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Physicochemical properties of phospholipids

Alfred Blume

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Georg Friedrich Händel

Lyonel Feininger

Halle, marketplace with "Red Tower" and Church of our Lady

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Outline

From historical results to modern modelling techniques

- Chemical structures of lipids
- Crystal structures of lipids
- Lyotropic and thermotropic behavior of phospholipids
- Methods: Differential scanning calorimetry, DSC, x-ray scattering
- Phospholipid head group charge
- Phospholipid mixtures
- Methods: FT-IR-spectroscopy
- Hydration of phospholipid headgroups
- Methods: Isothermal titration calorimetry, ITC
- External binding and partitiong processes
- pK-values of charged phospholipids
- Dynamics of phospholipids in bilayers
- Fluorescence imaging of giant unilamellar vescicles

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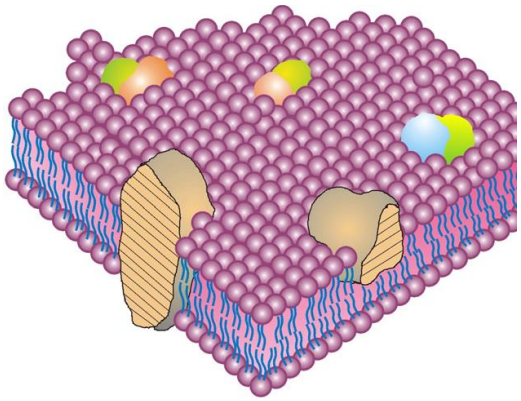
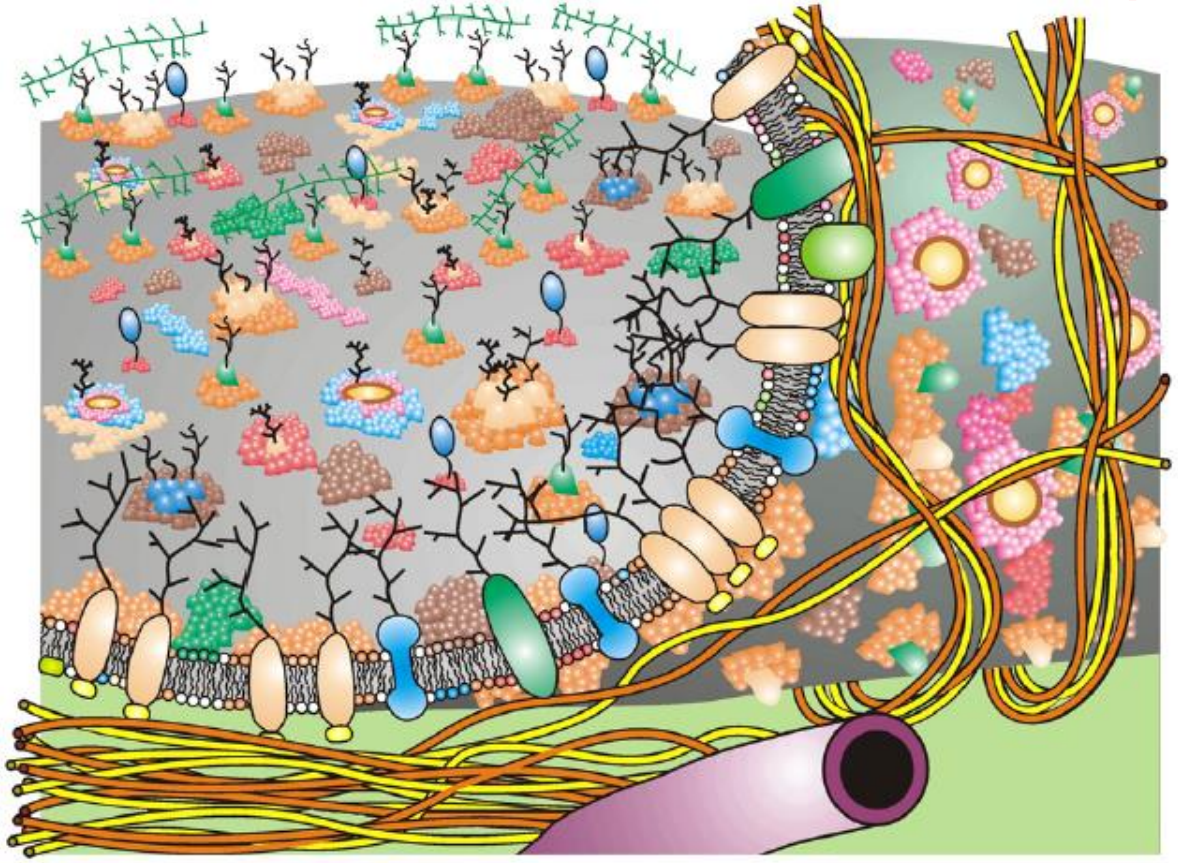
Updated fluid-mosaic membrane model

G.L.Nicolson, Biochimica et Biophysica Acta 1838 (2014) 1451–1466



old (1972)

new (2014)



An updated Fluid—Mosaic Membrane Model that contains information on membrane domain structures and membrane-associated cytoskeletal and extracellular structures. Different integral proteins, glycoproteins, lipids and oligosaccharides are represented by different colors, and where the membrane has been peeled-up to view the inner membrane surface cytoskeletal fencing is apparent that restricts the lateral diffusion of some but not all trans-membrane glycoproteins.

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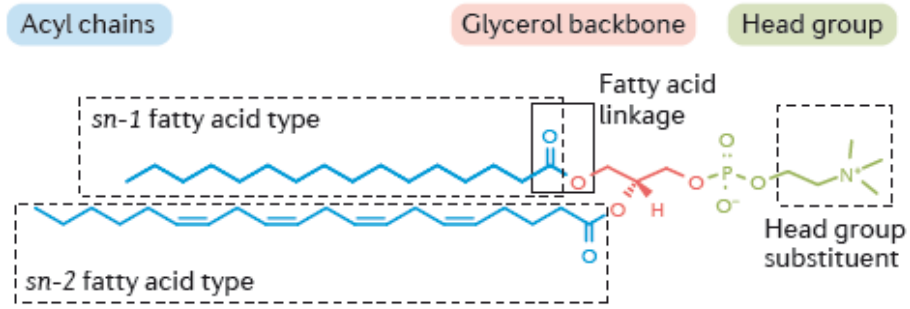
Chemical diversity of membrane phospholipids in mammals.

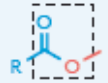
a GPL diversity



GPL	Head group substituent
Phosphatidic acid	–
PtdCho	Choline
PtdEtn	Ethanolamine
PtdSer	Serine
PtdIns	Inositol
PtdGro	Glycerol
Cardiolipin	PtdGro
LBPA	LPA
PtdGlc	Glucose

Fatty acid linkage

Ester (acyl)

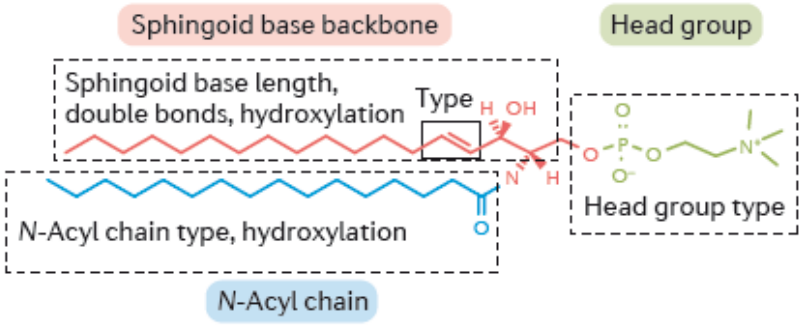


Ether GPLs

Ether (alkyl)

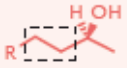
Vinyl-ether (alkenyl)

b Sphingolipid diversity

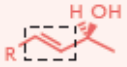


Sphingoid base type

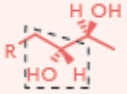
DHS



SPH



PHS



Sphingolipid	Head group
Cer	Hydroxyl
Sphingomyelin	Phosphocholine
CerPE	Phosphoethanolamine
GlcCer	Glucose
GalCer	Galactose
Complex GSLs	Oligosaccharides
C1P	Phosphate

Takeshi Harayama and Howard Riezman (2018) Nature Reviews Molecular Cell Biology

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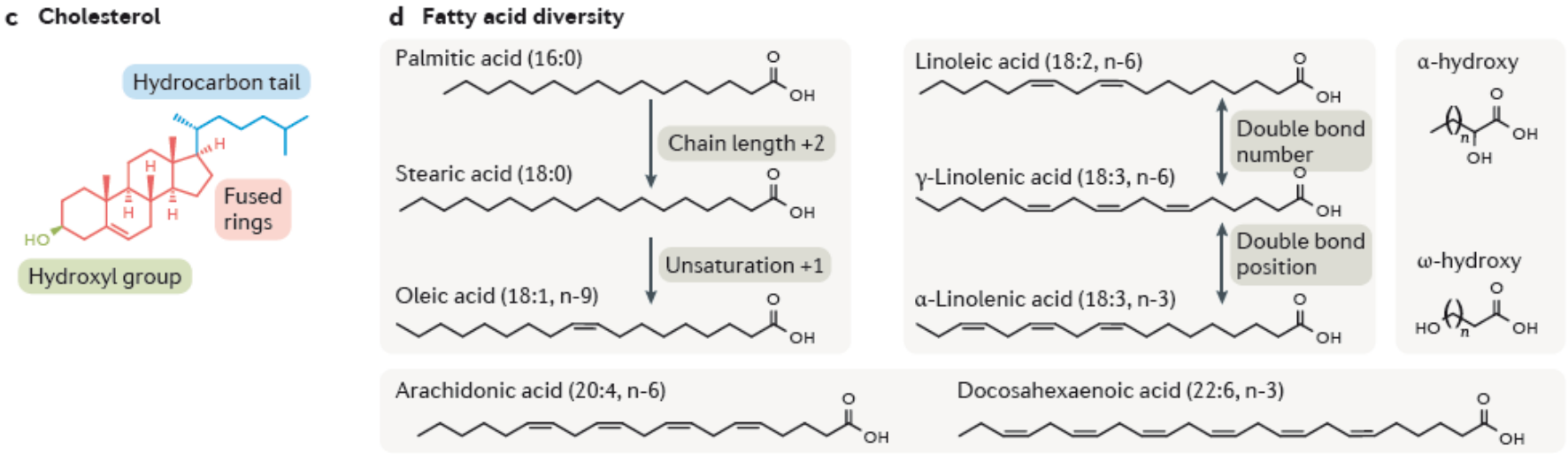
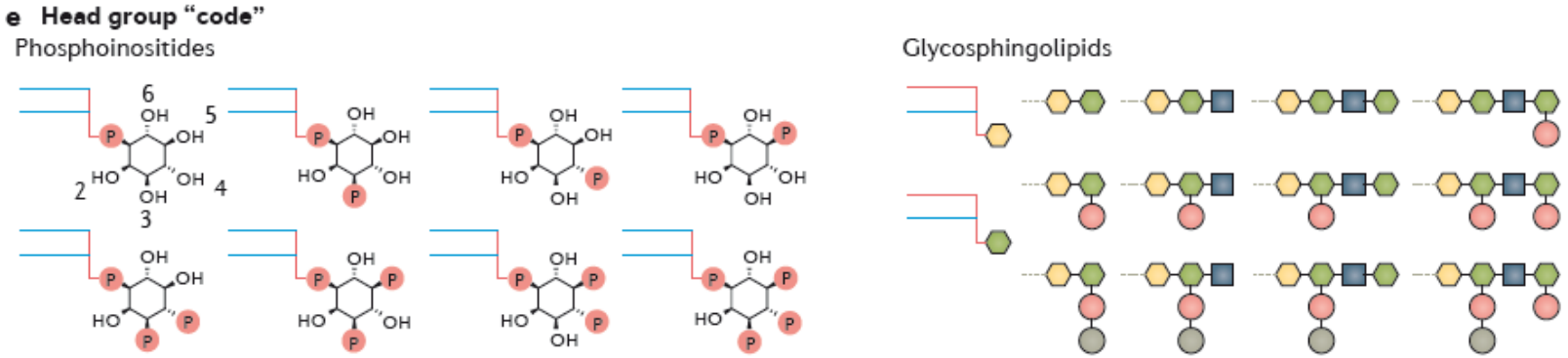
Chemical diversity of membrane lipids in mammals.





The majority of commercially in large quantities available natural phospholipids used for pharmaceutical applications are extracted from egg yolks or soy beans

Takeshi Harayama and Howard Riezman (2018) Nature Reviews Molecular Cell Biology

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Packing of lipids in crystals: DMPA

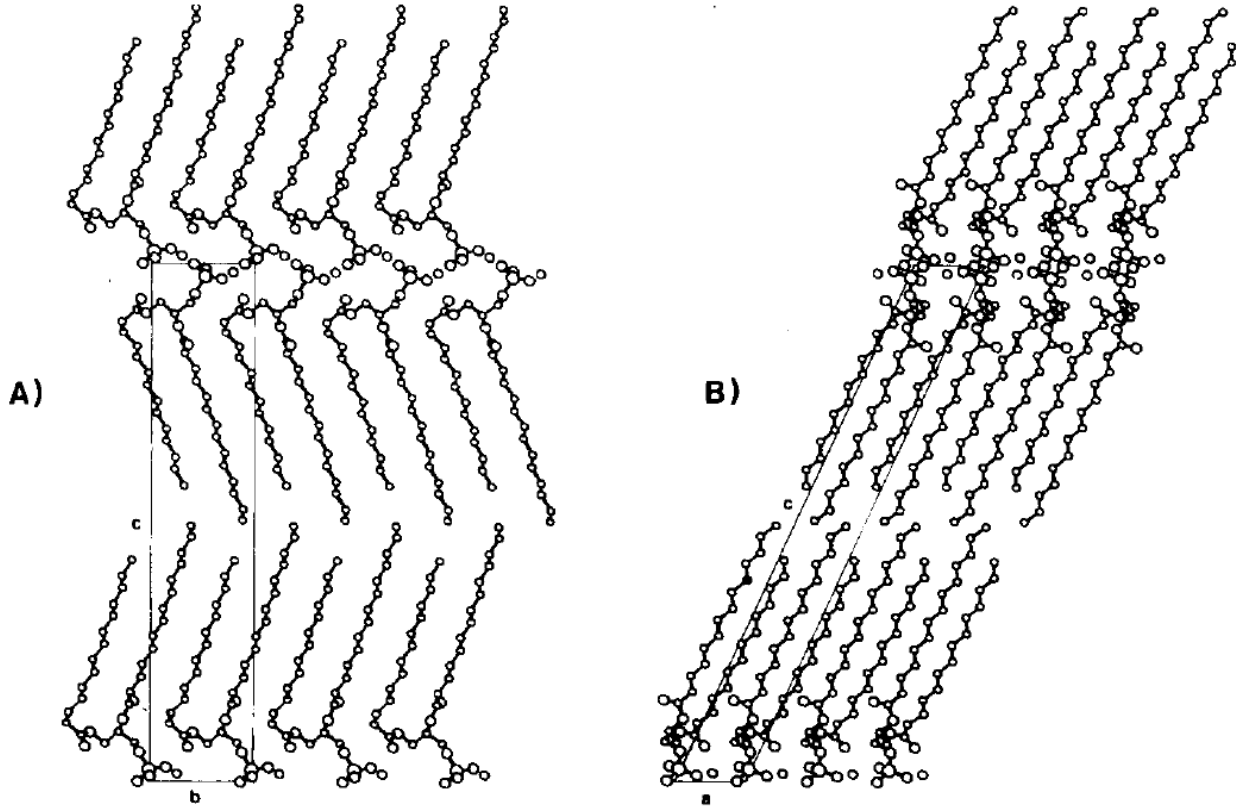

Fig. 2. Molecular packing of DMPA seen (A) along the *a*-axis and (B) along the *b*-axis.

Harlos, K.; Eibl, H.; Pascher, I.; Sundell, S. Conformation and packing properties of phosphatidic acid: The crystal structure of monosodium dimyristoylphosphatidate. *Chem. Phys. Lipids* 1984, *34*, 115-126.

Very few phospholipids have been crystallized

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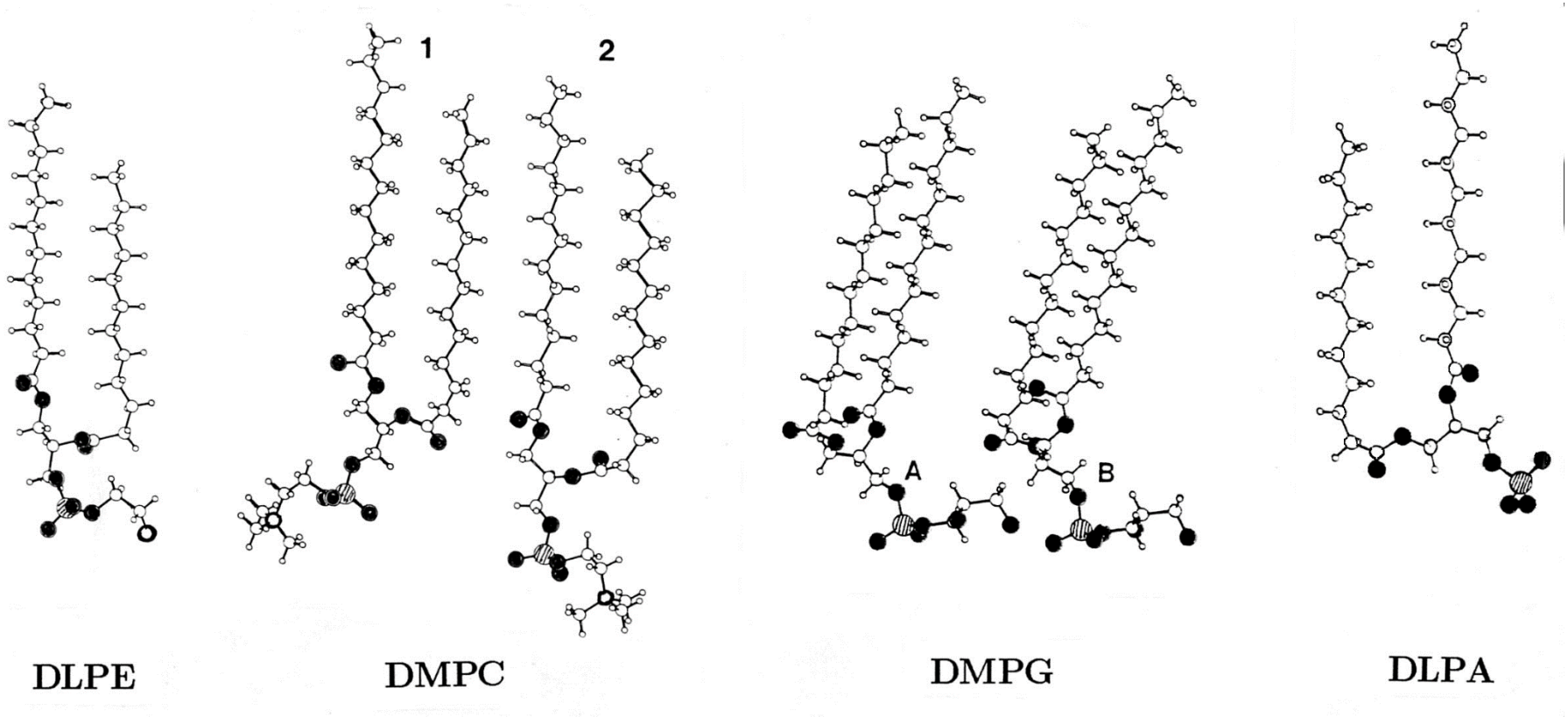
Conformation of lipids in crystals



The *sn*-1 chain is extended, the *sn*-2 chain is bent at the ester group

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Lyotropic and thermotropic mesomorphism of phospholipids

In contact with water, the lipid crystal structure takes up water. Upon further addition of water, different types of aggregates are formed (lyotropic phases), depending on temperature and concentration.
The driving force for the formation of these lyotropic phases is the binding of water to the polar groups and the hydrophobic effect, i.e. the tendency of hydrophobic residues to avoid contact with water.

