



## High-resolution $^{13}\text{C}$ NMR of sterols in model membrane

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Received 21 March 2005; accepted 6 June 2005

Available online 06 September 2005

### Abstract

The specific sterol dynamics in membranes (axial diffusion and small amplitude wobbling) leads to fairly long  $^1\text{H}$  and  $^{13}\text{C}$   $T_2$ s (5–25 ms). One can thus obtain high-resolution MAS  $^1\text{H}$  and  $^{13}\text{C}$  spectra, perform ge-HSQC experiments in an HR-MAS probe, assign completely the  $^1\text{H}$  and  $^{13}\text{C}$  spectrum of cholesterol, identify and quantify H-bond partners of cholesterol's OH. In oriented lipid bilayers, anisotropic interactions may be analysed and thus the sterol's orientation and dynamics.  $B_0$  field homogeneity is the main factor determining line width, and line width inferior to 0.2 ppm can be obtained after proper shimming. **To cite this article:** O. Soubias et al., C. R. Chimie 9 (2006).

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### Résumé

La dynamique spécifique des stérols dans les membranes biologiques (diffusion axiale rapide, oscillations de faible amplitude) se traduit par des temps de relaxation  $T_2$  des cohérences  $^1\text{H}$  et  $^{13}\text{C}$  relativement longs pour des solides (5–25 ms). Cela a permis d'obtenir des spectres à haute résolution en rotation à l'angle magique, de réaliser des expériences 2D ge-HSQC dans une sonde HRMAS, de réaliser l'attribution complète des spectres  $^1\text{H}$  et  $^{13}\text{C}$  du cholestérol en membrane, d'identifier et de quantifier ainsi les partenaires de liaison H de l'hydroxyle du cholestérol. Dans des bicouches lipidiques orientées, qui permettent d'analyser les parties anisotropes des interactions magnétiques et donc l'orientation et la dynamique des constituants membranaires, la résolution des spectres  $^{13}\text{C}$  du cholestérol est limitée par l'homogénéité du champ  $B_0$  et, après optimisation, des largeurs de raie inférieures à 0,2 ppm pour les résonances  $^{13}\text{C}$  du cholestérol ont été obtenues. **Pour citer cet article :** O. Soubias et al., C. R. Chimie 9 (2006).

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**Keywords:**  $^{13}\text{C}$  MAS; HR-MAS; Solid-state NMR; Oriented bilayers; Assignment; Sterol–membrane interaction; Hydrogen bonds

**Mots clés :**  $^{13}\text{C}$  MAS ; HR-MAS ; RMN du solide ; Bicouches orientées ; Attribution ; Interaction stérol–membrane ; Liaisons hydrogène

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## 1. Introduction

This article is a review of the results recently obtained in our group on increased resolution of  $^{13}\text{C}$  spectra of oriented lipid bilayers [1], and on complete  $^1\text{H}$  and  $^{13}\text{C}$  assignment of cholesterol in liposomes by HR-MAS [2–4], ultimately leading to a better quantification of cholesterol's hydrogen-bonding partners [4,5].

Solid-state NMR spectroscopy is a powerful tool to study membrane molecule structure and interactions because it allows the study of amorphous and partly mobile biological solids directly in the liquid-crystalline lipid bilayer. To the spectroscopist, perhaps the most important characteristic of the membrane's lipid bilayer is its high degree of local orientational order. In ordered systems the orientation dependent spin interactions dominate the NMR spectra and the relaxation behaviour of the spin system. Two complementary approaches have been developed: on one side, one can take advantage of the residual anisotropic interactions, which are due to the incomplete motional averaging and extract orientational information (static solid-state NMR on oriented samples) and on the other side, one can suppress the residual anisotropic interactions, leaving only the isotropic parts of the chemical shift and  $J$ -couplings (Magic angle spinning solid-state NMR).

