

P1**High Resolution ^{31}P NMR on CsH_2PO_4 : A One-Dimensional Ferroelectric***Tiglet Besara, Randall Achey, and Naresh Dalal*

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CsH_2PO_4 (CDP) is a unique member of the KH_2PO_4 (KDP) family of ferroelectrics. It undergoes a phase transition at 159 K, whose mechanism is still not well understood. A specific question is whether the transition is of the order-disorder or displacive type. Earlier studies by Blinc and coworkers [1,2] have discussed the phase transition in terms of an order-disorder system. We plan to present new high resolution ^{31}P NMR magic angle spinning measurements as a function of temperature on CDP and discuss the results in terms of the transition mechanism.

[1] R. Blinc, B. Zeks, A. Levstik, C. Filipic, J. Slak, M. Burgar, I. Zupancic, L.A. Shuvalov, and A.I. Baranov, *Phys. Rev. Lett.* **43**, 231 (1979)

[2] R. Blinc, I. Zupancic, G. Lahajnar, J. Slak, V. Rutar, M. Verbec, and S. Zumer, *J. Chem. Phys.* **72**, 3626 (1980)

P2

NMR Structure of *hv*DHFR1:Folate Complex from the Halophile *Haloferax volcanii*

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Environmental effects on an extremophile are under investigation in our research group. In particular, we want to understand how an extremely salty environment affects enzyme function, stability, solubility, and flexibility. Dihydrofolate reductase (DHFR) is an enzyme that catalyzes the reaction of dihydrofolate (DHF) to tetrahydrofolate (THF) with NADPH. This enzyme is found in halophilic as well as mesophilic organisms, providing a convenient means for comparison. *Haloferax volcanii* is a halophilic microorganism found in the Dead Sea. This microorganism has adapted to its extreme environment and even requires extremely high salt concentrations to survive.

The structure of *hv*DHFR1:folate has been investigated, using recently published triple resonance nuclear magnetic resonance (NMR) chemical shift assignments, with CNS (Crystallography and NMR System) structure calculations, suggesting an overall similarity to the comparable mesophilic ecDHFR:folate complex, and to the apo *hv*DHFR1 structure with the exceptions of some folate-interacting residues. These residues include ones found in the binding pocket of the enzyme, and also in other areas of the enzyme due to interaction with these folate-binding residues. The results of the structure calculation and secondary structural analysis of the chemical shift assignments suggest secondary structural features including a beta-sheet in the core of the enzyme composed of 8 beta-strands, surrounded by 4 alpha-helices, and 4 major loops.

P3**Solid-state NMR Studies of Wild-type and N27A Mutant Phospholamban in Lipid Bilayers**

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A 52 amino-acid transmembrane protein, phospholamban regulates the calcium transport across the sarcoplasmic reticulum (SR) of cardiac cells via a reversible inhibitory interaction with SERCA2a, the cardiac isoform of Ca²⁺-ATPase. Asn²⁷ to Ala (N27A) mutation in the hinge range of PLB has been proved to be a sufficient primary cause of heart failure and heart disease. In the present study, ¹⁵N, ²H and ¹³C solid-state NMR experiments have been conducted on wild-type PLB and N27A mutant reconstituted into phospholipids bilayers. The structural topology and dynamics of the cytoplasmic and transmembrane domains of PLB have been investigated. The structural and dynamic perturbation of the N27A mutation will be discussed upon comparison with that of the native form of PLB.

P4 **^2H and ^{31}P ssNMR Studies of the Effects of the N-Terminal Segment of Pulmonary Lung Surfactant Peptide-B on Lipid Dynamics***R. Suzanne Farver, Vijay C. Antharam, Douglas W. Elliot, Frank D. Mills, and Joanna R. Long*Department of Biochemistry and Molecular Biology & McKnight Brain Institute,
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Surfactant Protein-B (1-25) is an amphipathic peptide composed of the first 25 amino acids of the N-terminus of SP-B. Inadequate levels of SP-B result in respiratory distress syndrome (RDS). SP-B has been shown to effectively enhance the adsorption and dynamic surface activity of phospholipids found in lung surfactant, which coats the alveoli to minimize surface tension. This prevents the air sacs in the lung from collapsing and allows inflation of the lungs at physiologically attainable air pressures. The N-terminus of SP-B has been demonstrated to be sufficient in lowering surface tension. Using ^2H and ^{31}P solid-state NMR, the concentration dependent effects of SP-B (1-25) on lipid dynamics were measured. Lipid samples were composed of 4:1 DPPC:POPG and 3:1 POPC:POPG multilamellar vesicles. Based on these measurements, we will present a model of how this peptide interacts with lipid lamellae and affects their organization.

P5**The application of HYSCORE, pulsed ENDOR, and DFT to characterize carotenoid radicals**

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The experimental hyperfine coupling constants obtained from HYSCORE and pulsed ENDOR (Davies and Mims) spectroscopies are correlated with those predicted by Density Functional Theory (DFT) for a better characterization of the carotenoid radicals. The contour line shapes of the HYSCORE cross-peaks plotted as the squares of the frequencies ν^2_α and ν^2_β , are transformed into straight line segments. A simple extrapolation of these straight lines permits determination of two principal values of the hyperfine coupling tensor, which then are assigned on the basis of DFT calculations. The HYSCORE spectra are measured at different values of τ in order to resolve different pairs of cross-peaks at the same magnetic field setting. For spectra taken at different magnetic fields, we present a field correction method used to correlate the peaks by bringing them to a common nuclear Zeeman frequency. Also isotropic and anisotropic hyperfine coupling constants obtained by DFT have proved to be accurate enough to be used in simulation and interpretation of Davies and Mims ENDOR spectra. This work was supported by U.S. Department of Energy, Hungarian National Research Foundation, Alabama Supercomputer Center, Robert Ramsay Endowment of UA, DOE Catalysis Center Program and Pacific Northwest National Laboratory.

P6**Model Membrane Morphology and Acyl-Chain Dynamics of Bis(monoacylglycerol)phosphate***Thomas E. Frederick (1), Chad E. Mair (1) and Gail E. Fanucci (1)*

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Bis(monoacylglycerol)phosphate (BMP) is a phospholipid found primarily in late endosomes and has a unique structure due to single acyl chains located at the 3 and 1' positions on the glycerol components. BMP is believed to play an important role in late-endosome sorting functions and is also important in glycosphingolipid catabolism, such as the enhancement of enzymatic hydrolysis of GM2 to GM3, stimulated by activator proteins, in POPC/Chol/GM2 vesicles.¹ In an effort to understand the role that BMP plays in lipid catabolism and lysosomal storage disease, this work utilizes solid state ³¹P and ²H NMR spectroscopy, EPR spectroscopy, and dynamic light scattering to characterize the membrane morphology of BMP vesicles, and to investigate the effects that BMP has upon model membrane lipid morphology and phospholipid acyl-chain dynamics.

