Review

Molecular interactions of melatonin with lipid rafts

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ABSTRACT

Biological membranes are composed of a lipid bilayer with a heterogeneous structure and complex dynamics, both of which can be modulated by the presence of melatonin. The lateral heterogeneities in lipid bilayers, also known as lipid rafts, have unique molecular interactions with melatonin, which we review here. Specifically, we discuss the molecular-level, physicochemical influences of melatonin on dynamics of lipid rafts and their structural properties, including melatonin's propensity to preserve the structural integrity of lipid rafts at different length scales, as revealed through a range of experimental techniques and theoretical approaches.

Key words: lipid rafts, phospholipid membranes, melatonin, molecular interactions, structure, dynamics

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1. INTRODUCTION

The cell membrane is primarily a lipid bilayer with a heterogeneous composition of lipid molecules, including a range of a diversity of lipid head group and lipid tail configurations (1, 2). The biological membrane typically contains a wide assortment of other molecules including proteins, peptides, sterols, drugs, and small molecules such as melatonin (3–9). The heterogeneous lipid composition facilitates the formation of stable, sometimes transient, functional units within the membrane also known as "lipid rafts" (10, 11). These spatially assembled areas in biological membranes are thought to play an important role in their functionalities, including signaling, endo- and exocytosis, focal adhesion, membrane trafficking (12, 13), to name a few. It is thus of primary significance to understand physicochemical properties of the membrane lateral organization in the form of lipid rafts.

The biological functions of lipid rafts can be studied through molecular interactions between lipids and membrane-active molecules (14). However, the understanding of molecular interactions is precluded due to a limited number of experimental techniques able to probe them at the fundamentally

relevant molecular scale (15). Even for small molecules with known molecular interactions, such as melatonin, relatively little is known about how alike membrane-active molecules interact with complex heterogeneous lipid mixtures, where so-called liquid ordered (L_o) and liquid disordered (L_d) membrane environments coexist (16). Previous studies regarding the active molecule-lipid membrane molecular interactions have substantially been focused on their spatial orientation and configuration (17). As a result, the combined analysis of membrane lateral structure and multiscale dynamics of the lipid rafts has not been the focus, and often has not even been considered. Therefore, there is currently an incomplete description of multiscale molecular interactions between lipid rafts and associated molecules because the molecular mechanisms for the active molecule-lipid raft interactions involve cascade-like dynamic relaxation processes closely related to structural changes in biological membranes, partially due to spontaneous membrane curvatures (18). In this regard, variations in the lipid composition induce spontaneous curvatures in membranes, which can also influence the fundamental properties of the active molecule-lipid raft interactions at different time and length scales. As a result, the research field of multiscale melatonin-lipid raft molecular interactions is still piecemeal. Nevertheless, this field is rapidly developing, where the molecular scale interactions play the key role.

Previous studies on melatonin interactions with phospholipids have typically been focused on model membranes primarily observing membrane thinning effects attributed to the fluidity of these structures (19–24) and highlighting melatonin's protective properties (25–28). Model membranes are convenient systems to study since decades of extensive work have been spent to describe their phase behavior and lateral structure as a function of temperature and their lipid compositions (29). As a result, different thermodynamic phases and phase-separated states of model synthetic membranes are thought to represent important physicochemical aspects of lateral organization of biological membranes (28). Specifically, while much is known about the lipid membrane phases, including detailed phase diagrams, the multiscale molecular machinery of melatonin and its impact on these phases have largely been unexplored. Some related aspects have previously been studied via high-throughput computer simulations (30). However, information on the synergistic, molecular-scale framework comprising the dynamic and structural properties of the melatonin-lipid raft interactions is almost non-existent. Here, we evaluate the original studies on the multiscale structure and dynamics of lipid rafts in the presence of melatonin providing a comprehensive perspective based on X-ray and neutron scattering techniques, mathematical modelling and numerical analyses, and high-throughput molecular dynamics simulations. This framework may potentially provide complementary insights into molecular interactions, *i.e.*, structural properties of lipid rafts in the presence of melatonin and their dynamic properties such as collective motions in the form of acoustic and optical vibrational excitations, lipid self-diffusion, viscoelastic crossovers, and the molecular level stress propagation.

