Eur Biophys J (2007) 36:933–942
DOI 10.1007/s00249-007-0186-7

REVIEW

NMR of molecules interacting with lipids in small unilamellar vesicles

Grégory Da Costa · Liza Mouret · Soizic Chevance · Elisabeth Le Rumeur · Arnaud Bondon

Received: 15 February 2007 / Revised: 3 May 2007 / Accepted: 7 May 2007 / Published online: 13 June 2007

Abstract Detailed characterization of protein, peptide or drug interactions with natural membrane is still a challenge. This review focuses on the use of nuclear magnetic resonance (NMR) for the analysis of interaction of molecules with small unilamellar vesicles (SUV). These phospholipid vesicles are often used as model membranes for fluorescence or circular dichroism experiments. The various NMR approaches for studying molecule-lipid association are reviewed. After a brief survey of the SUV characterization, the use of heteronuclear NMR (phosphorous, carbon, fluorine) is described. Applications of proton NMR through transferred nuclear Overhauser effect to perform structural determination of peptide are presented. Special care is finally given to the influence of the kinetic of the interactions for the proton NMR of bound molecules in SUV, which can constitute a good model for the study of dynamical processes at the membrane surface.

Introduction

Many natural or drug-induced cellular processes involve protein, peptide or drug interactions with the membrane lipids. Because of the complexity of the native membrane, studies on such interactions are conducted with artificial models. Each model has its own limitations, ranging from the simplest type, involving organic solvent mixtures that solubilize integral proteins, through the use of micelles or bicelles, to more sophisticated approaches using small and large unilamellar or multilamellar vesicles of phospholipid mixtures (SUV, LUV and MLV, respectively). Nevertheless, combined with technological and methodological improvements, these approaches have already opened up avenues for the structural determination of peptides or proteins in a medium that mimics the membrane lipidic environment.

Two main models—organic solvents and micelles—for studying peptides and proteins under standard liquid conditions were reviewed (Henry and Sykes 1994; Sanders and Sonnichsen 2006). Another approach, enriched by numerous new developments, concerns the structural analysis of integral proteins by means of high-resolution solid state NMR. Recent reviews have discussed NMR experiments on aligned samples of membrane proteins (Angelis et al. 2005; Opella and Marassi 2004) and the use of MLV with high-resolution magic angle spinning NMR (Baldus 2006; Huster 2005; Luca et al. 2003; Watts 2005). Bicelles provide a membrane model system that can be used in studies with either solution or solid-state NMR (Prosser et al. 2006). Bicelles are discoid objects formed of long- and short-chain phospholipids. Their size and hence the tumbling time are related to the ratio of the two kinds of lipids. In this review, we focus on the use of small unilamellar vesicles (SUV) as a model membrane for NMR studies of proteins, peptides or drugs interactions with lipids. This system has so far been only partly reviewed.

In the first part, we briefly describe SUV and the origin of the visibility of the lipid signals under liquid-state NMR conditions. Then, we present the different approaches for NMR observation of molecules associated with SUV.

Presented at the joint biannual meeting of the SFB-GEIMM-GRIP, Anglet France, 14–19 October, 2006.

G. Da Costa · L. Mouret · S. Chevance · E. Le Rumeur · A. Bondon (✉)
RMN-Interactions Lipides Protéines, UMR CNRS 6026, IFR 140, PRISM, Université de Rennes 1, 35043 Rennes Cedex, France
e-mail: arnaud.bondon@univ-rennes1.fr

SUV: preparation and NMR detection of the lipids

The most usual method for making SUV is to sonicate a multilamellar vesicle (MLV) preparation. MLV are generally obtained by evaporating a solution containing the appropriate amount of lipids in an organic solvent, and then rehydrating the thin film of lipids thus obtained with the chosen buffer. Sonication must be applied beyond the phase transition temperature of the lipids. SUV with a diameter between 30 and 70 nm—depending on the lipidic composition and the conditions of sonication—are obtained. Centrifugation should then be applied to eliminate the titanium debris from the sonicator probe. SUV suspensions generally have a high optical clarity making them compatible with optical analysis of interactions with proteins or peptides. The suspension obtained remains stable for a few days, depending on the concentration, the pH conditions, the buffer and the lipidic composition of the vesicles. For further details about SUV preparation and properties (see for example Lentz et al. 1987; Pitcher and Huestis 2002 and references therein).

Despite the high curvature constraints, due to the small size of the vesicles, SUV have been widely used in a large number of biophysical studies and now constitute a standard model for the analysis of various biological processes involving membranes. However, only a few NMR studies have been performed using SUV, especially when compared with the large number of solid-state NMR developments over the last 20 years, to analyse the interactions of peptides or proteins with phospholipids.

The small dimensions of the SUV allow a fast tumbling motion of the entire vesicles, which coupled with the individual movements of the phospholipids, permits a good detection of the lipid NMR signals under standard liquid conditions. The origin of the sharp ^{1}H NMR signals observed in SUV (Fig. 1) was an important matter of debate in the first SUV NMR studies (Bloom et al. 1978; Sheetz and Chan 1972). It appeared that individual motions of the phospholipids remained nearly of the same order in SUV, LUV and MLV (Bloom et al. 1978). A very strong homonuclear dipolar coupling is involved in ^{1}H detection. The fast tumbling of the small-sized vesicles, combined with the phospholipid motions, isomerization of hydrocarbon chains, molecular rotation and wobble, lateral diffusion (Feller et al. 1999), enables the detection of the lipid signals. These two kinds of motion are required to efficiently average out the anisotropic interactions and act conjointly to give sharp lipid ^{1}H resonances in SUV. Two-dimensional cross relaxation experiments have been successfully performed on SUV demonstrating the limited spin diffusion in these systems and allowing the complete assignment of the phospholipid resonances (Ellena et al. 1985, 1987; Gabriel and Roberts 1987). Broadening of the ^{1}H resonances is produced either by slowing down the lipid motions or increasing the vesicle size. This must be carefully taken into consideration since a large number of parameters can modify the motional properties of the lipids and give rise to signal broadening, sometimes below the detection limits.

For example, when studying SUV containing cholesterol, a broadening of the acyl chains and the terminal methyl ^{1}H is observed (Fig. 1, Bhattacharya and Haldar 1996; Forbes et al. 1988; Haran and Shporer 1977). This has been interpreted as a consequence of cholesterol being able to slow down phospholipid motions and rigidify the membrane, thus yielding a poorer averaging of the dipolar coupling. No clear relation is defined between proton detection and the lipid composition or the incorporation of sterol into the vesicle, due to the complex phase behaviour of the lipids that can induce drastic variations of their individual movements.

