Probing Biomembranes with Positrons

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · FEBRUARY 2009

Impact Factor: 3.3 · DOI: 10.1021/jp809308j · Source: PubMed

7 AUTHORS, INCLUDING:

Filip Tuomisto
Aalto University
194 PUBLICATIONS **2,279** CITATIONS
SEE PROFILE

Juha M Holopainen
Helsinki University Central Hospital
154 PUBLICATIONS **3,490** CITATIONS
SEE PROFILE

Ilpo Vattulainen
Tampere University of Technology
283 PUBLICATIONS **7,877** CITATIONS
SEE PROFILE

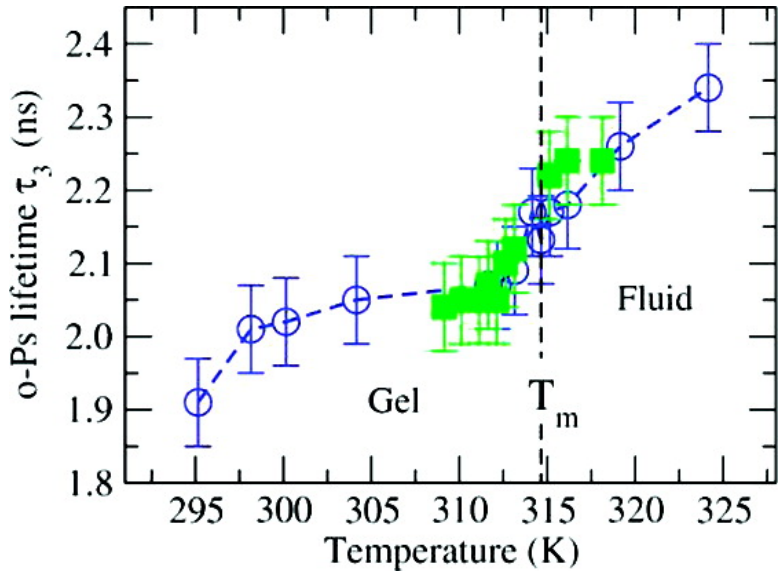
THE JOURNAL OF PHYSICAL CHEMISTRY B

Letter

Probing Biomembranes with Positrons

Petri Sane, Emppu Salonen, Emma Falck, Jarmila Repakova,
Filip Tuomisto, Juha M. Holopainen, and Ilpo Vattulainen

J. Phys. Chem. B, **2009**, 113 (7), 1810-1812• Publication Date (Web): 26 January 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

THE JOURNAL OF PHYSICAL CHEMISTRY B LETTERS

2009, *113,* 1810–1812
Published on Web 01/26/2009

Probing Biomembranes with Positrons

Petri Sane,[†] Emppu Salonen,[†] Emma Falck,[†] Jarmila Repakova,[†] Filip Tuomisto,[†] Juha M. Holopainen,[‡] and Ilpo Vattulainen*[,†,§,||]

Department of Applied Physics, Helsinki University of Technology, Finland, Department of Ophthalmology, University of Helsinki, Finland, MEMPHYS-Center for Biomembrane Physics, University of Southern Denmark, and Department of Physics, Tampere University of Technology, Tampere, Finland

Received: October 21, 2008; Revised Manuscript Received: January 15, 2009

Free volume pockets inside a cell membrane play a prominent role in a variety of dynamic processes such as the permeability of small molecules across membranes and the diffusion of, e.g., lipids, drugs, and electron carriers in the plane of the membrane. Nonetheless, by now the chances for characterizing free volume voids in a nonperturbative manner through experiments have been very limited. Here we use lipid membranes as an example to show how positron annihilation spectroscopy (PALS) together with atomistic simulations can be employed to gauge changes in free volume pockets in biological macromolecular complexes. The measurements show that PALS is a viable technique to probe free volume in biomolecular systems. As examples, we consider the gel-to-fluid transition and the role of increasing cholesterol concentration in a lipid membrane. Further applications proposed in this work for PALS are likely to provide a great deal of insight into the understanding of the role of free volume in the dynamics of biomolecular complexes.

