The School of Engineering & Applied Sciences
University at Buffalo *The State University of New York*

The University at Buffalo Department of Chemical & Biological Engineering

presents

The 16th Annual Graduate Student Research Symposium

October 18, 2013

University at Buffalo
Center for the Arts

The 16th Annual Graduate Student Research Symposium

Friday, October 18, 2013

1:00-3:30 p.m. Welcome and Opening Remarks
Stelios Andreadis, Ph.D.
Professor and Chair
Department of Chemical and Biological Engineering,
University at Buffalo, The State University of New York

Graduate Student Lectures
Kaustubh Rane, Ph.D. Candidate
Using Monte Carlo Simulation to Understand the Bulk and Interfacial Behaviors of Ionic Fluids
Faculty Advisor Jeffrey Errington, Ph.D.
Department of Chemical and Biological Engineering,
University at Buffalo, The State University of New York

Maoshih Liang, Ph.D. Candidate
Engineering Biomimetic Microenvironment for Vascular Grafts
Faculty Advisor Stelios Andreadis, Ph.D.
Department of Chemical and Biological Engineering,
University at Buffalo, The State University of New York

Keynote Speaker
Michael D. Reily, Ph.D.
Pharmaceutical Applications of Metabolomics
Applied and Investigational Metabolomics
Bristol-Myers Squibb Co.

Questions and Answers

3:30-5:00 p.m. Graduate Student Reception and Poster Competition

5:00 p.m. Announcing of the Winners of the Poster Competition
Stelios Andreadis, Ph.D.

5:30-7:00 p.m. Alumni and Student Reception

Abstracts for Graduate Student Speakers

Using Monte Carlo Simulation to Understand the Bulk and Interfacial Behaviors of Ionic Fluids

Kaustubh Rane, Ph.D. Candidate
Department of Chemical and Biological Engineering, University at Buffalo

Ionic fluids consist of a collection of dissociated ions. Notable examples are room temperature ionic liquids (RTILs) and molten alkali halides. This class has recently gained considerable attention, mainly due to the potential applications of RTILs in nanotechnology, energy storage and chemical processing. At the same time, studies on theoretical models continue to provide insight into the behavior of electrolyte solutions and colloids. This field has greatly benefitted from different types of molecular simulation due to the ability for one to use this tool to relate the macroscopic properties of a system to the underlying microscopic interactions. One type of simulation, called Monte Carlo, employs statistical thermodynamics to generate different configurations of molecules in order to calculate the thermophysical properties of a given system. In this presentation, we first describe how MC simulations are used to compute bulk and interfacial properties of interest. For the bulk behaviors, we present the vapor and liquid phase properties of different RTILs having a wide variety of structures and inter-ionic interactions. We examine thermodynamic properties, such as the saturated densities, vapor pressure, and enthalpy of vaporization, as well as metrics that describe the structure molecules adopt in the liquid and vapor phases. Regarding interfacial phenomena, we consider how different ionic fluids wet a particular solid surface. Here, we use a simple model for ionic fluids to systematically understand the influence of electrostatic interactions on wetting properties. Results are presented to show how the strength of the interaction between the fluid and solid as well as the system temperature affect the properties and microscopic structure of the fluid near the surface.

Engineering Biomimetic Microenvironment for Vascular Grafts

Maoshih Liang, Ph.D. Candidate
Department of Chemical and Biological Engineering, University at Buffalo

Cardiovascular disease (CVDs) is the leading cause of death in the developed world. Although the autologous replacement remains the golden standard, the limited supply and the pain caused by multiple surgeries necessitate the development of tissue engineered vascular constructs (TEVs) as alternatives. Previously, our group has made significant progress in engineering vascular grafts using natural materials such as fibrin and small intestine Submucosa (SIS). These grafts have been successfully implanted into an ovine animal model, where they remained patent for several months post implantation. However, certain challenges still remain such as diffusion limitations of growth factors through the vascular wall, heterogeneous distribution of cells, uneven collagen deposition and lack of elastin fibers. In order to address these issues, we developed biomimetic strategies to mimic the chemical and mechanical microenvironment of native arteries. Because supplement of TGF-β1 has been shown to promote myogenic differentiation and extracellular matrix (ECM) deposition in TEVs, we first engineered a fusion between TGF-β1 and a FXIII substrate peptide, which covalently anchored the fusion protein into fibrin during polymerization to simulate the local delivery of TGF-β1 in native vessels (Fig. a). Because mechanical stimulation also plays important roles in vascular tissue, we developed a 24-well based bioreactor (Fig. b) in which vascular grafts were cultured around a distensible mandrel in the middle of each well to mimic the cyclic pulsatile stimulation *in vivo*. TGF-β1 was either conjugated to fibrin or supplied in the culture medium and the fibrin-based constructs were cultured statically for a week followed by either another week of static culture or a week of cyclic distention. The tissues were examined for myogenic differentiation, vascular reactivity, mechanical properties and ECM content. Our results suggest that this two-prong biomimetic approach, immobilizing TGF-□1 in the 3D scaffold and controlling the mechanical microenvironment, can significantly improve cell distribution (Fig. c), ECM secretion, vascular reactivity and mechanical properties of vascular constructs. These findings suggest the importance of biomimetic strategies to engineer the tissue microenvironment for vascular grafts and for other tissue constructs as well e.g. cartilage, tendon or cardiac tissue, where TGF-□1 and mechanical loading play critical roles.

Abstract for Keynote Speaker

Pharmaceutical Applications of Metabolomics

Michael D. Reily, Ph.D.
Applied and Investigational Metabolomics
Bristol-Myers Squibb Co.

The metabolome, or the total compliment of small molecules in a living system that includes endogenous and introduced species, reflects the overall global biochemical state of an organism. Changes in the functional genome, transcriptome and proteome are closely tied to changes in the metabolome. Metabolomics (or metabonomics) is the comprehensive measurement the metabolome and how it changes in response to external stressors. In Pharmaceutical R&D, this information can be used deduce the relationship between a perturbation (such as disease or pharmacological intervention to disease) and the effected biochemical pathways, yielding mechanistic information and biomarkers that report upon the perturbation. These biomarkers can, in turn inform and accelerate the discovery of safe and efficacious drugs. This talk will provide a background on the technology and present several examples of how it has been employed in main stream pharmaceutical R&D.

Michael D. Reily, Ph.D. joined Bristol-Myers Squibb in September of 2007 as a Research Fellow in Bioanalytical and Discovery Analytical Sciences. He currently manages the Discovery Analytical Sciences NMR group and is co-leader of the Applied and Investigative Metabolomics (AIM) matrix team. Dr. Reily received his Bachelor of Science Degree in Chemistry from the University of West Florida in Pensacola and his Ph.D. in Bioinorganic Chemistry from Emory University in 1986. During his graduate work, Dr. Reily became interested in the application of high resolution NMR spectroscopy to answer structural questions about biomolecular interactions with drugs and he pursued this interest in postdoctoral with John Markley in the Biochemistry Department at the University of Wisconsin, Madison. In 1988, he came to Ann Arbor to the then Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company and has applied NMR spectroscopy to drug discovery and development in areas of medicinal chemistry, protein and nucleic acid structure determination and metabolite structure elucidation. His most recent career focus has been on the application of NMR and mass spectrometry-based metabolomics to study mechanistic toxicology and pharmacology and identify associated biomarkers. Dr. Reily is a member of the American Chemical Society and is author or co-author on over 80 peer-reviewed journal articles and book chapters.

School of Engineering and Applied Sciences
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Department of Chemical and Biological Engineering

- 2010 NRC rankings place UB CBE in 8th and 9th place for publications and awards per faculty, respectively, among 106 reviewed departments
- Outstanding funding from NIH, NYSTEM, NSF, USAF, AHA, DOE
- 7 NSF CAREER Awards
- 3 members of National Academy of Engineering

Bioengineering research

- **Andreadis** - Adult and induced pluripotent stem cells for cardiovascular tissue engineering, signaling pathways in cell-cell adhesion and wound healing, biomaterials for protein and gene delivery, lentiviral vectors and lentiviral microarrays for high-throughput gene expression analysis and gene discovery
- **Neelamegham** - Cell biomechanics, systems biology, thrombosis and hemostasis, glycosciences
- **Park** - Biotechnology, protein engineering, simulated dynamics, bioinformatics, drug discovery
- **Pfeifer** - Metabolic engineering, heterologous natural product biosynthesis, genetic vaccine design
- **Tzanakakis** - Tissue engineering, embryonic stem cells, adult stem cells, viral vectors, bio-chemical engineering

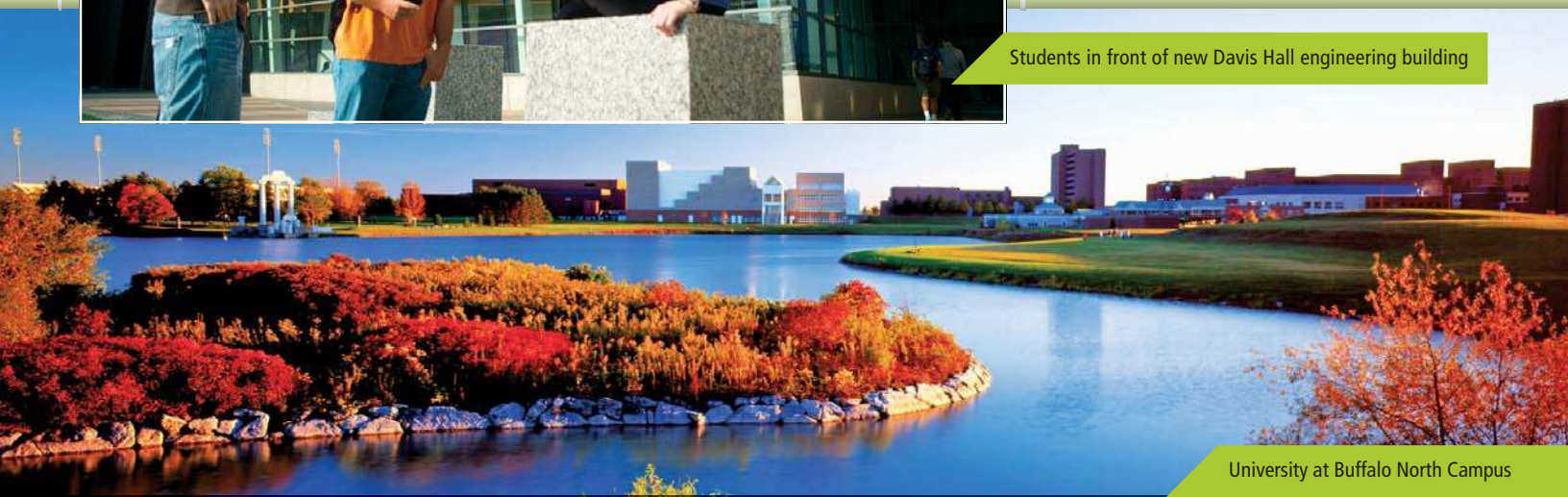
Modeling and computational research

- **Errington** - Molecular simulation, statistical thermodynamics, interfacial phenomena
- **Furlani** - Multidisciplinary modeling: microfluidics, computational fluid dynamics, mass/heat transfer, multiphase systems, MEMS, nanophotonics, biomagnetics
- **Hachmann** - Computational chemistry and materials science, virtual high-throughput and Big Data, machine learning, electronic structure theory and methods, quantum effects in catalysis and materials
- **Kofke** - Statistical physics, molecular modeling and simulation, software engineering
- **Lockett** - Mass/heat transfer, distillation, separations
- **Nitsche** -Transport phenomena, dermal absorption, biological membrane and pore permeability

Materials research

- **Alexandridis** - Self-assembly, directed assembly, complex fluids, soft materials, nanomaterials, interfacial phenomena, amphiphilic polymers, biopolymers, product design
- **Cheng** - Biodegradable functional polymers and nanostructures, new drug delivery systems, synthetic materials for tissue engineering
- **Lin** - Membrane materials and processes for gas and vapor separation and water purification
- **Lund** - Heterogeneous catalysis, chemical kinetics, reaction engineering
- **Ruckenstein** - Catalysis, surface phenomena, colloids and emulsions, biocompatible surfaces and materials
- **Swihart** - Synthesis and application of nanoparticles, reactor modeling, computational chemistry, particle nucleation and growth
- **Tsianou** - Molecularly engineered materials, self-assembly, interfacial phenomena, crystal engineering, bio-inspired materials
- **Zukoski** - Suspension mechanics, protein crystal-lization and nanoparticle self-assembly

Students in front of new Davis Hall engineering building



University at Buffalo North Campus

Acknowledgements

Welcome to the 16th Annual CBE Graduate Student Research Symposium! Over the years our research symposium has been an exciting event that showcases the high quality, multidisciplinary research that is conducted in CBE and spans diverse areas such as molecular engineering of novel materials, nanotechnology, bioengineering and molecular modeling. Every year our faculty and graduate students welcome the opportunity to present their work to their peers and alumni from CBE, other UB departments, and representatives from the local industry. As the Symposium has grown in ambition and scale, the effort needed to coordinate it has grown commensurately, and we owe many thanks to those whose time and hard work have brought it together this year.

First I would like to acknowledge the leadership and tireless efforts of CBE Graduate Student Association officer **Jacob Heltzel**, whose legwork and logistics on behalf of our students is commendable. Jacob truly cares about his colleagues and friends at CBE and how they fare in both their academic and free time while here at UB. We also owe much to the dedicated assistance of the CBE staff, most notably **Marlo Kerr, Andrew Schultz, Lori DuVall,** and **Joan Wilson**.

Second, I want to acknowledge the faculty members of the organizing committee, Professors **Sheldon J. Park**, **Marina Tsianou**, and **Haiqing Lin,** and members of the UB CBE Advisory Board, **Cindi Hoover**, Director of Healthcare and BioPharma R&D, Praxair; **Shawn Barrett**, Sr. Manager of New Product Introduction, Life Technologies; **Gregg Eagan**, Director of Manufacturing, Niacet Corporation; and **Weidong An**, Site Technology Manager, FMC, who have graciously agreed to join us today to serve as poster competition judges.

Finally, I would like to thank the speakers and other participants who highlighted this Symposium. I am grateful to our keynote speaker, **Dr. Michael D. Reily, Bristol Myers Squibb,** who, with a busy schedule, traveled here to present his research work and share his experience in industrial R&D with our graduate students. Special thanks to our senior graduate students **Maoshih Liang** and **Kaustubh Rane**, both of whom fearlessly agreed to present their work in this prominent venue. Thanks are also owed to all the CBE graduate students who worked so hard on their research over the years, and for presenting their work through the many carefully crafted posters that fill the CFA atrium.

The Graduate Student Research Symposium has continued to be an exciting occasion for our department. It is a showcase for the excellence that we strive for in our scholarship and graduate education. It is also a great occasion to reconnect with our alumni who graciously responded to our invitation and participated in this occasion with us. We look forward to many more years of this celebration of our research accomplishments.

Stelios Andreadis, Professor and Chair

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Biological Engineering

1. Nanog Enhances the Proliferation and Reverses the Effect of Senescence on Myogenic Differentiation of Human Mesenchymnal Stem Cells

Panagiotis Mistriotis[1], **Mao-shih Liang**[1], **Loukia G. Karacosta**[2], **Arthur M. Edelman**[2], **Stelios Andreadis**[1]

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Human Mesenchymal Stem Cells (hMSCs) show great promise as an autologous smooth muscle cell source for vascular tissue engineering and regenerative medicine. However senescence and organismal aging reduce the proliferative and myogenic differentiation capacity of MSCs. To address this issue, we recently reported that ectopic expression of Nanog can restore the lost contractile phenotype due to aging and enhance the proliferative potential of ovine MSCs (Han et al., 2012, Stem Cells). In the current study we aim in identifying the molecular mechanism that mediates the rejuvenating effects of Nanog in MSCs. MSCs isolated from human hair follicle were transduced with a tetracycline regulatable vector that carries the Nanog gene. This system enables Nanog expression upon Doxycycline (Dox) treatment. After serial passaging, MSCs were induced to senescence and Dox was added to the culture medium. Subsequently, the effect of Nanog on proliferative and myogenic capacity of MSCs were evaluated and compared with early passage (EP) MSCs. Ectopic expression of Nanog in late passage (LP) MSCs significantly increases the proliferation and decreases the percentage of senescent cells as indicated by SA--GAL staining .Furthermore, we show that Nanog controls the expression of key regulators of senescence such as cyclin inhibitors and DNA methyltransferases. Next we studied the myogenic differentiation upon Nanog expression. SMC-specific markers were highly upregulated in Nanog+LP MSCs resembling EP MSCs .In agreement, the contractility in response to vasoactive agonists was also enhanced . Interestingly, we also report that Nanog reverses the loss of the expression of key myogenic transcription such as SRF, MRTF-b, SMAD2 and MYOCD due to senescence . Notably, we identified that Nanog co-immunoprecipitatets with SRF , binds to the ACTA2 and SRF promoter and enhances the transcriptionaly activity of the SRF DNA binding site (CArG box) . At this framework, SRF knockdown abolished the effect of Nanog on myogenic differentiation. Taken together these data suggest that Nanog reverses the effects of senescence on MSC proliferation and myogenic differentiation capacity, thereby increasing the potential of MSC from aged donors for cellular therapy and tissue regeneration.