The second important aspect when studying cholesterol-bearing vesicles is that no cholesterol resonances are detected due to insufficient averaging of dipolar coupling. Accordingly, some ^{1}H cholesterol resonances have only been observed with SUV made up of perdeuterated lipids (Kingsley and Feigenson 1979), in which deuteration prevents the dipolar coupling between cholesterol and acyl chains protons. The severe broadening of the ^{1}H signals has often been reported for cholesterol either in SUV or MLV under magic angle spinning conditions (Epand et al. 2003; Forbes et al. 1988). Similar behavior was encountered for intrinsic proteins strongly inserted into the membrane (Brown and Wuthrich 1977). SUV are often thought to represent an inappropriate model for studying molecules associated with phospholipids by NMR because the signals of the molecules inserted into the membrane are considered to

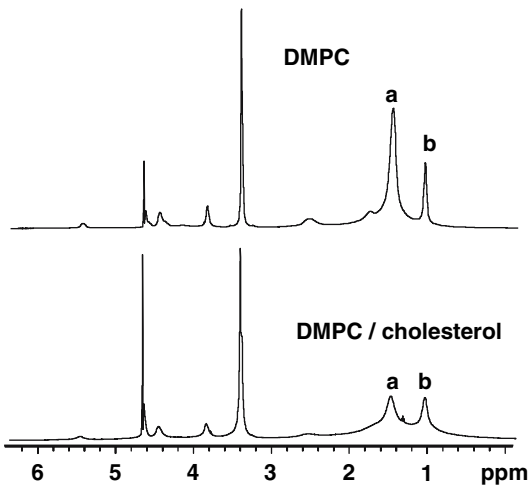

Fig. 1 ^{1}H NMR spectra recorded at 323 K of SUV; *top* DiMyristoylPhosphatidylCholine (DMPC), *bottom* (70/30) DMPC/cholesterol. Acyl chains are labelled (*a*) methylene groups and (*b*) terminal methyl

be invisible owing to the excessive contribution from anisotropic interactions that SUV tumbling motion is not sufficient to average out (Strandberg and Ulrich 2004), thus explaining why there have been rather few NMR developments involving SUV. However, only relatively limited additional movement is required for good proton detection. For example, when studying cholesterol incorporation into the membrane, changing the sterol structure to different cholesterol precursors such as desmosterol, which show an increased mobility inside the phospholipidic environment (see for example Magic Angle Spinning study of Scheidt et al. 2005), enables detection of most sterol protons (unpublished works).

In fact, as discussed further below, SUV remains an advantageous system to study the interactions of molecule with membrane, as far as the motions of the molecules are high enough to average out the anisotropic interactions. The same applies for membrane inserted molecules with a sufficient high mobility inside the bilayer and molecules that are involved in fast exchange dynamics with the membrane on the chemical shift NMR timescale. In such cases, the fast tumbling of the vesicles coupled with the interacting molecules mobility make the detection of sharp resonances under liquid NMR conditions possible.

Heteronuclear detection

Because of the relatively low lipid concentrations required for the preparation of SUV, only a few studies have been reported using nuclei with low abundance or low NMR sensitivity. Nevertheless, the advantage of using direct detection of such nuclei is related to the much smaller dipolar coupling when compared with proton detection. In this context, several approaches have been developed. In a first approach, the characterization of SUV association has been obtained by ^{31}P NMR observation of the lipid resonance modifications. Experiments with ^{19}F or ^{13}C NMR have also been performed with labelled or enriched phospholipids. The second approach is to directly observe the interacting molecule signals. This was performed by ^{19}F experiments, this time using labelled molecules or by ^{13}C studies with enriched molecules and more recently at natural abundance.

Phosphorous NMR

Concerning ^{31}P NMR studies, modification of the phosphorus lipid resonances was used to characterize and control the vesicle size (Hauser 1989; Jung et al. 1996; Traikia et al. 2000), the phospholipid distribution into the bilayer (Moreau et al. 2001; Nordlund et al. 1981) or the vesicle fusion process (Eum et al. 1989; Schmidt et al. 1981; Schullery et al. 1980). These properties were used to follow the addition of drugs upon the vesicles. For example, adriamycin ability to modify the lipid phases in SUV made of different lipidic composition (Nicolay et al. 1985) or the effect of different charge amphiphilic drug addition, sometimes conducing to SUV destruction have been evaluated (Eriksson 1987).

Phosphorus NMR experiments are often coupled with other techniques when studying molecule-membrane interactions. An example is the study of the interaction of four different amphiphilic polypeptides with SUV by ^{31}P NMR as well as ^{1}H NMR and compared to circular dichroism, turbidimetry, electron microscopy, and fluorescence techniques (Reynaud et al. 1993). When studying interactions of different molecules with the lipids, observation of the line width modifications as well as the ^{31}P chemical shift deviation are often used as a first control for association of the lipids with the molecule of interest. Phosphorus NMR was used in this way when studying a tridecapeptide pheromone, the α-factor of *Saccharomyces cerevisiae*. This peptide was shown to interact with the phosphorus headgroups of SUV (Jelicks et al. 1989). Another example is the study of two peptidic vector analogues to the third helix of antennapedia homeodomain interacting with SUV, phosphorus chemical shift deviation showed the association whereas the observation of only small linewidth modifications confirms that no vesicle fusion has occurred (Berlose et al. 1996). Different studies of glycophorin A—a major glycoprotein of the erythrocyte membrane—with small unilamellar vesicles were performed by ^{31}P associated with ^{19}F or ^{13}C NMR experiments (Ong 1984; Prestegard and Ong 1982). This will be developed in more details in the next parts concerning ^{19}F and ^{13}C NMR. One can also mention the study of the selective binding of annexin V with phosphatidic acid contained in phosphatidic acid and phosphatidylcholine SUV (Swairjo et al. 1994).

Fluorine NMR

Fluorinated fatty acids have been used to probe the intrinsic molecular motion and phospholipids packing in SUV (Gent and Ho 1978; Wu et al. 1985). As just mentioned, incorporation of various partially fluorinated phospholipids has also made the study of glycophorin A- SUV interactions possible. ^{19}F as well as ^{31}P NMR has been performed that enabled the probing of lipid-protein interactions (Ong 1984). Another approach consists of incorporating fluorine atoms directly into the drug, the peptide or the protein, with the aim of analysing the association with the phospholipids. One more application of fluorine NMR concerns the determination of the partition coefficients of drugs between phospholipid bilayer vesicles and water (Kitamura et al. 1999; Omran et al. 2002). Before the development of protein expression in artificial media through the improvements

in molecular genetics, the introduction of fluorine was performed by chemical modification of the natural proteins. For example, trifluoroacetylation of cytochrome *c* was carried out on several lysine residues. Fluorine NMR spectra of the cytochrome *c* derivatives bound to phospholipid vesicles were obtained with reasonably narrow linewidths (Staudenmayer et al. 1976). Conformation of the fluorinated analogue of gramicidin A was also studied by fluorine NMR. Fluorine atoms chemically introduced at the N- and C-terminus, combined with the use of soluble paramagnetic agent, have allowed definition of the N-terminus located at the interior of the bilayer, whereas the C-terminus remained at the membrane surface (Weinstein et al. 1985). Incorporation of fluorophenylalanine, fluorotyrosine or fluorotryptophan can be performed using protein expression in *E. coli*. This technique has been employed to study the nonlytic filamentous coliphage M13. It allows the localisation of fluorophenylalanines at the vesicle surface, whereas the fluorotyrosine are embedded in the bilayer (Dettman et al. 1982, 1984). Another example is the use of fluorotryptophans to monitor the membrane-bound D-lactate dehydrogenase of *E. coli* (Rule et al. 1987).