1. Werth et al., The Journal of Biological Chemistry, 2001, 276, 12685.

P7**Double Electron-Electron Resonance: Spin Label Choice and its Effects on Distance Distribution Profiles.**

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In the last 10 years, new pulsed EPR techniques, such as double electron-electron resonance (DEER) and double quantum coherence (DQC), have been developed in order to measure distances in peptides and proteins. With the introduction of deadtime-free DEER, we are currently able to measure distances in the range of 15-80 Å with great precision. Nonetheless, one of the main problems that the technique faces is that the introduction of an extraneous nitroxide spin label probe is needed to measure distances and distance distributions. This means that the distance distributions obtained report information about nitroxide-nitroxide distances, which may or may not correspond to alpha carbon distances obtained by X-Ray crystallography. It is clear then, that the internal motions of the spin label are of great importance when trying to assess whether changes in the distance distributions are due to backbone motion or to spin label internal rotations. In this framework, the work by Hubbell *et. al* on T4 lysozyme shed light on the behavior of the spin label on a solvent exposed site in an alpha-helix, under which the X₄-X₅ model was developed. Also, molecular dynamic simulations (MD) are being extensively used to investigate internal label motions.

In the present work, we utilize doubly spin-labeled HIV-1 protease to investigate the effect of the spin label choice on the distance distribution profiles obtained by DEER spectroscopy. Data was analyzed by either Tikhonov regularization or montecarlo (MC) fitting of the dipolar evolution data. By using a common available protease inhibitor, Ritonavir, we are able to effectively trap the backbone conformation of the flaps in a closed position, thereby removing large amplitude domain motions. The resultant EPR line-shapes are then a convolution of side-chain dynamics with beta-hairpin fluctuations.

From our results in both cw-EPR and DEER distance distributions, in the presence of inhibitor, we see that the mobility trend, from most to least mobile, follows: IASL > IAP ≈ MSL > MTSL. Upon removal of inhibitor, distance distribution widths (as determined by measuring the FWHM) become wider for all spin labels under study, with the exception of IASL, for which the change is negligible.

Comparing the absolute changes in FWHM for the inhibited vs uninhibited form of the protease, we can see that the change in the FWHM follows the trend MTSL > MSL > IAP > IASL. From this trend, MTSL seems to be the best choice in our particular case to monitor backbone motions, as it reflects the biggest change in the overall distance distribution widths.

P8

Studying the Peptide Backbone Dynamics of TOAC labeled Phospholamban using EPR Spectroscopic Technique*Harishchandra Ghimire and Gary A. Lorigan*

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Phospholamban (PLB) is a 52 amino acid membrane-bound protein which regulates the enzymatic activity of Sarco-endoplasmic reticulum calcium ATPase (SERCA). To study the structure and dynamics of PLB, a spin label 2, 2, 6, 6-tetramethylpiperidine-1 oxyl-4-amino-4 carboxylic acid (TOAC) was attached at different parts of the protein by solid phase peptide synthesis method. Amino acids Ile from the transmembrane domain (amino acids 30-52) and Ser-10 from the cytoplasmic domain (amino acids 1-20) of the full length PLB were replaced with TOAC spin label. Since the TOAC spin label is rigidly coupled to the peptide backbone, it reports more accurately on position, orientation, and dynamics of the peptide backbone. The protein was cleaved, purified by reverse phase HPLC and then inserted in a DMPC/DHPC bicelle to mimic its membrane-associated structure and studied by electron paramagnetic resonance (EPR) spectroscopic technique. The PLB bicelle spectrum at room temperature looks very abroad with Ile→TOAC replacement. It is because Ile-45 is in the transmembrane region and is motionally restricted. This is EPR spectra will be compared with the TOAC-10 labeled PLB in the cytoplasmic domain to see the difference in the peptide backbone dynamics using CW EPR. It is interesting to compare and see the kind of similarities and difference in the transmembrane and cytoplasmic domain dynamics using TOAC spin label.

P9

Relative Orientation of Imidazole Ligands in Cu(II) Complexes Revealed by ^{14}N ESEEM Spectroscopy of the Remote Nitrogen $\Delta m_1 = \pm 2$ Combination Line*Jessica Hernandez-Guzman, Jeffrey M. Canfield, Randahl C. Palmer, and Kurt Warncke*

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Alzheimer's Disease (AD) is characterized by the aggregation and fibrillization of the β -amyloid protein ($\text{A}\beta$), which leads to plaque deposition in the AD brain. The divalent metal ions, Zn(II) and Cu(II), are present at high concentrations ($>10^{-4}$ M) within the plaques. *In vitro* studies have shown that these metal ions accelerate or arrest fibrillization, depending upon the length and amino acid sequence in truncated and mutated $\text{A}\beta$ peptides, and that the metal ions can alter the fibril structure.¹ The coordination of Zn(II) and Cu(II) by peptide histidine imidazole sidechains is proposed to play an important role in determining the fibrillization "switch".¹ Our aim is to develop techniques of X-band ESEEM spectroscopy to determine the molecular structure of the Cu(II)-histidine imidazole coordination in cryotrapped soluble and fibrillar forms of $\text{A}\beta$ peptides, in order to gain insight into the factors that govern fibrillization. Here, we focus on a method to distinguish *cis*- from *trans*- imidazole coordination. The following three Cu(II) complexes have been prepared as models for different Cu(II)-histidine imidazole coordination states in $\text{A}\beta$ peptides: single imidazole [Cu(II) diethylenetriamine 2-methylimidazole], bis-*trans* imidazole [Cu(II) bis-histamine], and bis-*cis* imidazole [Cu(II) *cis*-bis(acetate)bis(2-methylimidazole)]. The Fourier transform of the three-pulse ESEEM from each model complex shows the remote imidazole ^{14}N nuclear quadrupole, ν_0 , ν_- , ν_+ , and double quantum, ν_{dq} , features. For the bis-*trans* and the bis-*cis* imidazole complexes, combination lines are displayed that are consistent with bis-imidazole coordination, including the double quantum harmonic, $2\nu_{dq}$. The $2\nu_{dq}$ line shape depends on the relative orientation of the remote ^{14}N in each imidazole. Powder ESEEM simulations for $\tau = 310$ ns, in which the modulation for each ^{14}N was combined prior to spherical averaging, gave the ^{14}N nuclear quadrupole and hyperfine tensors, and, for the bis-imidazole complexes, the Euler angles that specify the relative orientation of the two ^{14}N hyperfine principal axis systems (PAS). For the bis-*trans* and the bis-*cis* imidazole complexes, the Euler angles are $[\alpha \beta \gamma] = [156^\circ 30^\circ 219^\circ]$ and $[\alpha \beta \gamma] = [130^\circ 325^\circ 38^\circ]$, respectively. Transformation and assumed relations of the ^{14}N hyperfine PAS to the imidazole molecular axes are used to generate a physical model. The magnetic field dependence of the $2\nu_{dq}$ line shape will be used to relate the model to the *g*-tensor PAS. This approach will be useful for resolving the coordination geometry of Cu(II)- $\text{A}\beta$ complexes, and Cu(II)-protein complexes that are associated with other neurodegenerative diseases.

