Solid state NMR of membrane proteins: methods and applications

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Abstract

Membranes of cells are active barriers, in which membrane proteins perform essential remodelling, transport and recognition functions that are vital to cells. Membrane proteins are key regulatory components of cells and represent essential targets for modulation of cell function and pharmacological intervention. However, novel folds, low molarity and the need for lipid membrane support present serious challenges to characterisation of their structure and interactions. We describe the use of solid state NMR as a versatile and informative approach for membrane and membrane protein studies, which uniquely provides information on structure, interactions and dynamics of membrane proteins. High resolution approaches are discussed in conjunction with applications of NMR methods to studies of membrane lipid and protein structure and interactions. Signal enhancement in high resolution NMR spectra through DNP is discussed as a tool for whole cell and interaction studies.

Perspectives

- ***Importance of the field***: Membrane proteins represent estimated 30-50% of the human genome and over half of the pharmaceutically exploited targets. They perform key cellular functions as transporters, receptors, cell recognition markers and remodelling enzymes. Yet, membrane protein structural studies remain a major challenge due to their characteristic amphipathic architecture.

- ***Current thinking***: Solid state NMR is a tool uniquely suited for high resolution studies of membrane protein structure, dynamics and interactions with atomic level of detail. Challenges in solid state NMR analysis of membrane proteins include sample preparation protocols, including labelling, membrane models and mimetics, selection of experimental approach and pulse sequence that address a specific question and matching all components to the physical characteristics of the system of interest. Detailed high resolution information is then obtained by solid state NMR spectroscopy through fast sample spinning or through mechanical sample alignment and orientation in the magnetic field.

- ***Future directions***: Novel mimetic systems improve sample preparation and first insights into intact cell membranes and aided by DNP signal enhancement, whole cell solid state NMR spectroscopy is becoming a reality. Ultrahigh field NMR magnet developments combine with ultrafast sample spinning that enable high information content proton spectroscopy.

Introduction

Cell membranes are self-assembled structures, which enclose the principal compartments of life and define the cell as its fundamental unit. Membranes primarily consist of mixed phospholipid bilayers containing membrane proteins at a roughly equal mass ratio, as well as other, minor but important, biologically active molecular components. Membrane assembly, stability and remodelling are crucially dependent on a fine balance between the shape and properties of their constituent molecules, which may stabilise the bilayer structure or facilitate the formation of localised, transient intermediates that perform a specific biological functions. Cells modify their lipid and protein composition in response to a variety of factors such as environmental changes or stress, cell cycle phase or to carry out regular functions, such as endo- or exocytosis, vesicle fusion and division.

Balanced lipid composition is essential to membrane stability and function. Zwitterionic amphiphiles with roughly cylindrical molecular formfactors, such as diacyl phosphatidyl choline, spontaneously assemble into and stabilise bilayers structures. Diacyl phosphatidyl ethanolamine can also assemble into bilayer structures, prone to favouring negative curvature, which at lower hydration levels may convert to non-bilayer, inverted hexagonal phases. Conversely, anionic lipids, such as diacyl phosphatidyl glycerol, which also form bilayer structures, tend to favour positive bilayer curvature due to electrostatic repulsion between their headgroups. The structure of acyl chains of biomembrane lipids also plays a role in the stability of membrane, as well as roles in membrane protein function and dynamics (1). The presence of cholesterol tends to increase membrane stability, bending rigidity and to reduce membrane undulations without significantly affecting lipid axial mobility or membrane fluidity. The lipid composition of biomembrane is often highly complex, unique and crucial to the specific membrane and organism, adding to the difficulties of membrane protein studies.

