Possibilities of the neutron and synchrotron radiation for the characterization of the lipid nanosystems

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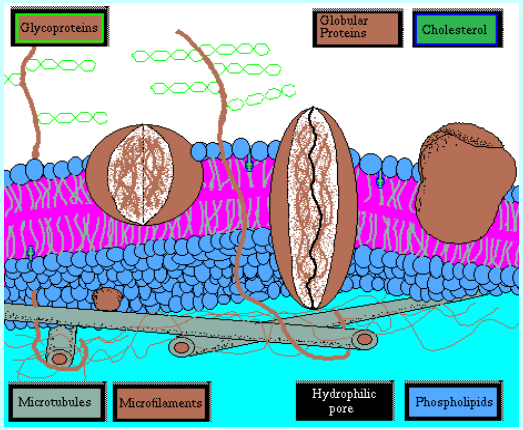
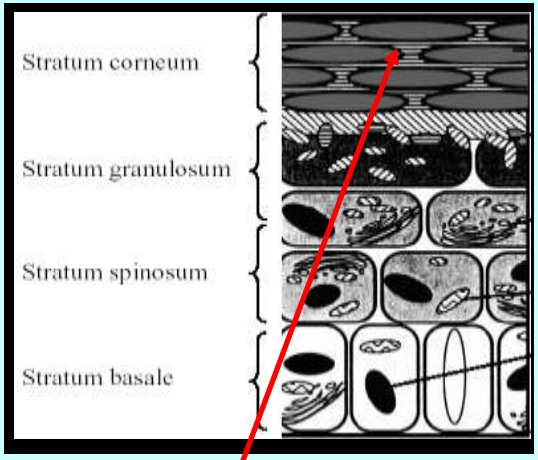
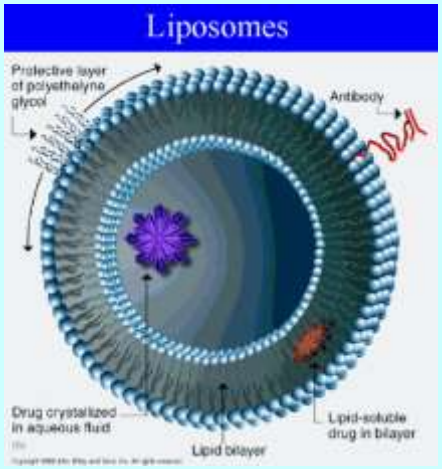
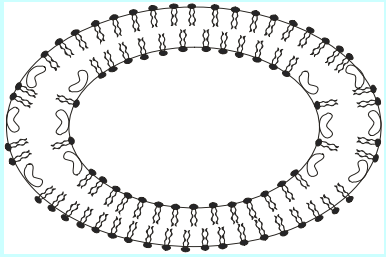
Phospholipids – major component of cell membranes.
Ceramides – major components of stratum corneum lipid matrix.
Phospholipids and ceramides – important materials for drug and cosmetic industry.

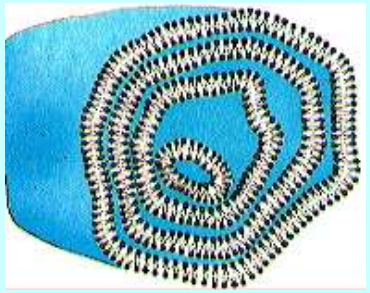






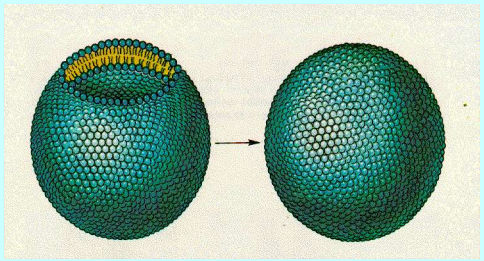


Objects and methods



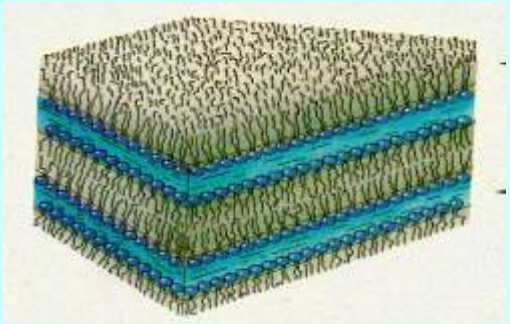
Multilamelar vesicles (Liposomes)

Small-angle and wide-angle powder X-ray diffraction at synchrotron source



Unilamellar vesicles

Neutron small-angle scattering



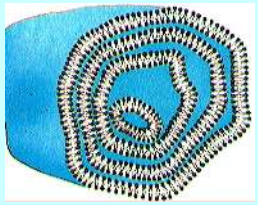
Oriented multilamellar planar system on quartz substrate

Neutron diffraction

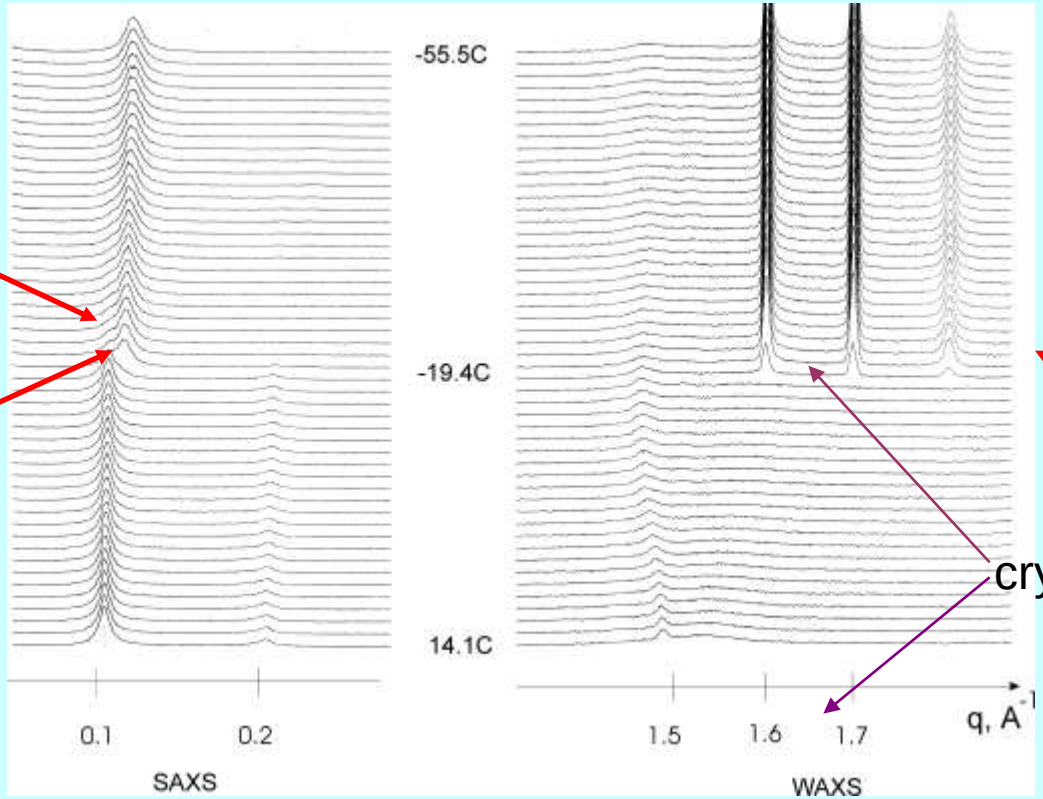
New methods developed for the characterization of the lipid nanosystems :

