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Only

A PHYSICAL STUDY OF DYNAMICS IN FULLY HYDRATED PHOSPHOLIPID BILAYERS

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Abstract

We have performed a study concerning the thermotropic phase behavior of phospholipid membrane bilayer of dimyristoylphosphatidylcholine (DMPC) in excess of water condition. We have also investigated the effect induced by the antimicrobial peptide Gramicidin D (GrD) inserted into the membrane bilayer. This study was carried out by using several techniques in order to collect information on different properties and on different spatial and temporal regimes. We have in fact used Differential Scanning Calorimetry (DSC), Fixed Energy Window (FEW) neutron scattering, Pulsed Field Gradient Nuclear Magnetic Resonance (NMR) and Molecular Dynamics (MD) simulations.

We have obtained information on the main phase transition (gel – liquid crystalline) temperature of bilayer system and on long range diffusion coefficient for both phospholipids and hydration water. Moreover, we have evidenced the effect of the transition on the microscopic molecular mobility perpendicular and parallel to the membrane plane. The influence of gramicidin on these properties are also commented.

Keywords: DMPC; lipid membrane; pore forming peptide;

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1. Introduction

Biological membranes are fundamental components of all living systems, forming the outer boundary of living cells or organelles. They consist of a lipid bilayer that confers a fluid character. Proteins embedded in the bilayer and carbohydrates attached to its surface make easy exchanges and transport across the membrane. The complexity of biological membranes and their interactions with intra- and extra-cellular environment makes difficult direct investigations. Consequently artificial model membranes are commonly used in order to gain insights about the physical and chemical characteristics of membranes and how these contribute to biological functions of natural membranes.

Lipids are amphiphilic molecules with a hydrophilic head group and usually two lipophilic hydrocarbon chains. In aqueous environment lipids can arrange themselves in a variety of morphologies depending on a large number of factors, e.g. the structure of the lipid, the nature of the lipid headgroup and its degree of hydration, temperature, concentration and osmotic pressure. Among the different structures there are multilamellar vesicles, continuous ordered bilayers and monolayers, liposomes and micelles [1]. In the bilayer structure, molecules are arranged in such a way that the hydrophilic head groups form the two lipid-water interfaces bounding the bilayer, whereas the hydrocarbon chains are confined inside the bilayer and have essentially no contact with water. In this frame, membrane proteins, which perform many important physiological functions for the cell activity, are incorporated by specific mechanisms, not yet fully understood, which depend on the peculiar properties of proteins and lipids (e.g. amphiphilic character, elasticity, charge distribution) and on the lipid composition of the bilayer. [2].

Fully hydrated phospholipid bilayers are largely employed as biomimetic model systems. Their structure is well known since it has been the object of many different studies in last years[3,4,5,6]. On the contrary, their dynamics is less elucidated and an in-depth investigation is needed since the mobility of biological membrane is strictly related to functional aspects. As a matter of fact, movements of the host lipid bilayer promote the activity of many integral transmembrane proteins [7]. Moreover, it is believed that the formation of gauche-trans-gauche kinks due to the collective thermal motion of hydrocarbon chains provides a possibility for the trans-membrane transport of small molecules such as water or ions [8].

Many structural and dynamic intrinsic properties of aqueous dispersions of lipid bilayers are governed by temperature. Indeed lipid bilayers present several lamellar phases as a function of temperature, namely the gel phase L'_β , the ripple phase P'_β and the liquid-crystalline phase L_α (also called fluid phase). In the L_α phase the hydrocarbon chains of lamellar bilayer structures are

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3 disordered [9]. This phase is considered the most important one, because many biologically relevant
4 processes occur in this phase. Indeed, lamellar bilayers in the fluid phase supply an efficient, planar
5 permeability barrier, which still allows functional flexibility and lateral diffusion motions of
6 associated membrane proteins. The L_{α} phase is favoured by lipid mixture containing unsaturated
7 hydrocarbon chains, higher temperatures and water content. [1].
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11 Because of their thermotropic and lyotropic behavior, phase transitions between different molecular
12 organization of aqueous lipid dispersions can be obtained by changing either temperature and
13 hydration. The transition from the gel phase to the ripple one is referred as pre-transition and the
14 transition from the ripple phase to the liquid one is called main transition or the chain order/disorder
15 transition. In the case of phosphatidylcholines, these phase transitions take place within the
16 temperature range of 283 K to 353 K, depending on the tail length of lipid, the head group
17 hydration and the unsaturation of the acyl chains [10]. Moreover, in pure phospholipids bilayer the
18 main transition is very sharp, while it is broadened in the presence of cholesterol, small molecules
19 and oligo or polypeptides interacting with the bilayer. These molecules also influence the structure
20 of the low temperature phases of the bilayer [7].
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24 As concerning the dynamics, many different kinds of motions, with correlation times ranging from
25 picoseconds (corresponding e.g. to the motion of lipid chain defects) to microseconds
26 (corresponding to collective excitations of the bilayer membrane), characterize bilayers and a large
27 variety of these is essential for the functionality of membranes [11,12,13]. Motions within the plane
28 of bilayer have been largely studied by NMR relaxation techniques [14,15,16] and neutron
29 scattering [11,12,13].
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33 Information on the dynamics along the membrane normal is scarce, although it is of particular
34 importance for a better characterization of the different phases. As a matter of the fact, binding via
35 hydrogen bonds between peptides and moieties in the choline headgroup is expected drastically to
36 restrict its motional freedom in the direction along the membrane normal.
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40 With the aim to gain information about the limitations induced by a pore forming peptide on the
41 motions of a lipid membrane along the in-plane and out of the plane directions, we have performed
42 a neutron scattering study of the thermotropic phase behavior of a DMPC/GrD mixture in excess of
43 water (with 0 and 2.4 mol % GrD). The results have been compared with differential scanning
44 calorimetry findings obtained on the same mixtures.
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48 Gramicidin has been chosen as a model of membrane proteins because of the relative ease with
49 which it partitions into membrane to form channels.
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53 NMR measurements have been also performed to investigate the lipid and water lateral diffusion in
54 the temperature region of the melting chain transition.
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3 Finally, in order to improve the molecular interpretation of experimental results, preliminary results
4 of molecular dynamic (MD) simulation are also presented.
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7 8 **2. Experimental details** 9

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11 DMPC and partially deuterated DMPC (DMPC-d54) samples have been purchased from Avanti
12 Polar Lipids. Gramicidin D (GrD) was purchased from Fluka. It is the natural mixture of
13 gramicidin A, B and C, consisting of 80% gramicidin A [17].
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16 Samples for different measurements have been prepared by following differentiated procedures in
17 order to better adjust them to specific experimental requirements.
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20 21 **2.1 Differential Scanning Calorimetry** 22