¹Dong, J.; Canfield, J. M.; Mehta, A. K.; Shokes, J. E.; Tian, B.; Childers, W. S.; Simmons, J. A.; Mao, Z.; Scott, R. A.; Warncke, K.; Lynn, D. G.; *Proc. Natl. Acad. Sci. U.S.A* 2007, *104*, 13313-13318.

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P10**Effect of Divalent Metal Ions on The Reaction between Uric Acid and Peroxynitrite Studied by X-Band EPR**

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The divalent metal ions, namely Ca^{2+} , Zn^{2+} , Mg^{2+} , Cu^{2+} , Ni^{2+} , Co^{2+} , Fe^{2+} , Cd^{2+} , and Mn^{2+} , have been used to study the stability of the urate derived radicals generated from the reaction between uric acid and peroxynitrite at pH 12. The reactions were monitored by X-band EPR and demonstrated that only the reaction mixtures containing Zn^{2+} and Cd^{2+} yielded stable radicals. The generation of the paramagnetic species detected by EPR can be blocked when ascorbate is present. The identification of the reaction products is still under investigation.

P11**Solution NMR structure of the 60 kDa metalloprotein complex between Putidaredoxin and cytochrome P450cam**

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Structural studies of protein complexes provide valuable information on highly regulated protein-protein interactions that control fundamental cell processes such as biological electron transfer. However, experimental high-resolution structures for large protein complexes can be quite challenging to obtain. Introduction of solution NMR methods such as TROSY and residual dipolar couplings (RDCs) have opened up new avenues for structure determination of large proteins and protein complexes. Here we present the results of applying the RDC methodology, supplemented with translational restraints from spin labeling on a ~60 kDa metalloprotein complex between the [2Fe-2S] ferredoxin, putidaredoxin (Pdx) and a heme protein, cytochrome P450cam. The Pdx-P450cam interaction required for camphor hydroxylation in the soil bacterium *Pseudomonas putida* is one of the best-characterized electron transfer couples and has long served as a model system for study of biological redox coupling between ferredoxins and cytochrome P450s. While the high-resolution individual structures of both Pdx and P450cam are known, no structural information on the complex is available. RDC measurements in two orienting media, bicelles and phage, were used to calculate the orientation of the two proteins relative to each other while overcoming the 180⁰ inherent symmetry problem. Translational restraints from cysteine spin labeling of one of the proteins along with knowledge of the binding interface from chemical shift perturbation and mutagenesis data allowed the docking of the two proteins while maintaining the relative orientation derived from RDC data. The resulting solution NMR structure of the complex is of sufficient accuracy to infer the role of key residues in complex interaction and explain the role of Pdx as an effector for P450cam function. Further confirmation of the complex structure is obtained by comparison with small angle scattering data. To our knowledge, this is the first successful application of a combined spin labeling and RDC approach within a restraint-based docking scheme to solve the structure of a large molecular weight protein complex by NMR, suggesting that such an approach could be an efficient way to provide reliable high-resolution structural information on protein complexes.

P12

Measuring Interspin Distances between Framework Metal Ion and Carotenoid or Myoglobin Radical in Mesoporous Materials by Pulsed EPR Relaxation Enhancement Methods

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Photoionization is the first step in many light driven reactions related to the storage of solar energy. The design of efficient artificial photoredox systems for storing light energy must be able to minimize back electron transfer. Mesoporous materials containing well-organized nanometer sized channels have been found to be good photoredox systems for solar energy conversion and storage. The material framework can act as an electron acceptor upon photoionization of embedded organic compounds. Replacement of some tetrahedral Si(IV) in the framework by transition metal ions was used for introducing active sites in these artificial systems.

In this work, Car^{•+} ($g = 2.003$) were generated upon photo-oxidation of canthaxanthin (Car) in MCM-41 and Ti(IV)-MCM-41 molecular sieves. An axial EPR signal with $g_{\parallel} = 1.956$ and $g_{\perp} = 1.903$ was assigned to Ti(III) ions from reduction of Ti(IV) at a framework tetrahedral site as a result of electron transfer between the Car and the metal ion. The myoglobin (Mb) peroxy radicals with $g_{\parallel} = 2.04$ and $g_{\perp} = 2.001$ were produced during oxidation of Mb incorporated in SBA-15 and Fe(III)-SBA-15 in the presence of H₂O₂.

The α - and β -proton hyperfine couplings determined from the analysis of 2D-HYSCORE and pulsed ENDOR spectra, respectively, indicated the formation of similar radicals in both siliceous and metal-substituted materials.

To obtain distances between the framework metal center and the radical center, the effect of a rapidly relaxing metal on T₁ and T_M of a slowly relaxing radical was measured as a function of temperature in both siliceous and metal-substituted materials. The 2-pulse echo decay and saturation recovery curves were best fitted by using a double exponential fit providing a slow and a fast component. The slow component was attributed to the Car or Mb radical. The fast component could be due to the metal ion relaxation or to the radical close to the metal ion. A significant decrease in the T_M value occurred near 35 K and 125 K for the Mb radical and the Car radical, respectively, is consistent with the interaction between the radical and the fast relaxing metal center. From the enhancement of T_M the interspin distances were estimated. They appeared to be $13 \pm 2 \text{ \AA}$ for Car and $8 \pm 2 \text{ \AA}$ for Mb.

P13**Magnetization damping in CoFeB multilayer systems**

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We report on measurements of the ferromagnetic resonance (FMR) for CoFeB systems with adjacent nonmagnetic layers of Ru, Ta and Cu. Broadband in-plane FMR measurements were performed using a shorted waveguide setup covering the frequency range from 7-40 GHz. The dependence of the resonance frequency on the external magnetic field is well described by the Kittel formula. Angular dependent measurements at 10 GHz show a uniaxial in-plane anisotropy with an easy axis parallel to the magnetic field direction present during growth. From the measured frequency dependence of the linewidth both the intrinsic (Gilbert damping constant) and the extrinsic contributions to the linewidth were determined. For samples with Ru and Ta layers on top and below the CoFeB layer we observe a contribution to the Gilbert damping constant inversely proportional to the thickness of the ferromagnetic layer consistent with spin-pumping theory. The sample with adjacent 20 nm thick Cu layers did not show a significant enhancement of the Gilbert damping constant. Due to the amorphous structure of the CoFeB layer the extrinsic contribution to the linewidth was found to be small for all samples.

P14**Magnetization damping in epitaxial CrO₂ (110)**

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Epitaxial CrO₂ thin films were grown on TiO₂ (110) substrates using chemical vapor deposition (CVD) using a CrO₃ precursor as described elsewhere [1]. In-plane angular dependent ferromagnetic resonance (FMR) measurements confirm a uniaxial in-plane anisotropy with the easy axis along the c-axis. Frequency dependent FMR measurements were carried out over a frequency range from 7-60 GHz along the easy axis of the film. The resonance field dependence on the microwave frequency is well described by the Kittel formula, enabling the determination of M_{eff} and γ of the films. The ferromagnetic resonance linewidth depends only weakly on the microwave frequency: the linewidth has a minimum around 20 GHz and increasing linearly for larger frequencies with a very small slope. Based on this we estimate the Gilbert damping constant (intrinsic) to be of the order 10^{-4} , i.e. very small. The main contribution to the magnetization relaxation is extrinsic in nature and can therefore be further optimized.

