Science Driver II - MagSci & Mag Res

On the interplay of High Magnetic Fields, NMR/MRI/EPR & Chem/Bio Research

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NAS MagSci Meeting

March 12, 2012

High Fields, Magnetic Resonance (NMR, MRI, EPR) & the MagLab's Role

OUTLINE

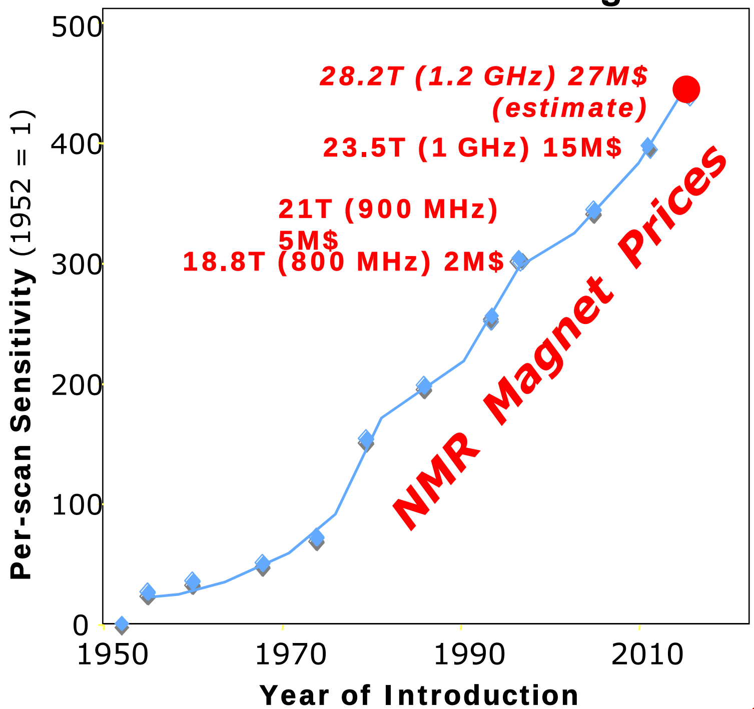
- Sensitivity Challenges and MagSci Promises for MagRes
- Promises Fulfilled: Animal MRI @ 21 T
- Promises on their way: The 36T SCH Solids NMR Project
- Orthogonal technologies #1: Electron-driven Dynamic Nuclear Polarization in solids & liquids NMR; in MRI
- Orthogonal Technologies #2: HTS- and Low-E based probes for ultra-high field solids, liquids and imaging MR
- Quasi-optical Electron MagRes technologies at ultra-high frequencies/fields

MR's Critical Challenge: SENSITIVITY

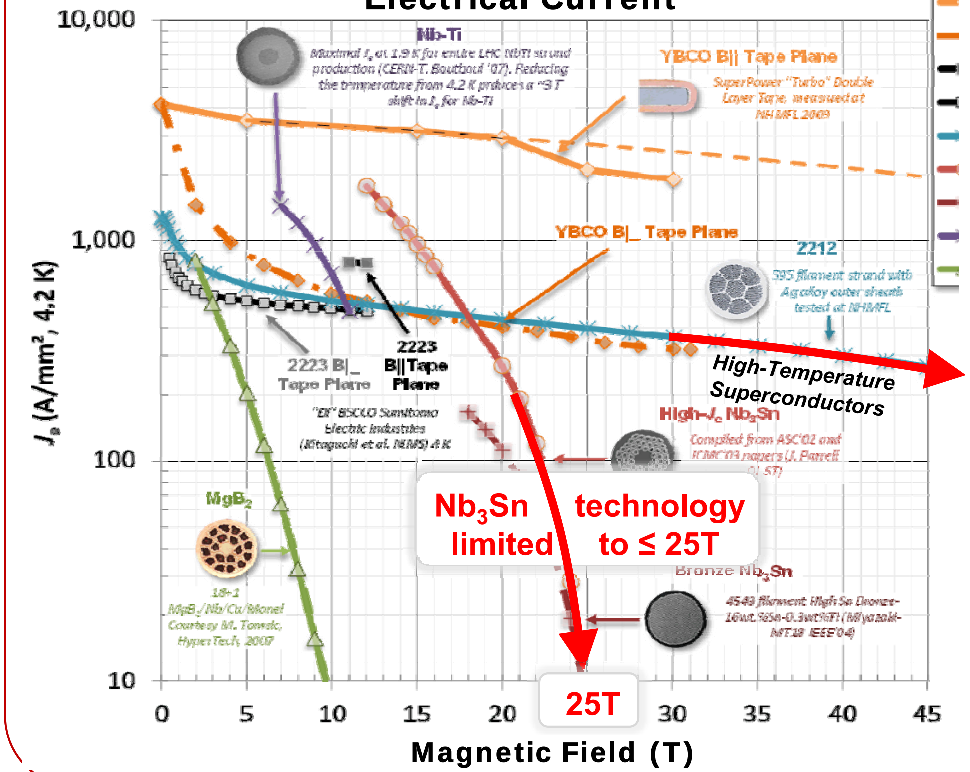
$$\text{Polarization} = \frac{N^{\uparrow} - N^{\downarrow}}{N^{\uparrow} + N^{\downarrow}} \approx \frac{\gamma \hbar B_0}{2k_B T}$$

*Because thermal energy exceeds spin-splitting of nuclear energy levels, **only 0.001 %** of the nuclei contribute to the NMR signal*

Nb-based superconducting magnets cannot address this challenge



Capacity to Carry Superconducting Electrical Current

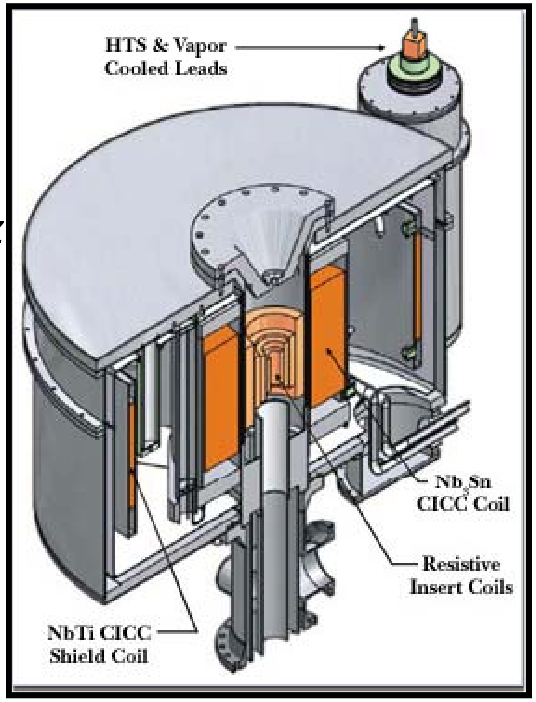


***MagLab MR Initiatives* face this challenges by multiple complementary solutions**

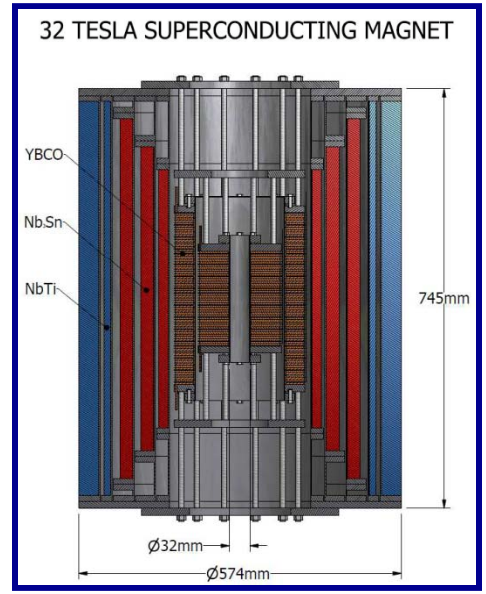
- _**New Magnet Designs:** Equivalent Sensitivity Enhancement ≥750. Achieving the highest fields remains the most general platform for chem/bio NMR/MRI studies
- _**Solids, liquids-state & Dissolution DNP:** Equivalent Sensitivity ≈700-100,000
- _**Emphasis on best probes for solids, liquids and MRI:** HTS cryocoils, low-E probes for high frequencies, single-coil double-tuned designs for MRI, etc

MR Magnets – Projects Within MagLab Reach

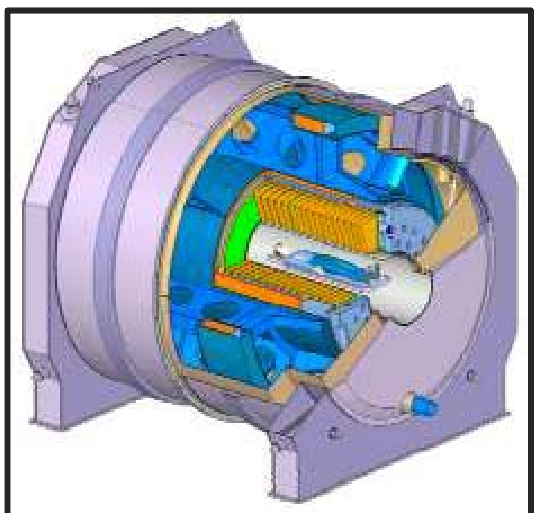
SERIES CONNECTED HYBRID
Expected to reach 1.6 GHz (36T) by 2015, operates with constant power supply (14MW) – will reach ppm stability:
Solids NMR only



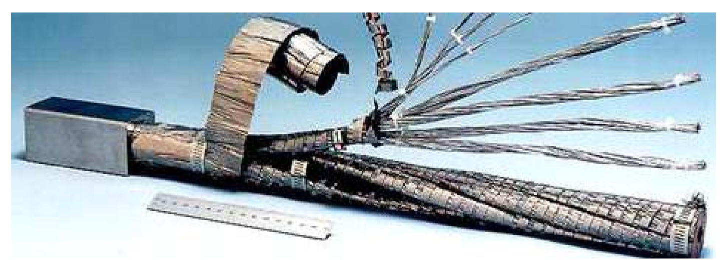
LTS/HTS HYBRID: Japan's NIMS; 1.03 GHz expected by 2015.



