Published in final edited form as:

Chem Phys Lipids. 2016 September ; 199: 39–51. doi:10.1016/j.chemphyslip.2016.05.001.

Cholesterol-Induced Suppression of Membrane Elastic Fluctuations at the Atomistic Level

Trivikram R. Molugu^a and Michael F. Brown^{a,b}

^aDepartment of Chemistry and Biochemistry, University of Arizona, Tucson AZ 85721, USA

^bDepartment of Physics, University of Arizona, Tucson AZ 85721, USA

Abstract

Applications of solid-state NMR spectroscopy for investigating the influences of lipid-cholesterol interactions on membrane fluctuations are reviewed in this paper. Emphasis is placed on understanding the energy landscapes and fluctuations at an emergent atomistic level. Solid-state ^2H NMR spectroscopy directly measures residual quadrupolar couplings (RQCs) due to individual C– ^2H labeled segments of the lipid molecules. Moreover, residual dipolar couplings (RDCs) of ^{13}C – ^1H bonds are obtained in separated local-field NMR spectroscopy. The distributions of RQC or RDC values give nearly complete profiles of the order parameters as a function of acyl segment position. Measured equilibrium properties of glycerophospholipids and sphingolipids including their binary and tertiary mixtures with cholesterol show unequal mixing associated with liquid-ordered domains. The entropic loss upon addition of cholesterol to sphingolipids is less than for glycerophospholipids and may drive the formation of lipid rafts. In addition relaxation time measurements enable one to study the molecular dynamics over a wide time-scale range. For ^2H NMR the experimental spin-lattice (R_{1Z}) relaxation rates follow a theoretical square-law dependence on segmental order parameters (S_{CD}) due to collective slow dynamics over mesoscopic length scales. The functional dependence for the liquid-crystalline lipid membranes is indicative of viscoelastic properties as they emerge from atomistic-level interactions. A striking decrease in square-law slope upon addition of cholesterol denotes stiffening relative to the pure lipid bilayers that is diminished in the case of lanosterol. Measured equilibrium properties and relaxation rates infer opposite influences of cholesterol and detergents on collective dynamics and elasticity at an atomistic scale that potentially affects lipid raft formation in cellular membranes.

Keywords

Solid-state NMR; area per lipid; cholesterol; lanosterol; membrane elasticity; lipid rafts

INTRODUCTION

Biomembranes display a tremendous complexity of lipids and proteins needed to perform the functions that cells require in the processes of life. Phospholipids are important

components of these cell membranes and have a variety of roles; for example, the formation of lipid bilayers provides structural integrity, and gives an energy reservoir and source of second messenger precursors. Representative examples of glycerophospholipids and sterols found in cellular membranes are provided in Fig. 1. It can be seen that the polar head groups of the phospholipids differ in their size, capacity for hydrogen-bonding, and charge, whereas the nonpolar acyl chains vary in their length and degree and position of unsaturation. For cholesterol, the polar hydroxyl group orients the molecule at the aqueous interface, and the four fused rings together with the hydrocarbon chain constitute the nonpolar core. Most strikingly, the methyl substituents are confined to the molecularly rough β -face and the opposite α -face is smooth, in arresting contrast to its metabolic precursor lanosterol (Fig. 1). Various membrane lipids exhibit lyotropic liquid-crystalline phases under physiological conditions, involving solid-ordered (s_0), liquid-disordered (l_d), and liquid-ordered (l_o) phases. Attractive and repulsive forces for the membrane lipids entails both the polar headgroups and the non-polar moieties, and yield a substantial polymorphism with both lamellar and nonlamellar phases (Amazon and Feigenson, 2014; Brown, 1994; Feigenson, 2006, 2015; Gruner, 1989; Krepiy et al., 2009; Phillips et al., 2009; Seddon, 1990; Seddon et al., 1997; van Meer et al., 2008; Zimmerberg and Gawrisch, 2006). Notably, the structural and dynamical properties of biomembranes are mediated by the lipid composition and interactions with the proteins, water, cholesterol, and surfactants (Brown and Chan, 2007; Coskun and Simons, 2011; Kaiser et al., 2009; Kaye et al., 2011; Kinnun et al., 2015; Leftin et al., 2014b; Mallikarjunaiah et al., 2011; Rheinstädter et al., 2004; Tyler et al., 2015). Membrane remodeling requires mesoscopic elastic deformations of the lipids (Kinnun et al., 2015) that can play a central role in biological functioning with regard to lipid-protein interactions, domain formation, and various nano- and microstructures implicated in key cellular functions (Brown, 1997; Brown, 2012; Liang et al., 2014; Soubias et al., 2014, 2015; Teague et al., 2013).

On the other hand, it is known that many of the molecular species of lipids and proteins in membranes do not mix ideally (Ackerman and Feigenson, 2015; Amazon and Feigenson, 2014; Armstrong et al., 2012; Armstrong et al., 2013; Eriksson and Henriksson, 2007; Eriksson et al., 2006; Escriba et al., 2008; Feigenson, 2015; Goñi et al., 2008; Konyakhina and Feigenson, 2016). Cholesterol is one such component that may not be uniformly distributed in cellular membranes, whose distribution entails liquid-ordered raft-like domains (Ackerman and Feigenson, 2015; Amazon and Feigenson, 2014; Epand, 1998; Epand, 2006; Feigenson, 2015; Goñi et al., 2008; Konyakhina and Feigenson, 2016; Scheidt et al., 2013; Sodt et al., 2014). Such raft-like domains have garnered considerable attention as platforms for signaling proteins in cellular biology and pharmacology (Brown and London, 1998; Day and Kenworthy, 2015; Golebiewska and Scarlata, 2010; Klose et al., 2013; Simons and Gerl, 2010; Simons and Sampaio, 2011; Song et al., 2014; Surma et al., 2012). The concept that biomembranes are two-dimensional fluids with randomly distributed proteins (the fluid mosaic model) is challenged by the hypothesis that cellular membranes may contain such areas of lateral segregation (Bartels et al., 2008; Camley and Brown, 2010; Edidin, 2003; Feigenson, 2015; Keller and McConnell, 1999; Korade and Kenworthy, 2008; Leftin et al., 2013; Leftin et al., 2014a; Lingwood and Simons, 2010; Meinhardt et al., 2013; Polozov and Gawrisch, 2006; Quinn, 2013; Simons and Gerl, 2010;

Veatch et al., 2007; Wassall and Stillwell, 2009). For instance, raft-like domains are believed to occur in lipid systems with coexisting liquid-disordered (L_d) and liquid-ordered (L_o) phases. The L_d phase in these systems typically contains highly unsaturated lipids with a low phase transition temperature, whereas the L_o phase predominantly consists of a saturated glycerophospholipids or sphingolipid component and cholesterol (Simons and Toomre, 2000). Moreover, certain proteins are endowed with the ability to interact with cholesterol via a cholesterol recognition/interaction amino acid consensus (CRAC) sequence motif (Baier et al., 2011; Fantini and Barrantes, 2013; Greenwood et al., 2008). In some cases, they include cationic clusters that allow interactions with phosphatidylinositol(4,5)bis-phosphate (PIP₂) in a cholesterol-dependent manner. Such CRAC domains are found in the *Rhodopsin* (Family A) G-protein-coupled receptors (GPCRs) (Jafurulla et al., 2011), and moreover post-translational lipid modifications (Vogel et al., 2007; Weise et al., 2013) can promote sequestration into cholesterol-rich regions or microdomains.

Improving our understanding of complex lipid mixtures is clearly an important target for research in pharmaceutical and physical chemistry, as well as in cellular biology. Various biophysical methods have been used to study lipid-cholesterol interactions, including electron spin resonance (ESR) (Cheng et al., 2014; Delmelle et al., 1980; Hubbell and McConnell, 1971; Lai and Freed, 2014; Manukovsky et al., 2013; Semer and Gelerinter, 1979; Stepien et al., 2015; Vitiello et al., 2015; Williams et al., 2013), Raman (Lippert and Peticolas, 1971; Mendelsohn, 1972; Tantipolphan et al., 2006), Fourier transform infrared (FT-IR) (Umemura et al., 1980), fluorescence spectroscopy (Xu and London, 2000; Yasuda et al., 2015a), atomic force microscopy (AFM), multidimensional NMR spectroscopy (Holland and Alam, 2006; Leftin et al., 2013; Leftin et al., 2014a; Warschawski and Devaux, 2005), solid-state ²H nuclear magnetic resonance (NMR) (Bartels et al., 2008; Brown, 1990; Bunge et al., 2008; Martinez et al., 2004; Martinez et al., 2002; Matsumori et al., 2012; Stockton et al., 1976; Vogel et al., 2016; Weisz et al., 1992; Yasuda et al., 2015a), and neutron diffraction methods (Armstrong et al., 2014; Toppozini et al., 2014). However, a thorough understanding of the physical basis for these observations in relation to the intricate lipid compositions of many biological membranes to some extent remains an enigma (Feigenson, 2015; McConnell, 2005; Meinhardt et al., 2013; Sodt et al., 2014; Stanich et al., 2013). This article covers recent developments in understanding lipid-cholesterol interactions in model membrane systems, and implications for cellular function as seen by solid-state nuclear magnetic resonance (NMR) spectroscopy. First, we give a brief introduction to lipid systems and solid-state NMR methods, and next we explain how solid-state NMR technology is applied for obtaining membrane structural and dynamical properties. We discuss the interactions of the phospholipids with cholesterol and lanosterol in model membranes, including the role of configurational entropy in lipid raft formation. Emphasis is placed on how the average material properties emerge from the atomistic level interactions in lipid bilayers as investigated by combining NMR spectroscopy with relaxation methods (Brown et al., 2001; Martinez et al., 2004; Martinez et al., 2002).

SOLID-STATE NMR SPECTROSCOPY OF BIOMEMBRANES

Deuterium Solid-State NMR Spectroscopy

Solid-state ^2H NMR spectroscopy (Brown, 1996; Kinnun et al., 2013; Leftin and Brown, 2011; Leftin et al., 2014b) of deuterated lipid molecules offers a versatile and non-invasive method for studying molecular organization within membranes. Isotopic substitution of ^2H for ^1H constitutes a minimal structural perturbation (Seelig, 1977) and interpretation of the spectra is relatively straightforward, due to the intramolecular nature of the quadrupolar interaction that dominates the spectral shape. There are a number of reviews that give a comprehensive treatment of ^2H NMR spectral analysis (Brown, 1996; Brown and Chan, 1996; Brown et al., 2006; Kinnun et al., 2015; Leftin and Brown, 2011; Seelig, 1977; Seelig and Macdonald, 1987; Seelig and Seelig, 1980). An essential feature of ^2H NMR spectroscopy is that one introduces site-specific ^2H -labels, corresponding to the individual C- ^2H bonds. In this way, we obtain atomistically resolved information for non-crystalline amorphous or liquid-crystalline systems. Because the coupling interactions in solid-state NMR are sensitive to orientation and/or distance, their values correspond to the average structure of the system of interest. On the other hand, molecular motions are manifested by the relaxation parameters that are also accessible in NMR spectroscopy. A unique feature is that solid-state ^2H NMR of biomolecular systems acquires both lineshape data and relaxation times for investigating the structural dynamics (Xu et al., 2014). Measurement of the ^2H NMR lineshapes yields knowledge of the average structure through the principal values of the coupling tensor, as well as the principal axis system. Yet, if we only determine the coupling tensors, then the method mainly provides us with structural knowledge as in X-ray crystallography. An important aspect of solid-state ^2H NMR is that information is also obtained regarding the molecular motions, encompassing a range of different time scales. Through the combined measurement of residual quadrupolar couplings (RQCs) and relaxation rates, we thereby obtain knowledge of the geometry, as well as investigate the multi-scale molecular motions and their amplitudes in the membrane systems of interest.

Deuterium NMR spectroscopy gives a particularly simple illustration of the principles of magnetic resonance as applied to molecular solids, liquid crystals, and biomembranes. This is because the very large electric quadrupolar interaction dominates over the magnetic dipolar couplings of the ^2H and ^1H nuclei, as well as the ^2H chemical shifts (Brown and Chan, 1996; Xu et al., 2014). The ^2H nucleus has a spin of $I = 1$, and hence there are three Zeeman energy levels due to projecting the nuclear spin angular momentum onto the magnetic field direction. The three eigenstates $|m\rangle = |0\rangle$ and $|\pm 1\rangle$ are given by the Hamiltonian $\hat{H}_Z$ for interaction of the nuclear magnetic moment with the static magnetic field. We learn in quantum mechanics that transitions between adjacent spin energy levels are allowed, which yields two single-quantum nuclear spin transitions. Moreover, the degeneracy of the allowed transitions in ^2H NMR is removed by the quadrupolar coupling. Here the perturbing Hamiltonian $\hat{H}_Q$ is due to interaction of the quadrupole moment of the ^2H nucleus with the electric field gradient (EFG) of the C- ^2H bond. It follows that for each inequivalent site two spectral branches are observed in the experimental spectrum.

