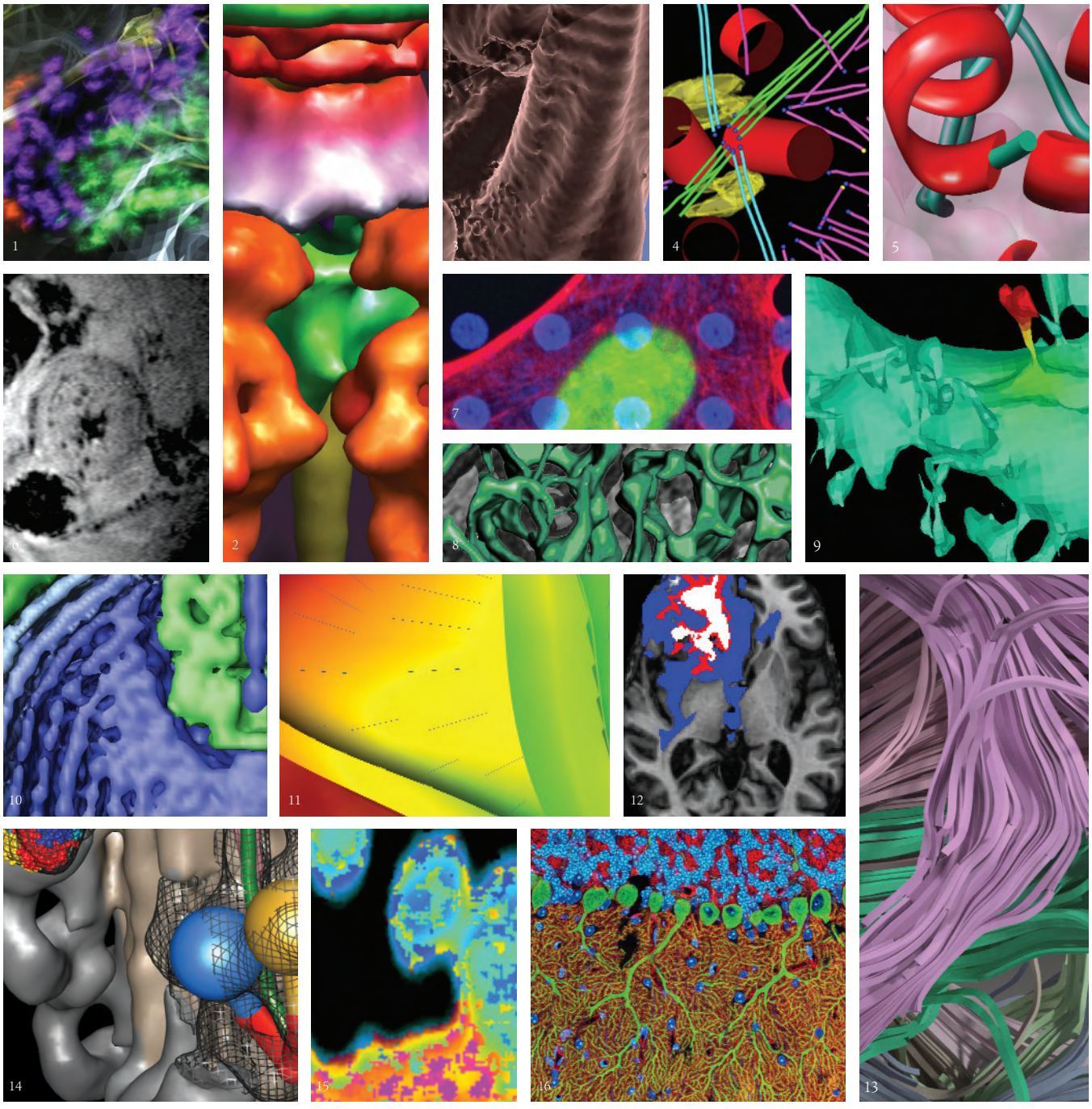
Transforming Biomedical Research & Health Care Through Technology Innovation

THE CATALYZING EFFECTS OF RESEARCH RESOURCES



NATIONAL CENTER FOR RESEARCH RESOURCES

NATIONAL INSTITUTE FOR BIOMEDICAL IMAGING & BIOENGINEERING

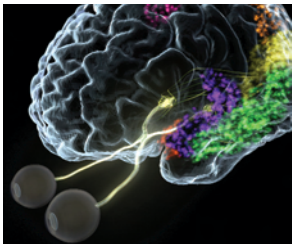
P41 PRINCIPAL INVESTIGATOR MEETING Bethesda, Maryland 19-20 JUNE 2007

Transforming Biomedical Research & Health Care Through Technology Innovation

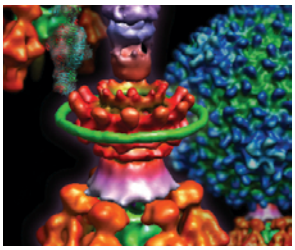
THE CATALYZING EFFECTS OF RESEARCH RESOURCES

NATIONAL CENTER FOR RESEARCH RESOURCES
NATIONAL INSTITUTE FOR BIOMEDICAL IMAGING & BIOENGINEERING
P41 PRINCIPAL INVESTIGATOR MEETING Bethesda, Maryland 19-20 JUNE 2007

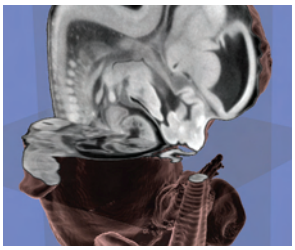
Table of Contents



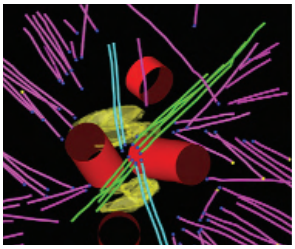
1. Complexity and variation in normal human brain anatomy and function. The Visual Systems image illustrates pathways of optical stimuli from the retina to the primary visual cortex. Laboratory of Neuro Imaging, UCLA, Arthur Toga (PI).



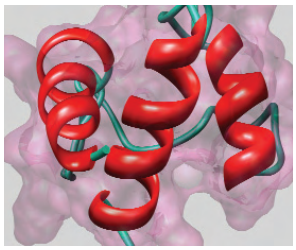
2. Bacteriophage P22 DNA Packaging and Infection Machinery: Visualization of the 1.7nm cryoEM reconstruction of the intact Bacteriophage P22. The portal complex (red) is hypothesized to change conformation when the virus is full of DNA, which signals the packaging motors to stop. Such a sensor may serve as a drug target in human viruses. Visualization by Gabriel Lander, National Resource for Automated Molecular Microscopy, The Scripps Research Institute, Bridget Carragher (PI).



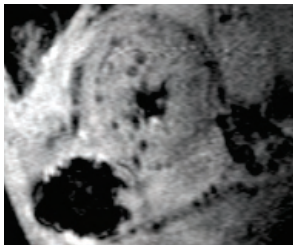
3. 3D microCT-based virtual histology allows for a fast, high-quality and inexpensive way to visually explore the 3D internal structure of mouse embryos so scientists can more easily and quickly see the effects of a genetic defect or chemical damage. J.T. Johnson III, M.S. Hansen, I. Wu, L.J. Healy, C.R. Johnson, G.M. Jones, M.R. Capecchi, C. Keller. "Virtual Histology of Transgenic Mouse Embryos for High-Throughput Phenotyping," *PLoS Genetics*, v.2, no.1, pp. 471-477. April 2006. The data were produced by Charles Keller, UT, San Antonio. The image was produced by the Center for Integrative Biomedical Computing, University of Utah, Chris Johnson (PI).



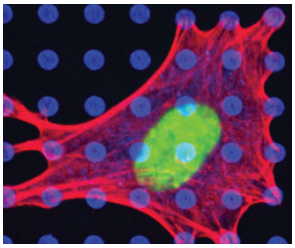
4. 3D projection of the microtubule-organizing center obtained by cryo-electron microscopy. Microtubules are organized by the MTOC, which is an organelle composed of a pair of centrioles surrounded by a matrix of pericentriolar material. Duplication of the MTOC is critical to formation of a spindle and the maintenance of genomic stability. Centrioles can also convert to basal bodies, needed for assembly of cilia, which are used for motility and signaling in all mammalian cells. Fixation of samples by high pressure freezing, followed by freeze-substitution, give improved preservation over conventional chemical fixation of cellular samples. Images are acquired in thick samples (›200 nm) using automated image acquisition on an intermediate voltage microscope. Dual-axis tomographic reconstruction using the IMOD software package generates 1.4nm thick tomographic slices for visualizing cellular details. A projection of the structures is reconstructed using the computational generated tomographic slices. Each slice can be rotated to obtain the best orientation. The model can be displayed and rotated to study the 3D geometry of structures. The work was conducted by Dr. Susan Dutcher, using the Boulder Laboratory for 3D Electron Microscopy of Cells, University of Colorado, Andreas Hoenger (PI).



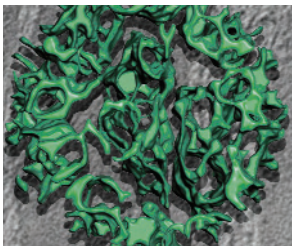
5. Structure of Cytochrome C, an essential component of energy transduction machinery in mitochondria, from a new stochastic protein sequence alignment method that vastly improves the quality of 3D protein models. The use of this method for comparative protein modeling was developed by the NRBSC Structural Bioinformatics group at the Pittsburgh Supercomputing Center (Marko, Stafford and Wymore, 2007, *J. Chem. Inf. Model.*, Vol. 47). Highly accurate models of protein structures are essential for rational structure-based drug design. National Resource for Biomedical Supercomputing, Pittsburgh Supercomputing Center. Joel Stiles (PI).



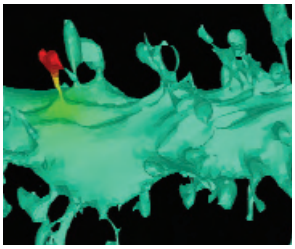
6. Transplanted Rat Heart MRI: As a non-invasive alternative to biopsy for the surveillance allograft rejection after heart transplantation, immune cell infiltration at the rejecting graft can be monitored by *in-vivo* MRI. Individual macrophages labeled with micrometer-sized superparamagnetic iron-oxide particles can be clearly seen as distinct punctate signal void in the *in-vivo* MR image of a rejecting heart. The MR image was acquired on a transplanted rat heart on day 6 post-transplantation, with 156 mm in-plane resolution obtained at 4.7-Tesla using a Bruker Biospec instrument housed in the Pittsburgh NMR Center for Biomedical Research, Chien Ho (PI).



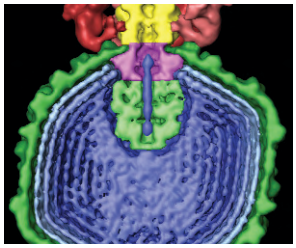
7. Cells generate traction forces upon adhesion to natural and synthetic scaffolds. This immunofluorescence image shows a smooth muscle cell (actin cytoskeleton in red and nucleus in blue) that has attached and spread across an array of elastomeric vertical micropillars (tips in blue). Deflections of the micropillars from the X-Y grid report forces generated by the cell against the substrate. This microfabricated system is being used to understand the role of mechanical forces in regulating cell adhesion and function, which is important for tissue regeneration and next generation implants. This image was produced by Chris Chen, one of the core collaborative projects for P41-EB 1046, Integrated Technologies for Polymeric Biomaterials, New Jersy Center for Biomaterials, Rutgers, the State University of New Jersey, Joachim Kohn (PI).



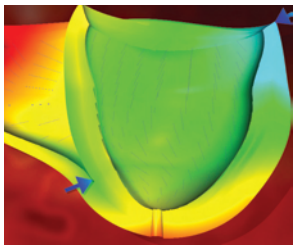
8. 3D membrane surfaces obtained by electron tomography of rat liver mitochondria. Electron tomography, developed at the Resource for Visualization of Biological Complexity, is revealing unexpected features of cellular structure and function. An example is how the topology of the energy-transducing inner membrane of mitochondria changes in different disease states and during cellular processes like apoptosis. The results suggest that diffusion pathways inside this organelle are regulated in response to stresses and energy demand and, conversely, that some disorders might be caused by aberrant membrane topology. Background: Slice from an electron tomogram of a section of frozen-hydrated rat liver. Resource for the Visualization of Biological Complexity, Wadsworth Center, Joachim Frank (PI).



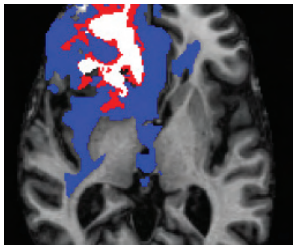
9. VCell simulation of membrane reactions coupled to lateral diffusion, showing a localized increase in PIP2 concentration in the membrane of a neuronal dendritic spine—a key mechanism underlying learning and memory. Understanding such mechanisms at the molecular and cellular level can ultimately lead to interventional strategies for both developmental problems in children and dementia in the aging brain. National Resource for Cell Analysis and Modeling, University of Connecticut Health Center, Leslie Loew (PI).



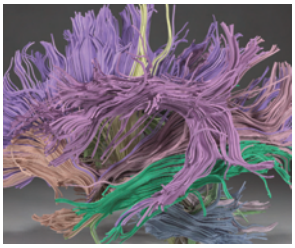
10. A cut-away surface view of the cryoEM 3D density map of epsilon15 bacteriophage. Each of the protein and dsDNA components is colored differently. Color gradient (light blue to darker blue) is used to indicate the likely packaging order of the dsDNA starting from outer layer towards inner layers and ending as the central straight segment of terminus (ref W. Jiang, J. Chang, J. Jakana, P. Weigele, J. King, and W. Chiu, 2006). Structure of epsilon15 bacteriophage reveals genome organization and DNA packaging/injection apparatus. *Nature,* 439: 612-6). National Center for Macromolecular Imaging, Baylor College of Medicine, Wah Chiu (PI).



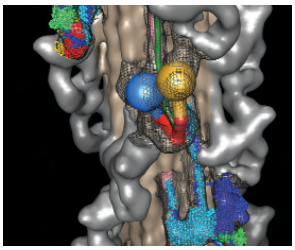
11. Multi-scale computational models of the heart that integrate from sub-cellular to organ system scales are being used by UCSD investigators to optimize protocols for cardiac resynchronization therapy (CRT) in congestive heart failure (CHF) patients, and to establish improved criteria for identifying patients most likely to benefit from CRT. The image is a cross-section of a finite element model of a dilated heart. The color code represents the activation time of a depolarization wave after bi-ventricular pacing with blue being 0 ms and red about 125 ms. National Biomedical Computation Resource, University of California San Diego. Peter Arzberger (PI).



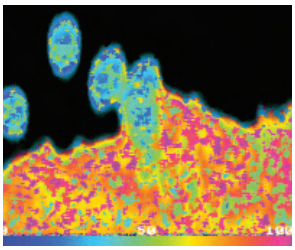
12. Tumor segmentation using multimodality MRI. The white area was tentatively attributed to tumor necrosis based on MRI properties. Its size correlated with tumor grade. Work by M.J. Donahue, J.O. Blakeley, J. Zhou, M.G. Pomper, J. Laterra, P.C.M. van Zijl, provided by the National Resource for Quantitative functional MRI, Peter van Zijl (PI), at the F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute and Department of Radiology, Division of MRI Research, Neurosection, Johns Hopkins University.



13. The white matter of the brain is the most recent addition to the growing list of brain regions from which functional information can be acquired non-invasively. Developing methods for post-processing for this very complex imaging data is one of the frontiers in medical image computing. This picture is the result of the analysis of diffusion tensor MR images, merged from a number of subjects and subjected to sophisticated statistical analysis, yielding a grouping of "virtual" white matter fiber tracts. One example for the use of this technology is in psychiatric research. In our collaboration with a psychiatry research group, this method is used to study fronto-temporal disconnectivity in patients with schizophrenia. Image generated by D. Banks; data provided by M. Shenton; analysis provided by C.F. Westin and L. O'Donnell. Neuroimaging Analysis Center, Brigham and Women's Hospital, Ron Kikinis (PI).



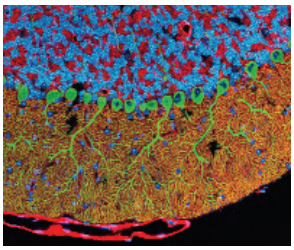
14. Detailed model of a muscle filament. An atomic-level model of a smooth muscle myosin protein was fit to the density map of a myosin thick filament fiber obtained via cryoelectron microscopy, thus yielding the first detailed model showing how myosin proteins are arranged in the filament. Models such as this are critical to understanding the structure of muscle filaments and how changes in the three-dimensional conformation of proteins cause muscle filaments to contract, thereby providing insight into diseases such as myasthenia gravis and myopathy. The model was created using the MultiScale extension to UCSF Chimera. Resource for Biocomputing, Visualization and Informatics, University of California San Francisco, Tom Ferrin (PI).



15. This image was generated using "multi-isotope imaging mass spectrometry (MIMS)," a technology that combines a novel type of secondary ion mass spectrometer (SIMS) with stable isotope labeling. A primary ion beam sputters the molecules of the surface of a sample, resulting in secondary ions, representative of the composition. After focusing with complex ion optics and separation and selection in the SIMS, the current of four ion masses is used to create four quantitative mass images of the same analyzed volume. One can then derive isotope ratio images to map the distribution, and quantify the accumulation, at the intracellular level, of molecules labeled with stable isotopes.

This image demonstrates for the first time in an adult mammal, the contrast between the high protein turnover in the hair cell body and the very low turnover in the stereocilia. The cochlea sample was taken from a mouse fed 15N leucine for 5 months. Turnover of the substrate increases the 15N/14N ratio, as denoted by the color-coded scale (see bottom). The 15N/14N isotope ratio observed in the main body of the hair cell was equivalent to that in the experimental diet, indicating 100% protein turnover (magenta). In contrast, stereocilia show ‹25% protein turnover (blue). The image is 5 x 5 microns. This opens a novel way to study inner ear functioning and deafness.

The image and the associated technology were generated at the "National Resource for Imaging Mass Spectroscopy" (NRIMS), P41 EB001974, Harvard Medical School and Brigham & Women's Hospital, Claude Lechene (PI).



16. Direct application of nanotechnology to bio-imaging: Mouse cerebellum* fluorescently labeled using 565 nm quantum dots to reveal the distribution of the IP3 receptors in the Purkinje neurons (shown green), phalloidin-rhodamine to label actin (shown red), and immunolabeling of glia cells** and DAPI staining of cell nuclei (combined in blue). High speed 2-photon fluorescence microscopy. Courtesy of Thomas Deerinck and Mark Ellisman (PI), the National Center for Microscopy and Imaging Research, University of California, San Diego.

** The cerebellum is a lobed structure located near the base of the brain that is primarily involved with the coordination of bodily movement and the development and recollection of physical skills. The size of the cerebellum in mammal species is a fairly reliable gauge of their physical aptitude and has been implicated in autism.*

*** Glia are the class of nerve cells that provide structural support for neurons. Also referred to as neuroglia, the cells are continuously capable of division and are more than ten times as abundant as neurons in the human brain. Glia, particularly astroglia, give rise to many forms of brain cancer.*

NATIONAL CENTER FOR RESEARCH RESOURCES
NATIONAL INSTITUTE FOR BIOMEDICAL IMAGING & BIOENGINEERING
P41 PRINCIPAL INVESTIGATOR MEETING Bethesda, Maryland 19-20 JUNE 2007

Agenda

Tuesday 19 June 2007
Location: Lister Hill Auditorium, NIH

0730

Departure - Suggested departure time from your hotel, planning for 60 minutes to get from the Hotel to Lister Auditorium (includes NIH security check). You need to take the Metro from the Bethesda Hyatt Regency Hotel to NIH Campus. Trains leave about every 5 minutes; a 3 minute ride; $1.35 fare. You can walk or take the NIH Shuttle from the Metro Station to Lister Hill Center (Bldg 38A). The NIH Campus Limited Shuttle takes 6 minutes to Lister Hill; the Red Line Shuttle leaves more often but takes about 20 minutes. Set up your posters on arrival. **Posters are to be set up during entire meeting.**

0845 - 0915

Welcome

- Dr. Barbara Alving, Director, NCRR
- Dr. Roderic Pettigrew, Director, NIBIB
- Overview of Meeting and Goals

0915 - 1015

Impact of NIH Investments (Session 1)

- Patrick Kochanek, M.D.: Director of the Safar Center for Resuscitation Research, Professor and Vice Chair of the Department of Critical Care Medicine, University of Pittsburgh School of Medicine
- Jonathan King, Ph.D.: Biology Department, Massachusetts Institute of Technology

1015 - 1045

Break

1045 - 1145

Impacts of NIH Investments (Session 2)

- Susan Dutcher, Ph.D: Professor and Interim Chair of the Department of Genetics at Washington University School of Medicine.
- David Drubin, Ph.D.: Professor and Chair of Cell and Developmental Biology at University of California at Berkeley.

1145 - 1225

Impact of NIH Investments (Session 3)

- James Chou, Ph.D.: Assistant Professor at Harvard Medical School

- Sean Mackey, M.D., Ph.D.: Co-Director, Pain Working Group, Neuroscience Institute, Stanford University

1225 - 1350

Lunch - Cafeteria

1350 - 1500

Impact of NIH Investments (Session 4)

- Joan Bailey-Wilson, Ph.D.: Co-Chief and Senior Investigator, Inherited Research Branch, Head, Statistical Genetics Section, National Human Genome Research Institute
- D. Branch Moody, M.D.: Brigham & Women's Hospital, Harvard University
- Julius Guccione, Ph.D.: Assistant Professor of Surgery, University of California at San Francisco
- Discussion

1500 - 1615

Break and poster viewing

1615 - 1735

New Technologies Developed by NCRR and NIBIB (Session 1)

- Mark Ellisman, Ph.D.: Director, National Center for Microscopy and Imaging Research, University of California, San Diego - Director, BIRN Coordinating Center
- Kamil Ugurbil, Ph.D.: Director, Center for Magnetic Resonance Research, University of Minnesota
- Carolyn Larabell, Ph.D.: Head, National Center for X-ray Microscopy, University of California, San Francisco / Lawrence Berkeley National Laboratory
- Kirk Shung, Ph.D.: Principal Investigator, Resource on Medical Ultrasonic Transducer Technology, University of Southern California

1735 - 1800

Poster removal. Lister Hill Bldg closes at 1800.

1900 - 2100

Dinner at Bethesda Hyatt Regency

Michael Rossmann, Ph.D.: Purdue University, Developing anti-viral strategies with the aid of synchrotrons, electron microscopes and super computer clusters.

Wednesday 20 June 2007
Location: Bethesda Hyatt Regency

0700 - 0800

Continental Breakfast

0800 - 0920

New Technologies Developed by NCRR and NIBIB (Session 2)

- Mehmet Toner, Ph.D.: Principal Investigator, Biomicroelectromechanical Systems (BioMEMS) Resource Center, Massachusetts General Hospital

- James Prestegard, Ph.D.: Principal Investigator, Resource for Integrated Glycotechnology, University of Georgia
- Stan Opella, Ph.D.: Director, Center for NMR Spectroscopy and Imaging of Proteins, University of California at San Diego
- Howard Halpern, M.D., Ph.D., Director, Very Low Frequency ERP Imaging in Vivo Physiology, University of Chicago

0920 - 0945
Break

0945 - 1100
NCRR and NIBIB separate meetings

1100 - 1200
Plenary Session: Overview by and Discussion with NIH on Status and Future for
- Clinical and Translation Science Awards (CTSA) See www.ctsaweb.org
- Roadmaps

1200 - 1400
Working Lunch ...
- Improvements for 2008
- Program Committee for 2008

Transforming Biomedical Research & Health Care Through Technology Innovation
THE CATALYZING EFFECTS OF RESEARCH RESOURCES

NATIONAL CENTER FOR RESEARCH RESOURCES
NATIONAL INSTITUTE FOR BIOMEDICAL IMAGING & BIOENGINEERING
P41 PRINCIPAL INVESTIGATOR MEETING Bethesda, Maryland 19-20 JUNE 2007

Organizing Committee

Scientific Program:

Peter Arzberger (Chair)	parzberg@ucsd.edu
Cathy Costello	cecmsms@bu.edu
Trisha Davis	tdavis@u.washington.edu
Chien Ho	chienho@andrew.cmu.edu
Wilfred Li	wilfred@sdsc.edu
John Markley	markley@nmrfam.wisc.edu

Local Arrangements

Peter van Zijl (Chair)	pvanzijl@mri.jhu.edu
Candace Herbster (Administrative contact)	herbster@kennedykrieger.org
Joe Gillen (Webmaster)	jgillen@mri.jhu.edu

Attendees (Listed Alphabetically)

Alving, Barbara alvingb@mail.nih.gov	NIH - NCRR
Arzberger, Peter parzberg@ucsd.edu	National Biomedical Computation Resource University of California, San Diego
Bailey-Wilson, Joan jebw@mail.nih.gov	NIH
Bennett, Brian bbennett@mcw.edu	National Biomedical Electron Paramagnetic Resonance Center Medical College of Wisconsin
Bigelow, Alan ab1260@columbia.edu	Radiological Research Accelerator Facility Columbia University
Bonetta, Laura bonettal@mail.nih.gov	NIH - NCRR
Brazhnik, Olga brazhnik@nih.gov	NIH - NCRR
Brenner, David djb3@columbia.edu	Radiological Research Accelerator Facility Columbia University
Brooks, Dana brooks@ece.neu.edu	Center for Integrative Biomedical Computing University of Utah
Brooks, Charles brooks@scripps.edu	Multiscale Modeling Tools for Structural Biology The Scripps Research Institute
Burlingame, Alma alb@cgl.ucsf.edu	Bio-Organic Biomedical Mass Spectrometry Resource University of California, San Francisco
Butler, Robert butlerrob@mail.nih.gov	NIH - NCRR
Camp, David dave.camp@pnl.gov	Proteomics Research for Integrative Biology Pacific Northwest National Laboratory
Capel, Malcolm capel@anl.gov	Synchrotron Radiation Structural Biology Resource Stanford University
Carragher, Bridget bcarr@scripps.edu	National Resource for Automated Molecular Microscopy The Scripps Research Institute
Castner, David castner@nb.engr.washington.edu	National ESCA and Surface Analysis Center for Biomedical Problems

	University of Washington
Cerione, Richard rac1@cornell.edu	Macromolecular Diffraction Biotechnology Resource Cornell University
Chait, Brian chait@rockefeller.edu	National Resource for Mass Spectrometric Analysis of Biological Macromolecules The Rockefeller University
Chance, Mark mark.chance@case.edu	Center for Synchrotron Biosciences Case Western Reserve University
Chen, Zhongping z2chen@uci.edu	Laser Microbeam and Medical Program University of California, Irvine
Chiu, Wah wah@bcm.edu	National Center for Macromolecular Imaging Baylor College of Medicine
Chou, James james_chou@hms.harvard.edu	Center for Magnetic Resonance Massachusetts Institute of Technology
Colleluori, Susan susan@mail.mmrrcc.upenn.edu	Resource for Magnetic Resonance and Optical Imaging University of Pennsylvania
Collier, Elaine ec5x@nih.gov	NIH - NCRR
Costello, Catherine cecmsms@bu.edu	Mass Spectrometry Resource for Biology and Medicine Boston University School of Medicine
Cynar, Skip scynar@ncmir.ucsd.edu	National Center for Microscopy and Imaging Research University of California, San Diego
D'Argenio, David dargenio@bmsr.usc.edu	Biomedical Simulations Resource (BMSR) University of Southern California
Dasari, Ramachandra rrdasari@mit.edu	Laser Biomedical Research Center Massachusetts Institute of Technology
Davis, Trisha tdavis@u.washington.edu	Yeast Resource Center University of Washington
Drubin, David drubin@berkeley.edu	Yeast Resource Center University of Washington
Dutcher, Susan dutcher@genetics.wustl.edu	Washington University School of Medicine
Ealick, Steven see3@cornell.edu	Undulator Resource for Structural Biology Cornell University
Eaton, Gareth geaton@du.edu	Very Low Frequency ERP Imaging In Vivo Physiology University of Chicago / University of Denver/ University of Maryland
Ellisman, Mark mark@ncmir.ucsd.edu	National Center for Microscopy and Imaging Research University of California, San Diego