Carbon NMR

^{13}C NMR has been used to monitor the association of glycophorin A with SUV composed of various proportions of selectively hydrocarbon chains labelled phosphatidylcholine and phosphatidylethanolamine. Although only small perturbations of ^{13}C linewidths and spin-lattice relaxation times were induced by the presence of the protein, there was some degree of lipid composition specificity. This is consistent with relatively weak interactions (Ong and Prestegard 1982). Glycophorin A was found to bind preferentially to either phosphatidylserine or phosphatidylethanolamine, in comparison with phosphatidylcholine in mixed vesicles (Prestegard and Ong 1982).

The addition of paramagnetic ions such as Mn^{2+}, Pr^{3+} and Dy^{3+} allows discrimination between the external and internal surfaces through modification of the exposed nucleus resonances. The absence of any induced broadening or shift can be associated with a deeply inserted molecule in the bilayer. With this objective in view, studies have been carried out by introducing carbon labelled membrane partner such as cholesteryl oleate (Malcolmson et al. 1996), galactosylceramide (Mattjus et al. 2002), monoacylglycerols (Boyle et al. 1996) and acyl carnitines (Ho et al. 2002).

Drug activity often requires membrane crossing and in this context analysis of the interactions with lipids were reported, for example, in the case of ^{13}C enriched phenothiazines (Kitamura et al. 2004) or the antibiotic trimethoprim (Painter et al. 1988). Other relevant drug-lipid interaction studies were performed using proton NMR. Substance P has been studied by ^{13}C NMR using isotopic labelling on its glycine residues. Two states have thus been defined, inserted into the bilayer or attached on the surface (Duplaa et al. 1992).

Spectrometer improvements (high field, sensitivity of the probeheads) enable the recording of ^{13}C NMR spectra of peptides at natural abundance. Further new developments linked to the availability of ^{13}C cryoprobe can be anticipated. Nevertheless, NMR experiments at ^{13}C natural abundance have already been applied to determine the binding site of peptides and proteins in lipid bilayer. Deep penetration in the bilayer of a transmembrane channel peptide gramicidin A was determined (Okamura et al. 2001). In the same report, the superficial binding of a model peptide formed from amphipathic helices of the plasma A–I apolipoprotein was defined (Okamura et al. 2001). More recently, Kimura reported the comparison of Met-enkephalin at natural ^{13}C abundance associated with large unilamellar vesicles (LUV), SUV or micelles (Kimura 2006).

Proton detection

NH–ND exchange

Several studies have made use of this technique, which allows characterization of the part of peptides or proteins in interaction with the lipids, as strong interaction prevents isotopic exchange of the amide protons with the deuterons of the heavy water. Such a strategy is commonly used for the study of proteins in solution to determine the amide protons deeply buried within the structure. A recent review describes the use of this technique with various peptides and proteins and membrane models (Dempsey 2001). In the case of membrane-bound protein, a perfect control of the association and dissociation of the phospholipid vesicles is required. Beside NMR, another approach very efficient for such an analysis is the use of mass spectrometry, because the proteoliposomes can be directly injected for H/D exchange analysis of labile hydrogen atoms (Demmers et al. 2000). Nevertheless, in the case of bovine alpha-lactalbumin, a good control of lipid bilayer release has been performed by varying the pH. The overall amide exchange behaviour of the membrane-bound state corresponds to a molten globule-like conformation, suggesting a global destabilization of the three dimensional structure. However, several amide protons have been shown to display significant resistance against solvent exchange (Halskau et al. 2002). A subsequent study has determined the influence of the liposome composition (Agasoster et al. 2003). Hydrogen–deuterium exchange experiments have also been performed on sapecin after lyophilization and pH jump treatments (Takeuchi et al. 2004).

Transfer-based experiments

The principle of transfer-based experiments, namely transferred NOE (trNOE) and saturation transfer difference (STD) consists of recording information on the lipid bound form through detection of the lipid free resonances (Fig. 2). Data collection requires an excess of ligand and a chemical exchange rate, between the free and the bound forms, higher than the free form magnetization relaxation rate R_1. Magnetization transfer occurs through cross-relaxation during the irradiation time. Using short irradiation time, the intensity of the dipolar interaction can be related to the interproton distance whereas spin diffusion is present only when using long saturation time. In the case of SUV, trNOESY with mixing times up to a few hundred milliseconds are devoid of spin diffusion (Ellena et al. 1985; Xu and Cafiso 1986). By contrast, spin diffusion can be efficient for STD experiments when using a long saturation time.

STD experiment does not allow precise structural characterization of any bound structure and it is rather widely used for NMR-based screening of protein ligands (Lepre et al. 2004). A closely related experiment using one isotopically labelled partner is called transferred cross saturation (TCS) (Takahashi et al. 2000). A first example demonstrating the efficiency of this methodology for the study of membrane-peptide was recently reported for the mastoparan interaction with bicelles (Nakamura et al. 2005).

Transferred NOE is generally abbreviated to trNOE, although this usage might lead to confusion with transient NOE experiments, we nevertheless use trNOE as the common abbreviation. TrNOE can be performed in one-dimensional or two-dimensional experiments (Clore and Gronenborn 1982; Forsén and Hoffman 1963). The influence of the exchange rate on the pattern of the NMR spectra depends on the differences in chemical shift between the free and the bound forms. Consequently, the extent of signal broadening and/or shift in the free form is variable (Ni and Scheraga 1994). In interaction studies of molecules with phospholipid vesicles, different cases can be observed. Narrow averaged resonances are detected with molecules in fast exchange, whereas broad averaged resonances or even two sets of signals can be observed with decreasing exchange rate.

The trNOE technique represents the most efficient use of proton NMR, not only for investigating the interaction of molecules with lipids but also, in several cases, to determine the structure of peptides bound to the vesicle. In the case of small peptides, only a few positive NOE cross-peaks are generally observed in the free form, whereas large negative NOE can be detected in the lipid-bound form. This allows a clear discrimination between the signals associated with both forms. The first trNOE experiments were applied for the study of mastoparan-X in association with deuterated lipids (Wakamatsu et al. 1983). Under these experimental conditions, the proton resonances of lipid-bound peptide were not observed due to significant broadening. Moreover, the proton resonances of free peptide molecules were broader in the presence than in the absence of vesicles, because of the chemical exchange with lipid-bound molecules (Nakajima et al. 1985; Wakamatsu et al. 1983, 1992). The membrane-bound conformation was found to be very similar to the previously reported G protein-bound conformation (Sukumar and Higashijima 1992).

