

Workshop on Interdisciplinary Biomedical Research

University of Notre Dame

March 1 - 3, 2009 (Sunday - Tuesday), Center for Continuing Education, University of Notre Dame

Organizers: Mark Alber, Holly Goodson, Danny Chen, Glen Niebur (Notre Dame) and Elliot Rosen (Indiana University School of Medicine)

Complex biomedical modeling efforts include predictive multiscale simulations consisting of different submodels at each scale, scalable parallelism for heterogeneous dynamical simulations, a data-rich environment with experimentally determined model parameters and, finally, distributed multidisciplinary research teams and resources. The goal of this workshop is to bring together researchers in biomedical field, biochemistry, bioengineering, biology, computational and mathematical biology and biophysics to discuss recent developments in biomedical modeling and experiment as well as current and possible future collaborations. Some of the topics to be discussed are: thrombus formation, bioimaging, study of osteoporosis.

Information: Tranberg.1@nd.edu, malber@nd.edu and http://www.nd.edu/~icsb/workshop_spr09.html

Sunday March 1, 2009

All Day Location - McKenna Hall Auditorium

11:30-12:15 Registration

12:15-12:30 Opening Remarks

12:30-1:00 Victoria Ploplis, Associate Director, W.M. Keck Center for Transgene Research, University of Notre Dame, *"Hemostasis Proteins in Nonhemostasis-related Functions – Lessons Learned from Gene Knock-out Models"*

1:00-1:30 Vikas Tomar, Department of Aerospace and Mechanical Engineering, University of Notre Dame, *"Hierarchy Correlations in Atomistic Mechanics of Collagen Hydroxypatite Biomimetic Composites and its Relations to Bone Modeling and Experiments"*

1:30-2:00 Jeffrey Peng, Department of Chemistry and Biochemistry, University of Notre Dame, *"Towards Flexibility Activity Relationships in Ligand Design"*

2:00-2:15 Coffee Break

2:15-2:45 Shahriar Mobashery, Department of Chemistry and Biochemistry, University of Notre Dame, *"Bacterial Cell Wall"*

2:45-3:15 Elliot Rosen, Division of Molecular Genetics and Gene Therapy, Department of Medical and Molecular Genetics, Indiana University School of Medicine, *"Why Do Thrombi Stop Growing? - Fibrin Polymerization May Limit Clot Growth"*

3:15-3:30 Mark Suckow, Director, Freimann Animal Care Facility, University of Notre Dame, *"Animal Modeling and Resources at Notre Dame"*

3:30-4:00 **Mary Prorok**, Department of Chemistry and Biochemistry and the W.M. Keck Center for Transgene Research, University of Notre Dame, *"The Structure and Activity of the Neuroactive Conantokin Peptides"*

5:00 **Public Lecture - Dr. Anantha Shekhar**, Director, Indiana Clinical and Translational Sciences Institute (CTSI); Associate Dean for Translational Research; Raymond E. Houk Professor of Psychiatry; Professor Pharmacology and Neurobiology; Indiana University School of Medicine; *"Clinical and Translational Research Cycle: Complex Biomedical Modeling Approach to Human Diseases"*

6:30 **Reception, McKenna Hall**

Monday March 2, 2009

Morning Location McKenna Hall Auditorium

7:45-8:15 **Registration and Coffee**

8:15-8:30 **Address by Robert Bernhard**, Vice President for Research, University of Notre Dame

8:30-9:00 **Hsueh-Chia Chang**, Department of Chemical and Biomolecular Engineering, *"The Biophysics of Blood Flow"*

9:00-9:30 **Yunlong Huo**, Department of Biomedical Engineering, IUPUI, *"Coronary Circulation in the Anatomically-Based Arterial Tree"*

9:30-10:00 **Philippe Sucusky**, Department of Aerospace and Mechanical Engineering, University of Notre Dame, *"Calcific Aortic Valve Stenosis: A Shear Stress-Dependent Mechanism?"*

10:00-10:30 **Coffee Break**

10:30-11:00 **Danny Chen**, Department of Computer Science and Engineering, University of Notre Dame, *"Segmentation, Reconstruction and Analysis of Blood Thrombi in 2-Photon Microscopy Images"*

11:00-12:00 **Special Lecture - Milan Sonka**, Professor of Electrical and Computer Engineering, Ophthalmology and Visual Sciences and Radiation Oncology, Department of Electrical and Computer Engineering, The Iowa Institute of Biomedical Imaging, University of Iowa, *"Medical Image Analysis – Methods and Applications"*

12:00-1:30 **Lunch Buffet, McKenna Dining Area**

Afternoon Location - Hesburgh Center Auditorium

1:30-2:00 **Alejandro Espinoza**, Department of Orthopaedic Surgery, Rush University Medical Center, *"Medical Imaging for Spine Biomechanics"*

2:00-2:30 **Brian Baker**, Department of Chemistry and Biochemistry, University of Notre Dame, *"T Cell Receptor Binding Solutions Directed by Peptide/MHC Conformation Dynamics"*

2:30-3:00 **Diane Wagner**, Department of Aerospace and Mechanical Engineering, University of Notre Dame, *"Culture Conditions Determine the Differentiation Response of Adipose-derived Mesenchymal Cells to BMP-6"*