Membranes are active structures, which perform key cellular functions such as solute exchange, signalling and molecular recognition. While the membrane lipid matrix provides a barrier, impermeable to ions and polar solutes, in order to maintain vital osmotic and electrostatic gradients, a diverse set of transmembrane and peripheral proteins performs membrane active functions, generating and maintaining electric polarization, asymmetry and essential transmembrane solute gradients. Membrane proteins are a class of protein found to interact or embed in the biomembrane, often containing either partial or largely hydrophobic domains, making them unstable without presence of lipid bilayer. Membrane protein accounts for about 30% of protein encoded in most genomes, and are targets of almost 60% of medical drugs (2). Membrane proteins and protein complexes preserve the homeostasis, drive and regulate membrane remodelling and can mediate selective toxicity that ensures access to extracellular resources.

Solid state NMR approaches to membrane protein studies include an arsenal of pulse sequences of increasing complexity, the utility of which is tailored to specific dynamic properties, structural characteristics and labelling schemes of the investigated systems. In addition, a palette of model systems for sample preparation ranging from membrane mimics and synthetic lipid membranes through native membrane isolates to whole cells, which provide different and unique insight into the structure, organisation and interactions of membranes or membrane constituents. Unlike crystallography and cryo-EM, where samples are fixed in static state either by crystal lattice or freezing, solid state NMR can reveal structural and dynamics information from membranes in physiological conditions or permit observation of molecules directly in their biological context.

Molecular studies of membranes and membrane proteins present particular challenges, which arise from the need of a membrane support to maintain protein fold and prevent aggregation. There are three main routes in addressing this problem – membrane mimetics such as detergent micelles can be used to solubilise individual proteins, which can then be studied by solution NMR (3); proteins embedded within small membrane patches enclosed by detergent molecules, called lipid bicelles, which can be aligned with the field of the NMR magnet and studied by orientation-selective methods (4); and, whole proteoliposomes can be subjected to mechanical rotation to yield high resolution magic angle spinning (MAS) (5). All the these approaches normally require additional experimentation to complement data sets and all frequently face a low signal challenge arising from the low protein molarity in the samples. One way of tackling this challenge is to carry out experiments under dynamic nuclear polarization (DNP) signal enhancement (6), which typically yields 10 to a 100-fold sensitivity gain with a trade-off in spectral resolution.

Solid state NMR and membrane proteins

Membrane proteins can be studied by solid state NMR directly, through their impact on membrane structure and organisation or through their interactions with other membrane components. Direct observation of proteins in membranes aims at resolving individual atomic environments on the protein and deriving structural information through distance and torsion angle constraints or through orientational constraints. In contrast to soluble protein, where high NMR spectral resolution of proteins is naturally achieved through free, fast, random molecular reorientation, membrane proteins embedded in lipid bilayer are motionally restricted by the membrane environment. As a result, the NMR spectra are dominated by strong, orientation-dependent nuclear interactions, which conceal the isotropic chemical shifts and scalar J-coupling that uniquely identify atomic environments in a protein and can be used to derive structural information.

Orientation-dependent, anisotropic nuclear interactions must either be removed by an averaging process to recover high resolution information from membrane proteins, or can be used to derive orientation-dependent constraints from spatially aligned membranes. High resolution NMR spectra can be acquired from membrane proteins solubilised in detergent micelles. Sufficiently small proteodetergent micellar structures undergo rapid molecular reorientation that removes anisotropic broadening when the reorientational correlation time τ_c of the micelle is much shorter than the NMR timescale τ_L. Molecular reorientation rate depends on the hydrodynamic radius of the protein r_h, aqueous viscosity η and temperature T:

$$1/\tau_c = k_B T / \eta r_h^3$$

This approach is limited by the stability of membrane proteins in detergent systems that assemble into sufficiently small micellar structures to satisfy the Debye-Stokes-Einstein equation.

The most commonly used method for high resolution solid state NMR studies of membrane proteins is magic angle sample spinning. Chemical shift-resolved spectra are obtained under MAS by averaging of anisotropic nuclear interactions. This is achieved through mechanical sample rotation about an axis inclined at $\theta_m \approx 57.4°$, the “magic angle”, to the external NMR magnetic field. Such rotation is aligned with [111] direction in a cubic crystallographic system and modulates all tension components with an equal statistical weight. The orientation dependence of nuclear interactions is proportional to the second Legendre polynomial

$$P_2(cos\theta) = {}^1/_2\,(3cos^2\theta - 1)$$

which vanishes at $\theta = \theta_m$. When sample rotation is faster or close to the strength of anisotropic nuclear interactions, the averaging removes orientation-dependent broadening, which leads to isotropic chemical shift/J-coupling dominated high resolution spectra.

