# Chapter 14
# Conformational Dynamics of Oligosaccharides Characterized by Paramagnetism-Assisted NMR Spectroscopy in Conjunction with Molecular Dynamics Simulation

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## Abbreviations

| | |
|---|---|
| Aβ | Amyloid β |
| HSQC | Heteronuclear single-quantum coherence |
| MD | Molecular dynamics |
| NMR | Nuclear magnetic resonance |
| NOE | Nuclear Overhauser effect |

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PCS Pseudocontact shift
REMD Replica-exchange molecular dynamics
αSN α-Synuclein

## Introduction: Static and Dynamic Views of Carbohydrate–Protein Interactions

The biological functions of oligosaccharides predominantly occur through their interactions with proteins (Drickamer and Taylor 1993; Sharon and Lis 2003). In cells, a series of high-mannose-type oligosaccharides present on secretory proteins serve as quality tags, which are decoded by intracellular lectins involved in the folding, transport, and degradation of glycoproteins (Kamiya et al. 2012). On the cell surface, oligosaccharides that modify membrane proteins and lipids act as acceptors for extracellular proteins, thereby mediating a variety of physiological and pathological events involved in cellular communication and development, cancer metastasis, viral infections, and the promotion of neurodegenerative disorders (Ernst and Magnani 2009). In order to elucidate the mechanisms underlying these oligosaccharide functions, an in-depth description of carbohydrate–protein interactions is essential, which will provide important clues for future therapeutic approaches (Kamiya et al. 2011a).

One of the most powerful means of characterizing carbohydrate–protein interactions is X-ray crystallography, which can provide the atomic coordinates of biomacromolecules and their complexes. For example, we recently determined the crystal

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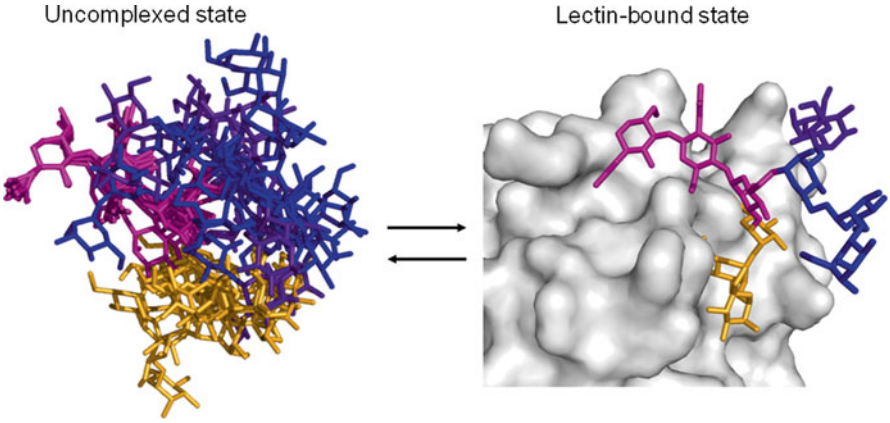


**Fig. 14.1** Static and dynamic views of carbohydrate–protein interactions. 3D-structure models of the high-mannose-type oligosaccharide $Man_9GlcNAc_2$ exhibiting conformational dynamics in solution (*left*) and accommodated in the sugar-binding pocket of ERGIC-53 (*right*)

structures of ternary complexes composed of the carbohydrate-recognition domain of ERGIC-53, its binding partner MCFD2, and α1,2 mannotriose (Satoh et al. 2014). This allowed us to build a 3D-structural model of the complex formed between this lectin and a high-mannose-type oligosaccharide $Man_9GlcNAc_2$, which visualizes atomic contacts mediating the carbohydrate–protein interaction (Fig. 14.1). This model demonstrates how the carbohydrate-binding pocket of ERGIC-53 accommodates the non-reducing terminal residues of the cognate ligand, thereby explaining the structural basis of the sugar-binding specificities of this lectin in comparison to its homologs. However, it should be noted that the sugar chains are highly flexible and mobile, at least in their uncomplexed forms (Fig. 14.1). This property endows the oligosaccharides with conformational adaptabilities upon interaction with proteins resulting in a loss of conformational entropy. For quantitative evaluation of the energetics of the carbohydrate–protein interactions, it is crucial to understand such oligosaccharide conformational dynamics. Nuclear magnetic resonance (NMR) spectroscopy has a powerful potential for characterizing the conformational dynamics of oligosaccharides in solution. However, several significant issues need to be addressed when applying NMR spectroscopy for detailed analyses of the dynamic conformation of oligosaccharides, as described below.

## How to Prepare NMR Samples

First, it is necessary to obtain sufficient quantities of homogeneous oligosaccharides. Here we primarily used the pentasaccharide moiety of gagnglioside GM1, Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc, as a model molecule because this glycosphingolipid is abundant in animal brains and commercially available as a source of

the homogeneous oligosaccharide. In general, however, glycoprotein glycans are structurally heterogeneous and much less abundant. Sophisticated synthetic approaches have proved useful in providing large quantities of oligosaccharides with complicated branched structures (Ando et al. 2010; Lepenies et al. 2010; Takeda et al. 2009; Wang et al. 2013). Another promising approach has been the use of eukaryotic glycoprotein expression systems along with genetic engineering (Kamiya et al. 2014). We used *Saccharomyces cerevisiae* strains with deletion of genes associated with *N*-glycan processing in the secretory pathway, for producing glycoproteins with homogeneous glycoforms (Fig. 14.2a) (Kamiya et al. 2011b, 2013; Nakanishi-Shindo et al. 1993). From the engineered yeast cells, high yields of specific high-mannose-type oligosaccharides could be isolated. Key advantages of this technique include the efficient production of stable-isotope-labeled oligosaccharides using yeast metabolic labeling, which is extremely useful for detailed NMR analyses (Fig. 14.2b, c).

