

# The dynamical Matryoshka model: 3. Diffusive nature of the atomic motions contained in a new dynamical model for deciphering local lipid dynamics

Tatsuhito Matsuo, Aline Cisse, Marie Plazanet, Francesca Natali, Michael Marek Koza, Jacques Ollivier, Dominique J. Bicout, Judith Peters

## ▶ To cite this version:

Tatsuhito Matsuo, Aline Cisse, Marie Plazanet, Francesca Natali, Michael Marek Koza, et al.. The dynamical Matryoshka model: 3. Diffusive nature of the atomic motions contained in a new dynamical model for deciphering local lipid dynamics. Biochimica et Biophysica Acta:Biomembranes, 2022, 1864 (9), pp.183949. 10.1016/j.bbamem.2022.183949. hal-03836694

# HAL Id: hal-03836694 https://hal.science/hal-03836694

Submitted on 2 Nov 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1	The dynamical Matryoshka model: 3. Diffusive nature of the atomic motions contained in a new
2	dynamical model for deciphering local lipid dynamics
3	
4	Tatsuhito Matsuo <sup>a,b,c</sup> , Aline Cisse <sup>a,b</sup> , Marie Plazanet <sup>a</sup> , Francesca Natali <sup>b,d</sup> , Michael Marek Koza <sup>b</sup> ,
5	Jacques Ollivier <sup>b</sup> , Dominique J. Bicout <sup>b,e</sup> , and Judith Peters <sup><math>a,b,f,\dagger</math></sup>
6	
7	<sup>a</sup> Univ. Grenoble Alpes, CNRS, LiPhy, F-38000 Grenoble, France
8	<sup>b</sup> Institut Laue-Langevin, 71 avenue des Martyrs, 38042 Grenoble Cedex 9, France
9	<sup>c</sup> Institute for Quantum Life Science, National Institutes for Quantum Science and Technology,
10	2-4 Shirakata, Tokai, Ibaraki, 319-1106, Japan
11	<sup>d</sup> CNR-IOM, OGG, 71 avenue des Martyrs, 38042 Grenoble Cedex 9, France
12	<sup>e</sup> Univ. Grenoble Alpes, CNRS, Grenoble INP, VetAgro Sup, TIMC, 38000 Grenoble, France
13	<sup>f</sup> Institut Universitaire de France
14	
15	<sup>†</sup> Corresponding author: Judith Peters, Ph.D.
16	Univ. Grenoble Alpes, CNRS, LiPhy, F-38000 Grenoble, France
17	Tel: +33 4 76 20 75 60
18	Email: jpeters@ill.fr
19	
20	
21	
22	
23	
24	

#### 25 Abstract

26 In accompanying papers [Bicout et al., BioRxiv 10.1101/2021.09.21.461198 (2021); Cissé et al., 27 BioRxiv 10.1101/2022.03.30.486370 (2022)], a new model called Matryoshka model has been proposed to describe the geometry of atomic motions in phospholipid molecules in bilayers 28 29 and multilamellar vesicles based on their quasielastic neutron scattering (QENS) spectra. Here, 30 in order to characterize the relaxational aspects of this model, the energy widths of the QENS spectra of the samples were analyzed first in a model-free way. The spectra were decomposed 31 into three Lorentzian functions, which are classified as slow, intermediate, and fast motions 32 33 depending on their widths. The analysis provides the diffusion coefficients, residence times, and geometrical parameters for the three classes of motions. The results corroborate the 34 35 parameter values such as the amplitudes and the mobile fractions of atomic motions obtained 36 by the application of the Matryoshka model to the same samples. Since the current analysis 37 was carried out independently of the development of the Matryoshka model, the present results enhance the validity of the model. The model will serve as a powerful tool to decipher 38 the dynamics of lipid molecules not only in model systems, but also in more complex systems 39 such as mixtures of different kinds of lipids or natural cell membranes. 40

- 41
- 42
- 4-
- 43
- 44
- 45

46 Keywords (maximum 6)

47 Matryoshka model, quasielastic neutron scattering, lipids, molecular dynamics, diffusion
48

#### 49 **1. Introduction**

50 Cell membranes play an essential role in cell function, including the boundary formation of 51 organelles and cells, and the transport of chemical substances exploiting their permeability. It is known that cell membranes consist of various types of lipids and alter their lipid composition 52 53 to adapt to extracellular environments such as temperature and pH so that they function 54 properly at given environments [1,2]. It is thus important to understand the physical and chemical properties of lipid molecules in detail to reveal the mechanism of their biological 55 functions. Lipid membranes can undergo phase transitions from crystalline, gel, to fluid phases 56 57 depending on temperature or pressure, which impact their dynamical properties as well as their 58 structures. The motions within membranes span a wide range of time- and space-scales: The 59 intramolecular motions, including the rotation of methylene groups, occur in the picosecond 60 regime, while large-scale motions, such as membrane undulations, take place from the 61 nanosecond to the millisecond regimes [3–7]. In order to understand their dynamical nature in 62 different physicochemical environments, various experimental techniques have been used such 63 as incoherent neutron scattering [8], nuclear magnetic resonance spectroscopy (NMR) [9], fluorescence recovery after photobleaching (FRAP) [10] depending on the time- and space-64 scale of interest. Together with the advancement in molecular dynamics (MD) simulation 65 techniques and their combination with experiments [11–13], a huge amount of knowledge of 66 individual and collective lipid motions has been accumulated. However, there does not still 67 68 exist a unified dynamical framework that describes comprehensively the lipid motions 69 occurring at different time domains.

70

Among the experimental techniques employed so far, quasielastic neutron scattering (QENS) is a powerful tool to study the dynamics of lipid molecules at the pico-nanosecond timescale

73 and Å-nm length scale, which correspond to the crossover region from localized vibrations to 74 various diffusional processes occurring at a much longer timescale involving bilayer bending 75 and membrane undulation. In particular, QENS provides detailed information on the motions of hydrogen atoms since the incoherent scattering cross-section of the hydrogen atom is much 76 77 larger than that of any other atom found in biomolecules or its isotope deuterium [14]. 78 Moreover, another advantage of this technique is that various motions which occur at different time scales can be focused on by changing the energy resolution of the neutron spectrometers, 79 which is equal to shifting the time window of the observation. 80

81

82 In order to interpret the QENS spectra, thereby capturing the dynamical properties of lipid 83 molecules in detail, establishment of a theoretical framework that describes relevant atomic 84 motions is indispensable. In 1989, the first theoretical model describing the lipid motions in bilayers was reported [4], where different motional processes were assumed depending on the 85 energy resolutions and the sample conditions. Similar approaches have widely been employed 86 87 not only for lipid molecules [5,15–17], but also for other molecules such as cholesterol inserted to lipid bilayers [18]. Later, a more sophisticated model has been presented, where QENS 88 spectra were decomposed into three kinds of motions with distinct time scales, i.e. slow, 89 intermediate, and fast motions [19,20]. In this model, each component of these three types of 90 motions was interpreted by various combinations of motions in lipid molecules. Although all 91 92 of these studies have significantly advanced our understanding of the dynamics of lipid 93 molecules and helped provide a physical picture, a more holistic description of the possible 94 motions contained in the lipid molecules was still missing.

