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MAG LAB REPORTS

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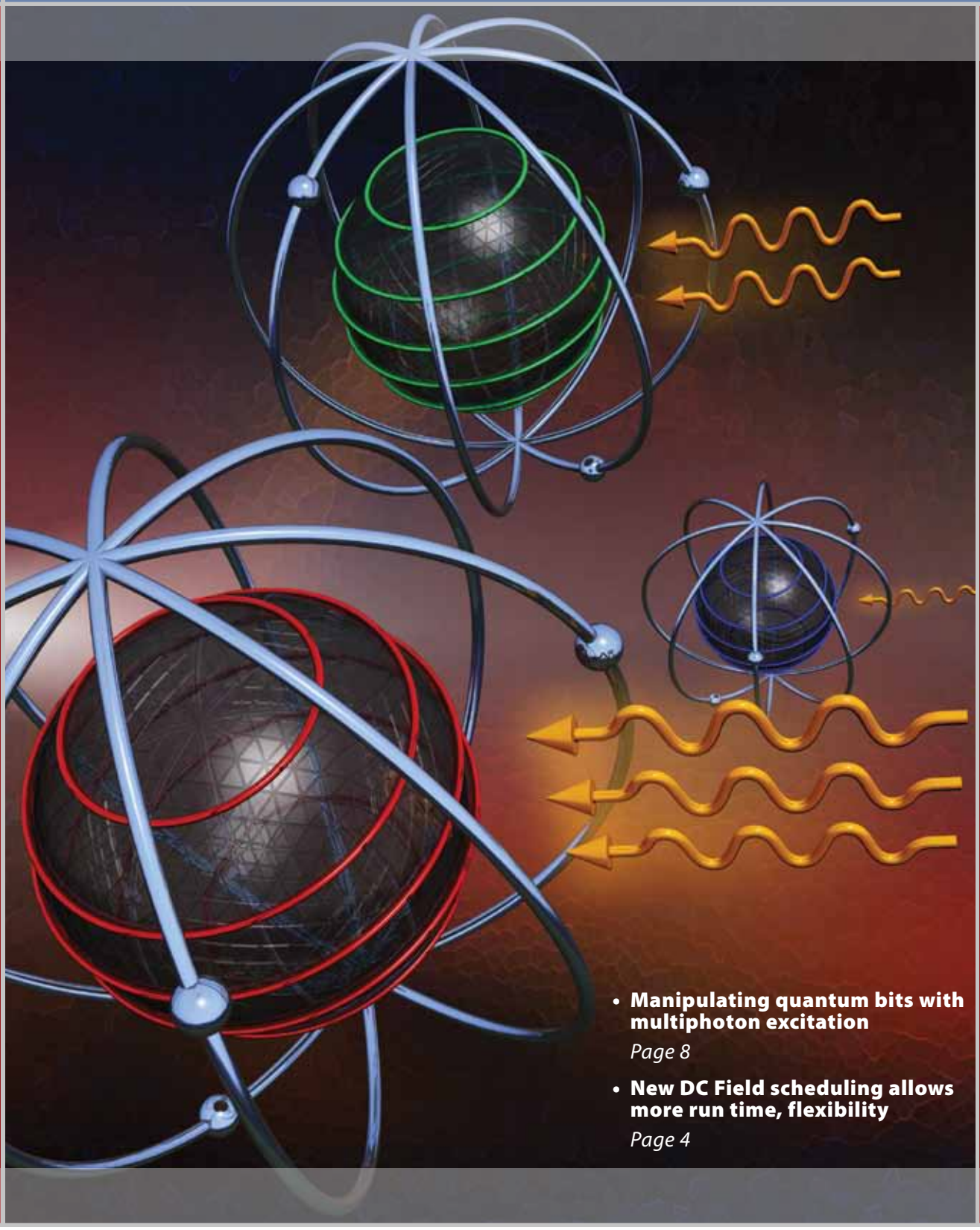

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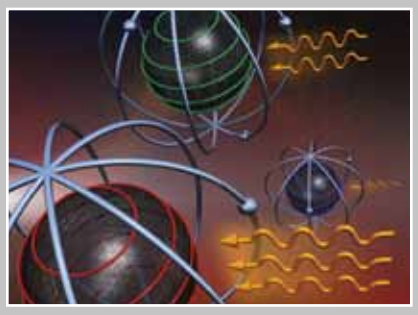

On the cover:
Photo illustration by Kevin John.
See page 8 for the article that inspired the cover.

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Scientists 'pull out the stops' for lab's annual Open House

By Greg Boebinger

We here at the Mag Lab are still happily recovering from our 14th annual Open House, which attracted more than 5,500 people (in just five hours) to the Mag Lab/FSU branch, shattering the previous record of 4,600 set just last year. Not only did we inspire a lot of "that is awesome!" "wow!" and "cool!" moments, we also collected more than 2,000 pounds of food for America's Second Harvest Food Bank of the Big Bend by asking our visitors to donate a canned good as the unofficial price of admission.

The headline in the newspaper summed it up well: "Mag Lab scientists pull out all the stops." Some scientists spent literally months building, tweaking and perfecting their demonstrations, much to the delight of our visitors. They shrunk quarters, froze flowers, launched potatoes out of pneumatic cannons and wrote "Magnet Lab" on the head of a fire ant with a focused ion beam. They gave our visitors, young and old alike, myriad opportunities for hands-on experimentation and a deeper understanding of the science concepts behind the bells and whistles.

It takes everyone – from grad student to scientist to administrative assistant to program directors – to pull off this event. I congratulate each and every one of them, as well as the leadership of our Public Affairs Department, on a job well done.

Open House master of ceremonies/court jester Greg Boebinger. Photo by Jim Berhalter.

SITE VISIT RECOGNIZES LAB'S CREATIVITY

The lab had its annual NSF site visit Dec. 9-11, and I'm encouraged by its conclusions and recommendations. The committee praised the lab for finding creative ways to do more with less in response to the budget cuts of 2008, and noted that such short-term, cost-saving measures are not sustainable over the long run.

The Magnet Lab cannot sustain a vital user program if our core budgets cannot support reliability, and we are teetering on the edge in this respect. As just one example, the cost of replacement parts for the flagship magnets in the Pulsed Field user program, the 60 T Long-Pulse and 100 T Multi-Shot Magnet Project, is several hundreds of thousands. We know with certainty that these magnets have a limited lifetime, because pulsed magnets are – in a very real sense – applied metal fatigue. These dramatic new and world-unique user capabilities have been brought online at the Mag Lab/LANL in a time of virtually flat budgets and rising costs … a very important Mag Lab accomplishment, but dependent on our core funding levels over the long term.

Improvements to our aging infrastructure – in particular a badly needed cryogenics upgrade to increase reliability and save on future liquid helium costs – have been put on hold, because resources must be diverted to other mission-critical areas (such as paying our utility and liquid helium bills!).

The site visit committee urged us to work with sponsors (and potential sponsors) to replace the lost funding, a recommendation we aggressively pursue to support the development of new capabilities, such as the much-appreciated and long-term U.S. Department of Energy support for the 100 T Multi-Shot Magnet Project. But we must recognize that new sponsors have been and will be reticent to pick up existing user program operations.

NEW SCHEDULE BENEFITS USERS

Switching to a decidedly more upbeat topic! In this issue, you'll read about the lab's new FlexTime schedule for the DC magnet program. This new schedule will allow users more run time and flexibility to use their assigned time. How much more? A full 10 hours per week more time to most efficiently use their allotted megawatt-hours. FlexTime also allows the Mag Lab to respond with more time and power if a user is getting exceptional (read: publishable) results. See page 5 for all the details.

Rock and roll,

Gregory S. Boebinger

GREGORY S. BOEBINGER

Schedule offers users flexibility, more run time

By Eric Palm

The DC Field Facility rolled out its FlexTime schedule for users in March, a scheduled that will allow users more run time and flexibility during the hours that the DC magnets are available.

Currently users receive 3½ hours of time on Mondays, and four, 7-hour-long shifts the rest of the week for a total of 31½ hours of available magnet time.

FlexTime will increase the available magnet time for each shift to a total of 41½ hours. The schedule for Mondays will stay the same, while the changes for Tuesday through Friday are illustrated in the table below.

	First Shift	Second Shift
Current Schedule	9:00 – 16:00	16:45 – 23:45
FlexTime Schedule	**6:30 – 16:00**	**16:30 – 2:30**

In addition to the extended shift times, if a user on the first shift is getting exceptional results (defined as publishable), we will allow him to keep running for up to 1 hour into the second shift's time. However, later in the week the users in the second shift will be able to start early to make up this time and maximize their experiment. The goal is to make the schedule as flexible as possible while guaranteeing that all users receive their magnet time during the week.

