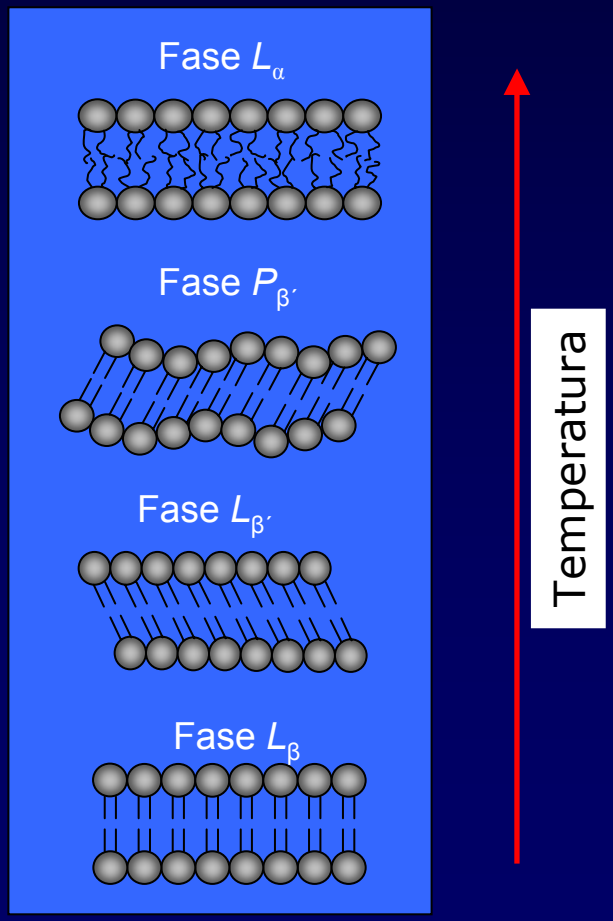
Dinámica molecular y propiedades viscoelásticas de membrana en liposomas

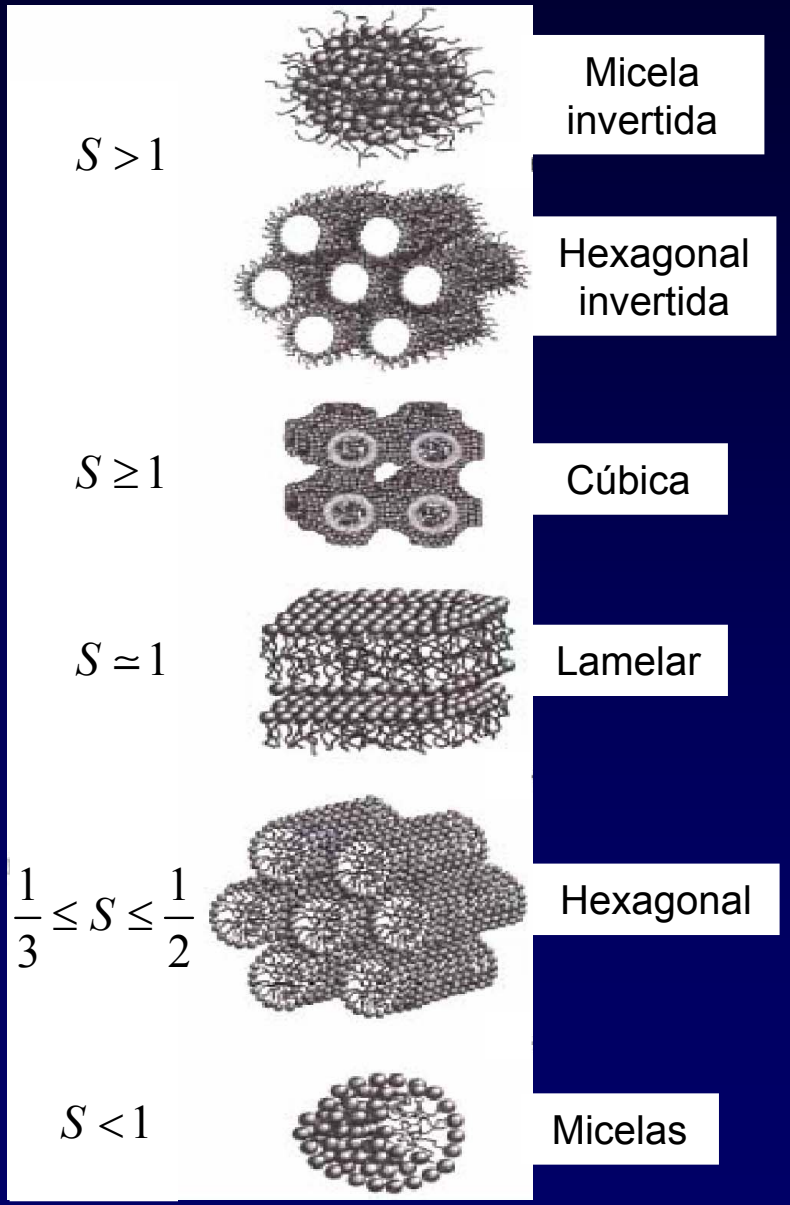
Esteban Anoardo

Lípidos anfifílicos en soluciones acuosas

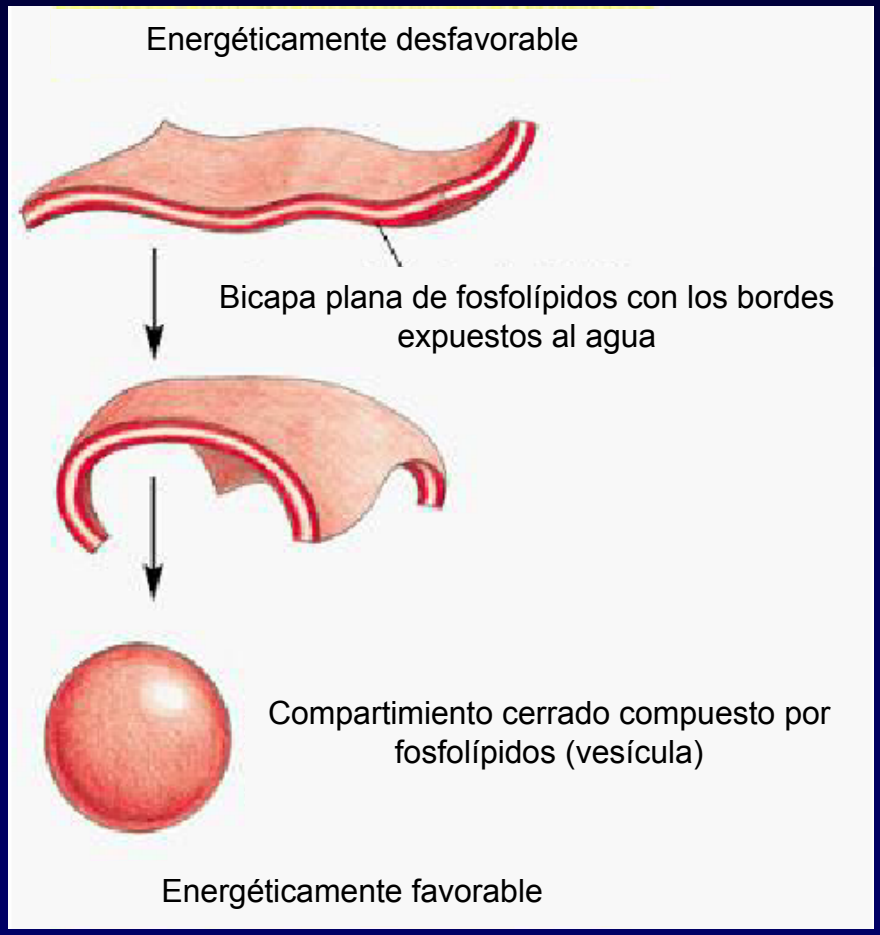
Organización
- Concentración de lípidos
- Temperatura
- Parámetro surfactante

