NSF MRI Preproposal: Development of a DNP NMR system with world-leading sensitivity for detecting metabolites and identifying natural products

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CoPI: Matt Merritt, BMB, COM & NHMFL
CoPI: William Brey, NHMFL
CoPI: Joaquin Casenova, Electrical and Computer Engineering (ECE), College of Engineering
Scientific Advisors: Stephen Hill, Physics, FSU & NHMFL; Jenshan Lin, ECE, Engineering; Johan van Tol, NHMFL
Major users at UF: Hendrik Luesch, Medicinal Chemistry, COP; Rebecca Butcher, Chemistry, CLAS; Robert Huigens, Med. Chem., COP; Yousong Ding, Med. Chem., COP; Steven Bruner, Chemistry, CLAS; Gail Fanucci, Chemistry, CLAS

1) Category of Application: Development

2) Brief description of instrumentation

We propose to develop the next generation of technology for nuclear magnetic resonance spectroscopy (NMR) which will provide unprecedented sensitivity for the detection, identification, and quantitation of natural products and metabolites. The instrumentation we are proposing will combine two orthogonal technologies developed at the National High Magnetic Field Laboratory (NHMFL) —1) high resolution probes utilizing high temperature superconducting (HTS) radiofrequency coils and 2) dynamic nuclear polarization (DNP) enhanced NMR at high magnetic fields—which individually have proven to enable significant gains in NMR sensitivity; concurrently we will move these technologies from 14.4 T to 18.8 T to realize further gains in sensitivity and resolution with magnetic field. Combining these technologies into an integrated high resolution NMR instrument at 18.8 T will usher in a new era of NMR applications enabled by 1-2 orders of magnitude gains in sensitivity relative to conventional NMR instruments at this field. Incorporating DNP and HTS technologies into a single instrument requires state of the art integration of terahertz microwave circuitry and recently patented radio-frequency coil designs utilizing Yttrium-Barium-Copper Oxide (YBCO) superconductors. To accomplish this, a team of experts in DNP (Long, Merritt), HTS NMR probes (Brey), and microwave circuit design and modeling for biological samples (Casenova) seek to develop a state of the art high field NMR DNP system through the NSF MRI development program. To drive the application of this technology in demanding systems, a cadre of five experts in natural products research (Luesch, Butcher, Huigens, Ding, and Bruner), an expert in metabolic flux (Merritt), and an expert in organic radicals for EPR and DNP applications (Fanucci) have been identified as major users of the instrument while it is under development. Once the technology is demonstrated and refined, it will be made available to a broader user community through the highly regarded NHMFL external user program. It is anticipated this instrumentation will ensure continued recognition of UF and the NHMFL as world leaders in the development of magnetic resonance technologies for chemical and biological applications. This instrument will allow the study of small molecules, particularly metabolites and complex natural products, which are only available in mass limited quantities. The technology developed and implemented will greatly increase the applicability of NMR to a wide range of research endeavors identifying the role of small molecules in chemical communication, metabolic flux regulation, and as drug development targets.

3) Impact of instrumentation on current/future research and research training activities

The faculty members pursuing this Major Research Instrumentation (MRI) grant have active research programs which develop and utilize NMR to solve fundamental biological and chemical questions. Understanding the structures, stereochemistry, and time-dependent concentrations of small molecule metabolites and natural products is critical to developing new drug therapeutics, understanding chemical communication, and evaluating healthy and diseased metabolic states. The development of an NMR instrument with unparalleled sensitivity will impact research at the University of Florida in several areas and will ensure our pre-eminence in drug development, metabolomics, and magnetic resonance in chemistry and biology enabling us to remain competitive for federal funding.

NMR is exceptional in its ability to measure molecular structures, stereochemistry, and concentrations non-destructively, including time-dependent chemical conversions in active biological samples. The biggest hurdle in utilizing NMR is its intrinsic low sensitivity relative to other analytical techniques. Development of the proposed technology would enable us to significantly expand the range of problems which can be addressed via NMR.

Approaches to enhancing NMR sensitivity—Magnets: NMR frequencies are directly proportional to the magnetic field which drives the development and purchase of larger and larger magnets. This is because the signal-to-noise (S/N) increases as the resonant frequency goes up. The exact improvement in S/N depends on many factors, but it is commonly accepted that the S/N increases approximately as $B_0^{3/2}$. Unfortunately, the price of magnets also increases significantly as the field strength increases. For example, an 800 MHz (18.8 T) superconducting system costs around $2 million whereas a 900 MHz (21.1 T) system is closer to $5 million, and a 1 GHz (23.5 T) system is over $10 million. Part of the non-linear increases in cost with field is that current conductors are inherently limited in current-carrying capacity at high fields and become non-superconducting above 25 T. The NHMFL is currently pursuing next generation HTS conductors for the development of magnets in the range of 28-36 T, but this technology is not presently available with the needed specifications for high resolution NMR spectroscopy. In this MRI application we seek to develop orthogonal technologies that increase sensitivity irrespective of magnetic field. They will be deployed in a conventional high field 18.8 T magnet we recently acquired, with plans to continue their development at higher magnetic fields as HTS magnets with NMR specifications are developed by the NHMFL magnet science and technology/ applied superconductivity groups.