- Method of lamellar and lateral synchrotron X-ray diffraction in real time for the cryoprotector characterization.
- Method of neutron diffraction in real time at pulse neutron source IBR-2 for study of diffusion processes in model stratum corneum membranes.
- Method of separated form factors for characterization of vesicular nanodrugs.
- Method of contrast variation of X-ray scattering by disaccharides.

Real-time X-ray diffraction at cooling DPPC/water system at synchrotron.



$\Delta d = 5.9 Å$



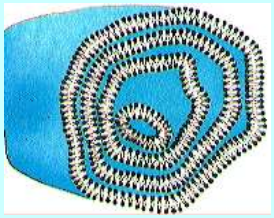
Free water Diffusion

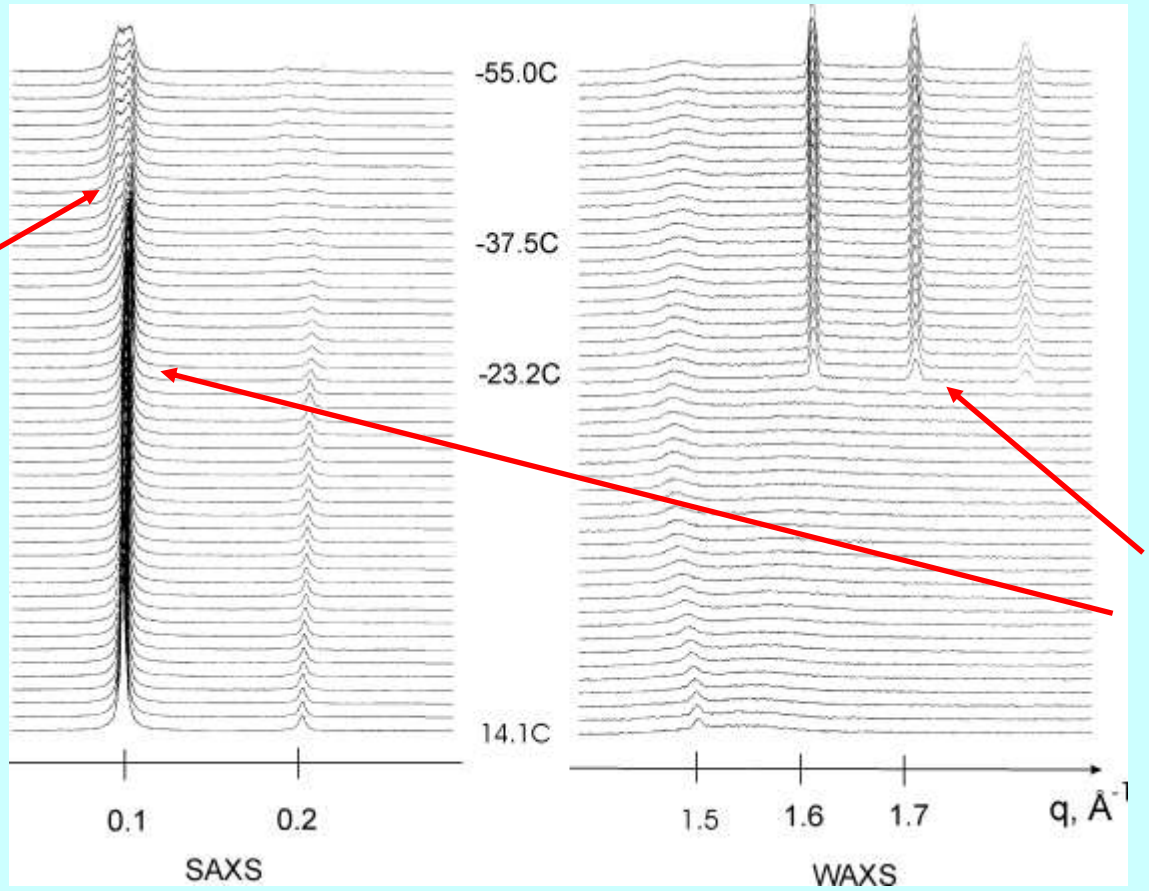
Ice crystal

crystallization $L_{\beta'} \rightarrow L_c$

Cooling with the rate of 1°C/min, acquisition - 1min/spectrum

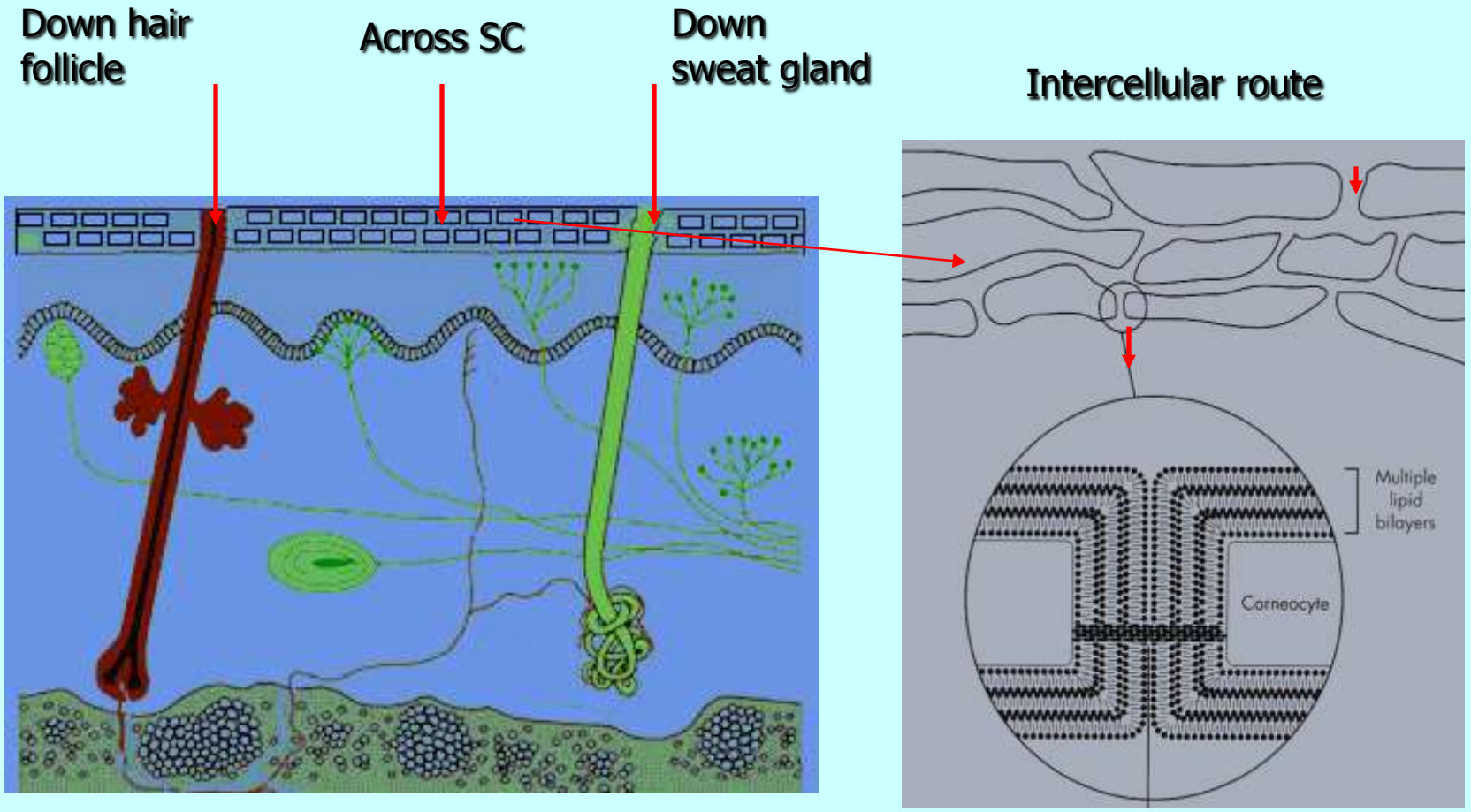
Low-temperature phase transition in DPPC/DMSO/water system at X_{dmso}=0.05. Synchrotron.



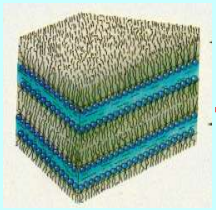


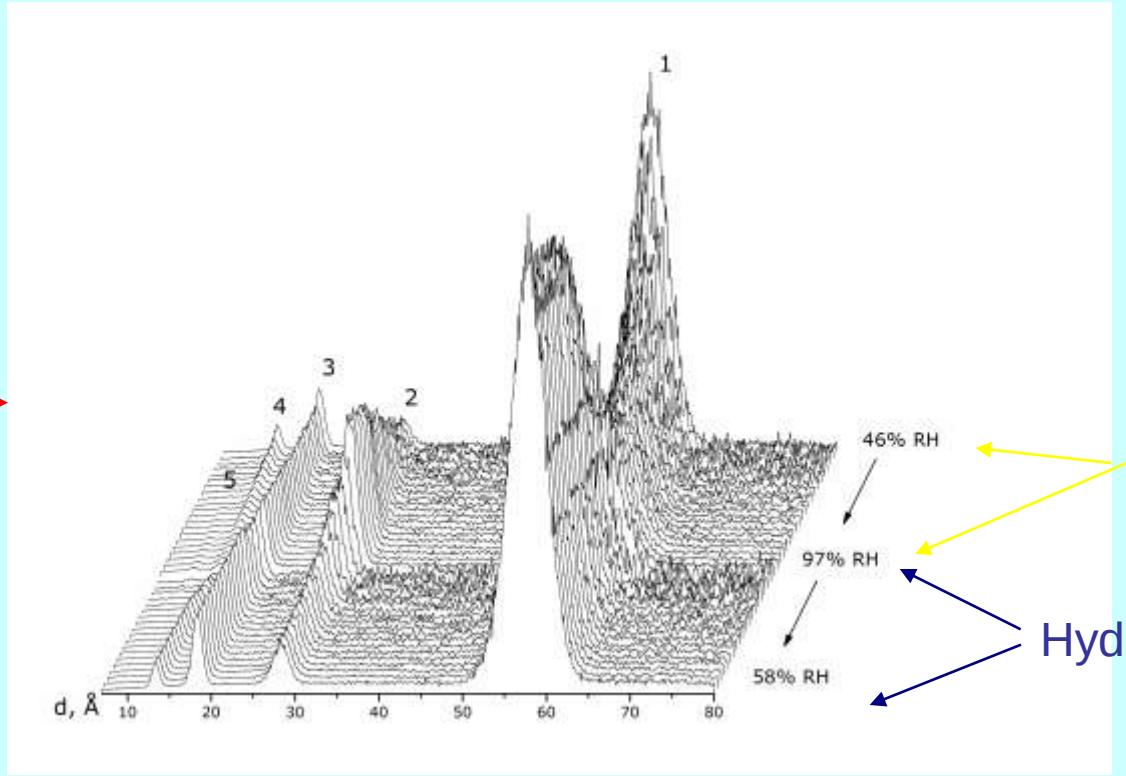
Cooling with the rate of 1°C/min, acquisition - 1min/spectrum

Drug penetration pathways across the skin.



Real- time neutron diffraction at DN-2, IBR-2. Time-of-flight.