## How to Obtain Atomic Long-Distance Information

NMR determination of biomolecular conformations are typically performed on the basis of the nuclear Overhauser effect (NOE) and scalar coupling (or *J*-coupling) (Peters and Pinto 1996; Wormald et al. 2002; Zhao et al. 2007). However, the major limitation of NMR analyses of oligosaccharides is the insufficiency of conformational restraints provided by these local conformational parameters because of the low number of protons that contribute to defining inter-glycosidic linkage conformation. To overcome this problem, we applied paramagnetic effects that can provide the long-distance information of oligosaccharides (Fig. 14.3) (Zhang 2014; Zhang et al. 2013). In this approach, lanthanide ions with unpaired electrons are attached to the reducing terminal of the oligosaccharide using a metal chelator (Yamamoto et al. 2011, 2012; Zhang et al. 2012). The magnetic dipole–dipole interaction of unpaired electrons with their spatially proximal nuclear spins perturb the chemical shifts and relaxation observed in NMR spectra of the tagged oligosaccharides, thus reflecting the geometrical arrangements of the individual $^{1}H$ and $^{13}C$ with respect to the paramagnetic probe (Luchinat and Parigi 2007). Figure 14.4 shows the method of introduction of a lanthanide ion at the reducing end of an oligosaccharide: The pentasaccharide moiety of GM1 was enzymatically cleaved from the ceramide part and chemically connected with a phenylenediamine-derived lanthanide-chelating group. Upon coordination of paramagnetic lanthanide ions, such as $Tm^{3+}$, spectral changes were induced due to pseudocontact shifts (PCSs), in comparison with the reference spectrum observed with a diamagnetic $La^{3+}$ ion as the source of the atomic long-distance information (Fig. 14.5). We also used reducing-terminal spin labeling with a nitroxide radical, which accelerates NMR relaxation of nuclei in its spatial proximity, for characterizing overall conformation of high-mannose-type oligosaccharides (Yamaguchi et al. 2013a).