3:00-3:15 **Coffee Break**

- 3:15-3:50** **David Brenner**, President and CEO, Innovation Park at Notre Dame, *“Transforming an Idea into a Marketable Innovation”*
- 3:50-4:25** **Gregory Crawford**, Dean, College of Science, University of Notre Dame, *“Starting a Biotech Company from a University Invention”*
- 4:25-4:50** **Brooke Pyne**, IEDC SBIR Director, Indiana Economic Development Corporation, *“Understanding Indiana’s SBIR/STTR Initiative”*
- 4:50-5:25** **Mayland Chang**, Assistant Director, Walther Cancer Research Center, University of Notre Dame, *“From Bench to Bedside: How to Bring a Therapeutic to Commercialization in an Academic Setting”*
- 5:45-6:30** **Panel Discussion on Biomedical Technology and Connection to Industry**
- Robert Bernhard, Vice President for Research, University of Notre Dame
 - David Brenner, President and CEO, Innovation Park at Notre Dame
 - Gregory Crawford, Dean, College of Science
 - Peter Kilpatrick, Dean, College of Engineering
 - Brooke Pyne, IEDC SBIR Director, Indiana Economic Development Corporation
 - Mayland Chang, Assistant Director, Walther Cancer Research Center, Notre Dame
 - Keith March, Director, Indiana Center for Vascular Biology and Medicine; Vascular and Cardiac Center for Adult Stem Cell Therapy

6:30pm **Workshop Dinner, Morris Inn Notre Dame Room**

Tuesday March 3, 2009

All Day Location - McKenna Hall Auditorium

- 8:00-8:30** **Registration and Coffee**
- 8:30-9:00** **Michael Ferdig**, Department of Biological Sciences, University of Notre Dame, *“Dissecting the Complexity of Malaria Drug Resistance: Integrating Gene Expression Levels and Chromosome Structural Variation”*
- 9:00–9:30** **Anthony Firulli**, Department of Pediatrics, Indiana University School of Medicine, *“A Twist on Limb Development”*
- 9:30–10:00** **Keith March**, Professor of Medicine, Physiology, and Biomedical Engineering; Director, Indiana Center for Vascular Biology and Medicine; Director, Vascular and Cardiac Center for Adult Stem Cell Therapy, *“TBA”*
- 10:00-10:15** **Coffee Break**
- 10:15-10:45** **Jawed Fareed**, Departments of Pathology and Pharmacology, Director, Special Coagulation Laboratory and the Hemostasis and Thrombosis Research Program, Loyola University Chicago, *“Contaminants in Heparin. What Role the Chemical Expertise Played?”*
- 10:45-11:15** **Paul Bohn**, Department of Chemical and Biomolecular Engineering, University of Notre Dame, *“Catalytic Transformations of Biological Macromolecules in Nanopores”*
- 11:15-12:15** **Plenary Lecture** – Kenneth Mann, Department of Biochemistry, University of Vermont - Burlington, *“Computational and Empirical Modeling of the Blood Coagulation System”*

- 12:15–1:30** **Lunch Buffet, McKenna Dining Area**
- 1:30-2:00** **Nitesh Chawla**, Department of Computer Science and Engineering, University of Notre Dame; “*CARE: Prospective Disease Prediction Based on Individual Disease Histories*”
- 2:00–2:30** **Eric Nauman** School of Mechanical Engineering, Weldon School of Biomedical Engineering, Department of Basic Medical Sciences, Purdue University, “*Multi-scale and Multi-physics Based Analysis of Insults to the Spinal Cord*”
- 2:30–3:00** **Sean Mooney**, Co-Director, School of Medicine Bioinformatics Core; Center for Computational Biology and Bioinformatics; Indiana University School of Medicine; “*Enabling the Next Generation of Biomedical Research with Translational Informatics*”
- 3:00-3:15** **Coffee Break**
- 3:15-3:45** **Scott Emrich**, Department of Computer Science and Engineering, University of Notre Dame, “*Sequencing Your Favorite Genome for \$1000*”
- 3:45-4:15** **Greg Madey**, Department of Computer Science and Engineering, University of Notre Dame, “*Modeling and Simulating the Transmission of Malaria*”
- 4:15-4:45** **Steve Buechler**, Department of Mathematics, University of Notre Dame, “*Low Expression of a Few Genes Indicates Good Prognosis in Estrogen Receptor Positive Breast Cancer*”

T Cell Receptor Binding Solutions Directed by Peptide/MHC Conformation Dynamics

Brian Baker

Department of Chemistry and Biochemistry
University of Notre Dame

Catalytic Transformations of Biological Macromolecules in Nanopores

Paul Bohn, Sean Branagan, Travis L. King and Zhen Wang

Department of Chemical and Biomolecular Engineering
University of Notre Dame

Studies of macromolecular reactivity in confined environments are challenging, but the confined environment can affect molecular recognition and catalysis. Our work is aimed at comparing the reactivity of biomolecules in geometrically confined spaces, such as the interior of cylindrical nanopores, with that in free solution. Single nanopores are constructed in membranes containing a layer of poly (methyl methacrylate)/poly (glycidyl methacrylate) (PMMA/PGMA) sandwiched between two layers of PMMA. The exposed glycidyl group is used to immobilize biomolecules through reaction with solvent-accessible primary amines. Uniform cylindrical nanopores are created by focused ion beam (FIB) milling. Horseradish peroxidase (HRP) is immobilized on the interior surface of PMMA/PGMA membrane, and laser induced fluorescence (LIF) is employed to monitor the enzymatic conversion of non-fluorescent amplex red to fluorescent resorufin in the presence of H₂O₂. Immobilized HRP exhibits very high activity, which is reflected in a ≥ 20 -fold increase in the reaction rate compared to free solution. The enzymatic reaction in nanopores proceeds at a rate that is closely coupled to the transport mechanism and the residence time in the nanopore.