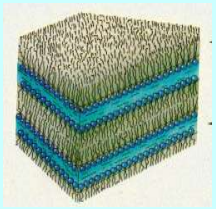


Dehydration 360min

Hydration 480min

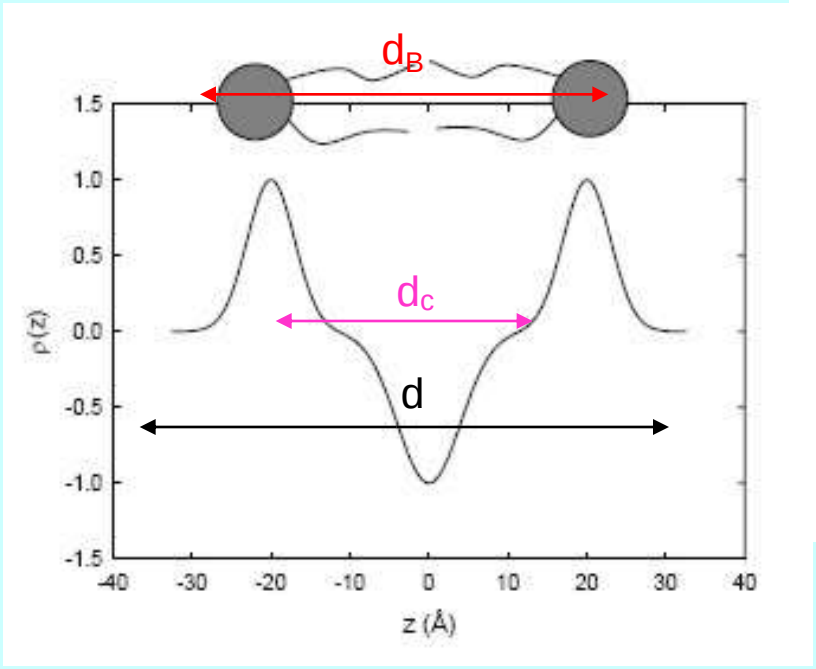
DPPC membrane hydration-dehydration in real time at T=20°C

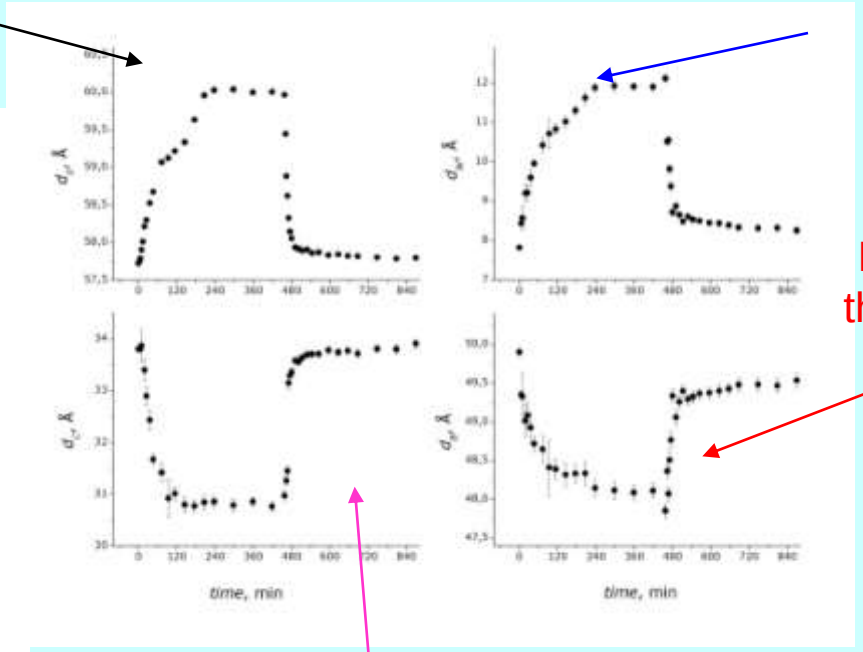
Nanostructure alteration during hydration-dehydration as result of fourier-synthesis in real-time. T=20°C.



Repeat distance d

Water layer d_w





Membrane thickness d_B

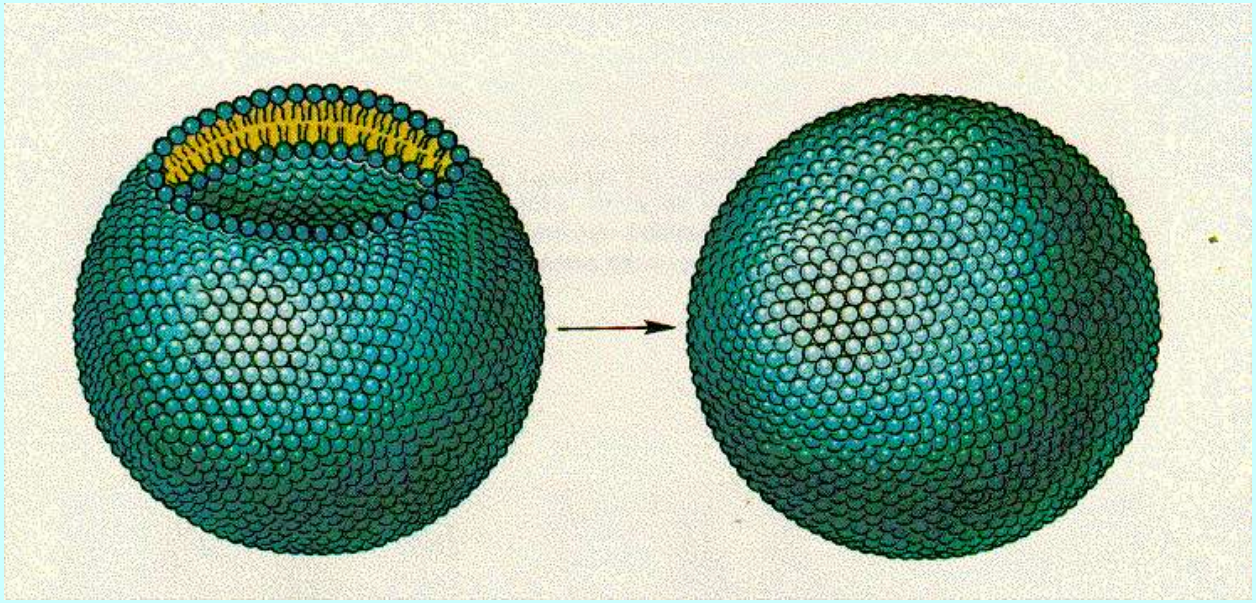
Thickness of the hydrocarbon chains region d_c

Н.Ю. Рябова, М.А. Киселев, А.И. Бескровный, А.М. Балагуров. Исследование структуры многослойных липидных мембран методом дифракции нейтронов в реальном времени. *Физика твердого тела,* 52 № 5 (2010) 984-991.

