X International Voevodsky Conference "Physics and Chemistry of Elementary Chemical Processes" (VVV-2022)

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LOW-FREQUENCY RAMAN SPECTROSCOPY OF PHOSPHOLIPID MEMBRANES

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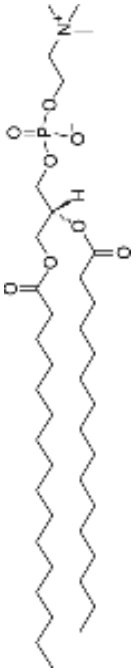
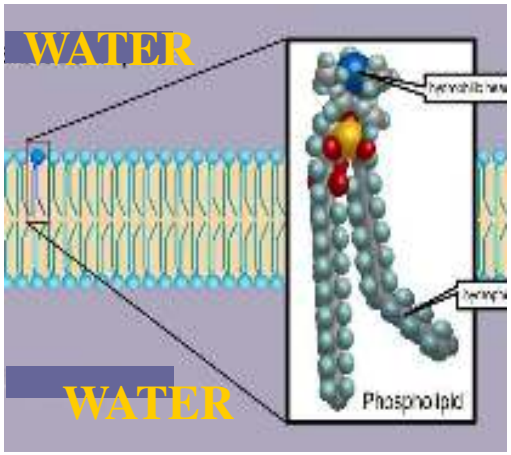
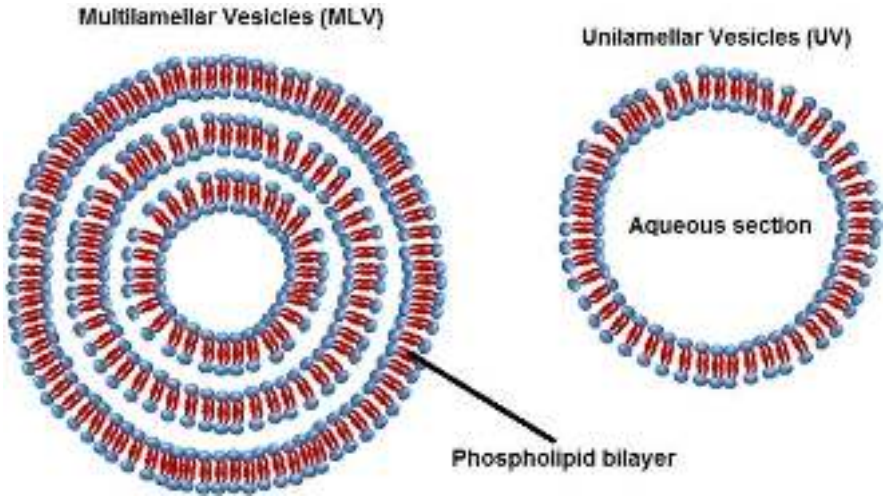
A number of peculiar features in the terahertz vibrational spectrum, which is manifested in the low-frequency Raman spectrum, are expected for 2D-like objects with the nanometer thickness as phospholipid bilayers. One can hope that the low-frequency Raman spectrum should have information about the thickness breathing mode of the phospholipid layers and about the acoustic-like excitations.

Phospholipid bilayers (membranes), vesicles







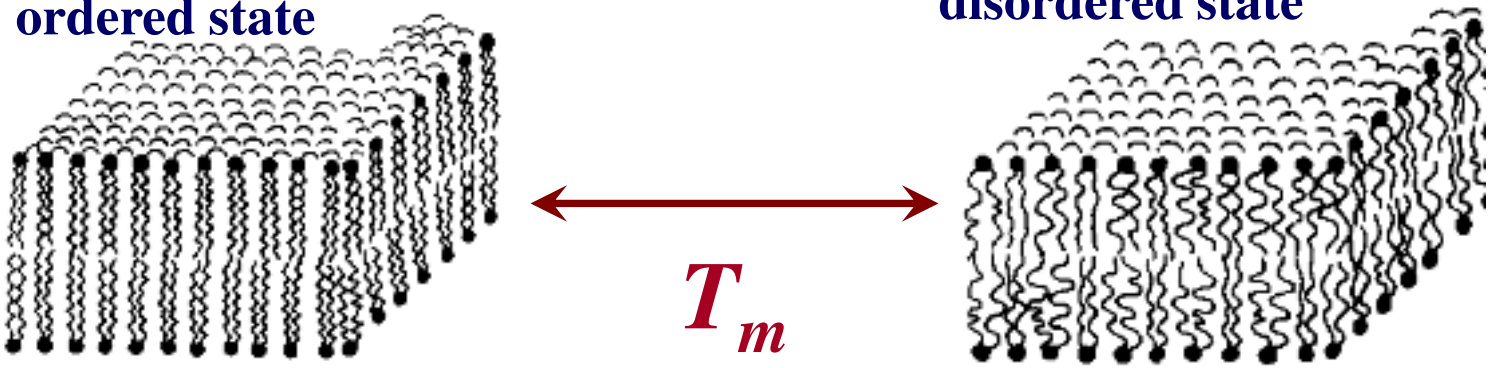
Phases of phospholipid bilayers

Gel state

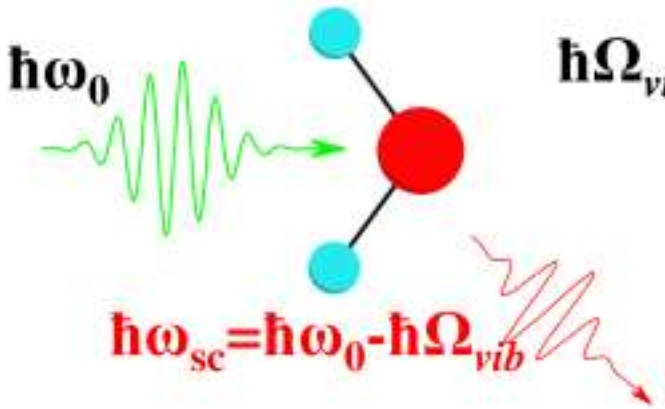
- conformationally and laterally ordered state