Transforming an Idea into a Marketable Innovation

David Brenner

President and Chief Executive Officer
Innovation Park at Notre Dame

Low Expression of a Few Genes Indicates Good Prognosis in Estrogen Receptor Positive Breast Cancer

Steven Buechler

Department of Mathematics
University of Notre Dame

Many breast cancer patients remain free of distant metastasis even without adjuvant chemotherapy. While standard clinical traits fail to identify these good prognosis patients with adequate precision, analyses of gene expression patterns in primary tumors have

resulted in more successful diagnostic tests. These tests use continuous measurements of the mRNA concentrations of numerous genes to determine a risk of metastasis in lymph node negative breast cancer patients with other clinical traits. Here we show that low expression levels of three genes, as assessed with Affymetrix GeneChip technology, identifies a group of estrogen receptor positive (ER+) breast cancer patients with a high enough long-term survival probability to make chemotherapy of questionable benefit. These genes are identified with a novel method of isolating genes that are both connected with relapse and have expression patterns that define subtypes that suggest distinct cellular states.

The Biophysics of Blood Flow

Hsueh-Chia Chang

Bayer Professor of Engineering
Department of Chemical and Biomolecular Engineering
Director, Center for Microfluidics and Medical Diagnostics
University of Notre Dame

We examine certain blood rheological properties relevant to blood diagnostic kit designs and human micro-circulation physiology. Due to elastic blood cell deformability, blood cells are shown to exhibit trajectories distinct from the streamlines in a blood capillary such that they favor low shear rate regions. A different classical explanation notwithstanding, this phenomenon is in fact responsible for the Fahraeus-Lynquist effect that different hematocrit is measured in blood vessels of different dimension. It is also responsible for a bottle-neck in the blood diagnostic industry: micro-needles for loading blood onto diagnostic kits by wetting fail when their radii are below 100 microns. We use the same segregation effect to develop blood cell-plasma separation units, a bacteria trap for blood samples and a simple flow assay for aged and diseased blood cells like leukemia and sickle cells. All are microfluidic units fabricated onto a single chip for point-of-care applications. By monitoring the micro-circulation dynamics of a trout model in response to imposed flow stimuli, we also show that the elasticity of veins, which specifies for the venous pressure, is as much responsible for cardiac output as the heart rate.

From Bench to Bedside: How to Bring a Therapeutic to Commercialization in an Academic Setting

Mayland Chang

Assistant Director, Walther Cancer Research Center
University of Notre Dame

Drug discovery and development is a lengthy and costly process, characterized by a high attrition rate. Although pharmaceutical companies have become better at weeding out failures early in the development process, only 21% of drugs that start phase I clinical trials get to market. Recent studies have shown that the major reason for failure in clinical development is poor pharmacokinetics. Apparently, too many drug candidates

are being developed with optimized *in vitro* biological activity, based solely on their ability to inhibit an enzyme or interact with a receptor, but fail in the clinic because of unfavorable pharmacokinetic properties. In this presentation, the stages from discovery, lead optimization, candidate drug, preclinical development, Investigational New Drug (IND), clinical trials, to New Drug Application (NDA) will be reviewed using Rescriptor™ Tablets (delavirdine mesylate) for the treatment of HIV as a case study. Widely accepted approaches in the pharmaceutical industry to improve the likelihood of clinical success will be discussed. Many of these approaches can easily be adapted in an academic setting to enhance research capabilities and add value to intellectual property that originates from university campuses. These efforts will become increasingly important in light of major changes in the pharmaceutical industry in the past several years, which demand substantial evidence for the likelihood of success of drug candidates that could be licensed from academia. A study from Notre Dame, exemplifying what can be done in the academic setting, will be discussed for the prototype mechanism-based gelatinase inhibitor SB-3CT. This compound exhibits excellent activity in animal models for cancer metastasis and stroke. No therapeutic recourse currently exists for prevention of cancer metastasis, which leads to fatality in 90% of patients. As for stroke, a single drug has been approved by the FDA, which is applicable to <5% of stroke patients.

CARE: Prospective Disease Prediction Based on Individual Disease Histories

Nitesh Chawla

Department of Computer Science and Engineering
University of Notre Dame

The monumental cost of health care, especially for chronic disease treatment, is quickly becoming unmanageable. This crisis has motivated the drive towards preventative medicine, where the primary concern is recognizing disease risk and taking action at the earliest signs. However, universal testing is neither time nor cost efficient. I present our work CARE, a Collaborative Assessment and Recommendation Engine, which relies only a patient's medical history using ICD-9- CM codes in order to predict future diseases risks. I will present experimental results on a large Medicare dataset comprising of 13 million patients and 32 million visits, demonstrating that CARE is efficient in capturing future disease risks.

Segmentation, Reconstruction, and Analysis of Blood Thrombi in 2-Photon Microscopy Images

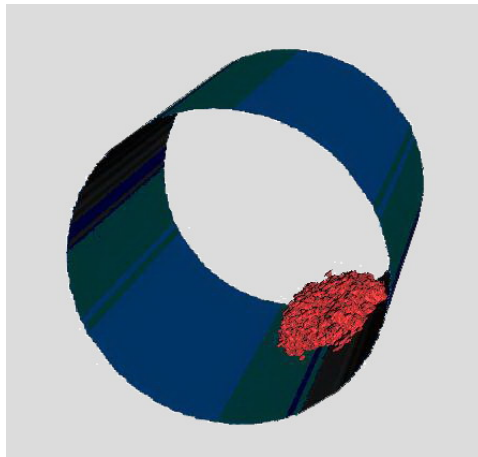
Jian Mu, Xiaomin Liu, Malgorzata M. Kamocka, Zhiliang Xu, Mark S. Alber, Elliot D. Rosen, Danny Z. Chen

Department of Computer Science and Engineering
University of Notre Dame

Upon vascular injury, to prevent blood loss following a break in blood vessel, components in the blood and vessel wall interact rapidly to form a thrombus (clot) to limit hemorrhage. The qualitative and quantitative analysis of the structure of developing

thrombi formed *in vivo* can help identify the factors altering thrombus growth and the structure affecting thrombus instability.