Solid-state NMR spectroscopy of oriented membrane samples provides a robust method for examining the structure, dynamics and orientation of membrane peptides [6–10] and sterols [11,12] and has emerged in recent years as a powerful technique to characterise structural constraints of small molecules at their binding sites within membrane proteins [13,14]. Static spectra of uniaxially oriented samples are characterised by one resonance frequency for each labelled site. Since the observed resonance frequencies depend on the orientation of the molecular sites relative to the magnetic field direction, they provide the orientational constraints used in structure determination. For instance, the orientation and the structure of a peptide in the membrane can be determined by  $^{15}\text{N}$  labelling of the amides or  $^{13}\text{C}$  labelling of the carbonyls in the peptide backbone [15,16], the sterol order parameters can be extracted from quadrupole splittings of specifically deuterated positions [17]. However,  $^{13}\text{C}$  and  $^{15}\text{N}$  resolution generally available on mechanically oriented samples is close to 1–2 ppm [7,18,19] for lipids (and even ~10 ppm for peptides) and it would be highly desir-

able to increase it by at least one order of magnitude. A part of our study was to understand the different sources of carbon line broadening in cholesterol/dimyristoylphosphatidylcholine (DMPC) oriented samples and to determine how to improve the resolution in the  $^{13}\text{C}$  spectra of oriented bilayer systems [1].

Magic angle spinning (MAS) techniques can be used to remove the residual anisotropic interactions, leaving only the isotropic parts of the chemical shift and  $J$ -couplings. In addition, it is possible to combine the two approaches, first removing the anisotropic interactions by MAS and then selectively reintroducing, for example, the dipolar interaction between selected spins and measuring the dipolar couplings. In  $^{13}\text{C}$  MAS NMR studies, it is common to combine cross-polarisation (CP), or insensitive nucleus enhancement by polarisation transfer (INEPT), with high-power proton decoupling to obtain high-resolution  $^{13}\text{C}$  spectra where only isotropic chemical shifts remain. The resolution obtainable in fluid membrane systems approaches that obtained in solution studies. This is especially true for membrane lipids, where line widths of less than 0.1 ppm can often be obtained [20–22]. In purely lipidic bilayers in the fluid phase, various rapid motions occur (fast axial diffusion, wobbling, internal segmental motions). In particular, fast axial diffusion converts the homogeneous proton dipolar broadening into an inhomogeneous broadening, which can be effectively averaged out by MAS [22]. This leads to an increase in  $^1\text{H}$  and  $^{13}\text{C}$   $T_2$ s, and it has been shown that common liquid state NMR techniques such as selective  $^1J$  scalar coupling INEPT are efficient [23–25]. The  $^{13}\text{C}$  NMR line widths for sterols, peptides or proteins in membranes can be significantly larger, however, depending on the size and the dynamics of the molecules [26–29]. Cholesterol dynamics is well described by a fast axial diffusion (which provides axial symmetry to all anisotropic interactions), combined with a small amplitude wobbling (the molecular order parameter of the diffusion axis is equal to 0.94 for DMPC/cholesterol in a 7/3 molar ratio at 310 K) [11,30,31]. But, contrary to protons of lipids, no internal motion within the four rings of cholesterol contributes further to the averaging of anisotropic interactions. We compared various pulse sequences in order to obtain well resolved  $^1\text{H}$ – $^{13}\text{C}$  2D correlation on the rigid part of cholesterol molecule, when it is inserted in a DMPC/cholesterol 7/3 lipid mixture, i.e. at a cholesterol molar ratio which is typically found in mam-

malian plasma membranes. In particular, we have compared dipolar (CP) and scalar coupling (INEPT) based transfers of coherences, and we have assessed the usefulness of homonuclear decoupling in the proton dimension [2]. We also compared the sensitivity of the liquid-like direct detection scheme (HETCOR) to the indirect detection scheme (ge-HSQC) of a  $^1\text{H}$ - $^{13}\text{C}$  2D chemical shift correlation on the rigid part of a cholesterol molecule [3]. Finally, the complete and unambiguous  $^1\text{H}$  and  $^{13}\text{C}$  assignment of cholesterol in DMPC membranes has been performed on the basis of a combination of 1D MAS carbon NMR spectra with various polarisation transfer schemes and 2D MAS  $^1\text{H}$ - $^{13}\text{C}$ , ge-HSQC and dipolar HETCOR [4,5].

## 2. High-resolution $^{13}\text{C}$ NMR spectra on oriented lipid bilayers

Bilayers mechanically oriented between glass plates provide an excellent model of natural membrane. We have recently determined cholesterol order parameters in DMPC- $^{13}\text{C}_4$ -cholesterol (see Fig. 5A for cholesterol structure and atom numbering) oriented bilayers from the C-H dipolar couplings using 2D PELF correlating  $^{13}\text{C}$  chemical shifts and H-C dipolar coupling [32] identical to those previously obtained with deuterium NMR using specifically deuterated cholesterol [11]. However, in this experiment we systematically observed that cholesterol- $\text{C}_4$  resonance had an apparent line width (1.5 ppm) much larger than expected from its transverse relaxation time ( $T_2$ ) from which we expect a refocused line width of 0.21 ppm.