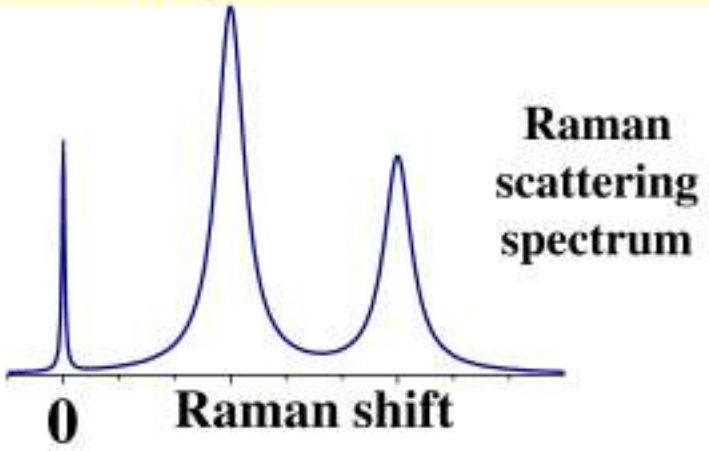
Fluid state

- conformationally and laterally disordered state



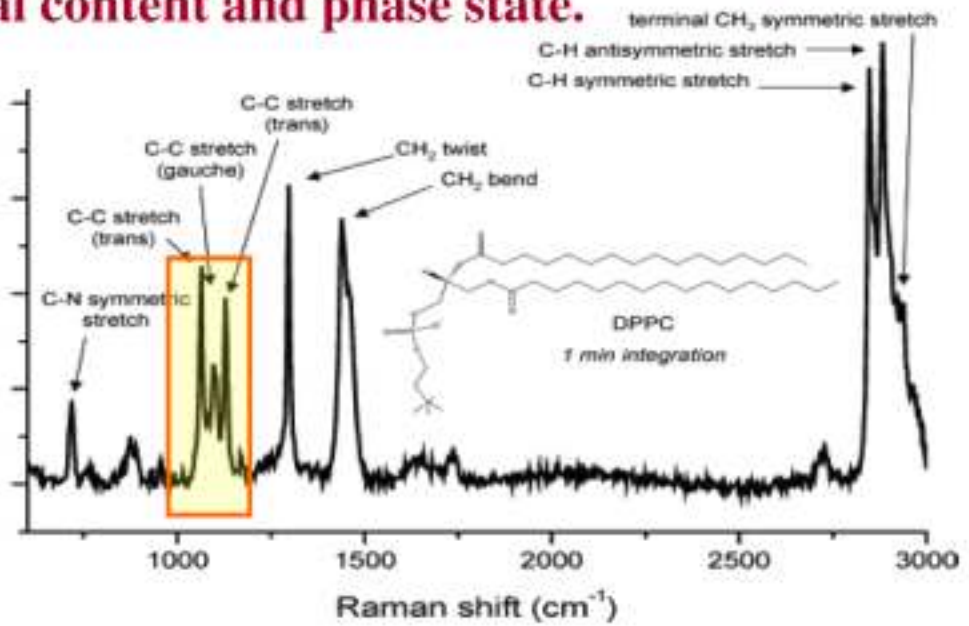
Raman spectroscopy





Raman spectrum reflects the vibrational spectrum of material and provides the information about its chemical content and phase state.

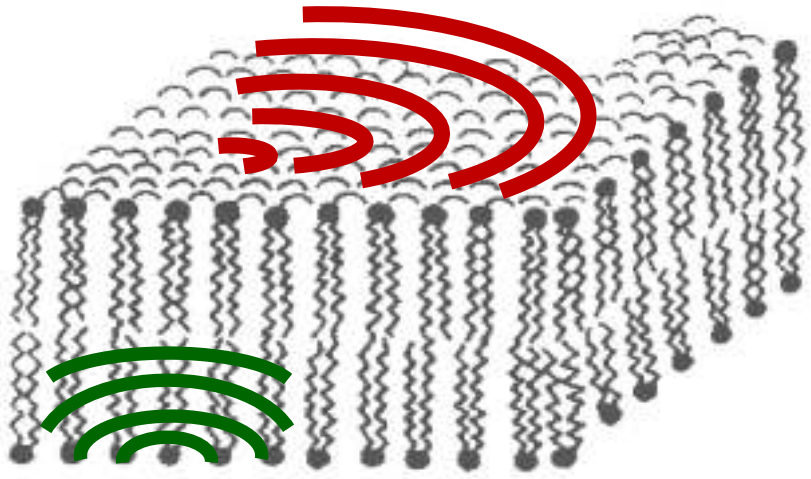
C.B. Fox *et al*
J.Phys.Chem.B
2007



Low-frequency (<100 cm^{-1}) Raman is in the acoustic-like range

Low-frequency Raman spectrum of 2D-like phospholipid layers

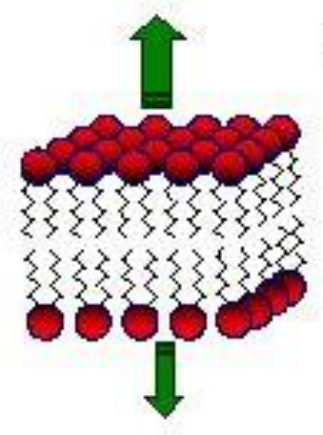
Acoustic-like vibrational modes along layers



Acoustic-like modes with the effective wavevector crossing layers or localized modes (e.g. the thickness breathing modes)

Low-frequency Raman spectra are promising for obtaining information about the thickness, modulus of elasticity, and lateral ordering on the nanometer scale.

Thickness breathing modes of phospholipid bilayers



Lowest vibrational mode of a thick plate with thickness of $2d$

$$\nu_N\left[cm^{-1}\right] = \frac{c_S}{4cd}$$

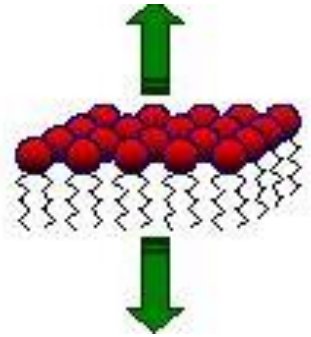
c_S **is sound velocity**
c **is speed of light**

