J|A|C|S COMMUNICATIONS

Published on Web 02/17/2010

Molecular Mechanism of Long-Range Diffusion in Phospholipid Membranes Studied by Quasielastic Neutron Scattering

Sebastian Busch,[†] Christoph Smuda,[‡] Luis Carlos Pardo,[§] and Tobias Unruh*,[†]

Forschungsneutronenquelle Heinz Maier-Leibnitz (FRM II), Technische Universität München, Garching bei München, Germany, Center for Radiopharmaceutical Science, Eidgenössische Technische Hochschule Zürich, Zürich, Switzerland, and Grup de Caracterització de Materials, Departament de Física i Enginyeria Nuclear, Universitat Politècnica de Catalunya, Barcelona, Spain

Received September 15, 2009; E-mail: tobias.unruh@frm2.tum.de

Phospholipid membranes have been the subject of intense research ever since the discovery of their function as cell membranes in 1925.[1] Not only their natural abundance (every human body contains several square kilometers[2]) but also their widespread application in agricultural, food, and pharmaceutical applications[3] makes them a worthwhile subject matter for structural and dynamical studies.

Whereas the opinions about the structure seem to converge,[4] the discussion about the dynamical properties is in full swing. The fundamental issue is that many processes in cell membranes take place on a scale of a few nanometers, as this is approximately the distance between two proteins,[5] and it turned out in the early 1990s that the dynamics on this scale differs drastically from the macroscopic (micrometer) behavior: Incoherent quasielastic neutron scattering (QENS) experiments[6,7] observed a fast localized motion of the whole molecule on the probed microscopic temporal and spatial scale of up to a few tens of picoseconds and few nanometers whereas macroscopic measurements observed a significantly slower long-range diffusion.[8,9]

This contradiction could be explained by adapting the free volume theory to membranes:[10] The phospholipid molecules are thought to rattle in a cage of neighbors (fast, localized motion) until a free volume of the size of a phospholipid molecule opens up and the molecule can slip into it. This random walk of the molecules from void to void is then observed as the macroscopic slow, long-range diffusive motion. It is clear that "all models are wrong but some are useful",[11] but the remarkable success of this description of macroscopic effects can be misleading, taking its microscopic image too literally. When increased computing power made Molecular Dynamics (MD) simulations of these complex systems feasible, it became more and more obvious that rattling and slipping motions could not be seen on a molecular scale[8,12] although the results were still sometimes interpreted in this frame.[13] Rather, simulation and experiment agreed that the molecular motions are collective in nature[14] and finally, a flow-like behavior as found in quasi-two-dimensional suspensions[15] was observed in MD simulations of phospholipids in the liquid phase.[16]

To resolve this discrepancy, we repeated the neutron scattering studies[6,7] that triggered the implementation of the free volume theory with highly improved instrumentation at the time-of-flight neutron spectrometer TOFTOF[17] at the neutron source FRM II, Munich, Germany. We will show that, in contrast to the former interpretation, QENS does not provide any indication for the picture of phospholipid molecules rattling and escaping from local cages on a molecular scale. Rather, the data agree well with the MD simulations of ballistic, flow-like motions as the means of long-range transport on a nanometer scale in the membranes.

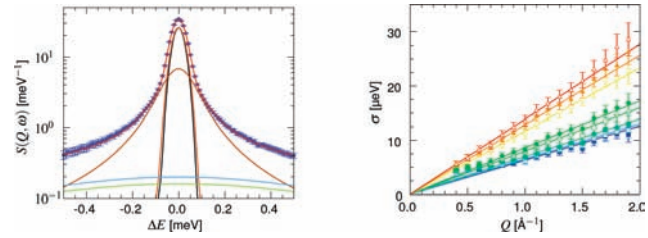

Figure 1. Left: QENS spectrum of DMPC shown exemplarily at 30 °C and $Q = 1.0$ Å^{-1}. The instrumental resolution is displayed as a black line, along with the best fit of eq 1 (red) and its components. Right: the extracted standard deviations σ of the long-range component as a function of Q for temperatures ranging from 5 °C (bottom) to 40 °C (top) in steps of 5 °C. The lines through the origin are fits to the data in the range 0.75 Å^{-1} < Q < 1.45 Å^{-1}. At temperatures below the main phase transition, σ levels off below $Q_c \approx 0.7$ Å^{-1}.