Approaches to enhancing NMR sensitivity —Probes: A far more practical solution to improve detection sensitivity is to improve the NMR probe. Through the radio frequency (RF) coil, the NMR probe is the interface between the sample and the spectrometer, and it is used to both excite nuclear spins and detect the electrical signals generated by precessing spins. For a fraction of the cost required to purchase a magnet, a fairly routine 500 to 700 MHz system can provide outstanding S/N with the right design of probe. The basic requirements for a probe are that it has an electrical conductor oriented to deliver a magnetic field B_1 that is perpendicular to the static field B_0 and that this conductor can detect the resulting fields generated by the sample. Standard commercial probes that are sold with virtually every NMR system have multiply resonant, copper-based coils. For soluble samples, typical high resolution NMR probes are designed to accommodate 5-mm tubes which allow for ~600 μL of liquid for analysis and provide good ^{1}H S/N for samples with concentrations of about 1 mM on modern instruments

NMR technology development at UF/NHMFL—HTS Probes for high resolution (solution) applications HTS materials can provide significant enhancements to NMR probe sensitivity. Despite the increased sensitivity, HTS materials are not routinely used for NMR probes. One of the challenges in probe design is the YBCO must be deposited as a highly oriented thin film (~300 nm thick) on a sapphire substrate and patterned into a resonator of the size and shape needed for a given NMR frequency via laser etching (Figure 1). Nonetheless, in the frequency range for NMR active nuclei (80-800 MHz for ^{15}N, ^{17}O, ^{2}H, ^{13}C, ^{1}H nuclei), resonators of this type have much lower loss than normal metals and so have the potential for significantly greater sensitivity. Dr. Bill Brey, a co-PI on this proposal, first demonstrated the promise of this technology by developing a probe optimized for 1H detection and 10 μL samples at 600 MHz (14.4 T). This probe was initially deployed in 2005 in the Advanced Magnetic Resonance Imaging &

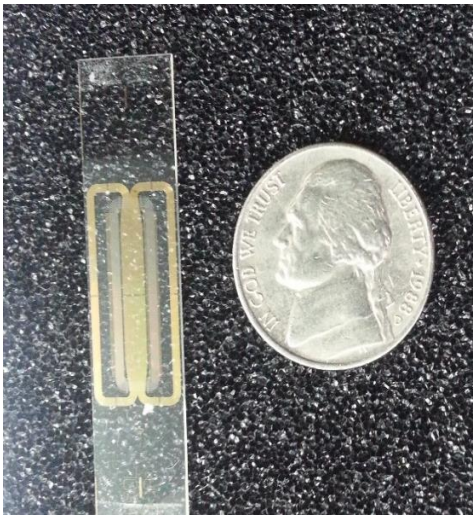

Figure. 1: Double tuned superconducting ^{13}C-^{1}H resonator for 1.5 mm samples.

Spectroscopy (AMRIS) Facility at UF, supporting a successful user program for mass-limited samples as it provided the highest ^{1}H mass sensitivity in the world at that time. This technology was licensed to Bruker Biospin, Inc., and became the basis for their line of 1.7 mm cryoprobes at mid- to high magnetic fields deployed in leading NMR facilities throughout the world. Dr. Brey then developed doubly resonant coils to enable both ^{13}C and ^{1}H detection in 1.5 mm (35 μL) samples. This technology was deployed in the AMRIS facility in 2012, again at 600 MHz ^{1}H (14.4 T), and was recently patented as well. This probe has demonstrated the highest ^{13}C mass sensitivity in the world and allows for simultaneous optimization for two frequencies in a single HTS probe. Currently he is developing, in collaboration with co PI Matt Merritt, a probe optimized for simultaneous direct detection of ^{13}C and ^{2}H frequencies at 14.4 T for application to metabolomics and fluxomics measurements, and he is developing an ^{15}N/^{1}H-optimized HTS probe for deployment at 18.8 T in the main user facility of the NHMFL at Florida State University (FSU) for direct detection of ^{15}N signals in proteins, and in particular characterizing intrinsically disordered proteins. Through this proposal we seek to develop HTS probe technology for ^{13}C/^{2}H/^{1}H NMR applications at 18.8 T and to redesign the sample space to enable simultaneous terahertz irradiation of samples to enable concurrent signal amplification by dynamic nuclear polarization (DNP).

Approaches to enhancing NMR sensitivity—Dynamic Nuclear Polarization: Dynamic nuclear polarization (DNP) overcomes the intrinsic insensitivity of NMR by transferring the higher polarization of electrons to the nuclei. The sensitivity gains observed for samples at cryogenic temperatures are truly impressive, with recent reports of DNP enhancements ranging from ~50 to a few hundred. The significant breakthroughs leading to high enhancements include the development of gyrotrons capable of delivering stable, high power (~10 W) microwaves and strategies for adding paramagnetic doping agents and transferring polarization. The advent of commercially available stable, high power microwave sources and auxiliary hardware compatible with high field NMR instruments has led to the recent transition of DNP-based research from proof of principle experiments to exciting new NMR applications enabled by DNP signal enhancements at cryogenic temperatures with spectroscopic elucidation of samples such as catalytic surfaces and membrane proteins via solid state NMR spectroscopy. More recently, it has been theoretically predicted and demonstrated at intermediate magnetic fields that DNP can enhance signals in solution samples at ambient temperatures via the Overhauser effect. It is predicted DNP enhancement at 18.8 T could add an order of magnitude in NMR sensitivity, making it as revolutionary a technique for high resolution NMR of small molecules as it has proven to be for magic angle spinning (MAS) DNP solid state NMR applications.

NMR technology development at UF/NHMFL—Dynamic Nuclear Polarization A specific goal of the NHMFL in its current funding period (2013-2017) is to develop a DNP user facility at high fields. Dr. Long has been leading an effort to combine expertise in NMR and electron magnetic resonance (EMR) at the NHMFL to develop DNP instrumentation for a wide variety of applications. Through this program, two instruments have been successfully developed and deployed in the NHMFL user program—a dissolution DNP polarizer in the AMRIS facility which is coupled to 4.7 T and 11.1 T MRI/S systems and a low temperature system at 14.4 T for MAS DNP solid state NMR applications at 600 MHz ^{1}H (395 GHz e^{-}). Of specific relevance to this proposal, Dr. Long,

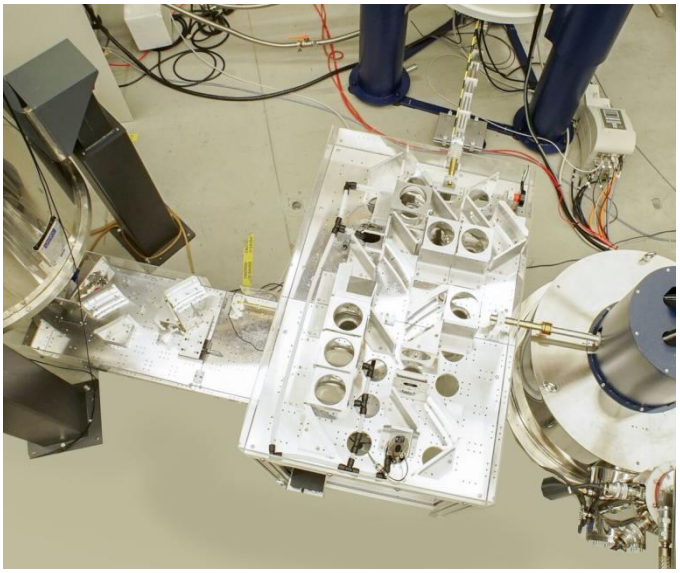

Figure 2 QO bench regulating the **μ**w beam from a 395 GHz gyrotron (right) to the DNP MAS ssNMR system (top) and the development instrument (left). A bench for selecting a circularly polarized beam and detecting electron signals is deployed for the development instrument.

