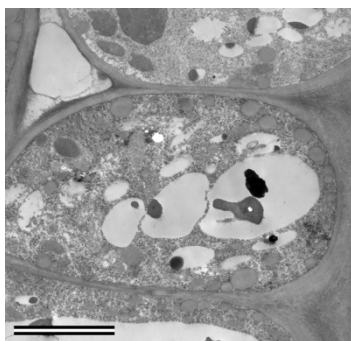
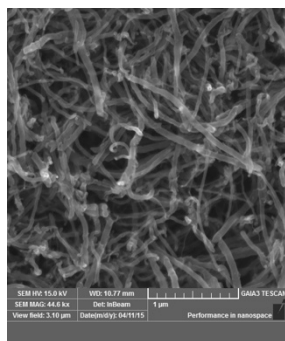
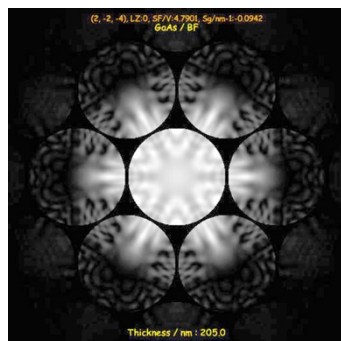




2015 Meeting
Friday, November 6, 2015
Oakland Center, Oakland University
2200 N. Squirrel Rd, Rochester, MI 48309



Our Sponsors:



Morning Talks in Gold Room C

8:30 – 8:55 Registration and Refreshments

8:55 – 9:00 Welcome and Opening Remarks – **Frank Giblin**, Director, Eye Research Institute, Oakland University

9:00 – 9:45 **John Mansfield**, Department of Materials Science and Engineering, Michigan Center for Materials Characterization, University of Michigan, MI:
Advanced Focused Ion Beam Techniques and 3D EDS and EBSD for Advanced Materials.

9:45 – 10:30 **Jay Jerome**, Vanderbilt University School of Medicine, Nashville, TN:
A Picture is Worth a Thousand Words but Quantitation is Worth a Thousand Micrographs

10:30 – 10:45 Break

10:40 – 11:30 **William Landis**, The G. Stafford Whitby Chair in Polymer Science, University of Akron, Akron, OH:
A Possible Role for the Small, Non-Collagenous Protein, Osteocalcin, in Collagen Mineralization of the Vertebrate Skeleton and Teeth

Gold Room B

11:30 – 13:30 Luncheon / Vendors / Poster Sessions

12:30 – 13:30 **Facilities Tours:** SEM – College of Engineering and Computer Science, Oakland University
TEM – The Ocular Structure and Imaging Facility, Eye Research Institute, Oakland University

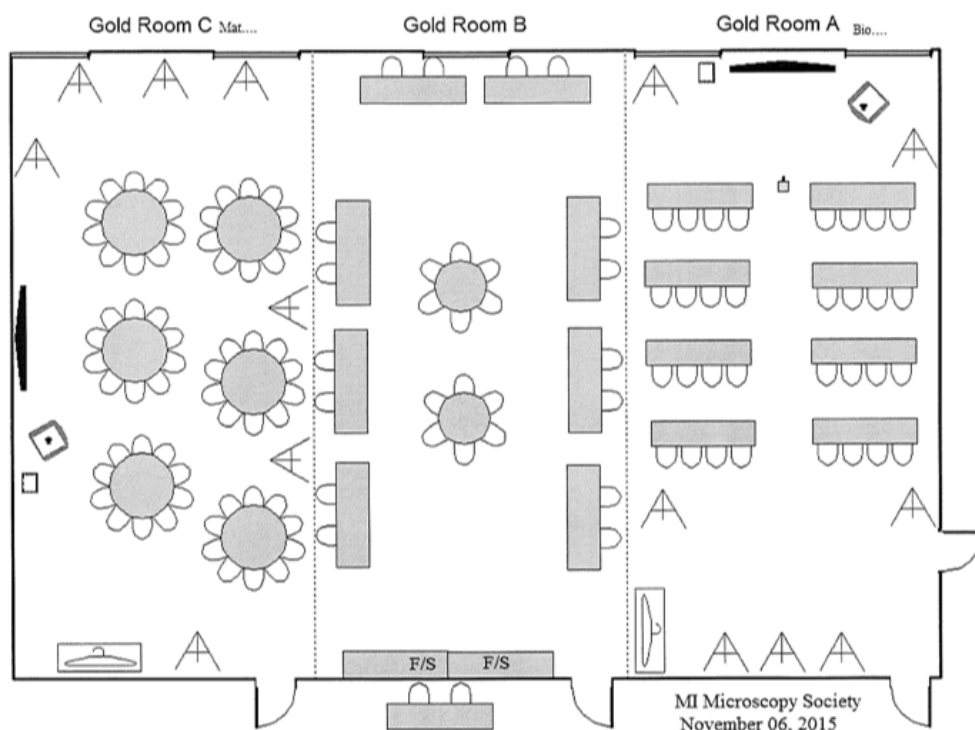
Afternoon Talks

Physical Science in Gold Room C 13:30 – 15:20

Biology in Gold Room A 13:30 – 14:50

16:25 – 16:40 Vickie Kimler: Awards and Closing Gold Room C

16:50 – 17:50 MMMS Business Meeting



Afternoon Talks**Physical Science in Gold Room C**

13:30 – 13:35 Moderator: Yi Liu

13:35 – 13:55 **Quantitative Characterization of Cellular Irregularities in Extruded Polystyrene Foam Using Digital Image Processing and Analysis**
V.A. Woodcraft, W.A. Heeschen, The Dow Chemical Company

13:55 – 14:15 **Ultralow kV Imaging and Microanalysis....Some Amazing Data and Some Words of Caution**
Vern Robertson, SEM Technical Sales Manager, JEOL USA, Inc

14:15 – 14:35 **Recent Advances in EBSD Acquisition, Analysis, and Applications**
Matt Nowell, EBSD Product Manager, EDAX Inc.

14:35 – 14:55 **TEM Studies in the Surface Oxide on the Activated Metal Hydride Alloys Suitable for Battery Applications**
K. Young, BASF/Battery Materials-Ovonix, and adjunct faculty Department of Chemical Engineering, Wayne State University, Detroit, MI

14:55 – 15:10 Break

15:10 – 15:30 **Aberration-Corrected Electron Microscopes in University of Michigan**
Kai Sun, Michigan Center for Materials Characterization & Department of Materials Science and Engineering, University of Michigan:

15:30 – 15:50 **Microscopic Study of Seed-Mediated Growth of Organic Crystals**
Guangzhao Mao, Professor and Chair, Department of Chemical Engineering and Materials Science, Wayne State University, Detroit, MI

15:50 – 16:10 **Probing Compositional Variation in Nanomaterials with Electron Microscopy**
Stephanie L. Brock, Department of Chemistry, Wayne State University

16:10 – 16:30 **New Techniques for Looking at Tricky Samples. High End S/TEM Developments**
Jan Ringnalda, FEI Company

Afternoon Talks**Biology in Gold Room A**

13:30 – 13:35 Moderator: Eric Linton

13:35 – 13:55 **Role of Vacuolar Protein Sorting 13C in Brown Adipocyte Lipid Metabolism**
Vanesa D. Ramseyer¹, Victoria A. Kimler² and James G. Granneman^{1,3} 1) Center for Molecular Medicine and Genetics, School of Medicine, Wayne State University; Detroit MI; 2) Ocular Structure and Imaging, Oakland University, Rochester, MI; 3) John D. Dingell VA Medical Center, Detroit, MI.