2. MEMBRANE STRUCTURE AND DYNAMICS

2.1. Lateral structure of membranes in the presence of melatonin.

The understanding of lateral heterogeneity in biological membranes remains highly incomplete despite decades of detailed studies (31–33). Broadly speaking, lipids commonly found in biological membranes often form different liquid phases, denoted as "ordered" and "disordered". In a heterogeneous mixture of lipids, these different phases tend to separate and often coexist. At equilibrium, lipid vesicles, *a.k.a.*, liposomes, tend toward a phase-separated state in which liquid ordered and disordered regions form distinct spatial domains. These domains tend to be *macroscopic*,

with length scales on the same order as the size of a membrane. The various phase-separated states have been studied extensively in model membrane systems, with detailed composition phase diagrams being available, for example, for ternary lipid mixtures (34). These synthetic lipid bilayers are thought to mimic certain physicochemical aspects of biological membranes, although the functional "lipid rafts" found in biological systems are much smaller in size than the phase-separated domains found in model membranes (35).

The connection between the phase separation dynamics in model membranes and the nanoscopic heterogeneities found in biological membranes remains unclear. Many proposals exist in the literature, including the possibility that the biological membrane is found in a so-called "microemulsion" phase (36) or it has a composition that is tuned to be near a critical point (37). Alternatively, it is possible that other cellular components including various proteins, such as the cytoskeleton, break up the domains (38). However, small molecules such as melatonin also have their unique structural impact on lipid heterogeneities.

To understand the phase behavior of a lipid membrane from a coarse-grained perspective, one may posit some field $\varphi \equiv \varphi(\mathbf{x}, t)$ which describes the fraction of a liquid ordered phase at location $\mathbf{x}$ and time t on the membrane surface, or any other particular lipid phase formed during lipid phase separation. Then, a wide range of possibilities for the phase behavior of the membrane is captured by a phenomenological free energy given, in appropriately scaled units of distance and energy, by

$$\mathcal{F} = \int dx \left[\frac{1}{2}\phi(\nabla^2 + \kappa)^2\phi + \frac{r}{2}\phi^2 + \frac{\mu}{3}\phi^3 + \frac{1}{4}\phi^4\right], \qquad (1)$$

where r is a control parameter that favors phase separation when $r < 0$ and lipid phase mixing when $r > 0$. The parameter μ describes an asymmetry between the liquid ordered and liquid disordered phases. Finally, κ is a parameter that describes a penalty for making domain walls between the liquid-ordered and liquid-disordered phases.

In regular phase separating systems, we expect that $\kappa > 0$ in Eq. (1). We would then find, for example, a second-order critical point describing the phase separation transition at $r + \kappa^2 = 0$ when $\mu = 0$ (or a first-order transition at $r + \kappa^2 \approx -\mu^2/9$ when $\mu \neq 0$). However, when competing interactions or small molecules acting as line-active agents stabilize domain walls between the lipid phases, the parameter κ may become negative. In this case, we find patterned (modulated) phases when the system tends toward phase separation (for $r < 0$) and disordered, microemulsion phases when $r > 0$. The various possible phases for this type of free energy (for $\mu = 0$) are given in Figure 1. As we will discuss below, melatonin may play a role in generating these structured phases with $\kappa < 0$ as it may serve to stabilize interfaces between liquid-ordered and liquid-disordered lipid phases.

When $\mu \neq 0$ and $r, \kappa < 0$ in the free energy in Eq. (1), we may find different patterned phases including hexagonal arrangements of domains. The various structured phases are diagrammed in Figure 2. Note that for small μ, we find the striped phases. For larger μ, the asymmetry between the two lipid phases will generate a hexagonal arrangement of domains, with one phase serving as the "matrix" and the other the lipid "domain." Finally, we also include another image of a microemulsion in Figure 2, on the right-hand-side where $r > 0$. Notethat the microemulsion, although a disordered phase, retains some domain-like structures as such a phase maintains a characteristic length scale. Although the free energy in Eq. (1) is informative regarding structured lipid phases, their description is incomplete.