Elston, Robert rce@darwin.case.edu	Human Genetic Analysis Resource Case Western Reserve University
Farber, Gregory farberg@mail.nih.gov	NIH - NCRR
Feld, Michael msfeld@mit.edu	Laser Biomedical Research Center Massachusetts Institute of Technology
Felix, Chris cfelix@mcw.edu	National Biomedical Electron Paramagnetic Resonance Center Medical College of Wisconsin
Freed, Jack jhf@msc.cornell.edu	National Biomedical Center for Advanced ESR Technology Cornell University
Freyer, James freyer@lanl.gov	National Flow Cytometry and Sorting Research Resource Los Alamos National Laboratory
Friedman, Fred fred.friedman@nih.hhs.gov	NIH - NCRR
Furenlid, Lars furen@radiology.arizona.edu	Center for Gamma-Ray Imaging University of Arizona
Gamble, Lara gamble@nb.engr.washington.edu	National ESCA and Surface Analysis Center for Biomedical Problems University of Washington
Gillian-Daniel, Annelynn gillian@nmrfam.wisc.edu	National Magnetic Resonance Facility at Madison University of Wisconsin-Madison
Glover, Gary gary.glover@stanford.edu	Center for Advanced Magnetic Resonance Technology at Stanford Stanford University
Gollub, Randy rgollub@partners.org	Center for Functional Imaging Technologies Massachusetts General Hospital
Gratton, Enrico egratton22@yahoo.com	Laboratory for Fluorescence Dynamics University of Califiornia, Irvine
Gray-mcguire, Courtney clg9@case.edu	Human Genetic Analysis Resource Case Western Reserve University
Gross, Michael L mgross@wustl.edu	Resource for Biomedical and Bio-Organic Mass Spectrometry Washington University
Guccione, Julius guccionej@surgery.ucsf.edu	National Biomedical Computation Resource University of California, San Diego
Halpern, Howard h-halpern@uchicago.edu	Very Low Frequency ERP Imaging In Vivo Physiology University of Chicago / University of Denver/ University of Maryland
Hao, Quan qh22@cornell.edu	Macromolecular Diffraction Biotechnology Resource Cornell University

Hedman, Britt hedman@ssrl.slac.stanford.edu	Synchrotron Radiation Structural Biology Resource Stanford University
Ho, Chien chienho@andrew.cmu.edu	Pittsburgh NMR Center for Biomedical Research Carnegie Mellon University
Hochstrasser, robin hochstra@sas.upenn.edu	Ultrafast Optical Processes Laboratory University of Pennsylvania
Hodgson, Keith hodgson@ssrl.slac.stanford.edu	Synchrotron Radiation Structural Biology Resource Stanford University
Hyde, James jshyde@mcw.edu	National Biomedical Electron Paramagnetic Resonance Center Medical College of Wisconsin
Irimia, Daniel dirimia@partners.org	Biomicroelectromechanical Systems (BioMEMS) Resource Center MGH / Harvard University
Irving, Thomas irving@iit.edu	Biophysics Collaborative Access Team Illinois Institute of Technology
Johnson, Chris crj@sci.utah.edu	Center for Integrative Biomedical Computing University of Utah
Kaplan, David david.kaplan@tufts.edu	Tissue Engineering Resource Center (TERC) Tufts University / MIT
Kapur, Tina tkapur@bwh.harvard.edu	National Resource Center for Image Guided Therapy Brigham and Women's Hospital, Harvard Medical School
King, Jonathan jaking@mit.edu	National Center for Macromolecular Imaging Baylor College of Medicine
Kochanek, Patrick kochanekpm@ccm.upmc.edu	Pittsburgh NMR Center for Biomedical Research Carnegie Mellon University
Kohn, Joachim kohn@rutgers.edu	Integrated Technologies for Polymeric Biomaterials Rutgers, the State University of New Jersey
Larabell, Carolyn carolyn.larabell@ucsf.edu	National Center for X-Ray Tomography University of California, San Francisco
Lawson, Kim klawson1@partners.org	National Resource Center for Image Guided Therapy Brigham and Women's Hospital, Harvard Medical School
Lechene, Claude nrims@rics.bwh.harvard.edu	National Resource for Imaging Mass Spectrometry Harvard University
Leigh, John jack@mail.mmrrcc.upenn.edu	Resource for Magnetic Resonance and Optical Imaging University of Pennsylvania

Levy, Abraham Levyabra@mail.nih.gov	NIH - NCRR
Li, Wilfred wilfred@sdsc.edu	National Biomedical Computation Resource University of California, San Diego
Loew, Leslie les@volt.uchc.edu	National Resource for Cell Analysis and Modeling University of Connecticut Health Center
Ludtke, Steven sludtke@bcm.edu	National Center for Macromolecular Imaging Baylor College of Medicine
Mackey, Sean smackey@stanford.edu	Center for Advanced Magnetic Resonance Technology at Stanford Stanford University
MacLeod, Rob macleod@cvrti.utah.edu	Center for Integrative Biomedical Computing University of Utah
Malloy, Craig craig.malloy@utsouthwestern.edu	Southwestern NMR Center for In Vivo Metabolism University of Texas Southwestern Medical Center
Mannella, Carmen carmen@wadsworth.org	Visualization of Biological Complexity Resource New York State Department of Health
Markley, John markley@nmrfam.wisc.edu	National Magnetic Resonance Facility at Madison University of Wisconsin-Madison
Marron, Mike marron@nih.gov	NIH - NCRR
Mastronarde, David mast@colorado.edu	Three-Dimensional Fine Structure of Cells and Tissues University of Colorado
McDermott, Gerry gerry.mcdermott@ucsf.edu	National Center for X-Ray Tomography University of California, San Francisco
McLaughlin, Alan mclaugal@mail.nih.gov	Other - non NIH or NCRR/NIBIB BTR program
Mechref, Yehia ymechref@indiana.edu	National Center for Glycomics and Glycoproteomics Indiana University
Merritt, Matthew matthew.merritt@utsouthwestern.edu	Southwestern NMR Center for In Vivo Metabolism University of Texas Southwestern Medical Center
Moody, Branch bmoody@rics.bwh.harvard.edu	Brigham & Women's Hospital, Harvard University
Morris, Scooter scooter@cgl.ucsf.edu	Resource for Biocomputing, Visualization, and Informatics University of California, San Francisco
Mukundan, Srini mukun001@mc.duke.edu	Integrated Center for In Vivo Microscopy Duke University Medical Center
Novotny, Milos novotny@indiana.edu	National Center for Glycomics and Glycoproteomics Indiana University

OConnor, Peter poconnor@bu.edu	Mass Spectrometry Resource for Biology and Medicine Boston University School of Medicine
Opella, Stanley sopella@ucsd.edu	Resource for NMR Molecular Imaging of Proteins University of California, San Diego
Orgel, Joseph orgel@iit.edu	Biophysics Collaborative Access Team Illinois Institute of Technology
Pettigrew, Roderic pettigrr@mail.nih.gov	NIH - NIBIB
Pierce, Michael hawkeye@uga.edu	Integrated Technology Resource for Biomedical Glycomics University of Georgia
Potter, Clint cpotter@scripps.edu	National Resource for Automated Molecular Microscopy The Scripps Research Institute
Prestegard, James H. jpresteg@ccrc.uga.edu	Resource for Integrated Glycotechnology University of Georgia
Puderbaugh, Ann puderba@mail.nih.gov	Resource for Magnetic Resonance and Optical Imaging University of Pennsylvania
Randers-Pehrson, Gerhard gr6@columbia.edu	Radiological Research Accelerator Facility Columbia University
Robinson, Howard hhr@bnl.gov	Macromolecular Crystallography at the National Synchrotron Light Source Brookhaven National Laboratory
Rosen, Bruce bruce@nmr.mgh.harvard.edu	Center for Functional Imaging Technologies Massachusetts General Hospital
Rossmann, Michael mr@purdue.edu	Purdue University
Sayre, Mike sayrem@mail.nih.gov	NIH - NCRR
Sheeley, Douglas sheeleyd@mail.nih.gov	NIH - NCRR
Shung, Kirk kkshung@usc.edu	Resource on Medical Ultrasonic Transducer Technology University of Southern California
Smith, Peter JS psmith@mbl.edu	BioCurrents Research Center Marine Biological Laboratory, Woods Hole
Stiles, Joel stiles@psc.edu	High Performance Computing for Biomedical Research Pittsburgh Supercomputing Center
Superfine, Richard rsuper@physics.unc.edu	Computer Integrated Systems for Microscopy and Manipulation University of North Carolina

Swain, Amy swaina@mail.nih.gov	NIH - NCRR
Sweet, Robert sweet@bnl.gov	Macromolecular Crystallography at the National Synchrotron Light Source Brookhaven National Laboratory
Tajkhorshid, Emad emad@life.uiuc.edu	Resource for Macromolecular Modeling and Bioinformatics University of Illinois at Urbana-Champaign
Tempany, Clare ctempany@bwh.harvard.edu	*National Resource Center for Image Guided Therapy Brigham and Women's Hospital, Harvard Medical School
Timpane, David timpaned@mail.nih.gov	NIH - NCRR
Tingle, Marjorie tinglem@mail.nih.gov	NIH - NCRR
Toga, Arthur toga@loni.ucla.edu	Laboratory of Neuro Imaging Resource UCLA School of Medicine
Toner, Mehmet mtoner@hms.harvard.edu	Biomicroelectromechanical Systems (BioMEMS) Resource Center MGH / Harvard University
Troxler, Thomas ttroxler@sas.upenn.edu	Ultrafast Optical Processes Laboratory University of Pennsylvania
Turteltaub, Ken turteltaub2@llnl.gov	National Resource for Biomedical Accelerator Mass Spectrometry University of California, LLNL
Ugurbil, Kamil kamil@cmrr.umn.edu	NMR Imaging and Localized Spectroscopy CMRR, University of Minnesota
van Zijl, Peter pvanzijl@mri.jhu.edu	Resource for Quantitative Functional MRI Kennedy Krieger Institute and Johns Hopkins University
Vicini, Paolo vicini@u.washington.edu	Resource Facility for Population Kinetics (RFPK) University of Washington
Wall, Joseph wall@bnl.gov	Stem Mass Mapping and Heavy Atom Labeling of Biomolecules Brookhaven National Laboratory
Wetzel, Arthur awetzel@psc.edu	High Performance Computing for Biomedical Research Pittsburgh Supercomputing Center
Woolfenden, James npreble@radiology.arizona.edu	Center for Gamma-Ray Imaging University of Arizona
Wymore, Troy wymore@psc.edu	High Performance Computing for Biomedical Research Pittsburgh Supercomputing Center

Zaia, Joseph
jzaia@bu.edu

Mass Spectrometry Resource for Biology and Medicine
Boston University School of Medicine

Zhu, Xiaofeng
xzhu1@darwin.case.edu

Human Genetic Analysis Resource
Case Western Reserve University

Zipfel, Warren
wrz2@cornell.edu

Developmental Resource for Biophysical Imaging Opto-Electronics
Cornell University

Speaker Abstracts

Tuesday 0915 - 0945
Patrick Kochanek
Pittsburgh NMR Center for Biomedical Research
Carnegie Mellon University

Tuesday 0945 - 1015
Jonathan King
National Center for Macromolecular Imaging
Baylor College of Medicine

Tuesday 1045 - 1115
Susan Dutcher
Washington University School of Medicine

Tuesday 1115 - 1145
David Drubin
Yeast Resource Center
University of Washington

Tuesday 1145 - 1205
James Chou
Center for Magnetic Resonance
Massachusetts Institute of Technology

Tuesday 1205 - 1225
Sean Mackey
Center for Advanced Magnetic Resonance Technology at Stanford
Stanford University

Tuesday 1410 - 1430
Branch Moody
Brigham & Women's Hospital, Harvard University

Tuesday 1430 - 1450
Julius Guccione
National Biomedical Computation Resource
University of California, San Diego

Tuesday 1615 - 1635
Mark Ellisman
National Center for Microscopy and Imaging Research

University of California, San Diego

Tuesday 1635 - 1655
Kamil Ugurbil
NMR Imaging and Localized Spectroscopy
CMRR, University of Minnesota

Tuesday 1655 - 1715
Carolyn Larabell
National Center for X-Ray Tomography
University of California, San Francisco

Tuesday 1715 - 1735
Kirk Shung
Resource on Medical Ultrasonic Transducer Technology
University of Southern California

Wednesday 0820 - 0840
James H. Prestegard
Resource for Integrated Glycotechnology
University of Georgia

Wednesday 0840 - 0900
Stanley Opella
Resource for NMR Molecular Imaging of Proteins
University of California, San Diego

Wednesday 0900 - 0920
Howard Halpern
Very Low Frequency ERP Imaging In Vivo Physiology
University of Chicago / University of Denver/ University of Maryland

MAGNETIC RESONANCE IMAGING APPLICATIONS TO RESUSCITATION RESEARCH

PM Kochanek, LM Foley, TK Hitchens, AM Dennis, M Manole, RSB Clark, H Bay r, C Ho
Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine and Pittsburgh NMR Center for Biomedical Research, Carnegie Mellon University
www.safar.pitt.edu
kochanekpm@ccm.upmc.edu

Our investigative team at the Safar Center for Resuscitation Research has carried out a number of studies at the Pittsburgh NMR Center for Biomedical Research applying novel magnetic resonance imaging (MRI) methods across a broad spectrum of models in the field of resuscitation research – including resuscitation from experimental traumatic brain injury (TBI), TBI combined with hemorrhagic shock, and experimental cardiopulmonary arrest. We have used rat and mouse models relevant to both adult and pediatric insults that carefully mimic the clinical condition through the insult phase and both pre-hospital and hospital phases of resuscitation. Our MRI applications have focused on cerebral resuscitation and have included serial, non-invasive, regional assessment of cerebral blood flow (CBF) using the arterial spin-labeling method, study of the inflammatory response in the brain using paramagnetic iron oxide particles to label macrophages, and assessment of blood-brain barrier permeability. Our work has produced novel insight into post-resuscitation events in the evolution of secondary damage and repair in the brain and these collaborative studies have provided important parallel physiological information to the molecular studies that are ongoing at the Safar Center in these models. In this presentation, findings from the three specific areas of investigation listed above will be presented. Specifically, using perfusion imaging, we have performed the first studies mapping CO_2 reactivity after experimental TBI in rats, assessed the effects of multiple anesthetics on CBF in normal and brain injured rats, quantified the effect of adenosine analogs on regional CBF in normal and brain injured rats, performed the first studies of regional CBF in mice after experimental TBI, and the first serial, regional study of CBF alterations after asphyxial cardiac arrest in immature (post-natal day 17) rats. We have also been assessing the impact of periods of hypotension produced by hemorrhagic shock in mice on regional CBF after TBI and the effect of novel resuscitation strategies in this setting. Recently, we have also used paramagnetic iron oxide articles to label macrophages and have tracked their accumulation after TBI in mice. This collaboration has served as a key resource for multiple clinician-scientists and scientists training at both institutes, including fellows in the fields of pediatrics critical care medicine and emergency medicine funded by a T-32 from NICHD. Collaboration with the Pittsburgh NMR Center has provided Safar Center investigators a resource that has been instrumental to acquisition of a program project and RO-1 awards in TBI from NINDS, and an RO-1 on asphyxial cardiac arrest in developing rats from NICHD. Finally, the application of MRI methods to study CBF and blood-brain barrier permeability after the combination of TBI and hemorrhagic shock is an important component of a newly funded program project award from the United States Army that is examining novel resuscitation strategies in a model that is highly relevant to the recent epidemic of blast-induced TBI in military and civilian terrorist attacks. Support P-41EB-001977, NS30318, NS38087, HD045968, T-32 HD040686 from NIH, and PR054755 W81XWH-06-1-0247 from the United States Army.

Advances in Revealing the Structure and Assembly of dsDNA Viruses

Jonathan King
Dept of Biology, MIT, Cambridge, MA 02139, jaking@mit.edu,
http://web.mit.edu/king-lab/www/index.html

Viral infections continue to be a major burden to human and animal health. Formation of infectious virions require complex intracellular assembly processes. For viruses with double stranded DNA, the original advances came from studies of bacterial viruses. As a result of the isolation of mutants which interfered with virion assembly, and the identification of phage proteins, it has been possible to identify all the steps in the assembly of dsDNA phages such as T4, T7, Lambda and P22. Among the unexpected features were that the capsid shells were assembled empty of DNA, with the DNA subsequently being pumped into the precursor shell. Assembly of these procapsids required scaffolding proteins, which are removed from the procapsids during DNA packaging and are absent from the mature virions. The machinery for the critical DNA packaging and DNA injection steps requires a ring or portal of 12 subunits, located at only one of the twelve icosahedral vertices.

Among the valuable tools enabling these findings were cryo-electron microscopy and image processing developed by the NCRR facilities at Baylor Medical Center. The interactions with NCRR scientists Wah Chiu, Juan Chang, and Wen Jiang (now at Purdue) opened up valuable and ongoing scientific collaborations, far broader than the initial projects.

The key features in dsDNA phage assembly – scaffolding proteins, packaging of DNA into a preformed shell, and a unique portal ring – have subsequently been discovered in dsDNA animal viruses such as Adenovirus and Herpesvirus. Steps in virion assembly are natural targets for the next generation of antiviral agents. Thus the most effective anti-HIV drugs act by inhibiting the protease critical for the processing and assembly of the viral capsid subunits. For other viruses, the steps in viral assembly involve predominantly non-covalent interactions, requiring a different class of inhibitors. Progress in characterizing the potential targets had been retarded by difficulties in determining the three dimensional structures of the proteins involved in intracellular assembly of virions, as well as difficulties in determining the organization of critical components, minor in number, but of great functional importance. Recent developments in cryo-electron microscopy and image processing have breached this barrier. These methods have revealed in unprecedented detail the organization of the DNA injection apparatus, and just recently the 3-dimenisonal fold of the capsid protein directly from cryo-electron microscopy. These advances are now being mobilized to characterize the structure of critical proteins in Herpesvirus assembly and infection, which should open new targets for therapeutic development.

Chang, J., Weigele, P., King, J., Chiu, W., and Jiang, W. (2006) Cryo-EM Asymmetric reconstruction of Bacteriophage P22 reveals organization of its DNA packaging and infecting machinery. *Structure*, **14**, 1073-1082.

Jiang, W., Chang, J., Jakana, J., Weigele, P., King, J. and Chiu, W. (2006) Structure of epsilon15 bacteriophage reveals genome organization and DNA packaging/injection apparatus. *Nature*, **439**, 612-616.

Using cryo-electron tomography to understand cilia and basal body function

Susan K. Dutcher[1], Eileen O'Toole[2], Thomas Giddings[2], and J. Richard McIntosh[2]
[1]Department of Genetics, Washington University St Louis, MO [2]MCD Biology, University of Colorado, Boulder, CO
Boulder Laboratory for 3-D Electron Microscopy of Cells
http://bio3d.colorado.edu; dutcher@genetics.wustl.edu

In the last five years the importance of cilia and basal bodies in human disease has become increasing clear. A variety of mutations that alter cilia and/or basal body-associated proteins in mammals give rise to embryonic lethality, polycystic kidney disease, retinal degeneration, obesity, diabetes, polydactyly, hydrocephaly, and primary ciliary diskinesia. Understanding the structure of these organelles in wild-type and mutant cells in model organisms is an important step to understanding their role in humans. In addition, basal bodies can convert to centrioles, which are a key component of the microtubule-organizing center of cells that play roles in chromosome segregation. We use the unicellular green alga, *Chlamydomonas,* as our model for understanding cilia and basal bodies. Cilia are complex machines that have over 400 polypeptides that are organized into a variety of structures in motile cilia. Motility is generated by the action of the dynein arms. The dynein regulatory complex, radial spokes and central pair microtubules regulate the activity of the multiple dynein arms. Mutations that block the assembly of these structures in *Chlamydomonas* have been analyzed by averaged electron microscopic images. These mutant strains will be excellent material for analysis by cryo-electron tomography.

Mutants that have defects in assembly rather than function often lack or have altered numbers of cilia. Cryo-electron tomography of wild-type cells has revealed several new features of basal bodies. An important structure is amorphous ring of material at the proximal end of the basal bodies that we hypothesize is required for initiation of basal body/centriole assembly. Mutants with defects in this ring have profound effects on basal body/centriole assembly. Two novel isoforms of tubulin, delta and epsilon-tubulin, are conserved in all organisms with triplet microtubules from *Giardia* to humans. Analysis of *Chlamydomonas* mutants suggests roles in basal body/centriole assembly. The mutants block the assembly of triplet and doublet microtubule blades.

Comparative genomics using ciliated and nonciliated organisms identifies a large subset of basal body and ciliary genes. This data set was used to identify the *BBS5* gene. Mutations in patients result in a wide spectrum of phenotype described above. Reduction in the Bbs5 protein in *Chlamydomonas* causes a loss of cilia and cryo-electron microscopy reveals a defect in the basal bodies. The analysis of other genes found by this analysis has revealed defects in the organization of other cytoskeletal fibers. Cryo-electron tomography provides an important tool for the analysis of basal body and ciliary defects that may have profound effects on human health.

Proteomics and functional genomics of the yeast cytoskeleton

David G. Drubin[1], Jonathan Wong[1], Yuko Nakajima[1], Iain Cheeseman[1], Stefan Westermann[1], Georjana Barnes[1], John Yates, III[3], Stanley Fields[2,5], Tony Hazbun[2,5]
1. Department of Molecular and Cell Biology, UC Berkeley
2. Department of Genome Sciences, University of Washington
3. Scripps Research Institute
4. Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University
5. Yeast Resource Center
drubin@berkeley.edu, http://mcb.berkeley.edu/labs/drubin_barnes/

The mitotic spindle is a complex network of proteins that drives the segregation of chromosomes in eukaryotes. To strengthen our understanding of the composition, organization, and regulation of the mitotic spindle, we have performed proteomic and functional genomic studies in collaboration with the Yeast Resource Center. We performed a system-wide two-hybrid screen on 94 proteins implicated in spindle function in *Saccharomyces cerevisiae*. We identified 605 predominantly novel interactions involving 303 distinct prey proteins. We uncovered a pattern of extensive interactions between spindle proteins reflecting the intricate organization of the spindle. Furthermore, we observed novel connections between kinetochore complexes and chromatin-associated proteins including 1) the Ino80 and SWI/SNF chromatin remodeling complexes; 2) histone acetyltransferases and deacetylases; and 3) subunits of the Mediator complex. The wealth of protein interactions provides a valuable tool for modeling the physical connections within the mitotic spindle and with other nuclear processes. The Aurora kinase Ipl1p plays a crucial role in regulating kinetochore-microtubule attachments in budding yeast, but the underlying basis for this regulation was not known. To identify Ipl1p targets, we first purified 28 kinetochore proteins from yeast protein extracts. These studies identified five novel kinetochore proteins and defined two novel kinetochore sub-complexes. We then used mass spectrometry to identify 18 phosphorylation sites in 7 of these 28 proteins. Ten of these phosphorylation sites are targeted directly by Ipl1p, allowing us to identify a consensus phosphorylation site for an Aurora kinase. Our systematic mutational analysis of the Ipl1p phosphorylation sites demonstrated that the essential microtubule binding protein Dam1p is a key Ipl1p target for regulating kinetochore-microtubule attachments in vivo. To determine how kinetochore proteins are organized to connect chromosomes to spindle microtubules, and whether any structural and organizational themes are common to kinetochores of distantly related organisms, we used affinity chromatography and mass spectrometry to generate a map of kinetochore protein interactions. The budding yeast CENP-C homolog Mif2p specifically co-purified with a Cse4p-containing nucleosome, providing direct biochemical evidence that the CENP-A homologue Cse4p completely replaces histone H3 at the centromere. A novel four-protein Mtw1 complex, the Nnf1p subunit of which we show has homology to the vertebrate kinetochore protein CENP-H, also co-purified with Mif2p. Interestingly, Mif2p itself was found to be a critical in vivo target of the aurora kinase Ipl1p. Based on our data, we proposed that a molecular core consisting of CENP-A, -C, -H and Ndc80/HEC has been conserved from yeast to humans to link centromeres to spindle microtubules.

Progress in Structure Determination of Membrane Proteins by NMR

James Chou, Ph.D.
Assistant Professor, Biological Chemistry & Molecular Pharmacology, Harvard Medical School
Center for Magnetic Resonance, Massachusetts Institute of Technology
http://chou.med.harvard.edu james_chou@hms.harvard.edu

Integral membrane protein is one of the final frontiers of structural biology. My research group focuses on exploring and developing the utility of solution NMR spectroscopy for structural determination and dynamic characterization of helical, integral membrane proteins. Over the years, our access to the P41 funded NMR facility has helped us greatly in achieving success in a number of membrane protein projects. They include 1) structure determination of human phospholamban, a 30 kD homo-pentameric, channel-like protein in sarcoplasmic reticulum membrane that plays an important role in regulating heart muscle contractility, 2) structural studies of the M2 proton-selective channel and its homologs from influenza viruses, and 3) structural studies of the mitochondrion proton uncoupling proteins for understanding the roles of these proteins in energy metabolism, thermoregulation and the maintenance of normal mitochondrial membrane potentials in mammals. These initial successes foresee a bright future of NMR spectroscopy in structure determination of the small- to medium-sized helical membrane proteins.

Development and Applications of Real time fMRI Technology

Sean Mackey MD, PhD[1], Gary Glover, PhD[2]
[1]Stanford Department of Anesthesia, Division of Pain Management, [2]Department of Radiology
Center for Advanced Magnetic Resonance Technology at Stanford

Our research program has focused on development of novel methods for monitoring and controlling regions of brain activation using real time functional magnetic resonance imaging (rtfMRI). We have initially applied this technology to train individuals to modulate activation in specific brain structures that have been found in previous research to be implicated in the perception of pain.

Attaining control over specific neural processes is the primary goal of neuropharmacology and neurostimulation; it is also a critical objective of biological psychiatry and psychology. Beyond exerting the control necessary to emit or suppress specific behaviors, however, it is not clear whether people can learn to control the many specific, localized processes that occur within their brains. Training people to modulate specific neural processes has the potential to enable them to have greater control over the resulting behavior, cognition, or associated disease.

Recently we have demonstrated in a small study that patients with chronic pain can learn to control a specific region of their brain – the rostral anterior cingulate cortex (rACC) – that is associated with pain processing and perception (PNAS, December 2005, 102:51; 18626-31). Importantly, we found in that study that the increased control in localized brain activity was associated with improvement in pain control. In this preliminary study, we investigated a total of 36 healthy individuals and 12 chronic pain patients and found that by using rtfMRI to guide training, subjects were able to learn to control activation in the rACC, a higher level 'cognitive' area putatively involved in pain perception and regulation. When subjects deliberately induced increases or decreases in rACC fMRI activation using strategies related to manipulation of pain sensation, there was a corresponding change in the perception of pain caused by an applied noxious thermal stimulus. Four control experiments demonstrated that this effect was not observed after similar training conducted without rtfMRI information, or using rtfMRI information derived from a different brain region, or sham rtfMRI information derived previously from a different subject. Chronic pain patients were also trained to control activation in rACC but without application of noxious thermal stimulus and reported decreases in the ongoing level of chronic pain after training. These findings show for the first time that individuals can gain voluntary control over activation in a specific brain region given appropriate training and that voluntary control over activation in rACC leads to control over pain perception.

We have recently extended this preliminary work to target other brain related illness and specific brain regions associated with these conditions. These illnesses and their associated targeted brain regions include: depression (subgenual ACC), addiction (nucleus accumbens), and cognitive development (prefrontal cortex). Successful development of this technology has the potential to significantly advance our understanding of the neural dynamics and mechanisms of cognitive, emotional and sensory states in healthy controls and patients. This can significantly advance our knowledge of how these clinical states are generated and maintained and how learned control of specific brain regions leads to subsequent improvement. This will ultimately lead to a translation in knowledge of these basic brain mechanisms into a potentially clinically-useful treatment strategy that will reduce the suffering and improve the quality of life in patients with a variety of chronic brain related illnesses.

Mass spectrometric identification of antigens and virulence factors in the cell wall of *M. tuberculosis*

D. Branch Moody MD, Steven A. Porcelli, David C. Young, Walter Muhlecker, Vernon Reinhold, Bruce Reinhold Tan-Yun Cheng, Ildiko van Rhijn, Isamu Matsunaga and Catherine E Costello.

Division of Rheumatology, Immunology and Allergry, Brigham and Women's Hospital, Harvard Medical School and Boston University Mass Spectrometry Resource.

For decades, it was thought that $\alpha\beta$ T cells solely recognized peptide antigens bound to MHC proteins. However, CD1 proteins fold in a three-dimensional structure that is similar to that of MHC class I, forming a groove, which can bind small molecules for display to T cells. Using human T cell lines as reporters, we have carried out collaborative studies using mass spectrometric methods to identify mycobacterial ligands for human CD1a (dideoxymycobactins) CD1b (glucose monomycolate) and CD1c l(mannosyl phosphoisoprenoids) as well as synthetic ligands for human CD1d (phenolic pentamethyldihydrobenzofuran sulfonates). The discovery lipid ligands for CD1 proteins shows how the T cell repertoire can recognize and react to lipid, glycolipid and lipopeptide antigens through direct contact with the variable regions of T cell receptor proteins. These surprising findings provide new approaches to use lipids in modulating T cell function for development of vaccines, adjuvants and diagnostic tests. After installation of high throughput liquid chromatography mass spectrometry systems, it is now possible to rapidly and sensitively interrogate changes in *M. tuberculosis* cell wall structures in response to defined variables. Using a new mass spectrometric profiling system, we have identified lipids altered during the transition to cellular growth and lipids regulated by polyketide synthases and sigma transcription factors. These studies are aimed developing a basic understanding of how host macrophages and T cells recognizes lipids and how these lipids function in the pathogenesis of tuberculosis, a disease that kills 2 million people worldwide each year.