13:55 – 14:15 **Subcellular Optogenetics: Optical Control of Subcellular Signaling and Cell Behavior**
Kanishka Senarath, Praneeth Siripurapu, Sabrina Cereceres and Ajith Karunarathne, Department of Chemistry and Biochemistry, The University of Toledo, Toledo, OH

14:15 – 14:35 **Using Microscopy and Video Analysis to Quantify Parasite Activity for Metabolic Modeling**
Jason P. Sckrabulis, Karie A. Altman, Ryan B. McWinnie, Thomas R. Raffel, Department of Biological Sciences, Oakland University

14:35 – 14:55 **Decreased Retinal Dopamine in Oxygen-Induced Retinopathy is Caused by Loss of Dopaminergic Amacrine Cells**
Nathan Spix¹, Zhi-Jing Zhang², Lei-Lei Liu¹, Christophe Ribelayga², and Dao-Qi Zhang¹, 1) Eye Research Institute, Center for Biomedical Research, Oakland University 2) Ruiz Department of Ophthalmology and Visual Science, Medical School, The University of Texas Health Science Centre at Houston

14:55 – 15:10 Break

15:10 – 15:30 **Morphologies of Naphthalene-Based Valine and Phenylalanine Assemblies**
Sanela Martić, Paul Savage, Department of Chemistry, Oakland University

15:30 – 15:50 **Multidisciplinary Microscopic Imaging of Osteoarthritic Cartilage**
Ji Hyun Lee and Yang Xia, Department of Physics, Center for Biomedical Research, Oakland University:

15:50 – 16:10 **Bimolecular Fluorescence Complementation Microscopy for Investigating Protein-Protein Interactions In Situ.**
Andrew F.X. Goldberg, Linda Ritter, Nidhi Khattree, Beatrice Tam, Loan Dang, and Orson L. Moritz, Eye Research Institute, Oakland University, Rochester, MI

Posters in Gold Rooms

Name	Affiliation	Department	Title
<u>Jose O. Esteves-Villanueva</u> and Sanela Martic	Oakland University	Chemistry, Center for Biomedical Research	Tau Protein Aggregation Inhibition and Aggregate Dissolution via Immunotherapies
<u>Milstein, Michelle L.</u> Goldberg, Andrew F. Ritter, Linda M. and Khattree, Nidhi.	Oakland University	Eye Research Institute	Structure-Function Analysis of the Peripherin-2/Rds Cytoplasmic C-Terminal Domain
<u>Joshua Hohlbein</u> , Nathan Spix, and Dao-Qi Zhang	Oakland University	Eye Research Institute, Center for Biomedical Research	All Amacrine Cells are Lost in a Mouse Model of Oxygen-induced Retinopathy
<u>Caroline Cencer</u> , Frank Giblin, Shravan Chintala, Daniel Feldmann, Mirna Awrow, Nahrain Putris, Mason Geno, and Vidhi Mishra	Oakland University	Eye Research Institute	UVB-Exposed Human Lens Epithelial Cells Exhibit Two Phases of DNA Damage and Repair by PARP-1
<u>Khadijah AlmohammedAli¹</u> , <u>Hannah Klug¹</u> , Paul V. Zimba ² , and Eric W. Linton ¹	1) Central Michigan University 2) Texas A&M University Corpus Christi.	1) Biology 2) Center for Coastal Studies	Morphological Evaluation of Four Possible New to Science Cyanobacteria Using Light and Scanning Electron Microscopy
<u>Brandon Metcalf</u> , Camryn DeLooff, Austen Knapp, Jennifer Felisky, Kirsten Laux, Mrinalini Deshpande, Taisiya Jacobs, Wendelin Dailey, Fang Yang, Quentin Tompkins, Mei Cheng, Ed Guzman, and Kenneth Mitton	Oakland University	Eye Research Institute, Pediatric Retinal Research Lab	Spectral Domain Optical Coherence Tomography for Non-invasive Imaging of Rodent Retinal Disease Models
<u>Sheldon R. Gordon</u> and Geoffrey H. Gordon	Oakland University	Biological Sciences	Corneal Endothelial Cells Can Migrate Along their Basement Membrane During Wound Repair Independent of an Organized Actin Cytoskeleton
<u>Quentin Tompkins</u> , Camryn DeLooff, Austen Knapp, Jennifer Felisky, Kirsten Laux, Mrinalini Deshpande, Taisiya Jacobs, Wendelin Dailey, Fang Yang, Brandon Metcalf, Mei Cheng, Ed Guzman, and Kenneth Mitton	Oakland University	Eye Research Institute, Pediatric Retinal Research Lab	Micron III Camera for Non-invasive Imaging of the Retinal Microvasculature and Image-guided Focal-Electroretinography of Retinal Disease Models
<u>Elise M. Wight</u> and Cynthia K. Damer	Central Michigan University	Biology	Investigating Copine A's Involvement in Endolysosomal Pathways in <i>Dictyostelium discoideum</i> using Confocal Microscopy Techniques
<u>Ilacqua, AN</u> , Graham BN, Price JE, Buccilli MJ, McKellar DM, and Damer CK	Central Michigan University	Biology	Investigation of Copine Protein Localization in <i>Dictyostelium discoideum</i>
<u>Pratima Pandey</u> , Yingxin Li, and Carol Heckman	Bowling Green State University	Biological Science	Role of Protein Kinase C Isoforms in Regulating Filopodia Dynamics
<u>Swati Naik</u> and Gabriel Caruntu	Central Michigan University	Chemistry & Biochem / Science of Advanced Materials	Shape and Size-Controlled Synthesis of Fe Doped TiO2 Nanocrystals
<u>Tommaso Costanzo</u> and Gabriel Caruntu	Central Michigan University	Chemistry	Study of Size and Shape of BaTiO3 Nanocrystals for Enhanced Physical and Chemical Properties
<u>Roshini Pimmachcharige</u> and Stephanie L. Brock	Wayne State University	Chemistry	The Growth Mechanism of MnAs Nanoparticles: Optimizing Properties for Magnetic Refrigeration

Abstracts Keynotes Gold Room C

John Mansfield

Advanced Focused Ion Beam Techniques and 3D EDS and EBSD for Advanced Materials.

