

John S. Dunn, Sr. Gulf Coast Consortium for Magnetic Resonance

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Protein Dynamics and Aggregation in Health and Disease: Imaging Tissues, Cells, and Molecules

Speaker Abstracts (in order of presentation)

Keynote Address

A tale of two proteins: Conformational dynamics in ribonuclease H and type II cadherin 8

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NMR spectroscopy is a powerful experimental approach for characterizing protein conformational dynamics on multiple time scales, while molecular dynamics (MD) simulation is the only method capable of describing full atomistic details of protein dynamics. Cadherin-mediated cell adhesion is achieved through dimerization of cadherin N-terminal extracellular (EC1) domains presented from apposed cells. Homologous mesophilic (*E. coli*) and thermophilic (*T. thermophilus*) ribonuclease H (RNase H) enzymes serve to illustrate how changes in protein sequence and structure that affect conformational dynamic processes can be monitored and characterized by NMR spectroscopy.² A Gly residue within a putative hinge between α -helices B and C is conserved among thermophilic RNases H. The dynamic properties of *T. thermophilus* RNase H are recapitulated in *E. coli* RNase H by insertion of a Gly residue between helices B and C. The Gly insertion may be conserved because reduced steric restrictions on backbone dihedral angles permit other interactions to control the kinetics and thermodynamics of conformational changes in RNase H enzymes. Specific intramolecular interactions that modulate backbone and sidechain dynamical properties of *E. coli* RNase H also can be characterized by joint analyses of MD simulations and NMR spin relaxation measurements. These studies emphasize the importance of hydrogen bonds and local steric interactions in restricting conformational fluctuations, and the absence of such interactions in allowing conformational adaptation to substrate binding. NMR spectroscopy has been used to characterize monomer and dimer states of the mouse type II cadherin-8 EC1 in solution.¹ Limited picosecond-to-nanosecond timescale dynamics of the tryptophan indole side chains for both monomer and dimer states are consistent with well-ordered packing of the N-terminal β -strands intramolecularly and intermolecularly, respectively. However, pronounced microsecond-to-millisecond timescale dynamics of the side chains are observed only for the monomer state, suggesting that monomer, but not dimer, species sample configurations in which the indole moieties are exposed. The results support a selected-fit mechanism for EC1 domain dimerization, where collisions between strand-exposed monomers lead to formation of the strand-swapped dimer state.

References:

1. Miloushev, V. Z., Bahna, F., Ciatto, C., Honig, B., Shapiro L. and Palmer, A. G. (2008) *Structure* **16**, 1195-1205
2. Butterwick, J. and Palmer, A. G. (2006) *Protein Sci.* **15**, 2697-2707

MRI detection of the migration of magnetically labeled immune cells in inflammation and autoimmune diseases

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Methods for imaging cell trafficking patterns in health and disease are useful in the study and development of novel cellular therapies, but have been hampered by toxicity of cell labels and limitations of detecting small numbers of cells. Immune cells alone or genetically engineered cells are being used for the treatment of various inflammatory or autoimmune diseases. It is necessary to develop non-invasive imaging approaches to assess the metabolic, physiological and functional capacity of the transplanted or infused cells in targeted tissue. Noninvasive imaging methods have been developed for tracking the migration and homing of immune cells or genetically modified cells in vivo. For example, reporter genes have been introduced into cells and detected by optical imager/fluorescent or confocal microscopy. Cells transfected to express the sodium iodide symporter or herpes simplex virus thymidine kinase can be detected by nuclear medicine or PET approaches. We have pioneered a technique to magnetically label cells in the GMP facility of the Department of Transfusion Medicine by combining two FDA approved agents ferumoxides (FE), a superparamagnetic iron oxide nanoparticle (SPIO) with protamine sulfate (Pro)(1). The combination drug FEPro does not alter T-cell or dendritic cell viability, function, response to antigenic stimuli, rate of apoptosis, or homing to targets providing researchers with the ability to monitor the temporal and spatial migration of immune cells in various experimental models or clinical vaccine studies by MRI (2-4). In an adoptive transfer EAE SJL mouse model, we were able monitor the migration of ferumoxides labeled encephalitogenic T cells to inflammatory lesions in the mouse spinal cord by MRI (3). We have also (4) been able to detect by MRI, the migration and incorporation of FEPro-labeled sensitized splenocytes in a glioma brain tumor model within 3 days after iv injection of cells and were able to demonstrate the presence of dendritic cells and CD 8 T-cells in and around the tumors by immunohistochemistry. In the first clinical study, De Vries et al (2) labeled dendritic cells with ferumoxides as part of a phase I vaccine study in melanoma patients. Direct injection of the magnetically labeled dendritic cells provided these investigators the ability to track the migration of the immunomodulatory cells through the lymph nodes by MRI. MRI could detect < 2000 FE labeled dendritic cells per voxel.