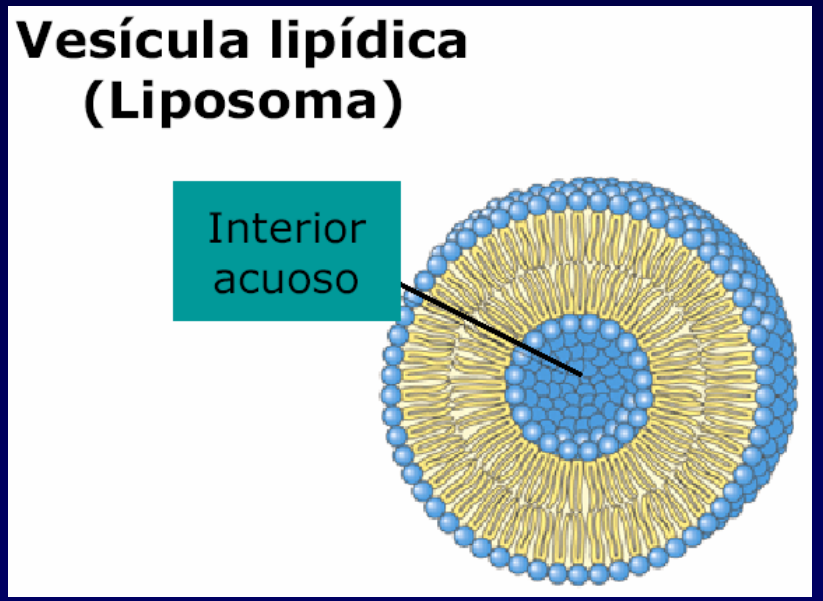
Fase lamelar DMPC



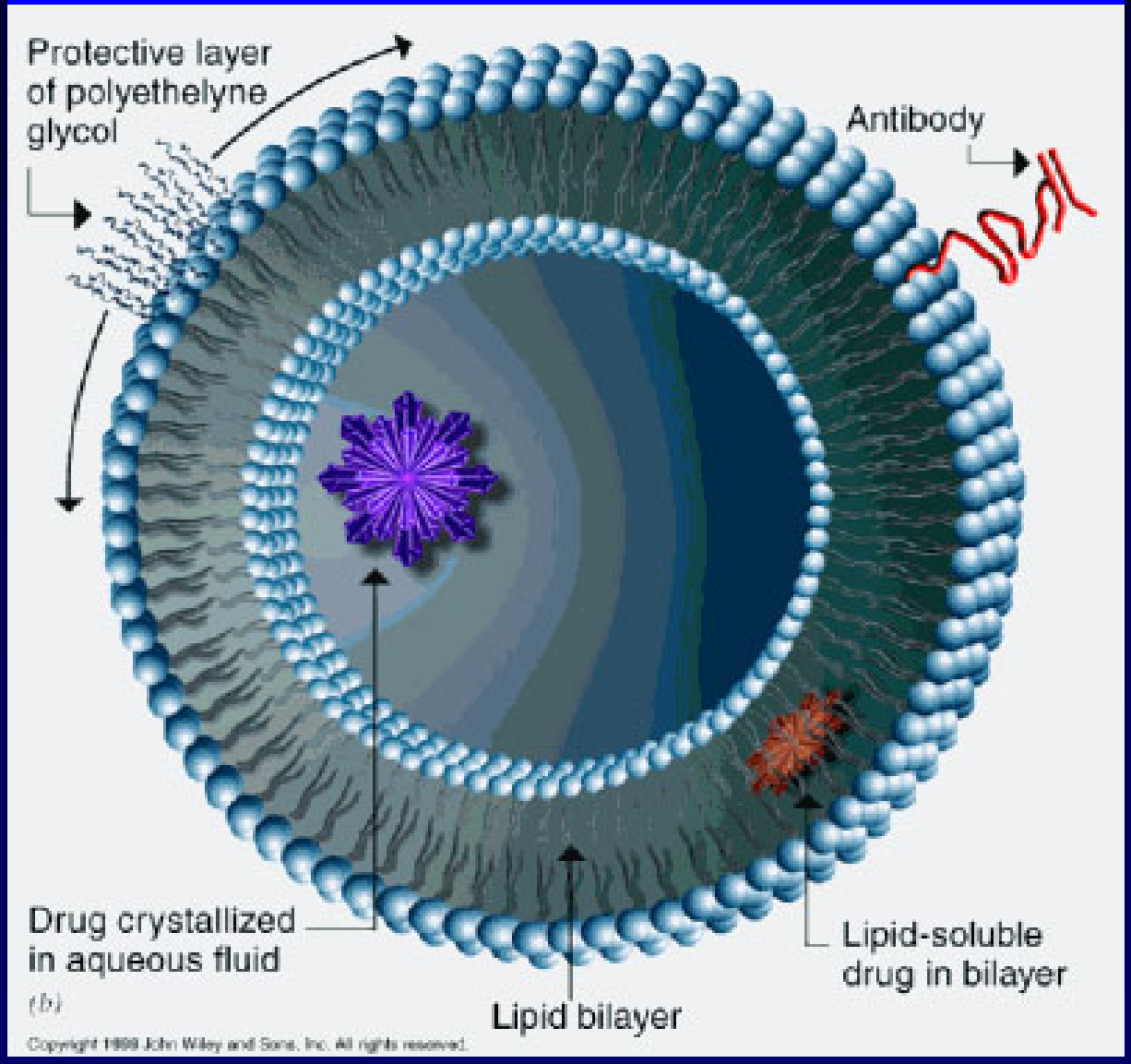


Formación de un Liposoma

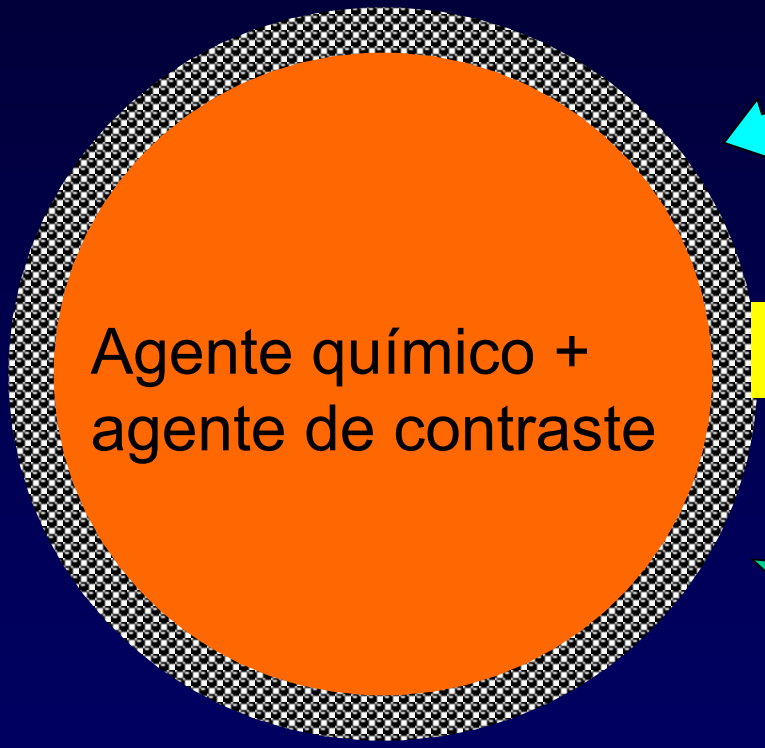




Liposomes


Protective layer of polyethelyne glycol
Antibody
Drug crystallized in aqueous fluid
(b)
Lipid bilayer
Lipid-soluble drug in bilayer
Copyright 1998 John Wiley and Sons, Inc. All rights reserved.

Aplicacion: liberacion controlada de quimicos encapsulados



Monitoreo: RMN, ultrasonografía, TAC, etc.