23 Multilamellar lipid vesicles for differential scanning calorimetry were prepared by dissolving
24 **appropriate amounts** of lipid and peptide in a mixture of chloroform and methanol (2:1 v/v) to
25 obtain the **40:1** peptide/ lipid ratio (corresponding to GrD in DMPC with 2.4 mol % peptide). The
26 solvent was then evaporated by gently blowing dry nitrogen gas over the sample, and the final
27 traces of solvent were removed by vacuum desiccation. The dry mixtures were hence hydrated by
28 adding 28 molecules of H₂O per mole of lipid/peptide to obtain samples in excess of water. Then,
29 mixtures were mixed and stored for 24 h at 323 K. Finally they were sonicated for 1-2 hours in a
30 bath type ultrasonicator.
31

32 Differential scanning calorimetry measurements were carried out with a Perkin-Elmer Pyris 1.
33 Samples were placed in a sealed aluminum pan to prevent dehydration during the **thermal**
34 analysis. Thermograms were recorded at a rate of 1K/min. To ensure that thermal equilibrium was
35 reached and to verify the occurrence of hysteresis, different scans on increasing and decreasing
36 temperature were carried out for each sample.
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39 40 **2.2. Neutron measurements** 41

42 The procedure for preparation of supported highly oriented D₂O-hydrated multilamellar
43 membranes of DMPC and of DMPC/GrD for neutron study was described in a previous paper [18].
44 Elastic neutron scattering at fixed energy window scans were performed on the thermal
45 backscattering spectrometer IN13 (ILL) that allows access to relatively high momentum transfers
46 Q , extending from $Q=0.4$ to $Q=4.7 \text{ \AA}^{-1}$ and corresponding to a probed spatial region ranging from ~
47 15 to 1 \AA . The instrumental energy resolution of 8 μeV allows to reveal movements occurring on
48 the time scale < **100 ps** [19]
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3 Due to the large number of hydrogen atoms in the system, incoherent scattering from the protons
4 represents the dominant contribution to the total neutron scattering. In practice, experiments probe
5 essentially the membrane thermal dynamics, since the contribution of D₂O hydration water to the
6 incoherent scattering signal is negligible. Expected motions in this time regime are chain defects,
7 fast head-group motions, and the local diffusion of the molecule in its solvation cage. In the
8 protonated mixtures, neutron experiments are sensible mainly to the chain dynamics; in these
9 samples in fact the scattering **from the chains** alone amounts to 77% of the total scattering. In the
10 DMPC-d54 sample, the deuteration of 54 out of 72 hydrogens in the acyl chains, makes possible a
11 partial discrimination of dynamics.

12 The anisotropy of motions has been resolved **by collecting data for two different sample**
13 **orientations forming 45° and 135°, respectively, between the normal to the membrane plane and the**
14 **incident neutron beam direction, with the membrane plane perpendicular to the scattering plane.**
15 **Experiments performed at the 135° orientation correspond to a momentum transfer predominantly**
16 **perpendicular to the membrane normal and mainly reveal membrane motions in the plane of the**
17 **bilayer such as lateral diffusion. Differently, experiments carried out at 45° orientation correspond**
18 **to a momentum transfer predominantly parallel to the membrane normal and are sensitive to the**
19 **out-of-plane motions.** In a typical measurement the temperature was varied from 27 to 325 K with a
20 precision of 1K and an acquisition time of about one hour per temperature was spent.

21 By using the software package ELASCAN available at ILL, the data were corrected to take into
22 account for incident flux, cell scattering, self-shielding and detector response, and normalized to the
23 sample at the lowest temperature T=27 K.

24 **2.3. Nuclear Magnetic Resonance**

25 Hydrogen Pulsed Field Gradient Spin Echo NMR (¹H-PGSE -NMR) [20,21] technique is able to
26 measure lateral diffusion coefficients of hydration water and lipid molecules. PGSE experiments on
27 aligned DMPC membrane deposited on mica sheets (following the same preparation procedure
28 adopted for the neutron analysis) have been performed. All measurements were carried out in fully
29 hydration (D₂O) condition at temperatures below, close and higher than phase transition
30 temperature, (T=283, 295 and 313 K), by using a Bruker AVANCE NMR spectrometer, operating
31 at 700 MHz ¹H-resonance frequency.

32 Self-diffusion measurements are based on NMR pulse sequences which generate a spin-echo of the
33 magnetization of the resonant nuclei. By the appropriate addition of two pulsed field gradients, in
34 the defocusing and refocusing period of the sequence, of duration δ and intensity g , separated by a
35 time interval of Δ , the spin-echo intensity becomes sensitive to the translational motion along the
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3 gradient direction for the tagged molecules. In stochastic processes, such as thermally excited
4 Brownian motion (self-diffusion), the spin-echo intensity $M(\delta g, \Delta)$ is attenuated. The attenuation
5 factor is given in terms of the mean square displacement $\langle z^2(\Delta) \rangle = 2D\Delta$ of the diffusing molecules,
6 along the gradient direction z , during the time interval Δ by
7

$$M/M_0 = \exp[-DQ^2\Delta] \quad (1)$$

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10 where $Q = \gamma \delta g$ and γ is the gyromagnetic ratio of ^1H . Q has the dimension of an inverse length, being
11 a measure of the spatial scale probed, and it is equivalent to the exchanged wave vector in a
12 scattering experiment.
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16 17 18 **2.4 Molecular Dynamics simulation**

19 A 3 ns simulation of a fully hydrated DMPC bilayer has been performed in the NVT ensemble at
20 the temperature of 313 K, controlled through the Berendsen method. A well-equilibrated initial
21 configuration was kindly supplied by Dr. Mounir Tarek [22]. The box dimensions were
22 (48.94x39.22x60.86) Å³. The membrane normal is oriented along the z -axis and the center of the
23 bilayer is at $z=0$. Orthorombic boundary conditions were applied in the x and y directions to
24 simulate an infinite planar layer and in the z direction to simulate a multilamellar system. The
25 program used was DL-POLY, version 2.16 [23]. An all-atom model has been employed to describe
26 the interactions among atoms using a force field from the DL-POLY package. Electrostatic
27 interactions were computed using the Ewald sum method and short range forces have been
28 evaluated using a cutoff radius of 10 Å. The time step employed was 2 fs and the final results were
29 computed over 1024 configurations selected over the whole simulation.
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40 41 **3. Experimental results and discussion**

