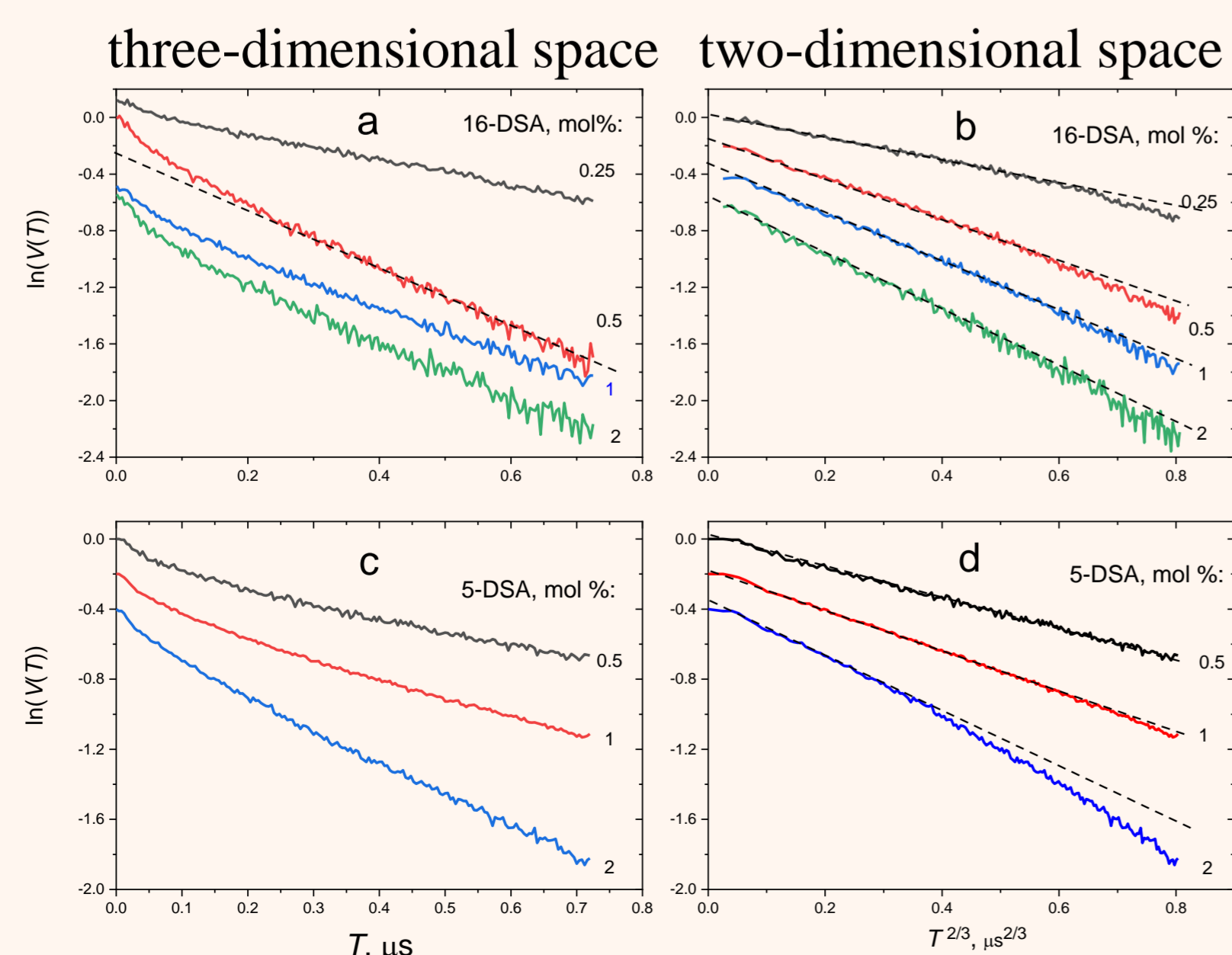


Oligomerization at high concentrations

Double Electron Electron Resonance (PELDOR)