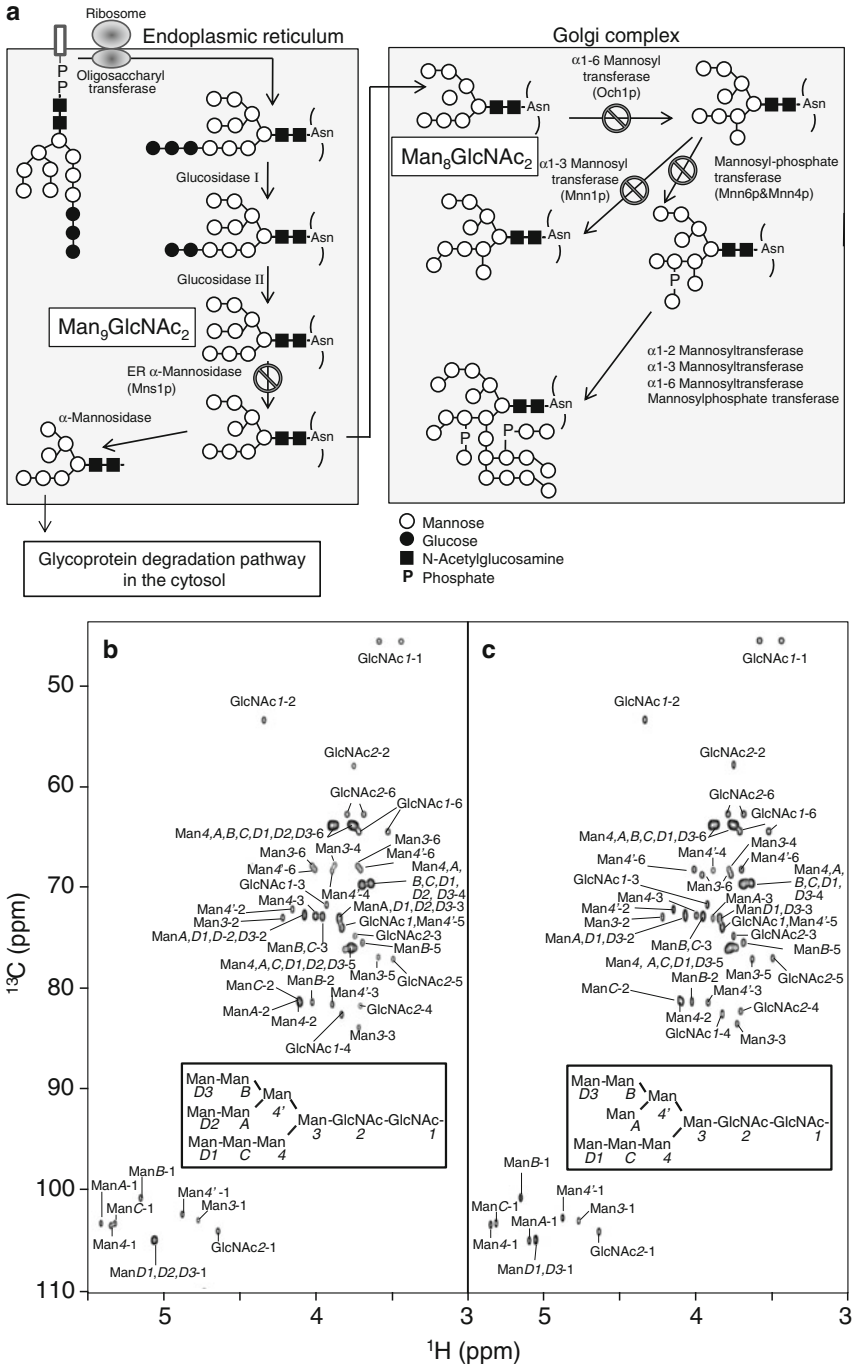


**Fig. 14.2** (**a**) Genetically engineered yeast cells for the overexpression of homogeneous high-mannose-type oligosaccharides $Man_9GlcNAc_2$ and $Man_8GlcNAc_2$. $^1H$-$^{13}C$ HSQC spectra of uniformly $^{13}C$-labeled (**b**) $Man_9GlcNAc_2$ and (**c**) $Man_8GlcNAc_2$, respectively, produced in the yeast cells. These figures were adapted from Kamiya et al. (2011b, 2013) with the permissions of Springer and MDPI

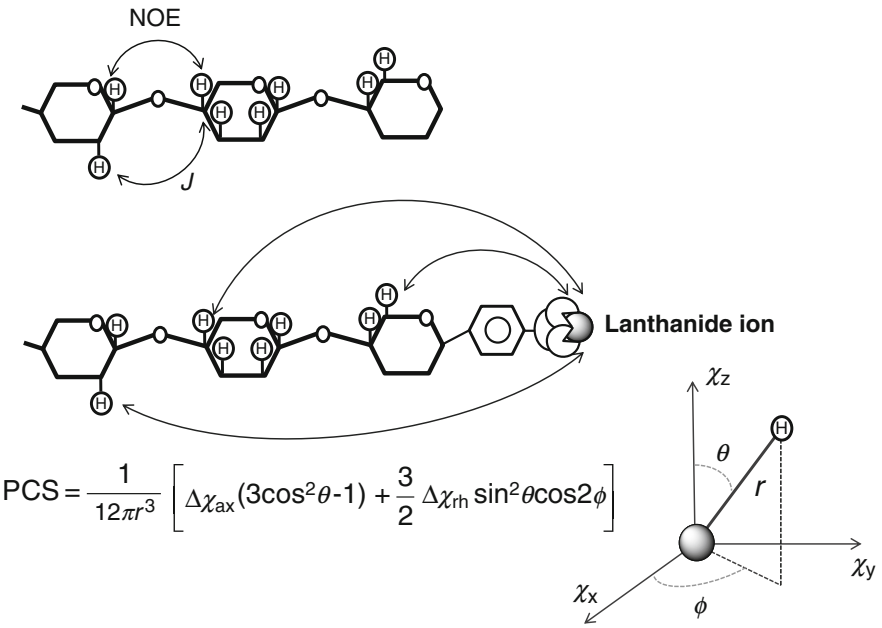


**Fig. 14.3** Conformational restraints provided by NOE, *J*, and PCS for characterizing glycosidic linkage conformation. In the equation for PCS, $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$ are the axial and rhombic components, respectively, of the anisotropic magnetic susceptibility ($\boldsymbol{\Delta\chi}$) tensor, and $r$, $\theta$, and $\varphi$ are the spherical coordinates of the nucleus defined with respect to the paramagnetic center and the principal axis of the $\boldsymbol{\Delta\chi}$ tensor

## How to Deal with Dynamic Conformational Ensembles

Structural information provided by NMR is averaged over dynamic conformational ensembles because conformational transitions of the free oligosaccharides occur very rapidly on the NMR time scale. By contrast, computational approaches including molecular dynamics (MD) simulation can describe the molecular behavior of an oligosaccharide in solution at the atomic level. However, the simulation results heavily depend on calculation protocols such as force field, initial state, and simulation time, and therefore have to be validated on the basis of experimental observations. We combined the paramagnetism-assisted NMR methods with MD simulation to explore the conformational space occupied by a flexible oligosaccharide in solution (Fig. 14.6) (Zhang 2014; Zhang et al. 2013; Yamaguchi et al. 2014). Here, we show the results obtained through this methodology using a series of ganglioside oligosaccharides, the GM3 trisaccharide, GM2 tetrasaccharide, and GM1 pentasaccharide (Yamamoto et al. 2012; Zhang 2014; Zhang et al. 2012).

To explore the conformational spaces of the carbohydarate moieties of GM3 and GM2, ten MD runs were performed for each oligosaccharide in explicit water with GLYCAM_06 force field for 12 ns at 300 K and combined after excluding the first