Of course if we gave you 33 percent more magnet time, you would naturally use more electrical energy (MWHrs). Since our budget is not increasing to match our expanded FlexTime schedule, we will be providing every user with an energy allocation. Each group for each visit will be given a set number of MWHrs to use per week. When users exceed that limit, their magnet time will be over. It is up to each group to use its energy budget in the way that best meets the users' own scientific agenda.

Some of our users primarily sweep the magnetic field (sweepers) and some primarily sit at high fields (sitters). Since sitters on average use 3 times the power of sweepers, they will be given a higher energy budget. Eventually we will become more sophisticated with our budgeting process to better manage our power bill while still enabling the highest quality scientific output by our users.

We expect that the overwhelming majority of our users will receive an energy budget that meets or exceeds what they have used in the past. Please remember that if you use power at the same rate as before, you will run out after 31 hours of magnet time. Our goal is provide the best and most flexible magnet time that allows our users to effectively manage their magnet time without breaking our budget. We anticipate that the extra hours of magnet time will allow users to recover from the inevitable problems that crop up during an experiment without feeling like they will not have enough time to get their experiment done.

If a user is approaching the end of his magnet time and realizes that he will need additional power to complete the data for the *Nature* article that he is on the verge of realizing, he can appeal to the Magnet Lab director for more MWHrs. The director in consultation with the DC Program directors will look at the quality of the science being done and the amount of money left in the budget (not necessarily in that order!) and make a decision about providing extra power. Please be aware that with the additional hours most users will be limited by their power budget and not the available time and we will not be able to provide users with the power necessary to use all 41 hours of magnet time during their shift.

This user driven management tool will allow the Mag Lab to control its power bill and still provide maximum flexibility to the user. If and when we receive an increase in our budget we will increase your energy budget correspondingly.

We're excited about the additional fluidity that FlexTime provides. Please be patient with us as we work out the bugs in this new schedule. We look forward to hearing from you during and after your visits here about aspects of the plan that worked well and how we can improve as we move forward.

Eric Palm is the director of the DC User Program. You can reach him at palm@magnet.fsu.edu or (850) 644-1325.

Newer, better, larger: Cryogenic 'System D' worthy of superlatives

By Tim Murphy

What would you say if you could get a ***larger*** temperature range, a ***much*** longer hold time, ***better*** temperature stability, ***improved*** ease of use and ***more*** sample space from the cryostats used in the DC Field Facility? Would you be interested? Did we mention the part about more space at field center?

While it may sound like an offer on one of the many infomercials on late night cable TV, enhancing service to users was the idea behind a new cryogenic system (System "D") that will soon be put into service in the 32- mm bore 35 tesla resistive magnet. This system has a temperature range of 0.3-300 K and a liquid helium bath hold time of better than five days. Figure 1 shows the new system sitting next to two of the older-style pumped bath dewars that have been in use since the lab first opened.

Figure 1.
The dewar on the left is one of the original 10" dia. dewars. The middle dewar is one of the newer 13" dia. dewars and the dewar on the right is System "D."

The system has two inserts that can be swapped depending on the temperature range desired: the first is a top loading, sorbtion pumped, sample in liquid, ^{3}He system with a temperature range of 0.3-70 K which has a hold time at base temperature of 60 hours. The second insert is a top loading, sample in dynamic gas, variable temperature insert (VTI) that has a temperature range of 1.5-300 K. Both inserts have a sample space I.D. = 17 mm (0.67") and use the same probes for both inserts, which enhances the reliability and allows the next user to mount samples while the current user is running in the system. These inserts eliminate the need for pumping the entire liquid helium bath to condense ^{3}He and go to low temperatures, and the VTI has excellent performance at higher temperatures without having to turn your probe into a superinsulation wrapped mini toaster oven. There are two rotating and two fixed orientation probes with the system. Each probe has nine twisted pair, four stainless steel coax and two high frequency coax. The probe thermometry is on a separate wiring bundle so all of the wires are available for experimental use. The probes use vacuum load locks so the days of lowering your cryostat into a plume of cold helium gas and freezing the probe into the dewar are over. This configuration also allows us to collect the helium boil-off from the dewar into the recovery system for recycling.

One of the rotating probes under construction is shown in Figure 2 alongside one of the older ^{3}He rotating probes for comparison. There are two thermometers (Cernox and Ruthenium oxide) permanently mounted to the rotator that cover the entire temperature range and a heater mounted to the bottom. The I.D. of the new rotator is 10.4 mm (0.41") as compared to 8.89 mm (0.35") on the old system. There is also a rotator body that will accommodate direct attachment of an 8-pin DIP header allowing for plug and play transport measurements.

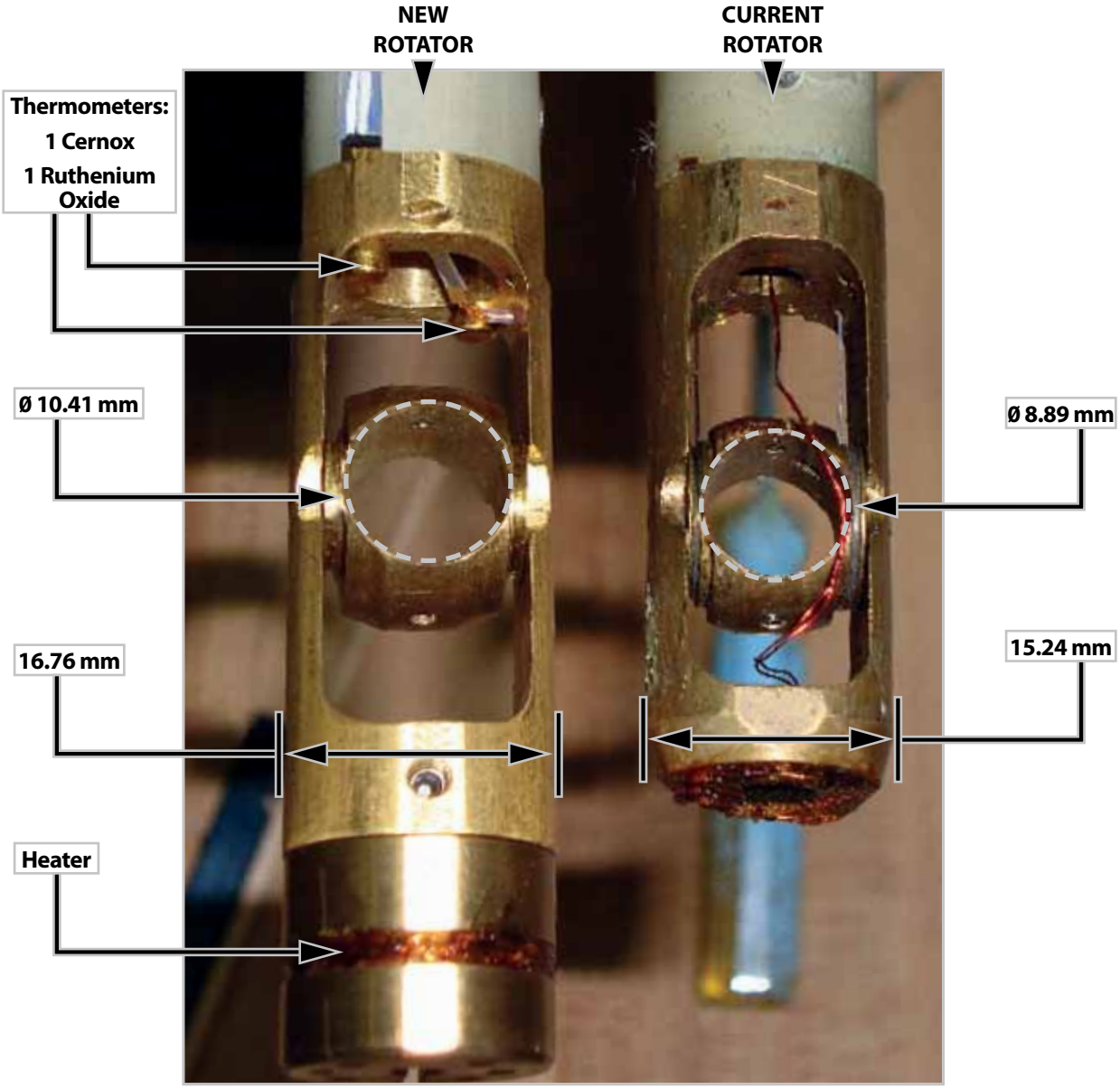

Figure 2.
The rotator on the left is for System D. The rotator on the right is what is currently used.