We performed a study on cholesterol/DMPC oriented sample in order to understand the different sources of carbon line broadening and to determine how to improve the resolution in the  $^{13}\text{C}$  spectra of oriented bilayer systems. We have specifically investigated the role of the sample geometry on  $B_0$  field inhomogeneities both theoretically and experimentally and the contribution of the quality of sample orientation (extent of liposomes and mosaic spread) [1]. We have prepared a DMPC [ $\gamma$ - $^{13}\text{C}$ ]- $^{13}\text{C}_4$ -cholesterol (7/3 mol/mol) sample, and oriented the lipid bilayers between glass plates so that the bilayer normal makes an angle of  $90^\circ$  (or of the magic angle) with  $B_0$ . The magnetic field distribution within the sample, i.e. glass plates and multi-layers of lipids was calculated using the surface current formal-

ism. It was interesting to observe that the overall field shape varies in a smooth manner over the whole sample. Therefore, we postulated that it should be possible to correct for induced field effects via careful shimming. The line shapes were simulated as a function of  $B_0$  field inhomogeneities and sample mosaic spread. Both effects contribute to the experimental line width. Using three signals of different CSA ( $^{13}\text{C}\gamma$ -choline,  $^{13}\text{C}_4$ -cholesterol and  $^{31}\text{P}$ -DMPC), we have quantified both contributions and measured the mosaic spread accurately. It appeared that for bilayers oriented at the magic angle mosaic spread can play a significant role on line widths. At  $90^\circ$  and  $0^\circ$  orientations however, the major parameter is  $B_0$  field inhomogeneities and careful shimming on an internal signal is necessary to obtain the highest resolution.  $^{13}\text{C}$ -labelled choline methyl resonance of DMPC is a good candidate for this task. Fig. 1 shows the  $^{13}\text{C}$  CP solid-state NMR spectrum of 3/7 mol/mol cholesterol/DMPC- $^{13}\text{C}\gamma$ -d54 oriented bilayers at  $90^\circ$  with respect to the external field. After optimisation of the important parameters (shimming on the choline resonance, mosaic spread of  $\pm 0.30^\circ$ ),  $^{13}\text{C}$  line widths of 0.2–0.3 ppm have been obtained. The most intense signal is due to the choline methyl at 55 ppm (taken as an internal reference of chemical shift). Natural abundance phospholipid polar head group signals are

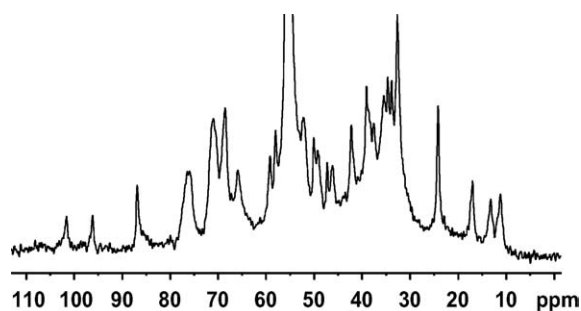


Fig. 1. 1D  $^{13}\text{C}$  CP spectrum on 30 mol% cholesterol/ $^{13}\text{C}\gamma$ -DMPC (2 mg cholesterol) oriented with the membrane normal perpendicular to the magnetic field, after shimming on the choline carbon resonance at 313 K.

$^{13}\text{C}$  spectrum was performed on a Bruker DMX narrow-bore spectrometer operating at 500 MHz for  $^1\text{H}$ , and recorded with a Bruker 7-mm double resonance probe with a solenoid coil oriented at  $90^\circ$  with respect to magnetic field. It was acquired with a standard cross polarisation experiment. A rf field of 55 kHz was used for cross polarisation (CP time 600  $\mu\text{s}$ ), whereas a proton rf field of 66 kHz was applied for decoupling during acquisition (20 ms, TD 4 K, DW 5  $\mu\text{s}$ ). The relaxation delay was 5 s and an exponential apodisation of 10 Hz was used prior to Fourier transformation. Carbon chemical shifts were referenced relative to the internal choline resonance taken at 55 ppm.

also visible from 62 to 78 ppm. All other signals are due to the cholesterol carbon and  $C_4$  can be assigned to the resonance at 49.9 ppm according to the result obtained previously [1].