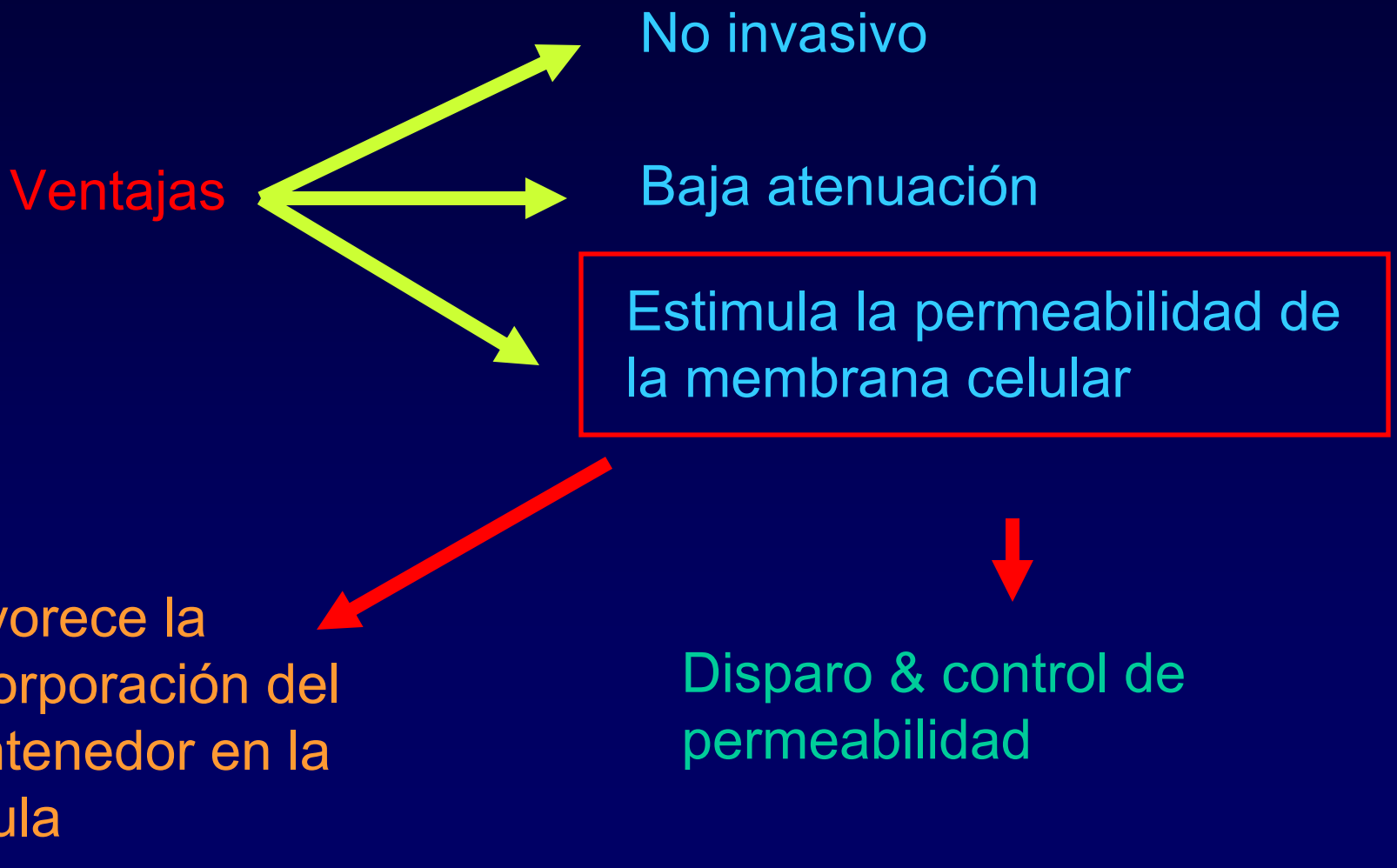
Target

Estímulo externo

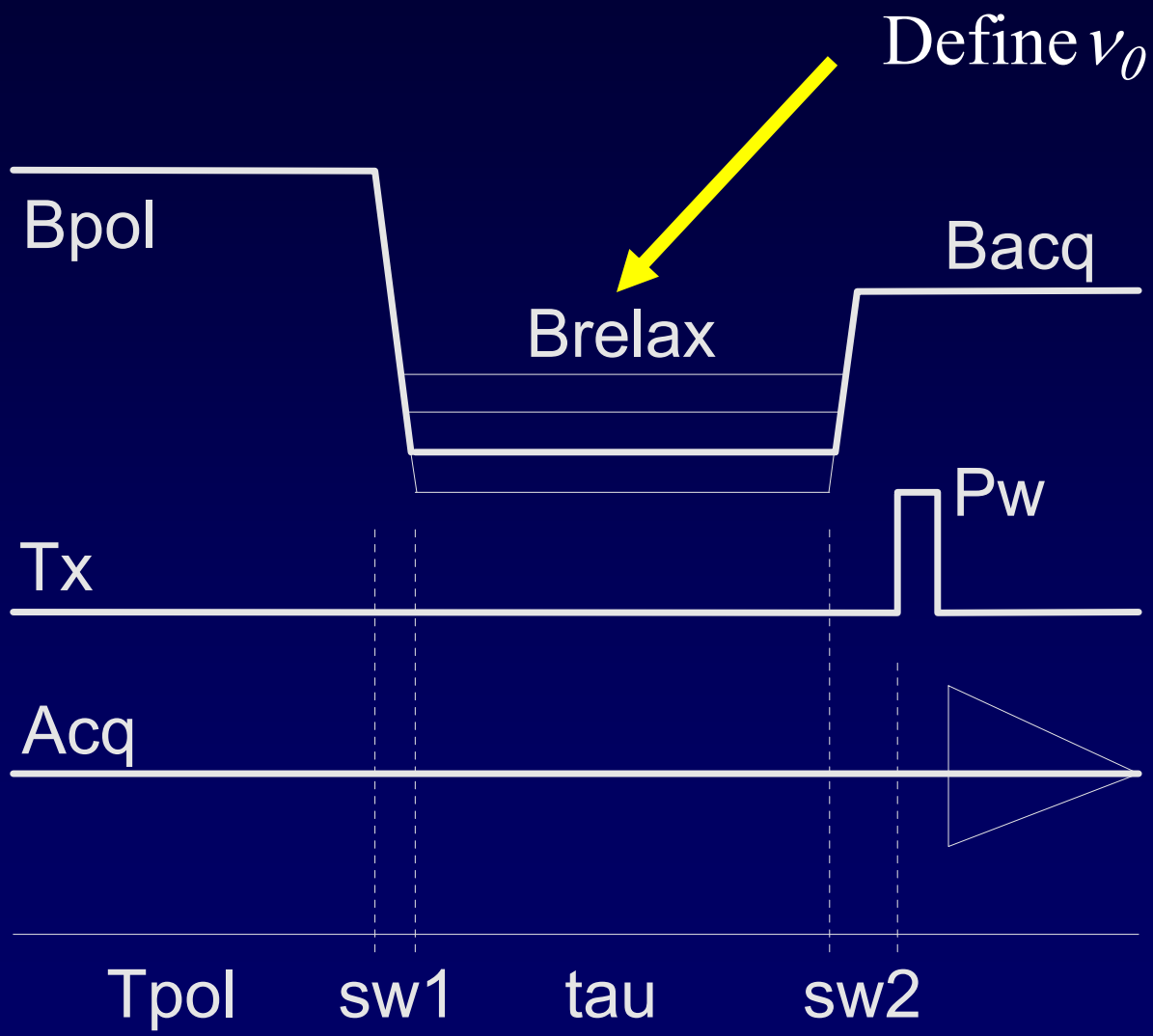
(trigger & control)

"The ideal scenario for targeted drug delivery would be to sequester the drug within a container where it could remain until released in a spatially and temporally controlled manner", G. A. Husseini et al., J. Contr. Release 69, 43 (2000).

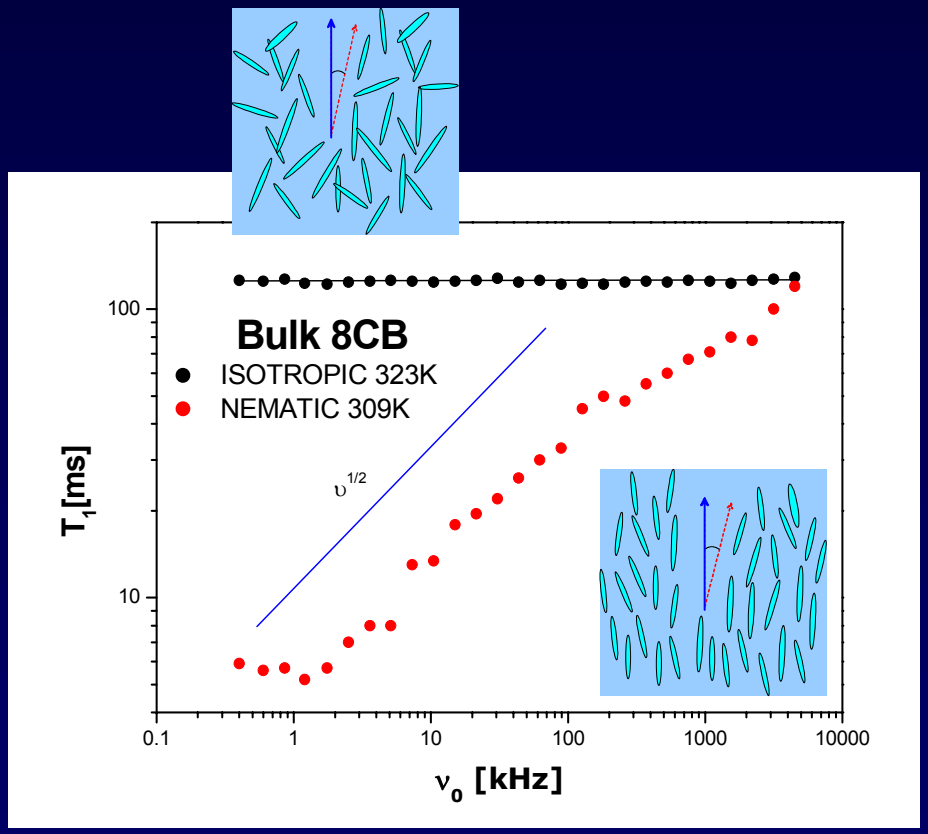
Estimulación acústica

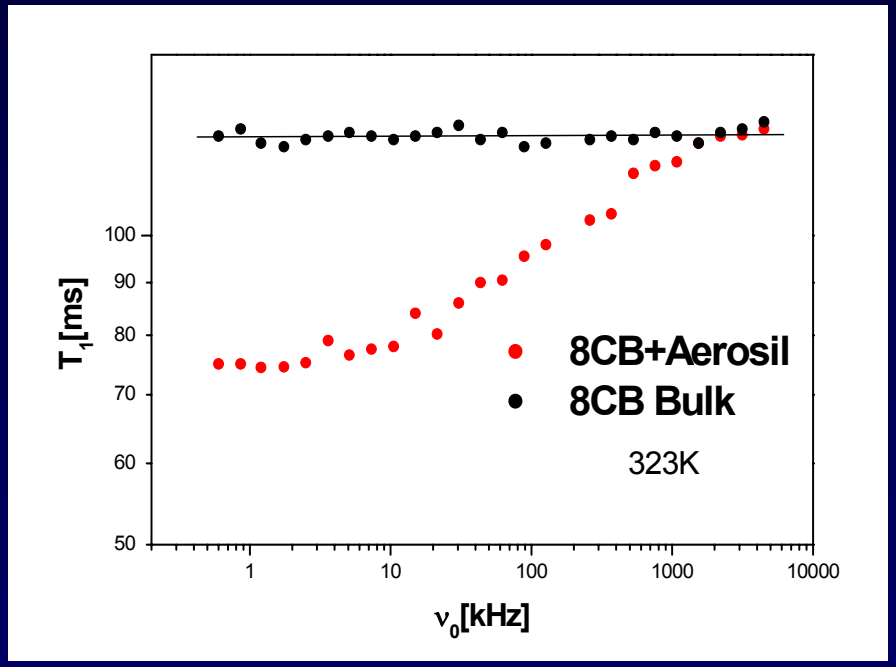


Field-cycling

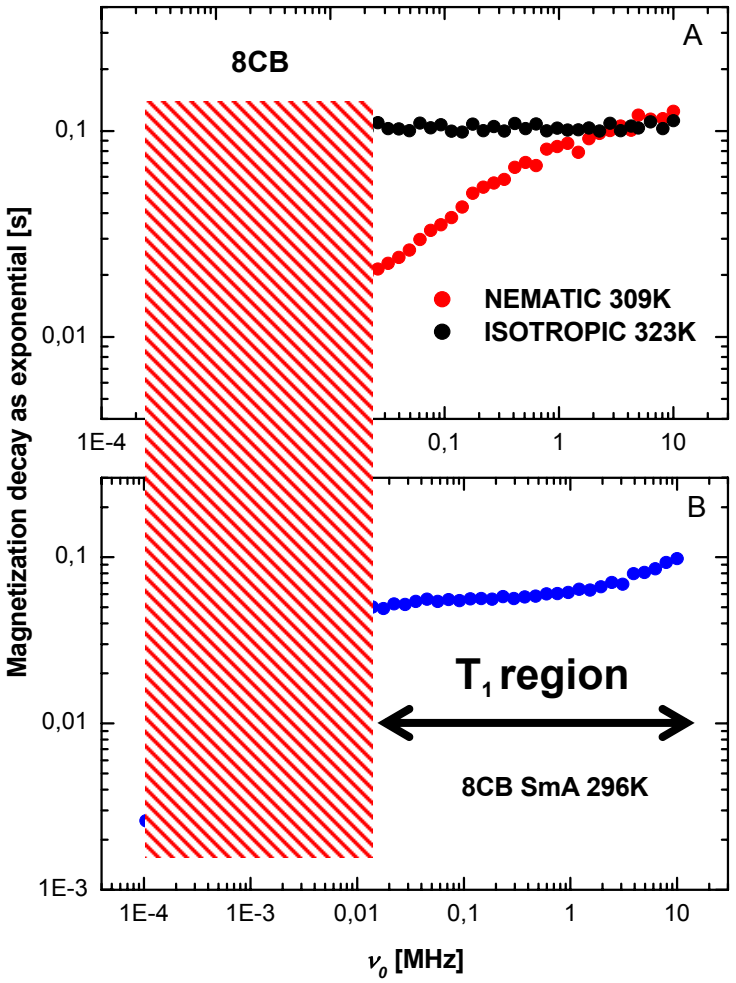


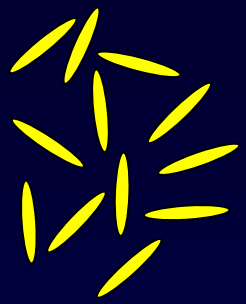
Relaxometría





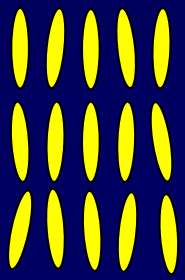
T_1 como sensor de orden





ISOTROPICA

NEMATICA



ESMECTICA A

J. Phys. Chem. **1988**, *92*, 2981–2987

Proton Spin Relaxation Dispersion Studies of Phospholipid Membranes

Eberhard Rommel, Friedrich Noack,*

Physikalisches Institut, Universität Stuttgart, Pfaffenwaldring 57, D-7000 Stuttgart 80, West Germany