Unilamellar vesicles from phospholipid molecules

From point of physics
it is nanospheres
with liquid crystal surface

R=250Å
$n \cong 2 \cdot 10^{14}$ vesicles/cm^3 for 1% DMPC (w/w)
6500 DMPC molecules in one layer for 30°C

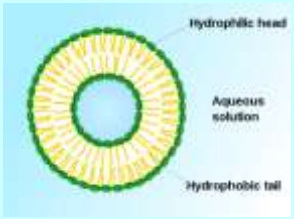


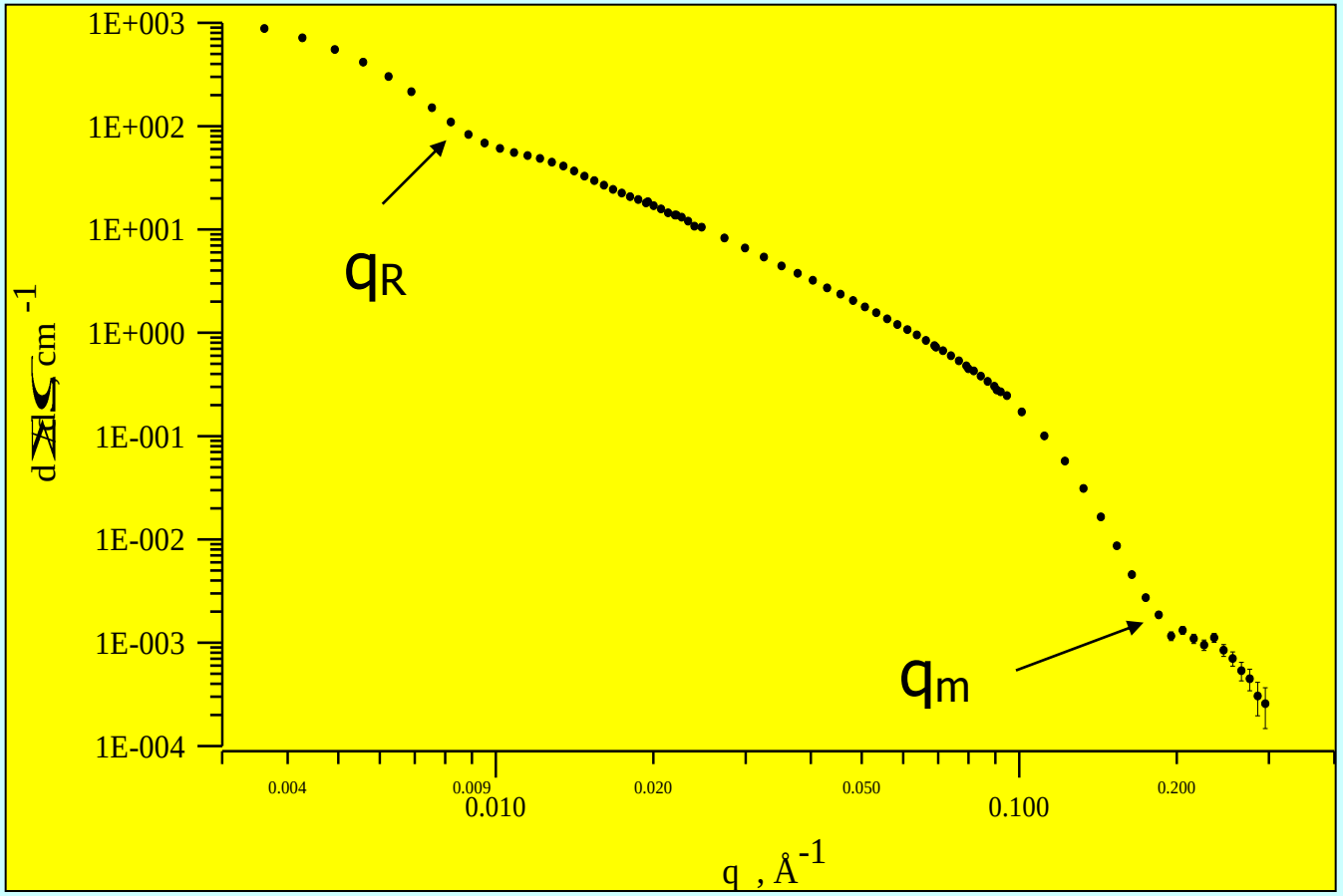
Neutron small-angle scattering in D_2O

X-ray small-angle scattering in sucrose solutions

M.A. Kiselev, P. Lesieur, A.M. Kisselev, D. Lombardo, M. Killany, S. Lesieur.
Sucrose solutions as prospective medium to study the vesicle structure:
SAXS and SANS study. J. Alloys and Compounds 328 (2001) 71-76.

SANS pattern at 30 °C of unilamellar DMPC vesicles prepared by extrusion through 500 Å pores at DMPC concentration of 1% (w/w).