Brey, and Hill have been developing, in collaboration with Lucio Frydman, a DNP instrument which capitalizes on quasi-optical approaches to deliver microwaves into an NMR magnet (Figure 2) and exploring the utility of DNP for polarizing solution NMR samples at ambient temperature. We recently demonstrated a gain of 60 in signal enhancement for ^{31}P at 14.4 T. A gyrotron tuned to 527 GHz, compatible with 800 MHz 1H (18.8 T) NMR instruments recently became commercially available, making the transfer of this technology to a higher field for improved spectral resolution feasible. However, at terahertz frequencies, efficient delivery of the necessary microwave polarization (i.e. the magnetic field component of the electromagnetic wave) to samples without the electric field component heating the samples and destroying them remains a significant challenge which must be addressed by careful engineering of the NMR probe and in particular the sample cavity. Thus for this application we are partnering with Joaquin Casenova, an expert in modeling and designing microwave hardware for biological applications, to explore new materials and geometries for the sample cavity. These designs will be specifically tailored to be compatible with the HTS RF coils needed for detection of nuclear signals and performing multidimensional NMR pulse sequences.

Impact of Instrumentation on Research at UF Following are brief descriptions of some currently funded grants at UF where high resolution NMR technologies are already being successfully employed and which would benefit substantially from the technology developed by this proposal.

Metabolic flux measurements (Matt Merritt): Hepatic glucose output, primarily through gluconeogenesis (GNG) from either glycerol or phosphoenolpyruvate (PEP) derived from oxaloacetate in the Krebs cycle, is a primary target of many pharmaceuticals used to treat diabetes. Metformin is the most widely used drug for the treatment of diabetes, yet its molecular mechanism remains hotly debated. This grant is focused on developing hyperpolarized (HP) [2-^{13}C]dihydroxacetone (DHA) as a metabolic imaging agent that is sensitive to the relative rates of hepatic glycolysis and GNG. In this project, the primary model for assessing the metabolic effects of DHA is the perfused mouse liver. This allows the luxury of collecting glucose from the liver effluent, providing a direct, quantitative measure of hepatic glucose output (HGO). By adding 2% D_2O to the perfusate, the relative contributions of glycogenolysis, GNG_{gly}, and GNG_{PEP} can be readily ascertained by ^2H NMR using the proposed instrumentation. This gold standard method can then be used to validate the HP data obtained with [2-^{13}C]DHA.

Marine natural products for drug discovery (Hendrik Luesch): Cyanobacteria are among the most ancient organisms on Earth and have evolved chemical weapons for defensive purposes, which we are exploiting for anticancer drug discovery. Particularly marine cyanobacterial secondary metabolites isolated so far have shown high hit rates in cytotoxicity assays. Our past research has exemplified that marine cyanobacteria contain compounds with exceptionally potent activity and/or possess unusual or first-in-class inhibitors with novel mechanisms of action. Due to the difficulty of capturing the bacteria in large numbers, mass sensitivity is put at a premium. This makes high mass sensitivity NMR one of the most essential components for successful completion of Dr. Luesch's research aims.

Chemical communication in nematodes (Rebecca Butcher): Similarly, Dr. Butcher's research identifying small molecules which regulate nematode development is characterized by a need for maximal mass sensitivity. Polyketides and nonribosomal peptides are structurally complex natural products that have been developed into many important therapeutics. Her group recently purified hybrid polyketide-nonribosomal peptides (that were named "nemamides") from *Caenorhabditis elegans* and structurally characterized them by NMR using the ^{13}C HTS cryoprobe described above. As microorganisms and plants are usually responsible for producing structurally complex natural products, it is quite remarkable to discover these types of natural products in an animal (nematode) species. Only very minute quantities of the nemamides could be purified from *C. elegans* (70 μg from 50L of worms).

4) Qualifications of the persons who will use or develop the equipment

The team of faculty members pursuing this MRI development grant consists of experts at UF and the NHMFL in DNP and NMR technology development (Long, Brey, Merritt), an expert in terahertz

microwave technology (Casanova), and expert in organic radicals for DNP applications (Fanucci) and expert users with demanding NMR applications (Merritt, Luesch, Butcher, Huigens, Ding, and Bruner). They have active research groups in which students and post-doctoral associates are trained in developing and/or using NMR and DNP techniques to solve fundamental questions in physical chemistry, metabolic flux, and natural products discovery for chemical communication and drug development. Several of the investigators participate in NSF REU and other undergraduate training programs.

As described above, the principal investigators in this proposal have already successfully pursued the development of new technologies which enable the study of systems previously rendered intractable due to sensitivity limitations. We have also worked with industrial partners to license previous instrumentation designs. Through the NHMFL user program we regularly offer workshops to train users of our unique instruments and AMRIS staff provide expert support for their experiments in the AMRIS facility. Dr. Long is the director of the AMRIS facility and many of the participants in this proposal serve on the internal advisory committee for AMRIS.

