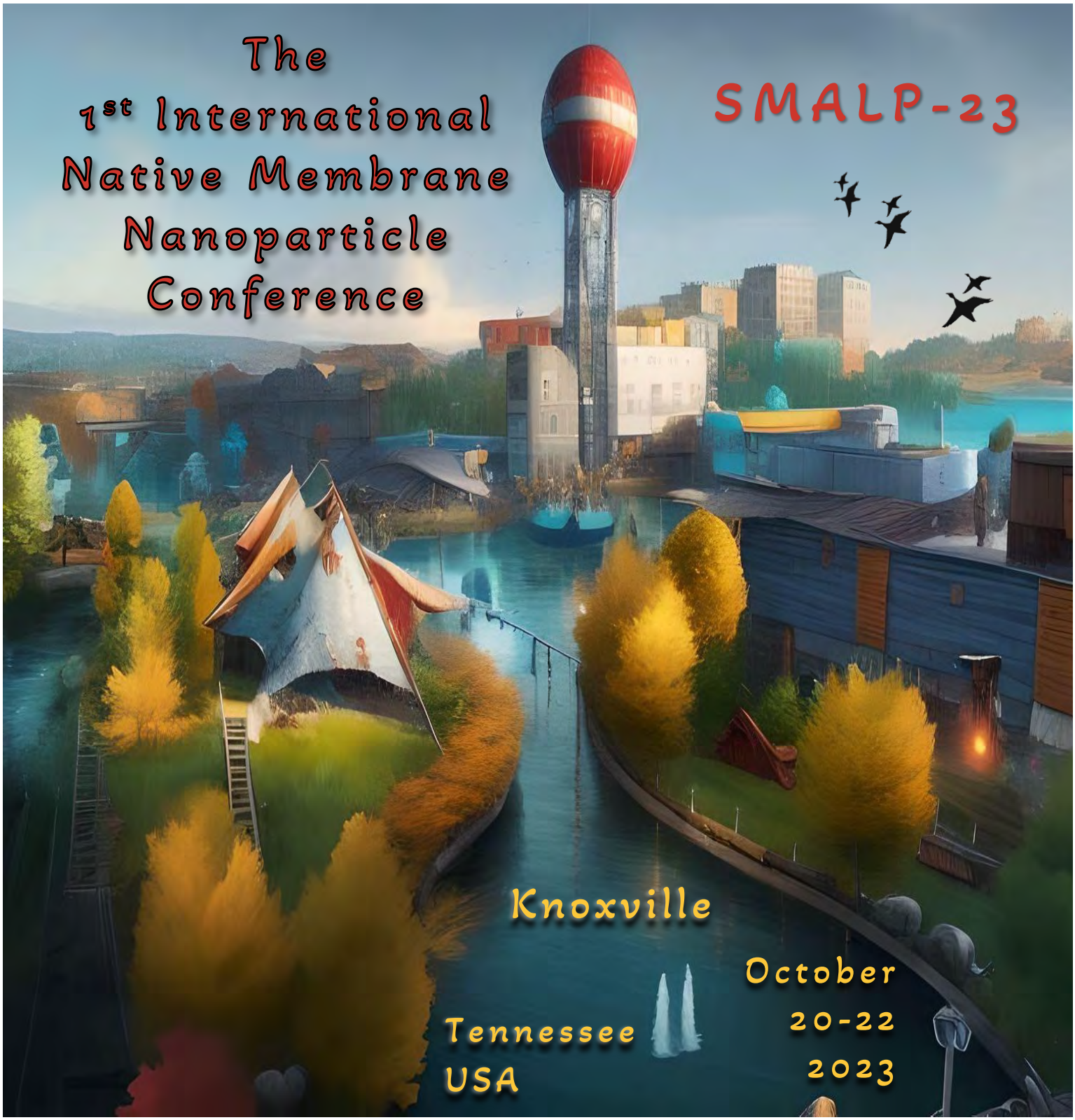
The
1st International
Native Membrane
Nanoparticle
Conference
SMALP-23
Knoxville
Tennessee
USA
October
20-22
2023

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SMALP-23 CONFERENCE

This inaugural **International Conference on Native Membrane Nanoparticles** is being held in Knoxville, Tennessee from Oct 19 -22, and will bring together leading scientists from various disciplines, including biochemistry, biophysics, spectroscopy, chemical biology, polymer chemistry, advanced imaging, and cell biology.

The development of innovative extraction methods, such as copolymers like SMA, membrane protein scaffolds, saposins, peptidiscs, and native vesicle extractionhas driven progress in this field. Amphipathic polymers fragment membranes into water-soluble discs, allowing the complex structures and interactions found in vivo to be discerned, without requiring synthetic detergents or artificial lipids. This is yielding new insights, tools and models of how membranes operate and can be manipulated.

Registration

- **Register for the Conference**
- Free Bus tour to Oak Ridge National Lab's **Spallation Neutron Source** on Oct 23 requires registration and visitor badging via the ORNL Personnel Access System prior to entrance. The bus will pick you up at Hotel and return ~12:30PM. If you did not register initially, please contact bbruce at utk.edu and we will see if you can still be added.

Accommodation

- **Hilton Knoxville Hotel** (1-865-523-2300) is a one minute walk, price is $150 per night for Oct 19-22 using Promotion Code: SMALP2
- **Cumberland House** (1-865-971-4663) is a 9 min walk, price is $103 per night for Oct 19-22 using Promotion Code: 90J
- **Note**: there are a limited number of discounted rooms, these will expire soon so please book ASAP

Venue: **University of Tennessee Conference Center**, Knoxville, TN

Dates: The scientific sessions run from 9am on Oct 20, 21 and 22. Also included are a workshop, poster sessions, coffee from 8am, lunches, conference dinner on Saturday

Travel: Taxi or uber from **McGhee Tyson Airport** (TYS) to the conference hotels in 20 minutes, parking vouchers available. Other airports in

SMALP News

FEBS Webinar on Oct 12, 17:40-19:00 West by Gisou can der Goot on *How S-acylation affects all major cellular processes: focus on SARS-CoV2 spike*

Stefan Scheidelaar is now Manager R&D Silicones at BRB International BV, having previously been Manager of Analytical SMA Chemistry at Aurorium (Polyscope), which **Giri Menon** left to become MD at Neuartis Pte Ltd.