42 43 **3.1. DSC**

44 DSC thermograms of the fully hydrated DMPC/GrD mixtures are shown in Fig. 1. Aqueous
45 dispersion of pure DMPC shows two endothermic peaks that correspond to a less energetic pre-
46 transition and a more energetic main transition occurring at about $T_{on}^1 = 286.7$ K and $T_{on}^2 = 297.8$ K,
47 respectively. The pre-transition describes the transformation from the L'_β to the P'_β phase and the
48 main transition describes the melting phase transition from the P'_β to the L_α phase. Both transition
49 temperatures well agree with the values of 287 K and 297 K reported in literature [24,25], the
50 minimal discrepancies revealed being likely due to the higher scanning rate used and to the different
51 methods of evaluation of the peak temperatures. In fact, we have taken the onset temperature, T_{on} ,
52 which is defined as the intersection between the tangent to the maximum rising slope of the peak
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3 and the extrapolated sample baseline [26], as indicative of the transition temperature. This
4 procedure leads to values for the phase temperature lower than those referring the experimental
5 peak positions. On the contrary, the scan rate at which the calorimetric data were recorded is rather
6 high and the possibility of a translation of transition temperature to higher values cannot be
7 excluded. More importantly, the smaller width of the main endothermic peak as compared with the
8 low temperature peak shows that the gel/liquid phase transition of the acyl chains is more
9 cooperative than the lipid pre-transition. When gramicidin is added to the lipid membrane, the pre-
10 transition disappears, while the melting peak shifts to lower temperatures ($\sim 3^{\circ}\text{C}$), showing a visible
11 broadening with an extended asymmetry at the high temperature end. These evidences are in good
12 agreement with calorimetric results reported by Kobayashi for the DMPC-Gramicidin A mixture
13 corresponding to a molar ratio of GrA to DMPC exceeding 0.03 [27]. Considering that GrD
14 includes a very high content (80%) of GrA and that the DMPC/GrD mixture we studied
15 corresponds to a molar ratio of GrD to DMPC of 0.024, we can deduce that GrD has a similar
16 behaviour to the one of GrA and the residual component B and D do not play any role in modifying
17 interaction with the host lipid. It is important to remark that, although the agreement between our
18 data and results concerning DMPC-Gramicidin A mixture [27] is very good, both evidences are in
19 partial contrast with the calorimetric results obtained by Ivanova et al.[28]. In fact, upon
20 incorporation of gramicidin A into DMPC, these authors observe that the melting transition is only
21 very slightly shifted to lower temperatures and shows a small asymmetry at the low temperature
22 end, even at high peptide concentration. The shift to lower temperatures is considered a
23 consequence of the higher peptide-lipid interfacial energy in the gel phase, while the asymmetry of
24 the profile results from the change in mixing behavior and reveals an unfavorable partitioning in the
25 low temperature phase.

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28 In comparison with the results by Ivanova et al [28], the shift of chain melting transition we observe
29 is relevant, the broadening of the peak is quite wide and more importantly, the peak shows an
30 asymmetry at the high temperature end. These findings do not find a simple explanation in terms of
31 the common interpretation. In fact, it is believed that changes in the calorimetric profile of melting
32 transition occurring in lipid membrane upon addition of integral peptide are descriptive of the
33 hydrophobic matching, that depends on the relative hydrophobic core lengths of both peptide and
34 the lipid membrane.

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37 The length of gramicidin A (2.6 nm) is shorter than the length of both gel and fluid phases of
38 DMPC [29], and fluid lipids are shorter than gel lipids. Hence, a better matching of fluid lipids with
39 gramicidin A and a good mixing of the peptide in this phase are foreseen [30], and as a consequence
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3 a shift of the main transition peak to lower temperatures with a extended wing at the low
4 temperature end is expected in the specific heat profile.

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6 The finding of an appreciable melting temperature shift ($\sim 3^{\circ}\text{C}$) indicates that peptide aggregates
7 preferably in the fluid phase, while Ivanova et al [28] supposed a good aggregation of peptide in
8 both gel and fluid phase (with a slight preference for the fluid phase) to justify the very slight shift
9 of the heat capacity profile upon addition of peptide. Moreover, our finding of a shoulder at the high
10 temperature end should be taken as an indication of a strong tendency to accumulate in the fluid
11 phase, which is enriched in peptide, in clear opposition to the conclusions by Ivanova et al [28]. It
12 is hard to explain these opposite evidences. However, it has to consider that it cannot be excluded
13 that the dissimilarities evidenced in the different calorimetric studies of DMPC/GrA mixture are
14 due to differences in the preparation procedure related to the use of buffer solutions [28] and /or
15 difference in the water content. At the same time, it cannot be excluded that the highly broadened
16 wing at the high temperature end masks a second component of main phase transition, as it occurs
17 in DMPC upon addition of Gramicidin S [31]. It is obvious that, to confirm such an eventuality it is
18 necessary to perform an in-depth study including the possibility to change the peptide content and
19 to verify the influence of different sample preparation procedures.

20
21 The other important feature coming from the present data concerns the absence of pre-transition,
22 indicating that acyl-chains confinement of lipid molecules is perturbed by the presence of the
23 peptide. Basing on the similarity with the X-ray scattering results obtained in the same lipid
24 membrane containing gramicidin A, it can be believed that the incorporation of gramicidin destroys
25 the ripple periodicity of DMPC [27]. DSC thermograms performed upon heating and cooling
26 cycles in the same temperature range have evidenced a slight shift in the peak temperatures,
27 ascribed to the occurrence of hysteresis in the gel/liquid- crystalline phase transitions .