5) Projected cost, plan for operation and maintenance of the instrumentation, and plan for 30% cost-share requirement

This project requires several major pieces of equipment in addition to the NMR probe and microwave technology we will be developing. For the purposes of this preproposal, we have initial budgetary quotes, but we will negotiate substantially discounted prices for the console and gyrotron which will make the budget substantially smaller. In particular, we need the following

18.8 T NMR quality magnet	donated to UF, cost of acquiring would have been	$1.5-2 M
Moving, energization of magnet	NHMFL cost share to this proposal	$160,000
800 MHz four-channel NMR console	projected NHMFL cost share to this proposal	$700,000
FTEs from AMRIS staff to support this proposal	UF cost share to this proposal	$175,000
527 GHz gyrotron	NSF MRI	$1,800,000
Design, machining and installation of quasioptics tables	NSF MRI	$150,000
FTEs, materials and supplies for terahertz modeling, hardware fabrication	NSF MRI	$500,000
FTEs, materials and supplies for HTS, cryogenic components in probe	NSF MRI	$500,000
Overhead	NSF MRI	$500,000
Total request to NSF MRI		**$3,450,000**
Total match from NHMFL and UF (30 %)		**$1,035,000**

**Note: I will need to have a detailed discussion with DSP and NHMFL to make sure cost share is appropriate as the NHMFL is also NSF funded. I do not anticipate this will be a problem (we have done similar projects in the past). The magnet is already delivered to UF and we are currently site planning before moving it to its final destination and bringing it to field.

Once the instrumentation is built and tested, it will be offered to users through the AMRIS Facility. The operating costs should be similar to other high field systems we support on a fee-for-service basis under federal costs accounting standards. The NMR magnet will have a lifetime of >20 years. NMR spectrometer consoles and gyrotrons typically have a lifetime of 10-12 years. To upgrade them, we will be able to apply to several different funding agencies. Installing an 800 MHz NMR instrument requires a space with sufficient physical dimensions and structural stability as well as adequate isolation due to the high magnetic fields. We have identified space in the physics building which would be well suited. We note that another MRI preproposal, submitted by Neil Sullivan, to acquire a high field/low temperature instrument discusses our shared vision for this space and growing the NHMFL facilities at UF. The deans of COM and CLAS, David Norton, and Greg Boebinger, the director of the NHMFL, are aware of our expansion plans and have expressed their support for our pursuing instrumentation proposals. If this pre-proposal moves forward we will formalize specific space and financial commitments.

6) Current NSF funding related to this project

1. NSF- DMR
   Title: National High Magnetic Field Laboratory 2013-2017
   PI: Greg Boebiner, UF PI Joanna Long
   Grant Period: 01/01/2013-12/31/2017
   Total Funding: $145,230,000; UF subaward to Long $ 3534588 (2013-2016)

2. NSF- CHE
   Title: CAREER: A multidisciplinary approach to the investigation of secondary metabolism in nematodes
   PI: Rebecca Butcher
   Grant Period: 05/01/2016-04/30/2021
   Total Funding: $433200 (2016)

3. NSF- MCB
   Title: Spin Labeling Insights into Hydration Effects on Macromolecular Flexibility and Function
   PI: Gail Fanucci
   Grant Period: 09/01/2013-08/31/2017
   Total Funding: $518,000 (2013-2016)

Other currently funded projects that will benefit

1. NIH R01DK105346
   Title: Imaging hepatic gluconeogenesis with hyperpolarized dihydroxyacetone
   PI: Matthew Merritt
   Grant Period: 07/30/2016-06/30/2021
   Total Funding: $353,957 (2016)

2. NIH R01CA172310
   Title: Novel targeted anticancer agents from marine cyanobacteria
   PI: Hendrik Luesch
   Grant Period: 03/06/2013-02/28/2018
   Total Funding: $1,731,389 (2013-2016)

3. NIH R01GM118775
   Title: Small-molecule signals controlling nematode development
   PI: Rebecca Butcher
   Grant Period: 04/01/2016-3/31/2021
   Total Funding: $237,173 (2016)

4. NIH R01CA188132
   Title: Studies of thymidylate synthase as a tumor promoting oncogene for development of new allosteric inhibitors for cancer Tx
   PI: Robert Huigens
   Grant Period: 01/01/2016-12/31/2020
   Total Funding: $25,031 (2016)

5. NIH R01GM105409
   Title: Elucidating molecular mechanisms of drug resistance in HIV-protease
   PI: Gail Fanucci
   Grant Period: 04/01/2013-03/31/2017
   Total Funding: $886920 (2013-2016)

6. US Air Force FA9550-16-1-0186
   Title: Engineering biosystems for aromatic nitration
   PI: Yousong Ding
   Grant Period: 05/15/2016-05/14/2019
   Total Funding: $120,000 (2016)

7. US Army W81XWH-15-1-0196
   Title: Engineering irisin for understanding its benefits to obesity
   PI: Yousong Ding
   Grant Period: 07/01/2015-12/31/2016
   Total Funding: $151,626 (2015-2016)

8. USDA 266675
   Title: Xenorhabdus bacteria on pheromone production by steinernema nematodes: impact on nematode fitness and form
   PI: Rebecca Butcher
   Grant Period: 02/01/2015-1/31/2017
   Total Funding: $99,464 (2015-2016)

9. Ellison Medical Foundation AG-NS-0963-12
   Title: Small-molecule cues that control development, metabolism, and lifespan in c. elegans
   PI: Rebecca Butcher
   Grant Period: 07/24/2012-07/23/2016
   Total Funding: $400,000 (2012-2016)

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A. Professional Preparation

U. of Arkansas; Fayetteville, AR	Chemistry	B.S.	1990
MIT; Cambridge, MA	Physical Chemistry	Ph.D.	1997
U. of Washington; Seattle, WA	Molecular Bioengineering	Postdoctoral	1997-2000

B. Appointments

2009-	Director, Advanced Magnetic Resonance Imaging and Spectroscopy Facility
2009-	Assoc. Professor, Biochemistry and Molecular Biology, University of Florida
2002-2009	Asst. Professor, Biochemistry and Molecular Biology, University of Florida
2000-2002	Staff Scientist, Chemistry, University of Washington.

C. Publications

Graduate student co-authors marked with an *; undergraduate/high school students are underlined.

