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# COHERENT COLLECTIVE DYNAMICS IN PHOSPHOLIPID MEMBRANES: WHERE ARE WE NOW?

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ABSTRACT. We review the main achievements accomplished in the study of the coherent collective dynamics of phospholipid membranes, *i.e.*, the collective vibrational excitations active on the sub-picoseconds to picoseconds time scale and terahertz frequencies, over a length scale comparable to the angstrom length scale. The first descriptions of the terahertz collective spectrum, mainly assigned to a single longitudinal acoustic-like mode similarly to simple liquids, have been progressively replaced by a complex scenario, with longitudinal and transverse acoustic-like and optic-like inelastic excitations. Complex phenomena (avoided crossing mechanisms between modes, a phonon gap introduced by the disorder and a slow- to fast-sound transition) have been highlighted in the dispersion curves of lipid bilayers. Systematic and comprehensive studies along the lines of the investigations reviewed here contribute to understand the role of the collective (coherent) chain dynamics in the passive transport across bilayers, in the formation of water wires inside dynamic defects in lipid bilayers and in other biological functions of biomembranes.

## 1. Introduction

Biological membranes are fundamental components of all living systems, forming the outer boundary of living cells or organelles. Membranes made up primarily of a bilayer of phospholipids with embedded proteins, carbohydrates, glycolipids, and glycoproteins, and, in animal cells, cholesterol. The complexity of biological membranes and their interactions with intra- and extra-cellular environments makes direct investigations highly difficult. Consequently, artificial model membranes, with a reduced number of components, are commonly used to gain insights into physical and chemical characteristics of natural membranes. Investigation of the dynamics of phospholipids in synthetic bilayers started in 1960s. A highly dynamic state of the lipid bilayer is a prerequisite for the function of biological membranes. As in other biomolecular assemblies, functionally relevant motions in biomembranes span a wide range of length and time scales (Pfeiffer *et al.* 1989; König and Sackmann 1996). Motions that have been detected experimentally include isomerizations in the hydrocarbon chains (10 ps to 100 ps), single lipid protrusions (10 ps to 1 ns), reorientation of the head groups (1 ns), rotations of the lipid molecules about their long axes (10 ns),

collective bilayer undulations ($> 10$ ns), and long range lipid diffusion in the plane of the bilayer (10 ns to 1 s, depending on the length scale) (Pfeiffer *et al.* 1989; Tarek *et al.* 2001). On the 100 ps time scale it is mostly believed that the lipid centers-of-mass are not freely diffusing laterally, nor are they undergoing large excursions in the direction normal to the bilayer due to long wavelength undulations, but rather are "rattling in a cage" formed by their neighbors, with roughly equal amplitudes in and perpendicular to the bilayer plane (Flenner *et al.* 2009). In addition to the isotropic center-of-mass motion, there is a considerable amount of motion in the internal degrees of freedom, especially in the acyl chains and, to a lesser degree, in the choline groups, involving the formation and disappearance of gauche bonds, gauche-trans-gauche kinks, and other chain defects. Notably, also the effects of other biomolecules (such as e.g. oligopeptides and alcohols) on the dynamics of the lipids have been widely investigated (Hofsäß *et al.* 2003; D'Angelo *et al.* 2008; Wanderlingh *et al.* 2008, 2010; Rifici *et al.* 2014, 2016).

## 2. Coherent collective dynamics of phospholipid membranes

In this contribution we aim to briefly review the main achievements accomplished in the study of the coherent collective dynamics of phospholipid membranes: with this terminology one refers to the collective dynamics on the sub-picoseconds and picoseconds time scale and terahertz frequencies, active over a length scale comparable to the inverse mean inter-particle distance, *i.e.*, the angstrom length scale. While a vast literature on single-particle motions of lipid membranes exists, very few studies have been focussed on their collective dynamics after the pioneering work by Chen *et al.* (2001). Importantly, the collective dynamics of the hydrocarbon chains at the picosecond time scale are believed to play a key role in the increased membrane elasticity (Bloom *et al.* 1991) and in the transport of small molecules and ions across the bilayer (Paula *et al.* 1996). A fundamental quantity, from both theoretical and experimental points of view, for the determination of the collective dynamics associated with density fluctuations is the dynamical structure factor, $S(Q,E)$, defined as the Fourier transform in space and time of the particle-density pair correlation function. The coherent collective dynamics in biosystems is studied