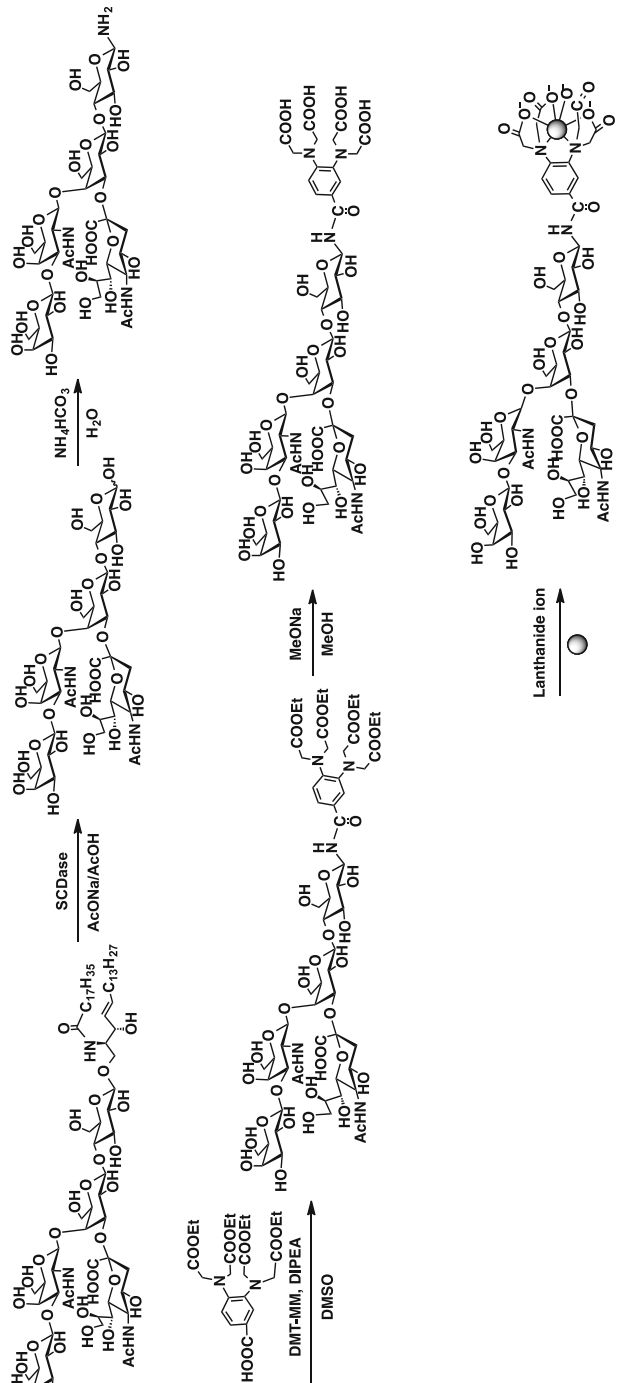


**Fig. 14.4** Scheme of lanthanide-tagging of the GM1 pentasaccharide. SCDase = sphingolipid ceramide *N*-deacylase, DIPEA = *N*,*N*-diisopropylethylamine, DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride

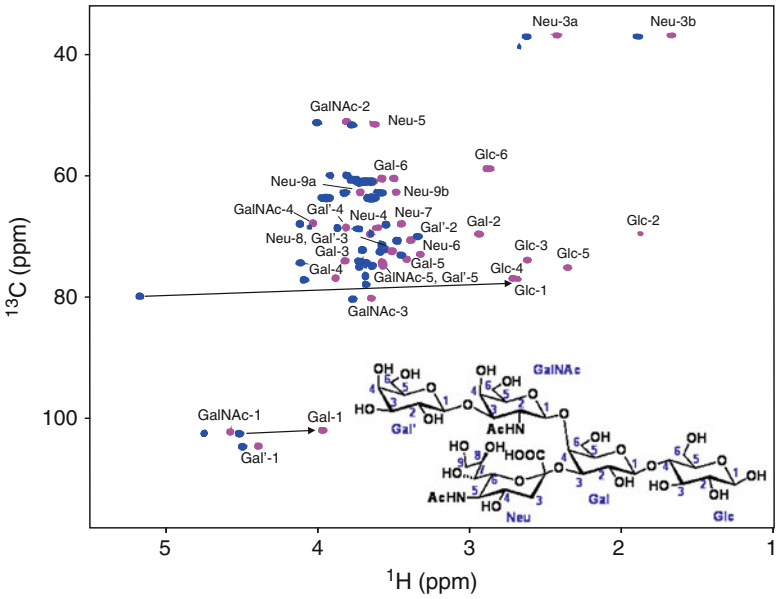


**Fig. 14.5** $^{1}$H-$^{13}$C HSQC spectral changes of the tagged GM1 pentasaccharide resulting from paramagnetic lanthanide-induced PCSs. The spectra of 1:1 complexes (0.1 mM each) of the pentasaccharide with paramagnetic $Tm^{3+}$ (*magenta*) and diamagnetic $La^{3+}$ (*blue*) in $D_2O$ were recorded at 300 K on a Bruker AVANCE 800 spectrometer equipped with a cryogenic probe (at Instrument Center, IMS)

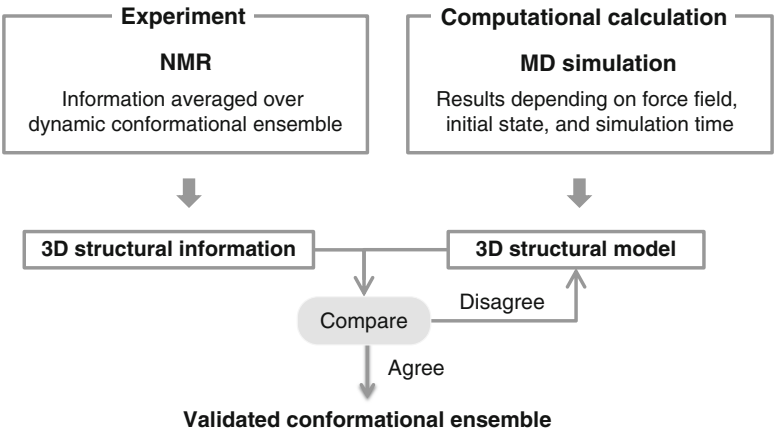


**Fig. 14.6** A coalition between NMR spectroscopy and MD simulation for characterizing dynamic conformational ensembles of oligosaccharides

2 ns trajectories. Theoretical PCS values were computed from ensemble models made of 2,000 conformers that were extracted from the MD trajectories at equal intervals. By confirming the close agreement between the experimentally observed and computationally calculated PCS values, the MD-derived conformational spaces were validated for the GM3 trisaccharide and GM2 tetrasaccharide.

