High-Field Magnetic Resonance Facility

The High-Field Magnetic Resonance Facility (HFMRF) brings a powerful synergy of creative scientific staff and unique instrumentation to bear on complex scientific problems. HFMRF is equipped with state-of-the-art nuclear magnetic resonance (NMR) and pulsed electron paramagnetic resonance (EPR) instruments, all of which play a role in determining molecular structures that are relevant to environmental remediation efforts, materials development for national energy needs, and biological health effects.

HFMRF offers unique tools and techniques designed in-house to enable novel research, including 1) *in situ* catalysis probes, 2) radionuclide NMR capabilities, 3) solid-state NMR cryogenic probes for direct observation of metals in macromolecules, 4) high-temperature probe technology, 5) laser-polarized gas for visualizing gas-filled spaces using magnetic resonance imaging (MRI), and 6) pulsed EPR techniques designed to follow conformational changes in membrane protein complexes containing metal clusters. In collaborative partnership with world-class scientists around the globe, we forge innovative approaches to some of the most pressing research needs in environmental molecular science and other national research priorities.

Staff and science consultants within this facility offer expertise in the areas of structural biology, solid-state materials characterization, and MRI techniques. Research activities include structure determination of large molecular assemblies such as protein-DNA (normal and damaged DNA) and protein-RNA complexes that model assemblies that may form as a cellular response to chemical or radiological insults; examination of conformational changes in membrane protein complexes involving metal clusters using pulsed EPR; NMR-based structural and functional genomics; multi-nuclear detection and catalyst and materials characterization using solid-state techniques; and non-invasive biological imaging, integrated magnetic resonance and confocal microscopy, and slow-spinning NMR to study cell systems.

Instrumentation & Capabilities

NMR and EPR

- 900-MHz NMR
- 800-MHz NMR
- 750-MHz NMR
- 600-MHz NMR (two systems)
- 500-MHz (two wide-bore systems and one narrow-bore system)
- 300-MHz wide-bore NMR (two systems – one radionuclide capable)
- Horizontal-bore 2-tesla NMR
- EPR spectrometer with electron nuclear double resonance (ENDOR)/electron-electron double resonance (ELDOR) capability

Additional Capabilities

- Combined optical and magnetic resonance microscope
- Low-temperature probes for metallo-protein chemistry and structure
- Virtual NMR capability enabling use and collaboration with EMSL scientists for remote users via secure shell over the internet

Since EMSL opened in October 1997, HFMRF has been one of the highest-volume experimental user facilities in support of local and external user research programs. During Fiscal Year 2006, HFMRF supported several projects in which 80 external scientists used the NMR spectrometers.

The research interests of staff and users include some of the most exciting areas in modern molecular biology and biochemistry:

- **Structural/Functional Genomics**. Determination of three-dimensional structures of DNA, RNA, proteins, and enzymes and their intermolecular associations. Particular interests and collaborations exist relative to protein fold classification and sequence-structure-fold relationships.

- **Biomolecular Complexes**. Understanding the molecular interactions of larger complexes of biomolecules (proteins, DNA, RNA, and mimetic membranes) that are key regulators in cell signaling and growth (e.g., DNA damage recognition and repair processes).

- **Biological Imaging**. Acquisition of imaging and corresponding chemical information in biological samples, with particular interest in development of combined magnetic resonance and optical spectroscopy techniques to observe and elucidate biological processes.

- **Solid State**. Low-gamma nuclei detection, ultra-low-temperature NMR for sensitivity enhancement, and slow-magic-angle-spinning (MAS) methodologies for nondestructive research of cells, tissues, small animals, and bacterial colonies.

- **Measurement Science and Instrumentation Development**. Development and application of novel and unique NMR instrumentation techniques for biological and environmental problems.

Capabilities

Varian INOVA 900. The Varian 900 (Figure 1) is an INOVA-based spectrometer utilizing an Oxford 21.1-tesla (T) magnet with a 63-mm room-temperature bore. This system is capable of high-resolution liquid- and solid-state NMR. There are four radio frequency (RF) channels with waveform generators and triple-axis pulsed-field gradients. The wide-line analog digital converters (ADCs) run at 5 MHz and the narrow ADCs have a maximum rate of 500 kHz. This console also has a solids variable-temperature (VT) control capability. We currently have a 5-mm HCN probe with X, Y, and Z axis gradients for liquids, a 5-mm orthogonal HX powder probe optimized

Figure 1. 900-MHz NMR spectrometer.

for low-gamma nuclides, a 5-mm orthogonal H-N-Zn triple-tuned probe for powders, and a 3.2-mm low-gamma HX MAS probe (24 kHz spinning).

Varian INOVA 800. The Varian 800 (Figure 2) is an INOVA-based spectrometer utilizing an Oxford 18.8-T magnet with a 63-mm, room-temperature bore. This system is capable of high-resolution liquid- and solid-state NMR. There are four RF channels with waveform generators and pulsed-field gradients. The wide-line ADCs run at 5 MHz, and the narrow-line ADCs run at a maximum rate of 500 kHz. This console also has a solids VT control capability. Available probes include a 5-mm HCN cryoprobe and two room-temperature 5-mm HCN probes with Z gradient for liquids, a 4-mm HXY MAS probe (25-kHz spinning, VT-capable), a 5-mm HX orthogonal powder probe optimized for low-gamma (38 to 65 MHz) nuclides, and a 5-mm HX static low-temperature probe (3.8 to 300 K). A 5-mm HX MAS probe (12-kHz spinning) is under construction.

Figure 2. Varian INOVA 800-MHz NMR spectrometer.

Figure 3. Varian INOVA 750-MHz NMR spectrometer.

Varian INOVA 750. The Varian 750 (Figure 3) is an INOVA-based spectrometer utilizing an Oxford 17.6-T magnet with a 51-mm, room-temperature bore. This system is capable of high-resolution liquid- and solid-state NMR. There are four RF channels with waveform generators and pulsed-field gradients. The narrow ADCs have a maximum rate of 500 kHz. We currently have two 5-mm HCN probes (Z gradient), a 5-mm HCP probe (Z gradient), a 5-mm HX MAS probe (X tuning range is 321 to 130 MHz; the spinning speed is rated to 12 kHz), and two 5-mm HX MAS probes (15-kHz spinning) with X tuning ranges of 60 to 120 MHz and 30 to 50 MHz.

Figure 4. Varian INOVA 600-MHz NMR spectrometer with Cryoprobe.

Varian INOVA 600. The Varian 600 (Figure 4) is an INOVA-based spectrometer utilizing an Oxford 14.1-T magnet with a 51-mm, room-temperature bore. This system is capable of high-resolution liquid-state NMR. There are four RF channels with waveform generators and pulsed-field gradients. The narrow-line ADCs run at a maximum rate of

500 kHz. We currently have a cryogenically cooled and a room-temperature 5-mm HCN probe with Z gradient and a 5-mm HX probe (X tuning range is 242 to 60 MHz).

Figure 5. Varian INOVA 600- MHz NMR spectrometer.