The form concept of Israelachvili provides a simple explanation for the occurence of different aggregate structures.

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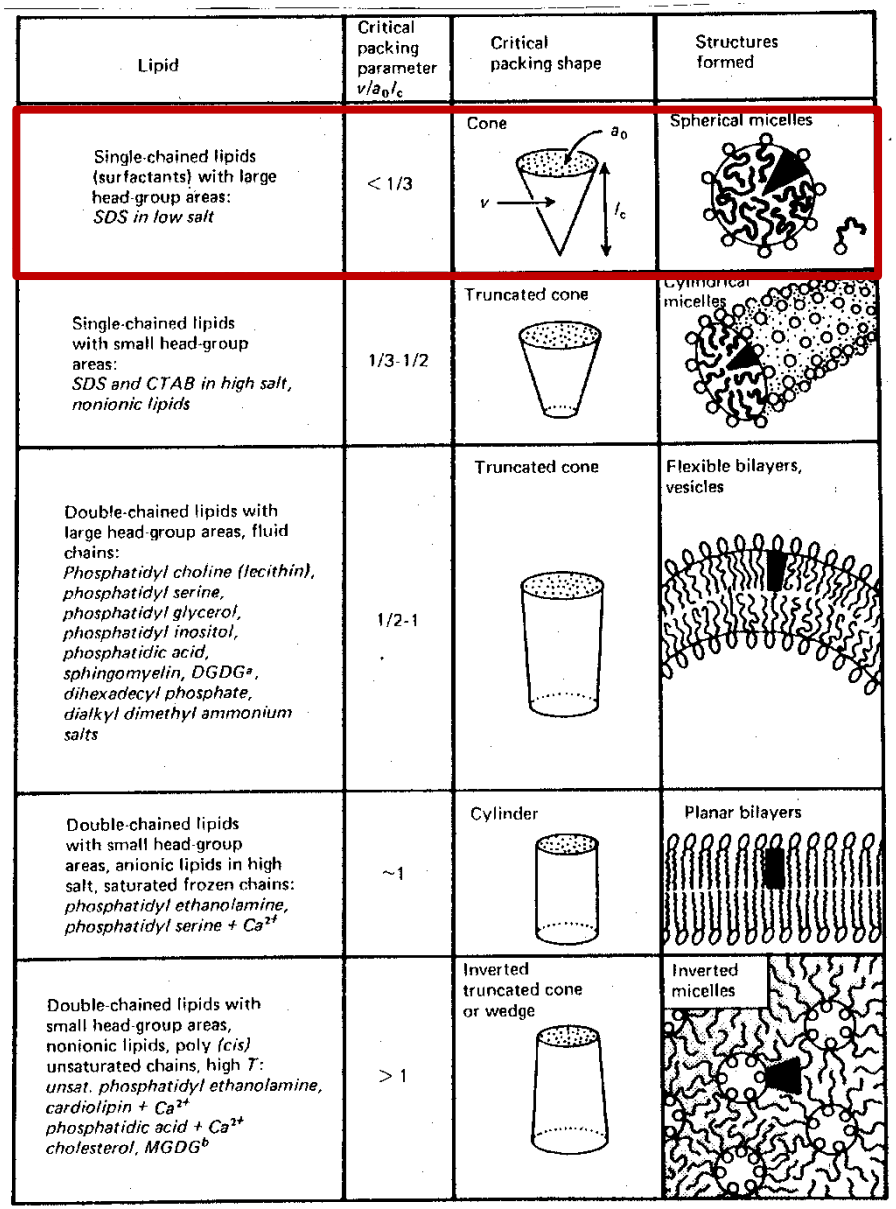

Lipid	Critical packing parameter v/a_0l_c	Critical packing shape	Structures formed
Single-chained lipids (surfactants) with large head-group areas: *SDS in low salt*	< 1/3	Cone (a_0, v, l_c)	Spherical micelles
Single-chained lipids with small head-group areas: *SDS and CTAB in high salt, nonionic lipids*	1/3-1/2	Truncated cone	Cylindrical micelles
Double-chained lipids with large head-group areas, fluid chains: *Phosphatidyl choline (lecithin), phosphatidyl serine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid, sphingomyelin, DGDG[a], dihexadecyl phosphate, dialkyl dimethyl ammonium salts*	1/2-1	Truncated cone	Flexible bilayers, vesicles
Double-chained lipids with small head-group areas, anionic lipids in high salt, saturated frozen chains: *phosphatidyl ethanolamine, phosphatidyl serine + Ca^{2+}*	~1	Cylinder	Planar bilayers
Double-chained lipids with small head-group areas, nonionic lipids, poly *(cis)* unsaturated chains, high *T*: *unsat. phosphatidyl ethanolamine, cardiolipin + Ca^{2+} phosphatidic acid + Ca^{2+} cholesterol, MGDG[b]*	> 1	Inverted truncated cone or wedge	Inverted micelles

Form concept for the formation of lyotropic phases according to Israelachvili

The packing paramter P is defined as $P = v/(a_0 l_c)$, where v = molecular volume, a_0 = cross sectional area of polar group, and l_c = length of extended alkyl chain

Double-chained phospholipids

Israelachvili, J.N. Intermolecular and Surface Forces. Academic Press, London, 1985

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Lyotropic phase behavior of phospholipids: spontaneous self-assembly in water into various lamellar structures

used for fluorescence and permeability studies

used for DSC, FT-IR or x-ray studies

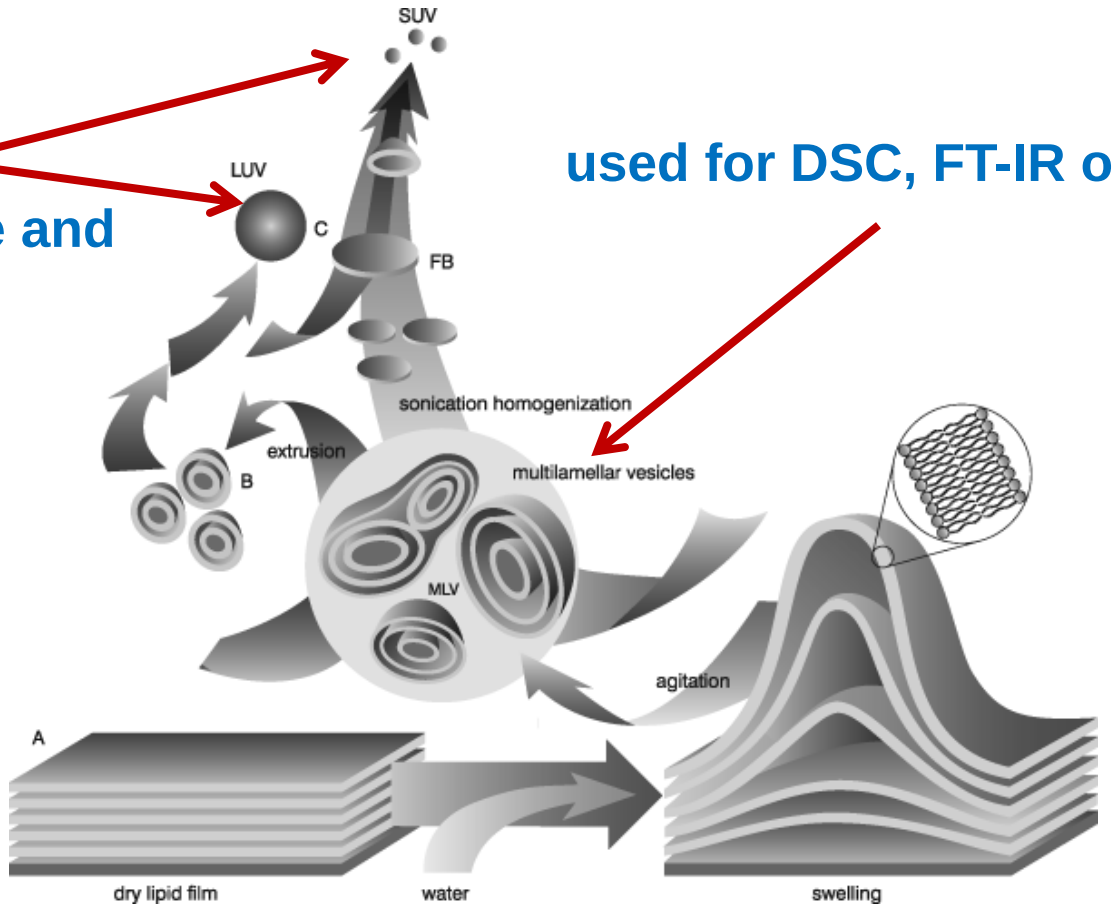

Image from Lasic, D.D., Recherche 20, 904, 1989

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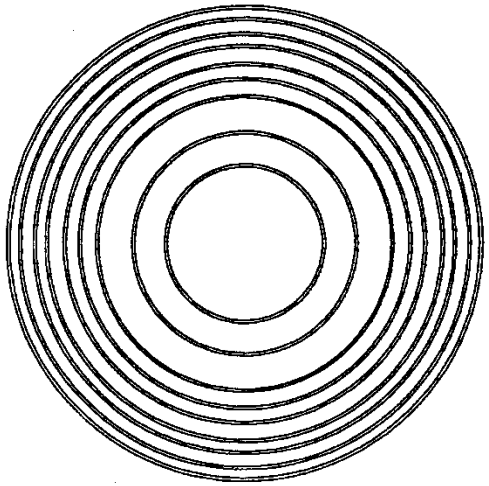
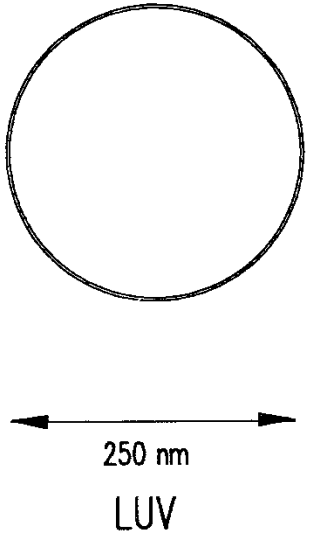
Lipid vesicles (LUV, IUV, SUV) are produced by ultrasonication and/or extrusion through filters with defined pore size

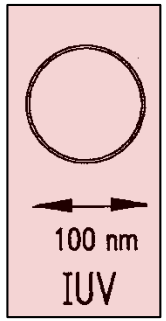
GUV
ca. 10 μm Ø



multi-lamellar



large uni-lamellar



intermediate uni-lamellar

small uni-lamellar

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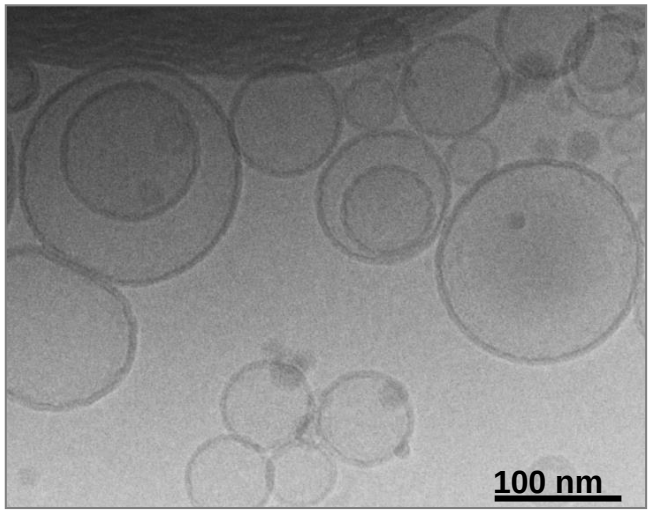
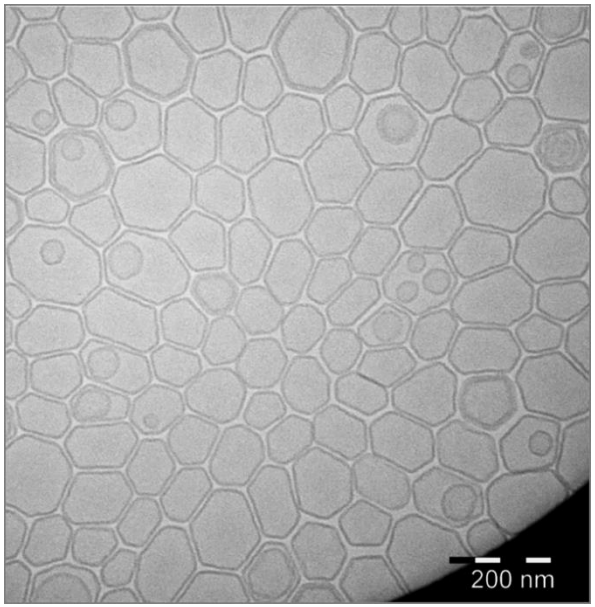
Sonication of the lyotropic lamellar phases with ultrasound or extrusion through filters with small pores leads to unilamellar liposomes or lipid vesicles

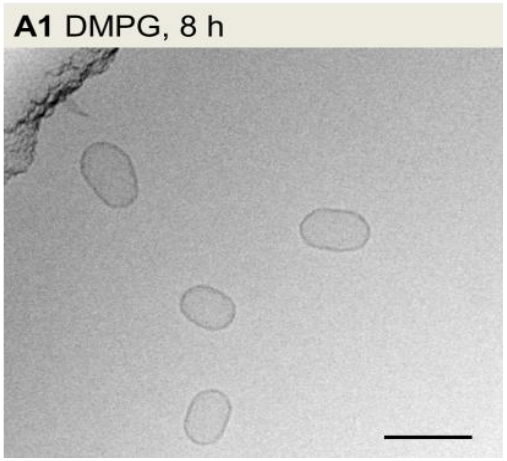


Cryo-EM-microscopy of POPC vesicles: fluid phase

fluid phase: smooth, round vesicles

Cryo-EM-microscopy of DPPC vesicles: gel phase





200 nm

Cryo-EM-microscopy of DMPG vesicles: isolated gel phase vesicles

gel phase: facetted vesicles

S. Finger, A. Meister, A. Blume, unpublished

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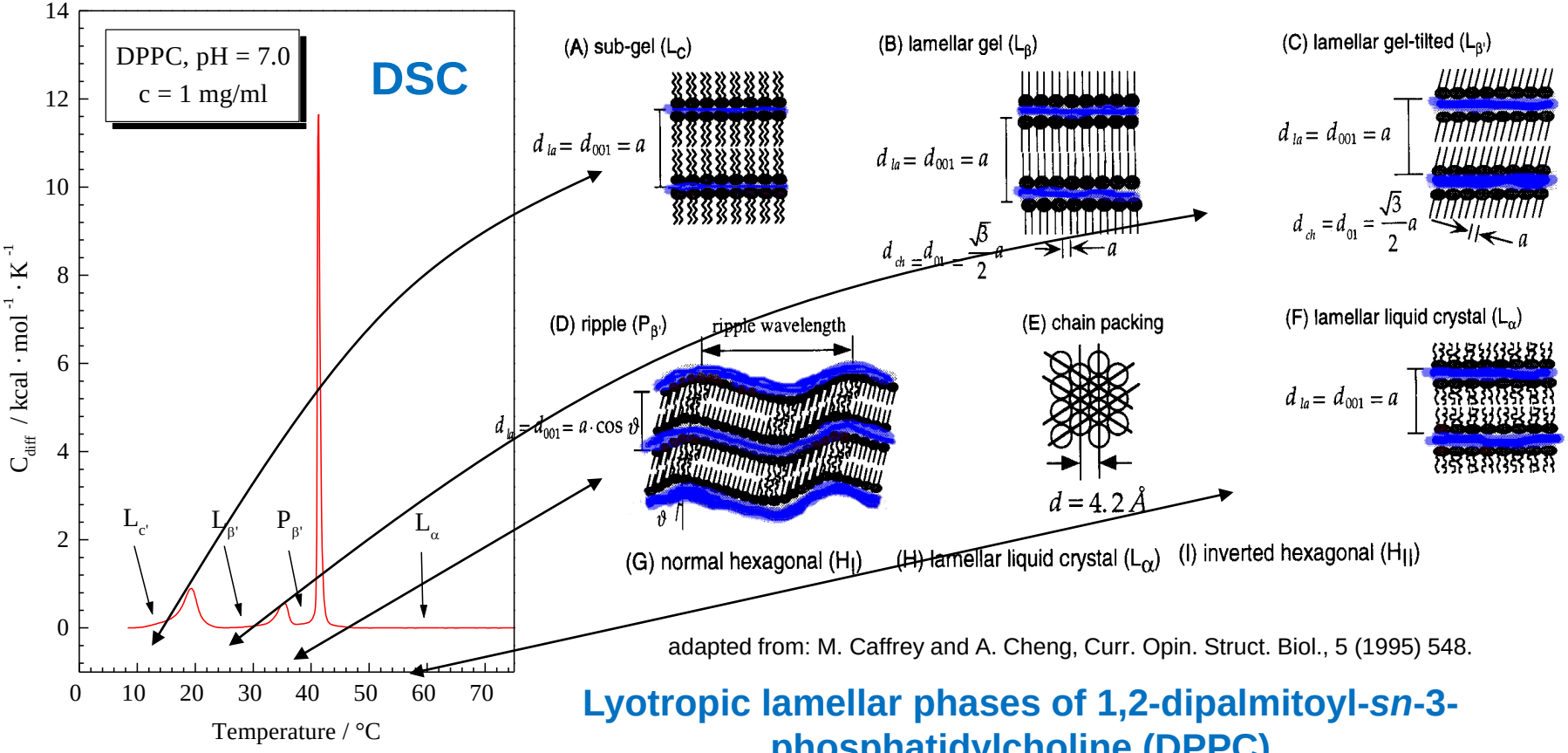
Lyotropic phases of phospholipids also show thermotropic behavior



adapted from: M. Caffrey and A. Cheng, Curr. Opin. Struct. Biol., 5 (1995) 548.

Lyotropic lamellar phases of 1,2-dipalmitoyl-*sn*-3-phosphatidylcholine (DPPC)

biological membranes are mostly in the liquid-crystalline phase just above the L_β => L_α phase transition.

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Methods:
Principle of Differential Scanning Calorimetry (DSC)

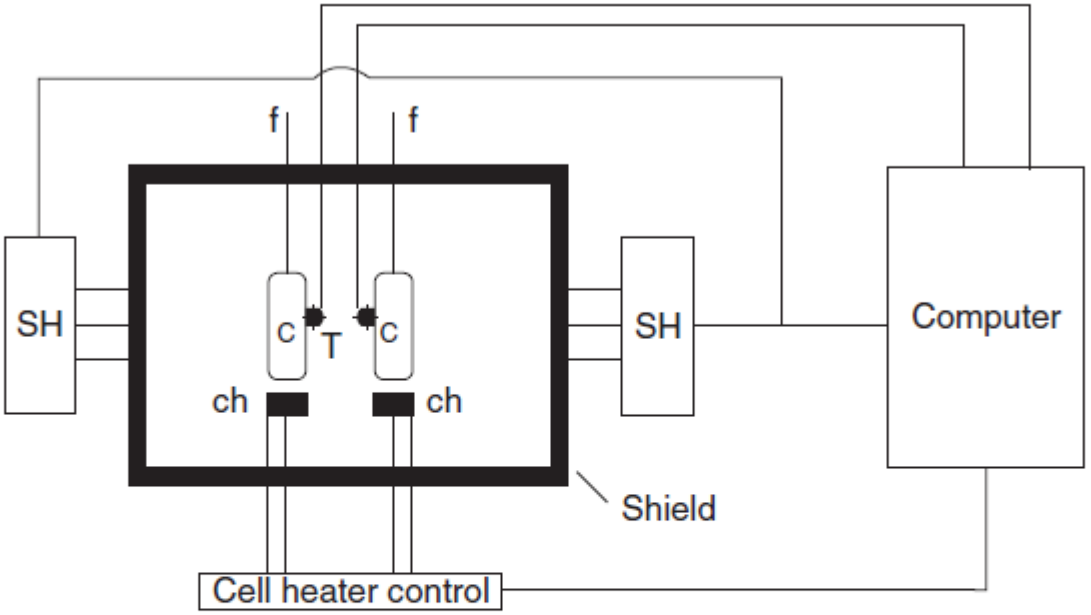

Fig. 1 General diagram for a differential scanning calorimeter. Cells (c) are located within a shield, which is in contact with shield heaters (SH). Individual cell heaters (ch) control the temperature of the sample and reference cells. Temperature sensors (T) are located on the cell surfaces, which determine if there is a temperature difference between the two cells, and through computer control apply appropriate compensating heat to the cells to keep temperature difference near zero. The compensating energy per unit time is recorded as the calorimetric signal.