This study put forward that shimming directly on a sample resonance is required to get the highest resolution on oriented bilayer  $^{13}\text{C}$  spectra of lipids. It should be stressed at this point that we have been using a DMPC/cholesterol sample in the fluid phase which is known to orient particularly well, in agreement with the  $0.3^\circ$  mosaic spread found herein, and that the carbon resonances observed have sharp intrinsic line widths ( $< 0.2$  ppm). The situation may be very different for peptide-membrane samples. First, it should be noted that all our analyses were performed in the assumption that the bilayer normal is a fast diffusion axis for all molecules, which is valid for cholesterol in DMPC, but certain peptides/proteins may behave differently (for example see [33,34]). The treatment of mosaic spread effect is very different in the rigid body hypothesis [35]. The typical  $T_2$ -dominated line widths of peptides and proteins in membranes are often higher than 1 ppm [7,18,19]. Peptides/proteins mosaic spreads are often in a  $5\text{--}20^\circ$  range, which combined with a  $^{15}\text{N}$  CSA of about 170 ppm make it a dominant parameter. In some cases, lipids and peptides even display a different mosaic spread within the same sample [13,14,16,36,37], or peptides may display topological equilibria related to their function [38]. With all these restrictions in mind, it may nevertheless be useful to pay more attention to shims whenever the  $T_2$ -dominated line widths are smaller than 1 ppm.

### 3. High-resolution $^{13}\text{C}$ MAS NMR in model membrane: complete $^1\text{H}$ and $^{13}\text{C}$ assignments of cholesterol in lipid bilayer

High-resolution 2D MAS NMR spectra of liposomes, in particular  $^1\text{H}\text{--}^{13}\text{C}$  chemical shifts correlations have been obtained on fluid lipid bilayers made of pure phospholipids for several years. We have investigated the possibility to obtain high-resolution 2D MAS spectra of cholesterol embedded in membranes, i.e. on a rigid molecule whose dynamics is characterised mainly by axial diffusion without internal segmental mobility [2]. The sample was cholesterol- $^{13}\text{C}_{3,4}$  inserted at 30 mol% in DMPC liposomes. The efficiency of various pulse sequences for heteronuclear HETCOR has

been compared in terms of resolution, sensitivity and selectivity, using either cross polarisation or INEPT for coherence transfer, and with or without MREV-8 homonuclear decoupling during  $T_1$ . At moderately high spinning speed (9 kHz), a similar resolution is obtained in all cases (0.2 ppm for  $^1\text{H}_{3,4}$ , 0.15 ppm for  $^{13}\text{C}_{3,4}$  cholesterol resonances), while sensitivity increases in the order: INEPT  $\ll$  CP ( $\times 4$ )  $<$  CP + MREV. Again due to the specific dynamics of cholesterol on model membranes, it was shown that homonuclear decoupling during the proton evolution is not an absolute requirement and that when an MREV-8 sequence is employed it improves the effective resolution and sensitivity only marginally. Both cross polarisation and INEPT can be used for coherence transfer, the former making the experiment more sensitive by a factor 4, the latter allow-

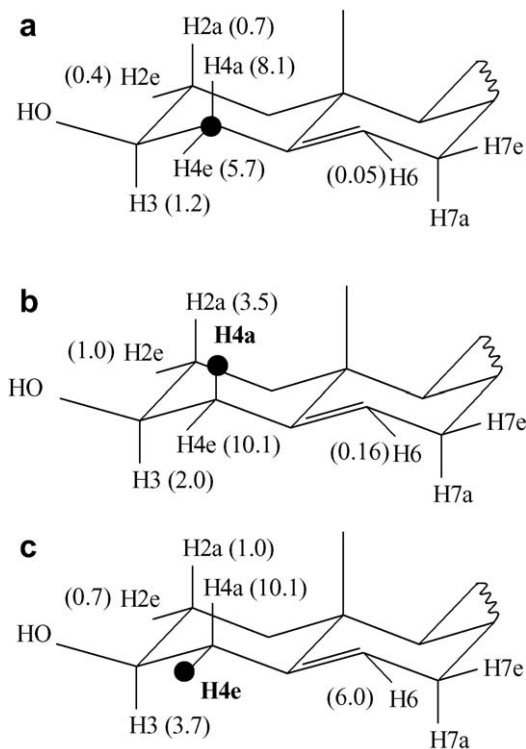


Fig. 2. Calculated values of dipolar couplings within cholesterol in fluid bilayers (these couplings, in kHz, are those expected for bilayer normal oriented at  $90^\circ$  with respect to the magnetic field). (a) Heteronuclear dipolar coupling between carbon  $C_4$  and the closest protons, (b) homonuclear dipolar couplings between  $H_{4a}$  and the closest protons, (c) homonuclear dipolar couplings between  $H_{4e}$  and the closest protons. These couplings were calculated from the average orientation and dynamics of cholesterol in DMPC/cholesterol 7/3 mol/mol bilayers at 310 K, which have been previously determined accurately using deuterium NMR [11].