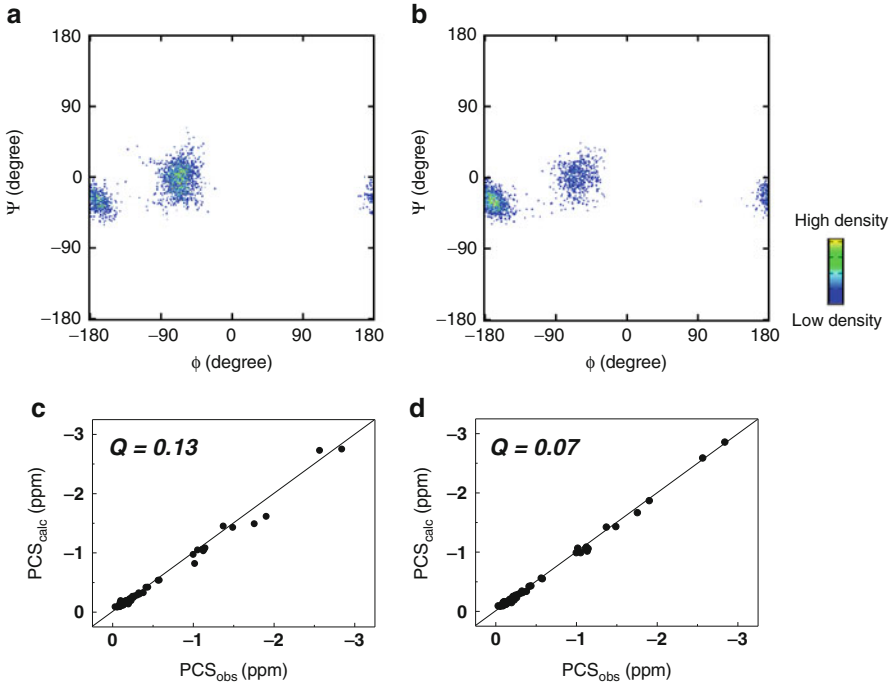


**Fig. 14.7** Torsion angle density maps of the Neu5Ac–Gal linkage of the GM1 pentasaccharides obtained by (**a**) the conventional MD and (**b**) the REMD simulation. The torsion angles **Φ** and **ψ** were defined as C1–C2–O′3–C′3 and C2–O′3–C′3–H′3, respectively. (**c**, **d**) Correlations between experimentally observed PCS values with $Tm^{3+}$ and computationally calculated PCS data for the GM1 pentasaccharide. The PCS values were back calculated from ensemble models derived from (**c**) the conventional MD and (**d**) the REMD simulation using $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$ determined based on the protocol described in the literature (Yamamoto et al. 2012). The REMD simulations of the carbohydrate moiety in explicit water were performed by using the AMBER12 program package. The simulations were carried out in *NVT* ensemble for 60 ns with 32 replicas with an exponential temperature distribution between 300 and 500 K. $Q$ values defined by the equation $Q = \mathrm{rms}(PCS_{calc} - PCS_{obs})/\mathrm{rms}(PCS_{obs})$ are criteria of agreement between experimental and calculated values

Using the same simulation protocol, an ensemble model of the GM1 pentasaccharide was created and subjected to the validation analysis. In contrast to the GM3 and GM2 cases, there was a significant disagreement between the experimental and theoretical PCS data, even though the total simulation time used for creation of the ensemble model was extended up to 240 ns (Fig. 14.7a). The discordance is most probably explained by insufficient sampling due to higher energy barriers between the multiple minima in the conformational energy-landscape of the large, branched oligosaccharides. To efficiently explore such a rough energy landscape dealing with the multiple-minima issue, we used replica-exchange MD (REMD) simulations, in which replicated simulations were run at different temperatures and exchanged during simulations to avoid being trapped into a local-energy-minimum state (Sugita and Okamoto 1999). We performed a REMD simulation using

GLYCAM_06 force field with a total simulation time of 1.9 μs and thereby obtained torsion angle density maps of the GM1 pentasaccharide. Significantly different conformational spaces were computed from the conventional MD and REMD simulations as exemplified by the Neu5Ac–Gal glycosidic linkage conformation (Fig. 14.7). The experimentally observed PCS data of this oligosaccharide are well represented in the ensemble model composed of 2,000 conformers that were extracted from the REMD simulation, demonstrating the utility of the method for the exploration of conformational spaces of large, branched oligosaccharides (Fig. 14.7).

Figure 14.8 compares the PCS-validated conformational spaces of the three ganglioside oligosaccharides, indicating similarities between the GM1 and GM2 oligosaccharides in terms of the conformational space of their common parts, i.e., the Gal-Glc, Neu5Ac-Gal, and GalNAc-Gal glycosidic linkage conformations. By contrast, the GM3 trisaccharide is distinct from the others regarding the Neu5Ac-Gal conformation. In the GM3 trisaccharide, the conformation of this linkage is most