The sample was chosen to be a typical phospholipid, dimyristoylphosphatidylcholine (DMPC), which was hydrated with D_2O to form a liquid crystal of fully hydrated multilayers without solid support. Its dynamics was measured with an observation time of 35 ps, corresponding to an instrumental resolution of 60 μeV (full width at half-maximum).[18] The sample temperature was varied between 5 and 40 °C, probing the dynamics below and in the region of interest above the main phase transition (at 24 °C).

At the spectrometer, neutrons with a well-known energy impinge on the sample and interact with the nuclei. If the sample was static, there would be no energy change $\hbar\omega$ of the neutrons and they would be detected smeared by the instrumental resolution. Aperiodic motions in the sample cause a quasielastic broadened energy spectrum that is measured at different momentum transfers $\hbar Q$ (cf. Figure 1). The shape and width of the broadening are essentially determined by the decay of the autocorrelation function of the hydrogen nuclei.[19] The simplest meaningful function describing the data in the present case was found to consist of a long-range motion of the whole molecule and two localized motions,[20]

$$\begin{aligned} S(Q,\omega) = {} & a(Q)\cdot G(\sigma(Q),\omega) \otimes \\ & [A_{\mathrm{fast}}(Q)\cdot\delta(\omega) + (1 - A_{\mathrm{fast}}(Q))\cdot L(\Gamma_{\mathrm{fast}},\omega)] \otimes \\ & [A_{\mathrm{slow}}(Q)\cdot\delta(\omega) + (1 - A_{\mathrm{slow}}(Q))\cdot L(\Gamma_{\mathrm{slow}},\omega)], \end{aligned} \tag{1}$$

where G denotes a Gaussian and L a Lorentzian with the Q-dependent standard deviation σ and the Q-independent half width at half-maximum Γ, respectively; $\otimes$ designates convolution in energy space. The use of a Gaussian follows from Maxwell–Boltzmann distributed ballistic motions. The function is finally convolved with the instrumental resolution to obtain the experimentally observed scattering function.

For a flow motion, the standard deviation of the Gaussian σ increases linearly with Q, the slope given by the flow velocity. If the

[†] Technische Universität München, FRM II.
[‡] Eidgenössische Technische Hochschule Zürich.
[§] Universitat Politècnica de Catalunya.

10.1021/ja907581s

molecule is trapped in a cage with a typical dimension r_c, the scattering function splits at Q values below $Q_c \approx (2\pi)/(r_c)$ into a quasielastic part with Q-independent width and an emerging $\delta(\omega)$ component which was however not resolved with these instrumental settings.

It can be seen in Figure 1 that, for temperatures below the main phase transition, $\sigma(Q)$ does level off at small values of Q, indicating that the phospholipid molecules are confined to a length of ~9 Å, approximately the size of the intermolecular distance. The same should hold above the main phase transition, following the picture where the fundamental step of phospholipid long-range diffusion is the escape from the cage: It can be calculated from the macroscopic diffusion coefficient that this event is so rare that 98% of the molecules are still confined during the observation time of 35 ps.[20] However, one can see in Figure 1 that this confinement is breaking down. While we cannot exclude that the leveling off below the phase transition is a limitation of the spectrometer, the absence of confinement above the phase transition is clear. This is a first indication that the molecules are not trapped in a cage of neighbors in the relevant high-temperature phase.