Introduction

For cell membranes, it is generally believed that free volume pockets inside a membrane play a prominent role in a variety of dynamic processes such as the permeability of small molecules across membranes and the diffusion of, e.g., lipids, drugs, and electron carriers in the plane of the membrane.[1] Changes in free volume distribution due to anesthetics partitioning into a membrane are also likely to perturb the lateral pressure profile, thus inducing a change in the ion channel state.[2] As for clinical applications, there are drugs that need to penetrate tissues,[3,4] such as in the topical application of eye drops and in transdermal drug delivery, implying that understanding molecular diffusion through membrane structures is crucial for the development of improved drug delivery carriers without a need for invasive intraocular injections.

Considering the above phenomena and applications, it is of exceptional interest to understand how the total amount and size distribution of free volume pockets depend on thermodynamic conditions and molecular composition. However, it is exceedingly difficult to gauge free volume pockets in membranes through experiments in a nonperturbative manner. The techniques used are largely based on probes such as fluorescent markers, which inevitably cause major perturbations in the vicinity of the probe.[5] Alternatively, X-ray and neutron scattering techniques yield information of mass and electron density distributions that may provide some insight into average free volume distribution in a sample. However, they do not characterize local free volume distribution, which would be decisive to understand diffusion phenomena.

Meanwhile, positron annihilation lifetime spectroscopy (PALS) is a well-established and exceptionally sensitive technique for probing subnanometre-sized local free volume pockets (voids) in solids and free-electron reactions in liquids.[6] It has been applied extensively to characterize, e.g., defects in semiconductors[7−10] for improving their materials properties. At the same time, positron emission tomography (PET)[11,12] has found applications as a diagnostic technique in, e.g., clinical oncology and neuroimaging, where the monitoring of positron annihilation signals through β^+ active radiotracers is applied to locate tumors and the blood flow in a brain. Very surprisingly, though, positrons have scarcely been applied to elucidate the unique structural and dynamical features of biological molecules or molecular complexes. The earlier work related to this broad theme is very limited and includes only studies of positronium lifetimes at selected temperatures for lipid bilayers.[13,14] However, due to the limited scope of earlier work and lack of alternative methods (e.g., atomistic molecular dynamics simulations) to gauge and confirm free volume changes, an understanding of how lifetimes measured in PALS could be used to yield insight into free volume distributions in biological matter has remained vague.

Here we show through an extensive study by combined PALS experiments and atomistic simulations how PALS can be employed to study changes in voids in biological systems. We focus on cell membranes and consider changes in free volume distribution in the gel-to-fluid transition and due to varying amounts of cholesterol, the key sterol in eukaryotic cell membranes.[15,16] These two scenarios are of exceptionally broad interest in the context of cell membranes, as they couple changes in phase behavior to the role of free volume. By coupling

* Corresponding author. E-mail: Ilpo.Vattulainen@tut.fi.
[†] Helsinki University of Technology.
[‡] University of Helsinki.
[§] University of Southern Denmark.
[||] Tampere University of Technology.