95

96 Based on the dynamical models above, a new theoretical model named Matryoshka model has

97 been presented in the accompanying papers [21,22], where the dynamic structure factor S(Q,  $\omega$ ) is analytically expressed by directly incorporating the scattering functions of various motions 98 99 that lipid molecules undergo in addition to the decomposition of all the possible motions into three classes, i.e. slow, intermediate, and fast motions. This model successfully accounts for the 100 101 elastic incoherent structure factor (EISF) and the guasi-elastic incoherent structure factor (QISF) 102 profiles, which are derived from the geometry of atomic motions in the samples, of 1,2dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in multi-lamellar bilayers (MLB) and multi-103 lamellar vesicles (MLV), and 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DMPG) in 104 105 MLB at various temperatures. This has enabled the detailed investigation of the geometry of 106 motions in lipid molecules placed in various physicochemical environments. On the other hand, 107 although the diffusive nature of the motions classified as the slow, intermediate and fast motions in the Matryoshka model is theoretically characterized through a series of analytical 108 109 equations of relaxation rates of each of these motions [21], it has not yet been experimentally 110 characterized. In the present paper, this aspect of each of the three types of motions are 111 reported and discussed.

112

113 2. Materials and methods

#### 114 *2-1. Sample preparation and neutron scattering experiments*

Details of sample preparations, QENS experiments, and the analysis of the QENS spectra are described in the accompanying papers [21,22]. Briefly, the following samples were used for neutron experiments:

118 - DMPC in MLB oriented at 135° relative to the incident neutron beam (DMPC MLB135),

119 which mainly focuses on the in-plane motions

120 – DMPC in MLB oriented at 45° to focus on the out-of-plane motions (DMPC MLB45)

DMPC in MLB oriented at 135° with hydrogen atoms in the tails perdeuterated (d54DMPC)
 DMPC in MLV (DMPC MLV)

DMPG in MLB oriented at 135° relative to the incident neutron beam (DMPG MLB135),
 focusing on the in-plane motions

All the samples except for DMPG MLB135 were measured on the IN6 spectrometer 125 126 (https://www.ill.eu/users/instruments/instruments-list/sharp/description/instrument-layout) at the Institut Laue-Langevin (ILL; in Grenoble, France) with the energy resolution of ~70 µeV, 127 128 which corresponds to a time window of ~20 ps. The spectra of DMPC MLB135 and DMPG 129 MLB135 measured the IN5 spectrometer were on (https://www.ill.eu/users/instruments/instruments-list/in5/description/instrument-layout) 130 at 131 the ILL with the energy resolution of ~90 µeV (~15 ps). Each spectrum was fitted by the following phenomenological equation [14] that contains three types of motions with different 132 133 relaxational times:

134

135 
$$S_{lipids}(Q,\omega) = C(Q) \left[ A_0(Q)\delta(\omega) + \sum_{i=1}^3 A_i(Q)L_i(Q,\omega) \right] \otimes R(Q,\omega) + B(Q), \tag{1}$$

136

137 where  $C(Q) = Ce^{-(u^2)Q^2}$  represents the scaling factor C including the Debye-Waller factor with 138  $\langle u^2 \rangle$  the mean square amplitude of vibration,  $A_0(Q)\delta(\omega)$  is an elastic component with  $A_0(Q)$ 139 being the elastic incoherent structure factor (EISF) and  $\delta(\omega)$  being the Dirac delta function.  $L_i(Q, \omega)$ 140  $\omega$ ) (=  $(1/\pi) \times (\Gamma_i(Q) / (\Gamma_i(Q)^2 + \omega^2))$ , and i = 1, 2, 3) is the i-th Lorentzian function describing the 141 atomic motion in lipids.  $\Gamma_i(Q)$  is the half-width at half-maximum (HWHM) of  $L_i(Q, \omega)$ . R(Q,  $\omega$ ) 142 refers to the instrumental resolution function, which is obtained from the Vanadium

measurement, B(Q) is a flat background, that includes the instrument contribution, and/or fast 143 vibrational motions outside the current time window. Finally,  $\otimes$  denotes the convolution 144 145 operation. In the following, the three Lorentzian functions are classified as the slow motions (the narrowest Lorentzian), the intermediate motions, and the fast motions (the broadest 146 Lorentzian). The spectra in the range of 0.37 Å<sup>-1</sup>  $\leq$  Q  $\leq$  2.02 Å<sup>-1</sup> (for the IN6 data) and 0.33 Å<sup>-1</sup> 147  $\leq Q \leq 1.82 \text{ Å}^{-1}$  (for the IN5 data) were used for the fitting. Note, however, that for the spectra 148 obtained on IN6, the HWHMs of the Lorentzian functions at the first two lowest Q values were 149 found to show unrealistically higher values than other data points probably due to multiple 150 scattering effects. Therefore, the spectra in the range of 0.72 Å<sup>-1</sup>  $\leq$  Q  $\leq$  2.02 Å<sup>-1</sup> were analyzed 151 for IN6 data and the data points at Q = 0.37  $[Å^{-1}]$  and 0.55  $[Å^{-1}]$  were excluded from the analysis 152 and not presented in this study. Fits were carried out in the range of -10 meV  $\leq \Delta E \leq 2$  meV 153 (for the IN6 data) and -10 meV  $\leq \Delta E \leq 1.3$  meV (for the IN5 data) using IGOR Pro software 154 155 (WaveMetrics, Lake Oswego, OR, USA). Some examples of fits are shown in Fig. 1.