In addition to the rotating sample holders, there is a non-rotating sample holder that can accommodate two 8-pin DIP headers as shown in Figure 3. The 8-pin DIP holders can be released via a screw to make it easier to plug headers into the sockets. There is a shield that slides around the lower assembly to protect it during probe load/unload operations. In addition the lower DIP socket assembly can be removed to allow custom user sample holders to be attached to the probe.

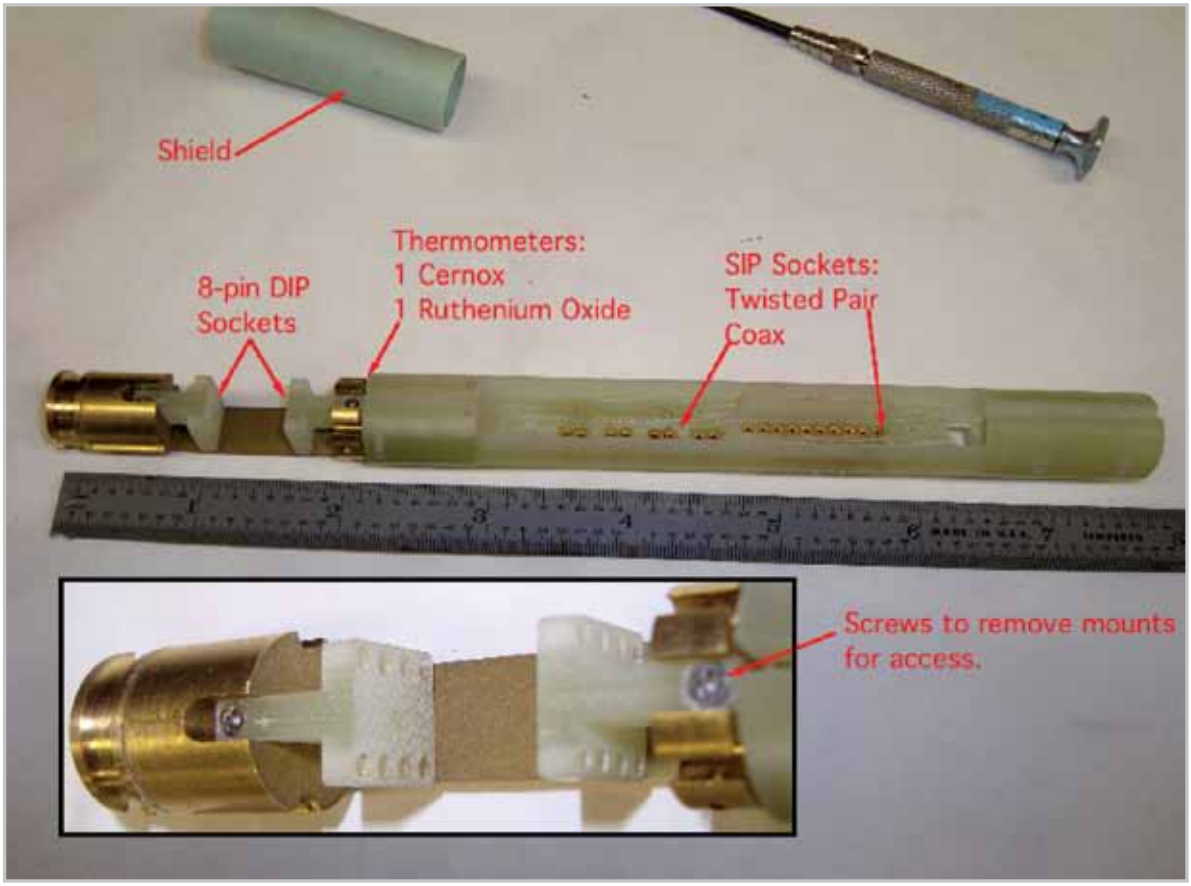

Figure 3.
System "D" fixed orientation 8-pin DIP socket sample holder

We also have two older siblings of System "D" and they are System "A" and "B" for the 20 T 50-mm bore superconducting and 31 T, 50-mm bore resistive magnets, respectively. These systems are identical in concept to System "D" but have different maximum fields and sample spaces. Table 1 below lists the relevant characteristics of each system. Systems "A" and "B" are both top loading and have rotating and fixed angle probes similar to System "D".

	^{3}He - Temperature - VTI		Field	Sample Space
System "A"	0.25-70 K	1.5-300 K	20 T	36 mm
System "B"	0.30-70 K	1.5-300 K	31 T	24 mm
System "D" (NEW!)	0.30-70 K	1.5-300 K	35 T	17 mm

Table 1: Comparison of top loading cryogenic systems.

Pictures and drawings for Systems "A" and "B" can be found online at:
http://www.magnet.fsu.edu/usershub/scientificdivisions/dcfield/facilities.html

...and on the operations wiki, which will soon be updated for all three systems at:
http://opsxserve.magnet.fsu.edu/groups/operations/wiki/cbb6d/Temperature_available.html

Multiphoton coherent manipulation in large-spin qubits

S. Bertaina, L. Chen, N. Groll, J. Van Tol, N.S. Dalal & I. Chiorescu

Quantum algorithms offer the possibility of solving certain problems with a tremendous increase of speed compared to their classical counterparts. Manipulating quantum bits (or qubits) however is a very delicate process, and building a quantum computer proves to be a challenging task. Photons are an ideal choice in such endeavor as they interact with quantum systems in predictable ways. As such, they are a versatile tool for manipulating, reading/coupling qubits and for encoding/transferring quantum information over long distances. As qubit implementations, spin-based systems are nanoscopic, potentially operable up to room temperature[1], less noise sensitive and benefit from single-spin detection schemes[2]. When diluted enough to avoid uncontrolled spin-spin interactions, a variety of spin qubits show long coherence times, e.g. the N-V color centers in pure diamonds, N atoms trapped in a C_{60} cage, Ho^{3+} and Cr^{5+} ions and molecular magnets. In this work[3] we present first observations of multi-photon spin coherent manipulation in a multi-level system (Mn^{2+}, S=5/2) diluted in a highly symmetric non-magnetic matrix. Decoherence effects on Mn^{2+} spins are strongly suppressed because of the system's high symmetry. We implemented a multi-photon/multi-level technique leading to Rabi oscillations, which offers a new way to operate spin qubits.

MgO is the host matrix of choice due to its quasi-isotropic crystal field (in cubic symmetry) leading to a small and tunable *in-situ* level anharmonicity. Such multi-state systems are proposed for quantum algorithms in either size-limited[4] or scalable schemes[5] and to study new exotic quantum phenomena, like the quantum antiresonances[6]. Aside the application in quantum computing, the spin 5/2 of a Mn^{2+} ion in a non-magnetic matrix of MgO allows studying quantum phenomena at the interface between the quantum and classical world. As known from quantum mechanics textbooks, two types of systems can exactly be treated analytically: the two-level system and the harmonic oscillator. The six electronic levels of Mn^{2+} can be tuned *in-situ* into a multi-level harmonic (or pseudo-harmonic) configuration. We show that its properties are strikingly different from those of a classical harmonic oscillator, which absorbs energy by continuously raising the occupation number. In our case, the finite number of equally spaced levels induces a coherent dynamics in which the spin projection shows an oscillatory motion, similar to the one of a two-level system.

