“Current Status and Perspective of Super-High Field NMRs Operated beyond GHz”

- ICMRBS2016 Satellite Workshop -

Organizers:
Hideaki Maeda, RIKEN, Japan
Yusuke Nishiyama, JEOL RESONANCE/RIKEN, Japan
Tatyana Polenova, University of Delaware, U.S.A.

Sponsors:
RIKEN, JEOL and JEOL RESONANCE

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Applied Superconductivity Research Group of the Cryogenics and Superconductivity Society of Japan
The NMR Platform of Japan

August 21, 2016

MASUKAWA Hall, Kyoto University, Japan
Preface

Welcome to the satellite workshop of ICMR 2016 on “Current Status and Perspective of Super-High Field NMRs Operated beyond GHz”, held on August 21, 2016, at Kyoto University, in Kyoto, Japan. The objectives of the workshop are (i) to overview current status and perspective of the super high field NMRs operated beyond 1 GHz, and (ii) to identify novel methodology and applications of NMR spectroscopy at super-high fields.

Magnets and probes: Conventional low temperature superconducting (LTS) NMR magnet is unable to exceed 1 GHz (23.5 T). The use of magnets, comprised of an HTS inner coil and an LTS outer coil, enables an NMR magnet to exceed 1 GHz. Such a super-high field NMR magnet has been developed by Japan with the demonstration of biomolecular NMR spectra at 1.02 GHz. Other super-high field NMR magnets operating beyond 1.2 GHz (28 T) are being developed by MIT and Bruker BioSpin. In addition, some EU and USA NMR facilities have developed super-high field NMR magnets, using a water cooled resistive inner coil and an LTS outer coil. Lectures at this workshop will cover these super-high field NMR magnets, in addition to the NMR probes.

NMR application: Super-high field NMR operating beyond 1 GHz provides ultra-high sensitivity and resolution, improving the efficiency of structure determination of biological molecules. Lectures presented at the workshop will cover the effect of super-high field NMR on the structure determination of intrinsically disordered proteins, non-coding RNA, membrane proteins, and large macromolecular assemblies: the effect on in-cell NMR will also be described. The intrinsic insensitivity of NMR spectroscopy of quadrupolar nuclei is largely overcome by using super high field NMRs; lectures will be given describing super high field NMR spectra of $^{17}$O, $^{35}$Cl, and $^{27}$Al.

The organizers will be pleased if the workshop promotes communication between the Applied Superconductivity Society and the NMR Society: such interaction is indispensable for the further development of super-high field NMRs.

Hideaki Maeda, RIKEN, Japan
Yusuke Nishiyama, JEOL RESONANCE/RIKEN, Japan
Tatyana Polenova, University of Delaware, U.S.A.
General Information

Subject:

Satellite workshop on “Current Status and Perspective of Super·High Field NMRs Operated beyond GHz”

[This Workshop is a part of the RIKEN Symposium Series.]

Objectives of the workshop:

(i) To overview current status and perspective of the super high field NMRs operated beyond 1 GHz.

(ii) To identify novel methodology and applications of NMR spectroscopy at super-high fields.

Date:

August 21 (Sun), 2016  9:00-15:10

Location:

Masukawa Hall, 1st floor, North Comprehensive Education and Research Bldg., North Campus, Kyoto University, Kyoto, Japan


Registration:
Register yourself at the on-site registration desk located in front of the Masukawa Hall, Kyoto University.

- **Contact address:**

  Hideaki Maeda;

  NMR Facility, RIKEN, 1·7-22, Suehiro-cho, Tsurumi, Yokohama, Japan;

  Tel +81·45-503-9267; Cell-phone 080-5049-9461(in Japan)

  maeda@jota.gsc.riken.jp; supermag12001@ezweb.ne.jp

- **Presentation:**

  - The lecturer can use his/her own PC for presentation, while the workshop prepares a Windows7-PC for presentation. Those who wish to use workshop-PC must download their file before the start of the session.