MagLab's 32T Project: Most promising option to break the 30T (≥1.25 GHz) barrier for high-res NMR applications



NEUROSPIN'S 11.7T (500 MHz) WHOLE-BODY MRI: Cable-In-Conduct Conductor (CICC) technologies of the kind in use at ITER and ATLAS. Supercon NbTi wires running powered inside super-fluid (1.8K) lHe and held by stainless pipe



Nb3Sn taken to the limit: 900 MHz, 105 mm bore

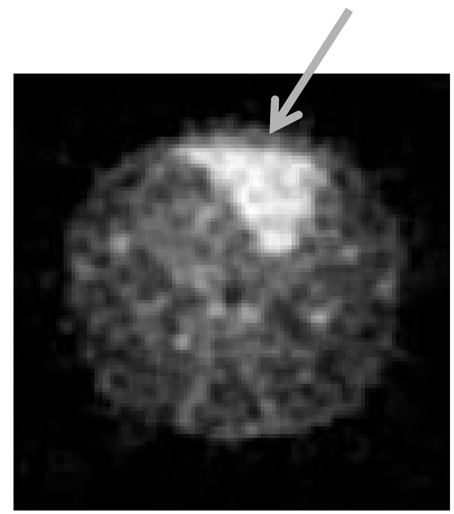
In vivo High-Field MRI in the 21T UWB Facility

Expanding conventional MRS/MRI. Unique opportunities to study biochemistry and physiology using low-γ nuclei.

Particularly for

31P	**($\gamma_{31P} = 0.40 * \gamma_{1H}$)**
23Na	**($\gamma_{23Na} = 0.26 * \gamma_{1H}$)**
13C	**($\gamma_{13C} = 0.25 * \gamma_{1H}$)**

^{23}Na MRI "lights up" tumors: intrinsic regions with high rates of cell death:
In vivo scan of mouse brain tumor.

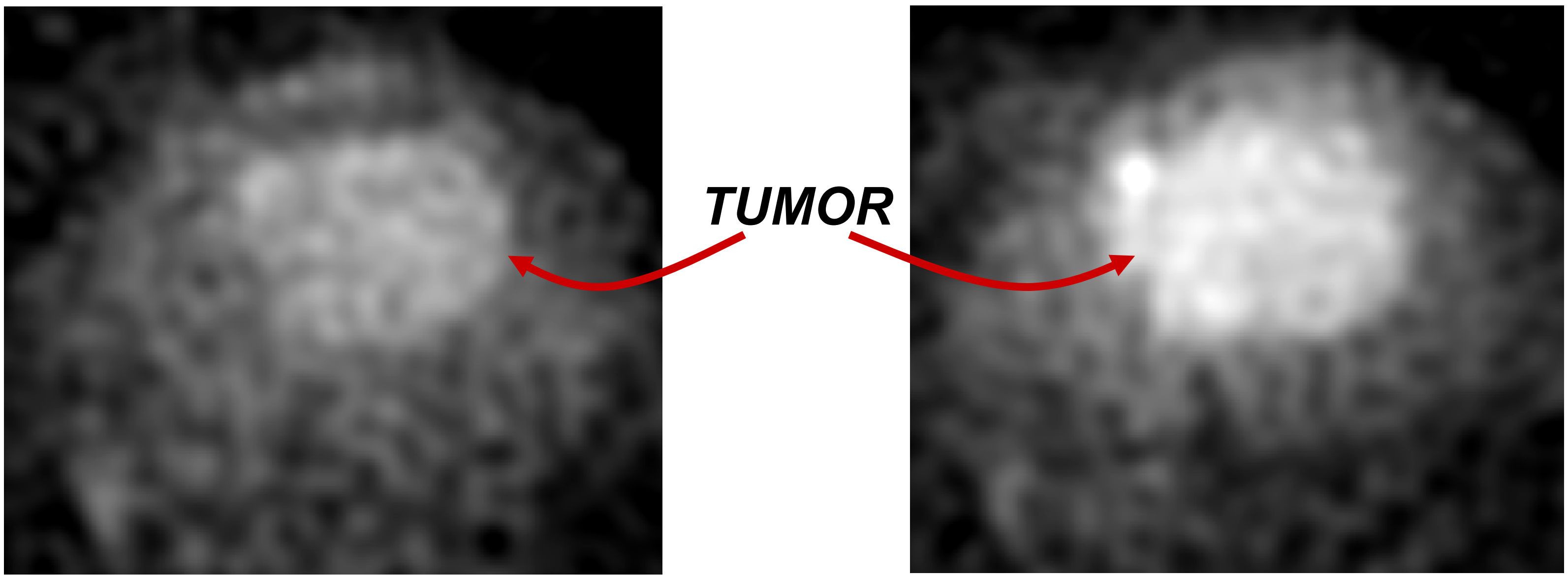


High magnetic field MRI goes hand-in-hand with the design of specialized RF coils and co-located multiple-resonance 1H-X probes: *specialized resonator development critical to all high-field MR work*

Rapid Assessment of Chemotherapeutic Efficacy: *Implanted tumor (9L Glioma) with treatment [BCNU (2x)]*

In vivo ^{23}Na Images of mouse brain

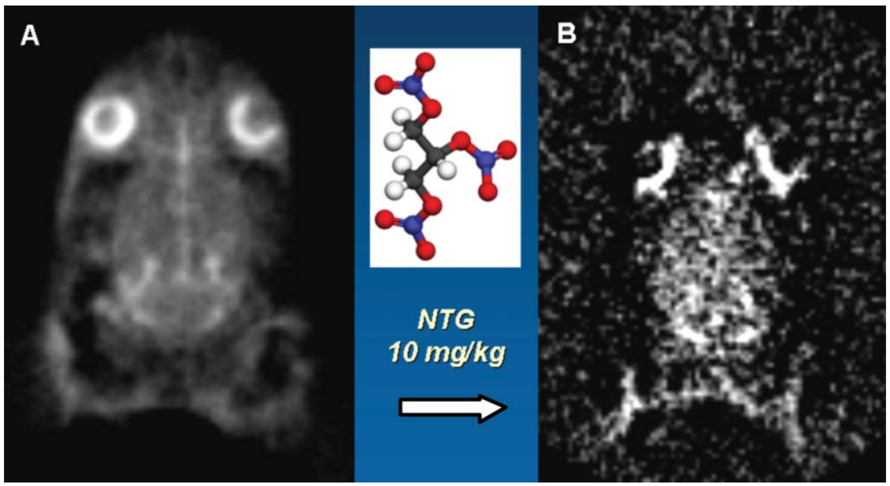
Before Therapy ***4 Days after Therapy***



Sodium (^{23}Na) in cytoplasm is a biomarker for imminent cell death.
The increase in ^{23}Na MRI signal after chemotherapy becomes visible weeks before ^{1}H MRI would show shrinkage of the brain tumor size. This reflects Sodium's sensitivity to minor functional alterations in mitochondrial glycolysis – and therefore in the tumor's resistance/growth

Functional Sodium MRI of Migraines at 21 T

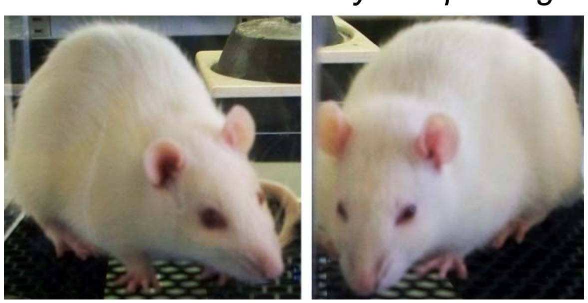
Experimental ^{23}Na MRI



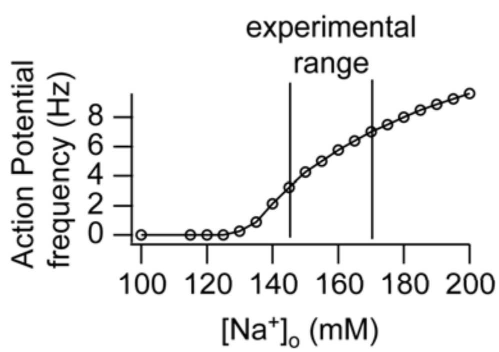
Behavior

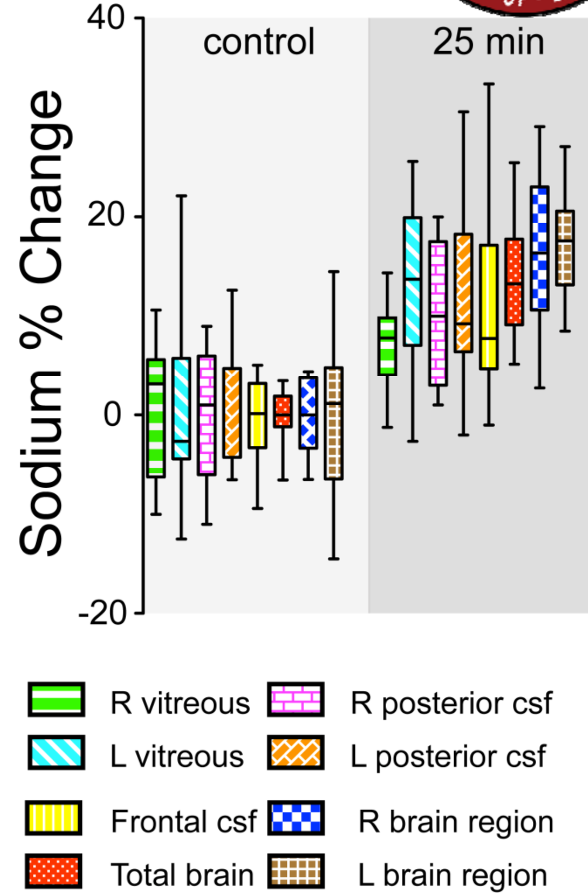
Control Rat
eyes wide open

Nitroglycerin Rat
eyes squinting



Theoretical Simulations

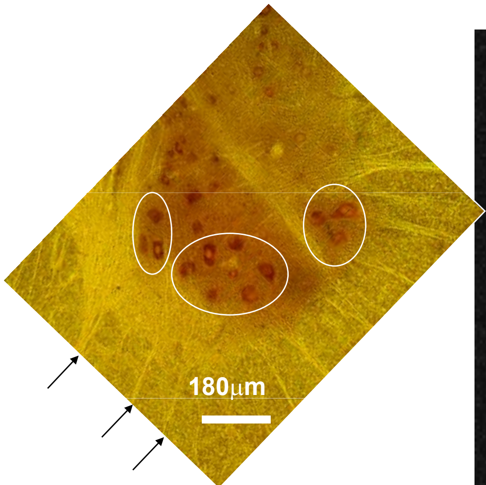




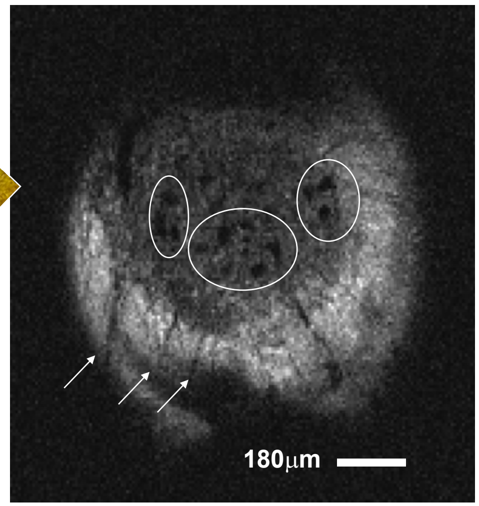
Harrington et al. Chephalalgia, 2011.

