Double Electron-Electron Resonance of Molecular Clusters in Biological Membranes

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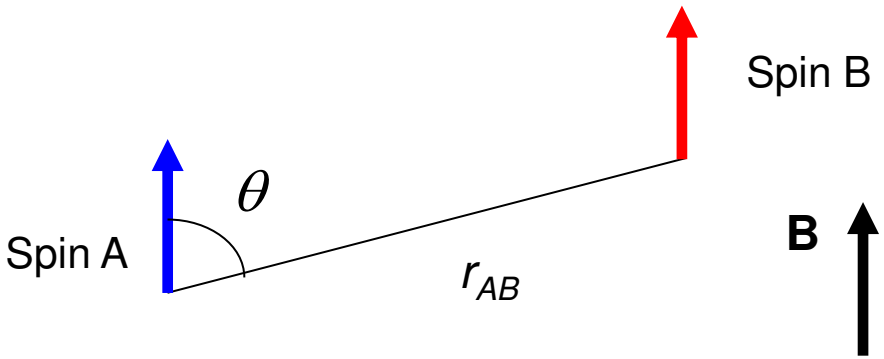
Double electron-electron resonance (DEER or PELDOR) is a pulsed EPR technique

which is based on electron spin echo spectroscopy and

allows studying magnetic dipole-dipolar interactions between electron spins separated by 1.5 – 8 nanometers

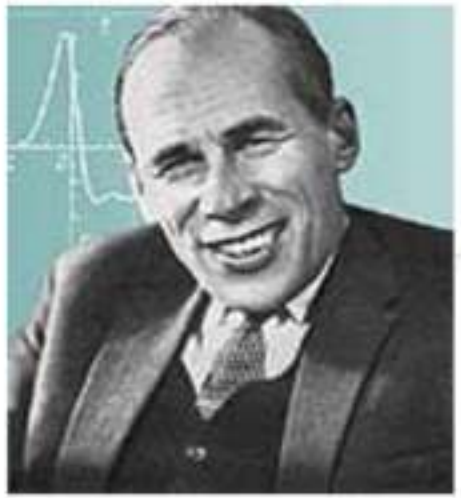


$(\omega_2 - \omega_1 >> \gamma\, b_{dip})$

Dipolar "magnetic field"

$$\mathbf{b}_{dip} = \frac{\mu_B}{r_{AB}^{\,3}}(1-3\cos^2\theta)m_B$$

Electron spin echo



V. V. Voevodsky. (1917-67)
Chemical applications of EPR

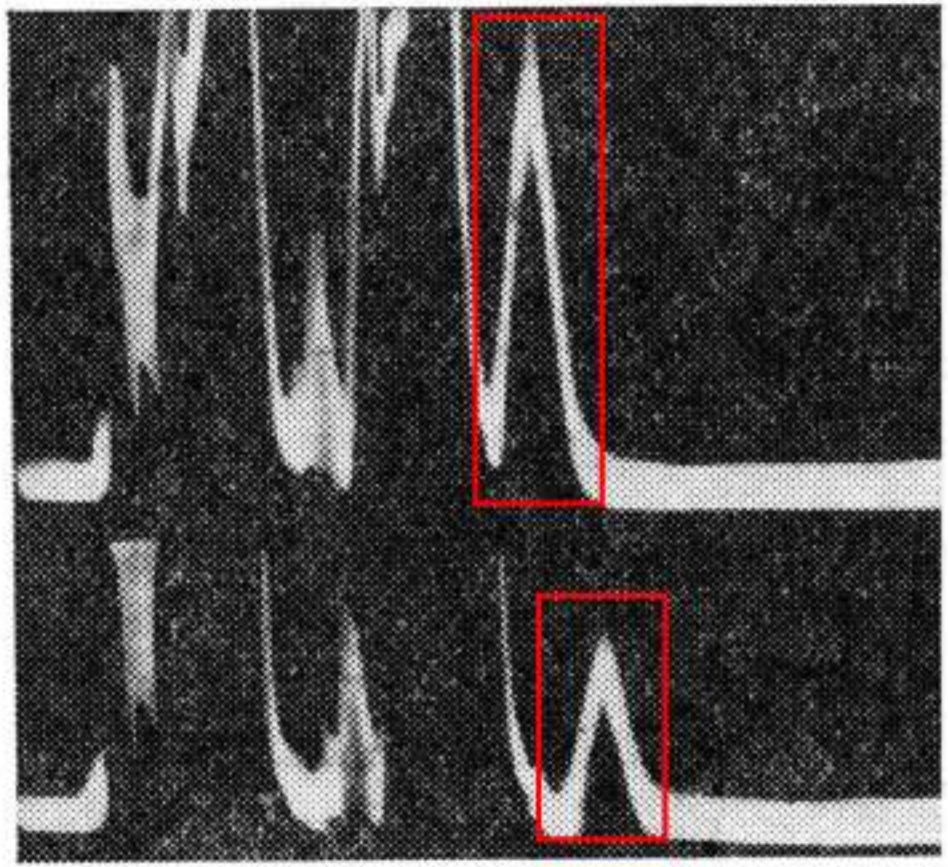


Рис. 3. Облученный метиловый спирт $5 \cdot 10^{-7}$ *сек*/деление, 77 °*K*, $T_2 = 2 \cdot 10^{-7}$ *сек*

First in the world:
electron spin echo signal from free radicals

Pribory i technika experimenta, 1967

A. G. Semenov (1924–90)
Developer of EPR spectrometers

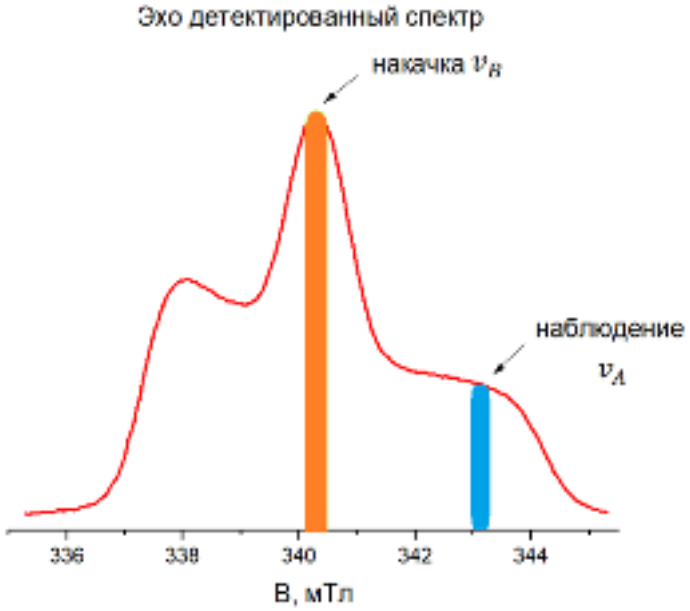
Издательство «Наука» 1976

Kev Salikhov

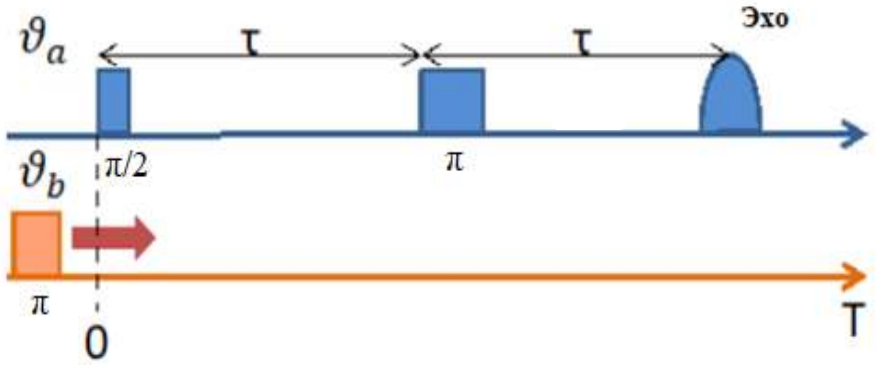
Yuri Tsvetkov
1934 - 2018

DEER signal appears after acting of microwave pulses at two EPR frequencies

Echo-detected EPR spectrum of nitroxide spin label

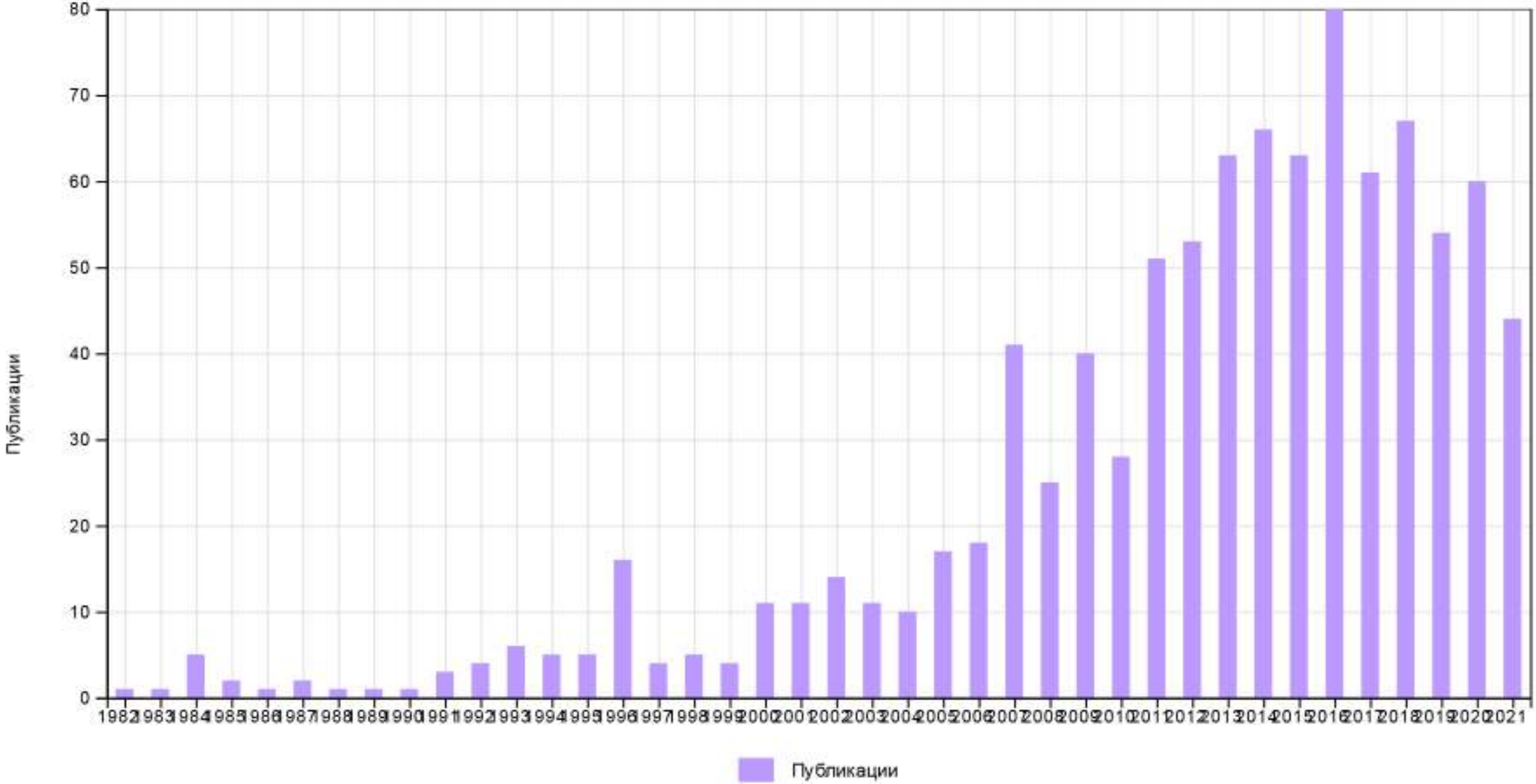


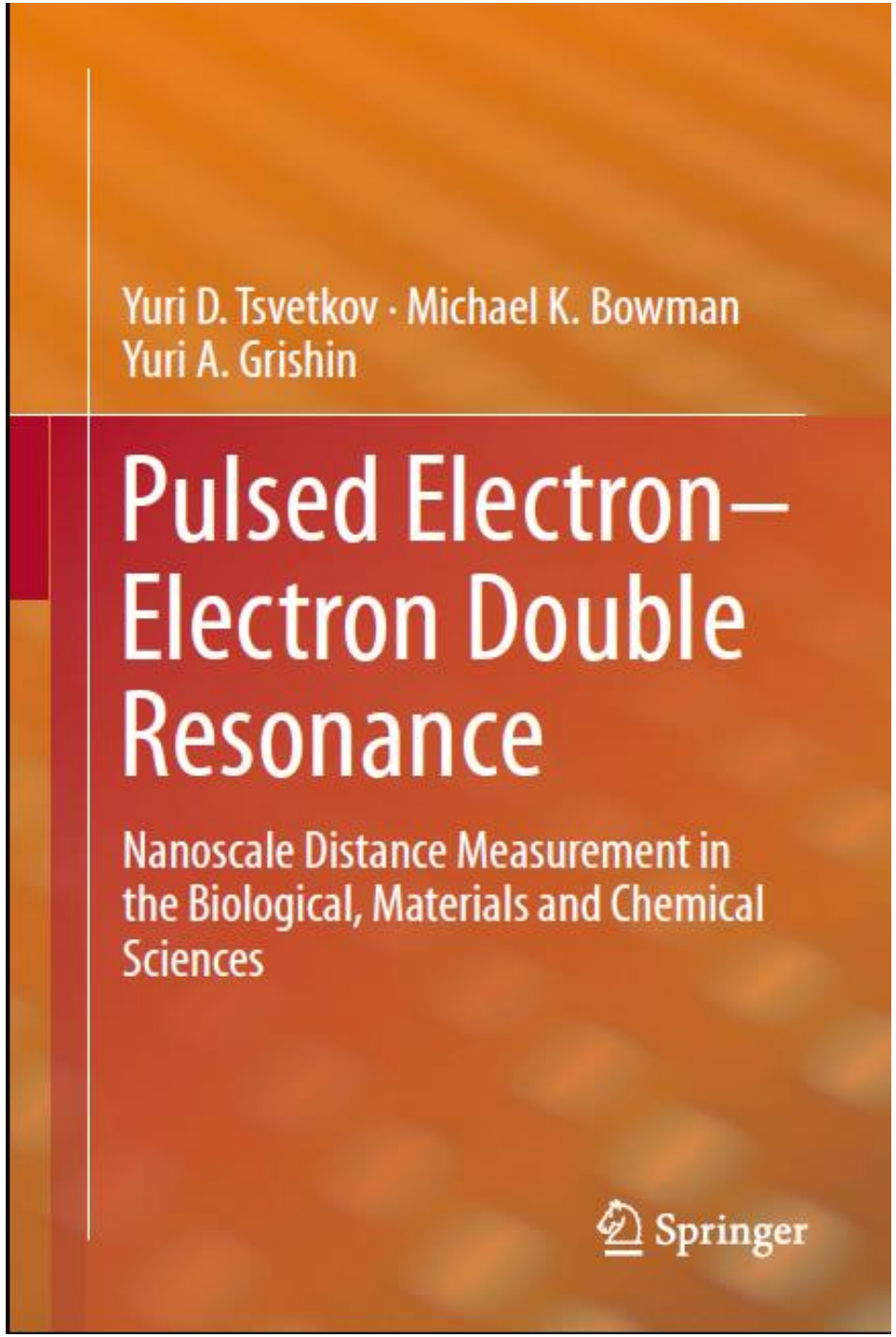
A. D. Milov et al. Soviet Physics Solid State, 1981