References:

[1]: X. W. Li, A. Gupta, and G. Xiao, Appl. Phys. Lett. **75**, 713 (1999).

P15**Sparse Isotopic Labeling and NMR Assignment of Proteins for Study by Paramagnetic Resonance Enhancement NMR**

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Structure determination of proteins by NMR often depends on metabolic, uniform isotopic labeling with ^{13}C , ^{15}N , and occasionally ^2H . This condition limits the use of NMR for structural studies of proteins that cannot be expressed in bacterial hosts, such as proteins with post-translational modifications. An alternative approach to isotopically label proteins from other sources is to sparsely label proteins using chemical methods such as reductive ^{13}C -methylation. Reductive ^{13}C -methylation is used after the protein is purified to specifically label the N-terminal amine and lysine ϵ -amines with two ^{13}C methyl groups. The difficulty in using these sparse isotopic labels is the assignment of their NMR signals. An assignment method is being developed for sparse isotopic labels of protein prepared using reductive ^{13}C -methylation. The method relies on site varying reaction rates to produce labeled sites with different $^{13}\text{C}/^{12}\text{C}$ ratios. Protein samples are prepared with substoichiometric amounts of ^{13}C -formaldehyde, the ^{13}C source, followed by excess ^{12}C -formaldehyde. FT-ICR MS/MS is used to identify and measure the ^{13}C content of the labels. This data is correlated with the ^{13}C content measured using the peak volumes in 2D ^1H - ^{13}C NMR spectra to assign the NMR peaks. Lysozyme and Concanavalin A are being used as model proteins to develop the assignment method. Progress on the method including evaluation of FT-ICR MS and tandem MS for measuring ^{13}C content of the sparse labels will be presented. In addition, preliminary data on the use of paramagnetic resonance enhancement NMR to measure distance restraints to the sparse ^{13}C labels using Mn^{2+} bound Concanavalin A will be presented.

P16**Relaxation and High Field Pulsed ENDOR Studies of Chromium(V) Doped Potassium Niobate.**

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Chromium(V) ($S = 1/2$, $I(^{52}\text{Cr}) = 0$) doped in the diamagnetic host potassium niobate has been proposed as an alternative standard for field calibration and g-standard for high-field EPR [1]. Furthermore, very recently it has been shown that this system can be a promising candidate for quantum computing applications [2], for which an understanding of the relaxation mechanisms in this system is necessary. The field and temperature dependence of the T_1 and T_2 relaxation was investigated. It is likely that the coupling of the electron spin with the neighboring ^{39}K nuclei ($I=3/2$) is one of the prominent mechanisms of spin-spin relaxation (T_2). The hyperfine and quadrupole interactions with ^{39}K nuclei was resolved by using the high-frequency (240 GHz) pulsed electron nuclear double resonance (ENDOR).

- [1]. B. Cage, A. Weekley, L. -C. Brunel and N. S. Dalal, *Anal. Chem.* **1999**, *71*, 1951.
[2]. S. Nellutla, K.-Y. Choi, M. Pati, J. van Tol, I. Chiroescu and N. S. Dalal, *Phys. Rev. Lett.* **2007**, In press.

P17**Coherent manipulation of electron spins up to Ambient temperatures in Cr⁺⁵ (S=1/2) Doped K₃NbO₈***M. Pati*², *S. Nellutla*¹, *K.Y. Choi*^{1,2}, *J. Van Tol*^{1,2}, *I. Chioresku*^{1,3}, *N.S. Dalal*^{1,2}

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Coherent spin manipulation on Cr⁺⁵ (S=1/2, I=0), constitutes a dilute two level model relevant for use as a spin qubit. Rabi oscillations are observed for the first time in a spin system of transition metal oxide even at room temperature. At liquid helium temperature spin-spin relaxation time (T_2) is $\sim 10 \mu\text{s}$ and with a Rabi frequency of 20 MHz, we get a single-qubit figure of merit Q_M of about 500. So, dilute ensemble of Cr⁺⁵ doped K₃NbO₈ is important in solid-state quantum information processing.

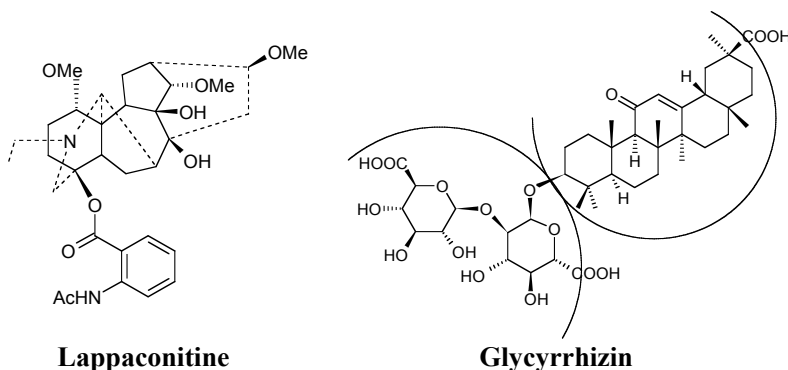
P18
CIDNP and NMR Study of Lappaconitine Phototransformation in Glycyrrhizin Micelle

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Chemically Induced Dynamic Nuclear Polarization (CIDNP) and NMR methods were used to demonstrate a significant change in the efficiency and direction of the phototransformation of alkaloid lappaconitine when it is incorporated into glycyrrhizin (GA) micelle.

Lappaconitine - the alkaloid isolated from aconite - shows bradycardic and hypotensive activity. The structure of lappaconitine (an ester of triatomic alcohol lappaconin and N-acetyl-anthranilic acid) predetermines its photochemical lability which is not only of fundamental interest but also affects its application as a drug.



This study is inspired by the establishment of a considerable strengthening of the therapeutic activity of lappaconitine in the presence of GA accompanied by a decrease in its toxicity. In the pharmacological literature the effect of GA is usually assigned to the formation of the complexes with drug molecules, however, the structure of these complexes is still a matter of debate. Our studies demonstrated the presence of two types of GA aggregates, a complex and a micelle. The complexes of GA with drug molecules with a stoichiometry of 2:1 were observed at low GA concentrations (10^{-3} - 10^{-5} M). At high concentrations ($\geq 10^{-3}$ M) GA forms large associates like micelles. In particular, the evidence of micelle formation was obtained by NMR relaxation techniques (measurement of spin-spin relaxation time T_2).

The free radical mechanism of lappaconitine phototransformation in homogeneous and GA micelle solutions was elucidated by using time-resolved CIDNP technique. Photolysis of lappaconitine results in cleavage of the ester bond with the elimination of N-acetyl anthranilic acid and/or its deacetylation. A high sensitivity of CIDNP effects to the nature of environment was found. Incorporation of lappaconitine into the micelle shifts the molecules from hydrophilic environment in homogeneous solution to hydrophobic one in the micelle, thus reducing their photodecomposition efficiency and suppresses the process of deacetylation.

