NMR and Cell Tracking Symposium

May 16, 2018

BioScience Research Collaborative
6500 Main St.
Houston, Texas
The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on **biomedical informatics, computational cancer biology, molecular biophysics, neuroengineering and pharmacological sciences**. GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include **chemical genomics, magnetic resonance, translational pain research, antimicrobial resistance, neuroengineering, regenerative medicine theoretical and computational neuroscience, mental health research, nano x, and alcohol and addiction research**. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.
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<td>8:30</td>
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<td>9:00</td>
<td>Welcoming and Introductory Remarks</td>
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<td><em>Catalyzing Clinical Translation of Hyperpolarized MR</em></td>
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<td>Craig Malloy, University of Texas Southwestern</td>
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<td>10:00</td>
<td>NMR Resources &amp; Projects in the Houston/Galveston area</td>
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<td>Robia Pautler, Baylor College of Medicine</td>
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<td><em>Rice University NMR facility and Application to Studies of RNA</em></td>
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<td>Ed Nikonowicz, Rice University</td>
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<td><em>Structure-based Design of Inhibitors of Flavivirus Replication</em></td>
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<td>Roopa Thapar, MD Anderson</td>
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<td><em>Protein NMR Spectroscopy: What Can You Do With It?</em></td>
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<td>Junji Iwahara, University of Texas Medical Branch</td>
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<td><em>Use of NMR to Study Intrinsically Disordered Proteins</em></td>
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<td>John Putkey, University of Texas Health Science Center, Houston</td>
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<td>11:00</td>
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<td><em>Visualizing Cytotherapy and Inflammation in vivo using Fluorine-19 MRI</em></td>
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<td>Eric Ahrens, University of California, San Diego</td>
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<td>12:00-1:15</td>
<td>Lunch and poster session</td>
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<td>1:15</td>
<td><em>Opportunities &amp; Obstacles in Cell Tracking</em></td>
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<td><em>Cell Tracking In Vivo – Some Clinical/Translational Perspectives</em></td>
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<td>Vikas Kundra, MD Anderson</td>
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<td>1:40</td>
<td><em>Monitoring immune cell delivery and persistence: An unmet clinical need</em></td>
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<td>Vidya Gopalakrishnan, MD Anderson</td>
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<td><em>Selected Abstracts</em></td>
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<td><em>Identifying Immunotherapy Resistance in Vitro and in Vivo in Melanoma Employing Magnetic Resonance</em></td>
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Hyperpolarized Magnetic Resonance Imaging Reveals Transformations in Pyruvate Metabolism Prior to Anatomic Changes in Patient-Derived Glioblastoma Models
Travis Salzillo, MD Anderson Cancer Center

Tracking VLA-4-Expressing Leukocytes Trafficking to Vulnerable Plaques in ApoE(-/-) Mice by MRI
Eric Tanifum, Baylor College of Medicine

3:05 Break

3:20 The Nanoengineering of T1 Contrast into Multifunctional Nanoparticles for Imaging and Therapy
Naomi Halas, Rice University

4:10 Divalent Europium in Magnetic Resonance Imaging
Matt Allen, Wayne State

5:00 Wrap-Up & Reception
Catalyzing Clinical Translation of Hyperpolarized MR
Craig Malloy, Professor
Advanced Imaging Research Center, University of Texas Southwestern

About Dr. Malloy:
Dr. Malloy graduated from Stanford University with a B.S. degree in Chemistry and he received his M.D. degree from the University of California at San Francisco. After training in Internal Medicine at Parkland Hospital and a cardiology fellowship at the Brigham and Women’s Hospital in Boston, he was an American Heart Association Clinician-Scientist at Oxford University. Dr. Malloy subsequently joined the faculty at UT Southwestern and served as Chief of Cardiology at the Dallas VA Medical Center. In 2006 he joined the Advanced Imaging Research Center as Medical Director. Dr. Malloy has a long-standing interest in analysis of intermediary metabolism in complex systems. Recently his focus has been integration of conventional 13C NMR isotopomer analysis with the exciting new opportunities provided by imaging hyperpolarized 13C in human patients.

Abstract:
There is renewed interest in understanding intermediary metabolism in cancer, neurological disorders, hepatic and many other diseases. 13C NMR is an extraordinarily rich method for probing metabolism. If the site of labeling is chosen carefully, the use of a 13C-enriched substrate combined with detection by NMR provides a wealth of information. This combination, 13C plus NMR, is in principle overwhelmingly superior to any other method for investigating carbon metabolism including 11C positron tomography, 14C tracer studies, or 13C combined with mass spectrometry. This advantage arise from information encoded in the chemical shift and scalar coupling. The limiting factor, of course, is the sensitivity of NMR for 13C detection. Three approaches can be taken to overcome the sensitivity limitation. One popular experimental method has been administration of highly-enriched 13C-containing substrates to a biological system ranging in complexity from cells in culture to human patients, followed by freeze-clamping, extraction and acquisition of a high-resolution spectrum in an analytical NMR systems. The multiplets encoded in each individual 13C resonance in such spectra can be analyzed in terms of relative fluxes of pathways, a processes that has become known as isotopomer analysis. A second approach is to push toward higher Bo magnetic field for direct detection in vivo. Although sensitivity may improve proportional to field or a little better, the equipment costs and other practical factors rapidly become prohibitive. A third approach, hyperpolarized (HP) 13C provides the greatest sensitivity enhancement of all. However, HP technology is complex and the relation between HP signals and the underlying biochemistry is not simple.