Spink, C. H. (2008). "Differential Scanning Calorimetry." Methods in Cell Biology 84: 115-141

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DSC with capillary cells

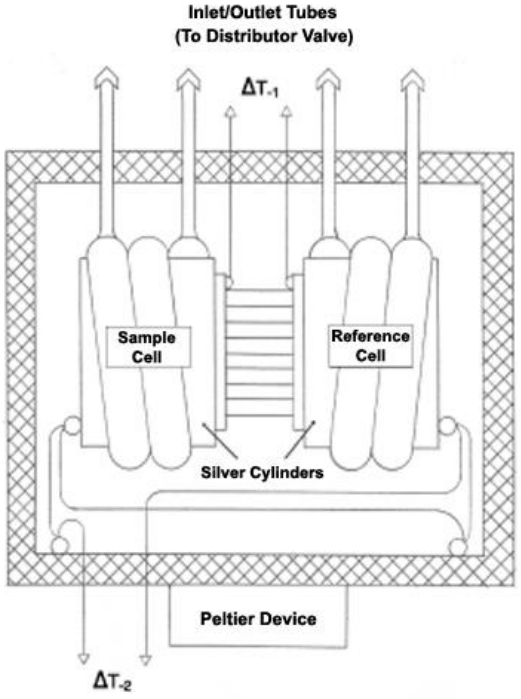

FIGURE 1. Schematic diagram of the thermal core of VP-Capillary DSC Platform, showing helical sample and reference cells each with inlet/outlet tubes, adiabatic jacket, Peltier device for heating/cooling the jacket, ΔT-1 sensor between sample and reference cells, and ΔT-2 sensor between cells and jacket.

MicroCal VP-DSC with "Lollipop"-cells

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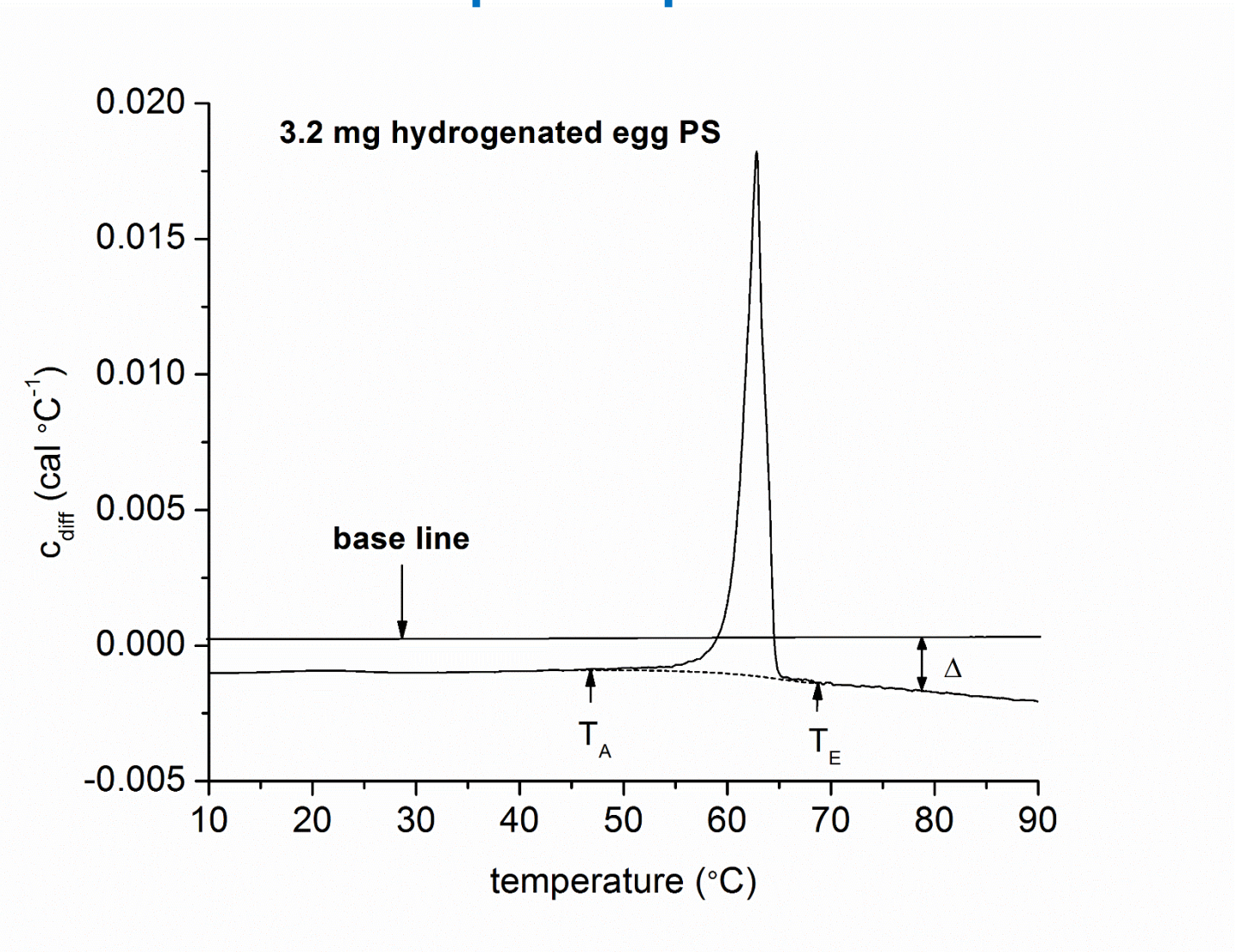
DSC curve of lipid suspension in excess water



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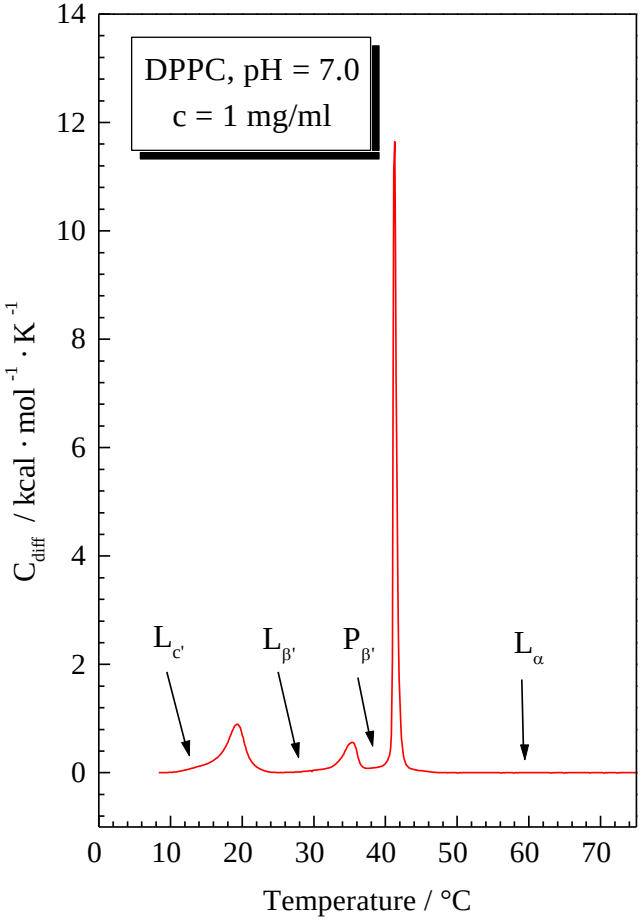
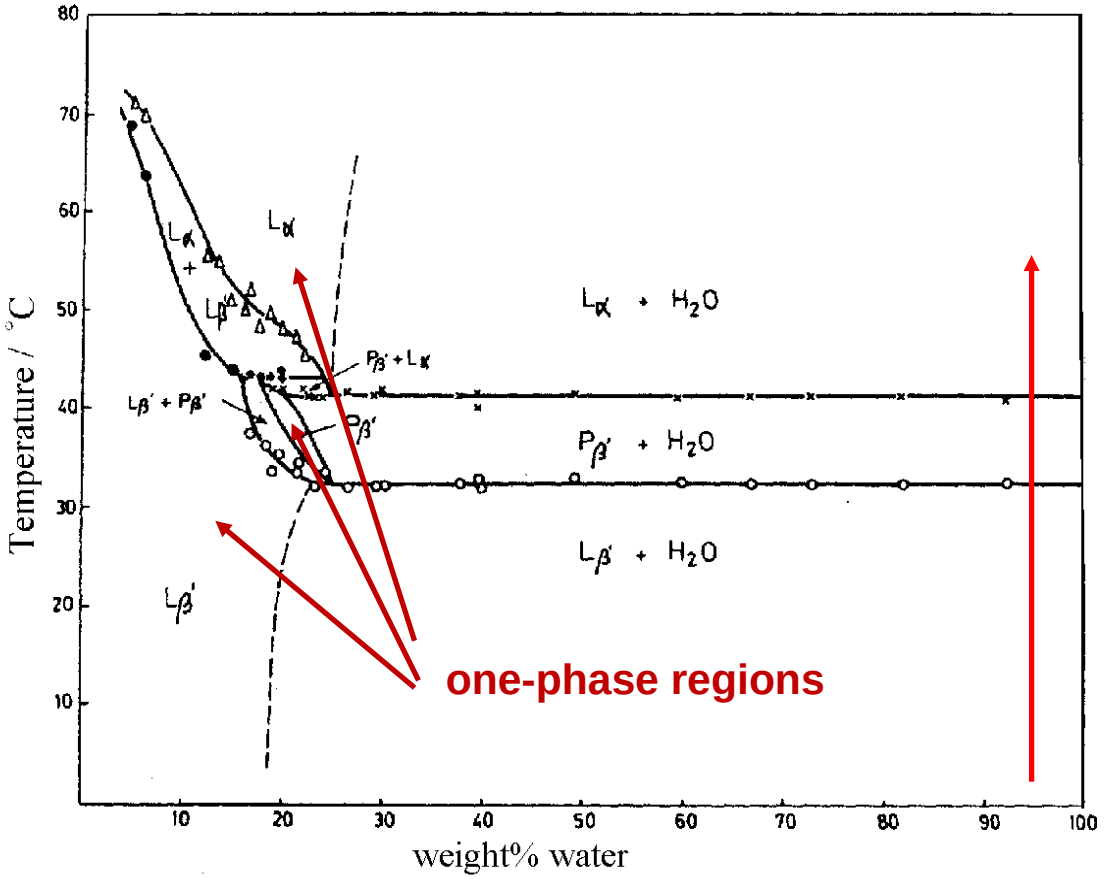
Binary phase diagram of DPPC/water mixtures: T_m depends on hydration

Phase diagram determined from DSC heating curves





C. Grabielle-Madelmont and R. Perron, J. Coll. Interf. Sci., 95 (1983) 471

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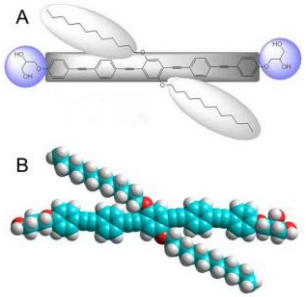
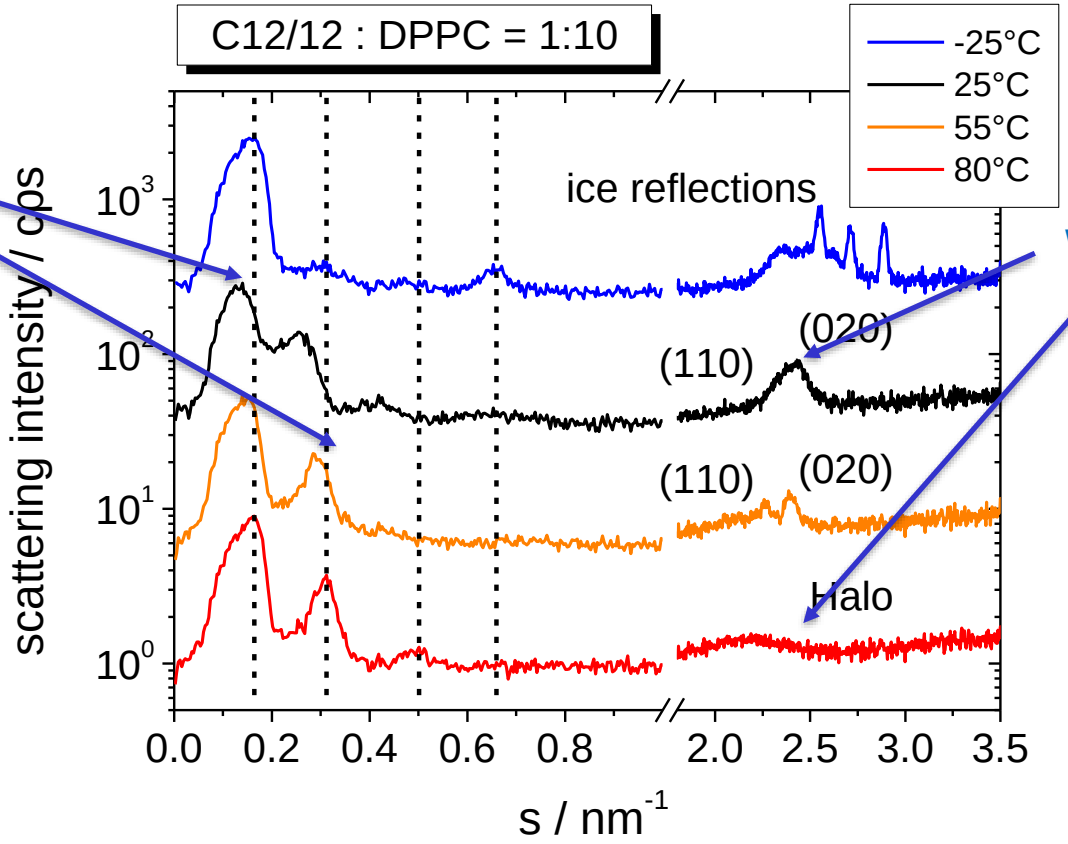
Methods:
X-ray powder diffraction of hydrated bilayers

small angle reflections from bilayer stacking



C12/12



reflections from chain packing in wide angle region

Powder diffractograms of hydrated mixture of C12/12 : DPPC = 1:10 with 50 weight% H_2O. Capillary probes

B.-D. Lechner, A. Blume unpublished

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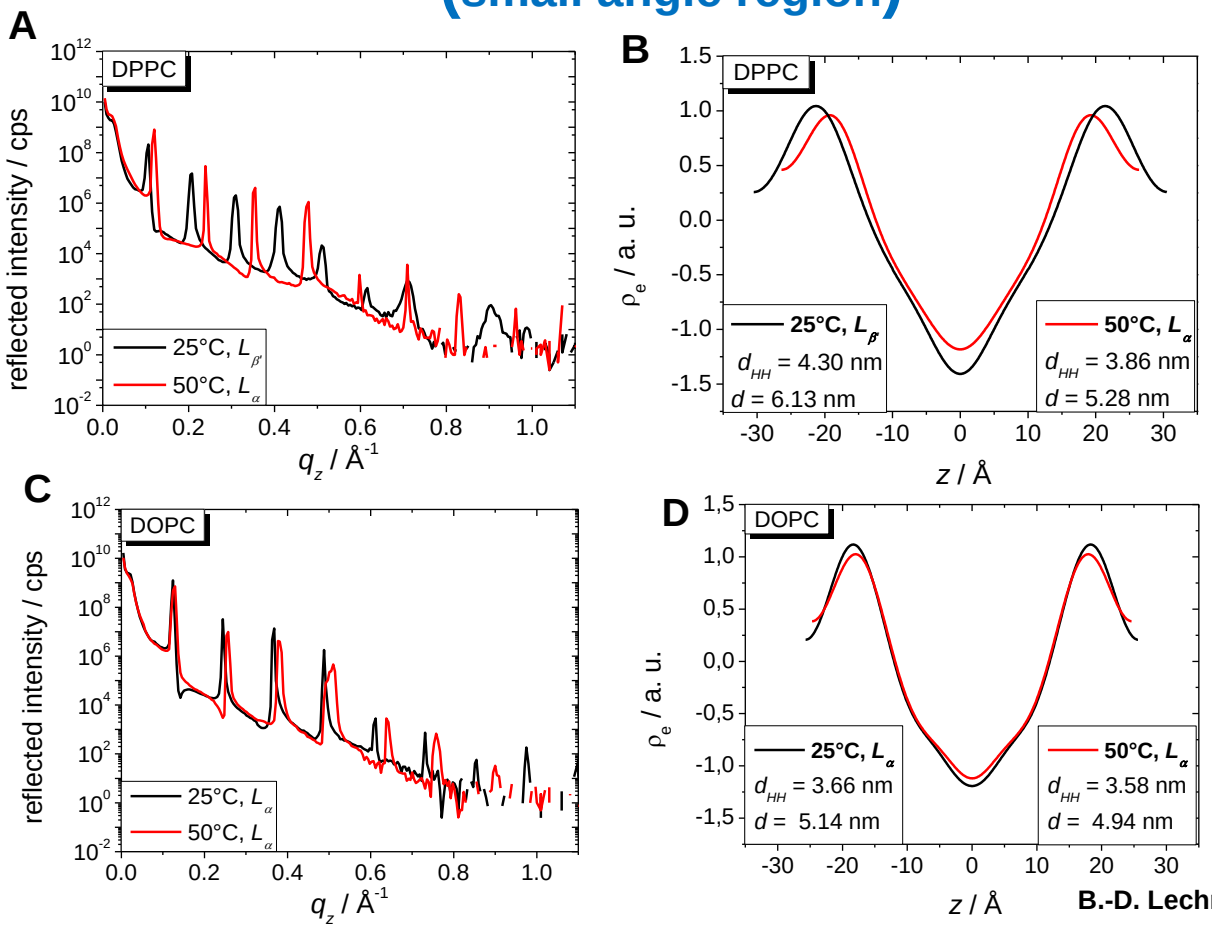
X-ray reflectivity curves of hydrated and oriented bilayers (small angle region)

scattering curves



electron density profiles

B.-D. Lechner, A. Blume unpublished

Reflectivity curves of oriented DPPC- (A) and DOPC-multilayers (C) Electron density profiles of DPPC-membranes (B) in the $L_{\beta'}$-(25 °C, black) or L_α-phase (50 °C, red) and DOPC-membrans (D) in the L_α-Phase at 25 °C (black) and 50 °C (red) at 99 % RH.