In this problem, we investigate the problem of segmenting, reconstructing, and analyzing blood thrombi (clots) in 2-photon microscopic images. It is an important and challenging problem in medical imaging. The algorithm that we used is based on the density-based clustering approach and also involves methods for dealing with image artifacts and for extracting information from the original images. In addition, we use a region-growing process to allow clots to capture nearby blood cells, and the union-of-balls (or alpha-shape) algorithm to reconstruct blood clots. Our algorithm produces results of biomedical significance. The results are valuable for the development of therapeutics for treating bleeding disorders. In addition, such algorithms can help us advance thrombus studies by combining the experimental models and multiscale computational models of thrombogenesis.



A reconstructed 3-D clot attached to the vessel wall

Starting a Biotech Company from a University Invention

Gregory Crawford
Dean, College of Science
University of Notre Dame

Sequencing Your Favorite Genome for \$1000

Scott Emrich
Department of Computer Science and Engineering
University of Notre Dame

Medical Imaging for Spine Biomechanics

Alejandro Espinoza and Nozomu Inoue

Spine Biomechanics Lab - Department of Orthopaedic Surgery
Rush University Medical Center

Low back pain is a serious clinical condition that affects most adults during their lifetime, with health and economic consequences to society. While many components of the spine have been implicated in it, only the effects are known, with no clear low back pain etiology. The intervertebral disc, which undergoes degeneration with aging, is one of the primary suspects. The ensuing altered disc biomechanics promote a cascade of events including abnormal spine loading, facet joint osteoarthritis, disc herniation, nerve compression, and disc height reduction among others.

Current clinical diagnostic methodologies to determine these distorted working conditions of the spine do not have the desired level of detail and precision, and have not looked into establishing a correlation between altered biomechanics and clinical conditions. Medical imaging has improved dramatically over the last decade, providing tools and methodologies to acquire high-resolution geometry of the spine and its soft tissue components. The objective of this work is to investigate the behavior of a degenerate spine compared to that of a non-symptomatic patient. Together with *in-vitro* kinematics and dynamics basic science studies, shapes, loads and motions were modeled jointly to characterize and study normal and clinical conditions of the spine. This work presents recent advances employing CT-based imaging and modeling to describe the spine biomechanics and diagnose bone and soft tissue conditions.

Contaminants in Heparin. What Role the Chemical Expertise Played?

Jawed Fareed

Departments of Pathology and Pharmacology

Director, Special Coagulation Laboratory and the Hemostasis and Thrombosis Research Program

Loyola University

In the first quarter of 2008, several batches of commonly used anticoagulants, namely heparin, were recalled by the USFDA due to an increased prevalence of serious adverse reactions and reported deaths. The recalled heparins were found to contain an unusual synthetic over sulfated chondroitin sulfate, which is usually not present in this anticoagulant. The contaminated heparins exhibited similar molecular and biologic properties and past the specifications. Synthetic over sulfated chondroitin sulfate has been synthesized earlier and tested for its biologic actions. The idea of adding this agent to heparin was rationally thought of as mixing of this contaminant does not alter heparins, molecular profiles and biologic actions. At the time when this contaminated heparin was used there were no astringent controls. Moreover, the regulatory agencies did not ask for structural characterization of heparin. Only after the reported adverse reactions, the regulatory bodies required additional chemical analysis. Thus, for both the introduction

of the contaminants in heparin and the characterization of the contaminants, Scientist with chemical expertise played an important role.

In order to further investigate the chemical and biological profiles of the contaminant in recalled unfractionated heparins (UFH) and low molecular weight heparins (LMWH), four recalled UFH preparations (3 finished products and 1 powder) were investigated. To obtain the contaminant, each material was treated by exhaustive depolymerization with nitrous acid and heparinase 1 to remove heparin followed by ethanolic precipitation and anion exchange chromatography. The amount of non-digested material ranged 10-30%, most of which was characterized to be hypersulfated chondroitin sulfate (HSCS) by proton and ¹³C NMR spectroscopy. The molecular weight profile exhibited a wider dispersity index in comparison to contaminant-free UFH with oligosaccharides ranging from 5-30 kDa (average 16.8 kDa). In addition, a well-characterized porcine cartilage HSCS preparation with average molecular weight of 17.2 kDa was used as a reference material. While varying degrees of dermatan sulfate (high molecular weight) and minor impurities were detected, the HSCS appeared to be the major contaminant in these preparations.