Figure 1. Examples of fits of the QENS spectra of DMPC MLB135 measured on IN6 (a) and IN5 (b). (a) and (b) are the data at Q =  $1.23 \text{ Å}^{-1}$  and T = 283 K, and at Q =  $1.25 \text{ Å}^{-1}$  and T = 280 K,

160 respectively. The grey circles are the experimental data points with corresponding statistical 161 errors. The total fit is represented by the black line. The green line corresponds to the elastic 162 peak convoluted by the resolution function, which is directly given by the Vanadium 163 measurements. The magenta, orange and blue curves are the Lorentzian functions convoluted 164 by  $R(Q, \omega)$  for slow, intermediate and fast motions, respectively. The upper panels show the 165 residuals of the fits.

166

#### 167 2-2. Lipid motions contained in the Matryoshka model

In the Matryoshka model [21] as shown schematically in Fig. 2, a lipid molecule is represented by a head group and a tail group (with only one tail instead of two for the sake of simplicity), which contain a fraction z and a fraction (1-z) of the total number of hydrogen atoms, respectively. The values of z are 0.25 and 0.18 for DMPC and DMPG, respectively. The slow, intermediate, and fast motions contain the following internal and/or collective motions of the lipid molecules (Table 1):

174 (i) The slow motions contain the following two movements:

- A whole lipid molecule undergoes two-dimensional diffusion (2D-diffusion)
   within the membrane plane. The space where the lipid molecule can move is
   represented by a cylinder with the radius of R<sub>II</sub>.
- The whole lipid molecule undergoes one-dimensional diffusion in the direction
   perpendicular to the membrane plane (in-out of the plane diffusion). This process
   is characterized by the force constant k, which characterizes the rigidity of the
   membrane in the normal direction of the membrane plane.
- 182 (ii) The intermediate motions include the following three motions:
- The whole lipid molecule undergoes rotational diffusion around its long axis. This
   process is characterized by the distance between the long axis and the center of
   inertia of all the hydrogen atoms R<sub>H</sub>.
  - 8

- 186 The head group performs a head-flip-flop motion between the angles  $\pm \alpha$  with 187 respect to the normal axis. The  $\alpha$  values are 32.3° and 30° for DMPC and DMPG, 188 respectively [23,24].
- The head group also performs a rotational motion around its own axis with the
   radius b<sub>H</sub>.
- 191 (iii) The fast motions include the following two motions:
- The tail group undergoes two-dimensional diffusion within the membrane plane 192 — 193 with its radius increasing as a function of the distance of the H atoms from the head group along the tail axis. This radius is defined as  $\sqrt{mR_1}$ , where R<sub>1</sub> is the 194 radius of the 2D-diffusion of H atoms closest to the head group and m denotes 195 196 the index number of a methylene/methyl group of the tail group. The range of m 197 is  $1 \le m \le M$ , where M is the total number of methylene/methyl groups on the tail (M = 14 for both DMPC and DMPG). Such model limits the radius at the 198 199 bottom of the tail, which is constrained by other lipids in a membrane.
- 200 Hydrogen atoms in the methylene and methyl groups in both the head and tail 201 groups undergo rotational jump-diffusion. Two-sites jump diffusion was assumed, 202 where the residence times of the two sites are  $\tau_1$  and  $\tau_2$ , respectively, with the jump 203 distance corresponding to the H-C-H distance of d = 2.2 [Å] [25].
- The above parameters  $R_{\parallel}$ , k,  $R_{H}$ ,  $b_{H}$ ,  $R_{1}$ , and  $\phi (= \tau_{1}/(\tau_{1}+\tau_{2}))$ , which characterize the geometry of motions of atoms in the lipids, were determined by the EISF/QISF analysis in the accompanying papers [21,22].



207

Figure 2. Schematic illustration of the Matryoshka model. A lipid molecule in membranes is drawn, on which parameters employed in the Matryoshka model are shown (for details of the parameters, refer to the main text). This figure is taken from Figure 2 of [21].

211

212 Table 1. Classification of various motions contained in the Matryoshka n	nodel.
--	--------

	Internal motion	Collective motion
Slow motions		2D-diffusion
		in-out of the plane diffusion
Intermediate motions	Head-flip-flop	Rotational diffusion
	Rotation (head)	
Fast motions	2D-diffusion (tail)	
	Jump diffusion (tail and head)	

213

### 214 3. Results and discussion

215 *3.1. Slow motions* 

The Q<sup>2</sup>-dependences of  $\Gamma_1(Q)$ , which corresponds to the slow motions, of all the samples are shown in Fig. 3. It is clearly seen that the  $\Gamma_1(Q)$  values of all the samples do not reach zero as Q approaches 0.0 [Å<sup>-1</sup>] while they show asymptotic behavior at higher Q<sup>2</sup> values, suggesting that the underlying process of this behavior is the jump diffusion within a confined space [14]. Note 220 that in the previous study [19], the narrowest Lorentzian function of the DMPC MLB135 was interpreted as a continuous diffusion within a confined space. This discrepancy is due to the 221 222 fact that the energy resolutions in the current study are much higher and the accessible Q-223 ranges are much larger than in the previous study, suggesting that the present data represent 224 faster and smaller-scale motions. Therefore, the jump-diffusional behavior of  $\Gamma_1(Q)$  indicates 225 that each step of the global jump diffusion of the entire lipid molecule is resolved here. In the case of DMPC MLB45 at 283 K and 311 K, the  $\Gamma_1(Q)$  values might be constant over the Q-range 226 measured, suggesting that these motions are described by a diffusion on a linear segment or 227 228 by rotational diffusion [26]. Since it is not possible to distinguish the type of motions due to 229 the limited data points and large error bars, analysis was conducted for both types of motions 230 for these two conditions.



232

Figure 3.  $Q^2$ -dependences of  $\Gamma_1(Q)$  of DMPC MLB135 (a), DMPG MLB135 (b) measured on IN5, 233 234 DMPC MLB135 (c), d54DMPC MLB135 (d), DMPC MLB45 (e), and DMPC MLV (f) measured on 235 IN6. (a, b): The data points at 280 K and 295 K are shown in cyan and magenta, respectively. (c-236 f): The data points at 283 K, 311 K, and 340 K are shown in purple, cyan, and magenta, respectively. The solid lines show the corresponding fits by the equation describing the jump-237 238 diffusion (Eq. (2)) and the dotted lines (DMPC MLB45 at 283 K and 311 K) are the fits by the 239 equation describing the diffusion on a linear segment ( $\Gamma_1(Q) = 1/\tau_1$ , where  $\tau_1$  is the correlation 240 time.). Error bars are within symbols if not shown. 241

The radius of the confined space (*a*), which is related to the center-of-mass diffusion, was estimated from the Q<sub>0</sub> value, at which  $\Gamma_1(Q)$  begins to increase, based on the relation  $a = \pi/Q_0$ [27]. Since some data points were missing for DMPC MLB45 due to the experimental 245 configuration, the *a* values were estimated for the other three samples, which were found to 246 be 2.9–3.5 Å and 3.6–6.6 Å for IN6 and IN5, respectively, as shown in Table 2. The estimated a values of DMPC MLB135 are largely different between IN6 and IN5. One possibility of this 247 difference would be the fact that it is not easy to determine the Q<sub>0</sub> value due to the limited 248 data points and fluctuations of the  $\Gamma_1(Q)$  values in the low-Q region. Overall, the estimated a 249 250 values here are smaller than the lateral distance between phospholipid molecules. However, since these values correspond to those resolved by the current time-windows, it is not 251 252 surprising to obtain such smaller values.