2012
A.D. Milov receives silver medal from International EPR society for invetion of DEER spectroscopy

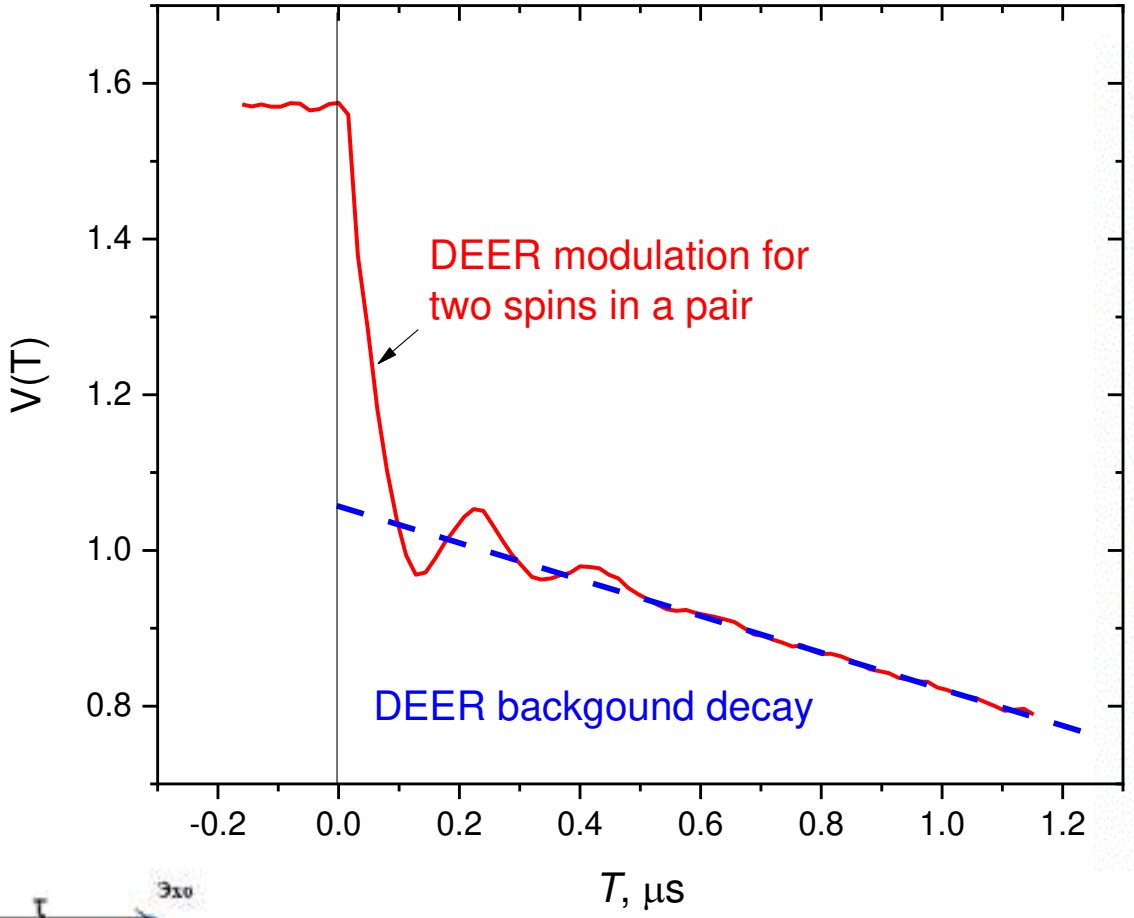
Double electron-electron resonance (Web of Science)



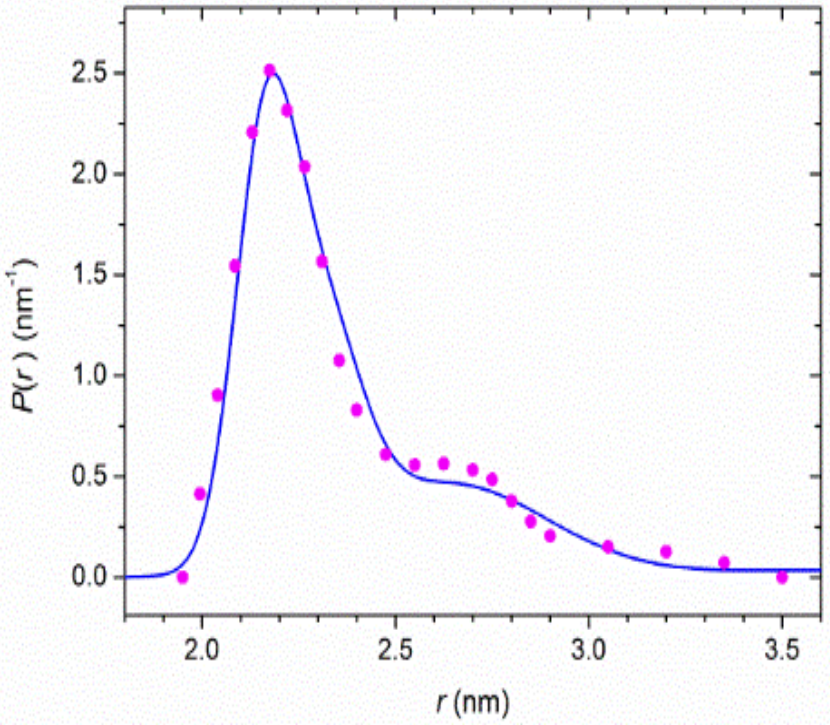


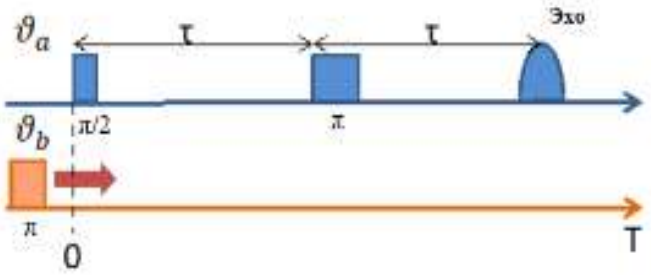
Springer, 2019

Typically, DEER (PELDOR) is applied to double-spin-labeled molecules



Mathematical treatment results in distance distribution function





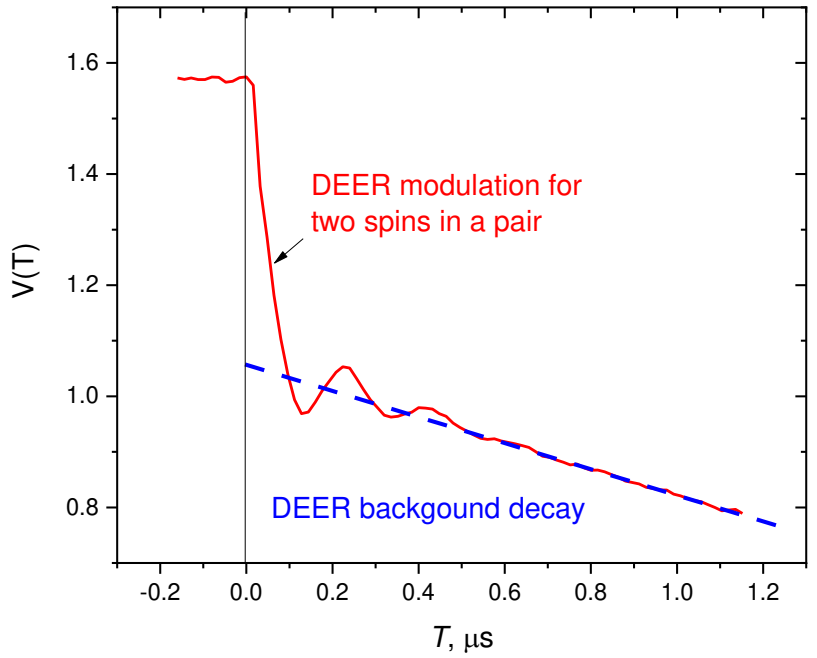
Molecular conformations are detected

Does the background signal also contain a useful information?

The answer is: Yes

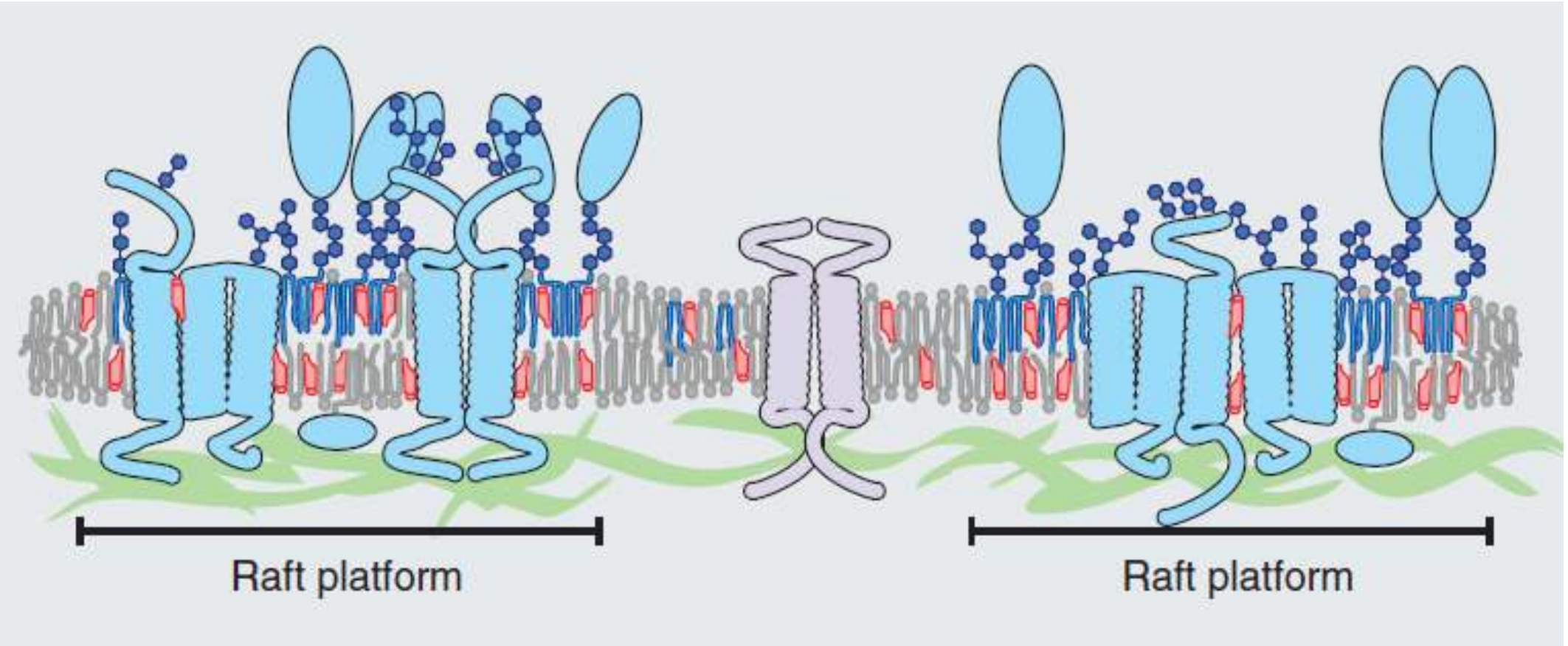
We study biological membranes:

1. Lipid rafts
2. Clustering of free fatty acids
3. Clustering of the antimicrobial peptide

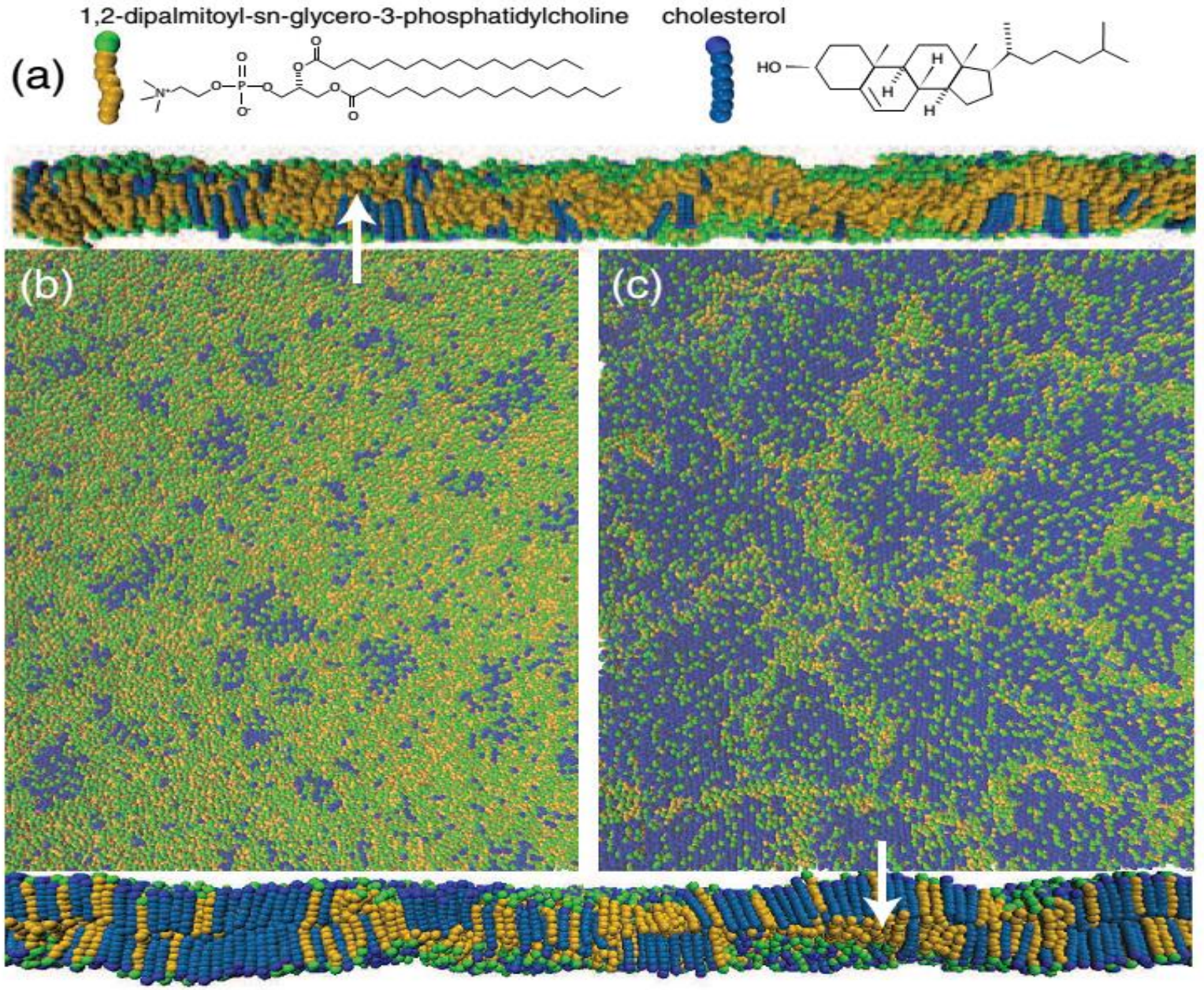


Topic 1: Lipid rafts in membranes

Mammalian membranes contains 10 – 40 % of cholesterol. Lipids and proteins in membranes are organized in specific domains, the so-called lipid rafts, which are enriched with cholesterol. Lipid rafts are small (10–200 nm), highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes.