In this era of scrutiny of clinical technologies, validation of new methods will be important. Eventually stakeholders will ask whether HP provides actionable clinical information. At the current stage of development, three biochemical validation methods could be considered. First, parallel experiments with 13C-enriched substrates, mimicking HP conditions can be performed and analyzed according to accepted metabolic models. Second, he insensitivity of conventional thermally-polarized 13C becomes an advantage when combined with HP 13C because thermally polarized 13C is undetectable on the time scale of the HP experiment. This combination may provide a conceptually new approach to studies of metabolism where one can combine the power of HP 13C to measure flux in real time with a more complete isotopomer analysis of the 13C NMR spectrum of the same tissue sample. Third, simultaneous or near-simultaneous experiments with PET tracers such as 11C pyruvate or lactate, or 18F deoxyglucose in a PET – MR system will also be valuable. It will be important to move HP from observational studies to more quantitative analysis of metabolism.
Visualizing Cytotherapy and Inflammation in vivo Using Fluorine-19 MRI

Eric Ahrens, Professor
Radiology, University of California, San Diego

About Dr. Ahrens:
Dr. Ahrens' research involves imaging innovations and adapting MRI to visualize molecular and cellular events in vivo. Currently, Ahrens is a Professor in the Department of Radiology at the University of California San Diego, Director of Stem Cell Molecular Imaging at the Sanford Consortium for Regenerative Medicine, and Director of the Molecular Imaging Center at Stanford. Formally, he was a Professor of Biological Sciences at Carnegie Mellon University. He has also served as a senior research fellow in the Department of Biology, California Institute of Technology. He holds a Ph.D. in physics from the University of California at Los Angeles and was a graduate fellow at Los Alamos National Laboratory. Ahrens is engaged in the design, characterization and application of novel contrast agents for clinical cell tracking and visualizing gene expression using MRI. He has pioneered the use of fluorine-19 probes for clinical cell tracking and has published widely on spin physics, imaging probe development, in vivo imaging in animal models using high-field MRI, and the conduct of clinical trials. He is an inventor on 9 patents related to MRI.

Abstract:
This talk will discuss an emerging approach for cell tracking called ‘in vivo cytometry.’ In this approach, cell populations of interest, such as immune cells or stem cells, are tracked and quantified in vivo. We formulate perfluorocarbon (PFC) emulsions to label cells ex vivo. The labeling process is observed to minimally disruption cell function and phenotype. The labeled cells are introduced into the subject and their migration can be monitored using fluorine-19 (19F) MRI. The 19F images are selective for the labeled cells, with no background signal from the host’s tissues. Moreover, the absolute number of labeled cells in regions of interest can be estimated directly from the in vivo 19F images. Additionally, the PFC emulsion reagents have bio-sensing properties that report on the absolute level of intracellular oxygen in vivo. In this talk we will provide an overview of preclinical data using in vivo cytometry to investigate inflammation, cancer, and stem cell models. We will describe in vivo cytometry results from a Phase-1 pilot clinical trial for colorectal cancer treatment using an immunotherapeutic dendritic cell vaccine, where 19F MRI was used to longitudinally visualize and quantify cells post-administration. This talk will also cover in situ labeling of macrophage using PFC emulsion. Upon intravenous injection, emulsion droplets are intrinsically taken up in situ predominately by monocytes and macrophages. As these in situ-labeled cells participate in inflammatory events in the body, the result is 19F accumulation at inflammatory sites. The in vivo 19F MRI signal is inflammation-specific and linearly proportional to the macrophage burden. In situ 19F labeling has been widely used for visualizing inflammation in a large number of preclinical models.
Cell Tracking In Vivo – Some Clinical/Translational Perspectives

Vikas Kundra, Professor
Diagnostic Radiology, MD Anderson Cancer Center

About Dr. Kundra:
Vikas Kundra, M.D., Ph.D. is Professor and Director of Molecular Imaging in the Department of Radiology, U.T.-M.D. Anderson Cancer Center with joint appointment in the Department of Cancer Systems Imaging. He received his M.D. and Ph.D. from Harvard University. He trained at Harvard Medical School's Brigham and Women’s Hospital. He is a Fellow of the Society of Body Computed Tomography-Magnetic Resonance Imaging and Distinguished Investigator of the Academy of Radiology Research. He practices as a clinical radiologist focused on body imaging primarily using CT and MRI. He has had multiple clinical and basic/translational science papers and grants. The latter includes from NIH, DOD, NSF, CPRIT, etc. Clinical research focus is body imaging/oncology including within GU imaging, prostate cancer. Basic/translational research focuses on imaging of gene expression, therapy response, imagable models of disease, delivery, and nanoparticles. Techniques range from basic cell and molecular biology to non-invasive imaging. Most non-invasive imaging modalities (such as CT, MR, PET, SPECT, Optical) are used in the research depending on the question being pursued.

Abstract:
Cell therapies have shown potential for various applications. Multiple clinical trials have shown promise regarding stem cell based therapies. Recently, T-cell therapies have been clinically approved. For furthering these, methods for cell tracking that can be translated to the clinic are needed. For these therapies, it is important to understand where the cells initially lodge after delivery, and if they replicate or differentiate. Thus, longitudinal evaluation is needed. Biopsy can lead to sampling error, only certain sites can be evaluated, and multiple rounds limit patient acceptance. Non-invasive imaging is needed. Labeling techniques have promise, but may be diluted as the cells divide. They can have utility at initial delivery to see where the cells lodge and initially divide/differentiate. Longer term studies require continuous production of the material to be imaged so that progeny can be imaged. Whichever imaging technique is used, it is important that the imaging method not interfere with normal cellular function or any additionally delivered gene of interest. Thus, the tag or the imaged protein should be relatively inert and if the latter should not need its own function (e.g. enzyme, pump) that may alter the cell in order to be imaged. In the case of receptors, a signaling deficient reporter such as based on somatostatin receptor type-2 is desirable. Non-invasive cell tracking systems have the potential to accelerate clinical translation of cell based therapies.
Monitoring Immune Cell Delivery and Persistence: An Unmet Clinical Need
Vidya Gopalakrishnan, Associate Professor
Pediatrics, MD Anderson