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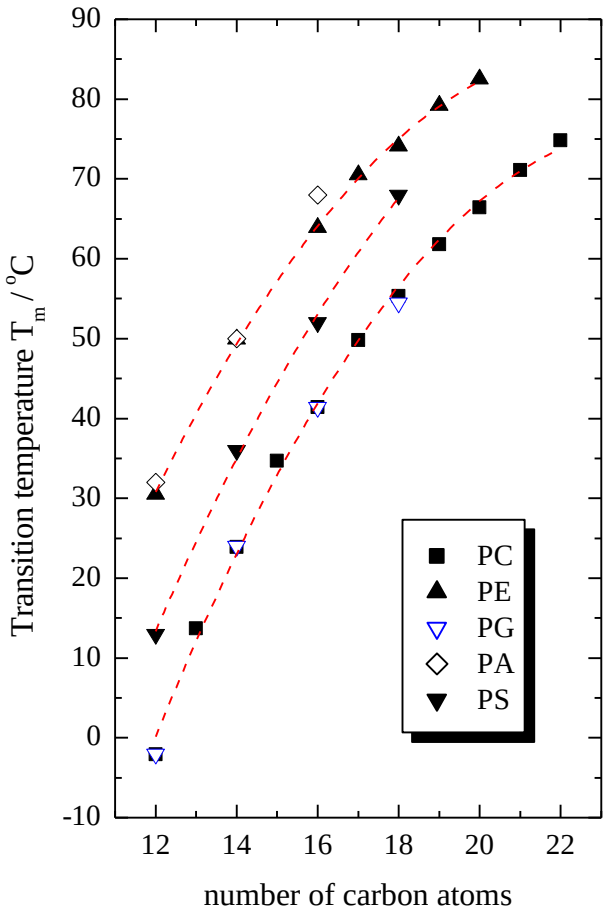
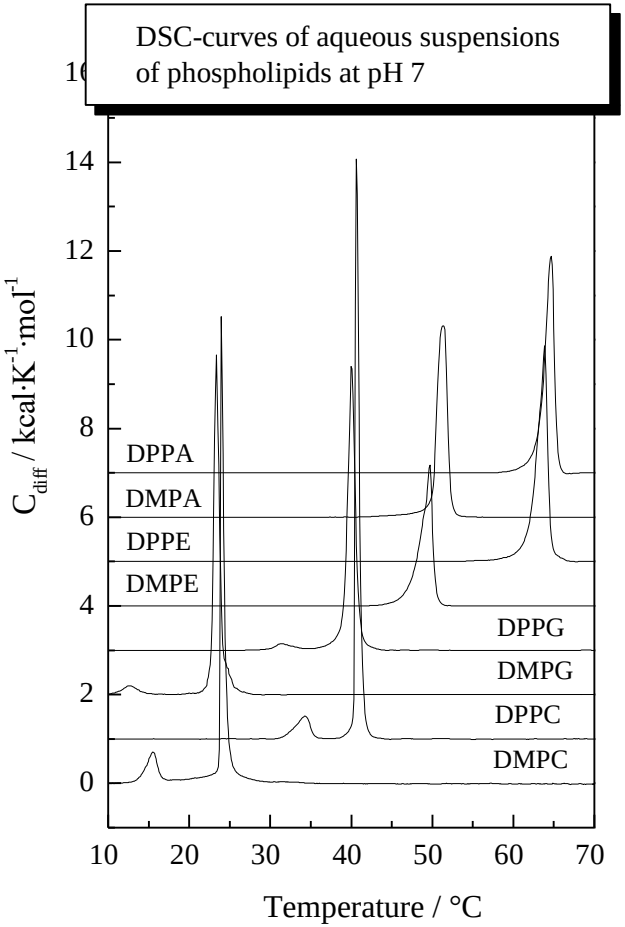
Phase transitions in water (pH 7) of pure phospholipids with different headgroups and saturated chain lengths: T_m depends on headgroup interactions and chain length





A. Blume und P. Garidel (1999) in: Handbook of Thermal Analysis and Calorimetry, Vol 4.: ”From Macromolecules to Man.” (R.B. Kemp, Ed.) Chpt. 3, ”Lipid Model Membranes and Biomembranes.” Elsevier Press, Amsterdam, pp. 109-173.

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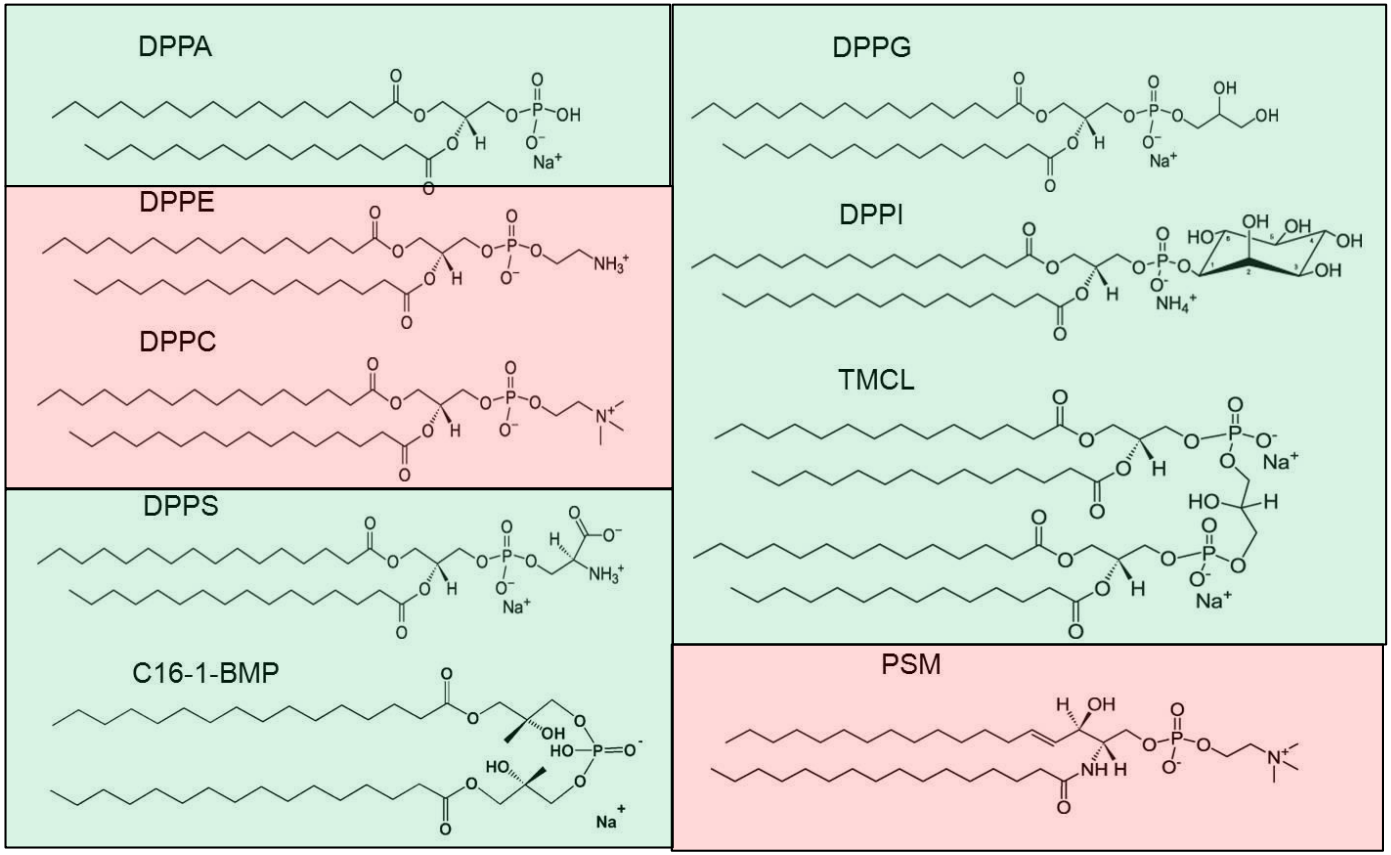
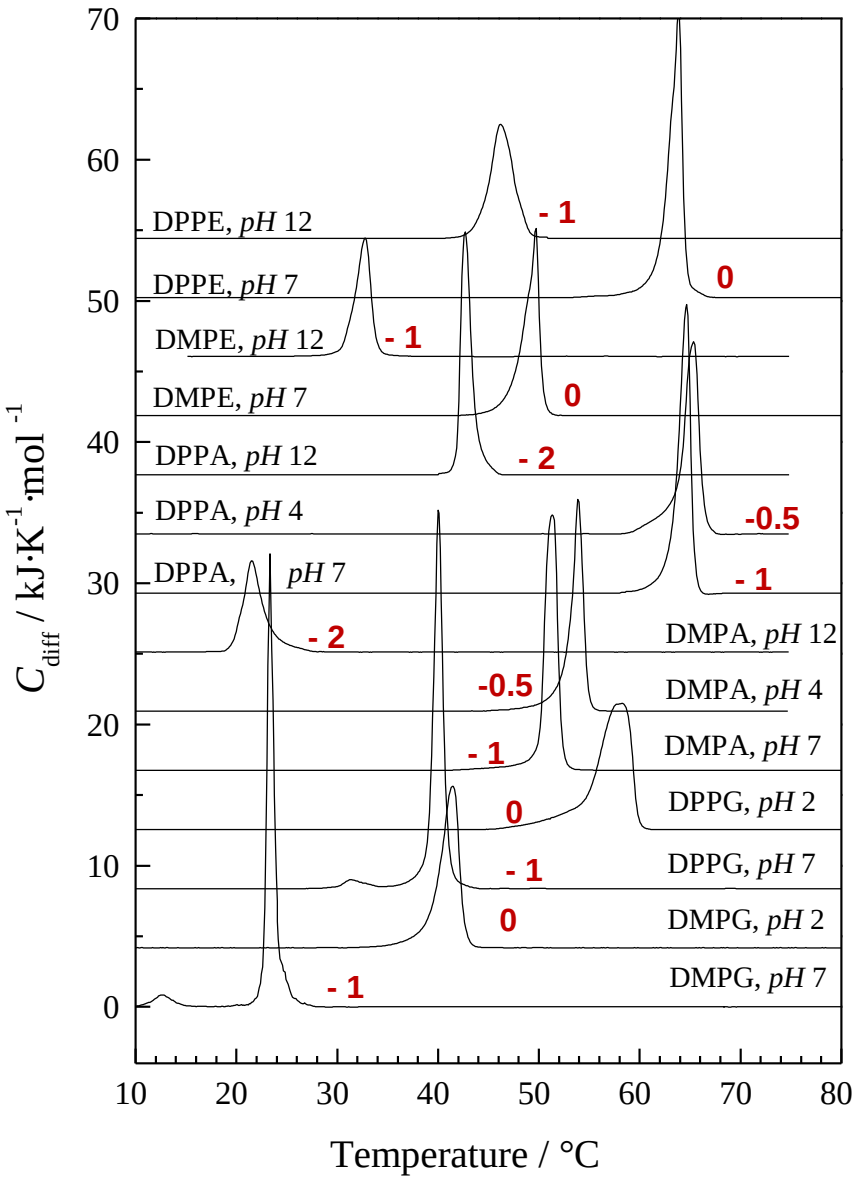
Common phospholipid classes: Charge state at pH 7



red: zwitterionic

green: negatively charged

all headgroups can act as H-bond acceptors and donors with the exception of PC (only acceptor)



Influence of headgroup structure and pH (charge):

DSC curves of dilute aqueous dispersions (1 mM) of different phospholipids (PGs in 0.1 M KCl)

Handbook of Thermal Analysis and Calorimetry, Vol 4: From Macromolecules to Man.
R.B. Kemp, editor
© 1999 Elsevier Press B.V., Amsterdam, pp 109 – 173

Chapter 3

LIPID MODEL MEMBRANES AND BIOMEMBRANES

A. Blume and P. Garidel

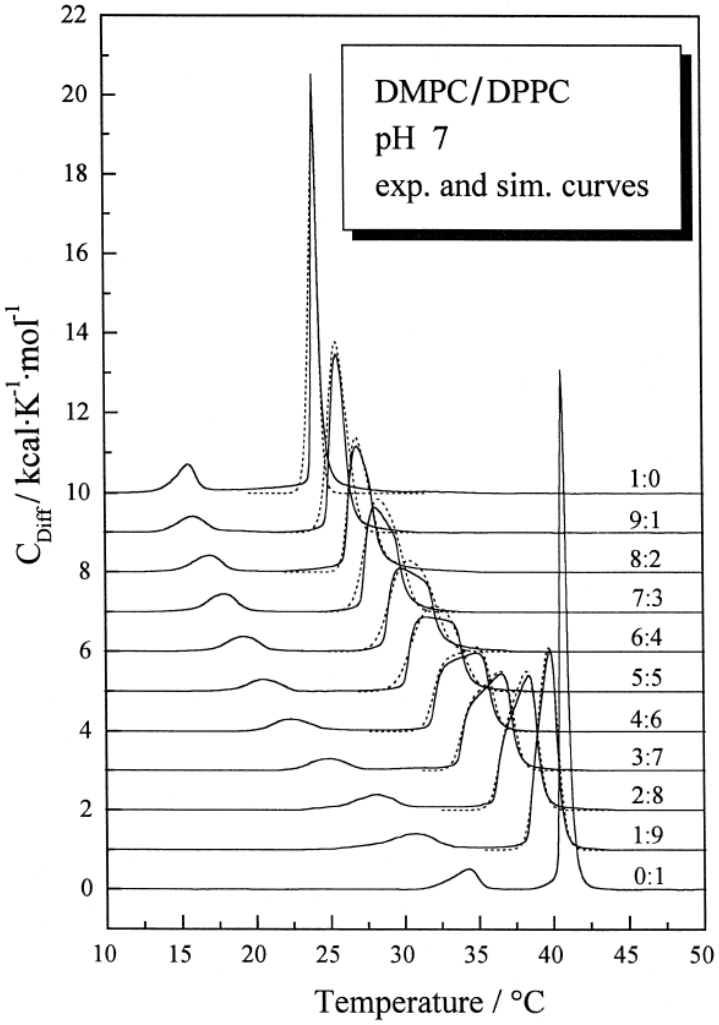
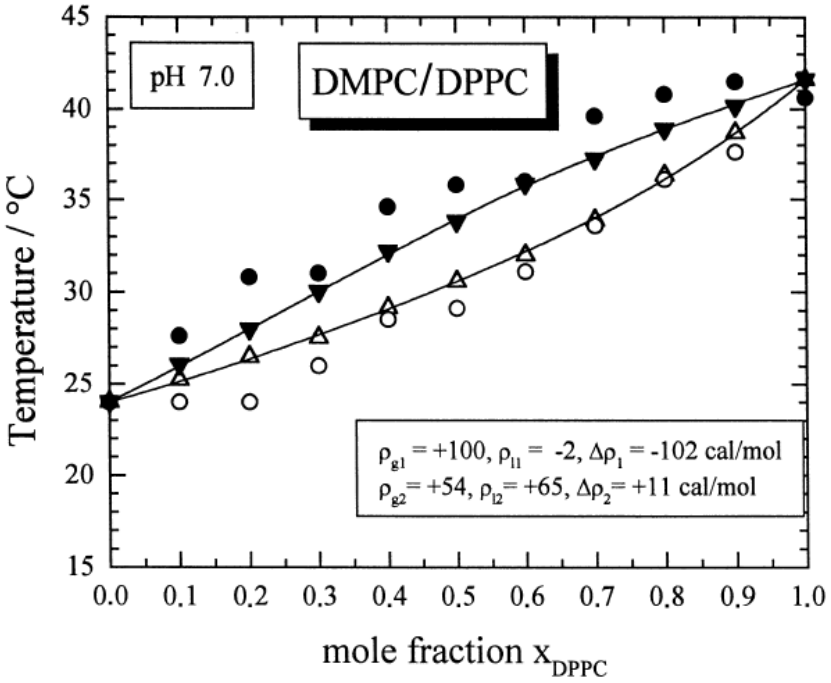


Fig. 3. DSC heating thermograms for DMPC/DPPC mixtures at various molar ratios at pH 7: Experimental cp-curves: solid line, simulated cp-curves: dotted line.

Pseudo-binary lipid mixtures with almost ideal miscibility



Garidel P, Blume A (1998) Miscibility of phospholipids with identical headgroups and acyl chain lengths differing by two methylene units: Effects of headgroup structure and headgroup charge. Biochim Biophys Acta 1371 (1):83-95.

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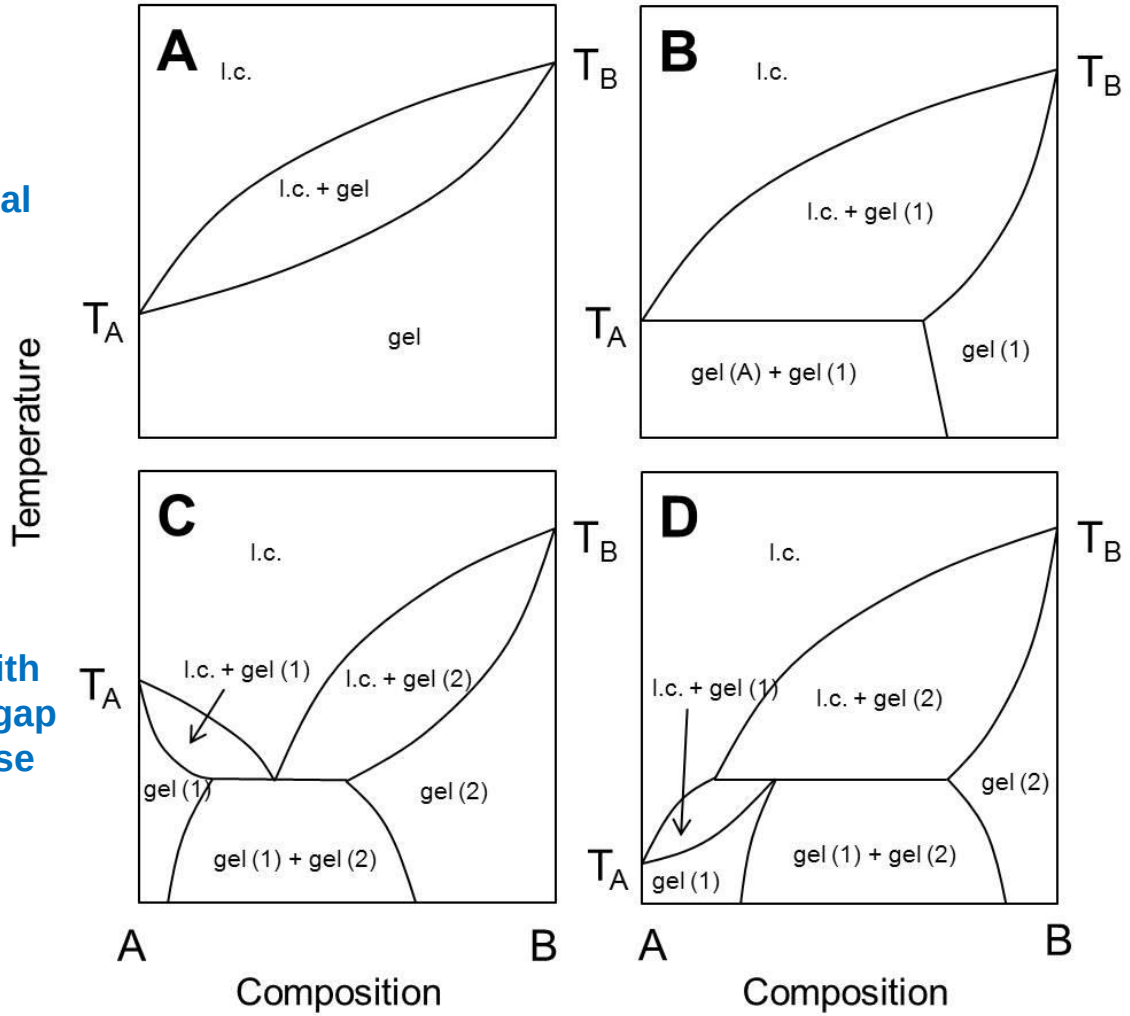
Simplification:
Schematic phase diagrams of pseudo-binary lipid mixtures

