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Capability of ganglioside GM1 in modulating interactions, structure, location and dynamics of peptides/proteins: biophysical approaches

Interaction of ganglioside GM1 with peptides/proteins

Ummul Liha Khatun · Anindita Gayen · Chaitali Mukhopadhyay

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Abstract Gangliosides, are glycosphingolipids, present in all vertebrate plasma membranes with particular abundance in nerve cell membrane. Gangliosides can act as portals for antimicrobial peptides, hormones, viruses, lectins, toxins and pathogens. They are strategically positioned on the outer membrane and hence can participate in a large number of recognition processes. Their abundance in nerve cell membrane makes them "likely" receptor candidates for neuropeptides. In this review we outline our work in the area of GM1-peptide/protein interaction. We have explored the effect of GM1 containing micelles/bicelles on structures of peptides, proteins as well as on denatured proteins. It has been observed that the peptides that are disordered or having random coil structure in aqueous solution, attained an ordered three-dimensional structure when interact with GM1. It is also observed that denatured proteins undergo refolding in presence of ganglioside. Peptides/proteins show stronger interaction with membrane lipid bilayer in presence of ganglioside than that without ganglioside. This review mainly focuses on capability of ganglioside GM1 in modulating interaction, structural, location and dynamics of peptides/proteins using a number of biophysical techniques–solution NMR, DOSY, CD, fluorescence *etc*.

Keywords Ganglioside · Neuropeptide · NMR · DOSY · Fluorescence · Bicelle · Micelle

U. L. Khatun · C. Mukhopadhyay (✉)
Department of Chemistry, University of Calcutta, 92, A.P.C. Road, Kolkata 700 009, India
e-mail: chaitalicu@yahoo.com

Present Address:
A. Gayen
Chemistry Department, New York University, New York, NY 10003, USA

Abbreviations

GM1	Ganglioside Monosialo 1
GD1	Ganglioside disialo 1
GT1	Ganglioside trisialo 1
GQ1	Ganglioside Quadra
SP	Substance P
LENK	Leucine-enkephalin
MLT	Melittin
NT	Neurotensin
BSA	Bovine Serum Albumin
Aβ	Amyloid Beta
CD	Circular Dichroism
AD	Alzheimer's disease
NMR	Nuclear Magnetic Resonance
CNS	Central Nervous System
DSC	Differential Scanning Calorimetry
SM	Sphingomyelin
Chol	Cholesterol
CMC	Critical Micelle Concentration
PC	Phosphatidylcholine
DMPC	1, 2- dimyristoyl-sn-glycero-3-phosphatidylcholine
DPC	Dodecylphosphocholine
CHAPS	3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate
NK_1R	Neurokinin-1 receptor
NTS-3	Neurotensin Receptor3
ROESY	Rotating-frame Overhauser Effect Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
DOSY	Diffusion Ordered Spectroscopy
STD	Saturation transfer difference
PFG-SE	Pulsed Field Gradient Spin Echo
Ksv	Stern–Volmer quenching constant
AFM	Atomic Force Microscopy

TEM	Transmission Electron Microscopy
REES	Red Edge Excitation Shift
FRET	Fluorescence Resonence Energy Transfer
SPR	Surface Plasmon Resonance
EPR	Electron Paramagnetic Resonance
FT-IR	Fourier Transform Infra Red
FCS	Fluorescence Correlation Spectroscopy
QCM	Quartz Crystal Microbalance Study
PISEMA	Polarization Inversion Spin Exchange at Magic Angle
PRE	Paramagnetic Relaxation Enhancement
MAS	Magic Angle Spinning
DMPS	Dimyristoyl phosphatidylserine
DMPG	1,2-Dimyristoyl-sn-glycero-3-phosphoglycerol
DMPE	Dimyristoyl phosphatidylethanolamine
SUV	Small Unilamellar Vesicles
GalNAc	−*N*-acetylgalactosamine
Neu5AC	−*N*-acetylneuraminic acid
Gal	Galactose
Glc	Glucose

Introduction

Biological membranes are complex assemblies and are subject of intense research since decades. Membranes function to organize biological processes by compartmentalizing them. The cell membrane is constituted by a lipid bilayer matrix having hydrophobic interior and hydrophilic exterior [1]. Major components of the bilayer are the phospholipids (*e.g.* phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine), cholesterol, sphingolipids, and different membrane proteins [1]. Low abundant lipids such as gangliosides reside in specific regions with considerable complexity and respond to various cellular functions [1]. The name ganglioside was first applied by the German scientist Ernst Klenk in 1930s to lipids newly isolated from ganglion cells of brain. Gangliosides are glycosphingolipids consisting of mono- to poly-sialylated oligosaccharide chains of variable lengths attached to a ceramide unit [2, 3]. Gangliosides comprise a large family; their constituent oligosaccharides differ in the nature of glycosidic linkages, sugar configuration, and the contents of neutral sugars and sialic acid (*N*-acetylneuraminic acid) [2–4]. As proposed by Svennerholm, based on the number of sialic acids (*N*-acetylneuraminic acid) gangliosides are classified into GM (mono-), GD (di-), GT (tri-) and GQ (quadra-sialylated) groups, G being used for ganglioside [3]. GM1 (ganglioside-monosialylated) {Gal β (1–3) GalNAc β (1–4) [Neu5Ac α (2–3)] Gal β (1–4) Glc β1-Cer} contains a pentasaccharide head group, of which four are neutral sugars and one sialic acid (Fig. 1). Depending on the difference in the position of sialic acids, the gangliosides are subdivided as

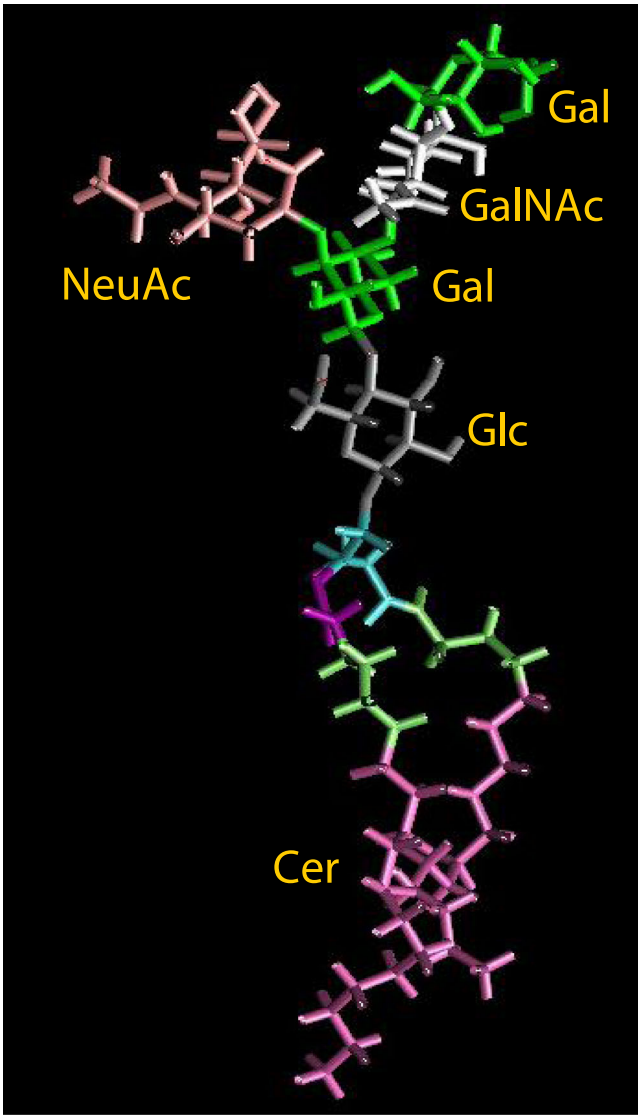

Fig. 1 Three-dimensional model of ganglioside monosialylated 1

GD1a/GD1b or GT1a/GT1b, the numbers 1, 2, 3, *etc*. referring to the order of migration of the gangliosides on thin-layer chromatography. For example, among two sialic acids in GD1a, one is attached to internal galactose and the other with terminal galactose where as in GD1b both the sialic acids are attached to internal galactose. Not only compositional diversity, the head groups of the gangliosides can attain a wide variety of conformations. NMR spectroscopic studies and simulations have shown that the trisaccharide core β-GalNAc-(1–4)-[α-Neu5Ac-(2–3)] β-Gal- can have a rigid conformation, and a more flexible β-Gal-(1–3)-β-GalNAc-terminal glycosidic bond is present in ganglioside GM1 [5, 6].