Varian Unity 600. The Varian 600 (Figure 5) is an INOVA-based spectrometer utilizing an Oxford 14.1-T magnet with a 51-mm, room-temperature bore. This system is capable of high-resolution liquid-state NMR. There are three RF channels with waveform generators and pulsed-field gradients. The narrow-line ADCs run at a maximum rate of 500 kHz. We currently have a 5-mm pentaprobe (proton, phosphorus, carbon, nitrogen and deuterium) with Z-gradient, a 5-mm HCN probe with Z gradient and a 5-mm HX probe (X tuning range is 242 to 60 MHz).

Figure 6. Varian NMR System 500-MHz wide-bore NMR spectrometer.

Varian NMR System 500 Wide Bore. The Varian 500 Wide Bore (Figure 6) has a new VNS-based spectrometer console and utilizes an Oxford 11.7-T magnet with an 89-mm room-temperature bore. This system is capable of a full range of solid-state NMR, experiments, including windo-less sequences and PISEMA. There are three RF channels with waveform generators. The wide-line ADCs run at 5 MHz. We currently have a new 4-mm HXY MAS probe capable of bio-solids triple resonance experiments and a new 7.5-mm HX MAS probe (8-kHz spinning), an HX single-crystal probe, a ^{1}H CRAMPS probe, a micro-coil imaging probe, a 40-mm imaging probe, and a static HX low-temperature probe (2 to 300 K).

Figure 7. Bruker Avance 500-MHz NMR spectrometer.

Bruker Avance 500 Wide Bore. The Bruker Avance 500 Wide Bore (Figure 7) is a micro-imaging system using an 89-mm vertical room-temperature bore. The system is capable of imaging mice and also has high-resolution liquid magnetic resonance capabilities with a Bruker 10-mm QNP probe. This liquid probe has a ^{1}H outer coil and an inner coil that is switchable among ^{13}C, ^{31}P, and ^{19}F with no gradients. The system is equipped with a combined confocal and magnetic resonance microscope capable of monitoring single layers of eukaryotic cells in a perfusion system simultaneously with both modalities.

Varian/Chemagnetics Infinity 500. The Chemagnetics 500 (Figure 8) is an Infinity-based spectrometer utilizing an Oxford 11.7-T magnet with a 51-mm, room-temperature bore. This system is capable of high-resolution liquid- and solid-state NMR. It has three RF channels and is equipped with both 16- and 14-bit ADCs. The solution state probes for this instrument include a 5-mm HCN gradient probe, a 5-mm DB gradient probe (X tuning range is 208.1 to 49.5 MHz), and a 10-mm HX probe (X tuning range is 218.6 to 21.2 MHz). There are two solid-state probes, a 5-mm HX MAS probe (X tuning range is 206.6 to 47 MHz; the spinning speed is rated to 12 kHz) and a 6-mm HX MAS probe (X tuning range is 218.6 to 48.7 MHz, H/F tuning range is 510.6 to 459 MHz; the spinning speed is rated to 9 kHz).

Figure 8. Varian/Chemagnetics Infinity 500-MHz NMR spectrometer.

Varian/Chemagnetics Infinity 300. The Chemagnetics 300 (Figure 9) is an Infinity-based spectrometer utilizing an Oxford 7.02-T magnet with an 89-mm room-temperature bore. This system is capable of high-resolution liquid- and solid-state NMR. It has three RF channels and is equipped with both 16- and 14-bit ADCs. The solution-state probes for this instrument include a 5-mm HX probe and a 10-mm HX probe. The solids probes are a 7.5-mm HX MAS probe (X tuning range is 136.7 MHz to 29.5 MHz; H tuning range is 274.7 to 349.1 MHz; spin rate is rated to 7 kHz) and a 5-mm HXY MAS probe (X tuning range is 129 to 57.4 MHz; Y tuning range is 85.1 to 21.2 MHz; spin speed is rated to 12 kHz). Further probes available are a 7-mm HX MAS probe (10-kHz spinning), an HX single-crystal probe, a ^{1}H CRAMPS probe, a single-tuned HX 5-mm, low-temperature MAS probe (35 to 300K, 12-kHz spinning), a 7-mm HX high-temperature probe (-100 to 500°C, 7-kHz spinning)

Figure 9. Varian/Chemagnetics Infinity 300-MHz NMR spectrometer.

Horizontal-Bore 2-T Magnet. The 2-T magnet (Figure 10) provides unique capabilities for the HFMRF. It is connected to a Varian Unity+ console with two RF channels and wideline 5-MHz

Figure 10. Horizontal-bore 2-T magnet.

ADCs. It has a 30-cm, room-temperature bore and is equipped with an imaging gradient set capable of 50 gauss/cm. It is suitable for small animal or large sample imaging and *in vivo* spectroscopy. Three homemade birdcage coil probes are available: 8-cm and 5-cm imaging/spectroscopy probes and a 5-cm ^{3}He probe.

Bruker Pulsed EPR/ENDOR/ELDOR Spectrometer. This multi-functional pulsed EPR spectrometer (Figure 11), operating in the X-band near 9.5 GHz, permits application of modern pulsed magnetic resonance techniques to systems containing unpaired electron spins. The system is based on the Bruker EleXsys console and SuperX-FT microwave bridge, which allow both ELDOR (electron-electron double resonance) and ENDOR (electron nuclear double resonance) measurements. A number of probes for both continuous-wave and pulsed spectroscopy are included, with an operating temperature ranging from room temperature to below liquid helium. System capabilities include measurement of g-tensors; hyperfine and nuclear quadrupole-coupling tensors for the study of electronic wavefunction of free radicals and metallo-proteins; and determination of small dipolar interactions by pulsed ELDOR and double electron-electron resonance methods for the measurement of distances between radicals in solids or between spin labels in proteins.

Figure 11. Bruker pulsed EPR/ ENDOR/ ELDOR spectrometer.

Upgrades

In 2006, the HFMRF has improved research capabilities both by upgrading current instrumentation and acquiring/developing new instrumentation.

- **Dynamic magic-angle spinning probe.** Capability development funds supported completion of the first dynamic magic-angle spinning probe, which, with a unique hardware and pulse sequence design, enables *in-situ* chemistry studies for users. The probe allows the flow of gases or liquids under controlled pressures to flow over solid-state materials for reaction chemistry studies and provides an exit for the products. An invention report has been submitted and accepted.

- **900-MHz high-temperature probe.** A 900-MHz high-temperature probe arrived at the end of September 2006. This probe will feature magic-angle spinning capability at temperatures of more than 400ºC, which will be an invaluable capability enhancement for catalysis work. Component testing and integration with the magnet system is still needed.

- **800-MHz cold probe.** This probe provides the highest sensitivity of any probe at PNNL for the study of biological molecules in the liquid state. The sensitivity gained is about four times that of a comparable 800-MHz probe. This probe will be of great use in the structure and dynamics characterization of large biomolecular complexes.

Future Directions

The HFMRF will continue to support researchers by providing state-of-the-art magnetic resonance resources and scientific consulting to enable top-tier research and publication in respected journals. DOE has challenged the facility to focus the power of its marquee instrument, the 900-MHz medium-bore magnet, on high-impact research that may contribute to a major article in *Science*, *Nature*, or the *Proceedings of the National Academy of Sciences*, or that could possibly lead to a Nobel Prize. In Fiscal Year 2006, the facility published five papers using data from the marquee instrument; three of these are in top-10 ISI journals, and one is in a top-5 journal, *Molecular Cell*. In addition, research accomplished on the 800- and 750-MHz magnets contributed to an article in *Proceedings of the National Academy of Sciences* in 2006. Facility staff will seek to continue the record of high-quality publications and have set a goal of continuing to publish 50% of articles in top-10 journals in 2007.