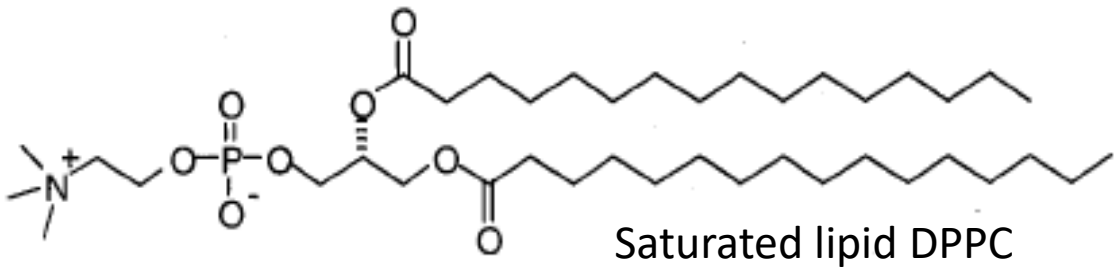
From D. Lingwood et al. Science 327, 46 (2009)



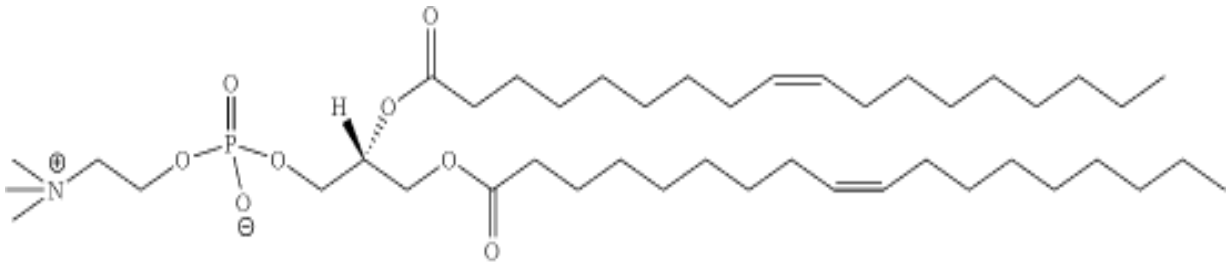
MD simulations in
L. Toppozini et al.,
Phys. Rev. Letters 2014

17 mol %
cholesterol

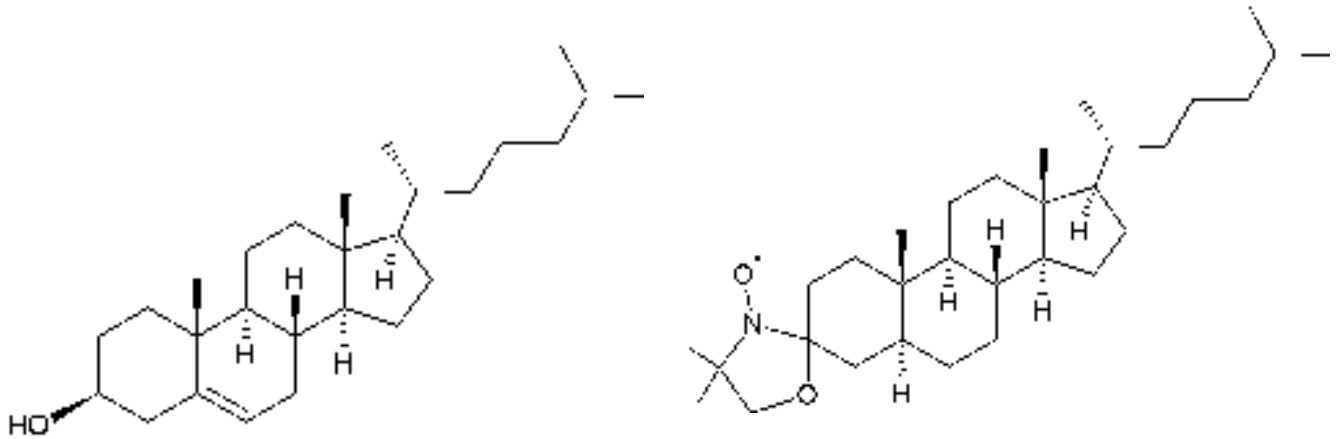
60 mol %
cholesterol



Saturated lipid DPPC

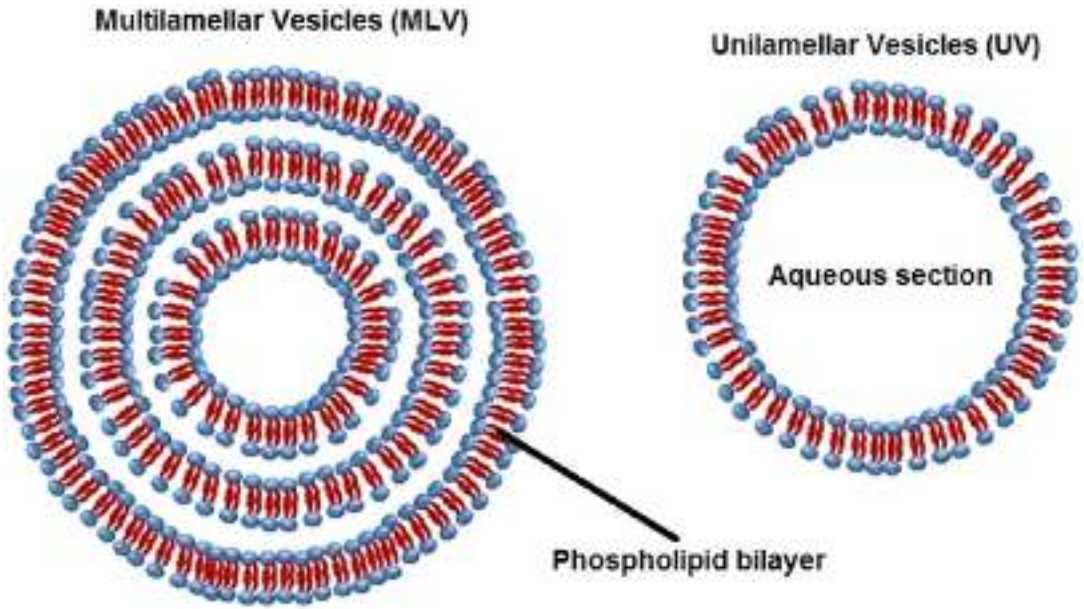


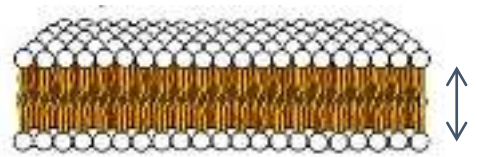
Double-unsaturated lipid DOPC



Cholesterol

Spin-labeled cholesterol analog (Doxyl-cholestane)



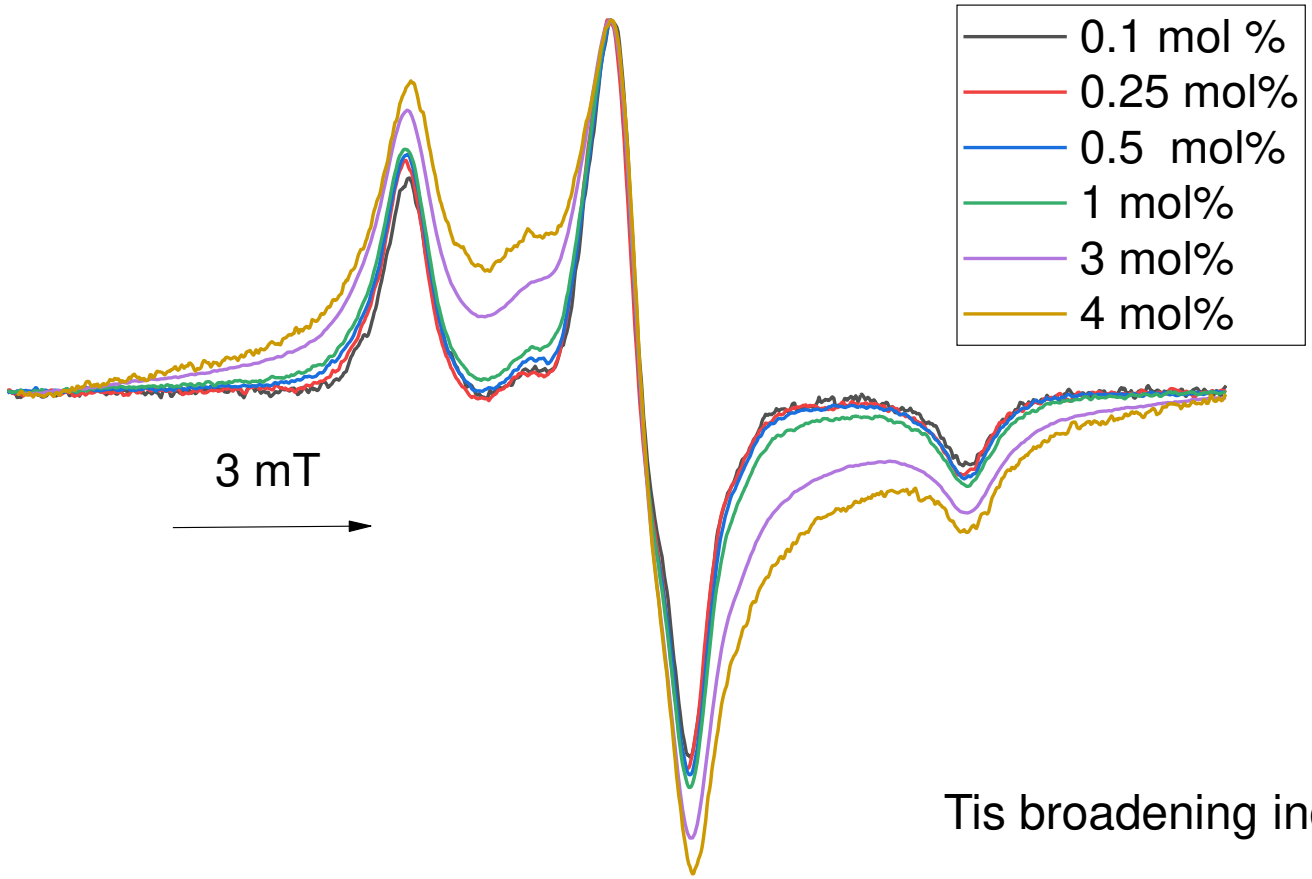


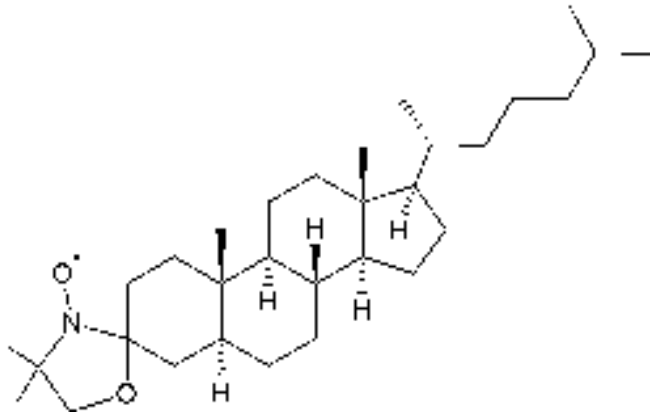
~ 5 nm

EPR spectra of spin-labeled cholesterol analog are concentration-broadened

DPPC/DOPC bilayer

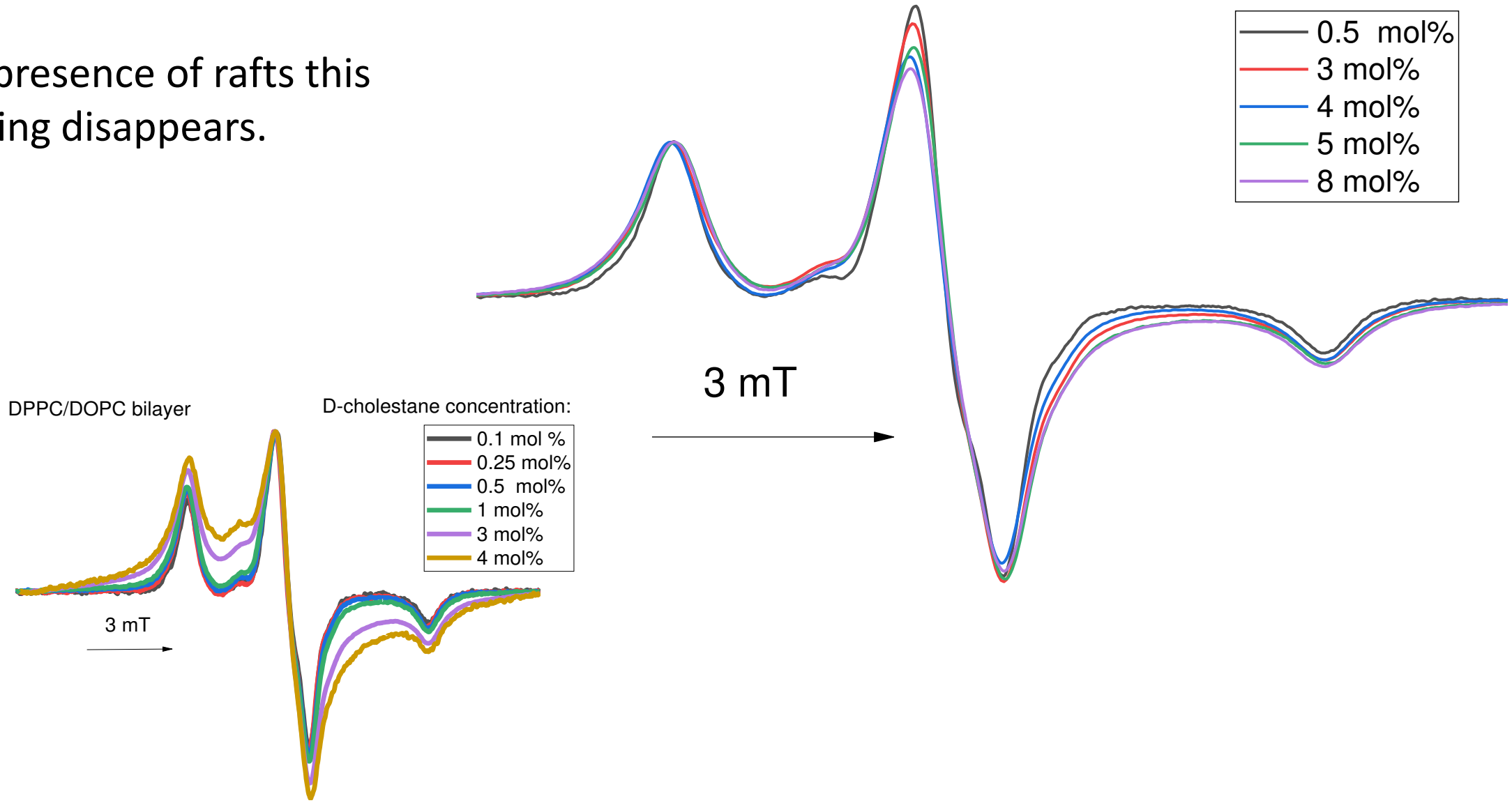
D-cholestane concentration:



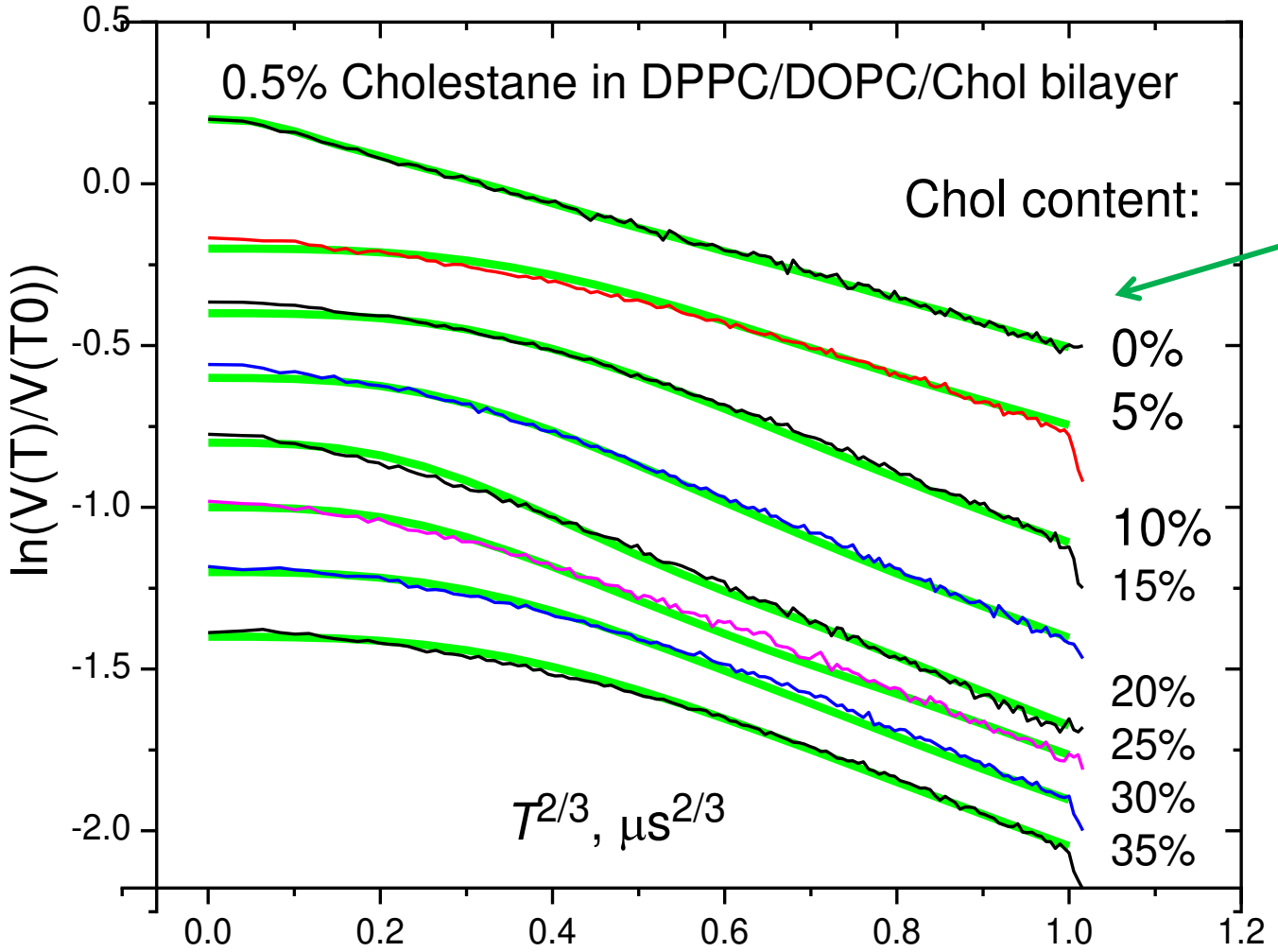


Tis broadening indicates molecular clustering

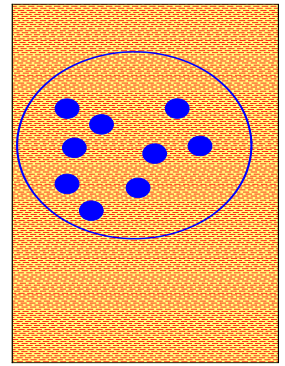
But in presence of rafts this clustering disappears.
Why?



