Expanding Nuclear Magnetic Resonance (NMR) Capabilities of 60 MHz permanent magnet systems through the development of novel pulse sequences for use in an undergraduate chemistry curriculum and incorporation into a course on Molecular Spectroscopy.

Sabbatical Leave Proposal Application Fall 2010–Spring 2011

Submitted by

Dr. R. Marshall Werner

October 9, 2009

Sabbatical Proposal

Summary

This sabbatical proposal involves three main components. First, novel software based pulse sequences will be developed that can expand the current capabilities of Nuclear Magnetic Resonance (NMR) instruments like the one we have here at LSSU. This portion of the sabbatical will require a collaborative effort with Anasazi NMR based in Indianapolis, IN and will involve both remote and on-site training and use of Anasazi instrumentation and PNMR software (*see attached letter of support*). Second, a series of advanced laboratory experiments will be developed based on the above activities. This portion of the sabbatical will involve collaboration with NMR research scientists at the Complex Carbohydrate Research Center, located in Athens, GA (*see attached letters of support*). Finally, the last component of the sabbatical will involve the incorporation of these experiments into a course on Molecular Spectroscopy aimed at junior and senior level chemistry and biochemistry students here at LSSU. The results of this work will permit me to seek external funding in the areas of NMR spectroscopy and undergraduate chemical education. I plan on publishing the experiments that are developed in either the Journal of Nuclear Magnetic Resonance or the Journal of Chemical Education. In addition, this work will be presented at both an American Chemical Society Meeting, and at a seminar series hosted by Anasazi Instruments.

Personal Background and Sabbatical Rationale

When I was hired at LSSU 8 years ago, my research focused on environmental and fish health issues due to limitations in chemical instrumentation. These interests were funded by two external grants (Great Lakes Fisheries Trust, $25K, United States Environmental Protection Agency, $715K). My research interests are now shifting allowing me to "return to my roots" as an organic and biochemical NMR spectroscopist. As both a graduate student at the University of Maryland College Park and as a post-doc at the Center for Advanced Research in Biotechnology, I utilized NMR as a technique for organic structure determination, and enzymatic reaction rate analysis. The instruments available in this graduate setting were state-of-the-art super-conducting Fourier Transform NMR (FT NMR) platforms. The problem with this sort of instrumentation is that it is both expensive to purchase and to maintain. These characteristics make super-conducting FT NMR instruments unsuitable for a setting like LSSU.

Since becoming a professor here at LSSU, I have led efforts in our department to purchase an NMR suitable for undergraduate purposes that meets the criteria of ease of use and low maintenance costs. In the Fall of 2006, a 60 MHz permanent magnet Fourier Transform NMR system was installed by Anasazi instruments (Indianapolis, IN) (Figure 1). This system has proven ideal for undergraduate use and has essentially no maintenance cost. Since this NMR was installed, the chemistry department has started to incorporate its use in several classes, most notably in Organic I and II as well as in Biochemistry I. In addition, a recent senior project focused on the development of a novel ^{31}P NMR experiment to assay a metabolic enzyme called pyruvate kinase (*see copy of Senior Project, manuscript in preparation for publication in the Journal of Chemical Education*).

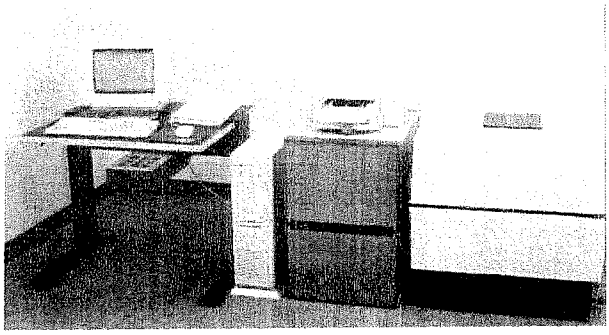


Figure 1. The Anasazi Instruments EFT-60 FT NMR system upgrade was installed in the Fall of 2006 here at LSSU. Our department provided the 1.4 Tesla permanent magnet shown on the right, while Anasazi installed the electronic upgrade and computer interface shown in the middle and left.

Recently, I have become intrigued by the idea of utilizing the Anasazi NMR as a tool to demonstrate chemical concepts to our students. Others have had similar ideas as evidenced by a recent publication of laboratory experiments specifically designed for permanent magnet systems such as the Anasazi NMR (1). This summer, I attended a National Science Foundation sponsored conference entitled, "The Center for Workshops in the Chemical Sciences NMR Conference". This conference was held at the Complex Carbohydrate Research Center, at the University of Georgia in Athens, GA. The material presented opened my eyes to the enormous untapped possibilities of using NMR not only

as a tool to solve chemical structures, but as a *tool to teach chemical concepts*. The instrumentation at the CCRC was not the 60 MHz permanent magnet FT NMR type that we have here at LSSU, but rather the expensive super-conducting FT NMR magnets. Upon inquiring, I discovered that most of the techniques and software based pulse sequences used to run the expensive super-conducting NMR magnets should in theory be possible to perform on the permanent magnet platform.

Most modern NMR researchers have not used permanent magnet platforms for their purposes because they generally have low resolution and high signal to noise ratios. However, these researchers are not typically using NMR as a teaching tool, but rather as a structural characterization tool. In order to *demonstrate* concepts such as chemical reaction rates, chemical shift, magnetic anisotropy, magnetization transfer, and Nuclear Overhauser Effects (NOEs), low resolution and higher signal to noise ratios are acceptable. Therefore, it should be possible to adapt many of these advanced pulse sequences to the Anasazi FT NMR system. It is estimated that there are over 6,000 functioning permanent magnet systems in the United States alone, approximately 3,000 of which are the Anasazi platform. Thus, the knowledge gained through these investigations should have broad impact particularly at institutions that focus on undergraduate chemical education.