Estimation for Bilayer eigenmode

if $2d$ = 5 nm, c_S = 2.4 km/s

⇒ **ν_1= 8 cm^{-1}**

The case of poor elastic contact between layers of a bilayer



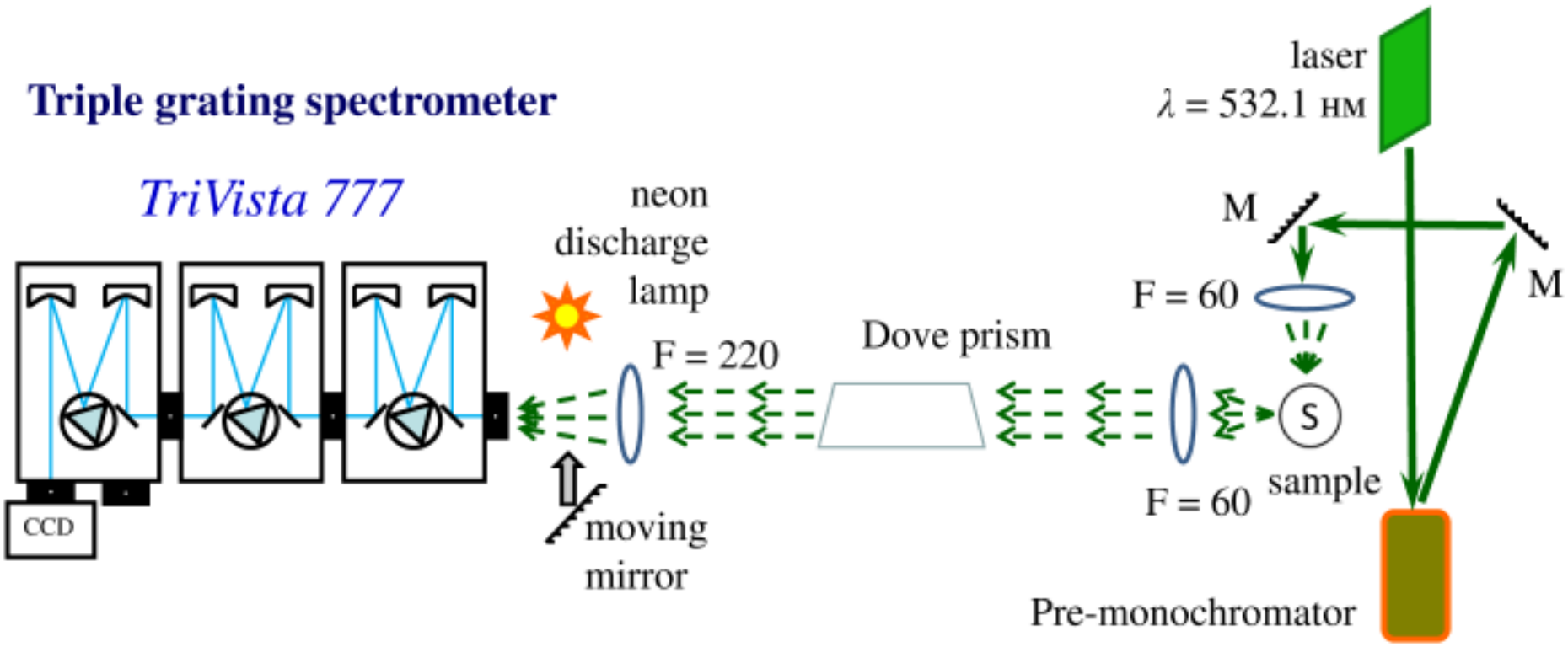
⇒ **ν_1= 16 cm^{-1}**

Monolayer eigenmode

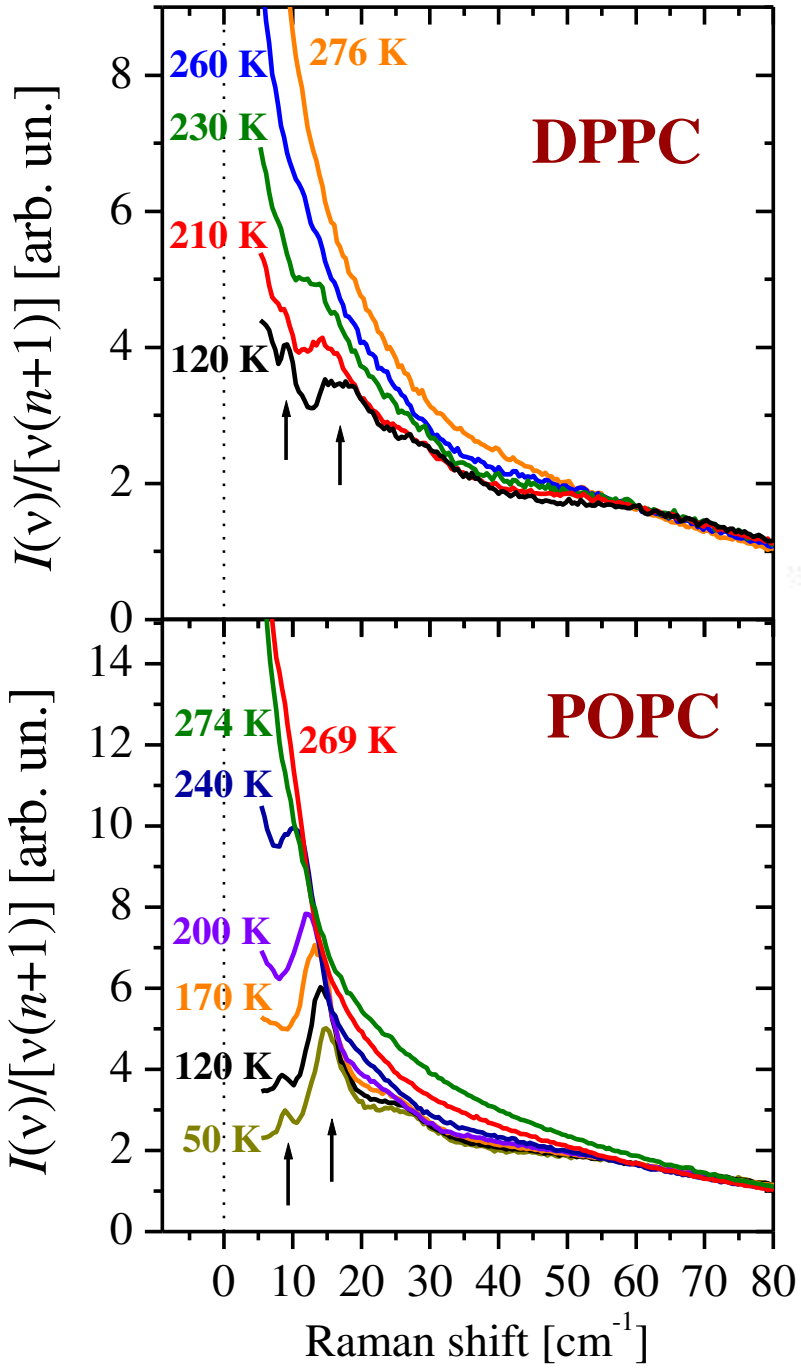
Low-frequency Raman set-up

Triple grating spectrometer

TriVista 777

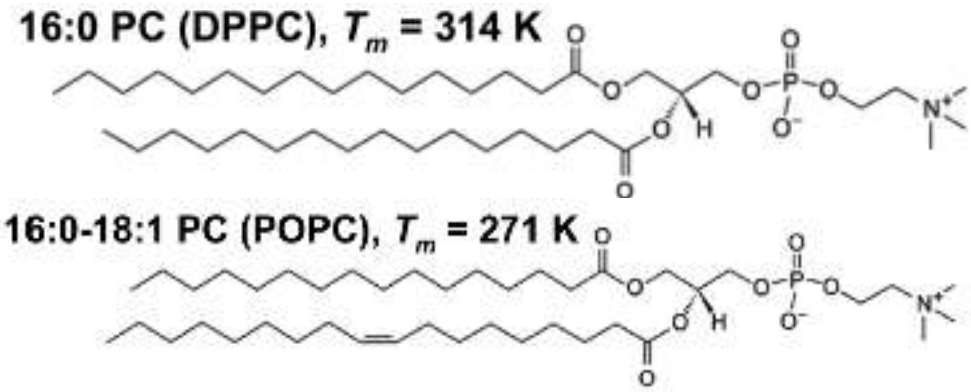


It is possible to measure low-frequency Raman spectrum of phospholipid suspensions down to 5 cm^{-1}.