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P19**Effects of Residue-Sequence Mutations in the Peptides Mimicking the Transmembrane Domain of Sindbis Virus: Spin-Labeling EPR Studies of Peptide Membrane Insertion**

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In Sindbis enveloped virus a host-derived membrane bilayer is “sandwiched” between the concentric protein shells and is penetrated by the transmembrane domain anchors of three glycoproteins. Those protein domains are capable of assembling in two strikingly different membranes: mammalian membranes that contain up to 40% of cholesterol and insects membranes that are composed from shorter unsaturated lipids and no cholesterol. Recently, it was shown that mutations in transmembrane domain of the Sindbis virus E2 protein produce differential alterations in the protein association with lipid bilayer: some mutants were able to grow in insect cells, but not in mammalian cells [1, 2]. The Sindbis virus with STM-16 deletion mutation of E2 transmembrane domain shows the most pronounced differential growth in mammal and insect cells. In an attempt to understand constraints placed upon membrane spanning domains for correct integration into the bilayer we have investigated the interaction of the synthetic peptides mimicking wild type transmembrane domain of E2 glycoprotein and its mutants with phospholipid bilayers. The phospholipid composition was chosen to represent mammalian and insects’ membranes. Results of EPR spin-labeling experiments show that STM-16 peptide adopts transmembrane configuration in phospholipid bilayer mimicking insect cell membranes. In cholesterol-containing bilayers of composition mimicking mammalian cell membranes the STM-16 peptide aggregates at the surface of the bilayer. T.I.S. acknowledges support from NSF grant MCB-0451510. B.R.D was supported by Undergraduate Research Fellowship from NCSU.

1. Hernandez, R., Sinodis, C., Horton, M., Ferreira, D., Yang, C., and Brown, D. T., J. Virol. 77, 12710-12719, 2003.
2. West, J., Hernandez, R., Ferreira, D., Brown, D.T., J. Virol. 80, 4458-4468, 2006.

P20**EPR and *dc* magnetization studies on multiferroic fluoride $K_3Fe_5F_{15}$**

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We present a brief report on the *dc* magnetic behavior and the electron paramagnetic resonance of $K_3Fe_5F_{15}$, a ferroelectric fluoride, which is also ferroelastic ($T_C=490K$). A sharp magnetic transition at 123K was observed in both zero field cooled and field cooled *dc* susceptibility of single crystal $K_3Fe_5F_{15}$. In the zero field cooled run, the transition is similar to antiferromagnetic one. The 9.6GHz EPR spectra showed a large increase in the line width starting below 125K, while cooling down the sample from room temperature in a liquid Helium cryostat. We also discuss the crystal orientation dependence of EPR line width measured at 135K.

P21

Mechanism of Cobalt-Carbon Bond Homolysis in Coenzyme B₁₂-Dependent Ethanolamine Ammonia-Lyase Investigated by Using Coenzyme Photolysis and Transient Optical Absorption and Electron Paramagnetic Resonance Spectroscopies

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Coenzyme B₁₂ (adenosylcobalamin, AdoCbl)-dependent enzymes enhance the rate of cobalt-carbon (Co-C) bond cleavage by $>10^{11}$ relative to the rate for coenzyme in solution. Substrate binding is required to trigger the cleavage, which produces low spin ($S=1/2$) Co(II) in cobalamin and the 5'-deoxyadenosyl radical. Our aim is to determine the quantum yield and recombination kinetics following photolysis of the Co-C bond in the enzyme, ethanolamine ammonia-lyase (EAL) from *Salmonella typhimurium*, to gain insight into how the protein promotes the cleavage reaction. The 532 nm output of a pulsed Nd-YAG laser was used to photolyze samples in aerobic solution at room temperature. The formation and decay of cob(II)alamin was monitored at 470 nm on the micro-to-millisecond timescale by using transient absorption spectroscopy. The quantum yield of cob(II)alamin formation for EAL-bound AdoCbl was 0.079 ± 0.006 . Forty percent of this fraction underwent biphasic recombination ($\tau_1 = 228 \mu\text{s}$, 27%; $\tau_2 = 2.2 \text{ ms}$, 13%) and 60% was stable on the $>1 \text{ s}$ time scale. The presence of the bound substrate analog, (*S*)-1-amino-2-propanol decreased the quantum yield of cob(II)alamin to 0.035 ± 0.006 and eliminated the fast phase of recombination ($\tau_2 = 2.2 \text{ ms}$, 21%). The quantum yields in the enzyme were substantially lower than measured for the coenzyme in solution (0.233 ± 0.014). Continuous-wave X-band electron paramagnetic resonance (EPR) spectroscopy of the samples at 170 K was also performed to characterize the photoproducts. The ¹⁴N superhyperfine features in the post-photolysis enzyme spectra showed enhanced resolution relative to cob(II)alamin in glassy medium, which confirmed that the cob(II)alamin was bound to the enzyme in a discrete confirmation. The concentration of radical species in the $g=2$ region was $<1\%$ of the Co^{II} concentration. Radical trap experiments showed that photo-generated radical equivalents were not accessible to 5,5-dimethyl-1-pyrroline 1-oxide (DMPO). The radical photoproduct(s) are therefore quenched by an, as yet, unidentified protein-internal process. The results show that the binding of substrate analog alters the quantum yield and recombination kinetics. This is consistent with a "substrate trigger" mechanism of Co-C bond cleavage in EAL. Supported by NIH DK54514.

P22

EPR and Heat Capacity Characterization of Mixed Ammonium Peroxychromates, $M'NH_4CrO_8$ ($M' = Rb, K, Na$).

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Mixed ammonium peroxychromate, $M'NH_4CrO_8$, are new Cr (5+) complexes, that are of interest as multiferroic materials. Heat capacity and dielectric measurements on these compounds revealed new structural and dielectric phase transition. The phase transition temperature (T_p) and the g values of the compounds are detailed in the following table.

Compound	T_p		ΔT_p (Thermal Hysteresis)	g_{parallel}	$g_{\text{perpendicular}}$	Ground state Energy level
	Exotherm up	Exotherm down				
$K_2NH_4CrO_8$	263.2	245.3	17.9	1.95619	1.97587	d_z^2
$Rb_2NH_4CrO_8$	266.8	249.5	17.3	1.97599	1.94520	$d_{x^2-y^2}$
$Na_2NH_4CrO_8$	271.3	258.31	13.0	--	--	--

In this poster the dielectric and EPR results of these compounds will be discussed.