10.1021/jp809308j CCC: $40.75

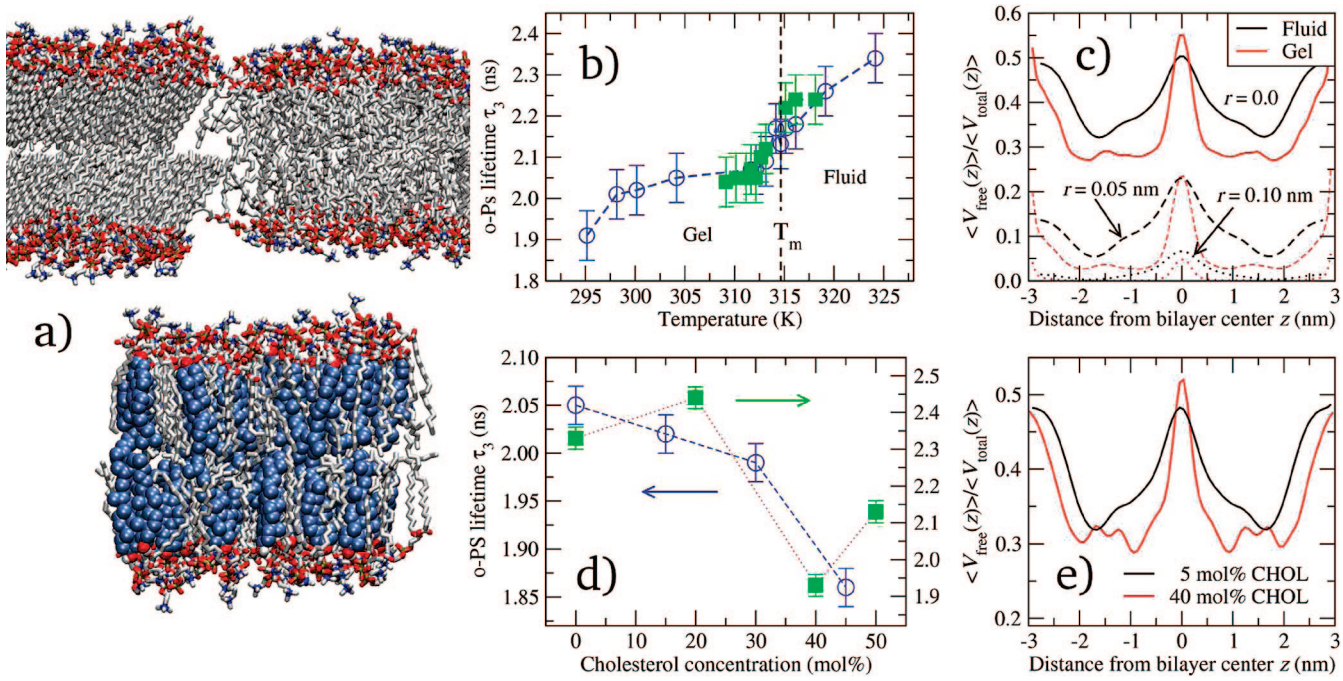


Figure 1. (a) Top: snapshots of the simulated gel (left) and fluid (right) phase DPPC membranes. Bottom: mixture of POPC (gray) and Chol (blue) with 40 mol % Chol concentration (water not shown). (b) Temperature dependence of the experimental lifetime of o-Ps in two DPPC samples indicated by different symbols. (c) Simulation results for free volume profiles in a DPPC membrane along the direction of the membrane normal (z-axis). The results include profiles in gel and fluid phases with two finite test particle radii $r = 0.05$ and 0.1 nm (Supporting Information). (d) Longest o-Ps lifetime component (τ_3) in POPC/Chol mixtures with varying Chol concentration. The data are for two different sets of measurements, in which the samples contained different water concentrations affecting the scale of lifetime changes. Hence, the right-hand axis indicates the lifetimes found in the second set shown by green markers. (e) Simulation results for POPC/Chol free volume profiles as in (C). The results are for two different Chol concentrations, and the simulation data are for a test particle of radius $r = 0.1$ nm. Data for DPPC/Chol yielded same conclusions.

atomistic simulations to PALS experiments, one is provided with a means to characterize free volume sizes in biological matter in a quantitative manner.

Free Volume Properties in Lipid Membranes

When a positron from a radioactive source enters molecular media, it thermalizes very rapidly. A fraction of positrons forms a bound state with spin-parallel electrons called ortho-positroniums (o-Ps) that preferentially localize in free volume pockets in the material. In the medium, the o-Ps prefer to undergo pick-off annihilation with an electron of opposite spin during collision with molecules in the cavity in which it is localized. The smaller the hole size, the shorter the lifetime of o-Ps that thus provides information on the free volume size.

In the measurements, we employed a digital lifetime spectroscopy setup consisting of two scintillation detectors with large scintillation heads for optimal detection efficiency of the decay gamma quantum (START-signal) of ^{22}Na and the annihilation gamma quanta of the positrons (STOP-signal). Pulses from the detectors were digitized and lifetime events (START-STOP) were filtered from the raw spectra and then forwarded to the measurement computer for actual lifetime analyses (see ref 17 for more details on the setup).

The PALS measurements were performed by injecting ^{22}NaCl (radioactive positron source) in an aqueous solution into a test tube containing multilamellar vesicles in purified water with lipid to water ratios fixed to approx. 40/60. The concentration of NaCl (<0.01 μM) was low enough not to have a significant effect on the properties of the lipid membranes. The temperature of the samples was controlled by placing the test tube in a heat bath.