Department of Materials Science and Engineering, Michigan Center for Materials Characterization, University of Michigan
jfmjfm@umich.edu

The integration of the scanning electron microscope with the focussed ion beam workstation in the 1993, first by FEI, Inc. and then by the other major electron microscope manufacturers, Zeiss, JEOL, Hitachi and Tescan, heralded the use of such instruments for a wide range of materials science applications, rather than limiting them to edit and repair applications in the semiconductor manufacturing industry.

The University of Michigan Electron Microbeam Analysis Laboratory houses three Dualbeam™ FIB systems and they are used for a wide variety of applications in materials science and engineering, chemical engineering, nuclear engineering, electrical engineering, aerospace engineering and biomedical engineering. This presentation will focus on a number of example applications.

Jay Jerome

A Picture is Worth a Thousand Words but Quantitation is Worth a Thousand Micrographs

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jay.jerome@Vanderbilt.Edu

Quantification of structural information provides a number of advantages compared to qualitative assessment. However, quantification does require additional effort. The good news is that when done correctly, the effort required can be minimized and the resulting benefits maximized. Using my own research, this talk will highlight some relatively easy methods for gaining quantitative structural information that can be implemented in almost any laboratory and provide insights into how to avoid some common mistakes I have run across over the years.

William Landis

A Possible Role for the Small, Non-Collagenous Protein, Osteocalcin, in Collagen Mineralization of the Vertebrate Skeleton and Teeth

The G. Stafford Whitby Chair in Polymer Science, University of Akron, Akron, OH
wlandis@uakron.edu

Mineralization of vertebrate tissues such as bone, dentin, cementum, and calcifying tendon involves type I collagen, which has been proposed as a template for calcium and phosphate ion binding and subsequent nucleation of apatite crystals. Type I collagen thereby has been suggested to be responsible for the deposition of apatite mineral without the need for non-collagenous proteins or other extracellular matrix molecules. Based on studies in vitro, the small, non-collagenous protein, osteocalcin, is thought to mediate vertebrate mineralization associated with type I collagen. Osteocalcin, as possibly related to mineral deposition, has not been definitively localized in vivo. The presentation here identifies osteocalcin and its localization in the leg tendons of avian turkeys, a representative model of normal vertebrate mineralization. Immunocytochemistry of osteocalcin demonstrates its presence at the surface of, outside and within type I collagen fibrils. The association between osteocalcin and type I collagen structure is revealed optimally when calcium ions are added to the antibody solution in the immunocytochemical methodology. In this manner, osteocalcin is found specifically located along the a4-1, b1, c2 and d bands defining in part the hole and overlap zones within type I collagen. From these data, while type I collagen itself may be considered a stereochemical guide for intrafibrillar mineral nucleation and subsequent deposition, osteocalcin bound to type I collagen may also possibly mediate nucleation, growth and development of platelet-shaped apatite crystals. Osteocalcin

immunolocalized at the surface of or outside type I collagen may also affect mineral deposition in these portions of the avian tendon. Possible direct involvement of osteocalcin with type I collagen is a novel role for this small protein in normal vertebrate mineralization events in vivo.

Physical Science Talks Gold Room C

Quantitative Characterization of Cellular Irregularities in Extruded Polystyrene Foam Using Digital Image Processing and Analysis

V.A. Woodcraft, W.A. Heesch

The Dow Chemical Company

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Flow-induced irregularities, or patterns, in cellular structure can be sometimes observed in products made through an extrusion foaming process. These resultant inhomogeneities can lead to either beneficial or undesired effects on product perception and performance. Ubiquity of the foam patterns in combination with apparent difficulty in characterizing them lends itself to development of a robust analytical method of measuring the foam pattern manifestation severity.

The present report offers characterization of the foam pattern severity via carefully-designed image collection and image analysis. The image collection, processing procedures, and analysis described in this report generate images and results that are consistent with human perception of the foam pattern, and can classify the different patterns according to type and magnitude. The key component is isolating the pattern bands in the context of the cellular structure. Close size similarity between the foam cells and pattern bands, coupled with differences in machine and human perception of the pattern bands result in local ambiguity about the assignment of bright/dark patterns to cells versus foam pattern bands. However, once the band structure has been isolated from the cell-to-cell variability, the foam pattern can be analyzed directly.

Several approaches to image collection, processing, and analysis were explored in order to isolate and characterize the pattern, including lighting conditions, image filtering algorithms, brightness profile measurement, and shape characterization. Strengths and weaknesses of the various approaches will be discussed in the context of developing the final solution. Finally, the approach to correlating visual classification with image analysis will be described.

Ultralow kV Imaging and Microanalysis....Some Amazing Data and Some Words of Caution

Vern Robertson

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In just the last few years there has been a quantum leap in the ability of the scanning electron microscopes (SEM) to observe and chemically analyze a wide variety of materials from various fields of interest. Field Emission (FEG) SEMs provide the capability to create a very small probe diameter (high resolution imaging) at very low accelerating voltage (high resolution microanalysis) with high beam currents required for analysis and with exceptional surface detail and reduced beam specimen interaction in a bulk sample with previously unattainable nanometer scale resolution at landing voltages as low as 10V. However, these extremely low voltages come with some clearly defined sample preparation and handling procedures. Low voltage imaging has also been successfully employed as a key technique for charge control and reduction both for imaging and analyzing nonconductive specimens. Improvements in electron column optics and x-ray spectrometers especially for low energy (soft) x-ray lines opens up new avenues for specimen observation. These new state-of-the-art microscopes, detectors and spectrometer technology today allows one to overcome many of the historical limitations associated with low kV imaging and microanalysis. HOWEVER, there are some considerations that

may not have been thought about in the past. Some case studies and examples of the good things (and some of the bad) that can result from ultralow kV imaging and analysis will be presented. As you will see from the images and analyses, ultralow kV is a VERY POWERFUL tool, and as with all powerful tools, it needs to be used with caution (or at least with keeping an eye out for the non-intuitive).

Recent Advances in EBSD Acquisition, Analysis, and Applications

Matt Nowell

EBSD Product Manager, EDAX Inc.

Matt.nowell@ametek.com

Electron backscattered diffraction (EBSD) has become a well-established microstructural characterization technique in the materials and earth sciences. This SEM-based technique uses spatially specific crystallographic information to create orientation-based micrographs, in an approach termed Orientation Imaging Microscopy (OIM). Recent developments in sensor technology have enabled faster acquisition of EBSD diffraction patterns, and when coupled with an efficient pattern analysis routine, allow for comprehensive microstructural characterization in a period of minutes. This presentation will detail how improvements in acquisition speed capabilities can be applied to a range of materials. In addition, the fast detector technology can also be used as an imaging supplement for an SEM. With this approach, the EBSD detector is used as an array of positional backscattered detectors, in an approach termed PRIAS, or Pattern Region of Interest Analysis. The imaging contrasts detected by PRIAS will be detailed and how selecting and combining different detection regions can isolate and enhance specific contrast mechanisms for enhanced image detail. Finally, developing applications in the areas of lightweight structural materials development and additive manufacturing will be addressed.