P23**Substrate Binding Changes Iron Coordination and Protein Function in Dehaloperoxidase from *Amphitrite ornata*: CW EPR and HYSCORE experiments**

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Dehaloperoxidase (DHP) from the terebellid polychaete *Amphitrite ornata* is the first known hemoglobin to exhibit efficient peroxidase activity in the oxidation of phenolic substrates. Here we describe the use of CW ERP spectroscopy and hyperfine sublevel correlation spectroscopic (HYSCORE) analysis of the ferric form of DHP to characterize effects of the substrate 2,4,6-trifluorophenol (TFP) binding on the iron coordination in order to elucidate molecular mechanism of the change in protein function from a globin to a peroxidase. CW EPR spectra show that heme iron of DHP at pH 6.0 exists in two high spin (HS) state forms that are characterized by different rhombicity. While upon substrate binding iron remains in the HS state, CW EPR spectrum indicates a change in the iron coordination. At high pH, however, iron exists in both LS (low spin) and HS states; upon substrate binding the equilibrium clearly shifts to the HS only state. HYSCORE spectra recorded at $g=2$ magnetic field provided additional information on iron coordination to nitrogen nuclei and also revealed the presence of exchangeable proton(s) with hyperfine coupling of *ca.* 6 MHz, consistent with water molecule being the sixth ligand in the iron coordination. The proton(s) spectral feature, observed at pH 6.0 and pH 9.0, disappeared upon substrate binding at both pH. This observation is in agreement with the proposed model of water being displaced from iron coordination upon substrate binding. This work is supported, in part, by NSF grants MCB-0451510 to TIS and MCB-9874895 to SF.

P24

Site-Specific DNA Adducts of *trans*-4-Hydroxynonenal

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The bifunctional electrophile *trans*-4-hydroxynonenal (*trans*-4-HNE) arises *in vivo* from peroxidation of ω -6-polyunsaturated fatty acids. The chemistry of the 6*S*,8*R*,11*S* and 6*R*,8*S*,11*R* exocyclic 1,*N*²-propanodG adducts arising from *trans*-4-HNE in the 5'-CpG-3' DNA sequence has been characterized. When placed into DNA opposite cytosine, both 1,*N*²-propanodG adducts spontaneously open to the corresponding *N*²-dG propyl aldehydes. The 6*S*,8*R*,11*S* adduct forms interstrand DNA cross-links in the 5'-CpG-3' sequence, whereas the 6*R*,8*S*,11*R* adduct does not. The slow rate of cross-link formation by the 6*S*,8*R*,11*S* adduct is attributed to the observation that in duplex DNA, the *N*²-dG propyl aldehyde arising from this adduct exists predominantly in the cyclic hemiacetal configuration, masking the reactive aldehyde. The 6*S*,8*R*,11*S* *N*²-dG propyl aldehyde orients in the 5'-direction in the minor groove, facilitating cross-link formation, while the 6*R*,8*S*,11*R* *N*²-dG propyl aldehyde orients in the 3'-direction. Supported by NIH Grants ES-05355 (M.P.S. and C.J.R.), and ES-00267.

P25**Experimental and Theoretical Studies of the Photoreduction of Copper(II) Dendrimer Complexes**

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Dendrimers have attracted considerable interest as nanoscale building blocks due to their highly branched three-dimensional structure and monodisperse nature. We present a photochemical method for synthesizing metal nanoparticles with narrow particle-size distributions by means of UV irradiation using amino-terminated PAMAM dendrimers as the coordinating template of precursor metal ions. The resultant nanoparticles have been characterized by high resolution TEM. The mechanism of photoreduction of various metals (Cu,Zn) has been characterized by UV-Vis and EPR techniques. EPR identifies one unpaired electron localized on tertiary nitrogen which is responsible for a one-electron reduction of the metal ion. DFT-level calculations have been performed to calculate g-values, ionization potentials and other data associated with possible model structures to help elucidate the experimental results.

P26

High-Field, High-Frequency EPR Studies on Dimeric Copper(II) Perfluorocarboxylates and Silylcarboxylates

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Perfluorinated copper(I) carboxylates have been often used in Chemical Vapor Deposition (CVD), an efficient method of growing high purity metal films that is particularly useful in the metallization of microelectronic and optical devices^{1,2}. Copper(I) complexes are obtained from copper(II) carboxylates, which can also be used directly in CVD. Simple copper(II) carboxylates tend to form dimeric entities.³ However, the nuclearity of copper(II) perfluorocarboxylates is much less clear. In vapors of volatile complexes of this kind, binuclear and trinuclear units have been detected,⁴ while polymers formed on condensation.⁵ EPR studies on dimeric copper(II) carboxylates have been performed mainly in X-Band and the published EPR parameters often appear to carry substantial errors.^{3,6}

In this work we synthesized copper(II) complexes of trifluoroacetate and its homologues: $C_2F_3COO^-$, $C_3F_7COO^-$ and $C_6F_{13}COO^-$. To investigate the effect of electron donating and electron withdrawing character of ligands on EPR parameters, the complex $[Cu((CH_3)_3SiCH_2COO)_2]$ was prepared and copper acetate monohydrate was also measured for comparative purposes. The high-field, high-frequency EPR spectra of solid samples taken over the frequency range 100-413 GHz revealed that the trifluoroacetate, pentafluoropropionate and $[Cu(C_6F_{13}COO)_2]$ complexes were monomeric. Signals due to both monomeric and dimeric molecules were observed in $[Cu(C_3F_7COO)_2]$. Polymeric complex $[Cu((CH_3)_3SiCH_2COO)_2]$ ⁷ exhibited spectra characteristic for isolated copper dimers. Upon recrystallization from acetonitrile each of the perfluorocarboxylates was converted to a dimeric species. For the dimeric systems, very pronounced changes in the zero-field splitting parameter D were observed depending on the ligand electron donating or withdrawing power.

¹ Grodzicki, A.; Łakomska, I.; Piszczek, P.; Szymańska, I.; Szłyk, E., *Coord. Chem. Rev.*, **2005**, 249, 2232.

² Szymańska, I. B.; Piszczek, P.; Bała, W.; Bartkiewicz, K.; Szłyk, E. *Surf. Coat. Technol.* **2007**, 201 9015.

³ Bleaney, B.; Bowers, K. D. *Proc. Roy. Soc. Ser A* **1952**, 214, 451, Abe, H.; Shimada, *J. Phys. Rev.* **1953**, 90, 316.

⁴ Szymanska, I. *Thesis*, Nicolaus Copernicus University **1999**

⁵ Cotton, F. A.; Dikarev, E. V.; Petrukhina, M. A. *Inorg. Chem.* **2000**, 39, 6072

⁶ Moreland, J. A.; Doedens, R. J. *J. Am. Chem. Soc.* **1975**, 97, 510.

⁷ Nakagawa, H.; Kani, Y.; Tsuchimoto, M.; Ohba, S.; Matsushima, H.; Tokii, T. *Acta Cryst. C* **2000**, 56, 12

P27***h*vDHFR1:NADPH Complex Triple Resonance NMR Assignments***Karthikeshwar Vangala (1) and John K. Young (1)*

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Extremophiles are organisms that survive in environments which are inhospitable to other creatures. Knowing the enigma behind their survival is always an interest among many research scientists. *Haloferax volcanii* is such a species of halophile which thrives in extreme saline conditions of the Dead Sea. Our research group mainly focuses on the enzyme DHFR1 from *H. volcanii* which catalyzes dihydrofolate (DHF) to tetrahydrofolate (THF) in the presence of the proton donor, NADPH. The analysis of the structure and protein behavior in high salt concentration of the *h*vDHFR1:NADPH complex is being carried out by three dimensional NMR. Triple resonance nuclear magnetic resonance spectra of the complex were obtained, and chemical shifts assignments are being interpreted. An HSQC titration series was carried out. Among the varying concentrations of NADPH to *h*vDHFR1, the 1:1 ratio was found to be optimal for binding. A comparison of *h*vDHFR1:NADPH complex and apo-enzyme HSQCs showed movement of a subset of peaks suggesting that the NADPH is binding to the enzyme.