(a) theoretically, by attempting to extend the hydrodynamics theory to small distances and short times by means of the generalized hydrodynamics and the molecular hydrodynamics theories;
(b) numerically, employing simulation methods based on the integration of the equations of motion of an ensemble of particles interacting via a specific model potential;
(c) experimentally, by using scattering methods such as inelastic X-rays and neutron scattering (IXS, INS) to determine the dynamic structure factor directly.

Chen *et al.* (2001) used high resolution inelastic X-ray scattering (IXS) to study the dynamical properties of multilamellar DLPC (dilaurylphosphatidylcholine) lipid bilayers close to full hydration in both the gel and liquid crystal phases. The analysis based on a generalized three effective eigenmode theory allowed them to construct the dispersion relation of the high frequency sound mode for the first time. They found a linear increase of the sound frequencies at small $Q$, with a sound speed of about 2580 m/s, and a marked softening of the excitation near $Q = 1.4\,\text{Å}^{-1}$, corresponding to the lipid chain-chain correlation peak in the structure factor that implies prevalent occurrences of short-wavelength in-plane

motions of lipid chains. Moreover, they found a minimum frequency in the fluid phase that is located below 1 meV, which is deeper in comparison with the minimum observed in the gel phase. These results were later compared to a molecular dynamics simulations study by Tarek *et al.* (2001). The authors of this work confirmed the existence of a highly dispersive sound mode, whose frequency and damping depend on the lipid phase, and demonstrated that the scattering arises mainly from in-plane motion of hydrocarbon chains. Additionally, they identified a nondispersive mode attributed to motions of the chain terminal carbons (located at 15 meV in the gel phase and at 7 meV in the liquid crystal phase).

In 2004 Rheinstädter et al. studied the collective short wavelength dynamics in DMPC bilayers in the fluid and the gel phases by inelastic neutron scattering (Rheinstädter *et al.* 2004). The temperature dependence of the inelastic excitations indicated a phase coexistence between the two phases over a broad range and lead to a different assignment of excitations from that reported in the preceding IXS study by Chen *et al.* (2001). In particular the authors found that the minimum in the dispersion relation is actually deeper in the gel than in the fluid phase (Rheinstädter *et al.* 2004). Finally, they could identify the nondispersive (optical) mode predicted by MD simulations (Tarek *et al.* 2001).

Hub *et al.* (2007) presented an extensive comparison between MD simulations and INS experiments concerning the short wavelength dynamics of a hydrated phospholipid bilayer. In the authors' opinion the interpretation in terms of the three-effective-eigenmode model is found to be only partly suitable to describe the complex fluid dynamics of lipid chains.

Brandt and Edholm (2009) calculated the dynamic structure factors for a lipid bilayer from MD simulations, extending the study performed by Tarek *et al.* (2001) to a larger system and lower wave vector (down to $0.34\,\mathrm{nm}^{-1}$). The results showed that, for low wave vectors, the lipid bilayer can be described by the linearized hydrodynamic equations, which lead to the well-known Rayleigh-Brillouin triplet shape of the dynamic structure factor; however, as the wave vectors increase, deviations from the behaviour predicted by these equations appear, suggesting that in the hydrodynamic limit the dynamical collective behaviour is different from the one theoretically estimated.