- **Continue to provide NMR expertise.** All advanced scientific instrumentation requires continuous effort to maintain peak performance of both hardware and software, and the science knowledge base to effectively and efficiently use the resource to obtain publishable data. Scientific consultants provide the operational knowledge base for users to efficiently use facility resources. The High-Field Magnetic Resonance Facility will continue to optimize user support efforts and provide scientific expertise, as budget allows, by providing researchers access to NMR experts who can assist with

experimental design and setup, data collection, processing, and analysis. The facility will require at least five individuals knowledgeable in solid-state NMR, liquid-state NMR, and imaging to provide the expertise needed for users to effectively use the facility's resources.

- **Obtain electronics experts.** Two trained electronics technicians are needed to provide instrumentation design and development for specific user needs, and to troubleshoot equipment failures and maintain equipment in optimum operating condition. These individuals are key to the creation of new probe designs for making innovative solutions to scientific problems addressed by magnetic resonance methods.

- **Provide support to calls and Scientific Grand Challenges**. EMSL management actively supported two High-Field Magnetic Resonance Facility-specific calls for proposals in 2006, but in 2007 this call will be transitioned to EMSL-wide science theme and capabilities-based calls. The facility will continue to support the EMSL Scientific Grand Challenges. In addition, as additional mission-critical projects are identified, appropriate staff must be added to address applicable research needs.

- **Add LC-NMR Spectrometry System.** The facility plans to add a 600-MHz LC-NMR spectrometry system dedicated to support the field of metabonomics, a rapidly growing area of research with much user interest. The system would enable accurate lineshape deconvolution and analysis of biomarkers in complex mixtures, supporting EMSL's Biological Interactions and Interfaces, Geochemistry/Biogeochemistry and Subsurface Science, and Science of Interfacial Phenomena science themes. User projects that address biological health effects, microbial production of alternative fuels, detection of biosecurity threats, and environmental remediation and protection are expected to benefit from use of this capability. The data generated would be complementary and integrated with other analytical techniques, especially LC-mass spectrometry.

- **Expand development of unique *in-situ* catalysis NMR probe designs.** The facility seeks to expand development of unique *in-situ* catalysis NMR probe designs developed at lower field and apply the technology to second-generation probes with variable temperature capabilities. Such a technology would be integrated with the 900-MHz NMR spectrometer to enable the study of catalytic reactions over solid-state transition metal-laden materials that benefit the most from study at the highest fields, such as titanium, molybdenum, strontium, and other important quadrupolar nuclei. These studies will have a major impact on the ability to follow reaction chemistries and lead to optimization of new materials for alternative fuel production and reduction of harmful emissions to the atmosphere.

NMR Structural Investigations of the Breast Cancer Susceptibility Protein, BRCA1

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BRCA1 is a complex protein that, when it malfunctions via mutations, can cause cancer. This research addresses how this protein interacts with other proteins in order to perform its various functions, including reducing the risk of breast and ovarian cancer.

Our work on the breast and ovarian cancer tumor suppressor protein, BRCA1, represents the convergence of two central research themes. The first relates to the importance of BRCA1 to a number of fundamental cellular processes, such as the cellular response to DNA damage, homologous recombination, and transcriptional regulation. Investigation of the function of BRCA1 in these pathways promises to yield insights into the role of BRCA1 in normal cellular development, and how loss of function results in the onset of breast and ovarian cancer. The second theme relates to mechanisms underlying ubiquitination. Protein ubiquitination provides a powerful regulatory mechanism for controlling pathways that include cell-cycle progression, transcriptional regulation, and responses to DNA damage. The importance of ubiquitin as a central biological process is underscored by the award of the 2004 Nobel Prize in Chemistry for research into the function of ubiquitination at the molecular level. It has recently been shown that BRCA1 can function as an E3-ubiquitin ligase. This activity is only observed when BRCA1 is complexed with a second protein called BARD1. Both BARD1 and BRCA1 have N-terminal RING-domains which mediate the interaction between the two proteins. Thus, work on BRCA1 allows us to integrate structural investigations on the role of BRCA1 in the development of breast and ovarian cancer as well as the study of the mechanisms of protein ubiquitination.

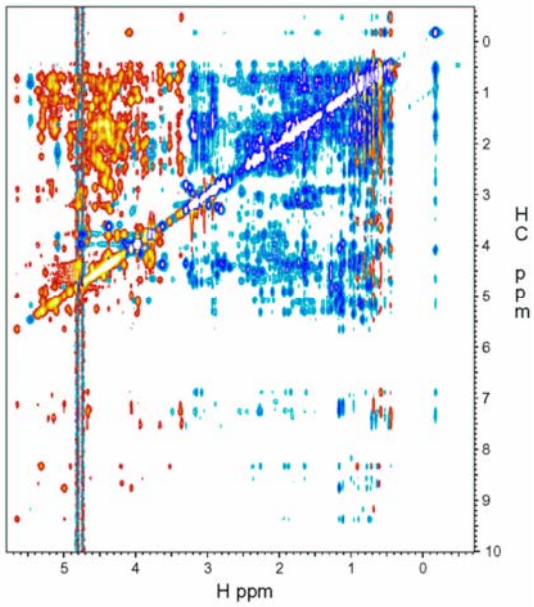

Figure 1. Two-dimensional projection of a three-dimensional Noesy-^{13}C-HSQC spectrum of unlabled ubiquitin bound to ^{15}N-^{13}C-labeled UbcH5c collected at 900 MHz.

BRCA1 is a large and complicated protein which is undoubtedly comprised of a multiplicity of functional domains. A growing body of literature suggests that BRCA1 interacts with at least 30 different macromolecules to accomplish its diverse functional roles. Our structural work involves characterization of macromolecular complexes involving the N-terminal RING domain and the C-terminal BRCT domain of BRCA1. Much of our recent work at EMSL has focused on understanding the ubiquitination activity mediated by the BRCA1 RING domain. As E3-ligases, RING domains are thought to facilitate the specificity of ubiquitination reactions by forming a multiprotein complex, binding both a ubiquitin conjugating enzyme (E2) covalently activated with ubiquitin and specific proteins targeted for ubiquitination. In addition, the BRCA1 RING domain mediates its interaction with BARD1. Cancer predisposing mutations found in the BRCA1 RING domain have been found to interfere with its ability to function as a ubiquitin ligase.

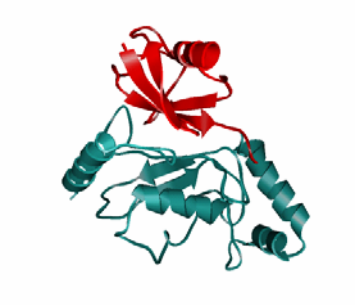

Figure 2. Structure of the noncovalent complex formed between UbcH5c and ubiquitin. Ubiquitin (red) binds to the exposed β–sheet region of UbcH5c (blue–green). The active site of UbcH5c is on the opposite side of the molecule.