Can't do this without the field!

MRI Microscopy: Non-Invasive Cellular and Subcellular Imaging in Tissues

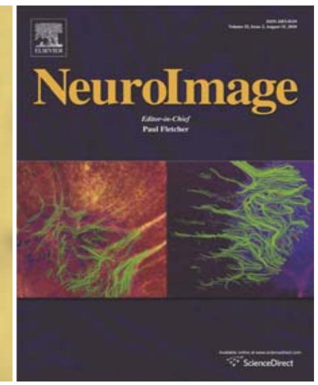


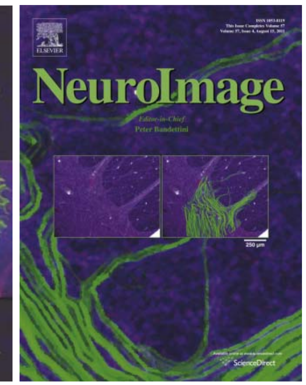
Optical image of rat hippocampal slice



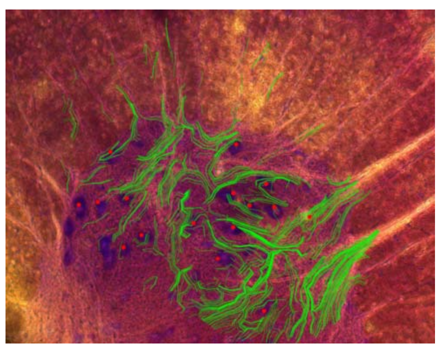
Corresponding MR image

The high fields available at the MagLab also enable frontier research in neuronal microimaging at the cellular level.





Tractography at the cellular level: Rat brain cells



Small surface coils have provided MRI images of individual cells from excised hippocampus. The diffusion of water can also be tracked, providing cellular connectivities. This allows us to understand the physics underlying fMRI.

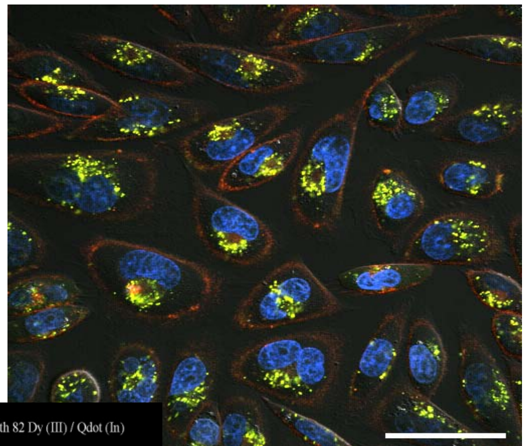
Collaboration between UF and Aarhus University, Denmark

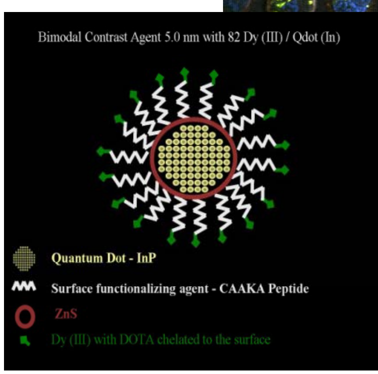
In Vitro to *In Vivo* to Confirmation & Treatment:

Exploiting MatSci & MRI to track stem cell fate & transport

Collaboration between FSU, Mayo Clinic & US Army Research Labs

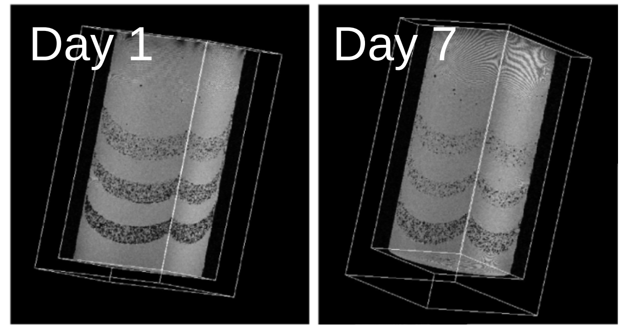
***In vitro* cell culture & modification**

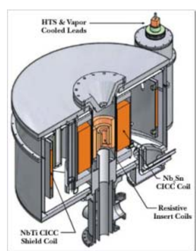




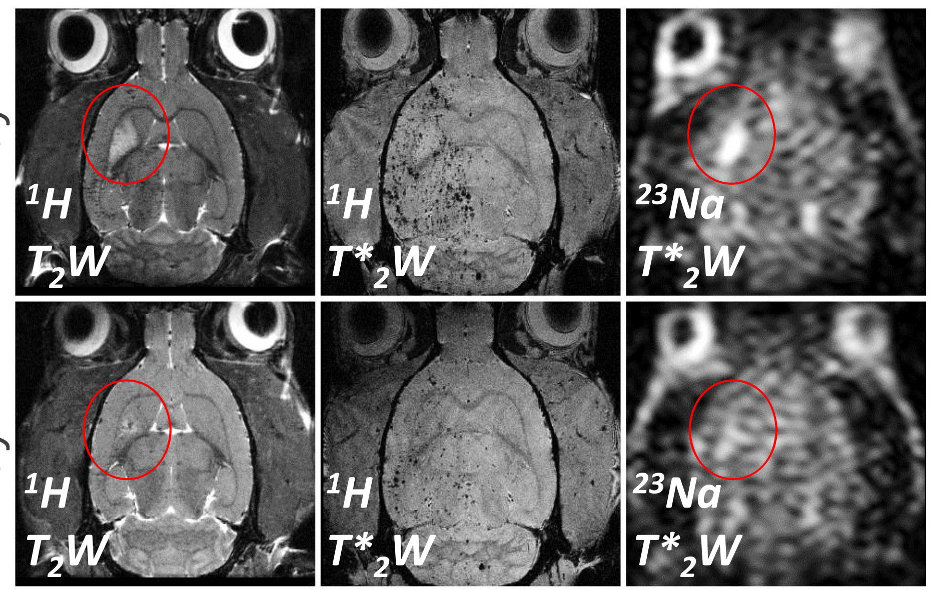
Nanoparticle engineering: multi-modal & functional

***In vitro* MRI of transfected & cultured adult stem cells to verify contrast and retention**





More S/N @ 36 T; ppm resolution enough for some characterizations; ideal for MRS/MRI on lower γ nuclei



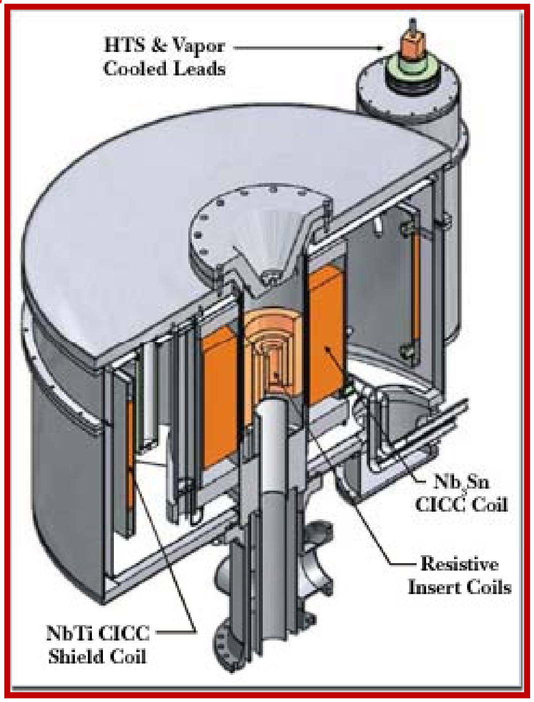
in vivo ^1H and ^{23}Na MRI to assess stem cell retention & stroke outcome

NAS MagSci Meeting *March 12, 2012*

MagLab Initiative: Unprecedented Ultrahigh-Field (36T) Solid State NMR Using a SCH Magnet

BREAKING THE Nb_3Sn SUPERCONDUCTING MAGNET BARRIER USING MAGNET TECHNOLOGY UNIQUE TO THE MAGLAB – AND MAKE IT AVAILABLE TO OUR USER PROGRAM

- *Compatible with "routine" solid state NMR of biological samples*
- *Unprecedented 36T NMR operation with sub-ppm continuous stability over hours and days to accommodate long-duration multidimensional NMR experiments*
- *Nearly doubles the Larmor frequency achieved today by state-of-the-art solid state NMR*
- *Room- or low-temperature operation: Fulfills all the needs in biomolecular solid NMR of mainstream ^{13}C, ^{15}N, and ^{2}H targets for structural and dynamic studies*
- *Integrates the latest in MagLab-designed multiple-resonance low-electric-field bio-Magic Angle Spinning NMR probes*