To investigate the biological profile of the isolated contaminant, it was subjected to chondroitinase A, B, and C and high potency heparinase 1 (2.5 U/ml) depolymerization. The material was resistant to the action of these enzymes. The contaminant was further profiled in routinely used anticoagulant and anti-protease assays. In the USP assay it exhibited a potency of 26.8 U/mg. It also produced a concentration dependent anticoagulant effect in the whole blood celite activated clotting time (ACT) and saline ACT tests but was weaker than heparin. In the PT assay (extrinsic coagulation system) the contaminant only exhibited very weak activity and did not affect the INR up to a 50 µg/ml concentration. However, in the global anticoagulant assays such as the aPTT (intrinsic coagulation system) and Heptest, in comparison to UFH, the contaminant produced varying degrees of concentration dependent anticoagulant activity (10-40 U/mg). In the amidolytic anti-thrombin assay it produced a concentration dependent inhibition of thrombin in citrated plasma (~ 25 U/mg). This contaminant did not exhibit any inhibition of FXa in the same systems. In antithrombin (AT) depleted plasma, while the anticoagulant and amidolytic activities of UFH were considerably reduced, the contaminant exhibited measurable concentration dependent effects indicating a non-AT dependence. In HCII depleted plasma the contaminant lost sizeable anticoagulant and anti-thrombin effects. The dermatan cofactor activity of pre- and post-heparinase digested contaminant was not different, whereas UFH showed a considerable decrease. The contaminant was readily neutralizable by protamine sulfate, polybrene, and PF4 in a similar fashion as UFH.

The contaminant mediated contact factor activation as measured by the generation of kallikrein and bradykinin was concentration dependent in plasma and whole blood. UFH also showed this activity in both systems. Significant differences in contact activation by UFH and the contaminant were noted between citrated and hirudinized whole blood. The contaminant also produced HIT mediated antibody activation of platelets; however, it had a faster onset of action and longer lasting time course of platelet aggregation than UFH. In the ¹⁴C-Serotonin Release Assay (SRA) the contaminant produced a strong release of

serotonin, which sustained at high concentrations and did not follow the parabolic response usually observed with UFH. Studies of the contaminant mixed with UFH in proportions of 3, 6, 12, 25, 50% (amount of contaminant) in plasma and whole blood revealed a non-additive assay dependent synergistic effect of the contaminant on the anticoagulant and anti-thrombin activities. At a 25% level, the contaminant produced a marked increase of the anticoagulant activity of the mixture mimicking the pharmacopoeial potency of ~ 150 U/mg; however, this increase was dependent on different contaminant preparations (different batches). Similar augmentation of the effects of UFH were noted in the thrombin and FXa generation tests as measured by amidolytic methods and measurements of such thrombin generation markers as FPA, TAT, and F1.2. Preliminary studies show that the contaminant may also exhibit direct anti-protease effects by complexing with FVIIa and the prothrombinase complex.

To study the in vivo effect of the contaminant, the effect of contaminated UFH and a potency equivalent contaminant-free preparation were studied in animal models of bleeding and thrombosis. In comparison to the contaminant-free UFH at an identical dosage of 200-800 U/kg, the contaminated UFH produced a marked increase in bleeding. Similarly, the antithrombotic effect in terms of ED₅₀ was markedly stronger with the contaminated preparation. Blood pressure measurements provided variable effects in which both the contaminated and contaminant-free preparations exhibited a hypotensive response in some rats. In contrast to the contaminant-free UFH, the contaminated preparation produced a stronger release of TFPI. Similar studies carried out on two hemi-synthetic HSCS preparations mixed with non-contaminated UFH provided comparable results. Since the contaminated batches of UFH also contain variable amounts of dermatan sulfate, additional studies are in progress at this time on the pharmacologic profile of the contaminated UFH, isolated contaminant, hemi-synthetic HSCS, hyper-sulfated dermatan sulfate, and their precursors. Additional investigations on the contaminant isolated from commercially available/branded and generic LMWHs are revealed molecular and biologic heterogeneity indicating that these products have originated from different sources. With the use of analytical tools such as, the NMR, capillary electrophoresis, and HPLC, contaminants can readily be rejected in heparins. The currently available heparins are free of these contaminants and the USP and USFDA requires chemical data on the purity of heparins.

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Dissecting the Complexity of Malaria Drug Resistance: Integrating Gene Expression Levels and Chromosome Structural Variation

Michael Ferdig

Department of Biological Sciences
University of Notre Dame

Classical genetics is invigorated by modern genomic tools. Linkage mapping can incorporate high resolution, high throughput genome data in a sort of ‘systems’ biology approach wherein perturbations of the system consist of the underlying segregating DNA variants that influence divergent traits inherited from the parent clones. We consider the global structural variation and its relationship to expression level polymorphisms and drug resistance traits in the progeny of the HB3 × Dd2 cross. In our earlier efforts to understand the genetic complexity of drug resistances we observed correlations among quantitative drug sensitivity profiles to a range of compounds for which shared QTL pinpoint interwoven genetic contributions. Here, we carry the linkage mapping approach a step further to explore the inheritance of the expression levels of all the genes in the genome. That is, rather than seeking only the genetic loci controlling an end-point drug response phenotype, we use the power of co-segregation to integrate the cascade of expression ‘traits’ along the path between genotype and drug sensitivity. We observe

extensive heritable variation in transcript levels and a preponderance of trans-regulation residing mostly in regulatory hotspots. The amplification on chromosome 5 that carries, */pfmdr1/*, associated with a variety of drug sensitivities, is also the strongest contributor to genome-wide transcriptional variation. Our studies highlight a potential role for copy number variation to have far reaching phenotypic and adaptive impact in malaria parasites, not only through the over-expression of amplified genes residing on the amplicon, but through subtle rewiring of the transcription network.