  - Please note that the time duration given to the lecture includes 5 min. for questions and discussion.

- **Transportation to the ICMRBS 2016:**

  - After the end of the workshop, two chartered-buses (50 passengers/ bus) will carry applicants to the Kyoto International Conference Center of the ICMRBS 2016: the bus will leave at 15:30 and arrive at the Kyoto International Conference Center at 16:00.

  - Please note that the chartered-bus stop is outside the university campus (see the campus map at p5), to which you have to walk for a few minutes.
Masukawa-Hall is located in the Kyoto University (building #13 in the campus)

The easiest way is to take a taxi from “Imadegawa” subway station or “Demachi-Yanaghi” station of Keihan railway to “Kitashirakawa” bus stop. It takes 5-10 min. by taxi and costs ~1,000 yen.

Another way can be seen in the following link.


You can directly type 35.031439, 135.786154 into Google map to get there.
Workshop Program

August 21, 2016

9:00-9:10
   Opening remarks; Hideaki Maeda (RIKEN)

   Chairperson: Hideaki Maeda (RIKEN)

9:10-9:40
   Ultrahigh magnetic fields and fast magic angle spinning for structure and
dynamics of protein assemblies; Tatyana Polenova (University of Delaware)

Super-high field NMR magnets operated beyond 1 GHz

9:40-10:10
   Super-high field NMR with a high temperature superconducting (HTS) magnet;
   Yoshinori Yanagisawa (RIKEN)

10:10-10:25
   NMR spectra achieved by the 1020 MHz NMR; Yusuke Nishiyama
   (JEOL RESONANCE/RIKEN)

10:25-10:55
   Development of High-Field HTS Magnets at MIT—MIT 1.3-GHz LTS/HTS
   NMR Magnet —; Yukikazu Iwasa (MIT)

10:55-11:35
   UHF magnets at Bruker; Gerhard Roth (Bruker BioSpin),
   Probes for ultra-high field NMR at Bruker; Rainer Kuemmerle (Bruker BioSpin)

11:35-12:30
   Lunch at a cafeteria (see the campus map, p5); a poster session will be held on
   superconductors and superconducting magnets, in addition to the NMR network
   of Japan.
Methodology and application of NMR spectroscopy at super-high magnetic fields

Chairperson: Yusuke Nishiyama (JEOL RESONANCE/RIKEN)
12:30-13:00
   High magnetic field for in-cell NMR; Lucia Banci (CERM and University of Florence)
13:00-13:30
   Solution NMR studies of intrinsically disordered proteins at ultrahigh fields; Peter E. Wright (The Scripps Research Institute)
13:30-14:00
   Prospects for ultra-high field NMR science at 1.5 GHz; Tim Cross (NHMFL)
14:00-14:30
   Ultra-high field NMR; Membrane protein and non-coding RNA; Toshio Yamazaki (RIKEN)
14:30-15:00
   Ultra-high field NMR: shining light on the dark sites of heterogeneous catalysts and advanced materials; Hiroki Nagashima, Frédérique Pourpoint, Julien Trébosc, Jean-Paul Amoureux, and Olivier Lafon (Ecole Nationale Supérieure de Chimie de Lille Cité Scientifique)

15:00-15:10
   Concluding remarks; Kiyonori Takegoshi (Kyoto University)

15:30
   Leaving for the Kyoto International Conference Center (ICMRBS 2016) by two chartered-buses (p 6). The bus stop is shown in the campus map at p 5.
Abstracts
In this presentation, I will discuss the critical importance of ultrahigh magnetic fields for studies of biological assemblies using MAS NMR spectroscopy. I will demonstrate that ultrahigh fields in conjunction with the emerging methodologies in the field of biological MAS NMR spectroscopy, including fast-MAS methods and dynamic nuclear polarization (DNP), enable atomic-level analysis of structure and dynamics of large macromolecular assemblies, such as those of HIV-1 viral and microtubule-associated proteins. These technologies are particularly powerful when integrated with other experimental and computational methods, yielding information inaccessible from any single technique.
Super-high field NMR with a high temperature superconducting (HTS) magnet