ing for a fully selective transfer between directly bound atoms. Since the four rings of cholesterol form a rigid part of the molecule and thus present the same dynamical properties [11], these conclusions, based mainly on the analyses of correlation peaks in position 3 (CH) and 4 (CH<sub>2</sub>), may safely be extended to other positions in the rings. Since most of the H–H dipolar couplings in cholesterol are smaller than 10 kHz (Fig. 2), they are effectively averaged out by MAS at 9-kHz spinning speed and virtually no rotational side bands were observed along the proton dimension. This was not the case at 5-kHz spinning speed, and these side band patterns could be observed with a larger spectral width in the proton dimension. At this reduced spinning speed, the homonuclear dipolar coupling between the two geminal protons attached to C<sub>4</sub> gives rise to spinning side bands from which one can estimate a H–H dipolar coupling of 10 kHz, which is in good agreement with the known dynamics of cholesterol in membranes.

We have also shown that HSQC experiments of natural abundance cholesterol in liquid-crystalline DMPC

membranes at 30 mol%, with a good resolution in both dimensions, can be obtained by HR-MAS experiments [3]. The inverse detection scheme is very suitable to enhance sensitivity on such samples and has allowed the detection of all the cholesterol expected cross peaks. Obviously, spinning rate is a key factor for an efficient transfer of coherence during the overall pulse sequence. The heteronuclear scalar composite pulse decoupling scheme GARP was found to be the most efficient technique for carbon decoupling during acquisition time. It can be foreseen that the gain in sensitivity brought by the use of a gradient HR-MAS probe (or a CPMAS probe with lock and gradients) and inverse detection in the HSQC experiment will play a key role in assignment efforts and analysis of fluid membrane components. A comparison between an HSQC of pure cholesterol typically obtained in organic solvent and the HSQC presently obtained, with cholesterol inserted in lipid membranes is represented in Fig. 3. Fig. 3B shows that, at 15-kHz spinning speed, several natural abundance cross peaks between directly bound carbon and