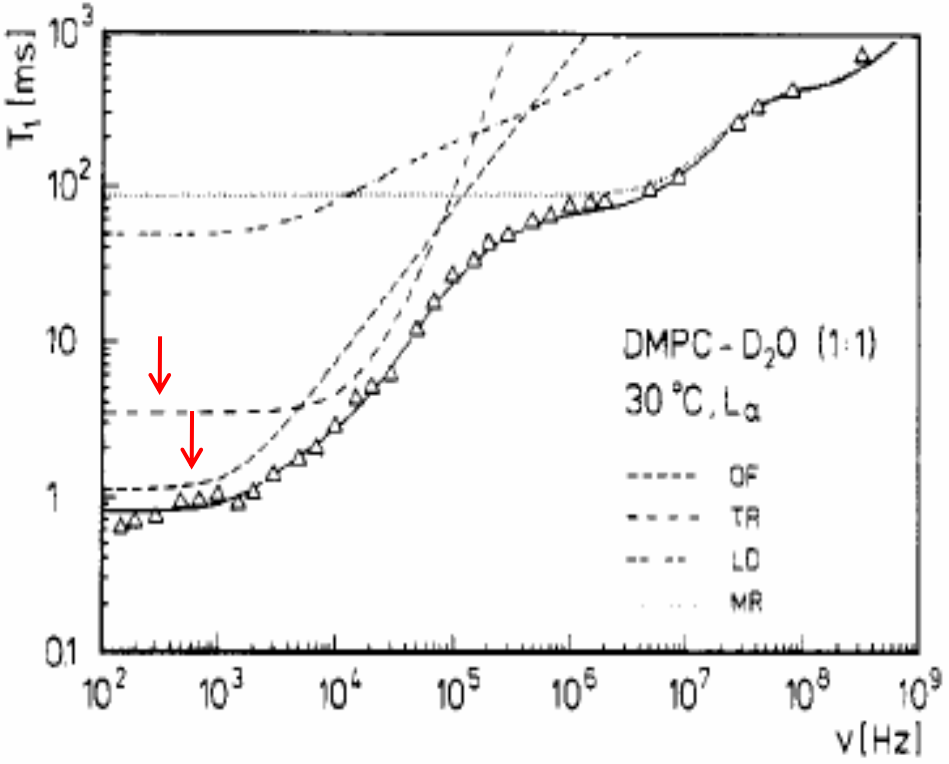
Peter Meier, and Gerd Kothe

Institut für Physikalische Chemie, Universität Stuttgart, Pfaffenwaldring 55, D-7000 Stuttgart 80, West Germany *(Received: November 3, 1987)*

This paper presents measurements of the proton spin T_1 relaxation dispersion of phospholipid membranes of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) over a very broad Larmor frequency range (100 Hz ≤ $\omega/2\pi$ ≤ 300 MHz). The results show that, in contrast to suggestions in the literature, collective molecular reorientations (order fluctuations) contribute to the proton relaxation process only at extremely low frequencies in the kilohertz regime, whereas the conventional high-frequency range is dominated by reorientation of individual molecules. The order fluctuations are observed by a characteristic $T_1(\omega) \sim \omega^{1}$ dispersion at low frequencies for both the liquid crystalline and intermediate phases of the model membranes, which is completely absent for the "crystalline" gel phase and for isotropic liquid phases of DMPC molecules.

DMPC: 1,2-Dimyristoyl-sn-glycero-3-phosphocholine- 1:1 in D_2O. Multilamellar

Following these arguments we present a quantitative evaluation of the data of the liquid crystalline phase in terms of relaxation by smectic *order fluctuations* of groups of molecules (relaxation rate $1/T_1^{(OF)}$),[11,12] internal and overall *molecular rotations* of individual molecules (relaxation rate $1/T^{(MR)}$),[30–33] *lateral diffusion* of molecules in the bilayer plane (relaxation rate $1/T_1^{(LD)}$),[44–46] and *translationally induced rotations* of molecules on curved bilayer regions (relaxation rate $1/T_1^{(TR)}$).[47] This model implies a cor-



- **Order fluctuations (smectic)**
- **Translationally induced rotations (diffusion on curved surfce)**
- **3 rotational terms (Lorentzian)**
- **Lateral diffusion (Vilfan's for smectic)**

Features

- Interpretations based on available theoretical models
- Too many fitting parameters

Alternatives

- Model-free approach
- Evidence-based interpretation. Fixed parameters from other experiments. Model refinement when needed.

~~Fitting~~ → **Simulation**

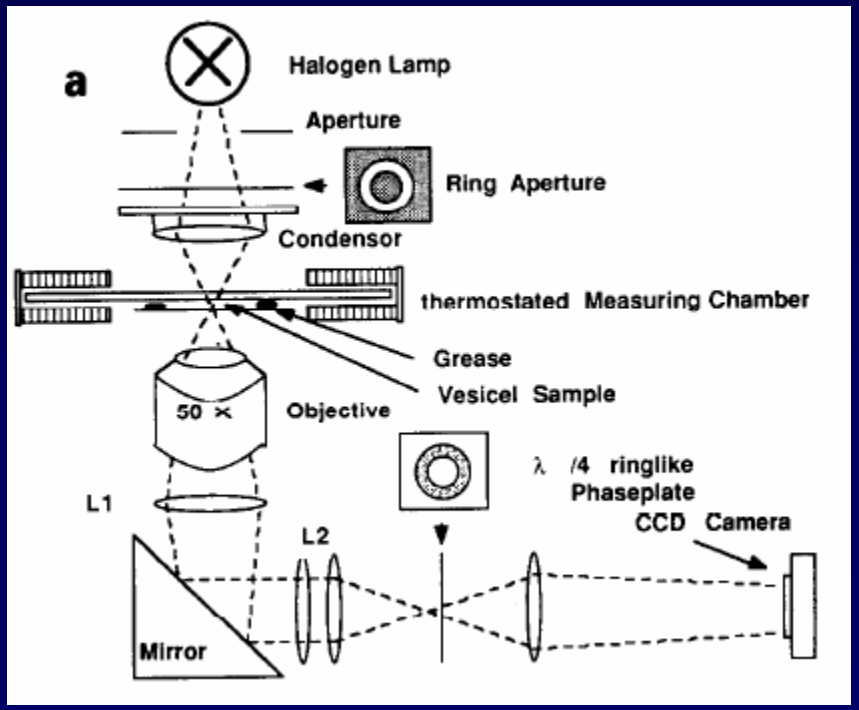
Physica A 163 (1990) 410–428
North-Holland

BENDING ELASTICITY AND THERMAL EXCITATIONS OF LIPID BILAYER VESICLES: MODULATION BY SOLUTES

H.P. DUWE and E. SACKMANN
Physics Department, Biophysics Group, Technische Universität München, D-8046 Garching, Fed. Rep. Germany

$$r(\vartheta, \varphi, t) = r_0\left(1 + \sum_{l,m}^{\infty} a_{l,m}(t) Y_{l,m}(\vartheta, \varphi)\right),$$

The thermal excitations of quasi-spherical vesicles are described in terms of a spherical harmonics expansion ($Y_{lm}(\vartheta, \varphi)$) of the middle surface separating the two monolayers. The latter is determined by the radius $r(\vartheta, \varphi)$ (ϑ: polar angle,



Eur Biophys J (2004) 33: 732–741
DOI 10.1007/s00249-004-0420-5

ARTICLE

Jonas Henriksen · Amy C. Rowat · John H. Ipsen

Vesicle fluctuation analysis of the effects of sterols on membrane bending rigidity

The vesicles undergo undulations in shape which can be observed using light microscopy and subsequently

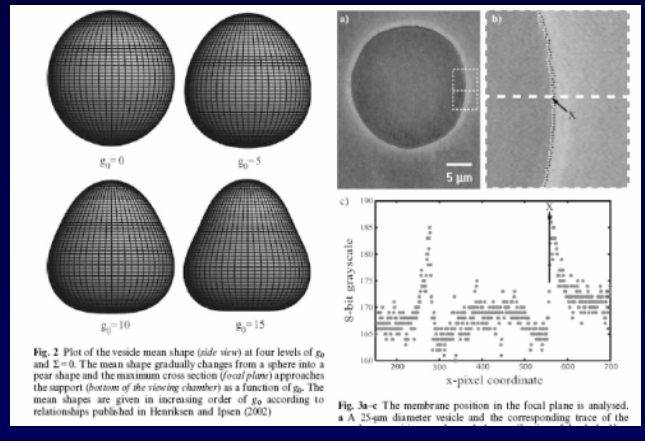


Fig. 2 Plot of the vesicle mean shape (*side view*) at four levels of g_0 and $\Sigma = 0$. The mean shape gradually changes from a sphere into a pear shape and the maximum cross section (*focal plane*) approaches the support (*bottom of the viewing chamber*) as a function of g_0. The mean shapes are given in increasing order of g_0 according to relationships published in Henriksen and Ipsen (2002)

Fig. 3a–c The membrane position in the focal plane is analysed. a A 25-µm diameter vesicle and the corresponding trace of the

PHYSICAL REVIEW E 71, 021905 (2005)

Viscoelastic dynamics of spherical composite vesicles

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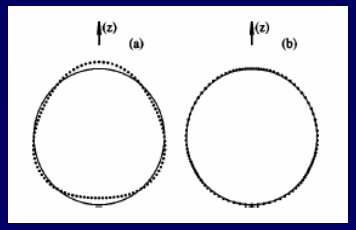
[2]*Physical Faculty, Rostov State University, 5 Zorge Street, 344090 Rostov-on-Don, Russia*
(Received 6 October 2003; revised manuscript received 21 October 2004; published 11 February 2005)



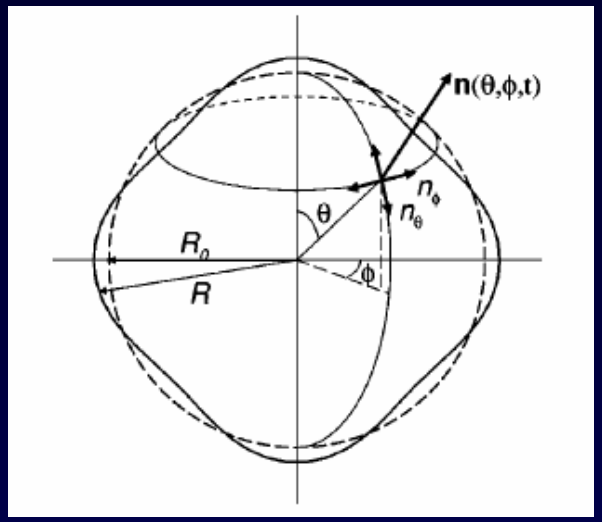
$$\mathbf{u}_\perp = \sum_{l=0}^{l=\infty} \sum_{m=-l}^{m=l} A_{lm}^{Y} Y_{lm}(\theta, \phi)\mathbf{e}_r,$$