Key words: aging, senescence, stem cells, tissue engineering, regenerative medicine, smooth muscle

2. High-throughput, Continuous Perfusion-based Microfluidic Cell Culture Device To Monitor Mesenchymal Stem Cell Differentiation

Pulari Thangavelu[1], **Hun Lee**[2], **Kwang Oh**[2], **Stelios Andreadis**[1]

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Mesenchymal stem cells (MSCs) are widely used in regenerative medicine and cell-based therapeutics due to their multipotency and proliferative capabilities. MSCs can be coaxed to differentiate along osteogenic, adipogenic, chondrogenic and myogenic lineages. MSCs are used in several current preclinical trials in addressing stroke, myocardial infraction, skin graft rejection, rheumatoid arthritis, graft-versus-host disease, etc. MSCs are one of the best-studied stem cells yet the differentiation process and the underlying signaling pathways remain poorly understood. Although recent omic studies have provided some understanding of MSCs differentiation, these studies are limited, as they do not capture the dynamics of the process and are laborious and expensive. To overcome this, we have engineered a dual promoter lentiviral vector (LVDP) that comprises of a transcriptional regulatory element that encodes for a green fluorescence protein enabling gene regulation monitoring, and a constitutive promoter encoding a red fluorescence protein that is used as an internal standard to quantify the gene dynamics. A library of the lineage specific promoters and their transcriptional factor response motifs have been cloned and 20 different chemical inhibitors were screened to study various regulatory pathways. LVDP allows rapid, real time and high-throughput monitoring of gene expression and pathway activation during MSC differentiation. However, the time duration of MSC differentiation spans days to weeks. During this time, the replenishment of differentiation factors along with the cell culture media is required every 2-3 days. The concentration of differentiation inducers decreases and suddenly increases with the next media change. In addition, this process prevents the continuous monitoring of the cells. To address this problem, we are developing a robust microfluidic device, which has the potential to mimic microenvironments found in vivo. Currently, we are working on the technical details of the design. The device consists of two layers: a top layer with a concentration gradient generator and a bottom layer that has six channels with ten wells each to culture cells. This device will enable us to conduct high throughput screening and real time monitoring of stem cell differentiation.

Key words: Stem cells, Differentiation, Microfluidic device

3. VEGF Mediated Capture of Endothelial Cells under Flow in Vitro

Randall Jay Smith Jr.[1], **Maxwell Koobatian**[2], **Daniel Swartz**[2], **Stelios Andreadis**[1]

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Advances in tissue engineering have allowed for the development of tissue engineered vessels, TEVs, which require autologous cell sources to provide a functional endothelial lumen. Recently, our lab has shown that TEVs comprised of pig small intestinal sub-mucosa, SIS, pre-seeded with endothelial cells have maintained patency when implanted into an ovine model. However, this requires an autologous endothelial cell source with extensive expansion of the cells to maintain patency and prevent thrombosis upon implantation. Autologous sources of endothelial cells are often difficult to obtain, as procedures often include additional surgery and extensive time to expand the limited cells acquired to the amount needed for even a small graft. Even after isolation, most cells have a limited ability for expansion.

Therefore, we propose to avoid the use of cells prior to implantation, and instead engineer a TEV which promotes capture of circulating endothelial cells while maintaining patency and preventing thrombosis. Using an immobilized growth factor, vascular endothelial growth factor, VEGF, we can selectively capture circulating endothelial cells upon the lumen wall. VEGF, a well-studied angiogenic growth factor, promotes proliferation and spreading of endothelial cells. Therefore, upon capture, cell growth and spreading is promoted. In order to test this concept in vitro, we have designed a microfluidic device with a functionalized surface in order to selectively capture endothelial cells under laminar flow. The device surface is functionalized with poly-L-lysine, PLL, a strongly positive molecule, which binds with high affinity to heparin. In addition, VEGF-165, the specific isoform we have produced, contains a highly specific heparin binding domain. Thus, our surface utilizes naturally occurring binding between PLL, heparin, and VEGF.

Using a microfluidic channel over a VEGF functionalized surface, we have selectively captured endothelial cells with high affinity under a range of shear rates. This study uses human umbilical vascular endothelial cells, HUVECs, as well as ovine pulmonary artery endothelial cells, OPAECs as our selected endothelial cells, and fibroblasts and hair follicle mesenchymal stem cells as non-endothelial cells. In both single cell type and mixed cell type scenarios, only endothelial cells were captured by the VEGF. Surfaces with only PLL and heparin did not capture any cell type. The mechanism in which endothelial cells are captured was tested by inhibiting VEGFR2, the main receptor for VEGF. Upon inhibition, no endothelial cells were captured, indicating that receptor-ligand binding of VEGF and VEGFR2 allows for the selective capture of cells expressing VEGF receptors on their surface.

This work has led the way for developing and testing an SIS-TEV immobilized with heparin and VEGF in an ovine model. Early results indicate that within a month to three months of implantation the acellular graft has a complete endothelial monolayer within the lumen, and remains patent throughout the process. These results are promising for the development of a completely off the shelf vessel for clinical use that no longer requires an autologous cell source.

Key words: VEGF, capture, TEV, endothelial cells, In Vitro, Microfluidic device

4. Time-course of Healing and Maturation of Implantable Vascular Grafts in an Ovine Model using Small Intestinal Sub-mucosa: Effect of Initial Smooth Muscle Seeding

Sindhu Row, **Carmon Koenigsknecht**, **Evan Schlaich**, **Haofan Peng**, **Daniel Swartz**, **Stelios Andreadis**

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Introduction: The purpose of this study is to engineer vascular grafts which have the ability to grow, remodel and function normally within the host. Tissue engineered arterial substitutes with cell-seeded biomaterial scaffolds have been proven to perform better at the blood-material-interface as compared to synthetic alternatives. In this study, we aim to study the time-dependent process of host cell migration, maturation and remodeling of graft tissue with fully functional and implantable arterial grafts made from hair follicle derived smooth muscle cells (HF-SMC), ovine pulmonary artery endothelial cells on a scaffold of Small Intestinal Submucosa (SIS). Early events of immune responses and fibrotic tissue formation around the implanted vessels as well as cell seeding effect on long-term patency and functionality of SIS based vessels are reported.

Materials and Methods: SIS sheets were rolled into tubular constructs with fibrin glue using a previously optimized technique (Peng 2011, Cell, Tissue and Organ). Using a customized bioreactor, arterial shear stress was achieved within our constructs using optimized parameters sustaining patency of our grafts with the use of an arterio-venous shunt bypass model that we developed in our laboratory. SIS grafts, with or without initial seeding of HF-SMC were evaluated when implanted as an inter-positional carotid graft in an ovine model at 1, 4 and 12 weeks.

Results and Discussion: The implanted tissues were monitored biweekly using Doppler ultrasound to document patency and measure blood flow rates through the grafts. At 1, 4 and 12 weeks we also performed angiography, which confirmed uniform bilateral blood flow rates through the carotids. We observed early cellularization of graft by host (before the 1 month time point starting at 1 week), followed by differentiation and maturation of permeated host cells, which remodel the TEVs in-vivo. Our data directly correlate with functionality and mechanical properties of explanted grafts. Over time in the host, SIS grafts developed architecture similar to native artery and vascular functionality due to infiltration of host cells.

Conclusions: The patency of the grafts and their capacity for remodeling and development of vascular function suggest that this approach is very promising and may be of clinical significance. Our grafts exhibited structural and functional properties similar to native artery and provided appropriate chemo-attractant signals for maturation in-vivo in a time-dependent manner. Biomaterial vascular grafts thus made provide an implantable arterial replacement for vascular regeneration, with potential for off-the-shelf design.

Key words: Animal model, Vascular Bypass, SIS, graft

5. Directing smooth muscle cell differentiation and functionality by engineering cell-cell adhesion pathways

Stella Alimperti[1], **Sindhu Row**[1], **Teresa George**[2], **Sandeep K. Agarwal**[2], **Stelios Andreadis**[1]

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The differentiation fate of a stem cell is controlled by its microenvironment. Although the role of soluble factors (e.g. TGF-1) in mesenchymal stem cell (MSC) differentiation towards smooth muscle (SMC) lineage has been studied, the role of inter-cellular adhesion in this process remains elusive. Our previous studies have shown that cell-cell contact is necessary to induce MSC differentiation into vascular fate, particularly through the cell surface molecule, OB-cadherin. To further investigate the role of OB-cadherin in MSC proliferation and differentiation potential, we employed a fusion protein of OB-cadherin with the Fc domain (OB-Fc), which allowed us to study cadherin-cadherin interactions in an isolated manner. In particular, OB-Fc is a chimeric homo-dimer consisting of CH2 and CH3 (Constant Heavy-chain) region of FC and is linked through the region of the antibody to all five extracellular domains of OB cadherin. Initially, IgG-1 which preferentially bound to FC fragment was coated on a hydrophobic plate and afterwards, BM-MSCs plated on OB-FC surface. To this end, these cells exhibited increased proliferation (5-fold) and suppressed expression of senescence-associated proteins such as p21 over several passages. At the same time, culture on OB-Fc surface enhanced the BM-MSCs differentiation potential towards the SMC lineage as evidenced by the levels of key myogenic markers such as SMA and CNN1. In particular, engagement of OB-cadherin increased SMA expression through ROCK pathway and its downstream effectors such as SRF. Knocking down OB-cadherin using shRNA reversed these effects. Similarly, blocking ROCK by chemical inhibition or shRNA suppressed the effects of OB-Fc, implicating ROCK in OB-cadherin signaling cascade. Finally, tissue constructs prepared from cells that were propagated on OB-Fc exhibited significantly increased vascular contractility and improved mechanical properties. Notably, these results were confirmed in vivo using OB-cadherin knockout mice (OB-/-). In particular, smooth muscle containing organs from OB-/- animals e.g. bladder exhibited diminished vascular reactivity, decreased collagen content and significantly decreased mechanical properties. To this end, our results demonstrate that engineering OB-FC containing substrates that mimic the in vivo microenvironment promotes MSC proliferation and differentiation potential into SMC, thereby suggesting that this may be a novel and potentially clinically applicable strategy for directing stem cell fate.

Key words: Mesenchymal Stem Cell, OBcadherin, Differentiation, Adherens Junctions

6. Non-viral and High-efficiency DNA Delivery for Transient Nanog Overexpression in Mesenchymal Stem Cells

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Nanog is divergent homeodomain transcription factor that contributes to pluripotency and self-renewal of embryonic stem cells (ESCs). Previously, our group demonstrated that overexpression of Nanog can reverse the effects of organismal aging on mesenchymal stem cells (MSCs) proliferation and myogenic differentiation. Here we tested the hypothesis that transient overexpression of Nanog in MSCs by DNA transfection may overcome the effects of cellular senescence. However, it has been proven difficult to deliver genes into MSCs. We could have efficient gene delivery to achieve our goal by using DNA magnetofection. Human hair follicle derived MSCs (HF-MSCs) were used in this study and HF-MSCs were first optimized with EGFP-expressing plasmid. The plasmids formed complexes with polyMAG nanoparticles and pulled down by magnetic field to the cells seeded in 24-well plates. Repeated addition of optimal magnetofection complex for tree time enhanced the transfection efficiency to 48.55%± 1.79 (total green fluoresce intensity; GFI increased by 3.09± 0.03 fold). This optimized protocol is applied to delivery Nanog-expressing plasmid to evaluate the effects of gene transfer on cellular senescence. Overexpression of Nanog in HF-MSCs cells leads to increased proliferation rate (1.15 day shorter than that of control cells) and enhanced myogenic diffetentiation potential. Overall, our results suggest that DNA magnetofection to overcome the difficulty of DNA delivery to MSCs and this strategy could be employed to reverse the effects of organismal aging or culture senescence on MSC without viral transduction and permanent genome modification.

Key words: Gene Delivery, Nanog, Magnetofection, Human hair follicle derived MSCs (HF-MSCs)

7. Role of hydrodynamic shear mediated platelet deformation on cell tethering

Changjie Zhang, **Sriram Neelamegham**

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Role of hydrodynamic shear mediated platelet deformation on cell tethering Changjie Zhang, Sriram Neelamegham Department of Chemical and Biological Engineering, University at Bualo, SUNY Email: czhang27@bualo.edu, neel@bualo.edu Platelets play an essential role in the hemostatic process by adhering to extra-cellular matrix (ECM) that is located on denuded blood vessel walls. Arterial blood flow enhances glycoprotein Ib binding to VWF, which initiates platelet adhesion to injured vessels. We found that platelets translocate on immobilized VWF exhibits a biphasic phenotype (Under optimal shear stress the translocation velocity decreased with increasing shear stress; beyond the optimal shear stress further increased shear stress accelerate platelets translocate on immobilized VWF). A possible mechanism for flow to reduce platelet rolling velocity on VWF is increased number of GPIb/VWF bonds. Higher flows cause larger compressive force, which may enlarge the contact area with the wall, enabling more bond formation. The alignment of translocation velocity curves when plotted against wall shear stress rather than shear rate support the enlargement of contact area by compressive area. To test this hypothesis further, we made GPIb beads to prevent global deformation and extrusion of membrane tether. Compared with platelets, GPIb beads rolled faster, especially exhibiting a monophasic translocation phenotype. Therefore platelet deformation might be the dominant mechanism underlying flow enhance translocation. The bond force tethering a translocating platelet to the surface has 2 components that are proportional to each other. The component along the flow direction balances the hydrodynamic force, whereas the component perpendicular to the flow direction compresses the platelet against the well. Under the same flow condition, the greater the GPIb/VWF bond strength the greater platelet deformation caused by the greater perpendicular component. We used Pro-VWF and DD3-VWF to introduce different strength of GPIb/VWF bond (DD3-VWF Pro-VWF), the result revealed that the greater GPIb/VWF bond strength the smaller shear stress required for the biphasic transition. Again this result supports the hypothesis that platelet deformation is an essential role in regulating flow enhance translocation.