Raman spectra of aqueous suspensions of phospholipid vesicles

N.V. Surovtsev et al // Phys. Rev. E 95 (2017) 032412



Raman peaks, attributed to bilayer and monolayer eigenmodes, are observed simultaneously!

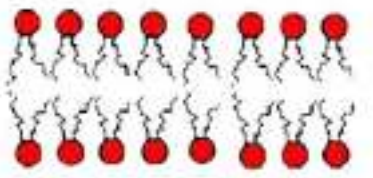
GOAL:
Can the low-frequency peak be used for revealing the coexisting domains in multicomponent membranes?

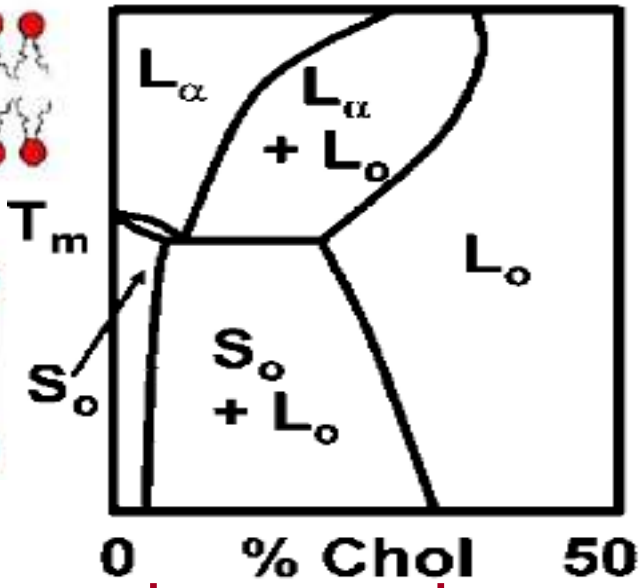
Phase diagram for phospholipid-cholesterol mixtures

phospholipid

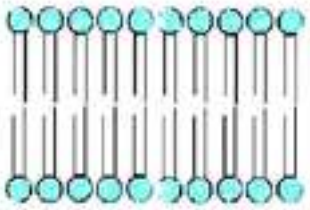
cholesterol

Liquid disordered

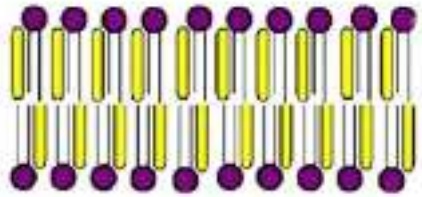




Gel (solid-ordered)



cholesterol poor



Liquid ordered (L_o)

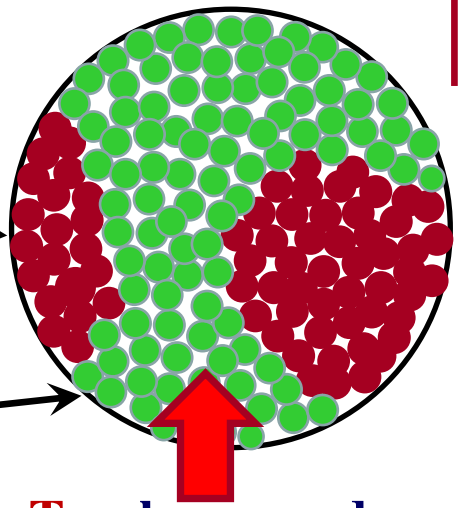
cholesterol rich

coexistence range

coexisting domains

cholesterol rich

cholesterol poor



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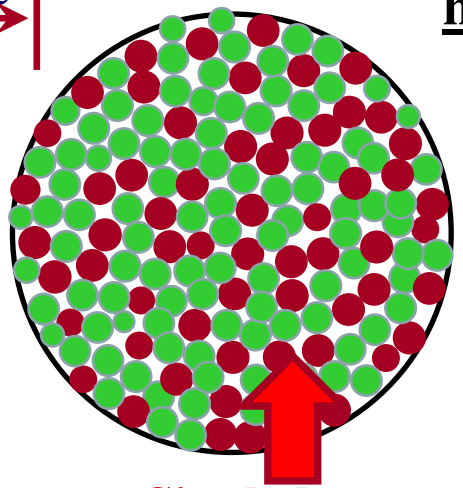