All the studies (Chen *et al.* 2001; Tarek *et al.* 2001; Rheinstädter *et al.* 2004; Hub *et al.* 2007; Brandt and Edholm 2009) thus agreed on the existence of a well-defined acoustic-like excitation attributed to the in-plane motions of the hydrocarbon chains: the main features of this excitation are a linear increase at the small in-plane wave vector $Q$ due to long-wavelength sound propagation, saturating at some maximum value at $Q_{\max}/2$, and, at higher $Q$, a pronounced minimum at $Q_{\max} = 1.4\,\text{Å}^{-1}$, corresponding to the nearest-neighbour distances of lipid acyl tails. The dispersion of the revealed excitation has been considered as indicative of a simple-liquid behaviour and described in the theoretical framework of hydrodynamics; however, despite this approach succeeded in predicting the thermal and elastic constants in the hydrodynamic limit, several findings suggest that the collective dynamics of lipid bilayers strongly differs from that outlined for simple liquids, such as the linear (instead of quadratic) momentum transfer $Q$ dependence of the width of the Brillouin lines (Brandt and Edholm 2009) and the existence of an opticlike mode found at 14 meV in the gel phase and at 7 meV in the liquid crystal phase (Tarek *et al.* 2001; Rheinstädter *et al.* 2004). These first interpretations of the collective motions in membranes in terms of simple liquids appeared to be oversimplified: actually, lipid bilayers are complex viscous fluids in which also propagating transverse (*i.e.*, shear) waves and optic-like modes arising from

mass-concentration fluctuations are expected to emerge even in the hydrodynamic region. This is the reason why Conti Nibali *et al.* (2014) further investigated the short-wavelength longitudinal and transverse collective dynamics of the fluid and gel phases of phospholipid bilayers by means of molecular dynamics simulations. Differently from the previous works focusing on the dynamical structure factor $S(Q,E)$ (Chen *et al.* 2001; Tarek *et al.* 2001; Rheinstädter *et al.* 2004; Hub *et al.* 2007), Conti Nibali *et al.* (2014) based their analysis on the direct investigation of the longitudinal and transverse current correlation spectra, computed from molecular dynamics simulations of fluid and gel phases of phospholipid bilayers. This alternative approach allowed to unveil collective modes that are otherwise hardly observable in the dynamical structure factor $S(Q,E)$. Conti Nibali *et al.* (2014) revealed for the first time:

i. propagating long-wave acoustic-like transverse modes,
ii. acoustic-like and optic-like (6, 12, 15, 20 meV) modes with a mixed symmetry character and
iii. a resonant interaction between the lowest frequency optical phonon (6 meV) and the longitudinal acoustic mode (*i.e.*, an avoided crossing mechanism between the two excitations with a consequent energy exchange) (Conti Nibali *et al.* 2014);
vi. a strong increase of the longitudinal mode velocity from the low wavector to high wavevector (from 2198 m/s to 2795 m/s, at $Q = 0.4^{-1}$), explained as a consequence of the above mentioned avoided crossing event: the so called slow- to fast-sound transition, originally considered as an anomalous characteristic of water, is thus extended to lipid membranes.

Importantly, this study highlights the importance of studying the interactions between collective modes -that likely play a major role in the energy-transfer processes in these biosystems. The new scenario proposed by MD simulations strongly challenged the previous descriptions of the coherent collective dynamics in phospholipid membranes and awaited experimental verification.

In 2016 by means of a high resolution inelastic X-ray scattering study of the in-plane phonon excitations in hydrated lipid multilayers Zhernenkov *et al.* (2016) and coauthors provided the first experimental evidence of a low frequency transverse mode in the gel phase, which exhibits a phonon gap when the membrane transitions into the fluid phase. They proposed that the observed phonon gap is attributable to the formation of short-lived nanometre-scale lipid clusters and transient pores, that play a role in the mechanism of passive molecular transport across the bilayer.