28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 **3.2 Neutron measurements**

44 In Fig. 2 the temperature dependence of scattered intensities integrated over a fixed energy window
45 centered at zero energy (FEW scans) integrated over all Q-range and normalized to the intensity at
46 the lowest temperature (I_N^{Tot}) for both in and out of plane components of DMPC (Fig. 2a) and
47 DMPC/GrD (Fig 2b) samples is shown. This representation is fit for giving a general view of
48 molecular mobility of the investigated lipids. As a matter of fact, a monotonic decrease of the FEW
49 intensity reflects a broadening of the quasielastic line due to an increased mobility, while sharp
50 changes reveal the unfreezing of dynamical modes, usually ascribed to conformational or melting
51 transitions. By analyzing the corresponding Q dependence, it is then possible to obtain insights on
52 the origin of such transitions.
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3 It can be observed that in both investigated samples the decrease in the intensity in the out of plane
4 orientation is more enhanced than **the one observed for the in plane configuration**. The differences
5 are more prominent above 270 K, where I_N^{Tot} decreases faster, revealing the occurrence of
6 accentuated dynamics. A previous detailed analysis of FEW scans at different Q values has revealed
7 the occurrence of two different transitions at $T_1=270$ K and $T_2=297$ K, particularly evident at
8 $Q_1=1.77 \text{ \AA}^{-1}$ and $Q_2=1.47 \text{ \AA}^{-1}$ respectively [18]. Basing on the correspondence between Q_1 and the
9 correlation peak of heavy bulk water and the ice Bragg peaks on one hand and between Q_2 and the
10 correlation peak of lipid acyl chains [32] on the other, the first transition has been believed to
11 describe the increase of mobility of membrane following the water unfreezing and the consequent
12 migration of aqueous solvent into intra-bilayer stacks, while the second one has been ascribed to
13 melting of the acyl chains (transition from the P'_β to the L_α phase) [11, 18].

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15 In Figs. 3a and 3b the I_N^{Tot} of DMPC, DMPC/GrD and DMPC-d54/GrD in the temperature range
16 affected by the transitions ($T>150\text{K}$) for both orientations are compared. It can be observed that the
17 dynamics of the membrane is hindered by the inclusion of GrD, **since the normalized FEW intensity**
18 **is higher than that of pure membrane**. Again, this hindrance is more enhanced in the out of plane
19 direction. Moreover, **the further increase of the FEW intensity observed in correspondence to the**
20 **acyl chain deuteration (DMPC-d54/GrD sample)** makes evident that a great participation to
21 observed dynamical processes comes from hydrocarbon chains. **The incorporation of GrD in DMPC**
22 **gives rise to a decrease of 3 K in the main transition temperature, in close agreement with the**
23 **observation from DSC measurements. In the inset of the figure is shown the detail of the FEW**
24 **scans at $Q = 1.47 \text{ \AA}^{-1}$ for DMPC/GrD and DMPC-d54/GrD samples in the region of the main**
25 **transition. In the deuterated sample a lowering of the transition temperature from 294 K to 289 K is**
26 **observed, in agreement with what is expected in a deuterated lipid [33].** It is known that already a
27 few degrees below the main transition the Gramicidin becomes immobile [34]. Inside the bilayer,
28 GrD interacts primarily with the polar headgroup and glycerol-backbone region of the phospholipid
29 molecules by electrostatic and hydrogen-bonding interactions and secondly with hydrocarbon
30 chains by hydrophobic and Van der Waals interactions. As a consequence of these interactions the
31 contiguous lipids are expected to be also motionless and the whole membrane mobility will slow
32 down. At the same time the presence of GrD shifts forward the transition related to the water
33 unfreezing from 270 K to 276 K imposing severe restrictions to out of plane dynamics. It is known
34 that the presence of gramicidin into bilayers is accompanied by dehydration of the lipid head groups
35 surrounding the peptide [35]. We can speculate that the dehydration of the lipid head groups and the
36 interactions between the GrD and the polar head groups hinder the transverse motions of membrane
37 and impose restrictions on the out plane dynamics (see Fig 3a).

3.3. NMR measurements

In our experiment the stack of mica sheets with deposited DMPC is placed parallel to the magnetic field so to sample the lateral (in plane) diffusion. An example of a ^1H -NMR spectrum from our sample is shown in the inset of Fig. 4: the intense peak at 4.2 ppm is due to residual ^1H from intralamellar water while the peaks at 3.1 and 1.1 ppm originate from ^1H in the methyl group and acyl chain methylene group respectively [15]. To record the decay of the ^1H components in these spectra a train of pulses at increasing gradient strength is used. Pulse parameters used to follow the diffusion of water molecules were $\delta=0.5$ ms and $\Delta=10$ ms, while for diffusion of phospholipids were $\delta=1$ ms and $\Delta=100$ ms.

In figs. 4 and 5 the decay of spin echo intensities for waters and for phospholipids are shown in function of $Q^2\Delta$ for the three different temperatures (283 K, 295 K, 313 K); in the same figures the fitting curves, obtained by a modification of eq. 1, are also shown. The fitting function, in fact, takes in account for a fraction of sample randomly aligned with respect to mica sheets, **while the misaligned fraction amounts to about 25%. The same indication has been found by measuring in the same sample the alignment by ^{31}P NMR spectra evaluated following the procedure described in ref. 27.** In the case of water molecules we find a diffusion coefficient of $D_w = 0.90 \cdot 10^{-9} \text{ m}^2/\text{s}$, $1.11 \cdot 10^{-9} \text{ m}^2/\text{s}$, $1.36 \cdot 10^{-9} \text{ m}^2/\text{s}$, from low to high temperatures. The obtained values are in good agreement with literature values for water in restricted geometry with hydrophilic boundaries [36,37]. In the case of lipid molecules we find $D_l = 0.80 \cdot 10^{-12} \text{ m}^2/\text{s}$, $1.3 \cdot 10^{-12} \text{ m}^2/\text{s}$, $15.2 \cdot 10^{-12} \text{ m}^2/\text{s}$, for the same temperatures. **It can be observed that the lateral diffusion coefficient strongly increases at 313 K, that is when the full system is surely in the liquid-crystalline phase, where an enhanced dynamics of the acyl chains is expected.** The value of D_l we found at 313 K is in agreement with values found from Molecular Dynamics simulations and with average values obtained in similar systems, while the flattening of D_l below the main transition was not observed before due to the difficult application of this techniques to gel-solid samples. Moreover we have to mention that analyzing PGSE decay at different values of Δ , we observed evidence for restricted diffusion due to obstacles or confinements both for water and lipid molecules at all the temperatures.

3.4. Molecular Dynamics simulation

We report the results of a MD simulation of a fully hydrated DMPC bilayer in liquid-crystalline phase **at 313 K**. The bilayer consists of 64 DMPC lipid molecules, fully hydrated with $n_w=25.7$ water molecules/lipid.

The electron density profile (EDP) along the bilayer normal axis (Fig. 6), averaged for 3 ns, let understand the structure of the membrane and the distribution of water molecules and of the different functional groups of the lipid molecules into the bilayer. In particular the distance between the two peaks in the total EDP, defined as the headgroup spacing D_{HH} , identifies the phosphate groups; instead, the EDP minimum value is observed in correspondence to the center of the bilayer, where the methyl groups of the lipid tails are located. Moreover, water density profile indicates a deep penetration into the head group region of the lipid in the fluid phase. Our data are in good agreement with x-ray diffraction data [6] and previous simulations [38,39].