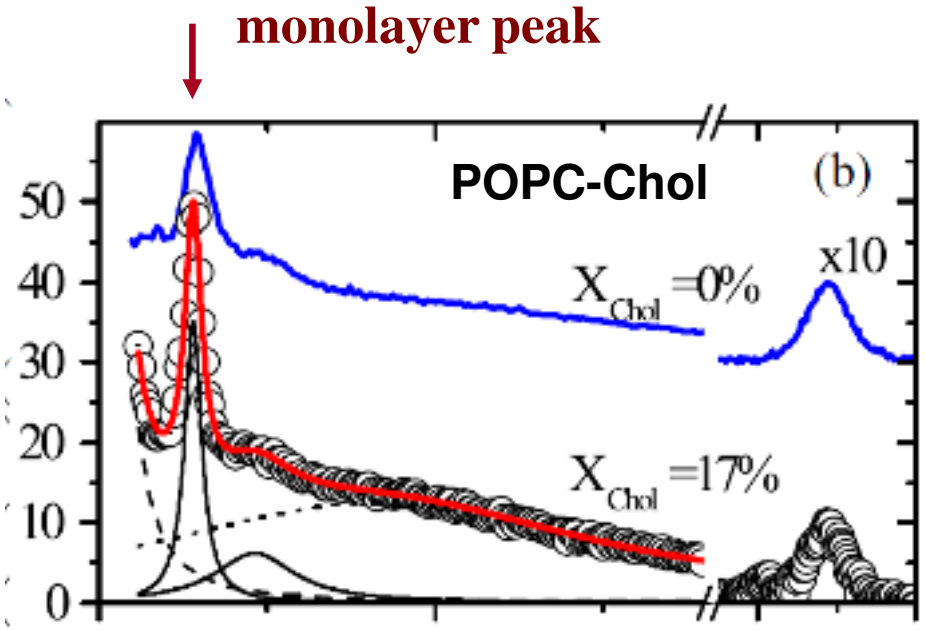
homogeneously distributed complexes

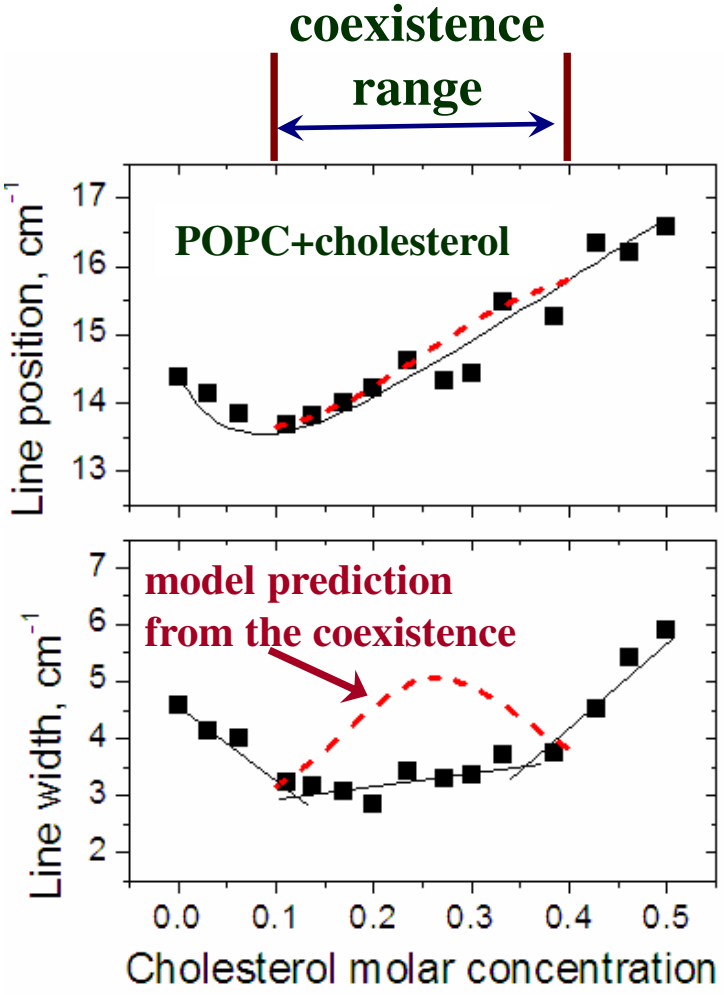
Two layer modes are expected in Raman spectrum

Single layer mode are expected in Raman spectrum

Low-frequency Raman spectra of frozen phospholipid vesicles

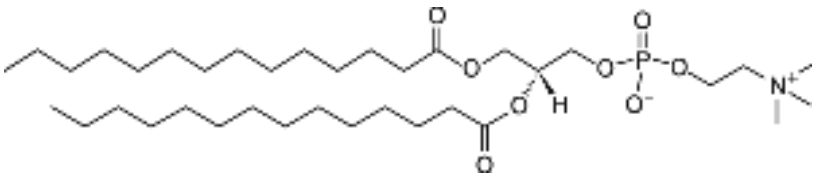
D.V. Leonov et al // Phys. Rev. E 99 (2019) 022417



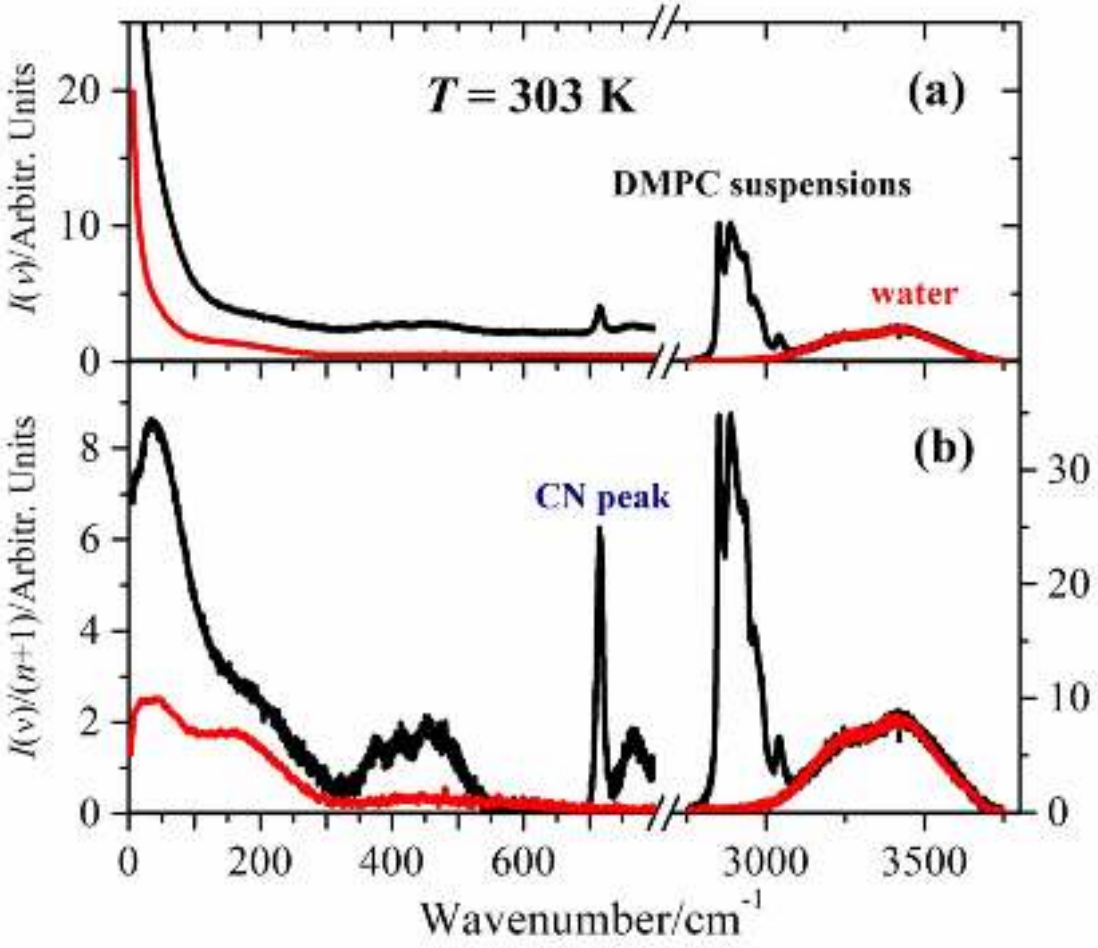


Parameters of the low-frequency peak (monolayer mode) contradict to the coexisting domains hypothesis for binary systems of phospholipid-cholesterol