A Twist on Limb Development

Anthony Firulli

Department of Pediatrics
Indiana University School of Medicine

Coronary Circulation in the Anatomically-Based Arterial Tree

Yunlong Huo

Department of Biomedical Engineering
IUPUI

Coronary heart disease remains the major cause of morbidity and mortality in the U.S. and is very much on the rise around the world. The principal mechanism in this problem is failure of sufficient blood supply to reach the heart muscle for its own metabolic needs and hence failure of the heart as a pump. In order to understand coronary blood flow, it is a logical starting point to investigate the blood flow in a healthy heart. The theoretical analysis, based on the experimental measurement, plays an important role in the study of coronary circulation. Here, I will describe two of my previous studies; i.e., 1) pulsatile blood flow in the entire coronary arterial tree down to the first capillary bifurcations and 2) complex flow patterns in the curved and branching epicardial coronary arteries. In the first example, I will show the spatial and temporal distribution of blood pressure and flow within the myocardium, which can predict the spatial heterogeneity of coronary circulation and the system of blood supply to the heart. In the second example, various spatial hemodynamic parameters will be discussed in the atherosclerotic-prone sites, which can help determine the abnormal biological response, such as dysfunction of endothelial cells, monocyte deposition, elevated wall permeability to macromolecules, and so on. These studies have both basic and clinical significance and further provide the rationales for future investigations.

Modeling and Simulating the Transmission of Malaria

Greg Madey

Department of Computer Science and Engineering
University of Notre Dame

Computational and Empirical Modeling of the Blood Coagulation System

Kenneth G. Mann, Thomas Orfeo, Saulius Butenas, Anetta Undas and Kathleen Brummel-Ziedins

Department of Biochemistry
University of Vermont - Burlington

Our studies involve mathematical (computer) simulations, a reconstructed plasma/platelet proteome, whole blood *in vitro* and blood exuding from microvascular wounds. All studies indicate that in normal hemostasis, tissue factor (Tf) in combination with plasma fVIIa provides an INITIATION PHASE through the *extrinsic fXase* (Tf-fVIIa) which is largely controlled by tissue factor pathway inhibitor (TFPI) in combination with antithrombin (AT) and the protein C (PC) pathway. The synergy between these inhibitors provides a threshold-limited reaction in which a stimulus of sufficient magnitude must be provided for continuation of the reaction. With sufficient stimulus, the fXa produced activates some prothrombin. This initial thrombin activates the procofactors and platelets required for presentation of the *intrinsic fXase* (fVIIIa-fIXa) and *prothrombinase* (fVa-fXa) which drive the subsequent PROPAGATION PHASE; continuous downregulation of which is provided by AT and the thrombin-thrombomodulin-PC complex. FXa generation during the PROPAGATION PHASE is largely (>90%) provided by the *intrinsic fXase*. Tf is required for the INITIATION PHASE of the reaction but becomes non-essential once the PROPAGATION PHASE has been achieved. The PROPAGATION PHASE catalysts (fVIIIa-fIXa and fVa-fXa) continue to drive the reaction as blood is resupplied to the wound site by flow. Ultimately, the control of the reaction is governed by the pro- and anticoagulant dynamics and the supply of blood reactants to the site of a perforating injury. Our systems have been utilized to examine the qualities of hypothetical and novel antihemorrhagic and anticoagulation agents in epidemiologic studies of venous and arterial thrombosis and the hemorrhagic pathology.

TBA

Keith March

Professor of Medicine, Physiology and Biomedical Engineering
Director, Indiana Center for Vascular Biology and Medicine
Director, Vascular and Cardiac Center for Adult Stem Cell Therapy
IUPUI

Bacterial Cell Wall

Shahriar Mobashery

Department of Chemistry and Biochemistry
University of Notre Dame

Cell wall is a polymer of approximately 3 GDa that encases the entire bacterium. Its health is critical for the survival of the organism and the enzymes for its assembly and the

cell wall itself are targets of antibiotics. Furthermore, cell wall is the source of signaling agents that regulate many aspects of bacterial existence. In this presentation, I will address our efforts toward synthetic preparations of representative fragments of the cell wall and some of these biochemical mediators that are derived from it. These reagents have been useful in unlocking the various aspects of the biochemical events that govern cell wall.

Enabling the Next Generation of Biomedical Research with Translational Informatics

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The impact that high throughput genomics, massive electronic clinical databases, and basic research will have on our approaches to identify, prevent and treat human disease cannot be understated. This new era should enable studies that will test hypotheses with both higher power and lower false discovery. Central to this endeavor is biomedical informatics, the electronic glue that will both coordinate administrative efforts to connect academic units, researchers, and biomedical services and, working closely with statisticians, it will enable new discoveries. Our group continues to focus playing a part in developing our national biomedical cyberinfrastructure, as well as testing specific hypotheses to understand the molecular mechanisms of genetic disease. We are interested in understanding the molecular effects of genetic changes, because we are entering an era of personalized genetics and personal human genome sequencing is on the horizon. This presents great opportunities and challenges in understanding biochemically how specific disease-causing and phenotype-altering genetic differences express their function. In this talk, I will give an overview of some current challenges and our efforts to address them. I will discuss our efforts for aiding identification of candidate genes using biochemical and genomic data. I will also present our efforts for developing and applying bioinformatics methods to identify molecular functions that can be disrupted by mutation and our approaches to understand the systems they operate upon. Finally, I will also include discussion of our efforts to build informatics systems to enable translational approaches in genetic, pharmacogenetic, and gene therapy studies.