Gangliosides are inserted in the outer layer of the plasma membrane with the hydrophobic ceramide moiety embedded in the bilayer and the oligosaccharide moiety protruding out [3]. Being abundant in the central nervous system (CNS)

gangliosides are involved in neuritogenesis, synaptic transmission, and other neural functions including brain development and maturation [4, 7]. They function as receptors in cell-cell recognition where specific glycan-binding proteins on opposing cells interact with the carbohydrate moiety of the ganglioside head-group [7], one of the well-studied examples being the recognition and binding of GM1 head-group by cholera toxin [8]. In addition, gangliosides act as receptor of numerous hormones [9], viruses [10], lectins [11] and toxins [12]. Recently, it has been reported that ganglioside can serve as a molecular portal for antimicrobial peptide [13]. Gangliosides are implicated in pathological states such as cancer [14] and neuro-degerative diseases like Alzheimer's, Parkinson's and Huntington's diseases, *etc*. [15, 16]. It has been reported that in presence of phospholipids, cholesterol and sphingomyelin, gangliosides form ordered phases known as "rafts" [17]. Such ordered phases or microdomains are suggested to be crucial sites for the binding and oligomerisation of amyloidogenic proteins [18].

The properties of such microdomains have been studied using a variety of ganglioside-containing ternary membranes. Influence of glycolipid oligosaccharide and long-chain base composition on the thermotropic properties of large unilamellar vesicles containing gangliosides was studied by Masserini *et al*. [19]. The thermotropic behavior of GM1 containing ternary mixtures, SM/Chol/GM1 were analyzed by DSC, showing the formation of separate GM1-enriched domains [19]. It is reported that the extent of ganglioside lateral phase separation depends on the length and unsaturation differences between the ganglioside long-chain base and phosphatidylcholine acyl chains [20].

In this review, an overview of the impact of ganglioside GM1 in modulating interaction, structure, location and dynamics of peptides and proteins will be presented using various biophysical techniques together with a summary of the different model membrane systems that are used in the different applications. We have studied the interaction of several peptides and proteins with GM1. The peptides that we have chosen are mostly neuropeptides, *e.g.*, Leu- and Met-enkephalins, Bradykinin, Substance P, Neurotensin, as well as Melittin, the bee venom toxin, which though not a neuropeptide, is a very well studied membrane-active peptide.

Membrane mimetics

Biological membranes are very complex and dynamic systems and are not easily amenable to experiments [21]. Thus membrane mimetics are used to understand the complexities of their structural and thermodynamic properties. Such model membranes are also used to study their effects on peptides/proteins. The altered structural and dynamic features of the interacting peptides/proteins could then be studied using solution spectroscopic techniques along with molecular modeling. Of the common membrane mimetics that we have used are GM1 micelles and binary and ternary bicelles without and with GM1 respectively.

Micelle

Among the conventional model membranes, micelles are the simplest. Micelles are aggregates of amphiphilic surfactant molecules that occur in spherical, cylindrical and ellipsoidal shapes [22]. A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with water, sequestering the hydrophobic single-tail regions in the micelle centre. At very low concentration of the surfactant, only monomers are present in the solution. At a broad threshold of monomer concentration called the critical micelle concentration (CMC), self-association occurs and micelles form [22]. This is a spontaneous equilibrium and this phenomenon is dependent on the concentration and temperature of the solution. Due to small size, reorientation time of micelles is short so it participates in high-resolution spectroscopy [23, 24]. Gangliosides show different aggregating properties in aqueous solution. It has been shown that most gangliosides, although double tailed, aggregate in the micellar form at room temperature (having CMC in the range 10^{-8} M) rather than in the bilayer form [25]. The properties of these micellar solutions have been studied [26, 27]. These micelles are fairly stable and are very useful to study the specific effect of gangliosides on the structure and dynamics of a particular protein/peptide.

Mixed micelle

Mixed micelles can be prepared by mixing two different amphiphilic molecules. It has been reported that mixture of gangliosides can segregate into mixed micelles [28]. Though micelle containing only one component is spherical but mixed micelles containing two components can deform from its spherical shape to ellipsoidal shape coupled to a redistribution of its components, the largest molecules being segregated in the region of high curvature [28]. Depending upon the nature and size of the head-group gangliosides will occupy different positions of high and low curvatures of the mixed micelles [29]. Mixed micelles can be formed by using ganglioside as one component and detergent as the other. Though DPC/GM1 is the popularly known mixed micelle containing GM1 [28], we have chosen 3-(cholamidopropyl)-dimethylammonio-2-hydroxyl-1-propane-sulfonate (CHAPS) instead of DPC (dodecyl phosphocholine) because CHAPS is a cholesterol mimic.

Bicelle

The rigorous search for a sensitive and innovative model membrane during the last 25 years has resulted in a new model

called 'bicelles'. Bicelles can be considered to be intermediate between classical vesicles and micelles and can be termed as 'binary bilayered mixed micelles' [30, and references therein]. But bicelles are more stable (bicelle samples without peptide were stable over months and the peptide containing bicelle were stable over weeks, if kept at−20 °C) than vesicles. Bicelles are composed of aliphatic long (12–18 carbons) chain lipids and either short (6–8 carbons) chain lipids or detergent. Long chain lipids reside in planer disk region and form bilayer whereas short chain lipids or detergent reside at the rim [30]. The properties of bicelles are strongly dependent on several physical parameters like composition, pH, ionic strength *etc*. However, the key parameter of bicelles is the q-value, which defined as the molar ratio of long chain lipids to that of short chain lipids or detergent.

$$q = [\text{long chain lipids}]/[\text{detergents or short chain lipids}]$$

The size and shape of bicelles are controlled by the q value [30–33]. If q value is high (q>1), the bicelles are called aligned bicelles and adopt lamellar bilayer morphology with ellipsoidal shape. Aligned bicelles are usually bigger in size, with longer reorientation time and exhibit a magnetic alignment with the membrane normal perpendicular to the static magnetic field, B_0. Generally, solid state NMR is applied to probe the morphology of aligned bicelles. In contrast, at smaller q value (q<1), bicelles are known to have fast tumbling and isotropic properties in aqueous solution with discoidal shape and they resist from magnetic field orientation. These isotropic bicelles are characterized by solution state NMR [30]. Zwitterionic phospholipids with different head groups are used to prepare zwitterionic bicelles while acidic phospholipids (such as DMPS, DMPG, and DMPE) are used to make anionic bicelle [31]. A large number of recent studies have shown the effective use of bicelles for elucidation of the structure of membrane-bound proteins/peptides [31–39]. Hydrophobic molecules like sterols can also be doped in bicelles. Experimentally, it is observed that a small amount of sterol can influence the packing, stability and alignment of bicelles [31]. Preparation of custom-made functional bicelles can serve not only as useful membrane mimetic but as delivery systems also [38]. The use of bicelles is not only limited in NMR now, it has been extended to EPR, CD, FTIR, AFM, TEM, fluorescence and other spectroscopic techniques [36, 37].

Use of ganglioside in model membranes

In order to achieve physico-chemical clarity, simple model membranes containing gangliosides are necessary [40]. Ganglioside doped bicelles can be prepared by incorporating ganglioside with phospholipid and cholesterol in definite mole ratio. We have prepared in our laboratory isotropic fast tumbling DMPC/CHAPS/ganglioside (1:4:0.3) bicelles followed by an extensive characterization. The protocol regarding the preparation of gangliosides-containing bicelles was standardized [41, 42]. CHAPS is used in this preparation as a mimic of cholesterol. Though CHAPS is a detergent and cholesterol is a lipid, both CHAPS and cholesterol are reported to increase the similar rigidity of membrane by increasing the order of acyl chains of phospholipids because of similarity in their molecular structure regarding the sterol region [31, 39]. Distribution of GM1 within DMPC/CHAPS/GM1 ternary bicelles has been shown from our FT-IR, fluorescence anisotropy as well as NMR experiments [41, 42].

Using high-resolution surface-sensitive techniques like atomic force microscopy (AFM), it was reported that GM1 is heterogeneously distributed in membranes among the gel-state phosphocholines depending on the concentration. While 2–5 mol% GM1 leads to clusters, 10 mol% leads to filaments [43]. Abundance of GM1 in membrane-ordered phases is supported by QCM, FTIR, SPR and fluorescence techniques, FRET, FCS and anisotropy [44, 45]. Report of DSC with PC membranes at low concentration of GM1 had shown that, phase separation does not occur suggesting GM1 is completely miscible with PC at low concentration [44].