36T, 14MW Series-Connected Hybrid Magnet: NMR/μMRI *@1.6GHz by 2015/6*

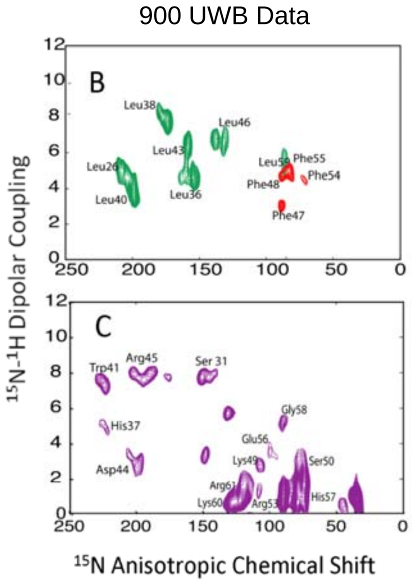
Scientific Opportunities:

- *ALL AREAS OF STRUCTURAL BIOLOGY*
- *Quadrupolar nuclei (e.g., ^{14}N and ^{17}O in carbohydrates)*
- *Diamagnetic metals (e.g., ^{23}Na, ^{39}K bound to nucleic acids, ^{67}Zn in Zinc fingers)*
- High-resolution NMR for samples that cannot be frozen *(e.g., nascent chains in ribosomes, proteins in proteosome, chaperones)*
- *Dynamics in biological systems (e.g. gating of membrane proteins)*

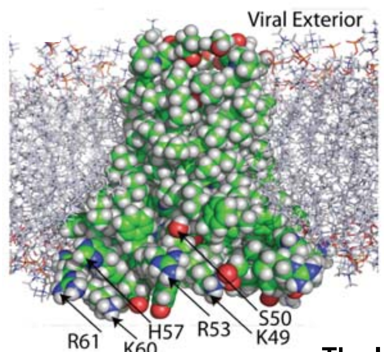
The First Fully Functional Membrane Protein Structure Characterized in a Native-Like Membrane Environment

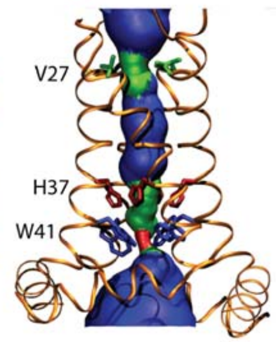
Sharma, M., Yi, M., Dong, H., Zhou, H.-X., Qin, H., Peterson, E., Busath, D.D. & Cross, T.A.; Science 2010 (*40 citations in 1st year*), TiBS 2011.

PISEMA Today - Structures of membrane proteins



- **M2 Protein (22-62) from Influenza A**
- **Structure determined in uniformly aligned liquid-crystalline lipid bilayers**





The largest structure solved by ssNMR (20 kDa)

Sharma et al., 2010 **Science**

A collaboration:
D. Busath, Dept. of Physiology and Developmental Biology, BYU;
H.-X. Zhou, Dept. of Physics, FSU

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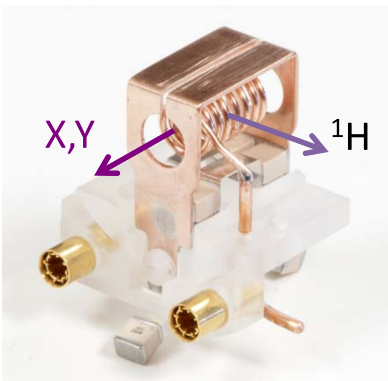
This would not be possible without the highest fields <u>and Low-E probes</u>.

The 36 T SCH will provide a 50% increase in available field for these studies, allowing even more and larger membrane proteins (≈30% of all proteins, ≈60% of all medicinal targets, ≈1% of deposited protein structures) to be studied.

Highly Sensitive, Low-E Probes Are Revolutionizing High-Field NMR in Biological Solids



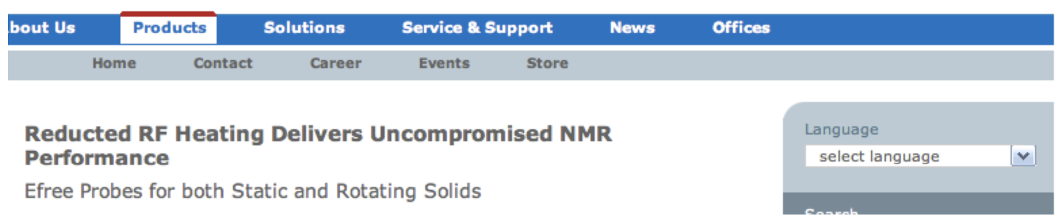
The MagLab is a world leader in developing Low-E probe technologies for biological solid state NMR that

- Minimize heating of conductive biological samples
- Increase S/N over conventional solid-state probes
- Best (only) design for B_0 fields > 1 GHz
- Membrane proteins and almost any other real biomolecular system were "cooked" before Low-E probes.

Unparalleled RF performance. A <u>must</u> as ω_{Larmor} becomes μwave!

Dissemination through Bruker: 50% of Bruker solid state NMR probes sold today are using our Low-E technology.
We also build probes for our users (Oxford, RPI, FZK, U.Minn, ...)

"Has MagLab Inside"

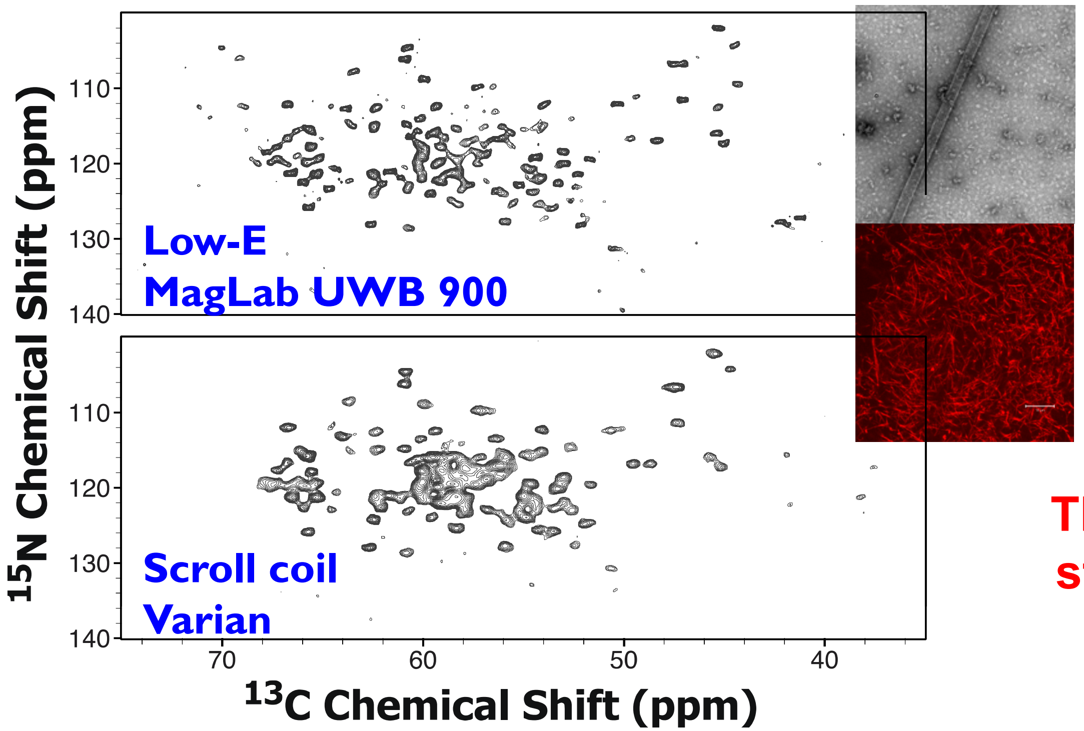


http://www.bruker-biospin.com/efree.html

HIV Capsid Protein Assembly: 900 MHz Spectra Comparison

HIV-1 CA capsid protein (26.6 kDa, 231 aa) organizes viral RNA for replication; assembles into cones, tubes, spheres

NCA



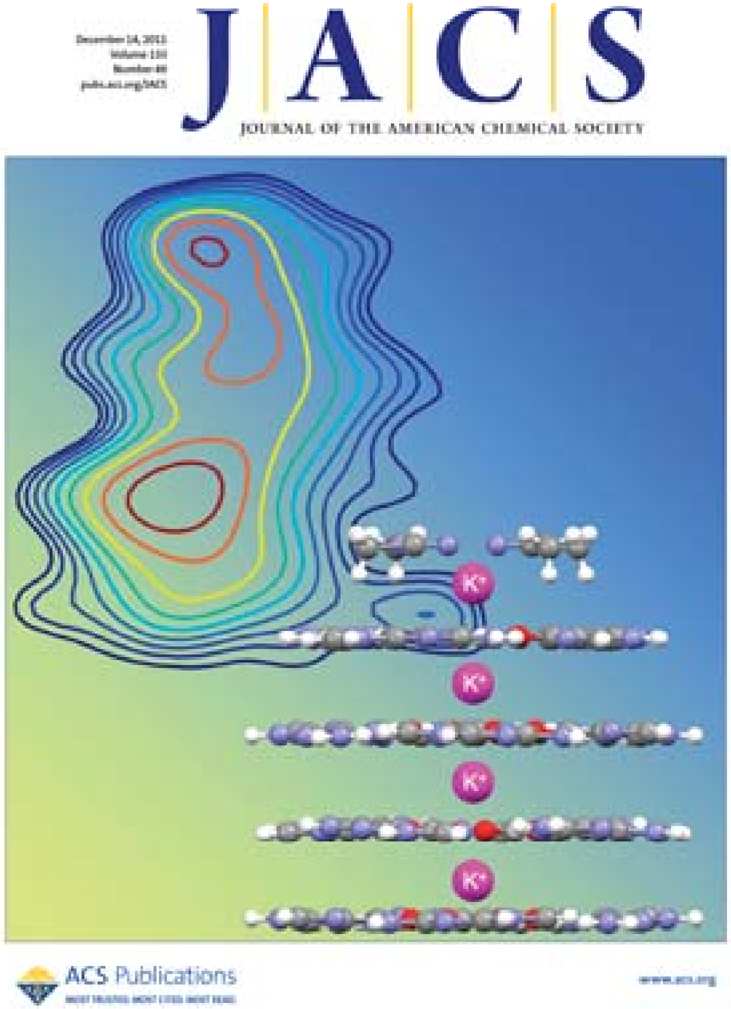
Same sample; same field; two instruments

21 T Triple Resonance (^{13}C, ^{15}N, ^{1}H) with MAS

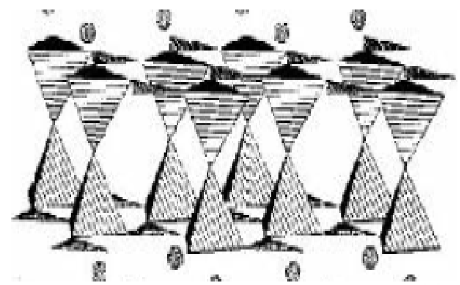
The MagLab Low-E probes enable structural determinations that are beyond the reach of contemporary commercial instrumentation.