Then other peptides have been successfully analysed by trNOE. In this way, it has been possible to elucidate the conformation of the membrane-bound N-terminal part of alpha-mating factor. The first five residues form a compact helical structure and the remaining residues were proposed to form an extended structure (Wakamatsu et al. 1986), 2D experimental evidence for a β-turn was later obtained (Jelicks et al. 1989). The conformations of Leu-enkephalin and its analogues have been elucidated by trNOE, with the inactive analogue [L-Ala2]Leu-enkephalin displaying a different conformation when bound to the lipid bilayer. This difference in the binding mode has been correlated with the inactivity of this peptide (Milon et al. 1990). As pointed

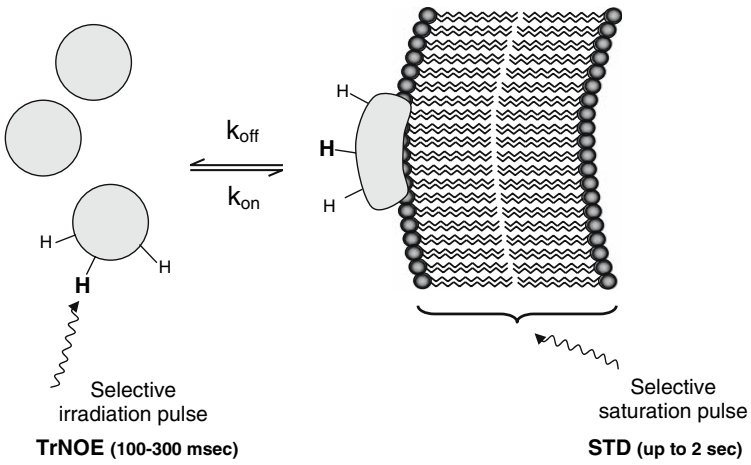

Fig. 2 Schematic representation of the transferred NOE (trNOE) and the saturation transfer difference (STD) experiments. Note that trNOE is based on the irradiation of the ligand whereas STD consists in a long saturation of the membrane signals

out, efficient trNOE requires a relatively fast exchange between the free and the bound forms, when compared to R_1. In a study of an analogue of the *E. coli* LamB protein, the binding affinity of the peptide for phospholipid vesicles was tuned by adjusting the proportion of acidic lipid in the vesicle (Wang et al. 1993). Another technique for controlling the exchange rate involves mutation of the peptide, as illustrated by the double mutation of sapecin (Takeuchi et al. 2004). Combined with distance geometry or molecular dynamics calculations, structure determination of some lipid bound polypeptides have been performed using NOE derived distance constraints.

Although micelles are often described as a poor model of complex lipid bilayers, well-defined conformation differences have not yet been documented between the micelle bound and vesicle bound peptides. Nevertheless, one example is melittin, the major constituent of honeybee venom, which adopts significant conformation differences of its C-terminal region when bound to lipid vesicles or dodecylphosphocholine micelles (Okada et al. 1994). Another example is magainin 2, a peptide, which dimerizes on binding to phospholipid vesicles, whereas it remains monomeric in micelles (Wakamatsu et al. 2002). Also to be mentioned is the structural difference observed between the conformation of HIV-1 Env peptide in bicelles and detergent micelles (Chou et al. 2002).

Two-dimensional trNOESY has been used to determine membrane-bound conformation by means of distance geometry and energy minimization calculations in the case of senktide, a potent analogue of neurokinin B (Bersch et al. 1993). This approach has also been adopted for a β-adrenoceptor-derived signal transducing peptide (Jung et al. 1996) as well as for substance P (Auge et al. 2000).

Beyond the scope of SUV studies, new developments of the trNOE methodology in the field of protein-lipid interaction have recently been reported with an implementation in solid-state NMR (Soubias and Gawrisch 2005). Another relevant use of trNOE was the analysis of molecular interactions using bead-linked proteoliposomes (Yokogawa et al. 2005).

Proton detection of the bound form

As pointed out in the introduction, the proton detection of lipid-bound molecules represents the most confusing aspect of studies with SUV. It has been reported that association with lipids can induce broadening beyond detection of the proton resonances. Nevertheless, well resolved resonances were sometimes observed.

NMR spectroscopy is very sensitive to the chemical exchange (Bain 2003). Some more explanations on the various dynamics involved in the broadening of the NMR signals are required. When the exchange rate between the free and bound forms is very low in the chemical shift timescale, and in the absence of fast motion of the inserted molecule (for example cholesterol), the signals are broadened beyond detection. At moderate exchange rate, a classical chemical exchange broadening is observed. Moreover, the overall tumbling of the bound molecule is under control of the SUV low tumbling. This slow motion is responsible of an additional severe broadening of the bound form signals when the molecule is devoid of high intrinsic mobility in the bilayer. This has been used to determine the amount of bound molecules by measuring the "disappearance" of their signals (de Alba et al. 2003). In the case of relatively high exchange rate, the broadening of the free-bound averaged signal can be associated to both the chemical exchange rate and the decrease of the mobility induced by the binding to the vesicles. Nevertheless, several reports concern ^{1}H NMR studies characterized by narrow proton signals of molecules fully bound to lipids, implying averaging of the anisotropic contributions.

Reviewing those works is quite difficult as experimental conditions and NMR data have not often been fully reported (percentages of bound molecules, variations of the molecule/lipid ratio, linewidth values and estimations of association and dissociation rate constants). Without detailed information on the studied systems the origins of the narrow proton resonances of SUV associated molecules are not obvious. An explanation often inferred for the visibility of SUV inserted molecules is generally associated with a strong mobility of these molecules inside the lipid bilayer. This has been proposed in the case of lipid interactions with different drugs (Debouzy et al. 1989; Enguehard-Gueiffier et al. 2005; Kuroda et al. 1996; Panicker and Mishra 2006) or anaesthetics (Eisenblätter et al. 2000; Schlieper and Michaelis 1983).

However, another parameter, the dissociation rate constant, could produce the same effect on the averaging of the anisotropic interactions. We recently demonstrated the role of this rate constant in the case of the interaction of porphyrin derivatives with SUV of phospholipids (Da Costa et al. 2006). At intermediate porphyrin/lipid ratio, broad resonances were observed corresponding to the chemical exchange of free and bound forms, in the chemical shift timescale. But increasing lipid concentration then permitted the detection of relatively narrow porphyrin resonances (Fig. 3). When sufficient lipids are present to prevent the occurrence of any free porphyrin, no more chemical exchange broadening can occur, although association-dissociation dynamic remains active.

Furthermore, when comparing the NMR data obtained at different pH values with the results obtained by fluorescence (Maman and Brault 1998), there was a clear relation between the proton linewidths and the pH dependence of the dissociation rate constant of the porphyrin (Da Costa

et al. 2006). This confirms, as previously mentioned, that broad resonance detection, at intermediate molecule/lipid ratio, is more related to the chemical exchange than to the tumbling of the molecule. The detection of averaged (membrane-bound and free) relatively narrow resonances of peptides has already been reported (Hall et al. 1998; Meers and Feigenson 1988). A careful study comprising detailed peptide/lipid ratio influence has been performed on three fragments of ACTH (Roux et al. 1997). The narrow line observation is also consistent with an averaging of the anisotropic interactions induced by the lipid association. This is further reinforced by the sharp resonances observed in the recent studies of achatin I (Kimura et al. 2004) and Met-enkephalin (Kimura 2006) with large unilamellar vesicles. Tumbling of the LUV is even slower than for SUV and phospholipid signals are quite broad. We believe that the narrow proton resonances of the bound peptide correspond to an additional process of anisotropic interaction averaging induced by the association-dissociation dynamic.