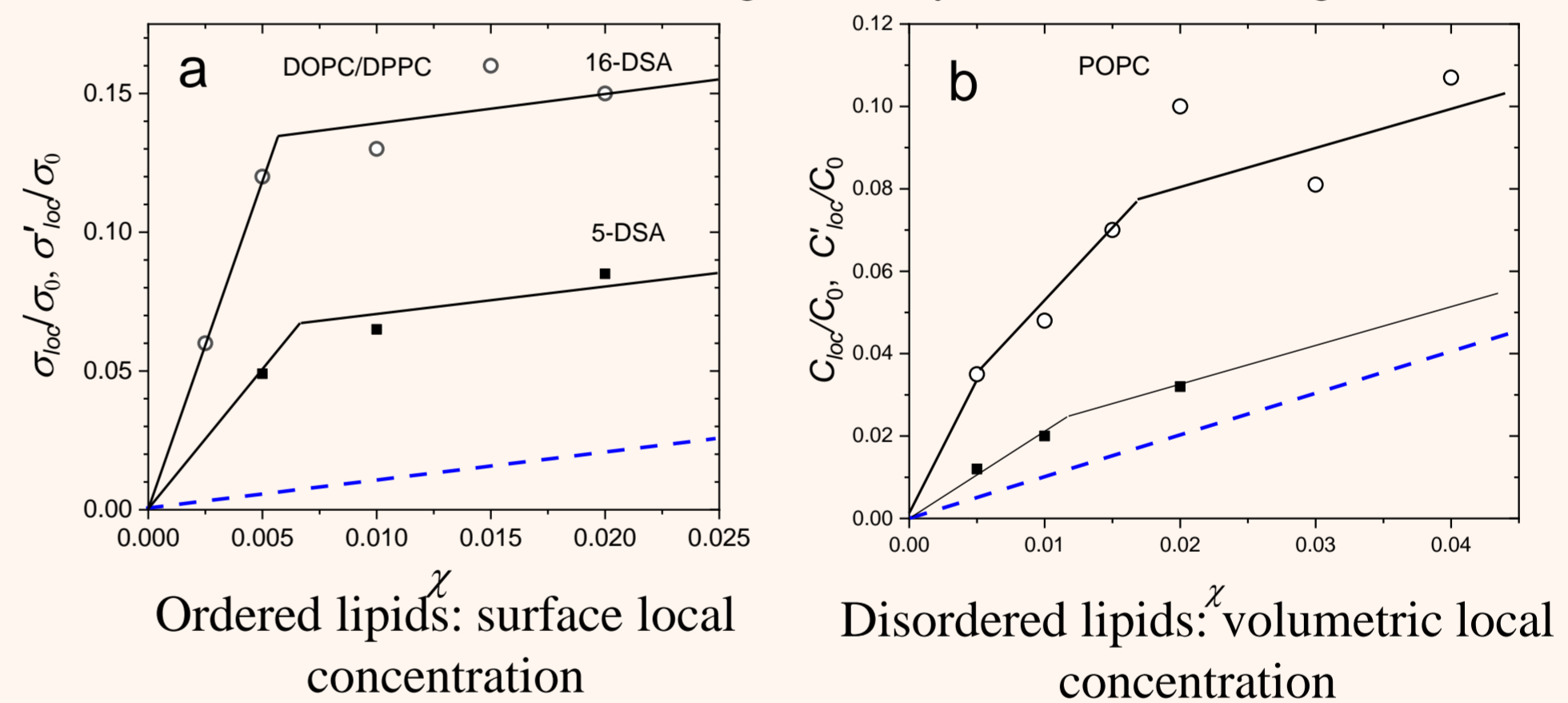
PELDOR Time Drops for DOPC/DPPC Bilayers



Stearic acid molecules occupy a certain vertical position, so that the spin labels belonging to different molecules are located in the same plane.