The main aim of the analysis of the MD trajectories was to study the diffusion of lipid molecules in the plane of the membrane, i.e. lateral diffusion. Molecular diffusion in complex systems usually occurs in three steps: a ballistic regime at very short times, a sub-diffusive rattling into a cage at intermediate times describing confined dynamics among the first neighbours and then a transition among different cages through a Fickian diffusion at long times. This picture has been also proposed to describe lateral diffusion of lipid molecules in membranes [1]. In this work the lateral diffusion coefficient has been evaluated from the trajectories of hydrogen atoms of the acyl chains considering the following equation:

$$D = \lim_{t \rightarrow \infty} \frac{1}{2d_f} \frac{d}{dt} \langle |r(t) - r(0)|^2 \rangle$$

where d_f is the system dimensionality ($d_f=2$ for lateral diffusion in the xy plane). In particular, we have evaluated the diffusion coefficient in two different regions, 300-500 ps and 1.5-3 ns, and we have obtained the values $D_l=21.7 \pm 1.0 \times 10^{-8}$ cm²/s and $D_l=22.0 \pm 1.0 \times 10^{-8}$ cm²/s, respectively. The closeness of these two values let us believe that they describe the same kind of diffusional motion, in agreement with Essmann's findings [40]. The obtained values are in good agreement with the experimental value of $D_l=18 \times 10^{-8}$ cm²/s determined by QENS technique [41], whose length and time scales match the ones of molecular dynamics simulation. Moreover, since these values also agree with our value obtained by NMR and literature ones [15] measured on longer time scale, we believe that lipids undergo ordinary diffusional motion in both short and long time scales.

Moreover, we have also studied the diffusion out of the membrane plane, i.e. the transversal diffusion along the z direction. Looking at Fig.7, where the mean squared displacements in the xy plane and in the z direction are reported, it is evident that, while the in plane MSD monotonically grows, the MSD along the normal to this plane shows an upper bound of $\sim 9 \text{ \AA}^2$, in agreement with neutron scattering results [1].

4. CONCLUSIONS

In closing, we note the importance of applying a number of physical techniques to studying various aspects of peptide-phospholipid interactions in order to arrive at a more complete understanding of these systems. Both Calorimetric and Neutron Scattering investigations reveal the destabilizing effect of gramicidin on membrane dynamics. In particular, for the first time neutron scattering shows that gramicidin promotes an increase in lateral mobility of acyl chains but also some restrictions on the in plane dynamics. ^1H -PGSE –NMR experiment provided information on long range lateral diffusion, up to some μm distances, of both inter-layer water and lipid component. In the case of water only a reduction of the diffusion coefficient is observed and assigned to the restricted geometry, while the phospholipid component shows as a novel and interesting result a nearly constant diffusion coefficient in the gel phase and a net increase on mobility in the liquid crystalline phase.

5. ACKNOWLEDGMENTS

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FIGURE CAPTIONS

Figure 1. DSC heating endotherms of fully hydrated DMPC/GrD mixtures in which mole ratio of GrA to DMPC are 0 (A) and 0.024 (B).

Figure 2. Temperature dependence of FEW scans integrated over all Q-range and normalized to the lowest temperature for both in plane (full symbols) and out of plane (empty symbols) components of DMPC (a) and DMPC/GrD (b)

Figure 3. Comparison among the temperature dependence of FEW scans of DMPC (full circle), DMPC/GrD (empty circle) and DMPC-D54/GrD (empty diamond) for both in plane (a) and out of plane (b) components. In the inset of panel (a) scans at $Q=1.47 \text{ \AA}^{-1}$ of DMPC/GrD and DMPC-D54/GrD, arbitrarily shifted along the intensity axis to make the comparison easier, are shown.

Figure 4. PGSE intensity decay with $\delta=0.5 \text{ ms}$ and $\Delta=10 \text{ ms}$ in fully hydrated DMPC bilayer at $T=283 \text{ K}$ (circle), 295 K (triangle) and 313 K (square). The continuous line are the fitting functions. In the inset ^1H NMR spectral lineshape is shown for $T=313 \text{ K}$.

Figure 5. PGSE intensity decay with $\delta=1.0 \text{ ms}$ and $\Delta=100 \text{ ms}$ in fully hydrated DMPC bilayer at $T=283 \text{ K}$ (circle), 295 K (triangle) and 313 K (square). The continuous line are the fitting functions.

Figure 6. The electron density profiles, $\rho(z)$, for the area of 60.7 \AA^2 as a function of distance from the centre of bilayer (z) along the bilayer normal for some components of DMPC.

Figure 7. Plot shows the mean square displacement (MSD) correlation function versus the time, for motion of the center-of-mass of the hydrogen atoms of the acyl chains in the direction parallel (dotted line) and normal (continuous line) to the membrane plane.