Concerning the study of proteins, we wish to introduce the work on α-synuclein, which compared the ^{1}H-^{15}N HSQC spectra of the protein in interaction with micelles or SUV (Eliezer et al. 2001). The N-terminal region binds to synthetic lipid vesicles and detergent micelles and the C-terminal part of the protein does not associate with either vesicles or micelles and remains free and unfolded. The C-terminal part gives cross peaks in the HSQC obtained in micelles and SUV, whereas the N-terminal is only visible in micelles. It can be concluded that the additional movement induced if the C-terminal by its unfolded character, is sufficient to allow signal detection of the SUV-bound protein. Another example corresponds to our recent data on the association of cytochrome *c* with SUV (Da Costa et al. 2006). Detection of the paramagnetic methyl protons of the heme was performed using a protein/lipid ratio of 1/100. Again, this ratio is important in order to avoid strong broadening of the signals due to fast chemical exchange if some free form remains in solution, as already been demonstrated on SDS micelles (Chevance et al. 2003).

To conclude on this part, we infer that in some cases, proton resonance broadening can be due to the chemical exchange rather than the slow tumbling time of the SUV. Such a broadening could have also occurred in different systems if attempts of detecting proton signals were only performed using a low lipid concentration that prevented the full binding of the molecule of interest to the SUV.

Summary

Several NMR approaches for studying drugs, peptides or proteins interactions with SUV as a model membrane have been presented. Whereas these kinds of studies have not been well developed, we think SUV does constitute an efficient model to mimic the membrane and to study certain molecule-membrane interactions under standard NMR liquid conditions.

The different motions: vesicle tumbling, lipid or molecule rotation and translation, dynamic of insertion of the interacting molecules, required to obtain sharp NMR resonances are summarized in Fig. 4. These different motions contribute together to yield detectable the lipid signals as well as the associated molecule signals.

Further work will be necessary to determine the minimum dissociation rate constant allowing proton NMR detection of fully bound molecules to the phospholipids in

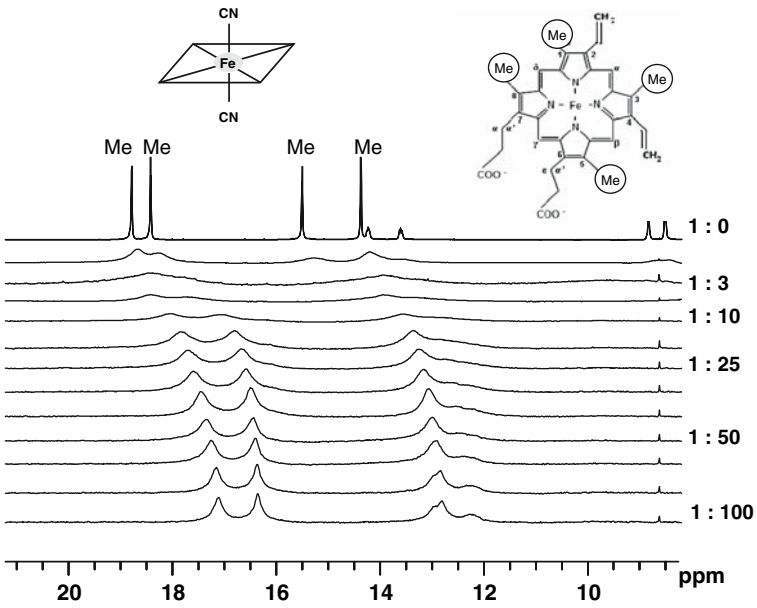

Fig. 3 ^{1}H NMR spectra of 0.5 mM heme Fe^{III}-$(CN^{-})_2$ in D_2O, pD 7.5, at 323 K, obtained by successive addition of 100 mM SUV (90/10) DiMyristoylPhosphatidylCholine/DiMyristoylPhosphatidylSerine. Some heme/SUV molar ratios are indicated. Intensities are arbitrary. Schematic representations of the heme and the hemedicyano complex are displayed above the spectra

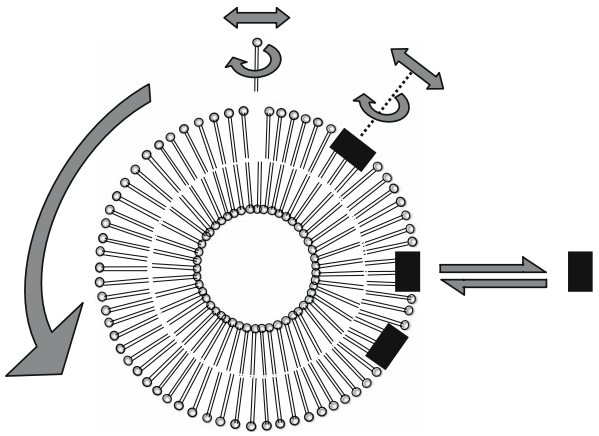

Fig. 4 Summary of the main motions responsible of the averaging of the anisotropic interactions in SUV

SUV. Detailed information about the molecule-membrane association-dissociation dynamic is not well documented. Only sparse data have been reported using surface plasmon resonance. Nevertheless, processes involving such kind of dynamics are involved in many physiological events for which SUV should constitute a relevant model membrane. This is particularly true when studying membrane interactions with various drugs, natural or modified peptides and membrane-binding peripheral proteins.

References

Agasoster AV, Halskau O, Fuglebakk E, Froystein NA, Muga A, Holmsen H, Martinez A (2003) The interaction of peripheral proteins and membranes studied with alpha-lactalbumin and phospholipid bilayers of various compositions. J Biol Chem 278:21790–21797

Angelis AAD, Jones DH, Grant CV, Park SH, Mesleh MF, Opella SJ, Thomas LJ (2005) NMR experiments on aligned samples of membrane proteins. Methods Enzymol 394:350–382

Auge S, Bersch B, Tropis M, Milon A (2000) Characterization of substance P-membrane interaction by transferred nuclear Overhauser effect. Biopolymers 54:297–306

Bain AD (2003) Chemical exchange in NMR. Prog Nucl Magn Reson Spectrosc 43:63–103

Baldus M (2006) Molecular interactions investigated by multi-dimensional solid-state NMR. Curr Opin Struct Biol 16:618–623

Berlose JP, Convert O, Derossi D, Brunissen A, Chassaing G (1996) Conformational and associative behaviours of the third helix of antennapedia homeodomain in membrane-mimetic environments. Eur J Biochem 242:372–386