Virtual Tools for Cardiac Ventricular Remodeling Surgery

Julius Guccione[1], Mark Ratcliffe[1], and Andrew McCulloch[2]
[1]UCSF and [2]UCSD
Contact for Research: Julius Guccione, guccionej@surgery.ucsd.edu
National Biomedical Computation Resource (http://nbcr.net/)
NBCR Contact: parzberg@ucsd.edu

The goal of this biomedical research (R 01 HL77921) is the quantification of regional myocardial material properties in the form of three-dimensional constitutive equations or stress-strain relationships through the development, validation and application of an easy-to-use software tool. Such a software tool is proving to be of great value in the design and evaluation of new surgical and medical strategies to treat and/or prevent infarction-induced heart failure. Once the constitutive equation for the myocardium is established the effect of therapeutic changes on regional geometry (i.e., surgical remodeling) and/or material properties (i.e., medicine, gene therapy, cell therapy) can be evaluated and the success or failure of a proposed therapy predicted. With clinical experience, such a tool could be used as a diagnostic modality to risk stratify patients early after a myocardial infarction who are at risk for adverse remodeling and the development of heart failure. The utility of the software tool that we are continuing to develop is therefore of extreme value.

Using finite element software developed specifically for the heart (*Continuity*), the PI and Co-Investigator Dr. Mark Ratcliffe, together with the staff of their Cardiac Biomechanics Laboratory at the UCSF/VA Medical Center, already have determined the effect of ventricular size and patch stiffness in surgical ventricular restoration (SVR). In the **S**urgical **T**reatments for **Isc**hemic **H**eart Failure (**STICH**) trial, a team of internationally known doctors is currently working with the NIH to determine the best treatment for patients with heart failure caused by coronary artery disease. The three treatments being compared in the STICH trial include: (1) medical therapy alone; (2) medical therapy with coronary artery bypass grafting (CABG); and (3) medical therapy with CABG and SVR.

Via the National Biomedical Computation Resource (P 41 RR08605), Co-Investigator Dr. Andrew McCulloch and his Cardiac Mechanics Research Group at UCSD are continuing to extend and improve *Continuity* for a wide range of applications in cardiovascular biomechanics and electrophysiology. *Continuity* is component-based using a very high-level scripting language for component integration, is easily linked to other packages and libraries and is designed for multi-scale structural integration from cellular biophysics to whole organ physiology as well as functional integration from mechanics to electrophysiology and regulatory mechanics. Moreover, detailed three-dimensional (3-D) models of ventricular geometry and muscle fiber architecture are already available.

An important advantage of *Continuity* over commercial finite element software is its ability to analyze stresses in anisotropic materials (mechanical properties depend on test specimen orientation) that undergo large deformation. We and others using *Continuity* have been exclusively modeling the ventricles as composed of such material for nearly twenty years. Moreover, these models have been based directly on measured 3-D muscle fiber angle and myocardial sheet angle distributions (using a variety of measurement techniques). Furthermore, these models have been validated experimentally under a wide range of conditions. More recent models now also include the transmural heterogeneity of cellular excitation-contraction coupling mechanisms.

Technology Developments and Applications at the National Center for Microscopy and Imaging Research: Multi-scale Imaging and of the Nervous System – Filling Gaps in our Knowledge Framework

Mark H. Ellisman
Professor, UCSD Departments of Neurosciences and Bioengineering
Director, the National Center for Microscopy and Imaging Research (NCMIR)
(http://www.ncmir.ucsd.edu/)

A grand challenge in neuroscience research is to understand how the interplay of structural, chemical, and electrical signals in nervous tissue gives rise to behavior. Experimental advances of the past decades have propelled the ability of the neuroscientists to obtain data from the level of molecules to the gross nervous system. Scientists have begun the arduous and challenging process of adapting and assembling neuroscience data at all scales of resolution and across disciplines into computerized databases. Techniques combining intracellular injection with subsequent photo-oxidation now permit the acquisition and correlation of light and electron microscopic images. These correlated data sets integrate gross structural imaging with high resolution electron microscopy of individual cells. Through the use of high voltage electron microscopy and electron microscopic tomography, high resolution data can now be imaged from thick sections, revealing large numbers of detailed structures situated across a wider and deeper contextual landscape.

In particular, these technological advances in bioimaging are providing new insights into three critical areas of neurobiology: 1) New understanding of the complexities of neurotransmission across the synapse is now possible from simulations of physiology using the precise geometry of synapse structures quantified by electron tomography, 2) Multi-scale examination of synaptic spines by correlated three-dimensional (3D) light and high voltage electron microscopy is helping to advance the study of neuroanatomical differences in the brain associated with mental retardation—with implications for the underlying mechanisms of learning and memory, 3) New views have been obtained leading to an important new understanding of the delineation of territories between the most abundant non-neuronal cell type in the brain, the astrocyte. This was achieved through the application of correlated light microscopy and high energy electron microscopy.

This brief presentation will review several new technologies and key findings in each of these activity areas and consider implications for future work to propel research to improve diagnosis and treatment of disorders of the nervous system.

IMAGING at ULTRAHIGH MAGNTETIC FIELDS

Kamil Ugurbil

University of Minnesota, Center for Magnetic Resonance Research (www.cmrr.umn.edu)

The central focus of the Core and Collaborative projects in the BTRR located at the University of Minnesota is to develop imaging methods and engineering solutions for the use of ultrahigh[1] magnetic fields for magnetic resonance imaging and spectroscopy, and the application of these techniques to obtain unique information on aspects of organ *function*, *perfusion*, *oxygen extraction*, *metabolism*, and *anatomy* non-invasively in humans, and intact animals. Ultrahigh fields pose numerous serious challenges for imaging biological objects the size of the human head and human torso. As the magnetic field increases, the frequency of the electromagnetic waves that must be employed for exciting magnetic nuclei in the body also increase. Early in the history of MRI, frequencies of ~10 MHz, well below ultrahigh field frequencies (300 MHz and above) were thought to be difficult because they were expected not to penetrate the human body significantly. While this concept was proven wrong, still, electrodynamic effects associated with frequencies of ~200 MHz and above were shown to indeed impose severe technical challenges. In addition, it was also expected, albeit with no experimental data, that with increasing magnetic fields, contrast mechanisms, especially T_1 contrast, that enabled differentiation of different tissues, and pathologies also diminished. As a result, after initial unpromising results obtained in the corporate research laboratories of major manufacturers (i.e. Siemens, GE, Philips) in the late eighties, industry abandoned the development of such technology for a decade an a half. However, starting at about 1990, many of the problems associated with ultrahigh fields were solved in this BTRR center. We demonstrated that with appropriate engineering and methodological solutions, high and ultrahigh fields provide numerous advantages that include improved signal-to-noise (SNR) ratio and, in many applications, significantly improved contrast-to-noise ratio (CNR). It was, for example, very rapidly demonstrated that T_1 images with excellent CNR can be obtained by designing sequences that were tolerant of RF inhomogeneities and that in many contrast mechanisms, including T_1 contrast, improved with increasing magnetic field.

One of the unique contrast mechanisms that improve with magnetic field is susceptibility contrast. This contrast provides the mechanism for functional neuroimaging with magnetic resonance (fMRI) that was introduced by independent and concurrent work in this BTRR and at MGH. Throughout its existence, fMRI has remained one of the central research foci in this BTRR. Most recently, work conducted in this BTRR has accomplished functional imaging in the human brain at the level of fundamental neuronal clusters composed of few thousands of neurons that form the most elementary computational units in the cortex. The same methods that enabled this accomplishment also led to the first time imaging of Alzheimer's plagues in genetically engineered AD mice.

This research has been pursued not only by developments in methodology and hardware, but also synergistically by computational methods as well as animal model experiments for elucidation of mechanisms underlying the MR measurements, for verification, and for quantification.

---

[1] The terminology is based on that used for classifying radiofrequency bands. The frequency range 300 MHz to 3 GHz is defined as Ultra high frequency (UHF) (see http://en.wikipedia.org/wiki/Ultra_high_frequency). The 30 to 300 MHz range is designated as Very High Frequency (VHF) (http://en.wikipedia.org/wiki/Very_high_frequency). The hydrogen nucleus resonance frequency at 7T is 300 MHz i.e. in UHF band. Therefore, 7T to 70T can be defined as Ultra High Field (UHF).

The National Center for X-ray Tomography

Carolyn Larabell
Department of Anatomy, UCSF, San Francisco, California
Physical Biosciences Division, Lawrence Berkeley National Laboratory
National Center for X-ray Tomography
Contact: Carolyn.Larabell@ucsf.edu

The National Center for X-ray Tomography (NCXT) is an NCRR supported Biotechnology Research Resource. One of our main research foci is the development of soft x-ray tomography as a cellular imaging modality with nanometer scale. A high performance soft x-ray microscope is one of the core technologies being developed by the NCXT, and made available to the biomedical research community. This new instrument is the first in the world to be designed specifically for biomedical imaging. Consequently, the images produced by this technique are unique in their information content, and offer a completely new view of cellular sub-structures and architecture.

Soft x-ray tomography relies on the physical properties of x-rays in a region of the spectrum known as the 'water window'. At these photon energies water is relatively transmissive towards x-rays, while the presence of carbon and nitrogen causes biological materials to absorb x-rays an order of magnitude more strongly. This difference in absorption gives rise to images with excellent contrast without the use of stains or other potentially disruptive contrast enhancing agents. These images reveal details of the sub-cellular architecture at a spatial resolution of 40nm or better. Furthermore, since soft x-rays can penetrate more deeply than electrons, there is no need to section the cell prior to analysis: cells are imaged intact and in their natural, fully hydrated state. Differences in the x-ray absorption coefficient can be used to identify and delineate individual organelles in the cell. Since these coefficients can be measured accurately, it is possible to differentiate species of organelle on this basis. An example of recent work is also shown in an accompanying poster (see Parkinson et al). In my talk I will present an update on soft x-ray tomography, together with details of our ongoing research efforts, and future goals for this imaging Resource.

High Frequency Ultrasonic Imaging

K. Kirk Shung, NIH Resource on Medical Ultrasonic Transducer Technology and Department of Biomedical Engineering, 136 DRB, University of Southern California, Los Angeles, CA 90089

High frequency ultrasonic imaging is considered by many to be the next frontier in ultrasonic imaging because higher frequencies yield much improved spatial resolution. The consequence is a reduced depth of penetration. It has many clinical applications ranging from visualizing internal and surface structures of the blood vessel wall and mapping anterior segments of the eye, to characterizing skin tumors. An added significance is the recent intense interest in small animal imaging for the purpose of evaluating the efficacy of drugs and gene therapy. Ultrasound is especially attractive in imaging the heart of a small animal like mouse which has a size in the mm range and a heart beat rate faster than 600 BPM. Commercial high frequency scanners often termed “ultrasonic backscatter microscope or UBM” all acquire images by scanning single element transducers at frequencies between 50 to 80 MHz with a frame rate lower than 30 frames/s. They are therefore not suitable for this application. Prototype annular and linear arrays at frequencies higher than 30 MHz are being developed. The engineering of such arrays are by no means trivial, hindered by the limitations of current technology. The development of a high frequency beam former with high frame rate is another challenge.

In this talk, current efforts in high frequency ultrasonic imaging and its medical applications will be reviewed and future strategies discussed.

New Approaches to Glycoprotein Structure: the Sialyltransferase, ST6Gal 1

J. H. Prestegard, K. W. Moremen, G-J Boons, R.C. Orlando, R.J. Woods
University of Georgia, Athens, GA, 30602
Resource for Integrated Glycotechnology
http://glycotech.ccrc.uga.edu, jpresteg@ccrc.uga.edu

Glycosylated proteins are very abundant with as many as 50% of all mammalian proteins carrying potential glycosylation sites. The properties of many of these proteins are also linked to diseases, including viral infection, autoimmune response, and cancer. Structural characterization is, therefore, key to the development of new therapeutic agents. Yet, three dimensional structures are poorly represented in databases such as the PDB. This results primarily because of difficulties in expressing and crystallizing the proteins. The Resource for Integrated Glycotechnology is providing new routes to structural information through development of a multidisciplinary approach that includes, expression of labeled glycosylated proteins in eukaryotic hosts, NMR detection of labels at the highest magnetic fields available, novel resonance assignment strategies that combine mass spectrometry with NMR, synthesis of resonance perturbing ligands to locate binding sites, and computational modeling to produce structures from sparse data sets. Progress will be illustrated with applications to the structural characterization of the sialyltransferase, ST6Gal 1, a protein involved in regulation of B cell signaling and a number of other phenomena dependent on the addition of sialic acids to the termini of cell-surface carbohydrates.

Membrane Protein Structure Determination at the Resource for NMR Molecular Imaging of Proteins

Stanley J. Opella
Department of Chemistry and Biochemistry, University of California, San Diego
Resource for NMR Molecular Imaging of Proteins
http://nmrresource.ucsd.edu/index.html
sopella@ucsd.edu

The overall objective of the Resource for NMR Molecular Imaging of Proteins at the University of California, San Diego is to develop the technology of NMR spectroscopy so that it can be applied to all of the proteins encoded in the human genome. As the most powerful form of spectroscopy, NMR has virtually unlimited potential for contributions to the field of molecular biology; however, in the past. successful applications have been largely limited to the same class of soluble globular proteins that is also most amenable to X-ray crystallography. The technology gap that needs to be filled is for proteins that are part of supramolecular assemblies, especially membrane proteins, an important class of proteins representing 20% - 30% of the information expressed from a genome and most drug receptors. Determining the structures of membrane proteins is a demanding undertaking: Core technology development includes instrumentation, experimental methods, and computer programs for the calculation of protein structures from experimentally measured spectral parameters.

The Resource played an essential role in a collaborative study with researchers at the Genomics Institute of the Novartis Research Foundation (Kim et al, 2007). Thrombopoietin (Tp0) is a glycoprotein growth factor that supports hematopoietic stem cell survival and expansion and is the principal regulator of megakaryocyte growth and differentiation. To understand how a small nonpeptidyl Tpo mimic interacts and activates the human Tpo receptor (hTpoR) we performed NMR experiments on constructs of this membrane protein. These experiments showed that the small molecule of interest interacts specifically with the extra cellular juxtamembrane region (JMR) and the transmembrane (TM) domain of hTpoR. This structural information provides insights into the mechanism of ligand-induced cytokine receptor activation. Moreover, publication of the results has attracted new collaborators to the Resource for studies of related systems.

The Resource provides the technological base for a Bioengineering Research Partnership with two biotechnology companies. G-protein coupled receptors (GPCRs) transduce signals from the outside of cells, by binding to small molecules, to the inside where they trigger a cascade of events starting with interactions with G-proteins. GPCRs play a role many human diseases due to their importance in maintaining proper function of living cells, and more than half of all currently available drugs bind to GPCRs. Promising initial results (Park et al, 2006) indicate that with further Core development we will be able to elucidate the three-dimensional structures of GPCRs in their native membrane bilayer environments, which will provide insight into their mechanisms of action and help to design drugs targeted at GPCR-related disorders including cancer, hear disease, and obesity.

Kim, M.J., Park, S.H., Opella, S.J., Marsilje, T.H., Michellys, P.Y., Seidel, H.M., and Tian, S.S. (2007) **NMR structural studies of interactions of a small, nonpeptidyl Tpo mimic with the Thrombopoietin receptor extracellular juxtamembrane and transmembrane domains**, *J. Biol. Chem.*, web published.

Park, S. H., Prytulla, S., De Angelis, A. A., Brown, J. M., Kiefer, H., and Opella, S.J. (2006) **High-resolution NMR Spectroscopy of a GPCR in Aligned Bicelles,** *J. Amer. Chem. Soc.* **128**, 7402-7403.

Imaging Oxygen Physiology with Electron Paramagnetic Resonance

PIs Howard Halpern, Gareth Eaton, Gerald Rosen
Unversities of Chicago, Chicago IL, Denver, Denver, CO and, Maryland, Baltmore, MD

Electron Paramagnetic Resonance (EPR) provides a signal with unusual sensitivity and specificity to crucial aspects of the fluid environment of life processes. Among these is the imaging of oxygen concentrations in the tissues of a living animal. To implement this magnetic resonance technique in the context of an animal image construction of an entire technology is required. Since unpaired electrons are rare or inaccessible in living systems, non toxic, injectable spin probes with extraordinarily narrow spectral lines (long electron relaxation times) are needed. MRI Fourier imaging is precluded by the six order of magnitude shortening of even the longest electron relaxation times relative to water protons (μs vs s timescales). Tomographic imagers obtaining field swept EPR spectra or magnetization evolution traces in the presence of fixed stepped gradients required their own design and construction. Finally, registration of EPR images (EPRI) is required to understand the anatomic correlates of the physiologic parameters was developed. This presentation will highlight these technical developments of the center including recent time domain acquisition images with better than 1 torr oxygen resolution. The use of such images in the determination of the effectiveness of radiation treatments for cancer will be shown as well.

Poster Abstracts - NIBIB

Biomedical Simulations Resource (BMSR)
University of Southern California
David D'Argenio

Biomicroelectromechanical Systems (BioMEMS) Resource Center
MGH / Harvard University
Mehmet Toner

Center for Gamma-Ray Imaging
University of Arizona
Lars Furenlid

Center for Synchrotron Biosciences
Case Western Reserve University
Mark Chance

Integrated Technologies for Polymeric Biomaterials
Rutgers, the State University of New Jersey
Joachim Kohn

National Biomedical Electron Paramagnetic Resonance Center
Medical College of Wisconsin
James Hyde

National ESCA and Surface Analysis Center for Biomedical Problems
University of Washington
Lara Gamble

National Resource for Imaging Mass Spectrometry
Harvard University
Claude Lechene

Pittsburgh NMR Center for Biomedical Research
Carnegie Mellon University
Chien Ho

Radiological Research Accelerator Facility
Columbia University
David Brenner

Resource Facility for Population Kinetics (RFPK)
University of Washington

Paolo Vicini

Resource on Medical Ultrasonic Transducer Technology
University of Southern California
Kirk Shung

Stem Mass Mapping and Heavy Atom Labeling of Biomolecules
Brookhaven National Laboratory
Joseph Wall

Tissue Engineering Resource Center (TERC)
Tufts University / MIT
David Kaplan

Pharmacokinetic/Pharmacodynamic Systems Analysis: Application of Bayesian Population Modeling to Preclinical/Clinical Translational Drug Development

David D'Argenio, Vasilis Marmarelis, Michael Khoo and Ted Berger
Department of Biomedical Engineering/University of Southern California
Biomedical Simulations Resource
bmsr.usc.edu
dargenio@bmsr.usc.edu

The use of mathematical modeling and associated computational methods is central to the study of the absorption, distribution and elimination of therapeutic drugs (pharmacokinetics) and to understanding how drugs produce their effects (pharmacodynamics). From its inception the field of pharmacokinetics and pharmacodynamics has incorporated methods of mathematical modeling, simulation and computation in an effort to better understand and quantify the processes of uptake, disposition and action of therapeutic drugs. These methods for pharmacokinetic/ pharmacodynamic systems analysis impact all aspects of drug development including *in vitro*, animal and human testing, as well as drug therapy. Core Project # 2 of the BMSR is investigating the following three significant and challenging problems in pharmacokinetic/pharmacodynamic modeling and therapeutic drug development: 1) pharmacokinetic/pharmacodynamic modeling for genetically polymorphic populations; 2) cellular PK and molecular PD modeling in drug development and 3) dose regimen design for molecular targeted therapies. Recent work described below illustrates work involving aims 1 and 2 as applied to a collaborative project with Dr. John Rodman (St. Jude Children's Research Hospital) on the cellular metabolism of the anti HIV-1 drug lamivudine in adolescent patients.

The pharmacologic variability of nucleoside reverse transcriptase inhibitors such as lamivudine (3TC) includes not only systemic pharmacokinetic variability but also inter-individual differences in cellular transport and metabolism. A modeling strategy linking laboratory studies of intracellular 3TC disposition with clinical studies in adolescent patients is described. Data from *ex vivo* laboratory experiments in peripheral blood mononuclear cells (PBMCs) from uninfected human subjects is first used to determine a model and population parameter estimates for 3TC cellular metabolism. Clinical study data from HIV-1 infected adolescents is then used in a Bayesian population analysis, together with the prior information from the *ex vivo* analysis, to develop a population model for 3TC systemic kinetics and cellular kinetics in PBMCs from patients during chronic therapy. The *ex vivo* modeling results demonstrate that the phosphorylation of 3TC is saturable under clinically relevant concentrations, there is a rapid equilibrium between 3TC monophosphate and diphosphate and between 3TC diphosphate and triphosphate, and that 3TC triphosphate is recycled to 3TC monophosphate through a 3TC metabolite that remains to be definitively characterized. The resulting population model developed from the HIV-1 infected adolescents shows substantial inter-individual variability in the cellular kinetics of 3TC with population coefficients of variation for model parameters ranging from 47--87 %. This two-step *ex vivo*/clinical modeling approach using Bayesian population modeling 3TC linking laboratory and clinical data has potential application for other drugs whose intracellular pharmacology is a major determinant of activity and/or toxicity. (Reported in: Z. Zhou, et al., *Antimicrobial Agents and Chemotherapy*. **50**:286-2694, 2006.)

BioMicroElectroMechanical Systems (BioMEMS) Resource Center

BioMEMS Resource Center, Center for Engineering in Medicine and Surgical Services, Massachusetts General Hospital and Harvard Medical School, Harvard-Massachusetts Institute of Technology, Division of Health Sciences and Technology, Cambridge, MA

www.BioMEMSrc.org; mtoner@hms.harvard.edu

The BioMEMS Resource Center has a mission to provide biomedical investigators with novel microsystems engineering tools for biological discovery, diagnostic, prognostic, and therapeutic applications. Thrust areas of interest for the BioMEMS Resource Center are the development of novel living cell-based, lab-on-a-chip type devices for sorting blood cells, for high-throughput biochemistry in small volumes, and for studying cellular behavior in controlled microenvironments, with direct applications in infectious diseases, trauma, cancer and immuno-inflammatory processes. Two specific examples will be presented in detail.

In the area of cancer diagnostic, we have developed a microfluidic platform technology for the identification of tumor-derived epithelial cells (circulating tumor cells or CTCs) in blood from patients with cancer. While CTCs comprise as few as 1 cell in a milliliter of blood of patients with known metastatic cancer (1CTC cell in 1 billion blood cells), their isolation presents a tremendous challenge for cell separation technologies, because both high cellular complexity of the blood and the scarcity of the target. Using the new CTC-chip technology we are able to detect CTCs in blood samples from patients with localized prostate cancer, in 7/7 cases (100%), ranging from 25-174 CTC/mL, with ~53% purity, indicating the potential use of our technology in early cancer detection. In a small cohort of metastatic lung, colorectal, pancreatic, and esophageal cancers, followed over several months we observed that temporal changes in CTC number exhibited a close correlation with clinical disease course and tumor volume as measured using standard radiographic monitoring. The development of an effective microchip for noninvasive identification of CTCs in patients with epithelial cancers raises the possibility of serial point-of-care monitoring, and has implications for early detection and molecular diagnosis of cancer.

In the area of basic science research, we developed new strategies based on functional genomics tools that combine GFP reporter technology and microfabrication techniques for the profiling of dynamic gene expression in living cell arrays. We demonstrate the approach in the context of hepatic inflammation by acquiring 5000 single-time-point measurements in each automated and unattended experiment. Experiments can be assembled in hours and perform the equivalent of months of conventional experiments. This and specific liver tissue engineering methods are used to develop microfabricated *in vitro* systems that mimic the *in vivo* organization of the liver acinus for investigating the dynamic sequence of expression of pro- and anti-inflammatory mediators following ischemia reperfusion of transplanted livers.

The core research efforts are supplemented with the activities of numerous collaborators in biological and clinical fields. For example, microfluidic gradient generators are being utilized to generate precisely controlled spatial and temporal chemotactic gradients to probe into the molecular and cellular mechanisms of leukocyte bi-directional motility in the immune response. Educational programs include ad-hoc on-site training on specific technologies, periodic workshops, and comprehensive courses targeted towards students and researchers, with the overall goal of rapid dissemination of new tools of microfabrication to the biomedical community. The BioMEMS Research Center also offers technological assistance for projects and for technology transfer, and is actively involved into organizing seminar series on BioMEMS topics.

Direct Electron Imaging of Radionuclide Emissions

L. Chen, L.S. Gobar, N.G. Knowles, G.D. Stevenson, A.F. Gmitro, and H.H. Barrett
Center for Gamma-ray Imaging and Department of Radiology
University of Arizona
http://www.gamma.radiology.arizona.edu, barrett@radiology.arizona.edu

Researchers within the Center for Gamma-Ray Imaging (CGRI) have invented a new method to directly image the distribution of electron-emitting radioisotopes at very high resolution and sensitivity. The method uses an ultra-thin scintillator, a monolayer of 3-μm P47 phosphor powder deposited on 3-μm clear Mylar foil, in close proximity to radioactive objects to convert the kinetic energy of emitted electrons into light, which is then transmitted to a digital light sensor by optical coupling means (Fig. 1). The images of the scintillation light patterns represent the isotope distributions under study.

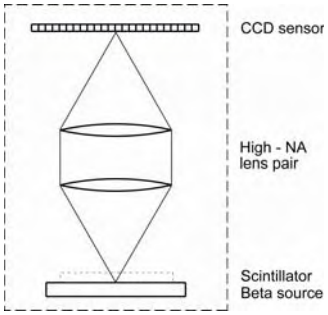


Figure 1. Schematic diagram of the electron imaging system.

A proof-of-concept prototype system has been completed using a large-area, low-noise CCD detector and various large-aperture imaging lenses. When using optics at unit magnification, the system has resolved details within the annular shape of a 3-mm 100-nCi Y-90/Sr-90 β source, marketed as a point source (Fig. 2). From the images, the spatial resolution is estimated at 60 μm, and the detection limit of this source is about 185 disintegrations.

Besides β emissions, the system is also very sensitive to other charged particles such as positrons and conversion electrons from γ emitters (Fig. 3). When imaging samples *in vitro* at unit magnification, the CGRI system can provide spatial resolution, sensitivity, and linear response range similar to state-of-art digital autoradiography systems using storage phosphors. The resolution is improved further with optics at higher magnifications. Unlike autoradiography, the new system is also capable of imaging small animals *in vivo.*

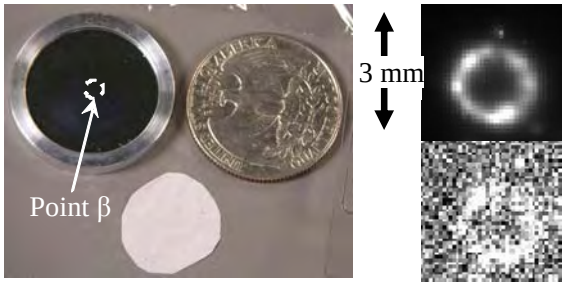


Figure 2. Photograph of the phosphor and the β source (A), the electron images of the source at 5-min (B), and 0.1-sec (C) exposures.

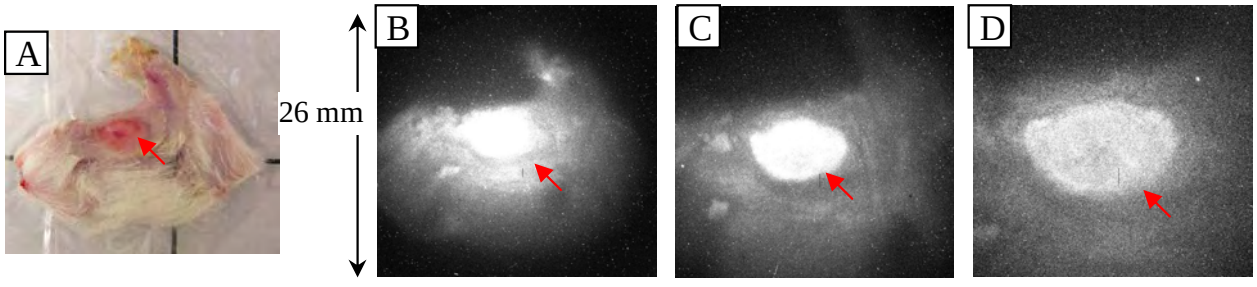


Figure 3. A mouse was implanted subcutaneously with a breast tumor and later injected with ^{99m}Tc--labeled tumor probe prior to euthanasia. Photograph of the mouse skin tissue with the cancer (A), and the images of the conversion electron emissions from the tissue at 1X (B), 1.7X (C), and 2.7X (D).

