Solid-State NMR for Molecular Science

Department of Materials Molecular Science
Division of Molecular Functions

NISHIMURA, Katsuyuki
Associate Professor
[nishimur@ims.ac.jp]

Education
1994 B.S. Himeji Institute of Technology (University of Hyogo)
1999 Ph.D. Himeji Institute of Technology (University of Hyogo)

Professional Employment
1999 Postdoctoral Fellow, National High Magnetic Field Laboratory, Florida State University
2001 Assistant Professor, Yokohama National University
2006 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies

Award
2002 The Young Scientist Poster Award, The Nuclear Magnetic Resonance Society of Japan

Member
Secretary
YOKOTA, Mitsuyo

Keywords Solid State NMR, Biomolecules, Developments

In order to elucidate functions of molecules, characterization of the molecule is the first step. There is a variety of important molecules, which are insoluble in any solvents and functional at amorphous state. Solid-state NMR enables us to obtain a variety of information at atomic resolution without damage to molecules and significant restrictions. Thus, solid-state NMR is one of the essential tools for the characterization of those molecules.

We have been working on methodology and hardware developments of solid-state NMR and their application to structural biology and materials science. We study characterization of membrane proteins and peptides, organic materials, natural products and synthetic polymers. Characterization of those molecules based on solid-state NMR is underway through collaborations with several research groups.

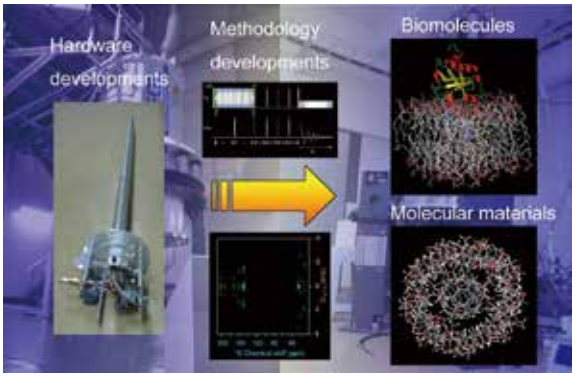

Figure 1. Outline of our studies.

Selected Publications

- N. Uekama, T. Aoki, T. Maruoka, S. Kurisu, A. Hatakeyama, S. Yamaguchi, M. Okada, H. Yagisawa, K. Nishimura and S. Tuzi, "Influence of Membrane Curvature on the Structure of the Membrane-Associated Pleckstrin Homology Domain of Phospholipase C-δ1," *Biochim. Biophys. Acta, Biomembr.* **1788**, 2575–2583 (2009).
- T. Iijima and K. Nishimura, "^{2}H Quadrupolar Carr-Purcell-Meiboom-Gill NMR for Paramagnetic Solids," *Chem. Phys. Lett.* **514**, 181–186 (2011).
- K. Yazawa, F. Suzuki, Y. Nishiyama, T. Ohata, A. Aoki, K. Nishimura, H. Kaji and T. Asakura, "Determination of Accurate ^{1}H Positions of Alanine Tripeptide with Anti-Parallel and Parallel β-Sheet Structures by High Resolution ^{1}H Solid State NMR and GIPAW Chemical Shift Calculation," *Chem. Commun.* **48**, 11199–11201 (2012).
- M. Tanio and K. Nishimura, "Intramolecular Allosteric Interaction in the Phospholipase C-δ1 Pleckstrin Homology Domain," *Biochim. Biophys. Acta, Proteins Proteomics* **1834**, 1034–1043 (2013).
- M. Yagi-Utsumi, K. Kato and K. Nishimura, "Membrane-Induced Dichotomous Conformation of Amyloid β with the Disordered N-Terminal Segment Followed by the Stable C-Terminal β Structure," *PLoS One* **11**, 0146405 (10 pages) (2016).
- N. Ousaka, F. Mamiya, Y. Iwata, K. Nishimura and E. Yashima, "'Helix-in-Helix' Superstructure Formation through Encapsulation of Fullerene-Bound Helical Peptides within a Helical Poly(methyl methacrylate) Cavity," *Angew. Chem., Int. Ed.* **56**, 791–795 (2017).

1. Characterization of Fluorinated Synthetic Polymer Based on Solid-State NMR[1)]

We have collaborated with a research group of Prof. Hiroki Fukumoto in Ibaraki university for the characterizations of fluorinated polymers newly designed in his group. Molecular design of polyaromatic hydrocarbons with fluorine unit is one of the central themes in investigation of functionalized organic materials such as *n*-type organic semiconductors and polymers for electronic devices. They have successfully developed new schemes of synthesis and copolymerization of fluorinated phenanthrene derivatives with hydroxyl groups based on the Mallory reaction using commercially available octafluorocyclopentene (OFCP). Since final polymers are insoluble in any organic solvents, we have utilized solid state NMR to characterize a polymerization state of this compound.

