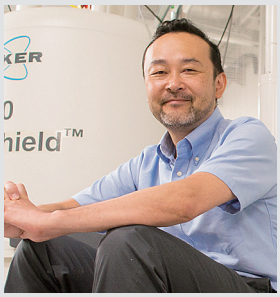
Solid-State NMR for Molecular Science

Department of Materials Molecular Science
Division of Molecular Functions



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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
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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 characterizations 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.

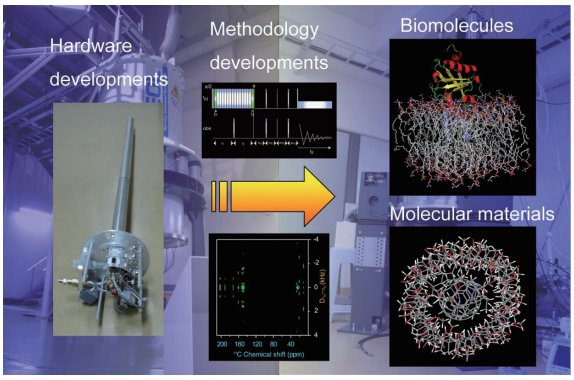

Figure 1. Outline of our studies.

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. Development of Solid-State NMR Probe

We have built a variety of solid-state NMR probes such as static and MAS ^{1}H-X double resonance probes for 400 MHz NMR, and a variable temperature ^{1}H-X double resonance MAS probe for 920 MHz ultra-high field NMR so far. Most of these probe buildings were achieved through major modifications of commercial probes. During the past few years, we have been working on building an original solid-state NMR probe which is fully compatible with commercial instruments currently used.

We have built original narrow bore solid-state NMR ^{1}H-X ("X" indicates variable resonant frequency) double resonance magic angle spinning (MAS) probe for 2.5 mm outer diameter (O.D.) sample tube used for 400 MHz (9.4 T) NMR spectrometer. The developed probe was built with originally designed parts except for spinning and spinning rate detection modules which were purchased from NMR company. The capacitive matching network design composed of commercially available non-magnetic variable capacitors was used. Balun type electric circuit was incorporated into ^{1}H channel, in which reduces to half the effective voltage of tuning capacitor and also minimize antenna effect of rf coil and rf inhomogeneity, especially at high field. Low frequency X channel was enabled to change largely its tunable frequency range to observe various nuclei by exchanging additional non-magnetic capacitors from bottom of the probe. The used network design may be compatible at higher fields by changing the parts related to resonant frequency.

Currently, the designs of individual parts are further updated and parts positions in the probe are further optimized to improve performance of the probe and access to the parts for the maintenance of the probe. ^{1}H-^{13}C-^{15}N triple resonance MAS probe was re-designed based on improved ^{1}H-X double resonance probe and is under building. We would like to replace two NMR modules purchased from NMR company to original ones. Therefore, we are currently attempting to design original spinning module for 4 mm sample tube in which a little bit easier than that for 2.5 mm sample tube. Probe developments enable to reduce cost for acquiring probes and open up possibilities to design new experiments which are tightly related to specifically designed hardware. In near future, we would like to incorporate special functions into our original probes.

2. 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.[1)] We have been collaborated with Prof. Kato group in IMS for those Aβ studies.

In the current study, Aβ (1-40) oligomer induced on lipid bilayers consisting of GM1 and DMPC have been attempted to characterize using solid-state NMR. All of essential solid-state NMR experiments such as ^{13}C-homonuclear- and, ^{13}C-^{15}N heteronuclear correlation experiments for signal assignments and dipolar coupling based ^{13}C-homonuclear correlation experiments to obtain distance information were completed.

As reported in last report, analysis of secondary structure of Aβ based on the chemical shifts of assigned signals revealed that disordered N-terminus followed by two β-sheet structures from middle region to C-terminus, in which differ from the one induced on DMPC bilayers.

During a year, the signal assignments were reconfirmed and dipolar coupling based ^{13}C-homonuclear correlation experiments were performed for the sample of [U-^{13}C,^{15}N] Aβ diluted with natural abundant Aβ at various mixing times to differentiate intra- and intermolecular correlations. Then intra- and intermolecular distance information was extracted through the analyses of those NMR data. By considering the result of paramagnetic relaxation enhancements (PRE) experiments as reported last year, promising intermolecular packing model was successfully obtained from the NMR data.

Currently, precise molecular structure of Aβ together with intermolecular packing configuration is under investigations based on NMR data with combination of computational science through collaboration with Prof. Okumura group in IMS.

3. Structural Characterizations of Molecular Materials Using Solid-State NMR

We have also been working on collaboration works with two other research groups, Prof. Yoshito Tobe in Osaka university and Prof. Nobuyuki Nishi in Aichi university of education for the characterizations of newly designed molecular materials based on solid-state NMR. Those projects are underway.

Reference

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