8. Glycosphingolipids Stabilize E-selectin Mediated Slow Rolling of Human Leukocytes on Endothelial Cells

Nandini Mondal, **Alexander Buffone Jr.**, **Sriram Neelamegham**

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E-, P- and L-selectin constitute a family of C-type lectins that mediate the first step of a cell adhesion cascade that eventually recruits leukocytes to sites of inflammation. Among the leukocyte cell-surface glycoconjugates, P-selectin glycoprotein ligand-1 (PSGL-1, CD162) represents the most prominent ligand for L- and P-selectin in both human and mouse leukocytes. The precise ligands for E-selectin remain unidentified on human granulocytes, and additionally the glycosyltransferases mediating E-selectin ligand biosynthesis on human leukocytes may be distinct from that in mice [1]. To better characterize the human E-selectin ligands, we developed HL-60 cells stably transduced with shRNA against PSGL-1, CD44 and UDP-Glucose Ceramide Glucosyltransferase (UGCG). The effect of gene silencing on the individual steps of the inflammation cell adhesion cascade was assessed. The results show that CD44 is not a significant human E-selectin ligand on HL-60 cells. Knocking down either PSGL-1 alone (PSGL-1$^-$ HL-60), or both PSGL-1 together with CD44 (PSGL-1$^-$CD44$^-$HL-60) resulted in reduced cell-surface HECA-452 epitope expression but no alteration in cell rolling on E-selectin bearing cells. Cells having 90-93% reduction in UGCG activity (UGCG$^-$HL-60) displayed dramatically reduced HECA-452 expression, and a moderate decrease in cell-surface CD15s and VIM-2 epitopes. Compared to wild-type HL-60, UGCG $^-$HL-60 displayed a 50% decrease in cell rolling density and 2-fold increase in cell rolling velocity on IL1-β stimulated HUVEC (Human umbilical vein endothelial cell) monolayers. UGCG $^-$HL-60 rolling was unstable compared to wild-type HL-60 since 60% of the tethering cells detached from HUVEC monolayers in a 1-minute interval compared to 20% for wild-type HL-60. The skipping nature of UGCG$^-$HL-60 cell rolling, with instantaneous velocities as high as 300m/sec, was also evident upon following individual cell trajectories. Treatment of wild-type and UGCG$^-$HL-60 cells with pronase resulted in reduced cell adhesion and increased cell detachment for both cell types, with the effects being more pronounced for the UGCG$^-$HL-60s. These data suggest a crucial role for human glycosphingolipids in stabilizing E-selectin mediated cell capture and slow rolling on endothelial cells.

References:

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Key words: cell adhesion, leukocyte, sialyl Lewis-X, fluid shear, selectin, glycolipid, carbohydrate,

9. The reverse sialylation properties of the human (2,3)sialyltransferase ST3Gal-I mediates sialoglycan biosynthesis

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The capping of mammalian cell-surface glycoconjugates by α(2,3)-linked sialic acid often regulates receptor-ligand recognition. Five α2,3 sialyltransferases, named ST3Gal-I, -II, -III, -IV and VI, catalyze this terminal modification on human glycoproteins. We systematically characterized the substrate specificity and activities of these enzymes by expressing them in soluble form using Chinese Hamster Ovary (CHO) cells. Both radioactivity based assays and liquid chromatography-mass spectrometry (LC-MS) analysis was carried out using natural acceptors, and also synthetic analogs that carry either sialylated or unsialylated Galβ1,3GlcNAc (Type-I), Galβ1,4GlcNAc (Type-II) or Galβ1,3GalNAc (Type-III) terminal structures. Such studies showed that sialylated Type-I glycans (Neu5Acα2,3Galβ1,3GlcNAc) are synthesized by ST3Gal -III, -IV, and -VI. ST3Gal IV and VI, and to a lesser extent ST3Gal-III, formed sialylated Type-II glycans (Neu5Acα2,3Galβ1,4GlcNAc). ST3Gal -I, -II and also ST3Gal-IV synthesized sialylated Type-III glycans (Neu5Acα2,3Galβ1,3GalNAc). In addition to the conventional forward sialylation reaction above, ST3Gal-I and to a lesser extent ST3Gal-II catalyzed the reverse synthesis of CMP-Neu5Ac from 5'-CMP in the presence of sialic acid donors containing the Neu5Acα2,3Galβ1,3GalNAc unit. This reaction is called reverse sialylation. ST3Gal-I also readily catalyzed the exchange/swapping of sialic acid residues between sialylated Type-III glycoconjugates and different forms of activated sialic acid, including the glycolyl form i.e. CMP-Neu5Gc. This reaction is called exchange sialylation. Finally, the analysis of soluble glycosyltransferases in human blood plasma reveals the presence of both reverse and exchange sialylation activity. Overall, in addition to the unidirectional transfer of sialic acid from CMP-Neu5Ac to various glycoprotein acceptors in the Golgi, the reversible enzyme activity of ST3Gal-I may also regulate the pattern of α(2,3)-linked sialoglycans in blood.

10. Calcium Regulates Intracellular and Extracellular Cleavage of VWF by ADAMTS13

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Introduction: VWF (von Willebrand factor) is a multimeric blood plasma protein which engages in extensive biomechanical interactions with blood platelets, endothelial cells and the sub-endothelium. By mediating platelet adhesion, this molecule is a key player during human thrombotic ailments and hemostasis processes. The multimer distribution of VWF is regulated by the metalloprotease ADAMTS13 (A Disintegrin and Metalloprotease with Thrombospondin type 1 motif 13) which cleaves VWF at its A2 domain. VWF and ADAMTS13 are both produced by endothelial cells. In this study, we investigated whether ADAMTS13 cleaves VWF intracellularly in HUVECs (human umbilical vein endotheial cells), and if this cleavage is regulated by calcium. This investigation provides basic science results on the nature of the interaction between VWF and ADAMTS13, the size regulation of VWF in circulation, and its relevance to the understanding and treatment of TTP (Thrombotic thrombocytopenic purpura).

Materials and Methods: Western blotting with HUVEC lysates and immnostaining with whole cells were employed in order to establish the occurrence of intracellular VWF cleavage by ADAMTS13. Western blotting was also employed to visualize VWF cleavage in HUVECs and HEK293T cells transfected to express either normal human multimeric VWF (VWF-Normal) or VWF-Lock, where the flexibility of the A2 domain is constrained by a cys disulfide linkage between the N- and C-terminus. The role of calcium in regulating VWF-A2 conformation and cleavage was analyzed using mammalian FRET-based constructs where Cerulean and Venus flank the VWF-A2 domain in either the isolated A2 domain (L-VWF FRET) or the multimeric protein (mVWF FRET).

Results and Discussion: In support of the concept that intracellular VWF cleavage occurs within endothelial cells even prior to protein secretion: i. 5 percent of intracellular cleavage of VWF by ADAMTS13 was detected in the HUVEC lysates with western blots. ii. Immunostaining of HUVECs showed partial colocalization of VWF with ADAMTS13. iii. Supernatants from HUVECs transfected with both VWF-Normal and VWF-Lock show presence of a cleavage band resulting from intracellular cleavage, while those from HEK293T cells do not. This intracellular cleavage may be calcium dependent since the structure of the VWF-A2 domain could be dynamically and reversibly tuned by changing media calcium concentrations. Here, variation of calcium in the intracellular range (0.1-0.4 mM) resulted in predominantly an open VWF-A2 domain that was readily cleaved by ADAMTS13. Higher calcium concentrations (1-1.5mM) that are reminiscent of the extracellular milieu resulted in VWF-A2 primarily being in the closed conformation where the protein was resistant to VWF-A2 cleavage.

Conclusions: The data support a model where calcium levels in the intracellular and extracellular environments of endothelial cells regulate the cleavage of VWF by ADAMTS13. While low calcium levels are sufficient to allow VWF cleavage in the intracellular milieu, high shear is needed in the extracellular environment for VWF cleavage since the A2 domain is protected by the higher extracellular calcium levels.

Key words: VWF, ADAMTS13, HUVEC, calcium, intracellular

11. Thio-bearing GalNAc analog abrogated O-glycan elongation and hence down-regulated cell rolling on P- and L-selectins

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Leukocytes help combat foreign organisms and infections by processes known as innate and adaptive immunity. In inflammation, the reddening and swelling of tissue which is associate with normal immunity, also affects normal human tissue. Injury associated with such abnormal high levels of inflammation in specific organs can contribute to diseases like multiple sclerosis, rheumatoid arthritis, asthma etc. Leukocyte binding and migration into affected tissue is a key step of the inflammatory cascade. The affected tissues send signals to endothelium and activate the cytokines and selectins. Then the leukocytes start tethering and rolling on the vessel wall before firm adhesion on and transmigration through the blood vessel to the inflammatory area. Thus, the binding of selectins to carbohydrate receptors is a key initial step that controls the entire cell adhesion cascade. Being able to regulate the selectin binding interaction would help reducing abnormal leukocytes trafficking that causes diseases.

A thio-bearing GalNAc analog, GalNTGc, has shown to have the capability to down-regulate the human promyelocytic leukemia cell trafficking. Surface-bound carbohydrate antibodies labeling on GalNTGc treated cells showed significant reductions on HECA and CSLEX1 which correspond to the major P- and L-selectin ligands. The systematically increased GalNTGc concentrations also altered the surface-bound carbohydrates accordingly. Furthermore, the microfluidic flow chamber assay which demonstrates the cell tethering and rolling under shears revealed reductions on number of cells interacting with P- and L-selectins. However, there was no significant effect on E-selectin rolling under shear. To study the mechanism of GalNTGc, the incorporation of GalNTGc was tested and confirmed with maleimide. Dramatic up-regulation on VVA lectins and reduction of PSGL-1 molecule weights after treatment with GalNTGc both suggested the possibility that GalNTGc may be a potential O-glycan inhibitor. Characterizing GalNTGc as a potential inhibitor for O-glycan extension and cell adhesion helps the development for metabolic oligosaccharide drugs in clinics toward multiple cell-adhesion based diseases.

Key words: GalNTGc, selectin, cell adhesion

12. High Throughput Engineering of Temperature Sensitive Intein and Split Intein with Improved *Trans*-Splicing Kinetics

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Inteins are protein elements that auto-catalytically excise from a precursor protein with subsequent linkage of the two flanking regions to reconstitute protein function. Inteins can also *trans*-splice, that is, two separate intein fragments associate to form the full length and active splicing intein. Alternatively, self-cleaving, rather than splicing, inteins have been engineered for affinity chromatography. Since inteins exert their effects post-translationally and can splice in many different contexts, they serve as valuable tools for a variety of applications. Two of these include the conditional control of protein function and the assembly of multiple protein domains. We have developed a reporter assay based on the subcellular localization of the reverse tetracycline-controlled transcriptional activator (rtTA) for the identification of temperature-sensitive (ts) self-cleaving inteins, as well as split inteins with improved *trans*-splicing rate. Through the use of nuclear localization signal (NLS) and nuclear export signal (NES), rtTA translocation into the nucleus and subsequent reporter expression in yeast is correlated with intein activity that is detected using flow cytometry. Because flow cytometry is inherently high-throughput, our assay can screen large libraries of mutants in contrast to other techniques such as replica plating. The identification of split inteins with faster splicing will facilitate the implementation of novel split intein mediated modular synthesis (SIMMS).

Key words: Temperature sensitive mutant; Intein; Protein splicing; Nuclear localization

13. Engineering and Applications of Soluble Monomeric Streptavidin

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Streptavidin is a homotetrameric protein that binds biotin with extraordinarily high affinity. Since streptavidin is both thermally and chemically stable, the streptavidin/biotin complex has been used for a variety of applications including labeling, purification, immobilization, and detection. However, as a tetramer, streptavidin can cause target aggregation during certain labeling applications, and recombinant fusions may be difficult to produce. To this end, our group recently reported engineered monomeric streptavidin (mSA2) with a biotin dissociation half-life ($t_{1/2}$) of 83 minutes. While the dissociation rate (k_{off}) is the lowest reported for monomeric streptavidin, it is still many orders of magnitude higher compared to wild type. Mutants with additional improvements in biotin dissociation would be more useful across biotechnology applications. Here we report the identification and characterization of mSA mutants based on improved biotin dissociation selected from a randomized mutant library displayed on the surface of yeast. While monomeric streptavidin can be expressed functionally on the yeast surface, it accumulates as inclusion bodies when expressed in bacteria, thus requiring exhaustive and time consuming *in-vitro* refolding. Even then, mSA yields remain low (3-5 mg/l culture). To address this, we have tested the expression of mSA2 as a genetic fusion to the well-known solubility enhancers GST, MBP, SUMO, and thioredoxin (trx). All fusion proteins can be expressed in soluble form in E. coli grown at 20 °C. In particular, MBP and trx fusions yielded 200 and 100 mg of fusion protein per liter of culture media, respectively, which corresponds to ~50 mg of mSA2 per liter of culture for both fusions. mSA2 can be isolated to high purity from the fusion tag through proteolytic cleavage followed by ion exchange chromatography. mSA2 purified from the soluble fraction exhibits identical thermal stability and biotin binding characteristics compared to mSA2 purified from inclusion body. Monomeric streptavidin is an ideal candidate for purification of biotinylated ligands as its lowered affinity for biotin compared to wild type would allow elution under mild conditions. To this end, coupling MBP-mSA2 to agarose resin led to capture of biotinylated ligands. Facile elution of the captured protein was accomplished with addition of free biotin and incubation at 37 °C.

Key words: Monomeric streptavidin; Solubility tag; Escherichia coli; Affinity chromatography; Biotinylation

14. Molecular mechanism of Crohn's disease associated protein, NOD2

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NOD2, nucleotide-binding oligomerization domain -like receptor protein, is a cytosolic bacterial sensor and coordinates the events following pathogen invasion which triggers NF-kB dependent proinflammatory response. NOD2 activation is important in innate immunity and dysregulation leads to inflammatory diseases, such as Crohn's disease (CD). Loss of function mutations in NOD2 linked to CD includes two missense mutation, R702W and G908R, and a frameshift mutation L1007fsinsC (L1007fs). The molecular mechanisms that connect these mutations to CD are not well understood. We modeled the activation mechanism of NOD2 using other related proteins that have been studied using biochemical and structural methods. Homology modeling suggests that the CD mutations may interfere with the activation process by causing abnormal intramolecular interaction. We tested this hypothesis by designing a helical peptide (α-6) that activates CD associated NOD2 variants by mimicking the region of NOD2-LRR domain. We introduced these peptides in HEK293T that express wt and mutant NOD2. Co-expression of α-6 increases NF-kB activity upon MDP stimulation by 2-3 folds. We intend to optimize the sequence and length of α-6 peptides to customize their activity towards NOD2 variants. Further to characterize the mode of interaction between Nod2 and the α-6 peptides, we will perform binding assays. We will also test synthetic α-6 peptide fused to cell penetrating peptide. Finally, we will test the *in vivo* activity of the engineered peptides in mononuclear cells isolated from peripheral blood of CD patients by measuring secreted cytokines. These peptides may reconstitute the loss-of-function NOD2 variants to wild type phenotype and could serve as a platform for other mechanism-based drugs to treat CD.

Key words: NOD2, activation mechanism, engineered peptide, conformational change, intramolecular interaction

15. Synthesis and Characterization of Mannose-capped Poly(beta amino esters) for Gene Delivery

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Advancements in gene therapy have promoted the development of new synthetic delivery agents to address failures of current viral-based vectors such as cytotoxicity, immunogenicity, and tumorigenicity. Polymeric vectors are generally considered as an excellent alternative for nonviral gene delivery due to the ability to finely tune/engineer bio stability, efficiency of cellular uptake, intracellular trafficking, and final gene expression. However, despite robust engineering strategies, polymeric vectors still require research to efficiently target and stimulate immune-specific cells. Thus, synthesis of a new library of mannose modified poly (beta amino esters) (Man-PBAE) will facilitate macrophage targeting through interactions with CD206. Synthesis is conducted by reacting allyl mannopyranoside with amine capped PBAE. The reaction steps include synthesis of: 1) allyl mannopyranoside, 2) amine capped PBAE, and 3) mannose-modified PBAE. The approach permits the analysis of a variety of structurally unique polymers for their gene transfection efficiency. Upon synthesis, newly generated polymers will be characterized for their chemical structure and composition using H1NMR and FTIR respectively. The thermal stability of Man-PBAE can be compared with unmodified PBAE using Thermogravimetric analysis (TGA). The degree of substitution of mannose to PBAE can be calculated by elemental analysis. Additional characterizations will precede using established polymer chemistry methodologies such as gel permeation chromatography and digital light scattering (DLS), prior to high-throughput gene delivery assays.

Each polymer will be complexed with reporter gene plasmid DNA (pDNA) and incubated with plated mammalian cells. Success is determined on the basis of luciferase and/or green fluorescent protein (GFP) expression. Our system offers the opportunity to not only investigate polymer specific influences upon gene delivery, but also the cell-specific gene delivery properties that would have otherwise been overlooked. The best performing polymers will be pulled and analyzed for structure similarity. Positive results of this assay can be exploited to use in conjugation with established gene therapy methodologies or provide an avenue towards better designed gene delivery vectors.