TEM Studies in the Surface Oxide on the Activated Metal Hydride Alloys Suitable for Battery Applications

K. Young

BASF/Battery Materials-Ovonix, 2983 Waterview Drive, Rochester Hills, MI 48309, USA and adjunct faculty in the Department of Chemical Engineering, Wayne State University, Detroit, MI 48202, USA

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The surface oxide on a few activated metal hydride alloys were thoroughly studied with transmission electron microscope. Various metallic alloy inclusions, voids, and channels were found. While the metallic inclusions are crucial catalysts for the surface electrochemical reactions, the voids and channels are good for electrolyte storage and transportation, respectively. Based on the findings, we were able to improve the ultra-low temperature (-40°C) nickel metal hydride battery performance.

Aberration-Corrected Electron Microscopes in University of Michigan

Kai Sun

Michigan Center for Materials Characterization & Department of Materials Science and Engineering, University of Michigan, Ann Arbor, Michigan 48109

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During the past decade, great achievement has been made in the development of aberration-corrected electron microscopes (ACEM). Nowadays, ACEMs have been installed in most TEM labs worldwide. In the Michigan Center for Materials Characterization (former Electron Microbeam Analysis Laboratory) at the University of Michigan, a JEOL JEM 2100F equipped with a probe Cs-corrector and a JEOL JEM 3100R05 equipped with both a probe and a TEM Cs-correctors have been installed. This talk firstly introduces the brief history of the development of ACEM and then the performances of the two ACEMs@UMich with several studies on the characterization of some advanced materials using the two ACEMs exemplified.

Microscopic Study of Seed-Mediated Growth of Organic Crystals

Guangzhao Mao, Professor and Chair

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Atomic force microscopy, transmission electron microscopy, and field-emission scanning electron microscopy have been used to investigate seed-mediated nucleation of organic crystals. We discovered and now are examining the nucleation of organic nanowires from inorganic nanoparticle seeds. The research tests the hypothesis that the small size of nanoparticles alters the shape/size of ensuing organic crystals. The hypothesis has been tested on a wide range of materials including long-chain carboxylic acids, tetrathiafulvalene charge-transfer salts, and tetracyanoplatinate salts. The electrodeposition of gold nanoparticles (nucleation seeds) on highly oriented pyrolytic graphite (HOPG) electrodes was studied as a function of the electrolytic conditions. The gold nanoparticle size increases with increasing gold salt concentration and decreasing applied overpotential. The electrocrystallization of tetrathiafulvalene charge-transfer salt on the nanoparticle-decorated HOPG was investigated as a function of the charge-transfer salt concentration and nanoparticle seed morphology. We observed a preferential nucleation of the charge-transfer salt on the nanoparticle seed. The seed-mediated organic crystals display confined crystal morphology (nanowires) in comparison to those nucleated on bare HOPG. We also observed preferential nucleation of the organic crystal on high-energy faces rather than on the most prominent face of the gold nanoparticle seed. The nanoconfinement effect is attributed to the local curvature of the seed that imposes an interfacial strain on the nucleated crystals. This work provides a fundamental understanding of seed-mediated crystallization – a widely used industrial material separation and purification process. The research addresses an issue at the heart of nanotechnology – what is the smallest space necessary for molecular ordering? This study also contributes a solution-based method to incorporate nanowires into nanopatterns and nanodevices.

Probing Compositional Variation in Nanomaterials with EM

Stephanie L. Brock

Department of Chemistry, Wayne State University

sbrock@chem.wayne.edu

The Brock research lab is focused on (1) synthesizing discrete nanoparticles of transition metal pnictide (pnictogen = Group 15 element) nanoparticles for magnetic and/or catalytic applications and (2) developing sol-gel methods for metal chalcogenide nanoparticle assembly into films and 3-D architectures for photovoltaics and environmental remediation. With respect to the first project, our current focus is on creating solid-solutions of ternary phosphides, $M'M''P$ ($M = \text{Mn, Fe, Co, Ni}$) and homogeneous anion doping in arsenides ($\text{MnAs}_{1-x}\text{Px}$) and antimonides ($\text{MnSb}_{1-x}\text{Asx}$). In the second project, we aim to strategically introduce compositional heterogeneity into semiconducting chalcogenide nanostructures to facilitate charge separation and migration for photovoltaics. Examples of how HAADF-STEM and EDS mapping enable probing of nanoscale composition variations and phase solubility in these two projects will be presented.

New Techniques for Looking at Tricky Samples. High End S/TEM Developments

Jan Ringnalda

FEI Company

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Modern electron microscope systems allow flexible methods for imaging and analyzing complex and fragile materials systems. This presentation will illustrate some of the new methods that can be utilized to reveal structural and elemental details of samples in two as well as three dimensions. Limitations of the different techniques will be discussed, and some myths will be dispelled.

Biology Talks

Gold Room A

Bimolecular Fluorescence Complementation Microscopy for Investigating Protein-Protein Interactions In Situ.

Andrew F.X. Goldberg, Linda Ritter, Nidhi Khattree, Beatrice Tam, Loan Dang, and Orson L. Moritz.

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Vertebrate retinal photoreceptors mediate vision via a G- protein mediated signaling cascade organized within the outer segment (OS), a sensory organelle derived from a non- motile cilium. OSs possess a highly membranous "stacked disk" structure that is elaborate, dynamic and required for organelle function and photoreceptor viability. The molecular basis for OS architecture is not well understood. We have developed and applied a bimolecular fluorescence complementation (BiFC) approach which offers an unprecedented glimpse at the formation and trafficking of protein complexes in transgenic *Xenopus laevis* photoreceptors. Using laser scanning confocal microscopy and genetic fusions with split GFP variants, we show that protein-protein interactions for a variety of photoreceptor proteins, including peripherin/rds (P/rds), cyclic nucleotide-gated cation channel (CNGB1), glutamic acid rich protein 2 (GARP2), rhodopsin, and RIBEYE, can be visualized in fixed ocular cyrosections. This method demonstrates that although most interactions are initiated within cell soma, those mediating dynamic renewal of the "stacked disk" structure take place at the site of new disk morphogenesis. This work also provides a general new strategy for localizing protein-protein interactions in adult sensory neurons.

Role of Vacuolar Protein Sorting 13C in Brown Adipocyte Lipid Metabolism

Vanesa D. Ramseyer¹, Victoria A. Kimler², and James G. Granneman^{1,3}

1) Center for Molecular Medicine and Genetics, School of Medicine, Wayne State University; Detroit MI; 2) Ocular Structure and Imaging, Oakland University, Rochester, MI; 3) John D. Dingell VA Medical Center, Detroit, MI.