(i) 5 publications most closely related to the proposed project

1. Smith AS* and Long JR, "Dynamic Nuclear Polarization as an Enabling Technology for Solid State Nuclear Magnetic Resonance Spectroscopy". *Anal. Chem*., 88(1):122-132 (**2016**).
2. Lama B, Collins JH, Downes D*, Smith AN*, Long JR, "Expeditious dissolution dynamic nuclear polarization without glassing agents". *NMR Biomed*., 29(3):226-31 (**2016**).
3. Smith, AN*; Twahir, UT*; Dubroca, T; Fanucci, GE and Long, JR, "Molecular Rationale for Improved Dynamic Nuclear Polarization of Biomembranes". *J. Phys. Chem. B*, 120(32): 7880-7888 (**2016**)
4. Smith AS*, Caporini M, Fanucci GE, Long JR, "A Method for Dynamic Nuclear Polarization Enhancement of Membrane Proteins". *Angew. Chemie*, 54(5):1542-1546. (**2015**).
5. Gor'kov PL, Brey WW, and Long JR, "Probe developments for biosolids NMR spectroscopy." *Encycl Magn Reson* In *Solid-State NMR Studies of Biopolymers*, McDermott, A.E and Polenova, T. (eds). John Wiley & Sons Ltd, Chichester, UK, pp 141-158 (**2010**).

(ii) 5 other significant publications,

1. Budinger, TF; Bird, MD; Frydman, L; Long, JR; Mareci, TH; Rooney, WD; Rosen, B; Schenck, JF; Schepkin, VD; Sherry, AD; Sodickson, DK; Springer, CS; Thulborn, KR, Ugurbil, K, Wald, LL, "Toward 20 T magnetic resonance for human brain studies:opportunities for discovery and neuroscience rationale", *Magma*, 29(3):617-640 (**2016**)
2. Kiswandhi, A; Lama, B; Niedsbalski, P*; Goderya, M*; Long, JR, Lumata, L, "The Effect of Glassing Solvent Deuteration and Gd^{3+} Doping on ^{13}C DNP at 5 T", *RSC Advances*, 6:38855-38860 (**2016**)
3. Tang W, Bhatt A*, Smith AN*, Crowley PJ, Brady LJ, Long JR, "Specific binding of a naturally occurring amyloidogenic fragment of *Streptococcus mutans* adhesin P1 to intact P1 on the cell surface characterized by solid state NMR spectroscopy". *J Biomol NMR,* 64(2):153-64 (**2016**).
4. Braide-Moncoeur O*, Tran NT*, Long JR, "Peptide-based synthetic pulmonary surfactant for the treatment of respiratory distress disorders". *Curr Opin Chem Biol.*, 32:22-28 (**2016**).
5. Farver S*, Smith AN*, Mills FD, Egri AG, Long, "Delineation of the dynamic properties of individual lipid species in native and synthetic pulmonary surfactants". *BBA-Biomembranes* 1848(1B):203–210 (**2015**).

D. Synergistic Activities (up to five)

Member of the National High Magnetic Field Laboratory (NHMFL): As assistant lab director, I am involved in strategic planning and prioritizing new initiatives with a particular focus on nuclear magnetic resonance, dynamic nuclear polarization, and new technologies for high stability/high homogeneity magnets. I serve as a member of the Executive Committee, the Science Council, and the Diversity Committee. I also direct one of seven user facilities offered by the NHFML—I am responsible for the

scientific, technical and fiscal administration of the AMRIS facility which has nine staff members, operates nine high field magnets for magnetic resonance imaging and spectroscopy, and supports a wide variety of federally-funded projects at the University of Florida and through the NSF-funded NHMFL external user program. This facility contains several unique superconducting magnets, including 11.1 T/40 cm and 17.6 T/8.9 cm magnets which both operate at 2.2 K and a custom built DNP polarizer operating at 5 T/1.2 K.

Peer Reviewer: Reviewer for Journal of the American Chemical Society, Biochemistry, Biophysical Journal, Journal of Physical Chemistry B, BBA, and several others; *ad hoc* reviewer for NSF, NIH, DOE, and Alzheimer's Association as well as panelist for NSF Graduate Research Fellowships, NIH Shared Instrumentations Grants, NRC postdoctoral fellowships.

Teacher: As a faculty member in a college of medicine, I regularly teach undergraduate, graduate, and medical students, including a graduate level course on biomolecular NMR with a laboratory component utilizing high field NMR instruments in the AMRIS facility. I also guest lecture in the departments of Materials Science & Engineering and Chemistry on topics in magnetic resonance.

Undergraduate Laboratory Mentor: I regularly have three-four undergraduate students from UF working in my lab. These students typically spend two years working on projects, before continuing on to either medical or graduate school. I host summer students through REU programs in the Department of Chemistry and the NHMFL and participate in the University Scholars Program and the HHMI Science for Life Undergraduate Research Program as well as high school students through the UF SSTP program.

Professional Service: Co-organizer (with Gail Fanucci) of the 2015 Southeastern Magnetic Resonance Conference in Gainesville FL, a regional conference with participation by more than 125 graduate students, postdocs, and faculty. Member of the executive committees for the Experimental NMR conference and the Rocky Mountain Solid State NMR Symposium. External advisory committee for NIH-COBRE grant at the University of Delaware.

E. Collaborators & Other Affiliations

(i) Collaborators (within last 48 months)

Prof. Manish Mehta (Oberlin College); Dr. Anil Mehta (Emory University), Prof. Nien-Hui Ge (UC, Irvine), Prof. L. Jeanine Brady (UF), Dr. William Brey (NHMFL), Peter Gor'kov (NHMFL), Prof. Thad Harroun (Brock University), Prof. Laurie Gower, (UF), Prof. Gail Fanucci, (UF), Prof. Mavis Agbandje-McKenna (UF), Prof. Scott Prosser (U Toronto), Prof. Lloyd Lumata (U Dallas)

(ii) Graduate Advisors and Postdoctoral Sponsors

Prof. Robert Griffin (Massachusetts Inst. Of Technology), Prof. Patrick Stayton (University of Washington), Prof. Gary Drobny (University of Washington)

(iii) Thesis Advisor and Postgraduate-Scholar Sponsor.