253

#### Table 2. Estimated radii of the confined space *a* [Å] for slow motions

	280 K	283 K	295 K	311 K	340 K
DMPC MLB135 (IN6)		2.9		2.9	2.9
DMPC MLB135 (IN5)	6.6		6.6		
d45DMPC MLB135 (IN6)		3.5		3.5	n/a
DMPC MLB45 (IN6)		n/a		n/a	n/a
DMPC MLV (IN6)		3.5		3.5	3.5
DMPG MLB135 (IN5)	3.6		4.2		

<sup>255</sup> 

Next, dynamics' parameters of the slow motions were estimated by the fitting of the  $\Gamma_1(Q)$ values using the equation describing the jump diffusion [14];

259 
$$\Gamma_1(Q) = D_1 Q^2 / (1 + D_1 Q^2 \tau_1),$$
 (2)

where D<sub>1</sub> is the jump diffusion coefficient and  $\tau_1$  is the residence time before the jump. Furthermore, based on these dynamics' parameters, the jump distance I<sub>1</sub> was also estimated by the relation I<sub>1</sub> =  $\sqrt{(4D_1\tau_1)}$  for in-plane motions, I<sub>1</sub> =  $\sqrt{(2D_1\tau_1)}$  for out-of-plane motions, and I<sub>1</sub> =  $\sqrt{(6D_1\tau_1)}$  for MLVs. In addition to this analysis, for DMPC MLB45 at 283 K and 311K, the correlation time was also calculated using the relation  $\tau_1 = 1/\Gamma_1(Q)$  assuming the diffusion on a

<sup>256</sup> 

265 linear segment. However, the obtained  $\tau_1$  values were the same within the errors as those 266 obtained by the jump-diffusion model (Table S1). Therefore, in the following, discussion is 267 made based on the dynamics' parameters obtained by the jump-diffusion model. The summary of the obtained dynamics' parameters is shown in Fig. 4 (the  $D_1$  and  $\tau_1$  values are tabulated in 268 269 Tables S1 and S2). The obtained parameters of DMPC MLB135 and DMPC MLB45 are found to 270 be similar within the errors except at 340 K. This is in agreement with a MD simulation study showing that the center-of-mass diffusion coefficients of DMPC at 303 K are essentially the 271 272 same between the in-plane and the out-of-plane directions (the difference is less than 10%) 273 [28].

274

275 When comparing the D<sub>1</sub> values of d54DMPC MLB135 and DMPC MLB135, there is a tendency that  $D_1$  of the former is slightly smaller at both temperatures although these differences are 276 277 within errors. The former mainly derives from the contribution of the head group and the latter 278 contains contributions of both the head and the tail groups. This result thus implies that the 279 diffusion of the head group might be slower than that of the tail group, which supports an assumption made in the Matryoshka-model, where the tail group motions are faster than the 280 281 head group motions [21,22]. The tail group would undergo diffusive motions around the hinge 282 between the tail and the head groups while the head group is less mobile, which increases the 283 D<sub>1</sub> value of the whole molecule compared with that of the head group as observed here. In a previous QENS study on the bilayers of dipalmitoyl-phosphatidylcholine (DPPC) with and 284 without deuteration of its tail group, the same tendency has been observed [29]. The EISF/QISF 285 286 analysis by the Matryoshka model on the same samples has shown that the diffusion radius of the head group (d54DMPC MLB135) on the membrane plane is larger than that of the whole 287 molecule [21,22]. Together with this finding, the present results imply that the head group of 288

289 DMPC takes more time to explore the available space than the tail group.

290

291 The dynamics' parameters of the slow motions of DMPC MLB135 were found to be larger for IN5 than for IN6. The geometry of motions for the slow motion of DMPC MLB135 is also slightly 292 293 different between the measurements on IN6 and those on IN5 [22]. Because MLBs measured 294 on IN6 were more hydrated than those measured on IN5 (27 wt% and 10 wt% hydration for IN6 and IN5 measurements, respectively) and thus enhanced dynamics is expected for IN6, the 295 differences in dynamics' parameters between IN5 and IN6 observed here are not due to the 296 297 difference in sample environments, but suggest that there is a distribution in the diffusive motions classified as the slow motions. The present results suggest that slight differences in 298 299 atomic motions are detected by QENS and seen by application of the Matryoshka model, which 300 proves their high sensitivity to decipher the dynamical nature of the samples.

301

302 The D<sub>1</sub> values and the jump distance of DMPC MLB135 obtained on IN5 were found to be 303 larger than those of DMPG MLB135, indicating that the diffusive motions are restrained in 304 DMPG. Since the head group of DMPC has a larger mass than that of DMPG (the molecular weights are 104 and 92 Da for the head groups of DMPC and DMPG, respectively), it is expected 305 that DMPC undergoes slower diffusive motions. However, the head group of DMPC possesses 306 a positive charge, but is more hydrophobic than that of DMPG, which has two hydroxyl groups 307 308 and thus polarity. It is likely that the hydration water molecules form less H-bonding with the 309 head groups of DMPC than those of DMPG, indicating that the atoms in the head group of 310 DMPC can move more freely. Because of these differences, the motions of the whole lipid 311 molecule would also be enhanced for DMPC compared with DMPG.

312



313

Figure 4. Temperature dependence of the residence time (τ<sub>1</sub>), the diffusion coefficient (D<sub>1</sub>), and
the jump distance (l<sub>1</sub>) of the slow motions. The parameter values of DMPC MLB135, d54DMPC
MLB135, DMPC MLB45, and DMPC MLV measured on IN6 are shown in cyan, magenta, orange,
and green, respectively. Those of DMPC MLB135 and DMPG MLB135 measured on IN5 are
shown in blue and pale orange, respectively.