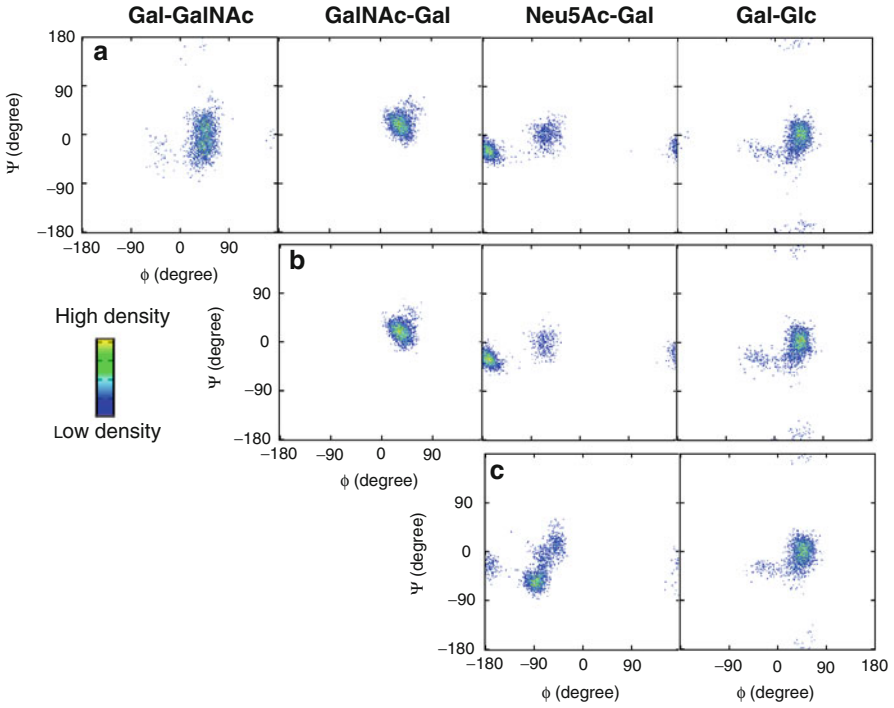


**Fig. 14.8** Torsion angle density maps of the experimentally validated MD trajectory of the (**a**) GM1, (**b**) GM2, and (**c**) GM3 oligosaccharides. The definitions of **Φ** and **ψ** were used for the Gal–GalNAc linkage, **Φ** = H1–C1–O′3–C′3 and **ψ** = C1–O′3–C′3–H′3, for the GalNAc–Gal and Gal–Glc linkages, **Φ** = H1–C1–O′4–C′4 and **ψ** = C1–O′4–C′4–H′4, and for the Neu5Ac–Gal linkage, **Φ** = C1–C2–O′3–C′3 and **ψ** = C2–O′3–C′3–H′3. This figure was from Zhang (2014) and parts of this figure were originally reproduced from Yamamoto et al. (2012) and Zhang et al. (2013) with the permissions of The Royal Society of Chemistry and MDPI

populated in the cluster ($\phi$, $\psi$) = ($-90° \pm 11°$, $-57° \pm 11°$), while the corresponding cluster is missing in the sialyl linkage conformations of GM1 and GM2. These data show that the GalNAc branch restricts the conformational freedom of the Neu5Ac-Gal glycosidic linkage, while the outermost Gal residue has no significant impact on the conformation of the remaining parts of the carbohydrate moiety.

## Concluding Remarks and Perspectives: Glycolipid clusters as a Platform for Protein Interactions

We developed the paramagnetism-assisted NMR approach in association with MD simulations, and this has been successfully applied to the characterization of the conformational dynamics of oligosaccharides derived from gangliosides. This methodology is applicable to the conformational NMR analyses of oligosaccharides in complex with proteins and would provide a new avenue toward atomic descriptions of dynamic oligosaccharide behaviors involved in interactions with proteins, which would in turn enable quantitative understanding of the energetics of carbohydrate recognition events. However, it should be noted that the gangliosides form clusters on cell surfaces and thereby promote sophisticated biomolecular functions.

Recently, growing evidence has indicated that ganglioside clusters on neuronal cell surfaces act as unique platforms for binding coupled with conformational transition of intrinsically disordered proteins involved in neurodegenerative diseases, e.g., amyloid β (Aβ) in Alzheimer's disease and α-synuclein (αSN) in Parkinson's disease (Fantini and Yahi 2010; Matsuzaki et al. 2010; Piccinini et al. 2010). These proteins interact with specific ganglioside clusters, and thereby undergo conformational changes resulting in the formation of their toxic, aggregated forms. To gain deeper insights into the molecular mechanisms underlying these cell-surface events, it is of vital importance to elucidate the dynamic conformation and interactions of the proteins and glycolipids involved therein by employing appropriate membrane models. In this context, it would be interesting to compare conformational dynamics between the liberated oligosaccharides and their assembled states.

At the hydrophilic/hydrophobic interfaces in membrane-like environments, Aβ and αSN have been reported to assume α-helical structures (Bodner et al. 2010; Ulmer et al. 2005; Utsumi et al. 2009; Yagi-Utsumi et al. 2010). Recently developed ganglioside-embedding small bicelles serve as nanoscale standardized membrane mimics for detailed NMR characterization of the carbohydrate–protein interactions on the glycolipid clusters (Fig. 14.9) (Gayen and Mukhopadhyay 2008; Khatun and Mukhopadhyay 2013; Yamaguchi et al. 2013b). By using these systems, we have successfully reveled that αSN interacts with GM1 and GM2 but not GM3, through its most ganglioside-philic site located in the N-terminal segment because of the limited sizes of the bicelles (Fig. 14.9). This success opens up new possibilities for probing the initial encounter complex transiently formed between proteins and glycolipids on membranes depending on their outer carbohydrate structure. Further developments of stable-isotope- and paramagnetism-assisted