PHYSICAL REVIEW E, VOLUME 64, 022902

Nuclear-spin relaxation induced by shape fluctuations in membrane vesicles

M. Vilfan,[1] G. Althoff,[2] I. Vilfan,[1] and G. Kothe[2]
[1]*J. Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia*
[2]*Department of Physical Chemistry, University of Freiburg, Albertstrasse. 21, D-79104 Freiburg, Germany*
(Received 31 March 2001; published 26 July 2001)

Nuclear-spin relaxation rates resulting from shape fluctuations of *unilamellar quasispherical vesicles* are calculated. We show that in the kHz range these fluctuations yield—in contrast to previous conclusions on planar membranes – a relaxation rate proportional to the inverse Larmor frequency and provide direct information on the bending rigidity of membranes.



$$R(\theta,\phi)=R_0[1+u(\theta,\phi)],$$

$$u(\theta,\phi)=\sum_{l}\sum_{m=-l}^{l}u_{l,m}Y_{l,m}(\theta,\phi).$$

$$J(\omega)=\frac{k_BT}{4\pi\kappa}\sum_{l=2}^{l_{\max}}\frac{l(l+1)(2l+1)}{(l^2+l-2)(l^2+l+\sigma)}\frac{2\tau_l}{(1+\omega^2\tau_l^2)}. \quad (10)$$

$$J(\omega)=\mathrm{Re}\int_{-\infty}^{+\infty}[\langle n_\theta(0)n_\theta^*(t)\rangle+\langle n_\phi(0)n_\phi^*(t)\rangle]e^{-i\omega t}dt.$$

$$n_\theta=-\frac{\partial u(\theta,\phi)}{\partial\theta},\qquad n_\phi=-\frac{1}{\sin\theta}\frac{\partial u(\theta,\phi)}{\partial\phi}.$$

$$\tau_l=\frac{\eta R_0^3}{\kappa}\frac{(2l+1)(2l^2+2l-1)}{l(l+1)(l+2)(l-1)(l^2+l+\sigma)}.$$

Fast motions

- BPP processes
- Arrhenius behavior

- vibrational fluctuations
- librations
- isomerizations
- fast rotational processes
- hydrocarbon chains fluctuations
- etc……

Manifested as a frequency independent offset

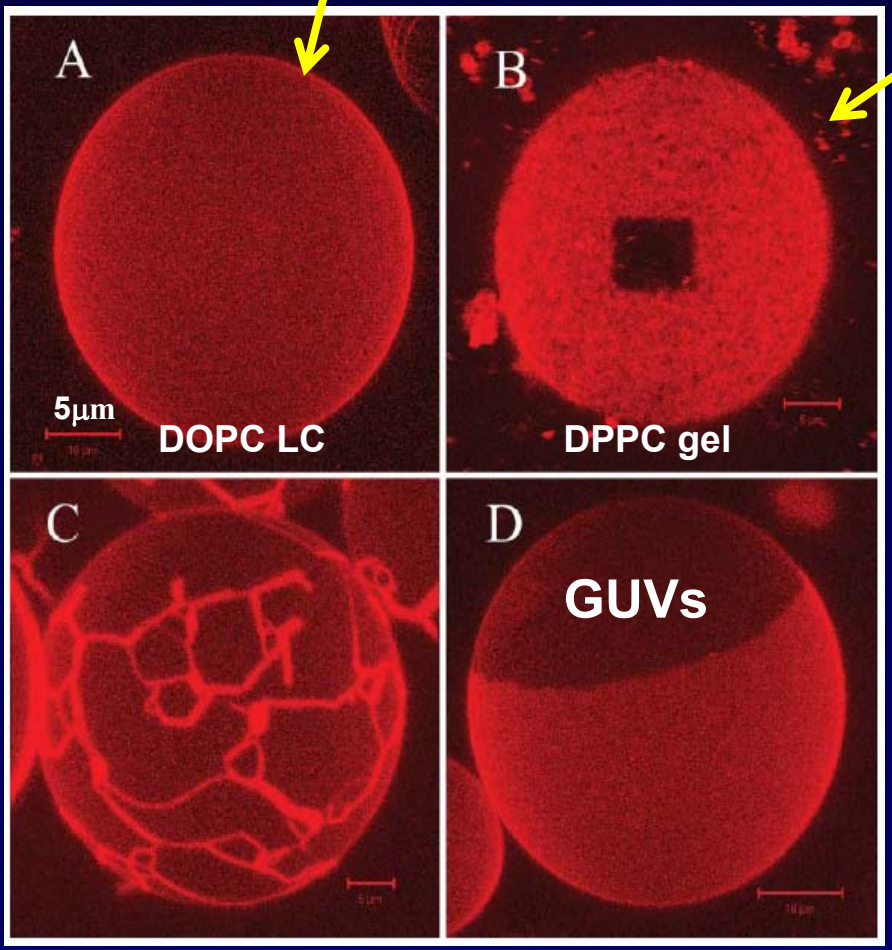
3758 Biophysical Journal Volume 85 December 2003 3758–3768

Lipid Dynamics and Domain Formation in Model Membranes Composed of Ternary Mixtures of Unsaturated and Saturated Phosphatidylcholines and Cholesterol

Dag Scherfeld,* Nicoletta Kahya,*† and Petra Schwille†
*Experimental Biophysics Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany; and †Dresden University of Technology, c/o Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

fast fluorescence recovery in a bleached spot.

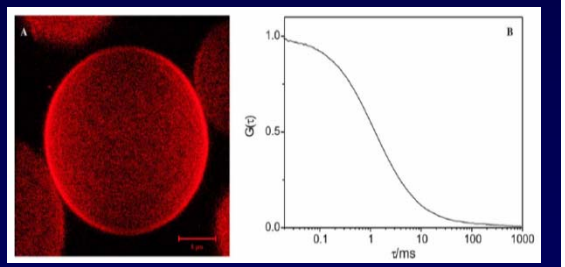
recovery after photobleaching of a squared spot. Fluorescence recovery did not occur within hours. GUVs made

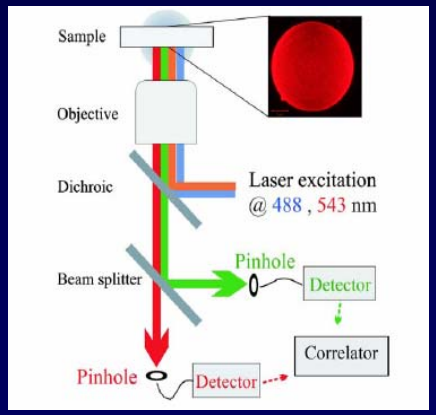


Confocal fluorescence microscopy

FRAP

Axelrod, D., Koppel, D.E., Schlessinger, J., Elson, E., Webb, W.W., 1976. Mobility measurements by analysis of fluorescence photobleaching recovery kinetics. Biophys. J. 16, 1055–1069.





FCS

$$G(\tau) = \frac{\langle \delta F(t+\tau)\delta F(t)\rangle}{\langle F(t)\rangle^2} = \frac{\langle \delta F(\tau)\delta F(0)\rangle}{\langle F\rangle^2}.$$

D~7E-12m²/s

$$G(\tau) = \frac{\left(\sum_i \langle C_i\rangle \left(\frac{1}{1+\tau/\tau_{di}}\right)\right)}{A_{\text{eff}}\left(\sum_i \langle C_i\rangle\right)^2},$$

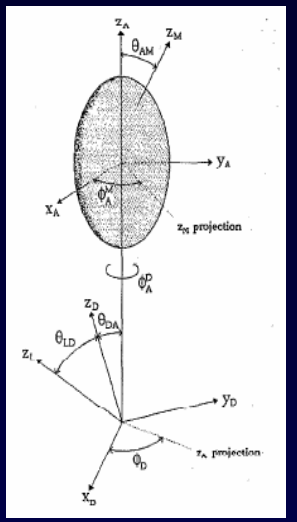
$$\tau_{d,i} = r_0^2/4D_i.$$

i: chemical specimen

Theory of spin relaxation by diffusion on curved surfaces

Bertil Halle
Physical Chemistry 1, University of Lund, Chemical Center, P. O. Box 124, S-22100 Lund, Sweden

$$\rho = \frac{1}{r} = \frac{a}{b}.$$



B. Isotropic micellar solutions

In isotropic systems, where the tensorial spin-lattice coupling is isotropically averaged by sufficiently fast motions, there is only one lab-frame spectral density function. According to (3.6)

$$j^L(\omega) = \frac{1}{5} S^2_{AM} \frac{\tau_0}{1+(\omega\tau_0)^2} + \frac{1}{5} \sum_{m=0}^{2} (2-\delta_{m0}) \times \int_0^\infty dt \cos(\omega t) \exp(-t/\tau_m) g_m^{AM}(t), \qquad (7.3)$$

where the symmetric top correlation times τ_m are related to the rotational diffusion coefficients $D_\parallel$ and $D_\perp$ through (3.5).

In the sphere limit, (3.5) and (5.14) show that (7.3) reduces to the Lorentzian

$$j^L(\omega) = \frac{1}{5} \frac{\tau_{rs}}{1+(\omega\tau_{rs})^2}, \qquad (7.4)$$

with the joint correlation time τ_{rs} being related to the rotational-diffusion correlation time τ_{rd} and the surface-diffusion correlation time τ_{sd} by

$$\frac{1}{\tau_{rs}} = \frac{1}{\tau_{rd}} + \frac{1}{\tau_{sd}}, \qquad (7.5)$$

$$\tau_{rd} = \frac{1}{6D_r} = \frac{4\pi\eta b^3}{3k_B T}, \qquad (7.6)$$

$$\tau_{sd} = \frac{b^2}{6D_s}. \qquad (7.7)$$

frequency spectral density $j^L(0)$.] Figure 7 shows that the departure from a Lorentzian dispersion can be substantial already at $\rho = 1.3$. For a prolate micelle, this corresponds to an increase in the aggregation number by 30%. While changes in aggregation number of this order of magnitude can probably be determined by other techniques, such as fluorescence quenching[41] and neutron scattering,[42] nuclear spin relaxation appears to be a unique method for revealing small deviations from spherical shape.