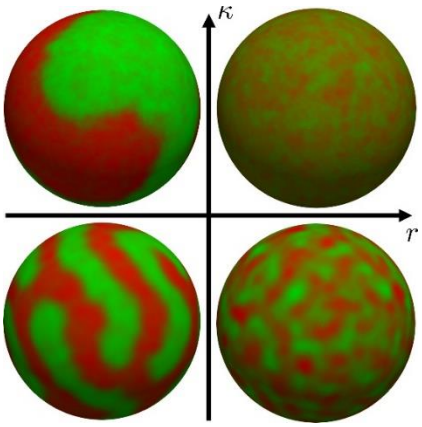

Fig. 1. Simulated lipid vesicles with free energies given by Eq. (1) with μ = 0 and fixed signs of κ and r as shown.

When κ, r > 0 (top right sphere), we find a homogeneous fluid phase, representing a mixed state of lipids. When κ > 0 and r < 0 (top left), the vesicle phase-separates into two distinct lipid phases (red and green), corresponding to liquid-ordered and liquid-disordered lipid regions. For κ < 0, we find states with a characteristic length scale $\lambda \approx \frac{2\sqrt{2}\pi}{\sqrt{-\kappa}}$. *For r > 0, we find a disordered phase called a "microemulsion," and it is thought to capture the properties of nanoscopic lipid heterogeneities in biological membranes (bottom right). Conversely, when r < 0, we find a phase-separated, patterned (spatially modulated) phase (bottom left). These patterns may be striped, as shown, or have hexagonal arrangements of domains when* $\mu \neq 0$. *All the lipidphase configurations are numerically generated by solving the dynamical equation Eq. (2) by using the FiPy finite volume solver Python package (39).*

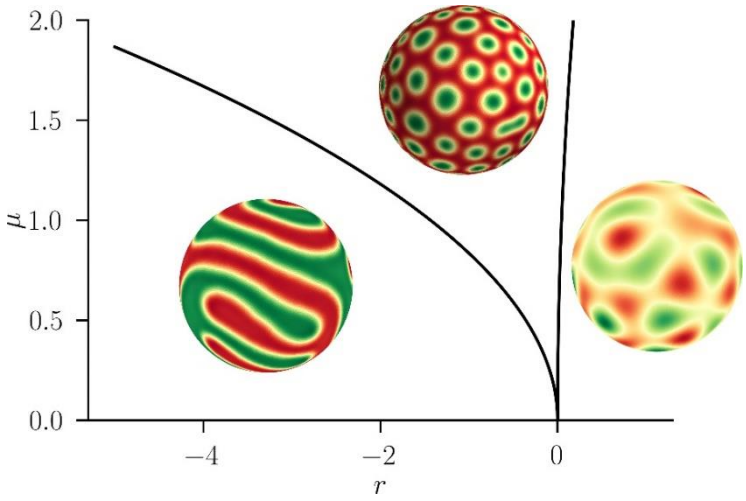

Fig. 2. Phase diagram for a fixed κ < 0 for the free energy given in Eq. 1.

We find three different phases corresponding to striped patterns (left), hexagonal domain arrangements (center), and a microemulsion (right). The phase boundaries are indicated with solid black lines. Note that the configurations shown are constructed by evolving the dynamical equation in Eq. (2) for a free energy F (see Eq. (1)) with the given parameters. The dynamical equation is solved using the FiPy finite volume solver Python package (39).