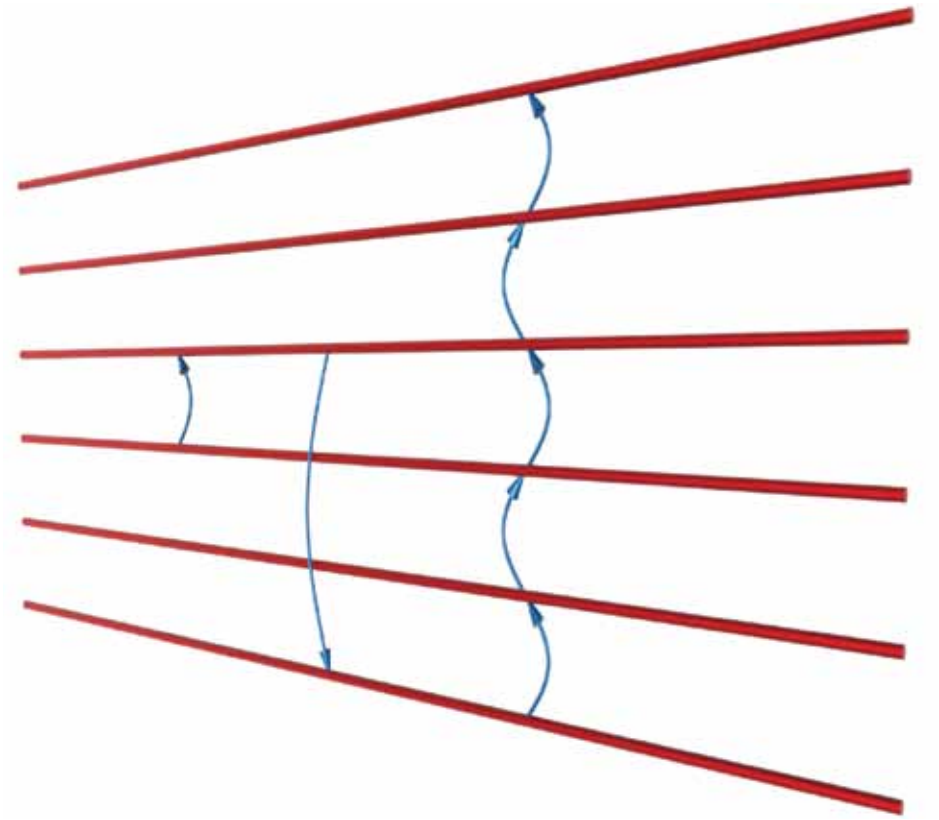

Figure 1.
Illustration showing the six levels of Mn^{2+} S=5/2 spin under the coherent drive of a single or triple photon excitation. The right-most arrows illustrate the situation when all the levels are equidistant and the pseudo-harmonic system shows the dynamics of a two-level system (see the oscillation in red in figure 2).

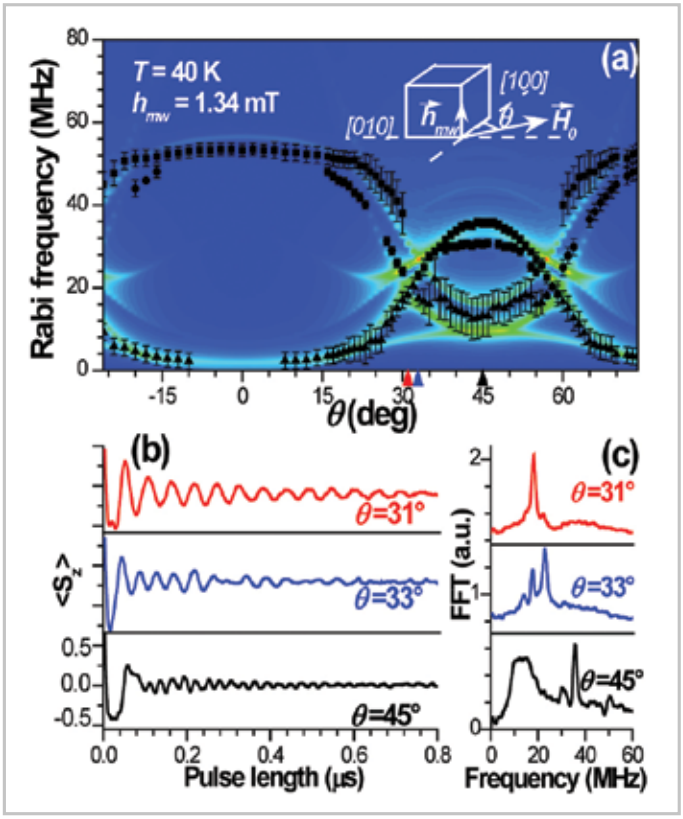

Figure 2.
(a) Fast Fourier Transform (FFT) peaks, in black, of Rabi oscillations recorded at T=40 K, as a function of θ (the inset shows the relative angles between the magnetic fields and the crystal axes). Error bars indicate peaks full-width at half max (FWHM). The contour plot represents FFT traces of simulated Rabi oscillations; the color code goes from 0 (blue) to 1 (red) with mixed colors for intermediate values. The markers on the ordinate axis indicate the angles used in (b) and (c).
(b) At the so-called compensation angle θ=31°, the equally spaced level system behaves as a two-level system showing a single-valued Rabi frequency. At θ=33°, beatings between two close Rabi frequencies are observed. At θ=45°, the averaged spin projection $\langle S_z \rangle$ undergoes a fast nutation superposed on a slower and less defined one.
(c) FFT of data in (b) [curves are shifted vertically, as in (b)].

These first observations of spin states dressed by coherent photons show the leading role of a small anisotropy on the dynamics of a spin qubit. Our results apply to other spin qubit implementations with quasi-harmonic energy diagram and in quasi-isotropic environments. The present study shows that multi-level spin systems can be coherently manipulated via multi-photon Rabi oscillations and therefore be exploited for use in quantum algorithms.

ACKNOWLEDGEMENTS

The measurements were performed by postdoc S. Bertaina and graduate students L. Chen and N. Groll at the facility operated by the Electron Magnetic Resonance division of National High Magnetic Field Laboratory. This work was supported by NSF Cooperative Agreement Grant No. DMR-0654118, NSF Grants No. DMR-0645408, No. DMR-0506946, and No. DMR-0701462, the FSU and the Magnet Lab (No. IHRP-5059), DARPA (No. HR0011-07-1-0031), and the Sloan Foundation.

REFERENCES

1. A.V. Akimov *et al.,* Nature, **450**, 402 (2007).
2. J. R. Petta *et al.,* Science, **309**, 2180 (2005).
3. S. Bertaina *et al.,* Phys. Rev. Lett., **102**, 050501 (2009)
4. M. N. Leuenberger and D. Loss, Nature, **410**, 789 (2001).
5. M. Grace *et al.,* New J. Phys., **8**, 35 (2006).
6. C. Hicke and M. I. Dykman, Phys. Rev. B, **76**, 054436 (2007).

Mechanism of drug resistance in gastrointestinal stromal tumor patients

Hui-Min Zhang[1,2], Mark R. Emmett[1,3], Alan G. Marshall[1,3], Ketan S. Gajiwala[4], Joe C. Wu[5], James Christensen[4], Gayatri D. Deshmukh[5], Wade Diehl[4], Jonathan P. DiNitto[5], Jessie M. English[5], Michael J. Greig[4], You-Ai He[4], Suzanne L. Jacques[5], Elizabeth A. Lunney[4], Michele McTigue[4], David Molina[4], Terri Quenzer[4], Peter A. Wells[4], Xiu Yu[4], Yan Zhang[5], Aihua Zou[4], and George D. Demetri[6]

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This report is a short overview of a paper, "KIT Kinase Mutants Show Unique Mechanisms of Drug Resistance to Imatinib and Sunitinib in Gastrointestinal Stromal Tumor Patients," recently published in the *Proceedings of the National Academy of Sciences*. The work was a collaboration with Pfizer Global Research and Development and the Dana-Farber/Harvard Cancer Center.

ABSTRACT

Most gastrointestinal stromal tumors (GISTs) exhibit aberrant activation of the receptor tyrosine kinase (RTK) KIT. The efficacy of imatinib mesylate and sunitinib malate in treatment of GIST patients has been linked to these compounds' inhibition of these mutant kinases. Often, patients on imatinib acquire secondary mutations in KIT that render the drug ineffective. Sunitinib has shown efficacy in some imatinib resistant mutants, but not all. Biochemical and X-Ray crystallography structural studies were performed to determine the molecular basis of sunitinib resistance. Due to the inability to form crystals of the full length KIT and some KIT mutants, solution-phase Hydrogen/Deuterium exchange (HDX) with high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) analysis was performed on these kinases under biological conditions. The HDX FT-ICR MS data was used to validate the truncated X-Ray data and provided conformational data complementary to the biochemical data. Together these data provide a structural and enzymatic explanation for the resistance profile observed with the KIT inhibitors. These results aid in the understanding of oncogenic mutants and for circumventing drug resistance.

INTRODUCTION

Most gastrointestinal stromal tumors (GISTs) exhibit aberrant activation of the receptor tyrosine kinase (RTK) KIT. The efficacy of the inhibitors imatinib mesylate and sunitinib malate in GIST patients has been linked to their inhibition of these mutant KIT proteins. Acquired resistance to systemic therapy is a critical problem in treating metastatic cancers. Improved understanding of the molecular mechanisms underlying resistance should provide insights leading to development of alternative treatment strategies or design of new therapeutic entities that could be used to circumvent desensitization. One example of acquired resistance is the secondary mutants of KIT identified in GIST. Most GISTs have primary activating mutations in the genes encoding the closely related RTKs KIT (approximately 85% of GIST patients) or platelet-derived growth factor receptor alpha (PDGFRA); [approximately 5% of patients][1].