About Dr. Gopalakrishnan:
Dr. Gopalakrishnan is an Associate Professor in the Department of Pediatrics-Research at the University of Texas MD Anderson Cancer Center. She completed her PhD at the University of Pittsburgh studying DNA replication in the human papilloma virus model system. Her subsequent post-doctoral fellowship at Johns Hopkins University examined molecular mechanisms that restrict DNA replication to a once per cell cycle event in the fission yeast model system. Current work in her group spans the spectrum of basic, translational and clinical research. The overall goals of her work are to delineate the molecular underpinnings of pediatric brain tumors and to leverage druggable pathways for therapeutic purposes. Her research is broadly divided into 3 areas: (A) Brain tumor epigenetics: with a major focus on the transcription factor REST and its role in driving initiation and progression of pediatric brain cancers, medulloblastoma (MB) and diffuse intrinsic pontine glioma (DIPG) (B) Brain tumor proteomics: with an emphasis on a component of the proteasomal machinery called USP37, and (C) Development of immunotherapy for pediatric brain tumors: She leads the efforts of a multidisciplinary team of scientists and physicians at MD Anderson Cancer Center to develop immunotherapy for recurrent/refractory pediatric brain tumors. Preclinical data from her group has been transitioned to the clinic through initiation of a Phase-I clinical trial to test the safety of intracranial administration of immune cells. To monitor the fate of infused cells, the team is developing novel imaging technologies, which are being tested in Dr. Gopalakrishnan’s laboratory. Her group is also investigating mechanisms of immune evasion in pediatric brain tumors. Over the years, Dr. Gopalakrishnan’s work has been supported by grants from the NIH, American Cancer Society, CPRIT, brain tumor foundations and philanthropy.

Abstract:
Pediatric patients with malignant pediatric brain tumors such as medulloblastoma (MB), ependymoma and atypical teratoid rhabdoid tumors (ATRT) are at significant risk for recurrence and metastasis. Current therapeutics are ineffective against recurrences and metastases, and young lives are unfortunately extinguished. Our multidisciplinary team has developed platform technologies to expand immune cells for clinical application. These technologies have allowed us to test the feasibility of using adoptive transfer of natural killer (NK) cells as a novel approach for the treatment of relapsed/refractory/metastatic brain tumors in mouse orthotopic models. Our observations from these studies as well as other data that therapeutics can be delivered into the intra-ventricular space of children using a surgically implanted catheter have provided the foundation for an ongoing investigator-initiated Phase-I clinical trial at MD Anderson Cancer center. The goal of this study is to assess feasibility, safety and maximum tolerated dose of autologous, ex vivo expanded NK cells that are infused directly into the fourth ventricle of pediatric patients with recurrent/refractory brain tumors. The trial, which has merged two important and emerging cutting-edge concepts in oncology and medicine: immunotherapy and loco-regional delivery of therapeutics, has shown promising results. However, it has also identified the need to understand response as a function of trafficking, persistence and ‘pharmacokinetics’ of cell based therapies. In preclinical studies, our team has demonstrated feasibility of monitoring delivery and localization of 19F-labeled NK cells and measuring in vivo cytolytic activity of these cells in mouse intracranial models of brain tumors. Our data will set the stage for designing trials to ask critical questions regarding delivery, dose, persistence and localization of immune cell based therapies for pediatric neuro-oncology.
Identifying Immunotherapy Resistance in Vitro and in Vivo in Melanoma Employing Magnetic Resonance
Shivanand Pudakalakatti, MD Anderson Cancer Center

About Dr. Pudakalakatti:
Dr. Pudakalakatti received his PhD degree in Chemistry from Indian Institute of Science, Bangalore and is currently GCC/Keck Center CCBTP postdoctoral fellow at Dr. Pratip Bhattacharya’s laboratory in the Department of Cancer Systems Imaging at MD Anderson Cancer Center. His current research interest is to apply NMR spectroscopy and 13C and 29Si MR hyperpolarization techniques for early detection of cancer and determining early efficacy of therapies in multiple cancer systems.

Abstract:
Introduction: Cancer immunotherapy stands unique among the other available cancer therapies since it triggers our own immune system to fight cancer. Cancer immunotherapy mainly works on blocking the immune checkpoint proteins: either Cytotoxic T-Lymphocyte-1 (CTLA4) or Programmed death-1 (PD1) or both1. The technique has witnessed successful application in melanoma. However, not all melanoma patients respond to immunotherapy. The biological molecular mechanisms which drive resistance to immunotherapy are elusive. To understand this crucial knowledge gap, immunotherapy resistant melanoma mouse strains are developed, and underlying biological molecular mechanisms to resistance are unraveled employing Nuclear Magnetic Resonance Spectroscopy (NMR) and Magnetic Resonance Imaging (MRI). The immunotherapy resistant melanoma (B16) strains were developed by an in vivo serial passage approach. The cell lines derived from parental (B16/TMT) and resistant (B16/F4) strains were used for in vitro NMR studies.

Methods: Metabolites were extracted from platelets using a methanol-water mixture by vortex and freeze-thawing process followed by centrifugation, rotary evaporation and lyophilization. The samples were prepared for NMR spectroscopy by dissolving the lyophilized sample in 600 µl of 2H2O and adding the NMR reference compound DSS of 0.5 mM. The data were acquired on a Bruker NMR spectrometer operating at 500 MHz 1H resonance frequency equipped with a cryogenically cooled triple resonance (1H, 13C, 15N) TXI probe2. Identification of metabolite peaks was done through Chenomx and the Human Metabolomic Database (HMDB). Hyperpolarized 1-13C pyruvate magnetic resonance (MR) spectroscopy was employed to study immunotherapy response and resistance in vivo in mice model. The dissolution DNP (HyperSense, Oxford Instruments) operating at 3T was employed to hyperpolarize (HP) 1-13C pyruvate. The 13C magnetic resonance spectra of HP 1-13C pyruvate were acquired in 7T Bruker MRI scanner on C57/BL6 mice models possessing responding and resistant melanoma tumor in flank by injecting 200 µL of 80 mM HP pyruvate via tail vein.

Results: NMR results revealed upregulation in concentration of lactate, acetate, alanine and glycine in resistant cell lines compared to responding cell lines. Whereas adenosine mono phosphate (AMP) and phosphocholine (PC) are downregulated in in vitro resistant cell lines compared to responding, the ex vivo tissue analysis of resistant and responding mice tumors confirmed the upregulation in concentration of lactate in the resistant mice. Metabolic imaging by hyperpolarized 1-13C pyruvate using MR revealed higher pyruvate to lactate conversion in immunotherapy resistant mice compared to responding ones in vivo.