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Lipid droplets (LD) are dynamic organelles that regulate fatty acid storage and mobilization through changes in protein composition and targeting. However, the identity, function and regulation of many LD proteins remain largely unknown. Using proteomic analysis of brown adipose tissue (BAT) subcellular fractions, we identified a novel protein, vacuolar protein sorting 13C (VPS13C). We hypothesized that VPS13C is a LD protein that regulates lipolysis in brown adipocytes (BA). Analysis of VPS13C tissue distribution showed that BAT contains the highest levels of VPS13C protein. In cultured BA, VPS13C mRNA and protein levels significantly increased during differentiation. Cell fractionation and immunofluorescence studies confirmed that VPS13C is highly targeted to LDs. Interestingly, confocal and electron-microscopy experiments showed that VPS13C localized in LDs in a unique hemispheric pattern suggesting the existence of a novel LD subdomain. In vivo experiments in mice showed that cold exposure or β 3-adrenergic agonist treatment decreases VPS13C in BAT ($p < 0.04$ and $p < 0.03$ vs control respectively). Silencing of VPS13C in BA cell cultures doubled basal lipolysis ($p < 0.03$), increased lipolytic potency of isoproterenol by 3 fold ($p < 0.05$) and decreased cellular triglyceride content by 18% ($p < 0.05$). Finally, adipose triglyceride lipase targeting to LDs was increased by 3 fold in silenced cells ($p < 0.04$). In conclusion, VPS13C is a novel LD protein that is highly expressed in BAT and acts as a negative regulator of basal and β -adrenergic-stimulated lipolysis. VPS13C is targeted to a distinctive LD subdomain and we hypothesize that it plays a novel role in trafficking of neutral lipids in oxidative tissues.

Using Microscopy and Video Analysis to Quantify Parasite Activity for Metabolic Modeling

Jason P. Sckrabulis, Karie A. Altman, Ryan B. McWinnie, Thomas R. Raffel

Department of Biological Sciences, Oakland University

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Video analysis of the movement of microscopic organisms can be difficult and costly. It can also be difficult to quantify the metabolisms of microscopic organisms, which is desirable for determining the temperature-dependence of physiological processes. We addressed these problems by developing a microscopy technique to quantify activity levels of cercariae, the free-living infective stage of trematode parasites, and used these measurements as a proxy for metabolism. Trematode parasites are snail-borne parasites with complex life cycles. Using *Ribeiroia ondatrae* (tadpole parasite), *Trichobilharzia stagnicolae* (waterfowl parasite), and *Schistosoma mansoni* (human parasite) trematode cercariae, we took high-framerate video recordings to quantify swimming behavior at eight temperatures across the parasites' normal range. Utilizing ImageJ, we were then able to analyze the videos and quantify a number of behaviors, including swimming speed, body bends per second, and proportion of time active. We then used these data to parameterize non-linear performance curves describing the temperature-dependence of parasite activity, yielding estimates of key metabolic parameters including the activation energy for metabolism.

Decreased Retinal Dopamine in Oxygen-Induced Retinopathy is Caused by Loss of Dopaminergic Amacrine Cells

Nathan Spix¹, Zhi-Jing Zhang², Lei-Lei Liu¹, Christophe Ribelayga², and Dao-Qi Zhang¹

1) Eye Research Institute, Center for Biomedical Research, Oakland University 2) Ruiz Department of Ophthalmology and Visual Science, Medical School, The University of Texas Health Science Centre at Houston

Dopamine is an important neurotransmitter and regulatory molecule in the retina. It has been shown that dopamine deficiency in the retina contributes to retinopathy. The purpose of this study was to determine whether dopamine deficiency is involved in retinopathy of prematurity (ROP), a potentially blinding disease that primarily affects prematurely born infants. Using a mouse model of ROP, we demonstrate that dopamine levels are reduced by nearly 50% in mice with oxygen-induced retinopathy. In addition, mice with oxygen-induced retinopathy exhibit a significant loss of retinal dopaminergic neurons. Since dopaminergic neurons are the sole source of retinal dopamine, we postulate that the low levels of dopamine observed are a direct result of the loss of retinal dopaminergic neurons. Our results suggest that the myopia (nearsightedness) and visual deficits commonly observed in patients with ROP may be partially explained by low levels of retinal dopamine.

Morphologies of Naphthalene-Based Valine and Phenylalanine Assemblies

Sanela Martic, Paul Savage

Department of Chemistry, Oakland University

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Hydrophobic peptides are prone to self-assembly into fibrillary a structure due to extensive N2-sheet content, which makes them promising biomaterials. By functionalizing natural amino acids with aromatic core, naphthalene, new bioconjugates may be formed with interesting self-association patterns, morphologies and photophysical properties. While examples of naphthalene-based peptides exist, little is known about dinaphthalene systems. The self-assembly of N-, C-, or N-/C- modified Valine or Phenylalanine amino acids was investigated as a function of solvent type and external stimuli (heat or sonication). The morphologies of the resulting precipitates or gels were characterized by scanning electron microscopy. Overall, dinaphthalene bioconjugates were prone to self-assembly into transparent gels (fibrillary networks), while N-naphthalene bioconjugates did not exhibit any ability to self-associate.

Multidisciplinary Microscopic Imaging of Osteoarthritic Cartilage

Ji Hyun Lee and Yang Xia

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Articular cartilage is a thin layer of specialized tissue covering the load-bearing ends of bones in joints, to absorb shocks and to distribute loads. The motivation to study cartilage is the critical role of the tissue in the development of osteoarthritis (OA) and related joint diseases - which affects about 50% of the adult. Our knowledge of tissue degradation, however, is still far from satisfactory. This is because an insidious process characterized by subtle changes in tissue's fine structure / function / composition / interaction precedes silently the degradation of cartilage as a clinical disease. Consequently, an accurate diagnosis of early OA remains elusive in clinical practice.

For the last twenty-one years, our lab has been focusing on the study of articular cartilage using multidisciplinary imaging techniques, including microscopic magnetic resonance imaging (μ MRI), polarized light microscopy (PLM), Fourier-transform infrared imaging (FTIRI), microscopic computer tomography (μ CT), and transmission electron microscopy (TEM). Together with biochemical assays and biomechanical testing, we examine the influence of the molecular and microscopic changes on tissue's functional integrity, providing critical information towards the understanding, and ultimately, management of early arthritic diseases. In this general presentation, we will introduce broadly the capabilities of the research tools in our lab and some recent results.

Acknowledgement

This work has been supported by three R01 grants (AR045172, AR052353) from National Institutes of Health and an instrument endorsement from R B and J N Bennett. The authors are grateful the students and our collaborators who carried out the original cartilage imaging work.