Key words: Gene therapy, gene delivery, polymeric vectors, poly(beta amino esters), mannose

16. Synthesis of Hybrid Biomaterial-Bacterial Gene Delivery Vectors

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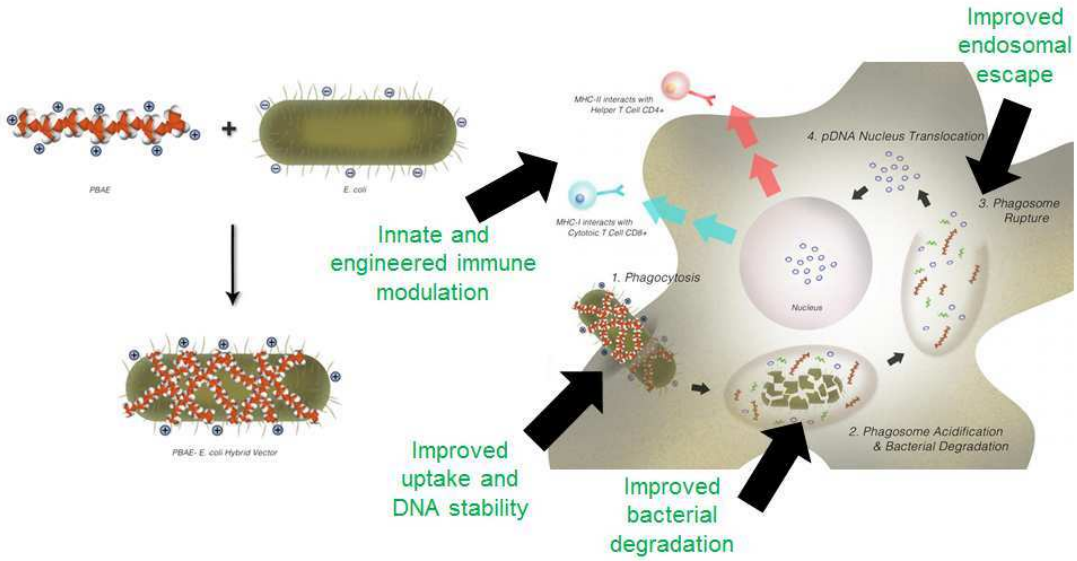
Since the advent of gene therapy in 1972, it has rapidly emerged as a viable option for both prophylactic and active treatments of diseases. Gene therapy is predicated on the usage of nucleic acids (DNA and RNA) to influence host cell expression patterns, resulting in alterations of cell phenotypes to contain more desirable traits. To elicit efficient responses, nucleic acids require use of carriers to overcome numerous in vitro and in vivo barriers, such as clearance and enzymatic degradation. Thus, a key end goal of most gene delivery research is to develop and characterize vectors that can achieve clinically-relevant levels of gene delivery. Currently the gold-standard of delivery vectors relies on the use of viral-based particles and/or strains. However, despite extensive development and numerous clinical trials, viral vectors have failed to adequately address concerns pertaining to potential insertional mutagenesis, cytotoxicity, immunogenicity, and tumorigenicity. These limitations of viral-based delivery vectors have given rise to the field of nonviral vectors that include the use of biocompatible materials (polymers, lipids, and inorganic materials) and biological agents. Of these, cationic polymers (CP) and *Escherichia coli* (*E. coli*) each represent potential alternatives. Each of these vectors possesses vector-specific properties that aid the gene delivery process. Specifically, CPs are easily synthesized and can be tailored to contain desired functionalities or biophysical properties (e.g. controlled release). Similarly, bacteria can naturally (or be engineered) to safely delivery an array of various biomolecules (nucleic acids, peptides, and small biomolecules). In this study, listeriolysin O (LLO) producing *Escherichia coli* BL21(DE3) strains were electrostatically complexed with cationic polymers to create a hybrid vector that synergistically combines positive elements of each individual vectors. Hybrid vectors were tested as delivery vector for the gene transfer to a murine RAW264.7 macrophage cell line using a 96-well high-throughput assay. The results suggest a complementation of each vector with increases in cellular uptake, internalization, and gene delivery. Overall, the approach presented provides a simple and effective way to prepare novel delivery vectors that offer the potential for a synergistic boost in final gene delivery beyond that provided by either biomaterial or biological vector in isolation.

Key words: Gene delivery, gene therapy, cationic polymers, bactofection



17. Natural Products: New Discoveries using Metagenomics, Structure Modification, and Production Optimization using Computational Methods

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Natural products have provided a range of potent antibiotics, anticancer agents, immunosuppressants and other therapeutic compounds. However, as ready access to new natural products diminished, priorities shifted away from natural product discovery and a rise in drug-resistant pathogens resulting in a lack of new antibiotic natural products. Screening-based discovery and structure variation of previous successful antibiotic compounds are the two strategies in this work to address the need for novel drugs. The fastidious nature of environmental microbes has spurred requests for technically convenient microbial host for the heterologous production of antimicrobial drugs. *Escherichia coli* (*E. coli*) strain BAP1 (*ara¡¿tetR, lacZ¡¿trfA-KanR, recA, codB¡¿pccAB*) was deliberately constructed with the purpose of supporting complex natural biosynthesis through metagenomics. Along with discovery of new natural products from environmental microbes, manipulating polyketides and tailoring biosynthetic pathways has been employed to expand erythromycin A diversity. The loading module of deoxyerythronolide B synthase (DEBS) was replaced by the loading domain of rifamycin synthase, which activates uptake of 3-amino-5-hydroxybenzoate (as well as benzoate and several benzoate derivatives) for polyketide synthesis. After screening six benzoate derivatives, 3-amino-benzoate and 3-hydroxy-benzoate 6-deoxyerythronolide B (6dEB) analogues productions were confirmed by High Performance Liquid Chromatograph-Mass Spectrometry (LC-MS). Meanwhile, substituting D-desosamine and L-mycarose on 6dEB with alternative 6-deoxyhexoses can achieve a myriad of erythromycin A analogues. Lastly, Flux Balance Analysis (FBA) and Minimization of Metabolic Adjustment (MoMA) was implemented in a *Aspergillus niger* (*A. niger*) stoichiometric computational model to predict genetic changes that will improve octatrienoic acid productivity and efficiency. We undertook FBA and MoMA to identify single gene-knockouts that resulted in increased octatrienoic acid production while maintaining cellular growth. The results demonstrated that several single gene-knockout mutants are computationally predicted to improve specific production up to 6.6-fold.

Key words: natural product, metagenomics, erythromycin, benzoate, 6-deoxyhexoses, FBA, MoMA, octatrienoic acid

18. *E. coli* Metabolic Engineering to Produce the Marine Anticancer Agent Lomaiviticin A

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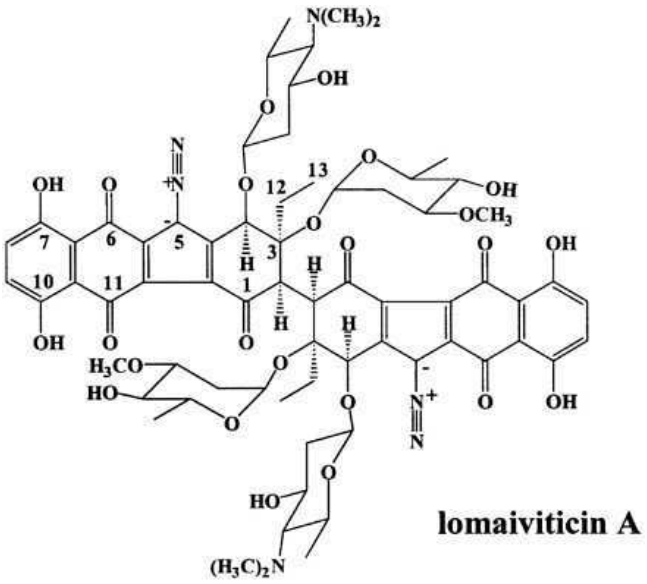
Polyketide represent a highly diverse family of natural products showing a wide range of biological activity including antibiotic, antitumor and antifungal activities. Polyketides are biosynthesized mainly through decarboxylative condensation of malonyl-CoA derived extender units and upon selection of different starting units in a similar process to fatty acid synthesis (a Claisen condensation). However, collaboration of a group of enzymes, known as polyketide synthase (PKS), cause more complicated synthase and post modifications compare to linear structure of fatty acids. However polyketides have been produced in extremely low concentration in their native hosts. Furthermore, structural complexity makes their organic synthesis come to failure or negligible production yield. Heterologous hosts provide an alternative to synthesize natural products. Among of heterologous hosts, because of easiness and availability of biological tools *E. coli* has found more attention.

Our research is devoted to heterologous production of a potent anticancer agent called lomaiviticin A. Lomaiviticin A is a highly novel marine natural product with potent antitumor properties through high ability to DNA cleavage in low concentration. The Lomaiviticin is produced by bacterial hosts in symbiotic co-existence with sea ascidians and it belongs to type II of polyketide synthase (PKS) family. It contains a dimeric polyketide structure with two diazo groups, both of which are critical for biological activity. In addition two sugar moiety are observed in each monomer, one amino sugar pyrrolosamine and a -oleandrose (figure 1).

Preliminary studies have shown soluble and active expression of enzyme teamwork is the main anticipating challenge. Using different chaperones and plasmid engineering techniques we could find some improvement for soluble expression of critical proteins. Our parallel approach on this system is dedicated to use native cosmid. Main challenge in this approach is transcription burden due to putative promoters in native host. We addressed this challenge by manipulation transcription machinery of *E. coli* through over-expression of transcription factors like alternative sigma factors. Having a constructed library of potential bacteria-plasmid systems we apply HPLC and LC/MS to looking for polyketide intermediates.

Key words: polyketide type II, heterologous host production, lomaiviticin A biosynthesis , *E. coli*



19. Polymyxin B Treatment Improves Bactofection Efficacy and Reduces Toxicity

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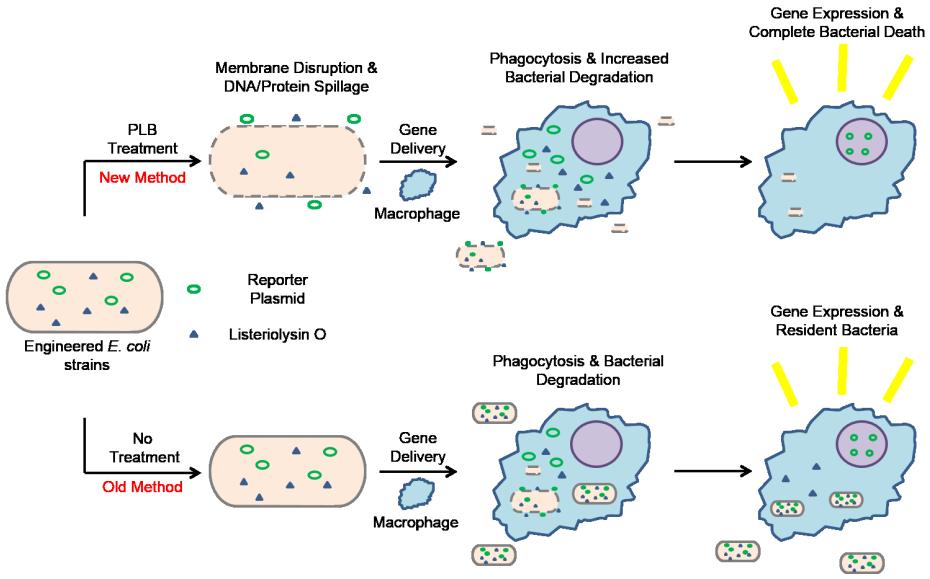
Bactofection is a technique wherein bacterial-maintained plasmid DNA is directly transferred and expressed in target mammalian cells. Despite extensive attempts to optimize the vector, lingering challenges related to cytotoxicity and immunogenicity has limited clinical relevance. To addresses these concerns, we investigated the use of a polymyxin B (PLB) pre-treatment upon listeriolysin O (LLO) producing Escherichia coli BL21 (DE3), and evaluated as a gene delivery vector to a murine RAW264.7 macrophage cell line using a 96-well high-throughput assay. Use of polymyxin B pre-treated delivery vectors resulted in statistically higher levels of gene delivery and reduced cytotoxicity, which can be attributed to PLBs ability to bind and inactivate endotoxin. This approach represents a facile way to elevate potency of bacterial-based gene delivery vectors while also limiting potential cytotoxic side-effects.

Key words: Escherichia coli, gene delivery, bactofection, PLB



20. Heterologous biosynthesis and modeling of terpenoids producing platform for botryococcene and Paclitaxel in *E.coli*

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Terpenoids represent a large and diverse group of natural compounds, and many of which have shown promising biological and therapeutic activity. However, desired molecules are found in trace amount and their native hosts complex metabolism contribute to the difficulty in massive harvesting of target compound. One way to address this issue is heterologous biosynthesis,which is, transferring the biosynthetic pathway in well- studied microbial systems such as *E.coli* . There are two biosynthetic upstream routes characterized leading to the common precursors IPP and DMAPP for terpenoids production, the classical acetate mevalonate (MVA) pathway and non-mevalonate pathway. Utilizing synthetic biology and metabolic engineering, efforts have been made in our group to reconstitute non-MVA upsteam pathway and partial downstream pathway to both anti-cancer drug Paclitaxel(taxol) and promising biofuel botryococcene by overexpressing dxs,idi,ispD,ispF and related terpenoid synthase.At the same time, identification of gene deletion and amplication by computational tools such as Flux balance analysis and Elementary Mode analysis based on minimal central metabolism of *E.coli* have also been studied. Further experimental challenges to quantify and redirect celluar flux to initiate production of desired compound involve gene expression,protein activity and analysis method development. Once product is produced, continuing efforts for enhanced production have to be made for in protein expression level balance, medium optimization,bioreactor scale-up and etc.

Key words: Taxadiene,Botryococcene,Heterologous biosynthesis,P450 Enzyme, *E.coli*,Biofuel

21. Signaling pathway modulation for directed cardiogenic differentiation of human pluripotent stem cells

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Introduction: Human embryonic and induced pluripotent stem cells (hPSCs) are an inexhaustible source of cardiomyocytes for treating myocardial infarction, a leading cause of morbidity and mortality. Recent advances in the development of patient-specific induced PSCs greatly improve the chances of clinical success. Yet, robust methods are lacking for generating cardiomyocytes across a variety of hPSC lines having varying lineage-specific differentiation propensities. Therapeutic realization also hinges on the transplantation of sufficient numbers of cells (~ 1.5x109 per damaged myocardium (1)) pointing to the need for large-scale bioprocess development. To resolve these issues, we have assessed particular signaling pathways with physiological implications in cardiac formation such as BMP, Wnt, Nodal/Activin, and FGF for efficient cardiac differentiation, and investigated methods for improving the yield of cardiomyocytes across a variety of hPSC lines. Materials and Methods: A serum-free, defined medium formulation is developed for having accurate account of the signaling mechanisms involved to be able to optimize the process and increase its reproducibility in a transplantable culture system. Our approach for developing a rapid cardiogenic differentiation method for hPSCs comprises three steps: (i) induction of mesoderm-oriented primitive streak, (ii) induction of cardiovascular cells, (iii) maturation of cardiomyocytes. Cells at each stage of differentiation were analyzed by quantitative reverse transcription-PCR (qRT-PCR), immunocytochemistry, western blotting analysis and flow cytometry. Functional characteristics of cardiomyocytes are determined by electrophysiology measurements of cardiac action potentials, and response of beating clusters to chronotropic drugs.