Interaction of peptides/proteins with ganglioside GM1

Study of cell-penetrating peptides in model membrane systems has been recently reviewed [46 and references therein]. We have used the following biophysical techniques to study the interaction of GM1 with peptides/proteins:

Fluorescence Spectroscopy

Fluorescence spectroscopy is amongst the most widely used techniques for studying lipid–protein interactions due to the intrinsic sensitivity, suitable timescale and minimal perturbation [46–48]. The characteristics of intrinsic fluorescence like peak position and intensity of aromatic amino acids of the proteins can be utilized to probe the changes in the protein environment upon membrane binding [46–48]. Fluorescence quenching, steady-state anisotropy and REES are routinely used to gain important information about the changes in the micro-environments of the aromatic residues of the peptides/proteins in presence of membrane mimics at a suitable lipid: peptide concentration ratio. For example, in case of Bradykinin, which contains phenylalanine, after addition of increasing concentration of GM1 micelle fluorescence intensity gradually increases and the fluorescence quenching efficiencies of iodide as well as acrylamide are substantially reduced, indicating a shielding of phenylalanine residue of bradykinin from aqueous environment, which implies the penetration of

phenylalanine within micellar core of GM1 *i.e.*, indicating the interaction of Bradikinin with ganglioside GM1 micelle [49]. We have also reported the changes in tryptophan fluorescence of Melittin in presence of GM1 micelles [50]. Recent report shows that ganglioside GM1 binding peptide P_3, having sequence VWRLLAPPFSNRLLP, has high affinity for GM1 with a dissociation constant of 1.2 μM. It was suggested that lateral assembly of GM1 molecules is essential for the recognition of carbohydrates head group by p3. Furthermore tryptophan, a core residue of the hydrophobic cluster, might be an essential residue for the recognition of the GM1 saccharides [51], which is in agreement with our previous report [50].

^{1}H NMR

Solution NMR studies of cell-penetrating peptides in model membrane systems have recently been reviewed by Lena Mäler [52] indicating the nature of changes observed in the spectra. Our studies on leu- and met-enkephalins, melittin, bradykinin peptides with GM1 micelles and interaction of Substance P and neurotensin with binary and ternary bicelles had shown that important information can be obtained even from 1D NMR experiments [41, 53–59]. Significant line broadening of ^{1}H NMR resonances of peptide protons is observed, suggestive of motional restriction due to interaction of peptides with membrane (in the present case membrane is either GM1 micelle or GM1 containing bicelle). Fig. 2 shows the 500 MHz 1-D proton NMR spectra of melittin (900 μM) in water, DMPC/CHAPS (1:4) bicelles and DMPC/CHAPS/GM1 (1:4:0.3) bicelles at pH 5.5 and 298 K. The changes in chemical shifts in membrane as compared to aqueous environment shed light on the possible structural alterations in the peptides. Such changes can be further explored in homonuclear 2D NMR like ROESY (Fig. 3) and in heteronuclear correlation spectroscopy *e.g.*, HSQC, HMBC, to extract information about the binding. Figure 4 shows the HSQC spectra of LENK taken in water, in presence of binary bicelles and ternary bicelles (containing GM1). The magnitude of $^1J_{CH}$ splitting, when shifting from the water to the bicelles increases or decreases, and for several $^{13}C^{\alpha}$-$^1H^{\alpha}$ pairs, the difference exceeds 10Hz (Table 1).

STD NMR

Saturation transfer difference (STD) NMR involves excitation and saturation of receptor signals and then transfer of the magnetization to the ligand [60]. The altered intensities of the ligand signals are used to gain information about their proximity to the receptor. In STD experiment the focus is on the signals of the ligand, without any need of assignment of receptor signals. The other advantage is that the experiment requires only small quantities of unlabeled receptor (macromolecule) [60 and references therein]. In our case the GM1 micelle acted as the receptor, while the peptides were ligands. Table 2 shows the interacting residues of melittin, bradykinin, leu-and met-enkephalins with GM1 micelle using STD NMR. It is interesting to see that in all the four cases, aromatic residues were directly involved in binding, which was also suggested from the fluorescence experiments discussed earlier [49, 50, 53].

Diffusion NMR

Diffusion NMR experiments *e.g.* pulsed field gradient spin echo (PFG-SE) experiment have gained tremendous attraction [61] since using this simple experiment, one can measure the diffusion coefficients as well as get information about the extent of binding between two species present in the solution. Magnetic field-gradient is used to spatially mark the diffusing molecules. Our observations with GM1 micelles and melittin, bradykinin, leu-and met-enkephalins using PFG-SE diffusion NMR studies are listed in Table 2. We observed that the diffusion coefficients of peptides are reduced depending upon their extent of binding with GM1 micelles [54–56].

DOSY

Diffusion Ordered Spectroscopy (DOSY) is a NMR experiment used widely for the determination of diffusion coefficients which are indicative of molecular size, shape and association between individual components in a mixture [61–64]. It is a two-dimensional version of PFG-SE NMR. We have used DOSY to study the interactions between peptides and lipid. The diffusion coefficient of Substance P (SP) in aqueous solution is 28.3×10^{-11} m^2/s which decreases in presence of both PC bicelle and GM1 containing PC bicelle indicating that SP interacts with both the bicelles [57]. Diffusion coefficient of neurotensin in water is 25×10^{-11} m^2/s, which decreases in either of the bicelles and the diffusion being comparatively slower in GM1 containing bicelle than that of the PC bicelle [58]. Similar is the result observed for the antimicrobial peptide melittin in presence bicelles [59]. Table 3 shows the summary of the results. Thus, translational diffusion results indirectly show the effect of ganglioside GM1 on MLT, NT and Substance P.

Peptide–lipid ROEs

Direct evidence of the interaction of a peptide with bilayer or micelle can be obtained by observing cross-relaxation between parts of a lipid molecule and the peptide as is observed in the case of substance P, melittin and Neurotensin. ROESY spectra of SP and NT in GM1

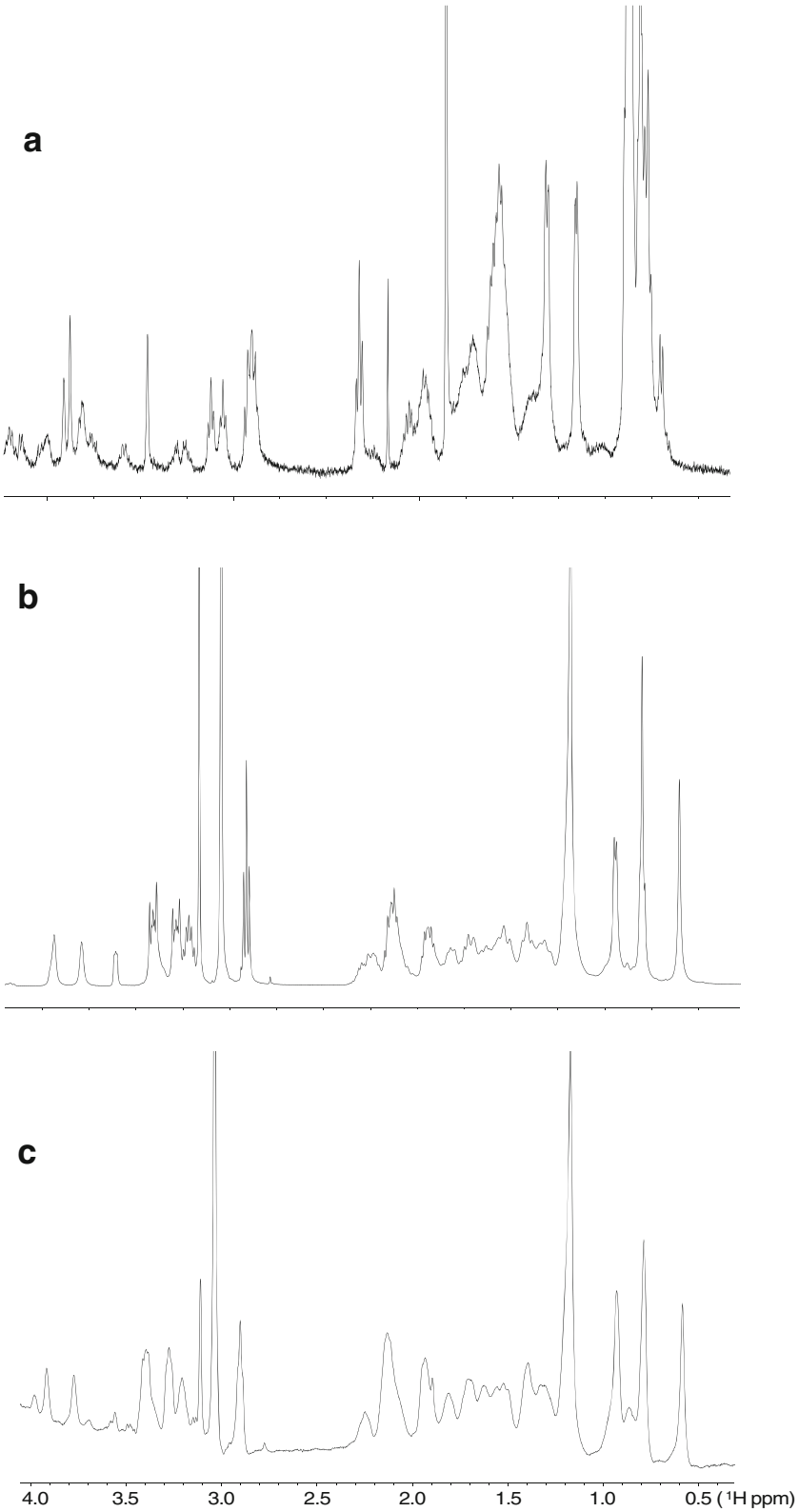

Fig. 2 500 MHz 1-D proton NMR spectra of melittin (900 μM) in water (A), DMPC: CHAPS (1:4) bicelles (B) and DMPC: CHAPS: GM1 (1:4:0.3) bicelles (C) at pH 5.5 and 298 K (peptide: lipid 1:100 mol/mol). The spectral region indicates the fingerprint aliphatic region (0.5–4.0 ppm). Broadening and signal attenuation of peaks occur in going from water to bicelles. Again broadening and attenuation is larger GM1 containing bicelles