In solid-state ^2H NMR spectroscopy, the experimentally observed quadrupolar coupling is given by the difference in the frequencies $\Delta\nu_Q \equiv \nu_Q^+ - \nu_Q^-$ of the spectral lines due to the perturbing Hamiltonian. The result for the quadrupolar frequencies ($\nu_Q^\pm$) thus reads:

$$\nu_Q^\pm = \pm \frac{3}{4} \chi_Q \left\{ D_{00}^{(2)}(\Omega_{\text{PL}}) - \frac{\eta_Q}{\sqrt{6}} [D_{-20}^{(2)}(\Omega_{\text{PL}}) + D_{20}^{(2)}(\Omega_{\text{PL}})] \right\} \quad (1)$$

In the above formula $\chi_Q \equiv e^2 q Q / h$ is the *static* quadrupolar coupling constant, η_Q corresponds to the asymmetry parameter of the EFG tensor, $D_{00}^{(2)}(\Omega_{\text{PL}})$ is a Wigner rotation matrix element, and $\Omega_{\text{PL}} \equiv (\alpha_{\text{PL}}, \beta_{\text{PL}}, \gamma_{\text{PL}})$ are the Euler angles (Rose, 1957) relating the principal axis system (PAS) of the EFG tensor (P) to the laboratory frame (L) (Brown, 1996; Leftin et al., 2014b; Xu et al., 2014). Furthermore, it turns out that the static EFG tensor of the C- ^2H bond is nearly axially symmetric ($\eta_Q \approx 0$), which leads us to the simpler result:

$$\nu_Q^\pm = \pm \frac{3}{4} \chi_Q D_{00}^{(2)}(\Omega_{\text{PL}}). \quad (2)$$

The experimental quadrupolar splitting is thus given by

$$\Delta\nu_Q = \frac{3}{2} \chi_Q D_{00}^{(2)}(\Omega_{\text{PL}}), \quad (3)$$

For liquid-crystalline membranes the motions of the constituent molecules are often cylindrically symmetric about the bilayer normal, an axis known as director. The overall rotation of the principal axis system of the coupling tensor to the laboratory frame, described by the Ω_{PL} Euler angles, can thus be represented by the effect of two consecutive rotations. First, the Euler angles $\Omega_{\text{PD}}(t)$ represent the (time-dependent) rotation from the principal axis frame to the director frame, and second the Euler angles Ω_{DL} represent the (static) rotation from director frame to laboratory frame. Using the closure property of the rotation group (Xu et al., 2014), and considering the cylindrical symmetry about the director, we can then expand eq. (3), which now reads

$$\Delta\nu_Q = \frac{3}{2} \chi_Q \langle D_{00}^{(2)}(\Omega_{\text{PD}}) \rangle D_{00}^{(2)}(\Omega_{\text{DL}}) \quad (4)$$

$$= \frac{3}{2} \chi_Q \frac{1}{2} \langle 3 \cos^2 \beta_{\text{PD}} - 1 \rangle \frac{1}{2} (3 \cos^2 \beta_{\text{DL}} - 1) \quad (4a)$$

Here $\beta_{\text{DL}} \equiv \theta$ is the angle of the bilayer normal to the static external magnetic field. The segmental order parameter S_{CD} is given by

$$S_{CD} = \frac{1}{2} \langle 3 \cos^2 \beta_{PD} - 1 \rangle \quad (5a)$$

where the angular brackets denote a time/ensemble average. It follows that

$$\Delta\nu_Q = \frac{3}{2} \chi_Q S_{CD} P_2(\cos \beta_{DL}) \quad (5b)$$

where $P_2(\cos \beta_{DL})$ is the second-order Legendre polynomial. The above expression describes the dependence of the quadrupolar splitting on the (Euler) angles that rotate the coupling tensor from its principal axes system to the laboratory frame, as defined by the main magnetic field.

We can now introduce simplifying precepts from statistical mechanics to explain the solid-state NMR lineshapes in terms of membrane structural dynamics. For detailed explanation and applications of the statistical mean-torque theory, the interested reader is referred to the literature (Bartels et al., 2008; Kinnun et al., 2015; Leftin et al., 2013; Leftin et al., 2014a; Petrache et al., 2000). The microscopic observables from ^2H NMR spectroscopy can then be related to the nano- or microstructure of the membrane lipid assembly. Structural quantities of interest for the lamellar state correspond to the mean interfacial area $\langle A \rangle$, together with the average thickness D_C of the bilayer hydrocarbon region, and the mean aqueous distance separating the lamellae (Brown, 1996; Jansson et al., 1992; Mallikarjunaiah et al., 2011; Nagle and Tristram-Nagle, 2000; Pastor et al., 2002; Thurmond et al., 1991). Clearly, the area per lipid molecule plays an important role in molecular dynamics (MD) simulations of lipid membranes (Huber et al., 2002; Klauda et al., 2008a; Klauda et al., 2008b; Klauda et al., 2010; Klauda et al., 2008c; Meinhardt et al., 2013; Pastor et al., 2002; Toppozini et al., 2014; Venable et al., 2014). The various nanostructures are the result of a balance of forces acting at the level of the polar head groups and hydrocarbon chains (Brown, 1994; Gawrisch, 2012; Gruner, 1989; Petrache et al., 2000; Seddon, 1990; Zimmerberg and Gawrisch, 2006). Notably, the deformation of a membrane film away from the equilibrium state is characterized by four material constants: (i) the surface tension γ (which is zero for a membrane bilayer at equilibrium), (ii) the area expansion modulus K_A or alternatively the lateral compressibility $C_A \equiv 1/K_A$, (iii) the bending rigidity K_C , and (iv) the monolayer spontaneous H_0 curvature. The above structural quantities are fundamental to the forces governing the nano- and microstructures of assemblies of membrane lipids and amphiphiles. Representative applications of solid-state ^2H NMR spectroscopy to lipid membranes include influences of cholesterol (Fig. 2) (Brown and Seelig, 1978; Oldfield et al., 1978; Trouard et al., 1999) as well as acyl chain polyunsaturation (Gawrisch, 2012; Huber et al., 2002; Huber et al., 2004; Petrache et al., 2001; Salmon et al., 1987; Shaikh et al., 2015; Teague et al., 2013; Wassall and Stillwell, 2009; Wiedmann et al., 1988).

Separated Local-field ^{13}C NMR Spectroscopy: ^{13}C - ^1H Dipolar Couplings Allow Calculations of Lipid Bilayer Structure

Lipid membrane systems clearly can benefit from precise structural characterization using solid-state NMR methods. As explained in the previous section, solid-state ^2H NMR spectroscopy is prominent among these methods. However, such applications can be foreshadowed by the need for ^2H -isotope labeling involving synthetic organic chemistry (Salmon et al., 1987; Williams et al., 1985). By introduction of dipolar-recoupling methods, it is possible to extend approaches originally developed with regard to solid-state ^2H NMR spectroscopy to other classes of biologically relevant lipids, as they occur in a membrane environment. In this regard, separated-local field (SLF) ^{13}C NMR spectroscopy (Cobo et al., 2012; Das and Opella, 2014; Gopinath et al., 2011; Jayanthi et al., 2010; Kharkov et al., 2012; Lobo et al., 2014; Lobo and Ramanathan, 2011; Lobo and Ramanathan, 2012; Ramamoorthy et al., 2004; Reddy et al., 2014; Yamamoto et al., 2015; Zhang et al., 2015) at natural abundance expands the range of applications of solid-state NMR spectroscopy in membrane biophysics (Ferreira et al., 2013; Gawrisch et al., 2002; Gross et al., 1997; Leftin et al., 2013; Leftin et al., 2014a). For example, sphingolipids and other natural lipids may be investigated, together with their interactions with cholesterol in raft-like lipid mixtures, as well as their interactions with membrane proteins. Additional applications of natural abundance ^{13}C NMR methods to polyunsaturated lipid bilayers (Brown et al., 1982; Gawrisch et al., 2002) have been described. In these examples and others, we are interested in how the molecular properties of membrane lipids explain their biological functions within the broad context of structural biophysics (Brown, 1994; Brown, 2012; Lee, 2004; Phillips et al., 2009).

Separated local-field experiments typically allow measurement of the direct ^{13}C - ^1H dipolar couplings in liquid-crystalline systems, such as lipid bilayers at natural isotopic abundance. The through-space direct ^{13}C - ^1H dipolar interactions report on the orientations of the individual ^{13}C - ^1H bonds with respect to the bilayer normal, and are mathematically isomorphous to the C - ^2H bond order parameters, measured in solid-state ^2H NMR spectroscopy. Various SLF methods include for example dipolar recoupling methods (Ferreira et al., 2013; Ferreira et al., 2008; Gross et al., 1997; Leftin et al., 2013; Leftin et al., 2014a; Warschawski and Devaux, 2005), switched-angle spinning, and off-magic-angle spinning experiments (Hong et al., 1995). Segmental order parameters can be unambiguously determined by SLF methods, and hence it is a useful technique for lipid structural studies. In lipid systems, correspondingly the segmental order parameters are defined as

$$S_{\text{CH}} = \frac{1}{2} \langle 3 \cos^2 \beta_{\text{CH}} - 1 \rangle \quad (6)$$

where β_{CH} is the instantaneous angle between the ^{13}C - ^1H bond direction and the bilayer normal. Based on geometrical considerations, the S_{CH} order parameters for a polymethylene chain are negative. Note that here we refer to the absolute order parameters $|S_{\text{CH}}|$, which are calculated from the relation

$$|S_{CH}| = \frac{|\Delta\nu_D|}{\chi_D \chi_P} \quad (7)$$

In the above formula $\chi_D = (-\mu_0 \gamma_H \gamma_C \hbar / 4\pi^2) \langle r_{CH}^{-3} \rangle$ is the dipolar coupling constant (40.783 kHz corresponding to $b_{CH}/2\pi = 20.392$ kHz for an aliphatic ^{13}C - ^1H bond), $\chi_P = 0.393$ is the pulse sequence scaling factor (Gross et al., 1997; Leftin et al., 2014a), and ν_D is the measured RDC evaluated at the $\theta=90^\circ$ orientation of the lineshape (Pake powder pattern). An illustration of the results obtained for mixtures of lipids and cholesterol using the dipolar recoupling with shape and scaling preservation (DROSS) experiment is shown in Fig. 3 (Leftin et al. 2014).

MOLECULAR DISTRIBUTIONS OF LIPIDS ARE REVEALED BY SOLID-STATE DEUTERIUM NMR SPECTRAL LINESHAPES

The solid-state ^2H NMR spectrum for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC- d_{54}) exemplifies applications to phospholipids that are perdeuterated throughout the chains in the physiologically relevant, liquid-crystalline state (Brown et al., 2001; Martinez et al., 2002) (Fig. 2). Because the sample consists of bilayers randomly oriented in an aqueous medium relative to the direction of the magnetic field, the spectrum is a powder-type pattern that is a superposition of signals from the lamellae at all orientations. At each angle, the signal consists of doublets from the chain methyl and methylene positions along the perdeuterated chains (Kobayashi et al., 2008). The resultant spectrum has well-defined edges at $\sim \pm 15$ kHz due to a roughly constant order for the acyl methylene segments nearest to the head group of the lipid molecule. Individual peaks within the spectrum arise from less ordered methylene groups in the lower portion of the chains, and the highly disordered terminal methyl group produces the central pair of peaks (Brown, 1996). From the distribution of RQCs, the structural parameters such as the mean area per lipid $\langle A \rangle$ and volumetric bilayer thickness D_C are readily derived by a mean-torque (MT) model in relation with the corresponding material constants or elastic moduli (Kinnun et al., 2015; Mallikarjunaiah et al., 2011; Petrache et al., 2000).

Bilayers Containing Cholesterol Enable Testing of Theories for Dynamical Structures of Membrane Assemblies

In general lipid bilayers containing cholesterol provide an excellent model for testing theories for the configurational ordering and structural dynamics of liquid-crystalline membranes (Kinnun et al., 2015). For example, considering the data shown in Fig. 2 for the DMPC- d_{54} bilayer, and its binary mixture (1:1) with cholesterol, a well-defined profile of the segmental order parameters $S_{CD}^{(i)}$ versus the acyl segment position (i) is obtained (Fig. 4). An approximate plateau occurs over the middle part of the chains, followed by a progressive decrease, which manifests the end effects within the bilayer central hydrocarbon core (Fig. 4). In addition, due to the orientation of the glycerol backbone approximately perpendicular to the membrane surface, the *sn*-1 and *sn*-2 acyl chains are inequivalent (Leftin and Brown,

2011; Leftin et al., 2014b; Seelig and Seelig, 1980). The initial chain geometry leads to smaller order parameters for the beginning of the *sn*-2 chain (Huber et al., 2002; Seelig, 1978). Beyond the first few segments, the order parameters of the *sn*-2 chain become larger than those of the *sn*-1 chain (Salmon et al., 1987). Smaller statistical fluctuations are associated with greater travel (flux) of the *sn*-2 chain to compensate for the initial position closer to the aqueous interface. The order profile clearly indicates that variations in the degree of acyl chain entanglement occur as a function of depth within the bilayer hydrocarbon region. As a result, it is unlikely that the phospholipids move individually within the bilayer, even in the presence of cholesterol (Brown, 1982; Trouard et al., 1992; Trouard et al., 1999).