Bersch B, Koehl P, Nakatani Y, Ourisson G, Milon A (1993) 1H nuclear magnetic resonance determination of the membrane-bound conformation of senktide, a highly selective neurokinin B agonist. J Biomol NMR 3:443–461

Bhattacharya S, Haldar S (1996) The effects of cholesterol inclusion on the vesicular membranes of cationic lipids. Biochim Biophys Acta 1283:21–30

Bloom M, Burnell EE, MacKay AL, Nichol CP, Valic MI, Weeks G (1978) Fatty acyl chain order in lecithin model membranes determined from proton magnetic resonance. Biochemistry 17:5750–5762

Boyle E, Small DM, Gantz D, Hamilton JA, German JB (1996) Monoacylglycerols alter the lipid composition and molecular mobility of phosphatidylcholine bilayers: 13C NMR evidence of dynamic lipid remodeling. J Lipid Res 37:764–772

Brown LR, Wuthrich K (1977) NMR and ESR studies of the interactions of cytochrome c with mixed cardiolipin-phosphatidylcholine vesicles. Biochim Biophys Acta 468:389–410

Chevance S, Le Rumeur E, de Certaines JD, Simonneaux G, Bondon A (2003) 1H NMR structural characterization of the cytochrome c modifications in a micellar environment. Biochemistry 42:15342–15351

Chou JJ, Kaufman JD, Stahl SJ, Wingfield PT, Bax A (2002) Micelle-induced curvature in a water-insoluble HIV-1 Env peptide revealed by NMR dipolar coupling measurement in stretched polyacrylamide gel. J Am Chem Soc 124:2450–2451

Clore GM, Gronenborn AM (1982) Theory and applications of the transferred nuclear overhauser effect to the study of the conformations of small ligands bound to proteins. J Magn Res 48:402–417

Da Costa G, Chevance S, Le Rumeur E, Bondon A (2006) Proton NMR detection of porphyrins and cytochrome C in small unilamellar vesicles: role of the dissociation kinetic constant. Biophys J 90:L55–L57

de Alba E, Weiler S, Tjandra N (2003) Solution structure of human saposin C: pH-dependent interaction with phospholipid vesicles. Biochemistry 42:14729–14740

Debouzy JC, Neumann JM, Herve M, Daveloose D, Viret J, Apitz-Castro R (1989) Interaction of antiaggregant molecule ajoene with membranes. An ESR and 1H, 2H, 31P-NMR study. Eur Biophys J 17:211–216

Demmers JA, Haverkamp J, Heck AJ, Koeppe RE 2nd, Killian JA (2000) Electrospray ionization mass spectrometry as a tool to analyze hydrogen/deuterium exchange kinetics of transmembrane peptides in lipid bilayers. Proc Natl Acad Sci USA 97:3189–3194

Dempsey CE (2001) Hydrogen exchange in peptides and proteins using NMR spectroscopy. Prog Nucl Magn Reson Spectrosc 39:135–170

Dettman HD, Weiner JH, Sykes BD (1982) 19F nuclear magnetic resonance studies of the coat protein of bacteriophage M13 in synthetic phospholipid vesicles and deoxycholate micelles. Biophys J 37:243–251

Dettman HD, Weiner JH, Sykes BD (1984) Phenylalanyl and tyrosyl side chain mobility in the M13 coat protein reconstituted in phospholipid vesicles. Biochemistry 23:705–712

Duplaa H, Convert O, Sautereau AM, Tocanne JF, Chassaing G (1992) Binding of substance P to monolayers and vesicles made of phosphatidylcholine and/or phosphatidylserine. Biochim Biophys Acta 1107:12–22

Eisenblätter J, Zenerino A, Winter R (2000) High-pressure 1H NMR on model biomembranes: a study of the local anaesthetic tetracaine incorporated into POPC lipid bilayers. Magn Reson Chem 38:662–667

Eliezer D, Kutluay E, Bussell R Jr, Browne G (2001) Conformational properties of alpha-synuclein in its free and lipid-associated states. J Mol Biol 307:1061–1073

Ellena JF, Hutton WC, Cafiso DS (1985) Elucidation of cross-relaxation pathways in phospholipid vesicles utilizing two-dimensional proton NMR spectroscopy. J Am Chem Soc 107:1530–1537

Ellena JF, Dominey RN, Archer SJ, Xu ZC, Cafiso DS (1987) Localization of hydrophobic ions in phospholipid bilayers using proton nuclear Overhauser effect spectroscopy. Biochemistry 26:4584–4592

Enguehard-Gueiffier C, Fauvelle F, Debouzy JC, Peinnequin A, Thery I, Dabouis V, Gueiffier A (2005) 2,3-Diarylimidazo[1,2-a]pyridines as potential inhibitors of UV-induced keratinocytes apoptosis: synthesis, pharmacological properties and interactions with

model membranes and oligonucleotides by NMR. Eur J Pharm Sci 24:219–227

Epand RM, Sayer BG, Epand RF (2003) Peptide-induced formation of cholesterol-rich domains. Biochemistry 42:14677–14689

Eriksson LE (1987) Interaction of charged amphiphilic drugs with phosphatidylcholine vesicles studied by NMR. Biophys Chem 26:9–18

Eum KM, Riedy G, Langley KH, Roberts MF (1989) Temperature-induced fusion of small unilamellar vesicles formed from saturated long-chain lecithins and diheptanoylphosphatidylcholine. Biochemistry 28:8206–8213

Feller SE, Huster D, Gawrisch K (1999) Interpretation of NOESY cross-relaxation rates from molecular dynamics simulation of a lipid bilayer. J Am Chem Soc 121:8963–8964

Forbes J, Bowers J, Shan X, Moran L, Oldfield E, Moscarello MA (1988) Some new developments in solid-state nuclear magnetic resonance spectroscopic studies of lipids and biological membranes, including the effects of cholesterol in model and natural systems. J Chem Soc Faraday Trans 84:3821–3849

Forsén S, Hoffman RA (1963) Study of moderately rapid chemical exchange reactions by means of nuclear magnetic double resonance. J Chem Phys 39:2892–2901

Gabriel NE, Roberts MF (1987) Short-chain lecithin long-chain phospholipid unilamellar vesicles: asymmetry, dynamics, and enzymatic hydrolysis of the short-chain component. Biochemistry 26:2432–2440

Gent MP, Ho C (1978) Fluorine-19 nuclear magnetic resonance studies of lipid phase transitions in model and biological membranes. Biochemistry 17:3023–3038

Hall MP, Burson KK, Huestis WH (1998) Interactions of a vesicular stomatitis virus G protein fragment with phosphatidylserine: NMR and fluorescence studies. Biochim Biophys Acta 1415:101–113

Halskau O, Froystein NA, Muga A, Martinez A (2002) The membrane-bound conformation of alpha-lactalbumin studied by NMR-monitored 1H exchange. J Mol Biol 321:99–110

Haran N, Shporer M (1977) Proton magnetic resonance study of cholesterol transfer between egg yolk lecithin vesicles. Biochim Biophys Acta 465:11–18