TABLE 1: Estimates for the Corresponding Free Volume Void Sizes in Gel (Red) and Fluid (Blue) Phases[a]

temp (°C)	o-Ps lifetime (ns)	mean void radius (Å)	mean volume (Å^3)
39	2.07 ± 0.02	2.91 ± 0.02	104 ± 1
40	2.09 ± 0.02	2.93 ± 0.02	106 ± 1
42	2.17 ± 0.02	3.00 ± 0.02	113 ± 1
43	2.18 ± 0.02	3.05 ± 0.02	115 ± 1

[a] Volume estimations are based on the Tao−Eldrup model (Supporting Information) for polymers.

The experiments clearly show (Figure 1b) that the lifetime of o-Ps in an aqueous solution of multilamellar DPPC vesicles increases with increasing temperature. At the main phase transition temperature of $T_m = 314.7$ K, where the membrane undergoes a gel−fluid transformation, the slope rises rapidly, indicating a clear change in free volume properties. Similar behavior has been found earlier by Jean and Hancock.[14] The difference in the lifetime of o-Ps below and above T_m, as well as for the subtransition at 296 K, proves that PALS is a viable technique to distinguish changes in free volume in lipid membranes. As for void sizes, the Tao−Eldrup model[6] based on positronium trapping in spherical voids in polymers predicts an average void radius of ∼2.9 and ∼3.0 Å in the gel and fluid phases, respectively (see Table 1). Although there is reason to take these numbers with some caution due to the approximations of the model, the 7% increase in void size in the gel-to-fluid transition (313 K → 315 K) is consistent with the 5% increase predicted by our atomistic simulations; see below and the Supporting Information.

Next, we performed isothermal PALS measurements in POPC/cholesterol (Chol) membranes above T_m with varying

Chol content. The results (Figure 1d) clearly depict that the o-Ps lifetime and consequently the average free volume size decrease substantially when the Chol content is increased above 30 mol %. Interestingly, these major changes in o-Ps lifetime coincide with the phase transition boundary: at ~30 mol % of Chol there is a transition from the coexistence region (between the liquid-disordered and liquid-ordered phases) to the liquid-ordered phase dominated by Chol.[18] Further, at the largest Chol concentration of 50 mol %, one finds a minor increase in lifetime, reflecting the formation of Chol crystals that takes place in this regime. These findings highlight the sensitivity of PALS for detecting changes in phase behavior as well as the structural differences in void distributions for fluid and raft-like membrane domains.

The PALS measurements were complemented by atomistic molecular dynamics simulations of a DPPC bilayer in gel (273 K) and fluid (323 K) phases, and of POPC/Chol (310 K) and DPPC/Chol[19] (323 K) membranes above T_m for Chol concentration between 0 and 40 mol % (see Figure 1a). Descriptions of the simulated systems and the analysis tools used in computing free volume profiles are given in the Supporting Information. The added value of atomistic simulations lies in the fact that they allow one to study the structure and dynamics of complex biological systems in full atomic detail; hence also the free volume voids can be computed with great accuracy, thus complementing PALS experiments. Analysis of voids in the simulated DPPC membranes indicated good agreement with experiments; see Figure 1c and the Supporting Information. This provides compelling evidence that both the amount of free volume and void number densities increase as the membrane transforms from gel to fluid. As for the role of Chol, simulation studies, as well as our PALS measurements for POPC/Chol membranes highlight the prominent role of Chol as a membrane stabilizer, reducing void sizes and densities (see Figure 1d,e and the Supporting Information).