containing bicelles, exhibit cross peaks between peptide residues to lipid resonances [57–59]. Comparison of, ROESY spectra of only bicelles and that of bicelles with peptides can be done to identify the lipid-peptide cross peaks [57, 58]. Fig. 5 is the 2D ^{1}H–^{1}H ROESY region showing the cross peaks of Neurotensin aromatic protons with lipid acyl chain –CH2– protons of DMPC in DMPC/CHAPS/GM1 bicelles.

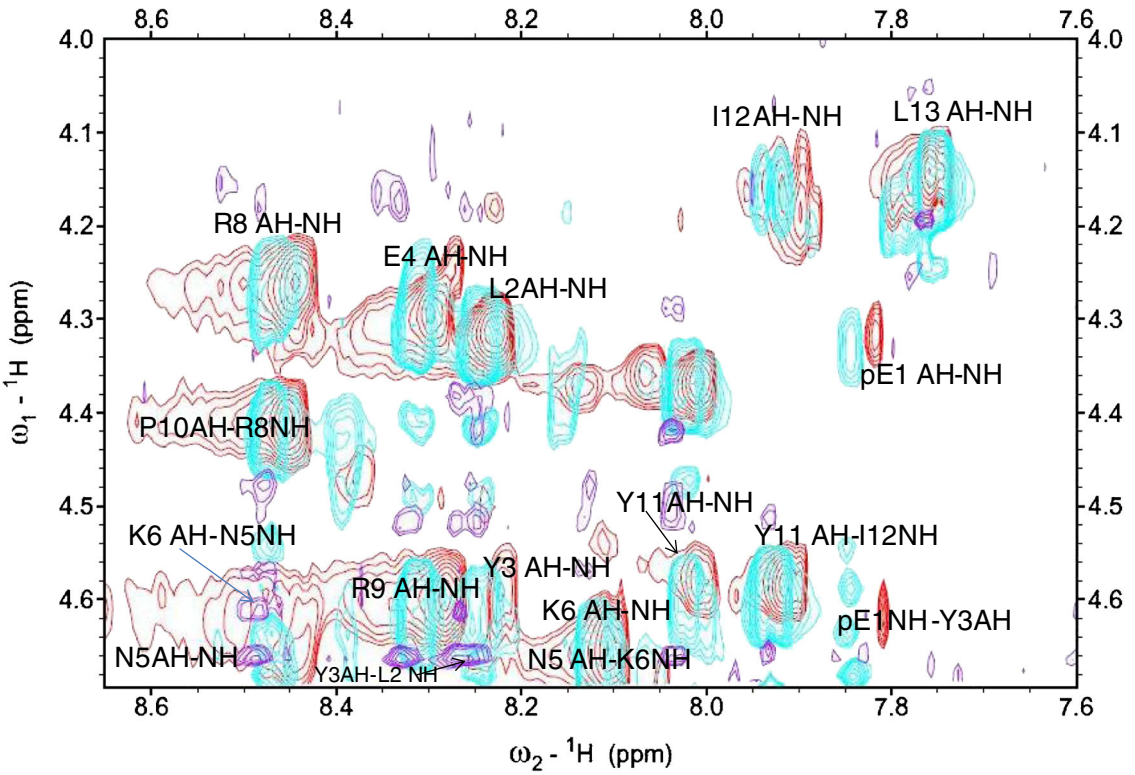

Fig. 3 Superimposed fingerprint region of 2D ^{1}H-^{1}H ROESY spectra of neurotensin (4.5 mM) showing contours in water (purple), DMPC:CHAPS(1:4) bicelles(cyan) and DMPC:CHAPS:GM1(1:4:0.3) bicelle (red) at pH 5.5 and 298 K (peptide: lipid 1: 20 mol/mol). The finger print region shows the alpha region of ROESY spectra

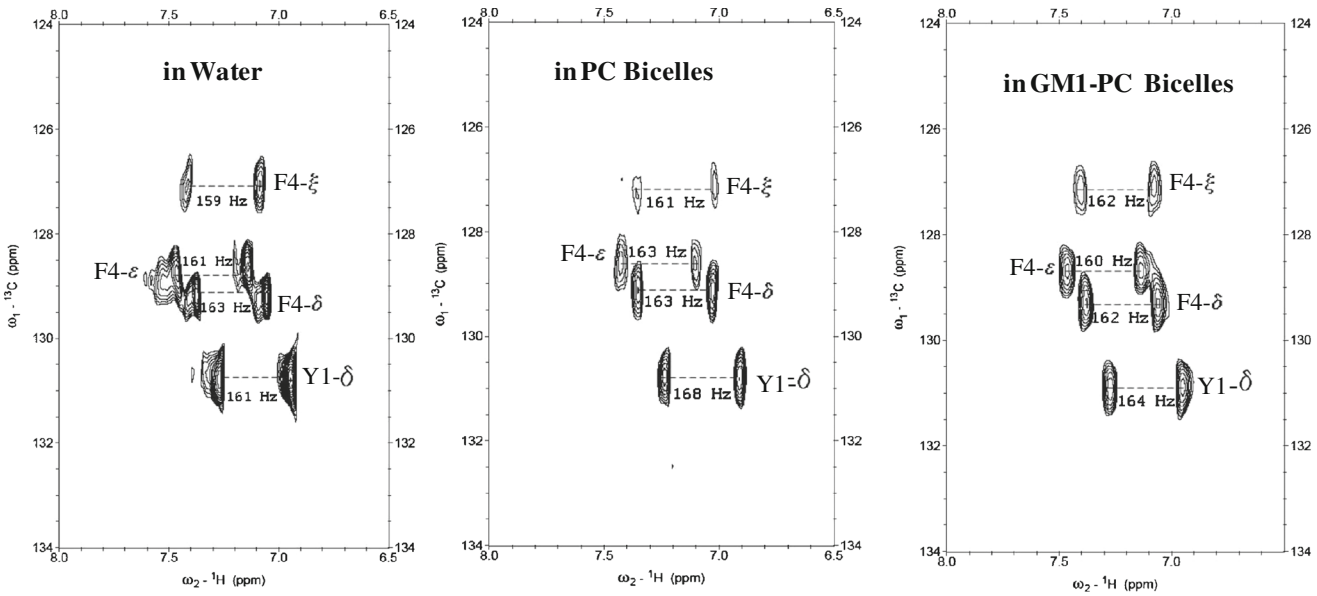

Fig. 4 F1-coupled [^{1}H-^{13}C]-HSQC spectrum showing aromatic region of peptide LENK associated to the PC bicelle and the GM1 containing PC bicelle. As can be seen, the magnitude of $^{1}J_{CH}$ splitting, when shifting from the water to the bicelles is quite increased or decreased, and for several $^{13}C^{\alpha}$-$^{1}H^{\alpha}$ pairs, the difference exceeds 10Hz (Table 1)

Table 1 ^{13}C-^{1}H coupling constants (in Hz) from HSQC

RESIDUE	13C-1H	water	PC bicelles	GM1 bicelles
Y1	Cα-Hα	140	143	149
	Cβ-Hβ	134	——	133
	Cδ-Hδ	164	164	163
	Cε-Hε	163	168	164
G2	Cα-Hα	153	140	142
G3	Cα-Hα	146	141	144
F4	Cα-Hα	143	145	146
	Cβ-Hβ1	132	139	140
	Cβ-Hβ2	112	103	82
	Cδ-Hδ	163	163	162
	Cξ-Hξ	159	161	162
	Cε-Hε	161	163	160
L5	Cα-Hα	150	146	142
	Cβ/γ-Hβ/γ	126	125	124
	Cδ-Hδ	123	125	120

Structure of the peptides in presence of GM1

Solution structure by circular dichroism (CD)

CD experiment gives the information about the secondary structure of the peptide induced by its surrounding environments [65]. The measure of circular dichroism (CD) is depicted as: $CD=\Delta A(\lambda)=A(\lambda)_L - A(\lambda)_R$, where λ is the wavelength at which absorption occur. CD spectroscopy is a spectroscopic technique where the CD of molecules is measured over a range of wavelengths. Due to high sensitivity and its ability to provide information about the secondary structures [65], CD is an excellent preliminary experiment that one can do prior to running more elaborate NMR experiments.