ideal
non-ideal



monotectic with miscibility gap in gel phase

eutectic with miscibility gap in gel phase

peritetectic with miscibility gap in gel phase

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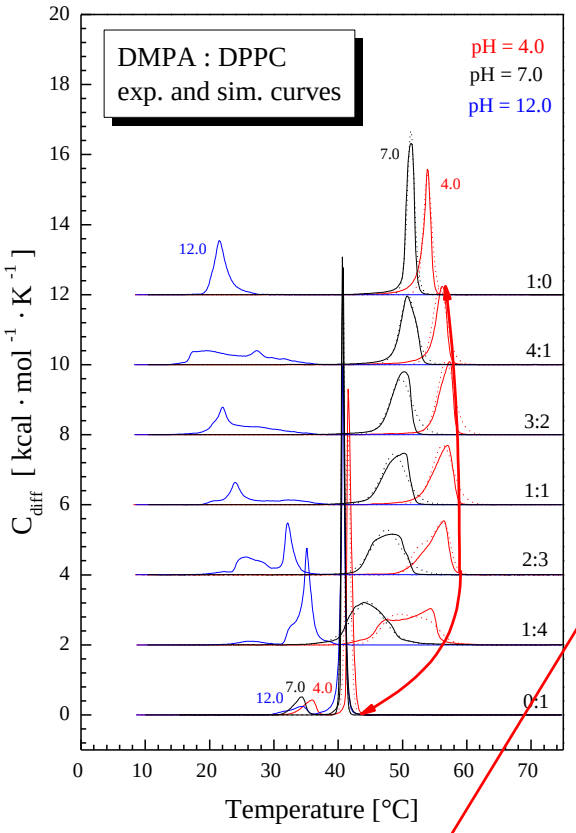
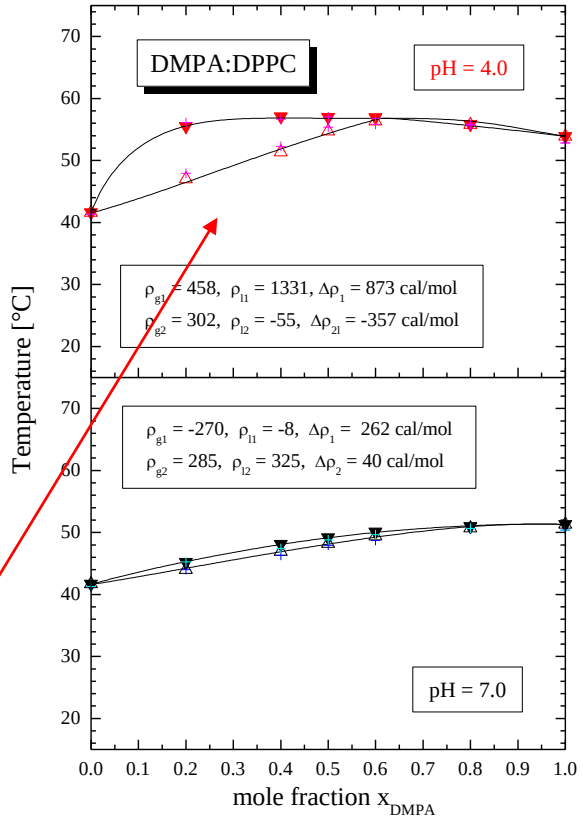
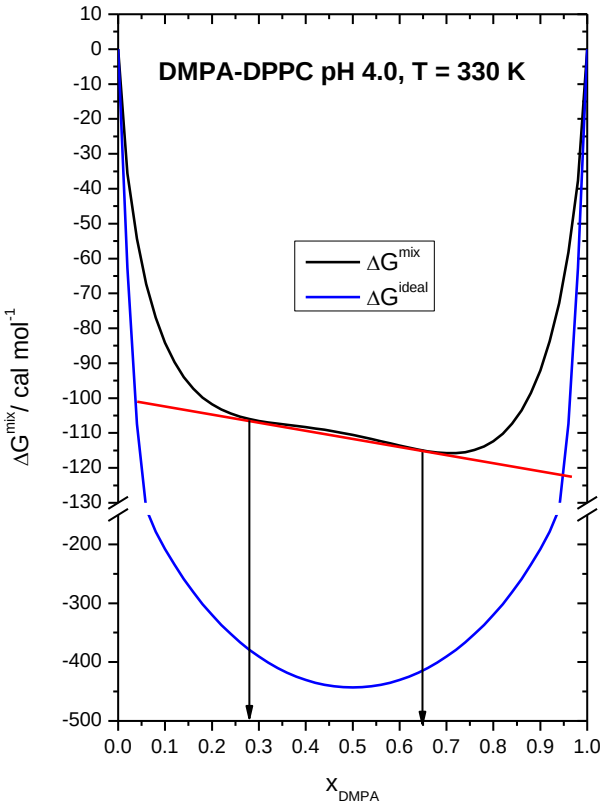
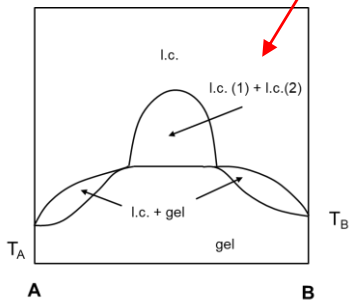
Demixing in the fluid phase in DMPA-DPPC mixtures at pH 4.0







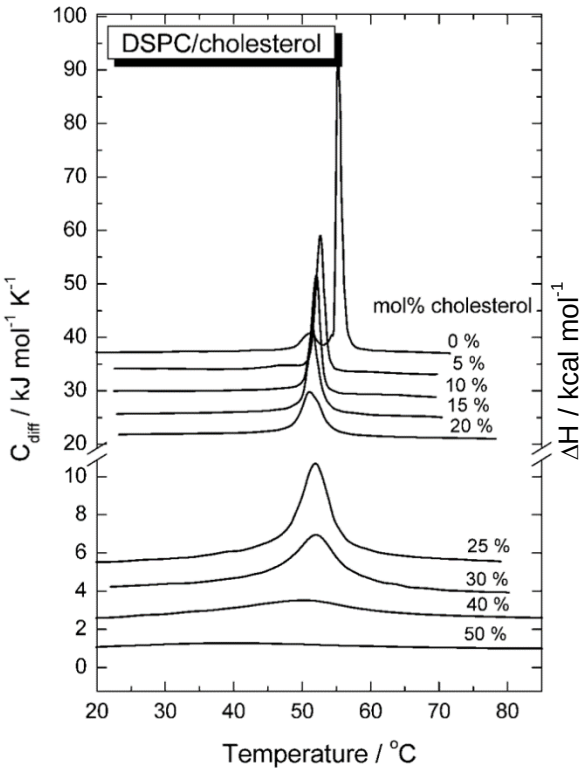


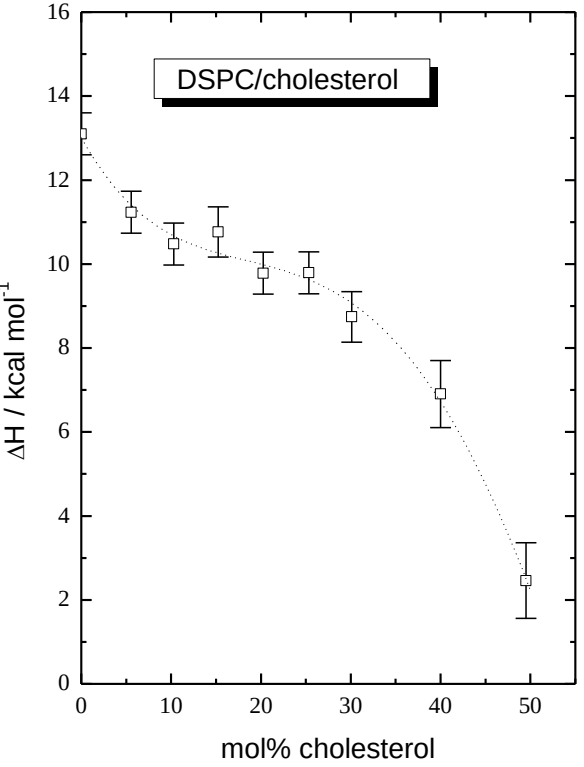
$$\Delta G^{E} = \Delta H^{E} = x(1-x)\left[\rho_1 + \rho_2(2x-1)\right]$$

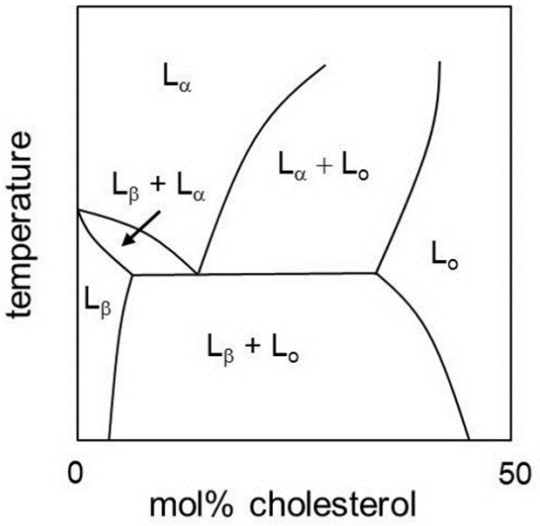
$$\Delta G^{mix} = \Delta G^{ideal} + \Delta G^{E} = RT\left[x\ln x + (1-x)\ln(1-x)\right] + x(1-x)\left[\rho_1 + \rho_2(2x-1)\right]$$

Garidel, P., Johann, C., Blume, A. (1997)
Non-ideal Mixing and Phase Separation in Phosphatidylcholine-Phosphatidic Acid Mixtures as a Function of pH and Chain Length. Biophys. J. 72, 2196-2210

A special case: phospholipid-cholesterol mixtures







Hypothetical generic phase diagram PC/chol

Left: DSC curves of DSPC–cholesterol mixtures with increasing cholesterol concentration. (Adapted from Huang, T. H. et al., Biochemistry 32, 13277–13287, 1993.)
Right: Generic phase diagram suggested for PC–cholesterol mixtures with the different phases as indicated. (From Ipsen, J. H. et al., Biochim. Biophys. Acta 905, 162–172, 1987.)

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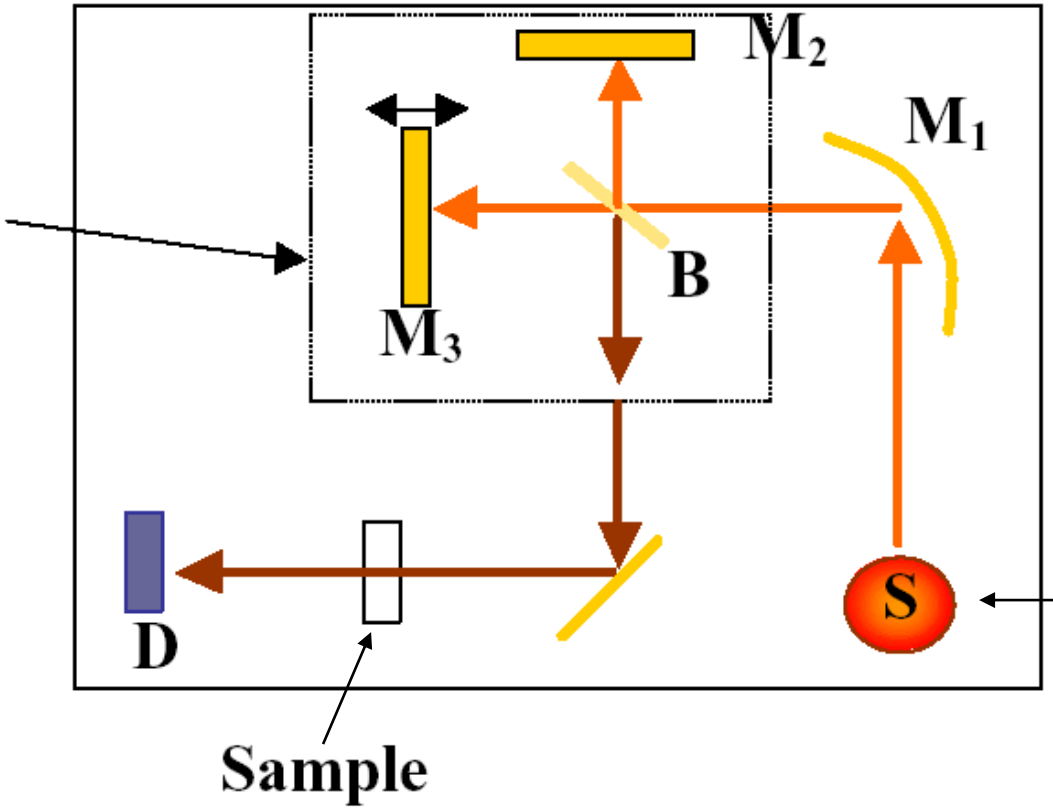
Methods:
Principle of a Fourier-Transform-Infrared-Spectrometer

Michelson interferometer



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FT-IR spectrometer and IR-cells

(a) Confocheck™ AquaSpec™ cell (CaF_2 windows)
(b) (b) AquaSpec™ cell in FT-IR spectrometer, transimission IR
(c) ConfocheckTM BioATR II™ (ZnSe+diamond), reflection IR

(http://www.brukeroptics.de/proteomics/ConfoCheck.html).

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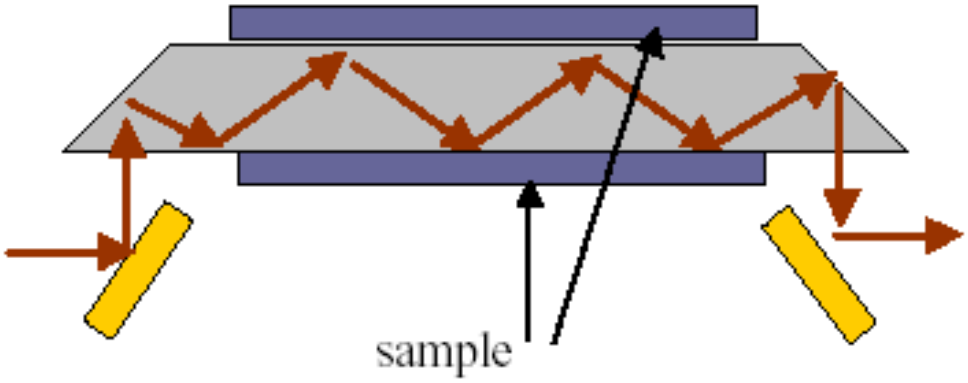
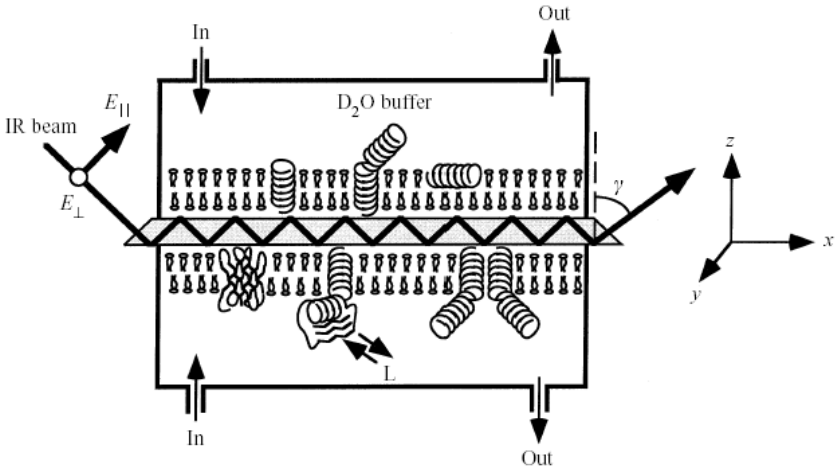
ATR (attenuated total reflection) technique

lipid film on ZnSe crystal

- Internal reflection (FTIR-ATR)





IR-beam

detector

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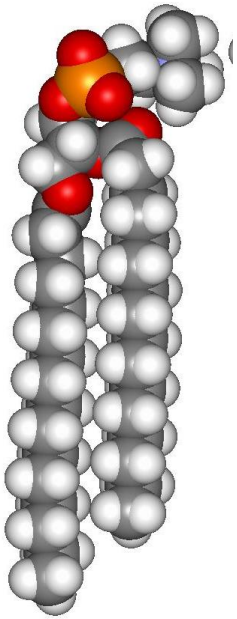
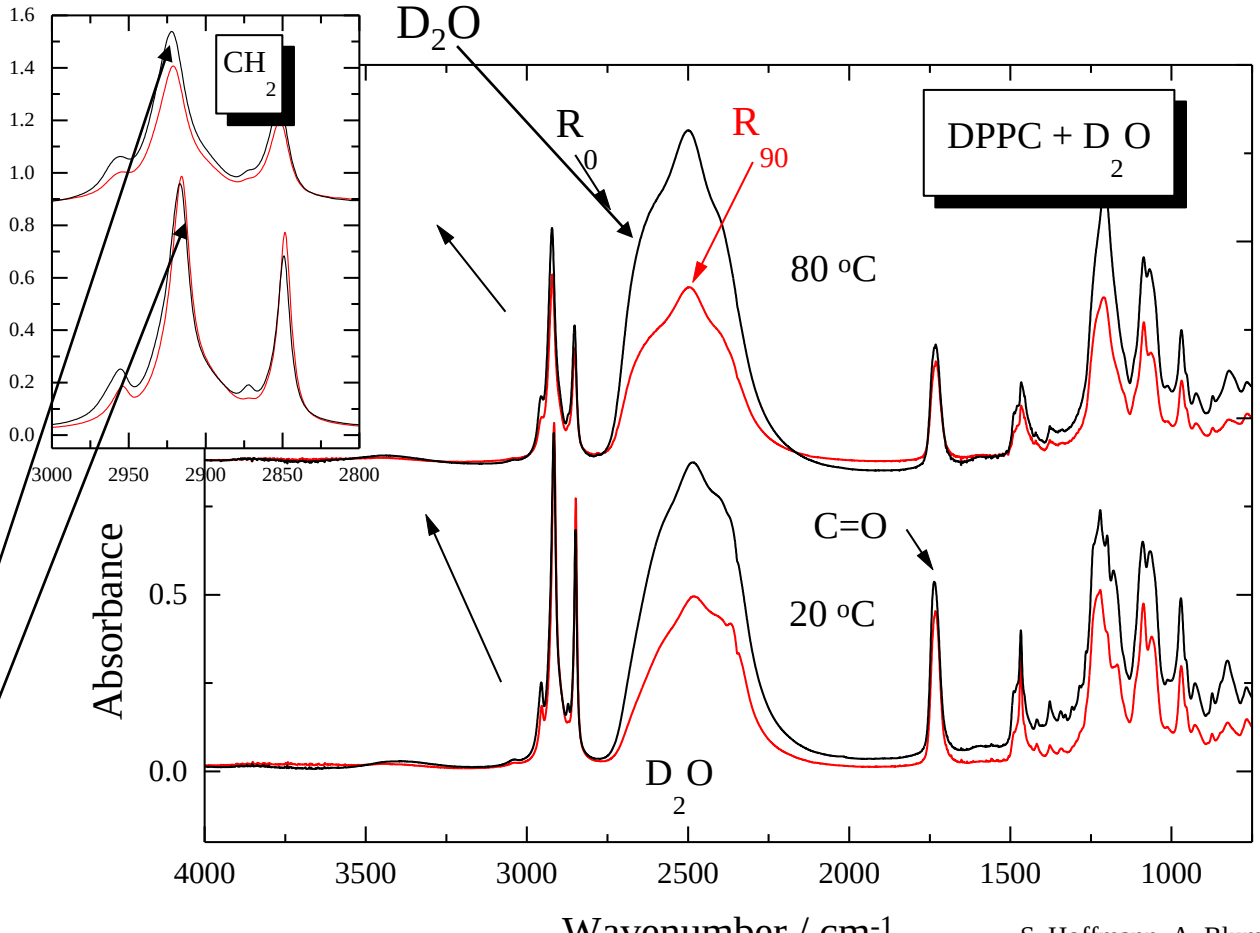
FT-IR-Spectroscopy