Regarding the comparison between DMPC MLB135 and DMPC MLV on IN6, it is found that while the  $\tau_1$  values are similar within the errors, the D<sub>1</sub> value and the jump distance are larger for MLVs at 283 K and 311 K. This could be due to the fact that the MLVs are generally more hydrated than MLBs [13]. The present results are also consistent with the results of the EISF/QISF analysis using the Matryoshka model, where the radius of a circle within which the whole lipid molecule can diffuse is larger for MLVs than MLBs [22]. 326

#### 327 *3.2. Intermediate motions*

The  $Q^2$ -dependences of  $\Gamma_2(Q)$  of all the samples are shown in Fig. 5. The profiles of all the 328 samples, except for DMPC MLB45, show non-zero values at low-Q regions, followed by an 329 330 asymptotic behavior, suggesting that these motions are described by jump diffusion within a 331 confined space [14]. On the other hand, the profiles for DMPC MLB45 do not show a Q<sup>2</sup>-332 dependent behavior, indicating that this motion is described by a diffusion on a linear segment 333 or rotational diffusion [26]. Since the measurement of DMPC MLB45 mainly observes the 334 motion perpendicular to the membrane plane, it is reasonable that only a one-dimensional 335 motion is observed.



Figure 5. Q<sup>2</sup>-dependences of  $\Gamma_2(Q)$  of the intermediate motions. The color scheme is the same as in Fig. 3. The solid lines show the fits by the equation describing the jump diffusion ( $\Gamma_2(Q)$ =  $D_2Q^2/(1 + D_2Q^2\tau_2)$ ) or by the equation describing the diffusion on a linear segment ( $\Gamma_2(Q) = 1/\tau_2$ ) for DMPC MLB45 (for the definition of the parameters, see the main text). Note that the scales on the ordinates are not the same in all the panels such that the fitting curves are better seen.

In order to obtain the dynamical parameters related to intermediate motions, the profiles in Fig. 5 were fitted by the equation describing the jump diffusion model,  $\Gamma_2(Q) = D_2Q^2/(1 + D_2Q^2\tau_2)$ , where D<sub>2</sub> is the jump diffusion coefficient and  $\tau_2$  is the residence time before the jump [14] in the Q<sup>2</sup>-range between 0.81 Å<sup>-2</sup> and 4.08 Å<sup>-2</sup> for the IN6 data and the Q<sup>2</sup>-range between 0.58 Å<sup>-2</sup> and 3.30 Å<sup>-2</sup> for the IN5 data. Furthermore, based on these dynamics' parameters, the jump distance  $l_2$  was also estimated by the relation  $l_2 = \sqrt{(4D_2\tau_2)}$  for in-plane motions and  $l_2 = \sqrt{(6D_2\tau_2)}$ for MLVs. The correlation time of DMPC MLB45 was calculated using the relation  $\tau_2 = 1/\Gamma_2(Q)$ .

The residence time (correlation time), jump diffusion coefficient, and the jump distance parameters obtained by the above fits are shown in Fig. 6 (a), (b), and (c), respectively (the parameter values are tabulated in Tables S1 and S2). It is found that as the temperature increases, the  $\tau_2$  values slightly decrease. There are no significant differences in the  $\tau_2$  values between DMPC MLB135 and DMPC MLB45, suggesting that the degree of interaction between atoms is similar in directions both parallel and perpendicular to the membrane plane.



Figure 6. Temperature dependence of the residence time ( $\tau_2$ ), the diffusion coefficient (D<sub>2</sub>), and the jump distance (l<sub>2</sub>) of the intermediate motions. The color scheme is the same as in Fig. 4.

362 The  $\tau_2$  values of DMPC MLV were found to be smaller than those of DMPC MLB135, especially 363 at 283 K and 311 K, while the D<sub>2</sub> values and the I<sub>2</sub> values were similar between them. This suggests that the atoms in MLVs move more rapidly than those in MLBs. Since lipid molecules 364 are generally more hydrated in MLVs than in MLBs [13], it is likely that more hydration water 365 366 enhances the frequency of the local motions in MLVs. Moreover, the mobile fraction of atoms, which is estimated by the Matryoshka model [22], is smaller for DMPC MLB135 than for DMPC 367 MLV, suggesting that there exist more hydrogen atoms in MLBs that undergo slower motions 368 369 than those in MLVs.

370

371 The D<sub>2</sub> values shown in Fig. 6 (b) were larger than the translational diffusion coefficients of bulk water, which are  $2.0-2.3 \times 10^{-5}$  cm<sup>2</sup>/s at 293–300 K [30–32] although the residence times were 372 373 larger than that of bulk water. The same phenomenon was previously reported for protein dynamics: A QENS study on human acetylcholinesterase (hAChE) has shown that the jump-374 375 diffusion coefficients of atoms in hAChE were lower or even higher than those of bulk water at 90 µeV and 50 µeV energy resolutions, respectively [33]. Since the time-scale and the length-376 377 scale of the observable motions depend on the energy resolution employed, the D<sub>2</sub> values obtained in this study for atomic motions in lipids should be regarded as "effective" jump-378 379 diffusion coefficients, which depend on the instrumental energy resolution. It would thus be crucial to study the diffusive nature of local atomic motions in a wide range of time- and length-380 scale to obtain the holistic physical picture of the dynamical behavior of lipid molecules. 381

382

383 The D<sub>2</sub> values of d54DMPC MLB135 were found to be much smaller than those of DMPC 384 MLB135 while the  $\tau_2$  values tend to be larger for the former, resulting in smaller l<sub>2</sub> values for

d54DMPC MLB135 than for DMPC MLB135. This suggests that the local atomic motions of the 385 386 tails are faster than those of the head groups. This is in good agreement with the Matryoshka 387 model [21], where it was necessary to ascribe the contribution of the tail groups to the fast motions in order to fully account for the EISFs and QISFs of the current samples. Since the 388 389 Matryoshka model was established independently of the width analysis of Lorentzian functions 390 described in this paper, this agreement further enhances the validity of the Matryoshka model. Moreover, it has been shown that the energetic barrier between conformational substates is 391 higher for the head groups than for the tail groups [34], which is consistent with the present 392 393 results.