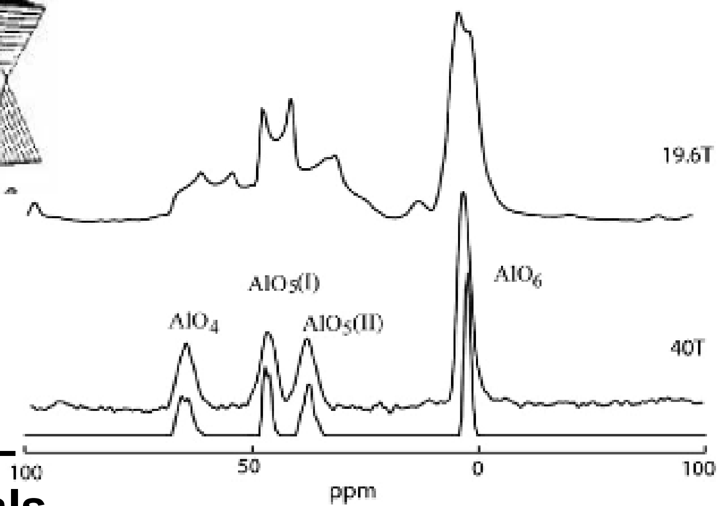
Unpublished magic angle spinning NMR data from Tatyana Polenova (U Del)

High-Fields Can Also Revolutionize Quadrupolar NMR Applied to Materials and Bio-Solids Research

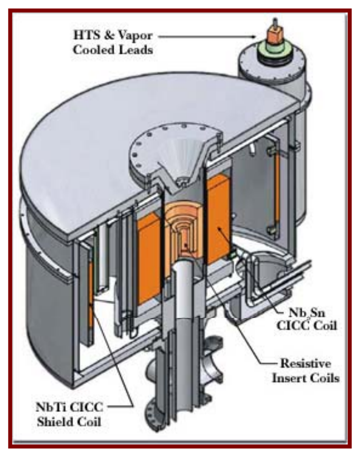


19.1 T (830 MHz 1H) ^{39}K MQ MAS NMR of G-Quadruplexes





40 T (1.68 GHz ^{1}Hs) **single-scan 27Al MAS NMR –incl. field stabilization– on Lasing Materials**



IN SUMMARY: Extra-ordinary opportunities but also new challenges in probe- and pulse-sequence design, will arise with the advent of 36 T SCH solid-state NMR system

Exploiting NMR, MRI, EPR and probe technology synergies:

Dynamic Nuclear Polarization and the MagLab

- First Initiative
  - Develop solids MAS DNP at 9.4 T
    - 400 MHz ^{1}H/263 GHz e^{-}
    - Low powers for T ≤ 25 K
    - Can work with HiPER: pulsed DNP possible
    - Will involve novel DNP NMR probes
    - Applications to materials, membrane structural biology, disordered amiloyds

The MagLab capitalizes its expertise in
- **EPR**
- **NMR/MRI**
- **Probe design**
- **Cryo- and Mechanical engineering**

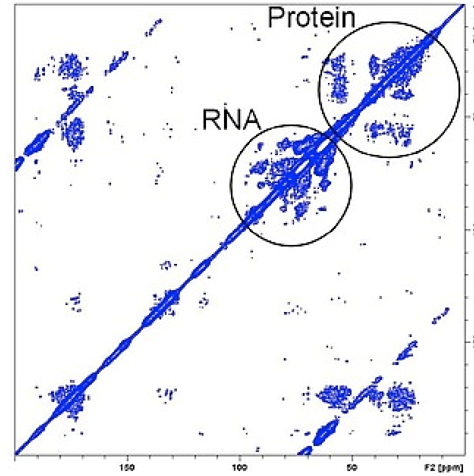
to provide unique resources and additional funding opportunities for exploiting the uses of DNP in chemical/biological/biomedical/clinical research

- Second Initiative
  - Develop liquid-state DNP by building two systems for *in vivo* and *in vitro* applications.
    - 5T dissolution home-built system for metabolomics, in-cell, and animal studies. UF match will provide personnel and supplies. NIH proposal for regional center submitted
    - Overhauser-based 14.1 T shuttling system for solution NMR of organics/pharmaceuticals/natural products. NSF MRI proposal submitted.
- Future Developments
  - Translate solids DNP to 18.8 or even 21 T systems
  - Extend animal dissolution studies to clinical investigations in partnership with UF/Shands and State of Florida

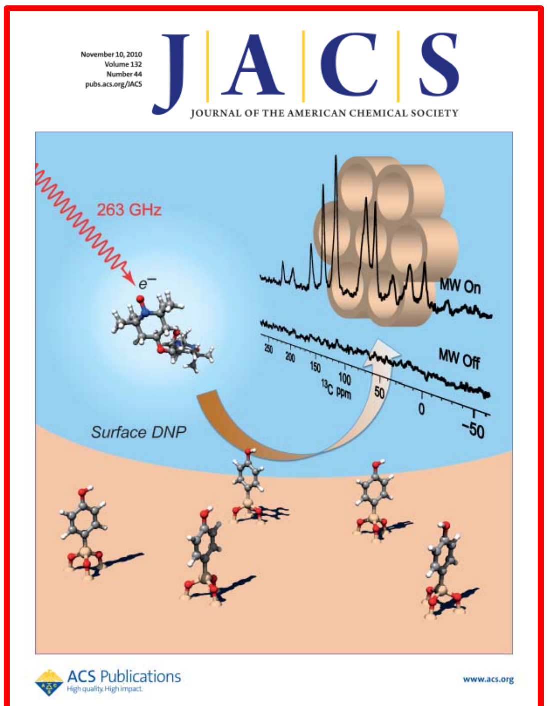
Solids DNP at High Fields & High Resolution

Gyrotron-based μwave sources
(MIT; also Fukui, St. Andrews, Frankfurt):

- *High-frequency (140-460 GHz) radiation sources*
- *High-powers (≈50 Watts): ≈60sec build up times*
- *Low temperatures (≈100 K) for accommodating T_1*
- *Stable over the long durations needed for biosolid NMR (though still facing broadening problems)*
- *Integrated with multiple-resonance MAS probes*



Biomolecular 2D ^{13}C-^{13}C DNP MAS NMR - Barnase in Ribosome (Oschkinat et al)



Emsley et al: Materials applications

- *Large enhancements enabling surface NMR*
- *Lines are usually broad – no resolution or sensitivity penalties*
- *Samples tend to be stable (no "cold denaturation")*
- *Radicals are generally "grafted"*

The First MagLab DNP Initiative:
DNP-Enhanced Solid-State NMR

Initial Solids DNP Hardware Effort *(Tycko et al, JMR 2010):*

- *Full capabilities for multiDNMR using DNP at 400 MHz*
- *Medium-frequency (263 GHz) microwave irradiation sources*
- *Hundred-fold lower microwave irradiation of sample than the commercial setup*
- *Low temperatures (≈ 20 K) for enhancing nuclear polarization*
- *Stable over the long times needed for biological solids NMR*
- *Integrating unique MagLab-designed NMR probes, including multiple- resonance Magic Angle Spinning NMR probes*

Scientific Opportunities:

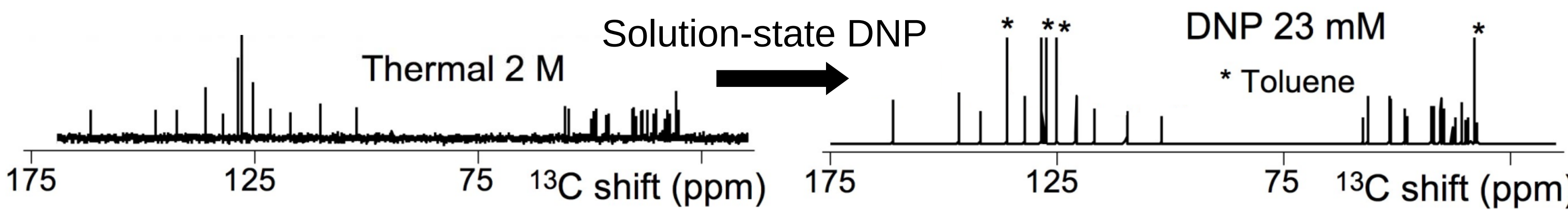
- *Large membrane-proteins*
- *Amyloids and other aggregating peptides*
- *Frozen intact cells, viruses, biofilms*
- *Paramagnetically-tagged protein-protein and protein-nucleic acid complexes*
- *NMR of surfaces and of materials (^{13}C, ^{29}Si) with enhanced sensitivity*

***Longer-term prospect*: porting this DNP know-how to the MagLab's ultra-widebore 900 MHz NMR – a world-unique user resource**

A Second MagLab Initiative: DNP-Enhanced Dissolution NMR/MRI

DNP Hardware to be sited at the MagLab AMRIS facility at University of Florida:

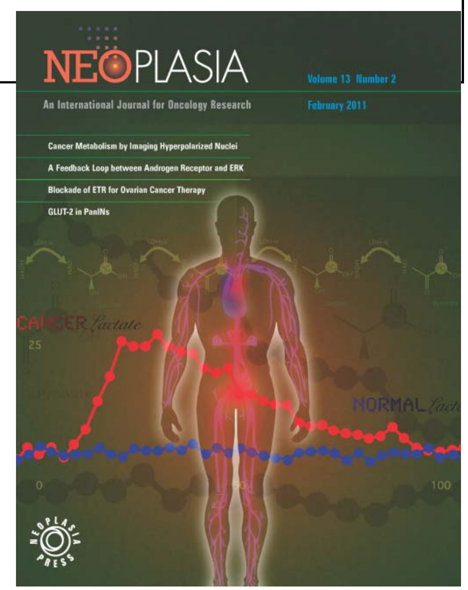
- *Aimed at solution-state single-scan NMR and MRI determinations* in cell *and* in vivo
- *Operates by mixing metabolites of interest with stable radical in glassing mixture*
- *Targets will polarize at 5 T (140 GHz) and at ≈ 1.2 K with typical DNP pumping of two hours*
- *Expected nuclear polarizations ≥30%   (>20,000-fold higher than NMR without DNP)*
- *Interfacing to 17.6 T vertical microimaging and 11.1 T horizontal imaging scanners*