Graduate Advisees (12): Vijay Antharam (PhD 2008), Frank Raucci (M.S. 2004), Seth McNeill (PhD 2009), R. Suzanne Farver (PhD 2011), Austin Turner (PhD 2011), Anna Kuznetsova (PhD 2012), Mark Bewernitz (PhD 2012), Kyle Heim (PhD 2014), Adam Smith (PhD 2015), Otonye Braide-Moncoeur (PhD 2014), Daniel Downes, Nhi Tran

Postdoctoral Advisees (7): Chris Williams, Mini Samuel-Landtiser, Douglas Elliott, Omjoy Ganesh, Wenxing Tang, James Collins, Bimala Lama

Undergraduate Advisees and RUI students (24): David Fleischman, Joy Wattawa, Ina Agaj, Robert Hartley, Zakia Sultana, Daniel Hillman, Sharon Aroda, Michael Ferrara, Stacy Lundstedt, Zahra Punjani, Neil Wargo, Andrea Orvieto, Everard Bellott, Erika Buechelmaier, Brad Miller, Richard Pardilla, Kyle Hannabass, Terrence Hornsby, Phil Goeff, Tom Frederick, Jacob Wilson, Nhi Tran, Michael Chiang, Daniel Canfield

High school students (5): S. Vellanki, A. Egri, F. Luciano, S. Wittig, E. Rowland

Biographical Sketch

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(a) Professional Preparation

Rice University	Houston, TX	Physics	B.A. 1983
University of Florida:	Gainesville, FL	Physics	Ph.D. 1994

(b) Appointments

- Research Faculty III, National High Magnetic Field Laboratory (August 2014—present)
- Associate Scholar/Scientist, National High Magnetic Field Laboratory (August 2006—August 2014)
- Assistant Scholar/Scientist, National High Magnetic Field Laboratory (May 1999—August 2006)
- Member of Technical Staff, Conductus, Inc., Sunnyvale, CA January (1994 – May 1999)
- Consulting Electronics Engineer, Tecmag, Inc. (1984-1986)
- Senior Research Assistant, University of Texas Health Science Center, Houston, TX (1988)
- Research Assistant, University of Texas Health Science Center, Houston, TX (1983—1987)

(c) Products

(i) most closely related to the proposed project

- Ramaswamy, V.; Hooker, J.W.; Withers, R.S.; Nast, R.E.; Edison, A.S. and Brey, W.W. 2016, *Development of a ^{1}H-^{13}C Dual-Optimized NMR Probe Based on Double-Tuned High Temperature Superconducting Resonators*, IEEE Trans. Appl. Supercond., 26, 3, 1-5, DOI: 10.1109/TASC.2016.2522302.
- Hooker, J.W.; Ramaswamy, V.; Arora, R.K.; Edison, A.S.; Withers, R.S.; Nast, R.E. and Brey, W.W. 2015, *An Empirical Expression to Predict the Resonant Frequencies of Archimedean Spirals*, IEEE Trans. Microw. Theory Techn., 63, 7, 2107-2114.
- Ramaswamy, V.; Hooker, J.W.; Withers, R.S.; Nast, R.E.; Brey, W.W. and Edison, A.S. 2013, *Development of a ^{13}C-Optimized 1.5-mm High Temperature Superconducting NMR Probe*, J. Magn. Reson., 235, 58-65.
- Brey, W.W.; Edison, A.S.; Nast, R.; Rocca, J.; Saikat Saha, S. and Withers, R.S., 2006, *Design, Construction and Validation of a 1 mm Triple Resonance High-Temperature Superconducting Probe for NMR*, J. Magn. Reson., 179 (2), 290-293.
- Brey, W.W.; Edison, A.S.; Ramaswamy, V. and Hooker, J.W. *NMR RF probe coil exhibiting double resonance.* U.S. Patent No. 8,779,768, July 15, 2014.

(ii) other significant products, whether or not related to the proposed project.

- Gor'kov, P.L.; Chekmenev E.Y.; Li, C.; Cotten, M.; Buffy, J.J.; Traaseth, N.J.; Veglia, G. and Brey, W.W. 2007, *Using low-E resonators to reduce RF heating in biological samples for static solid-state NMR up to 900 MHz*, J. Magn. Reson., 185, 77-93.

- Qian, C.; Masad, I.S.; Rosenberg, J.T.; Elumalai, M.; Brey, W.W.; Grant, S.C. and Gor'kov, P.L. 2012, *A volume birdcage coil with an adjustable sliding tuner ring for neuroimaging in high field vertical magnets: Ex and in vivo applications at 21.1 T*, J. Magn. Reson., 221, 110-116.
- Brey, W.W., Anderson, W.A., Wong, W.H., Fuks, L.F., Kotsubo, V.Y., Withers, R.S., *Nuclear magnetic resonance probe coil*. US Patent No. 5,565,778, Oct. 15, 1996.
- Brey, W.W., Johansson; M.E., Withers, R.S., *Method of making nuclear magnetic resonance probe coil*, US Patent 5,619,140, April 8, 1997.
- Lu, M.; Hou, G.; Zhang, H.; Suiter, C.L.; Ahn, J.; Byeon, I.-J.-L.; Perilla, J.R.; Langmead, C.J.; Hung, I.; Gor'kov, P.L.; Gan, Z.; Brey, W.; Aiken, C.; Zhang, P.; Schulten, K.; Gronenborn, A.M. and Polenova, T. 2015, *Dynamic allostery governs cyclophilin A-HIV capsid interplay*, P. Natl. Acad. Sci. U.S.A., 112, 47, 14617-14622.

(d) Synergistic Activities

- Leader of NMR Instrumentation Group at NHMFL, 1999-present. Group has developed a large number of probes that support solid state, solution and imaging user programs at UF and FSU facilities of NHMFL. These include static and magic angle spinning probes (including "Low E" coil insert), superconductive triple resonance probe for 1 mm samples, and fast spinning probes for 32 mm bore magnets.
- Project Leader for Sample Environment, IMR-MIP Series Connected Hybrid Construction Phase, Mark Bird PI, 2007-2016.
- NMR Project Leader for NHMFL UltraWideBore 900 MHz Facility in Commissioning Phase, 2004-2005.
- Reviewer for Journal of Magnetic Resonance, Magnetic Resonance in Medicine, Review of Scientific Instruments, PLOS ONE, IEEE Transactions on Applied Superconductivity, Scientific Reports.