394

395 The jump distances of the in-plane motions of the head group and whole molecule of DMPC were found to be ~2 Å and 3-4 Å, respectively. The latter value corresponds to the distance 396 397 between the neighboring alkyl chains in the lipid molecules. On the other hand, according to the analysis based on the Matryoshka model, the radii within which the whole molecules can 398 399 diffuse around are 1.8–2.4 Å (i.e. 3.6–4.8 Å in diameter) and 1.1–2.1 Å (2.2–4.2 Å in diameter) 400 for the corresponding samples, respectively [22]. The comparison of these values suggests that while the whole DMPC molecule undergoes a jump motion over a distance corresponding to 401 the neighboring alkyl chains, its head group moves by about half of the maximum distance 402 available for it. This behavior of the head group could be due to the head-flip-flop motion as 403 404 introduced in the intermediate motion of the Matryoshka model, which changes the distance 405 between the centers of mass of the head group and the tail groups projected onto the 406 membrane plane. Furthermore, the current study suggests that all of the above motions take 407 place during the timescale of 1–2 ps on average.

408

409 As for DMPC MLB135, all of the three parameter values obtained from the IN5 data were similar 410 to those obtained from the IN6 data, showing that the identical motions are resolved at both energy resolutions unlike the slow motions described above. When comparing the parameters 411 between DMPC MLB135 and DMPG MLB135 on IN5, the residence time of DMPC was smaller 412 413 than that of DMPG while the jump diffusion coefficient of DMPC is larger, resulting in a slightly larger jump distance for DMPC. This indicates that the local motions are enhanced for DMPC 414 compared to DMPG, which is in agreement with the findings for the slow motions. The 415 reduction in local dynamics of DMPG MLB135 compared with DMPC MLB135 is consistent with 416 the results obtained from the EISF/QISF analysis by the Matryoshka model where the radius of 417 418 the two-dimensional diffusion of the tail group on the membrane plane is smaller for DMPG 419 [22].

420

#### 421 *3.3. Fast motions*

As shown in Fig. 7 (a-f), the widths of the broadest Lorentzian functions corresponding to the 422 423 fast motions were found to be independent of Q for all the samples, suggesting that these 424 motions are mainly described by rotational jump diffusion [14] as seen in hydrogen atoms in the methylene and methyl groups. On the other hand, the Matryoshka model assumes another 425 kind of motions in addition to the rotational jump diffusion for the fast motions, which is the 426 two-dimensional diffusion of the hydrogen atoms in the tail groups within the membrane plane. 427 The width of the Lorentzian function describing this motion would increase as the Q<sup>2</sup> values 428 429 rise, which is not observed for  $\Gamma_3(Q)$  here, suggesting that the contribution of the rotational 430 jump diffusion to  $\Gamma_3(Q)$  is dominant. This is in line with the result of the analysis based on the Matryoshka model in that the integrated value of the A<sub>jump</sub>(Q) over the Q-range employed is 431 much larger than that of  $A_{tail}(Q)$  [21]. 432

433

434 The correlation time of these motions was calculated from the relation  $\tau_3 = 1/\Gamma_3(Q)$ , and its dependence on the temperature is shown in Fig. 7 (g) (the  $\tau_3$  values are tabulated in Tables S1 435 and S2). The τ<sub>3</sub> values of DMPC MLB135 on IN6 and DMPC MLV are found to be independent 436 437 of the temperature and to be the same within the errors, suggesting that the frequency of the 438 methyl and methylene rotations is independent of the local environments in which they reside 439 (i.e. in the MLB or in the MLV). The  $\tau_3$  values of d54DMPC MLB135 are larger than those of DMPC MLB135, indicating that the rotational jump diffusion is enhanced for the tail group 440 compared with for the head group, which is consistent with the basic assumption of the 441 442 Matryoshka model that the tail motions are faster than the head group motions [21,22]. The  $\tau_3$ values of DMPC MLB45 are found to be larger than those of DMPC MLB135, implying that the 443 444 fast motions show anisotropy (i.e. parallel or perpendicular to the membrane plane).

445

The  $\tau_3$  values of DMPC MLB135 measured on IN5 were found to be slightly smaller than those 446 of DMPG MLB135, suggesting that the frequency of the rotational jump diffusion of the methyl 447 448 and methylene groups is higher for DMPC MLB135, which is consistent with the enhanced dynamics of DMPC MLB135 for the slow and the intermediate motions. Furthermore, the  $\tau_3$ 449 450 values of DMPC MLB135 were similar between IN5 and IN6, suggesting that the same type of 451 motions is observed on both spectrometers as the intermediate motions. It thus appears that the different hydration state of the DMPC MLB135 between IN5 and IN6 as described above 452 453 does not significantly affect the intermediate and fast motions. In the Matryoshka model, it 454 was necessary to include the contribution of the rotational jump diffusion in the methyl and methylene groups in order to describe the QISF of the fast motion [21,22]. The present results 455 456 indicate that the rotational jump in these chemical groups occurs every 0.2 ps.



Figure 7. Summary of the parameters obtained for the fast motions. (a-f):  $Q^2$ -dependences of  $\Gamma_3(Q)$ . The color scheme is the same as in Figs. 3 and 5. The solid lines show the corresponding fits by the equation describing the rotational jump diffusion ( $\Gamma_3(Q) = 1/\tau_3$ ) (see also the text). (g): Temperature dependence of the correlation time ( $\tau_3$ ) of the fast motions. The color scheme is the same as in Figs. 4 and 6. 464

### 465 *3.4 Comparison of* $\Gamma_1(Q)$ , $\Gamma_2(Q)$ , and $\Gamma_3(Q)$ between experiments and Matryoshka model

466 All of the above analyses were carried out independently of the application of the Matryoshka

467 model and despite this fact, the present findings support the results obtained by the EISF/QISF

468 analysis using this model [21,22]. In order to see further whether the Matryoshka model can reproduce the energy widths of the QENS spectra, the experimental  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and  $\Gamma_3(Q)$ 469 470 values were compared with those based on the Matryoshka model. Analytical equations of 471  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and  $\Gamma_3(Q)$  based on this model, which are given in the Supplementary material, were 472 fitted to the measured corresponding profiles. Note that only the samples other than MLVs were 473 used for the fitting, because the derivation of the analytical expressions of  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and  $\Gamma_3(Q)$ 474 for MLVs is not straightforward. In the fitting, the values of the parameters (R<sub>II</sub>, k, R<sub>H</sub>, b<sub>H</sub>, R<sub>1</sub>, and φ), which were determined by the EISF/QISF analysis [22], were fixed except for the fitting of 475 476  $\Gamma_2(Q)$  with the following cases:

- 477  $R_H$  of DMPC MLB135 at 283 K on IN6
- 478  $R_1$  of DMPC MLB135 at 340 K on IN6
- 479  $b_H$  of d54DMPC MLB135 at 283 K and 311 K on IN6