FIGURE 1

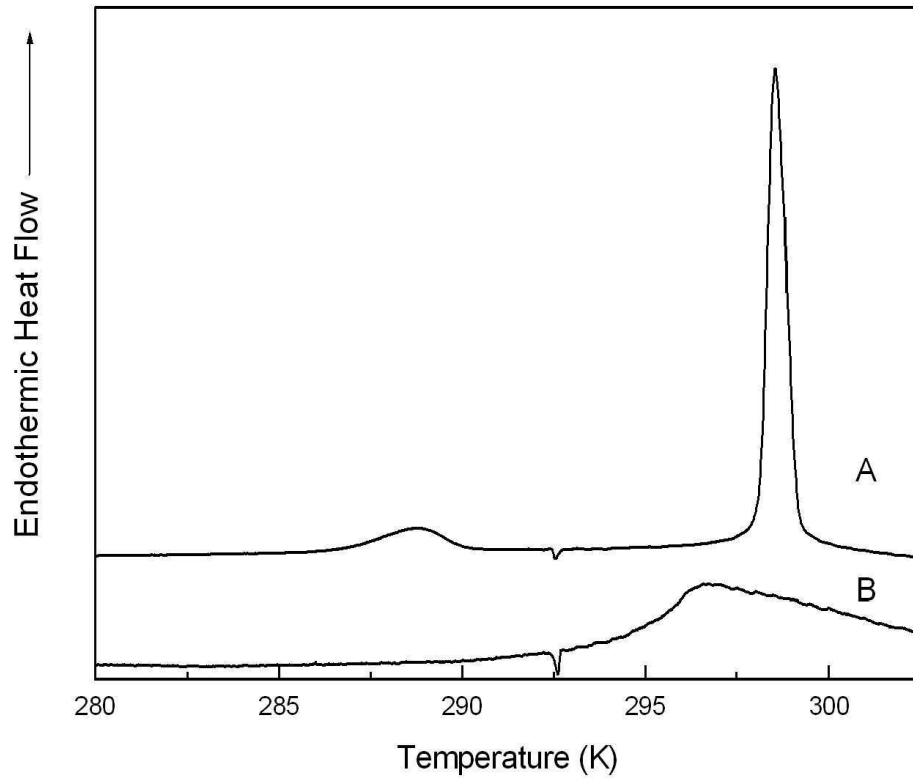


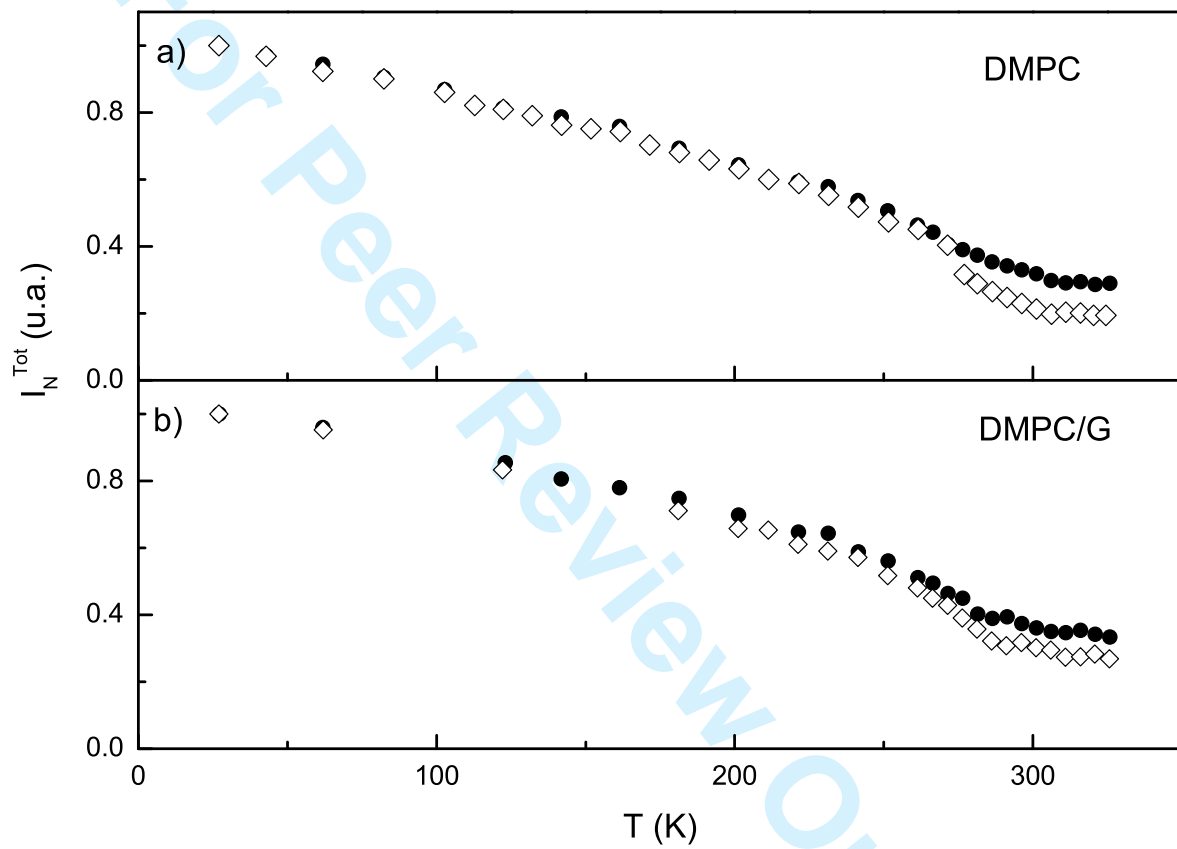
FIGURE 2