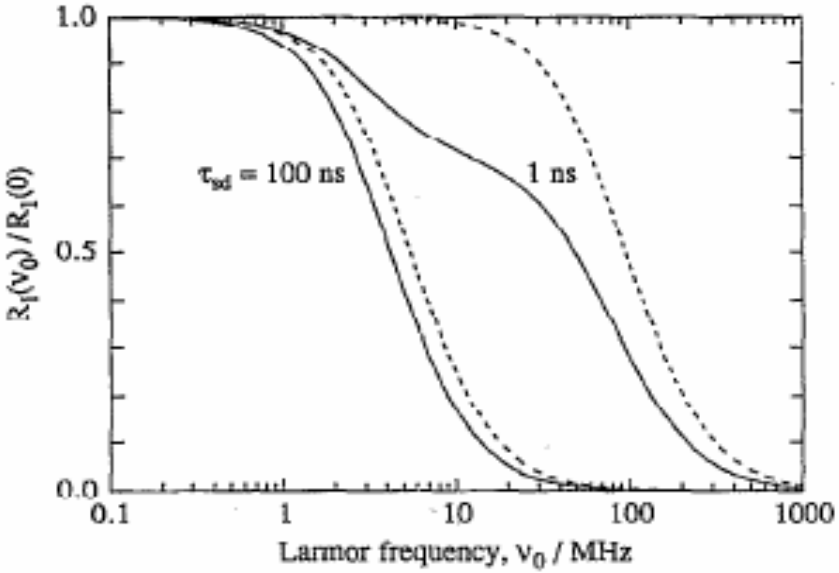


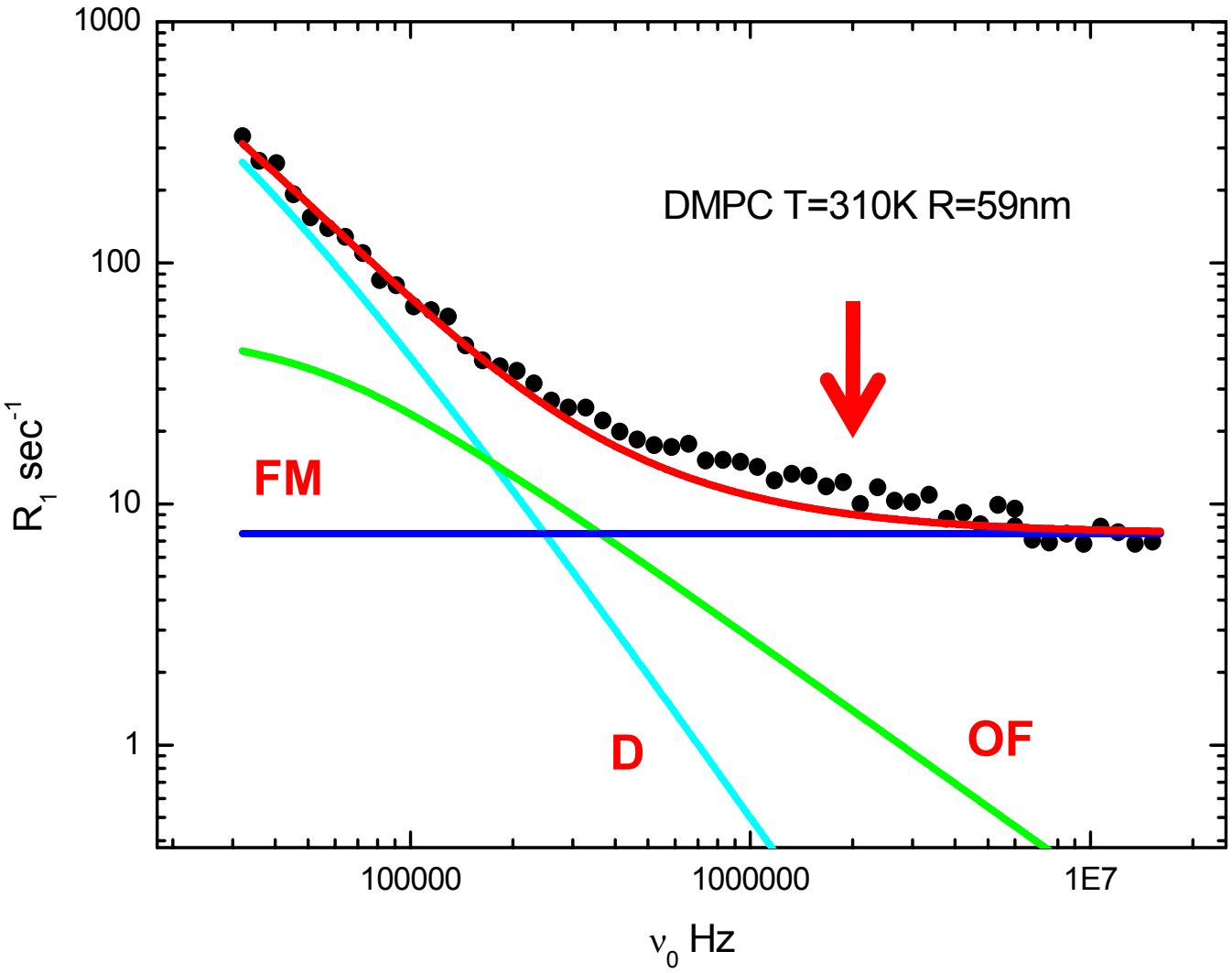
FIG. 7. Normalized frequency dispersion of isotropic longitudinal relaxation rate due to surface diffusion on freely rotating spheres (dashed curves) and prolate spheroids of axial ratio $\rho = 1.3$ (solid curves). The rotational correlation time is $\tau_{rd} = 20$ ns.

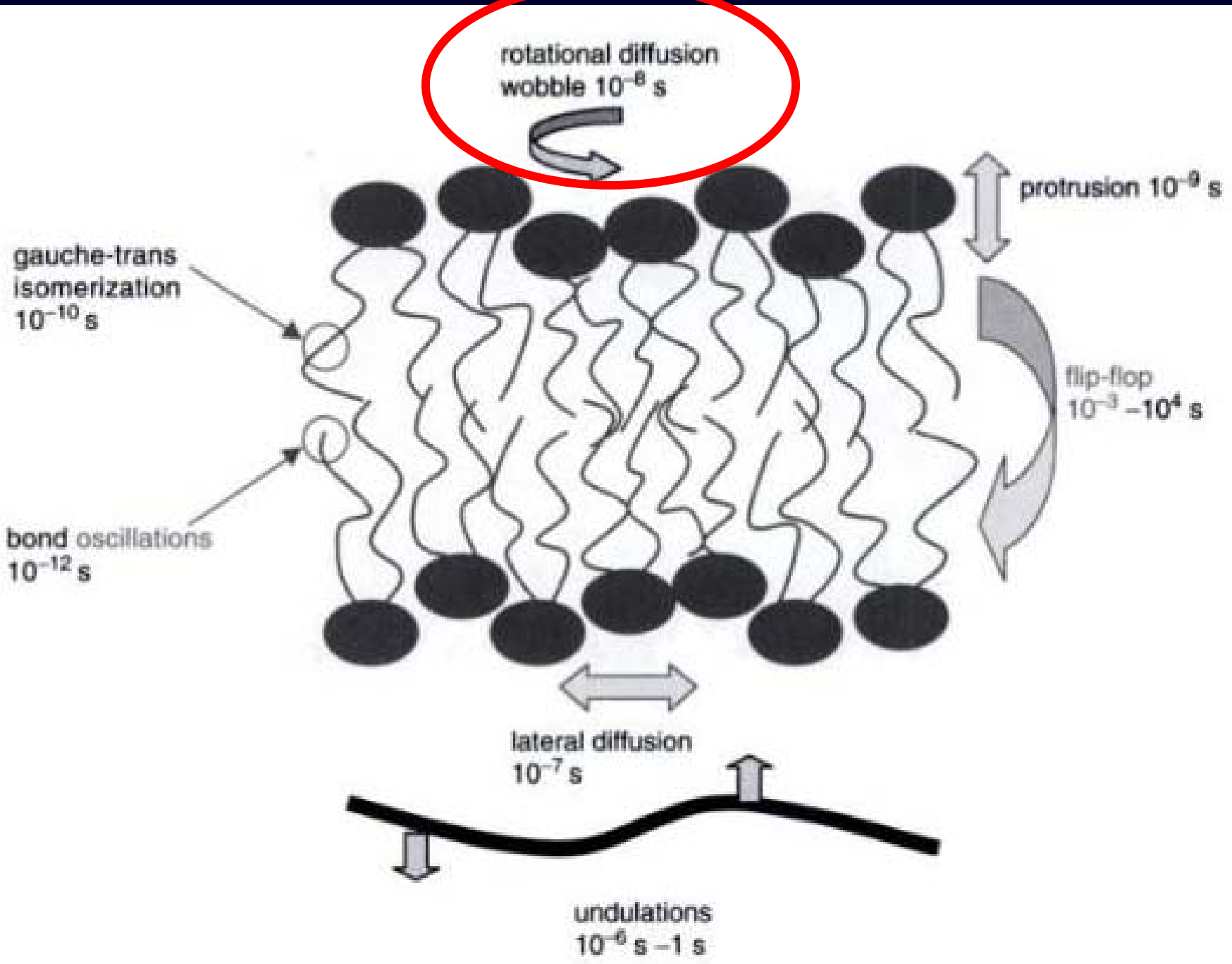
Interpretation of Molecular Dynamics on Different Time Scales in Unilamellar Vesicles Using Field-Cycling NMR Relaxometry

Carla J. Meledandri,[†] Josefina Perlo,[‡] Ezequiel Farrher,[‡] Dermot F. Brougham,*[,†] and Esteban Anoardo*[,‡]

National Institute for Cellular Biotechnology, School of Chemical Sciences, Dublin City University, Dublin 9, Ireland, and Larte - Famaf. Universidad Nacional de Córdoba and Instituto de Física Enrique Gaviola (CONICET), Córdoba, Argentina

Acknowledgment. This work was partially supported by funds from Foncyt (PICT25765), CONICET (PIP6420), and Secyt-UNC from Argentina. C.J.M. acknowledges the National Institute for Cellular Biotechnology and Enterprise Ireland (PC/2006/207) for financial support. D.B. also acknowledges Enterprise Ireland (IF/2001/364, PC/2004/0429) and the Higher Education Authority of the Republic of Ireland for supporting the purchase of NMR equipment. J.P. and E.F. acknowledge a fellowship granted by CONICET. The authors also acknowledge Dr. Guillermo Montich and CIQUIBIC - CONICET for support and access to infrastructure for preparing the liposome samples in Córdoba. C.J.M. and J.P. contributed equally to this work.