The majority of KIT mutations affect the juxtamembrane (JM) region of the protein encoded by exon 11 of the gene. Imatinib mesylate (an inhibitor of KIT and PDGFRs) is currently first-line treatment for advanced GIST. Unfortunately, the majority of patients eventually show resistance to the drug: approximately 14% of patients are initially insensitive to imatinib, and approximately 50% of patients develop resistance within 2 years[2-3]. The latter resistance commonly occurs via secondary gene mutations in the KIT TK domains. An effective second-line treatment is provided by sunitinib malate (*Sutent*®), which is approved multinationally for the treatment of advanced GIST following failure of imatinib due to resistance or intolerance. Sunitinib is an inhibitor of multiple RTKs, notably in this context, KIT and PDGFRA, and has been shown to be effective against certain imatinib-resistant KIT mutants, such as the ATP-binding-pocket mutants. However, certain imatinib-resistant mutants that are also resistant to sunitinib[4] are located in the activation loop (A-loop) of the KIT catalytic domain.

Previous structural studies[5-6] had shown that KIT can hold diverse conformations (Figure 1): the unactivated, auto-inhibited conformation (in which the A-loop is in the "DFG-out" orientation, and the JM domain binds into a pocket), the unactivated conformation (in which the A-loop is in the DFG-out

conformation with the JM domain oriented toward the solvent), and the activated conformation (in which the A-loop is in the "DFG-in" conformation and extends over the C-terminus of the catalytic domain). The wild type protein can be considered to be in equilibrium among these conformations, with a shift to the activated form upon phosphorylation.

The mechanism of KIT resistance to sunitinib could not be deduced in modeling studies based on the published structures described above[5-6]. Thus, solution-phase HDX FT-ICR MS was combined with other biochemical and biophysical methods to elucidate imatinib and sunitinib inhibition and the mutant KIT resistance mechanisms[7].

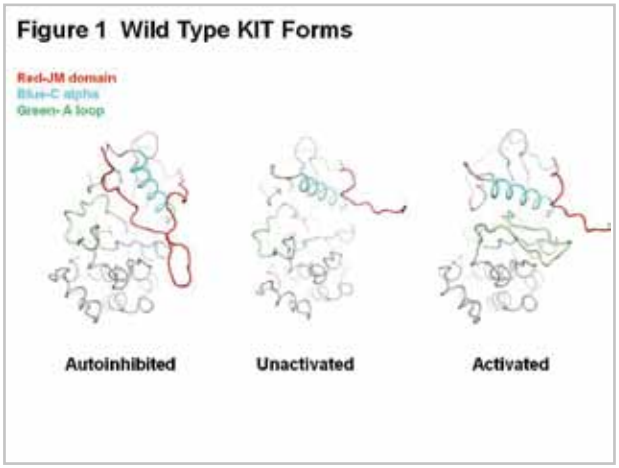

Figure 1.
The autoinhibited, unactivated and activated forms of wild type KIT. The JM domain (red), C α-helix (blue), and A-loop (green) are oriented differently in the autoinhibited and activated states. The JM domain (red) is much more solvent accessible in the unactivated and activated states.

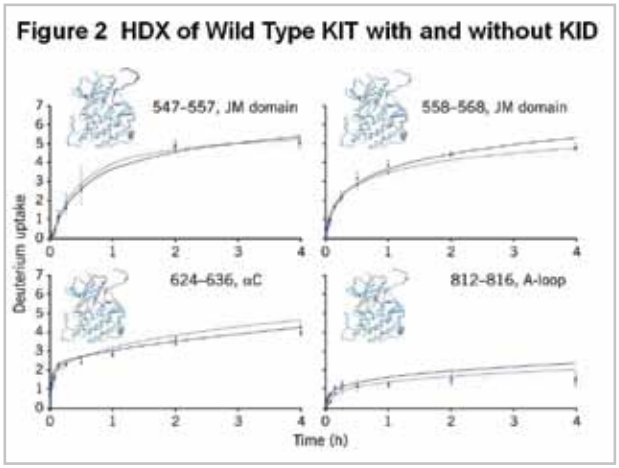

Figure 2.
HDX time courses for wild type KIT with (blue) or without the KID region (black) shows no significant conformational differences in the JM domain (residues 542–586), the α C segment (residues 624–636), or the A-loop (residues 810–834).

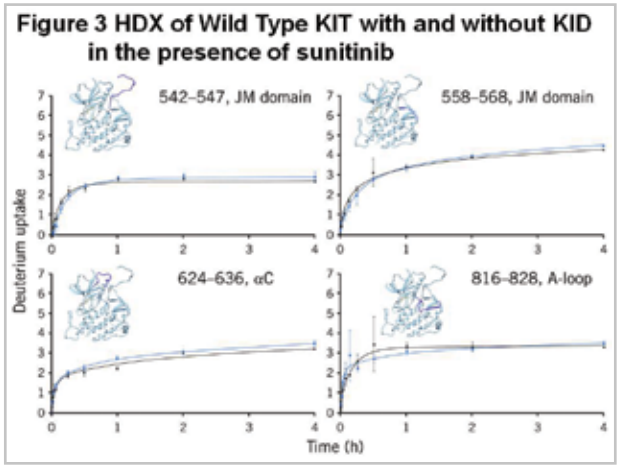

Figure 3.
HDX time courses for wild type KIT with bound sunitinib with (blue) or without the KID region (black) shows no significant conformational differences in the JM domain (residues 542–586), the α C segment (residues 624–636), or the A-loop (residues 810–834). These results confirm that the KID region plays no part in the binding of the drug to the KIT.

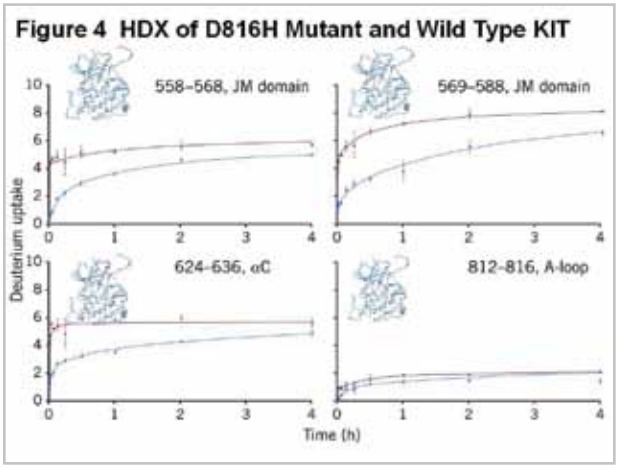

Figure 4.
HDX time courses for the D816H mutant (red) and wild type KIT (blue). Most of the D816H mutant peptides are more solvent exposed than those of wild type KIT, implying that the mutant protein is more flexible than the wild type protein, and supporting the predominant active conformation of the D816H mutant.

RESULTS

Kinase insertion domain (KID) has no influence on KIT conformation and function. KIT contains a flexible kinase insertion domain (KID) of ~50-60 amino acids as do other split TKs. All KIT wild type and mutant proteins investigated in the above-mentioned biochemical studies contained the KID. However, due to inability to crystallize KIT proteins with the KID intact, crystallographic experiments were necessarily limited to constructs in which the KID was deleted. Direct evidence of this domain's influence on the conformation of the whole kinase is therefore essential. Solution-phase hydrogen/deuterium exchange (HDX) experiments with high-resolution mass spectrometry analysis for two constructs of KIT (the wild type KIT kinase domain with and without the KID) were performed under the same conditions. HDX rates for each

of the covered peptides were similar in both proteins in the absence or presence of sunitinib. Thus, the KID has no major influence on KIT conformation (Figure 2) or on sunitinib binding (Figure 3) to KIT. Likewise, unactivated KIT with or without the KID showed similar affinity for sunitinib in protein fluorescence-quench experiments (data not shown).

KIT A-loop mutants auto-activate much faster than wild type KIT. Auto-activation experiments were performed for the wild type and mutant KIT kinases (data not shown). Auto-activation rates of KIT A-loop mutants D816H and D816V were substantially faster than that of wild type KIT (184- and 536-fold, respectively). The increase in the activation rate correlates well with loss of sunitinib potency during the course of auto-activation. Interestingly, although mutants harboring the V560D mutation exhibited the greatest increase in activation rates, they remained more sensitive to sunitinib through the study time course than the A-loop mutants D816H/V. This phenomenon may be related to the different conformations populated by the various mutants. HDX MS results showed that V560D adopted different conformations and thus can explain its different sensitivity to sunitinib from the D816H. A manuscript describing these results will be submitted to *Protein Science*.