FT-IR-ATR-spectra of oriented DPPC films on a ZnSe-crystal, hydrated with D_2O with light in two different polarizations





shift of CH_2-frequency to higher wave number above T_m

S. Hoffmann, A. Blume, unpublished

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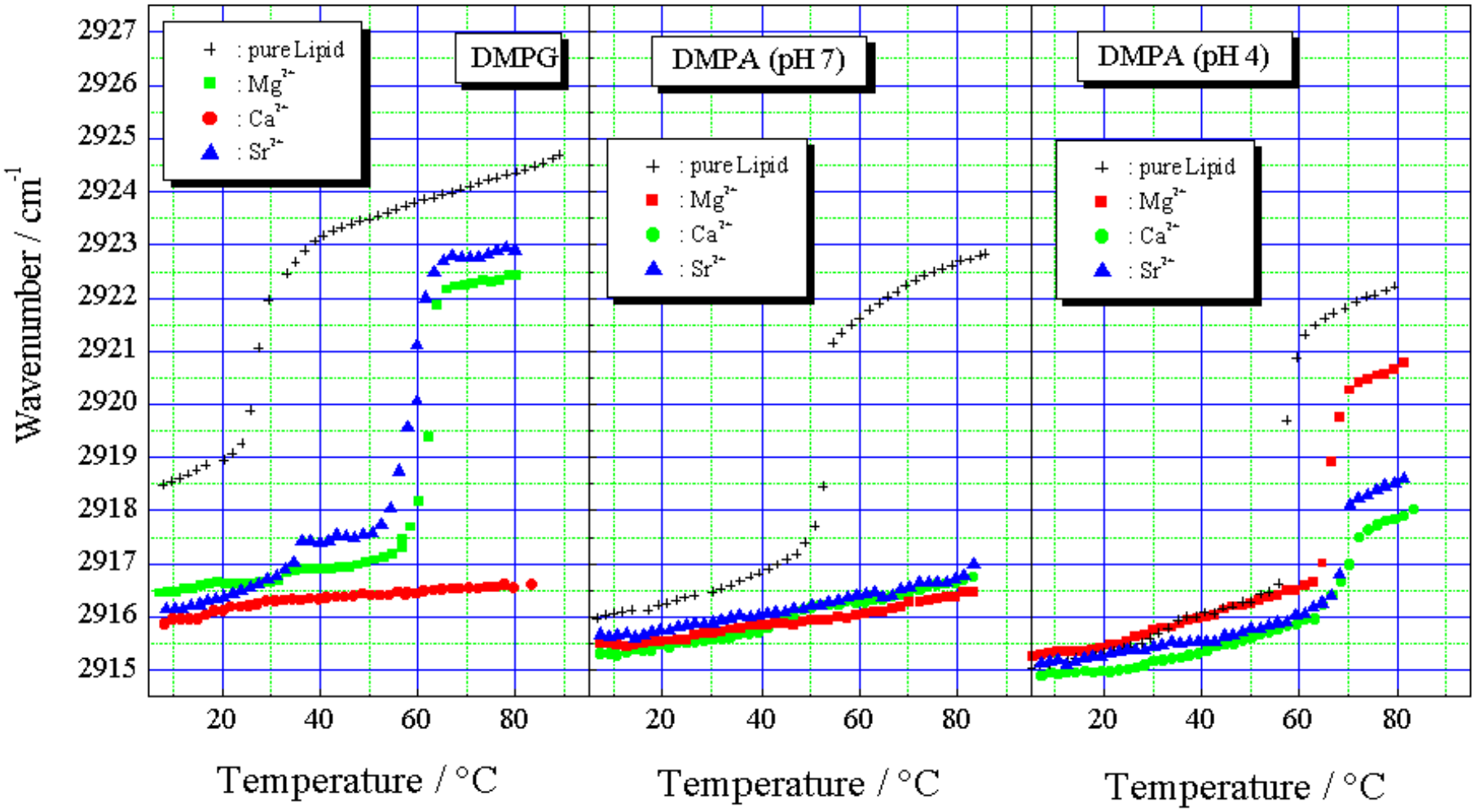
The transition temperature of lipids determined by the frequency of the symmetric and antisymmetric CH_2 stretching bands



Garidel and Blume, unpublished

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Schematic representation of a lipid bilayer indicating chain disorder

Hydration of lipid headgroup region: how far does water go into the bilayer?

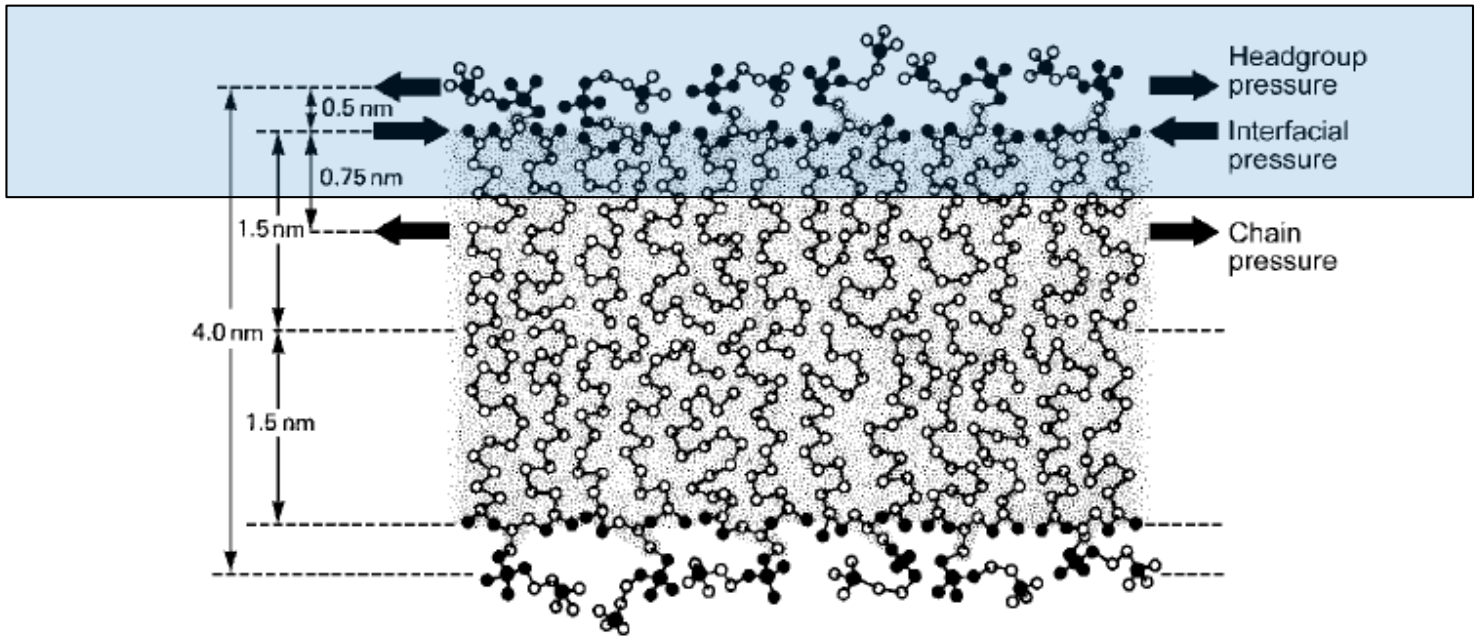

FIGURE 20.6 Lecithin (phosphatidyl choline, PC) bilayer in the fluid state drawn to scale. The lipid bilayer is the basic structure of biological membranes, and most membrane lipids contain two hydrocarbon chains. The lipids diffuse rapidly in the plane of the bilayer, covering a distance of about 1 μm in 1 s. They also cross the bilayer from one side to the other ("flip-flop"), as well as exchange with lipids in the solution, but the rates for these two processes are low, of the order of hours for double-chained lipids compared to 10^{-5} to 10^{-3} s for micelle forming single-chained lyso-lipids. *[Modified from Israelachvili et al., 1980a.]*

J.N. Israelachvili. Intermolecular and Surface Forces, 3rd edition, Academic Press 2011

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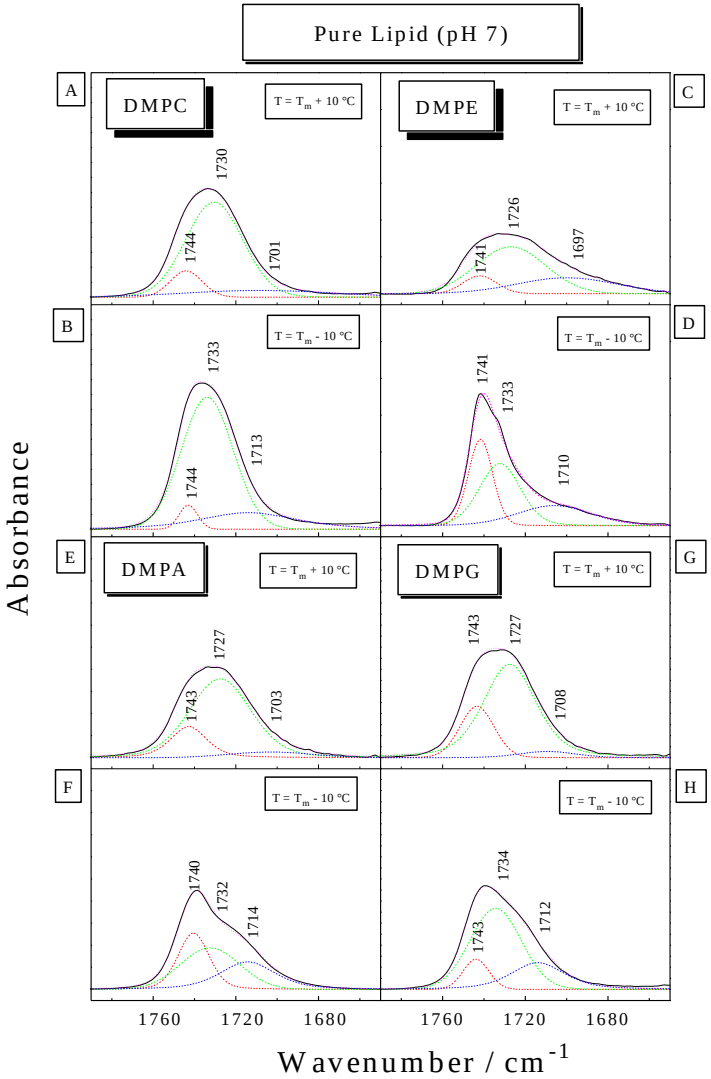
Determination of H-bonding to C=O groups by FT-IR-spectroscopy

$T > T_m$

$T < T_m$

$T > T_m$

$T < T_m$



The C=O band of all phospholipids contour shows 2 or 3 subbands corresponding to hydration, no hydration, or H-bonds from 1 or 2 H_2O molecules.

Hydration increases with higher temperature.

Blume, A., Hübner, W., Messner, G. (1988) Biochemistry 27, 8239-8249

Garidel and Blume, unpublished

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Determination of hydration of polar and hydrophobic moieties by MD simulations

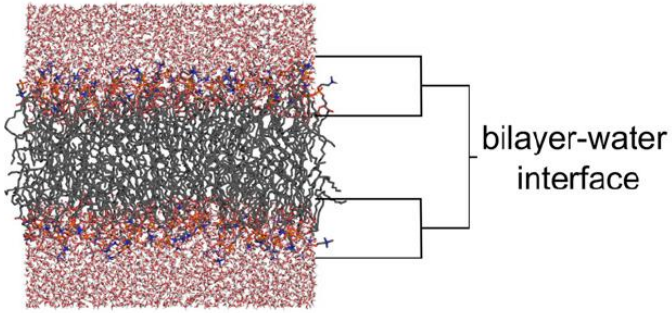

Fig. 1. Distinct regions of a liquid-crystalline lipid bilayer: the bulk water phase, the bilayer/water interface (marked), and the hydrophobic core. The lipid molecules are in the united atom representation and the atoms are represented in standard colours (carbon atoms are dark grey).

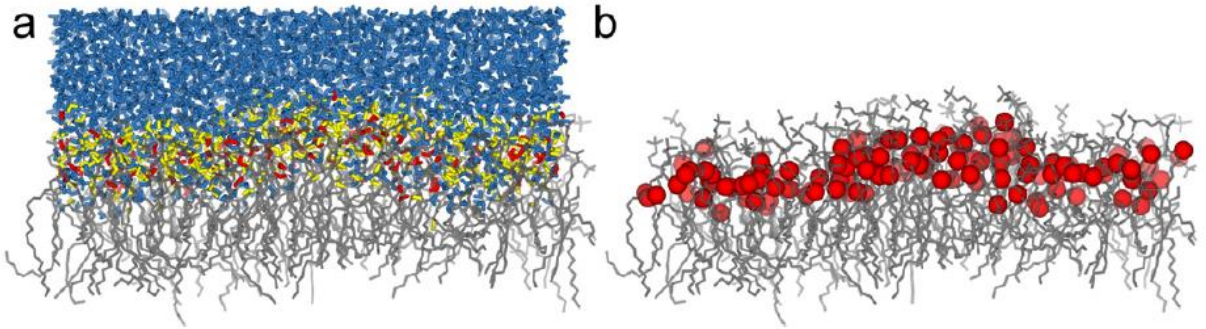

Fig. 5. (a) Different types of water molecules hydrating a PC bilayer. Blue, water molecules not H-bonded to PC; yellow, water molecules H-bonded to PC; red, water molecules bridging PC molecules (the water molecules that deeply penetrate the bilayer (blue) are not H-bonded to PC). For clarity, only one leaflet is presented. (b) The same leaflet without water molecules and with the carbonyl oxygen atoms (red) in the CPK representation.

Pasenkiewicz-Gierula, M.; Baczynski, K.; Markiewicz, M.; Murzyn, K. Computer modelling studies of the bilayer/water interface. *Biochim. Biophys. Acta* 2016, *1858*, 2305-2321.

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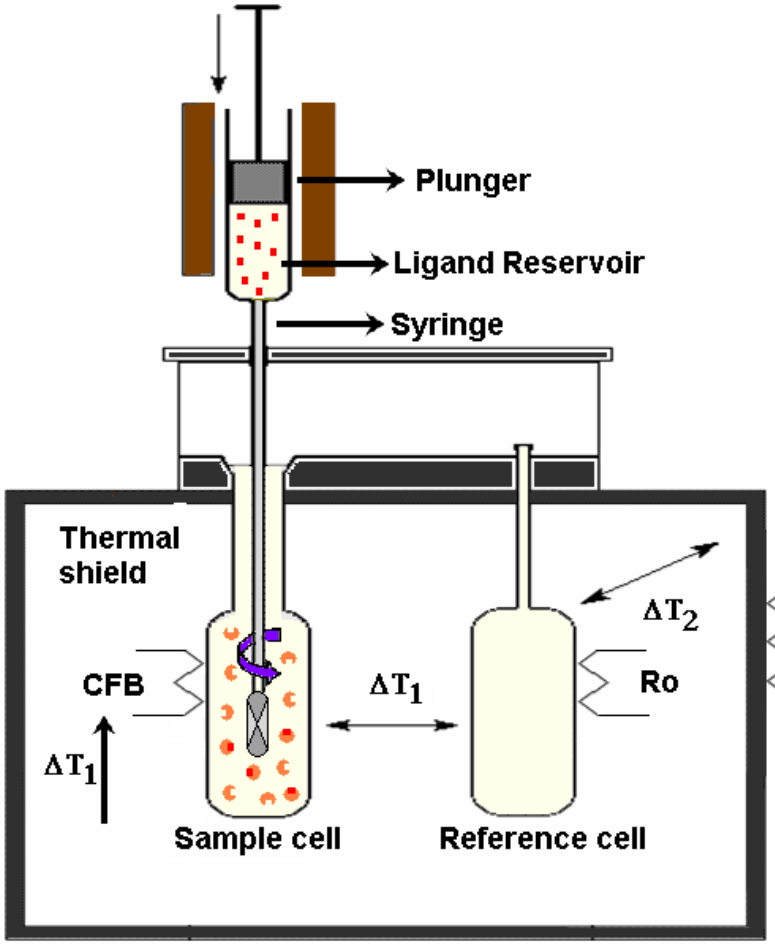
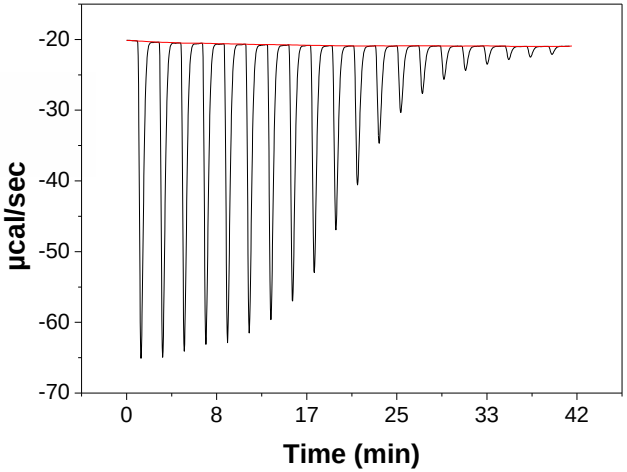
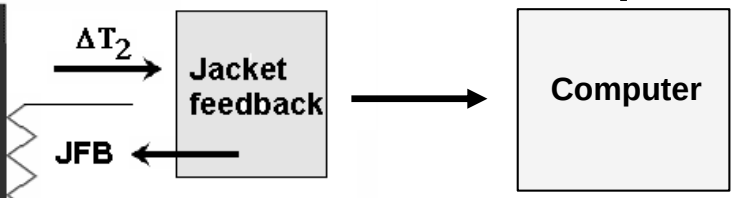
Methods: Isothermal titration calorimetry (ITC)







With ITC, the binding enthalpy and the binding constant (free energy of binding) can be determined from one and the same experimental titration curve