The approach of using a model membrane, comprising a lipid that forms a fluid bilayer together with a lipid known to form a more ordered bilayer, as well as cholesterol in varying amounts, is probably most accessible as a mimic of biomembranes with a vast number of components (Feigenson, 2006; van Meer et al., 2008). Interestingly, cholesterol has two different functions in model membrane systems: on the one hand, it increases the hydrophobic mismatch of the lipids at low concentrations, and thereby enhances phase separation. On the other hand, it functions as a mixing agent at high concentrations. Let us consider in greater detail the results in Fig. 4 for the DMPC-*d*₅₄ bilayer, both in the absence and presence of cholesterol. To calibrate our intuition, at this point it might be helpful for readers to consider some simple motional models as heuristic limiting cases. Referring again to Fig. 4, the first example is an all-*trans* rotating polymethylene chain, where equation (2) with $\beta_{\text{PD}} = 90^\circ$ then yields $S_{\text{CD}} = -1/2$ as a reference value. Next, we can consider a crankshaft model involving a polymethylene chain saturated with kink *gauche*[±]-*trans*-*gauche*[±] configurations, leading to $S_{\text{CD}} = -1/3$ for comparison. Lastly, the classical oil-drop model completely neglects tethering of the acyl chains to the aqueous interface, in which case the isotropic motion gives $S_{\text{CD}} = 0$ as a limit. One can then compare the experimental order profiles to the above limiting cases as benchmarks.

As we have already seen in Fig. 2, for the DMPC-*d*₅₄/cholesterol (1:1) binary mixture, in the liquid-ordered (*l_o*) phase there is a dramatic increase in the RQCs *versus* the liquid disordered (*l_d*) phase of DMPC-*d*₅₄ alone. This is due to a substantial reduction of the degrees of freedom of the flexible phospholipids, due to van der Waals interactions with the rigid sterol frame. The corresponding plateau in the order profile, *cf.* Fig. 4, can be understood in terms of a relatively constant probability of the acyl chain configurations, resulting from their tethering via the polar head groups to the aqueous interface, together with their travel (flux) toward the bilayer interior. For the top part of the acyl chains, the segmental order parameters approach the limiting value of $S_{\text{CD}} = -1/2$ when cholesterol is present, as expected for an all-*trans* rotating polymethylene chain (Martinez et al., 2004). However, there is still an approximate plateau indicating entanglement of the chain ends. Note that in the absence of cholesterol, the additional acyl disorder can arise from internal degrees of freedom of the phospholipids, *e.g.*, due to segmental isomerizations, molecular motions, or collective thermal excitations of the bilayer. These additional degrees of freedom lead to smaller absolute S_{CD} values for the DMPC-*d*₅₄ bilayer. Provided the disorder of the DMPC-*d*₅₄ bilayer is due mainly to rotational isomerism, then the acyl chains fall somewhere between the limiting crankshaft model with $S_{\text{CD}} = -1/3$, and the classical oil-

drop model for which $S_{CD} = 0$. For the DMPC- d_{54} bilayer, both in the presence and absence of cholesterol, the acyl chains are more disordered within the hydrocarbon core to fill in the free volume that would otherwise be present due to chain terminations, approaching the classical “oil-drop” limit only in the center of the bilayer.

Order Parameter Profiles of Binary Lipid-Cholesterol Mixtures of Phospholipids and Sphingolipids: Implications for Rafts in Cellular Membranes

In addition to glycerophospholipids, most eukaryotic cells contain sphingolipids and sterols as additional classes of lipids (30–40 mol % cholesterol and 10–20 mol % sphingomyelin). Notably, plasma membranes of animal cells are enriched in cholesterol, which is metabolically derived from lanosterol by removal of methyl groups from the α -face of the molecule, raising the question of their evolution and function in the organization of the bilayer. Despite extensive research, how the well-known ordering effect of cholesterol for sphingomyelin and other saturated glycerophospholipids leads to lateral phase segregation and microdomains remains under discussion, as the situation *in vivo* is far too complex to be exactly determined. Yet the structural properties and phase-transition temperatures of sphingomyelins near body temperature (37 °C) suggest they may play an important role in the formation of specialized domains in membranes such as lipid rafts (Barenholz and Thompson, 1999; Konyakhina and Feigenson, 2016; Lingwood and Simons, 2010; Simons and Gerl, 2010; Yasuda et al., 2015b).

Various studies have been reported on the comparison of order parameter profiles for sphingolipids and phospholipids, and their binary and tertiary mixtures with cholesterol (Bartels et al., 2008; Bunge et al., 2008; Leftin et al., 2014a; Morrison and Bloom, 1994; Oldfield et al., 1978; Trouard et al., 1999; Yasuda et al 2015b). A related study using solid-state ^{13}C NMR spectroscopy (Leftin et al., 2014a) has shown that the cholesterol-mediated structural perturbations are less pronounced for egg-yolk sphingomyelin (EYSM) than for POPC. In Fig. 5 the order parameter profiles derived using separated local-field (SLF) ^{13}C NMR spectroscopy for EYSM and POPC bilayers are shown. The influence of cholesterol for the L_o phase of POPC and EYSM are clearly distinguishable. The higher order parameter values for both the lipid bilayers at various carbon positions are an arresting indication of the L_o phase (Bartels et al., 2008; Brown, 2012; Bunge et al., 2008; Jiansong et al., 2009; Lai and Freed, 2014; Martinez et al., 2004; Martinez et al., 2002; Smith and Freed, 2009; Tong et al., 2009; Warschawski and Devaux, 2005; Yun-Wei et al., 2007), due to interaction with cholesterol (Chen and Rand, 1997; Filippov et al., 2003; Ipsen et al., 1990). In addition, the non-equivalence of the segments of the *sn*-1 and *sn*-2 chains (Seelig and Seelig, 1980) is clearly reflected in the case of the POPC bilayers (Figs. 5a and 5b). Monounsaturations of the oleoyl chain at the C9 and C10 sites renders the two vinyl ^{13}C - ^1H positions orientationally nonequivalent, both to each other and to the other saturated chain segments. The terminal methyl group of the acyl chain exhibits very small residual dipolar couplings (RDC), because of the reorientation and three-fold symmetry of the methyl ^{13}C - ^1H bonds. The largest couplings of these sites are observed at the C3 position, which may participate in interfacial exchange-type C3-OH hydrogen bonding, and/or C3-OH acceptor and NH donor hydrogen bonding. The large value is suggestive of a glycerol backbone conformation that is stabilized through lipid packing assisted by hydrogen-bonding in the L_d phase.

Most striking, upon addition of 50 wt% cholesterol, the increment in segmental order parameters is $|S_{CH}| \approx 0.25$ for POPC and ≈ 0.12 for EYSM, as indicated in Fig. 5 (comparisons are for the maximum absolute $|S_{CH}|$ values due to plateau region of the $|S_{CD}|$ profiles). Correspondingly, the increment in the hydrocarbon thickness and condensation of area per lipid for EYSM is lower than for POPC. Such a remarkable difference indicates that EYSM is in a relatively ordered state in the single-component membrane. The higher acyl segmental order parameters in single-component bilayers at a given temperature, for EYSM relative to POPC, indicates the high propensity of self-association for the hydrophobic moieties of sphingomyelin lipids (Lingwood and Simons, 2010; Simons and Gerl, 2010).

Several studies on phase diagrams of binary and ternary lipid mixtures with cholesterol have been reported (Chiang et al., 2004; Feigenson, 2006; Huang and Feigenson, 1993; James et al., 2014; Konyakhina and Feigenson, 2016; McConnell, 2005; Veatch and Keller, 2003). However, in a related solid-state ^2H NMR investigation (Bartels et al., 2008), results have been obtained from lineshapes of both POPC- d_{31} as well as PSM- d_{31} in a complementary way in a particular region of phase diagram of the corresponding ternary systems. These studies revealed that addition of cholesterol at 20 mole % has a major impact on the spectra of PSM- d_{31} in ternary mixtures with POPC (1:1). The range of phase coexistence is increased by the addition of POPC, thereby resisting the formation of the I_d phase from the I_o phase. By contrast, addition of PSM shows an increased ordering effect on the palmitoyl chain in POPC relative to the PSM-free POPC- d_{31} /cholesterol mixture. Analysis of solid-state ^2H NMR data using a first-order mean-torque model (Bartels et al., 2008; Petrache et al., 2000) has uncovered interesting insights into the effect of cholesterol on the lateral organization of lipid-cholesterol mixtures. As described by Bartels et al. (2008), the addition of cholesterol initially drives the phase separation (I_d to s_o) by inducing greater lateral order in sphingomyelin than in POPC lipids (Fig. 6). However, at 20 mol % cholesterol, discrete components due to POPC- d_{31} and PSM- d_{31} phase separations were observed. At physiological temperature the lipids in the ternary mixture (1:1 PSM/POPC with 20 mol % cholesterol) showed distinct structural parameters (bilayer thickness and area per lipid). Structural parameters of POPC are highly temperature dependent, whereas sphingomyelin shows resistance to thermally-driven structural deformation and stronger affinity of cholesterol that can drive the formation of membrane domains. In mixtures with high amounts of cholesterol (33 mole %), saturation of the ordering effect for PSM seems to facilitate ideal mixing of the components, and hence similar results are observed. These observations naturally bring the intuition that the phase separation is mostly driven by hydrophobic mismatch of the acyl chains of the various lipids, e.g. it is normally assumed that the thickness difference is induced by unequal sterol partitioning into the two phases.

Notably, these observations suggest that upon adding cholesterol, the entropic loss is less pronounced for EYSM than for POPC, as discussed by Leftin et al. (2014b). Mixing of cholesterol is more favorable for sphingolipids compared to phosphatidylcholines, potentially driving the formation of lipid rafts in multi-component biomembranes (Ohvo-Rekilä et al., 2002; Ramstedt and Slotte, 2006). That is to say, like dissolves like—as we learn in our introductory chemistry courses!

NUCLEAR SPIN RELAXATION REVEALS MULTISCALE DYNAMICS OF MEMBRANE LIPIDS

An important further aspect is that analysis of the nuclear spin relaxation rates yields experimental information about the molecular dynamics that is unobtainable with other biophysical methods (Brown, 1996). The possible types of motions that occur in lyotropic liquid crystals are: (i) segmental motions due to rotational isomerizations of the flexible surfactant or lipid molecules; (ii) slower effective rotations of the entangled molecules; and (iii) collective deformations of the bilayer which span a broad range, and can influence interactions involving the assembly (Brown et al., 2002; Leftin and Brown, 2011; Leftin et al., 2014b; Martinez et al., 2004; Martinez et al., 2002). At the high frequencies, bond stretching and bending vibrations are most likely too fast to influence significantly the nuclear spin relaxation, but rather lead to a pre-averaging of the coupling tensor (Brown, 1984b; Brown et al., 2002). Identifying the predominant contributions within the various motional regimes, and characterizing their energetic parameters, would reveal atomistic interactions leading to bulk material properties based on current NMR technology (Leftin et al., 2014b). For liquid-crystalline membranes, elastic deformations (modeled as splay, twist, and bend) within the hydrocarbon core are interpreted as collective lipid dynamics on the order of membrane dimensions (Brown et al., 2001). It follows that NMR relaxation studies of lipid membranes in the I_o and I_d phases can strongly benefit our understanding of the atomistic lipid-cholesterol interactions, leading to changes in bulk membrane physical properties, with striking biological consequences.