KIT D816H impacts the auto-inhibitory conformation of the JM domain. Despite extensive crystallization experimentation, D816H KIT could not be crystallized. In HDX experiments, the HDX rate of the D816H JM domain exceeded the already high exchange rate of the wild type JM domain (Fig. 4). The HDX rate for the N-lobe residues adjacent to the JM domain increased significantly, indicating less protection from the JM segment. Limited proteolysis (with Glu-C) experiments with D816H KIT and different proteases were also performed. The D816H variant was significantly more susceptible to Glu-C proteolysis than was the wild type protein, indicative of JM exposure to solvent, further supporting the HDX data. We therefore propose that the D816H substitution negatively influences the inhibitory conformation of the JM domain such that the equilibrium is shifted from the auto-inhibited state of the kinase to one with a solvated, disordered JM domain (activated form).

CONCLUSIONS

The influence of the KID on KIT either in the presence or absence of sunitinib has been elucidated by HDX MS. The inferences from the crystal structures of KIT without the KID are validated by extension to the full-length cytoplasmic protein. Solution-phase HDX further verifies that the JM domain in D816H is much more flexible or unstructured compared with wild type KIT. Other results described above comparing the D816H mutant with wild type KIT provide additional evidence supporting the solvated state of the JM domain in the mutated enzyme. Based on these observations, D816H substitution is demonstrated to negatively impact the inhibitory conformation of the JM domain such that the equilibrium is shifted away from the auto-inhibited state to the JM domain being released to solvent and disordered. These results demonstrate that HDX coupled with high resolution MS is complementary to X-ray crystallography and NMR, and in this system was essential for the elucidation of the molecular mechanisms of drug resistance.

ACKNOWLEDGEMENTS

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REFERENCES

1. C.L. Corless *et al.,* J. Clin. Oncol., **22**:3813–3825 (2004).
2. G.D. Demetri *et al.,* N. Engl. J. Med., **347**:472–480 (2002).
3. J. Verweij *et al.,* Lancet, **364**:1127–1134 (2004).
4. M.C. Heinrich *et al.,* J. Clin. Oncol, **26**:5352–5359 (2008).
5. C.D. Mol *et al.,* J Biol Chem, **278**:31461–31464 (2003).
6. C.D. Mol *et al.,* J Biol Chem, **279**:31655–31633 (2004).
7. K.S. Gajiwala *et al.,* Proc. Natl. Acad. Sci. U.S.A., **106**:1542-1547 (2009).

Going hybrid: Combining solution and solid-state NMR techniques to determine the structural topology of membrane proteins

By Gianluigi Veglia, Nathaniel J. Traaseth, Raffaello Verardi, and Shi Lei

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No, it is not the latest trumpeted slogan of Toyota, FIAT or Volkswagen. "*Going hybrid*" is a new necessity that structural biologists are facing when tackling larger and more complex biological systems. In fact, hybrid methods (i.e. the combination of several different biophysical approaches) are becoming more and more common in modern biophysics. For instance, Ad Bax and co-workers have initiated the use of small-angle X-ray scattering (SAXS) in combination with residual dipolar couplings from solution nuclear magnetic resonance (NMR) to refine the structures of large proteins. Also, electron paramagnetic resonance (EPR) and NMR data have been used in concert to achieve high-resolution structures of protein complexes. SAXS, X-ray and computational methods have also been combined to characterize protein complexes in solution.

While these approaches have enabled extraordinary steps toward the complete high-resolution structure determination of soluble macromolecular assemblies, the progress in the membrane realm has been sluggish. To date, only 100 unique folds have been identified for membrane proteins. The reason is what separates membrane proteins from soluble ones: the lipid membrane. Lipids surrounding these proteins are necessary to their function and often modulate their interactions with other proteins. Yet, the complex dynamic interplay between membrane proteins and lipids complicates crystallization and hinders classical structure determination approaches used with NMR spectroscopy.

This led to the flourishing of alternative spectroscopic approaches, among those, the efforts by the Cross laboratory at the Mag Lab in pioneering the use of solid-state NMR for the determination of membrane protein structures from anisotropic NMR observables (chemical shift anisotropy and dipolar couplings). My team and I at the University of Minnesota (Dr. Nate Traaseth, Raffaello Verardi, and Lei Shi) decided to combine the structural restraints of the classical solution NMR techniques with the methods developed by the Cross lab. The idea combines the assignments and distance restraints for backbone and side-chain atoms under isotropic conditions (solubilized in detergent micelles or isotropic bicelles) with orientational restraints with respect to the membrane bilayer (i.e. structural topology) derived from samples in mechanically aligned lipid membranes supported by glass plates or magnetically aligned lipid bicelle preparations. Our frequent trips to the Mag Lab and conversations with Drs. Cross, Fu and Bertram at Florida State University have inspired the combination of these structural constraints into a unique energy function that is implemented into XPLOR-NIH with a simulated annealing protocol.

The target function (E_{total}) is formulated as a linear combination of geometrical (E_{chem}), solution NMR ($E_{sol\text{-}NMR}$), and solid-state NMR (E_{ssNMR}) terms:

$$E_{total} = E_{chem} + E_{sol\text{-}NMR} + E_{ssNMR}$$

The geometrical and solution NMR potentials are available in XPLOR-NIH force field. E_{chem} is the sum of bonding (E_{bonds}, E_{angles}, $E_{improper}$) and non-bonding interactions (E_{vdw}).

$E_{sol\text{-}NMR}$ is the sum of restraints from solution NMR experiments such as NOEs (E_{NOE}), hydrogen bonds (E_{HBON}), torsion angles (E_{CDIH}), and an empirical potential from the database (E_{DB}). The penalty function E_{ssNMR} contains the restraints derived from chemical shift anisotropy (CSA) and dipolar coupling (DC) data obtained from the solid-state NMR experiment polarization inversion spin exchange at the magic angle (PISEMA). The solid-state NMR potential was modified from the flat-well penalty functions by Bertram and co-workers (Bertram, et al. 2000):

$$E_{ssNMR} = w_{CSA}E_{CSA} + w_{DC}E_{DC} = w_{PISEMA}\left(E_{CSA} + w_r E_{DC}\right)$$

Key elements in these experiments are the optimization of the sample preparations and the use of low-electric field (*low-E*) probes developed by the RF team (P. Gor'kov, W. Brey) at the Mag Lab, which allowed the acquisition of reproducible spectra and the derivation of valuable structural restraints for structure calculation. An example of the PISEMA data acquired for monomeric phospholamban (AFA-PLN), a 52 residue integral membrane protein, is reported in Figure 1. The sample was prepared in DOPC bilayers

supported on glass plates and was hydrated in deuterated water, depleting all of the exchangeable amide protons (cytoplasmic and loop residues), leaving only the resonances corresponding to the transmembrane portion of AFA-PLN.

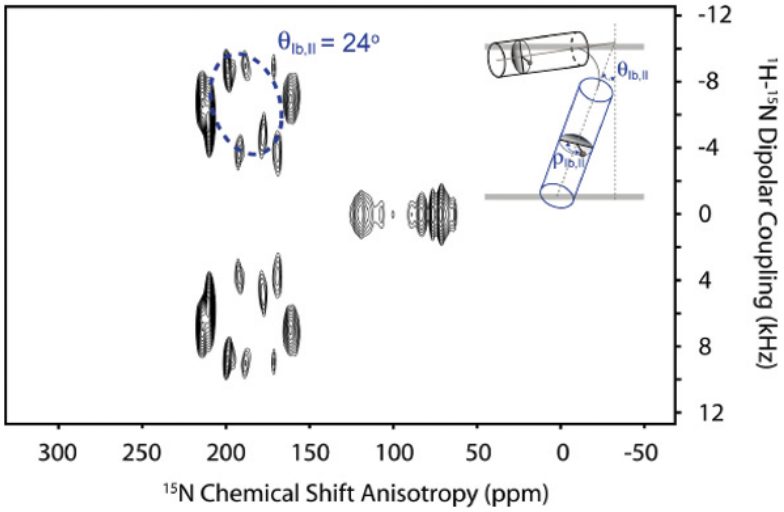


Figure 1.
[U-^{15}N] AFA-PLN PISEMA spectrum acquired in oriented DOPC lipid bilayers. Simulation of the PISEMA spectrum reveals a 24° angle that domains Ib and II make with respect to the lipid bilayer normal.