Cube Biotech introduced six new nanodisc copolymers: **Sulfo-SMA**, **Sulfo-DIBMA**, and the **SMALP BZ** series made by RAFT polymerization in collaboration with Bert Klumperman and **Nanosene** for better size definition and low dispersity.

Anatrace has released fluorinated Fos-Choline and amphipols for applications including **CryoEM structure determination**.

Biotherapeutics is a high growth industry, with biologics set to comprise most of the 100 top selling drugs in 5 years according to **Cytiva**.

Avanti offers webinars on Sept 12&13 about **Virodex**, a superior replacement for the now-banned and environmentally harmful Triton X-100 detergent.

David Swainsbury, U East Anglia talks about *Photosynthetic membrane proteins demonstrate the utility of SMA copolymers* at Cube Biotech's **Intelligent Infusions Masterclass** series on October 12 at 4pm CET.

Vertellus, which acquired Polyscope this year

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SMALP-23- Keynote Speakers

Sandro Keller
Univ. of Graz, AU

New Native Nanodiscs
for Membrane-Protein Biophysics

Alice Rothnie
Aston Univ., UK

My SMALP journey.
How it started....how it's going.

Chuck Sanders
Vanderbilt Univ., USA

How Amphipol Polymers and their
Applications in Membrane Research
Differ from SMALP Polymers

Stephen Sligar
Univ. Illinois, USA

Investigating cancer signaling
And vaccine development
with MSP-based nanodiscs

October 20 Friday (full Day)

		Keynote	30 min	15 min		
	8:00-9:00	Coffee			**Chair**	**Floor Aids**
	9:00-9:10	Welcome and Opening				
	9:10-9:40	I. Talk 1 Overduin	1		**S. Chair #1 Bruce/ Overduin**	**Ali & Rao**
Cell Biology	9:40-10:10	I. Talk 2 Booth	2			
	10:10-10:25	S .Talk 1 Schuck		1		
	10:25-10:40	S. Talk 2 Deitz		2		
	10:40-11:00	Coffee & Break				
	11:00-11:30	I. Talk 3 Klumperman	3			
	11:30-11:45	S. Talk 3 Long		3		
Polymers	11:45-12:00	S .Talk 4 Ball		4	**S. Chair #2 Long**	**Penneru/ Pruitt**
	12:00-12:15	S .Talk 5 Ghosh		5		
	12:15-12:30	S. Talk 6 Rotich		6		
	12:30-2:00	Lunch/posters				
	2:00-2:30	I. Talk 5 Nevzorov	4			
NMR	2:30-2:45	S. Talk 7 Ravula		7	**S. Chair #3 Liang**	**Zhao & Moore**
	2:45-3:00	S .Talk 8 Shah		8		
	3:00-3:15	S. Talk 9 Okorafor		9		
	3:15-3:30	Coffee Break				
	3:30-3:45	S .Talk 10 Hawkins		10		
	3:45-4:00	S .Talk 11 Aracrio		11		
Protein/lipid	4:00-4:15	I. Talk 6 Kubicek	5		**S. Chair #4 Barerra**	**Ward/ Ryback**
	4:15-5:00	KEYNOTE Sliger		1		
	5:00-6:30	Happy Hour/posters (ODD# ~15)			**Poster Judging**	

Dinner (Free)

October 21 Saturday Full Day

		Keynote	30 min	15 min		
	8:00-9:00	Coffee				
	9:00-9:10	Welcome and Updates				
	9:10-9:40	I. Talk 7	Gupta	7		
MS	9:40-10:10	I. Talk 8	Marty	8	**S. Chair #5 Kubicek**	**Ochola & Workman**
	10:10-10:25	S. Talk 12	Wei	12		
	10:25-10:45	Coffee & Break				
	10:45-11:15	I. Talk 9	Bhattcharyya	9		
	11:15-11:45	I. Talk 10	Schmidt	10	**S. Chair #6 Lamichhane**	**Wei & Gonneville**
	11:45-12:30	KEYNOTE	Rothnie	2		
Membrane Signalling	12:30-2:00	Lunch/posters				
	2:00-2:30	I. Talk 9	Liang	11		
	2:30-2:45	S. Talk 13	Brady	13	**S. Chair #7 Bruce**	**Russell & Khanal**
	2:45-3:00	I. Talk 14	Fiebig	14		
	3:00-3:15	Coffee Break				
	3:15-4:00	KEYNOTE	Keller	3		
Energetics	4:00-4:15	S. Talk 15	Chandrasekhar	15	**S. Chair #8 Kumperman**	**Houee & Maity**
	4:15-4:30	S. Talk 16	Nikfrajam	16		
	4:30-5:45	Happy Hour/posters (EVEN # ~15)				
	5:45	Transport/Walk to Riverboat				
	6:00-8:30	Conference Riverboat Dinner				
	>8:30	Free time to Explore Knoxville				

October 22 Sunday Half/Day

Keynote | 30 min | 15

	8:00-9:00	Coffee				**Chair**	**Floor Aids**
	9:00-9:30	I. Talk 14	Guo	12			
Cryo-EM	9:30-10:00	I. Talk 15	Matthies	13		**S. Chair #9 Oneill**	**Agyemang & Suresh**
	10:00-10:15	S .Talk 16	Umbach		17		
	10:15-10:30	S .Talk 17	Massemburg		18		
	10:30-10:45	Coffee & Break					
	10:45-11:15	I. Talk 16	Bao	14			
Misc.	11:15-12:00	KEYNOTE	Sanders		4	**S. Chair #10 Shuo**	**Adetunji & Rodriguez Garcia**
	12:00-12:30	Awards & Meeting Closure				**Overduin/ Bruce**	
	12:30	Box Lunch					
	>12:30	Free time and Departure					

ORNL SNS TOUR

Tour date/time:

Monday Oct 23

Start time: 9.00 AM

End time: 12.00 PM

Pick up/Drop off:

Downtown Hilton
501 West Church Avenue
Knoxville, Tennessee, 37902-2591, USA
https://www.hilton.com/en/hotels/knxkhhf-hilton-knoxville/

Participants are required to bring a photo identification and appropriate visa paperwork as detailed in the invitation sent by Oak Ridge National Laboratory.