The process of NMR relaxation is due to fluctuations of the coupling Hamiltonian, on account of the various possible motions of the lipid molecules within the bilayer. According to time-dependent perturbation theory, these fluctuations give rise to transitions between the various adjacent energy levels (Xu et al., 2014). Now in ^2H NMR relaxometry of liquid-crystalline membrane lipids, one is often interested in the spin-lattice (R_{1Z}) relaxation rates. Experimental R_{1Z} relaxation rate measurements involve perturbation of the magnetization away from the equilibrium value, and then following the attainment of equilibrium by observing the magnetization recovery as a function of time. The observable relaxation rates are related to the spectral densities of motion in the laboratory frame by:

$$R_{1Z} = \frac{3}{4} \pi^2 \chi_Q^2 [J_1(\omega_0) + 4J_2(2\omega_0)] \quad (8)$$

In the above expressions, R_{1Z} is the spin-lattice (longitudinal) relaxation rate, and $J_m(\omega_0)$ denotes the irreducible spectral densities of motion, where $m = 1, 2$, and ω_0 is the deuteron Larmor frequency. The spectral densities $J_m(\omega_0)$ describe the power spectrum of the motions as a function of frequency ω_0 in terms of fluctuations of the Wigner rotation matrix elements for transformation of the coupling (EFG) tensor from its principal axis system to the laboratory frame. They are the Fourier transform partners of the orientational correlation functions $G_m(t)$ which depend on time, and characterize the C- ^2H bond fluctuations.

Generalized Model-free Aspects of the Nuclear Spin Relaxation of Membrane Lipid Bilayers

Here we give a brief introduction to model-free interpretation of the relaxation rates for the benefit of general readers, while dealing with actual lipid relaxation data. For a more complete description of generalized model-free (GMF) analysis, readers are referred to the review by Xu et al. (2014). Contributions to the nuclear spin relaxation from motions with different characteristic mean-squared amplitudes and time-scales are possible in terms of a hierarchical energy landscape (Brown, 1982). They include: (i) the static coupling tensor that is modulated by rapid local segmental motions, such as *trans-gauche* isomerizations of the hydrocarbon chains of the lipid or surfactant molecules; and (ii) the residual coupling tensor (left-over from the fast motions) that is further modulated by slower motions. The slower motions in principle might include whole molecular motions of the flexible phospholipids, or alternatively collective thermal excitations involving various lipid molecules (Klauda et al., 2008a; Klauda et al., 2006). Clearly the segmental order parameters depend only on the amplitudes of the C–²H bond fluctuations. On the other hand, the relaxation rates depend on both the orientational amplitudes and the rates of the C–²H bond fluctuations. According to the GMF approach of relaxation rate analysis (Brown, 1984a, b; Xu et al., 2014), a simple square-law functional dependence of the R_{1Z} rates on the S_{CD} order parameters along the chain would result (Fig. 7). In the limit of short-wavelength excitations, on the order of the bilayer thickness and less, the spectral density reads (Nevzorov and Brown, 1997):

$$J_m(\omega) = \frac{5}{2} S_{CD}^2 D \omega^{-(2-d/2)} \left[\left| D_{-1m}^{(2)}(\beta_{DL}) \right|^2 + \left| D_{1m}^{(2)}(\beta_{DL}) \right|^2 \right] \quad (9)$$

Here ω is the angular frequency, D is the viscoelastic constant, d is the dimensionality, and $D^{(2)}$ indicates the second-rank Wigner rotation matrix (Rose, 1957). The irreducible spectral densities $J_m(\omega)$ depend on the square of the observed S_{CD} order parameters, and the slope of the square-law plot is inversely related to the softness of the membrane. For 3D quasielastic fluctuations, the viscoelastic constant is given by $D = 3k_B T \sqrt{\eta}/5\pi \sqrt{2K^3 S_s^2}$ where a single elastic constant K is assumed, in which η is the corresponding viscosity coefficient, S_s is the order parameter for the relatively slow motions, and other symbols have their usual meanings. No distinction is made between splay, twist, and bend deformations. In addition to the bending modulus K_C the compression modulus K_B may come into play (Nagle and Tristram-Nagle, 2000).

In the example presented here, a solid-state ²H NMR relaxation study of the effect of cholesterol on lipid bilayers shows a square-law functional dependence of the R_{1Z} rates *versus* the order parameters S_{CD} along the entire acyl chain for the multilamellar dispersions of DMPC-*d*₅₄/cholesterol bilayers (Fig. 7) (Martinez et al., 2002). This dependence on the motional amplitudes signifies relatively slow bilayer motions that modulate the residual coupling tensors left over from faster segmental motions (Fermi's golden rule). Given a simple composite membrane deformation model (Brown et al., 2002; Nevzorov et al., 1998), the R_{1Z} rates are due to a broad spectrum of 3D collective bilayer excitations, with effective rotations of the lipids. Transverse ²H NMR spin relaxation studies also provide evidence for

2D collective motions of the membrane film, albeit at lower frequencies (Althoff et al., 2002a; Althoff et al., 2002b; Bloom and Evans, 1991; Bloom et al., 1991; Molugu et al., 2012, 2013). By contrast, local *trans-gauche* isomerizations along the chains modulate the same *static* NMR coupling tensor, and do not yield such a square-law. With regard to splay deformations, the so-called bending rigidity is $\kappa \approx Kt$, where $t = 2D_C$ is the bilayer thickness, giving a $\kappa^{-3/2}$ dependence of the R_{1Z} rates (Brown et al., 2001). Moreover, 3D director fluctuations ($d=3$) yield a $\omega^{-1/2}$ frequency dispersion as a characteristic signature (Brown, 1982; Brown et al., 1983). In this case, the reduction in the square-law slope, *cf.* Fig. 7, reflects an increase in κ and/or S_s due to short-range cholesterol-phospholipid interactions. One should also note that at the molecular level, a dynamical protrusion of cholesterol across the midplane, *i.e.* between the apposed monolayers may occur as suggested by quasielastic neutron scattering (QENS) studies (Endress et al., 2002b; Kaye et al., 2011). Indeed the square-law functional dependence as first discussed (Brown, 1982) is a model-free correlation among the experimental observables, and it does not rest on any specific interpretation (Fig. 7). For the longitudinal relaxation rates of liquid-crystalline lipid membranes, the above analysis is expected to be generally applicable.

Influences of cholesterol on the physical properties of DMPC bilayers have been compared to its metabolic precursor lanosterol in a related study (Martinez et al., 2004) (Fig. 8). Notably, cholesterol is a two-faced molecule—the α -phase is smooth and the β -face is molecularly rough due to the methyl substituents. On the other hand, lanosterol is methylated on both the α -face and the β -face (Fig. 1), and it presents a more balanced countenance to the phospholipids (Rog et al., 2007). In terms of biomolecular NMR spectroscopy, it has been observed that the slope of square-law plot is greater for lanosterol than for cholesterol, consistent with the bilayer stiffness being less for lanosterol *versus* its metabolic product cholesterol (Martinez et al., 2004; Orädd et al., 2009; Shahedi et al., 2006). Again, it is found that the site-specific analysis of the solid-state NMR results based on atomistic observables matches the results for the macroscopic bilayer elasticity (Endress et al., 2002a; Endress et al., 2002b) (Fig. 9). The more molecularly smooth van der Waals surface of the α -face of cholesterol (Bloch, 1983; Filippov et al., 2003; Lai and Freed, 2014; Yeagle et al., 1977) enables a large increase in bilayer rigidity, and stabilizes the liquid-ordered phase to an even greater degree than lanosterol. The progressive increase in the bilayer rigidity on going from lanosterol to cholesterol parallels the metabolic pathway of sterol biogenesis (Brief et al., 2009; Cheng et al., 1986; Henriksen et al., 2006; Hsueh et al., 2005; Ling et al., 2002; Yeagle, 1985; Yeagle et al., 1990), and may be related to the optimization or evolution of the biophysical properties of cholesterol.

Conclusions

Solid-state NMR methods uniquely probe the biophysical properties of lipid membranes and provide information complementary to other spectroscopic methods. Current NMR technology provides knowledge of membrane properties that cannot be obtained with other existing biophysical methods. Interestingly, owing to their physiological liquid-crystalline nature, membrane elastic deformations together with their multi-scale molecular dynamics clearly fall in the solid-state ^2H NMR time and length scales. The membrane stiffening effect upon addition of sterols has been investigated at an atomistically resolved level,

showing a direct correspondence with bulk elasticity. Phase separation in bilayers of ternary lipid mixtures occurs mainly due to dissimilar affinities for different cholesterol concentrations. It is likely that configurational entropy plays a major role in the formation of the lipid domains called rafts (Leftin et al., 2014a). It has also been shown in model membranes (Ackerman and Feigenson, 2015; Feigenson, 2015; Huang and Feigenson, 1993; Konyakhina and Feigenson, 2016) that formation of such domains entails Gibbs free energies that are lower than those for protein-lipid interactions, which therefore may indicate a role for proteins in domain formation. Further detailed information regarding protein-lipid interactions can contribute to understanding the lateral organization of cellular plasma membranes, and how lipid rafts may be implicated in their functional mechanisms. Moreover, nuclear spin-lattice relaxation studies of fluid lipid bilayers manifest their quasi-elastic deformation on short length and time scales, on the order of the membrane thickness and less. Interpretation of the atom-specific NMR relaxation data is in broad agreement with molecular dynamics (MD) simulations of flexible surfactant films and lipid membranes (Hofsäß et al., 2003; Klauda et al., 2008a; Klauda et al., 2006; Pastor et al., 2002; Sodt et al., 2014). Molecular simulations (Hofsäß et al., 2003) show that local *trans-gauche* isomerizations are accompanied by concerted isomerizations about multiple bonds in the assembly of lipid acyl chains. The continuum model for NMR relaxation approximates the collective excitations in mathematical closed form, whereby the influences of cholesterol and the membrane thickness correspond to the bilayer bending energy. In this regard, a possible future direction is to compare the MAS-derived membrane structural parameters to those of oriented lipid bilayers to investigate the effects of curvature. Another interesting future aspect entails the study of lipid domains. Cholesterol-induced mixed l_o and l_a phases have been successfully observed simultaneously using ^2H solid-state NMR spectroscopy (Yasuda et al. 2015) of site specifically deuterated N-stearoylsphingomyelin (SSM) with ternary mixtures of dioleoyl-*sn*-glycero-3phosphocholine (DOPC) and cholesterol. The possibility of using separated local-field (SLF) methods for natural-abundance binary lipid mixture samples to inform such cholesterol-mediated phase separation remains to be explored (Ferreira et al. 2013, Leftin et al. 2013). Currently solid-state NMR ^2H spectroscopy uses selectively deuterated lipid samples to obtain such information. Investigations of NMR relaxation-derived viscoelastic properties of the mixed phases in selectively deuterated samples are particularly interesting in the context of this review article. A key remaining question is how the bilayer softness as studied with NMR relaxation may be significant to lipid-protein interactions in fluid membranes (Brown, 2012; Huster, 2014; Penk et al., 2011; Scheidt and Huster, 2009), where elastic curvature deformation may play an important role.

Acknowledgments

This research was supported by the U. S. National Institutes of Health. The authors are indebted to the members of our research group for their many contributions to the research in this article.

References

- Ackerman DG, Feigenson GW. Multiscale modeling of four-component lipid mixtures: domain composition, size, alignment, and properties of the phase interface. *J. Phys. Chem. B.* 2015; 119:4240–4250. [PubMed: 25564922]