D'Angelo *et al.* (2017) reported a far-infrared study of the molecular subpicoseconds motions of phospholipid bilayers at different hydration aimed to verify the existence of the optical modes predicted by the previous MD simulations (Conti Nibali *et al.* 2014). They showed that lipid membranes sustain several low frequency collective modes and that these modes arise from vibrations of different lipids interacting through intermolecular van der Waals forces. Moreover, they found that the frequencies of the vibrations of the lipids are very close to those of their hydration water and suggested a dynamical coupling between membranes and the solvent, as previously found in other biosystems, such as enzymes (Conti Nibali *et al.* 2014) and proteins (Paciaroni *et al.* 2013).

D'Angelo *et al.* (2018) performed Brillouin neutron scattering measurements and combined them with IXS data by Zhernenkov *et al.* (2016), with the aim of proposing a model for the collective dynamics of lipid membranes, *i.e.*, the model response function associated with the Hamiltonian of an interacting-phonon system. This powerful approach –that exploits complementary characteristics of the two techniques- allowed them to reveal a comprehensive scenario of the collective features of membranes. At low wavevectors, the dispersion relations have been interpreted in terms of two acoustic-like modes, one longitudinal and one transverse, plus a dispersionless optic-like mode at 13 meV. In agreement with Zhernenkov *et al.* (2016), they observe a phonon gap in the transverse mode in the liquid phase; however, their measurements suggest that this gap could be present also in the gel phase. At higher wavevectors, the interaction between the longitudinal acoustic mode and the optic-like mode at leads to an avoided-crossing mechanism, that activate an energy-transfer mechanism between these two modes. Additionally, an evidence for a slow- to fast-sound transition, similar to liquid water and other biomolecules: this phenomenon suggests an efficient dynamical coupling of the membrane with its hydration water, that could play a role in biomolecular mechanisms (Orecchini *et al.* 2009; Conti Nibali and Havenith 2014).

## 3. Conclusions

In 2001 the coherent collective dynamics of phospholipid membranes started to be investigated, with the main aim of understanding the precise mechanism of passive transport of molecules across the cell membrane. In the first experimental and theoretical studies the terahertz collective spectrum was mainly assigned to a single acoustic mode, an interpretation that later on turned out to be oversimplified and not appropriate. More recent experimental and theoretical efforts have shown that the scenario of the collective dynamics in biomembranes is much more complex and multifaceted. In the current state of the art in the field:

(1) multiple collective modes of different nature have been revealed (two acoustic-like modes, one longitudinal and one transverse, plus –at least- one optic-like mode) (Conti Nibali *et al.* 2014; Zhernenkov *et al.* 2016; D'Angelo *et al.* 2018);
(2) a phononic gap has been found in the transverse mode at low wavectors: it has been explained in terms of an increasing lipid chain disorder on long-length scale and linked to a passive transport mechanism through membranes (Zhernenkov *et al.* 2016; D'Angelo *et al.* 2018);
(3) avoided crossing mechanisms, due to interactions beween the longitudinal acoustic mode and optic-like modes, have been observed. These mode-mode interactions likely play a key role in the energy transfer processes in lipid membranes (Conti Nibali *et al.* 2014; D'Angelo *et al.* 2018);
(4) a slow- to fast-sound transition in the velocity of the longitudinal mode has been revealed: this evidence points to a bilayer-solvent dynamical coupling, that could play a key role in biomolecular mechanisms (Conti Nibali *et al.* 2014; D'Angelo *et al.* 2018).

Despite considerable progress has been made following the pioneering work by Chen *et al.* (2001), the study of the collective dynamics in phospholipid membranes is still in its infancy.

Complementary experimental and theoretical approaches will be needed to provide further insights in these complex dynamics and in the role they play in the passive transport and in other biological functions of biomembranes. In particular, investigating the interactions between collective modes could lead to a deeper understanding of the energy transfer phenomena in lipid membranes. Other significant challenges are those of understanding if these collective vibrational modes represent a channel for solvent-biomolecule dynamical coupling (Conti Nibali and Havenith 2014), known to ultimately play a role in biological activity, and if they induce and/or mediated the formation of water wires inside dynamic defects in lipid bilayers (Zhernenkov *et al.* 2016; D'Angelo *et al.* 2018).

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