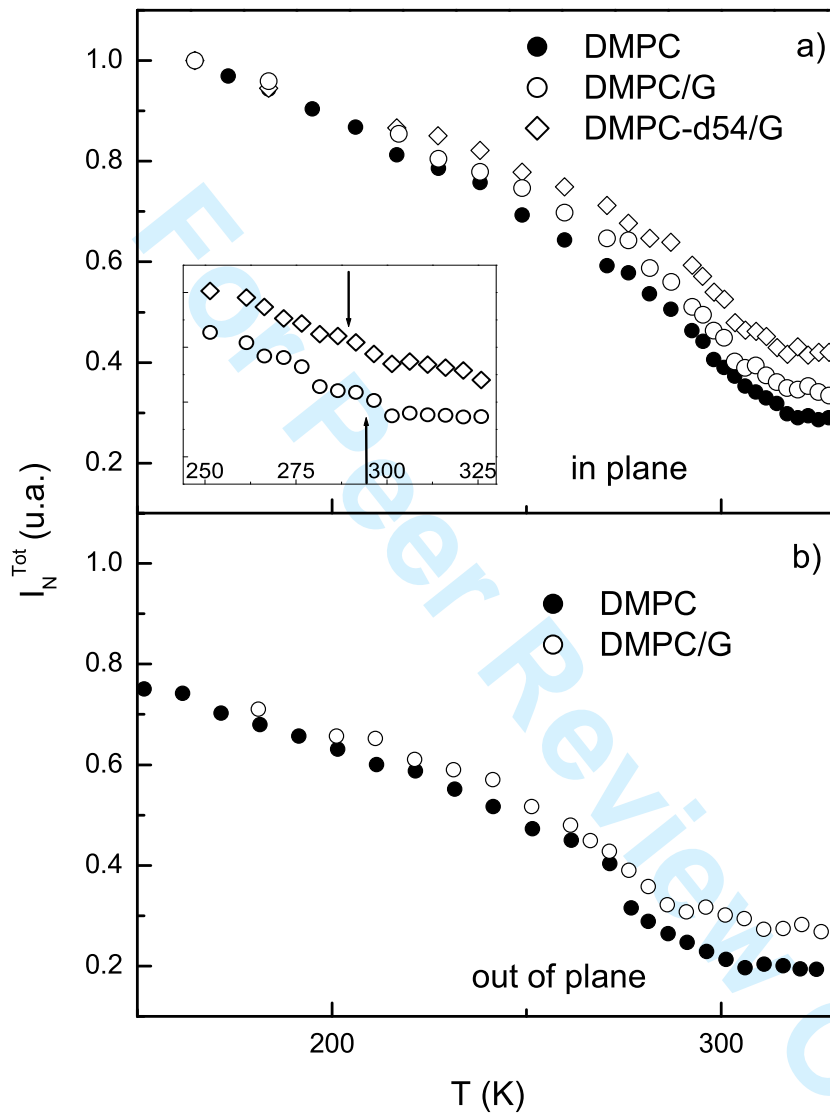
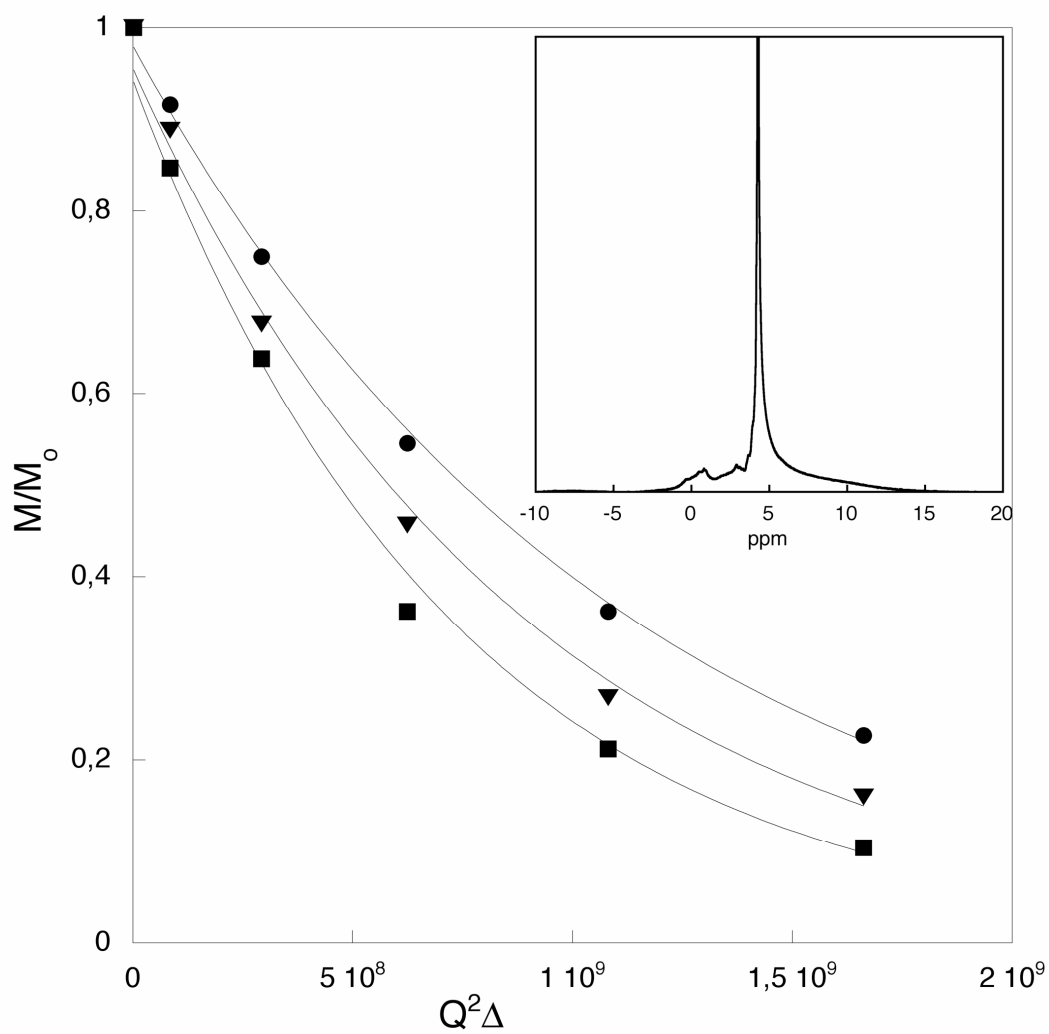
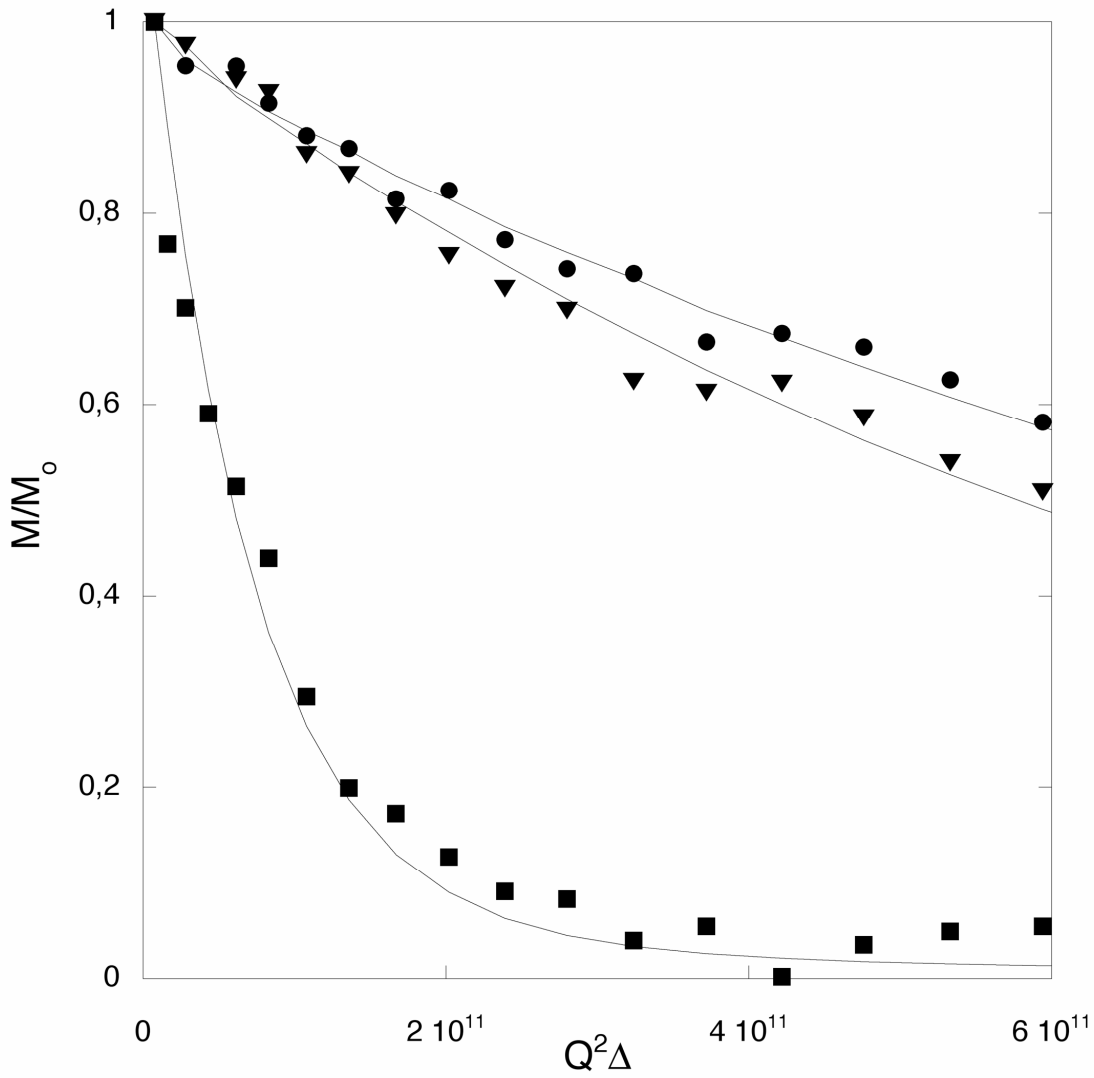
FIGURE 3