High resolution solid state NMR spectra can also be obtained in static samples, in which the orientational polydispersity is removed by mechanical alignment of stacked membranes or lipid bicelles containing the membrane proteins of interest. In the special case when the membrane normal is parallel to the NMR magnetic field, anisotropic chemical shift can reveal bond orientation. In ^{15}N-labelled proteins, this can combine with ^{1}H-^{15}N dipolar couplings and lead to orientationally resolved correlation spectra that are particularly informative in helical transmembrane domain (TMD) membrane protein studies (7).

Protons are the most abundant NMR sensitive nuclear system in proteins. They populate structurally informative sites in a protein and form extensive networks encoding multiple distance and torsion angle constraints. Interproton dipolar couplings form extensive connectivity networks that reflect local and long range structural detail in a protein. The magnitude of these homonuclear ^{1}H-^{1}H couplings is on the order of 100 kHz, which cannot be removed efficiently under commonly available MAS averaging and ^{1}H spectra incur homogeneous dipolar broadening, which can affect severely spectral resolution. While much effort has been put into ultrafast MAS instrument and method development, the utility of ^{1}H MAS NMR remains largely limited. Feasibility studies show promising results in spectral assignment of microcrystalline proteins (8). Resolution enhancement under ultrafast MAS can be aided by protein deuteration, which disrupts the interconnectivity of proton networks and attenuates dipolar coupling strength, which becomes manageable at 60 kHz MAS. With increasing MAS rates to over 100 kHz, the benefits of deuteration are gradually removed by increased efficiency of rotational averaging (9).

The most commonly employed reporter nuclear system in solid state NMR is ^{13}C. Typical ^{13}C linewidth in proteins is on the order of ppm, which offers good resolution compared to the typical chemical shift dispersion of almost 200 ppm, which combined with good population of diverse molecular sites in proteins makes ^{13}C an appealing structural reporter. Natural abundance of ^{13}C in biological system is low at 1.1%, which necessitates isotope enrichment either through uniform substitution or through strategic labelling of amino acid residues or chemical groups. Isotropic labelling is often done in recombinant protein expression through supplementation of bacterial growth media uniformly or through selective amino acid supplementation, as done in proteorhodopsin (10) but can be extended to whole bacterial cells (11) or even more complex protein production, such as labelling of spider silks through supplementation of the spider feed (12).

Nuclear excitation in ^{13}C solid state NMR experiments is commonly achieved by cross polarization (CP) (13), which involves Hartmann-Hahn magnetization transfer from ^{1}H to ^{13}C. This can be followed by ^{13}C observation under ^{1}H/^{13}C heteronuclear decoupling or an evolution period in more complex experiments. Commonly used decoupling sequences include TPPM (14) or SPINAL64 (15), which are more efficient than continuous wave proton irradiation. The use of CP provides signal enhancement benefitting from the higher ^{1}H polarization and faster T_1 relaxation, and is used as the excitation block in most ^{13}C solid state NMR experiments. In addition, CP can be used as a filter to select motionally restricted molecular sub-populations, such as bound ligands or membrane-associated molecules while suppressing mobile contributions, such as molecules in solution (16).

Heteronuclear $^{1}H,^{13}C$ correlation experiments would also require proton evolution and in some cases a period of mixing ahead of CP (17, 18).