Proposed Research and Specific Aims

I. Development of Advanced Pulse Sequences for the Anasazi NMR

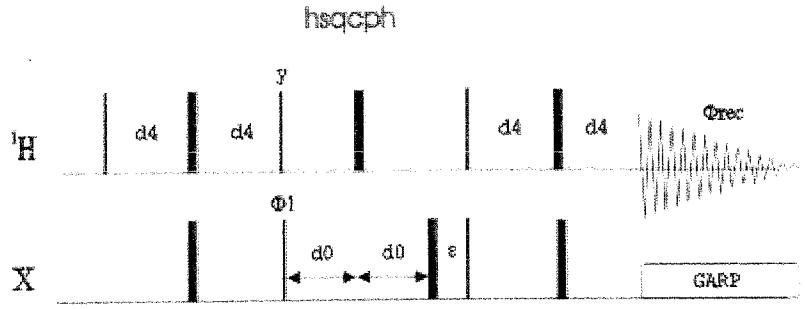
The software used to run the Anasazi 60 MHz NMR that LSSU currently has is called PNMR. This software already has some "canned" pulse sequences that were written by the manufacturer (Anasazi NMR) and are included as part of the purchase package. These pre-written pulse sequences include a series of standard 1-dimensional and simplistic 2-dimensional NMR experiments (i.e. COSY and HETCOR). Many of the more sophisticated experiments that are used on superconducting magnets do not have pulse sequences written for them in PNMR language. Furthermore, I cannot find literature precedence for trying these more advanced experiments on the permanent magnet platform. In talking with the technical staff at Anasazi, they are in agreement that many of these more advanced pulse sequences should in theory be possible using the permanent magnet platform. In particular there are several experiments that I will develop by writing pulse sequences in PNMR (non-PNMR software literature references for these pulse sequences are given:

1) Water Gate (2)
2) TOSCY (3)
3) Transfer NOE (4)
4) ROESY (4)
5) HSQC (5)

These pulse sequences represent both powerful structural characterization tools as well as powerful *teaching* tools. For example, the pulse sequence "Water Gate", aside from its

humorous name related to the activities of former President Nixon, is a sequence that suppresses the NMR signal due to water. This pulse sequence is typically used in the evaluation of bio-molecules such as proteins and DNA. This sequence has recently been used to examine live cell metabolism using what is called "In-Cell" NMR (2). **TO**tal **C**orrelation **S**pectroscop**Y** (TOSCY) provides a different mechanism of magnetization transfer than 2D-COSY correlation spectroscopy. In TOCSY, cross peaks are generated between all members of a coupled spin network. This type of pulse sequence can be use to demonstrate "magnetization-transfer" between nuclei in a compound (3). The 2D HSQC (**H**eteronuclear **S**ingle-**Q**uantum **C**orrelation) experiment permits allows a 2D heteronuclear chemical shift correlation map between directly-bonded ^{1}H and X-heteronuclei (commonly, ^{13}C and ^{15}N) to be generated. It is widely used because it is based on proton-detection, offering high sensitivity when compared with the conventional carbon-detected 2D HETCOR experiment (4).

Below is a schematic example of the HSQC pulse sequence shown using the "Bloch Format" diagram as a description of the details of the pulse sequence. Each pulse sequence that I plan on developing will need this type of "instructional figure" to be converted to PNMR software language for use on the Anasazi instrument.



The development of pulse sequences will require training on the PNMR software. (**Specific Aim I**). I will visit Anasazi NMR in Indianapolis, IN to receive this training (*see attached letter of support*). In addition, I will obtain a site license for the PNMR software for my personal use (**Specific Aim II**). To enhance training and communication with Anasazi, I will establish a remote communication method between our NMR at LSSU and instruments housed at Anasazi (**Specific Aim III**). Pulse sequences developed for PNMR will be written and evaluated by myself and the technical staff at Anasazi (**Specific Aim IV**).

II. Development of NMR experiments targeted for Undergraduate Chemical Education

Developing some of these advanced pulse sequences for use on the Anasazi NMR is only the first step. Once the feasibility of these is demonstrated, a series of experiments will be developed that can be used to *demonstrate* chemical concepts. To this end I will visit the Complex Carbohydrate Research Center, at the University of Georgia in Athens, GA. The researchers at this facility have a collaborative grant through the National Science Foundation (NSF) to train external scientists in NMR. The conference I attended last summer is part of this grant. The researchers at the CCRC are uniquely positioned to aid

in the development of new lab experiences based on the Anasazi pulse sequences that I propose. Both John Glushka and Jim Prestegard have access to NMR facilities in the University of Georgia Department of Chemistry, which houses two Anasazi NMR instruments. These instruments will be used to "test" the pulse sequences developed in a teaching setting. (**Specific Aim V**) The CCRC will also provide space, computer access, and technical assistance in developing these experiments.

Some of the specific experiments that I plan to develop are (chemical concept to be examined in parenthesis):

1. HSQC of galactose penta-acetate (chemical structure and spin coupling)
2. Water suppression of a nucleotide using Water Gate (difference between water and organic chemical NMR signals)
3. NOE of galactose penta-acetate (thru space interactions in molecules, Nuclear Overhauser Effect).
4. Enzymatic ligand binding using TOSCY (enzyme active site interactions, intermolecular interactions)
5. Drug screening using NMR and NOE transfer spectroscopy (dissociation constant of drug molecules, ligand binding)

Finally, I plan on training the remaining chemistry faculty in the use of more advanced NMR techniques so that they may choose to incorporate the use of NMR into their courses here at LSSU (**Specific Aim VI**). At present only two other faculty are using NMR in their courses (Dr. Iretski, and Dr. Westrick). I hope to encourage expanded use of this powerful teaching tool by the remaining faculty in the department. Courses such as Physical Chemistry, Instrumental Analysis, and General Chemistry would benefit from the incorporation of NMR into both their lecture and laboratory components.

III. Incorporation of NMR into a Molecular Spectroscopy course at LSSU

After developing the pulse sequences described and testing them in a teaching setting, an advanced course in molecular spectroscopy will be proposed for incorporation into the chemistry curriculum (**Specific Aim VII**). This course will focus on the main types of molecular spectroscopy: 1) Raman, Infrared, and Ultraviolet Spectroscopy, 2) Fluorescence Spectroscopy, and 3) Nuclear Magnetic Resonance Spectroscopy. This course would have a significant laboratory component that expands on the discussion of these types of spectroscopy students receive in Organic and Instrumental Chemistry. It would also have targeted experiments designed to *teach specific chemical concepts*. The development of this course will provide our chemistry, forensic, and environmental majors additional training in spectroscopy thus better preparing them for a future in their chosen discipline.