The direct imaging system has been used to image *in vivo* the ^{18}F-FDG uptakes in tumors implanted in dorsal skin chambers, revealing heterogeneity of FDG distribution inside tumors. First manuscripts to report the current findings and the methods are in preparation.

Three-dimensional structure of cofilin bound to monomeric actin derived by structural mass spectrometry data

J.K. Amisha Kamal (1); S. Benchaar (2); Keiji Takamoto (1); Emil Reisler (2); Mark R Chance;
1-Center for Synchrotron Biosciences, Case Center for Proteomics, Case Western Reserve University, Cleveland, OH. 2-Department of Biochemistry, UCLA, Los Angeles CA.
http://casemed.case.edu/proteomics/CSB/INDEX.HTM ; mark.chance@case.edu

Introduction

The cytoskeletal protein, actin, has its structure and function regulated by cofilin. In the absence of an atomic resolution structure for the actin/cofilin complex, the mechanism of cofilin regulation is poorly understood. Theoretical studies based on the similarities of cofilin and gelsolin segment-1 proposed the cleft between subdomains 1 and 3 in actin as the cofilin binding site. In the present study, radiolytic footprinting is used to map the binding surface of actin/cofilin. This method specifically identifies side chains experiencing changes in solvent accessibility as a result of ligand binding. Using the data, an atomic model of the binary complex is constructed with comparative modeling and protein-protein docking.

Methods

Radiolytic protein footprinting determines the change in the oxidation of amino acid probes due to a changed protein environment and attributes it to protein conformational change. Samples after synchrotron X-ray exposure (X-28C beam line of the National Synchrotron Light Source, Brookhaven National Laboratory) are subjected to enzymatic proteolysis followed by injection to liquid chromatography coupled electrospray ion source mass spectrometer. A plot of fraction-unmodified vs X-ray exposure time, called “dose response curve” is plotted for each peptide to extract the first order oxidation rate constant (k).

Results

We used radiolytic protein footprinting with mass spectrometry and molecular modeling to provide an atomic model of how cofilin binds to monomeric actin. Footprinting data suggest that cofilin binds to the cleft between subdomains 1 and 2 in actin and that cofilin induces further closure of the actin nucleotide cleft. Site-specific fluorescence data confirm these results. The model identifies key ionic and hydrophobic interactions at the binding interface, including hydrogen bonding between His87 of actin to Ser89 of cofilin that may control the charge dependence of cofilin binding. This model and its implications fill an especially important niche in the actin field owing to the fact that ongoing crystallization efforts of the actin/cofilin complex have so far, failed. In addition, this outlines a general method for determining the high-resolution structure of a binary complex derived from a combination of solution footprinting data and computational docking approaches.

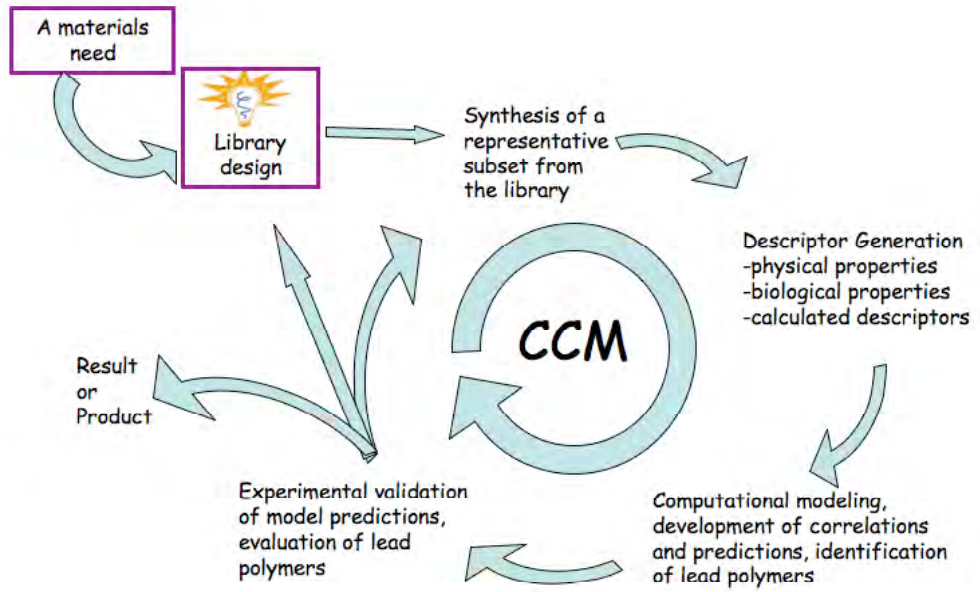
Relevant Publication: Kamal et al. *Proc. Nat. Acad. Sci.*, 2007, in press
Supported by P41-EB-01979.

Accelerated Biomaterials Discovery through a Combinatorial-Computational Approach

Joachim Kohn[1], Matthew Becker[2], Prabhas Moghe[1], Doyle Knight[1], Robert Latour[4]
[1]Rutgers, the State University of New Jersey, [2] National Institute of Standards and Technology, [3] University of Pennsylvania, [4] Clemson University

Technology Resource for Polymeric Biomaterials, RESBIO
http://njbiomaterials.org/RESBIO, kohnoffice@biology.rutgers.edu

Better biomaterials are often recognized as one of the important technology platforms pivotal to improving the quality of life of a large proportion of aging people as well as reducing the overall cost of health care. To address this need, RESBIO was funded to develop a combinatorial-computational method (CCM) to biomaterials discovery.



Schematic presentation of the CCM for biomaterials design and optimization: Development of the CCM requires (i) methods of parallel synthesis of polymer libraries, (ii) methods of rapid characterization and screening of key biomaterials properties, and (iii) methods of computational analysis and predictive modeling. .

The computational effort is directed toward the creation of models to analyze and predict polymer properties and the cellular response elicited by contact with artificial materials, while the experimental effort generates materials and creates datasets of biorelevant characterization that can serve either as the source of data for computational models or serve to validate the predictions made by computational models. RESBIO's CCM uses both molecular dynamics simulations and semi-empirical modeling, to achieve a complete characterization of the mechanical and bioresponse characteristics of entire polymer libraries. One of RESBIO's current research projects is to model a vast, virtual library of over 20,000 polymethacrylates with the goal of identifying those polymethacrylates that optimally support the growth of various cell lines in vitro. Another illustrative research project is the development of quantitative descriptors of the cytoskeletal organization of cells growing on different polymeric supports. Here, our long-term goal is to develop assays in vitro that can predict cell-material interactions in vivo.

Using the CCM, RESBIO has been able to discover new biomaterials for hernia repair (an FDA-approved device has been commercialized) and for use in fully degradable, cardiovascular stents (which will enter clinical trials in June 2007). Once fully implemented, RESBIO will disseminate the CCM to the biomaterials community and the medical device industry.

CHARACTERIZATION OF THE Cu(II)-BINDING SITES IN α-SYNUCLEIN

Jason Kowalski[a], Claudio O. Fernandez[b], Brian Bennett[a]
[a]Department of Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, and
[b]Instituto de Biologia Molecular y Celular de Rosario, University of Rosario, Rosario, Argentina
National Biomedical EPR Center, Milwaukee/PI: James S. Hyde/EB001980
www.mcw.edu, jshyde@mcw.edu

Late-onset Parkinson's disease is incurable and afflicts about 1% of the United States population over the age of 65 (*1, 2*). Various environmental factors have been linked to an increased risk of developing the disease (*3*). In particular, extended exposure to heavy metal ions has been linked to a significantly increased risk of developing Parkinsonism (*4*).

Late-onset Parkinson's disease is characterized by the presence of Lewy bodies in affected brain tissue. Lewy bodies consist primarily of fibrils of α-synuclein. One of a number of factors that promote fibrillation of α-synuclein is metal ions (*5*). NMR studies of Cu^{2+} adducts appeared to indicate that α-synuclein provides up to four distinct metal binding environments (*6*). However, the studies to date have provided no direct information on the ligand environment of the bound Cu^{2+} and there is some uncertainty as to the relative affinities of the sites for Cu^{2+}.

In the present study, we examine the binding of Cu^{2+} by native α-synuclein and by variants lacking one or more of the proposed binding sites, using EPR spectroscopy. We have characterized the binding sites in terms of their affinities for Cu^{2+} and their location in the α-synuclein molecule. With the aid of computer simulations, we have extracted spin Hamiltonian parameters and predict the ligand complement of the Cu^{2+} ion. We have thus constructed an overall model for Cu^{2+} binding that is consistent with these data and earlier studies.

1. Lotharius, J., Brundin, P., *Nat Rev Neurosci* 3, 932-942 (2002).
2. Siderowf, A., Stern, M. A*nn Intern Med* 138, 651-658 (2003).
3. Gorell, J.M., Peterson, E.L., Rybicki, B.A., Johnson, C.C. *J Neurol Sci* 217, 169-174 (2004).
4. Gorell, J.M., Johnson, C.C. Rybicki, B.A., Peterson, E.L., Kortsha, G.X., Brown, G.G., Richardson, R.J. *Neurotoxicology* 20, 239-247 (1999).
5. Uversky, V.N., Li, J., Fink, A.L. *J Biol Chem* 276, 44284-44296 (2001).
6. Binolfi, A., Rasia, R.M., Bertoncini, C.W., Ceolin, M., Zweckstetter, M., Griesinger, C., Jovin, T.M., Fernandez, C.O. *J Am Chem Soc* 128, 9893-9901 (2006).

Surface Modification and Characterization of Microarrays

Lara J. Gamble and David G. Castner
National ESCA and Surface Analysis Center for Biomedical Problems
Departments of Bioengineering and Chemical Engineering, University of Washington
www.nb.engr.washington.edu; email: castner@nb.engr.washington.edu

DNA-modified surfaces have received considerable attention in the fields of bio- and nanotechnology due to their importance in the development of biosensors and diagnostic devices. The construction of these surfaces often entails the attachment of presynthesized oligomers onto a derivatized surface. The surface properties of the immobilized oligomers assembled via different attachment schemes are expected to significantly influence their biological activity and interactions in the environment. Successful development and optimization of DNA-functionalized surfaces for microarray and biosensor applications requires the accurate and quantitative characterization of immobilized DNA chemistry and structure on various substrates. Surface analytical techniques are used here to develop a better understanding of the relationship between surface properties of immobilized DNA probes (e.g., composition, density, purity, and orientation) and the resulting hybridization efficiency. Surface analysis techniques used to study this system include x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). Orientation of the probe ssDNA was determined by near edge x-ray absorption fine structure (NEXAFS). Target DNA hybridization on probe DNA surfaces was monitored by surface plasmon resonance (SPR).

The affects of the microarray printing process on hybridization efficiency and spot uniformity is also investigated with a range of surface analytical techniques. The printing process involves delivering nanoliter drops of DNA solutions which dry within seconds. This rapid drying produces increased solution ionic strength and solute concentrations resulting in heterogeneous microarray spots. Irreproducible results are noticed in the fluorescence imaging of the spots (currently used to monitor DNA hybridization). Recent improvements in imaging photoelectron spectroscopy have allowed more detailed studies of micro-patterned surfaces. In this work, XPS imaging and time-of-flight secondary ion mass spectrometry (ToF-SIMS) is applied to the study of patterned DNA surfaces relevant to real world microarray applications. XPS was used for a quantitative comparison between spots while TOF-SIMS was used for a more detailed analysis of the uniformity within the micro-spots. Immobilized DNA probe and target surface compositions on two different commercially available microarray polymer slides are compared using microarray and macro-spot format as well as bulk modification.

A future application of DNA microarrays is in the reliable, direct capture of nucleic acid targets from complex media. This capability would greatly reduce the preparation time for use of DNA microarrays and biosensors and potentially make them more useful for real-time clinical applications. However, this goal remains a challenge for many current nucleic acid detection technologies attempting to produce assay results directly from complex real-world samples. We have investigated the surface structure and performance of single-strand DNA (HS-ssDNA) monolayers on gold backfilled with backfill diluents of 11-mercapto-1-undecanol (MCU) or 11-mercapto-undecyl tetra ethylene glycol (OEG)**.** The attachment chemistry and surface coverage of probe ssDNA were studied by X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). Orientation of the probe ssDNA was determined by near edge x-ray absorption fine structure (NEXAFS). Target DNA hybridization on probe DNA surfaces was monitored by surface plasmon resonance (SPR). Hybridization from buffer and serum was examined to determine the amount and effects of non-specific adsorption on DNA hybridization.

Quantitative imaging of nitrogen fixation by individual bacteria within animal cells

Claude P. Lechene[1], Yvette Luyten[2]. Gregory McMahon[1] & Daniel L. Distel[3]

1. National Resource for Imaging Mass Spectrometry, Harvard Medical School, and Brigham and Women's Hospital, 65 Landsdowne Street, Cambridge, MA 02139, USA
2. New England Biolabs, 240 County Rd, Ipswich, MA 01938-2723
3. Ocean Genome Legacy, Center for Marine Genomic Research, 240 County Rd, Ipswich, MA 01938-2723
Email: cpl@harvard.edu

Biological nitrogen fixation (BNF), the conversion of atmospheric nitrogen to ammonia that can then be used for biosynthesis, is exclusively performed by a few groups of bacteria and archeae. These microbes, found free living or in symbiotic associations with algae, higher plants and some animals, form a critical part of the global nitrogen cycle. Despite the essential importance of BNF, it has been impossible to observe and measure the incorporation of nitrogen by individual bacteria, or to map the fate of the newly fixed nitrogen in the symbiotic host cells. Here we used a powerful new method, multi-isotope imaging mass spectrometry (MIMS)[1], to show that individual symbiotic bacteria fix large quantities of ^{15}N both in pure culture and as inhabitants of dense bacterial communities within host cells (bacteriocytes) of a wood-eating marine bivalve, the shipworm Lyrodus pedicellatus. Strikingly, we also find newly fixed nitrogen within shipworm tissues that lack associated bacteria and that are remote from the bacteriocytes. This work describes the first direct demonstration of nitrogen fixation by individual bacteria within cells of a eukaryotic host and presents strong evidence that the fixed nitrogen is used for host metabolism. Our approach opens a powerful new way to study microbes, their biological associations, and their role in global nutrient cycles. Moreover, this novel technique for measuring the distribution of multiple stable isotope-labeled molecules at micron to nanometer scales can provide unique information in almost all fields of biomedical research. Applications include the study of intraand inter-cellular metabolic pathways, protein turnover, RNA and DNA expression and distribution, fatty acid transport, nucleo-cytoplasmic translocation, transplantation donorrecipient trafficking, stem cell lineage labeling, and drug localization.

1. Lechene et al. *High-resolution quantitative imaging of mammalian and bacterial cells using stable isotope mass spectrometry*. **J. Biol**. 5, 20 (2006).

A New Non-invasive Approach to Detect Heart Transplant Rejection by MRI

Yi-Jen Wu, Qing Ye, Lesley M. Foley, T. Kevin Hitchens, and Chien Ho
Carnegie Mellon University, A Multidisciplinary NMR Center for Biomedical Research,
cmu.edu/nmr-center, chienho@andrew.cmu.edu

Organ transplantation is the preferred medical treatment for patients with end-state organ failure. Two major challenges facing organ transplantation are graft rejection and shortage of donor organs. Graft rejection may result in the need for a second or third transplant or death of the patient. Thus, if graft rejection can be detected early and reversed by available immunosuppressive drugs, fewer organs will be needed and lives will be saved. There are two major types of graft rejection, namely acute and chronic. Although the frequency and severity of acute rejection are known to be the most predictive factors for developing chronic rejection later in time, the mechanistic relation between them is not well understood. But, if we can detect early signs of acute cardiac rejection, we may be able to reduce chronic rejection and organ loss. Since repeated tests are necessary, it would be desirable to have a non-invasive method for detecting acute rejection. MRI is non-invasive and advances in MRI methodology give promise of providing means for early detection of organ rejection. In this presentation, we summarize our recent research to develop a new non-invasive approach to detect heart rejection after transplantation using our heterotopic working rat heart models for both acute and chronic cardiac rejection. Our goal is to develop methods using MRI to detect graft rejection at a very early stage before irreversible damage has occurred to the transplanted heart.

We are applying a two-pronged approach to investigate cardiac rejection after heart transplantation in our rat models. First, by labeling immune cells, e.g., macrophages, with an MRI contrast agent like superparamagnetic iron-oxide particles ranging from nano to micron sizes, we can monitor the infiltration of these labeled immune cells at the rejection site as a non-invasive method to detect graft rejection. The iron-oxide particles are injected after transplantation and taken up by circulating macrophages. We are then able to follow with MRI the progression of cardiac rejection in detail throughout the entire heart as iron-oxide containing macrophages infiltrate the heart from the pericardium and penetrate into the endocardium. Macrophages labeled with iron-oxide particles can be observed in MR images for a long period of time after the injection. Second, we can monitor the function of the same transplanted heart by MRI, e.g., by measuring regional stress and perfusion, during various stages of rejection, with and without therapeutic intervention. The development of tagged MRI allows us to detect regional cardiac dysfunction at an early stage of cardiac rejection. We are searching for reliable biomarkers for early signs of both acute and chronic cardiac transplant rejection.

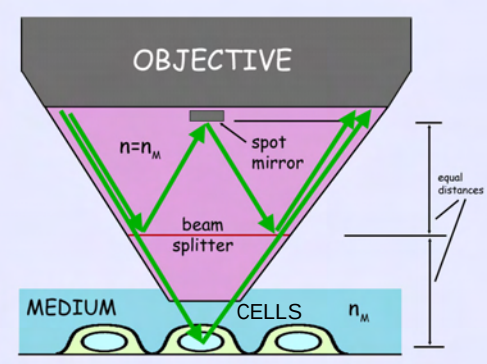
Our results suggest that our two-pronged approach by MRI has advantages over the conventional techniques (including myocardial biopsy) for detecting cardiac rejection. Thus, multi-parameter MRI measurements avoid the sampling errors and invasiveness of biopsy and can make accurate and non-invasive diagnosis of graft rejection possible. With further development, we are optimistic that our approach can be applied to detect both acute and chronic heart rejection in transplant patients.

The Columbia University Single-Cell / Single-Particle Microbeam

David Brenner, Alan Bigelow, Steve Marino, and Gerhard Randers-Pehrson
Radiological Research Accelerator Facility, Columbia University, New York, New York
www.raraf.org

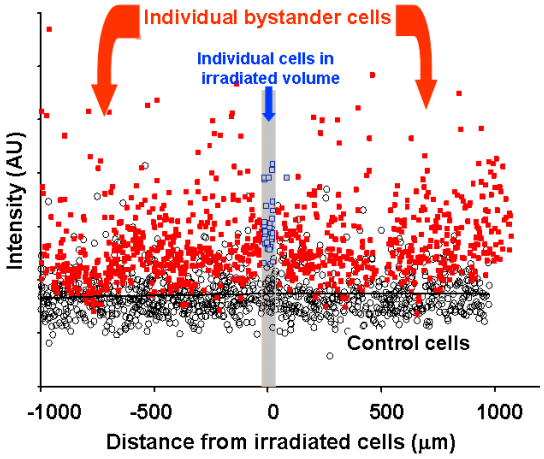
Our single-cell / single-particle microbeam at Columbia University's Radiological Research Accelerator Facility (RARAF) can deposit small or large doses of ionizing radiation damage in targeted microscopic or sub-microscopic regions of individual living cells. The microbeam is a tool for probing the nature of DNA damage signal transduction. For example, our investigators probe the response of cells to spatially targeted damage (e.g. nucleus vs. cytoplasm vs. mitochondria), as well as the effects of DNA damage signals on unirradiated neighboring (initially non-damaged) cells.

Our instrumentation initiatives are focused on improving the precision, accuracy, and usability of this technology for irradiating individual cells, either in a monolayer or within 3-D tissue. For example, there is a significant need for a high-resolution cellular / subcellular imaging system in which the cells of interested are not stained (because this may effect biological outcome), but which is based on reflected rather than transmitted light (the microbeam vacuum window precludes the of use transmitted light). In addition, the biological material typically has variable amounts of medium on it, which is problematic for any interference-based imaging technique. Therefore we have developed an immersion Mirau interferometry system (schematized here), in which the lens is filled with a material of the same refractive index as the cellular medium. This has proved to be an effective new imaging technique.



Our microbeam can irradiate defined regions of 3-D tissue and guarantee that adjacent regions will not be directly damaged. Using this system, our investigators have shown that non-irradiated "bystander" cells in 3-D normal human tissue that are located up to 2 mm away from irradiated cells show enhanced DNA damage, for a variety of endpoints. An example is shown here in which a very thin slice down the center of normal human 3-D skin tissue is microbeam irradiated, and individual cells are probed using immunohistochemical techniques for endpoints within different damage pathways. Each point corresponds to a measurement in an individual cell. Cells located within about 1.5 mm of directly damaged cells, but which themselves were not directly damaged (solid red symbols), show almost as much effect as those that were directly damaged by radiation (open blue symbols). DNA damage information from the directly irradiated cells must therefore have been transmitted over a distance of many tens of cells. This "bystander effect" has considerable importance in understanding the effects of low doses of mutagens, where most cells are not directly damaged by the mutagen.

JNK phosphorylation in 3-D tissue, measured in individual cells
Proton microbeam: 1 h post irradiation



New Nonparametric Methods for Population Kinetics

Paolo Vicini and the Resource Facility for Population Kinetics (RFPK)
Department of Bioengineering, University of Washington, Seattle, WA 98195-5061
Resource Facility for Population Kinetics (NIH/NIBIB P41 EB001975)
Website: http://www.rfpk.washington.edu, Email: vicini@u.washington.edu

Population kinetic analysis using nonlinear mixed effects models [1] has become the tool of choice for analysis of biomedical data affected by between-subject (BSV) and residual unknown (RUV) sources of variability. This approach was introduced by Beal and Sheiner [2] and is widely available through the software package NONMEM [3]. Use of population kinetic analysis software and model-based inference for such applications is increasingly common in the biomedical literature, and has been mentioned in FDA guidance documents and reports [4, 5]. The success of the approach stems from its ability to fit nonlinear models to sparse and noisy data, thus providing a rigorous framework for inference based on physiologically plausible models, which tend to be nonlinear in the parameters. One obstacle is the need to define a model for population-level variability. This requires the user to postulate a statistical distribution for biomedical model parameters, e.g. drug clearance or volume of distribution. Often, this choice is difficult and can be arbitrary, especially in absence of preliminary information about the likely shape of these distributions. Normal or log-normal distributions are typically chosen, both of which are unimodal. Although there have been efforts to develop nonparametric methods, where the population parameter density is not assumed a priori [6, 7], these approaches are currently not configured to inform the choice of parametric model.

At the RFPK, we have developed a novel approach for nonparametric population analysis, implemented in our System for Population Kinetics (SPK) web service, available to registered users at https://spk.rfpk.washington.edu/info/. The approach is based on the simultaneous estimation of both the weights and support points of the population density of the kinetic parameters using an interior point based method [8, 9]. The method was developed in the general context of statistical mixture models, and can be extended to model-based cluster analysis. We posed the nonparametric mixture density estimation problem as a maximum likelihood problem, allowing reconstruction of the unknown probability distribution without assumptions on its shape. This approach enables the SPK user to begin the data modeling process with a minimum number of assumptions. The nonparametric estimate of the distribution can then be used to build a data-informed, potentially multi-modal, parametric model for population variability and used as input to a mixed effects model.

References

1. Davidian M, Giltinan DM. *Nonlinear Models for Repeated Measurement Data*. 1995. London: Chapman & Hall.
2. Beal SL, Sheiner LB. Estimating population kinetics. Crit Rev Biomed Eng. 8: 195-222, 1982.
3. Beal SL, Sheiner LB. The NONMEM System. American Statistician. 34: 118-119, 1980.
4. FDA Guidance for Industry. Population Pharmacokinetics. FDA: Rockville, MD, 1999
5. FDA Critical Path Report. Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products. FDA: Rockville, MD, 2004.
6. Mallet A, Mentre F, Steimer JL, Lokiec F. Nonparametric maximum likelihood estimation for population pharmacokinetics, with application to cyclosporine. J Pharmacokinet Biopharm. 16: 311-27, 1988.
7. Jelliffe RW. The USC*PACK PC programs for population pharmacokinetic modeling, modeling of large kinetic/dynamic systems, and adaptive control of drug dosage regimens. Proc Annu Symp Comput Appl Med Care. 922-4, 1991.
8. Burke J. Statistical Mixture Models, Bender's Decomposition, and Interior Point Algorithms. US-Mexico Workshop on Optimization and its Applications. Huatulco, Mexico, January 8 - 12, 2007.
9. Baek Y. An Interior Point Approach to Nonparametric Mixture Models. Ph.D. Dissertation. Department of Mathematics, University of Washington, 2006.

Recent developments in high frequency ultrasonic transducers and arrays

K. Kirk Shung, NIH Resource on Medical Ultrasonic Transducer Technology, Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089-1111

High frequency (HF) ultrasonic imaging is considered by many to be the next frontier in ultrasonic imaging. It has many clinical applications ranging from visualizing internal and surface structures of the blood vessel wall and mapping anterior segments of the eye, and characterizing skin tumors. An added significance is the recent intense interest in small animal imaging for the purpose of evaluating the efficacy of drugs and gene therapy. Commercial high frequency scanners often termed “ultrasonic backscatter microscope or UBM” all use scanned single element transducers at frequencies between 30 to 60 MHz with a frame rate of 30 frames/s or lower. To alleviate problems with UBMs which include mechanical motion and fixed focusing, high frequency annular arrays at 40-50 MHz and linear arrays in the 20-30 MHz range are being developed. The engineering of arrays at such high frequencies has been problematic, hindered by the limitations imposed by current technology.

These problems may be overcome from the end of developing better piezoelectric materials that satisfy the specific needs of high frequency array design or/and from the end of developing novel fabrication methodologies. These issues are also relevant for the further improvement of UBM that can benefit to a great deal from the availability of superior single element transducers in terms of bandwidth and sensitivity. Various novel piezoelectric materials, including 2-2, 1-3 composites, fine grain materials, high or low dielectric constant materials, and fabrication methods that utilize conventional dice and fill, thin and thick films, and MEMS have been explored to fulfill this need.

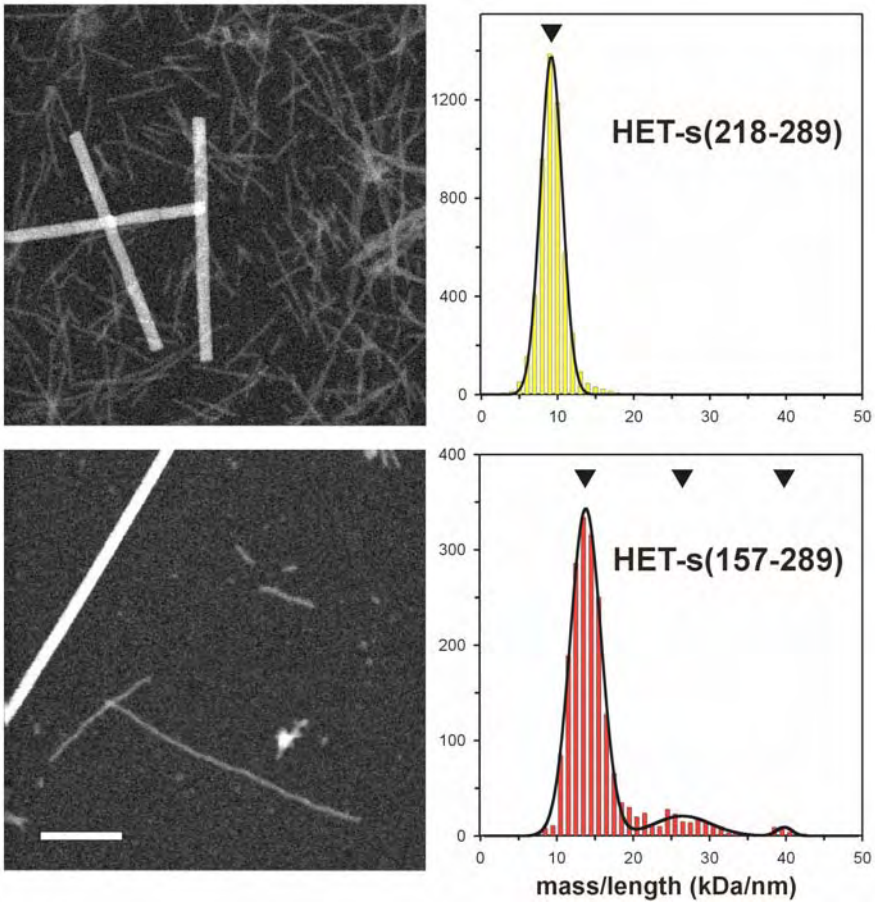
A prototype HF imaging system has also been developed to test the performance of the linear and annular arrays.