Isotope enrichment not only increases signal intensity, it also permits conformational analysis of proteins, ligand or substrates using homonuclear $^{13}C,^{13}C$ correlation methods, using chemical shift correlation to determine molecular interaction and connectivity. A number of examples include proton driven spin diffusion (PDSD) (19) and dipolar assisted rotational resonance (DARR) (20), both are common techniques used to probe ^{13}C correlation of membrane protein, as well as double quantum (DQ) experiments that utilises C or R-symmetry-based C^{n}/R^{n} pulse sequences (21) (Hohwy et al, 1998, J Chem Phys 108) and five-fold symmetry SPC5 (22) for direct spin pair observation. When the evolution of the indirect NMR dimension t_1 takes place in the single quantum state, the resulting correlation spectra have symmetric appearance of chemical shift resolved correlations, much like single quantum sequences. In such cases DQ excitation is followed directly by reconversion to single quantum state and serves as a filter to remove unpaired nuclear magnetizations, such as unlabelled membrane constituents. Homonuclear $^{13}C,^{13}C$ correlation spectroscopy with DQ filter was used to determine protein-cholesterol interaction using ^{13}C labelled TM helix of influenza M2 and ^{13}C enriched cholesterol (23). When evolution period t_1 is placed with the DQ state, the resulting spectra show pairwise chemical shift correlations that are expanded in the indirect dimension by the sum of chemical shifts (24). While this approach appears slightly more convoluted, the extended indirect dimension aids resolution and assignment.

In addition to homonuclear correlation experiments, ^{13}C can be used in conjunction with ^{1}H or ^{15}N and perform solution-like experiments, such as HSQC-MAS studies of lipids interacting with the K^{+} channel KcsA (25) or solid state correlation experiments to observe the membrane lipid component (17) or bistable states in Ca^{2+} ATP-ase (26). Combinations of homonuclear with heteronuclear and multidimensional correlation experiments enable following protein chain-specific magnetization routes through protein-specific sequences, such as HNCO/HNCA (27).

Membrane active proteins and peptides

A unique feature of solid state NMR is its ability to report on interactions between membrane-associated proteins and specific molecular receptors in membranes, as well as to reveal changes in membranes in response to protein binding. Specifically, the method can reveal direct information through coupling or dynamics that can selectively highlight interaction pairs even within polydisperse molecular environments.

Solid state NMR has been used to describe molecular recognition of membrane raft-associated ganglioside GM1 targets by amyloidogenic prion protein (28) [Figure 1], bacterial lipopolysaccharide by colicin N (29) and antimicrobial peptide nisin (30), membrane cholesterol by bacterial toxin penumolysin (31) and sphingomyelin by eukaryotic haemolysin equinatoxin (32).

The use of solid state NMR in membrane protein studies normally requires a combination of experimental approaches including both homonuclear and heteronuclear experiments, for example homonuclear DARR has been used in combination with HNCA in a structural study or diacylglycerol kinase (33) [Figure 2] or conformational transitions in a membrane reconstituted K^{+} channel (34).

Peptides can modulate the integrity and functions of membranes either by altering the permeability of the membrane hydrophobic barrier, or by regulating the functions and bioavailability of essential regulators of membrane function and assembly. Heteronuclear MAS NMR experiments can be used in a single dimension to study membrane-associated molecular complexes and to monitor distances within or between molecules by strategic use of reporter isotopes. Rotational echo double resonance (35) has been used to study interactions of antibiotic vancomycin with bacterial peptidoglycan (36) or between antimicrobial peptide nisin and conserved peptidoglycan membrane intermediate lipid II (37). Combinations of homonuclear and heteronuclear NMR approaches has been used to investigate interactions between antibiotic teixobactin and lipid II (38).