Magnetic cell labeling studies in inflammatory and autoimmune diseases can be translated from bench to bedside to monitor specific cellular phenotypes (e.g., T-cells, B-cells, monocytes, dendritic cells) or genetically engineered cells by MRI, advancing the understanding of the disease processes with the potential of directing therapy to specific sites of disease. Preclinical studies with FEPro cell labeling and noninvasive imaging will be required to move from experimental studies to filing disease specific IND's to monitor the trafficking and homing of immune cells in inflammatory and autoimmune diseases.

References: 1) Arbab, AS, et al, Magnetic Cell Labeling with Protamine Sulfate complexed to Ferumoxides for Cellular MRI. Blood 2004;104:1217-1223. 2) de Vries IJ et al, Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring cellular therapy Nature Biotech 2005;23:407-13. 3) Anderson SA, et al, Magnetic resonance imaging of labeled T-cells in a mouse model of multiple sclerosis. Ann Neurol. 2004 May;55(5):654-9. 4) Arbab AS et al Magnetically Labeled Sensitized Splenocytes to Identify Glioma by MRI: A Preliminary study. Magnetic Resonance in Medicine 2007;58:519-26.

MRI's role in the modern diagnosis and management of MS

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Magnetic resonance imaging (MRI) was first used to image living subjects with multiple sclerosis (MS) ~25 years ago. Despite limitations of early relatively low field scanners and crude imaging sequences it was immediately evident that MRI provided an important portal to the pathology of this disease; pathology that previously could only be adequately sampled once in a patient's lifetime. Early studies based completely on the constraints placed on the relaxation properties of water molecules imposed by the structural characteristics of their microenvironment provided an early look at well established MS lesions or plaques. The introduction of paramagnetic contrast agents and their use in longitudinal serial scanning of patients unveiled the very dynamic nature of lesion formation and evolution over time. MRI has secured an important place in the diagnosis of the disease, in monitoring the evolution of the disease process and in the investigation of the potential benefit of new treatments for the disorder. MRI also provides important information for the categorization of disease activity that may be crucial for understanding the results of investigations into the aberrant immune responses of MS patients; phenomena that appear to be fundamental to our current understanding and approaches to manage this potentially devastating disorder. At least in the early clinical phases of MS, MRI not only aids in securing the diagnosis of the disease, but also enables short-term prognosis as to the disease's course. While the specificity of MRI findings for the pathologic changes that are present in affected tissues places some limitations on the correlation between quantitative MRI findings and clinical disability that currently constrains the use of MRI as a true surrogate, they have not undermined the ability of serial MRI to measure the biological efficacy of new therapeutic agents. Central issues that are actively being addressed include whether composite measures of conventional MRI parameters can provide better concomitant and predictive correlations with clinical disease status in the short or long term, if better pulse acquisitions or higher field imaging can better delineate cortical pathology and its evolution, and do the more global changes in brain pathology as seen with advanced MRI approaches reflect distributed change in neuronal pathways that are the consequences of classic focal lesions or do they bespeak an independent neurodegenerative process.

Seeing the Invisible by Solution NMR Spectroscopy

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Many biochemical processes proceed through the formation of functionally important intermediates. For example, ligand binding, enzyme catalysis and protein folding may all involve the formation of one or more intermediates along the reaction coordinate connecting the initial and final protein states. A complete understanding of each process, requires, therefore, characterization of these intermediates in detail. While methods exist for studying the endpoints of these processes at atomic resolution in many cases, similar studies of the intermediates remain elusive. NMR methods for seeing such 'invisible' states will be described, along with a number of applications to protein folding illustrating the power of the methodology. A related problem is one where 'near invisible' systems are studied by solution NMR, such as supra-molecular structures, with molecular weights in the MDa range. New labeling approaches and NMR experiments will be described that bring such systems into focus and applications to a number of supra-molecular systems will be presented.