Discussion: Upregulation in the concentration of lactate, acetate, alanine and glycine and downregulation of AMP and PC observed in resistant cell lines compared to responding cell lines. This demonstrates adaptations in metabolic pathways of glycolysis, fatty acid synthesis and purine synthesis. These metabolomics data are correlated with upregulation of genes in resistant strains. Furthermore, the mice bearing resistant tumors showed higher real-time pyruvate to lactate conversion compared to responding ones in vivo in the HP metabolic imaging studies of intact animals. This suggests that tumor cells adapt to more glycolytic pathways which is one of the dominant mechanisms for immunotherapy resistance.

Conclusion: Altered metabolism in glycolysis, purine metabolism and fatty acid metabolism validated by NMR spectroscopy in vitro and hyperpolarized MR Spectroscopy in vivo are important metabolic pathways to be targeted for effective immunotherapy. The lactate to pyruvate ratio calculated from HP 1-13C pyruvate MR spectroscopy in vivo will serve as a metabolic biomarker to identify immunotherapy resistant mice.
Hyperpolarized Magnetic Resonance Imaging Reveals Transformations in Pyruvate Metabolism Prior to Anatomic Changes in Patient-Derived Glioblastoma Models

Travis Salzillo, MD Anderson Cancer Center

About Mr. Salzillo:
Mr. Salzillo is a fourth year graduate student studying medical physics at UTHealth here in Houston. I conduct research under Pratip Bhattacharya in the Department of Cancer Systems Imaging at MD Anderson. Our lab focuses on developing a novel metabolic imaging technique called hyperpolarized MRI and applying it to a variety of cancer models. I personally research the progression of tumor metabolism during the development and treatment of glioblastoma using both in vivo hyperpolarized MRI and ex vivo NMR techniques. Additionally he aims to incorporate these metabolic results with quantitative imaging and histological data to form a multimodality tumor profile of this disease. After graduation he plans on applying for postdoctoral positions as well as medical physics residency programs and hope to one day work in an academic hospital with both clinical and research responsibilities.

Abstract:
Objective Glioblastomas originate from a variety of cells such as astrocytes and neuronal stem cells which, along with their advanced stage, makes these tumors diverse in mutations. Thus, targeted therapies can rarely block all mechanisms of proliferation and survival, leading to median survival times of merely 15 months. The objective of this research is to characterize the evolution of tumor metabolism that either leads to or results from these somatic mutations. This work implements a novel metabolic imaging technique known as hyperpolarized magnetic resonance imaging (MRI) in order to interrogate tumor metabolism at different time-points of tumor growth. In these experiments, hyperpolarized pyruvate was injected and the concentration of it and its product, lactate, were quantified in vivo to infer the level of aerobic glycolysis in tumors. Increased intracellular glycolysis is associated with the transformation into malignant cells as observed in the Warburg effect. This study aims to capture this metabolic transition over the course of tumor development.

Methods Glioma sphere-forming cells (GSCs) were cultured from patient biopsies and intracranially injected into the brains of mice whose median survival was 35 days. Every 3 days, tumor volume was measured with T1-weighted, T2-weighted, and fluid-attenuated MRI sequences. At 20%, 30%, 40%, 60%, 80%, and 100% of median survival, hyperpolarized [1-13C] pyruvate MRI experiments were performed. Pyruvate-to-lactate conversion was quantified by the metric nLac. Tumors were then excised, flash-frozen, and prepared for nuclear magnetic resonance (NMR) assays. The resonances of 25 metabolites were identified and their concentrations quantified. Statistical significance of the different measurements was determined using ANOVA at 95% confidence with multiple comparison corrections.

Results From hyperpolarization experiments, nLac was significantly increased in the tumor-bearing mice compared to the controls beginning at the 40% time-point and continued to increase throughout tumor development. Tumor volume was not statistically different from its initial value until just after the 60% time-point. From the NMR experiments, glutamate and myo-inositol concentrations were significantly increased in tumors compared to controls beginning at the 60% time-point and glycine was significantly increased by the 100% time-point.

Conclusions Hyperpolarized MRI was able to reliably detect changes in tumor metabolism in vivo prior to changes in tumor volume measured with conventional MRI by over a week (~20% median survival time). Additionally, variations in several metabolite concentrations were identified over the course of tumor growth which are being further investigated with pathway analysis to identify additional metabolic processes that are up- or down-regulated during tumor development. Metabolic imaging has the potential to significantly impact diagnosis as well as treatment monitoring by identifying changes in tumor function before anatomic changes occur.
Tracking VLA-4-Expressing Leukocytes Trafficking to Vulnerable Plaques in ApoE(-/-) Mice by MRI

Eric Tanifum, Baylor College of Medicine

About:
Dr. Tanifum received BS and MS degrees in Chemistry from the Universities of Buea and Dschang in Cameroon respectively. He moved to the US in 2001, where he earned a Ph.D. in Organic Chemistry from Utah State University in 2006. Upon graduation, he conducted postdoctoral research in bioorganic chemistry at the same institution, and medicinal chemistry and small molecule library development at the University of Texas Medical Branch in Galveston. From there he moved to The University of Texas Health Science Center at Houston where he completed a two year fellowship in nanomedicine. He joined the Edward B Singleton Department of Pediatric Radiology where he currently serves as Basic Science Research manager in 2011, and was appointed assistant professor at Baylor College of Medicine in 2013.

His previous research efforts have resulted in the development of facile chemistries for the preparation of several complex bioactive molecules, small molecule libraries for the NIH Molecular Libraries-Small Molecule Repository, and a biotech startup company. His current research efforts are focused on the development of nanoparticle-based targeted drug delivery technologies for both diagnostic and therapeutic purposes.