Dynamic nuclear polarization MAS NMR

Despite its high information content and superb chemical and geometric resolution, the applications of NMR have been limited by its relatively low inherent sensitivity. The low NMR signal reflects a comparatively low nuclear magnetization and resulting polarizability compared to electrons. By contrast, unpaired electrons in radicals can yield 2-3 orders of magnitude greater signal, as observed in EPR spectroscopy but cannot convey the wealth of information normally available in NMR studies. The strength of both approaches is brought together in a combination approach, dynamic nuclear polarization (DNP). DNP relies on the coupling of highly polarised paramagnetic electron systems to the nuclear systems of interest, followed by observing the arising NMR signal. With the introduction of bespoke engineered biradicals, the resulting sensitivity enhancement in DNP NMR studies can range from 10s to 100s of times (39), which can reduce experimental acquisition times 100 to 10,000-fold and permit low signal experiments from low molarity systems, such as native membrane and whole cell studies, normally not achievable by solid state NMR approaches. DNP-enhanced MAS NMR has been used to study inhibitor bound to M2 proton transporter from influenza A (40), as well as in studies of drug binding to a multidrug efflux ABC transporter MsbA (41), and structural characterization of G protein couple receptor (GPCR) B_1R (42). DNP also allowed solid state NMR studies of more complex systems, often in combination with specifically designed biradicals and ligands, such as cell (43), whole bacterial cells (44) and human embryonic kidney cells (45). DNP provides opportunity to study membrane and membrane protein systems otherwise not possible with conventional solid state NMR methods, showing enormous potential to expand the study of membrane protein in native environments.

Conclusions

Membrane proteins play a variety of key cellular roles and present extremely valuable targets for drug design and pharmacological intervention. Despite recent success of membrane protein crystallography and technological advancements in cryo-EM, studies of structure, function and interactions of membrane proteins remain a major challenge. Solid state NMR offers novel insights into the molecular organisation and interactions in membranes and remains unique in delivering atomic level detail from ambient temperature membrane proteins and membrane protein assemblies. Despite complexity in sample preparation and method applications solid state NMR remains a powerful tool, providing rich insights into protein structure, protein-ligand, and protein-lipid interactions, as well as target recognition and targeted perturbation of membranes by antibiotics and antimicrobials. Recent developments in DNP-enhanced MAS NMR offer new

opportunities towards studying model systems with enhanced complexity, such as native membranes, as well as NMR studies of whole cells.

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References

1. Lee T-Y, Yeh V, Chuang J, Chung Chan Jerry C, Chu L-K, Yu T-Y. Tuning the Photocycle Kinetics of Bacteriorhodopsin in Lipid Nanodiscs. Biophysical Journal. 2015;109(9):1899-906.
2. Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? Nature Reviews Drug Discovery. 2006;5(12):993-6.
3. Warschawski DE, Arnold AA, Beaugrand M, Gravel A, Chartrand E, Marcotte I. Choosing membrane mimetics for NMR structural studies of transmembrane proteins. Biochimica Et Biophysica Acta-Biomembranes. 2011;1808(8):1957-74.
4. Prosser RS, Evanics F, Kitevski JL, Al-Abdul-Wahid MS. Current Applications of Bicelles in NMR Studies of Membrane-Associated Amphiphiles and Proteins. Biochemistry. 2006;45(28):8453-65.
5. Park SH, Das BB, Casagrande F, Tian Y, Nothnagel HJ, Chu M, et al. Structure of the chemokine receptor CXCR1 in phospholipid bilayers. Nature. 2012;491(7426):779-83.
6. Liao SY, Lee M, Wang T, Sergeyev IV, Hong M. Efficient DNP NMR of membrane proteins: sample preparation protocols, sensitivity, and radical location. Journal of Biomolecular NMR. 2016;64(3):223-37.
7. Jirasko V, Lends A, Lakomek NA, Fogeron ML, Weber ME, Malar AA, et al. Dimer Organization of Membrane-Associated NS5A of Hepatitis C Virus as Determined by Highly Sensitive H-1-Detected Solid-State NMR. Angewandte Chemie-International Edition. 2021;60(10):5339-47.
8. Marchetti A, Jehle S, Felletti M, Knight MJ, Wang Y, Xu ZQ, et al. Backbone Assignment of Fully Protonated Solid Proteins by 1H Detection and Ultrafast Magic-Angle-Spinning NMR Spectroscopy. Angewandte Chemie-International Edition. 2012;51(43):10756-9.
9. Cala-De Paepe D, Stanek J, Jaudzems K, Tars K, Andreas LB, Pintacuda G. Is protein deuteration beneficial for proton detected solid-state NMR at and above 100 kHz magic-angle spinning? Solid State Nuclear Magnetic Resonance. 2017;87:126-36.
10. Pfleger N, Worner AC, Yang J, Shastri S, Hellmich UA, Aslimovska L, et al. Solid-state NMR and functional studies on proteorhodopsin. Biochimica Et Biophysica Acta-Bioenergetics. 2009;1787(6):697-705.
11. Narasimhan S, Pinto C, Paioni AL, van der Zwan J, Folkers GE, Baldus M. Characterizing proteins in a native bacterial environment using solid-state NMR spectroscopy. Nature Protocols. 2021;16(2):893-918.
12. Bonev B, Grieve S, Herberstein M, Kishore A, Watts A, Separovic F. Orientational order of Australian spider silks as determined by solid-state NMR. Biopolymers: Original Research on Biomolecules. 2006;82(2):134-43.
13. Hartmann SR, Hahn EL. NUCLEAR DOUBLE RESONANCE IN ROTATING FRAME. Physical Review. 1962;128(5):2042-&.
14. Bennett AE, Rienstra CM, Auger M, Lakshmi KV, Griffin RG. Heteronuclear decoupling in rotating solids. The Journal of Chemical Physics. 1995;103(16):6951-8.