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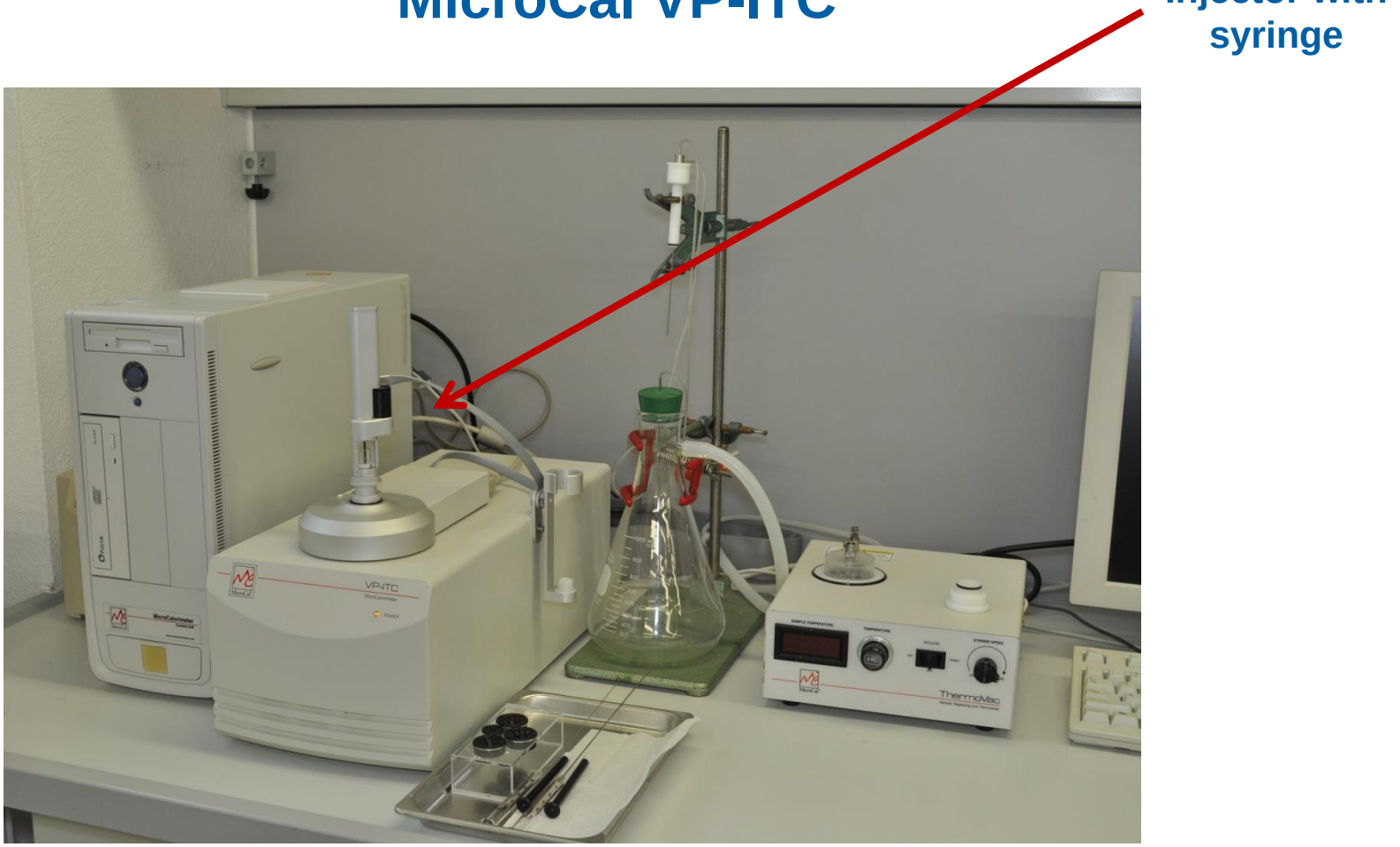
MicroCal VP-ITC



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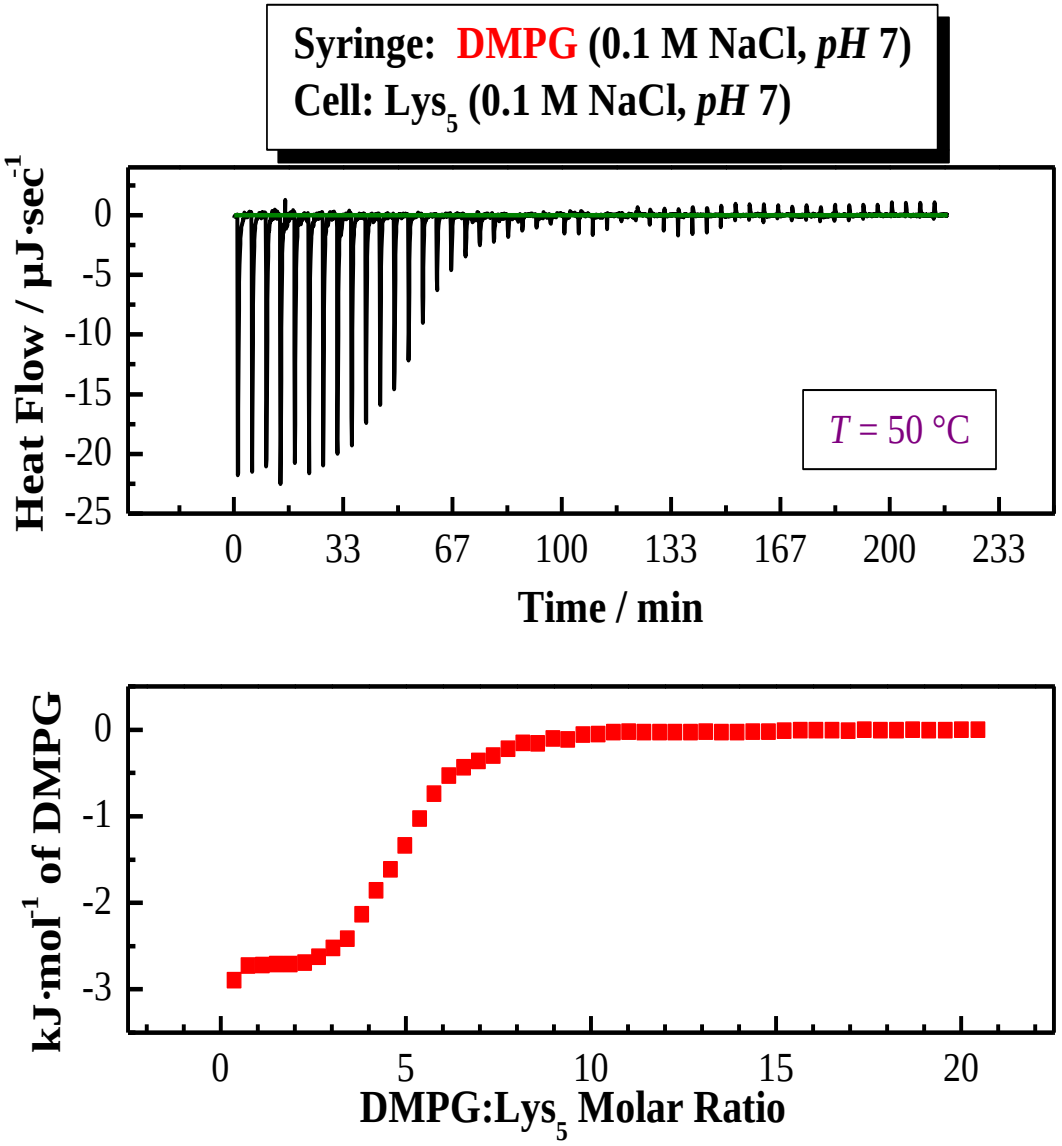
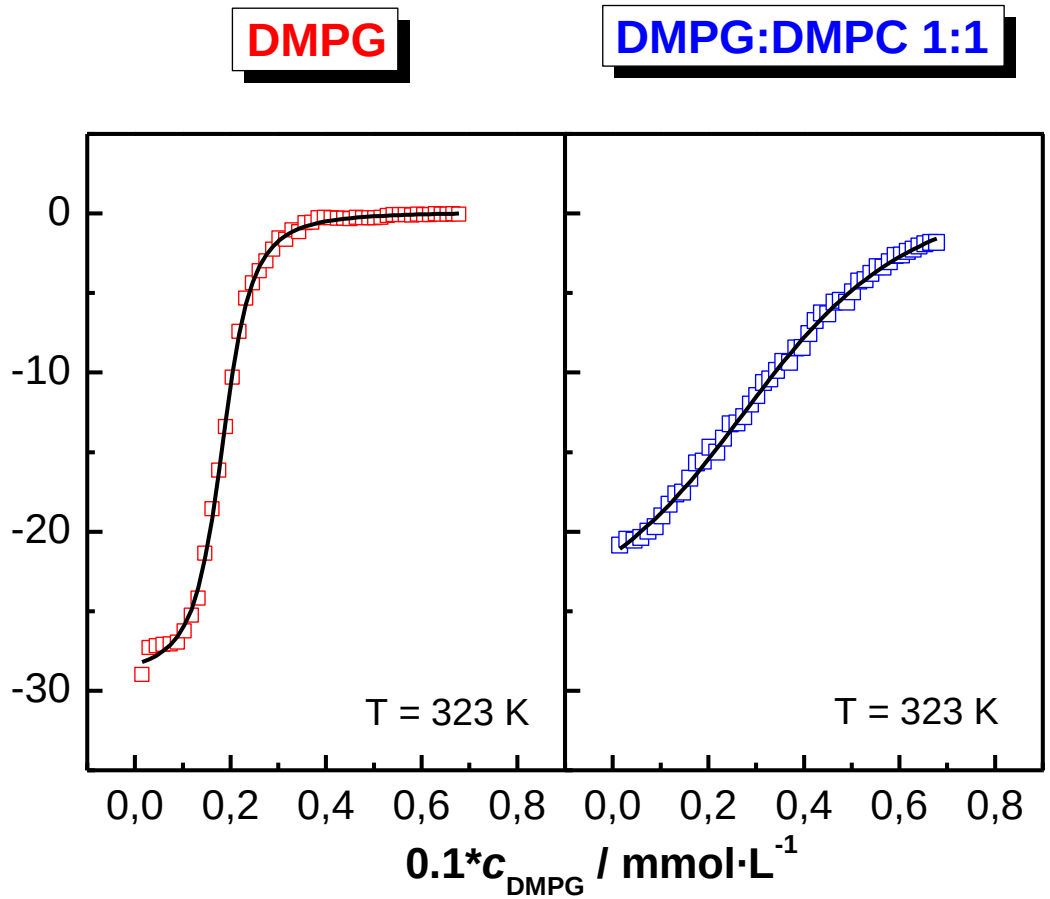


Electrostatic surface binding of an olipopeptide (pentalysine) to negatively charged DMPG-vesicles

From the titration curve the binding constant as well as the binding enthalpy can be determined

A. Erbe, W. Hübner, P. Garidel, A. Blume, unpublished

Fit of the titration curves with a binding model to determine the binding enthalpy and the binding constant



A simple 5:1 binding was assumed according to the equilibrium

$$5\ DMPG + K_5 \rightleftharpoons (DMPG_5K_5)$$

In addition it was assumed that a certain variable percentage of the inner vesicle surface is accessible

A. Erbe, W. Hübner, P. Garidel, A. Blume, unpublished

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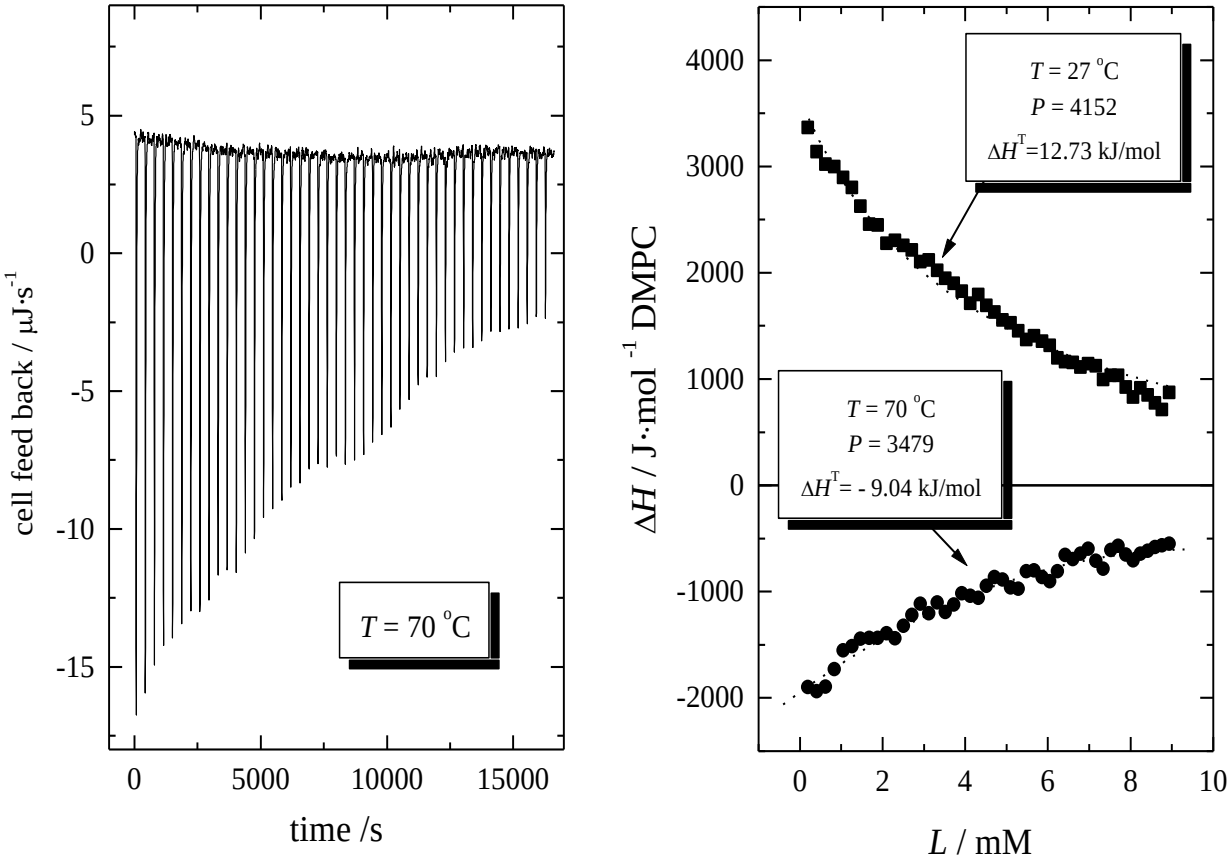
Partitioning of octyl glucoside (OG) into DMPC vesicles

Titration of DMPC vesicles into a OG solution



The change in sign of the partitioning enthalpy is caused by the hydrophobic effect

Keller, M., Kerth, A., Blume, A. (1997) Biochim. Biophys. Acta 1326, 178-192

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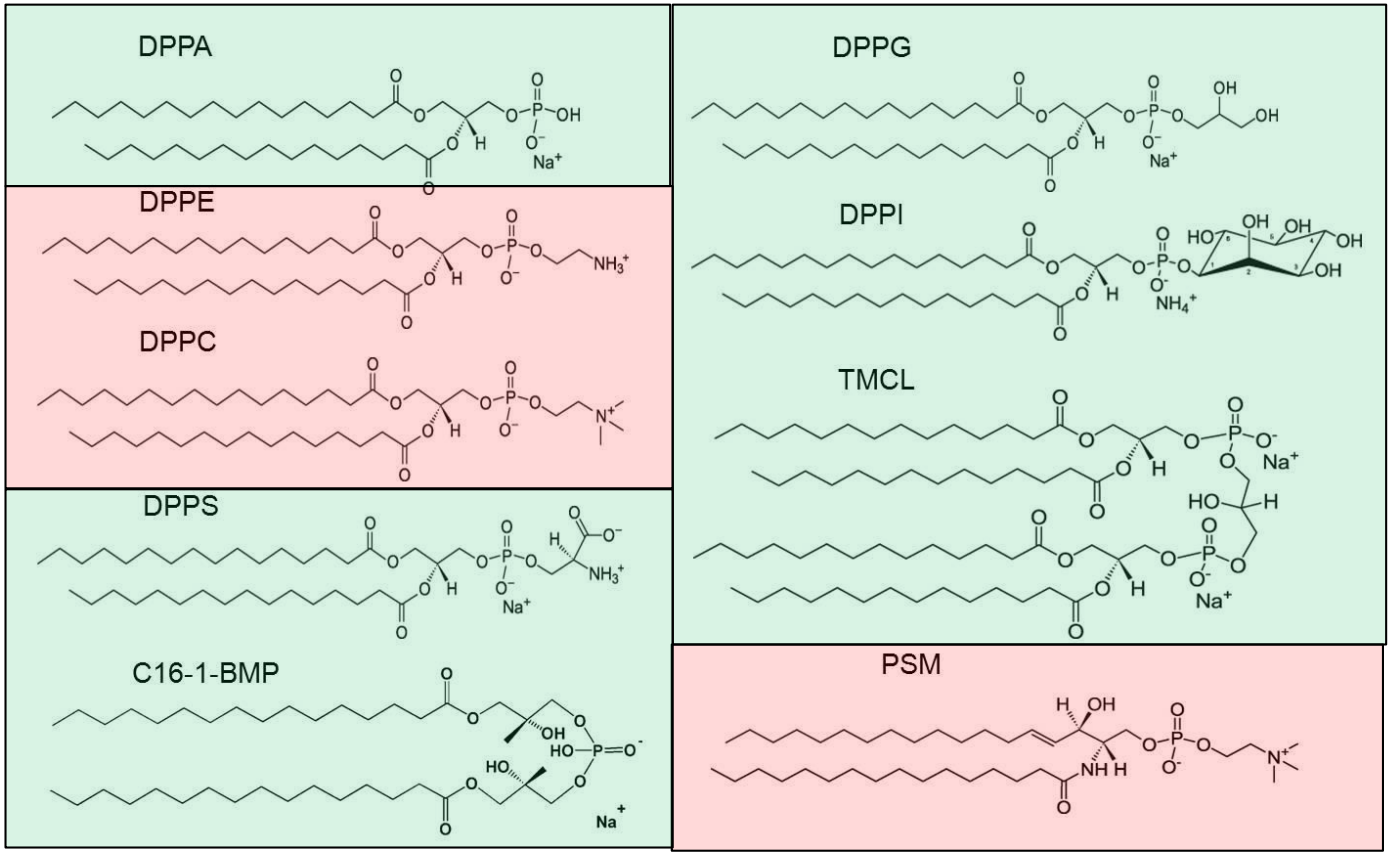
pK-values of charged phospholipids

Determination of the apparent pK-value



red: zwitterionic

green: negatively charged

all headgroups can act as H-bond acceptors and donors with the exception of PC (only acceptor)

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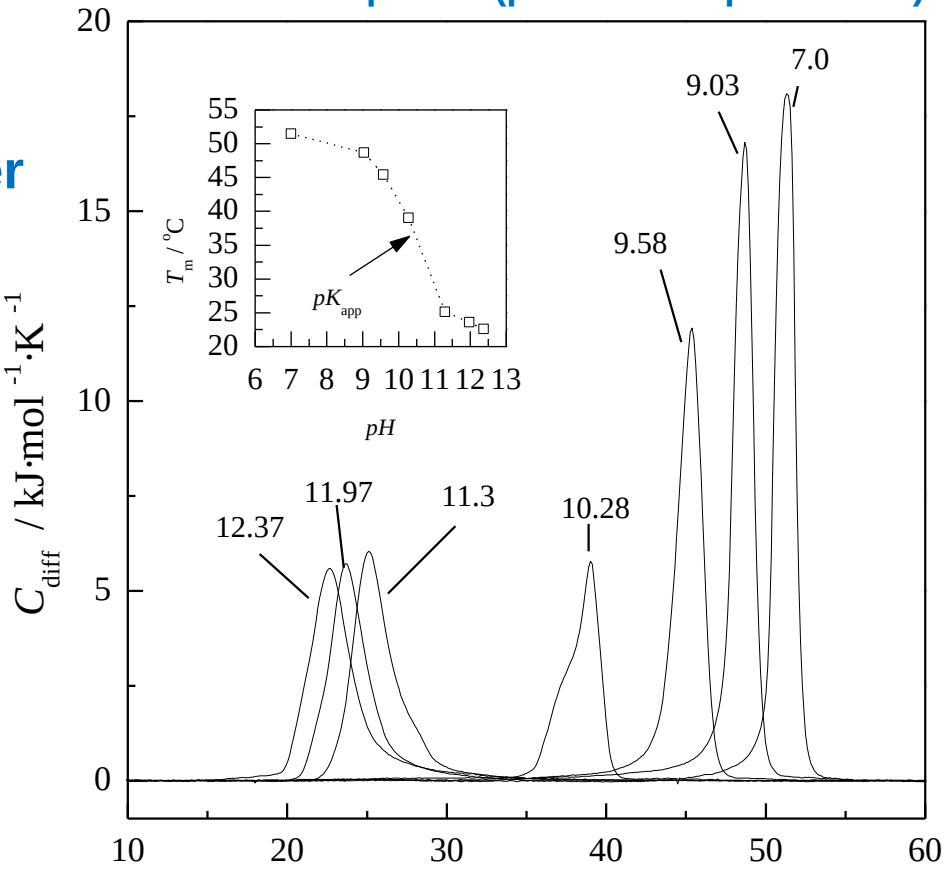
The apparent pK-value can be determined by the pH-dependence of the transition temperature T_m

DSC curves of dimyristoyl phosphatidic acid as a function of *pH*. The insert shows the *pH*-dependence of the transition temperature T_m and the apparent *pK*-value for the dissociation of the second proton (pK = 10.5 in pure water)

DMPA in water



apparent pK depends on ionic strength: increase in ionic strength decreases apparent pK

A. Blume and J. Tuchtenhagen, Biochemistry, 31 (1992) 4636.

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Salt depencence of the apparent pK of PAs

$$pK_s = pK_0 + 0.58 - \log n$$

where pK_0 is the pK in bulk and n the salt concentration.

