



(Review)
**Solid-state NMR Study on Membrane Protein Structure
in Biological Condition**

Su-Jin Kang and Bong-Jin Lee*

Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742,
Korea

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Abstract : Membrane proteins play a essential role in the biological systems and it is not easy to handle a membrane protein for its structural study. Solid-state NMR (ssNMR) can be a good tool to investigate the structures and dynamics of membrane proteins. In ssNMR, Magic Angle Spinning (MAS) and Cross Polarization (CP) can be utilized to reduce the line-broadening, leading to high resolution and sensitivity in the spectrum. ssNMR, if combined with other spectroscopic methods, can provide us a enough knowledge on structures and dynamics of membrane proteins in biological condition.

Keywords: Membrane protein, Solid-state NMR, Magic angle spinning, Cross polarization

INTRODUCTION

Membrane proteins, functioning at the interface between a cell and its surrounding, play a key role in cellular and physiological processes like drug receptors, ion channels and solute transporters.¹ In addition, membrane protein comprises approximately 30% of expressed gene products and 70% of all pharmaceutically relevant proteins. However, membrane protein itself has a large size and a much

Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742,
Republic of Korea

email: lbjnmr@nmr.snu.ac.kr

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larger size in membrane systems, and its handling, such as expression and purification, is not easy, unlike soluble protein. Thus, it is difficult to apply X-ray crystallography and solution Nuclear Magnetic Resonance (NMR) spectroscopy to the structural study of membrane proteins because of its large size and a difficulty in sample preparation.²

Even though a large part of protein and peptide structures have been determined by X-ray crystallography as shown in Figure 1, NMR spectroscopy has become one of the most successful method for the characterization of three-dimensional (3D) structure and function of biomolecules during the last two decades. Solution NMR is generally considered to be used to study relatively small molecules (molecular weight $< \sim 30,000$) in solution, providing beautiful spectra with high resolution and sensitivity by the development of various 3D NMR techniques. However, for very large proteins or membrane-bound assemblies, spectral line broadening by reduced tumbling rates and correspondingly longer rotational correlation times should be the most severe obstacle. In such cases, a good alternative is the solid-state NMR (ssNMR) technique, which provides unique possibilities to study insoluble or noncrystalline molecules at an atomic resolution. In particular, ssNMR is a method of choice to investigate the structure and dynamics of both the lipid and the proteins in biological and model membranes.³ Since ssNMR shows extreme line broadening and poor sensitivity, techniques such as Magic Angle Spinning (MAS), Cross Polarization (CP) and multiple-pulse sequences in the ultrahigh magnetic fields should be adopted.² During a few decades, progressive development of hardware and techniques in ssNMR makes it possible to determine individual structural and dynamical parameters in insoluble and non-crystalline systems. By

accompanying with solution NMR and crystallographic methods, ssNMR will give insight into the structure and function of membrane protein.

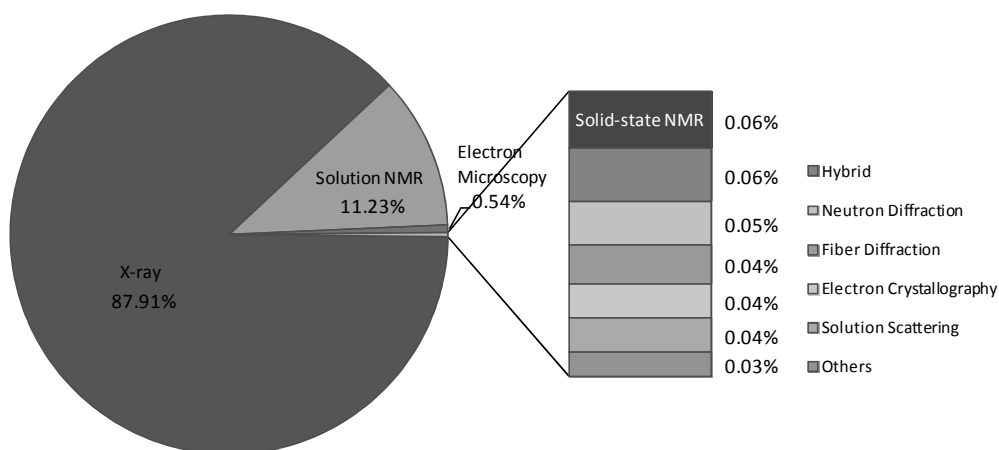


Figure 1. Composition of experimental methods in Protein Data Bank (data in Oct 2012, PDB)

Membrane Proteins and Solid-state NMR

In living cells, membrane proteins play a vital role in signal transduction, nutrient use, and energy exchange between cell and environment. A lot of proteins are associated with cellular membranes and serve as gates, channels, pumps, transporters, and enzymes in ion/molecule transport, energy regulation, signaling, etc.⁴ Moreover, the function of membrane protein often involves interactions with ligand molecules and represents an area of great pharmacological relevance. Therefore, membrane protein is leading to an enormous commercial interest as targets for drug

development. 3D structures of membrane proteins are invaluable to the understanding of their functions, especially mechanisms of disease.⁵⁻⁶