15. Fung BM, Khitrin AK, Ermolaev K. An improved broadband decoupling sequence for liquid crystals and solids. Journal of Magnetic Resonance. 2000;142(1):97-101.
16. Yeh V, Goode A, Eastham G, Rambo RP, Inoue K, Doutch J, et al. Membrane Stability in the Presence of Methacrylate Esters. Langmuir. 2020;36(33):9649-57.
17. Zorin V, Ciesielski F, Griffin DC, Rittig M, Bonev BB. Heteronuclear chemical shift correlation and J-resolved MAS NMR spectroscopy of lipid membranes. Magnetic Resonance in Chemistry. 2010;48(12):925-34.
18. Holland GP, Alam TM. Multi-dimensional H-1-C-13 HETCOR and FSLG-HETCOR NMR study of sphingomyelin bilayers containing cholesterol in the gel and liquid crystalline states. Journal of Magnetic Resonance. 2006;181(2):316-26.
19. Bloembergen N. On the interaction of nuclear spins in a crystalline lattice. Physica. 1949;15(3):386-426.
20. Takegoshi K, Nakamura S, Terao T. 13C–1H dipolar-assisted rotational resonance in magic-angle spinning NMR. Chemical Physics Letters. 2001;344(5):631-7.
21. Carravetta M, Eden M, Zhao X, Brinkmann A, Levitt MH. Symmetry principles for the design of radiofrequency pulse sequences in the nuclear magnetic resonance of rotating solids. Chemical Physics Letters. 2000;321(3-4):205-15.
22. Hohwy M, Rienstra CM, Jaroniec CP, Griffin RG. Fivefold symmetric homonuclear dipolar recoupling in rotating solids: Application to double quantum spectroscopy. The Journal of Chemical Physics. 1999;110(16):7983-92.
23. Elkins MR, Sergeyev IV, Hong M. Determining Cholesterol Binding to Membrane Proteins by Cholesterol 13C Labeling in Yeast and Dynamic Nuclear Polarization NMR. Journal of the American Chemical Society. 2018;140(45):15437-49.
24. Luca S, White JF, Sohal AK, Filippov DV, van Boom JH, Grisshammer R, et al. The conformation of neurotensin bound to its G protein-coupled receptor. Proceedings of the National Academy of Sciences. 2003;100(19):10706-11.
25. Zhang DY, Howarth GS, Parkin LA, McDermott AE. NMR studies of lipid regulation of the K+ channel KcsA. Biochimica Et Biophysica Acta-Biomembranes. 2021;1863(3).
26. Gopinath T, Veglia G. Probing membrane protein ground and conformationally excited states using dipolar- and J-coupling mediated MAS solid state NMR experiments. Methods. 2018;148:115-22.
27. Baldus M, Petkova AT, Herzfeld J, Griffin RG. Cross polarization in the tilted frame: assignment and spectral simplification in heteronuclear spin systems. Molecular Physics. 1998;95(6):1197-207.
28. Sanghera N, Correia BE, Correia JR, Ludwig C, Agarwal S, Nakamura HK, et al. Deciphering the molecular details for the binding of the prion protein to main ganglioside GM1 of neuronal membranes. Chemistry & biology. 2011;18(11):1422-31.
29. Johnson CL, Ridley H, Marchetti R, Silipo A, Griffin DC, Crawford L, et al. The antibacterial toxin colicin N binds to the inner core of lipopolysaccharide and close to its translocator protein. Molecular microbiology. 2014;92(3):440-52.
30. Lanne AB, Goode A, Prattley C, Kumari D, Drasbek MR, Williams P, et al. Molecular recognition of lipopolysaccharide by the lantibiotic nisin. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2019;1861(1):83-92.
31. Bonev BB, Gilbert RC, Andrew PW, Byron O, Watts A. Structural analysis of the protein/lipid complexes associated with pore formation by the bacterial toxin pneumolysin. Journal of Biological Chemistry. 2001;276(8):5714-9.
32. Bonev BB, Lam Y-H, Anderluh G, Watts A, Norton RS, Separovic F. Effects of the eukaryotic pore-forming cytolysin equinatoxin II on lipid membranes and the role of sphingomyelin. Biophysical journal. 2003;84(4):2382-92.