SELECTED TALKS: NUMBERS, PRESENTER, AND TITLES

ST-1 **Mark J. Arcario** PI Wash. Univ.
Larger nanodiscs support a more native-like membrane environment across a range of lipid types

ST-2 **Lauren Ball** Graduate Student Stellenbosch Univ.
Universal-RAFT mediated synthesis of double hydrophilic SMA-*b*-PVP copolymers for surface immobilization of SMALPs

ST-3 **Brian K. Long** PI UTK
Alternatives to SMA and DIBMA copolymers for membrane protein solubilization via nanodisc formation

ST-4 **Nathan G. Brady** Post.Doc Sandia Natl. Lab.
Small angle neutron scattering and lipidomic analysis of a native, trimeric PSI-SMALP from a thermophilic cyanobacteria

ST-5 **Soumya Chandrasekhar** Graduate Student Kent State Univ.
Polyethylene glycol-modified DNA-based nanodiscs for incorporation and characterization of membrane proteins

ST-6 **Lars N. Deutz** Undergraduate Student UT Austin
Membrane extraction in native lipid nanodiscs reveals dynamic regulation of Cdc42 complexes during cell polarization

ST-7 **Olivia C. Fiebig** Post-Doc Penn State Univ.
Exploring inter-protein energy transfer in the photosynthetic antenna network of purple bacteria with nanodiscs

ST-8 **Snehasish Ghosh** Post-Doc Yale Univ.
Membrane-active-polymers (MAPs): A platform to preserve membrane proteins in their local interactome

ST-9 **Olivia Hawkins** Post-Doc Aston Univ.
Investigation into the basis of protein-lipid interactions for ATP-Binding Cassette (ABC) transporters, using novel polymer-based solubilisation methods.

ST-10 **Lynnicia Massenburg** Graduate Student Penn. State Univ.
Structural organization of the *Physcomitrium patens* cellulose synthase 5 (PpCesA5) homo-trimeric assembly

ST-11 **Shakiba Nikfarjam** Post-Doc L.L. Natl. Lab.
Enhancing Solubilization and Stability of Membrane Proteins for High-Throughput Anti-body Screening Using Polymer Nanodiscs.

ST-12 **Evelyn Okorafor** Graduate Student Miami Univ.
The effect of lipid saturation on the structure of styrene maleic acid lipid nanoparticles (SMALP)

ST-13 **Thirupathi Ravula** Staff Scientist Univ. Wisc. Madison
Polymer-based lipid-nanodiscs for NMR studies

ST-14 **Nancy C. Rotich** Graduate Student Miami Univ.
A Comparison of the Effect of SMA Derivatives on the Structural Topology and Dynamics of two Bacteriophage Peptides.

ST-15 **Ryan J Schuck** Graduate Student UTK
Cholesterol inhibits oncogenic EphA2 assembly & activation

ST-16 **Muhammad Z. Shah** Graduate Student Miami Univ.
Vinyl Ether and Maleic Anhydride Copolymerization Forming Lipid Nano Discs to Study Membrane Protein

ST-17 **Simon Umbach** Graduate Student Goethe-University
Synergies of nanoparticles and cell-free expression for the cryo-EM analysis of GPCRs and their nanotransfer into living cells.

ST-18 **Shushu Wei** Graduate Student UTK
Single-Molecule Visualization of Human A_{2A} Adenosine Receptor Activation by a G Protein and Constitutively Activating Mutations

POSTERS: NUMBERS, PRESENTER, AND TITLES

P-1 **Fidaa Ali** Graduate Student UTK
PSI-SMALPs: Isolation, characterization, and simulation using novel SMA co-polymers

P-2 **Elaine Barnard** Graduate Student Stellenbosch Univ.
Novel amphiphilic terpolymers for the detergent-free isolation of molecular drug targets

P-3 **Caroline Brown** Graduate Student Yale Univ.
Capturing membrane snapshots: A quantitative proteome-wide guide for high-throughput spatially resolved extraction of membrane proteins for structural/ functional studies on native membranes

P-4 **Kaeli Bryant** Graduate Student Vanderbilt Univ.
Some assembly required: structural analysis of the *Helicobacter pylori* Cag type IV secretion system.

P-6 **Grayson B. Cobb** UG Student UTK
The Length of SMA Copolymers Influence the Extraction of Photosystem I from Thylakoids

P-6 **Alyssa Gonneville** Graduate Student UTK
Interaction between the human A_{2A} adenosine receptor and G protein in lipid-nanodiscs

P-7 **Sarvinaz Hajiyeva** Graduate Student Kent State Univ.
DNA-APols Nanodiscs: towards better uniformity and stability

P-8 **Gillian D. Hertslet** UG Student UTK
Single-molecule analysis reveals that a glucagon-bound extracellular domain of the glucagon receptor is dynamic.

P-9 **Ruby Huynh** UG Student Arizona State Univ.
Purification of ATP Synthase from *Heliobacterium modesticaldum* using Diisobutylene/Maleic Acid (DIBMA)

P-10 **Susmita Khanal** Graduate Student UTK
Conformational Dynamics of Glucagon Receptor Transmembrane Domain Revealed by Single-Molecule Fluorescence Microscopy

P-11 **Olena Korotych** Post-Doc UTK
Large supramolecular SMALPs from chloroplast thylakoids: native PAGE, proteomics, and TEM analysis

P-12 **Spencer Moore** UG Student UTK
Breaking Barriers: extracting respiratory supramolecular protein complexes with SMA co-polymers.