Sabbatical Benefits

The proposed sabbatical will benefit my own personal professional development, as well as the Department of Physical Sciences, and LSSU as a whole. The incorporation of NMR into more core chemistry classes and the proposed Spectroscopy course will strengthen the marketability of our students as they enter industry, graduate training, or government positions. I plan on publishing both the pulse sequences for PNMR software as well as several of the experiments derived from the application of these sequences. The proposed research strengthens the scientific relationship between our department and an academic institution (University of Georgia) and an industrial institution (Anasazi NMR). These collaborative efforts will offer our students better graduate and industrial opportunities by establishing relationships with these entities. Finally, the proposed research positions the department for successful external funding of undergraduate chemical education specifically in the areas of NMR spectroscopy.

References

1. "Modern NMR Spectroscopy in Education." 2007. D. Rovnyak and R. Stockland, eds. ACS Symposium Series 969. Oxford University Press, Cambridge.

2. "Looking into live cells with in-cell NMR spectroscopy." 2007. P. Selenko, G. Wagner. Journal of Structural Biology. Vol. 158., 244-253

3. A. Bax and D. G. Davis, *J. Magn. Reson.*, **65**, 355 (1985).

4. H. Friebolin. Basic One- and Two-Dimensional NMR Spectroscopy. 2005. Wiley-VHC, Weinheim.

5. G. Bodenhausen, and D.J. Ruben. Chem. Phys. Lett., 69, 185-189 (1980).

Curriculum Vitae

Robert Marshall Werner, Jr.

Contact Information

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Employment History

Lake Superior State University Department of Chemistry, Associate Professor. **(2001-present)**
Tenure awarded November 18, 2005.

Center for Advanced Research in Biotechnology (CARB) and National Institute of Standards and Technology (NIST) **(1998-2000)**

University of Maryland College Park (UMCP) **(1993-1998)**

Education and Honors

Post-Doctoral **(1998-2000)**

-Research with Dr. James T. Stivers on the mechanism of Uracil DNA Glycosylase (a DNA repair enzyme) at the Center for Advanced Research in Biotechnology (CARB), 1998-2000.
-National Institute of Standards and Technology (NIST) post-doctoral fellowship, 1998-2001.
-NIH post-doctoral fellowship, 2000-2001.

Graduate **(1993-1998)**

-Ph.D. in Bioorganic Chemistry, University of Maryland College Park (UMCP), 1993-1998. Title of thesis: "Mechanistic probes of oligosaccharyltransferase and peptide:N-glycanase."
-Research with Dr. Jeffery T. Davis on the mechanism of N-linked protein glycosylation.
-Outstanding undergraduate TA award, 1994, UMCP.

Undergraduate **(1989-1993)**

-B.S. in Biology (Ecology), Cornell University, 1989-1993.
-Undergraduate research with Dr. David B. Collum in aqueous organic chemistry, 1992-1993.
-New York undergraduate research fellow, 1992-1993.

Courses Taught at Lake Superior State University (Since Fall 2001)

Course #	Course Title	Semesters Taught
CHEM105	Life Chemistry II (GOB)	14 semesters
CHEM220	Survey of Organic Chemistry	2 semesters
CHEM225	Organic Chemistry I	2 semesters
CHEM226	Organic Chemistry II	8 semesters
CHEM451	Introductory Biochemistry	8 semesters
CHEM452	Biochemistry II	3 semesters
CH/EV499	Senior Thesis	multiple semesters
CH/EV 399	Junior Seminar	2 semesters
HONR 302	The Chemistry of Mind Altering Drugs	1 Semester

Publications

1. **R. Marshall Werner,** Austin Johnston. "^{31}P NMR of the Pyruvate Kinase Reaction: An Undergraduate Experiment in Enzyme Kinetics." *In preparation*, **2009**.

2. **R. Marshall Werner,** Rebecca Johnson, Danielle Pitman, Peter Bonneau. "Development of an ELISA assay for type II." *In preparation*, **2009**.

3. **R. Marshall Werner,** "Trends in thiamine status in Atlantic salmon from the St. marys River." *In preparation*, **2009**.

4. **R. Marshall Werner,** Ashley Moerke, Greg Zimmerman, Barb Keller, Judy Westrick, Barb Evans. "Summary of Environmental and GIS data for the Upper St. Marys River, Summer 2005." *In preparation*, **2009**.

5. **R. Marshall Werner,** Ashley Moerke, Greg Zimmerman, Barb Keller, Judy Westrick, Barb Evans. "Biotic Integrity and Habitat Assessment within the St. Marys River AOC." Final Report to USEPA. **2008**. EPA Project # GL-96538301-0.

6. **R. Marshall Werner**, Benjamin Rook, and Roger Greil. "Egg-Thiamine Status and Occurrence of Early Mortality Syndrome (EMS) in Atlantic Salmon from the St. Marys River, Michigan." *Journal of Great Lakes Research,* **2006**, 32, 293-305.

7. **R. Marshall Werner** and James T. Stivers, "Kinetic Isotope Effect Studies of the Reaction Catalyzed by Uracil DNA Glycosylase: Evidence for An Oxocarbenium Ion-Uracil Anion Intermediate." *Biochemistry*, **2000**, 39, 14054-14064.

8. **R. Marshall Werner**, Yu-Lin Jaing, Russell G. Gordely, G. Jayashree Jagedeesh, Jane E. Ladner, Gaoyi Xiao, Maria Tordova, Gary Gilliland and James T. Stivers, "Stressing-Out DNA? The Contribution of Serine-Phosphodiester Interactions in Catalysis by Uracil DNA Glycosylase." *Biochemistry*, **2000**, 39, 12585-12594.

9. James K. Coward, Tong Xu, Barbara S. Gibbs, **R. Marshall Werner** and Jeffery T. Davis, "Oligosaccharyltransferase: Recent Research on Catalysis and Ligand Binding." *FASEB Journal*, **1999**, 13, A1539, suppl. S.

10. **R. Marshall Werner**, Leonard M. Williams and Jeffery T. Davis, "The C-Glycosyl Analog of an N-Linked Glycoamino Acid." *Tetrahedron Letters*, **1998**, 39, 9135-9138.

11. Tong Xu, **R. Marshall Werner**, Jeffery T. Davis and James K. Coward, "Synthesis and Evaluation of Tripeptides Containing Asparagine Analogs as Potential Substrates or Inhibitors of Oligosaccharyltransferase." *Journal of Organic Chemistry*, **1998**, 63, 4767-4778.