480 - b<sub>H</sub> of DMPC MLB135 and DMPG MLB135 at 280 K and 295 K on IN5

The above parameters were allowed to change during the fitting because of their large errors 481 482 estimated by the EISF/QISF analysis [22]. The fitting results are shown in Fig. 8 (the values of 483 each parameter are tabulated in Tables S3 and S4). Although in some cases, the above parameter values were outside the range determined at the corresponding temperature by the 484 485 EISF/QISF analysis [22] (these values are shown with asterisk in Tables S3 and S4), all of these 486 parameter values were still within the range determined at other temperatures, suggesting that these values are not physically unrealistic ones. From Fig. 8, it is seen that the resultant fitting 487 488 curves are able to reproduce the experimental  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and  $\Gamma_3(Q)$  values quite well regardless of the instruments. Regarding the F<sub>1</sub>(Q) of in-plane motions, i.e. DMPC MLB135, DMPG MLB135, 489 and d54DMPC MLB135, as already discussed in the section 3.1 (see also Fig. 3), because of the 490 491 wide Q-range and the low energy resolutions, our experimental data appear to permit to see

the discrete steps of the global diffusion of the whole lipid molecules. Thus, description of such detailed motions, which are independent of EISF/QISFs, would improve further the quality of the fitting. All in all, the Matryoshka model can reproduce not only the EISF/QISF profiles, but also the energy widths of the QENS spectra, enhancing the reliability of this model to describe the lipid dynamics.

497

498



Figure 8. Comparison of the experimental  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and  $\Gamma_3(Q)$  profiles with those predicted by the Matryoshka model. Q<sup>2</sup>-dependences of  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and  $\Gamma_3(Q)$  on IN6 are shown in (a), (b), and (c), respectively. The data points at 283 K, 311 K, and 340 K are shown in purple, cyan, and magenta, respectively. (d), (e), and (f) show the Q<sup>2</sup>-dependences of  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and  $\Gamma_3(Q)$ on IN5, respectively. The data points at 280 K and 295 K are shown in cyan and magenta, respectively. The solid lines show the corresponding fits based on the Matryoshka model (Eq. (1-12) in the Supplementary material).

508

#### 509 4. Concluding remarks

510 In this study, the diffusive nature of the motions contained in the QENS spectra of DMPC and 511 DMPG in various forms was analyzed and thus relaxational aspects of the Matryoshka model were characterized. The three Lorentzian functions, which were obtained by decomposing the 512 QENS spectra, showed significantly distinctive relaxational features with their widths spanning 513 514 about two orders of magnitude. Regardless of this variety, the Matryoshka model can satisfactorily describe the various motions contained in each of these components. The current 515 516 results of the analysis of the widths of these Lorentzian functions, which was carried out 517 independently of the development of the Matryoshka model, not only provides information 518 on the relaxational aspects of the motions contained in this model, but also corroborates the 519 parameter values such as the amplitudes and the mobile fractions of atomic motions obtained 520 by the EISF/QISF analysis using the Matryoshka model, which enhances the validity of the 521 model. The Matryoshka model can thus serve as a powerful tool to decipher the intramolecular 522 motions of lipid molecules and demonstrate its power in characterizations of more complex 523 systems such as the mixtures of different kinds of lipids, protein-lipid complexes, and eventually 524 natural cell membranes in the near future.

525

#### 526 Acknowledgements

527 The authors thank the Institut Laue-Langevin for the allocation of the beam time to perform 528 the experiments. AC is supported by the JP Aguilar scholarship from the Foundation CFM for 529 her PhD thesis.

530

#### 531 Supplementary material

532 Parameter values obtained from the QENS spectra, analytical expressions of  $\Gamma_1(Q)$ ,  $\Gamma_2(Q)$ , and

533  $\Gamma_3(Q)$  based on the Matryoshka model are found in the Supplementary material.

535 <b>Re</b>	ferences
---------------	----------

- 536 [1] T. Heimburg, Thermal Biophysics of Membranes, Wiley-VCH Verlag GmbH & Co. KGaA,
- 537 2007.
- 538 [2] Ole G. Mouritsen, Life As a Matter of Fat, Springer-Verlag Berlin Heidelberg, 2005.
- 539 [3] W. Pfeiffer, G. Schlossbauer, W. Knoll, B. Farago, A. Steyer, E. Sackmann, Ultracold
- 540 neutron scattering study of local lipid mobility in bilayer membranes, J. Phys. Fr., 49
- 541 (1988) 1077–1082.
- 542 [4] W. Pfeiffer, T. Henkel, E. Sackmann, W. Knoll, D. Richter, Local Dynamics of Lipid
- 543 Bilayers Studied by Incoherent Quasi-Elastic Neutron Scattering, Europhys. Lett., 8
- 544 (1989) 201–206.
- 545 [5] S. König, W. Pfeiffer, T. Bayerl, D. Richter, E. Sackmann, Molecular dynamics of lipid
- 546 bilayers studied by incoherent quasi-elastic neutron scattering, J. Phys. II Fr., 2 (1992)
- 547 1589–1615.
- 548 [6] W. Pfeiffer, S. Konig, J.F. Legrand, T. Bayerl, D. Richter, E. Sackmann, Neutron Spin Echo
  549 Study of Membrane Undulations in Lipid Multibilayers, Europhys. Lett., 23 (1993) 457–
- 550 462.
- 551 [7] T.M. Bayerl, Collective membrane motions, Curr. Opin. Colloid Interface Sci., 5 (2000)
  552 232–236.

- 553 [8] V.G. Sakai, A. Arbe, Quasielastic neutron scattering in soft matter, Curr. Opin. Colloid
  554 Interface Sci., 14 (2009) 381–390.
- 555 [9] A.A. Nevzorov, M.F. Brown, Dynamics of lipid bilayers from comparative analysis of 2H
- and 13C nuclear magnetic resonance relaxation data as a function of frequency and
- 557 temperature, J. Chem. Phys., 107 (1997) 10288–10310.
- 558 [10] P.F.F. Almeida, W.L.C. Vaz, Lateral Diffusion in Membranes, in: R. Lipowsky, E. Sackmann
- 559 (Eds.), Handb. Biol. Phys., Elsevier Science, New York, 1995: pp. 305–357.
- 560 [11] S. Moradi, A. Nowroozi, M. Shahlaei, Shedding light on the structural properties of lipid
- bilayers using molecular dynamics simulation: a review study, RSC Adv., 9 (2019) 4644–
  4658.
- 563 [12] M. Doxastakis, V.G. Sakai, S. Ohtake, J.K. Maranas, J.J. de Pablo, A molecular view of
- 564 melting in anhydrous phospholipidic membranes, Biophys. J., 92 (2007) 147–161.
- 565 [13] B. Aoun, E. Pellegrini, M. Trapp, F. Natali, L. Cantù, P. Brocca, Y. Gerelli, B. Demé, M.
- 566 Marek Koza, M. Johnson, J. Peters, Direct comparison of elastic incoherent neutron
- 567 scattering experiments with molecular dynamics simulations of DMPC phase
- 568 transitions, Eur. Phys. J. E, 39 (2016).
- 569 [14] M. Bée, Quasielastic Neutron Scattering, Adam Hilger, Bristol and Philadelphia, 1988.
- 570 [15] S. König, T.M. Bayerl, G. Coddens, D. Richter, E. Sackmann, Hydration dependence of
- 571 chain dynamics and local diffusion in L-alpha-dipalmitoylphosphtidylcholine