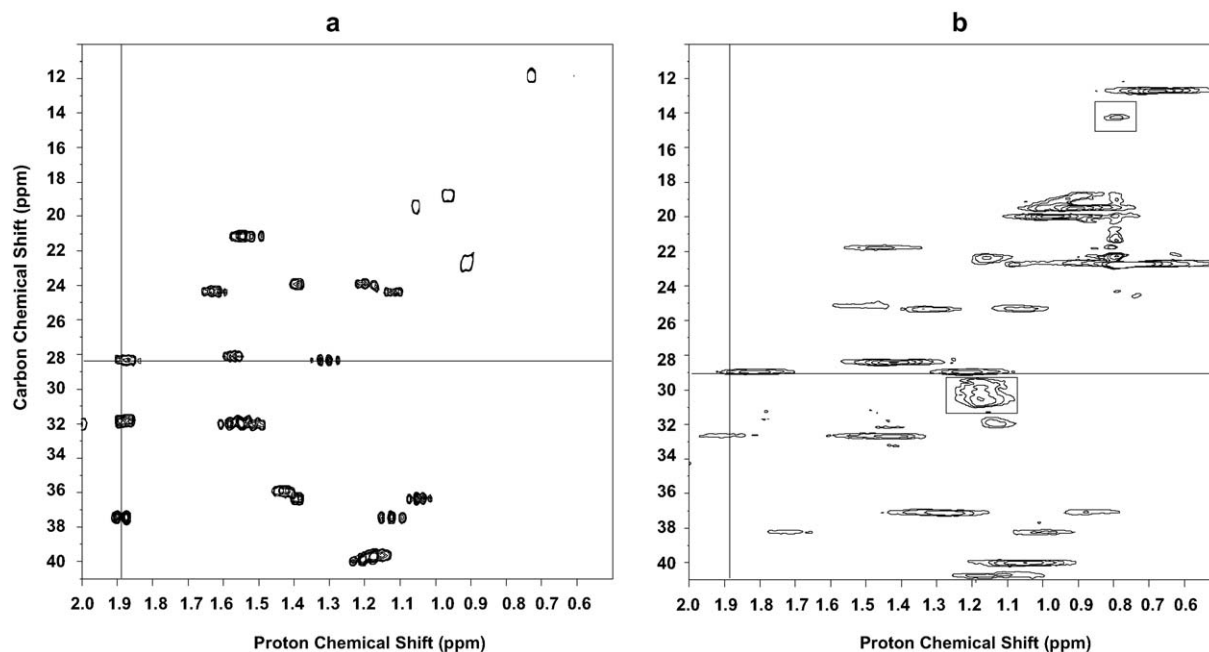


Fig. 3. (a) Typical 2D <sup>13</sup>C–<sup>1</sup>H HSQC spectrum of cholesterol in CDCl<sub>3</sub> centred around the aliphatic region and referenced to TMS; Temperature: 310 K. (b) Same region of a 2D <sup>13</sup>C–<sup>1</sup>H HSQC spectrum of cholesterol in DMPC-d<sub>54</sub>, at the same temperature and a MAS spinning rate of 15 kHz. Carbon spectral width 25 kHz. GARP carbon decoupling with a <sup>13</sup>C π/2 pulse of 100 μs. A total of 1024 T<sub>1</sub> increments with 64 scans each were collected. Square boxes surround cross peaks corresponding to the lipids. All the other cross peaks correspond to cholesterol resonances. Proton and carbon chemical shifts were internally referenced relative to the choline γ-CH<sub>3</sub> resonance taken at 55 ppm (<sup>13</sup>C) and 3.18 ppm (<sup>1</sup>H) [2]. The line widths were equal to 0.1 ppm (CH, CH<sub>3</sub>) and 0.2 ppm (CH<sub>2</sub>) in the proton dimension and 0.2 ppm in the carbon dimension to be compared with 0.005 ppm for the corresponding liquid-state spectrum.

protons can be observed with a good resolution and accuracy. Line widths obtained in the proton dimension were close to 0.1 ppm. In the carbon dimension,  $T_2$  relaxation time restricts the line width to 0.2 ppm. In this experiment, there was sufficient sensitivity to detect all the 24 expected natural abundance cross peaks, and the resolution was sufficient to resolve 20 out of them. Although a definitive assignment cannot be achieved with this experiment alone, detection of all heteronuclei and directly bound protons is usually the first step in this process. Since the relatively fast relaxation of coherences involved in the polarisation transfer would probably prevent the use of a liquid-like HMBC experiment, CP based HETCOR experiments with long contact time were required to establish connectivity between unbound atoms [4]. Fig. 4 illustrates the assignment of cholesterol from the combination of HSQC and dipolar HETCOR experiments. Using this ‘HMBC-like’ strategy, the complete proton and carbon assignment of cholesterol resonances in DMPC membrane was performed [4]. The variation between cholesterol carbon chemical shifts obtained in membrane and those obtained in anhydrous  $\text{CCl}_4$  is represented in Fig. 5B.

It has long been noticed that conformational effects (including dihedral angles, type of neighbouring atoms), hydrogen bonding or solvent effects influence the NMR chemical shifts. Therefore, it was interesting to analyse cholesterol’s interaction with its environment on the basis of carbon chemical shift variation. The observed variations for carbons 17, 20–23 probably reflect a different conformation distribution of the side chain in membrane and in solution. Furthermore, magnitude and sign of the variations for carbons 1–6 can be interpreted in terms of  $\text{C}_3\text{--O}_3$  rotameric distribution and in terms of hydrogen-bonding partners of the cholesterol hydroxyl group (these carbons are located in the first two rigid rings and no conformational effect is expected). We have first shown that the effects of  $\text{H}_3$  hydrogen-bonding and of  $\text{C}_3\text{--O}_3$  rotamer distribution on chemical shifts could be accurately calculated by using a quantum-chemical approach [5]. Several hydrogen-bonding partners for cholesterol’s hydroxyl group were considered, that is, water molecules (as hydrogen bond donor and acceptor), the acyl chain’s ester bonds (modelled by acetone [5]) and non-ester phosphate oxygen atoms of the lipid polar headgroup (modelled by dimethyl phosphate [5]). The experimen-