12. **R. Marshall Werner**, Ori Shokek and Jeffery T. Davis, "Preparation of 4-Oxo-L-Norvaline via Diazomethane Homologation of β-Aspartyl Aldehyde." ***Journal of Organic Chemistry*, 1997**, 62, 8243-8246.

13. **R. Marshall Werner**, Mike Barwick and Jeffery T. Davis, "C-Silylation of Secondary Amides: GlcNAc Peracetate Derivatives." ***Tetrahedron Letters*, 1995**, 36, 7395-7398.

Grants Awarded

$715,300, "Biotic Integrity and Habitat Assessment within the St. Marys River AOC." Funded by USEPA. Fall 2004-Fall 2007. PI. Extended to Summer 2008.

$24,960, "Thiaminase: New Tools in the fight against EMS." Funded by the Great Lakes Fishery Trust. Fall 2003-Spring 2005. PI.

$69,975, "CRIF/RUI: Acquisition of a High Pressure Liquid Chromatograph (HPLC) for Environmental Undergraduate Research and Education." Funded by NSF. Spring 2004-Spring 2007. Co-PI.

Recent Grants Submitted

$302,560, *Not Funded.* "Acquisition of a 300 MHz NMR to Enhance Undergraduate Research, Chemical Education, and Regional Cyber-Accessibility at Lake Superior State University." Submitted to NSF, Major Research Instrumentation. Winter 2006. Co-PI.

$331,747, *Not Funded.* "Acquisition of a 300 MHz NMR to Enhance Undergraduate Research, Chemical Education, and Regional Cyber-Accessibility at Lake Superior State University." Submitted to NSF, Chemical Instrumentation. Summer 2005. Co-PI.

$348,000, *Not Funded*, "Renovation of Lake Superior State's Aquatic Research Laboratory." Submitted to NSF. Summer 2005, Co-PI.

$415,241, *Not Funded*, " CAREER: Breaking the Functional Group Stranglehold: A Revitalized Bioorganic Approach to Chemistry." Submitted to NSF, CAREER, Summer 2003, PI.

$155,526, *Not Funded*, "Incorporation of FT-NMR into an Inquiry-Based Undergraduate Curriculum." Submitted to NSF, Dept. Undergraduate Education, CCLI A&I, Fall 2002, PI.

Invited Presentations

"Trends in egg-thiamine status of Atlantic salmon from the St. Marys River, Michigan." Lake Superior State University. Annual Meeting of the Michigan Chapter of the American Fisheries Society, Sault Ste. Marie, MI, March 3-5, **2008**.

"Patterns of coastal wetland fish communities in the St. Marys River, MI." Moerke, W. Werner, H. Potter, B. Keller, B. Evans, J. Westrick, and G. Zimmerman. North American Benthological Society Annual Meeting, Anchorage, AK, June3-10,**2006**.

"Mechanistic Studies of Uracil DNA Glycosylase." Northern Michigan University. Marquette, MI. March 18, **2005**.

"Thiamine status of St. Marys River (MI) Atlantic salmon and progress on the development of new techniques to study EMS/TDC. Early Mortality Syndrome Conference. Ann Arbor, MI. September 8-9, **2004**.

"LSSU's Award of a USEPA Funded Grant for the Aquatic Research Laboratory." St. Marys River Task Group. Cisler Center, LSSU. July 29, **2004**.

"Thiaminase: The little enzyme that could change the Great Lakes Forever." Michigan State University. Lansing, MI. June 23, **2004**.

"Thiaminase: New Tools in the Fight Against EMS in Great Lakes Salmonids." Central Michigan University. Mount Pleasant, MI. April 7, **2003.**

"Thiaminase: Progress Toward a Novel Fluorescent Micro-plate Assay." Early Mortality Syndrome Conference. Ann Arbor, MI. June 26, **2002**.

"Thiaminase: New Tools in the Fight Against EMS in Great Lakes Salmonids." Grand Valley State University. Grand Rapids, MI. March 22, **2002**.

"Mechanistic Studies of Uracil DNA Glycosylase." University of Michigan. Ann Arbor, MI. March 21, **2002.**

"A Picture of the Transition-State Structure of Uracil DNA Glycosylase. Studies Involving Kinetic Isotope Measurements." University of Maryland College Park, College Park , MD. Jeff Davis and Steve Rokita group meeting. May, **2000**.

"Probing the Reaction Mechanism of Uracil DNA Glycosylase with Kinetic Isotope Effects." Annual National Institute of Technology and Standards, Biotechnology Division conference. May, **2000**.

"Probing the Ser-Pinch Mechanism in Base-Flipping and Catalysis by Uracil DNA Glycosylase (UDG) Using Directed Mutagenesis and Phosphorothioate (Ps) Substitutions." American Chemical Society National Meeting in San Francisco, CA. Summer **2000**. Poster Presentation # 64, Division of Biological Chemistry.

"Towards Determination of the Transition-State Structure of Uracil DNA Glycosylase: Substrate Synthesis, Characterization, and KIE Measurements." Gordon Research Conference in Bioorganic Chemistry at Proctor Academy in Andover, NH. Summer, **1999**.

"Diazomethane Homologation of Amino Acid and Peptidyl Aldehydes." American Chemical Society Meeting in Las Vegas, Nevada. Summer, **1997**. Oral presentation #390.

"Choosing a Graduate School in Chemistry." American Chemical Society, MARM at Pace University. New York, NY. Summer, **1997**. Special volunteer speaker representing the University of Maryland.

"Synthesis of Methyl Ketone Tripeptides as Mechanistic Probes of Oligosaccharyltransferase by Aldehyde Homologation with Diazomethane." American Chemical Society National Meeting in Orlando, FL. Summer, **1996**. Oral Presentation # 168.

"Synthesis of a Novel Methyl Ketone Tripeptide as a Mechanistic Probe for Protein N-Glycosylation." American Chemical Society, MARM at Villanova University, PA. Summer, **1996**. Oral Presentation # 139.

"Synthesis and Conformation of Asparagine Linked Glycopeptides." American Chemical Society, MARM at University of Maryland Baltimore County, MD. Summer, **1994**. Poster presentation # 241.

Professional Conferences Attended

Center for Workshops in the Chemical Sciences NMR Workshop, NMR Fundamentals and Applications. Complex Carbohydrate Research Center, University of Georgia, Athens, GA. May 17-22, **2009**.