This system provides a unique opportunity for studying protein-protein interactions by nuclear magnetic resonance (NMR). It involves characterizing the structures and interactions among at least four different protein components: BRCA1, BARD1, an E2 (UbcH5c or UbcH7), and ubiquitin (Ub). The molecular weight of the fully assembled complexes approaches 60 kD. In previous years, we have been able to collect a great deal of data on the individual components of this system. During the last year, data collected on EMSL's 600-, 800-, and 900-MHz NMR spectrometers have allowed us to develop a model of the complex series of protein-protein interactions that are required to assemble an active BRCA1-dependent ubiqutin-ligase complex.

Recent work at EMSL allowed us to determine the structure of the noncovalent complex between UbcH5c and Ub. The high-field 800- and 900-MHz NMR spectrometers were critical to this effort and provided the necessary sensitivity and resolution (Figure 1) required to determine the solution structure of the 25-kD complex (Figure 2). Though a host of other structurally similar E2 exists, the ability to form a noncovalent complex with ubiquitin is unique to the UbcH5 family of ubiquitin-conjugation enzymes. Our structural work provided a foundation for investigating the mechanistic importance of this interaction. We were able to design a mutation, Serine 22 to Arginine (S22R), in UbcH5c that selectively abrogates the noncovalent binding of ubiquitin, leaving all other functions of UbcH5c intact. The integrity of the noncovalent ubiquitin binding interface is critical for the function of UbcH5c in ubiquitin transfer reactions. Though S22R-UbcH5c is capable of transferring a single ubiquitin to a target protein, it can no longer form poly-ubiquitin chains in BRCA1-directed ubiquitination reactions.

Part of the answer to this riddle is provided by our work on the activated UbcH5c~Ub covalent complex. UbcH5c carries activated ubiquitin via formation of a covalent thiolester bond between the active site cysteine of UbcH5c and the C-terminus of ubiqiutin. Formation of the activated UbcH5c~Ub complex in the context of S22R-UbcH5c allows us to investigate in molecular detail the structural changes caused by activation. This work is presently ongoing at EMSL. However, when we use wild-type UbcH5c, we find the presence of the noncovalent ubiquitin-binding site allows activated UbcH5c~Ub to self-assemble into higher molecular weight complexes. This ability to self-assemble is critical for the formation of poly-ubiquitin chains. Much of this work will be detailed in an upcoming publication of *Molecular Cell*.

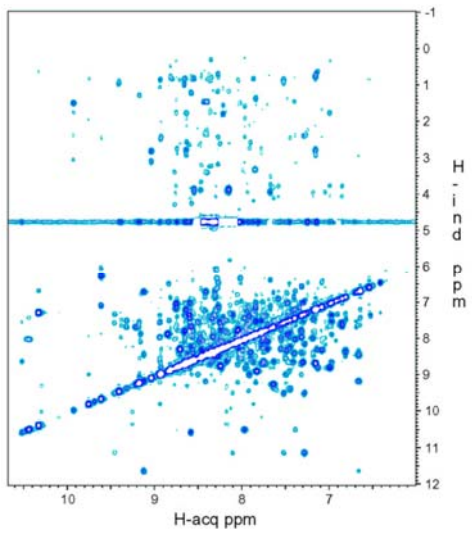

Figure 3. Two-dimensional projection of a three-dimensional Noesy-^{15}N HSQC spectrum of ^{2}H,^{13}C,^{15}N-labeled BRCT domain of BRCA1 in complex with an unlabeled domain of CtIP collected at 600 MHz. The molecular weight of the complex is ~35kD. Many of the peaks observed in the top half of the spectrum represent intermolecular interactions.

The recent installation of cold probes on 600- and 800-MHz NMR spectrometers has allowed us to investigate other BRCA1 protein-protein complexes. The C-terminus of BRCA1 contains an ~225 residue BRCT domain that also contains a number of cancer-predisposing mutations. This domain specifically binds a phosphopeptide motif found in a number of nuclear proteins. One of these, CtIP (CtBP Interacting Protein) is of particular interest as its association with both CtBP, a ubiquitous transcriptional repressor, and BRCA1, a transcriptional regulator, is believed to play a crucial role in the tumor suppressor function of BRCA1. As shown in Figure 3, we have been able to collect high-quality data on the BRCA1-CtIP complex, allowing investigation into the molecular details of this interaction.

Citations

Brzovic PS, A Lissounov, DE Christensen, DW Hoyt, and RE Klevit. 2006 "A UbcH5/Ubiquitin Noncovalent Complex Is Required for Processive BRCA1-Directed Ubiquitination" *Molecular Cell* 21(6): 873-880

Microscopic View of Strontium Interactions in Minerals

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Characterization of strontium in phyllosilicate minerals with solid-state nuclear magnetic resonance spectrometry (NMR) will contribute to an understanding of strontium/mineral interactions in soils exposed to leaking waste tanks, thus enabling the design of better models for predicting the fate of strontium in the environment and better cleanup technologies.

The interactions of strontium with clay minerals and zeolites are not well understood and must be researched so accurate models for predicting the environmental fate of radioactive strontium-90 released from sites such as Hanford can be developed. Solid-state NMR spectrometry is a useful tool for probing the molecular structure of materials, including the interactions of cations sorbed by mineral systems. However, there is only one NMR active isotope of strontium (i.e., strontium-87) and the direct study of strontium with solid-state NMR is experimentally challenging. Strontium-87 has similar chemistry to strontium-90 and is a quadrupolar nucleus (I = -9/2) with a low natural abundance (~7%), a low gyromagnetic ratio (γ = -1.163 x 10^7 $1^{-1}T^{-s}$), and large quadrupolar coupling constants (14-25 MHz) (Larsen et al. 2000; Bastow 2002; Bowers et al. 2006 a, b). These factors contribute to a lack of sensitivity that must be overcome to perform time-efficient studies of strontium in natural samples, such as environmentally relevant clay minerals and zeolites.

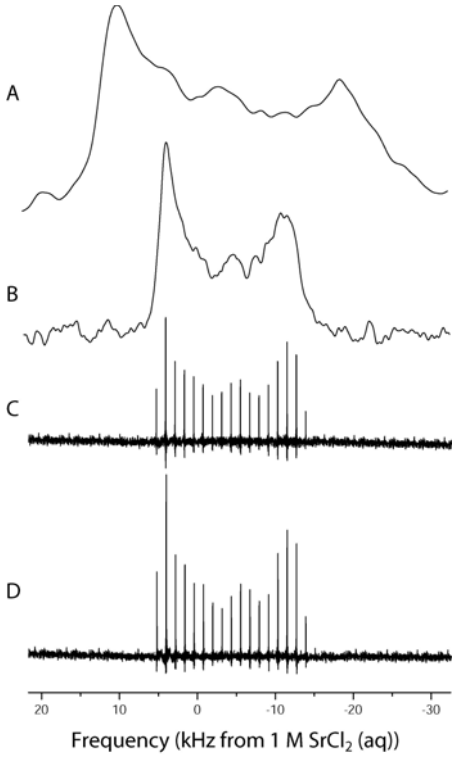

Figure 1. Strontium carbonate strontium-87 NMR spectra: (A) static echo at 11.74 T (800 MHz), (B) static echo at 21.14 T scaled by 1/37, (C) QCPMG at 21.14 T (900-MHz) scaled by 1/275, (D) DFS-QCPMG at 21.14 T scaled by 1/826.