Clean STD-NMR Spectrum for Improved Detection of Ligand-Protein Interactions with Low Concentration Protein

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Saturation transfer difference (STD)-NMR has been widely used to screen compound libraries for binding activity with proteins and to determine the binding epitopes of the ligands. However, false positives were observed in STD spectra due to power spillover from RF irradiation. A Clean STD-NMR method is proposed to improve the quality of the spectra by digital-editing of two STD spectra to generate a concatenated difference spectrum. The effect of concatenation of the two STD spectra is the reduction of the false positives. Clean STD-NMR method achieved higher degree of resonance saturation which results in stronger STD signals. Thereby, the sensitivity of the Clean STD signal was enhanced by about 3-times compared with that of original STD signal for a complex of DNA-protein (dCTGTCT-CUGBP1ab (25.2 kDa)). In the sample of the mixture of a small ligand and a protein BSA (bovine serum albumin), the interactions between the two molecules were detected at a BSA concentration as low as 0.1.

Ang-1 Reduces Blood-Spinal Cord Barrier Permeability and Lesion Volume in the Acute Phase of Spinal Cord Injury: MRI and Histological Studies

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Methods:

1. Two days following acute administration of Ang1 or vehicle alone (control) in experimental SCI, DCE-MRI studies were performed to noninvasively track spatial changes in BSCB permeability. DCE-MRI data was analyzed using a two compartment pharmacokinetic model. Immunohistochemistry for markers of vascular endothelium and BSCB breakdown was also performed on excised spinal cords.
2. High-resolution anatomical MRI scans were performed for quantification of lesion volume.
3. Neuromotor recovery was assessed using the Basso-Beattie-Bresnahan (BBB) scale.

Results:

1. At the injury epicenter and at sites 6 mm caudal/rostral to the lesion on DCE-MRI, Ang1-treated animals had improved vascular integrity via reduced BSCB permeability compared to vehicle controls ($p < 0.002$).
2. Ang1-treated animals had significantly reduced SCI lesion volume compared to vehicle controls ($p < 0.025$).
3. BBB scores of Ang1 treated animals were significantly greater than those of vehicle controls ($p < 0.04$).

Conclusion: This study suggests a beneficial role of acutely administered Ang1 in reducing BSCB permeability, reducing lesion volume, and improving neuromotor recovery in the acute phase of SCI. Longitudinal studies are underway to assess the long-term effect of Ang1 on recovery from SCI.

¹H MR Spectroscopy Markers of Alzheimer's Disease

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The development of new therapies for Alzheimer's disease and other neurodegenerative conditions has become of increasing societal importance given our aging population and increasing longevity, combined with the fact that this disease typically begins late in life. An important clinical group for early diagnosis and treatment of dementia are people with mild cognitive impairment, who have a higher risk of progression to Alzheimer's disease than their cognitively normal peers. Various imaging markers are being tested as the surrogates for the pathology of Alzheimer's disease, for early diagnosis in patients with mild cognitive impairment, which may allow new therapies to be developed more quickly, and increase the probability of success in the clinical trials. Non-invasive neuroimaging techniques such as ¹H magnetic resonance spectroscopy (¹H MRS) may have an important role in the clinical evaluation of Alzheimer's disease for early diagnosis and monitoring of disease activity. ¹H MRS is unique among diagnostic imaging modalities, because the signals from several different metabolites are measured within a single examination period. Each metabolite in turn is sensitive to a different aspect of in-vivo pathological processes at the molecular or cellular level. This presentation will give an overview of ¹H MRS findings in Alzheimer's disease. It will focus on the pathological correlates of antemortem ¹H MRS metabolite changes in Alzheimer's disease, longitudinal ¹H MRS changes and the clinical progression of Alzheimer's disease and ¹H MRS changes as a prognostic marker in patients with mild cognitive impairment.

Kinetics of macromolecular interactions by NMR

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NMR spectroscopy is a very powerful tool to analyze kinetics on a wide range of timescale. My presentation will focus on the relationship between kinetics of macromolecular interactions and NMR parameters. By showing numerical simulations based on the McConnell equations for N -site exchange models, I will explain theoretical background and experimental approaches to determine kinetic rate constants for various macromolecular processes. Topics include our recent studies of protein translocation between DNA molecules, protein-peptide association/dissociation, and protein redox reactions. Using the similar theoretical framework, I will also explain how macromolecular kinetics and apparent paramagnetic relaxation enhancement (PRE) are related.