P-13 **Matthew Necelis** Graduate Student Univ. of Virginia
Investigating lipid-lipid and lipid-protein interactions with bicelles and nanodiscs

P-14 **Godwin Ochola** Graduate Student UTK
Advancing Amphiphilic Copolymer-Mediated Membrane Protein Solubilization

P-15 **Sree Kavya Penneru** Graduate Student UTK
Usage of Styrene Maleic acid co-polymers (SMALPs) to isolate TOC-TIC complex in its Active & Native Form.

P-16 **P. Sundar Prakash** Graduate Student Kent State Univ.
DNA Peptidiscs

P-17 **Dylan Pruitt** Graduate Student UTK
Capture of peripheral, non-integral membrane proteins using mild detergent/ copolymer treatments: application to pigment-protein complexes from cyanobacterial thylakoids

P-18 **Shuo Qian** Staff Scientist ORNL
Characterization and mechanistic study of polymer-membrane nanoparticle formation by neutron scattering

P-19 **Mahipal S. Rao** Graduate Student UTK
SMA Copolymer Isolation of Functional Respirasome Sub-complexes: A Comparative Study

P-20 **Jennifer A Rybak** Graduate Student UTK
SMA derivatives differentially solubilize EGFR and EphA2

P-21 **Megan L. Shelby** Staff Scientist L.L. Natl. Lab.

A combined membrane memetic and cell-free expression approach to preparing membrane proteins for structural studies.

P-22 **Sriram Tiruvadi-Krishnan** Post Doc UTK
Kinetic analysis of the activation process of the human A_{2A} adenosine receptor in the lipid-nanodiscs

P-23 **Calum, Upton** Graduate Student Aston Univ.
Elucidating the molecular targets of bacterial nano-syringes

P-24 **Alyssa Ward** Graduate Student UTK
SiMPull-POP: A method for quantifying membrane protein oligomerization in native-like membrane environments

P-25 **Kevin L. Weiss** Post-Doc ORNL
Deuteration Capabilities of the Center for Structural Molecular Biology at Oak Ridge National Laboratory

P-26 **Qiu Zhang** Post-Doc ORNL
Biosynthesis of deuterated lipids for structural and biophysical characterization of biomembranes and membrane proteins

P-27 **Arba Zhao** UG/High School Student UTK
Unlocking Secrets of the Heart with Surface-Active Polymers

K E Y N O T E S P E A K E R S :

KS-2
Investigating cancer signaling and vaccine development with MSP based nanodiscs

Stephen G. Sligar[1]

[1]Departments of Biochemistry and Chemistry, University of Illinois, Urbana, IL 61801

Membrane proteins are involved in numerous vital biological processes. Unfortunately, membrane proteins are inherently recalcitrant to study using the normal toolkit for soluble proteins, and one is left with the challenge of finding inhibitors, activators and specific antibodies. Membrane proteins can be stabilized by a variety of amphipathic molecules and detergents. Particularly useful have been advances in generating nanoscale lipoprotein particles using a variety of polymeric materials. As discussed in the current and previous conferences in this series, these amphipathic materials include organic co-polymers, proteins, peptides and lipids that assemble with phospholipids to generate membrane bilayer mimetics. With the ability to self-assemble integral membrane targets into native-like bilayer structures these approaches have revolutionized the study of structural and functional properties of pharmacologically significant targets such as receptors, transporters, enzymes, and viral antigens. The membrane scaffold protein (MSP) based amphipathic polymers have also proven useful in providing a membrane surface of defined area and composition in order to reveal the structure and function of multi-component protein complexes that operate at the membrane surface. These include the blood coagulation cascade, integrin activation and cancer signaling cascades. In my presentation I will discuss our most recent results in the recruitment, activation and trafficking of the oncoprotein KRas4b to a MSP-based nanodisc surface as well as progress in universal vaccine development for oligomeric viral antigens. Supported by NIH MIRA GM118145.

KS-1
My SMALP journey. How it started…how it's going.

Alice Rothnie[1]

[1]Health & Life Sciences, Aston University, Birmingham, UK.

The application of styrene maleic acid co-polymers (SMA) to extract and purify membrane proteins, has enabled the study of membrane protein structure and function whilst maintaining their lipid bilayer environment. The SMA lipid particles (SMALPs) formed not only stabilise membrane proteins but are amenable to a wide range of downstream applications. Importantly they offer the opportunity to understand the local lipid environment and the interactions between proteins and lipids, which can often be important for protein function. As with any new technology there are still things we don't understand and some limitations to overcome. In this talk I will outline how I started in this field and how that has led to some of the current projects in our lab investigating both SMA variants and novel polymers to try to overcome these limitations, and some recent applications of SMALPs.

KS-3
New native nanodiscs for membrane-protein biophysics

Sandro Keller[1], David Glück[1], Lena Bauernhofer[1], Loretta Eggenreich[1], Annette Meister[2], Grégory Durand[3], and Carolyn Vargas[1]

[1]Biophysics, Institute of Molecular Biosciences (IMB), NAWI Graz, University of Graz, Graz, Austria

[2] HALOmem & Institute of Biochemistry and Biotechnology, Martin Luther University Halle–Wittenberg, Halle (Saale), Germany.

[3] Equipe Synthèse et Systèmes Colloïdaux Bio-organiques, Unité Propre de Recherche et d'Innovation, Avignon Université, 301 rue Baruch de Spinoza, 84916 Avignon cedex 9, France.