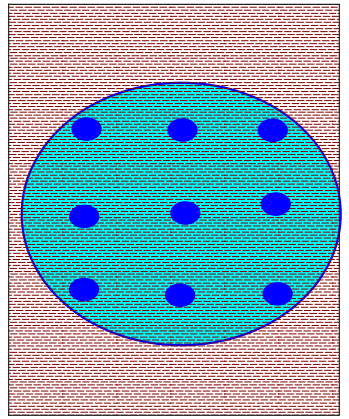
DEER provide the answer



Random spin distribution in absence of rafts

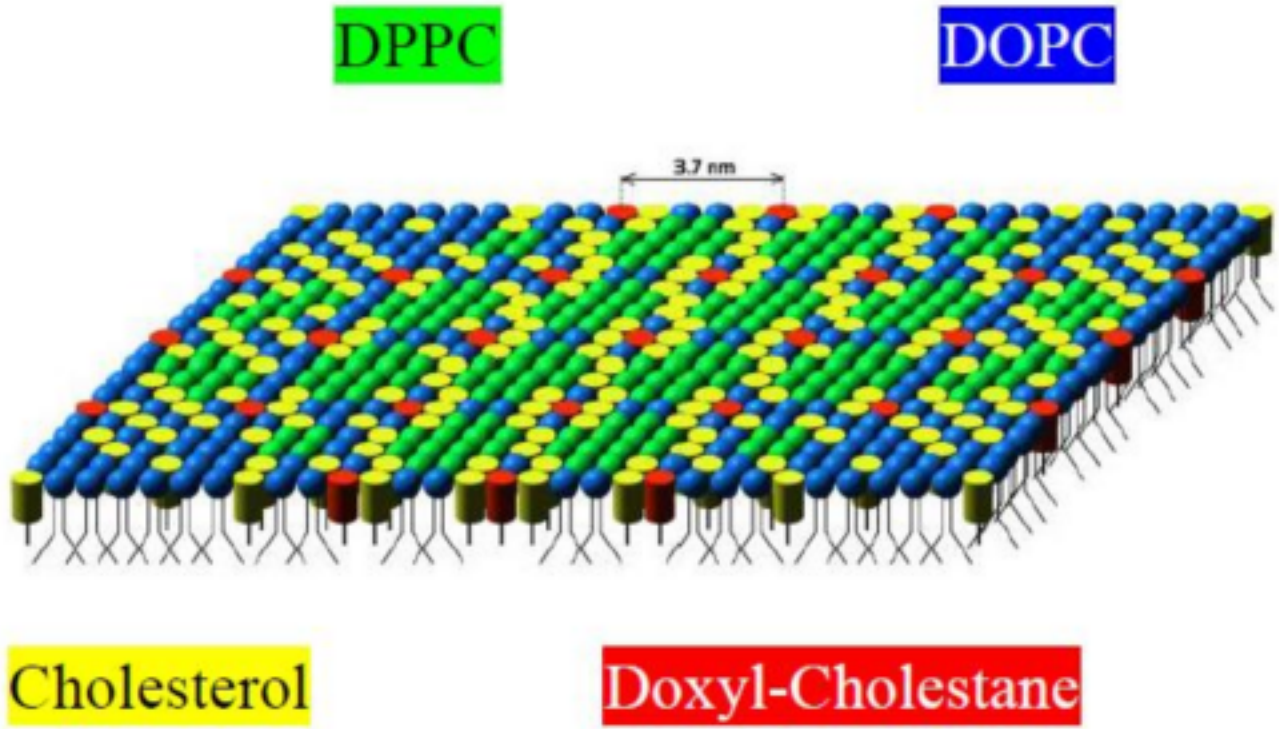


Rafts promote regular distribution

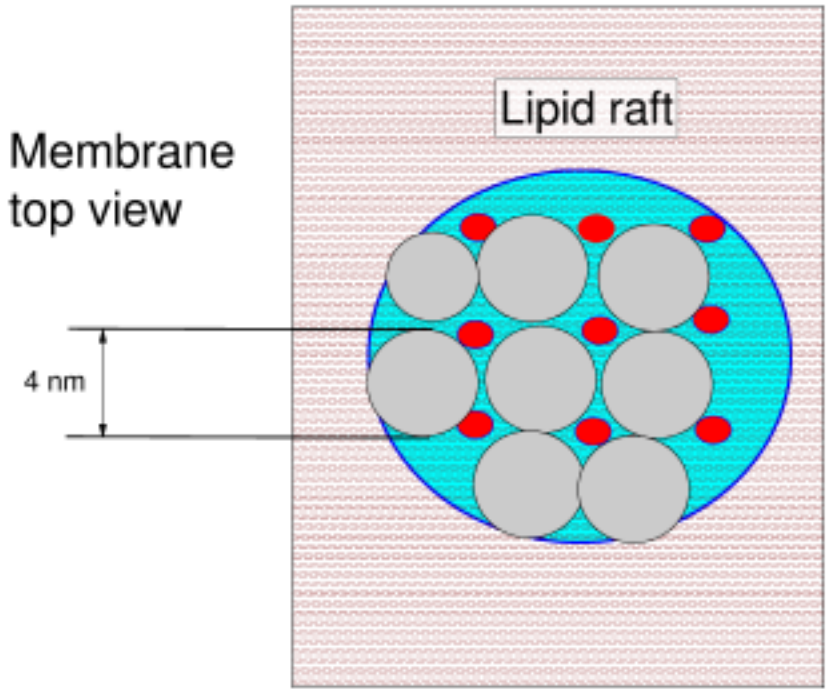


The membrane top view

The putative structure of lipid raft

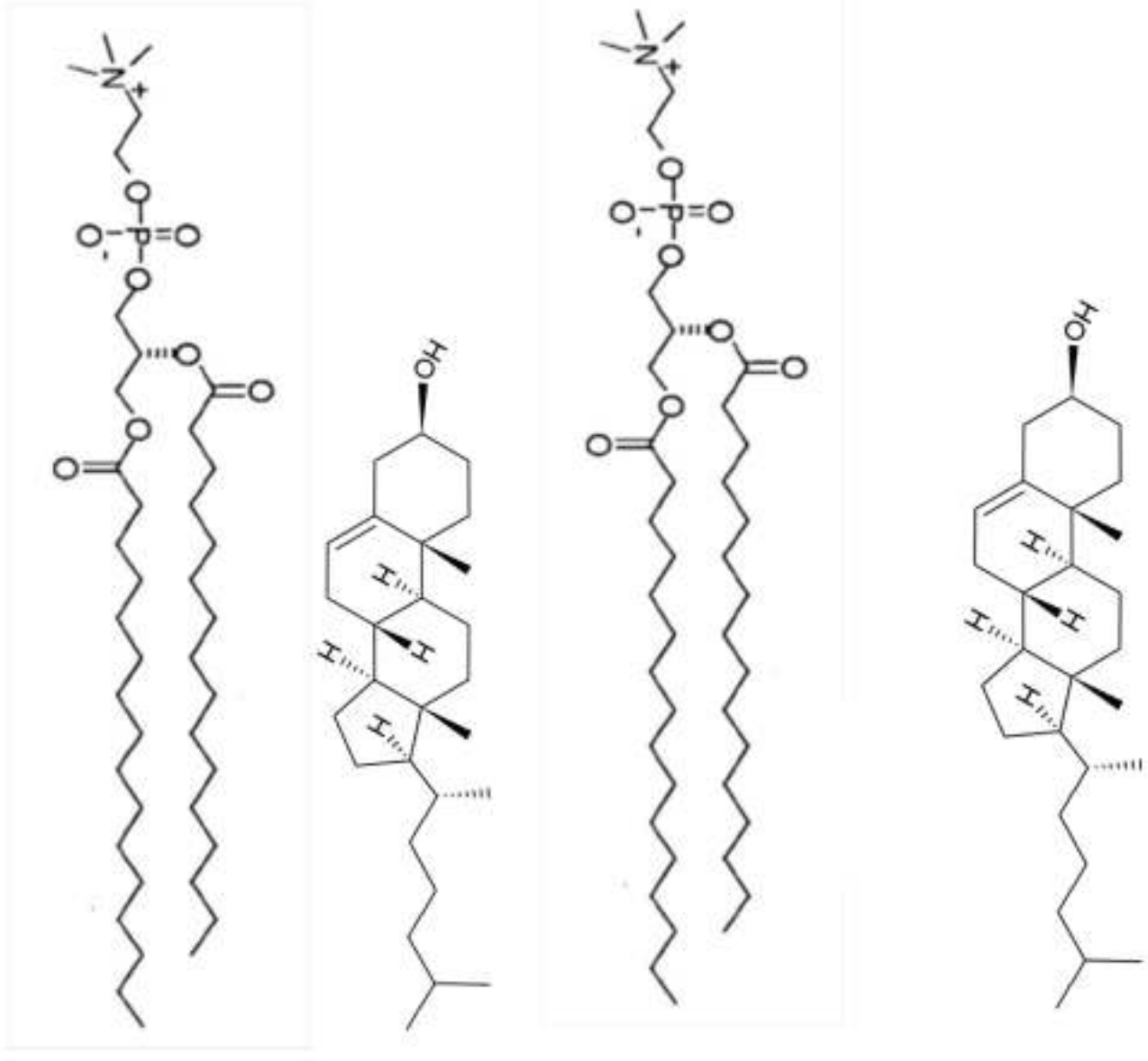


V.V. Unguryan, E.A. Golysheva, S.A. Dzuba, J. Phys. Chem. B, 2021, 125, 9557–9563.



Lipid raft (10 – 200 nm) consist of 4 nm sub-clusters

Suggested mechanism of sub-cluster formation is based on proximity of “structural step” for saturated lipids and cholesterol

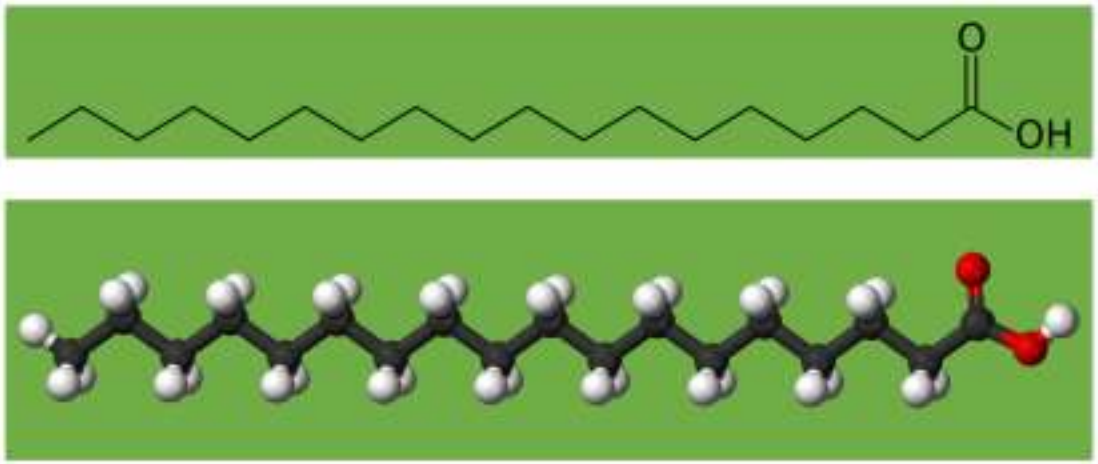


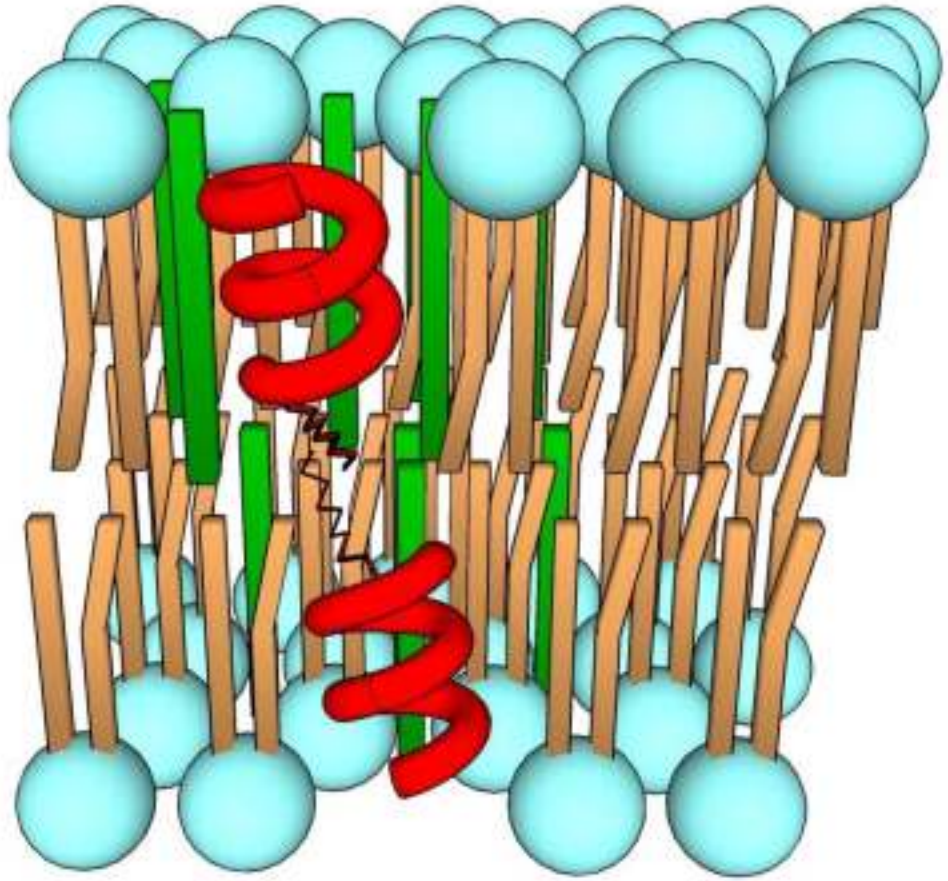
Topic 2: Clustering of free fatty acids

Free fatty acids play various roles in biological membranes: increase their fluidity, serve as energy supply and a source of structural components, participate in cell signaling, in cell fusion, and in many other physiological processes.

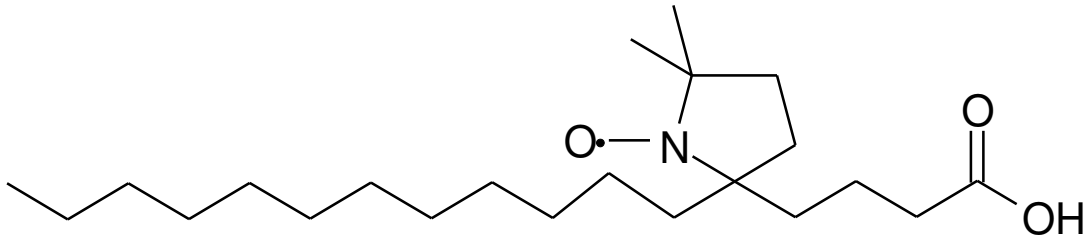
Their amount in mammalian membranes usually varies in the range of 0.3–10% of total lipids.