Yoshinori Yanagisawa¹,²

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²The NMR Facility, Division of Structural and Synthetic Biology, RIKEN Center for Life Science Technologies, Yokohama, Japan

NMR spectrometers that operate at a frequency higher than 1 GHz (i.e. a magnetic field strength of 23.5 T) are required for further breakthroughs in the fields of structural biology. The critical fields of metal low-temperature superconductors (LTS) used for conventional NMR magnets limited the maximum operating frequency to ~1 GHz. In combination with LTS outer coils, the use of inner coils made of oxide high-temperature superconductors (HTS), such as Bi₂Sr₂Ca₂Cu₃O₁₀₋ₓ (Bi-2223) and (RE)Ba₂Cu₃O₇₋ₓ (REBCO; RE = Rare Earth), is the effective option to realize a super-high field NMR since they have very high critical fields >> 23.5 T. Technical problems, however, appeared in developing working coils with the HTS conductors, which posed challenges for HTS magnet development. A Japanese team, including the author, performed basic research on the problems and then developed a 500 MHz (11.7 T)-class LTS/HTS (Bi-2223 or REBCO) NMR magnets. The performance of the magnets was evaluated through NMR measurements, which were the first demonstration of high-resolution NMR measurements obtained by a LTS/HTS NMR magnet. Eventually a 1.02 GHz (24.0 T) LTS/Bi-2223 NMR was developed based on those technologies, which was the world’s first NMR with an operating frequency higher than 1 GHz.

Acknowledgement:
This work was supported by SENTAN and the Strategic Promotion of Innovative Research and Development Program, JST. I would like to show my gratitude to the collaborators in JEOL RESONANCE, JASTEC, NIMS, Chiba University, Sophia University, and RIKEN.
NMR spectra achieved by the 1020 MHz NMR

Yusuke Nishiyama$^{1,2}$

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$^2$RIKEN CLST-JEOL Collaboration Center, Yokohama, Kanagawa, Japan

The combined use of HTS (high $T_c$ superconductor) and LTS (low $T_c$ superconductor) enables the static magnetic field higher than 23.5 T with NMR quality stability and homogeneity. Here we will present a set of solid-state NMR spectra observed at 24.0 T ($^1$H Larmor frequency: 1020 MHz). The high magnetic field is beneficial to improve both the resolution and sensitivity, especially, $^1$H NMR at fast MAS and central transition line of quadrupolar nuclei. Indeed the $^1$H NMR measured at 24 T under 60 kHz MAS gives comparable or even better resolution than that at 14.1 T under 110 kHz. The long term stability of the magnetic field is stable enough for time-consuming 3D experiments. Half-integer quadrupolar NMR are also demonstrated. The $^{35}$Cl NMR spectra measured at 24 T achieve 7 and 1.7 times better sensitivity and resolution, respectively, than those at 14.1 T. The high sensitivity allows us to measure $^{35}$Cl MQMAS spectra as well. The narrow central transition allows measuring $^{27}$Al double quantum/single quantum homonuclear correlation spectra. $^1$H/$^{35}$Cl and $^1$H/$^{27}$Al HMQC correlation spectra at ultra-high magnetic field are also discussed.
Development of High-Field HTS Magnets at MIT

— MIT 1.3-GHz LTS/HTS NMR Magnet —

Yukikazu Iwasa

Francis Bitter Magnet Laboratory, Plasma Science and Fusion Center, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.

In this presentation I describe development of high-field HTS magnets at MIT, focusing on the MIT 1.3-GHz LTS/HTS NMR magnet (1.3G). Discussion on 1.3G includes: 1) a brief history, 2) selected design details; and 3) technical challenges.