Subcellular Optogenetics: Optical Control of Subcellular Signaling and Cell Behavior

Kanishka Senarath, Praneeth Siripurapu, Sabrina Cereceres and Ajith Karunarathne

Department of Chemistry and Biochemistry, The University of Toledo, Toledo, OH

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Ability to control cell signaling is a crucial aspect of pharmacology and disease management. Lack of approaches to do so precisely in space and time in single cells to exert control over signaling and cell behavior has been an impediment. Interfacing research strategies in chemistry, biology and biophysics, we develop and employ optogenetic approaches to control signaling in subcellular regions of single cells, visualize molecular and cellular responses and dissect signal transduction pathways pertaining to pathologically important cell behaviors.

Intricate cell behaviors such as cell migration and neuron development require an asymmetric signaling activation and play crucial roles in immune system function, neurodegenerative diseases and cancer. They are governed by confined activation of G protein coupled receptors (GPCRs) on the plasma membrane by localized chemical gradients. We engineer opsin, cryptochrome and phototropin based optogenetic-signaling triggers and employ them to optically control (i) GPCR signaling networks in its entirety, (ii) specific G protein subunits and (iii) selected signaling proteins in desired subcellular regions. Precise spatiotemporal optical control over cell migration helps identify signaling activities and cross talk between GPCR active leading edge and inactive trailing edge. Optical control over signaling shows that, while neurite outgrowth and cell migration share a substantial molecular similarity, immune cells and neurons decode the same signal diversely to elicit different cellular responses. Overall, our subcellular optogenetic

approaches can have wide applicability in many disciplines experimentally and therapeutically.

POSTERS Gold Rooms

Biology 1

Tau Protein Aggregation Inhibition and Aggregate Dissolution via Immunotherapies

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Tau protein fibrils are pathological hallmarks of several neurodegenerative disorders such as Alzheimer's disease, dementia, and tauopathies. Tau cannot undergo aggregation, but phosphorylated tau forms neurofibrillary tangles and paired helical filaments, among other structures. In vitro tau aggregation may be induced by the presence of anionic agents. The interest in therapeutic targeting tau pathology has sparked drug design and development. Lately, immunotherapies in mouse models have succeeded in reducing tau aggregate levels and promoting aggregate clearance. Typical immunotherapies are mixtures of complex antibodies which make the identification of the inhibitor antibody challenging. To gain insight into the site-specific targeting of tau via antibodies, we have investigated four monoclonal and polyclonal antibodies and a commercially available immunotherapeutic formulation. The antibodies used were targeting specific tau domains: N-terminal, C-terminal, or R (R1-R4) domains. Two different approaches were used: 1) prevention of tau aggregation and 2) dissolution of tau aggregates in the presence of antibodies. The addition of specific antibodies to the reaction of tau441, longest tau isoform, and arachidonic acid disrupted the aggregation process. For dissolution studies, preformed tau aggregates were treated with antibodies. The transmission electron microscopy (TEM) was used to confirm tau aggregates, monitor tau aggregation, detect aggregation inhibition, and aggregate dissolution

Biology 2

Structure-Function Analysis of the Peripherin-2/Rds Cytoplasmic C-Terminal Domain

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Several inherited retinal diseases are caused by defects in peripherin-2/rds (P/rds), an integral membrane protein essential for proper formation of outer segment (OS) membranes of rod and cone photoreceptors. Although many pathogenic mutations are located within the protein's C-terminus, the normal function of this domain is not known and the means by which mutations cause retinal degenerations are not understood. This study investigates the importance of an inducible amphipathic helix (AH) within this region. Expression vectors for WT P/rds and a mutant lacking the AH (P/rds Δ AH) were used to generate stable HEK293 cell lines and transgenic *X. laevis*. Western blotting, ICC, IHC, LSCM, and TEM were applied to both models to characterize biosynthesis, subunit assembly, trafficking, localization, and membrane shaping activity of P/rds Δ AH relative to WT P/rds. Results: The P/rds Δ AH mutant properly folds and assembles into non-covalent tetrameric complexes and polymeric chains of disulfide-bonded tetramers in HEK293. This mutant, like WT P/rds, is largely released from the secretory pathway and accumulates within distinct intracellular membranes. TEM analysis demonstrated that both P/rds Δ AH and WT P/rds induce high curvature tubulo-vesicular membrane structures in stable HEK293 cells. In transgenic *X. laevis*, the P/rds Δ AH mutant is efficiently trafficked from its inner segment site of biosynthesis to its OS site of function, similar to WT. The inducible AH in the

P/rds C-terminus is not required for protein biosynthesis, subunit assembly, disulfide-mediated tetramer polymerization, generation of high curvature membranes, or trafficking to rod OSs in vivo. These results contrast with our previous finding that a peptide mimetic of the AH alone can induce high curvature membranes in vitro; thereby raising the question of whether this AH functions in concert with other protein domains for generating membrane curvature, or whether it plays an alternative primary role.

Biology 3

All Amacrine Cells are Lost in a Mouse Model of Oxygen-induced Retinopathy

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The retina is a patchwork of many different specialized neurons that all work together to pass visual stimuli to the brain for interpretation. Of all these neurons, this study focuses solely on the All amacrine cell, an interneuron that transmits rod signals from the outer to the inner retina. Specifically it looked at how the cell reacts when subjected to the low-oxygen environment caused by oxygen-induced retinopathy, a condition similar to retinopathy of prematurity seen in humans. It was hypothesized that these cells exhibit substantial cell loss due to the hypoxic atmosphere surrounding them. To test the hypothesis, one week old pups were subjected to a high- oxygen environment for five days. This caused the vasculature in the retina to regress, leaving the inner portion with very little blood and oxygen supply. The mice were then sacrificed at either postnatal day 17 or 42, their eyes removed, and the retinas isolated. The retinas were then immunohistochemically stained to visualize the All amacrine cells under a fluorescent microscope to test if there is cell loss present in the experimental group compared to the untreated control group. Once stained, the treated retinas exhibited All amacrine cell loss in the central retina where most a vascularization had occurred. The cell death was evident in the P17 models and remained consistent through P42, indicating that the cell loss is permanent. The data suggests that All amacrine cell death may play a vital role in the loss of scotopic vision exhibited by patients with retinopathy of prematurity.