Abstract:
Purpose: Anatomic imaging by coronary angiography is the present standard for diagnosing cardiovascular complications of atherosclerosis but it cannot delineate the cellular and molecular components that predict the stability of a plaques. Monocytes/macrophages and T-lymphocytes constitute about half the cellular makeup of vulnerable plaques. VLA-4 is expressed on the surface of leukocytes, including macrophages and T-cells. We present herein, a liposomal T1 contrast agent labeled with THI567, a ligand that labels circulating VLA-4-expressing leucocytes and enables noninvasive visualization of vulnerable atherosclerotic plaques in ApoE(-/-) mice using MRI.

Methods: A lipid-PEG-THI567 conjugate was synthesized and incorporated into a liposome formulation mixture including a DSPE-PEG-DOTA-Gd complex and Rhodamine DHPE. Flow cytometry was used to evaluate particle binding activity and receptor specificity in vitro, and leukocyte cell subset binding distribution in vivo. MR imaging in ApoE(-/-) mice was performed on a 1.0 T permanent magnet scanner. Images were acquired pre-contrast, immediately after contrast injection, and 72-96 hours post injection using a T1-weighted 3D gradient echo (GRE) sequence. Animals were sacrificed for histology after the last scan.

Results: VLA-4 targeted particles bind activated monocytes and T-lymphocytes both in vitro and in vivo with picomolar binding constants. In delayed phase imaging, 9/9 animals injected with the THI-567 targeted liposomes showed enhanced regions in the aortic arch or the descending aorta, with 4-fold greater mean signal than the animals injected with the untargeted agent. Histochemical analysis showed co-localization of the targeted particles and immune cells within the plaques. Sample data are shown in Figure 1.

Conclusions: Liposomal nanoparticles bearing a T1 contrast agent, targeted to monocytes/macrophages and T-lymphocytes allows for noninvasive visualization of vulnerable plaques in ApoE(-/-) mice by MRI.
The Nanoengineering of T1 Contrast into Multifunctional Nanoparticles for Imaging and Therapy

Naomi Halas, Professor
Biomedical Engineering, Chemistry, Physics and Astronomy
Director, Smalley-Curl Institute and Laboratory for Nanophotonics, Rice University

About Dr. Halas:
Dr. Naomi Halas is the Stanley C. Moore Professor of Electrical and Computer Engineering at Rice University, with joint appointments in Chemistry, Physics & Astronomy, Bioengineering, and Materials Science & Nanoengineering departments. Dr. Halas is a pioneer in the field of plasmonics, creating the concept of the “tunable plasmon” and inventing a family of nanoparticles with resonances spanning the visible and infrared regions of the spectrum. She pursues fundamental studies of plasmonic and nanophotonics systems and their applications in biomedicine, optoelectronics, photocatalysis, chemical sensing and, most recently, solar steam generation with applications in off-grid water treatment. She is the author of more than 300 refereed publications with more than 50,000 Web of Science citations, has more than 15 issued patents, and has presented more than 500 invited talks. Dr. Halas is co-founder of Nanospectra Biosciences, a Houston-based company developing ultralocalized photothermal therapies for cancer, currently in clinical trials. Halas has been elected to the National Academy of Engineering, the National Academy of Sciences, and the American Academy of Arts and Sciences, and is a fellow of the National Academy of Inventors.

Abstract:
Recently we have been focusing on various ultracompact nanoparticle designs that can be used as MRI T1 contrast agents that also function as contrast agents for other imaging modalities and, in addition, as active photothermal transducers that can be used for photothermal cancer therapy or light-induced drug delivery. We have shown that the incorporation of Gd(III) within a nanoparticle can result in surprisingly large T1 enhancements. In such a geometry the Gd(III) is, unlike the case of Gd(III) chelate compounds, isolated from the H2O protons in the surrounding tissue, relying instead on long-range interactions between the magnetic ion inside the nanoparticle and the H2O protons. The internalization of Gd(III) within the Au-coated NM (Gd-NM) substantially reduces the potential for release and exposure of Gd(III) to the patient, ameliorating potentially mounting Gd(III) toxicity concerns. We have recently shown that this same approach is even more effective in enhancing the T1 contrast for Fe(III). Nanoparticles that have encapsulated Fe(III) were found to possess relaxivities twice as large as those found for Gd(III) DOTA, and may provide a practical alternative for MRI image enhancement independent of Gd(III) use. We show that this nanostructure can not only enable tissue visualization with MRI, but with the incorporation of a fluorophore, also support enhanced fluorescence-based nanoparticle tracking. This type of multifunctional nanoparticle complex may be highly useful for cell tracking, for quantifying nanoparticle distributions in vivo, and for, when appropriate, inducing a near-infrared light-induced photothermal drug release or hyperthermic response.
Divalent Europium in Magnetic Resonance Imaging
Matt Allen, Professor and Chair
Inorganic, Wayne State University

About Dr. Allen:
Matthew Allen is currently Professor and Chair of the Chemistry Department at Wayne State University. Matt earned a B.S. in Chemistry from Purdue University, during which time he was also an intern at Eli Lilly. He earned a Ph.D. in Chemistry from the California Institute of Technology while on a National Defense Science and Engineering Graduate Fellowship. He was a National Institutes of Health postdoctoral fellow at the University of Wisconsin–Madison prior to joining WSU as an Assistant Professor in 2008. He was promoted to Associate Professor in 2013 and Professor in 2016. He has served as Chair of the Chemistry Department since July 2016. Matt’s research program at WSU involves the aqueous chemistry of the lanthanides and has implications in the diagnosis of cancer and in the development of environmentally benign ways to make new molecules. He has over 70 peer-reviewed publications and recently co-edited a book (Contrast Agents for MRI: Experimental Methods). Matt has received the WSU President’s Award for Excellence in Teaching and the University Safety Award. Additionally, while at WSU, Matt has received a Pathway to Independence Award from the National Institutes of Health, a CAREER Award from the National Science Foundation, the Elliot Lasser Award from the Contrast Media Research Symposium, and an Outstanding Teacher Award from the International Society for Magnetic Resonance in Medicine.