Dynamic behaviors are crucial to understand as they describe domain coarsening, fluctuations, coalescence, etc. These phenomena underlie the function of the lipid domains in biological membranes, where they are involved in active dynamic processes. These dynamics are typically quite involved, as the fluid nature of the membrane requires a hydrodynamic description. However, if we neglect the hydrodynamics, the dynamics of our coarse-grained field $\phi(\boldsymbol{r}, t)$ (indicating the fraction of one of the lipid phases) near equilibrium is, in the appropriately scaled units of time, given by

$$\partial_t \phi(\mathbf{r}, t) = \nabla_{\mathbf{r}}^2 \frac{\delta \mathcal{F}}{\delta \phi} + \xi(\mathbf{r}, t) \quad (2)$$

where $\xi(\mathbf{r}, t)$ is a Gaussian white noise describing thermal fluctuations. Here, we assume that the global fraction of lipid phases remains conserved throughout the evolution. The noise correlations are given by $\langle \xi(\mathbf{r}, t)\xi(\mathbf{r}', t)\rangle = -2Dk_BT\,\nabla_{\mathbf{r}}^2\delta(\mathbf{r}-\mathbf{r}')\delta(t-t')$. More sophisticated approaches, including the hydrodynamics, are possible (40). However, these simple "conserved" dynamics may capture the essential features of domain dynamics observed, for instance, in model membranes (41).

It should be noted that small molecules such as melatonin may play a role in the formation of the structured phases with $\kappa < 0$. For example, such small molecules may couple to the lipid membrane curvature and generate local deformations. Recent work (42) has shown that such interactions may yield a strong deformation in the membrane, including the double membrane formation. An example of such a local deformation is shown in Figure 3.

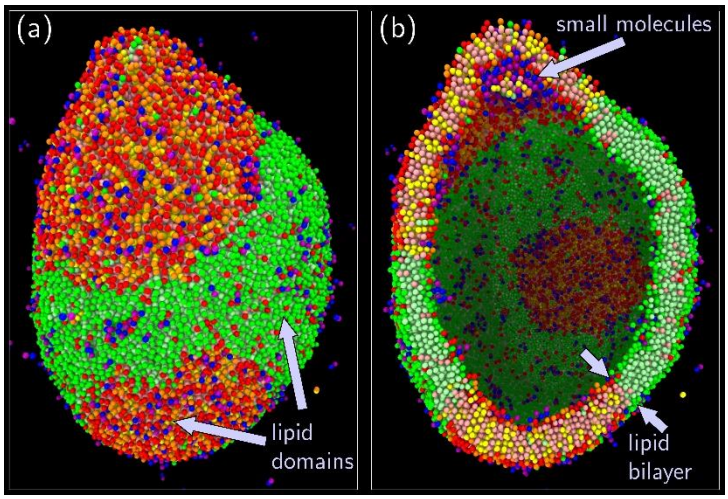

Fig. 3. Simulated lipid vesicle image (adapted from Ref. (42)) showing liquid ordered and liquid disordered domains (green/yellow versus red/orange regions) on the surface.

(a) and in a cross section (b). The data is obtained via high throughput molecular dynamics simulations, and more details are given in Ref. (42). The blue/purple particles represent small molecules which preferentially interact with the red/orange lipid phase. Note that the small molecules may aggregate and locally deform the membrane generating a spontaneous curvature (top region in (a) and (b)). Such preferential partitioning and membrane curvature generation are possible ways in which melatonin may influence the cell membrane properties and functions.

The spontaneous curvature formation may also generate an effectively negative value of the phenomenological parameter κ in Eq. (1). The membrane curvature-lipid composition coupling with generating a modulated phase was described previously by Leibler and Andelman (43). Recent

estimates of the parameters of this phenomenological theory suggest that the microemulsion phase may be responsible for generating the lipid heterogeneities in plasma membranes (44). It is of particular interest to consider how small molecules such as melatonin may assist us to explore the phase diagram described by the phenomenological free energy in Eq. (1).

One way to test the predictions of the theory is to study phase separation in liposomes using non-invasive experimental techniques such as small angle neutron scattering (SANS). By appropriately matching the contrast of the lipids (via acyl chains deuteration), it is possible to resolve lateral heterogeneities within the membrane (35). Recent work based on neutron scattering of large unilamellar vesicles (LUVs) in conjunction with fluorescence confocal microscopy of giant unilamellar vesicles (GUVs) (45) has provided compelling evidence that melatonin stabilizes the lipid rafts structure in phase-separating model membranes. One may also use SANS to decipher phases of the membrane (see Figure 1). Below, we review the details of the SANS analysis. A dilute solution of liposomes yields a neutron scattering spectrum with intensity given by