Results and Discussion: Human PSCs were efficiently directed to mesoderm-oriented primitive streak using BMPs and Wnts in 48 hours characterized by high expression levels of Brachyury (T), MIXL1, EOMES and MESP1 assessed by qPCR and immunofluorescence. Subsequently, manipulation of physiologically-relevant signaling pathways that interact and participate in mesoderm patterning such as Wnt, Nodal/activin, BMP and FGF resulted in different mesoderm derivatives. Therefore, a specific combination of these pathways is utilized to coax these cells along cardiovascular lineages expressing KDR, cKIT, Nkx2.5, GATA4 and MEF2C. Beating cardiomyocytes emerged within a period of 6 days, and cardiac markers such as β-MHC, TBX20, MLC2a and ANF were strongly upregulated. Expression was higher than that seen in cells treated with conventional protocols for cardiogenic differentiation. Upon further maturation, corresponding upregulation was observed by western blot analysis and immunofluorescence of cardiac proteins such as NKX2.5, GATA4, MEF2C, α-ACTININ, cardiac troponins and the cardiac gap junction protein CONNEXIN-43. Cells displayed cardiac action potentials and their contractile activity was modulated organotypically by incubation with diltiazem and phosphodiesterase inhibitors. The efficiency of cardiac differentiation was up to 70% as assessed by flow cytometry of cardiac markers, and a high number of cardiac cells per stem cell initially seeded was obtained.

Conclusions: An optimized protocol for cardiogenic differentiation of hPSCs was developed based on the rational manipulation of the core signaling pathways Wnt and BMP. Application of the method improved the efficiency and yield across different hPSC lines. Ongoing work concentrates on translating our findings to the generation of functional cardiomyocytes from hPSCs in scalable cultivation systems (2). Our findings will contribute to the development of stem cell-based therapies for heart diseases improving the quality of life of millions in the US.

Key words: Cardiomyocyte differentiation, bioprocessing human pluripotent stem cells

22. Evaluation of Nanog Reporter Systems in Pluripotent Stem Cells through Multiscale Modeling

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Nanog is a principal pluripotency regulator exhibiting a disperse distribution within stem cell populations in vivo and in vitro. Increasing evidence points to a functional role of Nanog heterogeneity on stem cell fate decisions. Besides the contributions of feedback loops and gene expression noise, a recent study [1] revealed that allelic control of Nanog in mouse embryonic stem cells could be a potential source of heterogeneity. We have previously shown that stochastic partitioning of cellular material during division could also contribute to Nanog heterogeneity [2]. It should be noted that studies on Nanog expression heterogeneity often employ knock-in reporters to reflect real-time Nanog protein amount [1,3,4]. The sources of heterogeneity can potentially affect the expected correlation between Nanog reporter and endogenous Nanog protein [5]. Here, we developed a multi-scale model to describe Nanog expression heterogeneity [6]. The model incorporates gene expression noise, stochastic division and allelic switching of Nanog expression. The results indicate that allelic regulation can give rise to three distinct Nanog states. With potential gene expression noise accounted for, a bimodal distribution is typically observed. The model simulations also reveal a different distribution of Nanog reporter compared to the native profile for Nanog. This led us to investigate the performance of different reporter systems in the scenario of allelic regulation. Finally, our findings show that deletion of one Nanog allele during reporter construction does not simply reduce Nanog uniformly for all ESCs but modulates Nanog heterogeneity directly. This work illustrates the significance of multi-scale models in furthering our understanding of stem cell physiology and providing tools for rational design of high-efficiency differentiation methods and relevant bioprocesses.

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23. Multiscale population balance equation model for heterogeneous human pluripotent stem cell populations: Determination of single-cell physiological state functions

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Cell population balance equations (cell-PBE) describe cell population dynamics by taking into account single-cell heterogeneity. The population balance equation comprises of physiological state functions representing variations in single cell behavior based on the cell intrinsic state and the differential response to environmental conditions at various cell states. Our goal in this study is to develop physiological state functions unique to self-renewing embryonic stem cells (ESCs) for single-cell growth and division rates and a probability density function for cellular material partitioning into new-born cells from the dividing cells. This requires cell distributions with respect to levels of intracellular components that represent intrinsic state of the cell, the newborn cell population, the overall cell population and the dividing cell population. The approach was tested by generating physiological state functions from analytical expressions. The closed-form approximations of the physiological state functions were derived by solving the inverse problem. There is an excellent agreement between the original analytical expressions and results from inverse problem.

24. The Role of Micro-RNAs in the Differentiation of Pluripotent Stem Cells into Cardiomyocytes

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Micro-RNAs are a group of small RNAs of around 22 nucleotides in length that regulate cell metabolism and differentiation(1, 2). There are many methods of cardiac differentiation using external elements such as growth factors available. Our group has developed cardiomyocytes from various cell lines using growth factors such as BMP4 and Wnt. Little is still known about the role of microRNAs in many processes including differentiation, although it is known that many microRNAs are lineage specific (3). As the cardiac lineage is of interest to our group, we would like to determine the microRNAs (miR) that play important roles toward cardiac differentiation of stem cells. This could potentially mean that we could induce differentiation by up-regulating a particular miR. A profiling of miRs during differentiation has been done in the past, and miR 124, miR-126, miR499-5p, miR-141, miR-214, miR 27a, miR-27b, miR-99b along with miR-1 and miR-133a have shown interesting trends. A further investigation of these miRs along with any other potential candidates needs to be carried out. In addition, the effect of the miRs on differentiation can be determined by inhibiting them.

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Key words: microRNA, cardiac, pluripotent stem cells

25. Xeno-Free Expansion and Differentiation of Human Pluripotent Stem Cell in Stirred Suspension Microcarrier Cultures

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A major challenge for the translation of human pluripotent stem cells (hPSCs) to clinical applications including tissue engineering and cell-based therapies is the development of scalable bioprocesses affording the generation of medically relevant quantities of cellular material free of xenogeneic factors. We employed a xeno-free stirred-suspension microcarrier bioreactor for the expansion of undifferentiated hPSCs and their directed commitment to particular fates. A vitronectin-derived peptide was conjugated on the surface of microcarriers. Initially different surface peptide densities were screened in static microcarrier cultures. Subsequently, cells were expanded for five successive passages in stirred suspension. The cells maintained a doubling time similar to that in regular dish cultures, with a 25-fold increase in cell concentration per passage, and exhibited higher than 85% viability. Moreover, the expression levels of pluripotency markers such as Nanog, OCT4 and SSEA4 were preserved as assessed by quantitative PCR, immunochemistry and flow cytometry. Cells cultured on the xeno-free microcarriers exhibited a normal karyotype. After multiple passages in the bioreactor, the cells were subjected to tri-lineage differentiation in static cultures. The resulting cells expressed markers of definitive endoderm (SOX17, FOXA2), mesoderm (MEOX1, KDR) and ectoderm (NES, TUBB3). Lastly, hPSCs propagated on peptide-conjugated beads were directed to mesoderm fate in stirred suspension. The fraction of differentiated progeny expressing KDR was higher than that of cells in dish cultures. These results suggest that the peptide-conjugated microcarrier culture system developed in this study is suitable not only for expansion of self-renewing hPSCs but also for their guided commitment to specific phenotypes. We expect that this culture modality will contribute to the design and development of stirred-suspension microcarrier bioreactors for the scalable propagation and differentiation of xeno-free hPSCs and their progeny intended for clinical therapies.

Key words: human pluripotent stem cells, microcarrier, xeno-free

Molecular and Multiscale Modeling

26. Interfacial Properties of CO_2-Water Systems by Transition Matrix Monte Carlo Simulations

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Carbon dioxide capture and storage via injection into saline aquifers is a promising option for reducing greenhouse gas emissions. In order to prevent leakage of sequestered gas through pores in the surrounding rock, the reservoir pressure must not exceed the breakthrough pressure, which depends on the interfacial properties of the rock-water-CO_2 system. Differences in experimental conditions and measurement techniques have precluded a consensus on the wetting behavior of surfaces in these systems, especially when supercritical CO_2 is present. The transition matrix Monte Carlo methods developed by our group can provide measurements of contact angles and interfacial tension. This poster presents our recent work in determining the bulk coexistence properties of water-CO_2 systems using a combination of grand canonical and activity fraction expanded ensemble calculations. We also outline the free energy-based methods that will be used to measure contact angles and interfacial tension.

Key words: carbon capture and storage, wetting, Monte Carlo simulations

27. Wetting and drying properties of systems using Isothermal Isobaric Monte Carlo Simulations

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We discuss a molecular simulation technique to compute the interfacial properties of a fluid in contact with a surface. The wetting and drying properties like contact angle and interfacial tension can be calculated using Isothermal Isobaric ensemble (NPT) Monte Carlo simulation. We have developed this approach to overcome the difficulties faced by Grand Canonical Monte Carlo (GCMC) simulation at low temperatures. We use free energy approach in which interface potential is associated with the surface excess free energy of the fluid film in contact with the surface. We employ spreading and drying approach to calculate the spreading coefficient and drying coefficient respectively. The contact angle and interfacial tension is calculated by combination of spreading and drying coefficient. Expanded Ensemble techniques are used to evaluate the contact angle over a range of temperatures and substrate strengths. This poster focuses on model system consisting of interactions described by Lennard-Jones Potential. We are interested in extending this approach to determine the interfacial properties of complex systems such as ionic liquids at low temperatures.

Key words: Interfacial properties, Isothermal Isobaric ensemble, Grand Canonical Monte Carlo, Expanded Ensemble

28. Understanding Interfacial Phenomena related to Enhanced Oil Recovery

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With increased oil prices, considerable research has been conducted to evaluate enhanced oil recovery strategies for improving oil recovery. One method that has received considerable attention is surfactant-based chemical flooding. Surfactant injection in the underground reservoirs has two key effects: reducing capillary pressure required for water to displace oil and reversing the rock surface wettability from oil-wet to water-wet conditions. To obtain a better understanding of these two issues, we will investigate oil-water and rock-fluid interfacial properties in the underground reservoir through molecular simulation. This poster focuses on bulk coexistence and wetting behavior of water on silica surface using Grand canonical Monte Carlo (GCMC) and temperature expanded ensemble (TEE) techniques. In the simulation, the free energy is obtained as density probability distribution, from which we can obtain wetting properties like contact angle and surface tension. These simulations enable us to learn more about interfacial properties of the rock-fluid interface. We hope the information collected will help improve chemical treatment for enhanced oil recovery.

Key words: interfacial property, wetting, Monte Carlo simulations, oil recovery

29. Numerical Analysis of Fully-Coupled Particle-Fluid Transport and Free-Flow Magnetophoretic Sorting in Microfluidic Systems

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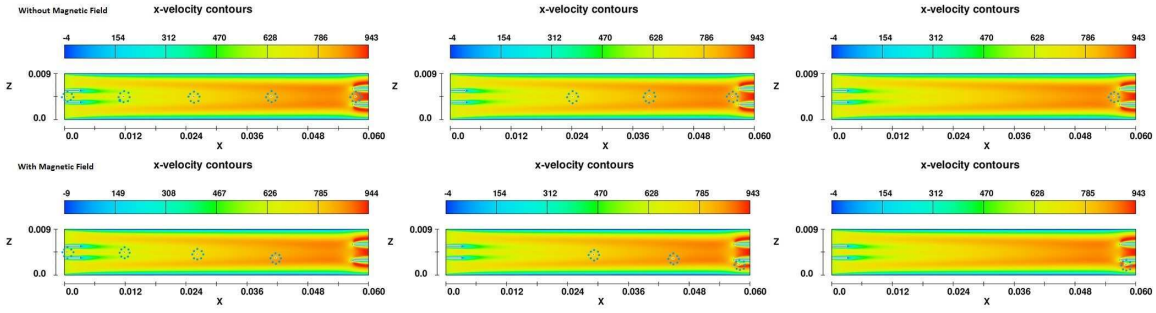
Magnetic particles are increasingly used in microfluidic systems to selectively separate and sort biomaterial from a specimen for a broad range of biomedical and clinical diagnostic applications. To date, most theoretical studies of such systems have been based on one-way particle fluid coupling wherein the fluid flow influences particle motion, but the flow is assumed to be constant, independent of particle motion. Relatively few groups have taken into account more rigorous two-way particle-fluid coupling wherein momentum is transferred from the particles to the fluid, thereby altering the flow. In this presentation a computational method is presented for predicting field-directed transport and continuous sorting of magnetic particles in multiport microfluidic systems taking into account two-way particle fluid coupling. Such systems consist of a flow channel with multiple inlets and outlets in close proximity to a magnetic field source. The source provides a magnetic force that permeates the microchannel and can be used to redirect (sort) incoming particles to desired outlets. We discuss a method for analyzing the free-flow sorting process using a hybrid numerical/closed-form modeling approach that combines numerical transport analysis with closed-form field analysis. Coupled particle-fluid transport is computed using computational fluid dynamic (CFD) analysis, while the magnetic force that governs particle motion is obtained in closed-form. The CFD analysis is based on a coupled Lagrangian-Eulerian formulation wherein the particle dynamics is predicted using Lagrangian analysis, while the fluid dynamics is predicted using Volume of Fluid (VOF) method in an Eulerian fixed-grid framework. This modeling accounts for two-way momentum transfer between the particles and fluid. It is demonstrated via application to various microfluidic sorting systems.

Key words: field-directed transport, magnetically functional microfluidic systems, particle-fluid interaction, magnetophoretic sorting, free-flow magnetophoreis.



30. Analysis of Pulsed-laser Plasmon-enhanced Photothermal Energy Transfer with Applications

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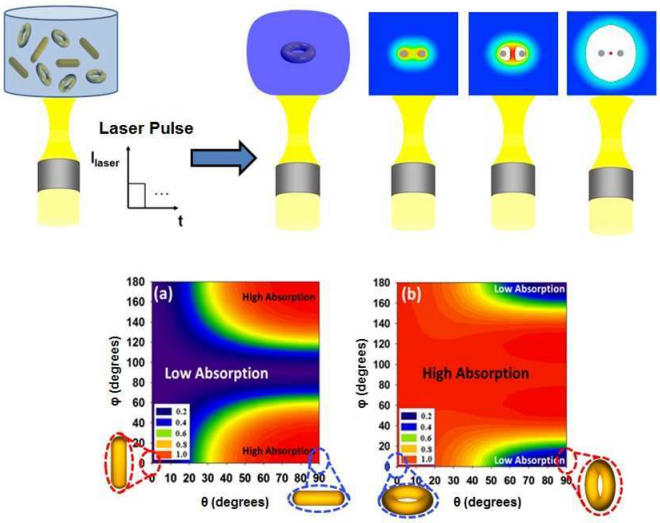
Laser-based plasmon-enhanced photothermal energy conversion has drawn increased interest in recent years as it can provide efficient heating with unprecedented (subwavelength) spatial resolution. In this approach, a pulsed laser is used to heat metallic nanostructures at their plasmon resonant frequency. In addition to enabling efficient nanoscale heating from a remote source, the optimal (resonant) heating wavelength can be tuned within the ultraviolet through near-infrared spectrum. In this presentation, we show fundamental aspects of plasmon-enhanced photothermal heating along with applications. We use computational models to demonstrate key photothermal effects associated with nanosecond-pulsed, laser-heated colloidal metallic nanoparticles. We simulate energy conversion within different nanoparticle structures at plasmon resonance, heat transfer from the particle to the surrounding fluid and phase change of the fluid leading to homogenous bubble nucleation. We consider various nanoparticle geometries including spheres, rods and tori. The analysis demonstrates that nanorings and nanotori have advantages over nanospheres and nanorods due to the tuneability of their resonant wavelength combined with relatively high absorption over a broad range of orientations relative to the incident polarization. We also show that parameters such as the laser intensity, incident wavelength, polarization, pulse duration and the orientation and particle shape can be tuned to optimize the photohermal process. We consider multi-particle systems and demonstrate effects of enhanced cooperative heating in such systems.