- Althoff G, Frezzato D, Vilfan M, Stauch O, Schubert R, Vilfan I, Moro GJ, Kothe G. Transverse nuclear spin relaxation studies of viscoelastic properties of membrane vesicles. I. Theory. *J. Phys. Chem. B.* 2002a; 106:5506–5516.
- Althoff G, Stauch O, Vilfan M, Frezzato D, Moro GJ, Hauser P, Schubert R, Kothe G. Transverse nuclear spin relaxation studies of viscoelastic properties of membrane vesicles. II. Experimental results. *J. Phys. Chem. B.* 2002b; 106:5517–5526.
- Amazon JJ, Feigenson GW. Lattice simulations of phase morphology on lipid bilayers: Renormalization, membrane shape, and electrostatic dipole interactions. *Phys. Rev. E.* 2014; 89
- Armstrong CL, Barrett MA, Hiess A, Salditt T, Katsaras J, Shi A-C, Rheinstaedter MC. Effect of cholesterol on the lateral nanoscale dynamics of fluid membranes. *Eur. Biophys. J. Biophys.* 2012; 41:901–913.
- Armstrong CL, Haeussler W, Seydel T, Katsaras J, Rheinstaedter MC. Nanosecond lipid dynamics in membranes containing cholesterol. *Soft Matter.* 2014; 10:2600–2611. [PubMed: 24647350]
- Armstrong CL, Marquardt D, Dies H, Kucerka N, Yamani Z, Harroun TA, Katsaras J, Shi A-C, Rheinstaedter MC. The observation of highly ordered domains in membranes with cholesterol. *PLoS ONE.* 2013; 8:e66162-1–e66162-10. [PubMed: 23823623]
- Baier CJ, Fantini J, Barrantes FJ. Disclosure of cholesterol recognition motifs in transmembrane domains of the human nicotinic acetylcholine receptor. *Sci. Rep.* 2011; 69:1–7.
- Barenholz Y, Thompson TE. Sphingomyelin: biophysical aspects. *Chem. Phys. Lipids.* 1999; 102:29–34. [PubMed: 11001558]
- Bartels T, Lankalapally RS, Bittman R, Beyer K, Brown MF. Raft-like mixtures of sphingomyelin and cholesterol investigated by solid-state ^2H NMR spectroscopy. *J. Am. Chem. Soc.* 2008; 130:14521–14532. [PubMed: 18839945]
- Bloch K. Sterol structure and membrane function. *CRC Crit. Rev. Biochem.* 1983; 14:47–92. [PubMed: 6340956]
- Bloom, M., Evans, E. Observations of surface undulations on the mesoscopic length scale by NMR. In: Peliti, L., editor. *Biologically Inspired Physics*. New York: Plenum; 1991. p. 137-147.
- Bloom M, Evans E, Mouritsen OG. Physical properties of the fluid lipid-bilayer component of cell membranes: a perspective. *Q. Rev. Biophys.* 1991; 24:293–397. [PubMed: 1749824]
- Brief E, Kwak S, Cheng J TJ, Kitson N, Thewalt J, Lafleur M. Phase behavior of an equimolar mixture of n-palmitoyl-d-erythro-sphingosine, cholesterol, and palmitic acid, a mixture with optimized hydrophobic matching. *Langmuir.* 2009; 25:7523–7532. [PubMed: 19563230]
- Brown DA, London E. Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* 1998; 14:111–136. [PubMed: 9891780]
- Brown MF. Theory of spin-lattice relaxation in lipid bilayers and biological membranes. ^2H and ^{14}N quadrupolar relaxation. *J. Chem. Phys.* 1982; 77:1576–1599.
- Brown MF. Theory of spin-lattice relaxation in lipid bilayers and biological membranes. Dipolar relaxation. *J. Chem. Phys.* 1984a; 80:2808–2831.
- Brown MF. Unified picture for spin-lattice relaxation of lipid bilayers and biomembranes. *J. Chem. Phys.* 1984b; 80:2832–2836.
- Brown MF. Anisotropic nuclear spin relaxation of cholesterol in phospholipid bilayers. *Mol. Phys.* 1990; 71:903–908.
- Brown MF. Modulation of rhodopsin function by properties of the membrane bilayer. *Chem. Phys. Lipids.* 1994; 73:159–180. [PubMed: 8001180]
- Brown, MF. Membrane structure and dynamics studied with NMR spectroscopy. In: Merz, J.K, M., Roux, B., editors. *Biological Membranes: A Molecular Perspective from Computation and Experiment*. Basel: Birkhäuser; 1996. p. 175-252.
- Brown MF. Influence of nonlamellar-forming lipids on rhodopsin. *Curr. Top. Membr.* 1997; 44:285–356.
- Brown MF. Curvature forces in membrane lipid-protein interactions. *Biochemistry.* 2012; 51:9782–9795. [PubMed: 23163284]

- Brown, MF., Chan, SI. Bilayer membranes: deuterium & carbon-13 NMR. In: Harris, RK., Grant, DM., editors. Encyclopedia of Magnetic Resonance. New York: John Wiley & Sons; 1996. p. 871-885.
- Brown, MF., Chan, SI. eMagRes. John Wiley & Sons, Ltd; 2007. Bilayer membranes: deuterium and carbon-13 NMR; p. 1-15.
- Brown MF, Deese AJ, Dratz EA. Proton, Carbon-13, and Phosphorus-31 NMR methods for the investigation of rhodopsin-lipid interactions in retinal rod outer segment membranes. *Methods Enzymol.* 1982; 81:709–728. [PubMed: 7098912]
- Brown, MF., Lope-Piedrafita, S., Martinez, GV., Petrache, HI. Solid-state deuterium NMR spectroscopy of membranes. In: Webb, GA., editor. Modern Magnetic Resonance. Heidelberg: Springer; 2006. p. 245-256.
- Brown MF, Ribeiro AA, Williams GD. New view of lipid bilayer dynamics from ^2H and ^{13}C NMR relaxation time measurements. *Proc. Natl. Acad. Sci. USA.* 1983; 80:4325–4329. [PubMed: 6576340]
- Brown MF, Seelig J. Influence of cholesterol on the polar region of phosphatidylcholine and phosphatidylethanolamine bilayers. *Biochemistry.* 1978; 17:381–384. [PubMed: 619997]
- Brown MF, Thurmond RL, Dodd SW, Otten D, Beyer K. Composite membrane deformation on the mesoscopic length scale. *Phys. Rev. E.* 2001; 64:010901-1–010901-4.
- Brown MF, Thurmond RL, Dodd SW, Otten D, Beyer K. Elastic deformation of membrane bilayers probed by deuterium NMR relaxation. *J. Am. Chem. Soc.* 2002; 124:8471–8484. [PubMed: 12105929]
- Bunge A, Mueller P, Stoeckl M, Herrmann A, Huster D. Characterization of the ternary mixture of sphingomyelin, POPC, and cholesterol: Support for an inhomogeneous lipid distribution at high temperatures. *Biophys. J.* 2008; 94:2680–2690. [PubMed: 18178660]
- Camley BA, Brown FLH. Dynamic simulations of multicomponent lipid membranes over long length and time scales. *Phys. Rev. Lett.* 2010; 105
- Chen Z, Rand RP. The influence of cholesterol on phospholipid membrane curvature and bending elasticity. *Biophys. J.* 1997; 73:267–276. [PubMed: 9199791]
- Cheng C-Y, Olijve LLC, Kausik R, Han S. Cholesterol enhances surface water diffusion of phospholipid bilayers. *J. Chem. Phys.* 2014; 141
- Cheng K-H, Lepock JR, Hui SW, Yeagle PL. The role of cholesterol in the activity of reconstituted Ca-ATPase vesicles containing unsaturated phosphatidylethanolamine. *J. Biol. Chem.* 1986; 261:5081–5087. [PubMed: 3007490]
- Chiang YW, Shimoyama Y, Feigenson GW, Freed JH. Dynamic molecular structure of DPPC-DLPC-cholesterol ternary lipid system by spin-label electron spin resonance. *Biophys. J.* 2004; 87:2483–2496. [PubMed: 15454445]
- Cobo MF, Achilles A, Reichert D, deAzevedo ER, Saalwaechter K. Recoupled separated-local-field experiments and applications to study intermediate-regime molecular motions. *J. Magn. Reson.* 2012; 221:85–96. [PubMed: 22750254]
- Coskun U, Simons K. Cell membranes: The lipid perspective. *Structure.* 2011; 19:1543–1548. [PubMed: 22078554]
- Das BB, Opella SJ. Multiple acquisition/multiple observation separated local field/chemical shift correlation solid-state magic angle spinning NMR spectroscopy. *J. Magn. Reson.* 2014; 245:98–104. [PubMed: 25023566]
- Day, CA., Kenworthy, AK. Functions of cholera toxin B-subunit as a raft cross-linker. In: Parmryd, I., editor. Membrane Nanodomains. 2015. p. 135-145.
- Delmelle M, Butler KW, Smith ICP. Saturation transfer electron-spin resonance spectroscopy as a probe of anisotropic motion in model membrane systems. *Biochemistry.* 1980; 19:698–704. [PubMed: 6243954]
- Edidin M. The state of lipid rafts: from model membranes to cells. *Annu. Rev. Biophys. Biomol. Struct.* 2003; 32:257–283. [PubMed: 12543707]
- Endress E, Bayerl S, Prechtel K, Maier C, Merkel R, Bayerl TM. The effect of cholesterol, lanosterol, and ergosterol on lecithin bilayer mechanical properties at molecular and microscopic dimensions: A solid-state NMR and micropipet study. *Langmuir.* 2002a; 18:3293–3299.

- Endress E, Heller H, Casalta H, Brown MF, Bayerl TM. Anisotropic motion and molecular dynamics of cholesterol, lanosterol, and ergosterol in lecithin bilayers studied by quasi-elastic neutron scattering. *Biochemistry*. 2002b; 41:13078–13086. [PubMed: 12390036]
- Epand RM. Lipid polymorphism and protein-lipid interactions. *Biochim. Biophys. Acta*. 1998; 1376:353–368. [PubMed: 9804988]
- Epand RM. Cholesterol and the interaction of proteins with membrane domains. *Prog. Lipid. Res.* 2006; 45:279–294. [PubMed: 16574236]
- Eriksson JC, Henriksson U. Bridging-cluster model for hydrophobic attraction. *Langmuir*. 2007; 23:10026–10033. [PubMed: 17760469]
- Eriksson JC, Henriksson U, Kumpulainen A. Hydrophobic attraction forces in asymmetric aqueous films between hydrophobized mica/bare mica surfaces. *Colloids Surf. A. Physicochem. Eng. Asp.* 2006; 282:79–83.
- Escriba PV, Gonzalez-Ros JM, Goni FM, Kinnunen PKJ, Vigh L, Sanchez-Magraner L, Fernandez AM, Busquets X, Horvath I, Barcelo-Coblijn G. Membranes: a meeting point for lipids, proteins and therapies. *J. Cell. Mol. Med.* 2008; 12:829–875. [PubMed: 18266954]
- Fantini J, Barrantes FJ. How cholesterol interacts with membrane proteins: an exploration of cholesterol-binding sites including CRAC, CARC, and tilted domains. *Front. Physiol.* 2013; 4
- Feigenson GW. Phase behavior of lipid mixtures. *Nat. Chem. Biol.* 2006; 2:560–563. [PubMed: 17051225]
- Feigenson GW. Pictures of the Substructure of Liquid-Ordered Domains. *Biophys. J.* 2015; 109:854–855. [PubMed: 26331243]
- Ferreira TM, Coreta-Gomes F, Ollila OHS, Moreno MJ, Vaz WLC, Topgaard D. Cholesterol and POPC segmental order parameters in lipid membranes: solid state ^1H - ^{13}C NMR and MD simulation studies. *Phys. Chem. Chem. Phys.* 2013; 15:1976–1989. [PubMed: 23258433]
- Ferreira TM, Medronho B, Martin RW, Topgaard D. Segmental order parameters in a nonionic surfactant lamellar phase studied with ^1H - ^{13}C solid-state NMR. *Phys. Chem. Chem. Phys.* 2008; 10:6033–6038. [PubMed: 18825291]
- Filippov A, Orädd G, Lindblom G. The effect of cholesterol on the lateral diffusion of phospholipids in oriented bilayers. *Biophys. J.* 2003; 84:3079–3086. [PubMed: 12719238]
- Gawrisch K. Tafazzin senses curvature. *Nat. Chem. Biol.* 2012; 8:811–812. [PubMed: 22987008]
- Gawrisch K, Eldho NV, Polozov IV. Novel NMR tools to study structure and dynamics of biomembranes. *Chem. Phys. Lipids*. 2002; 116:135–151. [PubMed: 12093539]
- Golebiewska U, Scarlata S. The effect of membrane domains on the G protein-phospholipase C β signaling pathway. *Crit. Rev. Biochem. Mol. Biol.* 2010; 45:97–105. [PubMed: 20128735]
- Goni FM, Alonso A, Bagatolli LA, Brown RE, Marsh D, Prieto M, Thewalt JL. Phase diagrams of lipid mixtures relevant to the study of membrane rafts. *Biochim. Biophys. Acta*. 2008; 1781:665–684. [PubMed: 18952002]
- Gopinath T, Mote KR, Veglia G. Proton evolved local field solid-state nuclear magnetic resonance using Hadamard encoding: Theory and application to membrane proteins. *J. Chem. Phys.* 2011; 135
- Greenwood AI, Pan J, Mills TT, Nagle JF, Epand RM, Tristram-Nagle S. CRAC motif peptide of the HIV-1 gp41 protein thins SOPS membranes and interacts with cholesterol. *Biochim. Biophys. Acta*. 2008; 1778:1120–1130. [PubMed: 18262490]
- Gross JD, Warschawski DE, Griffin RG. Dipolar recoupling in MAS NMR: a probe for segmental order in lipid bilayers. *J. Am. Chem. Soc.* 1997; 119:796–802.
- Gruner SM. Stability of lyotropic phases with curved interfaces. *J. Phys. Chem.* 1989; 93:7562–7570.
- Henriksen J, Rowat AC, Brief E, Hsueh YW, Thewalt JL, Zuckermann MJ, Ipsen JH. Universal behavior of membranes with sterols. *Biophys. J.* 2006; 90:1639–1649. [PubMed: 16326903]
- Hofsäß C, Lindahl E, Edholm O. Molecular dynamics simulations of phospholipid bilayers with cholesterol. *Biophys. J.* 2003; 84:2192–2206. [PubMed: 12668428]
- Holland GP, Alam TM. Multi-dimensional ^1H - ^{13}C HETCOR and FSLG-HETCOR NMR study of sphingomyelin bilayers containing cholesterol in the gel and liquid crystalline states. *J. Magn. Reson.* 2006; 181:316–326. [PubMed: 16798032]