$$I(q) = \frac{I_0}{4\pi} \int \langle |F_R(\mathbf{q})|^2 \rangle_{\text{th.,R}} \, d\Omega_{\mathbf{q}} + I_{inc.}, \qquad (3)$$

where $I_{inc.}$ is a constant incoherent scattering contribution, I_0 is a constant of proportionality dependent on the vesicle density in solution, where we integrate over all possible orientations $\mathbf{\Omega_q}$ of the wavevector $\mathbf{q}$. The average $\langle \dots \rangle$ indicates both a thermal average and an average over the various vesicle sizes in solution. The factor $F_R(\mathbf{q})$ is the Fourier-transformed scattering length density $\delta\rho(\mathbf{r})$ for a vesicle of a fixed radius R: $F_R(\mathbf{q}) = -\int d\mathbf{r}\ e^{-i\mathbf{q}\cdot\mathbf{r}}\delta\rho(\mathbf{r})$.

The factor $F_R(\mathbf{q})$ captures the effects of the lateral heterogeneities (lipid rafts) and can be made constant, when the lipids within the membrane are mixed, via an appropriate deuteration of the component lipids. A simple model of $F_R(\mathbf{q})$ assumes that the vesicle of radius R consists of an approximately uniformly dense, spherical lipid bilayer of thickness t. This shell model for $\delta\rho(\mathbf{r})$ reads

$$\delta\rho(\mathbf{r}) = \psi(\theta, \varphi)(\Theta(|\mathbf{r}| - R + t/2) - \Theta(|\mathbf{r}| - R - t/2)) - \rho_s, \quad (4)$$

with $\Theta(x) = 0$ for $x \leq 0$ and $\Theta(x) = 1$ for $x > 0$ the Heaviside step function and ρ_s the constant scattering length density of the solvent. Then, the lateral structure of nanoscopic lipid rafts can be captured by the function $\psi(\theta, \varphi)$ defined on the spherical surface of the vesicle.

The surface function $\psi(\theta, \varphi)$ can be expanded in spherical harmonics $Y_\ell^m(\theta, \phi)$. By fitting the shell model to SANS data, it is possible to extract the power spectrum of the spherical harmonic modes of $\psi(\theta, \varphi)$. This power spectrum has a different form depending on the lipid phase. For example, one can differentiate between a microemulsion and a simple phase-separating system by observing that the mode magnitudes $\langle |\psi_\ell^m|^2 \rangle$ are proportional to

$$\langle |\psi_\ell^m|^2 \rangle_{th.} \frac{1}{(\ell - \ell_0)^2 + \frac{R^2}{\xi_{micro.}^2}} \text{ or } \frac{1}{\ell^2 + \frac{R^2}{\xi_{ps.}^2}}, \qquad (5)$$

implying a microemulsion or a phase-separating system, respectively. Here ξmicro and ξps are correlation lengths of the corresponding phases. The mode ℓ_0 corresponds to the characteristic length scale of the microemulsion: $\lambda_0 \approx \frac{2\pi R}{\ell_0}$. The SANS data in Figure 4 is consistent with both scenarios, however, it is difficult to resolve the scattering peak corresponding to the lipid rafts. Further studies, including a more careful contrast matching that removes signal contamination from the melatonin itself, are necessary to understand the precise phase behavior of these model membranes in the presence of melatonin. Nevertheless, the SANS

data can be used to identify general trends, such as the increase of lipid raft size upon increasing concentration of melatonin.