Key words: Localized surface plasmon resonance, LSPR, photothermal energy conversion, plasmonic-enhanced photothermal energy transfer, LSPR-induced optical absorption, pulsed-laser photothermal heating



31. Modeling Field-directed Dynamics and Assembly of Magnetic Nanoparticles with Particle-Fluid Interaction

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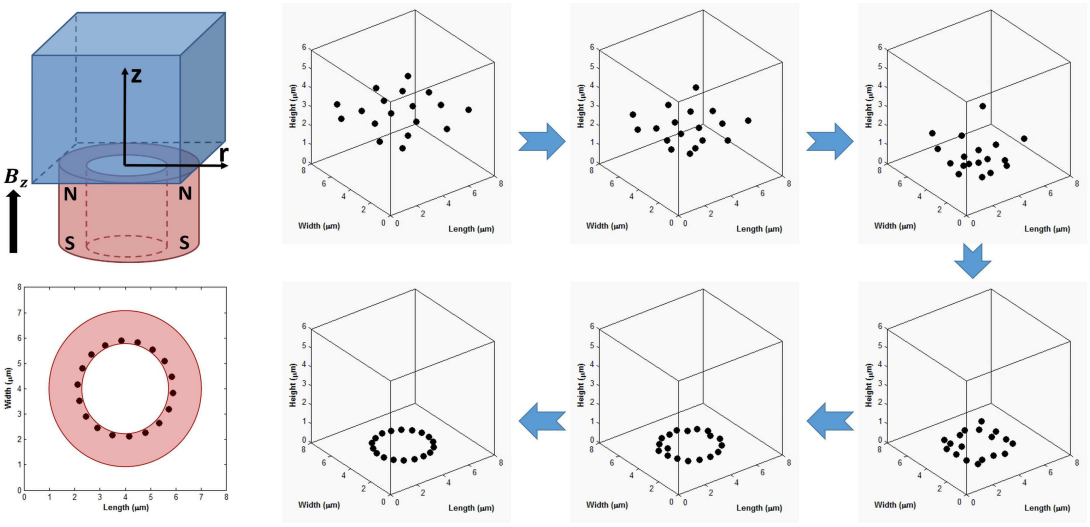
The interest in magnetic nanoparticles and ferrofluids has grown substantially in recent years as applications continue to proliferate. These include magnetic drug targeting, gene transfection, bioseparation and microfluidic mixing, among many others. However, despite the widespread and growing use of magnetic nanoparticles, there are many fundamental aspects of their collective behavior that remain unknown. In this presentation we demonstrate computational models to predict field-direct transport and assembly of magnetic particles for various applications. These models are based on a modified discrete element method and take into account several competitive effects including the applied-magnetic force, induced magnetic dipole-dipole interactions, Brownian dynamics, Van der Waals interaction, viscous drag and hydrodynamic interactions among the particles. A dynamic time-stepping approach is introduced to stabilize and accelerate the computation. Fully coupled particle-fluid interactions are also accounted for using a combined computational fluid dynamic (CFD)-based Lagrangian-Eulerian numerical formulation. The models are useful for predicting the self- and template-based assembly of particle micro- and nano-structures and the stability of such structures in a time-varying field. We demonstrate the models via various applications including fully-coupled self-assembly in a uniform field and template-based assembly in high-gradient magnetic fields.

Key words: magnetic nanoparticle, magnetic dipole-dipole interaction, field-directed assembly, template-based assembly, particle-fluid interaction



32. Virial Expansion Study of Selective Adsorption of Methane-Ethane Mixtures in Carbon

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The properties of fluids at interfaces have long been a problem of interest both from theoretical and experimental standpoints. The technological, environmental and biological importance of adsorption in porous materials is well established. For example, the ability to separate mixtures of gases is fundamental to a large number of industrial processes, and one method of effecting a separation is to selectively adsorb one component of the mixtures in porous material. While issues such as the relative permeabilities of the mixture components in the porous material are important, the viability of many separation processes involving adsorption in a porous material depends primarily on whether one component is sufficiently strong adsorbed relative to another.

We examine the utility of virial coefficient theory to predict and understand the adsorption of mixtures in slit pores with graphite properties. In order to apply the virial theory to the gas adsorption, it is necessary to derive the expression for the concentration of adsorbed molecules in powers of the pressure from the grand partition function. The coefficients appearing in this pressure expansion are the gas-surface virial coefficients, and they can be computed once both adsorbate-adsorbate and adsorbate-adsorbent intermolecular potentials are clearly defined. In this work we report a study of a prototype systems involving methane-ethane mixtures in carbon using this approach. Mayer-sampling Monte Carlo method is used to evaluate the necessary virial coefficients. The virial coefficients are then used to predict the adsorption isotherm for pure components and the selectivity of ethane relative to methane for a wide range of system parameters. We also study the effect of bulk pressure, mole fraction, temperature, and pore size on the selective adsorption. Conclusions are drawn about the deviation from Henry's law and the coverage, which can be described by virial expansions.

Key words: gas adsorption, virial expansion, Mayer-sampling Monte Carlo, Henry's law

33. Virial for highly size-asymmetric hard sphere mixtures

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An interesting question in statistical mechanics concerns whether mixtures of very different sized particles will demix, absent any energetic driving force. This question is usually posed in the context of two-component hard-sphere mixtures, for which parameters can be selected so they resemble colloidal suspensions. The behavior can be modeled using either an effective depletion potential, which simplifies the complex system to a pseudo single component system, or explicitly, as a true mixture of two species. The demixing behavior for small size ratios (small-sphere to large-sphere diameter) is of particular interest as different methods to examine the model provide contradicting conclusions regarding even the qualitative features of the behavior. For highly size-asymmetric hard sphere mixtures, integrating out the degrees of freedom of small species results in an effective hamiltonian for big species. Asakura and Oosawa (AO) calculated the effective potential between two big particles present in a sea of small particles, and this result is quite helpful in current case. In this work the aim is to calculate the virial coefficients for the AO model, as well as for explicit HS mixtures, to see how well the expansion converges and whether it is physically significant in explaining the fluid fluid separation.

Key words: Virial Coefficients, Binary Hard Sphere mixtures, Depletion potential, Demixing

34. Virial Coefficients Of Hydrogen And Nitrogen Including Quantum Effects Using Path Integral Monte Carlo Method

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Accurate virial coefficients are important for predicting properties of light gases such as hydrogen, helium etc., especially at low temperatures where the quantum effects aren't negligible. In addition to predicting properties of gases, virial coefficients (particularly the second virial coefficient) can be utilized as a tool to check the accuracy of various potential models against experimental data. Hence, the accuracy of such coefficients are of vital importance to many scientists and fellow engineers. The Mayer-sampling Monte Carlo method [1] provides an efficient route to calculating accurate virial coefficients. To incorporate quantum effects of light gases in which the positions of the atoms/molecules fluctuate by a significant amount at low temperatures, one needs to use path integral techniques. Path Integral Monte Carlo (PIMC) methods involve discretizing the quantum fluctuations of the atoms/molecules into beads that form closed paths or rings. Neighboring beads act as if they are harmonically connected to each other and the strength of the harmonic spring is directly proportional to temperature. The interaction energy is then computed as the average of the potential evaluated between corresponding beads of different rings. At very low temperatures, a large number of beads are required to accurately represent the path of the atoms/molecules.

PIMC methods have been applied by us and others to study quantum virial coefficients of He [2 - 5] and H_2 [6]. We now extend this approach to compute higher order virial coefficients using flexible potentials (if available) of small diatomic molecules such as H_2 and N_2. For diatomic molecules, the complexity in terms of the orientational sampling under the approximation of quantum rigid rotors involved in the PIMC methods can be greatly reduced by a new algorithm we introduce. We studied the probability distribution of placing each successive bead on the surface of a sphere as a function of the angle ϕ between the previous bead and the current bead position vectors. We noticed that for reasonably low ϕ, ($\phi < 15^\circ - 20^\circ$) we could analytically express the probability distribution functions for all the beads as one universal probability distribution function with a parameter k, that was different for each bead. The analytical nature of this universal probability distribution function is the key feature that makes the algorithm fast, as it can be computed on the fly. Also, since we are regrowing the entire ring from scratch for each Monte-Carlo move, the possibility of different starting points of the these rings is accounted for. The assumption of low ϕ is reasonable even at low temperatures where the harmonic springs become weak. We present preliminary results for the H_2 and N_2 systems.

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Key words: Virial Coefficients, Path Integral Monte Carlo, quantum effects

35. Evaluation of Thermodynamic Properties of Gas Mixtures via the Virial Equation of State with Accurate Molecular Models

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Experimental measurements of vapor pressures of heavy organic molecules can be performed via a gas saturation technique in a light carrier gas. The apparent vapor pressure based on an ideal-gas treatment of a vapor mixture is found to depend on the carrier gas, indicating that treatment of non-ideality is needed to properly interpret the experimental data. Semi-empirical methods can be applied, but a successful outcome is more likely if based on a treatment that considers the specific molecular features of the component molecules. The virial equation of state (VEOS) for mixtures presents a convenient approach to develop such an approach.

The VEOS for mixtures is given as a power series in density and mole fraction of each component, with coefficients relating to the interactions of corresponding number of molecules of each species. The pure virial coefficient of each component can be calculated via evaluation of cluster integral(s) dependent on the intermolecular interactions among molecules of the same species, whereas the cross virial coefficients are given as cluster integral(s) involving the interactions among unlike molecules species.

In previous work [1] we explored the Lennard-Jones binary mixtures using the Mayer sampling Monte Carlo method [2], computing virial coefficients and examining vapor-liquid critical behavior. In the present work, we focus on more realistic molecular models, which requires us to re-develop the virial series for mixtures of non-rigid molecules. For such systems, it is necessary to consider cluster integrals that are not present in conventional formulations of the virial series (applicable to rigid molecules), because molecular flexibility prevents cancellation of cluster integrals that is usually assumed in the conventional development [3,4].

We examine coefficients for carbon dioxide (CO_2), which is sometimes used as carrier gas in experimental measurements. For the organic component, we examine n-alkanes as prototype molecules. For short alkanes, both the pure virial coefficients and the cross virial coefficients of CO_2/n-alkanes are calculated up to the 4th order. For long alkanes, the virial coefficients are calculated up to the 3rd order. We examine the convergence behavior of the VEOS for these binaries, and compare the thermodynamic properties with available experimental data.

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Key words: gas mixtures, virial equation of state, cluster integral, flexibility

36. A Comprehensive Molecular-Based Study of the Stability of Clathrate Hydrates

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Clathrate hydrates are crystalline structures that consist of water molecules forming cages via a hydrogen-bonding network enclosing small guest molecules. Such structures normally form at a conditions of high pressure and low temperature. Clathrate hydrates have always been a problem in the gas and oil industry because their formation can block pipelines and processing equipment. Prevention of such flow-line blockages and motivates an increase level of study of fundamental mechanisms for gas hydrate formation and decomposition. Computational, molecularly-based modeling methods play an ever-growing role in helping us to understand the molecular processes and properties that contribute to clathrate hydrate formation and stability. There are many variable to consider, including hydrate crystal structure, the chemical species, concentration, and distribution of the solute, conditions of temperature and pressure, nuclear quantum effects, and the impact of co-solutes. Any understand of hydrate nucleation and growth must begin with an understanding of their thermodynamic stability, to ensure that the molecular model is one that corresponds to the known thermodynamic behavior of clathrate hydrates. This requires examination of the free energy of formation. We start this examination by considering defect-free lattice, i.e. pure water or cages each with one single identical solute molecule. We employ lattice dynamics to calculate the free energy at very low temperature. Then, the harmonically targeted temperature perturbation method, which is an efficient technique for evaluating the free energy as a function of temperature, will be used to calculate free energy up to temperatures of interest. With the knowledge of free energy we can identify the stable crystalline form and locate the conditions where phase transitions occur.

Key words: Clathrate hydrate, free energy, stability, phase equilibrium

Nanoscale Materials Science and Engineering

37. Polymer-Stabilized Colloidal Dispersions: Modulation of Interactions by Displacers

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We consider colloidal dispersions of silica nanoparticles in aqueous media containing poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) amphiphilic block copolymers (Pluronics), with a specific interest on how solution conditions such as pH, temperature, and the presence of displacers (electrolytes, polar organic solvents, or PEO homopolymers) affect the (i) organization of the amphiphiles on the nanoparticle surface and in the bulk solution, (ii) ensuing interactions between nanoparticles, and (iii) macroscopic properties of the dispersions. The fundamental knowledge thus gained guides the design of oil-in-water dispersants incorporating nanoparticles. We acknowledge Mrs. Ruksana Jahan for assistance with viscosity measurements.

Key words: Pluronics, PEO-PPO-PEO, Nanoparticles, PEO homopolymer, Dispersants

38. Thermodynamics of Surfactant Solutions

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The nanostructure emanating from the self-organization of polymers and/or surfactants in solvents is central to applications such as drug delivery and nanomaterials synthesis, where the self-assembled domains provide environments of varying and tunable shape, dimensions, short/long-range order, mobility, local polarity, concentration, and reactivity. Fundamental information on the thermodynamics, structure, and dynamics of polymer/surfactant assemblies is necessary in order to judiciously utilize these in various applications. In the present work we report on the use of isothermal titration calorimetry (ITC) to measure the enthalpies associated with the dissolution, micellization, and phase-separation of amphiphiles (surfactants or block copolymers) in selective solvents. We are further interested on how such thermodynamic quantities are modulated by the addition of electrolytes, cosolvents, or nanoparticles.

39. Dissolution Processing of Nanostructured Polymers

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Nanoscale and mesoscale order in polymers, manifested by chain crystallinity and organization in spherulites or fibers, significantly affects the dissolution of nanostructured polymers, and constrains their subsequent physical or chemical processing. Cellulosic biomass provides a nice example of a composite material that is recalcitrant to solvents. The efficient utilization of cellulose as a starting material for the synthesis of high value-added functional polymers and chemicals and also for biofuel production provides the motivation for the current project. We consider here the transport phenomena governing the dissolution of solid cellulose, e.g., solvent penetration, transformation from crystalline to amorphous domains, specimen swelling, and polymer chain untangling, and analyze the effect of various parameters on the kinetics of dissolution.

Key words: Biomass, Dissolution of cellulose, Nanostructured polymer, Transport phenomena

40. Phase Behavior and Structure in Polymer/Lithium Salt Mixtures

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Solid polymer electrolytes could improve the cost, stability, and safety of rechargeable lithium batteries, but there are several issues to overcome. Block copolymers allow rational molecular design and processing to provide desirable ion transport and mechanical stability. We consider phase behavior, structure, and dynamics in multi-component systems containing block copolymers and lithium salts, with a focus on the interplay between Li+ location/mobility and block copolymer organization. We employ block copolymers consisting of poly(ethylene oxide) (PEO) that enable three levels of organization: crystalline PEO blocks, liquid crystalline block copolymer assemblies, and long-range orientation of ordered domains. This research informs the design of new/improved electrolytes and batteries for alternative energy applications.

Key words: Solid polymer electrolytes, block copolymers, rechargeable lithium batteries

41. Structured Ionic Liquids

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Ionic liquids (ILs), organic salts that are fluid at ambient conditions, are a novel class of compounds with a combinatorially great chemical diversity and unique properties. The very low volatility and high thermal and chemical stability that many ILs exhibit, render them promising as solvents. We consider ionic liquid + nanoparticle hybrid systems, where the term "nanoparticle" encompasses materials that are hard/inorganic or soft/organic, macromolecular or supramolecular, natural or synthetic. This research supports and guides the next generation applications of ionic liquids in "formulated chemicals" or "functional products", i.e., multi-component systems that are rationally designed to meet specific end-use requirements.

Key words: Nanoparticle, Ionic Liquids, Self-assemble material

42. Poly(ethylene glycol)-block-Cationic Polylactides Nanocomplexes of Differing Charge Density for Gene Delivery

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Representing a new type of biodegradable cationic block copolymer, well-defined poly(ethylene glycol)-block-cationic polylactides (PEG-b-CPLAs) with tertiary amine-based cationic groups were synthesized by thiol-ene functionalization of an allyl-functionalized diblock precursor. Subsequently the application of PEG-b-CPLAs as biodegradable vectors for the delivery of plasmid DNAs (pDNAs) was investigated. Via the formation of PEG-b-CPLA:pDNA nanocomplexes by spontaneous electrostatic interaction, pDNAs encoding luciferase or enhanced green fluorescent protein were successfully delivered to four physiologically distinct cell lines (including macrophage, fibroblast, epithelial, and stem cell). Formulated nanocomplexes demonstrated high levels of transfection with low levels of cytotoxicity and hemolysis when compared to a positive control. Biophysical characterization of charge densities of nanocomplexes at various polymer:pDNA weight ratios revealed a positive correlation between surface charge and gene delivery. Nanocomplexes with high surface charge densities were utilized in an in vitro serum gene delivery inhibition assay, and effective gene delivery was observed despite high levels of serum. Overall, these results help to elucidate the influence of charge, size, and PEGylation of nanocomplexes upon the delivery of nucleic acids in physiologically relevant conditions.