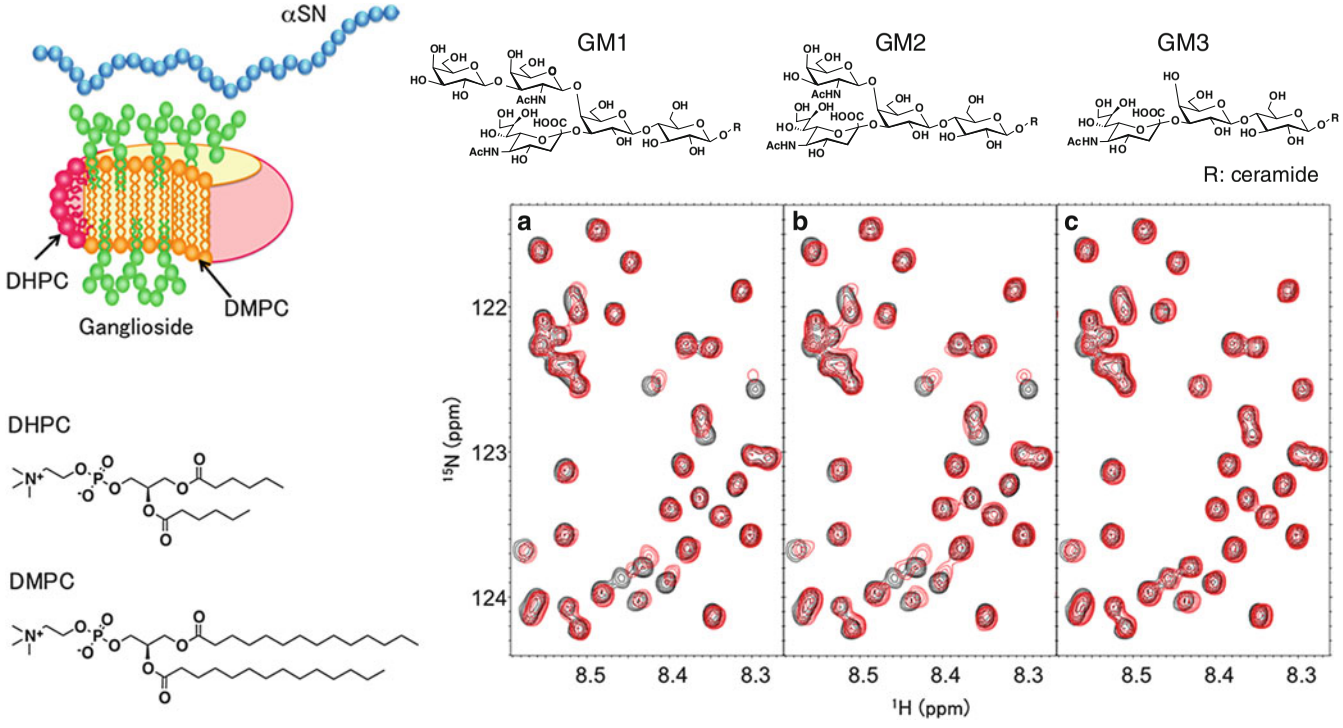


**Fig. 14.9** NMR characterization of interactions of **αSN** with the ganglioside-embedding small bicelles as nanoscale standardized membrane mimics for NMR analyses. $^{1}$H-$^{15}$N HSQC spectral changes of **αSN** induced by interactions with gangliosides (**a**) GM1, (**b**) GM2, and (**c**) GM3 embedded in the bicelles. This figure was from Zhang et al. (2013) and parts of this figure were originally reproduced from Yamaguchi et al. (2013b) with the permission of The Royal Society of Chemistry. DMPC = 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, DHPC = 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine

NMR techniques using neo-glycolipids will facilitate detailed characterization of the biomolecular systems on cell surfaces with dynamical ordering for the creation of integrated functions.

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## References

Ando H, Ishida H, Kiso M (2010) Renewed synthetic approach to gangliosides exploiting versatile and powerful synthetic units. Methods Enzymol 478;521–540

Bodner CR, Maltsev AS, Dobson CM, Bax A (2010) Differential phospholipid binding of α-synuclein variants implicated in Parkinson's disease revealed by solution NMR spectroscopy. Biochemistry 49:862–871

Drickamer K, Taylor ME (1993) Biology of animal lectins. Annu Rev Cell Biol 9:237–264

Ernst B, Magnani JL (2009) From carbohydrate leads to glycomimetic drugs. Nat Rev Drug Discov 8:661–677

Fantini J, Yahi N (2010) Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases. Expert Rev Mol Med 12:e27

Gayen A, Mukhopadhyay C (2008) Evidence for effect of GM1 on opioid peptide conformation: NMR study on leucine enkephalin in ganglioside-containing isotropic phospholipid bicelles. Langmuir 24:5422–5432

Kamiya Y, Yagi-Utsumi M, Yagi H, Kato K (2011a) Structural and molecular basis of carbohydrate-protein interaction systems as potential therapeutic targets. Curr Pharm Des 17:1672–1684

Kamiya Y, Yamamoto S, Chiba Y, Jigami Y, Kato K (2011b) Overexpression of a homogeneous oligosaccharide with $^{13}C$ labeling by genetically engineered yeast strain. J Biomol NMR 50:397–401

Kamiya Y, Satoh T, Kato K (2012) Molecular and structural basis for *N*-glycan-dependent determination of glycoprotein fates in cells. Biochim Biophys Acta 1820:1327–1337

Kamiya Y, Yanagi K, Kitajima T, Yamaguchi T, Chiba Y, Kato K (2013) Application of metabolic $^{13}C$ labeling in conjunction with high-field nuclear magnetic resonance spectroscopy for comparative conformational analysis of high mannose-type oligosaccharides. Biomolecules 3:108–123