Abstract:
Imaging of hypoxia has implications in the study of multiple diseases. The oxygen-sensitive ion EuII is isoelectronic with GdIII, resulting in both ions having desirable properties with respect to enhancing contrast in magnetic resonance imaging. Recently, the use of EuII as an in vivo contrast agent was reported. In this context, the contrast enhancement ceased upon oxidation, enabling the hypoxic core of a tumor to be imaged. However, the oxidized europium species did not produce enhancement in T1-weighted images, leading to a desire to couple a detection method for EuIII with EuII-based contrast agents. To detect Eu in both the +2 and +3 oxidation state, we used a variety of ligands to access properties amenable to imaging techniques other than T1-weighted 1H-magnetic resonance imaging. These methods include imaging other nuclei or using chemical exchange saturation transfer with magnetic resonance imaging and using other modalities such as photoacoustic imaging. Our results that I will discuss indicate that it is possible to detect both oxidation states of europium, but that the best method of detection depends on the ligand. Different strategies have been tried to detect EuIII after the oxidation of EuII-based contrast agents. Some of the strategies are promising for enabling the detection of Eu in both the +2 and +3 oxidation states.
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<td>Synthesis of a Hyper Fluorinated Hydrophilic Molecule for $^{19}$F MRI Application</td>
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<td>Nicola</td>
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<td>PLGA Particles Based Delivery System For Intrapericardial Delivery Of Prostaglandin through HeartPAS™ Device</td>
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**Synthesis of a Hyper Fluorinated Hydrophilic Molecule for $^{19}$F MRI Application**

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**Objective**  
$^{19}$F MRI using nanoparticle-based agents holds considerable promise for molecular imaging applications. However, current $^{19}$F MRI relies primarily on the use of hydrophobic perfluorocarbons (PFCs) and perfluoropolyethers (PFPEs), which limits tailoring of nanoparticle for specific targets. In this work, we describe the development of water-soluble, low molecular weight fluorinated molecule containing 24 magnetically equivalent fluorine atoms.

**Methods**  
The organofluorine compound with 24 magnetically equivalent fluorine atoms was efficiently synthesized and fully characterized. The MRI characteristics of the compound were determined on a 1T permanent magnet. $^{19}$F images were acquired using a spin echo sequence using the following parameters: TE=20 ms; TR=800 ms; slice thickness=5 mm; NEX=4, scan time=10 min. In vitro phantom containing $^{19}$F concentrations (250 mM to 6M) were imaged. Signal-to-noise ratio (SNR) were determined to study the effect of scan parameters, and to determine the minimal detectable concentration. Preliminary in vitro toxicity study of the compound were performed using RAW264.7 mouse macrophage cell line and Kupffer cell line (ImKC).

**Results**  
$^{19}$F MRI phantom study of the 0.25 M solution of the compound using spin echo sequence at 1 T instrument showed CNR >40. The Preliminary toxicity study of the compound on RAW264.7 macrophages using CellTiter 96® Aqueous Non-Radioactive Cell Proliferation assay kit showed that above 85% of the cells were metabolically active following treatment with the compound and at concentrations $\leq$ 30 mM, for up to 24 hours.

**Conclusion**  
A novel hydrophilic hyper-fluorinated organofluorine molecule suitable for the development of aqueous formulations for in vivo $^{19}$F MRI applications is synthesized, which demonstrated $>40$ CNR at 1 T MRI. Preliminary toxicity assessment suggests that the compound is safe at concentrations up to 20 mM. The preparation of aqueous formulations of this molecule for in vivo MRI assessment is in progress.
Our knowledge on drugs and on their capability to treat diseases is huge. However, there are a lot of problems due to administration methods. For example, adverse side effects are known to be a critical problem as a result of systemic delivery. After a Myocardial Infraction (MI), caused by sudden coronary artery occlusion, soluble factors are released by the myocardium into the coronary circulation and pericardial fluid. In patients after MI, enhanced secretion of cardiac hepatocyte grow factor (HGF) from the infarct region is associated with reduced ventricular remodeling and improved cardiac function. Prostaglandins stimulate the production of endogenous myocardial HGF and offer a therapeutic avenue to pursue. PGE-1 (Alprostadil) is an FDA approved small molecule drug that acts as a potent vasodilator with anti-platelet aggregation properties. Clinically, infusion of PGE-1 increases local HGF production. The HeartPAS™, our novel device, can be used to deliver PLGA-encapsulated Alprostadil into the pericardiac fluid. In this study, we investigate PLGA particle production, characterization, biocompatibility, and the interaction between PLGA particles and cells. Furthermore, the drug encapsulation and sustained release are evaluated by changing the PLGA polymer ration, which influences the degradation properties. The interaction of PLGA with cells is observed with Scanning Electron Microscopy imaging. Use of PLGA as a controlled release drug delivery system can be coupled with the HeartPAS™ to offer a powerful way to release a wide variety of therapeutics into the pericardium.
Identifying Immunotherapy Resistance *In Vitro* and *In Vivo* in Melanoma Employing Magnetic Resonance

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**Introduction:** Cancer immunotherapy stands unique among the other available cancer therapies since it triggers our own immune system to fight cancer. Cancer immunotherapy mainly works on blocking the immune checkpoint proteins: either Cytotoxic T-Lymphocyte-1 (CTLA4) or Programmed death-1 (PD1) or both\(^1\). The technique has witnessed successful application in melanoma. However, not all melanoma patients respond to immunotherapy. The biological molecular mechanisms which drive resistance to immunotherapy are elusive. *To understand this crucial knowledge gap, immunotherapy resistant melanoma mouse strains are developed, and underlying biological molecular mechanisms to resistance are unraveled employing Nuclear Magnetic Resonance Spectroscopy (NMR) and Magnetic Resonance Imaging (MRI).* The immunotherapy resistant melanoma (B16) strains were developed by an *in vivo* serial passage approach. The cell lines derived from parental (B16/TMT) and resistant (B16/F4) strains were used for *in vitro* NMR studies.