MASS ANALYSIS BY SCANNING TRANSMISSION ELECTRON MICROSCOPY (STEM) AND ELECTRON DIFFRACTION VALIDATE PREDICTIONS OF THE STACKED SOLENOID MODEL OF HET-S PRION FIBRILS*

Anindito Sen[1], Ulrich Baxa[1], Martha N. Simon[2], Joseph S. Wall[2], Raimon Sabate[3], Sven J. Saupe[3] & Alasdair C. Steven[1]
1 Laboratory of Structural Biology, National Institute of Arthritis, Musculoskeletal, and Skin Diseases, National Institutes of Health, Bethesda, MD 20892, USA;
2 Department of Biology,Brookhaven National Laboratory, Upton NY 11973, USA;
3 Laboratoire de Génétique Moléculaire des Champignons, Institut de Biochimie et de Génétique Cellulaire, UMR 5095 CNRS/Université de Bordeaux 2, 33077 Bordeaux, France.

STEM produces a quantitative image by scanning a small (0.25nm) electron probe over a specimen and measuring the fraction of electrons scattered. This gives a 2-D projection of the mass distribution of an unstained specimen on a thin (2nm) carbon substrate. Measuring mass of pixels along a defined length of filament gives mass per unit length directly, a parameter difficult to obtain by other means. This can be critical in testing various structural models.

Fungal prions are infectious filamentous polymers of proteins that are soluble in uninfected cells. In its prion form, the HET-s protein of Podospora anserina participates in a fungal self/nonself recognition phenomenon called heterokaryon incompatibility. Like other prions, HET-s has a so-cal led "prion domain" - its C-terminal region, HETs(218-289) - that is responsible for induction and propagation of the prion in vivo and for fibril formation in vitro. Prion fibrils are thought to have amyloid backbones of polymerized prion domains. A relatively detailed model has been proposed for prion domain fibrils of HET-s based on a variety of experimental constraints (Ritter et al., Nature 435, 844-848, 2005). To test specific predictions of this model, which envisages axial stacking of β-solenoids with two coils per subunit, we examined fibrils by electron microscopy. Electron diffraction gave a prominent meridional reflection at (0.47nm)-1, indicative of cross-β structure, as predicted. STEM mass-per-unit-length measurements yielded 1.02 + 0.16 subunits per 0.94 nm, in agreement with the model prediction (1 subunit per 0.94 nm). This is half the packing density of ~1 subunit per 0.47 nm previously obtained for fibrils of the yeast prion proteins, Ure2p and Sup35p, whence it follows that the respective amyloid architectures are basically different.



Sen et al. (2007) JBC 282, 5545-5550

Tissue Engineering Resource Center (TERC)

David L. Kaplan, Tufts U.; Gordana-Vunjak-Novakovic, Columbia U.; Robert Langer, MIT

The objective of the Tissue Engineering Resource Center (TERC) is to advance fundamental and translational aspects of functional tissue engineering through scientific discovery, collaborations, service, dissemination and training. The technical scope of the Center is focused on advances in biomaterial scaffolds, bioreactors and imaging in an integrated approach to functional tissue engineering. Specific areas of research and technological focus include: (a) fibrous protein scaffold designs to control human adult and embryonic stem cells with a focus on the role of scaffold chemistry, structure and mechanical properties on cellular differentiation, (b) new biomaterial scaffolds for tissue engineering with consideration for mechanical function, rates of matrix remodeling and molecular to macro-scale relationships between material properties and biological outcomes, (c) bioreactor systems with local control of cellular environments, multi-parametric signaling (hydrodynamic shear, mechanical and electrical forces) and imaging capability. Tissue-specific outcomes of focus in the Center include skeletal (bone, cartilage, ligament, adipose) and cardiovascular (heart muscle, vascular networks). Therefore, a systems approach to functional tissue engineering is the main focus of the program.

During the third year of the program, significant progress has been made on several fronts: (a) further characterization of relationships between protein-based biomaterial designs and cellular pathways toward functional tissue outcomes, (b) new modes of silk and collagen protein matrix processing, optimizing structure, morphology and chemistry to produce functional biomaterials and tissues in vitro, this control of matrix features permits the delivery of bioactive molecules to cells to foster improved functional tissue outcomes, resulting in the formation of bone, cartilage and adipose, among other systems, gradients and dual factor delivery, as well as sustained release are options pursued, as is the implementation of new tools for direct imaging of the matrices and cells to provide more dynamic readouts of the growing tissues, (c) small-size, modular, highly instrumented micro-array type reactors have been developed to enable high-throughput screening of culture conditions and cell preparations, two-dimensional (2D) and three-dimensional (3D) bioreactors from microscope slide scale to multi-well plate scales have been developed to foster more rapid screening while reducing the logistics of culturing. Some bioreactors consist of an array of individual cell culture wells that are perfused to provide environmental control and the application of physical signals. Some of these systems are also designed to interface directly with imaging modes. The system design is supported by mathematical modeling with the intent to engineer an array of functional tissues.

This significant progress has been made through synergistic collaborations between Core 1 (Tufts University) and Core 2 (Columbia University and MIT) teams, external collaborators from the academic and industrial communities, as well as by mobilization of substantial additional resources (expertise, engineering and clinical investigators, equipment) that are available to TERC through Tufts, Columbia and MIT. These dynamics, made possible through Center collaborations and service, provide new scientific and technical systems to establish baseline information for studies of specific functional tissues.

Poster Abstracts - NCRR / Informatics Resources

Center for Integrative Biomedical Computing
University of Utah
Chris Johnson

High Performance Computing for Biomedical Research
Pittsburgh Supercomputing Center
Joel Stiles

Human Genetic Analysis Resource
Case Western Reserve University
Courtney Gray-mcguire

National Biomedical Computation Resource
University of California, San Diego
Wilfred Li

National Resource for Cell Analysis and Modeling
University of Connecticut Health Center
Leslie Loew

Resource for Biocomputing, Visualization, and Informatics
University of California, San Francisco
Scooter Morris

Center for Integrative Biomedical Computing Research Collaborations

C.R. Johnson[1], R.S MacLeod[1,2], R. Whitaker[1], D. Weinstein[1], D. Brooks[3], J. Stinstra[1,2], and J. Cates[1]
Scientific Computing and Imaging Institute, University of Utah[1], Cardiovascular Research and Training Institute, University of Utah[2], Northeastern University[3]
NIH Center for Integrative Biomedical Computing (CIBC), www.sci.utah.edu/cibc

Computing Defibrillation Thresholds in Children using a Computer Model

Implantation of Implantable Cardiac Defibrillators (ICDs) in children is less frequent and less standardized than in adults so that determining efficient electrode placement is challenging and uncertain. We are collaborating with M. Jolley, M.D. and J. Triedman, M.D., Children's Hospital Boston, to develop interactive finite element (FEM) computational models to test defibrillation in children with interactive electrode location. The models come from CT-scans segmented into tissue types and then meshed for FEM. The system also includes a library of realistically shaped can and wire electrodes. An interactive interface allows the user to easily place and move the electrodes in the model to evaluate different implantation locations. To date we have fully segmented three CT scans, from 2, 10, and 27 year-old subjects, and have created a database of approximately 20 suitable electrode locations per model, which we are testing for bioelectric field strength and homogeneity. A key technical aspect is our scheme to refine the FEM mesh near the electrodes, which has produced results similar to a regular mesh 27 times as dense in about 7% of the computation time. Initial scientific findings have included evaluating the effectiveness of standard locations in adults and novel locations in children. A preliminary analysis indicates that electrode locations inside the lower abdominal wall, common in children to accommodate the can, may produce results that are sensitive to the air volume in the digestive tract.

Segmentation and Visualization for the Study of Cells and Tissues

The National Center for Microscopy and Imaging Research (NCMIR) at the University of California San Diego develops 3D electron tomography imaging technologies for the study of cells and tissues. Biomedical researchers need a variety of image analysis and visualization tools to extract structure and function from these images. The CIBC is developing a 3D volume segmentation and visualization tool for NCMIR researchers called Seg3D, which is designed to simplify their image analysis tasks. Seg3D combines a flexible manual segmentation interface with powerful higher-dimensional image processing and segmentation algorithms from the Insight Toolkit, such as nonlinear image denoising, edge-detection, statistical flood filling, morphological operations, and level-set segmentation algorithms. Users can explore and label image volumes using orthogonal slice view windows, cutting planes, and 3D volume rendering. Collaborative design of Seg3D with the researchers at NCMIR aims to address problems such as large datasets and complex image features. Label masking logic allows researchers to map ontological relationships between objects to the segmentation process to enforce concepts such as containment and adjacency. This important feature is missing from other segmentation tools, and is essential for cataloging and understanding the structural relationships between biological subsystems.

A Novel Excess Calcium Binding Site Model of Neurotransmitter Release

Dittrich M[1], Luo F[2], Cho S[2], King JD Jr [2], Pattillo JM[1,3], Meriney SD[2], and Stiles JR[1,2]
[1]National Resource for Biomedical Supercomputing, Pittsburgh Supercomputing Center, Carnegie Mellon University, Pittsburgh, PA 15213
[2]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260
[3]Natural Sciences and Math, Macon State College, Macon, GA 31206
National Resource for Biomedical Supercomputing, www.nrbsc.org, stiles@psc.edu

Neural function is predicated on neurotransmitter release at synapses. The mechanism by which Ca^{2+} ions trigger release cannot be observed directly, and hence remains poorly understood despite decades of physiological and molecular studies. As a result, there is presently a large discrepancy between physiological and biochemical models of neurotransmitter release. Classic *in vivo* physiology shows that release depends on the fourth power of extracellular Ca^{2+} concentration, and existing models therefore assume about 4 cooperative Ca^{2+} binding sites per synaptic vesicle. On the other hand, *in vitro* studies of presynaptic proteins indicate up to 8 SNARE-synaptotagmin complexes per vesicle. Each synaptotagmin molecule can bind up to 5 Ca^{2+} ions, so it appears that each vesicle may have up to 40 Ca^{2+} binding sites. In addition, it has been suggested that at least 2 sites on an individual synaptotagmin molecule may need to be occupied before the molecule can participate in triggering release.

We propose a novel “excess calcium binding site” model of neurotransmitter release to reconcile these disparate physiological and biochemical data**.** We hypothesize that up to 40 binding sites are associated with each vesicle, but that only a fraction of these sites (possibly in particular combinations) must be occupied to trigger release. To test our hypothesis, we used *MCell* and *DReAMM* software developed at the National Resource for Biomedical Supercomputing (www.mcell.psc.edu and www.nrbsc.org). We created a realistic 3D nerve terminal and used stochastic diffusion-reaction methods to simulate action potential-driven Ca^{2+} channel gating, ion permeation, and binding. We made no *a priori* assumptions about the number of Ca^{2+} binding sites on vesicles, and imposed no *ad hoc* cooperativity on binding and triggering of release. Instead, the number of binding sites and the conditions required for release were the principle unknowns. We found that excess binding sites are both *necessary and sufficient* for physiological Ca^{2+} sensitivity, with release triggered by occupancy of 6-8 sites corresponding to C(2) domains on a fraction of available synaptotagmin molecules. Our model also predicts that Ca^{2+} ions bound to fused vesicles mostly originate from more than one Ca^{2+} channel. The model was constrained by and validated against Ca^{2+} imaging to determine the distribution and opening probability of Ca^{2+} channels in nerve terminals, patch clamp recordings to determine the distribution of single Ca^{2+} channel current sizes evoked by an action potential, fluorescence microscopy and voltage clamp recordings to determine the probability of release from an active zone, the effect of partial Ca^{2+} channel block, the fourth-order relationship between release and Ca^{2+} concentration, and the normal physiological distribution of release latencies.

Our model provides the first quantitative mechanism of transmitter release that relates observed physiology to the molecular machinery of exocytosis. Furthermore, the presence of excess Ca^{2+} binding sites may be an important factor in synaptic plasticity and neuropathology. Many drugs and naturally-occurring toxins target the cellular machinery of neurotransmitter release, and the regulation and modulation of release is critical to virtually all somatic and cognitive behaviors. Thus it is critically important to understand the molecular mechanisms that regulate excitation-secretion coupling at the synapse.

The Human Genetic Analysis Resource: Mapping Genes for Complex Disease

Courtney Gray-McGuire[1,2], Robert C. Elston[1,2]

1- Department of Genetic and Molecular Epidemiology, 2- Case Center for Transdisciplinary Research in Energetics and Cancer, Case Western Reserve University, Human Genetic Analysis Resource, website: http://darwin.case.edu, email: clg9@case.edu

Perhaps the greatest challenge of studying common, multifactorial diseases today is how best to gather, analyze and synthesize the abundant data that has become available with a surge in technological advances. This is often the source of great debate. However, the Human Genetic Analysis Resource exists, in part, to serve as a conduit for this information, to assess the relevant techniques available in the field and offer them in the comprehensive, easy-to-use framework of the S.A.G.E. software suite. We present here two examples of the usefulness and success of this paradigm.

The isolation of genes responsible for baseline gene RNA expression levels has implications for many common, complex diseases. Morley et al (2004) recently found evidence of linkage to specific chromosomal segments for over 1,000, of 3,554 expression phenotypes. This was done using the sibling-based, model-free linkage method as implemented in SIBPAL in S.A.G.E. to analyze 14 large families from the Centre d'Etude du Polymorphisme Humain (CEPH). In addition to verifying the heritable component of expression levels, this study suggests that expression phenotypes are affected by both cis- and trans-acting factors within multiple genes working in concert.

Understanding the role of energy balance in humans has similarly broad implications for complex diseases, particularly metabolic syndrome and cancer. Results from both segregation and linkage analysis using S.A.G.E. supports the importance of genetic variants in obesity (Palmer et al, 2004) as do candidate gene studies investigating the role of C-reactive protein and Apolipoprotein E (Larkin et al, 2004 and 2005). Likewise, linkage analysis, using SIBPAL, of families enriched for colon cancer suggests a disease susceptibility gene on chromosome 9q22.2-31.2 (Weisner et al, 2003). The results of further linkage disequilibrium and association analyses using TDTEX and ASSOC in S.A.G.E. on both microsatellite and single nucleotide polymorphism (SNP) data affirm this finding.

Enabling Collaborative Advances towards Multiscale Modeling of Human Physiology and Disease Conditions

Wilfred W. Li[1], Chandrajit L. Bajaj[4], Nathan A. Baker[3], Yuhui Cheng[1], Peter N. Costandi[1], Lisa M. Eubanks[2], Mark H. Ellisman[1], Amarnath Gupta[1], Michael Holst[1], Kim D. Janda[2], J. Andrew McCammon[1], Andrew D. McCulloch[1], Anushka Michailova[1], Phil Papadopoulos[1], Art Olson[2], Michel Sanner[2], Peter W. Arzberger*[1]

University of California, San Diego[1], the Scripps Research Institute[2], Washington University, Saint Louis[3], University of Texas, Austin[4]

National Biomedical Computation Resource (NBCR), *http://nbcr.net*

*Principal Investigator, email: *parzberg@ucsd.edu*.

Abstract – The mission of the National Biomedical Computation Resource (NBCR) is to conduct, catalyze and enable multiscale biomedical research by harnessing advanced computation and data cyberinfrastructure through multidiscipline and multi-institutional integrative research and development activities. Here we report some recent advances enabled by tools from NBCR at multiple scales of biological investigation towards simulation based medicine for human diseases such as heart failure, neuromuscular dysfunction and Alzheimer's disease.

Continuity is a multiscale problem solving environment in bioengineering and physiology, especially cardiac biomechanics, biotransport and electrophysiology. Using noninvasive magnetic resonance imaging (MRI) and invasive catheter techniques, followed by finite element (FE) modeling of the obtained data using Continuity 6.3, Costandi *et al* were able to estimate the end diastolic volume pressure relationship (EDVPR) in the dilatation phase. The geometric data at the end-diastole from MRI and the pressure and volume data provided by the hemodynamic analysis were used to simulate computationally the systolic phase while maintaining the conservation of mass. The results together suggest that while there is initial compensation of heart function from dilatation in muscle LIM protein (MLP) deficient mice, eventually this fails (decompensation) completely in adults, possibly due to further increase beyond a certain threshold in the stiffness of the myocardium.

Neuromuscular dysfunction is a common pathological condition in many autoimmune diseases such as Lou Gehrig's or myasthenia gravis. To simulate physiological conditions where the ligand and enzyme concentrations are not at steady state but time-dependent, Cheng *et al* developed a FE solver based on the Finite Element ToolKit (FETK) for the time-dependent Smoluchowski equation to model the mouse Acetylcholinesterase (AchE) system, a diffusion-limited reaction with plenty of real experimental data for validation of models. The biomolecular mesh representation was generated using the Levelset Boundary Interior Exterior Mesher (LBIE). The calculations depend on the FETK and the Adaptive Poisson-Boltzmann Solver (APBS) for electrostatic potentials and for iterative mesh refinement to gain better accuracy in the binding constant calculation. This new tool establishes the foundation for integration of molecular scale studies into larger simulations at the cellular scale such as the neuromuscular junction, where AchE plays a critical role in neurotransmitter signaling pathways.

AutoDock is one of the most cited docking software in a recent literature survey, and AutoDockTools is an important user interface designed by NBCR to facilitate the preparation, execution and analysis of AutoDock studies. Eubank *et al* recently found that one of the major chemical ingredients found in marijuana, Δ^9–tetrahydrocannabinol (THC), binds the peripheral anionic site of acetylcholinesterase (AchE) using AutoDock, and the result is subsequently confirmed through enzymatic inhibition assays. As AchE is implicated in accelerating the formation of amyloid fibrils in Alzheimer's disease, this collaborative study suggests that THC and its analogues may lead to the design of better therapeutics which may improve neurotransmitter stability and prevent amyloid formation.

Additional information about the cyberenvironment for multiscale modeling and translational research from NBCR may be found at http://nbcr.net/tools.php and http://nbcr.net/news.php.

The Virtual Cell Project: New Algorithms for Modeling Cellular Complexity

James C. Schaff, Boris M. Slepchenko, Ion I. Moraru, Li Ye, Fei Gao, Frank Morgan, Igor L. Novak, Michael L. Blinov and Leslie M. Loew
Richard D. Berlin Center for Cell Analysis and Modeling
University of Connecticut Health Center
Farmington, CT 06030
National Resource for Cell Analysis and Modeling
http://vcell.org
les@volt.uchc.edu

The Virtual Cell is a general software environment for modeling cell biological processes that is deployed as a freely accessible distributed application over the Internet. Using a graphical user interface, kinetic, transport and electrophysiological data describing individual reactions are associated with 2D or 3D experimental microscopic images that describes their subcellular locations. From the resultant mechanistic model, the software automatically generates reaction-diffusion-advection partial differential equations. These equations are then solved numerically to produce simulations of the biological process. Non-spatial models may also be simulated by specifying the volumes and surface areas of idealized cellular compartments. The models are stored in a database that permits users to share the models with individual collaborators or make them completely public. The database also permits searching for model components either from other VCell models or from public pathway databases. More than 1100 users worldwide have created models and run simulations with the Virtual Cell.

During this past year, we have made significant progress in supporting models and simulations that comprise new physical mechanisms, especially with regard to more complex cellular processes. The largest project was the formulation, development and implementation of lateral diffusion along convoluted arbitrarily shaped surfaces representing the membranes of cells and their organelles. VCell now contains the first general purpose algorithm for coupling reactions and diffusion along a curved surface to diffusion and reactions in the adjacent volume. This is illustrated with a model of PIP_2 dynamics during activation of a neuronal synapse. Another important advance has been the ability to use spatially variable experimental data, such as might be derived from an immunofluorescence image, as inputs in a VCell model. Thus, not only can arbitrarily shaped cells be used to define the geometry of a model, now arbitrarily distributed molecular localizations can be used to define the initial state of a cellular system. A third advance has been the implementation of a stochastic solver using the Gibson-Bruck variant of the Gillespie algorithm; now users can create a physiology and then choose to non-spatially simulate it either deterministically, with solutions to ordinary differential equations, or discretely, with stochastic simulations. Polymerization reactions are particularly challenging because of the multitude of states that arise from the distribution of polymer lengths; we have dealt with this issue in a model of actin polymerization by developing a formulation that lumps the differently sized states of F-actin. Finally, to begin dealing with the combinatorial complexity of multi-state proteins systems, often involved in receptor kinase signaling, we have created a direct link within VCell to the rule-based modeling software system BioNetGen. Using SBML-based communication, BioNetGen can be called from within VCell to generate a model that can be simulated either stochastically or deterministically.

(supported by NCRR through NIH grant P41RR013186)

New Features in UCSF Chimera

Conrad Huang, Eric Pettersen, Greg Couch, Tom Goddard, Scooter Morris, Tom Ferrin
Department of Pharmaceutical Chemistry, University of California, San Francisco
Resource for Biocomputing, Visualization, and Informatics
http://www.rbvi.ucsf.edu/
scooter@cgl.ucsf.edu

Chimera is a major component of this Resource Center's technological research and development activities. Chimera was designed to provide standard molecular modeling functionality, as well as facilitate development of new methods for data analysis through the addition of extension modules. Recently, we have augmented Chimera functionality with two extensions, **DockPrep** and **Morph**, as well as bundling POV-Ray (http://www.povray.org/) as part of the Chimera distribution.

DockPrep is an extension for preparing input for **Dock** (http://dock.compbio.ucsf.edu/), a molecular docking system, and nicely complements the **ViewDock** extension, which is used for visualizing the results from Dock runs. **DockPrep** leverages the hydrogen-addition functionality of Chimera and adds atomic partial charge assignment using a combination of templates for standard residues and an external helper package, **antechamber** (http://amber.scripps.edu/antechamber/ac.html).

Morph, inspired by MolMovDB (http://www.molmovdb.org/), interpolates a trajectory of intermediate structures between two conformers. The computed trajectory is displayed using **Movie**, an extension for displaying molecular dynamics trajectories. When used in conjunction with the **MovieRecorder** extension, users can easily create movies illustrating the differences among conformers.

For publication-quality images, we have bundled POV-Ray, a ray tracing program, with the Chimera distribution. The Chimera Save Image dialog has been modified to provide access to the common POV-Ray options. Using the new interface, users can generate ray-traced images (which have many desirable aesthetic features such as shadows, smooth edges, and superior transparency effects) directly from Chimera with just a few mouse clicks.

Poster Abstracts - NCRR / Imaging Technology

BioCurrents Research Center
Marine Biological Laboratory, Woods Hole
Peter JS Smith

Center for Advanced Magnetic Resonance Technology at Stanford
Stanford University
Gary Glover

Integrated Center for In Vivo Microscopy
Duke University Medical Center
Srini Mukundan

NMR Imaging and Localized Spectroscopy
CMRR, University of Minnesota
Kamil Ugurbil

National Center for Microscopy and Imaging Research
University of California, San Diego
Skip Cynar

National Center for Microscopy and Imaging Research
University of California, San Diego
Mark Ellisman

National Center for X-Ray Tomography
University of California, San Francisco
Gerry McDermott

National Resource Center for Image Guided Therapy
Brigham and Women's Hospital, Harvard Medical School
Kim Lawson

Resource for Quantitative Functional MRI
Kennedy Krieger Institute and Johns Hopkins University
Peter van Zijl

Southwestern NMR Center for In Vivo Metabolism
University of Texas Southwestern Medical Center
Craig Malloy

Ionic profiles in the cellular boundary layer reveal single channel and non-electrogenic transporter activity

Mark A Messerli and Peter JS Smith
Marine Biological Laboratory
BioCurrents Research Center
www.biocurrents.org: psmith@mbl.edu

Many diseases, and avenues for disease treatment, involve malfunctioning or hyperactive ion channels or transporters. Conventional, real-time, methods have been instrumental in the characterization of normal and abnormal states of transport. However, they have not been useful for studying the vast majority of slow rate and no-current passing (electroneutral) transporters, as well as ion channels on cells that cannot be voltage clamped. It is for these reasons that we are exploring the use of extracellular electrochemical sensors for real time measurement of ion transport through channels and transporters. Ion-selective electrodes possess the speed and sensitivity to capture extracellular ion activity changes from moderate to large conductance, single channels. Measured events from a Ca^{2+}-activated K^{+} channel are a good fit with a model that takes into account diffusion, response time of the sensor, and distance from the source. This enables noninvasive, functional characterization of ion channels and mapping of their location. Used in a modulation mode, the signal to noise ratio is increased and can be used to measure small, relatively steady, fluxes from ion transporters. This provides real-time characterization of slow rate or electroneutral transporters. These approaches have clear applications for the high fidelity, noninvasive, long-term, monitoring of ion transport in the normal and diseased states.

Controlled inspiration depth reduces variance in breath-holding induced BOLD signal

Moriah Thomason Caires, Gary H. Glover,
Neurosciences Program & Dept. of Radiology,
Center for Advanced MR Technology @ Stanford University
http://rsl.stanford.edu/research/camrt.html gary.glover@stanford.edu

Introduction: One of the primary methodological challenges facing fMRI is that the blood oxygen level dependent (BOLD) signal is only an indirect measure of neuronal activity, because it derives from neurovascular processes. There is concern that inferences regarding cerebral activation may be confounded by regional variation in brain vasculature, or alterations in the cerebral vascular system that are due to age- or disease-related changes. Recent studies used BOLD contrast scanning during short periods (~18s) of breath holding (BH) to measure hemodynamic response differences between different age groups (1, 2). Because BH is easy to implement and noninvasive, and the effects of BH are global and robust, this paradigm is applicable for routine use in research studies. Measurement of neural activity has been shown to be improved by calibrating for fMRI signal variance that results from intrinsic properties of the local vasculature (3). Thomason and colleagues used BH to derive a correction map for each individual, then applied voxel-wise hemodynamic correction to working memory scan data taken in the same session. In the present study we have performed further studies to extend the precision of this technique with the express goal of attaining an effective and routine hemodynamic calibration metric.

Materials and Methods: It was hypothesized that BOLD signal amplitude is affected by BH depth of inspiration, and therefore could be controlled by giving participants real-time feedback to stabilize their inspiration depth. Thirteen healthy adults (ages 24-64) performed six BH scans. Three versions of BH scans were run, twice each, and all included seven repetitions of alternating periods of breath-holding and self-paced breathing in 18-s blocks. Versions differed in whether or not participants received feedback on the depth to which they should inspire. Feedback target depth was cued to either (i) a constant level or (ii) a varying level with three different set points (75%, 87%, and 100%), or (iii) included no feedback. Varied inspiration depth scans were averaged to test the correspondence of inspiration depth and BOLD signal effect. The average of controlled-depth scans and the average of no feedback scans were compared for more or less variance in (a) inspiration depth (a performance measure) and (b) BOLD signal. Activation maps were tested for increased statistical significance resultant from controlled feedback and reduced variance.

Results: BH-induced BOLD signal amplitude corresponded to depth of inspiration. Inspiration depth variation and BOLD signal variation were reduced with feedback that cued participants to the appropriate inspiration depth. Greater overall magnitude and extent was demonstrated in activation maps for scans with controlled feedback compared to the no-control scans.

Isotropic Four-Dimensional MR Imaging of the Mouse Heart

Elizabeth Bucholz[1,4], Ketan Ghaghada[1,2], Yi Qi[1], Catherine Hawley[4], Srinivasan Mukundan[1,2,3,4], G. Allan Johnson[1,2,4]

[1]Center for In Vivo Microscopy, Duke Univ. Medical Center, [2]Radiology, Duke Univ. Medical Center, [3]Chemistry, Duke Univ., [4]Biomedical Engineering, Duke Univ.

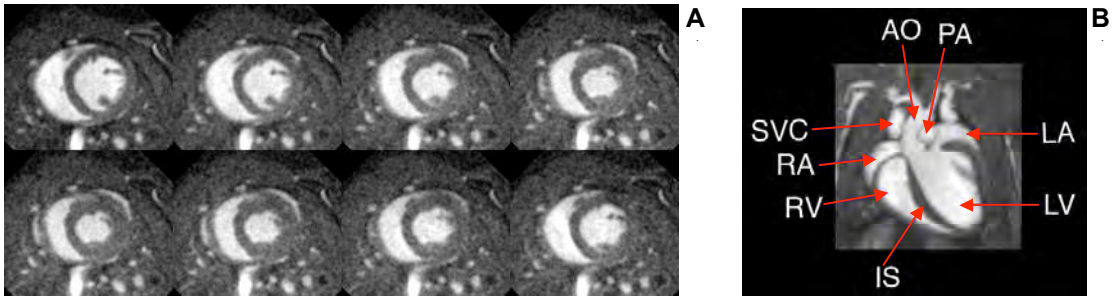
Resource Center: Center for In Vivo Microscopy, Duke Univ. Medical Center

Website: www.civm.duhs.duke.edu Email: gaj@orion.duhs.duke.edu

Introduction: MR imaging of the mouse heart has found wide acceptance in the study of both structural and functional phenotypes. The majority of the previous work has used 2D encoding methods that are limited in their ability to acquire isotropic resolution. We describe a 3D encoding technique, allowing acquisition of 4D data with temporal resolution of 10 ms and isotropic spatial resolution of 110 microns. A blood pool agent (liposomal-Gd) improves blood-myocardium contrast to support automated segmentation for quantitative functional measurements.