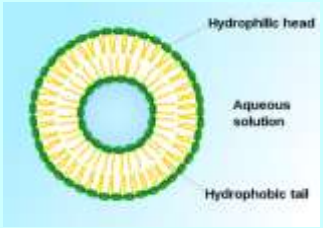


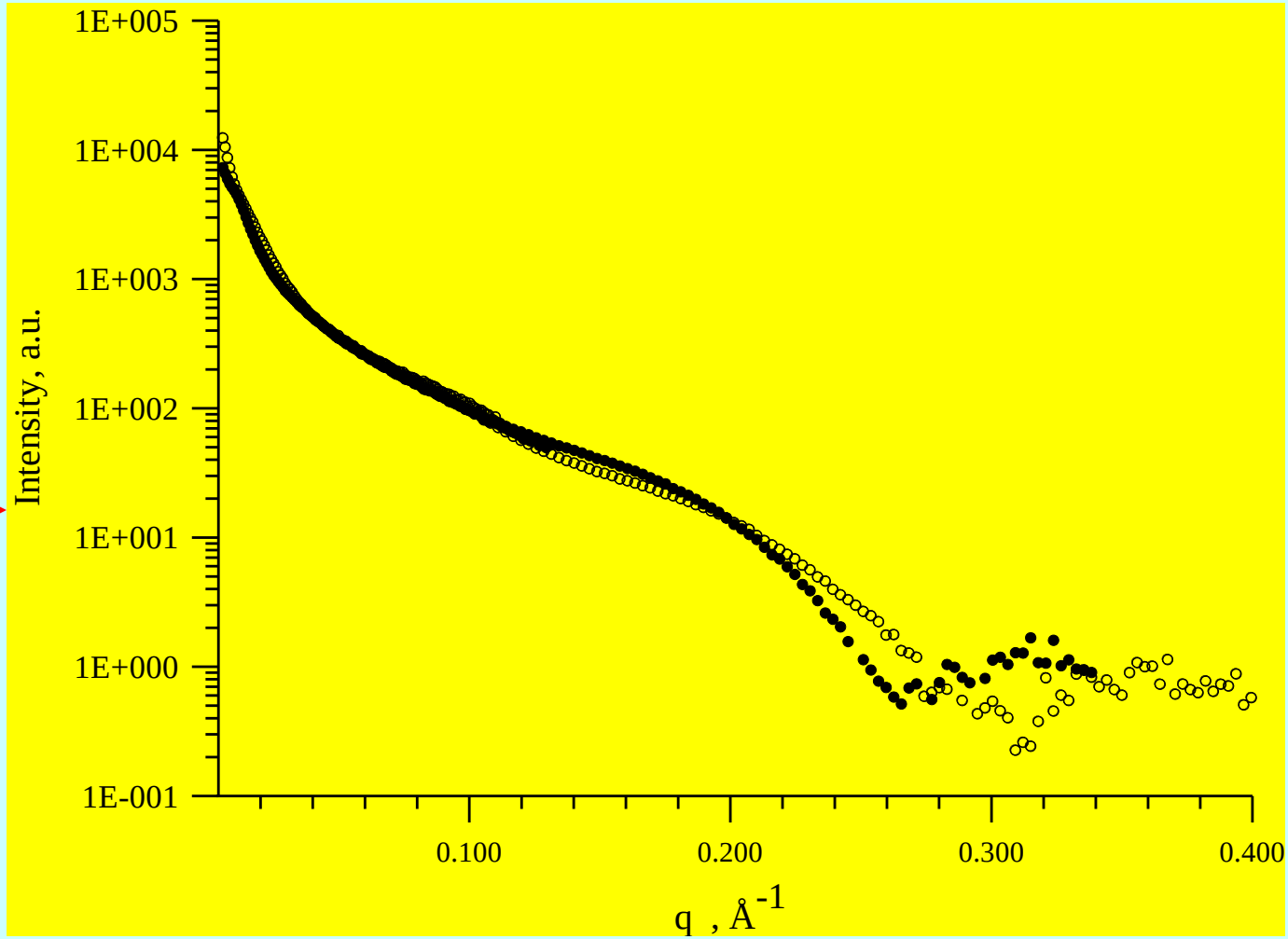


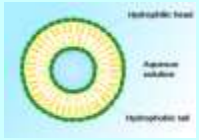
LURE

DESY

SAXS patterns at 10 °C (circles) and 30 °C (open circles) of unilamellar DMPC vesicles in 40% aqueous trehalose solution, DMPC concentration 3%(w/w)







Method of Separated Form Factors (SFF)

$$\frac{d\Sigma}{d\Omega}_{mon}(q) = n \cdot A^2(q) \cdot S(q) + IB$$

$$A_{ves}(q) = 4\pi \cdot \frac{R^2}{qR} \cdot \sin(qR) \cdot \int_{-d/2}^{d/2} \rho_c(x) \cdot \cos(qx) \cdot dx + 4\pi \cdot \frac{R}{qR} \cdot \cos(qR) \cdot \int_{-d/2}^{d/2} \rho_c(x) \cdot x \cdot \sin(qx) \cdot dx$$

$\rho_c(x)=\rho(x) - \rho_{D2O}$ is contrast, the difference between scattering length densities of the bilayer $\rho(x)$ and the heavy water ρ_{D2O}.

$$\frac{d\Sigma}{d\Omega}_{mon}(q) = n \cdot F_s(q,R) \cdot F_b(q,d) \cdot S(q) + IB$$

$$F_s(q,R) = \left(4\pi \cdot \frac{R^2}{qR} \cdot Sin(qR)\right)^2$$

$$F_b(q,d) = \left(\int_{-d/2}^{d/2} \rho_c(x) \cdot Cos(qx) \cdot dx\right)^2$$

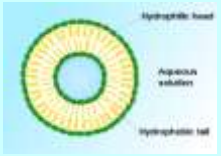
For the case of $\rho(x)$=Const

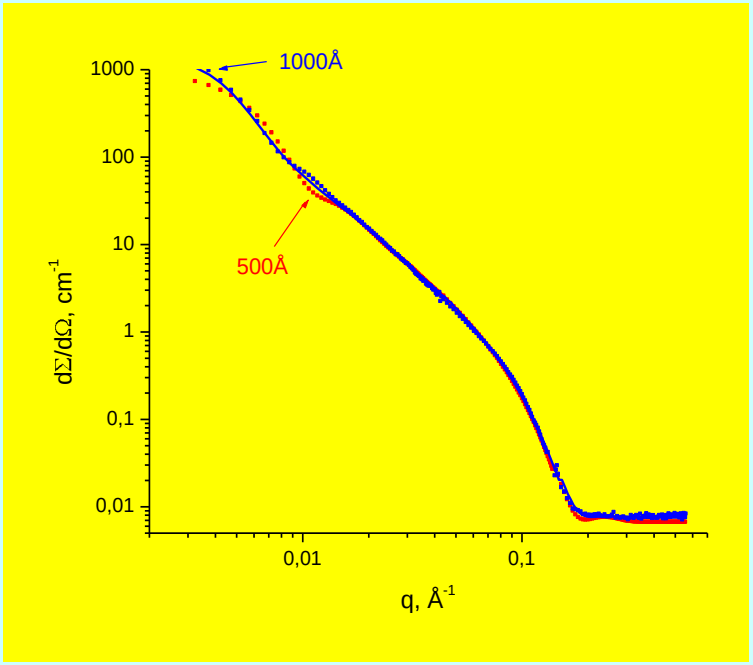
$$F_b(q,d) = \left(\frac{2\Delta\rho}{q} \cdot Sin\left(\frac{qd}{2}\right)\right)^2$$