Solution structure by NMR

The solution NMR studies of membrane-bound peptides depend critically on the molecular weight of the complex, as well as on the dynamics of the peptide in the bound form [34, 66]. The overall tumbling of a protein or a peptide–lipid complex should not exceed a critical correlation time, implying that the complex cannot be too large [66]. To determine the structure geometrical constraints are collected from a set of NMR experiments. These include interproton distances, derived from cross-relaxation rates, measured through quantification of ROESY cross-peak intensities and dihedral angles derived from J-coupling measurements. XPLOR-NIH [67] is one such software package that calculates protein three-dimensional structure using the distance and dihedral restrains. Peptide structures in the presence of fast-tumbling bicelles have been reported [68–71], and it has been shown that peptides may adopt different conformation in the more disc-like bicelles as compared to in detergent micelles [68–71]. In our laboratory, we have successfully investigated conformational transition of several peptides from disordered state in aqueous solution to more ordered state in presence of GM1 micelle and GM1 containing bicelle. CD and NMR in conjugation with molecular modeling show that kinin peptide bradykinin is unstructured in water while it adopts well defined turn conformation in presence of GM1 micelle [56]. Opioid peptides leu-enkephelin and met- enkephalin are intrinsically disordered in water where as they adopt turn-like (bent) conformation in presence of ganglioside GM1 micelle [54]. The small flexible peptide Leucine-Enkephelin was found to switch flexibly between several conformations in GM1 containing bicelles which resemble μ- or δ-receptor selective conformers of enkephalin [57]. Another randomly structured peptide Substance

Table 3 Measured translational diffusion coefficients of peptides and bicellar components DMPC and CHAPS in solution using DOSY (pH 5.5 and 298 K). $D_{obs}\pm10(\times10^{-11}\ m^2/s)$

SAMPLE	DMPC[a]	CHAPS[a]	SP[a]	NT[b]	MLT[c]	BICELLE
Water			28.3	25	18.6	
PC-bicelle	10.5	11.4	17.0	17.2	15.2	9.5
PC-GM1 bicelle	8.9	11	14.4	14.7	13.4	8.6

[a,b,c] Refs 40, 42 and 41

Table 2 Pulsed-field gradient-stimulated-echo diffusion NMR results obtained for the interaction of peptides with GM1 micelle and interacting residues of the peptides with micelles (marked in red) are obtained from STD- NMR result

Peptides	D_{free}	D_{bound}	% Bound
Melittin (GIGAVLKVLTTGLPALISWIKRKRQQ)	2.4	1.9	32.4
Bradykinin (RPPGFSPFR)	3.5	2.8	27.3
Leu-Enkephalin (YGGFL)	2.73	1.31	77.7
Met-Enkephalin (YGGFM)	2.48	0.986	94.9

Diffusion Coefficients are in $10^{-10}\ m^2/s$ [36, 38, 39]

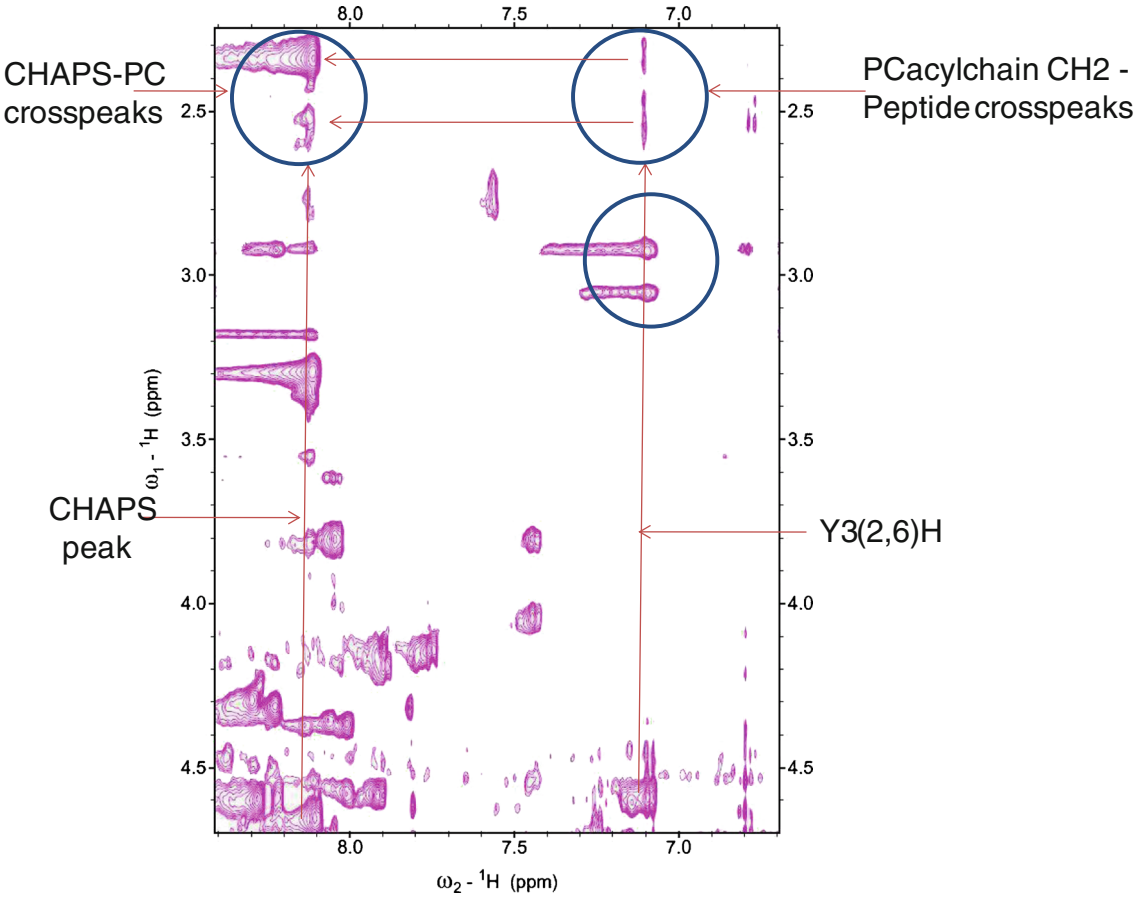

Fig. 5 2D ^{1}H–^{1}H ROESY region showing the cross peaks of Neurotensin aromatic protons with lipid acyl chain –CH2– protons of DMPC in DMPC:CHAPS:GM1 bicelles (1:4:0.3) at pH 5.5 and 298 K (peptide:lipid 1:20 mol/mol)

P, which is a neuromodulator, in GM1-containing bicelles was found to adopt its biologically active extended 3_{10} helical conformation, which is recognized by Neurokinin-1 receptor (NK_1R) where as in GM1/CHAPS mixed micelle the peptide adopt turn conformation [58]. Intrinsically disordered neuropeptide Neurotensin (found in central and peripheral nervous systems as well as gastrointestinal systems) was also revealed to have its receptor NTS-3 (sortilin)-recognized conformation in GM1 containing bicelles [59].

A very recent report has shown that the hydrophobic branching site of the carbohydrate chain of mono-sialo gangliosides GM1 and GM2 act as determining factor in the interaction with disordered peptides/proteins [72]. Chemical shift changes of the unstructured N-terminal segment (1–30) of α-synuclein are exhibited upon interaction with the GM1 bicelles, but the conformation was less ordered compared to α-helical conformation previously reported in ganglioside micelles or ganglioside-embedded vesicles. We have reported that in presence of binary PC bicelles melittin adopts fully α-helical structure where as in GM1 containing PC bicelles melittin adopt partially folded conformation [59].