- Hong M, Schmidt-Rohr K, Pines A. NMR measurement of signs and magnitudes of C–H dipolar couplings in lecithin. *J. Am. Chem. Soc.* 1995; 117:3310–3311.
- Hsueh YW, Gilbert K, Trandum C, Zuckermann M, Thewalt J. The effect of ergosterol on dipalmitoylphosphatidylcholine bilayers: a deuterium NMR and calorimetric study. *Biophys. J.* 2005; 88:1799–1808. [PubMed: 15596499]
- Huang JY, Feigenson GW. Monte-carlo simulation of lipid mixtures: finding phase separation. *Biophys. J.* 1993; 65:1788–1794. [PubMed: 8298012]
- Hubbell WL, McConnell HM. Molecular motion in spin-labeled phospholipids and membranes. *J. Am. Chem. Soc.* 1971; 93:314–326. [PubMed: 5541516]
- Huber T, Botelho AV, Beyer K, Brown MF. Membrane model for the G-protein-coupled receptor rhodopsin: hydrophobic interface and dynamical structure. *Biophys. J.* 2004; 86:2078–2100. [PubMed: 15041649]
- Huber T, Rajamoorthi K, Kurze VF, Beyer K, Brown MF. Structure of docosahexaenoic acid-containing phospholipid bilayers as studied by ^2H NMR and molecular dynamics simulations. *J. Am. Chem. Soc.* 2002; 124:298–309. [PubMed: 11782182]
- Huster D. Solid-state NMR spectroscopy to study protein lipid interactions. *Biochim. Biophys. Acta.* 2014; 1841:1146–1160. [PubMed: 24333800]
- Ipsen JH, Mouritsen OG, Bloom M. Relationships between lipid membrane area, hydrophobic thickness, and acyl-chain orientational order. The effects of cholesterol. *Biophys. J.* 1990; 57:405–412. [PubMed: 2306491]
- Jafurulla M, Tiwari S, Chattopadhyay A. Identification of cholesterol recognition amino acid consensus (CRAC) motif in G-protein coupled receptors. *Biochem. Biophys. Res. Commun.* 2011; 404:569–573. [PubMed: 21146498]
- James RCL, Litz JP, Keller SL. Lipid Membranes Containing Plant Sterols Separate into Coexisting Liquid Phases Over Broad Temperature and Compositional Ranges. *Biophys. J.* 2014; 106:92A–92A.
- Jansson M, Thurmond RL, Barry JA, Brown MF. Deuterium NMR study of intermolecular interactions in lamellar phases containing palmitoyllysophosphatidylcholine. *J. Phys. Chem.* 1992; 96:9532–9544.
- Jayanthi S, Sinha N, Ramanathan KV. 2(4)-SEMA as a sensitive and offset compensated SLF sequence. *J. Magn. Reson.* 2010; 207:206–212. [PubMed: 20880731]
- Jiansong T, Borbat PP, Freed JH, Yeon-Kyun S. A scissors mechanism for stimulation of SNARE-mediated lipid mixing by cholesterol. *Proc. Natl. Acad. Sci. USA.* 2009; 106:5141–5146. [PubMed: 19251653]
- Kaiser H-J, Lingwood D, Levental I, Sampaio JL, Kalvodova L, Rajendran L, Simons K. Order of lipid phases in model and plasma membranes. *Proc. Natl. Acad. Sci. USA.* 2009; 106:16645–16650. [PubMed: 19805351]
- Kaye MD, Schmalzl K, Nibali VC, Tarek M, Rheinstaedter MC. Ethanol enhances collective dynamics of lipid membranes. *Phys. Rev. E.* 2011; 83:050907-1–050907-4.
- Keller SL, McConnell HM. Stripe phases in lipid monolayers near a miscibility critical point. *Phys. Rev. Lett.* 1999; 82:1602–1605.
- Kharkov BB, Chizhik VI, Dvinskikh SV. Low rf power high resolution ^1H - ^{13}C - ^{14}N - separated local field spectroscopy in lyotropic mesophases. *J. Magn. Reson.* 2012; 223:73–79. [PubMed: 22967890]
- Kinnun JJ, Leftin A, Brown MF. Solid-state NMR spectroscopy for the physical chemistry laboratory. *J. Chem. Educ.* 2013; 90:123–128.
- Kinnun JJ, Mallikarjunaiah KJ, Petrache HI, Brown MF. Elastic deformation and area per lipid of membranes: Atomistic view from solid-state deuterium NMR spectroscopy. *Biochim. Biophys. Acta.* 2015; 1848:246–259. [PubMed: 24946141]
- Klauda JB, Eldho NV, Gawrisch K, Brooks BR, Pastor RW. Collective and noncollective models of NMR relaxation in lipid vesicles and multilayers. *J. Phys. Chem. B.* 2008a; 112:5924–5929. [PubMed: 18179193]
- Klauda JB, Ku erka N, Brooks BR, Pastor RW, Nagle JF. Simulation-based methods for interpreting X-ray data from lipid bilayers. *Biophys. J.* 2006; 90:2796–2807. [PubMed: 16443652]

- Klauda JB, Roberts MF, Redfield AG, Brooks BR, Pastor RW. Rotation of lipids in membranes: Molecular dynamics simulation, P-31 spin-lattice relaxation, and rigid-body dynamics. *Biophys. J.* 2008b; 94:3074–3083. [PubMed: 18192349]
- Klauda JB, Venable RM, Freites JA, O'Connor JW, Tobias DJ, Mondragon-Ramirez C, Vorobyov I, MacKerell AD Jr, Pastor RW. Update of the CHARMM All-Atom Additive Force Field for Lipids: Validation on Six Lipid Types. *J. Phys. Chem. B.* 2010; 114:7830–7843. [PubMed: 20496934]
- Klauda JB., Venable, RM., MacKerell, AD., Jr, Pastor, RW. Considerations for lipid force field development. In: Feller, SE., editor. *Computational Modeling of Membrane Bilayers*. 2008c. p. 1-48.
- Klose C, Surma MA, Simons K. Organellar lipidomics - background and perspectives. *Curr. Opin. Cell Biol.* 2013; 25:406–413. [PubMed: 23608594]
- Kobayashi M, Struts AV, Fujiwara T, Brown MF, Akutsu H. Fluid mechanical matching of H⁺-ATP synthase subunit c-ring with lipid membranes revealed by ²H solid-state NMR. *Biophys. J.* 2008; 94:4339–4347. [PubMed: 18310246]
- Konyakhina TM, Feigenson GW. Phase diagram of a polyunsaturated lipid mixture: Brain sphingomyelin/1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine/cholesterol. *Biochim. Biophys. Acta.* 2016; 1858:153–161. [PubMed: 26525664]
- Korade Z, Kenworthy AK. Lipid rafts, cholesterol, and the brain. *Neuropharmacology.* 2008; 55:1265–1273. [PubMed: 18402986]
- Krepkiy D, Mihailescu M, Freites JA, Schow EV, Worcester DL, Gawrisch K, Tobias DJ, White SH, Swartz KJ. Structure and hydration of membranes embedded with voltage-sensing domains. *Nature.* 2009; 462:473–479. [PubMed: 19940918]
- Lai AL, Freed JH. HIV gp41 Fusion Peptide Increases Membrane Ordering in a Cholesterol-Dependent Fashion. *Biophys. J.* 2014a; 106:172–181. [PubMed: 24411249]
- Lee AG. How lipids affect the activities of integral membrane proteins. *Biochim. Biophys. Acta.* 2004; 1666:62–87. [PubMed: 15519309]
- Leftin A, Brown MF. An NMR database for simulations of membrane dynamics. *Biochim. Biophys. Acta.* 2011; 1808:818–839. [PubMed: 21134351]
- Leftin A, Job C, Beyer K, Brown MF. Solid-state ¹³C NMR reveals annealing of raft-like membranes containing cholesterol by the intrinsically disordered protein α -synuclein. *J. Mol. Biol.* 2013; 425:2973–2987. [PubMed: 23583776]
- Leftin A, Molugu TR, Job C, Beyer K, Brown MF. Area per lipid and cholesterol interactions in membranes by separated local-field ¹³C NMR spectroscopy. *Biophys. J.* 2014a; 107:2274–2286. [PubMed: 25418296]
- Leftin A, Xu X, Brown MF. Phospholipid bilayer membranes: deuterium and carbon-13 NMR spectroscopy. *eMagRes.* 2014b; 3:199–214.
- Liang R, Li H, Swanson JMJ, Voth GA. Multiscale simulation reveals a multifaceted mechanism of proton permeation through the influenza A M2 proton channel. *Proc. Natl. Acad. Sci. USA.* 2014; 111:9396–9401. [PubMed: 24979779]
- Ling M, Nielsen M, Thewalt J, Ipsen JH, Bloom M, Zuckermann MJ, Mouritsen OG. From lanosterol to cholesterol: structural evolution and differential effects on lipid bilayers. *Biophys. J.* 2002; 82:1429–1444. [PubMed: 11867458]
- Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. *Science.* 2010; 327:46–50. [PubMed: 20044567]
- Lippert JL, Peticolas WL. Laser raman investigation of effect of cholesterol on conformational changes in dipalmitoyl lecithin multilayers. *Proc. Natl. Acad. Sci. USA.* 1971; 68:1572–1576. [PubMed: 5283948]
- Lobo NP, Das BB, Narasimhaswamy T, Ramanathan KV. Molecular topology of three ring nematogens from C-13-H-1 dipolar couplings. *Rsc Advances.* 2014; 4:33383–33390.
- Lobo NP, Ramanathan KV. Sensitivity Enhancement in Solid-State Separated Local Field NMR Experiments by the Use of Adiabatic Cross-Polarization. *Journal of Physical Chemistry Letters.* 2011; 2:1183–1188. [PubMed: 26295323]

- Lobo NP, Ramanathan KV. Combining adiabatic and Hartmann-Hahn cross-polarization for sensitivity enhancement in solid state separated local field 2D-NMR experiments of partially ordered systems. *Chemical Physics Letters*. 2012; 536:155–161.
- Mallikarjunaiah KJ, Leftin A, Kinnun JJ, Justice MJ, Rogozea AL, Petrache HI, Brown MF. Solid-state ^2H NMR shows equivalence of dehydration and osmotic pressures in lipid membrane deformation. *Biophys. J.* 2011; 100:98–107. [PubMed: 21190661]
- Manukovsky N, Sanders E, Matalon E, Wolf SG, Goldfarb D. Membrane curvature and cholesterol effects on lipids packing and spin-labelled lipids conformational distributions. *Mol. Phys.* 2013; 111:2887–2896.
- Martinez GV, Dykstra EM, Lope-Piedrafita S, Brown MF. Lanosterol and cholesterol-induced variations in bilayer elasticity probed by ^2H NMR relaxation. *Langmuir*. 2004; 20:1043–1046. [PubMed: 15803674]
- Martinez GV, Dykstra EM, Lope-Piedrafita S, Job C, Brown MF. NMR elastometry of fluid membranes in the mesoscopic regime. *Phys. Rev. E*. 2002; 66:050902-1–050902-4.
- Matsumori N, Yasuda T, Okazaki H, Suzuki T, Yamaguchi T, Tsuchikawa H, Doi M, Oishi T, Murata M. Comprehensive Molecular Motion Capture for Sphingomyelin by Site-Specific Deuterium Labeling. *Biochemistry*. 2012; 51:8363–8370. [PubMed: 23016915]
- McConnell H. Complexes in ternary cholesterol-phospholipid mixtures. *Biophys. J.* 2005; 88:L23–L25. [PubMed: 15695625]
- Meinhardt S, Vink RLC, Schmid F. Monolayer curvature stabilizes nanoscale raft domains in mixed lipid bilayers. *Proc. Natl. Acad. Sci. USA*. 2013; 110:4476–4481. [PubMed: 23487780]
- Mendelsohn R. Laser-Raman spectroscopic study of egg lecithin and egg lecithin-cholesterol mixtures. *Biochim. Biophys. Acta*. 1972; 290:15–21. [PubMed: 4640762]
- Molugu TR, Mallikarjunaiah KJ, Job C, Brown MF. Hydration-mediated slow dynamics in phospholipid membranes. *Biophys. J.* 2012; 102:389A–390A.
- Molugu TR, Mallikarjunaiah KJ, Job C, Brown MF. Suppression of cooperative motions in phospholipid membranes by osmotic stress: deuterium NMR relaxation study. *Biophys. J.* 2013; 104:81A–81A.
- Morrison C, Bloom M. Orientation Dependence of ^2H Nuclear Magnetic Resonance Spin-Lattice Relaxation in Phospholipid and Phospholipid:Cholesterol Systems. *J. Chem. Phys.* 1994; 101:749–763.
- Nagle JF, Tristram-Nagle S. Structure of lipid bilayers. *Biochim. Biophys. Acta*. 2000; 1469:159–195. [PubMed: 11063882]
- Nevzorov AA, Brown MF. Dynamics of lipid bilayers from comparative analysis of ^2H and ^{13}C nuclear magnetic resonance relaxation data as a function of frequency and temperature. *J. Chem. Phys.* 1997; 107:10288–10310.
- Nevzorov AA, Trouard TP, Brown MF. Lipid bilayer dynamics from simultaneous analysis of orientation and frequency dependence of deuterium spin-lattice and quadrupolar order relaxation. *Phys. Rev. E*. 1998; 58:2259–2281.
- Ohvo-Rekilä H, Ramstedt B, Leppimäki P, Slotte JP. Cholesterol interactions with phospholipids in membranes. *Prog. Lipid. Res.* 2002; 41:66–97. [PubMed: 11694269]
- Oldfield E, Meadows M, Rice D, Russel J. Spectroscopic studies of specifically deuterium labeled membrane systems. Nuclear magnetic-resonance investigation of effects of cholesterol in model systems. *Biochemistry*. 1978; 17:2727–2740. [PubMed: 687560]
- Orädd G, Shahedi V, Lindblom G. Effect of sterol structure on the bending rigidity of lipid membranes: A ^2H NMR transverse relaxation study. *Biochim. Biophys. Acta*. 2009; 1788:1762–1771. [PubMed: 19573518]
- Pastor RW, Venable RM, Feller SE. Lipid bilayers, NMR relaxation, and computer simulations. *Acc. Chem. Res.* 2002; 35:438–446. [PubMed: 12069629]
- Penk A, Mueller M, Scheidt HA, Langosch D, Huster D. Structure and dynamics of the lipid modifications of a transmembrane α -helical peptide determined by ^2H solid-state NMR spectroscopy. *Biochim. Biophys. Acta*. 2011; 1808:784–791. [PubMed: 21192915]