FIGURE 4

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FIGURE 5



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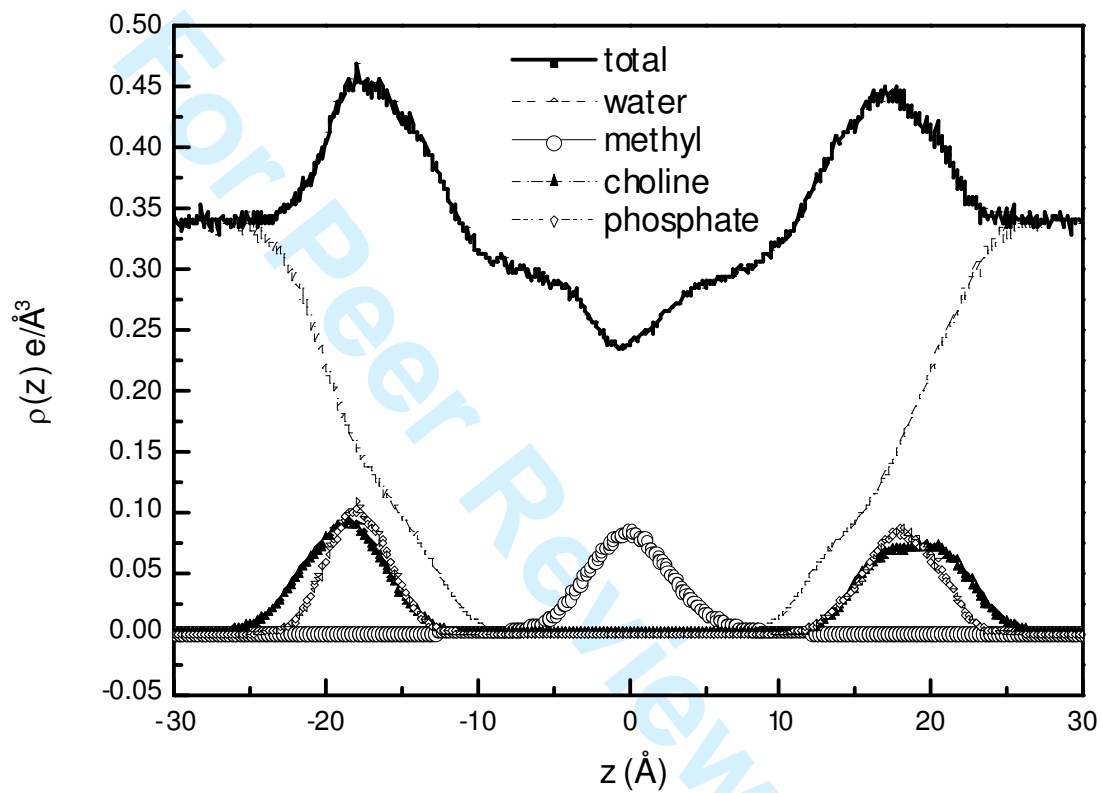
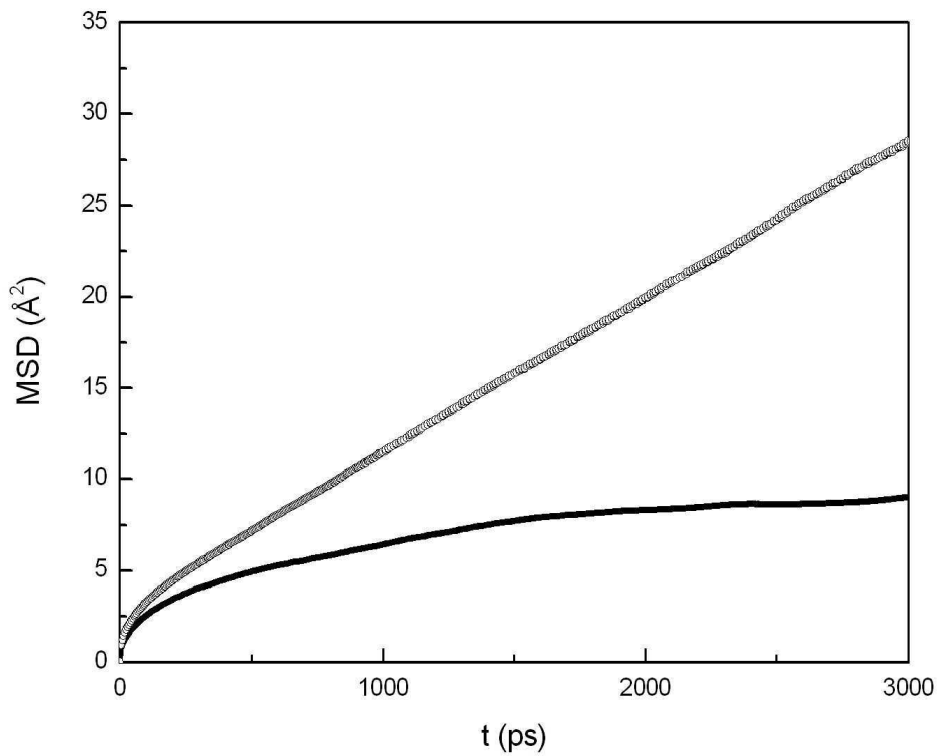
FIGURE 6

FIGURE 7



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