**Methods:** Metabolites were extracted from platelets using a methanol-water mixture by vortex and freeze-thawing process followed by centrifugation, rotary evaporation and lyophilization. The samples were prepared for NMR spectroscopy by dissolving the lyophilized sample in 600 µl of \(^2\)H\(_2\)O and adding the NMR reference compound DSS of 0.5 mM. The data were acquired on a Bruker NMR spectrometer operating at 500 MHz \(^1\)H resonance frequency equipped with a cryogenically cooled triple resonance (\(^1\)H, \(^13\)C, \(^15\)N) TXI probe\(^2\). Identification of metabolic peaks was done through Chenomx and the Human Metabolomic Database (HMDB). Hyperpolarized \(^1\)\(^3\)C pyruvate magnetic resonance (MR) spectroscopy was employed to study immunotherapy response and resistance *in vivo* in mice model. The dissolution DNP (HyperSense, Oxford Instruments) operating at 3T was employed to hyperpolarize (HP) \(^1\)\(^3\)C pyruvate. The \(^13\)C magnetic resonance spectra of HP \(^1\)\(^3\)C pyruvate were acquired in 7T Bruker MRI scanner on C57/BL6 mice models possessing responding and resistant melanoma tumor in flank by injecting 200 µL of 80 mM HP pyruvate via tail vein.

**Results:** NMR results revealed upregulation in concentration of lactate, acetate, alanine and glycine in resistant cell lines compared to responding cell lines. Whereas adenosine mono phosphate (AMP) and phosphocholine (PC) are downregulated in *in vitro* resistant cell lines compared to responding, the *ex vivo* tissue analysis of resistant and responding mice tumors confirmed the upregulation in concentration of lactate in the resistant mice. Metabolic imaging by hyperpolarized \(^1\)\(^3\)C pyruvate using MR revealed higher pyruvate to lactate conversion in immunotherapy resistant mice compared to responding ones *in vivo*.

**Discussion:** Upregulation in the concentration of lactate, acetate, alanine and glycine and downregulation of AMP and PC observed in resistant cell lines compared to responding cell lines. This demonstrates adaptations in metabolic pathways of glycolysis, fatty acid synthesis and purine synthesis. These metabolomics data are correlated with upregulation of genes in resistant strains. Furthermore, the mice bearing resistant tumors showed higher real-time pyruvate to lactate conversion compared to responding ones *in vivo* in the HP metabolic imaging studies of intact animals. This suggests that tumor cells adapt to more glycolytic pathways which is one of the dominant mechanisms for immunotherapy resistance.

**Conclusion:** Altered metabolism in glycolysis, purine metabolism and fatty acid metabolism validated by NMR spectroscopy *in vitro* and hyperpolarized MR Spectroscopy *in vivo* are important metabolic pathways to be targeted for effective immunotherapy. The lactate to pyruvate ratio calculated from HP \(^1\)\(^3\)C pyruvate MR spectroscopy *in vivo* will serve as a metabolic biomarker to identify immunotherapy resistant mice.

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Segregating the Metabolism of Malignant and Stromal Cells in Pancreatic Tumors using Fluorescence-Activated Cell Sorting (FACS) followed by NMR Metabolomics

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Tumor metabolism is often assessed by taking a chunk of the tumor, extracting the water-soluble metabolites, and measuring them using NMR or Mass spectrometry. A tumor chunk is a composite of malignant cells, stromal cells and the extracellular space, thus the metabolism of malignant cells alone gets buried under the population average. Pancreatic tumors are made of about 10% malignant cells and 90% stromal cells (majorly fibroblasts), and hence the issue of stromal contamination in metabolomics data is enormous. In this experiment, we isolated the malignant fraction of tumors using Fluorescence-activated Cell Sorting (FACS) and analyzed it using NMR metabolomics. This allows us to analyze the metabolism of malignant cells distinctly from stromal cells.

12 male C57 black mice (9-10 weeks old) were injected in the flank with 100,000 KPC-GFP+ cells to grow tumors. The tumors samples were collected and digested into a single cell suspension by mechanical pulverization, incubation with trypsin and collagenase at 37°C, and filtering through a 70µ strainer. This suspension was then separated into KPC (GFP+) cells and Stromal (GFP-) cells using FACSAriaII at the Flow Cytometry Core. The single cell pellets were snap frozen. During metabolomics study, the malignant cells were pooled to obtain ~10 million cells per sample. The cells were lysed using mechanical disruption with ceramic beads, and water-soluble metabolites were extracted into 2:1 v/v solution of methanol/ water. The solvents were removed by using rotavapor, followed by lyophilization, and the metabolite powder was dissolved in standard DSS/ D2O solution. The samples were analyzed on a 500 MHz NMR spectrometer, and the metabolites were measured by integrating the peaks on the 13C NMR spectrum using MestreNova software. The data was normalized by the precise number of cells in each sample.

Figure 1 demonstrates the relative concentrations of metabolites in the malignant and stromal populations. We don’t intend to compare the metabolite concentration of malignant cells vs stromal cells, as it would have no biological meaning. However, this would allow us to compare the metabolites of only malignant cells across multiple sample groups. Two major limitations of this methodology are:- (a) The metabolites’ pool may change during the digestion process due to ongoing enzymatic activity and (b) Metabolites in the extracellular space are lost. Nonetheless, this method described above may provide a direct readout of the metabolic contributions of different cellular components in a complex cancer system.

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Hyperpolarized Magnetic Resonance Imaging Reveals Transformations in Pyruvate Metabolism Prior to Anatomic Changes in Patient-Derived Glioblastoma Models

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Objective: Glioblastomas originate from a variety of cells such as astrocytes and neuronal stem cells which, along with their advanced stage, makes these tumors diverse in mutations. Thus, targeted therapies can rarely block all mechanisms of proliferation and survival, leading to median survival times of merely 15 months. The objective of this research is to characterize the evolution of tumor metabolism that either leads to or results from these somatic mutations. This work implements a novel metabolic imaging technique known as hyperpolarized magnetic resonance imaging (MRI) in order to interrogate tumor metabolism at different time-points of tumor growth. In these experiments, hyperpolarized pyruvate was injected and the concentration of it and its product, lactate, were quantified in vivo to infer the level of aerobic glycolysis in tumors. Increased intracellular glycolysis is associated with the transformation into malignant cells as observed in the Warburg effect. This study aims to capture this metabolic transition over the course of tumor development.