Nanodiscs that harbour individual membrane proteins or membrane-protein complexes in a lipid-bilayer environment hold great promise for biophysical investigations under well-controlled yet native-like conditions. Our laboratory focusses on new methods for the direct, detergent-free extraction of proteins from cellular membranes into native nanodiscs, which preserve both the overall bilayer architecture and the local lipid composition of the original cellular membrane. Thus, these native nanodiscs render membrane proteins amenable to *in vitro* biophysical investigations without ever removing the proteins from a lipid-bilayer environment. Recently, we have developed and used novel amphiphilic polymers with improved properties for forming nanodiscs that are compatible with a broad range of ensemble and single-molecule biophysical techniques. In this talk, I will present selected examples including antibody–receptor and peptide–lipid interactions studied by microfluidic diffusional sizing, membrane-protein complexes investigated by mass spectrometry, and cell-free translation of membrane proteins with cotranslational insertion into lipid-bilayer nanodiscs.

KS-4
How Amphipol Polymers and their Applications in Membrane Research Differ from SMALP Polymers

Charles R. Sanders[1]

[1]Department of Biochemistry, Vanderbilt University School of Medicine Nashville, TN 37240-7917

Amphipols are amphipathic polymers that were originally developed by Jean-Luc Popot and colleagues in the mid-1990's. They have a different history than the SMALPS and also tend to interact differently with membranes and membrane proteins. I discuss my lab's early contributions to the development of amphipols and also recent work suggesting that amphipols could possibly provide an enabling technology for the use of membrane proteins as "biologicals" for the treatment of human disease.

I N V I T E D S P E A K E R S :

IS-1
Circularized nanodiscs to characterize membrane perforation

Huan Bao[1], Qian Ren[1], and Shanwen Zhang[1]
[1]Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA, USA.

Despite extensive structural studies of many membrane pores, the molecule mechanism of pore formation remains elusive, mainly because they are driven by complex yet remarkably fast protein-lipid interactions. Although the rapid development of single-particle cryo-EM can reveal many intermediate states through state-of-art image processing methods, transient protein-lipid complexes during membrane pore formation only exist for a few milliseconds and are thus notoriously difficult to isolate for structural studies. To tackle this challenge, we recently developed a toolkit of nanodiscs (NDs) with expanded structures and functions, allowing for the isolation of pore-forming intermediates in membranes. Leveraging the power of this toolkit with single-particle cryo-EM, we have delineated the trajectory of membrane pore formation involved in bacterial pathogenesis.

IS-2
Determination of oligomeric organization of membrane proteins from native membranes at nanoscale-spatial and single-molecule resolution

Gerard Walker[1,2,3,#], Caroline Brown[2,3,#], Xiangyu Ge[4,5], Shailesh Kumar[1], Mandar D. Muzumdar[4,5,6,7], Kallol Gupta[2,3], **Moitrayee Bhattacharyya[1,*]**

[1]Department of Pharmacology, Yale University, New Haven, USA; [2]Department of Cell Biology, Yale University, New Haven, USA; [3]Nanobiology Institute, Yale University, New Haven, USA; [4]Department of Genetics, Yale University, New Haven, USA; [5]Yale Cancer Biology Institute, Yale University, New Haven, USA, [6]Department of Internal Medicine, Yale University, New Haven, USA, [7]Yale Cancer Center, New Haven, USA [#]These authors contributed equally

The protein-centricity of experimental biophysics has led to a view of membrane proteins devoid of two-dimensional native cell-membranes, which play pivotal roles in their regulation, structure, oligomerization, and function. Quantitation of membrane protein oligomeric states directly from native-membrane environments remains challenging. The main problems are: an inability to preserve the native-membrane environment while attaining precise spatial and high molecular resolution, insufficient sensitivity to detect and analyze membrane proteins at endogenous levels of expression, and dependence on bulk analyses that lack single-molecule information. We propose a single-molecule total internal reflection fluorescence microcopy (TIRF)-based imaging assay (Native-nanoBleach)[1] to quantify membrane protein oligomeric states directly from native-membrane environments at nanoscale spatial (~10 nm) and single-molecule resolution. We benchmarked and validated this experimental platform using several membrane proteins with well-established oligomeric states. We then applied this method to quantify as yet unknown oligomeric distributions of diverse membrane-proteins from *E. Coli,* mammalian cells, and patient-derived cell lines expressing target proteins at endogenous levels. We combined this innovative experimental approach with mutagenesis, truncations, antibody/nanobody binding, and inhibitor/drug treatments to ***(1)*** identify changes in oligomeric states and ***(2)*** map oligomeric interfaces in membrane proteins. We finally correlated our results with membrane-localized signaling, to connect physical modulation of protein oligomeric states to physiological outcomes. Our approach can be extended to identify compounds/antibodies that can modulate oligomeric distributions to ablate/alter signaling in disease-relevant membrane proteins. This general experimental pipeline ushers in a new era of studying membrane proteins *in their native-membrane environments* at an unprecedented spatial and molecular resolution.

IS-3
Co-translational membrane protein folding in native lipid environments

Paula Booth

King's College, London

The folding of newly synthesised proteins to their correct structure is essential to attaining functionally normal proteins that are vital to health. The majority of folding research studies artificially-denatured, full-length chains - a situation that is unrepresentative of cellular folding. Although folding is a highly topical area of research, membrane proteins are a particularly understudied class. Nearly all alpha helical membrane proteins fold co-translationally during biosynthesis, as the ribosome is translating mRNA, with insertion being assisted by translocase apparatus. Thus, the proteins fold in the membrane during elongation of the polypeptide chain, and not as full-length chains. We have shown that a lipid membrane (devoid of translocase components) is sufficient for successful co-translational folding. Folding is spontaneous, thermodynamically driven, and the yield depends on lipid composition. We have highlighted how Infra-Red spectroscopy can used to monitor co-translational folding in real time. We have also advanced our studies to native lipid compositions and the study of ribosome nascent chain complexes, where different length nascent chains are stalled whilst still attached to the ribosome.