Acknowledgement

This work was supported by the National Institute of General Medical Sciences.
UHF Magnets at Bruker

Gerhard Roth

Bruker BioSpin GmbH, Wikingerstr. 13, 76189 Karlsruhe, Germany

The presentation will give an overview over the current UHF magnet technologies at Bruker. These are based on low-temperature superconductors (LTS) and are suitable for ultra-high field, high-resolution NMR magnets of up to 1 GHz, as well as for UHF-FTMS or MRI magnets of up to 21 T.

Based on these technologies, current development focuses on the implementation of high-temperature superconductors (HTS) to break the 1 GHz barrier and to establish the appropriate magnet technologies for HTS conductors. Bruker is presently developing high-resolution, persistent 1.1 GHz wide-bore (89mm) and 1.2 GHz standard-bore (54mm) high-resolution NMR magnets for various European customers. For the new UHF magnets containing HTS conductors there will be no compromise with respect to homogeneity or drift. Deliveries are expected to start in late 2017.
As new UHF Magnets are becoming available, probe technology as well as NMR applications are facing new challenges and require optimization. Besides examples for solid state Bio-NMR as well as solution state Bio-NMR the presentation will focus around the impact of noise generated by the sample itself. RF design optimizations as well as possible optimizations for the NMR spectroscopist choosing different sample preparations will be addressed. Competitiveness and perspectives of $^{13}$C and $^{15}$N detection approaches in solution state Bio-NMR with respect to the typical first choice of indirect detection methods will be discussed.
High magnetic field for in-cell NMR

Lucia Banci
CERM and Department of Chemistry, University of Florence, Sesto Fiorentino, Italy

The availability of high magnetic field NMR spectrometers has made possible a series of experiments and types of applications not feasible, or hardly applicable, at lower fields.

Among these, one of the most challenging and of higher impact is in-cell NMR, i.e. collection of high resolution NMR spectra of biomolecules in intact, living cells. This type of experiments allows us to obtain information on the conformational and functional properties of biomolecules at atomic resolution in conditions as closer as possible to the physiological ones.

A few examples of the striking power of this approach will be presented for a few systems and functional aspects, with particular focus on the meaningful differences in biomolecules properties in living cells with respect to the in vitro features.


Barbieri L, Luchinat E and Banci L. Protein interaction patterns in different cellular environments are revealed by in cell NMR. Scientific Reports 5:14456: DOI: 10.1038/srep14456, 2015.


Solution NMR studies of intrinsically disordered proteins at ultrahigh fields

Peter E. Wright
Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA

35-50% of proteins in eukaryotic proteomes are either fully disordered (IDPs) or contain both folded domains and long, contiguous regions (IDRs) that are disordered. The unique characteristics of IDPs allow them to play a central role in dynamic regulatory and assembly processes in the cell. Disordered proteins mediate critical regulatory functions, including regulation of transcription, translation, the cell cycle, and numerous signal transduction events, and drive the assembly of cellular complexes and membrane-less cellular compartments. IDPs are directly involved in many debilitating diseases including cancer, leukemia, neurodegenerative diseases, cardiovascular disease, diabetes, and infectious disease. Elucidation of the structural ensembles, dynamics, interactions, posttranslational modifications, and function of IDPs represents a major challenge to which many of the traditional structural biology tools are poorly suited. A reductionist approach, in which IDPs are dissected into fragments, can be misleading and development of methods for analysis of full-length IDPs is essential. Because of its unique capabilities, ultrahigh field NMR promises to emerge as the central, enabling technology for characterization of IDPs. NMR analysis of IDPs is highly challenging because of (i) limited dispersion and pathological signal overlap, (ii) large variation in line widths, especially but not only in proteins that contain both structured and disordered regions, (iii) exchange broadening in spectra of free IDPs and their complexes, (iv) frequent need to work at low □M concentration to avoid self-association. Ultrahigh field NMR instruments promise to provide solutions to these problems and provide essential technology that will drive our understanding of IDP structure, dynamics, and functional interactions.
Prospects for Ultra-High Field Science at 1.5 GHz

Tim Cross

National High Magnetic Field Laboratory, Tallahassee, Florida, U.S.A.