In our ongoing studies at PNNL on the 21.14-tesla (T) (^{1}H resonance frequency of 900 MHz) instrument, we are using sensitivity-enhancing techniques to characterize the local electromagnetic environment of strontium nuclei in mineral systems.

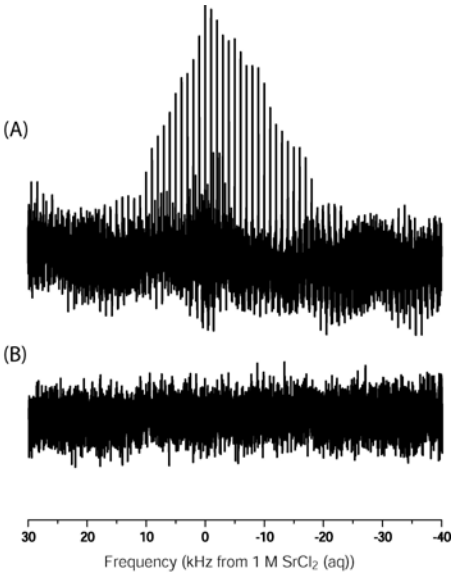

Figure 2. Strontium NMR spectra of Na-4 Mica after (A) and prior to (B) heat treatment at 500˚C for four hours. The heat-treated sample produces a single strontium resonance fit well by a quadrupolar line shape.

We have shown in recent work that the 21.14-T (900-MHz) field strength provides an order-of-magnitude enhancement to the signal-to-noise ratio for strontium-87 experiments and that quadrupolar Carr Purcell Meiboom Gill (QCPMG) analysis adds an additional order of magnitude (Bowers et al. 2006). Unfortunately, these enhancements were insufficient to observe strontium in complex minerals. In the past year, we were able to demonstrate that adding the double frequency sweep (DFS) preparatory scheme results in up to an additional fivefold enhancement to the signal-to-noise ratio (Figure 1), thereby permitting the detection of strontium resonances in soil minerals.

With DFS-QCPMG at 21.14 T (900 MHz), we were able to perform solid-state NMR studies of strontium in additional inorganic and organic systems, where these studies were impossible with QCPMG analysis alone. One important conclusion from our data is that water-strontium interactions have a profound effect on strontium-87 NMR spectra; in fact, we have been unable to observe strontium in any system where there is water in the strontium hydration sphere (Figure 2). The reasons for this are the subject of current investigations. DFS-QCPMG at 21.14 T has also been used to successfully examine the strontium-binding environment in a number of heat treated micas, montmorillonites, titanates, and titanosilicates. To perform such strontium-87 NMR analyses at more conventional fields (i.e., 11.74 T) would require on the order of 1800 days rather than the three days required to produce the spectrum shown in Figure 2. The library of quadrupolar parameters and their relationship to crystal structure prepared from our studies of simple systems now allows informed predictions of the strontium-binding environment based on strontium-87 NMR parameters and x-ray diffraction studies. This library could not have been developed over the past year without the use of DFS-QCPMG on the EMSL 21.14-T (900-MHz) NMR spectrometer. A detailed discussion of the strontium-binding environment in one of the mica samples mentioned earlier is the subject of a manuscript published in the *Journal of Physical Chemistry B* (Crosson et al. 2006). We also intend to publish the results of our mineral studies in a paper that will detail strontium binding in phyllosilicates and in another paper that will describe strontium binding in designer titanosilicate materials.

In the coming year, we intend to return to EMSL to study additional titanosilicate materials developed at Savannah River National Laboratory specifically to sequester strontium from Hanford-like wastes. Some of these materials have tunable selectivity for cesium and strontium, making this work highly important to the DOE mission. We will also continuing our investigations of mineral weathering under near-field exposure to simulated tank waste

leachate (Chorover et al. 2003; Crosson et al. 2006) by monitoring the kinetics of mineral dissolution and re-precipitation in samples of natural Hanford sediments.

Citations

Bastow TJ. 2002. "Electric Field Gradients at the M-site in MCO3: M=Mg, Ca, Sr and Ba." *Chemical Physics Letters* 354(1-2):156-159.

Bowers GM, AS Lipton, and KT Mueller. 2006 (a). "High-Field QCPMG NMR of Strontium Nuclei in Natural Minerals." *Solid State Nuclear Magnetic Resonance* 29(1-3):95-103.

Bowers GM, R Ravella, S Komameni, and KT Mueller. 2006 (b). "NMR Study of Strontium Binding by a Micaceous Material" *Journal of Physical Chemistry B* 110(14):7159-7164.

Chorover J, SK Choi, MK Amistadi, KG Karthikeyan, G Crosson, and KT Mueller. 2003. "Linking Cesium and Strontium Uptake to Kaolinite Weathering in Simulated Tank Waste Leachate." *Environmental Science & Technology* 37(10):2200-2208.

Crosson GS, SK Choi, J Chorover, MK Amistadi, PA O'Day, and KT Mueller. 2006. "Solid-State NMR Identification and Quantification of Newly Formed Aluminosilicate Phases in Weathered Kaolinite Systems." *Journal of Physical Chemistry B* 110(2):723-732.

Larsen FH, J Skibsted, HJ Jakcobsen, and NC Nielsen. 2000. "Solid-state QCPMG NMR of Low-γ Quadrupolar Metal Nuclei in Natural Abundance." *Journal of the American Chemical Society* 122(29):7080-7086.

Solution Structure of the Complex Between Poxvirus-Encoded CC Chemokine Inhibitor vCCI and Human MIP-1ß

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The chemokine system is critical for host defense in healthy individuals, but it also can lead to inflammatory diseases including asthma, arthritis, and atherosclerosis. This novel work used high-field nuclear magnetic resonance (NMR) spectroscopy to reveal the first known structure of a 35-kDa pox virus chemokine inhibitor:human chemokine complex, which is a stepping stone to future therapeutic drug design.

Chemokines (i.e., chemotactic cytokines) comprise a large family of proteins that recruit and activate leukocytes, giving chemokines a major role in both immune response and inflammation-related diseases. To date, about 50 chemokines have been identified, and these small proteins (7 to 14 kDa) are believed to function by binding with endothelial or matrix glycosaminoglycans (GaGs) to form a concentration gradient that is then sensed by high-affinity, 7-transmembrane domain G-protein coupled chemokine receptors on the surface of immune cells, leading to activation and chemotaxis. Chemokines play critical roles in the immune system, causing chemotaxis of a variety of cells to sites of infection and inflammation, as well as mediating cell homing and immune system development (Baggiolini 2001; Gerard and Rollins 2001). There are four subfamilies of chemokines, CC, CXC, C, and CX_3C, which are named for the position of conserved N-terminal cysteine residues; different subfamilies tend to function on different cell subsets (Baggiolini 2001).