1000
100
10
1
R_1 sec^{-1}
DMPC T=310K R=59nm
FM
D
OF
100000
1000000
1E7
ν_0 Hz


rotational diffusion
wobble 10^{-8} s
protrusion 10^{-9} s
gauche-trans
isomerization
10^{-10} s
flip-flop
10^{-3} –10^{4} s
bond oscillations
10^{-12} s
lateral diffusion
10^{-7} s
undulations
10^{-6} s –1 s

More on the motional state of lipid bilayer membranes: interpretation of order parameters obtained from nuclear magnetic resonance experiments

Nils O. Petersen, and Sunney I. Chan

Biochemistry, **1977**, 16 (12), 2657-2667 • DOI: 10.1021/bi00631a012

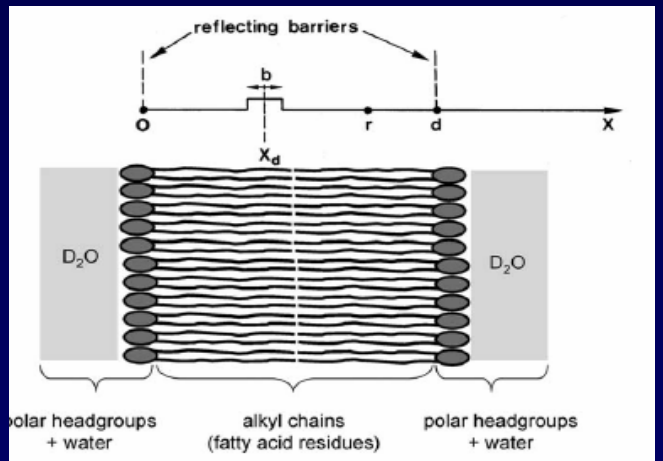
$$\left(\frac{1}{T_1}\right) \cong A\tau_{\parallel} + B\frac{1}{\omega_0^2\tau_{\perp}}$$

Kroon, 1976

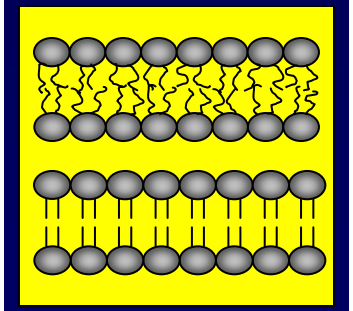
$$\tau_{\parallel} \approx 10^{-9}\text{–}2 \times 10^{-10}\ \text{s}$$

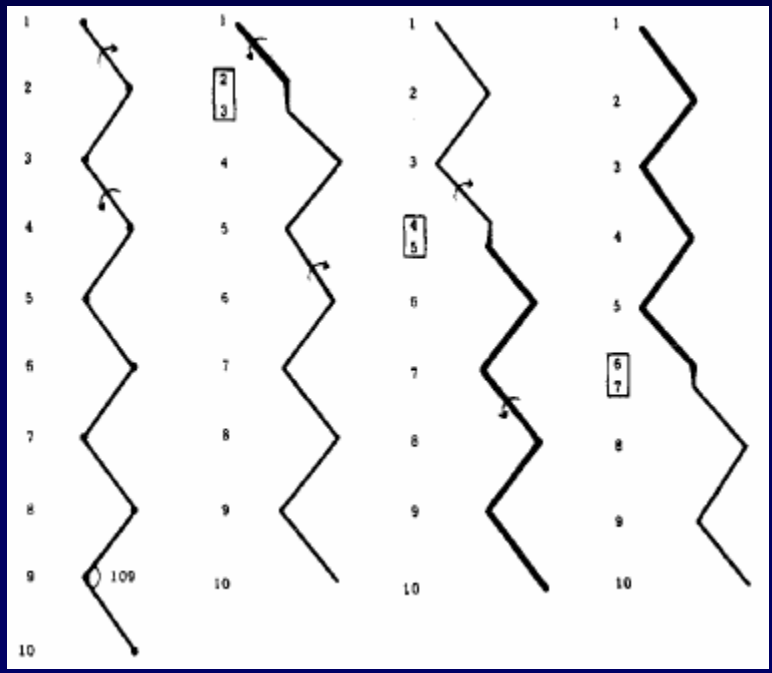
$$\tau_{\perp} \geq 10^{-7}\ \text{s}$$

However, the origins of these time scales were not fully understood at that time. We suggest now that the faster time scale, $\tau_{\parallel}$, is associated with rotational isomerization whereas the slower time scale, $\tau_{\perp}$, reflects primarily the rate of chain reorientation. For the bilayer systems under consideration here,



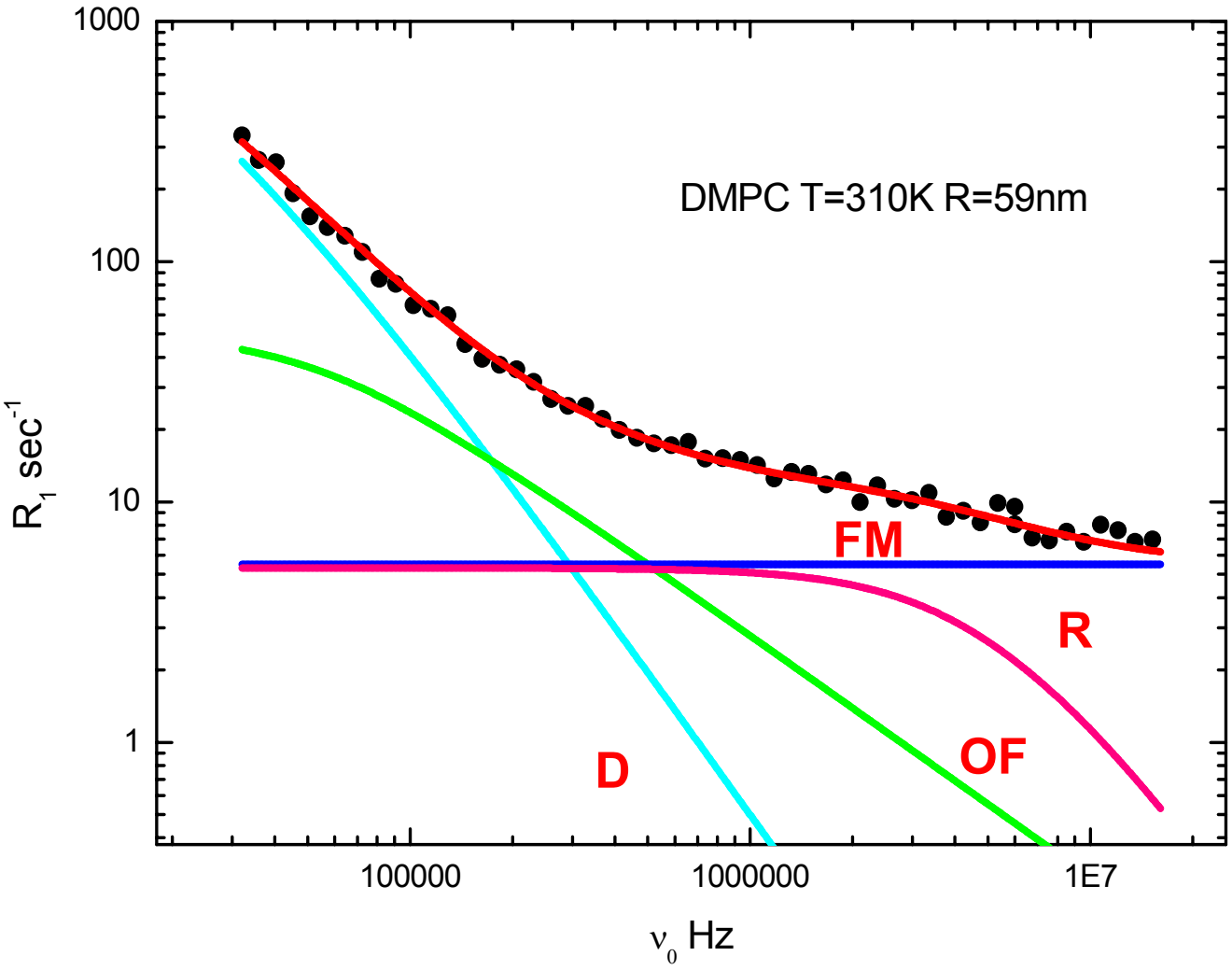
ig. 60. Schematic representation of the limited defect diffusion model used or the description of motions in the hydrocarbon part of individual nolecules in lipid bilayers. x_d is the instantaneous position of a structural efect (kink, torsion). r is the position of the reference nucleus, and b is the vidth of the diffusing structural 'defect' [227–229].





Kink diffusion

[227] R. Kimmich, Z. Naturforsch 31a (1976) 693.
[228] R. Kimmich, G. Voigt, Z. Naturforsch 33a (1978) 1294.
[229] R. Kimmich, A. Peters, J. Magn. Reson. 19 (1975) 144.