The 36T Series Connected Hybrid is nearing completion at the NHMFL in Tallahassee. This magnet has a 14T superconducting outsert that will operate at 4K and a 285K resistive insert that will provide 22T. 13MWatts of power will circulate through the cable-in-conduit outsert and through the Florida-Bitter plates of the resistive coil to generate a 36T field. As of August 8th the superconducting component is at 4K and an iron shield is being installed to minimize field fluctuations due to the 45T hybrid being ramped up and down frequently in a nearby magnet bay. By the end of August 5MW operation of the SCH will be tested to confirm complete compatibility between the magnet and power supply. Full field testing will occur in September along with field mapping. A triple resonance Bruker Avance IV console has been delivered with capability to operate at proton frequencies of 1.0, 1.2, and 1.5 GHz. Consequently, direct comparison between spectra in the Hybrid and in LTS superconducting magnets can be made. Stability of this powered system will be improved with either the enhanced Bruker Lock systems or with an advanced system that has been developed over the past decade between Bill Brey at the Magnet Lab and Jeff Schiano at Penn State.

There is no pretense that this magnet will deliver the high homogeneity high stability spectra of a persistent superconducting magnet, but in multiple scientific arenas we anticipate that it will provide very exciting data. Indeed, this will be the first time we will have preliminary data for grant proposals aimed at funding LTS/HTS magnets. Stability is the primary challenge and we anticipate that Schiano’s Cascade Compensation System that estimates fast and slow fluctuations separately using sensors better suited for each frequency regime will perform very well. This system has been shown to reduce field fluctuations by more than two orders of magnitude in purely resistive magnets. The technology coupled with the large inductance of the superconducting coil is anticipated to lead to significant improvements in field stability. With a bore of 40 mm a slim shim set having only a few gradient coils will be used along with ferro shims mounted on the probe cap to enhance homogeneity.
Multiple probes have been constructed for an initial set of experiments that range from structural biology to chemistry and materials science. One of the most exciting scientific arenas will be spectroscopy of odd-halves quadrupolar nuclei. Maybe, in particular, $^{17}$O spectroscopy of sites where so much chemistry and biochemistry takes place and yet so little spectroscopy has been performed will provide some of the most exciting results. We hope that the international scientific community will join with us to obtain these exciting results at such a high field to stimulate the flow of resources for developing HTS superconducting magnets at this field and beyond.
Ultrahigh field NMR: membrane protein and non-coding RNA

Toshio Yamazaki
RIKEN Center for Life Science Technologies, Yokohama, Japan

As examples of biomolecular application, a membrane protein for solid-state NMR and non-coding RNA fragments for solution NMR are presented. In RIKEN NMR facility, up to 900 MHz spectrometers are installed for solid-state and solution experiments. The benefits at high field (900 MHz) are resolution and sensitivity, which are needed for the analysis of complicated molecules. Simple extrapolation to ultrahigh field (1.4-fold jump) for the 2D experiments gives 2-fold spectral space. We expect to get resolving power for molecules of 2-fold size. On the other hand, we also start to observe difficulties of high field NMR. By observing field dependency (600-900 MHz), we can estimate the seriousness at ultrahigh field.

In cells, far more species of non-coding RNAs are transcribed than mRNAs coding proteins. They are expected to have structure and function as RNA. Because of low conservation even in vertebrate, it is difficult to extract essential regions. A simple NMR experiment can provide base-pairing information (number and stability), because imino protons are NMR-visible only in base pair. This is useful for screening of structured fragment. If fragment is appropriate for further analysis, then we can proceed to the stage of NMR measurements of NOE and RDC for structure determination. For small fragments, base-pair measurement and assignment is trivial, but functional unit of typical size (UCHL1 SINEUP:167 nt) is large enough to make the analysis more difficult. The spectral resolution provided by ultrahigh field is anticipated. The difficulty at higher field is sensitivity drop by salt concentration in the sample. Because RNA molecule has uniform negative charge, salt is required to stabilize the structure. The sensitivity loss is produced by eddy current in the sample. At ultrahigh field, dielectric loss of H$_2$O (even without salt) will be serious. We have to limit the volume of sample (the shape of container is important). Higher concentration is acceptable for RNA sample, because solubility is good.