Although about 20-25% of proteins are membrane proteins, out of a total of 43,500 structures in the Protein Data Bank (PDB), only about 615 (1.4%) unique membrane protein structures have so far been solved. One reason that deposition of membrane protein structures in the PDB lags far behind existing structures for soluble proteins is the difficulties in sample preparation, such as protein expression, purification and crystallization.² Sufficient amount of protein expression is more difficult compared to soluble proteins.⁷ Protein purification is also more complicated due to their low solubility in water, so that membrane-mimetic constructs such as detergent micelles and bicelles are required during purification. In addition, a proper detergent and/or lipid system must be decided to reconstitute the purified protein in a biologically active form. Even if overcoming these all difficulties in a sample preparation of membrane protein, membrane proteins reconstituted into micelles have a large size, that solution NMR cannot cover, leading to slow tumbling in solution. Although there are alternative methods such as cryo-electron microscopy (cryo-EM) and atomic force microscopy (AFM), those cannot provide a enough resolution to investigate structures and dynamics of membrane proteins. In this regard, ssNMR is considered to be a useful technique for the structural study of membrane proteins.⁸

In the last few years, ssNMR has been an alternative method to investigate the structures and dynamics of membrane proteins in model and biological membranes.⁹ As same as other spectroscopies, ssNMR also requires high spectral resolution and signal-to noise ratio. However,

unlike in solution, the spectral resolution and the overall sensitivity of ssNMR decrease due to the protein size and the orientation dependence of the nuclear spin interaction, resulting in extreme line-broadening and poor sensitivity.¹⁰⁻¹¹ Therefore, high resolution conditions should be established by combining the important techniques in ssNMR, such as MAS and CP in ultrahigh magnetic fields.¹²

High resolution ^1H NMR spectroscopy is a powerful method for protein structure determination in solution. However, this method has not been extensively applied to membrane protein systems since the strong homogeneous dipole-dipole broadening between neighboring hydrogen nuclei is not completely averaged away in anisotropic non-spinning membrane systems and leads to spectral line-broadening. In order to solve this problem, ssNMR adopts MAS technique, in which the sample rotates fast around an axis at a spinning speed typically between 1 and 15 kHz. This results in a line-narrowing effect in membrane protein systems similar to the effect of isotropic tumbling in solution.^{4,13} The orientational dependence of the magnetic interactions (chemical shift anisotropy and dipolar coupling), which varies with $(3\cos^2\theta-1)/2$, averages to zero at the magic angle, $\theta=54.74^\circ$ relative to the magnetic fields (Figure. 2). It is possible to obtain high resolution ‘solution-like’ ^1H spectra in NMR spectra of membrane proteins.^{3,14}

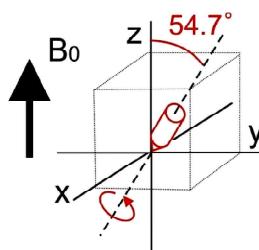


Figure 2. Magic angle, 54.7° , of sample rotor and direction of magnetic fields in ssNMR

In addition to MAS, cross polarization is one of the most important techniques in ssNMR. Biological ssNMR frequently use ^{13}C or ^{15}N nuclei with a low gyromagnetic ratio and long T_1 relaxation times, which require very long acquisition times to obtain sufficient signal. In CP technique, polarization from abundant spins such as ^1H or ^{19}F is transferred to dilute spins such as ^{13}C or ^{15}N , leading to enhance the signal and decrease the acquisition time. Polarization is transferred during the spin locking period, (the contact time) and a $\pi/2$ pulse is only made on protons (Figure 3).¹⁵ CP is widely used in ssNMR spectroscopy and frequently combined with other technique, such as MAS (CP MAS) NMR, to provide high resolution spectra.¹⁰

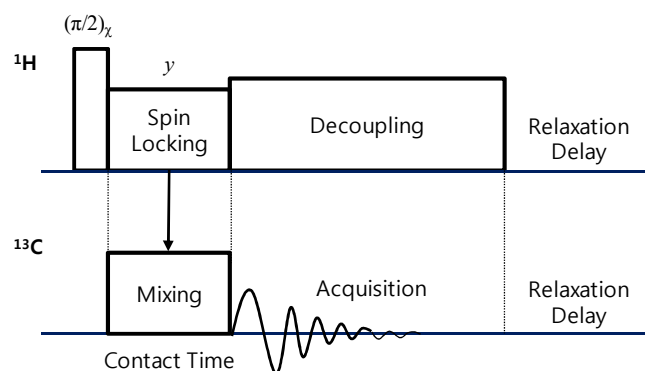


Figure 3. Pulse sequence of cross polarization. Polarization will be transferred during the spin locking period (the contact time).

Besides MAS and CP techniques, there are remarkable methodological improvements in ssNMR such as multiple-pulse sequence, hyperpolarization techniques and T_1 shortening experimental techniques. The techniques like segmental labeling and cell-free systems also can be helpful for studying membrane proteins using ssNMR.⁷

Conclusion

Membrane proteins are essential to signal transduction, nutrient use and energy exchange between the cell and environment. The knowledge on structures and functions of these membrane proteins has become a goal of modern structural genomics, especially considering its relation with disease. It has been demonstrated that ssNMR with solution NMR, X-ray crystallography and other spectroscopic methods can provide important information for protein folding, flexibility and function under biologically relevant condition, due to the advancements of techniques such as MAS and CP. However, it is clear that the methodology must be still much more developed for high resolution and sensitivity. In future, a big step will be taken towards the study of much more complex membrane protein systems thanks to the ssNMR.

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