Key words: cationic polymer, gene delivery, nanocomplex, PEGylation, polylactide

43. Synthesis of Functional PEGylated Polylactides for Therapeutic Delivery

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Gene therapy and chemotherapy show great promise to treat diseases such as cancer and HIV. However, these therapeutic agents need to be protected by carriers because degradation of gene/drug may occur before they are delivered into the target tissues. To design an ideal carrier, various factors should be taken into consideration, such as protection of plasmid DNA or drug from degradation, localization to disease tissue, avoiding off-target distribution, and efficient transport. In addition, carriers should possess remarkable water solubility or dispersibility. A broad variety of carriers for gene and drug delivery have been extensively studied. Among these materials, polylactides (PLAs) have gained significant attention because of their biodegradable and biocompatible properties. However, the inherent low water solubility of PLAs significantly limits their biomedical applications. Accordingly, it remains a challenge to use PLAs as delivery materials. Poly(ethylene glycol) (PEG), which is a water-soluble polymer, is able to enhance water solubility and circulation time of delivery systems. Incorporation of PEG with PLA can be utilized as a promising strategy to yield modified PLAs with improved applicability in therapeutic delivery. Toward this end, well-defined PLA variants exhibiting high delivery efficiency, low toxicity, degradability and water-solubility can be obtained through various PEGylation approaches. In this study, PEG was used as initiator in ring-opening polymerization of allyl-functionalized lactide for the synthesis of PEG-block-(allyl-functionalized PLA). Alternatively, PEGylated PLA can be obtained by grafting PEG chains on the backbone of alkyne-functionalized PLAs through azide-alkyne click functionalization, resulting in PLA-graft-PEG.

Key words: PEGylatation, polylactide, gene delivery, drug delivery

44. Synthesis of High-Quality InGaP-based Nanocrystals

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Photoluminescent quantum dots (QDs) have attracted much research attention because of their low scattering, tunable bandwidth, high brightness, and long-term photostability, leading to possible commercial applications as biological labels, phosphors for light-emitting diodes and gain-media for optically-pumped visible lasers. In recent years there has been emphasis on III-V-based QDs, such as InP, InAs and GaN, mainly due to their low toxicity and broad emission color range. Among them, InP-based QDs have been most intensively studied due to their tunable emission in the visible region of the spectrum. However, challenges still remain in synthesizing high quality InP-based QDs, including increasing the photoluminescence quantum efficiency (PLQE), controlling the size distribution and corresponding spectral emission width, increasing their environmental stability, and maintaining high PLQE under the conditions of high temperature and high excitation flux. To achieve high quality InP-based QDs, we create a highly confined structure, using InP as the core, InGaP as an intermediate shell layer, and ZnSeS as a graded ternary outer shell. Confinement of the electrons and holes to the InP/InGaP region reduces their interaction with surface, which results in higher PLQE, improved temperature stability, and a large suppression of Auger recombination. To date our PLQE at room temperature is up to 90%, spectral widths vary from 50 to 90 nm, PLQE drops by less than 5% when temperature increases from 25 to 145° C, and the PLQE is invariant to optical flux densities up to 20,000 W/cm^2. Currently and going forward, we will continue to optimize the optical properties of our QDs for usage as high performance down-conversion phosphors for solid state lighting and as gain media for continuous-wave (cw) optically pumped lasers.

Key words: nanocrystals, InP, optical properties.

45. Research Overview of the Lin Group on Energy-Efficient Membranes for Molecular Separations

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This presentation will provide an overview of the research activities in the Lin Membrane Laboratory. We are focused on the fundamental study of membrane technology for gas and liquid separations. More specifically, we will design the architecture of polymer based materials, synthesize these molecularly engineered materials, and systematically evaluate the effect of chemical structure and morphology on the separation performance, guided by the understanding of the practical applications and integral solutions with novel separation process designs. The high performance materials will be thoroughly characterized under simulated industrial conditions and in the practical thin film membrane form to fully evaluate their potential for practical applications. In parallel with experimental work, we will also develop predictive models illustrating the structure/function correlations, which can be used to guide the design and engineering of new materials. This presentation will also provide a glimpse of the current projects available for new students pursuing M.S./M.Eng. and Ph.D.

Key words: membrane, research overview

46. Eliminating the Pathway to Humins by Acid Catalyzed Hydrolysis of Glucose

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Fuels created from cellulose have garnered significant attention over the past decades. Currently research groups have moved away from traditional renewable fuels such as ethanol to fuels that are more compatible with current infrastructure. However, production of these fuels is accompanied by production of a solid carbon byproduct known as humins. Humins are undesired and lower the yield of the desired fuel products. Our group has studied the mechanism, and it appears that water is necessary for the production of humins. Consequently, our recent focus has involved the use of non-aqueous solvents combined with a desiccant to absorb the water generated by the reaction. The challenges are to find a stable solvent that will dissolve glucose and to find a suitable acid for use in that solvent. Three systems have been studied: gamma-valerolactone (GVL)/sulfuric acid, GVL/levulinic acid and formic acid (as both solvent and acid). The GVL systems suffer from a very low solubility of glucose while the formic acid system suffers from its decomposition.

Key words: Levulinic acid, hydrolysis, humins, 5-hydroxymethylfurfural, carbon spheres, morphology, selectivity

47. Synthesis and Characterization of Lead Tin Selenide Alloy Nanocrystals

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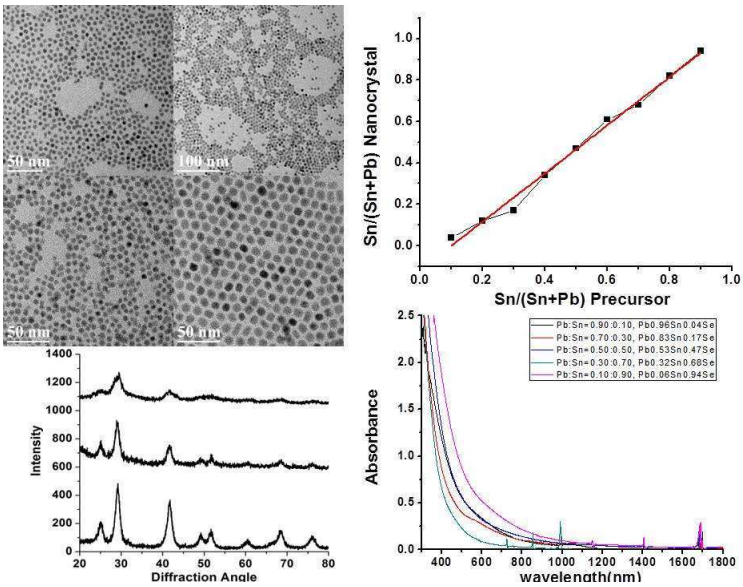
We present a convenient oleylamine-based solution phase synthesis process for producing lead tin selenide (Pb1-xSnxSe) alloy nanocrystals. Lead selenide (PbSe) nanocrystals have size-tunable optical and electronic properties, which make them interesting as a candidate for low-cost, solution-processed photovoltaic devices. However, their narrow intrinsic bandgap of PbSe means that very small nanoparticle size and extreme quantum confinement are required to produce materials with optical band gap at visible wavelengths. Substituting tin for lead to produce Pb1-xSnxSe alloy nanocrystals increases the intrinsic band gap allowing tunable of the band gap to visible wavelengths at a relatively larger size. Three different kinds of Se precursor for the synthesis were evaluated in this work. We found that Se precursor prepared by dissolving Se in oleylamine and a strong reducing agent was most effective and allowed for the simple and fast preparation of Pb1-xSnxSe at relatively low reaction temperature. The composition of Pb1-xSnxSe nanocrystals was determined by energy-dispersive x-ray spectrometry (EDS). The optical absorbance of the Pb1-xSnxSe nanocrystals was measured by UV-visible absorbance spectrometry. The morphology and crystal structure of the Pb1-xSnxSe nanocrystals were characterized by high-resolution transmission electron microscopy (HRTEM), scanning electron microscopy (SEM), and x-ray diffraction (XRD).

Key words: Lead Tin Selenide, Semiconductor Nanocrystals



48. Synthesis of Noble Metal Based Anisotropic and Heterogeneous Nanomaterial and the Applications

Dewei Zhu, **Xin Liu**, **Xianliang Wang**, **Mark T. Swihart**

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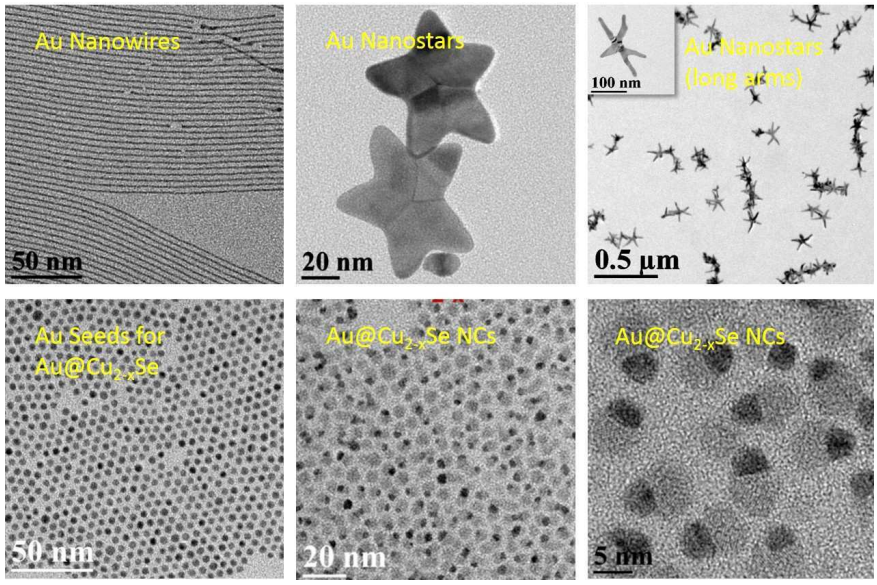
In this poster, we present our latest work on the synthesis of anisotropic noble metal nanoparticles (polygons, wires, and stars etc.) and heterogenous multicomponent nanoparticles (Au@Cu_{2-x}Se, Ag@Cu_{2-x}Se) and the application of these nanomaterials based on their Localized Surface Plasmon Resonance (LSPR). Metallic nanomaterials, especially gold, have well-studied and widely applied LSPR which can be tuned by the size and morphology of the nanoparticles. It provides us a broad range of possibilities to use our noble metallic nanomaterial with diverse size and shape in bio-imaging, sensing and detection, thermal therapy, catalysis, non-linear optics and many other intriguing fields. Here, we will principally demonstrate our latest work of gold nanostar and its potential application in Raman Scattering as well as the broaden LSPR of Ag@Cu_{2-x}Se and its application in bio-imaging which we have studied on Au@Cu_{2-x}Se in our former work[1].

[1] Liu, Xin, Changho Lee, Wing-Cheung Law, Dewei Zhu, Maixian Liu, Mansik Jeon, Jeehyun Kim, Paras N. Prasad, Chulhong Kim, and Mark T. Swihart. "Au-Cu_{2-x}Se Heterodimer Nanoparticles with Broad Localized Surface Plasmon Resonance as Contrast Agents for Deep Tissue Imaging." Nano Letters (2013).

Key words: noble metal, gold, localized surface plasmon resonance



49. Combustion-Driven One Step Synthesis of CZTS and CZTSe Nanoparticles and Films in a High Temperature Reducing Jet Reactor

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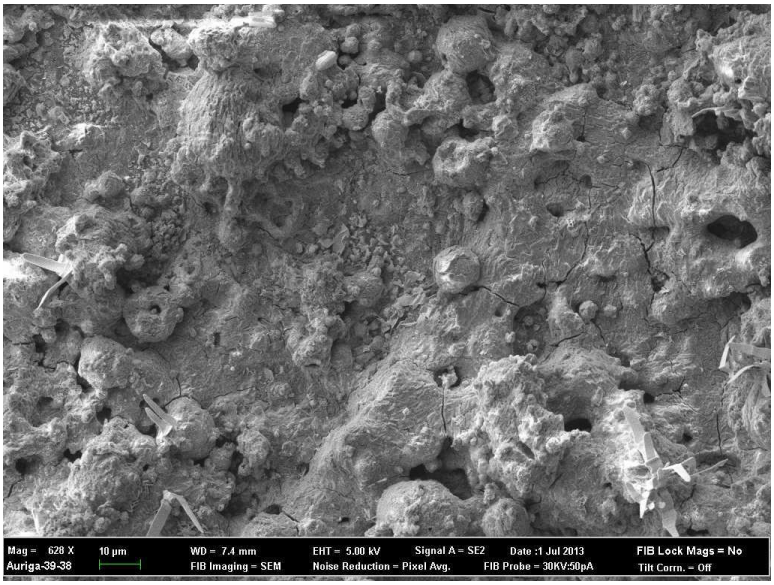
Solar energy is by far the most abundant among all energy resources. However, current solar technologies are rarely able to compete on cost with conventional energy sources, and manufacture of current solar cells requires substantial energy input and often uses rare or toxic elements. With a high absorption coefficient (10^4 cm^-1) a desirable bandgap (1.45 eV), and a composition based on abundant, non-toxic materials, Cu_2ZnSnS_4(CZTS), Cu_2ZnSnSe_4(CZTSe), and related materials have tremendous potential light-absorbing materials for use in solar cells. Theoretical calculations have shown that power conversion efficiency as high as 32% is possible for CZTS thin film solar cells (TFSCs). Actual devices based on these materials have reached 14% efficiency. One means of reducing the cost and environmental impact of CZTS/Se solar cells would be to manufacture solar cells using solution-based printing or coating processes with CZTS/CZTSe nanoparticle inks. Thus, we are interested in low-cost production of nanoparticles of these promising materials.

We present here the high temperature flame-based synthesis of CZTS thin films using the high temperature reducing jet (HTRJ) reactor developed by Scharmach et al (2010). We synthesized CZTS nanoparticles by injecting Zn(SO_4)$7H_2$O, Cu(SO_4)$5H_2$O, SnSO_4 and selenium precursors into this reactor system, where these low-cost, water-soluble precursors are thermally decomposed in the high-temperature reducing environment. We directly deposited CZTS nanoparticles on glass substrate by thermophoresis. The HTRJ process allows us to decouple the flame chemistry from the nanoparticle formation chemistry. Thus, we synthesized nanostructured CZTS thin films in one step by a low-cost and environmentally friendly route. The as-prepared films were sintered at 200oC inside the HTRJ reactor, eliminating the need of any post treatment methods to anneal and sinter the films. The multicomponent films were characterized by powder X-Ray diffraction, SEM and EDX for surface topology, crystal structure, and elemental composition. In this presentation, we will describe the HTRJ process briefly, and then focus on the synthesis and characterization of this low cost CZTS thin film as a promising PV material.