M.A. Kiselev, P. Lesieur, A.M. Kisselev, D. Lombardo, V.L. Aksenov. Model of separated form factors for unilamellar vesicles. J. Applied Physics A 74 (2002) S1654-S1656

Results for liquid phase at 30°C, HH approximation of ρ(x)

Model	D_F, Å	<R>, Å	σ, %	d, Å	D, Å	N_w	A, Å^2	IB, cm^{-1}
SFF	500	275.6 0.5	27	47.8 0.2	20.5 0.3	11.9±0.3	61.0±0.4	0.007
Full	500	275.7 0.4	27	47.8 0.2	20.5 0.3	11.9±0.3	61.0±0.4	0.007
SFF	1000	500*	48	45.5 0.6	20.8 0.4	10.8 0.4	62.6 1.0	0.007





Multilamellar (small curved) vesicles
d = 44.2 Å
A=59.6 Å^2
J.Nagle, S. Tristram-Nagle. Structure of lipid bilayers. BBA 1469 (2000) 159-195

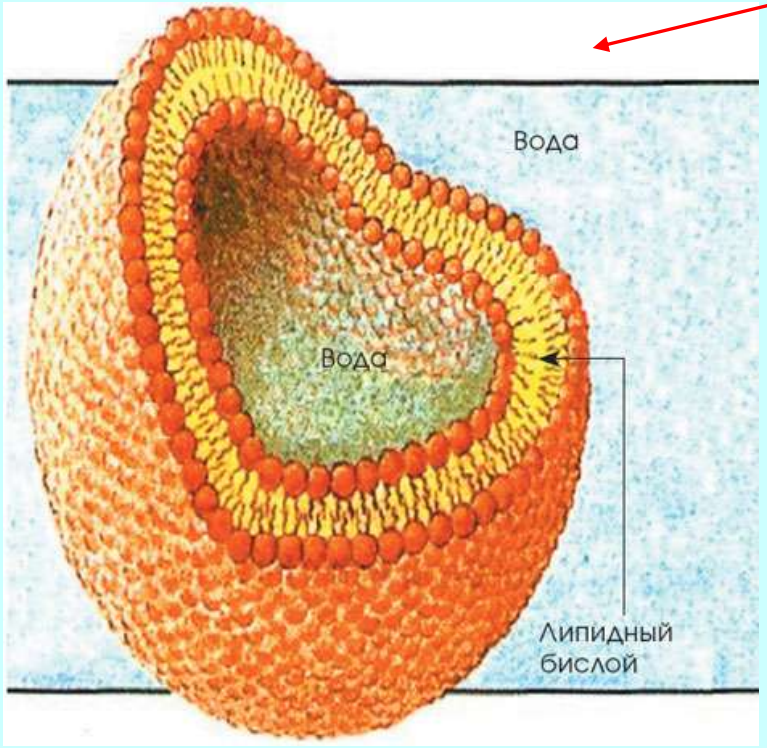
M.A. Kiselev, E.V. Zemlyanaya, V.K. Aswal, R.H.H. Neubert. What can we learn about the lipid vesicle structure from the small angle neutron scattering experiment? *European Biophysics J.* 35 (2006) 477-493.

Practical applications

Nanoparticles based on the phospholipids

Phosphogliv – 20-50 нм

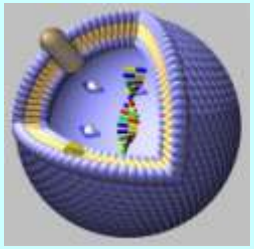
?
?
?

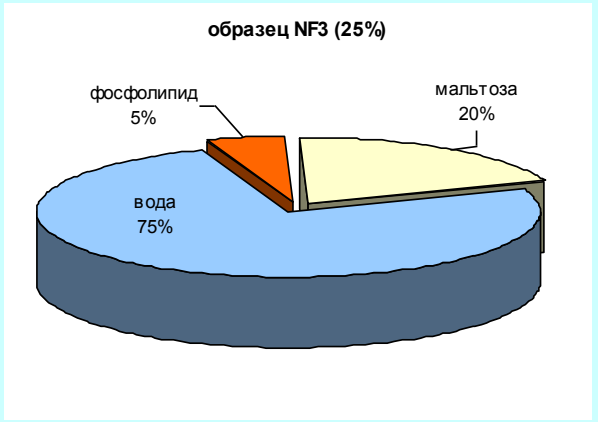


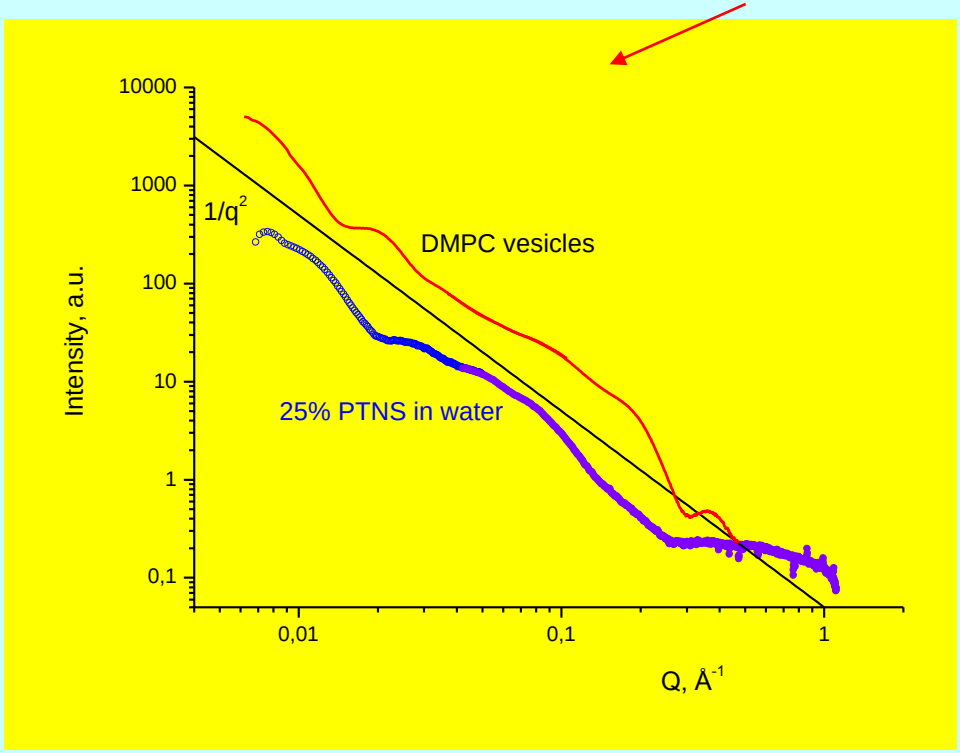
80% products in the bionanotechnology are drug delivery systems !!!