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A. Professional Preparation

North Carolina State University: Raleigh, NC	Chemistry	B.S.	1991
Washington University; St. Louis, MO	Physical Chemistry	Ph.D.	1996
U. of Washington; Seattle, WA	Chemistry	Postdoctoral	1997-1999

B. Appointments

2015-	Assoc. Professor, Biochemistry and Molecular Biology, University of Florida
2015-2015	Assoc. Professor, AIRC, UT Southwestern Medical Center
2008-2015	Asst. Professor (Tenure track), AIRC, UT Southwestern Medical Center
2004-2008	Asst. Professor (Research track), AIRC, UT Southwestern Medical Center
200-2004	Staff Scientist, Radiology, UT Southwestern Medical Center

C. Publications

Graduate student co-authors marked with an *; undergraduate/high school students are underlined.

(i) 5 publications most closely related to the proposed project

1. Marco-Rius I*, Cao P, von Morze C, **Merritt** M, Moreno KX, Chang GY, Ohliger MA, Pearce D, Kurhanewicz J, Larson PE, Vigneron DB. Multiband spectral-spatial RF excitation for hyperpolarized [2-^{13}C]dihydroxyacetone ^{13}C-MR metabolism studies. Magn Reson Med. 2016 Mar 28. PMID: 27017966
2. Bastiaansen JA*, **Merritt** ME, Comment A. Measuring changes in substrate utilization in the myocardium in response to fasting using hyperpolarized [1-^{13}C]butyrate and [1-^{13}C]pyruvate. Sci Rep. 6:25573, (2016) PMID: 27150735; PubMed Central PMCID: PMC4858671.
3. Bankson JA, Walker CM*, Ramirez MS, Stefan W, Fuentes D, **Merritt** ME, Lee J, Sandulache VC, Chen Y, Phan L, Chou PC, Rao A, Yeung SC, Lee MH, Schellingerhout D, Conrad CA, Malloy C, Sherry AD, Lai SY, Hazle JD. Kinetic Modeling and Constrained Reconstruction of Hyperpolarized [1-^{13}C]-Pyruvate Offers Improved Metabolic Imaging of Tumors. Cancer Res. 75(22):4708-17, (2015). PMID: 26420214; PubMed Central PMCID: PMC4651725.
4. Moreno, K. X.; Moore, C.; Burgess, S. C.; Sherry, A. D.; Malloy, C. R.; **Merritt**, M.E.; "Production of hyperpolarized $^{13}CO_2$ from [1-^{13}C]pyruvate in perfused liver does reflect total anaplerosis but is not a reliable biomarker of glucose production." Metabolomics, 11 (5), pp1144-56, (2015). PMCID 26543443; PubMed Central PMCID: PMC4629494.
5. Purmal, C*.; Kucejova, B.; Sherry, A. D.; Burgess, S. C.; Malloy, C. R.; **Merritt**, M. E.; "Propionate Stimulates Pyruvate Oxidation in the Presence of Acetate." American Journal of Physiology – Heart and Circulatory Physiology, 307 (8), H1134-41, (2014). PMID: 25320331; PMCID: PMC4200343

(ii) 5 other significant publications,

1. Lumata L.L,; Ratnakar S.J.; Jindal A,; **Merritt** M.E.; Comment A.; Malloy C.R.; Sherry A.D.; Kovacs Z.; BDPA: An efficient polarizing agent for fast dissolution dynamic nuclear polarization NMR spectroscopy. Chem Eur J. (2011), 17(39):10825-7. PMID: 21919088
2. Moreno K.X.; Harrison C.; Sherry A.D.; Malloy C.R.; **Merritt** M.E.; Transfer of hyperpolarization from long T_1 storage nuclei to short T_1 neighbors using FLOPSY-8. J Mag. Res. (2011), 213(1):187-191. PMID: 21974998; PMCID: PMC3212847
3. **Merritt** M.E.; Harrison C.; Sherry A.D.; Malloy C.R.; Burgess, S.C.; Flux through hepatic pyruvate carboxylase and phosphoenolpyruvate carboxykinase detected by hyperpolarized ^{13}C magnetic resonance. Proceedings of the National Academy of Sciences (2011), 108(47):19084-9. PMID: 22065779; PMCID: PMC3223470
4. **Merritt**, Matthew E.; Harrison, Crystal; Storey, Charles; Jeffrey, F. Mark; Sherry, A. Dean; Malloy, Craig R. "Inhibition of Carbohydrate Oxidation During the First Minute of Reperfusion After Brief Ischemia:

NMR Detection of Hyperpolarized $^{13}CO_2$ and $H^{13}CO_3^-$" Magnetic Resonance in Medicine, (2008), 60(5), 1029-1036. PMCID: PMC2696889

5. **Merritt**, Matthew E.; Harrison, Crystal; Storey, Charles; Jeffrey, F. Mark; Sherry, A. Dean; Malloy, Craig R. "Hyperpolarized ^{13}C allows a direct measure of flux through a single enzyme-catalyzed step by NMR" Proceedings of the National Academy of Sciences of the United States of America, (2007), 104(50), 19773-19777. PMCID: PMC2148374

D. Synergistic Activities (up to five)

Member of the National High Magnetic Field Laboratory (NHMFL): As NHMFL faculty, I am involved in strategic planning and prioritizing new initiatives with a particular focus on nuclear magnetic resonance, dynamic nuclear polarization, and their applications to metabolomics and metabolic flux measurements.

Peer Reviewer: Reviewer for Nature Communications, Scientific Reports, Analytical Chemistry, NMR in Biomedicine, Magnetic Resonance in Medicine, Journal of Magnetic Resonance, Circulation Research, FEBS Letters, etc. Peer reviewer for American Chemical Society Petroleum Grant.

Teacher: As a faculty member in a college of medicine, I regularly teach undergraduate, graduate, and medical students, including graduate level courses entitled Advanced Biochemistry and Structural Biochemistry.