Methods: Glioma sphere-forming cells (GSCs) were cultured from patient biopsies and intracranially injected into the brains of mice whose median survival was 35 days. Every 3 days, tumor volume was measured with T1-weighted, T2-weighted, and fluid-attenuated MRI sequences. At 20%, 30%, 40%, 60%, 80%, and 100% of median survival, hyperpolarized [1-13C] pyruvate MRI experiments were performed. Pyruvate-to-lactate conversion was quantified by the metric nLac. Tumors were then excised, flash-frozen, and prepared for nuclear magnetic resonance (NMR) assays. The resonances of 25 metabolites were identified and their concentrations quantified. Statistical significance of the different measurements was determined using ANOVA at 95% confidence with multiple comparison corrections.

Results: From hyperpolarization experiments, nLac was significantly increased in the tumor-bearing mice compared to the controls beginning at the 40% time-point and continued to increase throughout tumor development. Tumor volume was not statistically different from its initial value until just after the 60% time-point. From the NMR experiments, glutamate and myo-inositol concentrations were significantly increased in tumors compared to controls beginning at the 60% time-point and glycine was significantly increased by the 100% time-point.

Conclusions: Hyperpolarized MRI was able to reliably detect changes in tumor metabolism in vivo prior to changes in tumor volume measured with conventional MRI by over a week (~20% median survival time). Additionally, variations in several metabolite concentrations were identified over the course of tumor growth which are being further investigated with pathway analysis to identify additional metabolic processes that are up- or down-regulated during tumor development. Metabolic imaging has the potential to significantly impact diagnosis as well as treatment monitoring by identifying changes in tumor function before anatomic changes occur.

This work was supported by Brain SPORE Developmental Research Award and CPRIT Research Training Award (RP170067).
Tracking VLA-4-Expressing Leukocytes Trafficking to Vulnerable Plaques in ApoE(-/-) Mice by MRI

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Purpose
Anatomic imaging by coronary angiography is the present standard for diagnosing cardiovascular complications of atherosclerosis but it cannot delineate the cellular and molecular components that predict the stability of a plaques. Monocytes/macrophages and T-lymphocytes constitute about half the cellular makeup of vulnerable plaques. VLA-4 is expressed on the surface of leukocytes, including macrophages and T-cells. We present herein, a liposomal T₁ contrast agent labeled with THI567, a ligand that labels circulating VLA-4-expressing leucocytes and enables noninvasive visualization of vulnerable atherosclerotic plaques in ApoE(-/-) mice using MRI.

Methods
A lipid-PEG-THI567 conjugate was synthesized and incorporated into a liposome formulation mixture including a DSPE-PEG-DOTA-Gd complex and Rhodamine DHPE. Flow cytometry was used to evaluate particle binding activity and receptor specificity in vitro, and leukocyte cell subset binding distribution in vivo. MR imaging in ApoE(-/-) mice was performed on a 1.0 T permanent magnet scanner. Images were acquired pre-contrast, immediately after contrast injection, and 72-96 hours post injection using a T₁-weighted 3D gradient echo (GRE) sequence. Animals were sacrificed for histology after the last scan.

Results
VLA-4 targeted particles bind activated monocytes and T-lymphocytes both in vitro and in vivo with picomolar binding constants. In delayed phase imaging, 9/9 animals injected with the THI-567 targeted liposomes showed enhanced regions in the aortic arch or the descending aorta, with 4-fold greater mean signal than the animals injected with the untargeted agent. Histochemical analysis showed co-localization of the targeted particles and immune cells within the plaques. Sample data are shown in Figure 1.

Conclusions
Liposomal nanoparticles bearing a T₁ contrast agent, targeted to monocytes/macrophages and T-lymphocytes allows for noninvasive visualization of vulnerable plaques in ApoE(-/-) mice by MRI.

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Radio-Enhancement of Gold Nanoparticles *In Ovo* and *In Vivo* as a Therapeutic for Lung Cancer

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It has been demonstrated that the use of gold nanoparticles (AuNPs) in tumor treatments provides advantages due to radiative enhancement effects. Intratumoral injection of AuNPs combined with X-ray radiation as therapy for Lewis Lung carcinoma is presented for C57BL/6 mice inoculated with cells expressing luciferase (LLC-Luc). In this work, results are also presented for the chick chorioallantoic membrane (CAM) as tumor model. This study showed that the CAM can be used as preliminary *in vivo* model to study the effects of different treatments, using a simpler and faster system in which the influence of the immune system is not present and which does not require the IACUC approvals. Funding was provided by the Simmons’s foundation and Golfers Against Cancer.
Magnetic resonance imaging (MRI) is a well-established imaging modality that has been used for in vivo human diagnostics for over 30 years. $^{19}$F MRI, as an emerging technique, allows non-invasive image of whole organisms with negligible background signal. $^{19}$F has favorable NMR properties, including a nuclear spin of $\frac{1}{2}$, a Larmor frequency that differs only 6% from $^1$H, and an 83% sensitivity relative to $^1$H. The large ppm range of $^{19}$F provides an opportunity to do multicolor imaging. In a biological context, there is minimal endogenous fluorine MR signal in the body, since all fluorine is present in solid ionic form in bones and teeth. This makes this modality especially promising for sensing applications as all the observed signal will come from exogenous imaging agents. Paramagnetic metals can be used to modulate the relaxation and chemical shift properties of interacting fluorines via Paramagnetic Relaxation Enhancement (PRE) and Pseudocontact Shift (PSC) effects. In the Que lab, we are exploiting both metal redox chemistry and coordination changes to design $^{19}$F MR-based sensors for specific biological analytes. Our general design relies on a switch from a paramagnetic to a diamagnetic state to give changes in fluorine signal intensity and chemical shift.

This work was supported by start-up funds from the University of Texas at Austin and the Welch Foundation (F-1883).