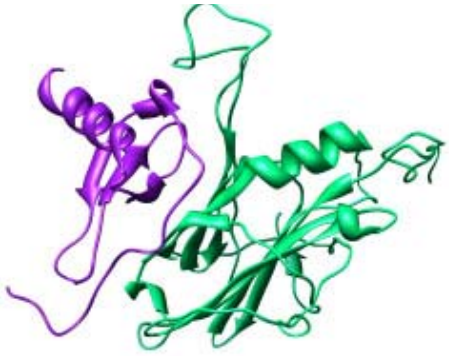

Figure 1. Solution structure of the vCCI:MIP-1β complex, with vCCI backbone in green and MIP-1β backbone in violet.

All known pox and herpes viruses encode proteins that interfere with the host chemokine network, probably as part of a strategy to manipulate and subvert the immune system (Boomker et al. 2005). Such virally encoded proteins include chemokine mimics, chemokine receptor analogs, and a group of secreted, soluble chemokine binding proteins (CKBPs) that exhibit little similarity to any mammalian protein (Seet and McFadden 2002). CKBPs competitively bind to chemokines and disrupt chemokine interactions with the host cell surface receptors or GAGs. Although some CKBPs interact with a very broad spectrum of chemokines across several chemokine subfamilies, the poxvirus-encoded viral CC chemokine inhibitor (vCCI) proteins bind selectively to the CC family of pro-inflammatory chemokines (Seet and McFadden 2002; Lalani et al. 1998). The vCCI proteins have been shown to be potent inhibitors of chemokine action *in vitro* (Lalani et al. 1998) and effective anti-inflammatory agents in vivo (Dabbagh et al. 2000).

Although the structures of several chemokines (Clore et al. 1990; Mayer and Stone 2000; Crump et al. 1997; Fernandez and Lolis 2002), as well as the individual structure of vCCI (11) are known, no structure had previously been solved of a vCCI:chemokine complex, so the structural basis of vCCI's interaction with chemokines was not clear. We have used heteronuclear multidimensional NMR to determine the first structure of an orthopoxvirus vCCI in complex with a human CC chemokine MIP-1ß variant. Access to high-field spectrometers at the EMSL High-Field Magnetic Resonance Facility was critical to obtaining the spectral data that enabled structure determination work on this 35-kDa complex.

Our structural studies reveal that the vCCI and MIP-1ß form a complex in a 1:1 stoichiometry, and that vCCI occludes the regions in the chemokine that are important for chemokine homo-dimerization, receptor binding, and glycosaminoglycan interaction (Figure 1). The structure also defines key interactions that form the basis for the affinity and selectivity of vCCI towards certain CC chemokines. The insights gained from this groundbreaking work will be critical to development of therapeutic agents to modulate immune response. This work was published in *Proceedings of the National Academy of Sciences of the United States of America* 103(38):13985-13990.

Citations

Baggiolini M. 2001. "Chemokines in Pathology and Medicine." *Journal of Internal Medicine* 250(2):91-104.

Boomker JM, LF de Leij, TH The, and MC Harmsen. 2005. "Viral Chemokine-Modulatory Proteins: Tools and Targets." *Cytokine & Growth Factor Reviews* 16(1):91-103.

Clore GM, E Appella, M Yamada, K Matsushima, and AM Gronenborn. 1990. "Three-Dimensional Structure of Interleukin 8 in Solution." Biochemistry 29(7):1689-1696.

Crump MP, JH Gong, P Loetscher, K Rajarathnam, A Amara, F Arenzana-Seisdedos, JL Virelizier, M Baggiolini, B Sykes, and I Clark-Lewis. 1997. "Solution Structure and Basis for Functional Activity of Stromal Cell-Derived Factor-1; Dissociation of CXCR4 Activation from Binding and Inhibition of HIV-1." *EMBO Journal* 16(23):6996-7007.

Dabbagh K, Y Xiao, C Smith, P Stepick-Biek, SG Kim, WJ Lamm, DH Liggitt, and DB Lewis. 2000. "Local Blockade of Allergic Airway Hyperreactivity and Inflammation by

the Poxvirus-Derived Pan-CC-Chemokine Inhibitor vCCI." *Journal of Immunology* 165(6):3418-3422.

Fernandez EJ and E Lolis. 2002. "Structure, Function, and Inhibition of Chemokines." *Annual Review of Pharmacology and Toxicology* 42:469-499.

Gerard C and BJ Rollins. 2001. "Chemokines and Disease." *Nature Immunology* 2(2): 108-115.

Lalani AS, TL Ness, R Singh, JK Harrison, BT Seet, DJ Kelvin, G McFadden, and RW Moyer. 1998. "Functional Comparisons among Members of the Poxvirus T1/35-kDa Family of Soluble CC-Chemokine Inhibitor Glycoprotei." *Virology* 250(1):173-184.

Mayer KL and MJ Stone. 2000. "NMR Solution Structure and Receptor Peptide Binding of the CC Chemokine Eotaxin-2." *Biochemistry* 39(29):8382-8395.

Seet BT and G McFadden. 2002. "Viral Chemokine-Binding Proteins." *Journal of Leukocyte Biology* 72(1):24-34.

A Solid-State ^{95}Mo NMR and Computational Investigation of Dodecahedral and Square Antiprismatic Octacyanomolybdate(IV) Anions: Is the Point-Charge Approximation an Accurate Probe of Local Symmetry?

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Solid-state nuclear magnetic resonance (NMR) studies of low-gamma nuclei have been hampered in the past because of inherent difficulties associated with observing low-frequency quadrupolar nuclei. Such studies are becoming feasible and productive with the use of the highest possible magnetic field strengths coupled with sensitivity-enhancement experiments.

Because of the experimental challenges they present, solid-state NMR studies of quadrupolar nuclei with small magnetic moments represent a relatively undeveloped area of research. The inherently low NMR sensitivity of such nuclei means that considerable time is required to obtain NMR spectra of adequate signal-to-noise characteristics—a problem that is often compounded by the fact that solid-state NMR spectra, particularly of quadrupolar nuclei, may span several hundred kilohertz. Recent technological advances have enabled NMR data collection for challenging quadrupolar nuclei that have previously been refractory to observation. For example, the development of high-field magnets and new-and-improved pulse sequences facilitate data collection; high magnetic fields result in increased sensitivity and amplification of the magnetic shielding, σ, interaction and, in addition, substantially reduce probe ringing, which is often an experimental hindrance when observing low-frequency quadrupolar nuclei. New pulse sequences include a variety of sensitivity-

enhancement techniques that further improve the quality of the NMR experiment and result in significant reductions in experimental time.

One important isotope that falls into the small magnetic moment category is ^{95}Mo. Despite the prevalence of molybdenum in modern chemistry, ^{95}Mo has received little attention from the solid-state NMR community, primarily because of its unfavorable properties: Ξ = 6.547 MHz, N.A. = 15.92 %, Q = -0.022×10^{-28} m^2. Unfortunately, the small quadrupole moment, Q, of ^{95}Mo creates an additional impediment for NMR studies in the solid state to long spin-lattice relaxation times. Nevertheless, by employing the highest possible magnetic field strengths and available sensitivity-enhancement techniques, we have been able to undertake ^{95}Mo NMR studies. Through determination of the molybdenum σ and electric field gradient (EFG) tensors, a wealth of information on molecular and electronic structure can be obtained.