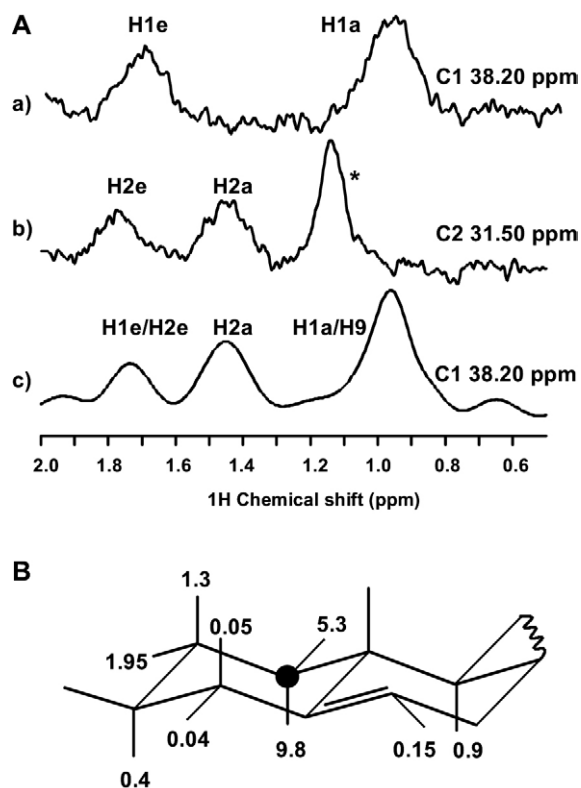


Fig. 4. Combination of *ge*-HSQC and dipolar HETCOR for the assignment of cholesterol. (A) Cross-sections along the proton dimension at carbon chemical shifts corresponding to  $\text{C}_1$  (a, c) and  $\text{C}_2$  (b), extracted from *ge*-HSQC (cf. figure caption 3B for experimental conditions) (a, b) and Dipolar HETCOR (5-mm DOTY scientific XC-5 MAS probe, 9-kHz spinning rate, 2.5-ms cross polarisation contact time, 66-kHz  $^1\text{H}$  decoupling field and TPPM decoupling scheme during acquisition time) (c). Comparison of cross sections and favourable chemical shift dispersion allow assigning  $\text{C}_1$ . \* This signal arises from natural abundance lipid methylene. (B) Dipolar couplings between  $\text{C}_1$  and neighbouring protons (in kHz), calculated from the known structure and dynamics of cholesterol [11].

As shown in Fig. 4B the only protons displaying a significant ( $> 1$  kHz) dipolar coupling with  $\text{C}_1$  are  $\text{H}_{1\text{a,e}}$  and  $\text{H}_{2\text{a,e}}$ . Correspondingly, the two HSQC traces in Figs. 4A reveal one-bond connections between  $\text{C}_1$  and the two  $\text{H}_1$  protons (a) and between  $\text{C}_2$  and the two  $\text{H}_2$  protons (b). The dipolar HETCOR trace along  $\text{C}_1$  contains connections with both  $\text{H}_1$  and  $\text{H}_2$  protons, thus guiding the assignment from  $\text{C}_1$  to  $\text{H}_2$  and  $\text{C}_2$  (c). It can be observed that, due to the 2.5-ms contact time, the signal intensities are not simply related to the dipolar couplings in the 1–10-kHz range. Shorter mixing times (i.e. 100–500  $\mu\text{s}$ ) provide a more selective transfer, and give complementary information.

tal chemical shift variations observed in membranes for carbons 1–6 were then analysed by combining the individual calculated chemical shifts variation for the three