A total of 44 CSA and 41 DC were extracted from several PISEMA spectra carried out on selectively labeled samples and combined with the NOEs and torsion angle restraints derived from our previous studies in dodecylphosphocholine detergent micelles. The structural ensemble was calculated using a simulated annealing protocol at the Minnesota Supercomputing Institute. The average structure was then equilibrated in an explicit DOPC lipid bilayer shown in Figure 2. Unlike our previous deposited structure (1N7L), these new coordinates for AFA-PLN contain a realistic depth of insertion for all structural domains of AFA-PLN with respect to the lipid membranes. We should point out that in the final structure refinement we implemented the DeGrado depth of insertion potential that allowed us to model the membrane around phospholamban. The molecular dynamics in explicit lipids were carried out using the CHARMM force field, providing a comprehensive picture of both protein and membranes. These results are now submitted for publication.

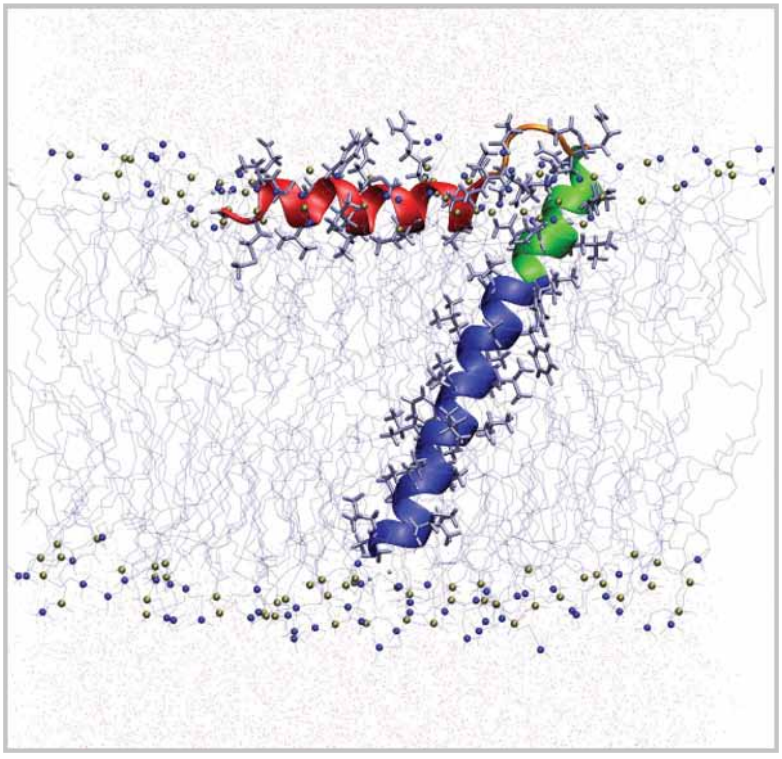


Figure 2.
Hybrid structure of AFA-PLN equilibrated in an explicit DOPC lipid bilayer. This structure was minimized with both solution and solid-state NMR restraints in DPC micelles and DOPC lipid bilayers, respectively. All of the restraints are satisfied in the proposed hybrid structure.

Center extends its outreach via the Internet

Educators at the Magnet Lab's Center for Integrating Research & Learning (CIRL) continue to find ways to engage K-12 students, teachers, undergraduates and graduate students in enhancing science education in an increasingly complex world of classrooms and schools. New outreach experiences build on revised state standards in science and connect students and teachers to real-world applications of science concepts.

Recently, Magnet Lab Webmaster Kristen Coyne worked with CIRL educators to develop audio slide shows featuring entertaining demonstrations with an explanation of the science behind them. The shows are posted on YouTube, TeacherTube (a site that is often accessed by K-12 teachers and students), and of course the Mag Lab Web site. As of this writing, the slide shows have received well over 18,000 hits among the three locations.

In an age of advanced technology, exciting scientific discoveries and new areas of research, many teachers and students still cling to old misconceptions about science and scientists, sometimes perpetuating stereotypes of what a scientist looks like and what scientists do in their laboratories. CIRL works to provide role models and strategies for teachers that will inform and excite the next generation of science, technology, math and engineering professionals.

Increased focus on science education has led to a spike in requests for outreach. CIRL reached more than 2,500 K-12 students in North Florida and South Georgia in the fall of 2008 alone. By January, the entire academic year was scheduled for Carlos Villa, CIRL's outreach coordinator. In addition to classroom visits, the CIRL staff has conducted Science Night for 250 elementary-aged students and their parents, continues its signature workshops in schools, is serving as science fair adviser to several schools, as well as participating in community events.

Four high school students interned at the Magnet Lab during the fall semester: Carolyn Kim with Stan Tozer, Daniel Watkins with Jim Brooks, Daniel Chang with Tom Painter, and Matthew Stolz with Scott Hannahs. Ms. Kim continues her internship for spring semester along with students from Women in Math, Science, and Engineering at FSU who also are working with Tozer.

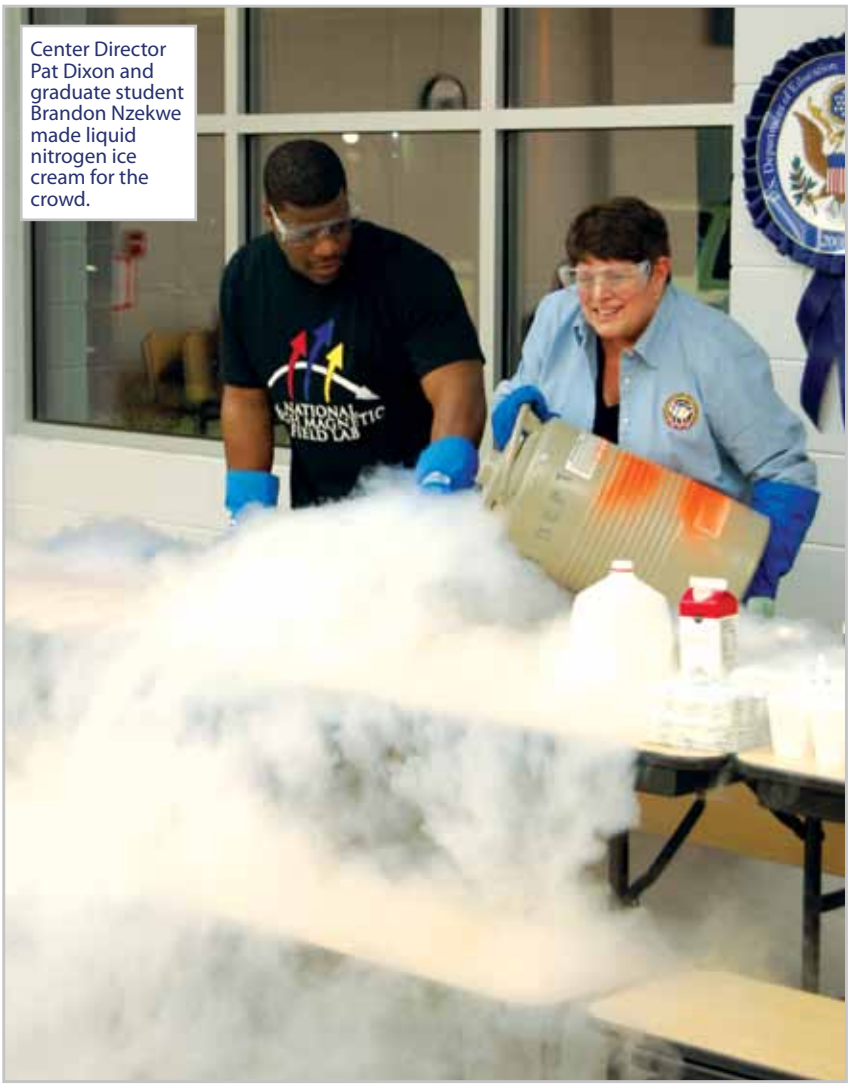
Center for Integrating Research & Learning educators wowed the crowed at Sealey Elementary School's Science Night in late January. The Mag Lab has been a Partner In Education with Sealey, a math and science magnet school, for several years. Science Night is geared toward 3-5 graders and their parents.

Left: Center Assistant Director Jose Sanchez uses a bell jar, vacuum pump and a balloon to demonstrate pressure.

Below: Participants don special glasses that show fingerprints of light and reveal the visible spectrum.

Carlos Villa and Jose Sanchez will attend major science conferences representing the Magnet Lab: The National Science Teachers' Association national meeting and the National Association for Research in Science Teaching international conference. In addition, two papers based on CIRL research were accepted at the Association for Science Teacher Education and the National Association for Research in Science Teaching. North Carolina State University Assistant Professor Margareta Pop, who completed the research while a doctoral candidate at FSU, presented the ASTE paper. Both papers dealt with teachers' motivation to change their practice based on participation in a Research Experiences for Teachers (RET) program. Roxanne Hughes, graduate research assistant, will present a poster at the American Educational Research Association conference on CIRL's girls in science program, SciGirls.