In this study, we have demonstrated the efficiency and effectiveness of solid-state ^{95}Mo NMR spectroscopy as a tool for analyzing two symmetry forms of the diamagnetic octacyanomolybdate(IV) anion: 1) the approximate dodecahedral, D_{2d}, symmetry and 2) the approximate square antiprismatic, D_{4d}, symmetry. The success of this study is attributed to the use of the highest available magnetic field strengths, 21.15 tesla (T), which corresponds to 900-MHz proton frequency; 17.63 T (750 MHz); and 11.75 T (500 MHz), and the quadrupolar Carr-Purcell Meiboom-Gill (QCPMG) and double-frequency sweep (DFS)/QCPMG sensitivity-enhancement techniques. By examining the D_{2d} and D_{4d} $Mo(CN)_8^{4-}$ anions in the solid state, we have studied each structure independently and have characterized the molybdenum σ and EFG interactions, thereby avoiding problems associated with solution NMR studies, such as ligand exchange or interconversion between symmetry forms. The acute sensitivity of the molybdenum σ and EFG tensors to small changes in the local structure of these anions has allowed the approximate D_{2d} and D_{4d} $Mo(CN)_8^{4-}$ anions to be readily distinguished.

Our results indicate that the magnitudes of the molybdenum σ and EFG interactions are comparable for the D_{2d} and D_{4d} $Mo(CN)_8^{4-}$ anions; however, the relative values and orientations of the principal components of the molybdenum σ and EFG tensors for the two symmetry forms are different, resulting in ^{95}Mo NMR line shapes that are distinctly different at the fields employed here (Figure 1). Quantum chemical calculations of the molybdenum σ and EFG tensors, using zeroth-order regular approximation density functional theory (ZORA DFT) and restricted Hartree-Fock (RHF) methods, have also been carried out, and the results obtained are in good agreement with experimental results. The most significant and surprising result from the DFT and RHF calculations is a substantial EFG at molybdenum for an isolated $Mo(CN)_8^{4-}$ anion possessing an ideal square anti-prismatic structure; this is contrary to the point-charge approximation, which predicts a zero EFG at molybdenum for this structure.

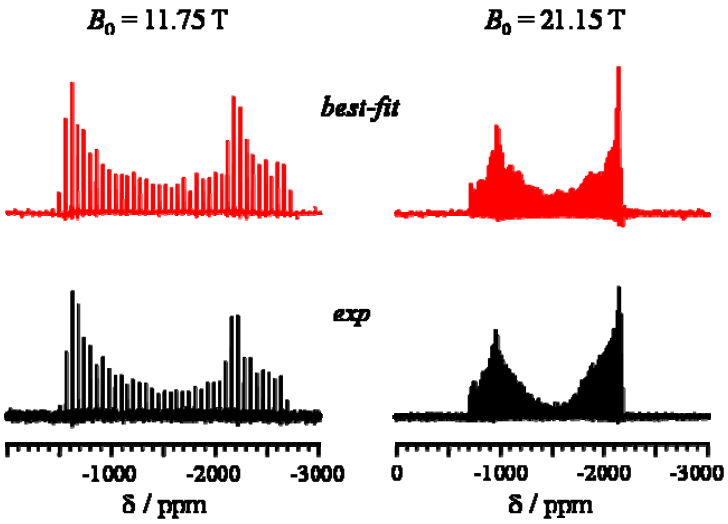

Figure 1. Experimental and best-fit simulated ^{95}Mo central-transition NMR spectra of a solid, stationary sample of $Tl_4Mo(CN)_8$ acquired using the QCPMG pulse sequence at 11.75 T (left) and the DFS/QCPMG pulse sequence at 21.15 T (right). A total of 31 760 transients were summed for $Tl_4Mo(CN)_8$ at 11.75 T and 8192 at 21.15 T.

Citation

Forgeron MAM and RE Wasylishen. 2006. "A Solid-State ^{95}Mo NMR and Computational Investigation of Dodecahedral and Square Antiprismatic Octacyanomolybdate(IV) Anions: Is the Point-Charge Approximation an Accurate Probe of Local Symmetry?" *Journal of the American Chemical Society* 128:7817-7827.

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MA Kennedy, S Ni, Miami University, Oxford, Ohio

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Structure of Telomerase RNA and Telomeric Proteins

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Application for 800MHz NMR Spectrometer Time to Facilitate the Structural Study of the Complex Formed by Pox Virus Encoded Protein vCCI and Human CC Chemokine MIP-1beta

PJ LiWang, L Zhang, Texas A&M University, College Station, Texas

alphaB-crystallin - the Core and the Oligomer : A Structural Investigation

P Rajagopal, R Klevit, University of Washington, Seattle, Washington

Determination of the Three-dimensional Solution Structure of NosL, a Potentially Novel Copper(I) Metal Transporter

V Copie, LM Taubner, GA Jacobs, Montana State University, Bozeman, Montana

Structure and Interactions of a Domain of Dynein Intermediate Chain--Protein Folding Coupled to Binding

EJ Barbar, Oregon State University, Corvallis, Oregon

Slow MAS of Lipids in Mouse Fast and Slow Skeletal Muscle

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Solid State MAS NMR of High-Valent Cation Exchanged H-MFI

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Study of the Network Structures of Polymer-Derived Amorphous SiAlCN Ceramics

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Hydrogen Storage Materials

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Hydrogen Storage Materials

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Hydrogen Storage Materials

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Structural Genomics of Eukaryotic Model Organisms

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Study of the Binding Mechanism of Mutant SN-15 to Hydroxyapatite using $^{15}N\{^{31}P\}$REDOR

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Structure of PR Domain of RIZ1 Tumor Suppressor

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Routine $_1$H and $_{13}$C NMR Analysis of Functionalized Semiconductor and Metallic Nanoparticles Synthesized for Biodetection Studies

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Routine ^{1}H and ^{13}C NMR Analysis of Functionalized Semiconductor and Metallic Nanoparticles Synthesized for Biodetection Studies

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NMR Structural Studies of Clustered DNA Damage

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Structural Investigations of Solid Materials by High Resolution Solid State NMR at Very High Field

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NMR Structural Studies of a 180 kDa HDL Particle

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Structural Studies of Lipid-free Apolipoprotein A-I

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Investigating Molecular Recognition and Biological Function at Interfaces Using Antimicrobial Peptides

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Correlation of Structure and Function of Zinc Metalloproteins Via Solid-state NMR Methods

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Magnetic Resonance Microscopy of Environmental Lung Injury

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Investigation of the Role of Mg_2^+ in DNA Repair Proteins APE1, Pol, and FEN1

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Investigation of Catalyst Reaction Mechanisms by In-situ High Field, High Resolution NMR Spectroscopy

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Deposition of Cobalt-doped Oxides for Spintronic Applications

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NMR Study of Effects and Mechanisms of Mechanical Activation on Hydrogen Sorption/Desorption of Nanoscale Lithium Nitrides

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NMR Study of Effects and Mechanisms of Mechanical Activation on Hydrogen Sorption/Desorption of Nanoscale Lithium Nitrides

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An Extended Study of the Molybdenum(IV) Octacyanide Anion: Comparison of Dodecahedral versus Square Antiprismatic Structural Forms via Solid-State ^{95}Mo Nuclear Magnetic Resonance Spectroscopy