Demonstration of >10,000-fold increase in NMR signal using Dissolution DNP

Scientific Opportunities (Proposed to NIH as Facility)

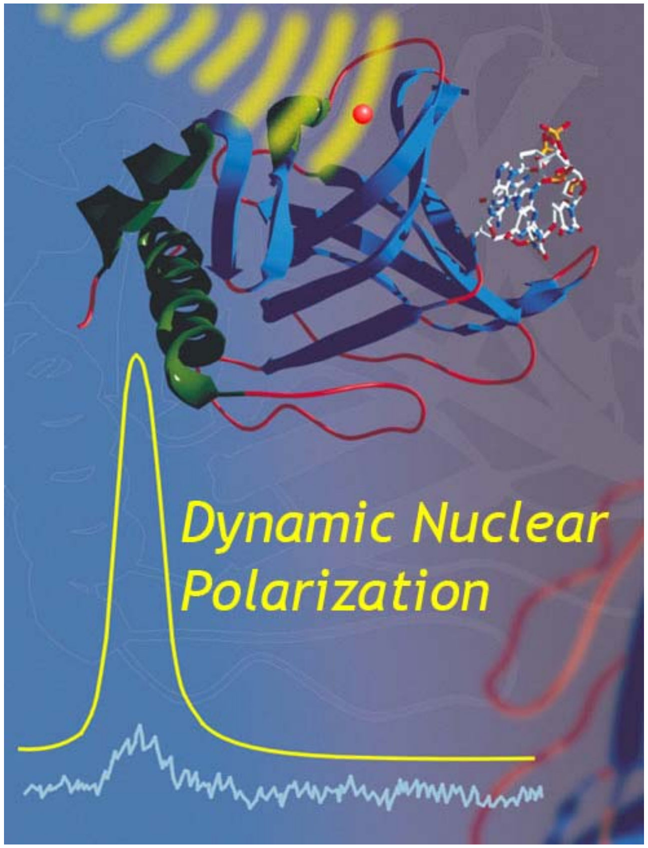
- *Metabolomics on extracts (in combination with Mass-Spec)*
- *In-Cell Metabolic Studies of disease*
- In vivo *metabolism in animal models*
- *Monitor therapeutic responses*
- *Functional Metabolic Studies: Brain, liver, heart, muscular*
- ***Potential to translate to humans for detailed* in vivo *molecular imaging***



A Third MagLab-Proposed Initiative:
DNP-Enhanced Solution-Phase Organic NMR in Liquids

Let e^- polarize / saturate e^- transitions / e^--n^+ X-relaxation polarizes nuclei

Prisner et al – In Situ Liquid DNP NMR at 260 GHz (9.4 T): ≈30x Enhancement on 5 nL sample



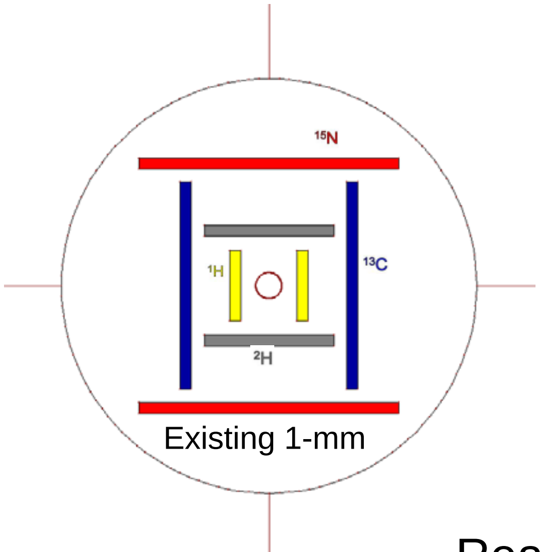
Features of the MagLab Proposal:

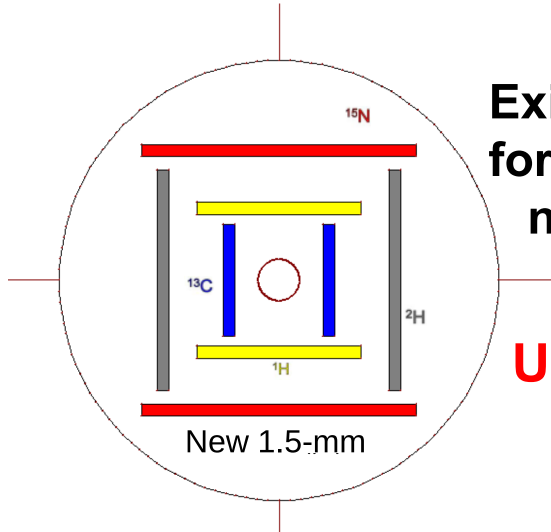
- *Joint EPR/NMR collaboration at FSU/UF (MagLab)*
- *Shuttling between DNP and high-res NMR regions*
- *Both DNP & NMR take place @ ≈14.1 T*
- *Gyrotron-equipped to bypass low DNP efficiencies*
- *Low-viscosity media bypass X-relaxation bottlenecks*
- *Non-aqueous solvents bypass μwave losses*
- *Expected enhancement factors: 10-50x on ≈50 μL*
- *Upgrading to higher fields possible*
- ***Interfacing planned to MagLab-based HTS probes***
- *NSF MRI application pending*

Scientific Drivers:

- *Organic Structure Elucidation*
- *Metabolomics*
- *Natural Products*
- *Pharmaceuticals*
- *Drug Developments*
- ***Fast Multidimensional NMR implementations***
- *New Avenues in THz-oriented DNP*

Coupling the High Sensitivity that High Fields and DNP can Provide, with Solution State NMR High Temperature Superconducting Probes





Existing HTS probes are optimized for mass-limited samples: ideal for natural products/metabolomics

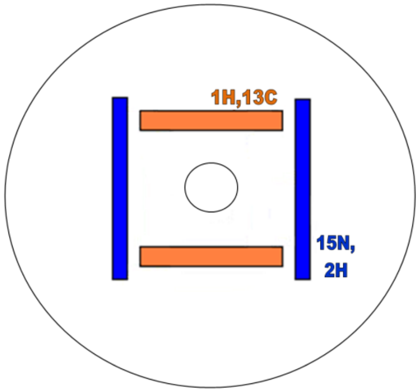
Up to 10x greater ^{13}C S/N.

Ready for NMR Users Mid-2012

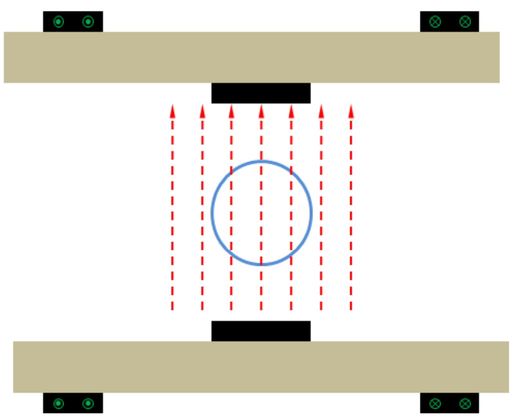
Agilent Technologies

The MagLab is the only non-commercial group in the world currently developing HTS solution NMR probes. The previous 1-mm set records for sensitivity of mass-limited samples. All of the HTS projects at the MagLab have been funded by the NIH and are collaborations with industrial partners. Results are disseminated to the broader NMR community through new commercial products and publications.

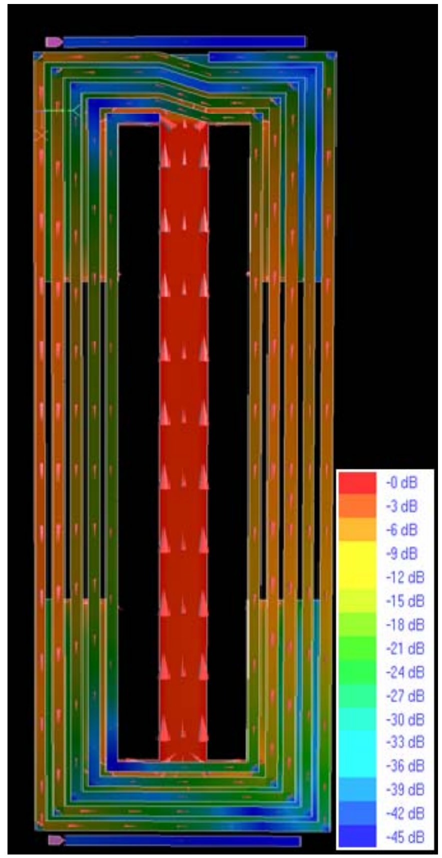
Future HTS Plans: Optimization of Sensitivity for Two Nuclei on the Same Sapphire Substrate