33. Li JP, Shen Y, Chen YK, Zhang ZF, Ma SJ, Wan QF, et al. Structure of membrane diacylglycerol kinase in lipid bilayers. Communications Biology. 2021;4(1).
34. Lange A, Giller K, Hornig S, Martin-Eauclaire MF, Pongs O, Becker S, et al. Toxin-induced conformational changes in a potassium channel revealed by solid-state NMR. Nature. 2006;440(7086):959-62.
35. Gullion T, Schaefer J. Rotational echo double resonance NMR. Journal of Magnetic Resonance. 1989;81(1):196-200.
36. Cegelski L, Kim SJ, Hing AW, Studelska DR, O'Connor RD, Mehta AK, et al. Rotational-echo double resonance characterization of the effects of vancomycin on cell wall synthesis in Staphylococcus aureus. Biochemistry. 2002;41(43):13053-8.
37. Bonev BB, Breukink E, Swiezewska E, De Kruijff B, Watts A. Targeting extracellular pyrophosphates underpins the high selectivity of nisin. The FASEB Journal. 2004;18(15):1862-9.
38. Shukla R, Medeiros-Silva J, Parmar A, Vermeulen BJA, Das S, Paioni AL, et al. Mode of action of teixobactins in cellular membranes. Nature Communications. 2020;11(1).
39. Song C, Hu K-N, Joo C-G, Swager TM, Griffin RG. TOTAPOL: A Biradical Polarizing Agent for Dynamic Nuclear Polarization Experiments in Aqueous Media. Journal of the American Chemical Society. 2006;128(35):11385-90.
40. Andreas LB, Barnes AB, Corzilius B, Chou JJ, Miller EA, Caporini M, et al. Dynamic Nuclear Polarization Study of Inhibitor Binding to the M2(18-60) Proton Transporter from Influenza A. Biochemistry. 2013;52(16):2774-82.
41. Spadaccini R, Kaur H, Becker-Baldus J, Glaubitz C. The effect of drug binding on specific sites in transmembrane helices 4 and 6 of the ABC exporter MsbA studied by DNP-enhanced solid-state NMR. Biochimica Et Biophysica Acta-Biomembranes. 2018;1860(4):833-40.
42. Joedicke L, Mao J, Kuenze G, Reinhart C, Kalavacherla T, Jonker HRA, et al. The molecular basis of subtype selectivity of human kinin G-protein-coupled receptors. Nature Chemical Biology. 2018;14(3):284-90.
43. Viennet T, Viegas A, Kuepper A, Arens S, Gelev V, Petrov O, et al. Selective Protein Hyperpolarization in Cell Lysates Using Targeted Dynamic Nuclear Polarization. Angewandte Chemie International Edition. 2016;55(36):10746-50.
44. Yamamoto K, Caporini MA, Im S-C, Waskell L, Ramamoorthy A. Cellular solid-state NMR investigation of a membrane protein using dynamic nuclear polarization. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2015;1848(1, Part B):342-9.
45. Albert BJ, Gao C, Sesti EL, Saliba EP, Alaniva N, Scott FJ, et al. Dynamic Nuclear Polarization Nuclear Magnetic Resonance in Human Cells Using Fluorescent Polarizing Agents. Biochemistry. 2018;57(31):4741-6.