Argonne Undergraduate research Symposium. Argonne National Laboratories, Argonne, IL, Nov. 2-4, **2006**.

Early Mortality Syndrome Conference. Ann Arbor, MI. September 5-6, **2006**.

Early Mortality Syndrome Conference. Ann Arbor, MI. September 22-23, **2005**.

Lake Huron Technical Committee, Sault St. Marie, Ontario. July 19-21, **2005**.

Early Mortality Syndrome Conference. Ann Arbor, MI. September 8-9, **2004**.

Restoration of Native Species Workshop, Ann Arbor, MI. June 21-22, **2004**.

USGS - Thiamine Deficiency Complex Conference, Ann Arbor, MI. September 16, **2003**.

American Chemical Society Regional Meeting, Michigan Technical University. Houghton, MI. April 11-12, **2003**.

American Chemical Society Annual Meeting, Boston, MA. August 18-22, **2002**. Presented poster entitled, "Thiaminase: Progress Toward a Novel Fluorescent Micro-plate Assay." Early Mortality Syndrome Conference.

Early Mortality Syndrome Conference. Ann Arbor, MI. June 25-26, **2002**.

International Thiamine Conference, Rutgers University. Newark, NJ. May 17-21, **2002**.

American Chemical Society National Meeting. San Francisco, CA. Summer **2000**

Gordon Research Conference in Bioorganic Chemistry. Proctor Academy. Andover, NH. Summer, **1999**.

American Chemical Society National Meeting. Las Vegas, NV. Summer, **1997**.

American Chemical Society Mid-Atlantic Regional Meeting. Pace University. New York, NY. Summer, **1997**.

American Chemical Society National Meeting. Orlando, FL. Summer, **1996**.

American Chemical Society Mid-Atlantic Regional Meeting. Villanova University, PA. Summer, **1996**.

American Chemical Society Mid-Atlantic Regional Meeting. University of Maryland Baltimore County, MD. Summer, **1994**.

Senior Projects Mentored at Lake Superior State University

Peter Bonneau, (**Honors**) Spring 2009
"The Development and Application of an ELISA for Thiaminase II."

Peter Bonneau, Spring 2009
"The Development of an ELISA for Thiaminase II."

Austin Johnston, Fall 2008
"Determination of Enzyme Kinetics: ^{31}P NMR."

Alex Mwai, (**Honors**), Spring 2007
"Genetic analysis of perch from the St. Marys River."

Alex Mwai,, Fall 2006
"50/50 Superposition of the F2 and F1 hyperfine levels of the $5^2 S_{1/2}$ energy levels of Rubidium-87 atoms."

Anthony Bruni, Fall 2005
"Measurement of coenzyme-Q10 from vitamin supplement pills."

Danielle Pitman, Spring 2005
"Development of and ELISA for bacterial derived thiaminase."

Justin McCleod, Spring 2005
"Egg Thiamine and Early Mortality Syndrome in Great Lakes Basin Walleye *Sander vitreus*."

Benjamin Rook (**Honors**), Fall 2004
"Rapid Reversed-Phase Solid-Phase Extraction Analysis of Egg-Thiamine Levels in St. Mary's River Atlantic Salmon (*Salmo salar*)."
"Relationship of Egg-Thiamine Levels in St. Mary's River Atlantic Salmon (*Salmo salar*) with Atlantic Salmon Population Factors and Forage-Base Population Factors."

Becca Johnson, Spring 2004
"Purification of Thiaminase I for the production of anti-Thiaminase antibodies."

Richard Federley, Spring 2004
"Assay Development and Large Scale Purification of Thiaminase."

Adam Nanninga, Fall 2003
"Analysis of Procure® in Fish Tissue."

Michael O'Toole (**Honors**), Spring 2003
"An improved Method for Isolation of *Bacillus thiaminolyticus* from Alewife."
"Purification of Thiaminase I From Alewife-Derived *Bacillus thiaminolyticus*."

Nick Gresick, Spring 2002

"Thiamine Deficiency in Atlantic salmon: Analysis of Egg-Thiamine Levels and Determination of Fry Survival Rate in Response to Thiamine Treatment."

Personal Honors and Interests

-Volunteer assistant coach and Team Academic Advisor, Lake Superior State University men's basketball team.
-Men's varsity basketball, Cornell University.
-Coach youth basketball (2001-present).
-Eagle Scout, Boy Scouts of America.

Professional References

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Letter of Support from Anasazi Instruments

Monday September 21, 2009

Hi Marshall,

We are happy to help you develop and evaluate advanced pulse sequences. It would be a particular interest in the laboratory applications to develop a measurement with a particular chemical system in mind.

Please send me a short list of experiments you'd like to try to develop and I'll be happy to work with you since I am the primary person in the area of application development. Thanks for your interest and support.

Don Bouchard

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On Mon, Sep 21, 2009 at 1:26 PM, Robert Werner

Donald,

Thanks for your consideration of my ideas of trying to develop more advanced pulse sequences to be used on the Anasazi instrument and the PNMR Software. As I had indicated, we have been successful at incorporating the NMR into several of our classes. I would like the opportunity to work with your company to develop more advanced pulse sequences to extend the capability of the instrument. My main goal is to develop advanced laboratory experiments for our upper division classes and to disseminate these new approaches via publication.

If you are in fact interested in establishing a collaborative relationship for this upcoming year, please let me know. I am interested in visiting your facility and establishing a way to "remotely" try some of these pulse sequences on instruments at your facility.

Thanks, Marshall

Education is not the filling of a pail,
but the lighting of a fire. Yeats

Marshall Werner
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Department of Chemistry
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Letter of support from John Glushka

Friday September 25, 2009

Dear Marshall,

I'd be happy to help you in any way – I think it is a potentially very useful project. Just give us some visiting times when you have things planned. Is there an Anasazi user group on the web? I take it PNMR is the nmr software – let me know what are the computer requirements to run it.