Stearic acid

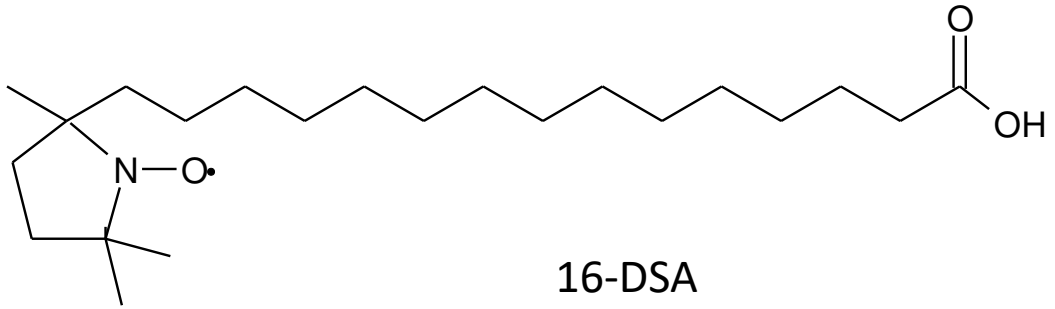




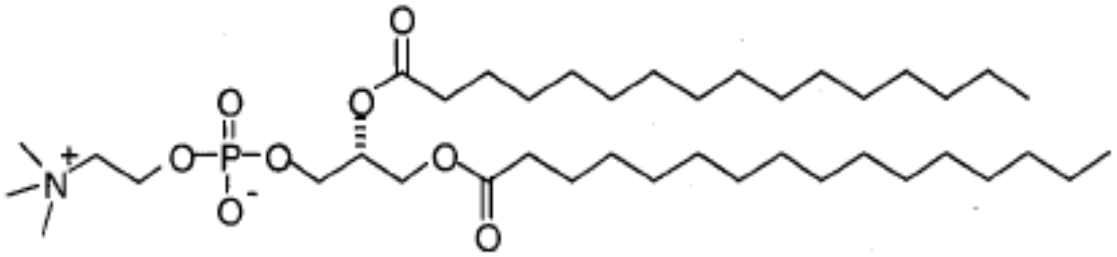
Spin-labeled stearic acids



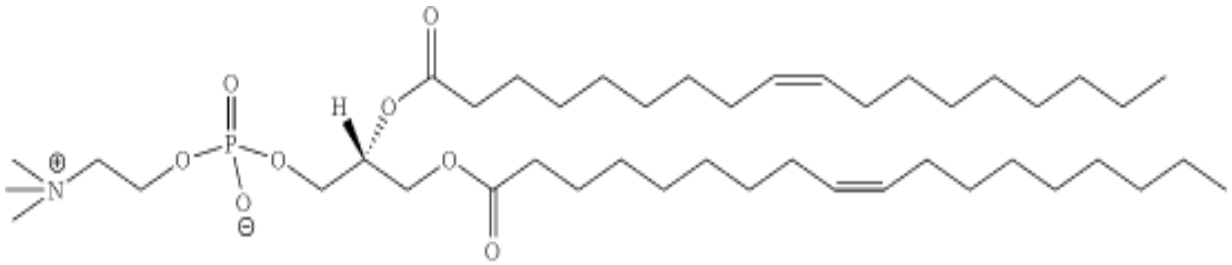
5-DSA



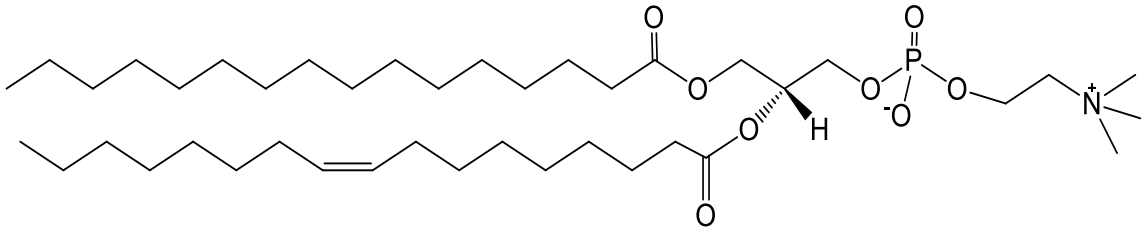
16-DSA



DPPC lipid

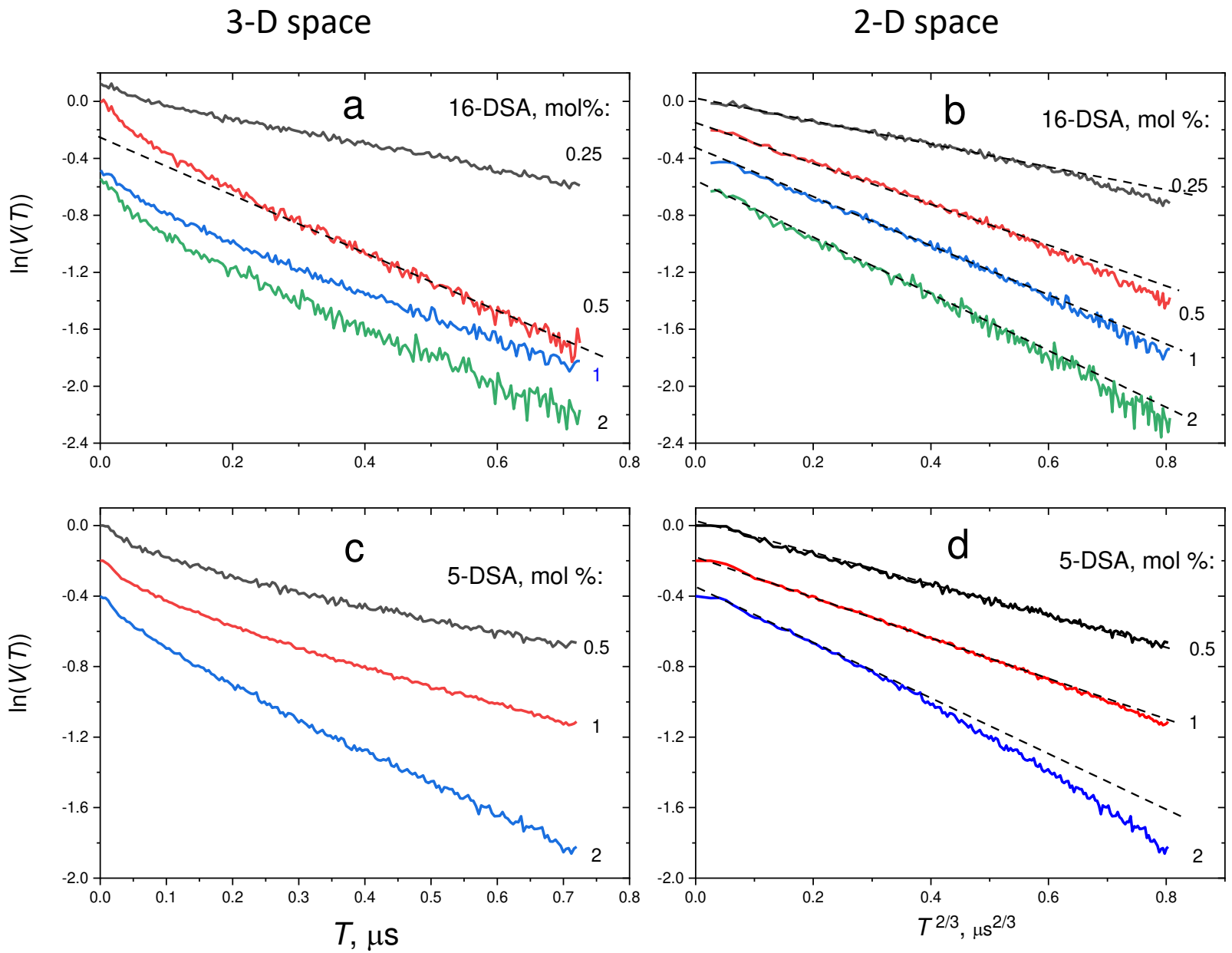


DOPC

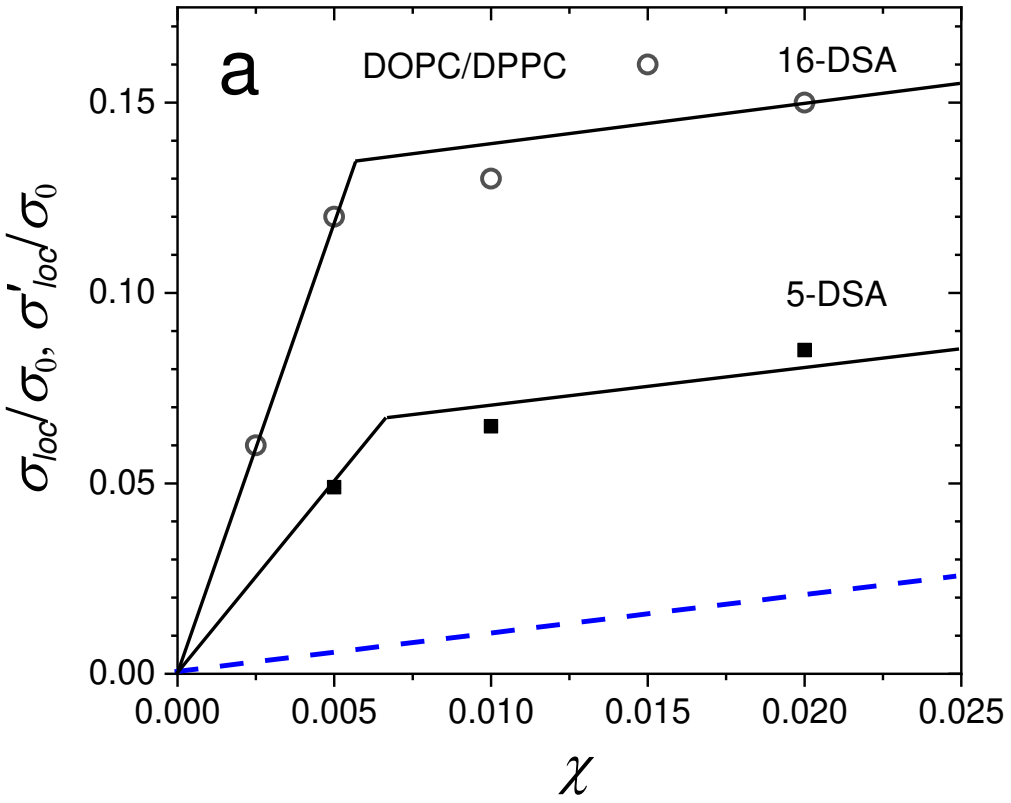


POPC

DEER time traces for DOPC/DPPC bilayers



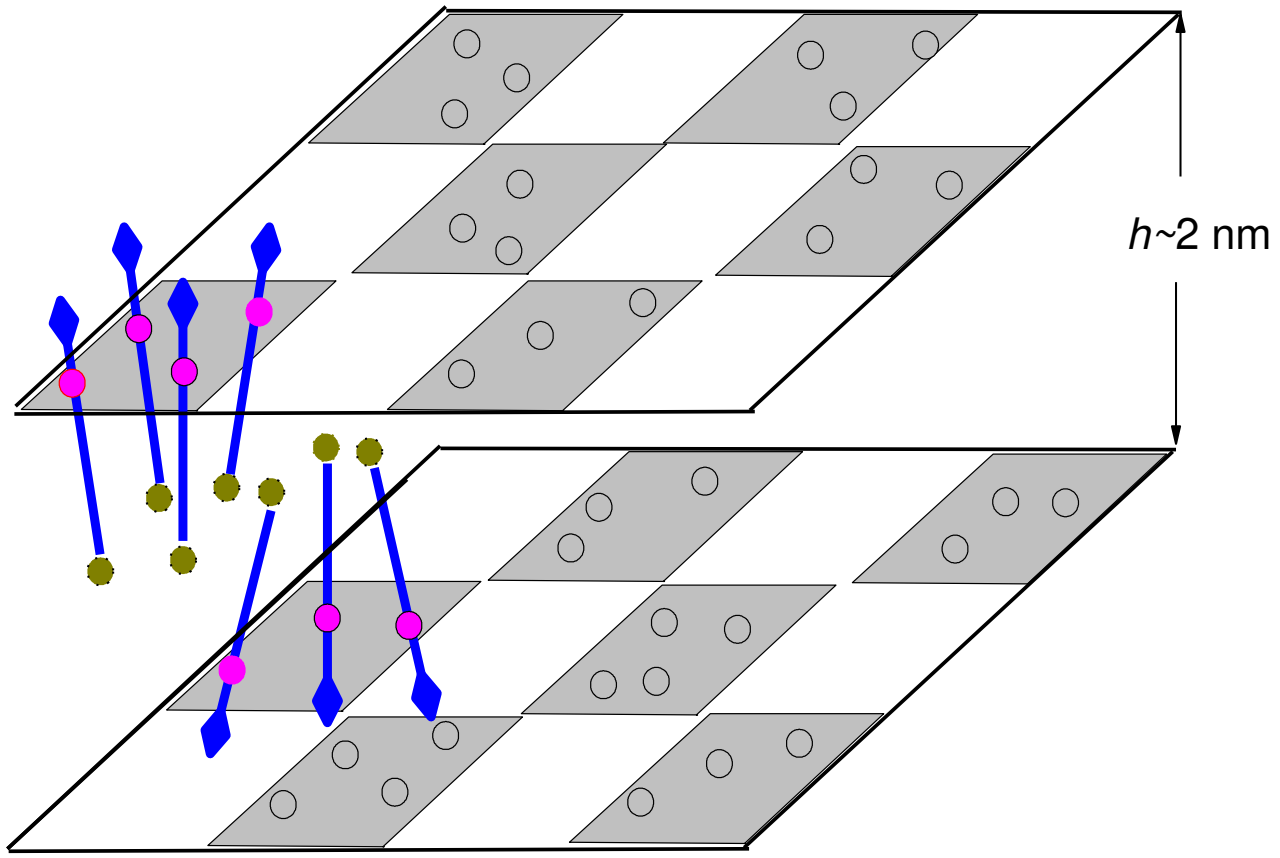
Local concentrations are much higher than average: clustering



$$V(T) = V(0)e^{-const*\sigma p_b T^{2/3}}$$

For 5-DSA local concentrations are lower than those for 16-DSA. Why?

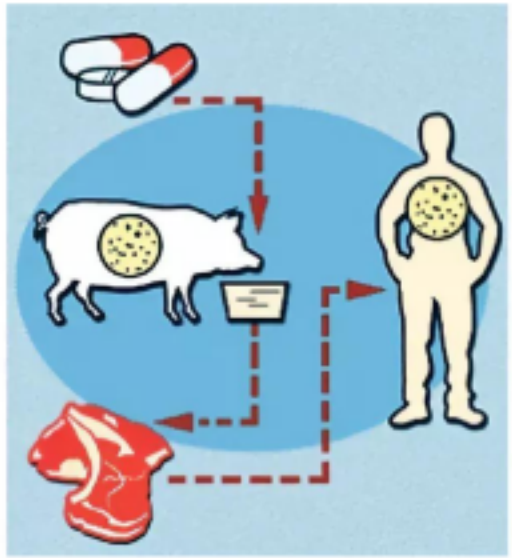
Our model of alternative sub-clusters



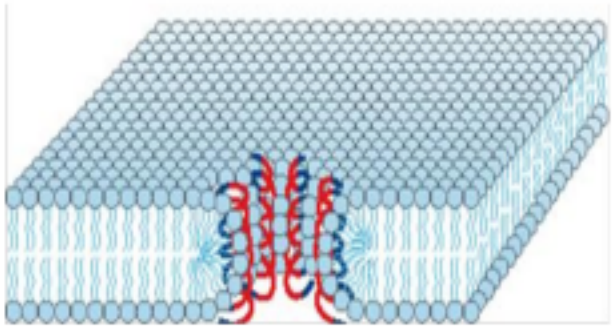
A.S. Smorygina, E.A. Golysheva, S.A. Dzuba, Langmuir, 2021.