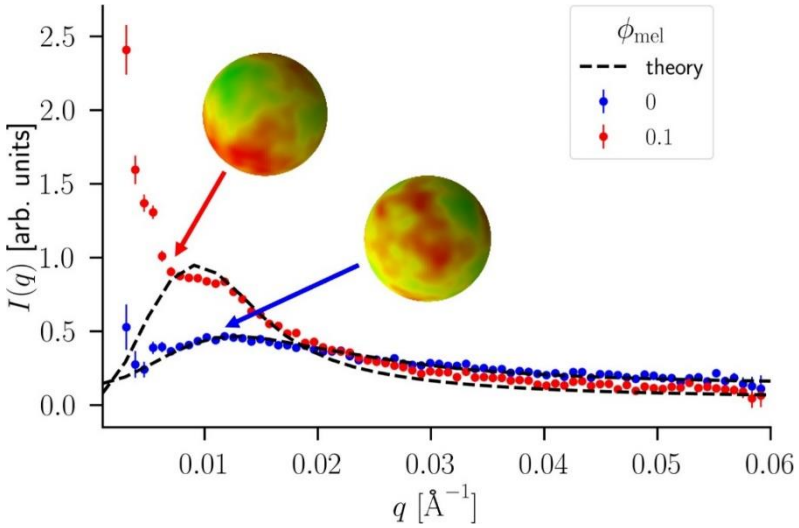

Fig. 4. Small-angle neutron scattering data from LUVs (50 nm in diameter) in the presence of 10% melatonin (red points) and in the absence of melatonin (blue points), reproduced from Ref. (45).

The black dashed lines are fits to scattering data based on predictions from the theory related to the microemulsion phase (see Eq. (5)) taking into account the scattering signal from the lipids. Note that the addition of melatonin is consistent with the lipid raft formation of larger size as the scattering intensity peak shifts to smaller q values and becomes more prominent for $\varphi_{mel} = 0.1$.

2.2. Molecular insights into the force from lipids and lipid raft vibrational patterns.

Phonons are quantized collective excitations of molecules that can propagate in biological materials (15, 46). Phonons exist in biological membranes due to density-density fluctuations in the form of compression and shear waves, also known as phonon excitations, stemming from a well-known principle, namely the force from lipids (FFLs) (47). The FFLs is thought to play a central role in many biological processes (47–49). Specifically, the FFLs modulates collective molecular displacements, which enable interactions and coupling with sound and electromagnetic waves at corresponding frequencies in the long wavelength hydrodynamic limit ($E \rightarrow 0$, $Q \rightarrow 0$). In an inelastic X-ray scattering (IXS) geometry (Fig. 5a) an incident beam of energy E_i and momentum $\mathbf{k}_i$ scatters with energy E_f and momentum $\mathbf{k}_f$ because of their interaction predominantly with the electrons in the lipid membrane acyl chains. Therefore, the scattered beam carries information of the molecular motions within the scanned energy $E = E_f - E_i$ transfer and momentum $\mathbf{Q} = \mathbf{k}_f - \mathbf{k}_i$ transfer, respectively.

Figure 5b demonstrates phonon dispersion relationships of a single component 1,2- dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membrane in the gel (20 ˚C) and fluid (45 ˚C) phase, as measured by IXS. Despite numerous experimental attempts, the existence of transverse phonon excitations with (low-*E*, low-*Q*) gaps were observed for the first time by Zhernenkov *et al.* (50) (Figure 5b) and the experimental data is in good agreement with the phonon theory of liquids (51–53). The speed of sound in the DPPC membrane was estimated as 2532 m/s in the gel phase, and as 2241 m/s in the fluid phase.