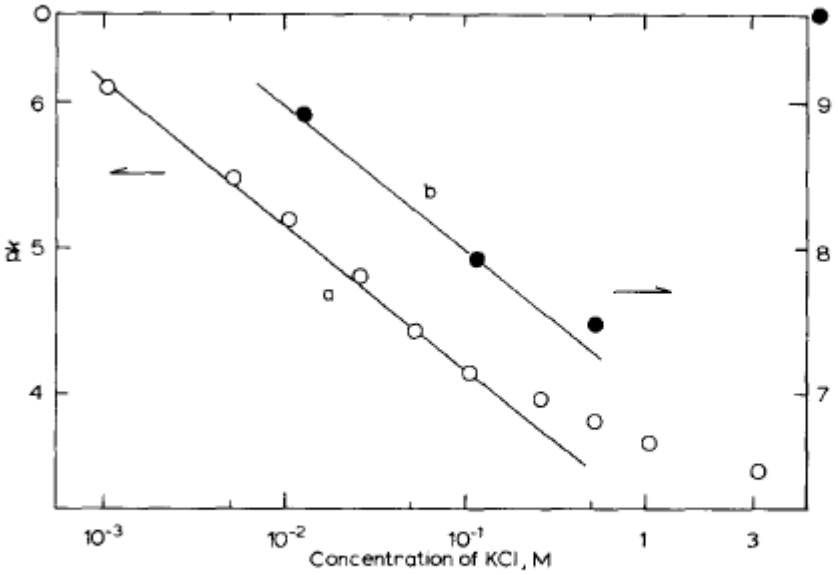

Fig. 5. (a) Apparent pK for the proton-induced phase separation against salt concentration. (b) Surface pK of sonicated phosphatidic acid vesicles against salt concentration. The straight lines are drawn by Eqns. 1 and 2 based on Gouy-Chapman theory.

Tokutomi, S.; Ohki, K.; Ohnishi, S.-i. Proton-induced phase separation in phosphatidylserine/phosphatidylcholine membranes. Biochim. Biophys. Acta 1980, 596, 192-200.

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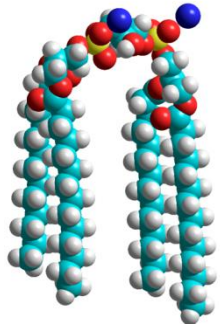
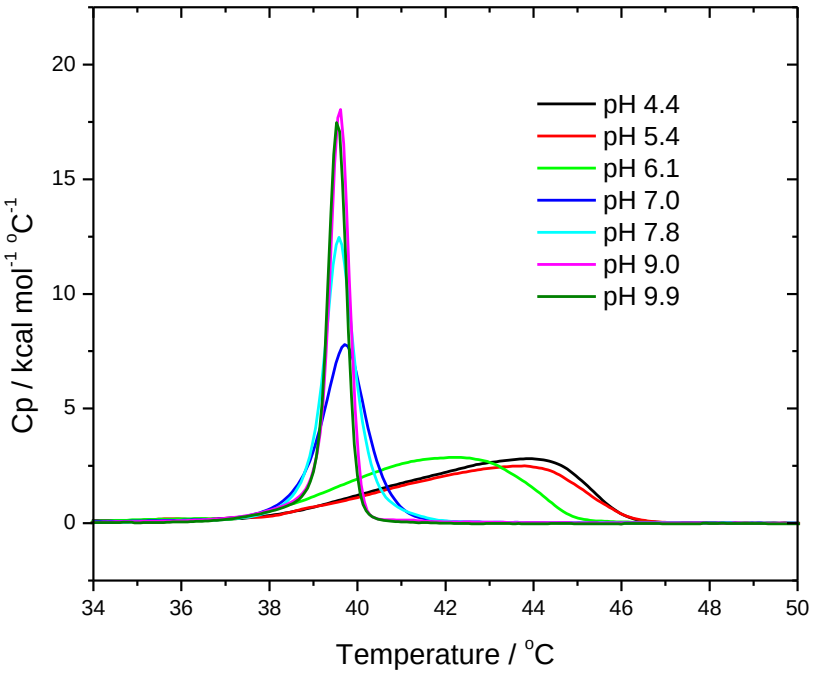
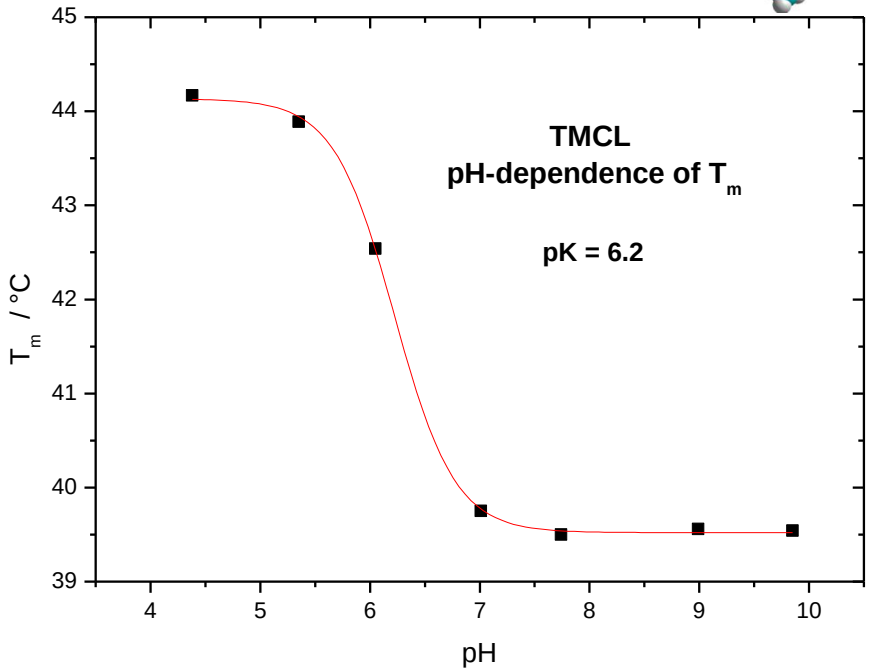
The question of the headgroup pK-values of cardiolipin (CL)

Determination of T_m-values as a function of pH by DSC







Finger and Blume, unpublished

TMCL is doubly charged at pH 7 (0.1 M NaCl)

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Methods for the determination of dynamics in liquid-crystalline (fluid) lipid bilayers

NMR-spectroscopy: ^{1}H, ^{2}H, ^{13}C, ^{31}P, ^{19}F

Fluorescence spectroscopy:
Photobleaching
Fluorescence resonance energy transfer (FRET)

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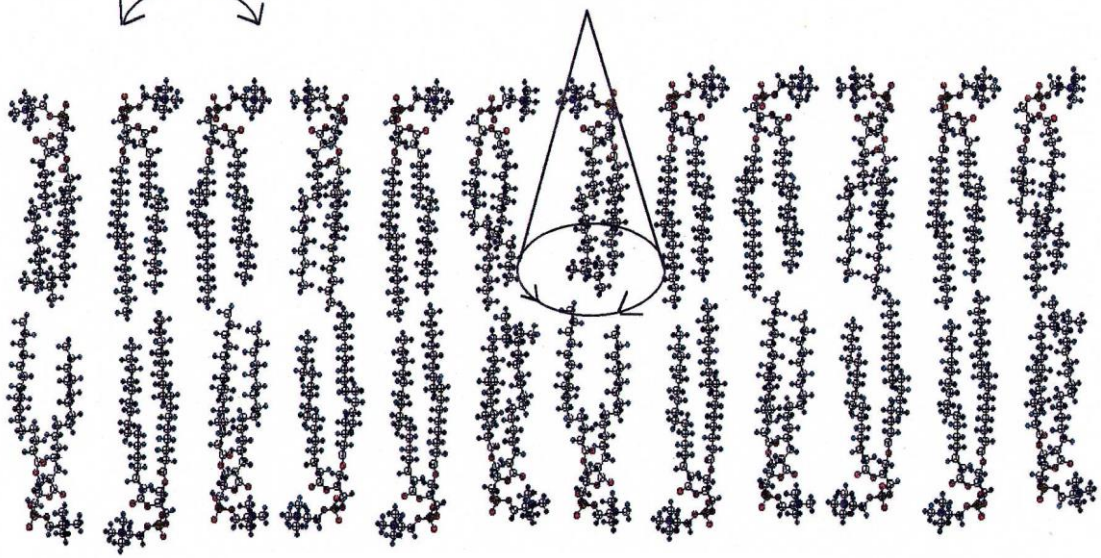
Dynamics in liquid-crystalline bilayers

$\tau_c = 10^{-8} - 10^{-7}$ s

hopping (jump diffusion)

$\tau_c = 10^{-9} - 10^{-4}$ s

wobbling
rotational diffusion in a cone

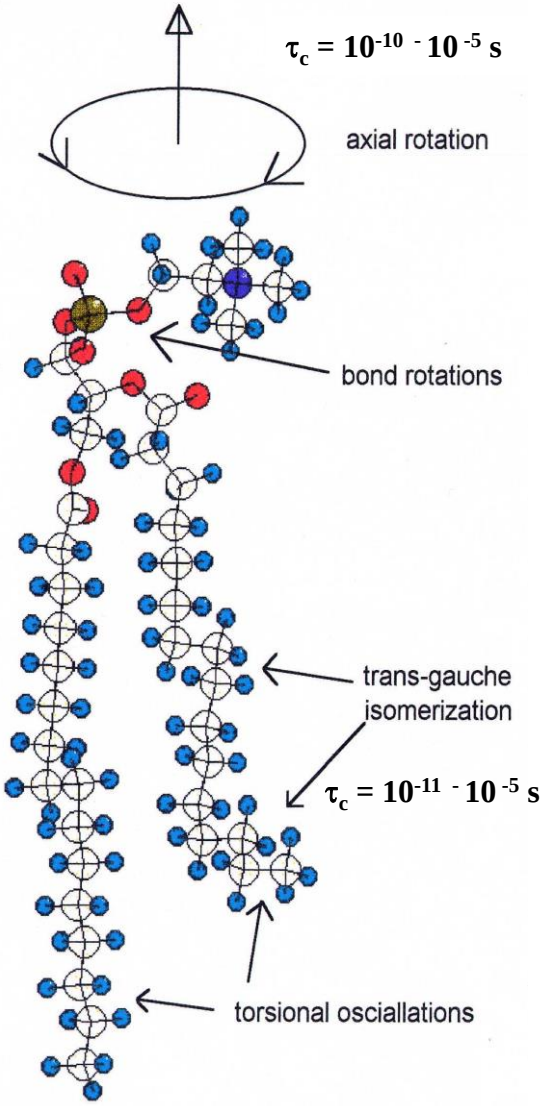


$\leftarrow D_T = 10^{-8} - 10^{-7}\ cm^2\ s^{-1} \rightarrow$

lateral diffusion

$x^2 = 4D_T t = 4 \cdot 10^{-7}\ cm^2 = 4 \cdot 10^{7}\ nm^2$

in 1 second: $\sqrt{\overline{x^2}} = 6.6 \cdot 10^3$ nm



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Visualization of giant unilamellar lipid vesicles by laser scanning fluorescence microscopy (LSFM) using fluorescence probes

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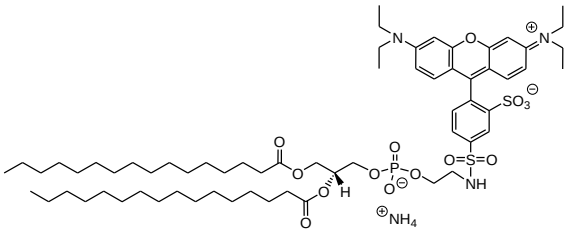
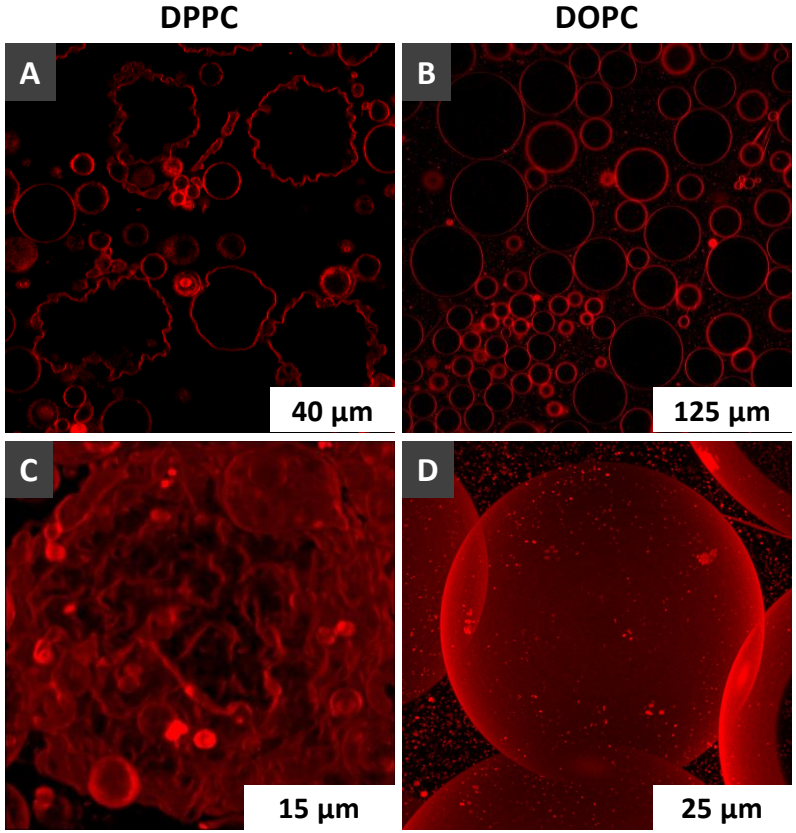
Laser scanning fluorescence microscopy (LSFM) of GUVs



Rh-DHPE



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PhD-Thesis
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Top: LSFM-images of DPPC-GUV in the $L_{\beta'}$-phase (A, C) and DOPC-GUV in the L_{α}-phase (B, D); with 0.5 weight% Rh-DHPE at 20°C;
Bottom: volume image reconstructed from 78 (C) or 70 (D) cuts at different focus plains; scales: xy-plain: 40 µm (A), 125 µm (B), 15 µm (C), 25 µm (D), z-direction: 40.82 µm (C), 30.4 µm (D).

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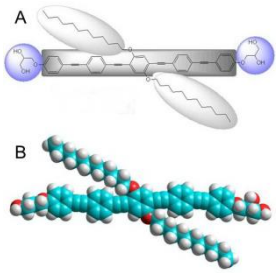
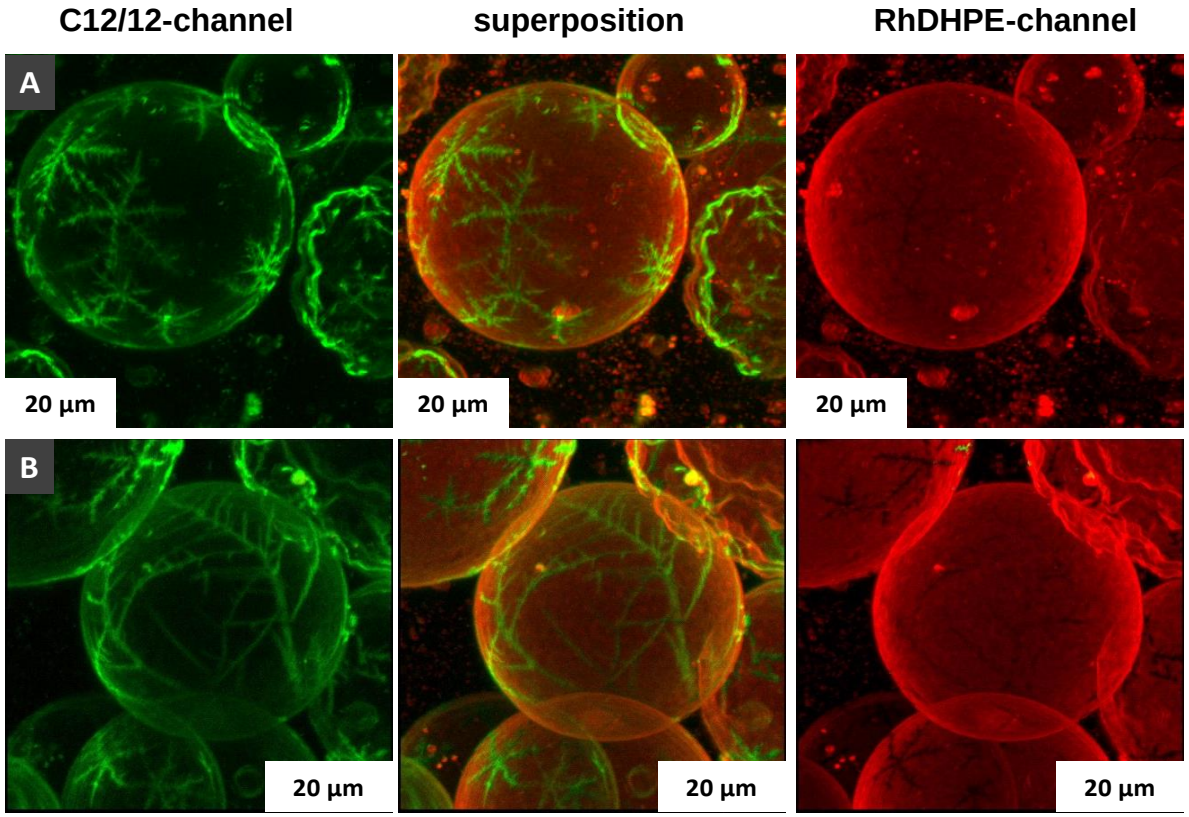
Laser scanning fluorescence microscopy (LSFM) of GUVs



C12/12



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Volume images of GUVs of C12/12 : DPPC = 1:10 (0.5 weight% Rh-DHPE) with 42 (A) or 117 (B) cuts at different focus planes; left: green channel (fluorescence of C12/12), right: red channel (Rh-fluoresceence), middle: superposition of both channels; scales: xy-plain: 20 µm, z-direction: 20.05 µm (A), 44.02 µm (B).

C12/12 phase separates into star-like domains

B.-D. Lechner et al., Langmuir 2015, 31, 2839−2850

Summary

The physico-chemical properties of lipids have been studied in detail for many phospholipids as a function of temperature and hydration using various physico-chemical methods.

The lyotropic behavior of lipids and lipid mixtures containing charged lipids depends on pH and the ionic strength of the suspension.

For applications of phospholipids as drug-delivery systems and in emulsions, the knowledge of the physico-chemical behavior of these amphiphilic molecules is essential for successful formulations.

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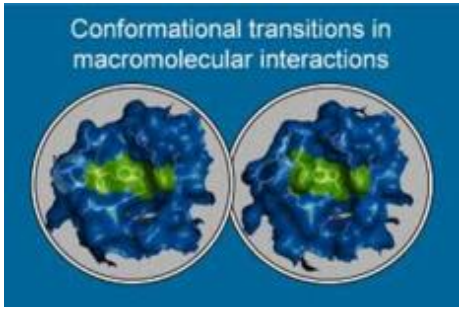
SACHSEN-ANHALT



Boehringer Ingelheim

DFG Deutsche Forschungsgemeinschaft

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