Hauser H (1989) Mechanism of spontaneous vesiculation. Proc Natl Acad Sci USA 86:5351–5355

Henry GD, Sykes BD (1994) Methods to Study Membrane-Protein Structure in Solution Nuclear Magnetic Resonance. Methods Enzymol 239:515–535

Ho JK, Duclos RI Jr, Hamilton JA (2002) Interactions of acyl carnitines with model membranes: a 13C-NMR study. J Lipid Res 43:1429–1439

Huster D (2005) Investigations of the structure and dynamics of membrane-associated peptides by magic angle spinning NMR. Prog Nucl Magn Reson Spectrosc 46:79–107

Jelicks LA, Broido MS, Becker JM, Naider FR (1989) Interaction of the *Saccharomyces cerevisiae* alpha-factor with phospholipid vesicles as revealed by proton and phosphorus NMR. Biochemistry 28:4233–4240

Jung H, Windhaber R, Palm D, Schnackerz KD (1996) Conformation of a beta-adrenoceptor-derived signal transducing peptide as inferred by circular dichroism and 1H NMR spectroscopy. Biochemistry 35:6399–6405

Kimura T (2006) Human opioid Peptide met-enkephalin binds to anionic phosphatidylserine in high preference to zwitterionic phosphatidylcholine: natural-abundance 13C NMR study on the binding state in large unilamellar vesicles. Biochemistry 45:15601–15609

Kimura T, Okamura E, Matubayasi N, Asami K, Nakahara M (2004) NMR study on the binding of neuropeptide achatin-I to phospholipid bilayer: the equilibrium, location, and peptide conformation. Biophys J 87:375–385

Kingsley PB, Feigenson GW (1979) H-1 Nmr observation of small molecules in perdeuterated phosphatidylcholine vesicles. Febs Lett 97:175–178

Kitamura K, Takegami S, Kobayashi T, Makihara K, Kotani C, Kitade T, Moriguchi M, Inoue Y, Hashimoto T, Takeuchi M (2004) Dissociation constants of phenothiazine drugs incorporated in phosphatidylcholine bilayer of small unilamellar vesicles as determined by carbon-13 nuclear magnetic resonance spectrometric titration. Biochim Biophys Acta 1661:61–67

Kitamura K, Yamamoto M, Takegami S, Sigiura M (1999) Determination of the partition coefficient of triflupromazine between phosphatidylcholine vesicles and water by 19F nuclear magnetic resonance spin-lattice relaxation time measurement. Talanta 49:261–265

Kuroda Y, Ogawa M, Nasu H, Terashima M, Kasahara M, Kiyama Y, Wakita M, Fujiwara Y, Fujii N, Nakagawa T (1996) Locations of local anesthetic dibucaine in model membranes and the interaction between dibucaine and a Na+ channel inactivation gate peptide as studied by 2H- and 1H-NMR spectroscopies. Biophys J 71:1191–1207

Lentz BR, Carpenter TJ, Alford DR (1987) Spontaneous fusion of phosphatidylcholine small unilamellar vesicles in the fluid phase. Biochemistry 26:5389–5397

Lepre CA, Moore JM, Peng JW (2004) Theory and applications of NMR-based screening in pharmaceutical research. Chem Rev 104:3641–3676

Luca S, Heise H, Baldus M (2003) High-resolution solid-state NMR applied to polypeptides and membrane proteins. Acc Chem Res 36:858–865

Malcolmson RJ, Higinbotham J, Beswick PH, Reed D, Parkinson JA, Sadler IH (1996) 13C-NMR determination of the solubility and molecular dynamics of cholesteryl oleate in dimyristoylphosphatidylcholine vesicles. Chem Phys Lipids 84:93–104

Maman N, Brault D (1998) Kinetics of the interactions of a dicarboxylic porphyrin with unilamellar lipidic vesicles: interplay between bilayer thickness and pH in rate control. Biochim Biophys Acta 1414:31–42

Mattjus P, Malewicz B, Valiyaveettil JT, Baumann WJ, Bittman R, Brown RE (2002) Sphingomyelin modulates the transbilayer distribution of galactosylceramide in phospholipid membranes. J Biol Chem 277:19476–19481

Meers PR, Feigenson GW (1988) Location valinomycin. Biochim Biophys Acta 938:469–482

Milon A, Miyazawa T, Higashijima T (1990) Transferred nuclear Overhauser effect analyses of membrane-bound enkephalin analogues by 1H nuclear magnetic resonance: correlation between activities and membrane-bound conformations. Biochemistry 29:65–75

Moreau C, Cavalier A, Floch ML, Segalen J, Rocher C, Traikia M, Leray G, Bondon A, Thomas D, Rumeur EL (2001) Sarcolemma phospholipid structure investigated by immunogold electron microscopy and 31P NMR spectroscopy with lanthanide ions. FEBS Lett 509:417–422

Nakajima T, Yasuhara T, Uzu S, Wakamatsu K, Miyazawa T, Fukuda K, Tsukamoto Y (1985) Wasp venom peptides; wasp kinins, new cytotrophic peptide families and their physico-chemical properties. Peptides 6:S425–S430

Nakamura T, Takahashi H, Takeuchi K, Kohno T, Wakamatsu K, Shimada I (2005) Direct determination of a membrane-peptide interface using the nuclear magnetic resonance cross-saturation method. Biophys J 89:4051–4055

Ni F, Scheraga HA (1994) Use of the transferred nuclear Overhauser effect to determine the conformations of ligands bound to proteins. Acc Chem Res 27:257–264

Nicolay K, van der Neut R, Fok JJ, de Kruijff B (1985) Effects of adriamycin on lipid polymorphism in cardiolipin-containing model and mitochondrial membranes. Biochim Biophys Acta 819:55–65

Nordlund JR, Schmidt CF, Thompson TE (1981) Transbilayer distribution in small unilamellar phosphatidylglycerol-phosphatidylcholine vesicles. Biochemistry 20:6415–6420

Okada A, Wakamatsu K, Miyazawa T, Higashijima T (1994) Vesicle-bound conformation of melittin: transferred nuclear Overhauser enhancement analysis in the presence of perdeuterated phosphatidylcholine vesicles. Biochemistry 33:9438–9446

Okamura E, Kimura T, Nakahara M, Tanaka M, Handa T, Saito H (2001) 13C NMR method for the determination of peptide and protein binding sites in lipid bilayers and emulsions. J Phys Chem B 105:12616–12621

Omran AA, Kitamura K, Takegami S, Kume M, Yoshida M, El-Sayed A-AY, Mohamed MH, Abdel-Mottaleb M (2002) 19F NMR spectrometric determination of the partition coefficients of some fluorinated psychotropic drugs between phosphatidylcholine bilayer vesicles and water. J Pharm Biomed Anal 30:1087–1092

Ong RL (1984) 31P and 19F NMR studies of glycophorin-reconstituted membranes: preferential interaction of glycophorin with phosphatidylserine. J Membr Biol 78:1–7