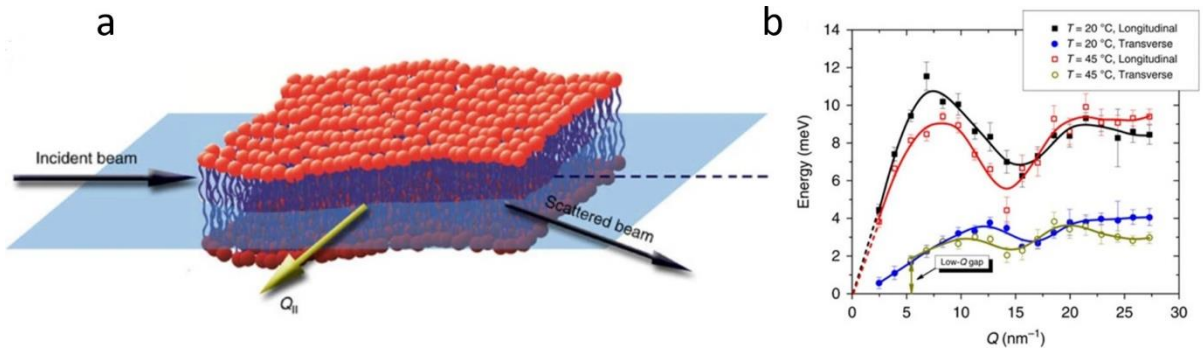

Fig. 5. (a) *A schematic of an inelastic X-ray scattering experiment probing the transverse phononic excitations of a lipid bilayer. E(Q) profiles of phonon dispersion relationships of a DPPC membrane in gel (20 ˚C) and fluid (45 ˚C) phases.* **(b)** *The compelling experimental evidence of the phonon gap in the phospholipid membrane at low-energy and low-momentum values. Figure was adapted from Zhernenkov et al. (50).*

Importantly, the presence of compression and shear forces with the re-emergent behavior of transverse phonon gaps are responsible for the formation of lipid rafts at nanometer length scale. Therefore, nanometer-sized lipid rafts are transient existing at the specific (picoseconds) time scale (15, 46). Indeed, longitudinal phonon excitations can exist and propagate in perfectly arranged molecular assemblies such as crystalline solids as well as in highly disorderedmaterials (54). In contrast, transverse phonon excitations are unable to propagate in disordered materials at low frequencies including phospholipid membranes since their shear restoring forces get overdamped beyond a few nanometers (15). In this regard, permanent existence of compression waves and transient behavior of shear forces at high frequencies is responsible for the formation and dissolution of lipid rafts, making molecular-level stress propagation possible. The molecular stress transfer is enabled within the biological membranes, and it includes the following sequence of dynamic events: elastic formation of lipid rafts, picosecond time nearest lipid neighbor rattling, followed by lipid raft relaxation processes as a result of the elastic to viscous crossover in the vibrational spectrum (55) due to emergent transverse phonon gaps making nanoscopic lipid rafts diffusive (46). The formation and dissolution of transient lipid rafts occurs randomly throughout the membrane. Therefore, the vibrational landscape of the membrane at this fundamental time scale is reminiscent of a surface of a boiling soup.

The molecular stress propagation in biological membranes is akin to a pulse propagation due to the transient nature of nanoscopic lipid rafts. The acoustic properties of transient lipid rafts can be changed upon melatonin inclusion, as observed in DMPC-Cholesterol membranes via IXS. Specifically, in the presence of melatonin the speed of sound in the DPPC-Chol membrane was increased from ~1860 ± 300 m/s to ~2128 ±124 m/s, and the lifetime of transient lipid rafts also increased from ~4.6 ps to ~8.3 ps. This means that the presence of melatonin makes lipid rafts stiffer protecting their structural integrity. The melatonin-enhanced rigidity of lipid rafts also implies their increased mobility due to increased overall membrane fluidity and decreased viscosity at macroscopic length scale (56). In other words, the membrane becomes more fluid at longer time scales in the presence of melatonin, and at the microscopic scale lipid rafts become more rigid and mobile, sustaining their biophysical functionalities (15, 46).

Figure 6 shows phonon excitations of a DPPC-Chol system. Interestingly, both acoustic and optical phonon modes are insensitive to temperature change and independent of cholesterol concentrations. The existence of a truncated optical branch at Q_{cutoff} =7 nm^{-1} implies the existence

of nanoscopic cholesterol-enriched lipid rafts with a size of $\sim 9\mathring{A}$ in diameter. Figure 6d shows the coexistence of liquid ordered and liquid disordered lipid phases. The ordered phase consists of strongly correlated lipid-cholesterol molecules. The optical phonon branch is truncated at $Q \sim 7$ nm^{-1} due to *the finite size effect*, which was also detected in vibrational patterns of liquid crystals (58), and nanostructured germanium (59).