The extreme of high concentration is solid state. We are also working on membrane protein with solid-state NMR. Higher the magnetic field, higher MAS rate is required to suppress chemical shift anisotropy. The sample rotor becomes smaller. At the
ultrahigh field, only ultrafast MAS will be acceptable and $^1$H detection experiments will be superior to $^{13}$C detection experiments in many cases. In this presentation, however, we focus on $^{13}$C detection experiments. The size of basic membrane proteins is about 300 amino acids. The number of signals is already too big to resolve all of them in the $^{13}$C detection 3D experiments. For the water channel, aquaporin Z (231 amino acids), we achieved 30% assignments of backbone CA, CB, CO, N atoms from data of 700 MHz NMR. More unassigned signals were left because of ambiguity of connection and loss of amino acid type information. We need enhancement of resolution by magnetic field. We can observe the improved resolution at 900 MHz and also at 1020 MHz. In the solid-state NMR, the effect of lossy sample is again serious. Because of $^1$H high power, the electric current in the sample produces heat. The probe designed for minimal electric field is required.
Ultra-high field NMR: shining light on the dark sites of heterogeneous catalysts and advanced materials

Hiroki Nagashima, Frédérique Pourpoint, Julien Trébosc, Jean-Paul Amoureux, Olivier Lafon*
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The rational development of improved heterogeneous catalysts and advanced materials can be undertaken in a rational way by a better understanding of their structures. Because it can give information on the local structure and dynamics at atomic scale, Solid-State Nuclear Magnetic Resonance (SSNMR) spectroscopy is very well suited to the study of heterogeneous catalysts as well as disordered or amorphous materials.

Nevertheless, about 74% of NMR-active isotopes, including $^{11}$B, $^{14}$N, $^{17}$O, $^{27}$Al, $^{45}$Sc or $^{67}$Zn, have a spin number $I \geq 1$ and their signal is broadened by the quadrupolar interaction. This broadening limits both sensitivity and resolution, thus hampering the extraction of site-specific structural information. As this broadening, when expressed in ppm, is inversely proportional to the square of the gyromagnetic ratio, the SSNMR detection of low-$\gamma$ quadrupolar nuclei, such as $^{14}$N, $^{67}$Zn or $^{25}$Mg, with $\gamma \leq \gamma(^{15}$N) is especially challenging and SSNMR studies of these isotopes are relatively sparse even if they represent almost 40% of NMR active nuclei. Furthermore, the lack of sensitivity and resolution is exacerbated in the case of quadrupolar nuclei located at the surface of heterogeneous catalysts since the surface sites often represent a small fraction of all sites and correspond to asymmetric environments subject to large

Figure 1. Simulated NMR spectra of $^{17}$O surface sites at different magnetic field. The estimated acquisition time is indicated on the right-hand
quadrupolar interaction.

As the second-order quadrupolar broadening in ppm is inversely proportional to the square of the static magnetic field, $B_0$, the use of high magnetic field is highly beneficial for SSNMR of quadrupolar nuclei. We will present recent high-field SSNMR studies of quadrupolar nuclei in heterogeneous catalysts, such as Metal-Organic Frameworks, amorphous silica alumina and zeolites. In recent years, our group has notably developed original techniques to probe proximities involving quadrupolar nuclei in solids. These techniques are powerful tool for the selective observation of surface nuclei and for probing the interactions between surface sites and reagents. We will also discuss how the advent of high-resolution NMR magnet with $B_0 > 23.5$ T (i.e. $\nu_0(^1\text{H}) > 1$ GHz) opens new avenues in studies of quadrupolar nuclei in heterogeneous catalysts and advanced materials.