Larger Probes: Optimized for concentration-limited samples

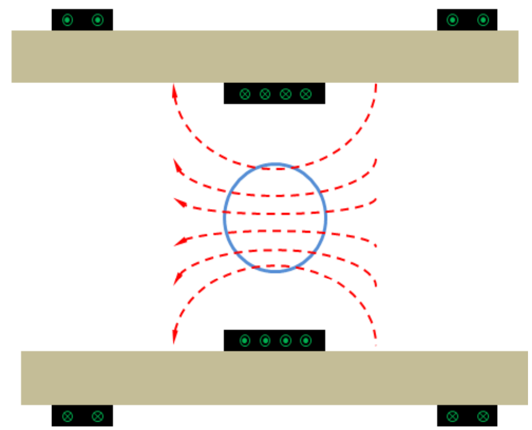


B_1 field schematic for ^{13}C mode



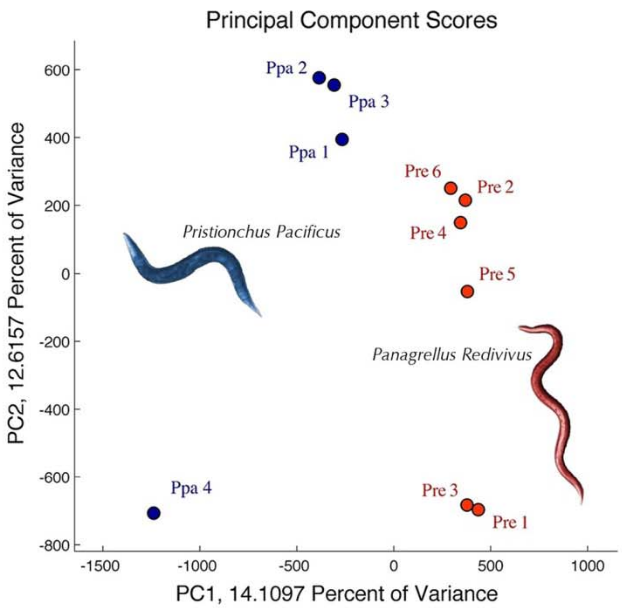
Sample coil configuration demonstrating dual resonance

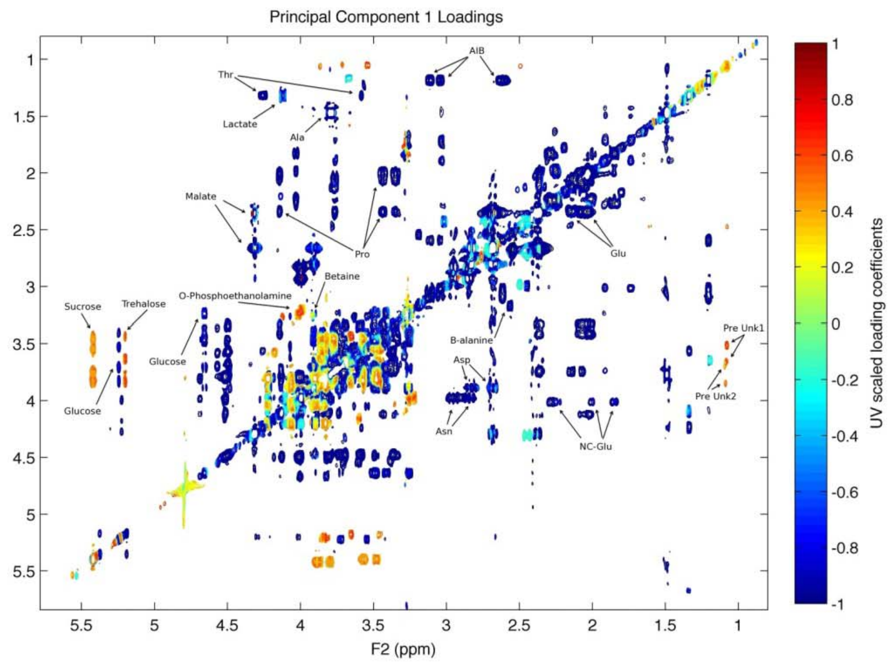
- **Sensitivity optimization of two nuclei simultaneously**
- **Provide more room in probe body for larger samples**

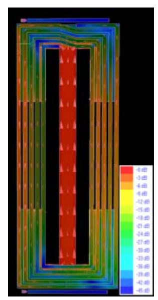


B_1 field schematic for ^{1}H mode

DNP NMR in "omics": Better Identification of Components in Complex Biological Mixtures







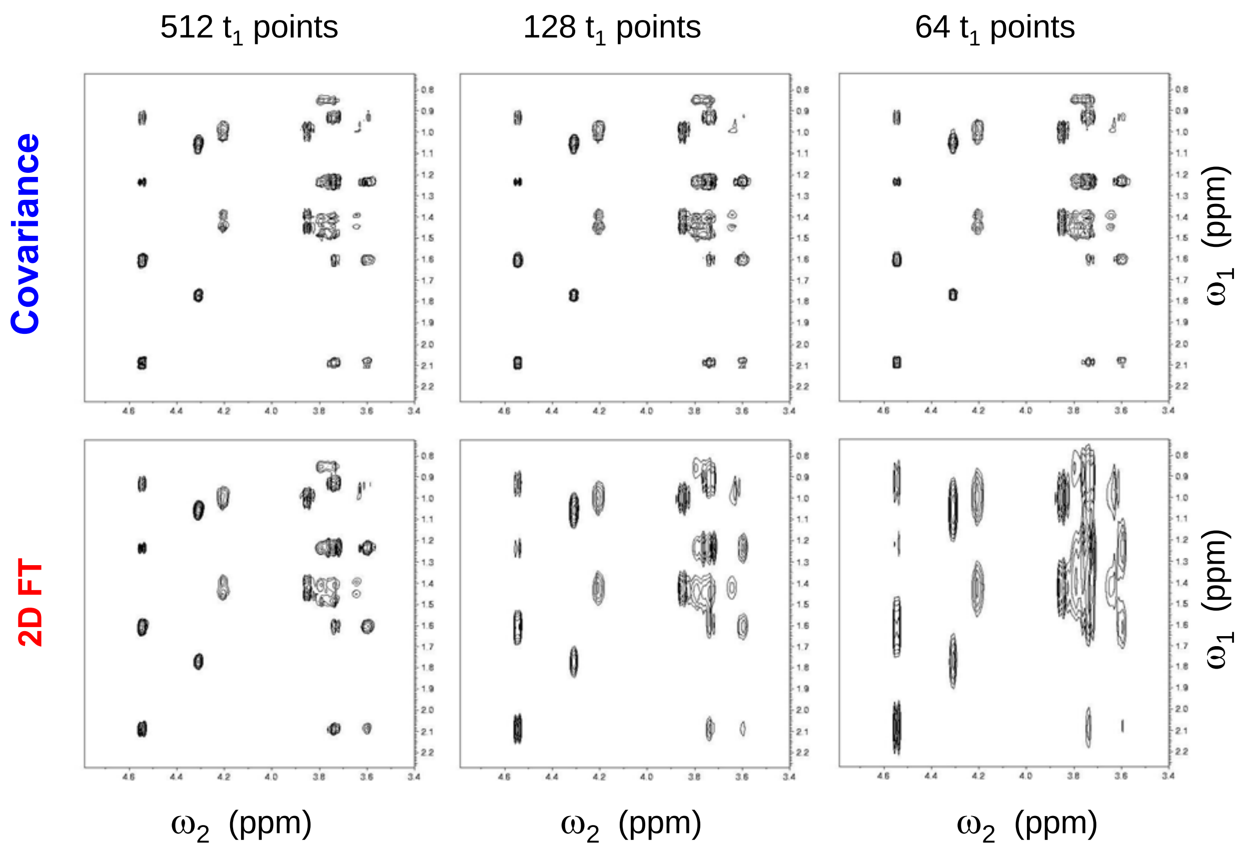
New HTS probes enhance NMR metabolomics by allowing for an order of magnitude better ^{13}C detection, dramatically increasing the number of molecules that can be detected.

A combination of the Tallahassee/UF-developed HTS probes, with both dissolution and shuttling DNP, could increase the sensitivity of such experiments by ≥100x over what is currently possible at the highest magnetic fields

Optimal Sensitivity Per Unit Time:

Covariance NMR and Other Fast/Ultrafast nD Acquisition methods for High Resolution Spectroscopy in Solids (36 T) and in the DNP Shuttling Solution System

Spectral region of antamanide TOCSY



- **Multidimensional NMR methods are the basis of complex mixture analysis software**
- **Many of these studies are done in organic solvents: compatible with high-field DNP in non-aqueous media**
- **Useful in solid-state NMR Correlations as well**

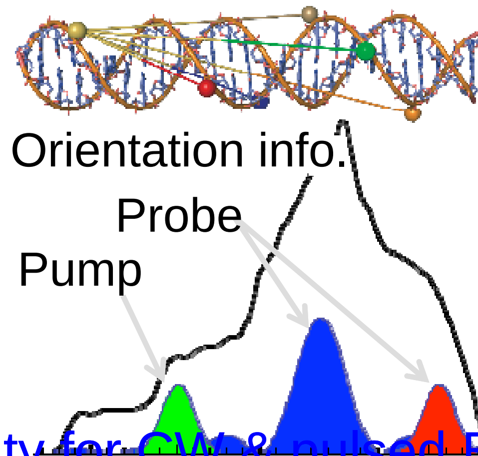
Covariance NMR: Over 500 virtual users! http://spinportal.magnet.fsu.edu/

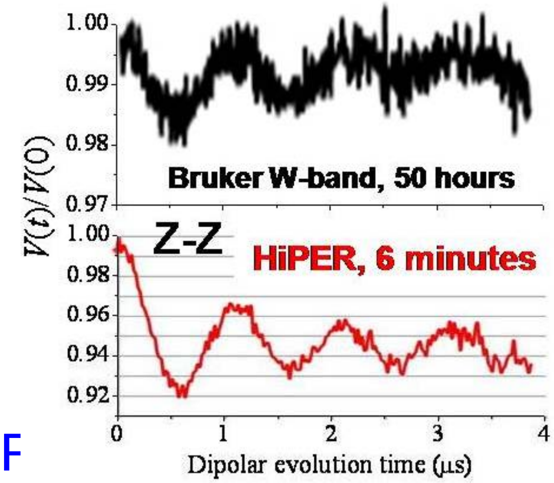
- **Maximal resolution and sensitivity in minimal acquisition times.**
- **Fast nD NMR/MRS methods become increasingly important as the number of scans becomes limited --either because of power supply or mechanical limitations.**

HiPER (nanosecond EPR + DNP)

Steve Hill, FSU, in collaboration with the University of St. Andrews and Thomas Keating LtD, UK

Quasi-optical mm-wave technologies (95 – 350+ GHz) can provide a quantum leap in the performance of pulsed ESR systems