IS-5
Cryo-EM structures of a mechanosensitive channel, *E.coli* MscS, in the native cell membrane nanoparticles

Youzhong Guo[1, 2], Thi Kim Hoang Trinh[1, 2], Claudio Catalano[1, 2], Yi-Lun Lin[1, 2], Weihua Qiu[1, 2]

[1.] Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, VA23219, USA.
[2.] Institute for Structural Biology, Drug Discovery and Development, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298, USA.
Mechanosensitive channels play a variety of crucial roles in the membranes of cells. The *E.coli* mechanosensitive channel MscS (*Ec*MscS) has been used as a model to study the gating mechanisms of mechanosensitive channels. Numerous attempts have been made to determine the atomic structure of this protein. However, all reported structures of *Ec*MscS have been solved using detergent-based techniques. Due to detergent's inability to retain the native cell membrane lipids associated with the membrane protein, the natural protein-lipid interactions have always been compromised. Utilizing the technological advancements of the Native Cell Membrane Nanoparticles (NCMN) system, we determined the high-resolution cryo-EM structures of *Ec*MscS in its closed and open states as NCMN particles. We discovered native cell membrane lipids associated with both the transmembrane and soluble domains of this mechanosensitive channel, distinct from all previously reported structures. The natural protein/lipid interaction suggests a direct lipid-mediated gating mechanism.

IS-6
Deciphering molecular organization of membrane proteins in their native membrane

Kallol Gupta[1,2]

[1]Nanobiology Institute, Yale University, West Haven, Connecticut, United States
[2]Department of Cell Biology, Yale School of Medicine, New Haven, CT, United States

The local membrane environment is crucial in governing the behavior of membrane proteins (MP). Conventional methods that use detergents to solubilize MPs disrupt the natural bilayer environment which often plays an essential role in regulating the functional organization of MPs and associated lipids. This creates a significant gap in our knowledge of how the nanoscale organization of MPs influences their ability to trigger cellular responses. Addressing this, we have developed a native mass spectrometry-based platform that enables detection of membrane protein-lipid complexes directly from customizable lipid membrane. Coupling this with top-down mass spectrometry our platform enables unambiguous determination of membrane-embedded oligomeric state, bound lipids, and heteromeric architectures of MPs directly from lipid membranes. Moving ahead, the application of this platform for targeted analysis of any MP of interest demands high-throughput strategies that can rapidly yield spatially resolved extraction and enrichment of the membrane domains containing the target MP. Meeting this demand, we've created a comprehensive, quantitative protocol for efficiently and spatially extracting MPs into customizable native nanodiscs ranging from 8-20nm in diameter. This allows for subsequent structural and functional studies of target MPs directly from their natural membranes. By combining established libraries of membrane-active copolymers (MAPs) with custom-designed chain-controlled polymers, we've devised a high-throughput, fluorescence-based test for swiftly evaluating the capability of synthesized MAPs to form native nanodiscs (referred to as MAPdiscs) against specific cells. Following this, we integrate MAP extraction with label-free quantitative proteomics to produce a high-throughput, proteome-wide quantitative guide for determining the most favorable conditions for extracting an endogenously expressed target MP into MAPdiscs with spatial precision. Applying this, we have determined the most optimized extraction conditions for 2100 distinct MPs, along with their complexes. We've consolidated this information into a user-friendly WebApp, allowing users to obtain the optimized nanoscale spatially resolved extraction conditions for their target MP, as well as multi-MP complexes, into native nanodiscs. We demonstrate the ability of the platform by directly extraction several multipass MPs from different cellular organellar membranes and further determining the oligomeric states of various MPs at their endogenous expression level.

IS-7
Beyond Boundaries: The Crucial Role of Method Spectrum in Membrane Protein Studies

Jan Kubicek and Barbara Maertens

Cube Biotech GmbH. Creative-Campus-Allee 12, 40789 Monheim, Germany.

Membrane proteins perform important cellular functions. They account for about one third of all proteins encoded in the human genome and are the target of about 70% of all approved drugs. Unlike soluble proteins, membrane proteins are integrated into, anchored to, or associated with the hydrophobic environment of a lipid bilayer. This makes their characterization and investigation difficult. Extraction from the membrane is key, and success in the investigation of membrane proteins can be achieved with a broad spectrum of methods. Whether detergent solubilization, co-translational insertion or reconstitution into MSP-based nanodiscs, or polymeric extraction, all methods have a specific field of application. However, they strongly differ in their stabilizing character.

IS-8
RAFT-mediated polymerization to enhance functionality in the SMALP technology

Bert Klumperman, Lauren E Ball, and Gestél Kuyler

Stellenbosch University, Department of Chemistry and Polymer Science, Private Bag X1, Matieland 7602, South Africa

The discovery in 2009 of the ability of poly(styrene-*co*-maleic acid) (SMA) to interact with phospholipid membranes and break them up in nanodiscs with a diameter in the order of 10 nm has revolutionized the field of membrane protein (MP) research. Commercially available copolymers such as SMA2000, SMA3000 have been used extensively to isolate and stabilize MPs for subsequent investigations. It was later found that other amphiphilic copolymers are also able to isolate MPs in nanodiscs, where disc size, stability over wider pH ranges and at higher ionic strength could be influenced. Relatively little is known about the role of the polymer in creating a native membrane-like environment for the MP. Increasing knowledge over structure-activity relationships of polymers used in the SMALP technology is crucial in moving the field forward. In addition to that, the polymer can play a larger role in the downstream investigations of MPs through the introduction of added functionality in the amphiphilic polymers.

IS-9

From Detergent to Membrane-Scaffold Polymers: A Look into the Structural Heterogeneity of SMALPs Through the Lens of SMA Detergency

Hongjun Liang

Department of Cell Physiology and Molecular Biophysics, Center for Membrane Protein Research, School of Medicine, Texas Tech University Health Sciences Center, Texas 79430, USA.