Multi-scale and Multi-physics Based Analysis of Insults to the Spinal Cord

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According to the National Spinal Cord Injury Statistical Center, approximately 11,000 spinal cord injuries (SCIs) occur each year in the United States, not including SCI-related fatalities. In July 2004 an estimated 250,000 Americans were living with some form of SCI and lifetime costs for a 25-year-old afflicted with high tetraplegia will likely exceed \$2.5 million. Spinal cord injuries (SCIs) cause neurological deficit through the compression, contusion, stretch, or laceration of the spinal cord. Physical trauma occurs at two distinct strain rates. Subacute compression injury occurs at a quasi-static strain rate and can be caused by a herniated disc, insertion of an implant, tumor or bone growth impinging on the spinal cord, or an abscess. High strain rate injuries arise from falls, blunt trauma, and burst fractures.

Despite the severity of these injuries, little is known about the mechanisms by which mechanical insults result in neurological deficit. Mechanical loads applied to the whole cord are transmitted to the myelin and individual axons. The insult then initiates a cascade of signals that diffuse through the tissue to create a secondary injury. Herein we will discuss an ongoing series of experiments and computational models that integrate multi-scale mechanics, transport, and electrical signaling in an effort to elucidate those factors that are most important to the progression of SCI.

Towards Flexibility Activity Relationships in Ligand Design

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Hemostasis Proteins in Nonhemostasis-related Functions – Lessons Learned from Gene Knock-out Models

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Hemostasis, the process by which bleeding from an injured blood vessel is arrested, involves a number of vascular, platelet, and plasma factors. Diminishing the extent of this process and maintaining vascular patency are mediated through the activation of a number of regulatory pathways. Abnormalities in maintaining this balance can lead to fatal bleeding or thrombotic episodes. While much is known about the specific role of hemostasis proteins in this process, recent advances in transgenic and gene knock-out technology have enhanced an understanding of other functions these proteins play in a wide variety of physiologies and pathophysiologies. Two regulatory mechanisms that control the extent of clot formation or its degradation are the Protein C (PC) pathway and plasminogen activator inhibitor-1 (PAI-1), respectively. Our laboratory has performed studies in mice in which genes for these regulatory components have been altered. Results from these studies have revealed nonhemostasis functions for these proteins in other biological events. Using primary aortic endothelial cells from PAI-1 deficient

(PAI-1^{-/-}) mice, studies have demonstrated that PAI-1 regulates endothelial cell function, *i.e.*, cell proliferation, by altering the activation of a number of cell signaling pathways. This supports earlier observations in our laboratory that demonstrated altered vessel formation in PAI-1^{-/-} mice using tumor and nontumor challenge models. The protein C pathway is critical for arresting clot formation following the injury and provisional repair process. However, a number of investigations have implicated a role for this pathway in regulating inflammation. While a complete inactivation of the PC gene is embryonic lethal, our laboratory has developed transgenic mice that express 1-20% PC. In a dextran sodium sulfate (DSS)-induced model of Inflammatory Bowel Disease (IBD), disease progression and severity were enhanced in mice expressing low (3%) levels of PC relative to wild-type (WT) mice. This correlated with increased colonic permeability and diminished epithelial tight junction protein in low PC mice, even in the absence of DSS challenge. Results from this IBD study support the working hypothesis that PC mediates PAR-1 signaling resulting in cytoskeleton rearrangement and vascular barrier protection. Future studies using gene modified mice expressing specific functional domain mutations of PC, and other proteins of hemostasis, should further elucidate complex structure/function relationships that dictate their biological properties.

The Structure and Activity of the Neuroactive Conantokin Peptides

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Understanding Indiana's SBIR/STTR Initiative

Brooke Pyne

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Why Do Thrombi Stop Growing? – Fibrin Polymerization May Limit Clot Growth

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Current models of thrombogenesis include the binding of platelets in flowing blood onto the surface of the developing thrombus. The newly incorporated platelets release factors promoting further platelet activation and provide a surface supporting surface-dependent coagulation reactions. Since the new surface of the developing clot provides a

prothrombotic environment for continued growth, it is not obvious why thrombi stop growing.

We recently developed a vascular injury model using multiphoton intravital microscopy. Following laser-induced injury of mouse mesenteric venules, the developing thrombus is monitored by collecting stacks of images of optical planes through the thrombus. Multichannel image acquisition enables one to monitor fluorescently labeled fibrinogen and platelets. By including fluorescently labeled dextran in the blood one can monitor flow as well as unlabeled cells that appear as black holes by excluding the labeled dextran. This system produces high resolution structural information revealing the changing, heterogeneous sub-domain structure of the developing thrombus.

Using newly developed image processing algorithms we are able to follow the changing composition of the thrombus surface. Thrombus growth is greatest when the thrombus surface is composed primarily of platelets. The cessation of clot growth corresponds with an increasing percentage of fibrin. At later times the thrombus surface is covered by cells. We hypothesize that fibrin polymerization on the surface of the developing clot provides a barrier between resting platelets in the flowing blood and the prothrombotic surface of platelets within the thrombus. Thrombin binding sites on fibrin may also interfere with the diffusion of thrombin generated on platelets within the clot to the new thrombus surface. Thus, fibrin polymerization not only increases thrombus stability but may also inhibit continuous thrombus growth.

Clinical and Translational Research Cycle: Complex Biomedical Modeling Approach to Human Diseases

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Medical Image Analysis – Methods and Applications

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Medical imaging has brought frequent use of a number of imaging modalities like X-ray, CT, MR, PET, SPECT, ultrasound, and OCT to routine clinical care. Yet, daily interpretation of these images is still typically performed visually and qualitatively, with quantitative analysis being an exception rather than the norm.