^{19}F possesses as large a gyromagnetic ratio as ^{1}H. Therefore, ^{19}F-X hetero-, and ^{19}F homonuclear dipolar couplings are as large as those for ^{1}H. In order to observe ^{13}C spectra in the presence of ^{19}F, it is essential to decouple ^{19}F during ^{13}C detection, which requires a ^{1}H-^{19}F-^{13}C triple resonance probe. Since a ^{19}F resonance frequency is very close to that for ^{1}H, it is usually difficult to isolate ^{1}H and ^{19}F channels well. Therefore, although a ^{1}H-^{19}F-X triple resonance probe is commercially available, it is commonly significantly expensive and their performances are limited. In addition, we do not possess such a probe. In order to overcome those problems, we have used high-speed magic angle spinning (MAS) techniques to attenuate ^{19}F homonuclear- and ^{19}F-^{13}C heteronuclear dipolar couplings as much as possible.

^{13}C observed solid-state NMR measurements for all the raw materials and polymers were carried out at spinning speed of 20 kHz using a ^{1}H-^{13}C-^{15}N triple resonance probe with an outer diameter of 2.5 mm at the ^{1}H-^{13}C double resonance mode. ^{13}C solid-state NMR spectra for the raw materials exhibited quite sharp signals, suggesting homogeneous local structures. In contrast, those for polymers showed broad signals, suggesting inhomogeneous local structures. Unfortunately, 2D correlation experiments were unable to be performed due to low sensitivity to the polymers. Only ^{13}C signals for the carbons directly attached to fluorine disappeared due to the strong ^{13}C-^{19}F heteronuclear dipolar couplings. However, most of the observed ^{13}C signals were successfully assigned based on a variety of 1D NMR spectra in combination of spectral editing techniques. Based on those analyses, the polymerization state of fluorinated phenanthrene derivatives was successfully characterized.

In addition, we have been working on collaboration work with two other research groups for the characterization of newly designed materials based on solid-state NMR.

2. Development of Solid-State NMR Probe

We have built a variety of solid-state NMR probes such as static and MAS probes for 400 MHz NMR, and a variable temperature MAS probe for 920 MHz ultra-high field NMR so far. Most of these probe buildings were achieved through major modifications of commercial probes. Since last year, we have been working on building an original solid-state NMR probe which is fully compatible with commercial instruments currently used. Probe developments enable us to reduce costs for acquiring probes and open up possibilities to design new experiments which are tightly related to specifically designed hardware. Currently, we are building a solid-state ^{1}H-X double resonance MAS probe. It will be extended to triple resonance probes for ^{1}H-^{13}C-^{15}N. In the near future, we would like to incorporate special functions into our original probes.

3. Structural Characterization of Amyloid β Protein Oligomer Promoted on Lipid Bilayers Using Solid-State NMR

Amyloid β (Aβ) protein is disordered in solutions under diluted conditions, however it conforms insoluble amyloid fibrils, which are found in senile plaque as a hallmark of Alzheimer's disease. Although molecular structures of amyloid fibrils have been determined, its molecular process for fibrillation in vivo has not been clarified yet. However, accumulated evidences suggest that the fibrillation process may be promoted on neuronal cell membrane. Especially, it has been reported that Aβ specifically interacts with ganglioside GM1 which is one of the key lipids in lipid raft. Therefore, GM1 embedded into lipid bilayers composed of neutral lipid DMPC may be regarded as the most simplified model neuronal cell membrane. In order to clarify the role of GM1 in the fibrillation process, first, we have successfully determined the oligomeric structure of Aβ (1-40) induced on DMPC bilayers based on solid-state NMR.[2)]

In the current study, to clarify the contribution of GM1 in the fibrillation process, we have attempted to characterize the oligomeric structure of Aβ (1-40) bound to lipid bilayers consisting of GM1 and DMPC. In order to achieve reliable signal assignments, we have re-examined ^{13}C-^{15}N heteronuclear solid-state NMR correlation experiments because of low sensitivities for previously obtained spectra. Then the signal assignments have been completed together with 2D ^{13}C homonuclear correlation data. Analysis of the secondary structure of Aβ based on the chemical shifts of assigned signals revealed that a disordered N-terminus was followed by two β-sheet structures from the middle region to the C- terminus. Comparison of secondary structures of Aβ in the absence and presence of GM1 in lipid bilayers suggests that the β-sheet structure in the middle region may be promoted through the interaction with GM1. In order to clarify intermolecular packing, ^{13}C space through homonuclear correlation experiments were carried out at various mixing times for the sample with spin labeled Aβ at the C-terminus. The correlation spectra for spin labeled sample, Ser^{26} signal clearly disappeared, suggesting the close location of Ser^{26} residue and C-terminal spin center. Based on the obtained data, a molecular packing model is currently under construction.

References

1) S. Kataoka, H. Fukumoto, T. Kawasaki-Takasuka, T. Yamazaki, K. Nishimura, T. Agou and T. Kubota, *J. Fluorine Chem.* **218**, 84–89 (2019).

2) M. Yagi-Utsumi, K. Kato and K. Nishimura, *PLoS One* **11**, 0146405 (10 pages) (2016).