Extracting membrane proteins (MPs) with minimum perturbation to their native states is a challenge in membrane biology. Detergent solubilization has been widely used but proven to be too disruptive for many MPs. Amphipathic styrene-maleic acid (SMA) copolymers solubilize biomembranes into SMA-lipid particles, or SMALPs, that are often regarded as nanodiscs or native nanodiscs, a minimum viable bilayer that presumably supports MPs in their native states. The promising outlook of SMALPs inspires discovery of many SMA-like copolymers that also solubilize biomembranes into putative nanodiscs, but a fundamental question remains on how much the SMALPs or SMALP analogues truly resemble the bilayer membrane structure. How biomembranes are sliced and diced is critically dependent on the detergency of the solubilization agents. Unlike small detergents, the detergency of amphipathic copolymers is defined by their microstructures such as chain size, hydrophobic/ hydrophilic ratio, and sequence. Using the polymer-to-lipid (*P*/*L*) stoichiometric ratios in fractionated SMALPs consisting of well-defined SMAs and lipids as a yardstick, we identified the structural heterogeneity of SMALPs and confirmed the different SMALP structures with complementary characterization tools. We attribute the structural heterogeneity of SMALPs to the microstructure variations amongst individual polymer chains that give rise to their polydisperse detergency. We demonstrate that proteorhodopsin, a light-driven proton pump, is distributed among different nanostructures solubilized within the same SMALPs to display different photocycle kinetics. Our discovery challenges the native nanodisc notion of SMALPs or SMALP analogues and highlights the necessity to separate and identify the structurally dissimilar SMALPs components in membrane biology studies.

IS-10

IDENTIFYING Membrane Protein-Lipid Interactions with Lipidomic Lipid Exchange-Mass Spectrometry

Michael Marty[1]

[1] Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, 85721, USA.

Lipids can play important roles in modulating membrane protein structure and function. However, it is challenging to identify natural lipids bound to membrane proteins in complex bilayers. Here, we developed lipidomic lipid exchange-mass spectrometry (LX-MS) to study the lipid affinity for membrane proteins on a lipidomic scale. We first mix membrane protein nanodiscs with empty nanodiscs that have no embedded membrane proteins. After allowing lipids to passively exchange between the two populations, we separate the two types of nanodiscs and perform lipidomic analysis on each with liquid chromatography and mass spectrometry. Enrichment of lipids in the membrane protein nanodiscs reveals the affinity of individual lipids for binding the target membrane protein. We apply this approach to study three membrane proteins. With the E. coli ammonium transporter AmtB and aquaporin AqpZ in nanodiscs with E. coli polar lipid extract, we detected binding of cardiolipin and phosphatidylglycerol lipids to the proteins. With the acetylcholine receptor in nanodiscs with brain polar lipid extract, we discovered a complex set of lipid interactions that depended on the head group and tail composition. Overall, lipidomic LX-MS provides a detailed understanding of the lipid binding affinity and thermodynamics for membrane proteins in complex bilayers and provides a unique perspective on the chemical environment surrounding membrane proteins.

IS-11

Cryo-EM structures of *E. coli* mechanosensitive channel MscS and bovine mitochondrial complex III in styrene maleic acid nanodiscs

Elissa Moller[1,2,3,*], Fei Zhou[1,*], Sergei Sukharev[2,3,4], **Doreen Matthies**[1]

[1] Unit on Structural Biology, Division of Basic and Translational Biophysics, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.
[2] Department of Biology, University of Maryland, College Park, MD 20742, USA.
[3] Biophysics Graduate Program, University of Maryland, College Park, MD 20742, USA.
[4] Institute for Physical Science and Technology, University of Maryland, College Park, MD 20742, USA.

Membrane protein structure determination is complicated by the removal of stabilizing lipids, which can result in non-native conformations or a strong preference for certain states at the exclusion of others. This is especially applicable to mechanosensitive channel MscS, which gates in response to subtle changes in membrane tension. MscS structures have been solved in detergent as well as with added lipids which resulted in very different conformations of the channel. Here we used styrene maleic acid to extract mechanosensitive channel MscS from *E. coli* membranes as well as complex III from inner mitochondrial membranes and used negative staining as well as cryo-electron microscopy to analyze their structures.

IS-12

Magnetically aligned peptoid-stabilized lipid macrodiscs for structural studies of membrane proteins by solid-state NMR

Alexander A. Nevzorov[1], Azamat Galiakhmetov[1], Adit Shah[1], Addison Lane[1], Carolynn Davern[1], and Caroline Proulx [1]

[1]Department of Chemistry, North Carolina State University, 2620 Yarbrough Drive, Raleigh, NC 27695-8204.

Oriented-sample solid-state NMR allows for studying structure and function of membrane proteins in planar, lipid-rich bilayer environments and at the physiological temperature. We have recently demonstrated that novel magnetically aligned lipid mimetics composed of DMPC lipids and peptoid belts provide magnetic alignment and NMR spectroscopic resolution comparable or superior to those observed in detergent-containing bicelles. Peptoids can be synthesized from polyglycines with amide protons being substituted by alternating hydrophobic and hydrophilic moieties, thereby

forming amphipathic belts that can wrap themselves around the hydrophobic lipid interior. It was shown that 15-mer peptoids composed of alternating carboxy-ethyl and phenyl-ethyl side chains at the 1:2 molar ratio can solubilize large (>30 nm) lipid nanodiscs possessing sufficient magnetic susceptibility anisotropy to yield uniform magnetic alignment. Here we demonstrate that both magnetic alignment and NMR resolution of peptoid-based macrodiscs can be considerably improved by employing a mixture of the zwitterionic and negatively charged lipids (DMPC/DMPG at the 85/15 molar ratio). The resulting linewidths are about 30% sharper due to the electrostatic repulsion between the discs, thus resulting in a higher orientational order parameter. Moreover, highly aligned, detergent-free macrodiscs can be formed with a longer-chain lipid, DPPC. Interestingly, the spectra of Pf1 reconstituted in DMPC vs. DPPC lipid mimetics are almost indistinguishable, which would mean that the overall transmembrane helix tilt could be governed by the anchoring of the lysine and arginine residue side chains to the membrane interface, and not just by the hydrophobic matching alone. Supported by NSF MCB 1818240 to AAN.