Key words: CZTS, thin film, solar cell, flame aerosol reactor



50. Mechanistic and Synthetic Research on $Fe_3O_4 - Au - Cu_{2-x}S$ Multi-component Nanocrystals

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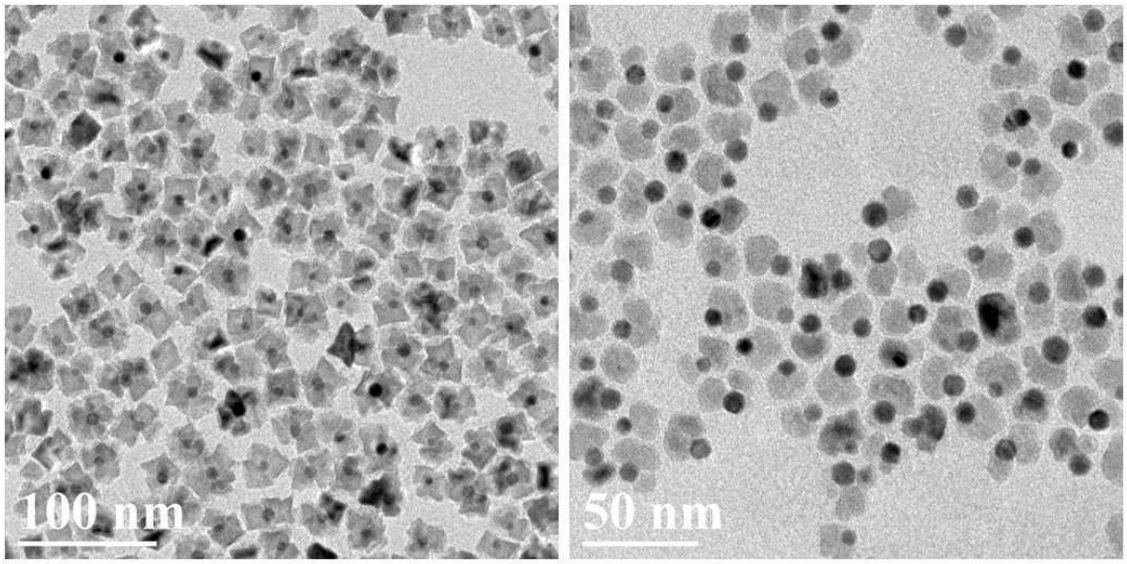
Multi-component nanoparticles that combine different materials in a single nanostructure are of interest for a variety of applications and show particular promise as multimodal contrast agents for biological imaging. In this study, we are investigating the preparation of $Fe_3O_4 - Au - Cu_{2-x}S$ and analogous species. These hetero-trimer particles synergistically combine key properties of their constituent materials. The Fe_3O_4 component exhibits superparamagnetism, which allows the nanostructures to be manipulated by magnetic fields and to serve as contrast agents for magnetic resonance imaging. Both the Au domain and the $Cu_{2-x}S$ domain exhibit Localized Surface Plasmon Resonance (LSPR), which produces strong absorbance and scattering of light. Au nanoparticles, alone, exhibit strong LSPR absorbance near 530 nm, while $Cu_{2-x}S$ nanoparticle exhibit LSPR absorbance in the near-infrared (NIR). When the two plasmonic materials are combined, their interaction produces a broad, flat LSPR absorbance and scattering across the visible and NIR spectral regions. This allows the nanoparticles to serve as contrast agents for photoacoustic imaging and dark-field optical imaging and to be used in photothermal therapy. In the hetero-trimers, all of these physical properties are combined in single nanostructure. If they are used in multimodal bioimaging, with a single injection, multiple imaging techniques can be applied simultaneously, minimizing the side-effects while facilitating the accuracy of diagnosis. Our current focus in this project is on optimizing the synthesis of these ternary nanostructures and on understanding the mechanisms of their formation. Our general approach is to start with Au seed nanoparticles, and then produce binary and ternary nanoparticles by heterogenous nucleation and growth of the other materials on the Au nanoparticle seeds. The images below show two varieties of $Au - Fe_3O_4$ binary nanoparticles that we have prepared in the course of this project to date.

Key words: Mechanism, synthesis, nanocomposite, localized surface plasmon resonance



51. Synthesis of Copper-Nickel Nanoparticles and Conductive Nanoparticle Films using High Temperature Reducing Jet (HTRJ) Reactor

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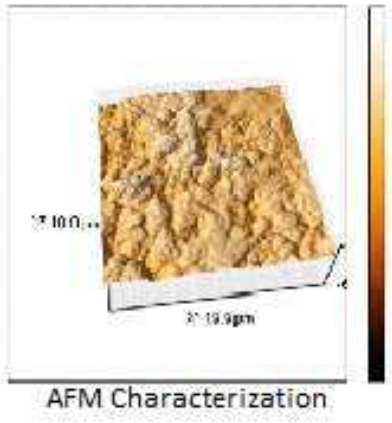
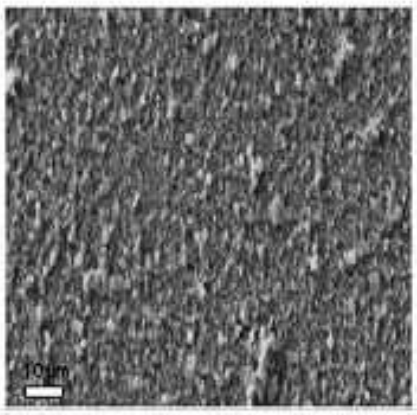
Copper-nickel alloys have excellent resistance to corrosion and good electrical conductivity. Cu-Ni nanoparticles therefore have potential as components of conductive metal inks for printable electronics. Our group recently developed a new method of producing metal nanoparticles and nanostructured metal coatings, which we apply here to copper-nickel nanoparticles. These are produced in a high temperature reducing jet (HTRJ) environment. This environment is achieved by using hydrogen as a fuel in a fuel-rich flame. The hot combustion products are accelerated through a converging-diverging nozzle within which an aqueous metal salt solution is introduced into the hot reducing jet. Copper-nickel nanoparticles and coatings have been synthesized by thermally decomposing water soluble nitrate precursors using the HTRJ process. We also analyzed oxidation resistance of these films as well as electrical conductivity at different compositions.

Key words: Copper-Nickel Alloy, High Temperature Reducing Jet reactor, Metal nanoparticles



AFM Characterization



SEM Image of coating

52. Cu2-xS1-ySey Alloy Nanocrystals with Broadly Tunable Near-Infrared Localized Surface Plasmon Resonance

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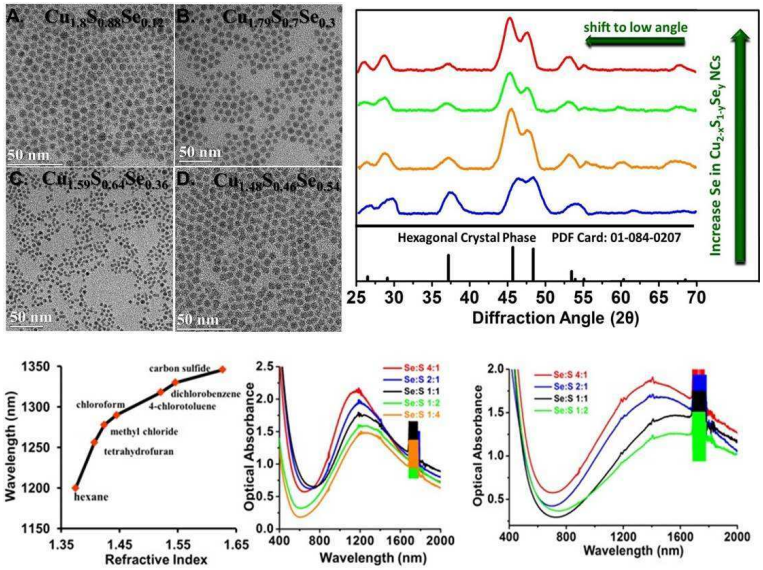
We report facile methods for synthesizing monodisperse Cu2-xS1-ySey alloy NCs with tunable composition and size. The near infrared (NIR) localized surface plasmon resonance (LSPR) in these self-doped Cu2-xS1-ySey alloy NCs can be tuned over a broad range from 975 nm to 1750 nm. The LSPR shifted to longer wavelength with increasing sulfur content and with increased concentration of oleic acid used in the synthesis. This method provides new possibilities for broadly tuning LSPR wavelength by controlling the anion composition, cationic vacancy concentration, and surface ligands in heavily-doped Cu2-xS1-ySey alloy NCs. This opens up access to LSPR absorbance across a broad portion of the near-IR using small colloidal quasi-isotropic NCs.

Key words: alloy nanocrystals , localized surface plasmon resonance, cationic vacancy



53. Au-Cu2-xSe Heterodimer Nanoparticles with Broad Localized Surface Plasmon Resonance as the Contrast Agent for Deep Tissue Imaging

Xin Liu[1], **Changhoo Lee**[2], **Wing-Cheung Law**[3], **Chulhong Kim**[2], **Paras N. Prasad**[3], **Mark T. Swihart**[1]

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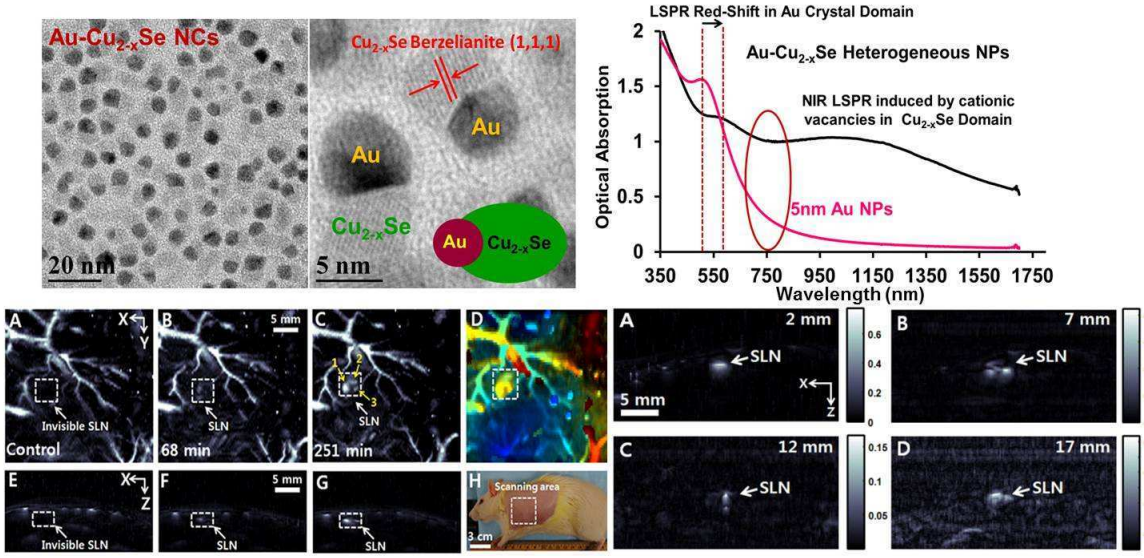
Metal nanostructures exhibit localized surface plasmon resonance (LSPR) based on the coupling of free electrons with the oscillating electromagnetic field of light. This strong light-matter interaction can generate strong local fields and induce thermal expansion that facilitates their applications in theranostics, nanophotonics and nanoelectronics. However, engineering the plasmonic absorbance of these metal nanostructures to the near infrared (NIR) spectral region is required for their biomedical applcations, because the visible light significantly interferes with tissues and biological molecules that depress the light penetration and imaging depth. This, in turn requires finely controlling the shape and size of metal nanostructures, to produce rods, cages, rings or shells. In this work, we report a new type of heterogeneous nanoparticles (NPs) composed of a heavily doped semiconductor domain (Cu2-xSe) and a metal domain (Au), which exhibit a broad localized surface plasmon resonance (LSPR) across visible and near-infrared (NIR) wavelengths, arising from interactions between the two nanocrystal domains. We demonstrate both in vivo photoacoustic imaging and in vitro dark field imaging, using the broad LSPR in Au-Cu2-xSe hybrid NPs to achieve contrast at different wavelengths. The high photoacoustic imaging depth achieved, up to 17 mm, shows that these novel contrast agents could be clinically relevant. More broadly, this work demonstrates a new strategy for tuning LSPR absorbance by engineering the density of free charge carriers in two interacting domains.

Key words: heterogeneous nanoparticles, localized surface plasmon resonance, contrast agent, photoacoustic imaging,



54. Synthesis, Characterization and Catalytic Applications of Peptide-Capped Au Nanomaterials

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Peptide-derived nanomaterial synthesis represents a new approach to achieve inorganic structures that are prepared under sustainable biological conditions. In addition, gold nanomaterials have been of great interest due to their optical properties that arise from the localized surface plasmon resonance, leading to a various applications including catalysis, energy capture and storage and sensors. To this end, we demonstrate the templated-synthesis of gold nanoparticles using a series of peptide sequences that are specifically known to bind inorganic materials. Based on recent studies that have isolated surface binding structures and thermodynamic parameters for a library of Au binding peptides, 10 peptide sequences with various lengths, binding strengths, spatial structures and isoelectric values were selected to direct the fabrication of the biomolecule-functionalized gold nanoparticles through a simple and straightforward approach. The as-prepared nanomaterials exhibited robust stability in aqueous solution, and were characterized using multiple techniques including UV-Vis, TEM, CD, DLS and zeta-potential. A 4-nitrophenol to 4-aminophenol reduction reaction model was further employed to study the catalytic properties of the biomolecule-capped gold nanoparticles. The rate constants at different temperatures were measured and the activation energies of these peptide-capped gold nanoparticles were determined. We also correlate the effects of peptide sequence, binding strength, specificity and surface morphology over the particle size, structure, and their catalytic, and optical properties. As a part of our ongoing project on biomolecular assembly of nanomaterials, this study gives us the fundamental understanding of biomolecule-capped nanomaterials fabrication that will be developed as the building blockings to achieve functional hybrid bio-assembly systems.

Key words: Gold nanoparticles, peptides, specific binding, bio-assembly, bio-nanocombinatoric

55. Calcium Oxalate Crystallization in Silica Hydrogels - Experimental Investigation and Theoretical Predictions

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The deposition of inorganic biominerals typically takes place in gel-like extracellular matrix environments. These matrices are mainly composed of organic macromolecules that play an important role in controlling the nucleation, growth, and the final morphology of biominerals. Hydrogels can serve as excellent matrix models for biomineralization. The hydrogel matrix provides a template for crystallization, while at the same time controls the diffusion of ions, regulates the nucleation and growth rates, and directs the hierarchical organization of the minerals. In this work, we studied the crystallization of calcium oxalate, the primary mineral constituent of kidney stones in silica hydrogels. The experimental setup consists of a double-diffusion system where calcium and oxalate ion reservoirs are separated by a silica hydrogel column. The solubility product is not the only criterion for precipitation in gel media. The concentration of both ions precipitating should be equal to a critical concentration. This is demonstrated experimentally using calcium oxalate-silica gel (pH~6) double diffusion system. Addition of model anionic polymer, poly(styrene sulfonate) (PSS), as an additive at low concentrations alters the morphology and polymorphism of calcium oxalate in the silica gel medium. The interaction of PSS with specific faces of the calcium oxalate crystal containing calcium ions changes the growth rate and affects the way crystals aggregate. At certain concentrations, PSS was also found to inhibit the formation of calcium oxalate monohydrate (thermodynamic stable form). Precipitation patterns in calcium oxalate-silica gel double diffusion system vary for silica gels formed at different pH and the pattern formations are explained using theoretical predictions based on the relative diffusion coefficients of both ions. A basic understanding of the diffusion and precipitation processes in silica hydrogels can aid in predicting the time scales of nucleation and growth of the crystals and can facilitate further use of such gel matrices as templates for biomineral formation or development of bio-inspired materials.

Key words: Biomineral, Nucleation, Growth, Morphology, Polymorphism, Hydrogels

56. Effect of Polymer Molecular Weight on the Assembly and Disassembly of Polyelectrolyte Multilayers

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Layer-by-Layer (LbL) assembly is a versatile technique for fabricating polyelectrolyte multilayers (PEMs) with nano-scale level controlled structure, composition, and morphology. Multilayer assembly and triggered disassembly can find a variety of applications ranging from surface modification and surface protective coatings to drug delivery. PEMs incorporating biocompatible components have been a focus of current research because their disassembly behavior can be stimulated by different external conditions.

In this work, we investigated the assembly and disassembly of multilayers composed of a strong polycation, poly(diallyldimethylammonium chloride) (PDDA) and a weak polyanion, poly(acrylic acid) (PAA) as model drug carrier systems. Our results showed that the film thickness, surface morphology and elastic modulus are dramatically affected by the molecular weight of PDDA. The disassembly kinetics of the PDDA-PAA multilayers with the variation of pH was different for two kinds of molecular weights of PDDA. The multilayers composed of higher molecular weight PDDA, which has greater affinity towards PAA, disassembled at a slower rate. The influence of solution pH is also very significant during disassembly. At higher pH values, the disassembly of high and low molecular weight systems was gradual, while both systems quickly collapsed upon exposure to solutions of lower pH.

Our findings can aid in optimizing the use of tunable multilayers in controlled drug delivery applications.

Key words: Layer-by-Layer (LbL) assembly, Tunable disassembly, Polyelectrolytes, Multilayers, Drug delivery systems