Undergraduate Laboratory Mentor: As a new Biochemistry faculty member, I have 5 undergraduate students from UF working in my lab. These students typically spend two years working on projects, before continuing on to either medical or graduate school. Currently a senior will graduate and apply to medical school, while 4 sophomores have also joined the lab.

Professional Service: President of the Hyperpolarized Media Study Section, a member group within the Society for Magnetic Resonance in Medicine. This position is held due to an international election, and I was selected from approximately 200 members worldwide. I am also a member of the UF Biochemistry and Molecular Biology Admissions committee.

E. Collaborators & Other Affiliations

(i) Collaborators (within last 48 months)

Prof. Dan Vigneron (UCSF), Prof. Cornelius von Morze (UCSF), Prof. John Kurhanewicz (UCSF), Prof. Arnaud Comment (Cambridge), Prof. William Brey (NHMFL), Prof. James Bankson (MD Anderson), Prof. Shawn Burgess (UT Southwestern).

(ii) Graduate Advisors and Postdoctoral Sponsors

Prof. Jacob Schaefer (Washington University in St. Louis), Prof. Gary Drobny (University of Washington)

(iii) Thesis Advisor and Postgraduate-Scholar Sponsor.

Graduate Advisees (1): Crystal Harrison (PhD 2013),

Postdoctoral Advisees (4): Crystal Harrison, Lloyd Lumata, Mukundan Ragavan, Ram Khattri

Undergraduate Advisees and RUI students (5): Chris Moore, Hana Kayaleh, Kevin Sanchez, Adam Behar, Alan Carter

JOAQUIN CASANOVA

A. Professional Preparation

UF, Gainesville	Electrical Engineering	PhD, May 2010
UF, Gainesville	Agricultural and Biological Engineering	ME, December 2007
UF, Gainesville	Agricultural and Biological Engineering	BS, December 2006

B. Appointments

Research Assistant Professor, University of Florida	August 2016-present
Senior Engineer, University of Florida	November 2013-June 2016
Research Engineer, USDA	May 2010-October 2013

C. Products

30 technical publications in peer-reviewed journals and conference proceedings. 2 patents awarded.

Most closely related to the proposed project:

Schwartz, R. C., Casanova, J. J., Bell, J. M., & Evett, S. R. (2014). A reevaluation of time domain reflectometry propagation time determination in soils. *Vadose Zone Journal*, *13*(1).

Casanova, J. J., Schwartz, R. C., & Evett, S. R. (2014). Design and field tests of a directly coupled waveguide-on-access-tube soil water sensor.*Applied Engineering in Agriculture*, *30*(1), 105-112.

Casanova, J. J., Evett, S. R., & Schwartz, R. C. (2012). Design and field tests of an access-tube soil water sensor. *Applied Engineering in Agriculture*,*28*(4), 603-610.

Casanova, J. J., Evett, S. R., & Schwartz, R. C. (2012). Design of access-tube TDR sensor for soil water content: Testing. *Sensors Journal, IEEE*,*12*(6), 2064-2070.

Casanova, J. J., Evett, S. R., & Schwartz, R. C. (2012). Design of access-tube TDR sensor for soil water content: Theory. *Sensors Journal, IEEE*,*12*(6), 1979-1986.

Garnica, J., Casanova, J., & Lin, J. (2011, May). High efficiency midrange wireless power transfer system. In *Microwave Workshop Series on Innovative Wireless Power Transmission: Technologies, Systems, and Applications (IMWS), 2011 IEEE MTT-S International* (pp. 73-76). IEEE.

Casanova, J. J., Taylor, J. A., & Lin, J. (2010). Design of a 3-D fractal heatsink antenna. *Antennas and Wireless Propagation Letters, IEEE*, *9*, 1061-1064.

Low, Z. N., Casanova, J. J., Maier, P. H., Taylor, J. A., Chinga, R. A., & Lin, J. (2010). Method of load/fault detection for loosely coupled planar wireless power transfer system with power delivery tracking. *Industrial Electronics, IEEE Transactions on*, *57*(4), 1478-1486.

Casanova, J. J., Low, Z. N., & Lin, J. (2009). Design and optimization of a class-E amplifier for a loosely coupled planar wireless power system.*Circuits and Systems II: Express Briefs, IEEE Transactions on*, *56*(11), 830-834.

Casanova, J. J., Low, Z. N., & Lin, J. (2009). A loosely coupled planar wireless power system for multiple receivers. *Industrial Electronics, IEEE Transactions on*, *56*(8), 3060-3068.

Casanova, J. J., Judge, J., & Jang, M. (2007). Modeling transmission of microwaves through dynamic vegetation. *Geoscience and Remote Sensing, IEEE Transactions on*, *45*(10), 3145-3149.

Other significant products:

Casanova, J. J., O'Shaughnessy, S. A., Evett, S. R., & Rush, C. M. (2014). Development of a wireless computer vision instrument to detect biotic stress in wheat. Sensors, 14(9), 17753-17769.

Casanova, J., O'Shaughnessy, S., & Evett, S. (2013, November). Wireless computer vision system for crop stress detection. In ASA-CSSA-SSSA Annual Meeting Abstracts (p. 123). ASA-CSSA-SSSA Annual Meeting Abstracts. Session 196-7.

D. Synergistic Activities

Main Activities

Dr. Casanova is a research assistant professor in the Department of Electrical and Computer Engineering at the University of Florida. His main research activities are electromagnetic sensors, instrumentation design, and machine intelligence applications. Previously he did research with the USDA in these areas and developed chemistry instrumentation for UF's Chemistry Department.

Professional Leadership

2004–present Member American Society of Agricultural and Biological Engineers (ASABE)
2006–present Member Institute of Electrical and Electronics Engineers (IEEE)

E. Collaborators & Other Affiliations

Collaborators and Co-Editors (within the last 48 months)

C. Li (TTU), R. Miyamoto (Oceanit), R. Yost (U. Florida), T. Casey (U. Florida), S. Anderson (Acclima), R. Schwartz (USDA), S. Evett (USDA), S. O'Shaughnessy (USDA).