Topic 3. Clustering of antimicrobial peptides

Antimicrobial peptides are antibiotics of new type which may help to overcome the problem of bacterial resistance



Antimicrobial peptides disturb bacterial membrane



The problem exists of elucidation of molecular mechanisms of action of peptides on the bacterial membranes

Antimicrobial peptide tilopeptin

Ac-Trp-Val-Aib-Aib-Ala-Gln-Ala-Aib-Ser-Aib-Ala-Leu-Aib-Gln-Lol Native tilopeptin

Ac-Trp-Val-**TOAC^{3}**-Aib-Ala-Gln-Ala-Aib-Ser-Aib-Ala-Leu-Aib-Gln-Lol

Ac-Trp-Val-Aib-Aib-Ala-Gln-Ala-**TOAC^{8}**-Ser-Aib-Ala-Leu-Aib-Gln-Lol

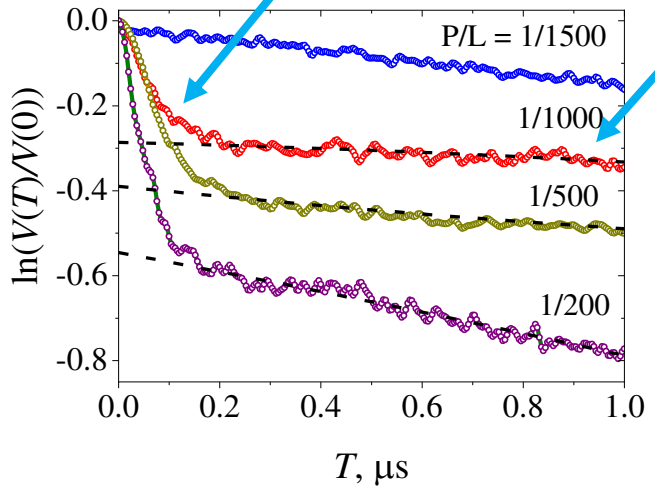
Ac-Trp-Val-Aib-Aib-Ala-Gln-Ala-Aib-Ser-Aib-Ala-Leu-**TOAC^{13}**-Gln-Lol

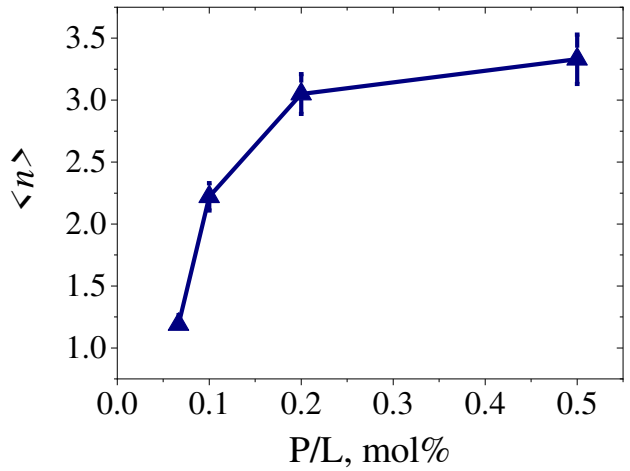
Synthesis: Marta De Zotti, Marina Gobbo, Fernando Formaggio
Department of Chemical Sciences, University of Padova, Padova, Italy

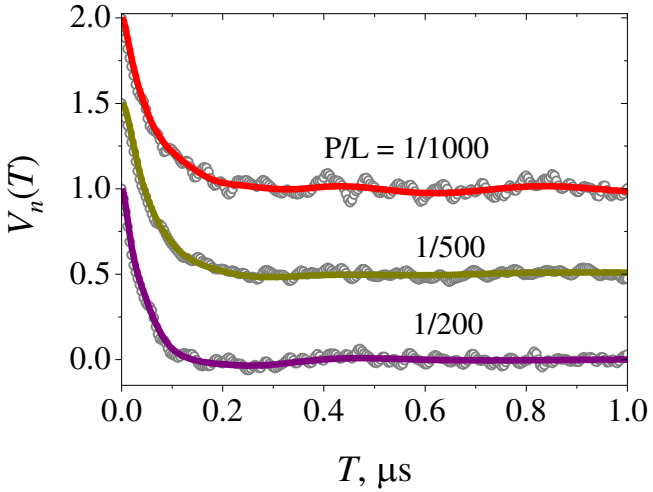
DEER of spin-labeled tilopeptin in the membrane

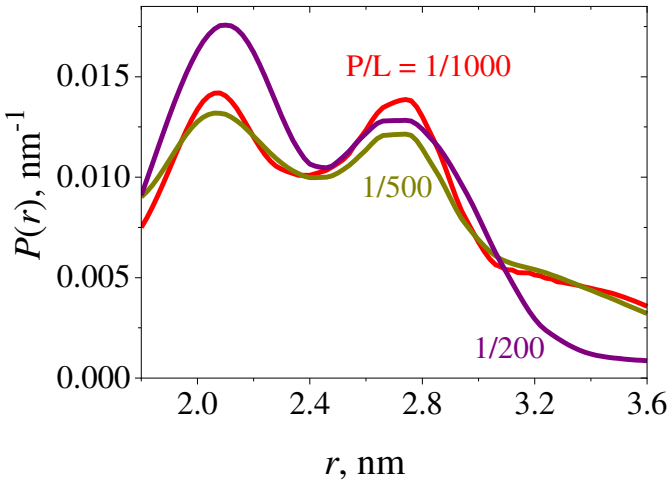
Fast decay implies clustering

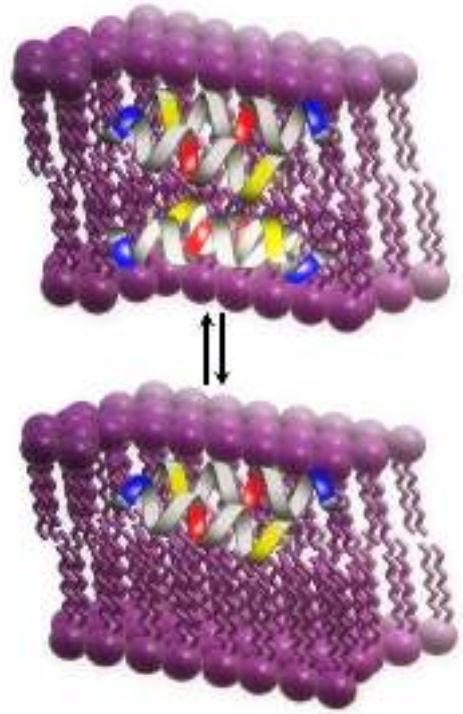
Background signal decays slowly: mutual repulsion of the clusters







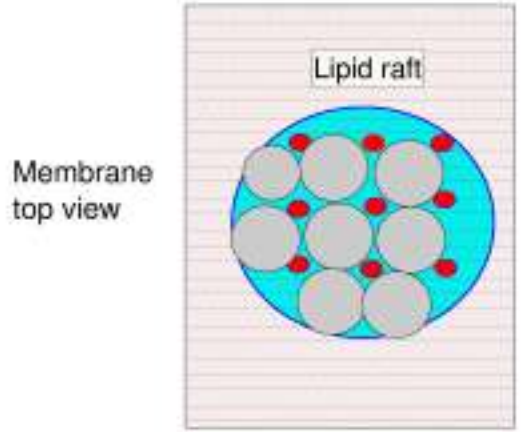


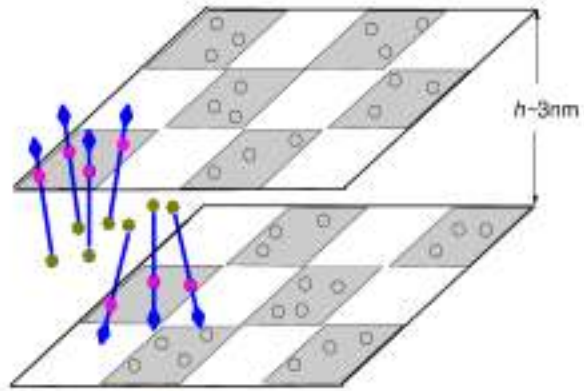


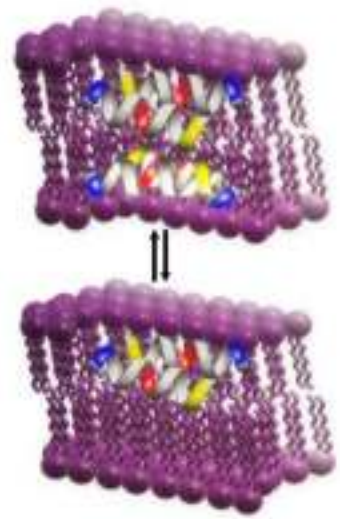
V. N. Syryamina, N. E. Sannikova, M. De Zotti, M. Gobbo, F. Formaggio, S. A. Dzuba, Biochim. Biophys. Acta - Biomembranes, 2021.

Conclusion

DEER background signal decay allows obtaining information of clustering of spin-labeled molecules: formation of cluster and their structure







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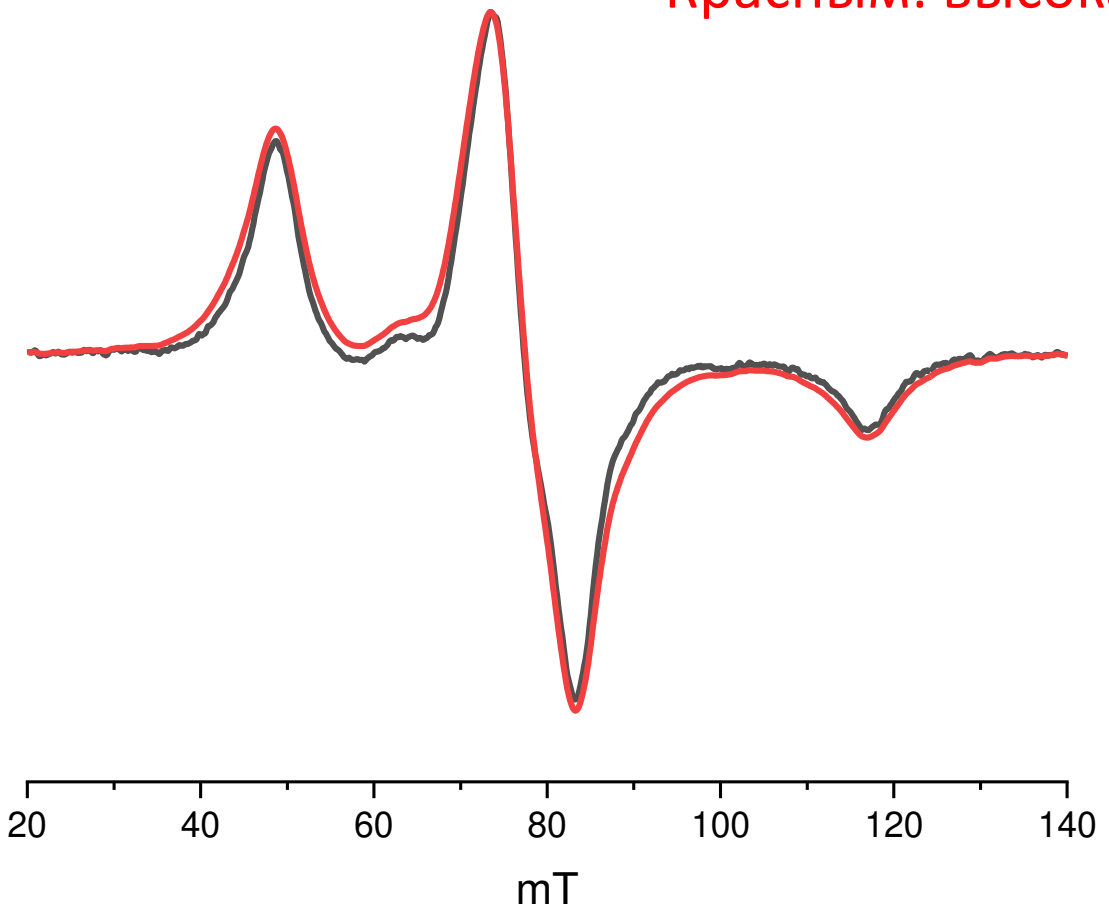
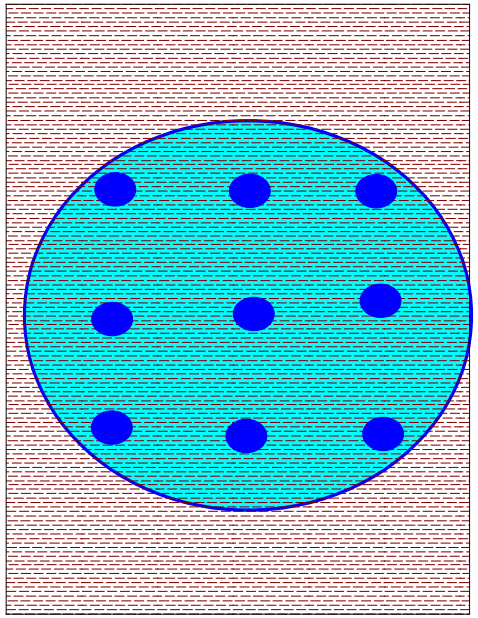
Marta De Zotti
Marina Gobbo

Thank you for your attention

Спектры ЭПР для «квадратной модели» также показывают отсутствие видимого уширения

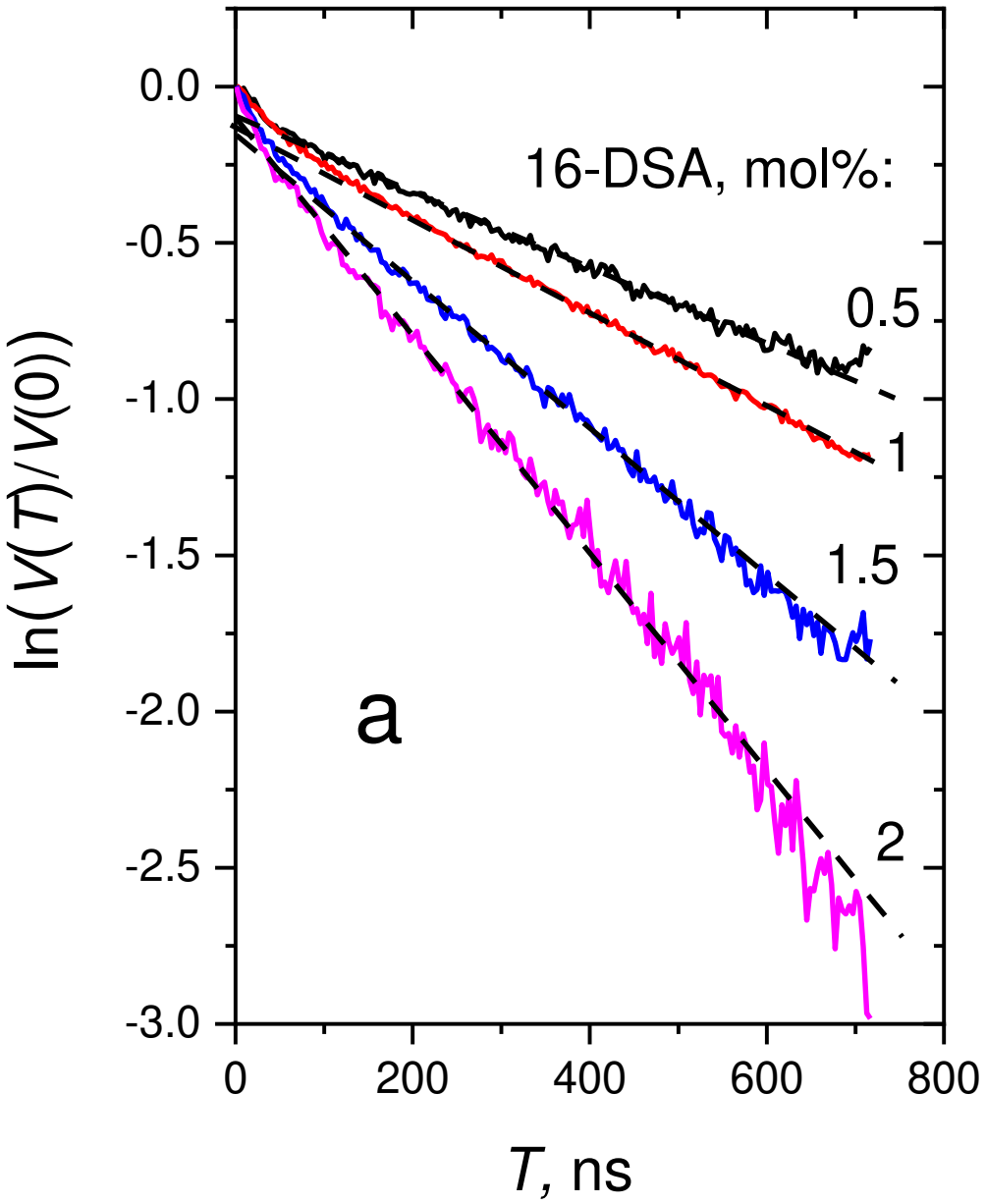
Красным: высокая концентрация спиновых меток