According to a report of Fujitani *et al.* [73] peptide, p3 shows a structural alteration in going from water to GM1 micelles. The reported solution structure shows that p3 peptide in water exists in two conformers due to exchange of cis and trans forms at Pro (7)-Pro (8) linkage. In presence of GM1 micelle p3 peptide exists only in trans conformer. Martinez *et al.* [74] examined the interactions of alpha-synuclein with several brain sphingolipids. They found that alpha-synuclein specifically binds to ganglioside small unilamellar vesicles (SUVs) containing GM1. Depending on the amount of GM1 present, alpha-helical structure is induced leading to inhibition as well as loss of alpha-synuclein fibril formation. Alzheimer's disease (AD) is triggered by the deposition of amyloid beta-protein (Aβ) in brain. Yamamoto *et al.* [75] suggested that the age-dependent high-density GM1 clustering at presynaptic neuritic terminals is a critical step for Aβ deposition in AD. GM1 clusters interact with unstructured amyloid beta monomer result in the formation of β-sheet structure, which is then deposited in brain. Recently we have seen by molecular dynamics simulation [76] that ganglioside GM1 promotes the structural conversion of amyloid beta-protein and increase the rate of peptide aggregation.

Peptide location in a bilayer

A number of biophysical techniques can be used for studying the orientation and location of membrane-bound peptides [77]. These methods are mainly based on magnetic resonance spectroscopy (solid state and liquid state NMR, measurements of ROEs/NOEs between peptides and lipids) and EPR [77]. Solution-state NMR offers several possibilities to monitor the location of peptides and proteins in a membrane mimetic, although these methods are not as direct as the ones used in solid-state NMR [33, 35]. Other spectroscopic techniques are fluorescence, IR, oriented CD and alternative approaches like colorimetry, Quartz crystal microbalance and interface-sensitive X-ray and neutron scattering are also frequently used [77].

Steady-state fluorescence and quenching

We measured the intrinsic tryptophan fluorescence of melittin (MLT) in water, in the presence of PC bicelles and in the presence of GM1 containing PC bicelles to evaluate the degree of penetration of the peptide into the membrane bilayer [59]. Blue spectral shift is indicative of a more hydrophobic environment and is larger in case of PC bicelles than that of GM1 containing bicelles. This result indicates that melittin undergoes deeper insertion in the hydrophobic core of the membrane in PC bicelles than that of GM1 containing PC bicelles [59]. We have studied the accessibility of the tryptophan residue of membrane bound peptide towards acrylamide, a water-soluble highly efficient quenching molecule, which is unable to penetrate into the hydrophobic core of the lipid bilayer [59]. The more deeply a tryptophan residue is buried, the less strongly it can be quenched by acrylamide. K_{SV} value is lowest for melittin in binary PC bicelle than that of GM1 containing PC bicelle indicating a much deeper insertion of the peptide within bilayer in absence of GM1. The more superficial localization of melittin in ganglioside containing PC bicelles than that of control PC bicelles can be explained as follows:

It has been observed by AFM and molecular modeling that head group of ganglioside GM1 protrudes out (1 nm) of the phospholipid head group [42]. It may be expected that highly positive melittin (+5) will be attracted by the negative charge containing sialic acid of ganglioside and hence melittin will remain closer to the head group region of gangliosides [which is also confirmed from its relatively low % bound conformation Table 2]. Recently Miyazaki *et al.* [13] have reported that antimicrobial peptide Magainin2 specifically binds to the head-group oligosaccharide region of GM1 and remains far above the hydrophobic region. Consequently pore formation by Magainin 2 becomes much less effective in GM1/PC bilayer rather than only PC bilayer.

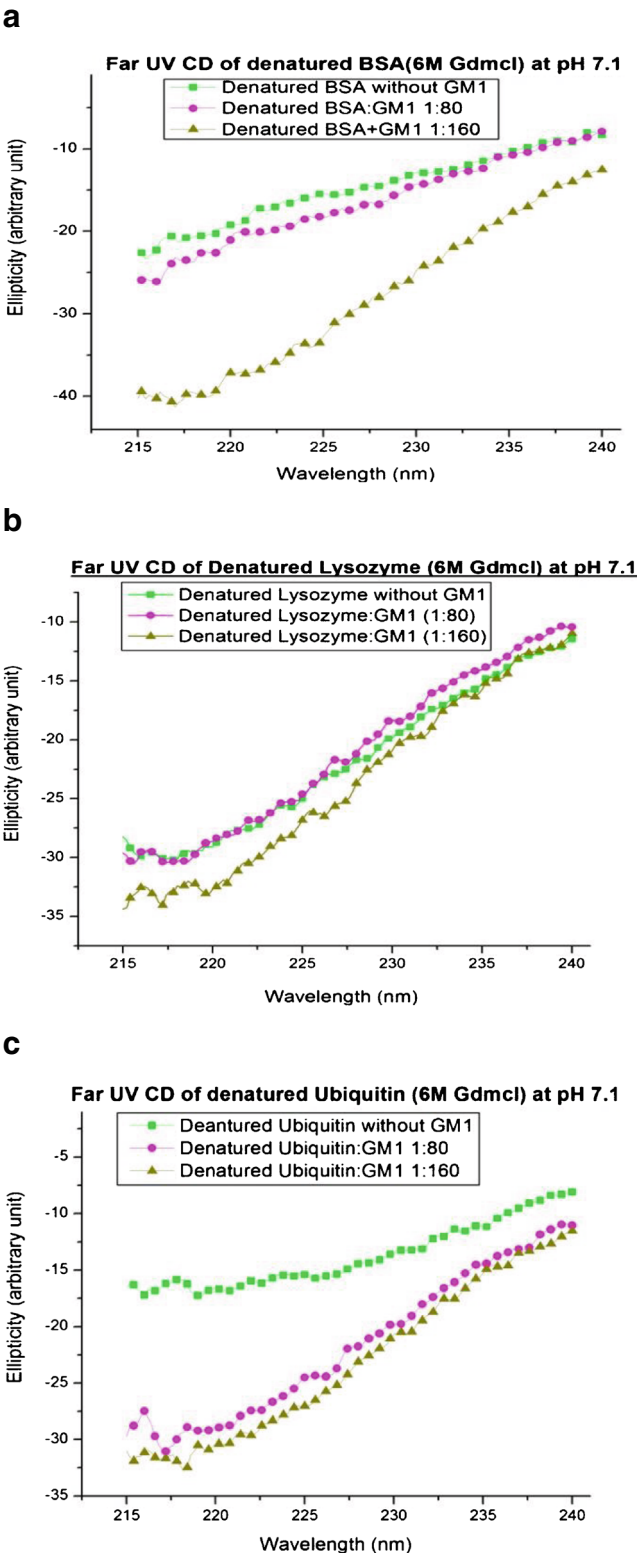

Fig. 6 Circular dichroism spectra of BSA, Lysozyme and Ubiquitin, all denatured by 6 m GdmCl in presence of GM1 micelles

Peptide–lipid ROEs

As mentioned earlier in section 3.6, direct evidence of the location of a peptide in bilayer or micelle can be obtained from a ROESY experiment. It is seen in Fig. 5 that in the aromatic region of ROESY spectra of Neurotensin in GM1 containing bicelles strong cross peaks exist between Y3 (2,6) (7.13 ppm) and DMPC acyl chain protons (~2.3 ppm, ~2.5 ppm and ~3 ppm) [58]. This type of ROESY cross peak was absent in PC bicelle. Similar result was obtained with Substance P [57]. This result demonstrates that insertion of the NT/SP within the hydrophobic core of the membrane and deeper insertion of peptides is achieved in GM1 containing bicelles. Opposite is the case observed for melittin [59]. For melittin, cross peaks are observed between amide hydrogen of peptide and DMPC acyl chain H2 in PC bicelles, which is absent in GM1 containing bicelles indicating deeper insertion of the peptide in absence of GM1. Hence the location of melittin is consistent with our steady-state fluorescence, quenching result *i.e.*, probable location of melittin in GM1 containing bicelles is near the head group of GM1.

Interaction of denatured proteins with GM1 Micelles

We have observed that GM1 can induce structure into unstructured peptides like melittin, substance P, neurotensin, *etc*. It is likely that since the head-group of gangliosides protrude outside the cell membrane and they have strategic positions to interact with both native and denatured proteins. We have studied the interaction of GM1 micelles with native and denatured forms of different proteins. Ubiquitin, BSA and lysozyme are proteins of different lengths and different classes *i.e.*, partially denatured lysozyme at pH 1.5 shown to have more secondary structure components in the presence of increasing GM1 micelle concentration. Interactions of heat denatured BSA [78], gdmcl denatured BSA, lysozyme as well as ubiquitin all had (Fig. 6) shown that in presence of GM1 micelles retain their secondary structure to a large extent.