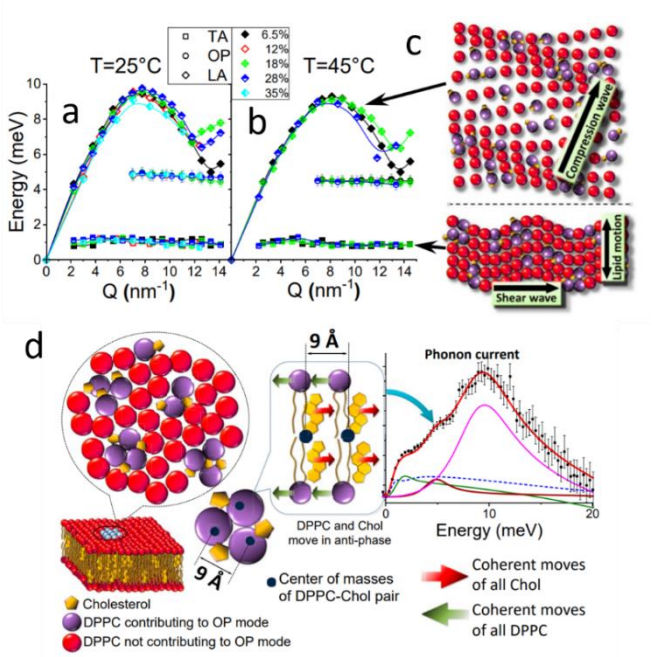

Fig. 6. Phospholipid vibrational patterns.

At 25 ˚C (a) and 45 ˚C (b) in the presence of cholesterol. ***(c)*** *A schematic representation of longitudinal acoustic (LA) and transverse acoustic (TA) modesin the phase-separated membranes. An optical phonon branch at E(Q) ~5.5 meV is truncated atlow-Q end at ~7 nm^{-1} at different cholesterol concentrations (6.5 up to 35 mole%). (d) Coexistence of liquid ordered and liquid disordered lipid phases. The figure was adapted from Soloviov et al.(57).*

Interestingly, the presence of melatonin increases the speed of sound in lipid rafts of DMPC-Cholesterol biomembranes, as already above, from ~1860 ± 300 m/s to ~2128 ± 124 m/s because of strong melatonin-lipid interactions increasing both the lipid tail packing and area per lipid (46). Additionally, the self-diffusion coefficient of lipids is reduced due to the presence of melatonin facilitating molecular level dynamic barrier that depresses the permeation of small molecules such as oxygen practically functioning as an antioxidant (15, 46). Finally, optical phonon excitations in DMPC-Chol membranes form a lattice-like vibrational landscape comprising longitudinal and transverse non-propagating waves, also known as standing waves. Melatonin at high concentrations eliminates vibrational patterns which also implies no energy transfer. However, melatonin has no effect on truncated optical modes generated by lipid-cholesterol pairs. In this regard, this is the molecular level evidence that melatonin strongly affects the water-membrane interface slowing down the permeation of small molecules such as water and oxygen (15, 46). In summary, structure and multiscale dynamics of lipid membranes can strongly be influenced by melatonin. Importantly, heterogeneous lateral structure of lipid rafts can be preserved in the presence of melatonin at high concentrations as revealed by different experimental techniques and theoretical approaches.

3. CONCLUSIONS

Herein, we reviewed the aspects of lipid rafts structure and dynamics that can be implicated in melatonin function. It remains an outstanding challenge to connect these concepts to specific biological processes. One possibility is to study the effects of melatonin on intracellular membranes, such as those of the endoplasmic reticulum and mitochondria. These membranes will have different lipid compositions which may possibly have different interactions with intracellular melatonin (60). Examining these processes may be important, for example, in elucidating the effects of melatonin in cancer cell lines (61). The intracellular concentration of melatonin may be quite appreciable when considering pharmacological applications, reaching 1 μM in some cases. Although this concentration seems too small to lead to the drastic membrane rearrangements discussed in this review, it would be important to understand the distribution of the melatonin within the cell to see if certain membranes more readily recruit the melatonin through, for example, preferential binding to receptors or certain lipid species.

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AUTHORSHIP

All authors contributed to the conceptualization, writing, and editing of this manuscript.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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