Low-frequency Raman spectrum – sensitivity to the phase state of phospholipid bilayers



Raman spectrum of the aqueous suspension of DMPC vesicles



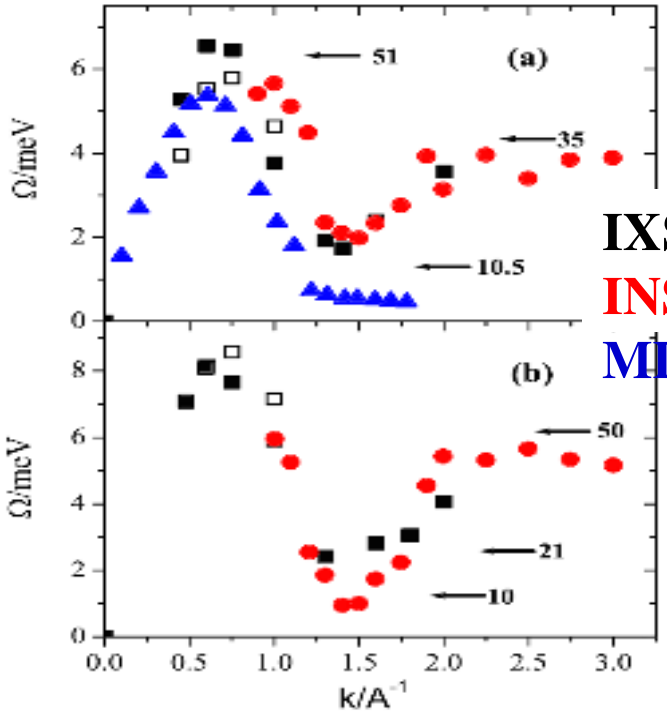
N.V. Surovtsev, S.V. Adichtchev // J. Raman Spectrosc. 51 (2020) 952

Susceptibility presentation

Low-frequency Raman spectrum – sensitivity to the phase state of phospholipid bilayers

N.V. Surovtsev, S.V. Adichtchev
// J. Raman Spectrosc. 51 (2020) 952

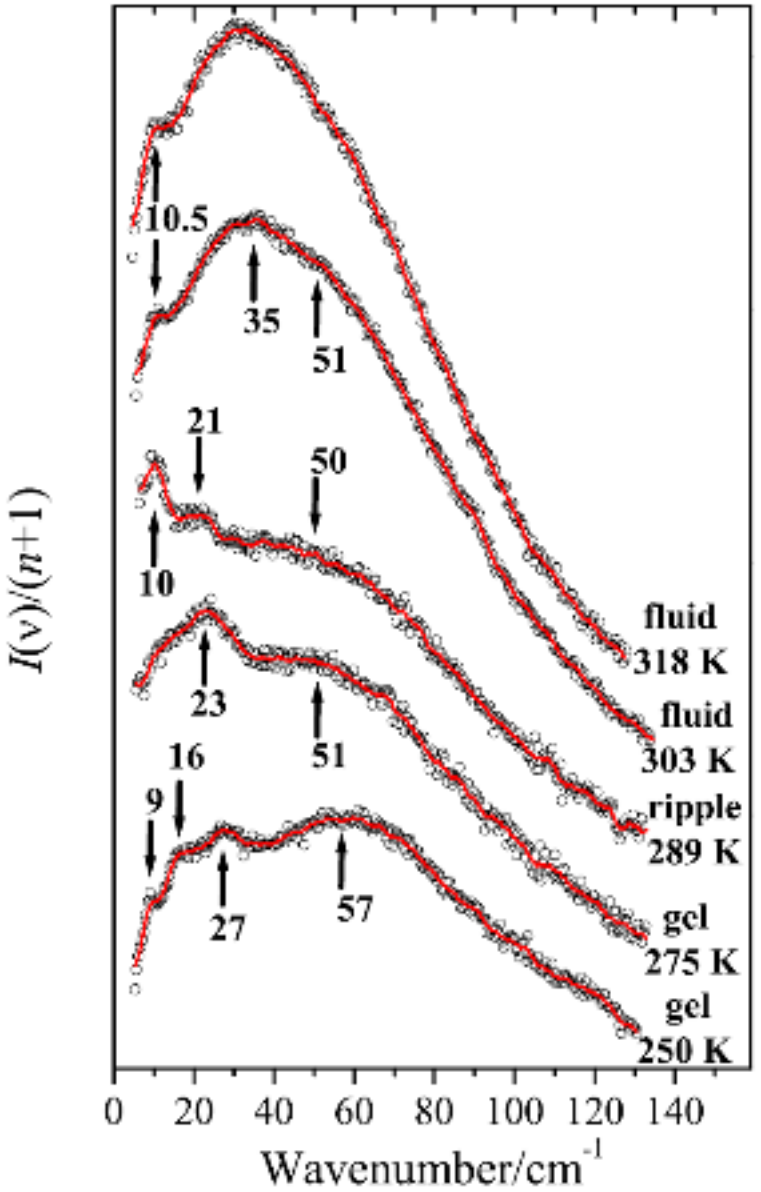
Raman susceptibility of the aqueous suspension of DMPC vesicles



fluid phase

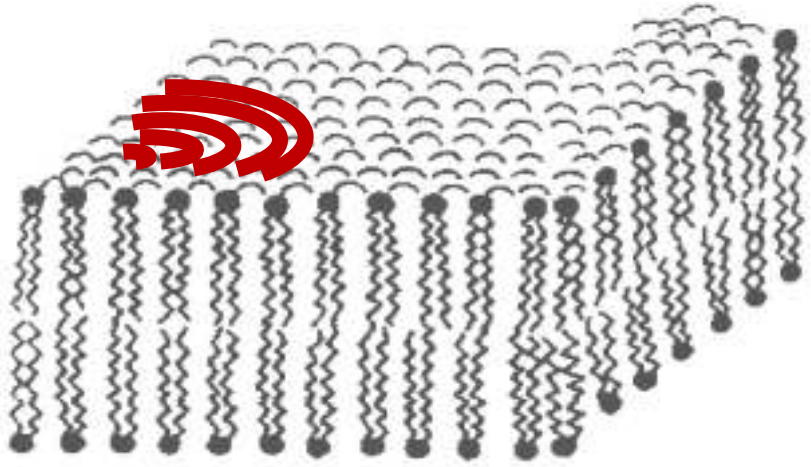
IXS
INS
MD

ripple phase



Interpretation:

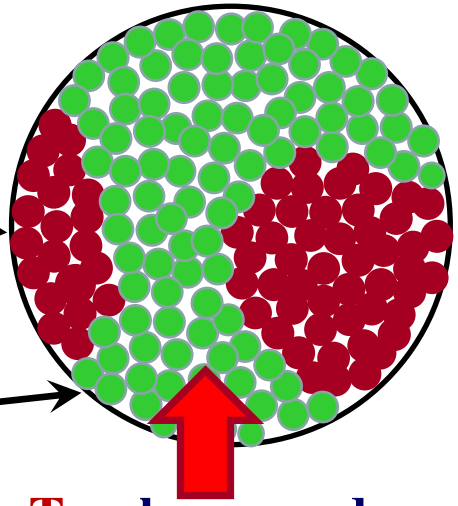
Acoustic-like excitations propagating along layers.



coexisting domains

cholesterol rich

cholesterol poor



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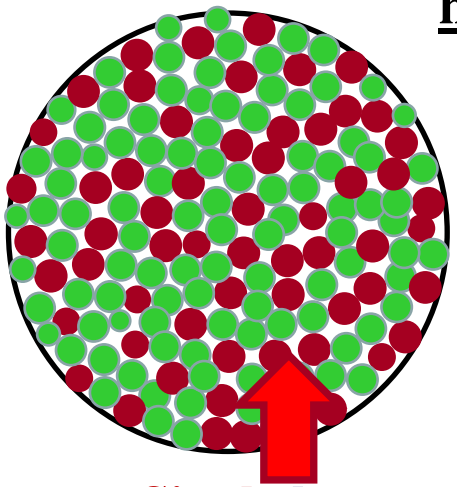
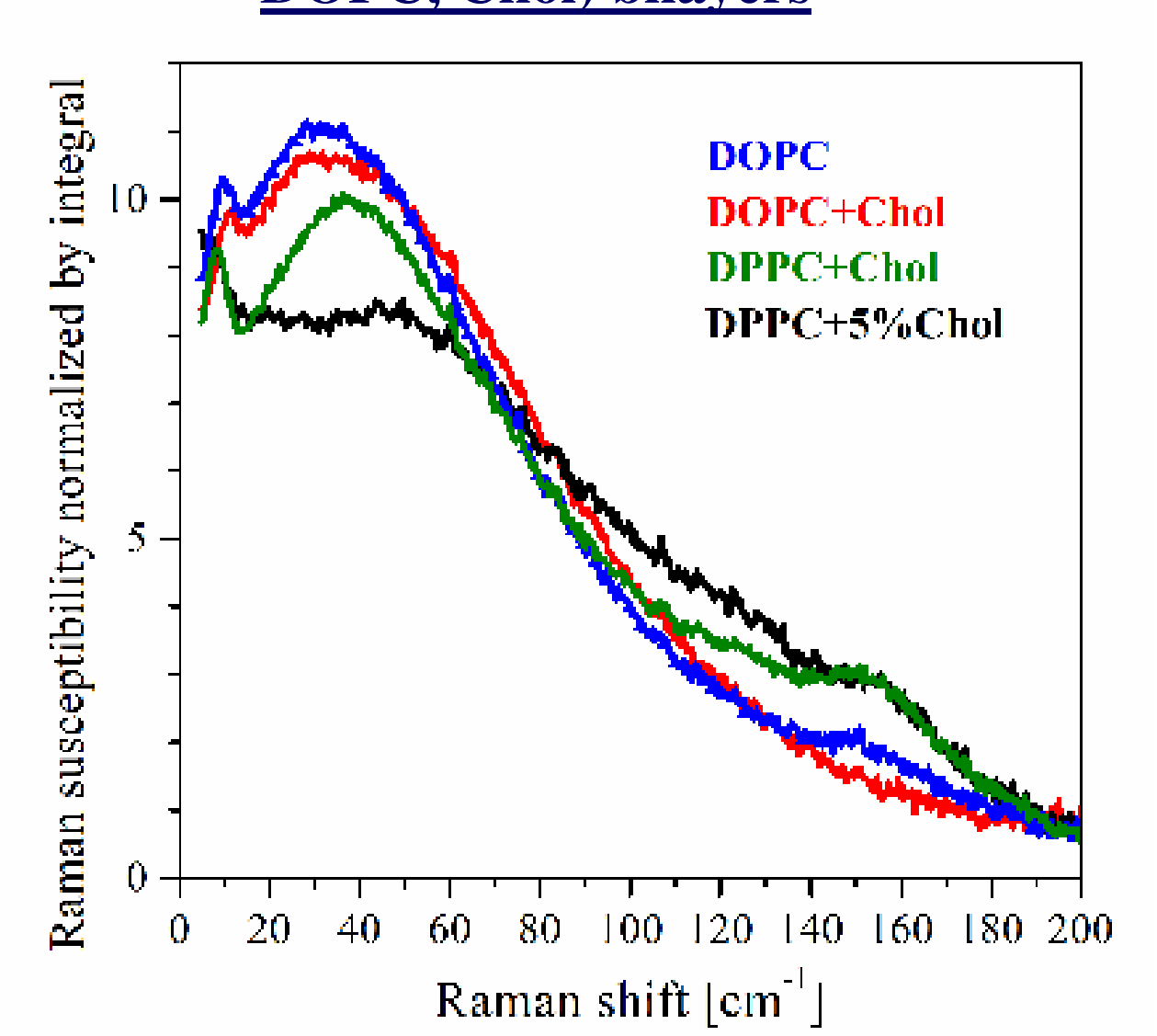