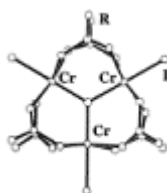
P28

Pulsed EPR studies of Mononuclear and Multinuclear Cr(III) Complexes

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Chromium(VI) complexes are potent mutagens and carcinogens when inhaled, while the potential of these complexes to generate similar effects when taken orally is an area of active debate. A potential mechanism is based on the generated Cr(III) binding to DNA to form binary and ternary complexes, which ultimately give rise to the mutagenic and carcinogenic effects. Unfortunately, while these Cr-DNA complexes have been studied intensely the last circa 15 years, virtually no data on the molecular level structure of these Cr(III)-DNA complexes exists. The aim of the current project is to determine the strength of the interaction in double-stranded DNA between Cr(III) and the components of the DNA and the 3-D structure of the Cr-binding sites. To accomplish these goals, a unique combination of techniques is being applied: synthesis and characterization of nucleic acid oligomers, Cr(III) coordination chemistry including Cr(III)-biomolecules and model synthetic compounds, and high-field and pulsed EPR spectroscopy. These laboratories have recently examined the potential for pulsed EPR methods for use in characterizing mononuclear and multinuclear model Cr(III) complexes. In particular, a number of "basic carboxylate"-type trinuclear Cr(III) complexes of the general formula



$[\text{Cr(III)}_3\text{O}(\text{O}_2\text{CR})_6\text{L}_3]^+$ (left) have been examined (A, $[\text{Cr}_3\text{O}(\text{OAc})_6(\text{H}_2\text{O})_3]\text{Cl}$ in EtOH; B, $[\text{Cr}_3\text{O}(\text{O}_2\text{CCD}_3(\text{H}_2\text{O})_3)\text{Cl}$ in EtOH; C, $[\text{Cr}_3\text{O}(\text{OAc})_6(\text{py})_3]\text{ClO}_4$ in EtOH/DMSO; and D, $[\text{Cr}_3\text{O}(\text{OAc})_6(\text{d}^5\text{-py})_3]\text{Cl}$ in EtOH/DMSO). These trinuclear cations have been well characterized by a variety of techniques and can readily be prepared with a variety of carboxylates and terminal ligands (L). Herein, we report the preliminary characterization of several of these compounds. For example, pulse Mims ENDOR shows that proton couplings can be detected from complex A and that near-by protons occur around the Cr center. Relaxation measurements of complex A at 14 K indicate $T_2 = 109$ ns. Two and three pulse ESEEM was detected which show proton ESEEM (Figure 1) couplings from distant protons of complex C. These measurements demonstrate that pulse ENDOR, ESEEM and relaxation measurements can be made of the model Cr centers which are coordinated with different ligand substituents.

P29**Electrostatics of Micelle and Membrane Protein Systems as Accessed by EPR of pH-Sensitive Nitroxide Probes***Maxim A. Voinov and Alex I. Smirnov*

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Local polarity is considered to be one of the most essential physical parameters affecting the function of membrane proteins. Specifically, a precisely balanced interplay of polar and hydrogen bonding effects of the amino acid residues is thought to govern thermodynamics of the polypeptide chains on each step of its interaction with cellular membrane - partitioning of unfolded peptides at the interface, protein folding, and, consequently, the bilayer insertion. Phospholipid bilayers represent highly heterogeneous and anisotropic environment that subject membrane proteins to rather large gradients of effective dielectric constant, ion concentration, and hydrogen bonding. The dielectric profile of protein-membrane complexes (*i.e.*, its local dielectric permittivity, $\epsilon(l)$) could be ascertained from changes in the ionization constant of the molecular probe placed at the specific depth in the lipid bilayer. pK_a values of the ionizable groups are well known to be strictly dependent upon the dielectric permittivity of the surrounding medium. More importantly, there is a feasible range of $\epsilon(l)$ values where a linear relationship between pK_a and $\epsilon(l)$ is observed. We suggest and describe an EPR-based approach to assess the local dielectric constant of both homogeneous and heterogeneous environment with specially designed pH-sensitive nitroxide probes. We also report on EPR titration experiments carried out in the mediums of various polarities, micelles formed from ionic and nonionic surfactants, and also transmembrane α -helical peptides.

P30

Kinetics and Thermodynamics of Co^{II}-Substrate Radical Pair Formation in Coenzyme B₁₂-Dependent Ethanolamine Ammonia-Lyase Determined by using Time-Resolved, Full-Spectrum Electron Paramagnetic Resonance Spectroscopy in a Cryosolvent System*Miao Wang and Kurt Warncke*

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The reaction of coenzyme B₁₂ (adenosylcobalamin)-dependent ethanolamine ammonia-lyase (EAL) from *Salmonella typhimurium* to form the Co^{II}-substrate radical pair catalytic intermediate has been studied by using time-resolved, full-spectrum electron paramagnetic resonance (EPR) spectroscopy in a cryosolvent system. The 41% v/v DMSO/water cryosolvent system allows mixing of the holoenzyme and substrate, (S)-2-aminopropanol, at 230 K. At 230 K, the reaction is arrested for a time $>6 \times 10^3$ s. Temperature step to 234-248 K initiates the cleavage of the cobalt-carbon bond and the mono-exponential rise (rate constant, k_{obs} ; characteristic time, τ_{obs}) of the EPR-detected Co^{II}-substrate radical pair state. The EPR spectrum acquisition time is $\ll \tau_{\text{obs}}$, which allows continuous full-spectrum monitoring during progress of the reaction, and leads to a $\tau_{\text{obs}}: \tau_{\text{obs}}$ ratio that is reduced by $>10^2$, relative to millisecond rapid mixing experiments at ambient temperatures. The k_{obs} values and Co^{II}-substrate radical pair amplitudes are independent of substrate concentration at each temperature. Therefore, the reaction occurs from the {enzyme/coenzyme/substrate, or ternary, complex. The constant value of the Co^{II}-substrate radical pair amplitude at long times ($>3\tau_{\text{obs}}$), and the temperature dependence of the amplitude, indicates an equilibrium with the ternary complex. The reaction is thus treated as a relaxation to equilibrium by using a linear two-step, three-state mechanism. The Co^{II}-5'-deoxyadenosyl radical pair intermediate state in this mechanism is not detected by EPR at signal-to-noise ratios of $\leq 10^3$. Therefore, the free energy of the Co^{II}-5'-deoxyadenosyl radical pair state is ≥ 3.3 kcal/mol (at 243 K; ≥ 4.2 kcal/mol at 298 K), relative to the ternary complex. The free energy difference, $\Delta G(\text{Co}^{\text{II}}\text{-substrate radical pair}) - \Delta G(\text{ternary complex})$, is +0.25 to -0.29 kcal/mol over the T range, 238 to 250 K. Extrapolation of the van't Hoff plot shows that the difference is -2.6 kcal/mol at 298 K. The results show that the protein selectively destabilizes the Co^{II}-5'-deoxyadenosyl radical pair state, which minimizes damage from accumulation of the highly reactive and mobile primary C5' radical, and selectively stabilizes the Co^{II}-substrate radical pair state, which biases the reaction in the forward direction of cobalt-carbon bond cleavage and radical pair separation. Supported by NIH DK54514.

