# II-B Development of a Novel Solid-State NMR Technique

In order to alleviate an adversary effect of heating problem by irradiation of strong rf field for long duration, a novel solid state NMR technique has been explored as a means useful for a cross-polarization and separated local field NMR which enable to enhance sensitivity and determine relative orientation of the principal axes of the chemical shift and the heteronuclear dipolar interaction tensors, respectively. In the conventional approach, however, continuous rf irradiation of millisecond long rather than microsecond pulse used for conventional NMR is required. We have successfully developed a novel technique using weak rf fields without any serious loss of spectral quality. Therefore, this approach is essential for a study on biologically important molecules near under physiological conditions.

## II-B-1 Remarkable Reduction of RF Power by ATANSEMA and DATANSEMA Separated Local Field in Solid-State NMR Spectroscopy

**NISHIMURA, Katsuyuki; NAITO, Akira[1]**
*([1]Yokohama Natl. Univ.)*

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We proposed a novel approach to markedly reduce rf power for both $^{1}$H and observed nuclei during spin exchange for separated local field experiments. The rf power to satisfy the Hartmann-Hahn matching conditions during spin exchange for observed nuclei was arbitrarily reduced by alternating the directions of effective fields for $^{1}$H nuclei with unequal duration times and amplitudes. The proposed techniques were compared experimentally with those developed previously by the authors. The rf power for observed nuclei and average $^{1}$H were reduced by factors of 9 and 2, respectively, for $^{13}$C NMR signals of liquid crystalline 5CB.

**References**

1) K. Nishimura and A. Naito, *Chem. Phys. Lett.* **402**, 245–250 (2005).
2) C. H. Wu, A. Ramamoorthy and S. J. Opella, *J. Magn. Reson. A* **109**, 270–272 (1994).

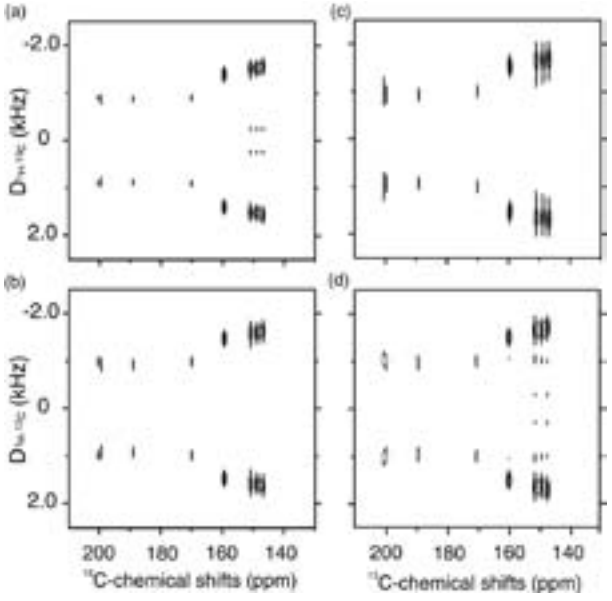


**Figure 1.** 2D-Separated local field $^{13}$C-NMR spectra aromatic region of 5CB in the liquid crystalline state at 20 °C obtained by (a) TANSEMA[1)] ($^{1}$H, $^{13}$C = 57.7, 11 W), (b) ATANSEMA ($^{1}H_{av}$, $^{13}$C = 34.4, 11 W), (c) DATANSEMA ($^{1}H_{av}$, $^{13}$C = 25.4 , 11 W), (d) PISEMA[2)] ($^{1}$H, $^{13}$C = 57.7, 97 W).

## II-B-2 Reduction of RF Power by Duration and Amplitude Time-Averaged Nutation Cross Polarization in Solid State NMR Spectroscopy

**NISHIMURA, Katsuyuki; NAITO, Akira[1]**
*([1]Yokohama Natl. Univ.)*

We have developed a new approach to markedly reduce rf power for both $^{1}$H and observed nuclei during cross-polarization under static condition. The rf power to satisfy the Hartmann-Hahn matching condition for observed nuclei was arbitrary reduced by alternating the direction of effective fields with unequal-duration times and -amplitudes. The proposed technique was compared theoretically and experimentally with the previously developed duration time averaged technique with and without $^{1}$H-homonuclear dipolar decoupling. The rf power for observed nuclei and averaged power for $^{1}$H were shown to be reduced experimentally by factors of 9 and 2, respectively, as manifested from $^{13}$C-NMR signals of MBBA in the liquid crystalline state without expense of reduced signals.