Biology 4

UVB-Exposed Human Lens Epithelial Cells Exhibit Two Phases of DNA Damage and Repair by PARP-1

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Purpose: DNA present in the human lens epithelium is a target for UVB-induced damage, which can result in cataract formation. The nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) aids in the repair of DNA strand breaks by producing polymers of ADP-ribose units (PAR) from NAD⁺. Here, we investigate PARP-1 and PAR repair of damaged DNA in UVB-exposed cultured human lens epithelial cells (LECs). Methods: Cells were exposed to UVB light for 2.5 min (280- 380 nm wavelength, 312 nm peak, 0.9 mW/cm²), and then cultured normally. At various times after exposure, fluorescence immunocytochemistry was employed to detect DNA strand breaks, reactive oxygen species (ROS), and levels of PARP-1 and PAR in fixed cells. Results: UVB-exposed cells appeared stressed, but did not suffer extensive cell death. Levels of PARP-1 fluorescence in the cell nucleus remained constant in control and UVB- treated cells at all time periods. DNA strand-breaks and increased levels of PAR were evident at 5 min, and again at 90 min after exposure, but not at 30 and 60 min. Inhibition of PARP-1 blocked all PAR formation. Interestingly, ROS were detected only at the 90 min time period. Conclusion: Biphasic damage and repair of DNA in UVB- exposed human LECs were observed to occur at 5 min and 90 min post irradiation. This may be due to direct and indirect effects of UVB

on the DNA. The results indicate that PARP-1 and PAR play active roles in protecting the human lens against UVB light.

Biology 5

Morphological Evaluation of Four Possible New to Science Cyanobacteria Using Light and Scanning Electron Microscopy

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Cyanobacteria are bacteria that gather energy through photosynthesis and live in aquatic (freshwater and marine) as well as terrestrial environments. Because of this diversity many different species of cyanobacteria exist. While limited, morphology is one of the key factors considered when identifying and classifying species. This study looked at the morphology of four possible new to science species of cyanobacteria by using light and scanning electron microscopy. The four species were: *Hildebrandia triangulus* Gen. nov. & sp. nov. found in a Colorado marine rearing facility; *Plankothrix* sp. nov. from a shrimp farm in Cartagena, Columbia; an unidentified species from Lonestar pond, Texas; and an unidentified filamentous species. Data on each species size, shape, colony and mucilage forming ability was collected. This data will be combined with TEM studies, DNA sequence and fatty acid analysis to determine whether each is a known or new to science species.

Biology 6

Spectral Domain Optical Coherence Tomography for Non-invasive Imaging of Rodent Retinal Disease Models

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Study of retinal development and disease relies heavily on the use of animal models, particularly rodent models. Spectral Domain Optical Coherence Tomography (SD-OCT) uses magnification through the eye's physiological optics to construct a detailed three-dimensional image as well as a cross sectional image of the eye. Resolution along the scan beam axis is 0.001 mm. Several rodent models of retinal disease involving photoreceptor degeneration, ischemic injury, death of the inner retina, and neovascularization are routinely imaged with SD-OCT instrument. The non-invasive nature of this imaging permits visualization in the living eye. Combined with software analysis of three-dimensional topography, retinal layer thickness can be monitored covering a large retinal area over time. In this study, we evaluated effects of VPA treatment on different retinal degeneration mice including RD-1 RD-10 and RD-S. Both acceleration and slowing of receptor loss were seen. We also used SD-OCT imaging to observe physical changes in retinal vein dilation during testing with different isoforms of VEGFA.

Biology 7

Corneal Endothelial Cells Can Migrate Along their Basement Membrane During Wound Repair Independent of an Organized Actin Cytoskeleton

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The role of actin reorganization during wound repair following a central freeze injury to organ cultured corneal endothelial cells has been examined. Normally, these cells reorganize their peripheral actin bands into stress fibers and migrate into the injured area. In order to ascertain the significance of this

rearrangement relative to wound repair, some tissues were incubated in 4 μ M TRITC- conjugated phalloidin overnight and then injured. Observations up to 24h after wounding indicated that despite a lack of actin organization, cells responding to the injury appeared morphologically identical to their control counterparts. Tissues were also cultivated in the presence of either cytochalasin B (CB), soybean agglutinin (SBA) or fluorouracil (FU), any of which also resulted in actin cytoskeletal disruption. Under these conditions cell movement continued despite the absence of detectable stress fibers. For SBA, CB and FU treated tissues; wound repair rates did not significantly differ from control preparations although FU treated tissues showed a slightly slower rate of repair. Electron micrographs confirmed an absence of organized actin in migrating cells of the injured tissues treated with any of the agents. Therefore, results presented here indicate that although corneal endothelial cells employ actin reorganization and normally form stress fibers during injury-induced cell movement, this response does not appear to be an absolute requirement for these cells to migrate along their basement membrane during wound repair.

Biology 8

Micron III Camera for Non-invasive Imaging of the Retinal Microvasculature and Image-guided Focal-Electroretinography of Retinal Disease Models

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Using the Micron-III camera system we have developed methods to monitor the rodent retinal microvasculature and disruption of the blood retinal barrier, in vivo. These non-invasive techniques can be used to examine the effects of various stresses as well as biological agents delivered systemically or via intraocular injection. Fluorescein Angiography permits the live real-time imaging of the smallest capillaries in both the mouse and the rat eye. Zones of vascular ablation in the mouse Oxygen Induced Retinopathy model (OIR) were visualized in the immature eye, to predict regions that subsequently suffered the most severe loss of bipolar neurons due to retinal ischemia. We have also developed a method to use Evan's Blue angiography to compare the permeability of the blood retinal barrier as affected by challenges with different isoforms of VEGFA, in vivo. Retinal imaging was also developed with dim red-light illumination to enable the image-guided targeting of focal-ERG testing in the mouse eye. Using this new technique we can test the electrophysiological response of very small, and different, retinal locations within the same eye of live animals. Furthermore, we combined SD-OCT imaging during real-time visualization by Fluorescein Angiography to correlated ischemic zones with the loss of bipolar neurons and the focal-ERG B-wave response. This has established that surviving inner retinal neurons are capable of establishing and recovering synaptic functionality after severe ischemic damage to the neural retinal network.

Biology 9

Investigating Copine A's Involvement in Endolysosomal Pathways in *Dictyostelium discoideum* using Confocal Microscopy Techniques

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Copines are phospholipid membrane binding proteins found in eukaryotic organisms, such as the single-cell amoeba, *Dictyostelium discoideum*. This family of proteins bind to membranes in a calcium-dependent manner, but their specific functions are unknown. Our previous studies indicated that CpnA tagged with green fluorescent protein (GFP-CpnA) specifically associates with the plasma membrane and several organelles including endosomes, lysosomes, phagosomes, and contractile

vacuoles. Previous studies also indicated that CpnA is able to bind actin filaments in a calcium-dependent manner. Actin filaments function in the biogenesis of post-lysosomes, a pH neutral secretory lysosome and we are using cpnA- cells to investigate the function of CpnA in this process. We used immunofluorescence and confocal microscopy to image and count the number of post-lysosomes in cpnA- cells and wild-type cells, with cpnA- cells having 26% fewer post-lysosomes. In addition, we treated live cells with fluorescein isothiocyanate (FITC)- and tetramethylrhodamine (TRITC)-labeled dextran and imaged cells every 15 minutes over a 2-hour period. The FITC is only fluorescent in the neutral post- lysosome, so that lysosomes appear red and post-lysosomes appear yellow. The number of post-lysosomes per cell were counted at each time interval. Both cpnA- and WT cells developed post-lysosomes at a similar rate with similar numbers of post-lysosomes peaking at 0.5 hours. However, the cpnA- cells appeared to have smaller post-lysosomes and to expel them at a faster rate with 53% fewer post-lysosomes per cell than WT at 1.75 hours. These results indicate that CpnA has a role in lysosomal and post-lysosomal maturation and/or trafficking.