- Petrache HI, Dodd SW, Brown MF. Area per lipid and acyl length distributions in fluid phosphatidylcholines determined by ^2H NMR spectroscopy. *Biophys. J.* 2000; 79:3172–3192. [PubMed: 11106622]
- Petrache HI, Salmon A, Brown MF. Structural properties of docosahexaenoyl phospholipid bilayers investigated by solid-state ^2H NMR spectroscopy. *J. Am. Chem. Soc.* 2001; 123:12611–12622. [PubMed: 11741426]
- Phillips R, Ursell T, Wiggins P, Sens P. Emerging roles for lipids in shaping membrane-protein function. *Nature.* 2009; 459:379–385. [PubMed: 19458714]
- Polozov IV, Gawrisch K. Characterization of the liquid-ordered state by proton MAS NMR. *Biophys. J.* 2006; 90:2051–2061. [PubMed: 16387785]
- Quinn PJ. Structure of sphingomyelin bilayers and complexes with cholesterol forming membrane rafts. *Langmuir.* 2013; 29:9447–9456. [PubMed: 23863113]
- Ramamoorthy A, Wei YF, Lee DK. PISEMA solid-state NMR spectroscopy. *Annu. Rep. NMR Spectrosc.* 2004; 52:1–52.
- Ramstedt B, Slotte JP. Sphingolipids and the formation of sterol-enriched ordered membrane domains. *Biochim. Biophys. Acta.* 2006; 1758:1945–1956. [PubMed: 16901461]
- Reddy MK, Varathan E, Lobo NP, Das BB, Narasimhaswamy T, Ramanathan KV. High-Resolution Solid State C-13 NMR Studies of Bent-Core Mesogens of Benzene and Thiophene. *Journal of Physical Chemistry C.* 2014; 118:15044–15053.
- Rheinstädter MC, Ollinger C, Fragneto G, Demmel F, Salditt T. Collective dynamics of lipid membranes studied by inelastic neutron scattering. *Phys. Rev. Lett.* 2004; 93:1–4.
- Rog T, Pasenkiewicz-Gierula M, Vattulainen I, Karttunen M. What happens if cholesterol is made smoother: Importance of methyl substituents in cholesterol ring structure on phosphatidylcholine-sterol interaction. *Biophys. J.* 2007; 92:3346–3357. [PubMed: 17293396]
- Rose, ME. *Elementary Theory of Angular Momentum.* New York: Wiley; 1957.
- Salmon A, Dodd SW, Williams GD, Beach JM, Brown MF. Configurational statistics of acyl chains in polyunsaturated lipid bilayers from ^2H NMR. *J. Am. Chem. Soc.* 1987; 109:2600–2609.
- Scheidt HA, Huster D. Structure and Dynamics of the Myristoyl Lipid Modification of Src Peptides Determined by H-2 Solid-State NMR Spectroscopy. *Biophys. J.* 2009; 96:3663–3672. [PubMed: 19413971]
- Scheidt HA, Meyer T, Nikolaus J, Baek DJ, Haralampiev I, Thomas L, Bittman R, Mueller P, Herrmann A, Huster D. Cholesterol's aliphatic side chain modulates membrane properties. *Angew. Chem. Int. Ed.* 2013; 52:12848–12851.
- Seddon JM. Structure of the inverted hexagonal (H_{II}) phase, and non-lamellar phase transitions of lipids. *Biochim. Biophys. Acta.* 1990; 1031:1–69. [PubMed: 2407291]
- Seddon JM, Templar RH, Warrender NA, Huang Z, Cevc G, Marsh D. Phosphatidylcholine–fatty acid membranes: effects of headgroup hydration on the phase behaviour and structural parameters of the gel and inverse hexagonal (H_{II}) phases. *Biochim. Biophys. Acta.* 1997; 1327:131–147. [PubMed: 9247174]
- Seelig J. Deuterium magnetic resonance: theory and application to lipid membranes. *Quart. Rev. Biophys.* 1977; 10:353–418.
- Seelig J. General features of phospholipid conformation in membranes. *Z. Physiol. Chem.* 1978; 359:1049–1050.
- Seelig J, Macdonald PM. Phospholipids and proteins in biological membranes. ^2H NMR as a method to study structure, dynamics, and interactions. *Acc. Chem. Res.* 1987; 20:221–228.
- Seelig J, Seelig A. Lipid conformation in model membranes and biological-membranes. *Quart. Rev. Biophys.* 1980; 13:19–61.
- Semer R, Gelerinter E. Spin label study of the effects of sterols on egg lecithin bilayers. *Chem. Phys. Lipids.* 1979; 23:201–211.
- Shahedi V, Oradd G, Lindblom G. Domain-formation in DOPC/SM bilayers studied by pfg-NMR: Effect of sterol structure. *Biophys. J.* 2006; 91:2501–2507. [PubMed: 16829566]

- Shaikh SR, Kinnun JJ, Leng X, Williams JA, Wassall SR. How polyunsaturated fatty acids modify molecular organization in membranes: Insight from NMR studies of model systems. *Biochim. Biophys. Acta*. 2015; 1848:211–219. [PubMed: 24820775]
- Simons K, Gerl MJ. Revitalizing membrane rafts: new tools and insights. *Nat. Rev. Mol. Cell Biol*. 2010; 11:688–699. [PubMed: 20861879]
- Simons K, Sampaio JL. Membrane organization and lipid rafts. *Cold Spring Harb. Perspect. Biol*. 2011; 3
- Simons K, Toomre D. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol*. 2000; 1:31–39. [PubMed: 11413487]
- Smith AK, Freed JH. Determination of tie-line fields for coexisting lipid phases: an ESR study. *J. Phys. Chem. B*. 2009; 113:3957–3971. [PubMed: 19673072]
- Sodt AJ, Sandar ML, Gawrisch K, Pastor RW, Lyman E. The molecular structure of the liquid-ordered phase of lipid bilayers. *J. Am. Chem. Soc*. 2014; 136:725–732. [PubMed: 24345334]
- Song Y, Kenworthy AK, Sanders CR. Cholesterol as a co-solvent and a ligand for membrane proteins. *Protein Science*. 2014; 23:1–22.
- Soubias O, Teague WE, Hines KG, Gawrisch K. The role of membrane curvature elastic stress for function of rhodopsin-like G protein-coupled receptors. *Biochimie*. 2014; 107:28–32. [PubMed: 25447139]
- Soubias O, Teague WE Jr, Hines KG, Gawrisch K. Rhodopsin/lipid hydrophobic matching–rhodopsin oligomerization and function. *Biophys. J*. 2015; 108:1125–1132. [PubMed: 25762324]
- Stanich CA, Honerkamp-Smith AR, Putzel GG, Warth CS, Lamprecht AK, Mandal P, Mann E, Hua T-AD, Keller SL. Coarsening Dynamics of Domains in Lipid Membranes. *Biophys. J*. 2013; 105:444–454. [PubMed: 23870265]
- Stepien P, Polit A, Wisniewska-Becker A. Comparative EPR studies on lipid bilayer properties in nanodiscs and liposomes. *Biochim. Biophys. Acta*. 2015; 1848:60–66. [PubMed: 25306967]
- Stockton GW, Polnaszek CF, Tulloch AP, Hasan F, Smith ICP. Molecular-motion and order in single-bilayer vesicles and multilamellar dispersions of egg lecithin and lecithin-cholesterol mixtures - deuterium nuclear magnetic-resonance study of specifically labeled lipids. *Biochemistry*. 1976; 15:954–966. [PubMed: 943179]
- Surma MA, Klose C, Simons K. Lipid-dependent protein sorting at the trans-Golgi network. *Biochim. Biophys. Acta*. 2012; 1821:1059–1067. [PubMed: 22230596]
- Tantipolphan R, Rades T, Strachan CJ, Gordon KC, Medlicott NJ. Analysis of lecithin–cholesterol mixtures using Raman spectroscopy. *J. Pharm. Biomed. Anal*. 2006; 41:476–484. [PubMed: 16469466]
- Teague WE Jr, Soubias O, Petrache H, Fuller N, Hines KG, Rand RP, Gawrisch K. Elastic properties of polyunsaturated phosphatidylethanolamines influence rhodopsin function. *Faraday Discuss*. 2013; 161:383–395. [PubMed: 23805751]
- Thurmond RL, Dodd SW, Brown MF. Molecular areas of phospholipids as determined by ^2H NMR spectroscopy: comparison of phosphatidylethanolamines and phosphatidylcholines. *Biophys. J*. 1991; 59:108–113. [PubMed: 2015377]
- Tong J, Borbat PP, Freed JH, Shin Y-K. A scissors mechanism for stimulation of SNARE-mediated lipid mixing by cholesterol. *Proc. Natl. Acad. Sci. USA*. 2009; 106:5141–5146. [PubMed: 19251653]
- Toppozini L, Meinhardt S, Armstrong CL, Yamani Z, Kucerka N, Schmid F, Rheinstaedter MC. Structure of cholesterol in lipid rafts. *Phys. Rev. Lett*. 2014; 113
- Trouard TP, Alam TM, Zajicek J, Brown MF. Angular anisotropy of ^2H NMR spectral densities in phospholipid bilayers containing cholesterol. *Chem. Phys. Lett*. 1992; 189:67–75.
- Trouard TP, Nevzorov AA, Alam TM, Job C, Zajicek J, Brown MF. Influence of cholesterol on dynamics of dimyristoylphosphatidylcholine as studied by deuterium NMR relaxation. *J. Chem. Phys*. 1999; 110:8802–8818.
- Tyler, AI., Clarke, J., Seddon, J., Law, R. Solid state nmr of lipid model membranes. In: Owen, DM., editor. *Methods in Membrane Lipids*. New York: Springer; 2015. p. 227-253.