Keep in touch –

John

John Glushka
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315 Riverbend Rd. Athens, GA 30602
706-542-4483 fax:706-542-4412
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On Sep 24, 2009, at 8:45 AM, Robert Werner wrote:

> Dear Jim and John,
>
> I attended your NMR conference this summer and wanted to thank you for
> such a great experience. While at the conference you may recall a
> number of the undergraduate faculty were interested in using some of
> the advanced pulse sequences you presented (TOSCY, Watergate, NOE
> transfer) on our permanent magnet Anasazi systems. One big question
> was the limitations of the hardware of these systems. I have
> contacted
> Anasazi and they have agreed to work with me both on site and via
> remote connection during a proposed sabbatical for next academic year
> (2010-2011) to address these hardware limitations.
>
> Since attending your conference, I have become interested in expanding
> the capabilities of these permantent magnet systems with an eye on the
> use of NMR in the undergraduate curriculum. I would like to ask for
> your input and expertise when it comes to the details of these pulse
> sequences. I would also like the opportunity to visit the CCRC at UGA
> during 2010-2011 academic year for a week or two. This visit would be
> at no cost to you, I would only ask for access to the computer
> workstations, the ability to load PNMR on one of these stations, and
> finally your availibility to ask advice and questions when writting
> these pulse sequences.
>
> Please let me know what you think.
>
> Best Regards, Marshall
>
> ---
> Education is not the filling of a pail,
> but the lighting of a fire. Yeats
>
> Marshall Werner
> Lake Superior State University
> Department of Chemistry
> 906-635-2281
>

Response from Jim Prestegard

Friday September 25, 2009

Marshall,

Glad to hear that you will be pushing this forward. We would certainly be happy to have you here for a week or two and will do what ever is necessary to help you out.

Jim

James H. Prestegard
Complex Carbohydrate Research Center
University of Georgia
315 Riverbend Rd
Athens, GA 30602-4712
706-542-6281
jpresteg@ccrc.uga.edu

-----Original Message-----
From: Robert Werner [mailto:mwerner@lssu.edu]
Sent: Thursday, September 24, 2009 8:45 AM
To: John Glushka; Jim ?Prestegard
Cc: Marshall Werner
Subject: Pulse Sequence Development for Anasazi

Dear Jim and John,

I attended your NMR conference this summer and wanted to thank you for such a great experience. While at the conference you may recall a number of the undergraduate faculty were interested in using some of the advanced pulse sequences you presented (TOSCY, Watergate, NOE transfer) on our permanent magnet Anasazi systems. One big question was the limitations of the hardware of these systems. I have contacted Anasazi and they have agreed to work with me both on site and via remote connection during a proposed sabbatical for next academic year (2010-2011) to address these hardware limitations.

Since attending your conference, I have become interested in expanding the capabilities of these permantent magnet systems with an eye on the use of NMR in the undergraduate curriculum. I would like to ask for your input and expertise when it comes to the details of these pulse sequences. I would also like the opportunity to visit the CCRC at UGA during 2010-2011 academic year for a week or two. This visit would be at no cost to you, I would only ask for access to the computer workstations, the ability to load PNMR on one of these stations, and finally your availibility to ask advice and questions when writting these pulse sequences.

Please let me know what you think.

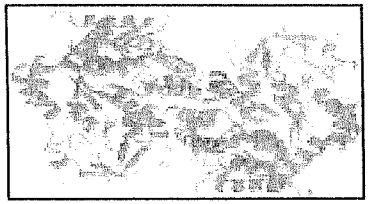
Best Regards, Marshall

Education is not the filling of a pail,
but the lighting of a fire. Yeats

Marshall Werner
Lake Superior State University
Department of Chemistry
906-635-2281

Determination of Enzyme Kinetics: ^{31}P NMR

Austin Johnston, Dr. M. Werner
Department of Chemistry and Environmental Sciences



Pyruvate Kinase

Purpose and Introduction:

The reason for this experiment is to determine the enzyme kinetics of pyruvate kinase using two different methods. These two methods are the previously accepted and widely documented continuous spectrophotometry, and the new and experimental P31 NMR. The NMR, when changed to the P31 probe, can identify each independent phosphate of several different compounds. Three methods will be outlined here including: The spectrophotometric assay, the NaOH quenched method, and the "one pop" method. The spectrophotometric assay will be done with a spectrophotometer and will be very similar to the P31 NMR methods, only less concentrated. Next is the base quenched method where the reaction will be stopped at several different pre determined increments of time. Using a strong base to halt the reaction, each based eluent will have an NMR ran on it. Finally, there is the "one pop" method in which all the reagents are added together and NMR is ran on the same reaction while the reaction is underway.

Materials:

"One Pop" Method P31 NMR:
16 mM $MgCl_2$
8 mM KCl
21 mg Adenosine Diphosphate (ADP)
10 mg Phosphoenol Pyruvate (PEP)
1 M imidizole buffer, pH at ~ 7.5
Pyruvate Kinase (kept cold)

Continuous Spectrophotometric Rate Determination:
(Adapted from Sigma Aldrich)
•100 mM Potassium Phosphate Buffer, PH 7.6 at 37°C
•17 mM Phosphoenol Pyruvate (PEP)
•1.3 mM ß-Nicotinamide Adenine Dinucleotide (NADH)
•100 mM Magnesium Sulfate Solution (MgSO4)
•44 mM Adenosine Diphosphate Solution (ADP)
•L-Lactic Dehydrogenase Solution (LDH)(5000 units/mL)
•Pyruvate Kinase (PK) (.25 units/mL)

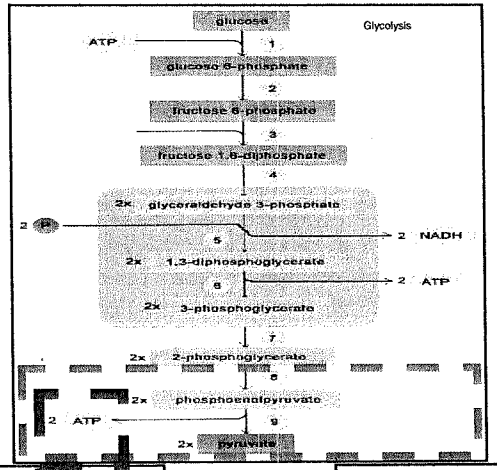
NaOH Quenched P31 NMR:
•16 mM MgCl2
•8 mM KCl
•.170 g ADP
•.80 g PEP
•1 M imidizole buffer, pH at ~ 7.5
•10 N NaOH
•Pyruvate Kinase (kept cold)

Methods:

Procedure to Run "One Pop" Reaction With P31 NMR:

- This method is very similar to the NaOH quenched method, only instead of 8 stopped and separated reactions and NMR tubes, there is only 1 reaction, 1 tube, no base quenching, and one-eighth the reagents.
- Add 10 mg PEP, 22 mg ADP, 12.5 µL KCl, 10 µL $MgCl2$, and finally .71 µL imidizole buffer into a single reaction vessel.
- Take this reaction without the enzyme and place it in an NMR tube and run, this will serve as the time 0, where all PEP is still present and no ATP. Run the NMR on this t=0 tube using the following NMR specifications:
  - Number of scans (NS), 128
  - Size (SI), 32k
  - Receiver Gain (RG), 90
  - Relaxation Delay (RD), 2 seconds
  - F1, 24.292
  - Width (W1), 6983.2
  - Pulse Width (PW), 12.2 µsec
  - When importing into NUTS, Line broadening (LB) must be 4.0
  - Decouple? **YES**
- After the spectrum is obtained, remove the reaction from the NMR tube and place back into its original vial. Now it is time to add the enzyme.
- This all must be done while trying to be quick and efficient. Add enough enzyme to have the reaction linear over a 60 minute window, suggestion would be 20 µL of stock 2000 units/ml solution.
- As quickly as possible, place the started reaction in an NMR tube and into the NMR and run, recoding when the scans begin.
- As soon as the first run is done, which was hopefully started around 1 minute, run 128 scans again. Keep doing this for 60 minutes or 7 spectras, as soon as the program is ready, run another one and so forth.
- Transfer these spectras into the NUTS program and integrate the visible peaks but always setting the peak around 0 (PEP) to integrate for 1.
- Come back 24 hours later to obtain a "completed" spectra where the reaction was allowed to go to completion, this ensures that the reaction finished.

Fig. AJ.1



Results:

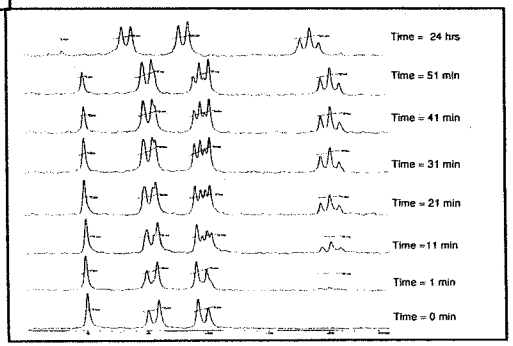
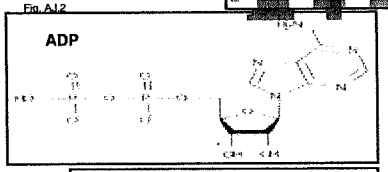


Fig. AJ.6

Fig. AJ.2



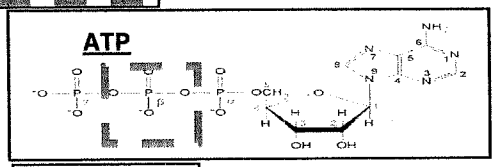


Fig. AJ.3

Fig. AJ.4

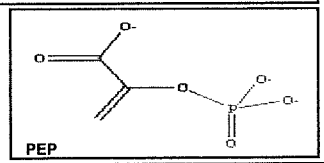


Fig. AJ.5

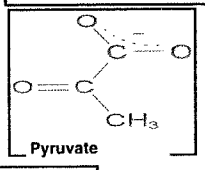
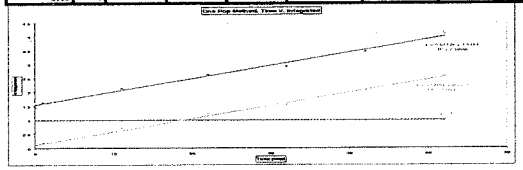


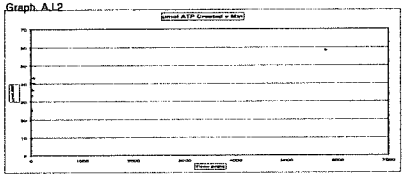
Fig. AJ.7

Time (min)	PEP			Integral 4	% PEP	% ATP		µmol ATP	µmol ATP/min
0	1			0	1	0		0	0
1	1			0.10	0.840338134	0.159663866		6.804307426	6.804307426
11	1			0.73	0.578034662	0.421965318		25.28260378	2.307509434
22	1			1.24	0.446428571	0.553571429		33.29914483	1.513597492
32	1			1.65	0.302155663	0.697843137		36.56376667	1.142617715
42	1			2.04	0.328947368	0.671052632		40.26062615	0.961086861
52	1			2.57	0.280112045	0.719887955		43.30363173	0.832762149
5760	1			31.5	0.030709231	0.969290769		58.3024233	0.010121948

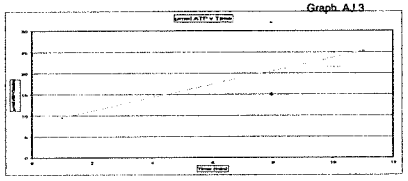
Graph. AJ.1



Graph. AJ.2



Graph. AJ.3



Procedure to Run Continuous Spectrophotometric Rate Determination:

- Pipette (in milliliters) the following reagents into suitable cuvettes:
  - De-Ionized Water 1.40 mL
  - Reagent A (Buffer) 0.80 mL
  - Reagent B (PEP) 0.10 mL
  - Reagent C (ß-NADH) 0.25 mL
  - Reagent D ($MgSO4$) 0.20 mL
  - Reagent E (ADP) 0.10 mL
  - Reagent F (LDH) 0.002 mL
- Mix by inversion and equilibrate to 37°C. Monitor the A340nm until constant.
- Record this reading and this will serve as your starting point.
- Use a suitably thermostatted Spectrophotometer if available to keep the reaction at the correct body temperature.
- Then add reagent G (enzyme solution, PK) 0.10 mL.
- Immediately mix by inversion and place in spec. Record the decrease in A340nm for approximately 5 minutes.
- Obtain the A340nm/minute using the maximum linear rate for both the test and blank.