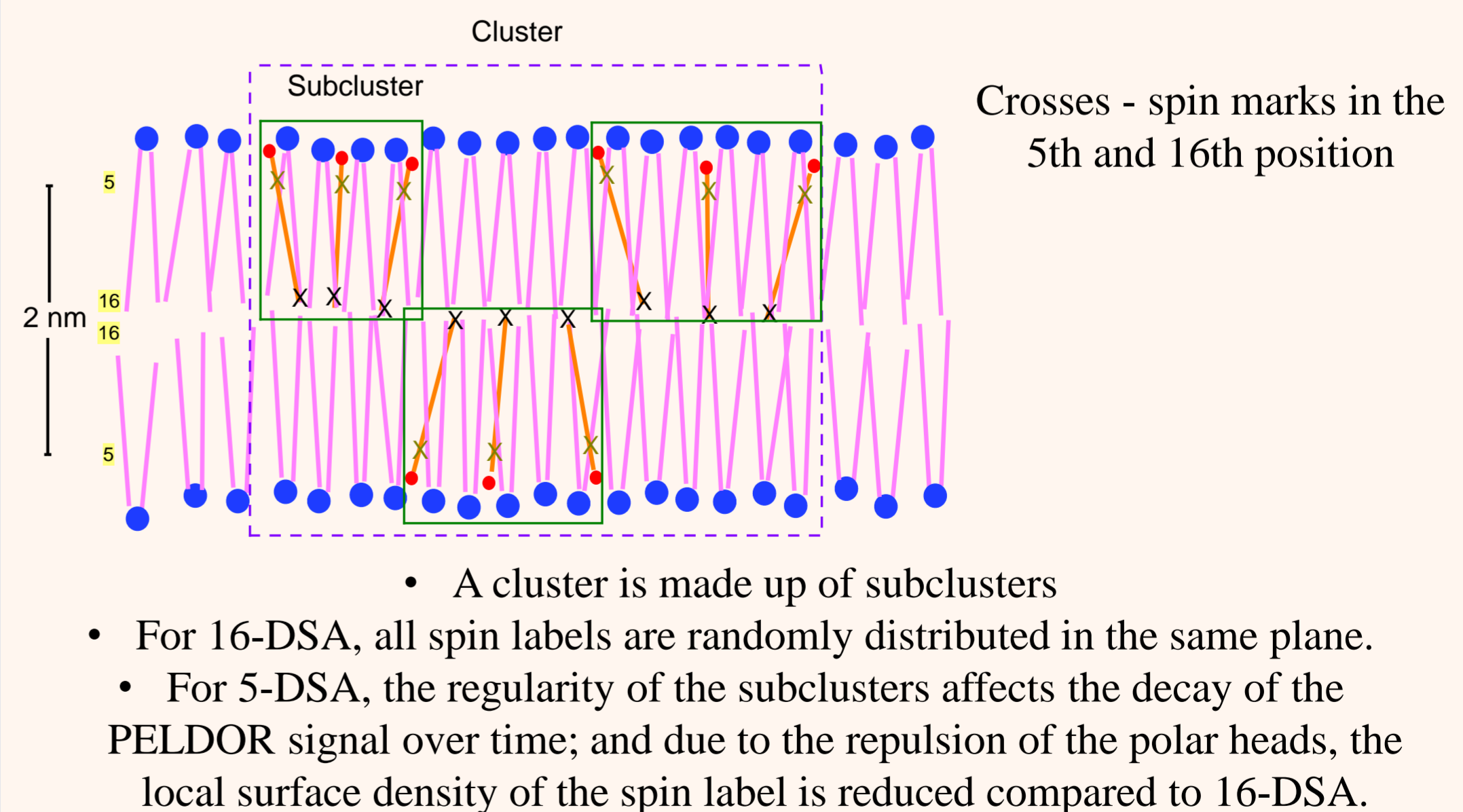
Clustering

The found local concentrations significantly exceed the average: clustering



For 5-DSA, local concentrations are less than for 16-DSA

Clustering model



Conclusion

- The found local concentrations significantly exceed the average, which indicates clustering
- Clusters are made up of subclusters
- The DOPC/DPPC bilayer is characterized by a two-dimensional distribution of stearic acid in the membrane, while POPC has a three-dimensional distribution.
- The CW EPR spectra at room temperature also indicate clustering

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