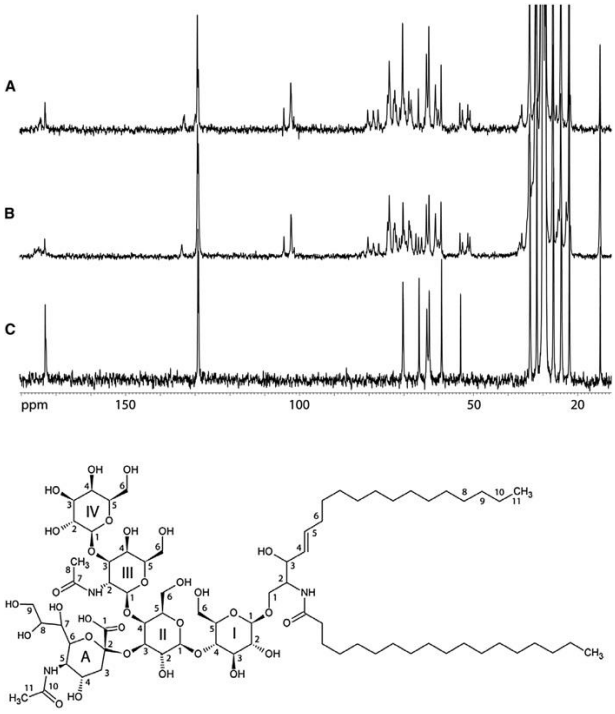

Figure 1: High resolution 13C MAS NMR spectra from prion protein interaction with ganglioside GM1 in lipid membranes (reproduced with permission from (28), copyright 2011 Elsevier).

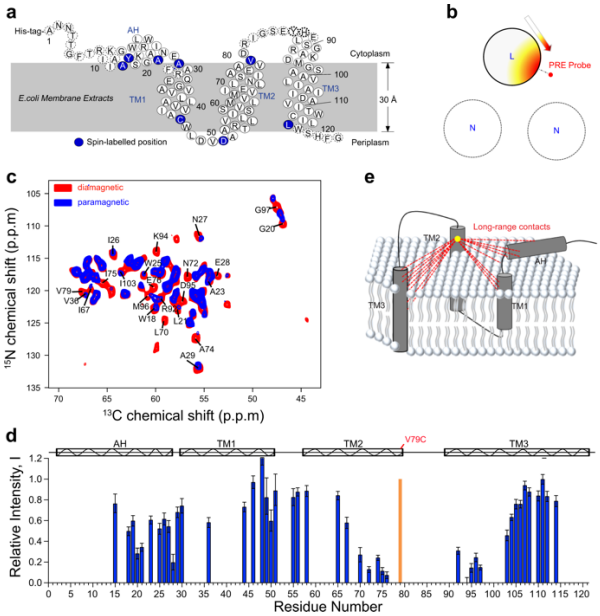

Figure 2: Diacylglycerol kinase studied by heteronuclear correlation MAS NMR (adapted with permission from (33), copyright 2021 Springer Nature).