The talk will focus on several methods, approaches, and associated projects that bring quantitative medical image analysis closer to daily clinical routine. In the context of cardiovascular, pulmonary, and ophthalmologic imaging, methods for segmentation,

morphologic and functional assessment, and disease characterization of human image data from intravascular ultrasound and angiography of coronary arteries, MRI of the aorta and heart, vascular ultrasound, MDCT of the lungs, and OCT of the retina will be presented and its promise for routine use in clinical care will be discussed. The presentation will be accompanied by on-line examples of program packages supporting these application areas that were developed in the Iowa Institute for Biomedical Imaging and associated spinoff companies as a result of multiple NIH research grants.

Animal Modeling and Resources at Notre Dame

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Calcific Aortic Valve Stenosis: A Shear Stress-Dependent Mechanism?

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Purpose: Calcific aortic stenosis is the most common indication for valvular disease in the United States [1]. This degenerative disorder characterized by the accumulation of calcific nodules contributes to the obstruction of the left ventricular outflow which can lead ultimately to heart failure. Valvular calcification preferentially occurs on the aortic side of the leaflets where they are exposed to complex and unstable hemodynamic conditions [2]. The reasons for this side-specific response potentially associated with the local shear stress environment are not completely understood. In addition, while it has been shown that exposure of vascular endothelial cells to oscillatory shear stress induces inflammatory responses by bone morphogenetic protein (BMP)-4-dependent mechanisms [3], it is not clear whether BMP plays a role in the inflammation of aortic valve leaflet exposed to altered mechanical environment. In the present study, we hypothesized that inflammation occurs preferentially on the aortic surface of aortic valve leaflets in a BMP- and TGF- β 1-dependent manner due to the local hemodynamic loading conditions. This hypothesis was tested by investigating the effects of normal and altered shear stress on the initiation of valvular inflammation, and characterizing the respective role of TGF- β 1 and BMPs in this response.

Methods: The ventricular and aortic sides of porcine aortic valve leaflets were exposed for 48 hours to pulsatile and oscillatory shear stresses in an *ex vivo* culture system. Three culture media were used: standard culture medium, medium supplemented with TGF- β 1, and medium supplemented with the BMP antagonist noggin. Immunohistochemistry was performed to detect expressions of two well-known inflammatory markers VCAM-1 and ICAM-1, and the novel marker BMP-4.

Results: The results showed an increased inflammatory response of the aortic surface exposed to pulsatile shear stress (altered hemodynamics) as compared to fresh tissue

(four-fold increase in ICAM-1 and VCAM-1 expressions, six-fold increase in BMP-4 expression). Under similar shear stress conditions, the use of osteogenic medium resulted in a eight-fold increase in BMP-4 expression as compared to fresh tissue, while noggin significantly reduced the inflammatory response observed with standard and osteogenic media.

Conclusions: The results demonstrate that altered shear stress regulates the inflammatory response of aortic valve leaflets in a TGF- β 1- and BMP-dependent manner, providing some potential directions for future drug-based therapies for aortic valve disease. In addition, the knowledge of the shear stress-induced calcification pathway could aid in the development of a fluid-based model to predict the patient-specific progression of the disease and the improvement of its management.

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Hierarchy Correlations in Atomistic Mechanics of Collagen Hydroxyapatite Biomimetic Composites and Its Relation to Bone Modeling and Experiments

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One of the motivations in developing biomimetic materials is the use of complex structural hierarchy to obtain materials with fault tolerance. Another interest is in using hierarchy to couple with additional functional properties. In this work, we present our extensive atomistic hierarchical analyses of tropocollagen (COL) and hydroxyapatite (HAP) nanocomposite interfaces. Focus is on understanding the role of hierarchy in peak interfacial strength for fracture and in determining the extent of the localization of peak fracture stress. We find that the crystalline orientation, supercell dimensions, collagen

residue sequence, and volume fraction are important factors crucial to the overall hierarchical fault tolerant design. We also analyzed COL-HAP nanocomposites in three different chemical environments: vacuum, water, and calcinated water. Simulations show a clear correlation between the concentration of the surrounding environment and the predicted mechanical properties. We also found that environment could be coupled with multitude of functional properties in such bio-nanocomposites. Finally, we performed work in bone experiments and modeling which will be shown and related to the presented biomolecular modeling work.

Culture Conditions Determine the Differentiation Response of Adipose-derived Mesenchymal Cells to BMP-6

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Cells isolated from adipose tissue are an attractive cell source for tissue engineering due to their accessibility and multi-lineage potential, but strategies for driving the adipose-derived mesenchymal cells (AMCs) to a particular lineage have yet to be elucidated. We have recently developed a bipotent medium that supports both osteogenic and chondrogenic differentiation in murine AMCs depending whether the cells are cultured in monolayer or in pellet culture. Key to our medium formulation is bone morphogenetic protein 6 (BMP-6), a growth factor which differentially induces osteogenesis or chondrogenesis. However, the mechanisms driving the effect of BMP-6 in these culture conditions remain poorly understood. To elucidate the cellular mechanisms employed by BMP-6 during differentiation, we examined the activation of FAK, the MAP kinase (MAPK) pathway, and the canonical BMP-6 pathway via SMAD signaling in both monolayer and pellet culture. Future experiments are planned to optimize the medium formulation and to explore the role of FAK in directing the differentiation of the cells. We anticipate that a thorough understanding of how culture conditions influence the differentiation of AMCs will be key in engineering complex tissues composed of multiple cell and tissue types.