As it is crucial that the observed line broadening is not a fit artifact, a Bayesian data analysis[21] was performed. It could be shown with clear statistical significance that, above the main phase transition, the model incorporating a line broadening is superior to one with only a $\delta(\omega)$ line. This result indicates again that the lateral transport process of phospholipid molecules within a membrane is not governed by decaging events on a picosecond time scale.[20] This finding is in full agreement with recent MD simulations.[16] We conclude therefore that the rattling and escape model cannot describe the dynamical processes on the time scale of some tens of picoseconds.

To check whether it is possible to support the proposed mechanism of flow-like motions of dynamically assembled patches of molecules also on a nanosecond time scale, an experiment probing these times was conducted. In contrast to coherent neutron spin echo spectroscopy, it is not possible with incoherent QENS to check the collectivity of motions directly, but the motions of the single particles can nevertheless be compared to the simulation. As the MD simulations[16] observed a decay of the velocity autocorrelation function in this time regime, one would expect to measure a lower flow velocity than on a time scale of some tens of picoseconds.

The sample was prepared the same way, but the spectrometer was tuned to a much longer observation time of 0.9 ns, corresponding to an instrumental resolution of 4 μeV (full width at half-maximum).[18] The data were analyzed using eq 1, shown in Figure 2. The low temperature measurements (not displayed) have a $\delta(\omega)$ component, indicating confinement[20] which breaks down above the main phase transition. The analysis of the fluid phase at 30 °C allows us to extract the most probable velocity of the Maxwell−Boltzmann distribution of 0.3(nm)/(ns). This value and the decay of the flow velocity compared to the 35 ps measurement are in good agreement with the simulation.[20]

Summarizing, the following picture of phospholipid motion on a pico- to nanosecond time scale emerges: The head and tail of the molecule perform localized diffusive motions visible as two broad components in the scattering function (Figure 1) and a negative part of the velocity autocorrelation function[13,14] at times of a few picoseconds. This is not the footprint of a rattling and escape motion; the whole molecule rather flows with its neighbors as a dynamically assembling patch across the membrane.

This effect should have important consequences for living cells where the distance between proteins is on the here probed nanometer scale.[5] Recently, it could be demonstrated that ballistic motion is the most effective search strategy.[22] This could for example influence G protein coupled signal transduction cascades where two proteins have to come into contact.[23] For such processes, only the microscopic transport mechanism is relevant.

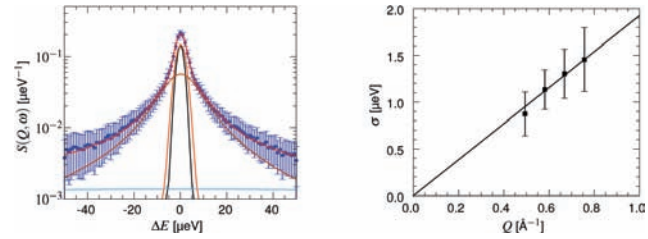

Figure 2. Left: QENS spectrum of DMPC at 30 °C and $Q = 0.76$ Å^{-1}. The instrumental resolution is displayed as a black line, along with the best fit of eq 1 (red) and its three components. Right: the extracted σ as in Figure 1. The line through the origin is a fit to the data points.

Acknowledgment. The authors thank S. Kurmulis for her help during the measurements, Prof. W. Petry for funding, and both him and Prof. E. Sackmann for their readiness for discussions. This work was partly supported by Grants FIS2008-00837 and 2009SGR-1251.