In addition to the research on SciGirls and RET, a new study was undertaken by Brandon Nzekwe, graduate research assistant, to gather data on participants in the Magnet Lab's Research Experiences for Undergraduates (REU) program 1999-2008. Of 180 participants, data was gathered on 103 former participants. Currently 74 are enrolled students; 5 serve as science educators; 17 work in private industry; and 7 have government-based professions. Forty-two former participants are graduate students and 24 indicate that they are pursuing a Ph.D. Of the 103 participants that provided information, 32 have 91 science based publications between them; 15 of these were published on research conducted while participating in the REU program. Three REU alumni continue working at the Magnet Lab: Rick Clinite (REU 2001) returned to the lab and FSU to pursue a graduate degree after graduating from Cornell. He continues to work in Condensed Matter Science with Jim Brooks. Kenneth Purcell (REU 2001) worked with former Director Jack Crow both before and after graduating from Western Kentucky University and is currently working in Condensed Matter Science. Nicole Tibbetts (REU 2000) recently received her Ph.D. in Geochemistry while working with Roy Odom. Kelly McKirahan (REU 2005) reported that she received an NSF Academic Excellence award for her REU program; Paul Egan (REU 2006) credits the REU in part for his National Goldwater Scholarship award.



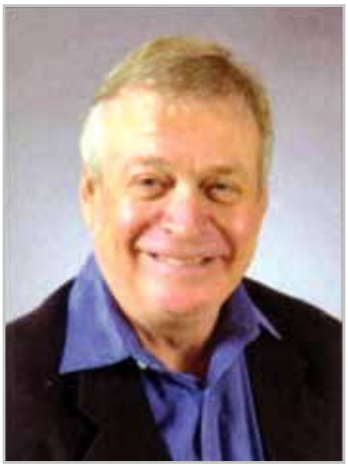
Center Director Pat Dixon and graduate student Brandon Nzekwe made liquid nitrogen ice cream for the crowd.

The first CIRL Advisory Board meeting via conference call was held in January and provided valuable input on future directions for CIRL programs. Advisory Board members committed to assisting with program development, identifying possible funding opportunities, and to working together to broaden CIRL's national presence. Members are: VieVie Baird, Florida Department of Education; John Bruno, Flagler College Tallahassee; Mary Jo Koroly, University of Florida; Fiona Goodchild, University of California Santa-Barbara; Jay Dubner, Columbia University; and Diandra Leslie-Pelecky, University of Texas Dallas.

The diversity of educational programming at the Magnet Lab coupled with the commitment of scientists who mentor students, teachers, undergraduates, and graduate students continues as a model of informal science education.

People

Katye E. Altieri, an oceanography graduate student at Rutgers University and an external user of the Magnet Lab's Ion Cyclotron Resonance (ICR) facility, is the 2008 winner of the Desert Research Institute's prestigious Peter B. Wagner Memorial Award for Women in Atmospheric Sciences. Altieri was chosen for her study of secondary organic aerosol formation through cloud-processing reactions. The Wagner Award is the culmination of rigorous competition among female graduate students who submit papers reflecting original research in atmospheric sciences. The winning paper, "Oligomers formed through in-cloud methylglyoxal reactions: Chemical composition, properties, and mechanisms investigated by ultra-high resolution FT-ICR Mass Spectrometry," was published in the journal *Atmospheric Environment*.



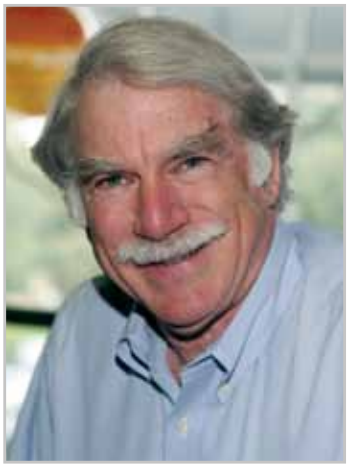
The World Association of Theoretical and Computational Chemists has awarded the 2008 Schroedinger Medal to **Rod Bartlett**, Magnet Lab affiliate and graduate research professor of chemistry and physics at the University of Florida. The Schroedinger Medal is the association's prestige for an outstanding senior theoretical/computational chemist. The selection is the result of a secret ballot of the entire board and is highly competitive. Bartlett was awarded the medal for the systematic development of correlated wave function methods, especially many-body perturbation theory and coupled cluster theory.

Two Magnet Lab researchers were recently elected fellows of the American Physical Society:

Rafael P. Brüschweiler, professor, Department of Chemistry and Biochemistry at Florida State University, and associate director for Biophysics at the Mag Lab, "for fundamental contributions to methodology and applications of nuclear magnetic resonance spectroscopy in combination with novel computational approaches for the dynamic characterization of proteins in solution."

Alexander V. Gurevich, scholar/scientist and principal investigator, Applied Superconductivity Center, "for significant contributions to the theory of superconductivity, particularly the effect of crystalline defects on critical currents, vortex dynamics, and upper critical fields of high-temperature superconductors and MgB_2."

Florida State University's Council on Research and Creativity awarded **Susanne Cappendijk**, an assistant professor in the Department of Biomedical Sciences and user of the lab's 900 MHz magnet, a First Year Assistant Professor award for her work, "Nicotinic Acetylcholine Receptors in the Zebra Finch Brain: How Receptor Topography Can Help in Understanding Neurodegenerative Diseases. "The award, one of 47 bestowed university-wide, comes with $17,000 in salary support.



Alan G. Marshall, director of the ICR user program and Robert O. Lawton Professor of Chemistry and Biochemistry at FSU, has been selected to receive the 2009 Eastern Analytical Symposium Award for Outstanding Achievements in Mass Spectrometry. The award consists of a plaque and an honorarium that will be presented at a one-half day symposium in Marshall's honor during the upcoming EAS meeting, to be held Nov. 15-18 in Somerset, New Jersey. Marshall is just the third recipient of this relatively new award, his 14th national/international award during his 15 years at FSU.

Wei Pan, a long time collaborator with the Mab Lab's High B/T facility in the Microkelvin Laboratory at UF, has been named as a winner of the Presidential Early Career Award. Wei Pan was one of eight recipients named by the Department of Energy for this year's awards. He was cited for leadership in the field of experimental many-particle physics, especially non-Abelian states in ultra-clean two-dimensional systems; and for broad scientific community outreach activities and leadership. Pan is a principal member of the technical staff in the Physical, Chemical and Nano Sciences Center at Sandia National Laboraties, where he leads Sandia's DOE basic Energy Sciences project on quantum electronic phenomena and structures.

Science Starts Here

NAME:

Andrew Christianson

POSITION:

Shull Fellow
Oak Ridge National Laboratory
High Flux Isotope Reactor

"The Magnet Lab has achieved one of the best balances between serving the user community and nurturing a strong internal science program."

Andrew Christianson

TIME AND ROLE AT THE MAGNET LAB:

2000-2003, graduate research assistant at Pulsed Field Facility

CURRENT WORK:

Andrew studies strongly correlated electron systems with the aim of understanding the fundamental interactions that give rise to the novel magnetic and superconducting ordered states displayed by these materials. The technique he currently uses to investigate strongly correlated electron systems is neutron scattering often in conjunction with applied magnetic fields. Recent work includes studies of new superconducting materials and the structure and dynamics of magnetic nanoparticles.

IN HIS OWN WORDS:

"My experience at the Magnet Lab gave me an early introduction to many current problems in the field of strongly correlated electrons and an enthusiastic and highly motivated group of experts to interact with.

"Throughout my career I have worked at many user facilities in the U.S. and around the world. The Magnet Lab has achieved one of the best balances between serving the user community and nurturing a strong internal science program."

HOW MENTORS MAKE A DIFFERENCE:

"During my time at the Magnet Lab, I had the privilege of working closely with Alex Lacerda. He not only served as my mentor at the Los Alamos campus, but also served on my Ph.D. committee at Colorado State. Alex was a pleasure to work with as he always injected excitement and encouragement about the science we were doing. I am truly grateful for a mentor who was encouraging and never lost sight of the fact that the goal of every graduate student is to do great science that enables the achievement of a Ph.D. in a reasonable amount of time."

"Science Starts Here" showcases young scientists whose career paths have been greatly shaped by their experiences at the Magnet Lab.

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