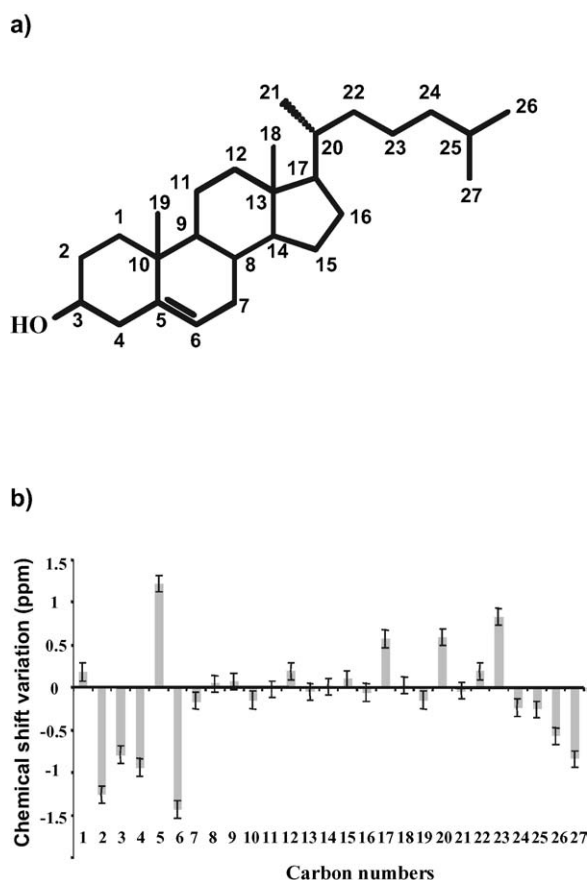


Fig. 5. (a) Structure of cholesterol and classical carbon numbering. (b) Experimental chemical shift variation pattern between cholesterol in DMPC membrane and in  $\text{CCl}_4$  solution.

$\text{C}_3\text{--O}_3$  rotamer states and the four hydrogen-bonding partners [4]. We have thus directly demonstrated for the first time the interaction of the cholesterol  $\text{H}_3$  proton with the phosphatidylcholine phosphodiester, and quantified the percentage of this interaction in the membrane state ( $18 \pm 3\%$ , meaning that the OH group is bound to the phosphodiester 18% of the time). Other hydrogen-bonding partners have also been demonstrated, although their statistics have been quantified less accurately (Fig. 6). Our results show that less than one partner is in interaction with  $\text{H}_3$  (total H-bond via  $\text{H}_3 = 61 \pm 13\%$ ). This indicates that within our theoretical approach, lipids are not always H-bonded to cholesterol and that  $\text{H}_3$  can be free of any interaction, which may be related to the highly dynamic behaviour of cholesterol/DMPC system and its hydroxyl group. If one calculates the total number of H-bonded water, one

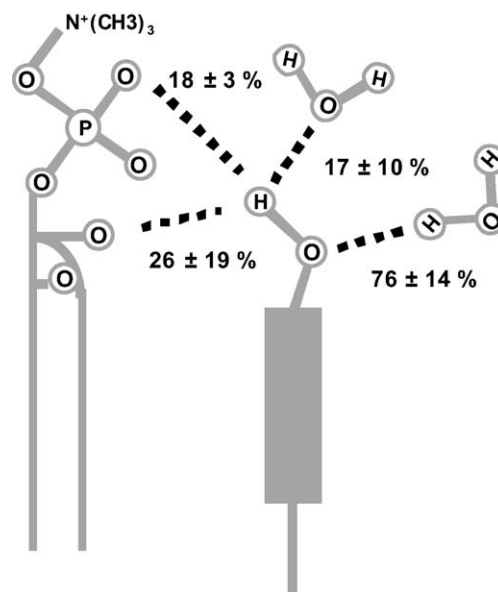


Fig. 6. The number of hydrogen bonds per OH group was limited to one (or 100%) involving the hydrogen atom as a donor and two (or 200%) involving the oxygen atom as an acceptor (since the oxygen atom has two lone pairs).

Since the fitting procedure only imposed a maximum value of hydrogen-bonding partners, the final optimum result shows a certain percentage of OH groups free of hydrogen bonds. Note that the amount of hydrogen bonds with phosphate oxygen is particularly well defined.

obtains almost 1 water molecule per cholesterol, which is close to the value obtained by molecular dynamics simulations ( $1.1 \pm 0.1$ ) [39]. The total number of interactions (lipid esters, phosphatidylcholine phosphodiester, and water) obtained by our theoretical and statistical analysis is equal to 1.4 per cholesterol, which is again close to the 1.3 proposed by Pasenkiewicz-Gierula et al. [39]. Finally, we found in our data no convincing evidence that the rotamer distribution of the OH group is very different from what it is in solution, i.e. equal population of the three major rotamers.

We have thus established a new strategy, which allows to quantify the H-bonding behaviour of cholesterol in a membrane. This can now be applied to a series of interesting issues in membrane biophysics, such as the specific interaction of cholesterol with unsaturated lipids, with sphingomyelin in the context of raft formation, or the details of cholesterol's hydration as a function of the lipid mixture.

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