Conclusion and Explanation:

Above there are three different graphs. The first graph is the integrations of the NMR spectras over time. Each color represents each individual spike. The dark blue line is the PEP peak and is always set at 1. This is done so that the other peaks, especially the light blue one (β-phosphate of ATP), can be compared against it. The other two lines are the α-phosphate and the γ-phosphate, which are only here to indicate that there are changes. The reason that these phosphates are not used in the calculations is because they are the same on ADP and ATP, which does not indicate if and how much ATP was created. The graph AJ.2 was obtained by calculations on the findings of the first graph. The integral for PEP was placed over the integral of PEP plus the integral of the ATP β-phosphate. This gives a ratio of PEP left and ATP created in the entire reaction. These ratios are then converted into µmol ATP and µmol PEP. Then taking the µmol ATP and placing it over time in minutes, provides units of enzyme. A unit of enzyme is defined as 1 µmol of substrate converted per minute. When done over the total time of this reaction (5760 minutes), we can see that it makes the parabolic shape that is expected with enzymes kinetics. This graph gives us an indication of PK activity, but only shows that the enzyme is working as expected. The final graph is the activity of the enzyme from minute 1 to minute 11. This provides a straight line for which the slope of the line will be the enzyme activity, in this case 1.5778 units.

Bottom Line and Further Experiments:

This experiment was done successfully in triplicate, each time yielding similar enzyme kinetics. The NMR is a way to assess the kinetics of an enzyme efficiently. This method can achieve that goal as well as introduce students to a very useful instrument, the NMR. Further research would include successfully using the base quenched method. Although using more of the reagents, it would result in a more accurate and time controlled assay that a whole class could easily participate in.

Special Thanks to:
Dr. M. Werner
LSSU Department of Chemistry and Environmental Sciences

MEMO

To: Sabbatical Leave Committee

From: Dr. R. Marshall Werner

Date: October 9, 2009

Subject: Statement of intent to return to LSSU after 2010-2011 Sabbatical

I agree to return to Lake Superior State University for one full academic year immediately following the full academic year sabbatical or reimburse in full monetary value encumbered by Lake Superior State University during the period of my leave. Further, I agree to submit a written summary of my accomplishments before 12/1/11.

M. Wenn

Lake Superior State University

Report on Sabbatical Leave

1. Name of Professor: Dr. R. Marshall Werner

2. Department: Chemistry and Environmental Sciences

3. Time Frame of the Sabbatical Leave: F2010-S2011

4. Title of Sabbatical Leave Proposal:

   Expanding Nuclear Magnetic Resonance (NMR) Capabilities of 60 MHz permanent magnet systems through the development of novel pulse sequences for use in an undergraduate chemistry curriculum and incorporation into a course on Molecular Spectroscopy.

5. Narrative of Sabbatical Leave:

My recent sabbatical had three primary aims:

I. Development of Advanced Pulse Sequences for the Anasazi NMR
II. Development of NMR experiments targeted for Undergraduate Chemical Education
III. Incorporation of NMR into a Molecular Spectroscopy course at LSSU

Progress on these Aims:

I. Development of Advanced Pulse Sequences for the Anasazi NMR

In the summer of 2010, I visited Anasazi Instruments (Indianapolis, IN) to receive training on the user interface, programming language, and interpretation of NMR pulse sequences. Upon return to LSSU, I tested several new programs that were developed to assess their

feasibility on our 60 MHz system. These tests were successful for the most part, however, due to the limitations in electronics and hardware of our current system, not all of the more advanced pulse sequences could function properly. Two senior thesis projects are currently continuing these efforts.

II. Development of NMR experiments targeted for Undergraduate Chemical Education

During the Fall of 2010 and Spring, I worked on developing a series of experiments aimed at illustrating concepts associated with nuclear magnetic resonance. These include measurement of spin relaxation times (i.e. T_1 and T_2 values) and the effect of viscosity on these parameters as it relates to molecular tumbling in solution. In addition, several experiments involving ^{31}P NMR for use as an illustrative biochemical example were investigated. These experiments will be incorporated into the chemistry curriculum.

During the course of my sabbatical, I formulated the idea of hosting a scientific conference here at LSSU to highlight issues surrounding NMR education. Thus, the conference "Bootcamp for NMR Educators" was held on May 22-23, 2011. A total of 22 scientists attended representing numerous academic institutions and 4 industrial entities. The conference was sponsored by 7 international companies.

<u>Conference Sponsors</u>

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415-287-0727

*Attendee at conference

This conference included demonstrations of new NMR instrumentation, presentations by academic and industrial representatives, and presentations by two key-note speakers. A more complete analysis of the global interest in this conference is provided as a hardcopy attachment detailing the visits to the conference website. To summarize, there were 617 unique conference web site visitors from 43 different states and 48 different countries.

III. Incorporation of NMR into a Molecular Spectroscopy course at LSSU

Due to the recent accreditation of our Chemistry program by the American Chemical Society, our department is currently in the process of evaluating our entire curriculum to meet accreditation standards. There have been informal discussions of the development of such a course to include NMR concepts as well as the incorporation of NMR laboratory experiences throughout the curriculum. Until these discussions are completed, the development of this course will remain on hold.

Additional Activities:

I have been serving as the guest associate editor for the International Journal of Great Lakes Research special issue highlighting the contributions of LSSU to research on the St. Marys River. During my sabbatical this issues was finalized and has now been published.

Publications during sabbatical:

1. Moerke, A.H., Werner, R.M. Ecological status of the St. Marys River: Foreword. 2011. *International Journal of Great Lakes Research*, **37**, 1-4.
2. Keller, B.J., Back, R.C., Westrick, J., Werner, R.M., Evans, B., Moerke, A., Zimmerman, G., Wright, D.D., Grenfell, E., Courneya, J. Sediment quality at selected sites in the St. Marys River area of concern. 2011. *International Journal of Great Lakes Research*, **37**, 12-20.
3. Werner, R.M., Southwell, B., Rook, B. Five year trends in the egg-thiamine status of Atlantic salmon from the St. Marys River, Michigan. 2011. *International Journal of Great Lakes Research*, **37**, 43-46.

Future Activities and Benefits of Sabbatical

The NMR conference that was held here at LSSU was especially fruitful in establishing contacts with both academic and industrial scientists with interest in NMR. I am currently working with several sponsors to investigate continuing this conference on an annual basis. Due to the remote location of LSSU, this may involve a rotating schedule in collaboration with other mid-west institutions. A grant opportunity may present itself that may allow this conference to become externally funded. In addition, further grant opportunities may allow LSSU to upgrade its NMR instrumentation. Specifically, a more powerful permanent magnet system (90 MHz vs. our current 60 MHz) would be a significant upgrade and would allow more advanced experiments to be performed due to advances in instrument electronics and program capabilities.