Conclusion

Thus it has been observed that solution state NMR including DOSY, STD NMR, PFG-SE as well as fluorescence and CD– all these biophysical techniques can collectively explain the interaction, structure, location and dynamics of small peptides and proteins within GM1 containing membranes very well. It is clear that ganglioside (which is a crucial component of nerve cell membrane and also present in all vertebrate cells) has a capability in modulating the interaction, structure, location and dynamics of different classes of peptides and proteins. Use of GM1- containing membranes which are brain-membrane mimics that induce biologically relevant conformation not only in the disordered peptides but also in the randomly structure peptides like melittin and denatured proteins. All these peptides do not interact with ganglioside in the same way, or to the same extent, some appear to interact tightly while others adapt to different membrane conditions. Thus the combined studies of interaction, structure, dynamics and location in a model ganglioside containing bilayer provides us with an understanding of how they may act on real membranes.

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References

1. van Meer, G., Voelker, D.R., Feigenson, G.W.: Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol. **9**, 112–124 (2008)
2. Sandhoff, K., Harzer, K.: Gangliosides and gangliosidoses: principles of molecular and metabolic pathogenesis. J. Neurosci. **33**, 10195–10208 (2013)
3. Svennerholm, S.: The Gangliosides. J. Lipid. Res **5**, 145–155 (1964)
4. Yu, R.K., Tsai, Y.-T., Ariga, T., Yanagisawa, M.: Structures, biosynthesis, and functions of gangliosides—an overview. J. Olec. Sci. **60**(537–544) (2011)
5. Brocca, P., Berthault, P., Sonnino, S.: Conformation of the Oligosaccharide Chain of GM1 Ganglioside in a Carbohydrate-Enriched Surface. Biophys. J. **74**, 309–318 (1998)
6. Patel, R.Y., Balaji, P.V.: Characterization of the conformational and orientational dynamics of ganglioside GM1 in a dipalmitoylphosphatidylcholine bilayer by molecular dynamics simulations. Biochim. Biophys. Acta **1768**, 1628–1640 (2007)
7. Lopez, P.H., Schnaar, R.L.: Gangliosides in cell recognition and membrane protein regulation. Curr. Opin. Struct. Biol. **19**, 549–557 (2009)
8. Seo, J.H., Kim, C.S., Cha, H.J.: Structural evaluation of GM1-related carbohydrate–cholera toxin interactions through surface plasmon resonance kinetic analysis. Analyst **138**, 6924–6929 (2013)
9. Kielczynski, W., Harrison, L.C., Leedman, P.J.: Direct evidence that ganglioside is an integral component of the thyrotropin receptor. Proc. Natl. Acid. Sci. USA **88**, 1991–1995 (1991)
10. Markwell, M.A., Svennerholm, L., Paulson, J.C.: Specific gangliosides function as host cell receptors for Sendai virus. Proc. Natl. Acid. Sci. USA **78**, 5406–5410 (1981)
11. Caron, M., Joubert-Caron, R., Cartier, J.R., Chadli, A., Bladier, D.: Study of lectin-ganglioside interactions by high-performance liquid affinity chromatography. J. Chromatogr. A **646**, 327–333 (1993)
12. Fishmann, P.H.: Role of membrane gangliosides in the binding and action of bacterial toxins. J. Membr. Biol. **69**, 85–97 (1982)
13. Miyazaki, Y., Aoki, M., Yano, Y., Matsuzaki, K.: Interaction of antimicrobial peptide Magainin 2 with gangliosides as a target for human cell binding. Biochemistry **51**, 10229–10235 (2012)
14. Deng, W., Li, R., Ladisch, S.: Influence of cellular ganglioside depletion on tumor formation. J. Natl. Cancer Inst. **92**, 912–917 (2000)
15. Doria, G., Tettamanti, G.: Gangliosides and neurological diseases. Brit. Med. J. **306**, 204–205 (1993)

16. Behan, P.O., Haniffah, B.A.G.: Neurological disorders: GM1 ganglioside improves motor function. Nat. Rev. Drug Discov **11**, 274 (2012)
17. Sonnino, S., Mauri, L., Chigorno, V., Prinetti, A.: Gangliosides as components of lipid membrane domains. Glycobiology **17**, 1R–13R (2006)
18. Park, J.Y., Kim, K.S., Lee, K.B., Ryu, J.S., Chung, C.H., Choo, Y.K., Jou, I., Kim, J., Park, S.M.: On the mechanism of internalization of α-synuclein into microglia: roles of ganglioside GM1 and lipid raft. J. Neurochem. **110**, 400–411 (2009)
19. Masserini, M., Palestini, P., Freire, E.: Influence of glycolipid oligosaccharide and long-chain base composition on the thermotropic properties of dipalmitoylphosphatidylcholine large unilamellar vesicles containing gangliosides. Biochemistry **28**, 5029–34 (1989)
20. Ferraretto, A., Pitto, M., Palestini, P., Masserini, M.: Lipid domains in the membrane: thermotropic properties of sphingomyelin vesicles containing GM1 ganglioside and cholesterol. Biochemistry **36**, 9232–9236 (1997)
21. Seddon, A.M., Curnow, P., Booth, P.J.: Membrane proteins, lipids and detergents: not just a soap opera. Biochim. Biophys. Acta **1666**, 105–117 (2004)
22. Tanford, C.: The hydrophobic effect: formation of micelles and biological membranes, p. 233. Wiley-Interscience, New York (1980)
23. Mukerjee, P., Mysels, K.J.: Critical micelle concentrations in aqueous surfactant systems. NSRDS-NBS 36, Washington, DC (1971)
24. Fernández, C., Wüthrich, K.: NMR solution structure determination of membrane proteins reconstituted in detergent micelles. FEBS Lett. **555**, 144–150 (2003)
25. Cantu, L., Corti, M., Acquotti, D., Sonnino, S.: Aggregation properties of gangliosides: influence of the primary and secondary structure of the headgroup. J. Phys. IV **3**, 57–64 (1993)
26. Cantu, L., Corti, M., Zemb, T., Willams, C.: Small angle X-ray and neutron scattering from ganglioside micellar solutions. J. Phys. IV **3**, 221–227 (1993)
27. Brocca, P., Cantù, L., Sonnino, S.: Aggregation properties of semisynthetic GD1a ganglioside ($IV^3Neu5AcII^3Neu5AcGgOse_4Cer$) containing an acetyl group as acyl moiety. Chem. Phys. Lipids **77**, 41–49 (1995)
28. Cantù, L., Corti, M., Sonnino, S., Tettamanti, G.: Evidence for spontaneous segregation phenomena in mixed micelles of gangliosides. Chem. Phys. Lipids **55**, 223–229 (1990)
29. Sonnino, S., Cantù, L., Corti, M., Acquotti, D., Venerando, B.: Aggregative properties of gangliosides in solution. Chem. Phys. Lipids **71**, 21–45 (1994)
30. Dürr, U.H.N., Soong, R., Ramamoorthy, A.: When detergent meets bilayer: birth and coming of age of lipid bicelles. Prog. Nucl. Magn. Reson. Spectrosc. **69**, 1–22 (2013)
31. Andersson, A., Biverståhl, H., Nordin, J., Danielsson, J., Lindahl, E., Mäler, L.: The membrane-induced structure of melittin is correlated with the fluidity of the lipids. Biochim. Biophys. Acta **1768**, 115–121 (2007)
32. Suzuki, Y., Buer, B.C., Al-Hashimi, H.M., Marsh, E.N.G.: Using fluorine nuclear magnetic resonance to probe changes in the structure and dynamics of membrane-active peptides interacting with lipid bilayers. Biochemistry **50**, 5979–5987 (2011)
33. De Angelis, A.A., Opella, S.J.: Bicelle samples for solid-state NMR of membrane proteins. Nat. Protoc. **2**, 2332–2338 (2007)
34. Glover, K.J., Whiles, J.A., Wu, G., Yu, N.-J., Deems, R., Struppe, J.O., Stark, R.E., Komives, E.A., Vold, R.R.: Structural Evaluation of Phospholipid Bicelles for Solution-State Studies of Membrane-Associated Biomolecules. Biophys. J **81**, 2163–2171 (2001)
35. Diller, A., Loudet, C., Aussenac, F., Raffard, G., Fournier, S., Laguerre, M., Grelard, A., Opella, S.J., Marassi, F.M., Dufourc, E.J.: Bicelles: a natural 'molecular goniometer' for structural, dynamical and topological studies of molecules in membranes. Biochimie **91**, 744–751 (2009)
36. Durr, U.H.N., Gildenberg, M., Ramamoorthy, A.: The magic of bicelles lights Up membrane protein structure. Chem. Rev. **112**, 6054–6074 (2012)
37. Marcotte, I., Auger, M.: Bicelles as model membranes for solid- and solution-state NMR studies of membrane peptides and proteins. Concepts Magn. Reson. **24A**, 17–37 (2005)
38. Rubio, L., Alonso, C., Rodríguez, G., Cócera, M., López-Iglesias, C., Coderch, L., De la Maza, A., Parra, J.L., López, O.: Bicellar systems as new delivery strategy for topical application of flufenamic acid. Int. J. Pharm. **444**, 60–9 (2013)
39. Ohvo-Rekila, H., Ramstedt, B., Leppimaki, P., Slotte, J.P.: Cholesterol interactions with phospholipids in membranes. Prog. Lipid Res. **41**, 66–97 (2002)
40. Cantu, L., Corti, M., Brocca, P., Del Favero, E.: Structural aspects of ganglioside-containing membranes. Biochim. Biophys. Acta **1788**, 202–208 (2009)
41. Gayen, A., Mukhopadhyay, C.: Evidence for effect of GM1 on opioid peptide conformation: NMR study on leucine enkephalin in ganglioside-containing isotropic phospholipid bicelles. Langmuir **24**, 5422–5432 (2008)
42. Khatun, U.L., Gayen, A., Mukhopadhyay, C.: Gangliosides containing different numbers of sialic acids affect the morphology and structural organization of isotropic phospholipid bicelles. Chem. Phys. Lipids **170–171**, 8–18 (2013)
43. Yuan, C., Johnston, L.J.: Distribution of ganglioside GM1 in L-alpha-dipalmitoylphosphatidylcholine/cholesterol monolayers: a model for lipid rafts. Biophys. J. **79**, 2768–2781 (2000)
44. Weng, K.C., Kanter, J.L., Robinson, W.H., Frank, C.W.: Fluid supported lipid bilayers containing monosialoganglioside GM1: a QCM-D and FRAP study. Colloids Surf. B Biointerfaces **50**, 76–84 (2006)
45. Ravichandra, B., Joshi, P.G.: Gangliosides asymmetrically alter the membrane order in cultured PC-12 cells. Biophys. Chem. **76**, 117–32 (1999)
46. Lee, A.G.: Lipid–protein interactions in biological membranes: a structural perspective. Biochim. Biophys. Acta **1612**, 1–40 (2003)
47. Protein-Lipid Interactions: New Approaches and Emerging Concepts (Springer Series in Biophysics) C. Reyes Mateo, Javier Gómez, José Villalaín, José Manuel González Ros (Eds.)
48. Zhao, H., Lappalainen, P.: A simple guide to biochemical approaches for analyzing protein–lipid interactions. Mol. Biol. Cell **23**, 2823–2830 (2012)
49. Chatterjee, C., Mukhopadhyay, C.: Conformational alteration of bradykinin in presence of GM1 micelle. Biochem. Biophys. Res. Commun. **315**, 866–871 (2004)
50. Chatterjee, C., Mukhopadhyay, C.: Melittin–GM1 Interaction: A Model for a Side-by-Side Complex. Biochem. Biophys. Res. Commun. **292**, 579–585 (2002)
51. Matsubara, T., Iijima, K., Nakamura, M., Taki, T., Okahata, Y., Sato, T.: Specific binding of GM1-binding peptides to high-density GM1 in lipid membranes. Langmuir **23**, 708–714 (2007)
52. Mäler, L.: Solution NMR studies of cell-penetrating peptides in model membrane systems. Adv. Drug Deliv. Rev. **65**, 1002–1011 (2013)
53. Chatterjee, C., Mukhopadhyay, C.: Structural alterations of enkephalins in the presence of GM1 ganglioside micelles. Biopolymers **70**, 512–521 (2003)
54. Chatterjee, C., Majumder, B., Mukhopadhyay, C.: Pulsed-field gradient and saturation transfer difference NMR study of enkephalins in the ganglioside GM1 micelle. J. Phys. Chem. B. **108**, 7430–7436 (2004)
55. Chatterjee, C., Mukhopadhyay, C.: Binding and Folding of Melittin in the Presence of Ganglioside GM1 Micelles. J. Biomol. Struct. Dyn. **23**, 183–92 (2005)
56. Chatterjee, C., Mukhopadhyay, C.: Interaction and structural study of kinin peptide bradykinin and ganglioside monosialylated 1 micelle. Biopolymers **78**, 197–205 (2005)