Ong RL, Prestegard JH (1982) High-resolution carbon-13 nuclear magnetic resonance studies of small unilamellar vesicles containing glycophorin A. Biochim Biophys Acta 692:252–262

Opella SJ, Marassi FM (2004) Structure determination of membrane proteins by NMR spectroscopy. Chem Rev 104:3587–3606

Painter GR, Grunwald R, Roth B (1988) Interaction of the antifolate antibiotic trimethoprim with phosphatidylcholine membranes: a carbon-13 and phosphorus-31 nuclear magnetic resonance study. Mol Pharmacol 33:551–558

Panicker L, Mishra KP (2006) Nuclear magnetic resonance and thermal studies on the interaction between salicylic acid and model membranes. Biophys Chem 120:15–23

Pitcher WH III, Huestis WH (2002) Preparation and analysis of small unilamellar phospholipid vesicles of a uniform size. Biochem Biophys Res Commun 296:1352–1355

Prestegard JH, Ong RL (1982) NMR studies of the interaction of lipids with glycophorin A in small unilamellar vesicles. Biophys J 37:80–81

Prosser RS, Evanics F, Kitevski JL, Al-Abdul-Wahid MS (2006) Current applications of bicelles in NMR studies of membrane-associated amphiphiles and proteins. Biochemistry 45:8453–8465

Reynaud JA, Grivet JP, Sy D, Trudelle Y (1993) Interactions of basic amphiphilic peptides with dimyristoylphosphatidylcholine small unilamellar vesicles: optical, NMR, and electron microscopy studies and conformational calculations. Biochemistry 32:4997–5008

Roux M, Neumann JM, Toma F (1997) ACTH binding to phospholipid bilayers: A H-1-NMR study of the 5–14, 1–14, and 1–24 derivatives. Biopolymers 42:731–744

Rule GS, Pratt EA, Simplaceanu V, Ho C (1987) Nuclear magnetic resonance and molecular genetic studies of the membrane-bound D-lactate dehydrogenase of Escherichia coli. Biochemistry 26: 549–556

Sanders CR, Sonnichsen F (2006) Solution NMR of membrane proteins: practice and challenges. Magn Res Chem 44:S24-S40

Scheidt HA, Huster D, Gawrisch K (2005) Diffusion of cholesterol and its precursors in lipid membranes studied by 1H pulsed field gradient magic angle spinning NMR. Biophys J 89:2504–2512

Schlieper P, Michaelis L (1983) Interaction of local anesthetics with small phospholipid vesicles investigated by proton NMR spectroscopy. Eur Biophys J 10:1–9

Schmidt CF, Lichtenberg D, Thompson TE (1981) Vesicle-vesicle interactions in sonicated dispersions of dipalmitoylphosphatidylcholine. Biochemistry 20:4792–4797

Schullery SE, Schmidt CF, Felgner P, Tillack TW, Thompson TE (1980) Fusion of dipalmitoylphosphatidylcholine vesicles. Biochemistry 19:3919–3923

Sheetz MP, Chan SI (1972) Effect of sonication on the structure of lecithin bilayers. Biochemistry 11:4573–4581

Soubias O, Gawrisch K (2005) Probing specific lipid-protein interaction by saturation transfer difference NMR spectroscopy. J Am Chem Soc 127:13110–13111

Staudenmayer N, Smith MB, Smith HT, Spies FK Jr, Millett F (1976) An enzyme kinetics and 19F nuclear magnetic resonance study of selectively trifluoroacetylated cytochrome c derivatives. Biochemistry 15:3198–3205

Strandberg E, Ulrich AS (2004) NMR methods for studying membrane-active antimicrobial peptides. Concepts Magn Res Part A 23A:89–120

Sukumar M, Higashijima T (1992) G protein-bound conformation of mastoparan-X, a receptor-mimetic peptide. J Biol Chem 267:21421–21424

Swairjo MA, Roberts MF, Campos MB, Dedman JR, Seaton BA (1994) Annexin V binding to the outer leaflet of small unilamellar vesicles leads to altered inner-leaflet properties: 31P- and 1H-NMR studies. Biochemistry 33:10944–10950

Takahashi H, Nakanishi T, Kami K, Arata Y, Shimada I (2000) A novel NMR method for determining the interfaces of large protein-protein complexes. Nature Struct Biol 7:220–223

Takeuchi K, Takahashi H, Sugai M, Iwai H, Kohno T, Sekimizu K, Natori S, Shimada I (2004) Channel-forming membrane permeabilization by an antibacterial protein, sapecin: determination of membrane-buried and oligomerization surfaces by NMR. J Biol Chem 279:4981–4987

Traikia M, Warschawski DE, Recouvreur M, Cartaud J, Devaux PF (2000) Formation of unilamellar vesicles by repetitive freeze-thaw cycles: characterization by electron microscopy and P-31-nuclear magnetic resonance. Eur Biophys J 29:184–195

Wakamatsu K, Higashijima T, Fujino M, Nakajima T, Miyazawa T (1983) Transferred NOE analyses of conformations of peptides as bound to membrane bilayer of phospholipid; mastoparan-X. FEBS Lett 162:123–126

Wakamatsu K, Okada A, Miyazawa T, Ohya M, Higashijima T (1992) Membrane-bound conformation of mastoparan-X, a G-protein-activating peptide. Biochemistry 31:5654–5660

Wakamatsu K, Okada A, Suzuki M, Higashijima T, Masui Y, Sakakibara S, Miyazawa T (1986) Nuclear-magnetic-resonance studies on the conformation of membrane-bound alpha-mating factor. Transferred nuclear Overhauser effect analysis. Eur J Biochem 154:607–615

Wakamatsu K, Takeda A, Tachi T, Matsuzaki K (2002) Dimer structure of magainin 2 bound to phospholipid vesicles. Biopolymers 64:314–327

Wang Z, Jones JD, Rizo J, Gierasch LM (1993) Membrane-bound conformation of a signal peptide: a transferred nuclear Overhauser effect analysis. Biochemistry 32:13991–13999

Watts A (2005) Solid-state NMR in drug design and discovery for membrane-embedded targets. Nat Rev Drug Discov 4:555–568

Weinstein S, Durkin JT, Veatch WR, Blout ER (1985) Conformation of the gramicidin A channel in phospholipid vesicles: a fluorine-19 nuclear magnetic resonance study. Biochemistry 24:4374–4382

Wu WG, Dowd SR, Simplaceanu V, Peng ZY, Ho C (1985) Fluorine-19 NMR investigation of molecular motion and packing in sonicated phospholipid vesicles. Biochemistry 24:7153–7161

Xu ZC, Cafiso DS (1986) Phospholipid packing and conformation in small vesicles revealed by two-dimensional H-1 nuclear-magnetic-resonance cross-relaxation spectroscopy. Biophys J 49: 779–783

Yokogawa M, Takeuchi K, Shimada I (2005) Bead-linked proteoliposomes: a reconstitution method for nmr analyses of membrane protein-ligand interactions. J Am Chem Soc 127:12021–12027