Turnover - 6 billion USD/year

Phospholipid transport nanosystems (FTNS)





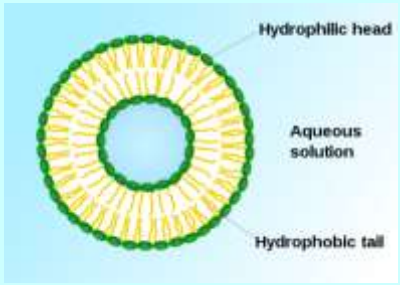


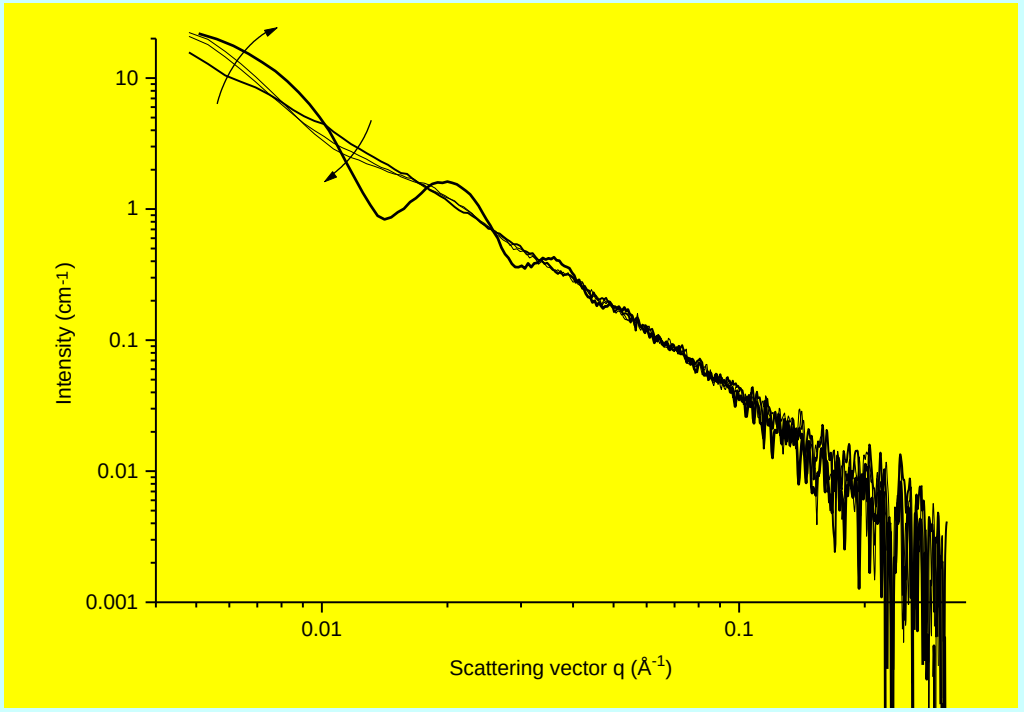
SAXS patterns. DMPC vesicles in 40% sucrose buffer – red line (LURE, France). 25% solution of FTNS in water – blue line (DIKSI, Sibirea-2, Moscow). Black line - $1/q^2$ law.

LURE

Formation of the low-polydispersity vesicles in the DMPC/sodium cholate system at the rate 100oC/min.

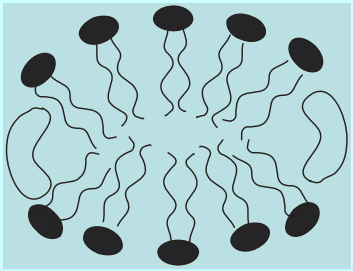
Synchrotron study.





P. Lesieur, M.A. Kiselev, L.I. Barsukov, D. Lombardo. Temperature induced micelle to vesicle transition: kinetic effects in the DMPC / NaC system. *J. Appl. Cryst*. 33 (2000) 623-627.

Membrane self-assembly in the mixed DMPC/ sodium cholate system induced by the temperature-jump. Synchrotron.

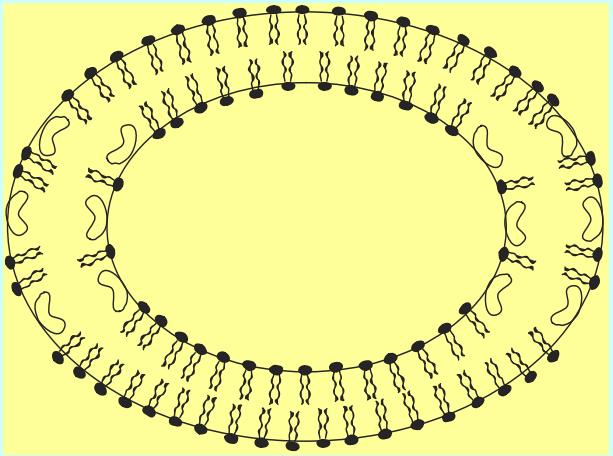


20°C.
Initial state.

100°C/min

? ? ?

What are intermediates ???



60°C.
Final state.

New X -ray sources are required !!!

Conclusions.
Possibilities to study in future:

- Drug diffusion through model SC membranes.
- Nanostructure of the magnetovesicles.
- Elastic properties of the transdermal drug delivery vesicles and magnetovesicles.
- Effectiveness of the cryoprotectors applied for bacteria storage.

Participants from Russia

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- E.V. Ermakova JINR Russia
- N.Y. Ryabova JINR Russia
- A.M. Kisselev JINR Russia
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- A.B. Zabelin KI Russia
- O. M. Ipatova IBMC Russia
- L.I. Barsukov IBC Russia

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- J. Zbytovska MLU Germany
- D. Kessner MLU Germany
- A. Schroter MLU Germany
- M. Janich MLU Germany
- T. Gutberlet HMI Germany
- Th. Hauss GCB Germany
- S. Dante GCB Germany
- V.K. Aswal PSI Switzerland
- G. Klose LU Germany

Thank you for the attention