Biology 10

Investigation of Copine Protein Localization in *Dictyostelium discoideum*

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Copines are calcium-dependent phospholipid binding proteins. Copines have been suggested to be involved in calcium-dependent signaling pathways that regulate a variety of cellular processes. We chose the model organism *Dictyostelium discoideum* to investigate the function of the six copine proteins, CpnA-CpnF. Cell lines expressing CpnA, CpnB, CpnC, CpnE, and CpnF were tagged with GFP at the N-terminus (GFP-Cpn) or the C-terminus (Cpn-GFP). We used confocal microscopy to visualize the location of GFP- tagged copines in fixed and live cells. We fixed cells with either 3.7% formaldehyde in phosphate-buffered saline (PBS) or 1% formaldehyde in methanol. In general, the GFP- tagged copine localized to the cytoplasm in live cells and cells fixed with PBS fixative. When live cells were treated with a calcium ionophore or cells were fixed with methanol fixative, the GFP-tagged proteins localized to the plasma membrane and intracellular vacuoles. GFP-tagged CpnB, CpnC, and CpnF were found in the nucleus. To further investigate the binding characteristics of copines, we performed membrane binding assays and found that all of the GFP-tagged copines bind to membranes and all, except CpnF- GFP, exhibit calcium-dependent membrane binding. To determine the specific types of lipids copines bind, GFP- tagged copines were purified and used in membrane lipid dot blot assays. GFP-tagged copines bound to a variety of acidic phospholipids. The calcium-dependent localization of copines to the plasma membrane and intracellular vacuoles suggests copines play a role in cell signaling, membrane trafficking, and the localization of CpnB, CpnC, and CpnF to the nucleus suggests they function in transcriptional regulation.

Biology 11

Role of Protein Kinase C Isoforms in Regulating Filopodia Dynamics

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Filopodia are sensors. They act like "antennae" of the cell, sensing both the soluble ligands in the environment and the substrate. Filopodia have an important role in directing motility and axon pathfinding. Filopodia are sites for adhesion and signaling, and their composition is well understood. Previous work shows that oncogenic transformation of epithelial cells was accompanied by the loss of filopodia. Therefore, the mechanisms of assembly and disassembly were interesting. Our hypothesis was that PKC activation affects the parallel arrays of actin

filaments and disassembles them. PMA is a type of tumor promoter, which is also known to be an activator of PKCs (classical and novel). Synthetic peptides of hydrophobic segment of PKC and the phosphorylation sites on MARCKS (myristoylated alanine-rich C kinase substrate) were designed to block PKC action and see how it affects the filopodia. Phase contrast microscopy was used to analyze the periphery of the cells after the treatment with various PKC and MARCKS peptides in presence and absence of PMA. Two variables were measured: Percentage periphery of cells covered with filopodia and percentage of cells in a population showing filopodia. When cells were treated with peptides alone, the effects were very small, but when treated with PMA in addition, some peptides rescued the filopodia from destruction. With some treatments, PMA had the unusual effect of enhancing filopodia formation or stability. In this case, the same peptides that had rescued them now counteracted the enhancement by PMA. We conclude that one isoform of PKC, PKC epsilon, exerts negative control. Mechanisms of positive control remain to be tested.

Physical Sciences 1

Shape and Size-Controlled Synthesis of Fe Doped TiO₂ Nanocrystals

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Anatase TiO₂ is one of the important semiconductors and finds application in fields ranging from photocatalysis, solar cells, gas sensors etc. Controlling shapes and sizes of TiO₂ particles is crucial to construct materials with desired properties and doping with impurity ions in-turn allows tuning of the electronic states. The coupling of impurity ion doping and hydrothermal synthesis has not been explored previously. In this work, we employ hydrothermal synthesis for obtaining uniform monodisperse nanocrystals of TiO₂ as well as dope with different Iron (Fe) concentrations. The effect of using two distinct capping agents, oleic acid and oleylamine results in different shapes and sizes of TiO₂. The morphology obtained in pristine TiO₂ is retained in Fe doped TiO₂. The phase purity is obtained using X-Ray diffraction and Raman spectroscopy. FTIR is employed to understand surface functionalization of the nanoparticles. Transmission Electron Microscopy is used to monitor the shape and size of nanocrystals obtained.

Physical Sciences 2

Study of Size and Shape of BaTiO₃ Nanocrystals for Enhanced Physical and Chemical Properties

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As well-known, chemical and physical properties of nanomaterials are strongly affected from the particles morphology. For example the Curie temperature of BaTiO₃ decrease from 120°C to lower temperature when the crystals becomes smaller in size. The morphology, on the other hand, can affect chemical behavior of the materials in application like catalysis. In fact, different particles shape are related to different lattice plane terminations, and the reactivity of the surface is mostly related to the specific termination. We have studied the effect of different capping ligand in the hydrothermal synthesis of BaTiO₃ nanoparticles by TEM. We have also compared the morphology observed with calculated Wulff shape of different low index lattice plane, and we found that other than the usual cubic shape of BaTiO₃ terminated on the {001} family of plane, it is possible to synthesize nanoparticles with a dodecahedron shape with facets terminated on the {110}. Furthermore, HRTEM revealed the lattice fringes, from which is possible to confirm the surface termination as a function of the capping ligand.

Physical Sciences 3

The Growth Mechanism of MnAs Nanoparticles: Optimizing Properties for Magnetic Refrigeration

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MnAs is known to have a large magnetocaloric effect associated with a first order magnetostructural phase transition making it potentially an interesting phase for magnetic refrigeration applications, but its high thermal hysteresis prohibits efficient cycling and the sharpness of the transition limits the temperature range for operation. Reduction of particle size and doping of MnAs can be used to tune the phase transition temperature and degree of hysteresis, but size control alone can be challenging if little is quantitatively known about crystallization process. Here we describe quantitative determination of particle growth during the MnAs synthesis reaction revealing a pathway to establish a reliable, reproducible method to control the size and composition of nanoparticles. The effect of size and composition on the phase change temperature is probed by powder X-ray diffraction and the effect of size on the thermal hysteresis is evaluated by magnetic susceptibility measurements. The morphology and the composition of the nanoparticles are analyzed by electron microscopy techniques. These data will be presented and the mechanism of particle growth will be discussed.

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