Materials and Methods: All MR studies were performed on a 7T, 21-cm bore, magnet interfaced to a GE EXCITE console (EPIC 12.4). A 3-cm surface coil was used for transmit and receive. A 3D cine sequence excites with a hard-pulse excitation followed by gradients, which sample the FID along radial trajectories of k-space. A short TR (2.5 ms) allows sampling of 50 views per cardiac cycle (~100 ms). In a typical acquisition, 62,720 views are acquired with the trajectory end points distributed uniformly on a sphere of k-space. The sequence allows flexible tradeoff of the distribution of the views in any cycle in both space and time. Six C57BL6 mice were induced with isoflourane and given a tail vein injection of 0.2 mmol/kg liposomal-Gd contrast agent. Functional metrics were extracted using a Vitrea workstation (Vital Images, Inc., Minneapolis MN) functional cardiac package.

Results and Discussion: The ultra-short TE (0.3 ms) and TR-enabled acquisition of eight phases of the cardiac cycle in 34 minutes. CNR between blood and myocardium (10.5) was sufficient to allow automated segmentation of chambers and extraction of quantitative functional metrics (Typical values: LV @ diastole = 59.55 ± 8.28 µL; LV@ systole = 22.24 ± 2.81 µL, Ejection fraction = 0.625 ± .024).



(A) Short axis view of 8 phases of the cardiac cycle at 110-micron isotropic resolution. (B) Coronal maximum intensity view demonstrating the cardiac/vascular structures. RA–right atrium; RV–right ventricle; IS–interventricular septum; LV–left ventricle; LA–left atrium; AO–aorta; PA–pulmonary artery; SVC–superior vena cava.

Conclusion: We have described a 4D MR imaging technique that allows isotropic imaging at 110 microns with temporal sampling down to 10 ms. The use of a blood pool agent enhances the blood signal, so chamber volumes can be automatically segmented. The resulting acquisition, reconstruction, and analysis pipeline is now being used to support high-throughput assessment of cardiac structure and function in the mouse.

All work was performed at the Duke Center for In Vivo Microscopy, an NCRR/NCI National Resource (P41 RR005959/U24 CA092656).

Applications of Advanced Multiscale Microscopy to Heart Disease and Brain Cancer at the National Center for Microscopy and Imaging Research

Hayashi T[1], F Hughes[1], G Perkins[1], DL Price[1], M Berlanga[1], B Smith[1], T Deerinck[1], H Hakozaki[1], K Kim[1], V Phung[1], M Terada[1], A Thor[1], B Hegedus[4], R Bash[3], K Natalie[3], C Yin[3], Q Zhang[3], ME Martone[1], M Hoshijima[2], T Van Dyke[3], D Gutmann[4], and MH Ellisman[1]

[1]NCMIR, Univ. Calif. San Diego, La Jolla, CA 92093; [2]Dept. Medicine, UCSD; [3]Univ. of North Carolina, Chapel Hill, NC 27599; [4]Washington University St. Louis, St. Louis, MO 63110

National Center for Microscopy and Imaging Research (ncmir.ucsd.edu) mellisman@ucsd.edu

The NCMIR is a recognized authority in the development of technologies for high throughput multi-scale imaging and analysis of biological systems at the mesoscale, the dimensional range that encompasses macromolecular complexes, organelles, and multi-component structures such as synapses. With an emphasis on advancing our understanding of the mechanisms underlying diseases of the nervous system, NCMIR is also helping to investigate the cellular and molecular underpinnings of biological systems relevant to cancer, heart disease, and neurodegenerative disease, among others. Home to more than 70 active projects, interdisciplinary research teams continually leverage and hone NCMIR's expertise in 3D and 4D imaging technologies, innovative labeling chemistries, software systems for instrument automation and control, and knowledge-engineered databases. This poster highlights developments and applications from two of these projects.

NCMIR's electron tomography and correlated light and electron microscopy are being applied to characterize an animal model of dilated cardiomyopathy, a major cause of severe heart failure and associated sudden death in humans. By employing NCMIR's advanced high resolution electron tomography workflow, Drs. Hoshijima and Hayashi of UCSD's Dept. of Medicine have discovered abnormalities in the mesoscale structures constituting membrane systems underlying excitation-contraction (E-C) coupling in the heart. Their research has revealed disease-related changes in a critical component of E-C coupling and identified possibly unique modes of interaction among T-tubules, the endomembrane system, and other organelles. Their detailed mesoscale characterization is helping to buttress a growing body of evidence that suggests that excitation-contraction coupling defects and abnormality in calcium handling play a dominant role in the development of cardiomyopathy and its transition to heart failure.

Several collaborative investigations take advantage of NCMIR's advanced wide-field imaging capabilities to directly observe detailed subcellular structures across large regions and explore how higher order structures, such as cellular networks, are assembled out of finer building blocks. Currently, a pair of related studies are helping to drive the development of large scale mapping techniques and semi-automated measurement and segmentation procedures to characterize the morphology, distribution and molecular constituents associated with normal, and tumor-associated glial cells. Recognizing that little is understood about the properties of glial cells elicited by cancer-promoting lesions in distinct brain microenvironments, collaborators from the Univ. of North Carolina and Washington University are developing high-throughput procedures to acquire quantitative multiscale images of tumor-associated glial cells of astrocytoma and optic glioma, respectively. An understanding of tissue alterations, including how these glial associations change during the course of tumor formation, will help the researchers to identify potential diagnostic and therapeutic targets. These studies extend our previous work on astrocytes and leverages NCMIR's new automated ultra-large field LM technique for quantifying pathological changes in transgenic mice across millimeter- to nanometer-sized structures.

Quantitative 3-Dimensional Imaging of Eukaryotic Cells

D.Y. Parkinson[2,4], M.A. Le Gros[2,4], L.D. Etkin[3], G. McDermott[1,4] W. Gu[1,4] & C.A. Larabell[1,2,4]
[1]Department of Anatomy, UCSF, San Francisco, California
[2]Physical Biosciences Division, Lawrence Berkeley National Laboratory
[3]Department of Molecular Genetics, M.D. Anderson Cancer Center, Houston, Texas
[4]National Center for X-ray Tomography
Contact: Carolyn.Larabell@ucsf.edu

The National Center for X-ray Tomography (NCXT) is an NCRR supported Biotechnology Research Resource. One of our main research foci is the development of soft x-ray tomography as a cellular imaging modality at the nanometer scale. This technique relies on the physical properties of x-rays in the region of the spectrum known as the 'water window'. At these photon energies, water is relatively transmissive towards x-rays, while the presence of carbon and nitrogen causes biological materials to absorb x-rays an order of magnitude more strongly. This difference in absorption gives rise to images with excellent contrast without the use of stains or other potentially disruptive contrast enhancing agents. These images reveal details of the sub-cellular architecture at a spatial resolution of 40nm or better. Furthermore, since soft x-rays can penetrate more deeply than electrons, there is no need to section the cell prior to analysis: cells are imaged intact and in their natural, fully hydrated state. Differences in the x-ray absorption coefficient can be used to identify and delineate individual organelles in the cell. Since these coefficients can be measured accurately, it is possible to differentiate species of organelle on this basis. Using the eukaryote *Schizosaccharomyces pombe* as our model system, we have begun a study to identify and quantify organelles in 3-D volumetric representations of the cells. Examples of this process can be seen in Figure 1 below. We will present these results, together with details of other NCXT research efforts at the meeting.

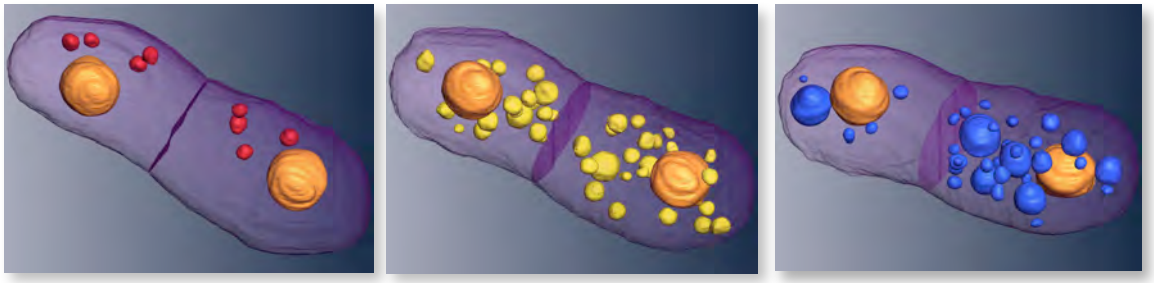


Figure 1: Organelle architecture in a dividing *S. pombe* cell, derived from the x-ray absorption coefficients. **Key**: Red, the most absorbing organelles, yellow intermediate, and blue the least absorbing. The nucleus is shown in orange.

Improved Targeting and Assessment of Image-Guided RF Liver Ablation

Neculai Archip, Servet Tatli, Paul R. Morrison, Simon K. Warfield, Stuart G Silverman, Ferenc A. Jolesz
Brigham and Women's Hospital, Harvard Medical School, Boston, MA
National Center for Image-Guided Therapy (www.ncigt.org)
narchip@bwh.harvard.edu

MRI or contrast-enhanced CT are used to plan and assess the radiofrequency ablation (RFA) of liver tumors, while unenhanced CT is often used to guide the intervention. However, tumor margins are not always visible on unenhanced CT images, a reality that can make accurately placing the RF electrode difficult.

To counteract this phenomenon, we enhance the information available to the interventional radiologist during RFA through deformable registration of pre-procedural MRI with the intra-procedural CT. Accurate position of the RF electrode with respect to the tumor margin is thus known. We also propose the quantitative assessment of the RFA treatment outcome by precisely aligning pre- and post-procedural MRI (coagulation necrosis and pre-procedural tumor volume).

Thirteen patients (7 women and 6 men; mean age: 58.5 years) who underwent CTguided RF ablation of liver tumors (8 metastasis from various primary and 5 hepatocellular carcinomas) were included in this retrospective study. We introduce a novel non-rigid registration algorithm based on a biomechanical model. For all patients, we apply our new registration algorithm for (1) pre-procedural contrastenhanced MRI and intra-procedural unenhanced CT images of liver, and (2) preprocedural enhanced MRI and post-procedural enhanced MRI of liver. We compare the results of our new registration technique with the results of three other standard registration techniques (rigid, b-spline, demons). The accuracy of the registration methods was evaluated using two metrics: (1) mutual information (MI) and (2) mean value of distance between the edges of anatomical landmarks of the liver. Using the contrast-enhanced T1-weighted images, tumor volume was segmented manually from pre-procedural MRI. Coagulation necrosis volume was segmented using the postprocedural T1w-enhanced MRI. Dice Similarity Correlation coefficient (DSC) that compares spatial relationships of two volumes was then computed.

Overall, for registration between pre-procedural enhanced MRI and intra-procedural unenhanced CT images, the mean error for our method was 1.64 mm compared with 12.2 mm for the rigid, 2.4 mm for b-spline, and 3.0 mm for the demons method. MI calculations were 0.13 for rigid registration, 0.25 for b-spline, 0.18 for demons, and 0.44 for the biomechanical non-rigid registration techniques (with 0 minimum and 1 maximum overlap). The mean error for registration between pre- and post-procedural MR images was 1.7 mm for our registration method. The mean DSC between tumor and coagulation necrosis volumes was 0.61 (range: 0.12-0.88). Our novel technique was significantly better than rigid ($p < 0.0001$), non-rigid b-spline ($p < 0.0001$) and demons ($p < 0.0001$) registration methods.

We demonstrated that our non-rigid registration method allows pre-procedural contrast enhanced MR images to depict liver tumors accurately during CT-guided RF ablation. It also improves the accuracy of DSC measurements, thereby improving three-dimensional assessment of RF ablations of liver tumors. Therefore, the new methodology may prove useful to early detection of recurrences.

Image-Based Calibration and Scan-Control for Transperineal Prostate Needle Placement Robot in MRI Scanner

Simon DiMaio, Nobuhiko Hata, Clare M.C. Tempany
Brigham and Women's Hospital and Harvard Medical School, Boston MA
National Center for Image-Guided Therapy (www.ncigt.org)
ctempany@bwh.harvard.edu

Background: Prostate cancer is a very significant health concern for men, their families and for Society. As we see the aging of the baby boomers in the United States, it is estimated that the number of men diagnosed per year will more than double, to 450,000 new diagnosed cases by 2015. Combined with an aging population, these factors have made prostate cancer a major medical and socioeconomic problem. Some of these deaths may be due to inadequate methods of detection; however, the majority is likely due to inadequate treatment. Currently, there are no reliable methods to detect biologically aggressive tumors and, therefore, many men have detected indolent tumors that will never reach clinical significance.

To address this challenge, researchers are investigating the use of Magnetic Resonance Imaging (MRI) and other imaging modalities to detect focal prostate cancer and, more importantly, to assess its biological activity in vivo. This is already a major effort of ours. However, we are also working to improve the diagnosis and biopsy "hit rate" and, ultimately, to allow for focal therapy to "index" cancers under imaging control and guidance. To this end, we illustrate one of our key projects involving our external collaborators, *Johns Hopkins and the CMS Image Guidance Division*. The project aims to develop a novel image-based method for calibrating and tracking the robotic mechanism in the MRI coordinate frame. This project is part of an ongoing effort to develop enabling technologies to improve MR-guided prostate interventions using a MRI-compatible robotic mechanism. It is funded by NIH Biomedical Research Partnership mechanism (1R01CA111288, NCI, PI Tempany).

Methods and Results: We have developed a method that tracks a set of MR-visible fiducials and takes advantage of the multi-planar imaging capabilities of MRI to dynamically control the scan plane for optimal device localization and visualization. The fiducial frame was constructed using acrylic plastic with seven glass cylinders embedded and filled with an NiCl solution in water (<1%), each forming a 3mm diameter, MR-visible line fiducial. The position and orientation of the Z-frame can be computed from a single intersecting 2D image. In experiments, the Z-frame was placed in a 3T MR scanner (GE Signa EXCITE 3T), on a rotating platform with marked angle gradations, initially aligned at the isocenter. The intersection points of the seven line fiducials were automatically detected by a fast k-space template matching algorithm and used to compute the position and orientation of the Z-frame relative to the scan plane. We found that the mean angular error of the tracking was found to be less than 1º, with a maximum error of 1.59º after convergence of the scan plane.

Conclusion: We present an image-based method for calibrating and tracking the robotic mechanism in the image coordinate frame using a localization approach that does not rely upon additional instrumentation. The proposed method is highly desirable for navigating instruments in MRI-guided interventions. In conclusion, this work demonstrates that it is possible to use passive fiducial detection in 3T MRI images for dynamically locating and navigating the robotic mechanism and its needle driver.

Targeted delivery of doxorubicin to the rat brain at therapeutic levels using MRI-guided focused ultrasound

Lisa Hsu Treat, Nathan McDannold, Natalia Vykhodtseva, Yongzhi Zhang, Karen Tam, Kullervo Hynynen
Department of Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA and the Health Sciences and Technology, Harvard University–Massachusetts Institute of Technology, Cambridge, MA, USA
National Center for Image-Guided Therapy (www.ncigt.org)
njm@bwh.harvard.edu

The clinical application of chemotherapy to brain tumors has been severely limited because antitumor agents are typically unable to penetrate an intact blood-brain barrier (BBB). Although doxorubicin (DOX) has been named as a strong candidate for chemotherapy of the central nervous system (CNS), the BBB often prevents cytotoxic levels from being achieved. In this study, we demonstrate a noninvasive method for the targeted delivery of DOX through the BBB such that drug levels shown to be therapeutic in human tumors are achieved in the normal rat brain. Using MRI-guided focused ultrasound with preformed microbubbles (Optison) to locally disrupt the BBB and systemic administration of DOX, we achieved DOX concentrations of 886 ± 327 ng/g tissue in the brain with minimal tissue effects. Tissue DOX concentrations of up to 5366 ± 659 ng/g tissue were achieved with higher Optison doses but with more significant tissue damage. In contrast, DOX accumulation in non-targeted contralateral brain tissue remained significantly lower for all paired samples ($p < 0.001$). These results suggest that targeted delivery by focused ultrasound may render DOX chemotherapy a viable treatment option against CNS tumors despite previous accessibility limitations. In addition, MRI signal enhancement in the sonicated region correlated strongly with tissue DOX concentration ($r = 0.87$), suggesting that contrast-enhanced MRI could perhaps indicate drug penetration during image-guided interventions. Our technique using MRI-guided focused ultrasound to achieve therapeutic levels of DOX in the brain offers a large step forward in the use of chemotherapy to treat patients with CNS malignancies.

Adaptive Cellular Imaging With 3D MRI

Seung-Schik Yoo, Dimitrios Mitsouras, Krisztina Fischer, Jong-Hwan Lee,
Robert V Mulkern, and Frank J Rybicki
Brigham and Women's Hospital, Harvard Medical School, Boston, MA
National Center for Image-Guided Therapy (www.ncigt.org)
yoo@bwh.harvard.edu

Cell-replacement therapy (CRT) refers to a set of therapeutic strategies to replace damaged or defective cells with cells with functional potency. As a result of the advancement of stem-cell research and related biotechnologies, CRT for various disorders/injuries is anticipated. Non-invasive imaging techniques will play key roles; (1) one is to guide the surgical implantation of particular cells of interest by providing physicians the exact location of the surgical probes/tools with respect to the patient's anatomy during surgery, and (2) the other is to monitor the survival, growth, and migration of implanted cells, as well as to monitor any potential tumor formation to promote the patient's safety under CRT.

With the advent of MR-sensitive cell-labeling agents, cellular imaging using MRI (referred to as cellular MRI in this text) has risen as a new modality for in vivo monitoring of cell characteristics and behavior. An advantage of using MRI for the cellular imaging in the context of CRT is that MRI, which provides excellent soft tissue contrast, can be used as a means to guide the transplantation procedure (such as in MRI-guided therapy). Image-guided, frameless, surgical intervention can ensure the correct location of a target site with real-time feedback to surgeons and doctors. Cellular MRI in clinical setting will cast a unique set of technical challenges since the visualization of the magnetically labeled cells are desired at near-microscopic spatial resolution from a localized image volume, all in clinically acceptable imaging time.

The objective of the current research is to develop and implement an adaptive MRI method addressing these challenges by combining (1) real-time MRI data acquisition and processing and (2) ability to adapt/change the MR imaging parameters/sequences to zoom in/out onto the region-of-interest with maintaining the image quality to ensure the detection of the implanted labeled cells. Using inner volume MRI approach (Mitsouras et al. Med Phys. 2006 Jan;33(1):173-86.), regions surrounding the magnetically-labeled cells located within the object can be selectively imaged at extremely high spatial resolution in conventional 3T MRI hardware less than 20 minutes of scan time.

Tract-Based Morphometry

Lauren J. O'Donnell, Carl-Fredrik Westin, and Alexandra J. Golby
Brigham and Women's Hospital, Harvard Medical School, Boston, MA
National Center for Image-Guided Therapy (www.ncigt.org)
agolby@partners.org

By measuring water diffusion in the brain, diffusion tensor MRI (DTI) gives information about the directionality and integrity of white matter fiber tracts. Quantitative analyses of DTI data in specific white matter tracts have commonly measured the mean value (in the entire tract) of a scalar invariant such as the fractional anisotropy (FA) or mean diffusivity (MD). However, FA and MD vary spatially along fiber tracts due to neuroanatomical factors such as crossing fibers and nearness to CSF, as well as to abnormal processes such as tumor infiltration. Therefore we propose to study DTI data *along* the white matter tracts. We call this approach tract-based morphometry (TBM), the statistical analysis of tensors or scalar invariants along the length of fiber tracts. The goal of the method is to detect white matter changes in support of a particular neuroscientific hypothesis. Our pilot study quantified diffusivities along affected and contralateral corticospinal tracts in several tumor patients, finding white matter changes past the apparent border of the tumor in T2-weighted images. We then developed an automatic TBM method for group analysis. First DTI tractography from multiple subjects is analyzed to produce common arc length coordinates (point correspondences) across subjects, then DTI statistical analysis is performed in this tract-based coordinate system. Initial TBM results from an experiment comparing right- and left-hemisphere FA in normal right-handed subjects showed significant differences in regions of the cingulum bundle and arcuate fasciculus. Overall, the TBM approach brings analysis of DTI data into the clinically and neuroanatomically relevant framework of the tract anatomy.

Magnetic Resonance Imaging of Alzheimer's Pathology in the Brains of Living Transgenic Mice: A New Tool in Alzheimer's Disease Research
Magnetic Resonance Imaging of Alzheimer's Pathology in the Brains of Living Transgenic

Clifford R. Jack[#], Jr, Malgorzata Marjanska, Thomas M. Wengenack[#], Denise A. Reyes[#], Geoffrey L. Curran[#], Joseph Lin, Gregory M. Preboske[#], Kamil Ugurbil, Joseph F. Poduslo[#] and Michael Garwood
University of Minnesota, Center for Magnetic Resonance Research (www.cmrr.umn.edu) and The Mayo Clinic

This is a collaborative project between the BTRR located in the University of Minnesota (in Center for Magnetic Resonance Research) and the Mayo Clinic.

Alzheimer's disease (AD) is the fourth leading cause of death for those over 65. By the year 2040 an estimated 14 million will be living with the disease. One of the cardinal pathologic features of AD is the formation of both senile and amyloid plaques (basically protein deposits) in the brain. Until recently, these plaques were too small to see, so definitive diagnosis of Alzheimer's disease could occur only at autopsy or with the use of cognitive testing that is influenced by many other factors, often making it inaccurate. Together with scientists from the Mayo Clinic, CMRR investigators have developed novel magnetic resonance imaging (MRI) technology permitting visualization and quantification of individual AD plaques for the first time in a living creature. Early studies are being conducted on transgenic mice which express one or more of the genes responsible for familial AD in humans. Double transgenic mice develop "human-like" plaques, providing a mechanism to study amyloid plaque biology in a controlled manner. The research has shown that histologically verified plaques as small as 35 micrometers in diameter can be visualized in living animals. Although imaging of labeled plaques has also recently been accomplished with positron emission tomography (PET), only MRI has sufficient spatial and contrast resolution to visualize individual plaques noninvasively. In addition, age-dependent changes in metabolite concentrations analogous to those that have been identified in human AD patients can be detected in these transgenic mice using proton magnetic resonance spectroscopy (^{1}H MRS). These novel MR-based techniques provide a new set of tools to the scientific community engaged in studying the biology of AD in transgenic models of the disease. An obvious application which is being undertaken is to evaluate therapeutic modification of disease progression, with the goal of accelerating drug discovery for Alzheimer's disease.

1. C.R. Jack, M. Garwood, T.M. Wengenack, B. Borowski, G.L. Curran, J. Lin, G. Adriany, O.H.J. Gröhn, R. Grimm, and J.F. Poduslo, *Magn. Reson. Med*. *52*, 1263-1271, 2004.
2. M. Marjanska, G.L. Curran, T.M. Wengenack, P.-G. Henry, R.L. Bliss, J.F. Poduslo, C.R. Jack, Jr., K. Ugurbil, and M. Garwood, *Proc. Natl. Acad. Sci. USA 102*, 11906-11910, 2005.
3. C.R. Jack, Jr., T.M. Wengenack, D.A. Reyes, M. Garwood, G.L. Curran, B.J. Borowski, J. Lin, G.M. Preboske, S.S. Holasek, G. Adriany, and J.F. Poduslo, *J. Neurosci. 25*, 10041-10048, 2005.
4. C.R. Jack Jr, M. Marjanska, T.M. Wengenack, D.A. Reyes, G.L. Curran, J. Lin, G.M. Preboske[1], J.F. Poduslo, and M. Garwood (2007) *Neuroscientist 13*, 38-48.

IMAGING THE DEVELOPING BRAIN

S. Mori, A. Qiu, J. T. Ratnanather, J. Pekar, P. Barker, P. van Zijl, M. I. Miller
Resource for Quantitative functional MRI
Kennedy Krieger Institute, F.M. Kirby Research Center for Functional Brain Imaging,
Johns Hopkins University; Center for Imaging Science, Dept. of Radiology.
http://mri.kennedykrieger.org/; contact: pvanzijl@mri.jhu.edu

The Resource for Quantitative Functional Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) is an interdepartmental and interdisciplinary laboratory combining facilities of the F.M. Kirby Research Center for Functional Brain Imaging at the Kennedy Krieger Institute (KKI) and the Center for Imaging Science (CIS) at Johns Hopkins University (JHU). This resource is dedicated to using its unique expertise to design novel MRI and MRS data acquisition and data processing technology with a special focus on clinical and neuroscience-based pediatric and neurodevelopmental applications. One of the main issues to be addressed is the need to assess tissue properties and brain anatomical features and apparent alterations in brain activation and/or pathology, when the brain is changing size during development. The problem of changing brain size will be tackled by using landmark, curve, surface and volume based brain warping technologies to be able to derive a unified and comprehensive whole-brain anatomical reference frame for multi-modality MRI data.

Two types of imaging for which this approach is especially important are high-resolution anatomical imaging ($1x1x1mm^3$ true spatial resolution) and diffusion tensor imaging (DTI, ($2.2x2.2x2.2mm^3$ true spatial resolution), in which white matter connections can be identified. While conventional anatomical image approaches (water density and relaxation time, T1, T2, based) are problematic for studying development due to lack of tissue contrast within white matter and between white and gray matter during several stages of brain development, DTI contrast is unique in that it can distinguish such structures at any time point, allowing identification of corresponding brain structures throughout the development process.

Currently we are collaborating with St. Luke Hospital in Belgium and Yonsei University in Korea to establish DTI database of developing brain (www.pediatricDTI.org). Based on this database, we are working on quantitative characterization of the normal brain development. The first level of the analysis was performed by manually delineating corresponding structures and measuring their size, T2, apparent diffusion constant, and diffusion anisotropy (1). In the second level of the analysis, we used a linear normalization method to transform all the data to a common template. After the normalization, we performed pixel-by-pixel analyses to investigate time dependent changes in white matter anatomy. The study clearly indicates that the superior longitudinal fasciculus is one of the tracts that emerges latest during the development. In the third level analysis, our goal is to investigate the development of white matter via non-linear transformation based on Large Deformation Diffeomorphic Metric Mapping (LDDMM) and tensor statistical analysis.

(1) Hermoye, L, Saint-Martin, C., Cosnard, G., Lee, S-K, Kim, J., Nassogne, M-C., Menten, R., Clapuyt, P., Donohue, PK, Hua, K., Wakana, S, Jiang, H, van Zijl, PCM., Mori, S, “Pediatric diffusion tensor imaging: Normal database and observation of the white matter maturation in early childhood”, Neuroimage, 29, 493-504 (2006)

Proton Spectroscopy of Human Skeletal Muscle at 7 Tesla

Craig Malloy, Ivan Dimitrov, Jimin Ren, Piyu Zhao, Tom Cull, James Murdoch, and Dean Sherry
Philips Medical Systems and the University of Texas Southwestern Medical Center
Southwestern NMR Center for *In Vivo* Metabolism
http://www.utsouthwestern.edu/utsw/home/research/AIRC/index.html
Email: Matthew.Merritt@utsouthwestern.edu or Craig.Malloy@utsouthwestern.edu

Intramyocellular lipids (IMCL) may play a specific role in type 2 diabetes. Recent evidence also suggests that the chemical composition, particularly a high concentration of saturated fat in the intracellular compartment, may predispose to insulin resistance. However, composition of the IMCL compartment has been difficult to assess by ^{1}H spectroscopy at 1.5 to 3 T because the available information, areas of the resolved methyl and methylene resonances from each compartment, are sensitive to both chain length and degree of saturation. At 7 Tesla, improved chemical shift dispersion may allow resolution of other resonances due to unsaturated bonds. This information should permit measurement of the composition of intramyocellular fats.

Healthy adults were studied supine in a 7T Achieva using a partial volume quadrature T/R imaging and spectroscopy coil. TSE images were acquired and STEAM (10x10x10 mm, TR 2000, TE 16, TM 22, NA 80) was used for spatial localization. The study was well-tolerated by all subjects. The chemical shift of the methyl resonance of creatine was assigned to 3.02 ppm.