1000
100
10
1
R_1 sec^{-1}
DMPC T=310K R=59nm
FM
R
OF
D
100000
1000000
1E7
ν_0 Hz

Relaxometría estimulada por sonicación

Fundamento físico

Orden molecular

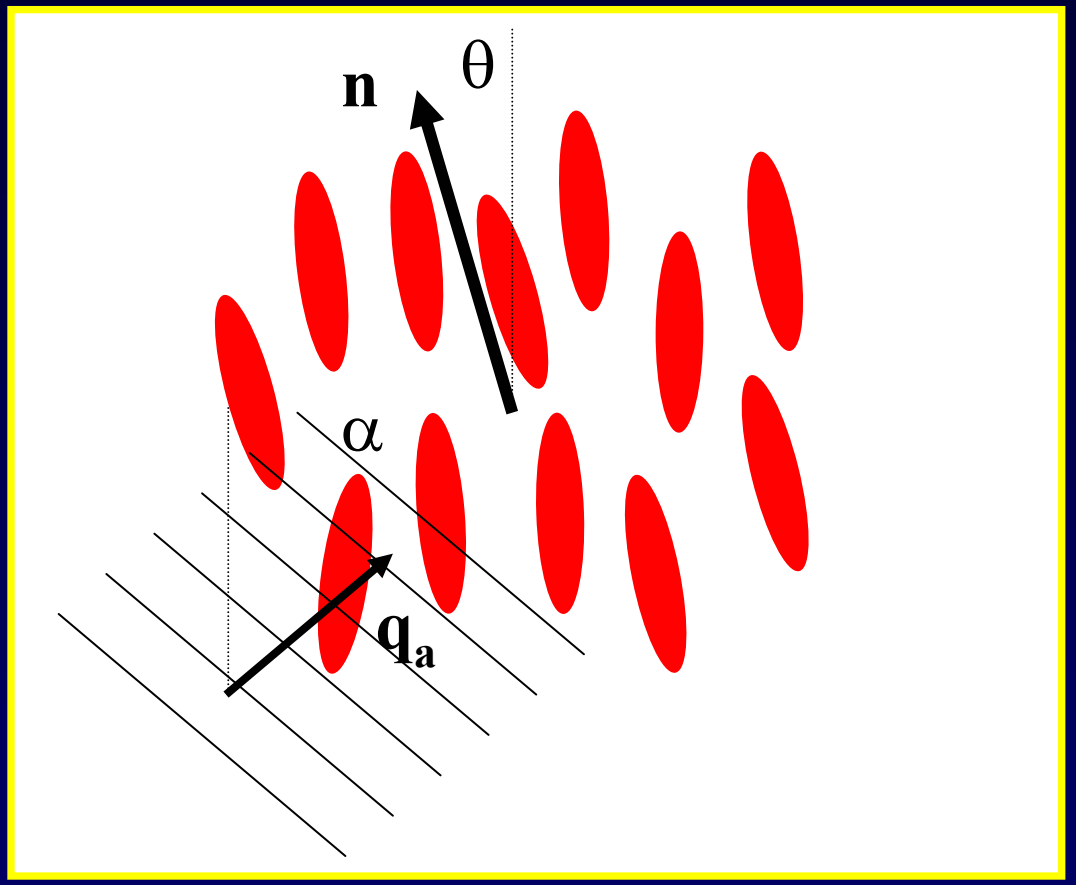
Dinámica molecular

I – Interacción sonido - director

$$\langle V_{\text{int}} \rangle = \frac{1}{2} Q(\mathbf{n}.\mathbf{q_a})^2$$

$$= \frac{1}{2} Q.q_a^2 \cos^2(\theta - \alpha)$$

$$Q = \frac{2\xi\rho_0 I}{v^3}$$



Bonetto-Anoardo-Kimmich (2002)

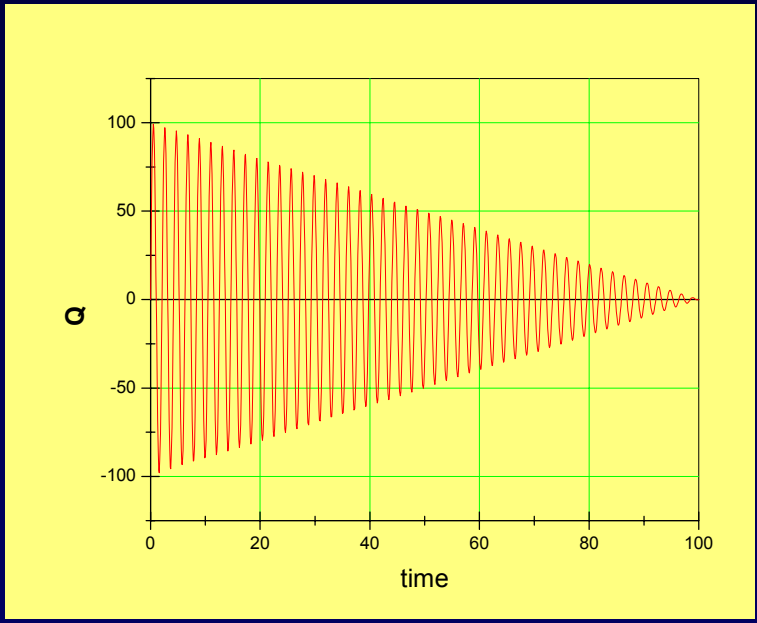
Selinger-Spector-Greanya-Weslowski-Shenoy-Shashidhar (2002)

II – Resonancia hidrodinámica?

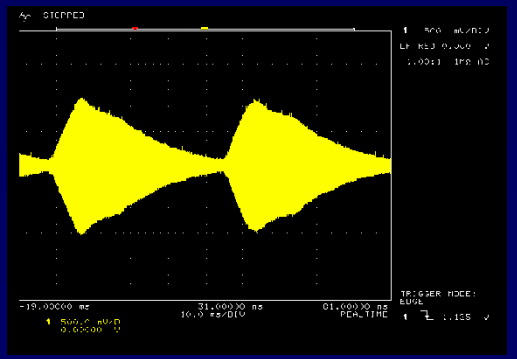
$$F = \frac{1}{2V}\sum_{\mathbf{q}}\sum_{\alpha=1}^{2}\left|\mathbf{n}_\alpha(\mathbf{q})\right|^2\left[Kq^2 - Q\right]$$

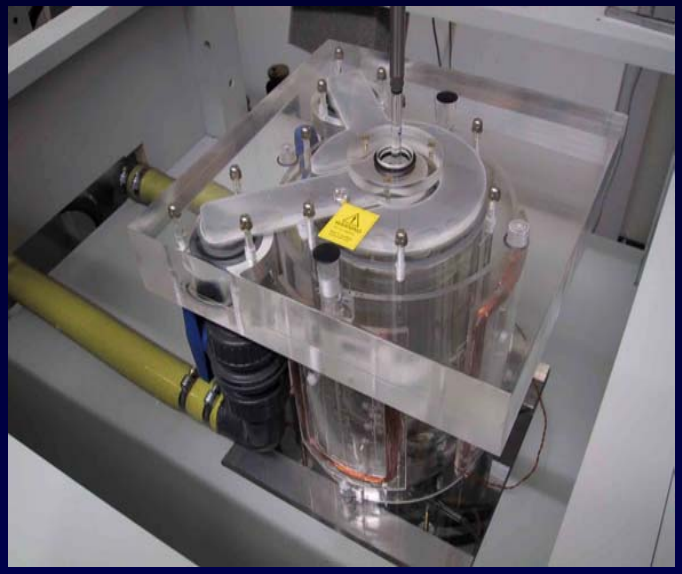
$$Q = \frac{2\xi\rho_0 I}{v^3}$$

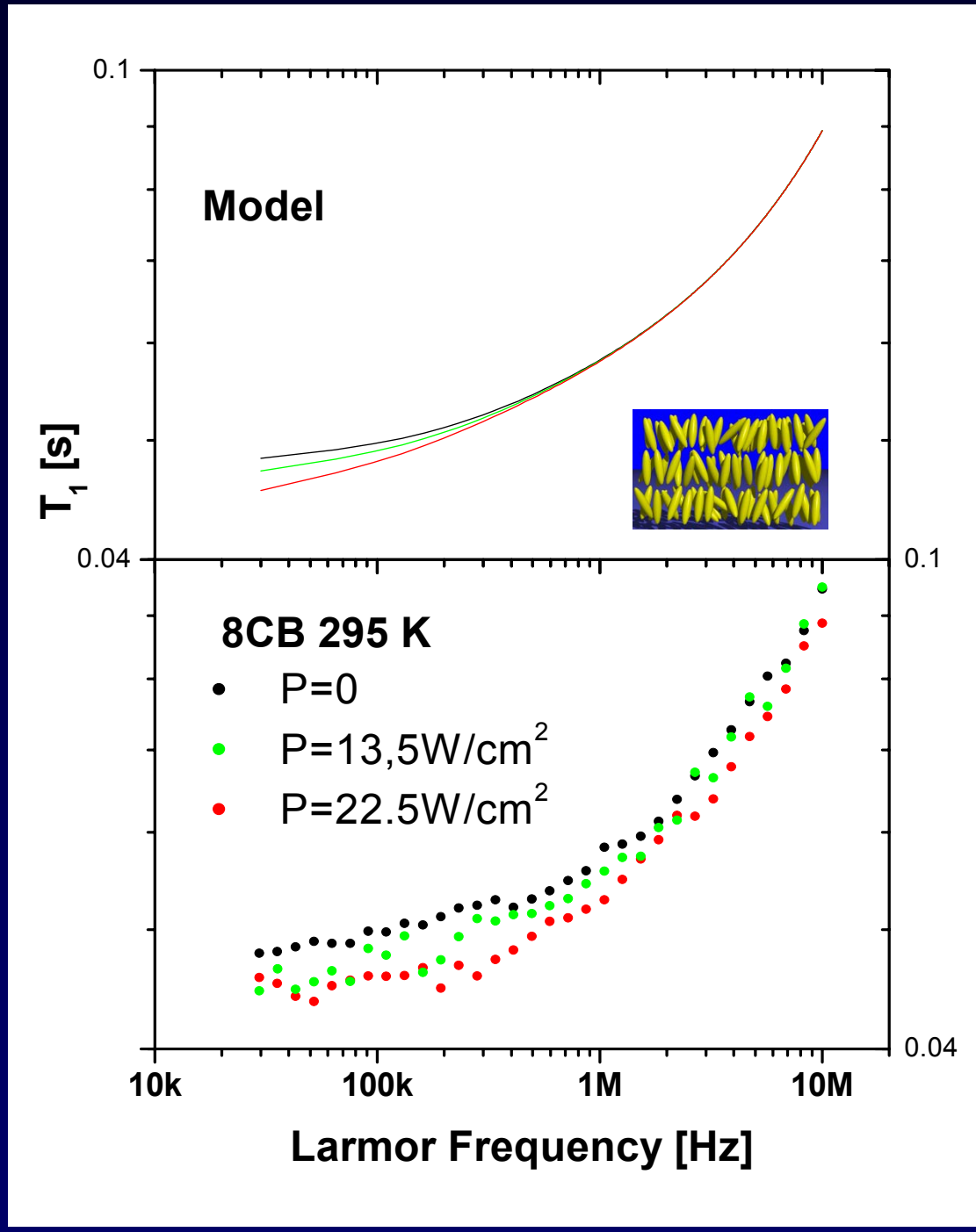
$$\left\langle \left|n_\alpha(\mathbf{q})\right|^2 \right\rangle = \frac{KTV}{Kq^2 - Q}$$



$$\tau_\alpha(\mathbf{q}) = \frac{\eta_\alpha}{Kq^2 - Q}$$







Bonetto-Anoardo (2002-2004)

Conclusiones

- La técnica experimental empleda ofrece la posibilidad de caracterizar liposomas con diámetros en el rango 50 – 200nm.

- Permite estudiar procesos de interacción acústica a baja potencia.