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Thrust Area 1: Purification and Biophysical Characterization of MR-1 Redox Proteins
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Thrust Area 1: Metal-reducing *Shewanella* Believed to be Involved in the Dissimilatory Reduction of Solid Phase Iron (III)

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Dissolution of Borosilicate Waste Glass: Effect of Al and B Coordination on Alkali Ion Exchange

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Structure and Function of the Membrane Protein OEP16

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Structure of Trityls

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Interrogation of Glucose Metabolism by Oral Biofilms Using Combined NMR/Optical Spectroscopy and Stable Isotope Labeling

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Sensitivity Enhancing NMR of Strontium-87 Nuclei in Clays, Zeolites, and Waste Cleanup Technologies

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***In vivo* and *ex vivo* High Resolution Slow-MAS MR Spectroscopy in Mice**

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Characterization of *in vivo* ^{1}H-NMR Biomarkers for Pulmonary Phospholipidosis

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MR Imaging of Respiratory Structure and Function

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A Multinuclear MAS NMR Investigation of Environmentally Relevant Materials: Lanthanum Strontium Gallium Magnesium Oxide and Magnesium Aluminum Layered Double Hydroxides

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Structural Investigation of alphaB-Crystallin Core Domains

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Structural Biology of the Human High Mobility Group A (HMGA) Proteins: Characterizing the Hub of Nuclear Function

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Structural Studies of a Novel Family of Manganese Uptake Proteins in Cyanothece Containing a Repeated Five-Residues Domain (RFR).

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Structural Studies of a Family of Proteins from the Diurnal Cyanobacteria Cyanothece 51142 that Contain an Unusual Repeated Five-Residues Domain (RFR).

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Identify Biomarkers for COPD (Chronic Obstructive Pulmonary Disease) in Humans using Metabonomic Analysis of Serum and Urine by NMR.

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Investigation of Crystalline to Amorphous Phase of Cellulose by using Slow Magic Angle Spinning NMR Spectroscopy

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Identifying Value Added Products from Biomass Conversion Reactions by NMR

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Mistranslation Fragment of an *in silico* Designed Novel-fold Protein Forms and Exceptionally Stable Symmetric Homodimer with a High-affinity Interface

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Structure of Designer Proteins

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Solid State NMR to Investigate Protein Interactions at Interfaces

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The Iron Binding Environment of the Cyanobacterial Ferric Ion Transporter, FutA1

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Characterization of *in-vivo* ^{1}H-NMR Biomarkers for Pulmonary Phospholipidosis

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Pulsed EPR Studies of Nanocrystalline Zeolites and Hollow Zeolite Structures

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High-resolution ^{27}Al MAS NMR of Weathered Clays and Hanford Sediments

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Binding Environment of Strontium-87 Nuclei in DOE Waste Remediation Materials

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NMR Assignment and Structure of the C-Terminal Domains of Human Villin

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Sintering of Hydroxyapatite for Advanced Orthopedic Applications

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^{99}Tc NMR Study of Tc(V) Solids

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Magic Angle Spinning NMR Experiments on Radioactive Samples with a Microcoil Spinner

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Structure of Complex Proanthos\Cyanidins by High Field NMR Spectroscopy

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Solid State NMR Studies of Nano-materials

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NMR and Computational Studies of Chemical Transformations at Complex Interfaces

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Clusters of Damage in Irradiated DNA

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Free Radical Reactions in the Catalytic Cycle of Cytochrome bc Complexes

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Solution Structure of a 42 kDa "Metal Sensor" CzrA-DNA Complex

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Protein Interactions and Interfaces

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Solid-State NMR Spectroscopy on Radioactive Samples with Microcoils

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NMR Studies of Human Apolipoprotein-E

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Molecular Probes of Quinol Oxidation by the Cytochrome b6f complex

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Ultra-High Field NMR Studies of Stable Isotope Applications

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Structural Proteomics of Myobacterium Tuberculosis

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NMR Structural Investigations of BRCA1

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Solid-State ^{67}Zn NMR of Synthetic Metalloprotein Models

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NMR Microscopy of Diffusive Transport in Natural Porous Mineral Grains

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Free Radical Processes in g- and Heavy Ion Irradiated DNA

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Solid-State NMR Characterization of Metal Phosphines

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Probing the Mechanism of the Alkaline Phosphatase Reaction by ^{67}Zn and ^{25}Mg NMR

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NMR Structural Investigations of BRCA1

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Distance Measurements in RNA using DEER Spectroscopy with Site-directed Spin Labeling

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Structural Proteomics: Annotating the Genome using 3D Structure

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Defect Dynamics on Crystalline Quartz

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Microscopic Characterization of Porosity, Diffusivity, and Tortuosity in Single Particles of Hanford Sediments using Nuclear Magnetic Resonance (NMR) Technique

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Special Purpose Re-configurable ASIC Hardware for Accelerating Protein Structure Analysis Software

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Studies of Ligand-Induced Conformational Change in CD44 receptor

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Solid State NMR Studies of Chloropropyl Silica Gels

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Composite Gadolinium Oxide and Yttrium Phosphate Nanoparticles for Managing Cancer Therapy with Magnetic Resonance Imaging

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Interaction of *Escherichia coli* Formamidopyrimidine-DNA Glycosylase (Fpg) with Damaged DNA Containing an 7,8-Dihydro-8-oxoguanine Lesion

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TRAPDOR Experiments on Siliceous Sinters from Thermal Springs

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Magnetic Resonance Microscopy of Water Dynamics at Hydrophilic Surfaces

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Complexation of Th(IV) by Organic Acids in Aqueous Solution

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Kinetics of Polyphosphate Decomposition in Heterogeneous Environments

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Nuclear Magnetic Resonance Detection of Radiation Damage in Ceramics

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NMR Analysis of Synthesized Organic Compounds for Modification of Nanostructures

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Pulsed-EPR studies to Investigate a New Family of Free Radical Spin Traps

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Study of the Structures of Thermally Formed Oxides on Amorphous SiAlCN Ceramics

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Development of Multipurpose Tags and Affinity Reagents for Rapid Isolation and Visualization of Protein Complexes

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Stabilization of Soil Organic Matter: Land Use, Erosion and Burial

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Structure of the PR Domain of RIZ1 Tumor Suppressor

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Separation of ^{47}Ti and ^{49}Ti Solid-State NMR Lineshapes by Static QCPMG Experiments

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PNNL/NESG Structural Genomics

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Structure and Dynamics of aB57

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Structural Studies of Lipid-free Apolipoprotein A-I

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Structural Studies of Apolipoprotein A-I/preb-HDL Particles

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Solid State ^{183}W MAS NMR at High and Ultra High Magnetic Fields

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Investigation of the Role of Mg_2^+ in DNA Repair Proteins

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Structural Genomics: Determining the Structure of Proteins from the Infectious Agent *Pseudomonas aeruginosa*

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Correlation of Structure and Function of Zinc Metalloproteins via Solid-state NMR Methods

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Drug Interactions of Human and Bacterial Cytochrome P450s Probed by Pulsed Electron Paramagnetic Spectroscopy

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Investigation of Soot Morphology and Microstructure with Respect to the Oxidation

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