57. Gayen, A., Goswami, S.K., Mukhopadhyay, C.: NMR evidence of GM1-induced conformational change of substance P using isotropic bicelles. Biochim. Biophys. Acta. **1808**, 127–139 (2011)
58. Khatun, U.L., Goswami, S.K., Mukhopadhyay, C.: Modulation of the neurotensin solution structure in the presence of ganglioside GM1 bicelle. Biophys. Chem. **168–169**, 48–59 (2012)
59. Khatun, U.L., Mukhopadhyay, C.: Interaction of bee venom toxin melittin with ganglioside GM1 bicelle. Biophys. Chem. **180–181**, 66–75 (2013)
60. Viegas, A., Manso, J., Nobrega, F.L., Cabrita, E.J.: Saturation-transfer difference (STD) NMR: a simple and fast method for ligand screening and characterization of protein binding. J. Chem. Educ. **88**, 990–994 (2011)
61. Sørland, G.H., Aksnes, D., Gjerdåker, L.: A pulsed field gradient spin-echo method for diffusion measurements in the presence of internal gradients. J. Magn. Reson. **137**, 397–401 (1999)
62. Malliavin, T.E., Louis, V., Delsuc, M.A.: The DOSY experiment provides insights into the protegrin-lipid interaction. J. Chim. Phys. **95**, 178–186 (1998)
63. Morris, G. A.: Diffusion-Ordered Spectroscopy. In: Grant, D. M. and Harris, R. K. (eds.) Encyclopedia of Magnetic Resonance, Supplementary Volume. John Wiley and Sons (2002)
64. Johnson Jr., C.S.: Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. Prog. Nucl. Magn. Reson. Spectrosc. **34**, 203–256 (1999)
65. Kobayashi, N.; Muranaka, A.: Circular Dichroism and Magnetic Circular Dichroism Spectroscopy for Organic Chemists. Royal Society of Chemistry (2012)
66. Andersson, A., Maler, L.: NMR solution structure and dynamics of motilin in isotropic phospholipid bicellar solution. J. Biomol. NMR **24**, 103–112 (2002)
67. Schwieters, C.D., Kuszewski, J.J., Tjandra, N., Clore, G.M.: The Xplor-NIH NMR molecular structure determination package. J. Magn. Reson. **160**, 65–73 (2003)
68. Whiles, J.A., Brasseur, R., Glover, K.J., Melacini, G., Komives, E.A., Vold, R.R.: Orientation and effects of mastoparan X on phospholipid bicelles. Biophys. J. **80**, 280–293 (2001)
69. Bárány-Wallje, E., Andersson, A., Gräslund, A., Mäler, L.: NMR solution structure and position of transportan in neutral phospholipid bicelles. FEBS Lett. **567**, 265–269 (2004)
70. Poget, S.F., Cahill, S.M., Girvin, M.E.: Isotropic bicelles stabilize the functional form of a small multidrug-resistance pump for NMR structural studies. J. Am. Chem. Soc. **129**, 2432–2433 (2007)
71. Poget, S.F., Girvin, M.E.: Solution NMR of membrane proteins in bilayer mimics: small is beautiful, but sometimes bigger is better. Biochim. Biophys. Acta **1768**, 3098–3106 (2007)
72. Yamaguchi, T., Uno, T., Uekusa, Y., Yagi-Utsumi, M., Kato, K.: Ganglioside-embedding small bicelles for probing membrane-landing processes of intrinsically disordered proteins. Chem. Comm. **49**, 1235–1237 (2013)
73. Fujitani, N., Shimizu, H., Matsubara, T., Ohta, T., Komata, Y., Miura, N., Sato, T., Nishimura, S.-I.: Structural transition of a 15 amino acid residue peptide induced by GM1. Carbohydr. Res. **342**, 1895–1903 (2007)
74. Martinez, Z., Zhu, M., Han, S., Fink, A.L.: GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. Biochemistry **46**, 1868–77 (2007)
75. Yamamoto, N., Matsubara, T., Sato, T., Yanagisawa, K.: Age-dependent high-density clustering of GM1 ganglioside at pre-synaptic neuritic terminals promotes amyloid β-protein fibrillogenesis. Biochim. Biophys. Acta **1778**, 2717–2726 (2008)
76. Manna, M., Mukhopadhyay, C.: Binding, conformational transition and dimerization of amyloid-β peptide on GM1-containing ternary membrane: insights from molecular dynamics simulation. PLoS ONE **8**, 1–13 (2013)
77. Hohlweg, W., Kosol, S., Zangger, K.: Determining the orientation and localization of membrane-bound peptides. Curr. Protein Pept. Sci. **13**, 267–279 (2012)
78. Gayen, A., Chatterjee, C., Mukhopadhyay, C.: GM1-induced structural changes of bovine serum albumin after chemical and thermal disruption of the secondary structure: a spectroscopic comparison. Biomacromolecules **9**, 974–983 (2008)