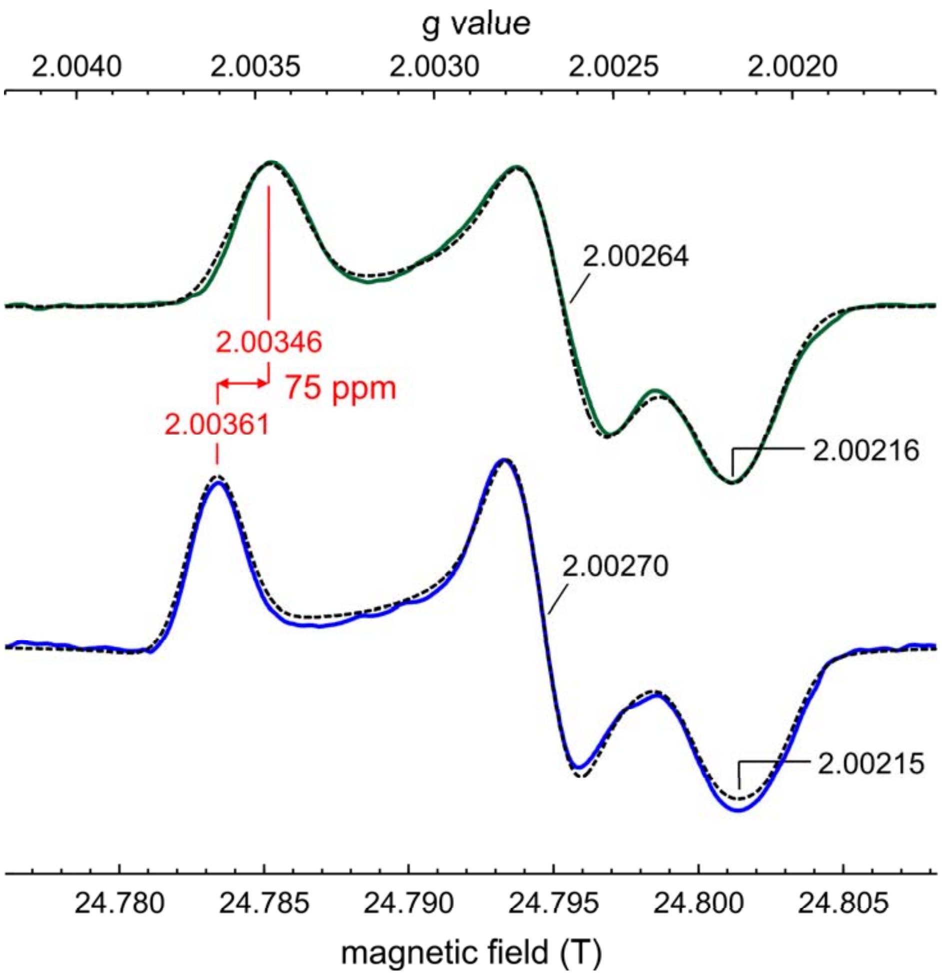


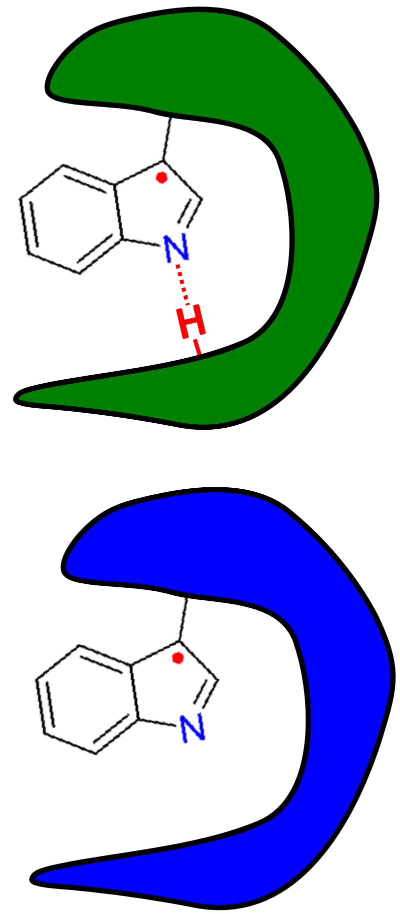


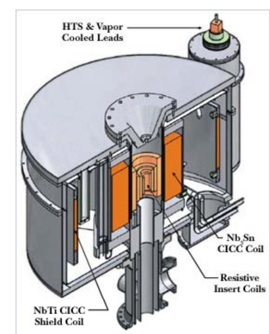
- Remarkable gains in sensitivity for CW & pulsed EPR (~1 ns)
  - Dramatically improved S/N and reduced data acquisition times
  - Much longer evolution times in DEER, i.e., much longer distance measurements
  - Will enable biophysical EPR much closer to physiological conditions
- FT-EPR on nanosecond timescales, thereby bringing the NMR paradigm to EPR

THIS IS ONLY POSSIBLE OPTICALLY AND ONLY POSSIBLE AT HIGH FIELDS/FREQUENCIES

Also in Biological CW EPR, High Fields Enable New Science







Structural studies of Azurin utilizing Tryptophan radicals. This was done at 25T (EPR at ≈700 GHz) and could not have been done at lower fields: Ultrahigh fields/frequencies required to resolve small g-anisotropies.

SCH could provide even greater resolution

Our Hope: The MagLab can help to improve NMR/MRI/EPR sensitivities by a combination of several "orthogonal" approaches:

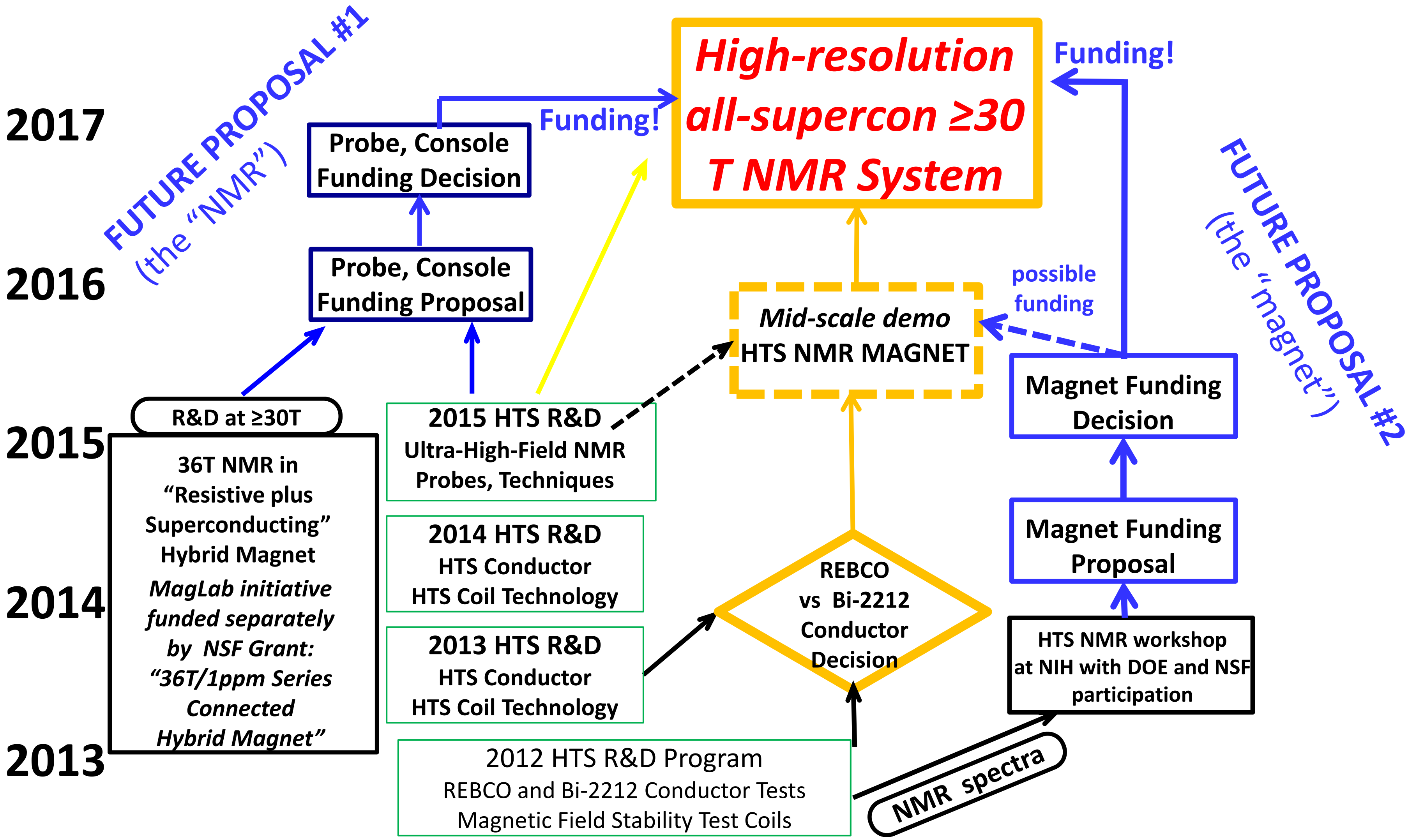
Our Challenge: Overcome MR's Low Sensitivity

- **Magnet technologies**: current Nb-based LTS's yield diminishing returns, but breakthroughs loom on the horizon: LTS/HTS inserts, CICC tech, hybrid designs
- Operating at higher frequencies can (and has to!) be complemented by probe technologies that exploit lower thermal noises, "silent" HTS electronics, closer proximity to the spins –with no sample µwaving
- By using thermal equilibrium states MR uses –even at the highest achievable fields– only a small fraction of the polarization that is potentially available: *plenty of room to play and to invent in hyperpolarization!*
- Alternative irradiation/detection technologies –optical, magnetometric, spintronics, force microscopy– could give the ultimate per-spin sensitivity

An example of how this could work for

Breaking the high-resolution ≥30 T NMR barrier

Only the enabling R&D (green boxes) are funded from the NSF MagLab Core...
Then there is the Magnet; the Consoles; the Probes...



Whether used for studying

- Nuclei or Electrons
- Chemistry or Biophysics
- Structure or Dynamics
- Liquids or Solids
- Pharmaceuticals or Materials
- Spectroscopy or Imaging
- Preclinical or Clinical
- Physics or Psychology

few methods can match the current capabilities of magnetic resonance.

The potential that could open up by operating at higher magnetic fields (36T solids NMR, ≥30T liquids NMR, ≥35T animal MRI/MRS, >10T humanMRI) is bound-less