The water soluble metabolites, carnitine, creatine, carnosine and taurine, were easily detected as reported previously. Multiple resonances were observed in the proton spectrum between 0.8 and 3.0 ppm from physiological fats: -CH_2- α to -COO, -CH_2- β to -COO, -CH_2- α to a double bond, -CH_2- between two double bonds, -$(CH_2)_n$-, and -CH_3. Resonances assigned to -CH=CH- from the intracellular and extracellular compartments were also detected at ~5.5 ppm. The concentration of physiological polyunsaturated fatty acids, linoleic and linolenic acid, appears low in the intracellular compartment, since the characteristic resonance due to =CH-CH_2-CH= was observed only in some subjects in the expected region, 2.7 - 2.9 ppm. The resonance at 2.22 ppm is consistent with -CH_2- α to a double bond in the intracellular compartment of fat. If all fat is assumed to be composed of 18 carbon-fatty acids, then the fraction of intracellular fat that is stearic acid is about 20% and the remainder of intracellular fats are largely monounsaturated. ^{1}H spectra from soleus were similar to the gastrocnemius, although the area of the IMCL methylene signal compared to carnitine generally was larger in the soleus than in the gastrocnemius.

The ability to resolve specific proton resonances in the intracellular compartment relies on the coincidence that two resonances in a single compartment (such as the methylenes α to a double bond and methylenes β to the carbonyl) have approximately the same chemical shift difference as that induced by susceptibility effects between the two compartments. Further, it was assumed that these resonances do not overlap significantly with other metabolites. Therefore, these assignments should be interpreted with caution. Nevertheless, the low signal from diallylic protons at 2.7 ppm suggests that the concentration of polyunsaturated fats is low. If all fat is assumed to be composed of 18 carbon-fatty acids, then the fraction of fat that is stearic acid is about 20% based on comparison of resonances C and H, and that the remainder of fats are monounsaturated. There is intense public and scientific interest in the interactions among disease, fat composition, and fat distribution between the intra- and extracellular muscle compartment. The ability to obtain information on fat composition and distribution in distinct muscle groups offers improved technology for understanding insulin resistance in human subjects.

Poster Abstracts - NCRR / Technology for Systems Biology

Integrated Technology Resource for Biomedical Glycomics
University of Georgia
Michael Pierce

Mass Spectrometry Resource for Biology and Medicine
Boston University School of Medicine
Catherine Costello

Mass Spectrometry Resource for Biology and Medicine
Boston University School of Medicine
Peter OConnor

Mass Spectrometry Resource for Biology and Medicine
Boston University School of Medicine
Joseph Zaia

National Center for Glycomics and Glycoproteomics
Indiana University
Milos Novotny

National Flow Cytometry and Sorting Research Resource
Los Alamos National Laboratory
James Freyer

Proteomics Research for Integrative Biology
Pacific Northwest National Laboratory
David Camp

Resource for Integrated Glycotechnology
University of Georgia
James H. Prestegard

Yeast Resource Center
University of Washington
Trisha Davis

Development of technologies for relative quantitation of cell-derived glycoprotein glycans: Applications to stem cell differentiation and clinical specimens

Michael Pierce, Ron Orlando, James Atwood, Gerardo Alvarez-Manilla, Michael Tiemeyer, and Lance Wells.
Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602
NCRR Integrated Technology Resource for Biomedical Glycomics,
http://cell.ccrc.uga.edu/world/glycomics/glycomics.php; hawkeye@uga.edu

Purification of glycoproteins from cells for further analysis is a challenge, since detergents and chaotropic agents required for the solubilization of membrane proteins are incompatible with downstream separation and purification steps. A protocol has been developed, based on extraction and removal of lipids using organic solvents, that yields trypsin-digested glycopeptides with high yield and yet does not introduce detergents or denaturing agents, allowing further fractionation of glycopeptides by several techniques. One of the first analyses to be performed is identification of the glycans expressed on the glycopeptides. Glycans are released by enzymatic or chemical treatments, depending whether they are N- or O-linked, respectively. The released and purified glycans are then subjected to permethylation using methyl iodide, separated, and identified by NSI-MS analysis. Our Center is focused on developing technologies to allow relative quantitation of glycans expressed in cell populations during differentiation or after the onset of disease. We have developed three technologies for relative quantitation. These include analysis of glycans by a type of TIM, total ion mapping, and permethylation of two populations of glycans to be compared using either standard CH_3I or $^{13}CH_3I$: Aoki et al., Dynamic developmental elaboration of N-linked glycan complexity in the *Drosophila melanogaster* embryo. 2007 *J. Biol. Chem.* 282:9127-42; Alvarez-Manilla, et al., Tools for glycomics: relative quantitaion of glycans by isotopic permethylation using $^{13}CH_3I$. 2007 *Glycobiol.* Mar 23 Epub. In addition, we have developed a novel technology for **QU**antitation by **I**sobaric **L**abeling (**QUIBL**) that involves the use of $^{13}CH_3I$ or $^{12}CH_2DI$ to generate isobaric pairs of per-*O*-methylated glycans. The exact masses of $^{13}CH_3I$ and $^{12}CH_2DI$ differ by 0.002922 Da, and thus isobaric analyte pairs containing a single label are difficult to resolve using current mass spectrometers. However, glycans contain multiple methylation sites (*i.e.,* –OH and NH_2 groups), increasing the Δm between differentially labeled analytes and allowing them to be separated at a resolution of ~30,000 $m/\Delta m$. As the number of hydroxyl groups increases, the mass difference for differentially labeled isobaric species and the total mass of the glycan all increase in parallel. Hence, the resolution ($m/\Delta m$) needed to resolve isobarically labeled glycans is practically independent of the glycan's molecular mass. Using these technologies, we can routinely determine relative glycan expression of even minor species using less than 500,000 cells. Our focus on applying this technology, and many others developed in our Resource, is the glycomics of stem cell differentiation in order to identify cell lineage-specific markers. Furthermore, we are also focused on increasing significantly the sensitivity of these analyses for application to clinical specimens of small populations of cells, such as cells from human tumors that can be obtained using laser capture microscopy.

Comparison of Fragmentation Patterns for Oligosaccharides Obtained with Various MS/MS Methods; Application to Structural Analysis of Unknown Oligosaccharides in Glycoproteins

Bo Xie, Cheng Zhao, Giuseppe Infusini, Nancy Leymarie, Shiu-Yung Chan, Joseph Zaia, John F. Cipollo, Peter B. O'Connor and Catherine E. Costello

Dept. of Biochemistry, Boston University School of Medicine, Boston, MA 02118-2646
Mass Spectrometry Resource for Biology and Medicine
http://www.bumc.bu.edu/msr
cecmsms@bu.edu

Mass spectrometry (MS) is undergoing rapid expansion in glycomics because it meets the technical demand for elucidation of the structural complexity and functions of the MS and tandem MS methods. Oligosaccharides released from glycoproteins were permethylated and analyzed by several MS methods, to establish reference standards and demonstrate capabilities. In addition, unknown and novel glycan structures released from glycoproteins related to Urinary Tract Infections (UTIs), inherited Peripheral Neuropathies and Congenital Disorders of Glycosylation (CDGs) have been successfully determined.

Experimental: For MALDI-MS, permethylated glycans were spotted onto the target plate with 2,5-DHB. For ESI-MS, samples were dissolved in $NaOH/CH_3OH$. ESI or MALDI Collisionally-Activated-Dissociation (CAD) tandem mass spectra were acquired on a QSTAR Pulsar *i* Q-o TOF MS (Applied Biosystems), and ESI-LTQ-Orbitrap MS (Thermo Fisher Scientific). CAD and "hot" ECD were performed on a home-built ESI-FTICR MS.

Results: For permethylated *N*-linked glycans released with PNGase F from RNase B and Fetuin, MALDI and ESI with CAD MS/MS on a Q-o-TOF MS produced similar, extensive glycosidic cleavages (B, C, Y) with isobaric fragments originating from the non-reducing and reducing ends, fragments generated by cross-ring cleavages and many internal fragments. Cleavage of higher charged species in ESI produced more extensive fragmentation. Parallel CAD and "hot" ECD in FTMS provided further, yet consistent, structural information, especially "golden" complementary cross-ring pairs; "hot" ECD generated fewer internal fragments, an advantage for the *de novo* sequencing of oligosaccharides.

The highly reproducible fragmentation patterns produced by different MS/MS methods can simplify spectral assignment and enable determination of the detailed structures of unknown glycans present on disease-related glycoproteins. For example, in order to study the molecular basis of UTIs, analysis by ESI CAD MS/MS of *N*-glycans, permethylated after release from Uroplakins, provided the structures of high mannose and novel complex type glycans [1]. When ESI CAD FTMS was used to study glycans released from myelin Protein Zero in *Xenopus laevis* peripheral nerves, the major structure was determined to be an asymmetric hybrid type with an *N*-acetyl neuraminic acid residue at one of the non-reducing termini [2]. Negative-ion tandem MS enabled assignment of the structure of the glycan characteristic of a new CDG [3].

Acknowledgements: T.T. Sun provided the Uroplakins; D. Kirschner, the myelin xP0; H. H. Freeze, the CDG glycan. This research is supported by NIH P41 RR10888 and N01 HV28178 (CEC), S10 RR20946 and R01 HL74197 (JZ).

References:

[1] B. Xie *et al., J. Biol. Chem*, **2006**, *281*, 14644-14653. Epub 2006 Mar 27.
[2] B. Xie *et al*, submitted.
[3] C. Kranz *et al*., *Hum. Mol. Genet*. **2007***16*, 731-741. Epub 2007 Mar 1.

Estimating Relative Isoaspartyl/Aspartyl Abundances Using ECD

Jason J Cournoyer, Peter B O'Connor;
Boston University School of Medicine, Boston, MA

Introduction:
Deamidation of asparaginyl residues to a mixture of isoaspartyl and aspartyl residues is believed to be a common degenerative pathway for proteins and peptides. Differentiating the two forms by mass spectrometry is challenging because they are isomeric but ECD (electron capture dissociation) has been shown to differentiate the two forms based on fragments unique to each form. When coupled with HPLC, ECD methods can quantify the two forms as well. However, lengthy HPLC method development is required to achieve the separation required for accurate relative quantitation of the two forms. For three peptides, ECD spectra of their isoaspartyl and aspartyl versions demonstrate a common fragmentation trend suggesting a method for estimating their relative abundance directly from their ECD spectra.

Methods:
ECD analysis was performed on a home built qQq-FTMS (Fourier transform mass spectrometer with mass filtering front-end quadrupoles and CAD cell) equipped with a nano-spray source and 7T actively shielded magnet. For each experiment, the multiply charged precursor ions were isolated in Q1, externally accumulated in Q2 and then transmitted to the ICR cell for ECD and subsequent detection. The peptides VFDKDGNGYISAAELR and TGPNLHGLFGR were from the tryptic digest of calmodulin and cytochrome *c*, respectively, and were allowed to completely deamidate under basic conditions. The aspartyl and isoaspartyl versions of the peptides were then separated by HPLC and collected for ECD analysis. The peptide WAF(isoD/D)SAVAWR-NH_2 was synthesized by solid phase synthesis using standard FMOC chemistry.

Preliminary Results:
The relative abundances of ECD fragments for the isoaspartyl and aspartyl versions for the three peptides were compared. All three isoaspartyl versions showed the diagnostic ions (z_r-57 and/or c_{l-r}+57, where r is the aspartyl or isoaspartyl residue and l is the length of the peptide) while the aspartyl versions did not. For peptides with c-terminal basic residues, electron capture generating the z_{r-1} and z_r-57 fragment ions should originate from the same dissociation channel. The ECD spectrum of TGP(isoD)LHGLFGR shows concomitant decrease and increase of z_7 and z_8-57, respectively, compared to the ECD spectrum of the aspartyl version therefore demonstrating a diverging fragmentation pathway with isoaspartyl substitution. Likewise, the ECD of WAF(isoD)SAVAWR-NH_2 shows the same trend for its z_6 and z_7-57 fragment ions. However, the ECD of VFDKDG(isoD)GYISAAELR shows the trend for its z_9 and z_{10}-57, as well as compliment ions c_7 and c_6+57, but the change in abundances compared to the aspartyl version is much less than the other two peptides. A reason may be that the multiple glycine residues affords flexibility for hydrogen bonding between basic and acidic residues therefore influencing fragment detection and relative fragment abundances.
Another trend observed for all three peptides is the increase in the z_r fragment ion abundance for the isoaspartyl peptide compared to their aspartyl versions. The only difference is the position of the radical on the z• fragment ions; for isoaspartyl, the radical resides on the β-carbon position and the α-carbon for the aspartyl z• fragment ion. The close proximity of the electron-withdrawing carboxylic acid group may account for increased stability and thus abundance for the isoaspartyl z• fragment ion compared to that of the aspartyl form.
In conclusion, the two fragmentations trends discussed suggest that it may be possible to estimate the ratio of deamidation products directly form an ECD spectrum.

The Utility of Multiplexed Stable Isotopic Labeling of Carbohydrates and Quantitative Analysis by Mass Spectrometry

Michael J. Bowman; Joseph Zaia
Mass Spectrometry Resource for Biology and Medicine, Boston University School of Medicine, Dept. of Biochemistry
www.bumc.bu.edu/msr
jzaia@bu.edu

The increasing interest in glycomics has led to the widespread use of mass spectrometry to determine the carbohydrate components expressed in biological systems. However, the quantification of carbohydrates within different samples remains a daunting task, due to instrument and sample variability. Herein, we show cusyom-synthesized multiplexed stable isotope labeled tags that have broad applicability for the simultaneous quantitation of four samples during the same mass spectrometry experiment. Results exploring the utility of multiplexed stable isotopic labeling from a variety of sources have been demonstrated.

A stable isotope-labeled tag in four forms (+0,+4,+8,+12) was synthesized for the purpose of labeling the reducing end of glycans. Glycosaminoglycans (GAGs) from the chondroitin sulfate proteoglycan (CS-PG) class and pharmaceutical low-molecular weight (LMWT) heparins were partially depolymerized using enzymes to form oligosaccharide distributions. These oligosaccharides were labeled with the tetraplex stable isotope-containing tags by reductive amination. The resulting tagged GAGs were combined, separated by high performance size exclusion chromatography (Superdex peptide 3.2/30, Beckman Gold 125 solvent module), and analyzed in the negative electrospray mode using an Applied Biosystems QSTAR Pulsar-I (Q-ToF) mass spectrometer. In addition, the PNGase F released □-1-acid glycans from four species were also multiplex-labeled and analyzed.

A series of glycomics tags that incorporate stable isotope modules into the oligosaccharide structure were used to label the glycans released from CS-PGs, LMWT heparins, and *N*-linked glycans from □-1 acid glycoproteins. The abundances of ions in the MS mode serve to quantify ions produced from a given sample in each of the four samples. These results further demonstrate the principle of quantitation by multiplex analysis of carbohydrate using stable isotope tags.

Chondroitinase ABC was used to depolymerize the released GAG chains from aggrecan, biglycan, cartilage extract, and decorin. The CS-proteoglycans were normalized for concentration allowing for a mass spectrometric analysis of relative CS quantities. Additional information on the isomeric content of the CS chains was obtained via tandem mass spectral analysis utilizing the distinctive fragmentation patterns of each glycoform (CSA, CSB, and CSC).

Additional experiments were performed to demonstrate greater utility of the tags in examination of GAGs. Low molecular weight heparins (Lovenox and Fragmin) were depolymerized to the greatest extent possible with heparin lyases

I, II, and III. A significant fraction of heparin (and LMWT heparin) resistant to the lyase treatment should be composed of a tetrasaccharide containing a trisulfated GlcN residue that derives from the pentasaccharide sequence required for anti-thrombin III binding. The quantities of lyase resistant structures for each LMWT heparin preparation were determined. In addition, using tandem mass spectrometry, the differential fragmentation patterns between porcine heparin, Lovenox, and Fragmin were determined for all component composition. This method provides an alternate method to examine the structural differences between heparin preparations.

A final demonstration of the utility of multiplex tagging examined the *N*-linked glycans released from □-1-acid glycoprotein from four species. Glycoprotein quantities were normalized to quantitate the abundances of glycans present and the tandem MS of each composition was analyzed. Support was provided by NIH grants P41RR10888, R01HL74197.

Glycomic and Glycoproteomic Technologies and Applications Developed by the National Center for Glycomics and Glycoproteomics

Department of Chemistry, Indiana University, Bloomington, IN 47405
National Center for Glycomics and Glycoproteomics
http://ncgg.indiana.edu/
novotny@indiana.edu

The development of new technologies in the areas of glycomics and glycoproteomics has been the main mission of the National Center for Glycomics and Glycoproteomics since its inception 19 months ago. Our activities have been focused on the development of reliable, reproducible and quantitative glycomic and glycoproteomic approaches to be utilized in biomedical research. The utility of these approaches in investigating the aberrant glycosylation of proteins associated with cancer development and progression will be highlighted and presented in this poster. The biomedical application of another quantitative glycomic approach based on stable-isotope labeling will also be presented. A glycoproteomic approach based on lectin enrichment and glycoprotein fractionation prior to the conventional proteomic analysis was developed for monitoring glycoproteomic changes associated with cancer. The Center has also developed several bioinformatic tools for glycomic and glycoproteomic studies, including effective determination of the glycosylation sites and their microheterogeneity, as well as a tandem MS algorithm allowing differentiation of structural isomers. Additional technologies developed under the Center, which include rapid and reproducible quantification and characterization of glycomic maps derived from biomedical samples, include ion-mobility spectrometry, vUV-induced photofragmentation, and microfluidic approaches.

Recent Instrument Development at the National Flow Cytometry Resource

Gregory Goddard, Mark A. Naivar, Steve W. Graves, James H. Jett, Robert Habbersett, John C. Martin, John P. Nolan, James Parson, Mark E. Wilder and James P. Freyer
National Flow Cytometry Resource, Bioscience Division, Los Alamos National Laboratory
http://nfcr.lanl.gov/
freyer@lanl.gov

The National Flow Cytometry Resource (NFCR) has a long-standing tradition of developing new instrumentation to improve the application of flow cytometry in biomedical research. In this presentation, we will describe two recent instrumentation development efforts: a high-resolution, full spectral flow cytometer and an integrated hardware and software system for completely digital data acquisition and analysis.

While conventional multiparameter flow cytometers have proven highly successful, there are several types of analytical measurements that would benefit from a more comprehensive and flexible approach to spectral analysis. We have developed a new full spectral resolution instrument that utilizes a diffraction grating to disperse fluorescence and side-scattered light from cells or microspheres over a rectangular charge-coupled-device image sensor (1). The flow cell and collection optics are taken from a conventional flow cytometer with minimal modifications to assure modularity of the system. Calibration of the prototype spectral analysis flow cytometer demonstrated a spectral resolution of <1 nm over the range of 300-1090 nm. We have demonstrated a single particle/cell intensity sensitivity of ~150 molecules of Fluorescein isothiocyanate. Single particle spectra taken with our instrument were validated against bulk solution fluorimeter and conventional flow cytometer measurements. We have demonstrated that the flow spectrometer has sufficient sensitivity and wavelength resolution to detect single cells and microspheres, including multifluorophore labeled microspheres. The capability to use standard mathematical deconvolution techniques for data analysis, coupled with the feasibility of integration with existing flow cytometers, will improve the accuracy and precision of ratiometric measurements, enable the analysis of more discrete emission bands within a given wavelength range, and allow more precise resolution of the contribution of individual fluorophores in multiply-tagged samples.

We have developed an Open, Reconfigurable Cytometry Acquisition System (ORCAS) based on digital data acquisition hardware to support both novel flow cytometry development efforts and to upgrade existing commercial flow cytometers (2). The system is flexible in how it detects, captures and processes event data. Custom data capture boards utilizing analog to digital converters (ADCs) and field programmable gate arrays (FPGA) detect events and capture correlated event data. A commercial digital signal processing (DSP) board processes the captured data and sends the results over the IEEE 1394 bus to the host computer that provides a user interface for acquisition, display, analysis and storage. We have collected list mode data, correlated pulse shapes, and streaming data from a variety of custom and commercial instruments using Linux, Mac OS X and Windows host computers. We have analyzed signals from photomultiplier tubes (PMT), photodiodes, and photon counting avalanche photodiodes (APD), extracting pulse features not found on commercial systems. List mode data are saved in FCS 3.0 formatted files while streaming or captured waveform data are saved in custom format files. ORCAS is compact, scaleable, flexible, and modular. Programmable feature extraction algorithms have exciting possibilities for both new and existing applications. The recent availability of a commercial data capture board will enable easier implementation of this new system on existing flow cytometers.

1. Goddard et al., *Cytometry* 69A: 842, 2006.
2. Naivar et al., *Cytometry*, in press.

A Proteomics Research Resource for Integrative Biology

Richard D. Smith, Pacific Northwest National Laboratory
Pacific Northwest National Laboratory
NCRR Biomedical Technology Resource Center
https://ncrr.pnl.gov/, rds@pnl.gov

The long-term goal of this Center is to develop and make advanced proteomic technologies more accessible to and useful for the biomedical research community. Collaborative projects drive the technology development to further extend state of the art proteomics for resolving medically important biological questions.

Increased sensitivity in LC-ESI-MS. Improving the sensitivity for detecting and identifying biomolecules continually opens the door for new applications. The sensitivity of electrospray ionization mass spectrometry (ESI-MS) is largely determined by the efficacy with which gas-phase ions are created from analyte molecules in solution (ionization efficiency) and the ability to transfer the charged species from atmospheric pressure to the low-pressure region of the mass analyzer (transmission efficiency). We have significantly increased the ionization efficiency for ESI by constructing an array of individual electrospray emitters, which divides the 1-2 μL/min flow rate from liquid chromatography (LC) separations into multiple < 100 nL/min flows that generate an array of stable nano-electrosprays. This development provides the benefits of nanoESI at higher LC flow rates and increases the overall spray current. To take full advantage of the increased electrospray currents, we have concurrently developed a multi-capillary inlet to improve transmission efficiency and to effectively sample the expanded electrospray plume from the multi-ESI emitter array into the mass spectrometer. Combining these technologies with an electrodynamic ion funnel interface has increased the sensitivity by more than an order of magnitude compared to a standard ESI interface. Robust electrospray operation is ensured by an intelligent electrospray feedback control. By monitoring the electrospray current, the electrospray voltage is continuously adjusted according to the solvent composition so that a stable electrospray in cone-jet mode can be maintained throughout the gradient elution of an LC separation.

Spatial and quantitative brain proteomics. Integrated temporal, spatial, and quantitative protein maps of the mammalian brain aid in understanding brain function and molecular etiologies in neurodegenerative diseases. However, challenges associated with proteome complexity, throughput, the sensitivity of analytical methodologies, and accurate protein quantification have hampered brain imaging efforts. To address these challenges, we combined voxelation technology (for preparing tissue samples of 1 mm^3) with a high throughput proteomics approach to achieve comprehensive spatial mapping of mouse brain protein abundances at 1 mm resolution. The approach included robotic micro-scale sample processing to handle the extremely small samples prior to high sensitivity and high throughput LC-FTICR analyses. Quantitative measurements of a coronal section of C57BL/6J mouse brain divided into 71 voxels were made from stable isotopic intensity ratios to obtain measures of relative protein abundances. 1,028 proteins were localized with ~50% of the proteins distributed to >65 voxels at sufficient resolution to distinguish major brain structures, i.e., cerebral cortex, striatum, diencephalon, white matter, and olfactory tubercles. Protein and gene localizations generally agreed for comparisons among this dataset, the Allen Brain Atlas, and the Gene Expression Nervous System Atlas (GENSAT). Unlike the Allen Brain Atlas and GENSAT databases that required brain sections from thousands of mice, only two mice were needed for this study. Concordance of the gene and protein distributions validates this highly sensitive, high throughput proteomics approach and provides new opportunities for further study of the spatial brain proteome and its dynamics during the course of disease progression in animal models.

New Approaches to Glycoprotein Structure: the Sialyltransferase, ST6Gal 1

J. H. Prestegard, K. W. Moremen, G-J Boons, R.C. Orlando, R.J. Woods
University of Georgia, Athens, GA, 30602
Resource for Integrated Glycotechnology
http://glycotech.ccrc.uga.edu, jpresteg@ccrc.uga.edu

Glycosylated proteins are very abundant with as many as 50% of all mammalian proteins carrying potential glycosylation sites. The properties of many of these proteins are also linked to diseases, including viral infection, autoimmune response, and cancer. Structural characterization is, therefore, key to the development of new therapeutic agents. Yet, three dimensional structures are poorly represented in databases such as the PDB. This results primarily because of difficulties in expressing and crystallizing the proteins. The Resource for Integrated Glycotechnology is providing new routes to structural information through development of a multidisciplinary approach that includes, expression of labeled glycosylated proteins in eukaryotic hosts, NMR detection of labels at the highest magnetic fields available, novel resonance assignment strategies that combine mass spectrometry with NMR, synthesis of resonance perturbing ligands to locate binding sites, and computational modeling to produce structures from sparse data sets. Progress will be illustrated with applications to the structural characterization of the sialyltransferase, ST6Gal 1, a protein involved in regulation of B cell signaling and a number of other phenomena dependent on the addition of sialic acids to the termini of cell-surface carbohydrates.

Machine learning aids peptide identification in shotgun proteomics datasets.

Lukas Käll, Aaron Klammer, Xianhua Yi, Jesse Canterbury, Jason Weston, Michael J. MacCoss, and William Stafford Noble
Yeast Resource Center and Department of Genome Sciences, University of Washington, Seattle Washington.
depts.washington.edu/yeastrc, tdavis@u.washington.edu

Interpretation of shotgun proteomics datasets requires correctly identifying which peptides from a large protein database correspond to a given set of spectra. SEQUEST, developed by the YRC, is remarkably successful at solving this problem, but it is not perfect. We have taken two approaches to improve peptide identification. One approach uses semi-supervised machine learning. The other includes peptide chromatographic retention time as part of the identification process.

A limitation of existing machine learning efforts to improve peptide identification in shotgun proteomics datasets are that they are based on fixed training sets [J Proteome Res. 2:137][Anal. Chem. 74:5383] and are hence unable to compensate easily for variations in mass spectrometry conditions. Instead of curating representative training sets for individual conditions, which in most cases is not practically feasible, we have devised an algorithm called Percolator that is capable of learning directly from the individual shotgun proteomics datasets that we want to classify. We have implemented the algorithm as post-processing software that can be appended to any existing database search algorithm.

Percolator uses an iterative semi-supervised learning method that eliminates the need for constructing a separate training set. The peptide-spectrum matches (PSMs) from searching a decoy database consisting of shuffled protein sequences are used as negative examples for the classifier, and a subset of the high-scoring PSMs from searching the normal database are used as positive examples. We then train a machine learning algorithm called a support vector machine (SVM) to discriminate between positive and negative PSMs. This approach is fully automated and significantly improves the sensitivity of existing database search algorithms for a fixed false discovery rate. We demonstrate that incorrect results from database searching algorithms can be correctly re-ranked, and in some cases multiple peptides can be accurately assigned to a single fragmentation spectrum. Furthermore, our algorithm produces as output statistically meaningful q-values, estimated using the distribution of scores from the decoy database search.

Most database search algorithms use information only from the product ion spectrum to determine peptide sequence, ignoring information such as peptide chromatographic retention time. Efforts to exploit retention time are often frustrated by its variability across experimental conditions and instruments. We control for this variability by training a model of retention time on individual chromatography runs, using a machine learning technique known as the support vector regressor (SVR). We train six different SVRs on high-confidence identifications from each step of a six-step MudPIT, and then use these regressors to filter out peptide identifications with large discrepancies between observed and predicted RT. This increases the number of unique peptide identifications by 14%.

Poster Abstracts - NCRR / Optical & Laser Technology

Laboratory for Fluorescence Dynamics
University of Califiornia, Irvine
Enrico Gratton

Laser Biomedical Research Center
Massachusetts Institute of Technology
Ramachandra Dasari

Laser Biomedical Research Center
Massachusetts Institute of Technology
Michael Feld

Ultrafast Optical Processes Laboratory
University of Pennsylvania
Thomas Troxler

Intrinsic markers of breast tumors

Kukreti, S.[1,2,4], A. Cerussi[1], V. W. Tanamai[1], B. Tromberg[1,3], E. Gratton[1,2,3,4].

[1]Beckman Laser Institute, University of California, Irvine; [2]Laboratory of Fluorescence Dynamics, University of California, Irvine; [3]Department of Biomedical Engineering, University of California, Irvine; [4]Biophysics and Computational Biology, University of Illinois, Urbana-Champaign.