P31**High-field measurements on molecular clusters with very large spin: Mn_{25} with $S=51/2$, $61/2$ and $65/2$**

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High field EPR (up to 14T) at various high frequencies (up to 329 GHz) was used to test Mn_{25} clusters with $S=51/2$, $61/2$ and $65/2$ at various temperatures (5-290 K). We report the typical EPR spectra and temperature and spin dependence of FWHM, absorption intensity, peak position and Lorentzian to Gaussian ratio of the peaks. We hope to get further understanding of quantum tunneling of magnetization (QTM) and other classic and quantum behaviors in single molecular magnets (SMMs).

P32**Characterization of the UBA Domain of E2-25K, a Ubiquitin-Conjugating Enzyme**

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Malfunctions in the ubiquitin/proteasome system of protein degradation have been implicated in a number of neurodegenerative diseases, including Huntington's Disease, Alzheimer's Disease, and Parkinson's Disease. More specifically, the ubiquitin-conjugating enzyme E2-25K has been identified as a huntingtin binding partner, and has been shown to play a role in mediating the toxicity of both polyglutamine-expanded huntingtin and A β (the key protein involved in Alzheimer's pathogenesis). Because the activity of E2-25K is implicated in the development of at least two major neurodegenerative diseases, it is an interesting target for drug therapy. E2-25K has a conserved ubiquitin-conjugating (UBC) region (which catalyzes the formation of a covalent bond between the c-terminal glycine of a ubiquitin molecule and the ϵ -amine of a lysine residue on the acceptor protein), as well as a ubiquitin-associated (UBA) domain. In order to examine its value as a potential drug target, the importance of the contribution of the individual domains to the overall function of the enzyme needs to be explored. As a first step towards determining the role played by the UBA domain, we have examined the non-covalent interactions with monoubiquitin by solution nuclear magnetic resonance (NMR) spectroscopy. Additionally, we have determined that interactions between the E2-25K catalytic domain and the UBA domain do not induce significant structural changes in the UBA domain.

P33**Solid-State NMR Studies of Polypropylene/Clay Nanocomposites**

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Structure/property relationships have been constructed for a large number of polymer/clay nanocomposites. Little is known, however, about how the structures evolve with processing and develop under large-scale deformation. We are using solid-state NMR to examine the structure, dynamics and morphology of polymer/clay nanocomposites as a function of processing and during large-scale deformation. To date, we have conducted ^1H and ^{13}C solid-state NMR experiments on isotactic polypropylene (iPP) and iPP/clay nanocomposites before and after biaxial stretching at elevated temperatures. ^1H Bloch decays were analyzed with respect to the crystallinity of the samples and the presence of an interphase between amorphous and crystalline regions. Only minor differences were found between the pure iPP and the iPP/clay nanocomposite. Spin-lattice (T_1) relaxation data revealed some differences in the samples as a function of the thermal and mechanical treatment. To some extent this is attributed to differences in the dispersion of the clay nanoparticles, which contain Fe^{3+} impurities. ^{13}C cross-polarization and direct polarization (DP) MAS spectra were recorded from room temperature to 90 °C. The DP spectra exhibited distinct differences in the range of 25-50 ppm which are attributed to variations in the ratios of gauche to trans backbone segmental conformers. We hope to ascertain whether these differences are morphological or orientational in origin.

P34 **^{13}C - and ^{19}F -NMR Spectra of Perfluoro Divinyl Ethers and Their Precursors**

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Both ^{13}C - and ^{19}F -NMR spectral data have been obtained for several perfluoro divinyl ethers and their precursors. The need for ^{13}C NMR spectroscopy turned out to be critical in several of the structure determinations based upon what was learned from the analysis of ^{13}C - ^{19}F coupling constants. New trends in F-F spin-spin coupling were also developed and used in the confirmation of the structures and the assignments of the resonances. Long-range couplings between fluorine atoms in the vinyl groups and fluorine atoms in the alkyl groups were interpreted in terms of molecular geometry, i.e., small, through-space couplings between certain fluorine atoms of the alkyl groups and the fluorine atoms cis and gem to the ether oxygen atom in the vinyl groups, but not the fluorine atom trans to the ether oxygen atom, can account for the observed spectra.

P35

Nitrogen-pair defect in 4H and 6H Silicon Carbide*M. E. Zvanut (1), J. van Tol (2)*

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Silicon Carbide (SiC) is one of the leading contenders in the search for a suitable semiconductor for high power, high temperature devices. Although commercial substrates are available, incorporation of nitrogen during growth remains a critical issue. We have studied a nitrogen-related point defect present in a wide variety of 4H and 6H polytypes of SiC using electron paramagnetic resonance (EPR) spectroscopy. High frequency EPR measurement and the dependence of the defect concentration on nitrogen incorporation supports the identification as a nitrogen-donor pair as has recently been suggested by others [1,2].

The samples studied were grown by physical vapor transport by three different vendors. The amount of nitrogen, as determined by secondary ion mass spectroscopy (SIMS), ranged from 10^{16} – 10^{18} cm⁻³, and the electrically measured net carrier concentration ($N_d - N_a$) varied between 5×10^{15} to 7×10^{17} cm⁻³. EPR was performed between 15 and 30 K in the dark and after illumination.

Originally, the spacing of the EPR lines lead to the suggestion that the spectrum was due to the low-probability nuclear spin transitions of the isolated nitrogen donor. However the measurement of the g-tensor clearly refutes this claim. Furthermore, measurements at 10 GHz and 240 GHz show that the center in 7×10^{16} cm⁻³ N-doped 4H SiC is a pair center involving nitrogen on a site of cubic symmetry and a second, as yet unidentified defect. Comparison of data obtained from 4H and 6H material hints at the nature of the second center. A consistently higher concentration of the pair in the 4H polytype compared to that in the 6H material is consistent with a model in which the second half of the pair is a nitrogen atom substituting at a site of hexagonal symmetry. The talk will focus on partial identification of the center as a nitrogen-related pair and interpretation of the pair concentration dependence on the nitrogen doping density.

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1. U. Gerstmann, et. al., European Conference on Silicon Carbide and Related Materials, abstract We6.2, September, 2006.
2. S. Greulich-Weber, "EPR and ENDOR Investigations of Shallow Impurities in SiC Polytypes", Phys. Stat. Sol. a **182**, 95 (1997).