- Umemura J, Cameron DG, Mantsch HH. A Fourier transform infrared spectroscopic study of the molecular interaction of cholesterol with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine. *Biochim. Biophys. Acta*. 1980; 602:32–44. [PubMed: 6893416]
- van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* 2008; 9:112–124. [PubMed: 18216768]
- Veatch SL, Keller SL. Separation of liquid phases in giant vesicles of ternary mixtures of phospholipids and cholesterol. *Biophys. J.* 2003; 85:3074–3083. [PubMed: 14581208]
- Veatch SL, Soubias O, Keller SL, Gawrisch K. Critical fluctuations in domain-forming lipid mixtures. *Proc. Natl. Acad. Sci. USA.* 2007; 104:17650–17655. [PubMed: 17962417]
- Venable RM, Sodt AJ, Rogaski B, Rui H, Hatcher E, MacKerell AD Jr, Pastor RW, Klauda JB. CHARMM all-atom additive force field for sphingomyelin: Elucidation of hydrogen bonding and of positive curvature. *Biophys. J.* 2014; 107:134–145. [PubMed: 24988348]
- Vitiello G, Falanga A, Alcides Petruk A, Merlino A, Fragneto G, Paduano L, Galdiero S, D'Errico G. Fusion of raft-like lipid bilayers operated by a membranotropic domain of the HSV-type I glycoprotein gH occurs through a cholesterol-dependent mechanism. *Soft Matter*. 2015; 11:3003–3016. [PubMed: 25734956]
- Vogel A, Scheidt HA, Baek DJ, Bittman R, Huster D. Structure and dynamics of the aliphatic cholesterol side chain in membranes as studied by ^2H NMR spectroscopy and molecular dynamics simulation. *Phys. Chem. Chem. Phys.* 2016; 18:3730–3738. [PubMed: 26762541]
- Vogel A, Tan K-T, Waldmann H, Feller SE, Brown MF, Huster D. Flexibility of ras lipid modifications studied by ^2H solid-state NMR and molecular dynamics simulations. *Biophys. J.* 2007; 93:2697–2712. [PubMed: 17557790]
- Warschawski DE, Devaux PF. ^1H – ^{13}C Polarization transfer in membranes: a tool for probing lipid dynamics and the effect of cholesterol. *J. Magn. Reson.* 2005; 177:166–171. [PubMed: 16125427]
- Wassall SR, Stillwell W. Polyunsaturated fatty acid-cholesterol interactions: domain formation in membranes. *Biochim. Biophys. Acta.* 2009; 1788:24–32. [PubMed: 19014904]
- Weise K, Huster D, Kapoor S, Triola G, Waldmann H, Winter R. Gibbs energy determinants of lipoprotein insertion into lipid membranes: the case study of Ras proteins. *Faraday Discuss.* 2013; 161:549–561. [PubMed: 23805758]
- Weisz K, Gröbner G, Mayer C, Stohrer J, Kothe G. Deuteron nuclear magnetic resonance study of the dynamic organization of phospholipid/cholesterol bilayer membranes: molecular properties and viscoelastic behavior. *Biochemistry.* 1992; 31:1100–1112. [PubMed: 1734959]
- Wiedmann TS, Pates RD, Beach JM, Salmon A, Brown MF. Lipid-protein interactions mediate the photochemical function of rhodopsin. *Biochemistry.* 1988; 27:6469–6474. [PubMed: 3219348]
- Williams GD, Beach JM, Dodd SW, Brown MF. Dependence of deuterium spin-lattice relaxation rates of multilamellar phospholipid dispersions on orientational order. *J. Am. Chem. Soc.* 1985; 107:6868–6873.
- Williams JA, Wassall CD, Kemple MD, Wassall SR. An electron paramagnetic resonance method for measuring the affinity of a spin-labeled analog of cholesterol for phospholipids. *J. Membr. Biol.* 2013; 246:689–696. [PubMed: 23982160]
- Xu X, Struts AV, Brown MF. Generalized model-free analysis of nuclear spin relaxation experiments. *eMagRes.* 2014; 3:275–286.
- Xu XL, London E. The effect of sterol structure on membrane lipid domains reveals how cholesterol can induce lipid domain formation. *Biochemistry.* 2000; 39:843–849. [PubMed: 10653627]
- Yamamoto K, Percy P, Lee D-K, Yu C, Im S-C, Waskell L, Ramamoorthy A. Temperature-Resistant Bicelles for Structural Studies by Solid-State NMR Spectroscopy. *Langmuir.* 2015; 31:1496–1504. [PubMed: 25565453]
- Yasuda T, Matsumori N, Tsuchikawa H, Lonnfors M, Nyholm TKM, Slotte JP, Murata M. Formation of gel-like nanodomains in cholesterol-containing sphingomyelin or phosphatidylcholine binary membrane as examined by fluorescence lifetimes and ^2H NMR spectra. *Langmuir.* 2015a; 31:13783–13792. [PubMed: 26639840]

- Yasuda T, Tsuchikawa H, Murata M, Matsumori N. Deuterium NMR of raft model membranes reveals domain-specific order profiles and compositional distribution. *Biophys. J.* 2015b; 108:2502–2506. [PubMed: 25992728]
- Yeagle PL. Lanosterol and cholesterol have different effects on phospholipid acyl chain ordering. *Biochim. Biophys. Acta.* 1985; 815:33–36. [PubMed: 3986200]
- Yeagle PL, Albert AD, Boesze-Battaglia K, Young J, Frye J. Cholesterol dynamics in membranes. *Biphs. J.* 1990; 57:413–424.
- Yeagle PL, Martin RB, Lala AK, Lin H-K, Bloch K. Differential effects of cholesterol and lanosterol on artificial membranes. *Proc. Natl. Acad. Sci. USA.* 1977; 74:4924–4926. [PubMed: 270726]
- Yun-Wei C, Costa-Filho AJ, Freed JH. Dynamic molecular structure and phase diagram of DPPC-cholesterol binary mixtures: a 2D-ELDOR study. *J. Phys. Chem. B.* 2007; 111:11260–11270. [PubMed: 17760438]
- Zhang R, Damron J, Vosegaard T, Ramamoorthy A. A cross-polarization based rotating-frame separated-local-field NMR experiment under ultrafast MAS conditions. *J. Magn. Reson.* 2015; 250:37–44. [PubMed: 25486635]
- Zimmerberg J, Gawrisch K. The physical chemistry of biological membranes. *Nat. Chem. Biol.* 2006; 2:564–567. [PubMed: 17051226]

Figure 1.

Chemical structures of representative glycerophospholipids, cholesterol, and lanosterol: the polar head groups vary in size, hydrogen-bonding, and charge. Examples are shown for zwitterionic phosphocholine (PC) and phosphoethanolamine (PE) head groups, and for the anionic phosphoserine (PS) head group. Non-polar acyl chains differ in length and degree of unsaturation, as illustrated by oleic acid (18:1 ω -6) and docosahexaenoic acid (22:6 ω -3). Cholesterol differs in the absence of methylation at the α -face relative to its biological precursor lanosterol. [90 % of the single column width]

Figure 2.

Solid-state ^2H NMR spectra manifest reduction in configurational degrees of freedom due to cholesterol: (a) DMPC- d_{54} in the liquid-disordered (L_d) phase, and (b)–(d) DMPC- d_{54} containing various mole fractions of cholesterol in the liquid-ordered (L_o) phase. Data were acquired at a magnetic field strength of 11.7 T (76.8 MHz) at $T = 44^\circ\text{C}$. Powder-type spectra of randomly oriented multilamellar dispersions were numerically inverted (de-Paked) to yield sub-spectra corresponding to the $\theta = 0^\circ$ orientation. Note that a distribution of residual quadrupolar couplings (RQCs) corresponds to the various C^2H_2 and C^2H_3 groups

with a progressive increase due to cholesterol. Data are taken from Ref. (Martinez et al., 2004). [90 % of the single column width]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Site-specific ^{13}C - ^1H residual dipolar couplings (RDCs) are measured using two-dimensional separated local-field ^{13}C NMR (DROSS) spectrum for POPC/cholesterol (1:1) binary mixture at $T = 30^\circ\text{C}$. (a) Selected recoupled powder patterns, showing experimental (grey) and simulated (red) line shapes. (b) Oblique view of aliphatic fingerprint region of the DROSS spectrum of binary system POPC/cholesterol. (c) 2D plane of the spectrum shown in (b). The ^{13}C isotropic chemical shift (δ) spectrum is on the F_2 (horizontal) axis (red). The peak separation of the Pake doublet yields the site-specific ^{13}C - ^1H dipolar coupling along F_1 (vertical axis). Figure modified from Ref. (Leftin et al., 2014a). [92 % of the two column width]

Figure 4.

Bilayer dimensions are given by the interfacial membrane area per lipid $\langle A \rangle$ and volumetric thickness D_C . Structural parameters are calculated from the acyl chain volume V_C and moments $\langle \cos \beta \rangle$ and $\langle \cos^2 \beta \rangle$ obtained from the order parameter plateau, where β is the angle between the virtual bond connecting two neighboring carbons of the i th segment and bilayer normal (Petrache et al., 2000). Profiles of absolute segmental order parameters $|S_{CD}^{(i)}|$ as a function of acyl chain position (i) for DMPC- d_{54} and DMPC- d_{54} /cholesterol (1:1) at $T = 44^\circ\text{C}$ (Martinez et al., 2002). Filled and open symbols refer to inequivalent $sn-1$ and $sn-2$

acyl chains, respectively. Reference order parameters are indicated for limiting cases of an oil drop model with $S_{CD} = 0$, a crankshaft model having $S_{CD} = -1/3$, and an all-*trans* rotating chain with $S_{CD} = -1/2$. Data are taken from Ref. (Trouard et al., 1999). [90 % of the single column width]

Figure 5.

Segmental order parameter S_{CH} profiles from solid-state ^{13}C NMR spectroscopy (separated local-field) indicate lipid-specific loss of conformational disorder due to cholesterol. Absolute order profiles are plotted for (a) the *sn*-2 oleoyl chain of POPC, (b) the *sn*-1 palmitoyl chain of POPC, (c) the *sn*-2 sphingosine chain of EYSM, and (d) the *sn*-1 palmitoyl chain of EYSM. Circles represent pure lipids and squares represent lipid mixtures with cholesterol (1:1). For POPC data are shown at two temperatures, $T = 28^\circ\text{C}$ (gray-filled symbols) and $T = 48^\circ\text{C}$ (solid symbols), and for EYSM at $T = 48^\circ\text{C}$. Note that upon adding cholesterol the absolute S_{CH} order parameters increase more for POPC than in EYSM. Figure adapted from Ref. (Leftin et al., 2014a). [90 % of the single column width]

Figure 6.

Semi-logarithmic plots of (a, b) the average area per lipid $\langle A \rangle$ and (c, d) the hydrophobic volumetric thickness D_C versus temperature. The effect of cholesterol on the phase transitions of (a, c) PSM- d_{31} and (b, d) POPC- d_{31} in binary mixtures is shown. Increasing the cholesterol concentration diminishes the phase transition of PSM- d_{31} but may affect POPC- d_{31} at cholesterol mole fraction $X_C = 0.33$, as indicated by a small curvature of the plots. Note that the plots are steeper for POPC- d_{31} than for PSM- d_{31} in the one-phase regime. Figure adapted from Ref. (Bartels et al., 2008). [90 % of the single column width]

Figure 7.

Solid-state 2H NMR relaxation analysis shows emergence of membrane elastic fluctuations and their suppression by cholesterol at the atomistic level. Dependence of spin-lattice relaxation rates $R_{12}^{(i)}$ on squared order parameters $S_{CD}^{(i)}$ for resolved 2H NMR splittings of $DMPC-d_{54}$ showing influences of cholesterol in the liquid-ordered (l_o) phase at $T = 44$ °C. Data at 76.8 MHz (11.8 T) are shown. The presence of cholesterol leads to a large decrease in the square-law slopes, corresponding to a progressive reduction in bilayer elasticity. An opposite increase is seen for bilayers containing the $C_{12}E_8$ nonionic detergent at $T = 42$ °C. Data are taken from Ref. (Leftin and Brown, 2011) [90 % of the single column width]

Figure 8.

Solid-state ^2H NMR relaxation uncovers striking differences in membrane elastic fluctuations from lanosterol *versus* cholesterol at the atomistic level. Dependence of spin-lattice relaxation rates $R_{1Z}^{(i)}$ on squared order parameters $S_{CD}^{(i)}$ in liquid-ordered phase (I_o) for resolved ^2H NMR splittings of DMPC- d_{54} showing influences of lanosterol and cholesterol at $T = 55^\circ\text{C}$. Data at 76.8 MHz (11.8 T) are shown: DMPC- d_{54} alone, lanosterol/DMPC- d_{54} (1:1), and cholesterol/DMPC- d_{54} (1:1). Note the decrease in the square-law slopes is consistent with a gradual reduction in bilayer elasticity on going from lanosterol to cholesterol. Data are taken from Ref. (Martinez et al., 2004). [90 % of the single column width]

Figure 9.

Comparison of solid-state ^2H NMR square-law slopes to bending rigidity κ obtained from thermal shape fluctuation data for lipid vesicles. Results for homologous series of PCs with acyl carbon lengths of $n_C = 12, 14$, and 16 in the liquid disordered (l_d) phase at 55.4 MHz (squares); data at 76.8 MHz for DMPC/cholesterol mixtures with $X_C = 0, 0.33$, and 0.50 in the liquid-ordered (l_o) phase (triangles). The inset shows the values of κ estimated from the ^2H NMR model for the homologous series of PCs. Bending rigidity estimates from solid-state ^2H NMR are in good agreement with the values obtained from thermal shape fluctuation data. Figure adapted from Ref. (Martinez et al., 2002). [90 % of the single column width]