Laboratory for Fluorescence Dynamics; http://www.lfd.uci.edu; lfd@uci.edu

Beckman Laser Institute; http://www.bli.uci.edu; jcora@uci.edu

We have developed a new methodology based on high resolution spectroscopy in thick tissues that provide clinicians with a “noninvasive view” of the molecular changes due to the presence of a lesion. Preliminary results from 24 lesions suggest that this “non-invasive” approach detects tumors and discriminates different type of lesions. This technology is based on the frequency-domain spectroscopy of tick tissues that we pioneered at the LFD. In thick tissues, light in the near infrared (700 to 1100 nm) scatters multiple times and is absorbed by specific tissues chromophores such as hemoglobin, water and lipids. The amount of light that reaches a given distance from the point of illumination depends both on the scattering and absorption in the tissue. To obtain the absorption spectrum free from the scattering contribution we must have a method that independently determines at teach wavelength these two parameters. In the frequency domain spectroscopy approach we illuminate the tissue using a high frequency modulated light. After traveling several centimeters in the tissue, the sinusoidally modulated intensity is phase shifted and attenuated. Since the phase shift is mainly due to scattering, we can recover the absorption free of scattering. Since it is easy to modulated the intensity of a laser diode, but much more difficult to modulate a source that emits in the entire near-it spectrum, we constructed in collaboration with the Beckman Laser Institute in Irvine an instrument that has a tungsten light source as the spectral source and few modulated laser diodes used to separately measure the scattering and absorption parameter at few selected wavelengths. Once the scattering is known, we extract the true spectrum of the light transmitted through the breast. By carefully studying this spectrum, we observed that there were some spectral features that were seen only in the regions of the breast affected by the tumor. The problem was that there was a very large variability between subjects due to different lipid, water and hemoglobin content. While the research in this filed (in other labs) has concentrated in using these tissue content variability to diagnose the lesion, we took a radically different approach. We develop a method that fully accounts for the inter-subject variability and only reveals the changes that are due to the lesion. For the first time we were able to show that there are subtle spectral variations due to the tumor. These spectral variations are principally due to differences in lipid composition in the tumor with respect to the surrounding tissue.

MIT Laser Biomedical Research Center

Professor Michael S. Feld
Director, G.R. Harrison Spectroscopy Laboratory, MIT, Cambridge, MA 02139
Website: http://web.mit.edu/spectroscopy E-mail: msfeld@mit.edu

The MIT laser biomedical research center pioneers the development of a number of optical technologies for the advancement of real-time medical diagnostics and the fundamental understanding of cell structure and dynamics. Central program objectives include spectral diagnosis of disease, imaging techniques, and cellular biophysics. Experimental techniques that merge optical spectroscopy, imaging, scattering, and interferometry are applied to study the biophysics and biochemistry of healthy and diseased biological structures from the subcellular to the entire-organ scale.

We have recently combined Raman spectroscopy with other modalities, such as diffuse reflectance and intrinsic fluorescence, to improve quantitative analysis and diagnosis. For example, we have improved the accuracy of Raman measurements in turbid media by developing intrinsic Raman spectroscopy, which utilizes diffuse reflectance to correct for intensity and spectral distortions from bulk absorption and scattering. Relating to tissue diagnosis, we have improved diagnostic accuracy in discriminating malignant breast tissue from normal and benign lesions through the combination of Raman, fluorescence and reflectance spectroscopy. We have also demonstrated the ability to detect morphological features of vulnerable atherosclerotic plaque by using the complementary information provided by the multiple modalities.

Furthermore, we are advancing in the direction of wide-area tissue characterization via a recently constructed *in-vivo* tri-modal spectroscopy (TMS) imaging system. This system acquires diffuse reflectance, fluorescence, and φ/LSS spectra from epithelial tissue using a raster-scanning approach. With this method we have extended TMS from a single-point, small-area (1mm by 1mm) modality to a wide-area (2cm by 2cm) imaging modality. The efficacy of the imaging system for precancer detection is being tested in both the cervix and oral cavity.

Quantitative Phase Microscopy continues to be the major thrust in the studies of cell structure and dynamics. We have developed diffraction phase microscopy, a highly sensitive optical interferometric technique, to quantify the red blood cell membrane fluctuations at the nanometer and millisecond scales. The results reveal significant properties of both temporal and spatial coherence associated with the membrane dynamics. We show that these correlations can be accounted for by the viscoelastic properties of the cell membrane. In addition, we are developing other interferometric techniques for measuring activity-dependent optical path shifts in neurons and other excitable cells. We have used a heterodyne Mach-Zehnder interferometer to image nanometer-scale voltage-dependent motions in single cells expressing the membrane motor protein prestin, and observed oscillations transverse to the stimulation pipette which cannot be detected by single-point measurements. Recently, we have developed tomographic phase microscopy which can map the 3D structure of refractive index in live cells and tissues showing structures of individual organelles and nucleoli. It is of interest to note that the regions in nucleus excluding nucleoli have refractive index either lower or comparable to that of cytoplasm.

2D IR Spectroscopy Advances from Small Peptides to Membrane Bound Proteins

S. Bagchi, C. Fang, Y.-S. Kim, T. Troxler and R. M. Hochstrasser
Ultrafast Optical Processes Laboratory
Department of Chemistry, University of Pennsylvania, Philadelphia PA 19104
rlbl.chem.upenn.edu

Although the new infrared multidimensional spectroscopy cannot access as many parameters as NMR, they have ultrafast time resolution and combine structural information with dynamics: this advantage makes them promising. These characteristics are exactly what are needed to advance our understanding of molecular liquids such as water, where clusters of molecules are undergoing rapid fluctuations, photoprocesses such as isomerization and energy conversion in photosynthesis, and ultrafast atomic scale motions in polypeptides, membranes, nucleic acids and other complex environments.

The diagonal regions of these 2D spectra exhibit peaks determined by the vibrational anharmonicity. The cross peaks are sensitive to the relative polarization of the infrared pulses in the sequence and when two modes are excited in a pulse sequence, the outcome depends on the angle between the transition dipoles involved in the two steps.

The structural preferences of the backbone of peptides can be characterized by the distance and angular constraints from 2D IR of amides. Isotope replacement enables 2D IR experiments to characterize the interactions between specific pieces of structures that achieve considerable complexity. In our recent research we have characterized the structure and dynamics of some simple peptides to verify these ideas and used the results to advance to much larger systems. One example of a simple system is a tryptophan dipeptide for which cross peak and polarization analysis identified the equilibrium structure as C7 type in nonpolar solvents but totally different in water.(1) These structural effects are easily visualized in 2D IR spectra and the ideas are transferrable. The proximities of the various pieces of the backbone structure are manifested also in the energy transfer between units and in the making and breaking of hydrogen bonds within the backbone and with the surrounding water that cause fluctuations in the amide frequencies. The spectral shapes and frequencies are determined by the fluctuating electrostatic fields at the mode from the moving charges in the peptide and the surrounding solvent as verified in our experiments with isotopically labeled 12 residue tryptophan zipper structures.(2)

Moving toward even larger systems we found there is no requirement for the coupled modes to be part of the same molecule or be connected by conventional chemical bonds. For example there is coupling between the amide modes associated with the different helices of a transmembrane dimer of GpA.(3) The 2D IR of GpA in micelles allowed tertiary interactions between amide-I vibrators on the separate helices of the transmembrane (TM) helix dimers to be probed by ultrafast two-dimensional vibrational photon echo spectroscopy. The 2D IR proved to be a useful method for the study of membrane bound structures and Integrins are now being investigated. The isotopomers and their 50:50 mixtures formed helical dimers in sodium dodecyl sulfate (SDS) micelles whose difference 2D IR spectra showed components from homodimers, where both helices had either $^{13}C=^{16}O$ or $^{13}C=^{18}O$ substitution, and the heterodimer where one had $^{13}C=^{16}O$ and the other $^{13}C=^{18}O$. The cross peaks in the pure heterodimer 2D IR spectrum and the splitting of the homodimer peaks in linear IR show that the amide-I mode is delocalized across a pair of helices. The excitation exchange coupling in the range 4.3 - 6.3 cm^{-1} was verified to arise from through-space interactions between amide units on different helices. The angle between the two Gly_{79} amide-I transition dipoles, estimated as 110^{o} from 2D IR, combined with the coupling led to a structural picture of the hydrophobic interface that is remarkably consistent with results from NMR on helix dimers. The helix crossing angle in SDS is estimated as 45^{o}. The 2D IR also set limits on the range of geometrical parameters for the helix dimers from an analysis of the coupling constant distribution. Recent results on even larger systems involving 160 kD enzymes will be shown on the poster.

1. Bagchi, S., Kim, Y. S., Charnley, A. K., Smith, A. B., III and Hochstrasser, R. M. (2007) *Journal of Physical Chemistry B* **111,** 3010-3018.
2. Wang, J., Chen, J. and Hochstrasser, R. M. (2006) *Journal of Physical Chemistry B* **110,** 7545-7555.
3. Fang, C., Senes, A., Cristian, L., DeGrado, W. F. and Hochstrasser, R., M. (2006) *Proc Natl Acad Sci USA* **103,** 16740-16745.

Poster Abstracts - NCRR / Technology for Structural Biology

Biophysics Collaborative Access Team
Illinois Institute of Technology
Thomas Irving

Biophysics Collaborative Access Team
Illinois Institute of Technology
Joseph Orgel

National Biomedical Center for Advanced ESR Technology
Cornell University
Jack Freed

National Center for Macromolecular Imaging
Baylor College of Medicine
Wah Chiu

National Magnetic Resonance Facility at Madison
University of Wisconsin-Madison
John Markley

Synchrotron Radiation Structural Biology Resource
Stanford University
Malcolm Capel

Synchrotron Radiation Structural Biology Resource
Stanford University
Britt Hedman

Visualization of Biological Complexity Resource
New York State Department of Health
Carmen Mannella

X-ray studies of neurofribillary tangles in Alzheimer's disease brain tissue

S. Rice[1], A. Bongaarts[1], E. Landahl[2], O. Antipova[3], R. Barrea[3,4], T. Irving[3,4], J. Orgel[3,4]
1: Northwestern University. 2: Argonne National Lab. 3: Illinois Institute of Technology. 4: BioCAT, APS.
Biophysics Collaborative Access Team (BioCAT): http://www.bio.aps.anl.gov/
Contact: orgel@iit.edu

The visible neuronal pathology that occurs in Alzheimer's disease (AD) and a number of other dementia-causing diseases is composed of a polymerized form of the microtubule-binding protein, tau. Collectively, these diseases are termed "tauopathies" and represent the predominant cause of human dementia. This pathology occurs when tau, a molecule that possesses little ordered structure in solution, polymerizes into an apparently well-ordered filament. Tau filaments can take on "straight" (single) or paired helical forms. This self-assembly phenomenon is of interest in its own right as a biophysical process, but this interest is made more acute by the potential relevance of its mechanism to a large number of human neurodegenerative disorders. Similar to the tauopathies (AD, Pick disease, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), FTDP-17 dementias, and others)(Lee, V., et al Ann. Rev. Neurosci. 24:1121-1159, 2001), aggregation of other proteins by demonstrably similar mechanisms is a characteristic of several other neurodegenerative diseases. Thus, understanding how and why tau polymerizes could open avenues for the development not only of therapeutic agents directed at AD, but at compounds to treat the broader base of neurodegenerative diseases.

The question of whether tau is ordered and how it is ordered in NFTs is ideal for small-angle x-ray diffraction or microdiffraction experiments, similar to those that have been performed on muscle fibers and other fibrous tissues (e.g. Irving and Maughan 2000). The particular challenges of performing x-ray diffraction experiments on NFTs are exceptionally well suited for the Bio-CAT beamline at APS. Preliminary data taken March 1-2, 2007 at Bio-CAT identified diffraction patterns seen previously in purified NFTs, and also identified a number of new structural features, as well as a correspondence between tissue morphology and NFT orientation. Supported by NIH P41 RR008630.

Determining the molecular structure of type II collagen by X-ray diffraction

O. Antipova[1], R. Barrea[1,2], T. Irving[1,2], J. Orgel[1,2]
1: Illinois Institute of Technology. 2: BioCAT, APS.
Biophysics Collaborative Access Team (BioCAT): http://www.bio.aps.anl.gov/
Contact: orgel@iit.edu

The fibrous collagens are the most prominent proteins of mammalian connective tissues and are essential to their structure and function. Of these, type II collagen forms the structural basis for cartilage, bones and teeth. Physiological structure, turnover and component synthesis have been studied in much greater detail than that of the three-dimensional arrangement of collagen molecules in fibrils. The native conformation of the fibrillar form of collagen is crucial to proper tissue assembly, extracellular interactions and other biological processes and disease. Understanding this structure could be expected to throw significant light on these normal and pathological processes.

The natural crystalline structure of collagen fibrils in some tissues allows the use of fiber diffraction methods for its study and the following visualization of its fibrillar sub-structure. SAXS and WAXS experiments at the BioCAT facility at ANL provided unique diffraction images of collagen type II with resolution up to 12 Å for the native and isomorphusly derivatised samples. These data will prove crucial for the forthcoming refinement of the *in situ* electron density map of collagen type II's macromolecular structure via Multiple Isomorphous Replacement (MIR). Analysis of this map may help reveal the mechanisms of interactions between collagen type II and other connective tissue molecules, involved in tissue development, turnover and diseases such as osteoarthrytis and chondrodysplasias. Supported by NIH P41 RR008630.

The Study of Chemotactic Receptor Interactions with Histidine Kinase CheA and the Coupling Protein CheW by Pulsed Dipolar ESR Spectroscopy

Jaya Bhatnagar*, Peter P. Borbat*†, Abiola M. Pollard*, Alexandrine M. Bilwes*, Brian R. Crane*, and Jack H. Freed*†
*Department of Chemistry and Chemical Biology and †National Biomedical Center for Advanced ESR Technology (ACERT), Cornell University, Ithaca, New York 14853

Bacterial chemotaxis refers to the movement of bacteria in response to "tasting" molecular cues of surrounding media; and it represents a primitive, yet complex, self-regulated motional response apparatus that is able to sense and process stimuli, and adapt to the environment by swimming in the proper direction. This sensing and response mechanism appears very early on the phylogenic tree, and to understand it is important to our knowledge of how it may have evolved into much more complex sensing functions of higher organisms. The main building block of the chemotactic sensor is a ternary complex of chemoreceptors, the histidine kinase, CheA, and the coupling protein, CheW. Subtle conformational changes of the receptor in response to the bound substrate are transduced downstream via a complex network of structural changes in the signaling complex to regulate autophosphorylation of CheA that ultimately controls the flagellum motor; with the whole regulating process involving a number of mobile proteins of known structure. The structure of this ternary complex, as well as its changes in response to a stimulant, is a goal of our study. At ACERT, pulse dipolar ESR spectroscopy (PDS) is intensively applied to yield accurate distances between introduced spin-labels that are used as constraints to define structures[1]. Hitherto, we solved the ternary structure of CheA with CheW by PDS[2], which was confirmed by the crystal structure of CheW in complex with P4-P5 domains of CheA[2]. The PDS study is currently focused on ternary complexes of chemoreceptors, CheA, and CheW from *Thermotoga maritima*. A soluble cytoplasmic fragment of TM0014 receptor is used, and due to the dimeric nature of the receptor and CheA, engineered tandem dimers are often employed to simplify the analysis of dipolar signals. The protomer of CheA is organized into five distinct domains, P1-P5, each responsible for specific function. Our preliminary results on Δ289CheA (i.e. just P3-P5 domains) indicate stabilization of otherwise rather mobile P5 domains into a fixed relative position in presence of receptors. We do find the P5 domain on each monomer of CheA binds CheW with the high affinity and that two CheW's on "top" of mushroom-like CheA dimer have a wide range of flexibility that is not markedly affected by the receptor. The TM0014 and Δ289CheA heterodimers, each labeled with a single spin label, yield dipolar signals that begin to tell how they assemble into the functional complex.

1. Borbat PP, Freed JH (2007) Measuring distances by pulsed dipolar ESR spectroscopy: spin-labeled histidine kinases. Methods in Enzymology 423: 52-116.

2. Park S-Y, Borbat PP, Gonzalez-Bonet G, Bhatnagar J, Pollard AM, et al. (2006) Reconstruction of the chemotaxis receptor-kinase assembly.[see comment]. Nature Structural & Molecular Biology 13: 400-407.

Single Particle CryoEM Reveals Protein Backbone Of Apo-Groel And Expanded Conformation Of Single Ring GroEL-GroES With Substrate

Steven J. Ludtke, Dong-Hua Chen, Matthew L. Baker, Jiu-Li Song[+], David T. Chuang[+] and Wah Chiu

Verna McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX77030. [+]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390.

National Center for Macromolecular Imaging, http://ncmi.bcm.edu, wah@bcm.edu

GroEL is the prototypical molecular chaperonin (~800 kD), a protein nanocage that assists other proteins in proper folding by reducing the formation of misfolding aggregates. Assisted protein folding, triggered by ATP hydrolysis, occurs in one of the two rings of GroEL followed by the release of refolded protein, ADP and GroES. All high-resolution studies thus far of chaperonins have utilized X-ray crystallography, often with mutations and crystallization conditions that subtly alter and stabilize the structure. Here, we reveal a solution conformation of native apo-GroEL determined using electron cryomicroscopy (cryoEM) and single particle analysis at a resolution sufficient to trace the protein backbone. The map clearly shows an asymmetry between the subunits of the two rings as expected from the negative co-operativity between the two rings. These results show for the first time that the protein backbone of a molecular machine can be built directly from a CryoEM single particle reconstruction.

In addition, studies of the SR398 single ring mutant of GroEL in the presence of GroES and ATP have revealed an unprecedented expanded conformation of this assembly. This conformation is promoted by the presence of substrate. When an unusually large substrate, an 86-kDa heterodimeric ($\alpha\beta$) assembly intermediate of mitochondrial branched-chain α-ketoacid dehydrogenase is used, ~80% of the particles are found in this expanded state. The volume of the folding chamber in this conformational state is ~ 80% larger than the X-ray structure of the equivalent *cis* cavity in the GroEL-GroES-$(ADP)_7$ complex. In the absence of substrate, the SR398-GroES-Mg-ATP complex is still found to exist in the expanded state ~20% of the time. In addition, previous studies of GroEL had shown that large substrates would interact with the uncapped end of GroEL, rather than being fully encapsulated. We have now directly observed encapsulation of a substrate with almost twice the mass of the previously identified limiting mass. Furthermore, biochemical studies have indicated encapsulation of this substrate in the native GroEL-GroES system as well. This implies that some expansion may occur with native GroEL-GroES as well. This study demonstrates the ability of single particle reconstruction to separate a heterogeneous population of particles to produce multiple 3-D reconstructions.

Acknowledgement: This research has been supported by NCRR, NIGMS and NSF.

Fast and Accurate Identification and Quantification of Metabolites from Two-Dimensional ^{1}H-^{13}C NMR Spectra

Ian A. Lewis, Qiu Cui, Seth C. Schommer, Brendan Hodis, Kate A. Robb, Marco Tonelli, William M. Westler, Hamid Eghbalnia, Fariba Assadi-Porter, Michael R. Sussman and John L. Markley*
Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison Wisconsin 53706-1544, USA

Resource: National Magnetic Resonance Facility at Madison (http://www.nmrfam.wisc.edu)

*Contact person: Phone 608-231-2059. Fax: 608-262-3759. E-mail: markley@nmrfam.wisc.edu

One-dimensional ^{1}H Nuclear Magnetic Resonance (1D ^{1}H NMR) has been used extensively as a high-throughput analytical tool for investigating metabolites in unfractionated biological fluids and tissue extracts. In this role, NMR spectra are usually treated as multivariate statistical objects rather than as collections of quantifiable metabolites. As a result, NMR-based analyses have been largely restricted to generating spectral "fingerprints" for the purpose of classifying samples. We have recently developed a practical two-dimensional (2D) ^{1}H-^{13}C NMR strategy for identifying and quantifying approximately 80% of the NMR-observable metabolites present in biological samples. We have developed a web-based tool (MMCD) to assist in NMR- and mass spectrometry-based identifications of metabolites in biological samples; MMCD is freely accessible from the NMRFAM website (http://mmcd.nmrfam.wisc.edu/). We validated our protocol for quantifying metabolites by using 2D ^{1}H-^{13}C HSQC (heteronuclear single quantum correlation) NMR to analyze mixtures of synthetic compounds and extracts from *Arabidopsis thaliana*, *Saccharomyces cerevisiae*, *and Medicago sativa*. Our results demonstrate that accurate (technical error < 3%) molar concentrations of more than 40 metabolites per cell extract can be determined in 12 minutes using our quantitative 2D ^{1}H-^{13}C NMR strategy. In contrast, traditional 1D ^{1}H NMR analysis of the same mixtures resulted in more than 16% technical error, despite nearly ideal conditions.

We recently completed biological studies that made extensive use of the quantitative metabolomics strategy to measure steady-state metabolite concentrations. One of these studies was an extensive genetic, transcriptional, and metabolomic investigation of genetic mutants that affect osmotic tolerance in *Arabidopsis thaliana*. We showed that plants that over-express a membrane histidine kinase, ATHK1, are resistant to osmotic challenge. We attributed the salinity tolerance of these plants to their extensive up-regulation of osmolyte production, primarily glutamine and sucrose, relative to wildtype plants. Our ability to measure accurate molar concentrations was critical because it allowed us to evaluate the contribution of abundant metabolites to overall osmolarity.

We are currently applying our quantitative strategy to a number of clinically relevant disorders including: a predictive model for acute inflammatory response, an early detection method for polycystic ovary syndrome (PCOS) and the metabolic effects of sirtuin-mediated epigenetic regulation. Our quantitative metabolomics strategy has also proved useful for measuring metabolic flux. In a recent study of metabolic flux regulation in red blood cells (collaboration with Prof. Philip S. Low, and Maria Estella Campanella, Purdue University), we showed that band 3, and bicarbonate/chloride exchange protein, is critical for control of metabolic flux through the pentose shunt and glycolysis. This study required precise quantification of the isotopic labeling patterns in lactate to determine flow of metabolites through the pentose shunt and glycolysis in band 3 knockout versus wild type mice.

Supported by NIH grants: 21 DK070297 (MRS, PI) and P41 RR02301 (JLM, PI).

Microcrystallography Facility at the Advanced Photon Source

Malcolm Capel, Kanagalaghatta Rajashankar & Steven Ealick
Dept. Chemistry & Chemical Biology, Cornell University
Synchrotron Radiation Structural Biology Resource
url:necat.chem.cornell.edu contact: capel@anl.gov

The North Eastern Collaborative Access Team (NECAT) has constructed and operates 2 undulator beam lines optimized for macromolecular microcrystal diffraction at the Advanced Photon Source, (APS) using the APS tandem offset undulator as the X-ray source and incorporating Kirkpatrick-Baez focusing. Our Phase I beam line is tunable over a spectral range of 5-25 KeV (focus spot size 60H x 20V microns, FWHH), while our Phase II beam line is a fixed energy beam line operating at 12.662 or 14.76 KeV (focus spot size 100H x 20V micron FWHH). Both beam lines produce very high flux density and use x,y mirror steering to dynamically stabilize the beam position at the sample position. A Maatel MD2 micro diffractometer has recently been installed on the Phase II beam line which provides shaped X-ray beams down to 10 microns and the ability to visualize and center 5-10 micron crystals. Hardware and software systems supporting microcrystallography techniques at NECAT are described, along with recent experimental results. We also describe plans for improving the flux density and beam position stability of our beam lines intended to make data collection from microcrystals a low risk and routine endeavor.

SSRL Synchrotron Radiation Structural Biology Resource Science

Keith O. Hodgson and Britt Hedman
Stanford Synchrotron Radiation Laboratory, SLAC, Stanford University, Stanford CA 94025
A Synchrotron Radiation Structural Biology Resource
http://www-ssrl.slac.stanford.edu
hodgson@ssrl.slac.stanford.edu

The SSRL Synchrotron Radiation Structural Biology Resource develops new technologies primarily in three areas – macromolecular crystallography, x-ray absorption spectroscopy and small angle x-ray scattering. Collaborations between core scientists and outside groups are a key paradigm in guiding these developments and enabling them to mature so as to be rapidly disseminated to the broader biomedical community. In this poster, we illustrate three scientific examples of such core-collaborative research – two involving large complex enzyme structures and the second a study of radiation-induced x-ray damage and metalloprotein active site structure.

Resource developed robotics and computational technologies have enabled solution of a number of large and complex macromolecular structures. One example, from the laboratory of Chaitan Khosla, contained the largest structure (582 kDa per asymmetric unit) solved by MAD phasing to date. Over 600 crystals were screened and ninety selenium sites were located for phasing. This enzyme, 6-deoxyerythronolide B synthase (DEBS), is a polyfunctional, multi-subunit enzyme that catalyzes the biosynthesis of structurally complex and medicinally important natural products. The 2.7 Å structure reveals important details about the catalytic active site. Another large macromolecular machine (MW of about 0.5 MD) whose structure was revealed using Resource technologies is that of RNA polymerase II. Key details about how this polymerase reads DNA and writes a message (messenger RNA) have been revealed through the elegant work of Roger Kornberg and collaborators. This work was recognized by the award of the 2006 Nobel Prize to Roger Kornberg.

By using a novel combination of polarized protein single crystal x-ray absorption spectroscopy and crystallography, two key advances were made. First, through systematic study of photoreduction as a function of x-ray dose, as monitored by XAS, the speed of radiation damage was established for the Mn_4Ca cluster active site in Photosystem II (in an international collaboration headed by V. Yachandra, LBNL). This information was then applied in the structural determination of the active site (via EXAFS), and when combined with the enhancement of polarization, enabled the establishment of three structural models.

The SSRL resource is supported by NIH-NCRR (RR-01209) with additional funding for the general user support program being provided by NIH NIGMS and DOE BER. SSRL operations is funded by DOE BES.

TOPOLOGY OF ENERGY TRANSDUCING BIOLOGICAL MEMBRANES

Carmen Mannella, Chyongere Hsieh, Christian Renken, Karolyn Buttle, Michael Marko
Wadsworth Center, Albany NY
Resource for Visualization of Biological Complexity
www.wadsworth.org/rvbc, carmen@wadsworth.org

Electron tomography is being used to explore the topology of the inner membrane of mitochondria and of the photosynthetic membranes of cyanobacteria through a series of collaborative projects at the RVBC.

Mitochondria are the organelles in eukaryotic cells that generate the ATP needed to power the cell's molecular machinery. The chemiosmotic reactions involved in ATP production occur on and across the mitochondrion's inner membrane which has invaginations called cristae. Electron tomography has shown that cristae are not random infoldings (as depicted in older textbooks) but internal micro-compartments connected to the periphery of the inner membrane by narrow tubular junctions. Computer modeling indicates that inner membrane topology can influence efficiency of ATP production by its effects on internal diffusion of metabolites (1). Electron tomography is helping to define the topological transitions in the inner membrane associated with different osmotic and metabolic states, internally generated reactive oxygen (2,3), and protein release during apoptosis (4), as well as mutations in proteins associated with mitochondrial diseases (5,6). The results suggest that diffusion pathways inside this organelle are regulated by proteins that control inner membrane curvature and dynamics, and, conversely, that some mitochondrial disorders might be caused by aberrant membrane topology.

Cyanobacteria are environmentally important photosynthetic bacteria, with one species, *Prochlorococcus*, contributing nearly half of the net primary oxygen production in certain open ocean regions. Electron tomography is being used to define differences in cell wall structure and internal, photosynthetic membrane topology between older and more recently evolved strains of this cyanobacterium (7). The internal membranes in both strains form a concentric, multi-lamellar network around the cell periphery, interconnected by short inter-membrane junctions. There are also fenestrations through the lumens defined by pairs of membranes. These fenestrations are more prominent in the older species, in which the number of membrane layers is greater, suggesting that the fenestrations play a role in the internal diffusion of metabolites

Specimens used for these studies include both conventionally fixed/plastic-embedded, and unfixed, frozen-hydrated cells and tissue. A problem encountered with ice-embedded specimens is the generation of severe defects during mechanical sectioning, employed to reduce specimen thickness for maximum resolution. An alternative approach that we are pioneering is focused ion beam milling of frozen-hydrated cells. This methodology has achieved artifact-free thinning of the bacterium *E. coli,* with subsequent tomographic analysis revealing numerous small invaginations in the cytoplasmic membrane not previously described.

Collaborators:
1) I. Moraru, L. Loew, Virtual Cell Resource, Univ. Connecticut
2) Y. Deng, Natl. Univ. Singapore
3) S. Benzer, Caltech
4) L. Scorrano, S. Korsmeyer, Harvard Univ.
5) M. Palladino, Univ. Pittsburgh
6) A. Van der Bliek, UCLA
7) C. Ting, Williams Coll.

Index