Kamiya Y, Satoh T, Kato K (2014) Recent advances in glycoprotein production for structural biology: toward tailored design of glycoforms. Curr Opin Struct Biol 26:44–53

Khatun UL, Mukhopadhyay C (2013) Interaction of bee venom toxin melittin with ganglioside GM1 bicelle. Biophys Chem 180–181:66–75

Lepenies B, Yin J, Seeberger PH (2010) Applications of synthetic carbohydrates to chemical biology. Curr Opin Chem Biol 14:404–411

Luchinat C, Parigi G (2007) Paramagnetic systems in biochemistry: solution NMR studies eMagRes. Wiley, New York

Matsuzaki K, Kato K, Yanagisawa K (2010) Abeta polymerization through interaction with membrane gangliosides. Biochim Biophys Acta 1801:868–877

Nakanishi-Shindo Y, Nakayama K, Tanaka A, Toda Y, Jigami Y (1993) Structure of the *N*-linked oligosaccharides that show the complete loss of α-1,6-polymannose outer chain from *och1*,

*och1 mnn1*, and *och1 mnn1 alg3* mutants of *Saccharomyces cerevisiae*. J Biol Chem 268:26338–26345

Peters T, Pinto BM (1996) Structure and dynamics of oligosaccharides: NMR and modeling studies. Curr Opin Struct Biol 6:710–720

Piccinini M, Scandroglio F, Prioni S, Buccinnà B, Loberto N, Aureli M, Chigorno V, Lupino E, Demarco G, Lomartire A, Rinaudo MT, Sonnino S, Prinetti A (2010) Deregulated sphingolipid metabolism and membrane organization in neurodegenerative disorders. Mol Neurobiol 41:314–340

Satoh T, Suzuki K, Yamaguchi T, Kato K (2014) Structural basis for disparate sugar-binding specificities in the homologous cargo receptors ERGIC-53 and VIP36. PLoS One 9:e87963

Sharon N, Lis H (2003) Lectins, 2nd edn. Springer, Dordrecht

Sugita Y, Okamoto Y (1999) Replica-exchange molecular dynamics method for protein folding. Chem Phys Lett 314:141–151

Takeda Y, Totani K, Matsuo I, Ito Y (2009) Chemical approaches toward understanding glycan-mediated protein quality control. Curr Opin Chem Biol 13:582–591

Ulmer TS, Bax A, Cole NB, Nussbaum RL (2005) Structure and dynamics of micelle-bound human α-synuclein. J Biol Chem 280:9595–9603

Utsumi M, Yamaguchi Y, Sasakawa H, Yamamoto N, Yanagisawa K, Kato K (2009) Up-and-down topological mode of amyloid beta-peptide lying on hydrophilic/hydrophobic interface of ganglioside clusters. Glycoconj J 26:999–1006

Wang Z, Chinoy ZS, Ambre SG, Peng W, Mcbride R, De Vries RP, Glushka J, Paulson JC, Boons GJ (2013) A general strategy for the chemoenzymatic synthesis of asymmetrically branched *N*-glycans. Science 341:379–383

Wormald MR, Petrescu AJ, Pao YL, Glithero A, Elliott T, Dwek RA (2002) Conformational studies of oligosaccharides and glycopeptides: complementarity of NMR, X-ray crystallography, and molecular modelling. Chem Rev 102:371–386

Yagi-Utsumi M, Kameda T, Yamaguchi Y, Kato K (2010) NMR characterization of the interactions between lyso-GM1 aqueous micelles and amyloid beta. FEBS Lett 584:831–836

Yamaguchi T, Kamiya Y, Choo YM, Yamamoto S, Kato K (2013a) Terminal spin labeling of a high-mannose-type oligosaccharide for quantitative NMR analysis of its dynamic conformation. Chem Lett 42:544–546

Yamaguchi T, Uno T, Uekusa Y, Yagi-Utsumi M, Kato K (2013b) Ganglioside-embedding small bicelles for probing membrane-landing processes of intrinsically disordered proteins. Chem Commun (Camb) 49:1235–1237

Yamaguchi T, Sakae Y, Zhang Y, Yamamoto S, Okamoto Y, Kato K (2014) Exploration of conformational spaces of high-mannose-type oligosaccharides by an NMR-validated simulation. Angew Chem Int Ed Engl in press

Yamamoto S, Yamaguchi T, Erdélyi M, Griesinger C, Kato K (2011) Paramagnetic lanthanide tagging for NMR conformational analyses of *N*-linked oligosaccharides. Chem Eur J 17:9280–9282

Yamamoto S, Zhang Y, Yamaguchi T, Kameda T, Kato K (2012) Lanthanide-assisted NMR evaluation of a dynamic ensemble of oligosaccharide conformations. Chem Commun (Camb) 48:4752–4754

Zhang Y (2014) Paramagnetism–assisted NMR analyses of conformational dynamics of ganglioside glycans. The Graduate University for Advanced Studies

Zhang Y, Yamamoto S, Yamaguchi T, Kato K (2012) Application of paramagnetic NMR-validated molecular dynamics simulation to the analysis of a conformational ensemble of a branched oligosaccharide. Molecules 17:6658–6671

Zhang Y, Yamaguchi T, Kato K (2013) New NMR tools for characterizing the dynamic conformations and interactions of oligosaccharides. Chem Lett 42:1455–1462

Zhao H, Pan Q, Zhang W, Carmichael I, Serianni AS (2007) DFT and NMR studies of $^{2}J_{COH}$, $^{3}J_{HCOH}$, and $^{3}J_{CCOH}$ spin-couplings in saccharides: C-O torsional bias and H-bonding in aqueous solution. J Org Chem 72:7071–7082