572		multilayers studied by incoherent quasi-elastic neutron scattering, Biophys. J., 68
573		(1995) 1871–1880.
574	[16]	S. König, E. Sackmann, Molecular and collective dynamics of lipid bilayers, Curr. Opin.
575		Colloid Interface Sci., 1 (1996) 78–82.
576	[17]	J. Swenson, F. Kargl, P. Berntsen, C. Svanberg, Solvent and lipid dynamics of hydrated
577		lipid bilayers by incoherent quasielastic neutron scattering, J. Chem. Phys., 129 (2008)
578		3–10.
579	[18]	C. Gliss, O. Randel, H. Casalta, E. Sackmann, R. Zorn, T. Bayerl, Anisotropic motion of
580		cholesterol in oriented DPPC bilayers studied by quasielastic neutron scattering: The
581		liquid-ordered phase, Biophys. J., 77 (1999) 331–340.
582	[19]	U. Wanderlingh, G. D'Angelo, C. Branca, V. Conti Nibali, A. Trimarchi, S. Rifici, D.
583		Finocchiaro, C. Crupi, J. Ollivier, H.D. Middendorf, Multi-component modeling of
584		quasielastic neutron scattering from phospholipid membranes, J. Chem. Phys., 140
585		(2014) 174901.
586	[20]	U. Wanderlingh, C. Branca, C. Crupi, V.C. Nibali, G. La Rosa, S. Rifici, J. Ollivier, G.
587		D'Angelo, Molecular dynamics of POPC phospholipid bilayers through the gel to fluid
588		phase transition: An incoherent quasi-elastic neutron scattering study, J. Chem., (2017)
589		vol. 2017, Article ID 3654237, 8 pages.
590	[21]	D.J. Bicout, A. Cissé, T. Matsuo, J. Peters, The dynamical Matryoshka model: 1

- 591 Incoherent neutron scattering functions for lipid dynamics in bilayers, BioRxiv
- 592 10.1101/2021.09.21.461198 (2021).
- 593 [22] A. Cissé, T. Matsuo, M. Plazanet, F. Natali, M.M. Koza, J. Olivier, D.J. Bicout, J. Peters,
- 594 The dynamical Matryoshka model: 2 A new modelling of local lipid dynamics at the
- 595 sub-nanosecond timescale in phospholipid membranes, BioRxiv
- 596 10.1101/2022.03.30.486370 (2022).
- 597 [23] A. Watts, K. Harlos, D. Marsh, Charge-induced tilt in ordered-phase
- 598 phosphatidylglycerol bilayers Evidence from x-ray diffraction, Biochim. Biophys. Acta -
- 599 Biomembr., 645 (1981) 91–96.
- 600 [24] S. Tristram-Nagle, Y. Liu, J. Legleiter, J.F. Nagle, Structure of gel phase DMPC

601 determined by x-ray diffraction, Biophys. J., 83 (2002) 3324–3335.

- 602 [25] NIST Computational Chemistry Comparison and Benchmark Database, NIST Standard
- 603 Reference Database Number 101 Release 21, August 2020, Editor: Russell D Johnson III
- 604 http://cccbdb.nist.gov/, (n.d.).
- 605 [26] A.J. Dianoux, M. Pineri, F. Volino, Neutron incoherent scattering law for restricted
- diffusion inside a volume with an anisotropic shape: Application to the problem of
- 607 water absorbed in nafion® membranes, Mol. Phys., 46 (1982) 129–137.
- 608 [27] F. Volino, A.J. Dianoux, Neutron incoherent scattering law for diffusion in a potential of
- spherical symmetry: general formalism and application to diffusion inside a sphere,

- 610 Mol. Phys., 41 (1980) 271–279.
- 611 [28] F.Y. Hansen, G.H. Peters, H. Taub, A. Miskowiec, Diffusion of water and selected atoms
- 612 in DMPC lipid bilayer membranes, J. Chem. Phys., 137 (2012).
- 613 [29] J. Tabony, B. Perly, Quasielastic neutron scattering measurements of fast local
- 614 translational diffusion of lipid molecules in phospholipid bilayers, BBA Biomembr.,
- 615 1063 (1991) 67–72.
- 616 [30] J. Qvist, H. Schober, B. Halle, Structural dynamics of supercooled water from
- 617 quasielastic neutron scattering and molecular simulations, J. Chem. Phys., 134 (2011)
  618 144508.
- 619 [31] P. Ben Ishai, E. Mamontov, J.D. Nickels, A.P. Sokolov, Influence of ions on water

620 diffusion--a neutron scattering study, J Phys Chem B, 117 (2013) 7724–7728.

- 621 [32] T. Matsuo, T. Arata, T. Oda, K. Nakajima, S. Ohira-Kawamura, T. Kikuchi, S. Fujiwara,
- Difference in the hydration water mobility around F-actin and myosin subfragment-1
- 623 studied by quasielastic neutron scattering, Biochem. Biophys. Reports, 6 (2016) 220–
- 624 225.
- 625 [33] M. Trapp, M. Tehei, M. Trovaslet, F. Nachon, N. Martinez, M.M. Koza, M. Weik, P.
- 626 Masson, J. Peters, Correlation of the dynamics of native human acetylcholinesterase
- and its inhibited huperzine A counterpart from sub-picoseconds to nanoseconds, J. R.

628 Soc. Interface, 11 (2014).

629	[34]	J. Peters, J. Marion, F. Natali, E. Kats, D.J. Bicout, The Dynamical Transition of Lipid
630		Multilamellar Bilayers as a Matter of Cooperativity, J. Phys. Chem. B, 121 (2017) 6860-
631		6868.
632		