Solid-State NMR for Molecular Science

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Education
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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.

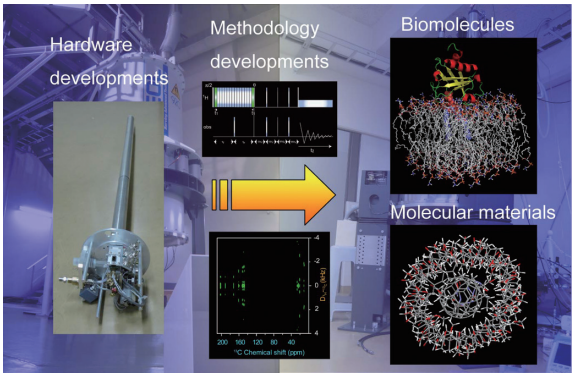

Figure 1. Outline of our studies.

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. Structural Characterization of Protein Using Solid-State NMR

Water bear possess extreme tolerance for environments. So far, the molecular mechanisms to protect their cells in such environments have not been clarified yet. Especially, under extremely dry environment, water bear takes torpor, then recovers under wet environment. It has been reported that several specific proteins have been expressed in water bear before torpor. Secretary abundant heat soluble protein (SAHS) is one of those proteins, and its biological functions have not been clarified yet. However, it has been considered that SAHS plays important roles to conserve their tissues, thus cells of water bear during torpor. The molecular structure of SAHS under hydrated state has been characterized precisely. In contrast, their structural information is limited under dry state. Therefore, we have been attempted to clarify the SAHS structure at dry state using solid-state NMR spectroscopy. This is collaboration project with Prof. Kato group in IMS and Prof. Yagi group in Nagoya city university.

Any proteins under dry condition may be expected to exhibit inhomogeneous structure. In order to investigate local structural and those homogeneity of SAHS at dry state, 2D dipolar assisted rotational resonance (DARR) ^{13}C-homonuclear correlation solid-state NMR measurements were carried out for the dry-state SAHS protein which only isoleucine residues are isotopically enriched by ^{13}C and ^{15}N. Those samples were newly prepared by improved procedures. Together with solid-state NMR analyses for several mutants for SAHS, signal assignments were successfully achieved. Then local secondary structures were identified through investigations of obtained ^{13}C chemical shifts of individual sites. Consequently, the result suggests that essentially most of secondary structure of SAHS are conserved even under dry state.

2. Developments of Spectral Editing Solid-State NMR Techniques

For the characterization of molecules using NMR, signal assignment is the first important step. Especially for biomolecules, such as proteins, ^{13}C and ^{15}N uniform isotope enrichments of samples is common and essential approach. Those isotope enrichments of samples enable to detect homo-, and heteronuclear correlation peaks among ^{13}C and ^{15}N nuclei at reasonable sensitivities. Those analyses enable structural characterizations of molecules. However, those isotope enrichments are not generally applicable, such as natural products and synthetic molecules via complicated processes. For such unlabeled samples, the efficient approach is quite limited due to the difficulties of observation of ^{13}C and ^{15}N correlation signals among natural abundant nuclei. Therefore, totally different approaches must be applied for their characterizations.

We have been working on developments of efficient spectral editing techniques to support accurate signal assignments for such unlabeled organic samples in solid-state NMR spectroscopy under both rigid and mobile sample conditions. The applicability of the developed technique has been verified for reference samples and their applications are under study.

3. Development of Solid-State NMR Probes

We have been working on developments of totally original solid-state NMR probes during a couple of years. In those probes, only spinning module and spinning counting module are remained as commercial parts. In order to replace those remained commercial parts to our original ones, we have been working on developments of original sample spinning modules for magic angle spinning (MAS) solid-state NMR probes which are fully compatible with currently using Bruker spectrometer and commercial sample tubes. As an initial stage, we started the design of a spinning module for standard 4.0 mm sample tube for Bruker. After two times of version up, our original spinning module reached to the spinning performance of commercial one from Bruker. The experimental performance of developed spinning module was evaluated by installing the module to the originally developed bench spinner system. By using Bruker automated spinning controller using standard parameters of drive- and bearing gas pressures for commercial 4.0 mm sample tube, our spinning modules achieved maximum sample spinning rate of 15 kHz for Bruker commercial sample tube. In addition, by using originally built manual spinning controller system with original drive-, and bearing-gas pressures, our spinning module achieved higher spinning rate over maximum spinning rate for Bruker commercial spinning module. Currently, a shrink version of the spinning module is under development in order to enable installation of the module to a narrow bore solid-state NMR probe with outer sleeve diameter of 38 mm.