homogeneously distributed complexes

Two layer modes are expected in Raman spectrum

Single layer mode are expected in Raman spectrum

Low-temperature Raman spectra of mono- and binary (DPPC, DOPC, Chol) bilayers



Difference spectra – the test for the coexistence of domains of different phases

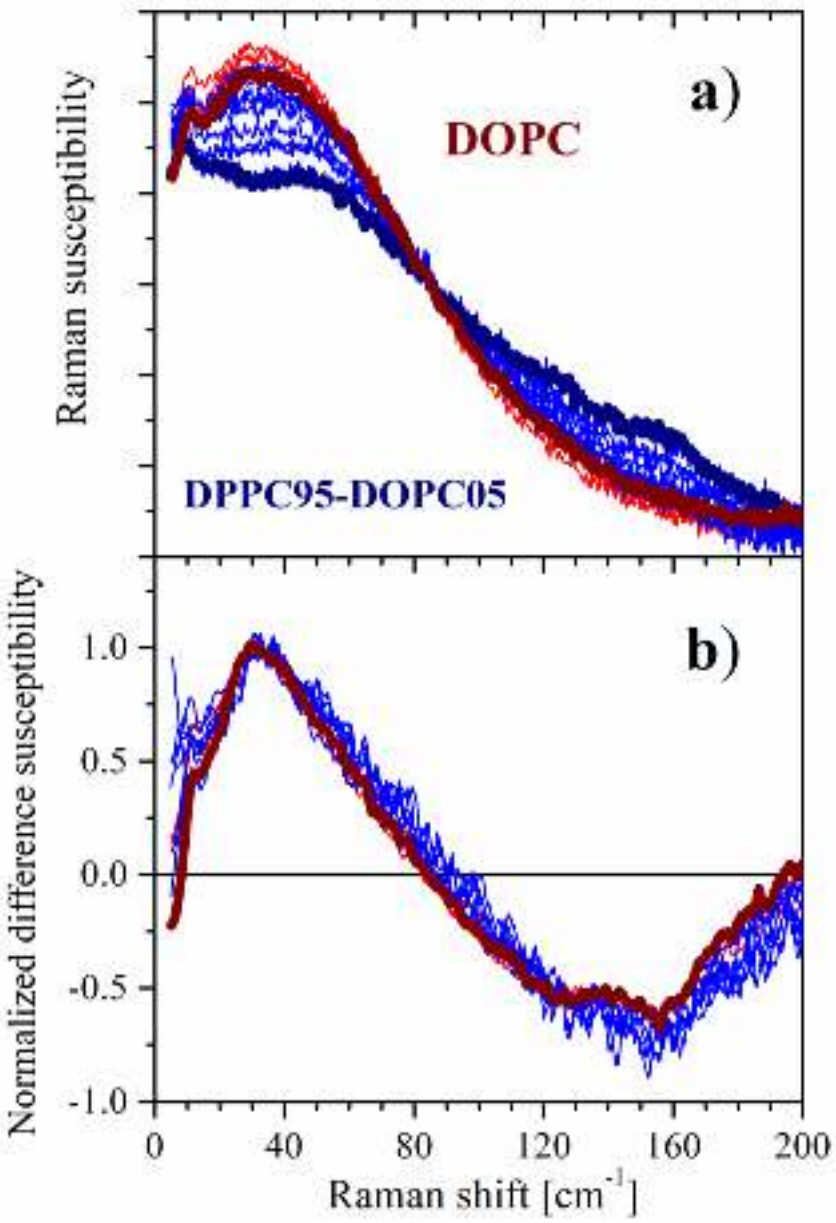
Если: $S_{AB}(\omega) = aS_A(\omega) + (1-a)S_B(\omega)$

То: $S_{AB}(\omega) - S_B(\omega) = a(S_A(\omega)-S_B(\omega))$

Difference spectra should have the same spectral shape

"Magnitude" of the difference spectrum corresponds to the contribution of one of the component

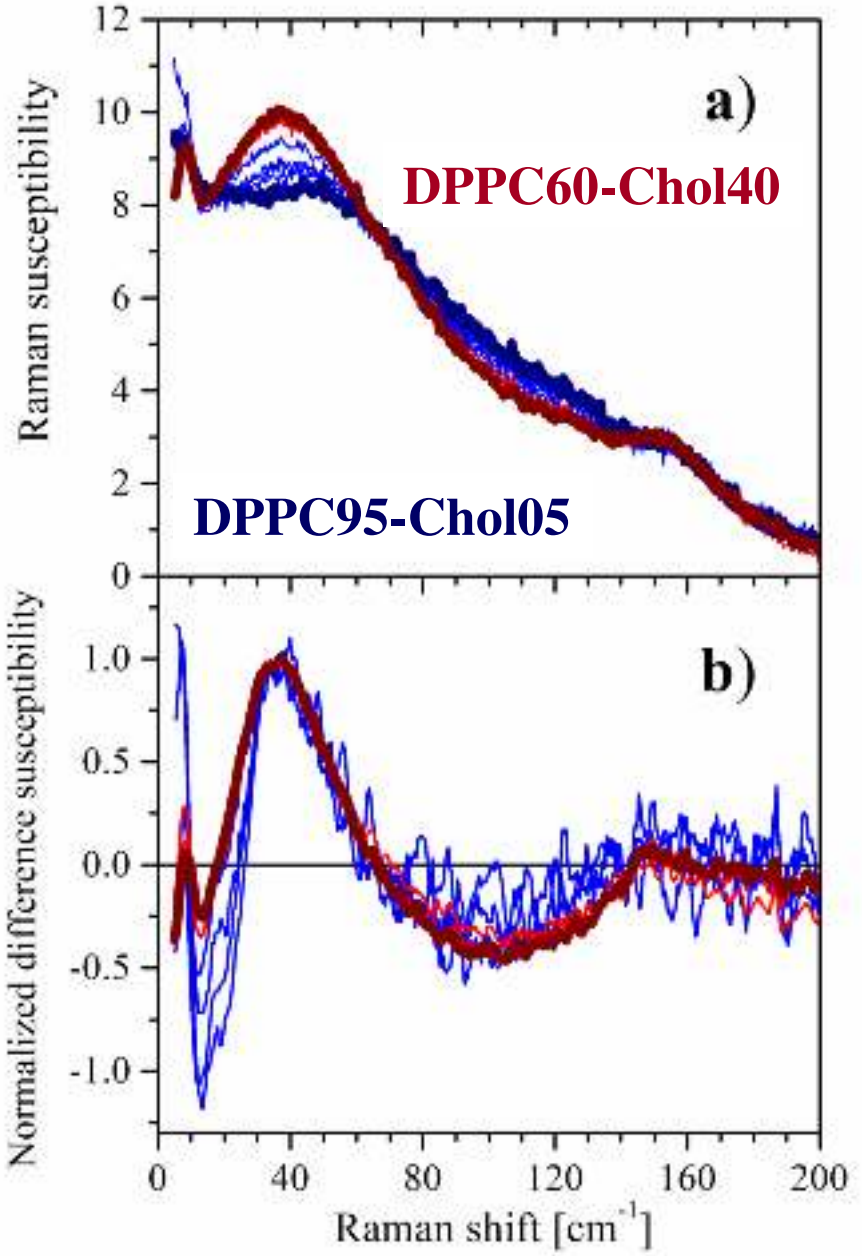
Difference spectra – the test for the domain coexistence



DOPC-DPPC mixtures

The master curve as evidence of the domain coexistence

Difference spectra – the test for the domain coexistence



DPPC-Chol mixtures

The master curve works for frequencies above 30 cm^{-1}

For sound velocity 2 км/с,

30 см$^{-1}$ corresponds to

the wavelength of 2 nm

Coexistense of phospholipid-cholesterol complexes, but homogeneity for the scales above 2 nm

Conclusions

The low-frequency Raman spectrum of phospholipid membranes has contributions from the thickness breathing modes of the phospholipid layers and the acoustic-like excitations.

These spectra should be useful for characterizing the elastic modulus and layer thickness, their temperature dependences, the sensitivity to the phase state and for the domain coexistence problem.