**References**

1) K. Nishimura and A. Naito, *Chem. Phys. Lett.* **380**, 569–576 (2003).
2) R. K. Hester, J. L. Ackerman, V. R. Cross and J. S. Waugh, *Phys. Rev. Lett.* **34**, 993–995 (1975).

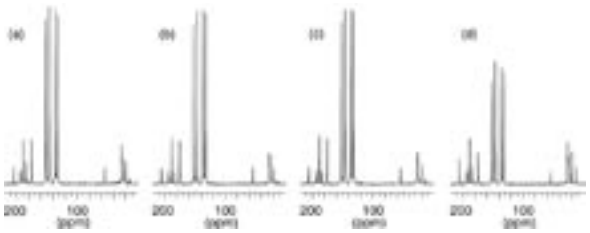

**Figure 1.** $^{13}$C-NMR spectra of MBBA in the liquid crystalline state at 20 °C obtained by (a) TANMA-CP[1)] ($^{1}$H, $^{13}$C = 57.7, 11 W), (b) ATANMA-CP ($^{1}H_{av}$, $^{13}$C = 34.4, 11 W), (c) DATANMA-CP ($^{1}H_{av}$, $^{13}$C = 25.4, 11 W), and (d) LG-CP[2)] ($^{1}$H, $^{13}$C = 57.7, 97 W).

# II-C Structural Characterization of Biomolecules by Solid State NMR

Solid state NMR is an excellent technique to examine structures and dynamics of biomolecules including membrane proteins or membrane-associated peptides at physiological temperature. In particular, we have explored to reveal the functional role of membrane proteins or peptides inside or at the membrane surface of fully hydrated lipid bilayers by using solid state NMR at ambient temperature.

## II-C-1 Histidines, Heart of the Hydrogen Ion Channel from Influenza A Virus: Toward an Understanding of Conductance and Proton Selectivity

**HU, Jun[1]; FU, Riqiang[1]; NISHIMURA, Katsuyuki; ZHANG, Li[1]; ZHOU, Huan-Xiang[1]; BUSATH, David D.[2]; VIJAYYERGIYA, Viksita[2]; CROSS, Timothy A.[1]**
*([1]Florida State Univ. and Natl. High Magnetic Field Laboraory; [2]Brigham Young Univ.)*

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The heart of the $H^+$ conductance mechanism in the homotetrameric M2 $H^+$ channel from influenza A is a set of four histidine side chains. Here, we show that protonation of the third of these imidazoles coincides with acid activation of this transmembrane channel and that, at physiological pH, the channel is closed by two imidazole–imidazolium dimers, each sharing a low-barrier hydrogen bond. This unique construct succeeds in distributing a pair of charges over four rings and many atoms in a low dielectric environment to minimize charge repulsion. These dimers form with identical $pK_{as}$ of 8.2±0.2, suggesting cooperative $H^+$ binding and clearly illustrating high $H^+$ affinity for this channel. The protonation behavior of the histidine side chains has been characterized by using solid-state NMR spectroscopy on the M2 transmembrane domain in fully hydrated lipid bilayers where the tetrameric backbonestructure is known. Furthermore, electrophysiological measurements of multichannel and single-channel experiments confirm that these protein constructs are functional.

## II-C-2 Conformational Changes of Adrenocorticotropic Hormone, ACTH (1-24), Bound to Lipid Bilayers, Dependent upon Proportion of Lipids Composition, as Studied by Solid State NMR

**NISHIMURA, Katsuyuki; MYOGA, Hiroki[1]; NARITA, Muneto[1]; KIRA, Atsushi[2]; NAITO, Akira[1]**
*([1]Yokohama Natl. Univ.; [2]ULVAC Inc.)*

Local conformations of ACTH (1-24), bound to fully hydrated multibilayers consisting of neutral and anionic lipids (DMPC/DMPG) at liquid crystalline, were examined by $^{13}C$-solid state NMR. It turned out that ACTH (1-24) exhibited conformational changes depending upon the lipid composition of DMPC to DMPG. The binding constants of ACTH (1-24) to the lipid bilay ers were determined by a quartz crystal microbalance (QCM) for various DMPC/DMPG proportion. The local maximum was seen at the proportion of DMPG/(DMPC + DMPG) = 0.25, which is close to the composition of neutral and anionic lipids occurring in human cells. This result suggests that ACTH (1-24) has favorable lipid composition in order to bind tightly lipid bilayers pior to reach receptor, together with changing its conformation depending on the lipid composition.