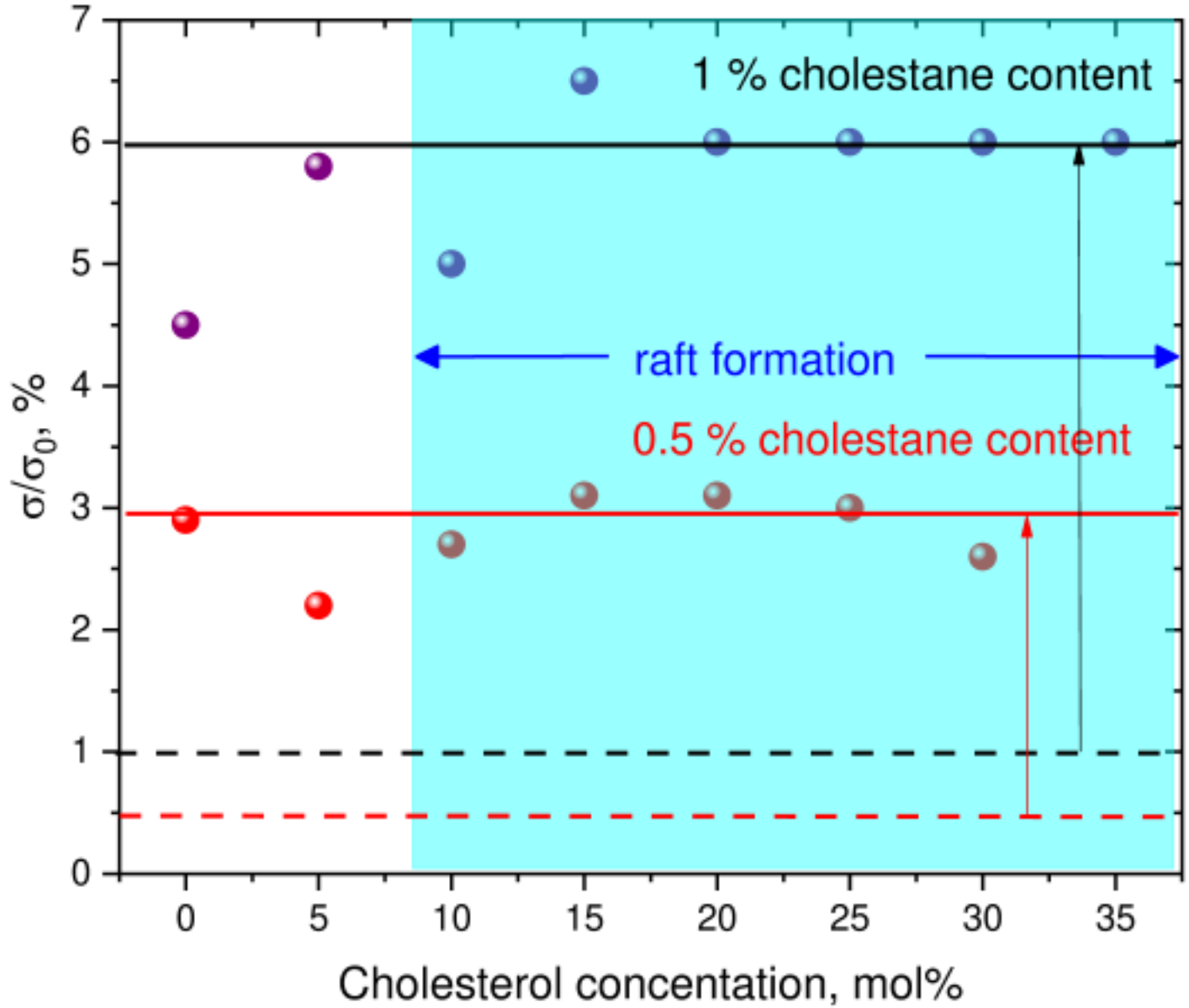


The membrane top view

For single-labeled molecules only background decay:

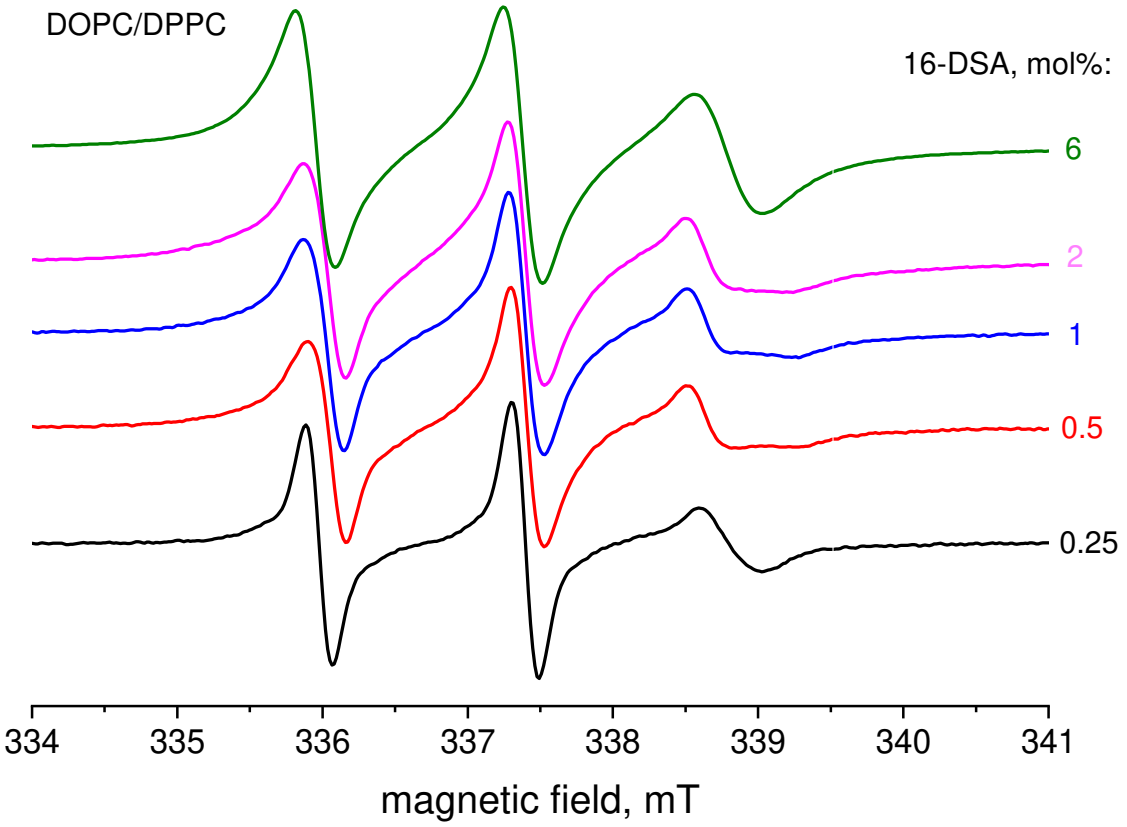


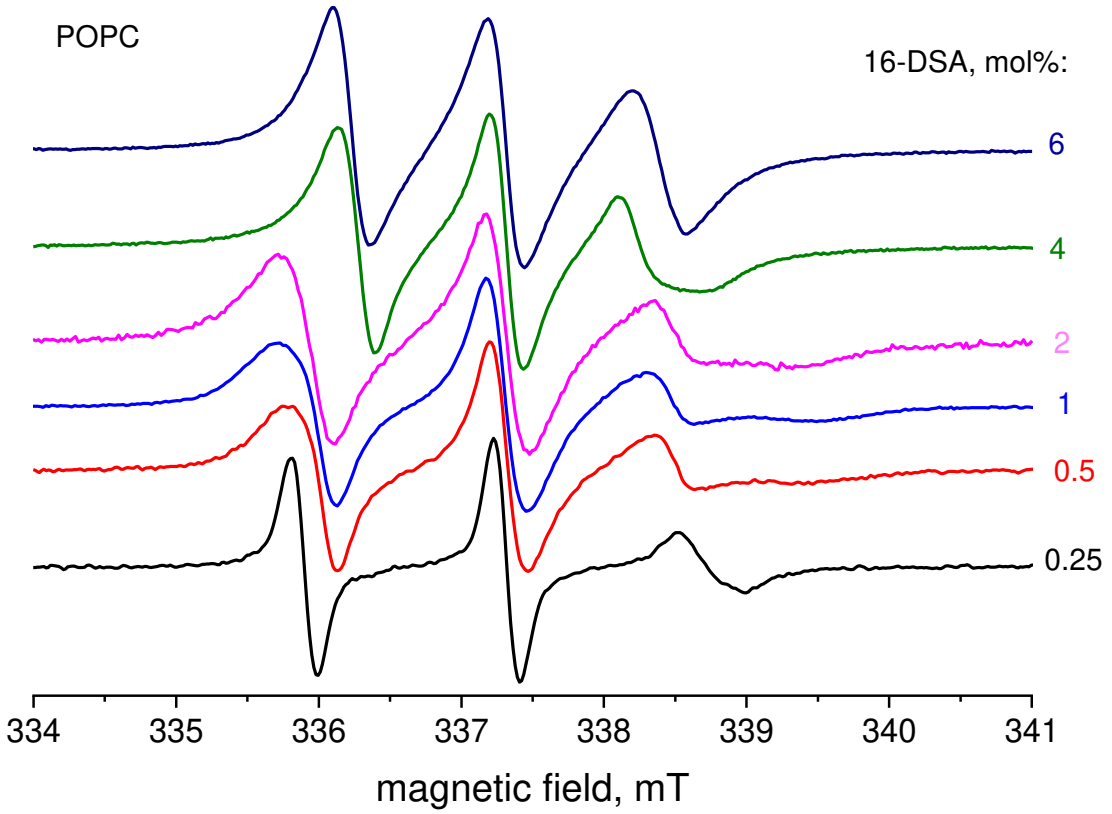
The local D-cholestane surface concentrations obtained as a function of the cholesterol concentration



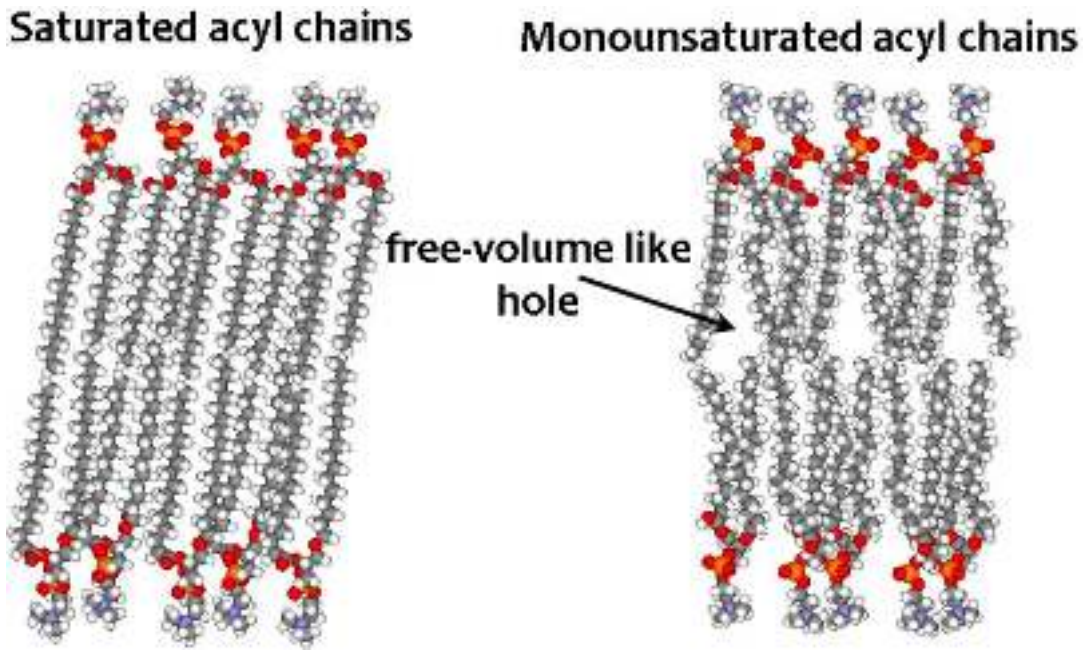
V.V. Unguryan, E.A. Golysheva, S.A. Dzuba, J. Phys. Chem. B, 2021

CW EPR spectra at room temperature for 16-DSA of different concentration in the DOPC/DPPC and POPC bilayers

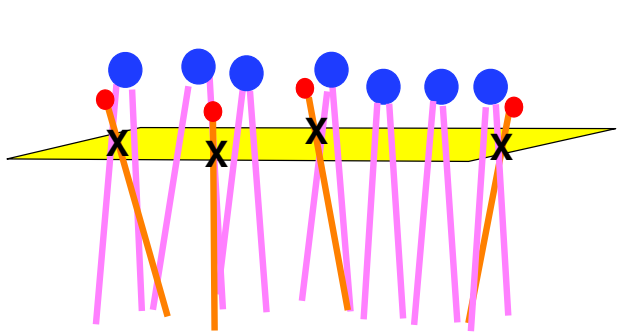




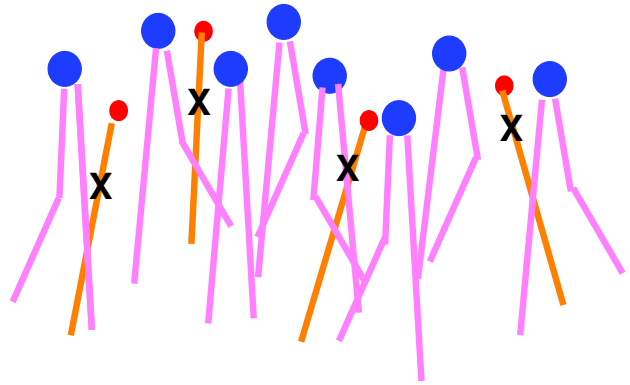
Итак, «двумерность» для одного бислоя и «трехмерность» для другого.
Объяснение этой разницы:

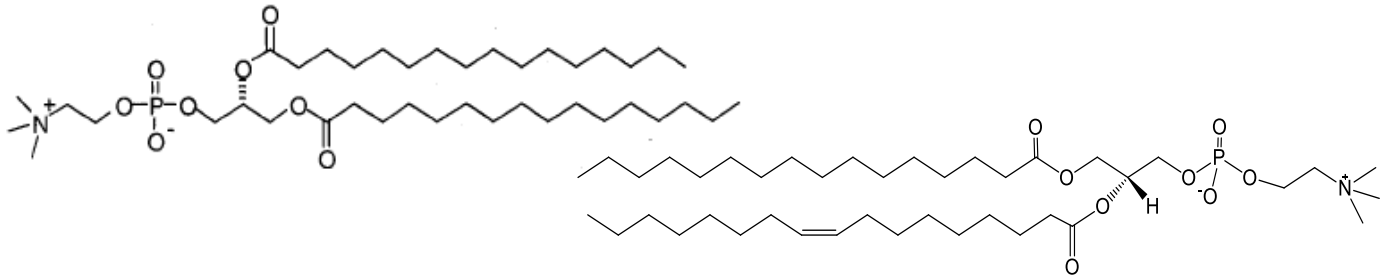


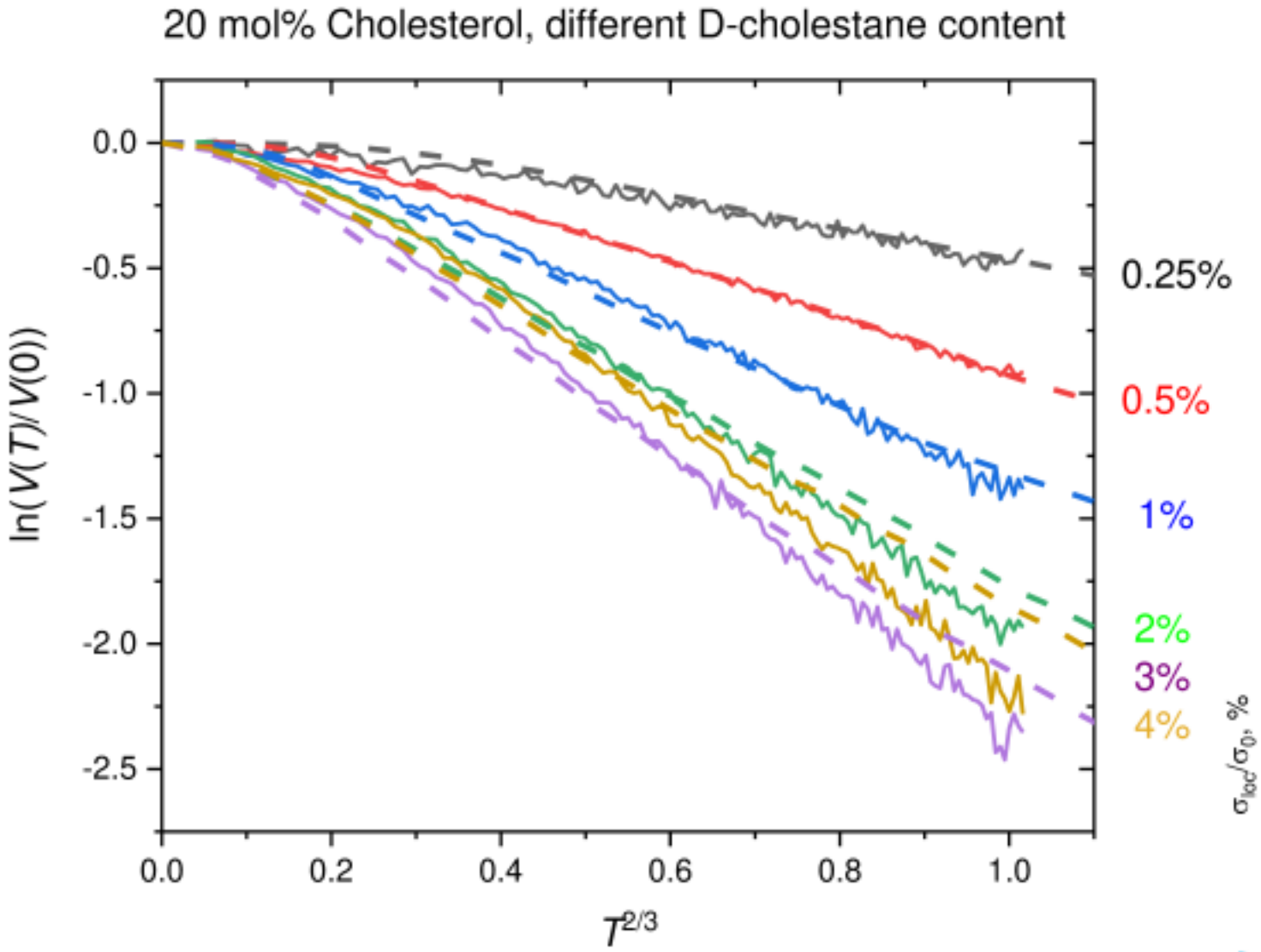
Бислой DOPC/DPPC:
высокая упорядоченность липидов

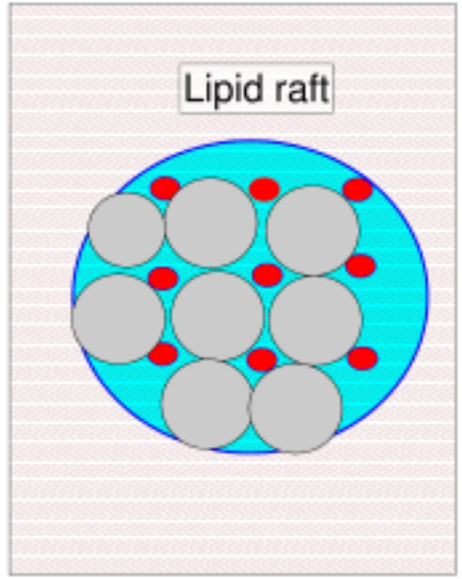


Бислой POPC:
липиды разупорядочены

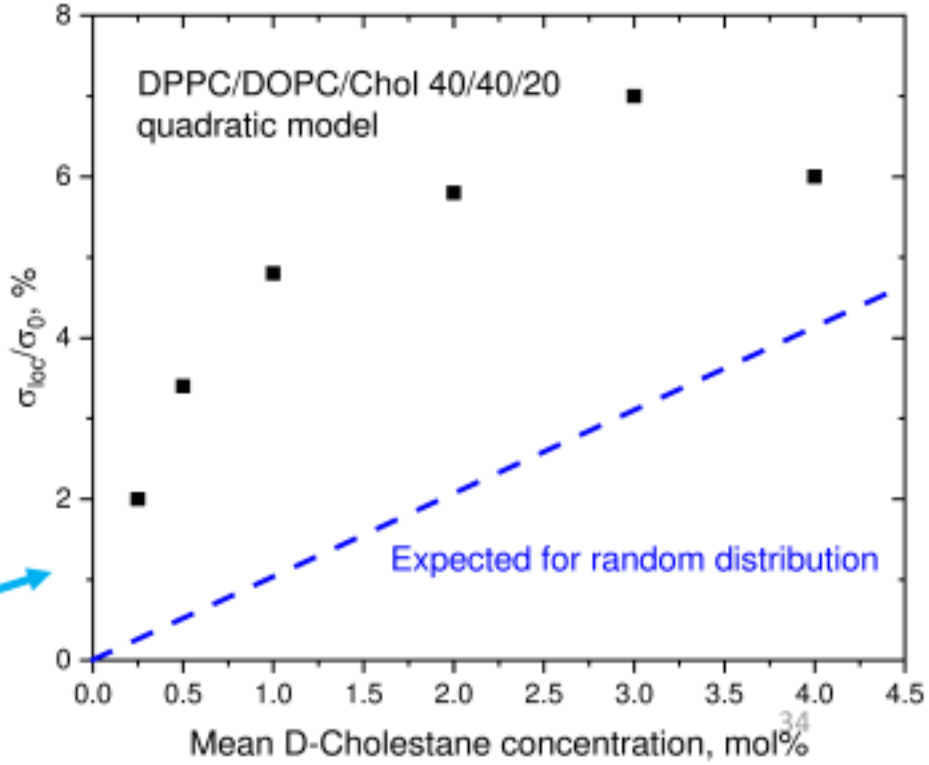






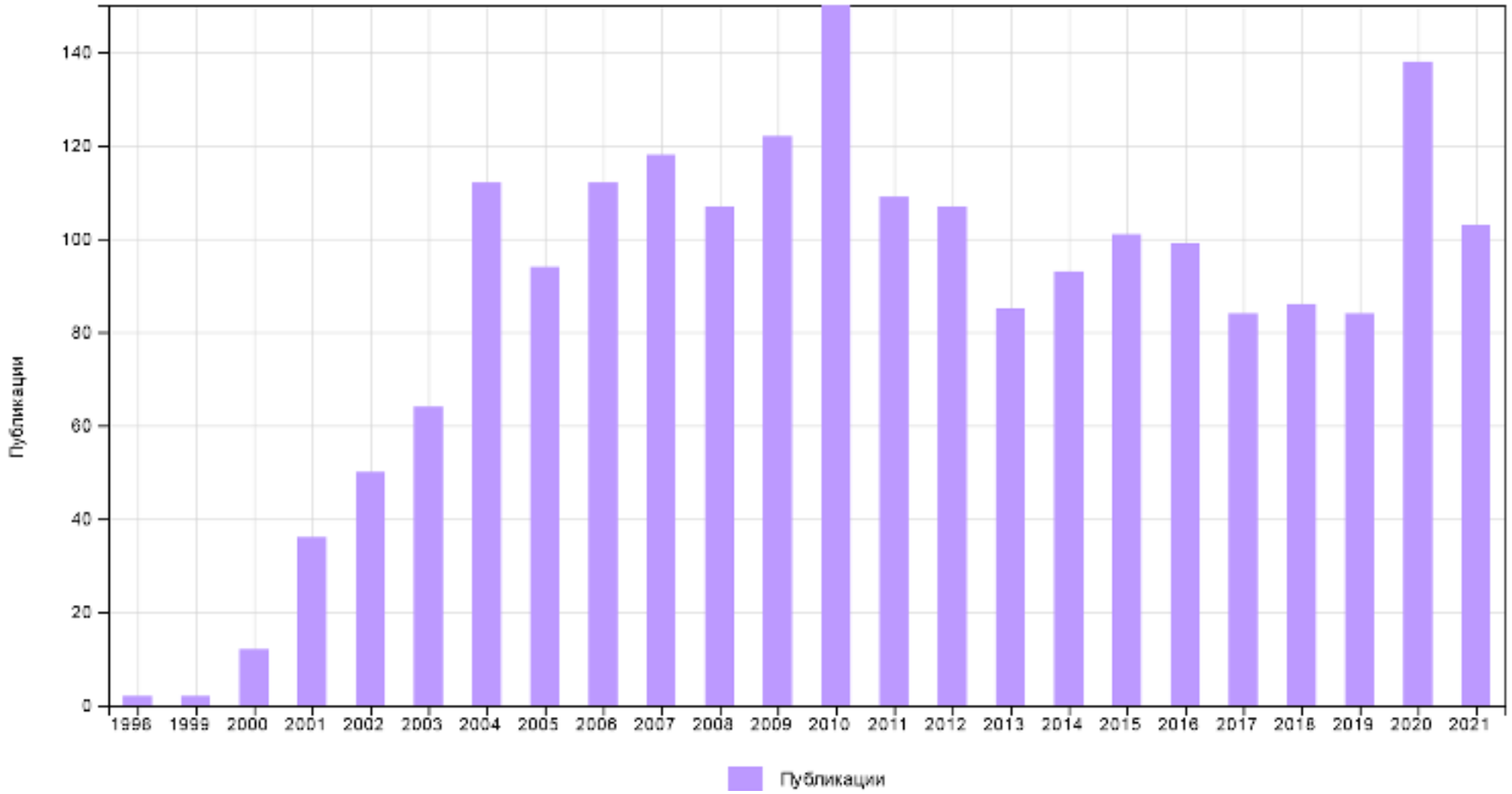


Membrane
top view

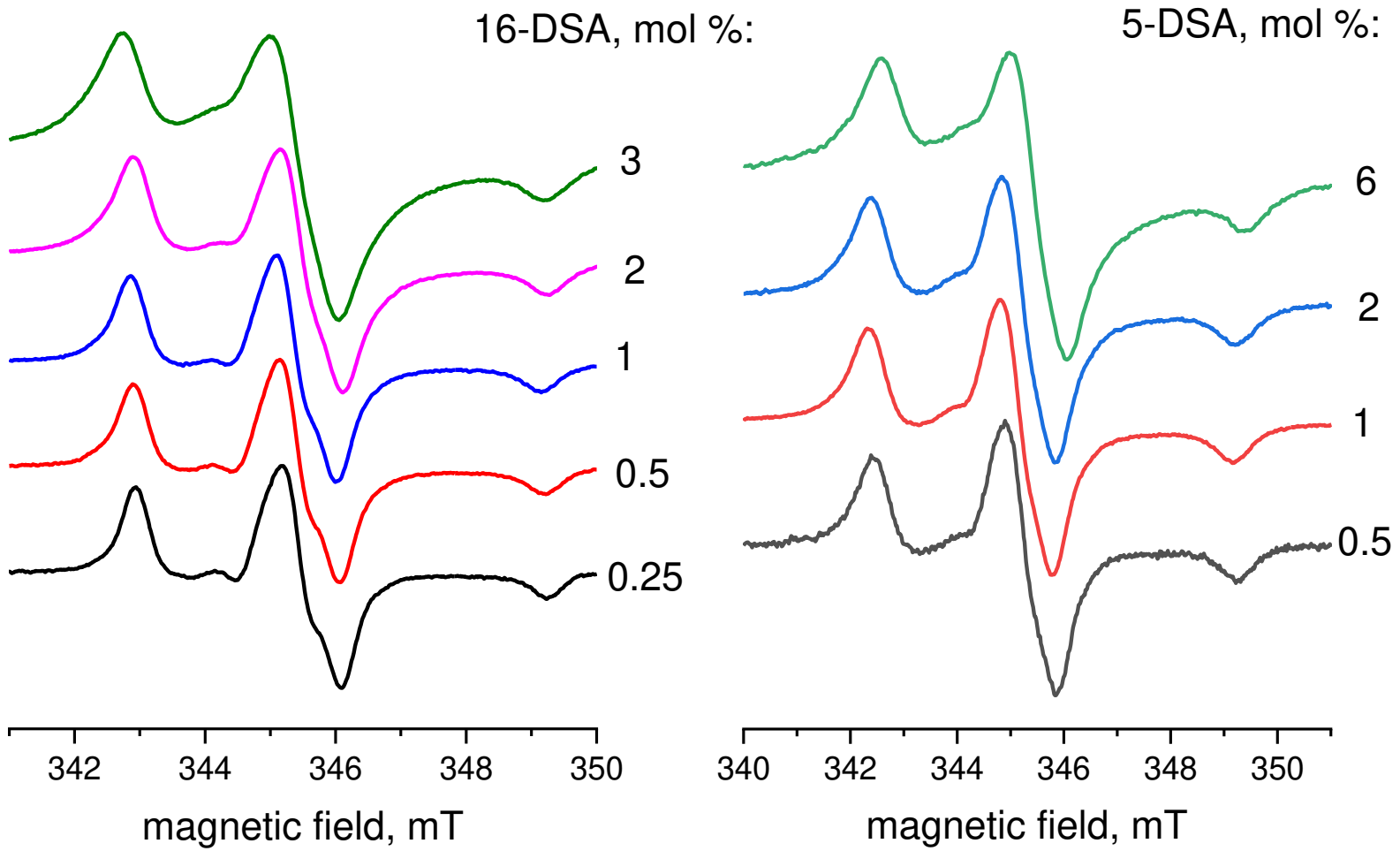


Excess of local concentration over the average values means **clustering**

Reviews on lipid rafts (Web of Science)

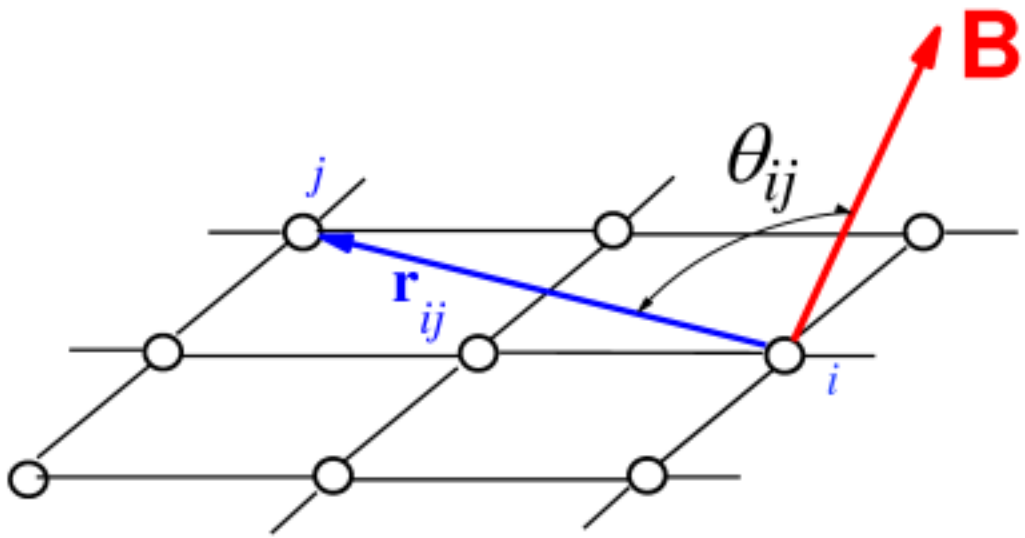


Спектры ЭПР при 200 K для 16-DSA and 5-DSA при разных концентрациях в бислое



Олигомеризация при высоких концентрациях

В расчетах мы используем простую модель квадратной «сверхрешетки»



$$V(T) = \prod_{i \neq j} \cos \frac{g^2 \mu_B^2}{\hbar r_{ij}^3} (1 - 3\cos^2 \theta_{ij}) T$$

Theory predicts for random space distribution of spin labels:

For 3-D random distribution : $V(T) = V(0)e^{-const*Cp_bT}$

C – local volume concentration,
p_b - excitation efficiency

For 2-D random distribution (as expected for bilayers): $V(T) = V(0)e^{-const*\sigma p_b T^{2/3}}$

σ – local surface concentration,

Временные спады DEER для бислоя POPC

анаморфоза для трехмерного пространства

анаморфоза для двумерного пространства