Supporting Information Available: Material characterization, data reduction and analysis details, further comparison with previous work, additional results and complete ref 17. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Gorter, E.; Grendel, F. *J. Exp. Med.* **1925**, *41*, 439.
(2) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biolology of the Cell*, 5th ed.; Taylor & Francis Group: 2007.
(3) Taylor, T. M.; Davidson, P. M.; Bruce, B. D.; Weiss, J. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 587–605.
(4) Nagle, J. F.; Tristram-Nagle, S. *Biochim. Biophys. Acta (Reviews on Biomembranes)* **2000**, *1469*, 159.
(5) Ramadurai, S.; Holt, A.; Krasnikov, V.; van den Bogaart, G.; Killian, J.; Poolman, B. *J. Am. Chem. Soc.* **2009**, *131*, 12650–6.
(6) Tabony, J.; Perly, B. *Biochimica et Biophysica Acta (Biomembranes)* **1991**, *1063*, 67.
(7) König, S.; Pfeiffer, W.; Bayerl, T. M.; Richter, D.; Sackmann, E. *J. Phys. II France* **1992**, *2*, 1589.
(8) Böckmann, R. A.; Hac, A.; Heimburg, T.; Grubmüller, H. *Biophys. J.* **2003**, *85*, 1647.
(9) Cevc, G., Ed.; *Phospholipids Handbook*; CRC: 1993.
(10) (a) Cohen, M. H.; Turnbull, D. *J. Chem. Phys.* **1959**, *31*, 1164. (b) Galla, H.-J.; Hartmann, W.; Theilen, U.; Sackmann, E. *J. Membr. Biol.* **1979**, *48*, 215. (c) Vaz, W. L. C.; Almeida, P. F. F. *Biophys. J.* **1991**, *60*, 1553–1554.
(11) Box, G. E. P. *J. Am. Stat. Assoc.* **1976**, *71*, 791–799.
(12) (a) Falck, E.; Patra, M.; Karttunen, M.; Hyvönen, M. T.; Vattulainen, I. *Biophys. J.* **2004**, *87*, 1076–91. (b) Almeida, P. F. F.; Vaz, W. L. C.; Thompson, T. E. *Biophys. J.* **2005**, *88*, 4434–8. (c) Falck, E.; Patra, M.; Karttunen, M.; Hyvönen, M. T.; Vattulainen, I. *Biophys. J.* **2005**, *89*, 745–52.
(13) Wohlert, J.; Edholm, O. *J. Chem. Phys.* **2006**, *125*, 204703.
(14) (a) Ayton, G. S.; Voth, G. A. *Biophys. J.* **2004**, *87*, 3299–311. (b) Rheinstädter, M. C.; Das, J.; Flenner, E.; Brüning, B.; Seydel, T.; Kosztin, I. *Phys. Rev. Lett.* **2008**, *101*, 248106.
(15) (a) Perera, D. N.; Harrowell, P. *J. Chem. Phys.* **1999**, *111*, 5441. (b) Diamant, H.; Cui, B.; Lin, B.; Rice, S. A. *J. Phys.: Condens. Matter* **2005**, *17*, S2787.
(16) Falck, E.; Róg, T.; Karttunen, M.; Vattulainen, I. *J. Am. Chem. Soc.* **2008**, *130*, 44–5.
(17) (a) Teixeira, S.; et al. *Chem. Phys.* **2008**, *345*, 133–151. (b) Unruh, T.; Neuhaus, J.; Petry, W. *Nucl. Instrum. Methods Phys. Res., Sect. A* **2007**, *580*, 1414–1422. (c) Unruh, T.; Neuhaus, J.; Petry, W. *Nucl. Instrum. Methods Phys. Res., Sect. A* **2008**, *585*, 201.
(18) Unruh, T.; Šmuda, C.; Busch, S.; Neuhaus, J.; Petry, W. *J. Chem. Phys.* **2008**, *129*, 121106.
(19) (a) Boon, J. P.; Yip, S. *Molecular Hydrodynamics*; McGraw-Hill Inc.: 1980. (b) Bée, M. *Quasielastic Neutron Scattering*; Taylor & Francis: 1988.
(20) See Supporting Information.
(21) Pardo, L. C.; Rovira-Esteva, M.; Busch, S.; Ruiz-Martín, M. D.; Tamarit, J. L.; Unruh, T. http://arxiv.org/abs/0907.3711v3, 2009.
(22) James, A.; Plank, M. J.; Brown, R. *Phys. Rev. E* **2008**, *78*, 051128.
(23) Simon, M. I.; Strathmann, M. P.; Gautam, N. *Science* **1991**, *252*, 802.

JA907581S