Concluding Remarks

We have shown how the interplay of PALS and atomistic computer simulations allows one to develop means to quantify free volume pocket sizes from complex biomolecular systems using the lifetime of o-Ps as it is already routinely possible in polymer systems.[20] Here, we have applied this approach to one- and two-component membranes to elucidate how void sizes inside membranes change in phase transitions and due to increasing concentrations of cholesterol. More generally, the present studies pave the way for a number of applications in biosciences. In cells, voids affect the dynamics of all molecules embedded in and permeating through the cell membrane, and they are immensely important for understanding the viability of the cell. Yet, diffusion dependent transport is not only necessary on a cellular level but also essential for the health of entire organs. For example, the viability of corneal epithelium depends on nutrients that diffuse through the cornea from the anterior chamber. Likewise, in mammalian lungs, breathing largely depends on oxygen diffusion into the blood, whereas carbon dioxide diffuses into the alveoli in a reciprocal exchange. A detailed molecular-level understanding of these processes in terms of the void properties will aid in developing therapeutic interventions to diseases such as corneal scarring and respiratory distress syndrome. In other contexts, PALS is expected to provide insight into changes in void distributions and microstructure of a variety of biomolecular complexes as they undergo structural changes, including viruses, DNA-encapsulating carrier particles, as well as lipoproteins and protein complexes. Overall, the extraordinary nature of positron−matter interactions gauged by PALS makes it a very attractive technique to probe biological matter.

Acknowledgment. We thank the Finnish IT Centre for Science, The HorseShoe computing cluster at the University of Southern Denmark, Academy of Finland, and Sigrid Juselius Foundation.

Supporting Information Available: Description of the lipid systems considered, and the experimental and computational methods used to study these systems. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Marrink, S. J.; Berendsen, H. J. C. *J. Chem. Phys.* **1996**, *104*, 9090.
(2) Cantor, R. S. *Biochemistry* **1997**, *36*, 2339.
(3) Barry, B. W. *Nat. Biotechnol.* **2004**, *22*, 165.
(4) Karande, P.; Jain, A.; Mitragorti, S. *Nat. Biotechnol.* **2004**, *22*, 192.
(5) Repakova, J.; Holopainen, J. M.; Morrow, M. R.; McDonald, M. C.; Capkova, P.; Vattulainen, I. *Biophys. J.* **2005**, *88*, 3398.
(6) Jean, Y. et al. *Principles and Applications of Positron and Positronium Chemistry*; World Scientific: Singapore, 2003.
(7) Saarinen, K.; Hautojärvi, P.; Corbel, C. In *Identification of Defects in Semiconductors−Semiconductors and Semimetals*; Stavola, M., Ed.; Academic Press: New York, 1998; Vol. 51A, pp 209−285.
(8) Tuomisto, F.; Ranki, V.; Saarinen, K.; Look, D. C. *Phys. Rev. Lett.* **2003**, *91*, 205502.
(9) Tuomisto, F.; Pennanen, K.; Saarinen, K.; Sadowski, J. *Phys. Rev. Lett.* **2004**, *93*, 055505.
(10) Tuomisto, F.; Pelli, A.; Yu, K. M.; Walukiewicz, W.; Schaff, W. J. *Phys. Rev. B* **2007**, *75*, 193201.
(11) Phelps, M. E.; Hoffman, E. J.; Mullani, N. A.; Ter-Pogossian, M. M. *J. Nucl. Med.* **1975**, *16*, 210.
(12) *PET−Molecular Imaging and Its Biological Applications*; Phelps, M. E., Ed.; Springer: Berlin, 2004.
(13) Wang, Y. Y.; Hancock, A. J.; Jean, Y. C. *J. Am. Chem. Soc.* **1983**, *105*, 7272.
(14) Jean, Y. C.; Hancock, A. J. *J. Chem. Phys.* **1982**, *77*, 5836.
(15) Simons, K.; Ikonen, E. *Nature* **1997**, *387*, 569.
(16) Niemelä, P.; Ollila, S.; Hyvönen, M. T.; Karttunen, M.; Vattulainen, I. *PLoS Comput. Biol.* **2007**, *3*, 304.
(17) Sane, P.; Kilpeläinen, S.; Tuomisto, F. *Mater. Sci. Forum* **2009**, *607*, 254.
(18) Vist, M. R.; Davis, J. H. *Biochemistry* **1990**, *29*, 451.
(19) Falck, E.; Patra, M.; Karttunen, M.; Hyvönen, M.; Vattulainen, I. *J. Chem. Phys.* **2004**, *121*, 12676.
(20) Mogensen, O. E. *Positron annihilation in Chemistry*; Springer-Verlag: Heidelberg, 1995.

JP809308J