IS-13
Functionalization of Membranes by the Proteolipid Code

Michael Overduin[1] and Troy A Kervin[2]

[1] Department of Biochemistry, University of Alberta, Edmonton, Alberta Canada
[2] Nuffield Department of Medicine, University of Oxford, Oxford UK.

Membranes are organized fluid structures that incorporate many different proteins and lipids. Lipid-lipid, protein-lipid, and protein-protein interactions establish their basic architecture, which consists of a lipid bilayer with embedded integral membrane proteins (IMPs) as well as peripheral membrane proteins (PMPs) that associate with a single leaflet. While more than 50 years have passed since this architecture was revealed, there is no consensus on how membranes are divided into distinct regions that allow the compartmentalization of different functions within the same continuous fluid. Commonly used terms such “raft” lack universal applicability, while “lipid domain” ambiguates with protein domains. To resolve this quandary, we analyzed structures and interactions of native membrane protein assemblies from various organelles. An explicit framework for membrane structure centered is formulated around the concept of a “zone”, which we define as a region of membrane enclosing a contiguous group of molecules capable of working together to perform a cellular function. The structural, functional, and regulatory dimensions of membrane zones are presented in light of a decade of work on native membrane particles by the research community.

IS-14
DNA-based lipid bilayer mimetics

Thorsten Schmidt[1]

[1]Kent State University, Physics Department, Kent, OH 44242.

DNA is a unique polymer. It is the information storage molecule of all known life forms but can also be used to form arbitrary nanoscale structures that are not found in nature. Our group is leveraging this programmability to engineer nanoscale architectures and tools for applications in Biophysics and Structural Biology. One goal of our lab is to establish DNA-lipid nanodiscs (Iric *et al.* Nanoscale 2018, 18463–18467.) as a new customizable nanoscale lipid bilayer mimetic for single-particle cryo-EM of membrane proteins. Our DNA nanotechnology-based approach will overcome some existing limitations of established bilayer mimetics and offer unprecedented control over structural, chemical, and physical design parameters. We expect DNA-based systems to enable qualitatively new types of experiments in structural biology and single-molecule biophysics.

S E L E C T E D T A L K S :

ST-1

Larger nanodiscs support a more native-like membrane environment across a range of lipid types

Mark J. Arcario [1], Vikram K. Dalal[1], and Wayland W.L. Cheng[1]

[1]Department of Anesthesiology, Washington University in Saint Louis, St. Louis, Missouri 63110, USA.

In the current structural revolution brought about by cryo-electron microscopy, nanodiscs have become an indispensable tool in understanding how the membrane environment affects membrane protein structure. Recent studies, however, have described subtle structural differences to cryo-EM structural models depending on the nanodisc scaffold size. While it is well-known that nanodiscs alter certain physical properties of lipid bilayers, it has been hypothesized that these alterations are due to the edge effect necessitated by the nanodisc scaffold and that large enough nanodiscs would recover more native-like properties. With the advent of nanodisc circularization technology which makes nanodiscs up to 200 nm thermally stable, we have been able to test this hypothesis. Using nanodiscs circularized with the SpyCatcher-SpyTag modification we have tested the phase behavior of pure lipids and lipid mixtures in nanodiscs ranging from 11 nm in size to 50 nm in size using the environment-sensitive fluorophore, laurdan. When compared to a 100 nm liposome of the same lipid composition, we have found that nanodiscs 25 nm and under demonstrate a more ordered bilayer and have a higher melting temperature. Importantly, however, increases in size mitigate these perturbations to the lipid properties. At the point of a 50 nm nanodisc, we are able to recover native-like membrane properties, supporting a long-standing hypothesis in the field and providing crucial guidance for structural biologists who lean heavily on nanodisc technology.

ST-2

Universal-RAFT mediated synthesis of double hydrophilic SMA-*b*-PVP copolymers for surface immobilization of SMALPs

Lauren Ball [1], Rueben Pfukwa[1], and Bert Klumperman[1]

[1]Department of Chemistry and Polymer Science, Stellenbosch University, Western Cape, South Africa.

The advent of membrane protein (MP) solubilisation using a variety of amphiphilic copolymers, generally based on poly(styrene-*co*-maleic acid) (SMA), has led to the successful solubilisation of MPs in their stable and active conformations, within nanodisc structures termed SMA lipid particles (SMALPs). This affords opportunities regarding the study of binding interactions between isolated MPs (desirable drug targets) and designed therapeutics. Surface plasmon resonance (SPR) is a powerful tool for high-throughput screening of drug candidates against MPs, as the binding affinity and binding kinetics between MP and drug candidate can be quantified. This technique requires the receptor molecule (i.e. the SMALP) to be immobilized on a gold chip, a process that generally requires modification of the protein to facilitate interactions with a chemically modified gold surface (e.g. streptavidin modified carboxymethylated dextran surface, binding to a biotinylated MP). This study aimed to develop a universal method for SMALP immobilization whereby the copolymer utilized in the solubilisation of the MP, also facilitates the binding of the SMALP to a gold surface, thereby eliminating the need for MP modification. This is achieved via synthesis of a novel poly(styrene-*alt*-maleic acid)-*block*-poly(*N*-vinylpyrrolidone) (SMA-*b*-PVP) copolymer which undergoes post-polymerisation aminolysis to produce a thiol at the PVP chain end. The SMA block facilitates solubilisation of MPs while the PVP block acts as a hydrophilic spacer creating distance between the SMALP and the gold surface. Preliminary tethering studies, using atomic force microscopy, have shown that the block copolymer and corresponding DMPC SMALPs can be tethered successfully to a gold surface.

ST-3

Alternatives to SMA and DIBMA copolymers for membrane protein solubilization via nanodisc formation

Brian K. Long[1], Cameron E. Workman[1], Pushan Bag[2], Bridgie Cawthon[2], Fidaa H. Ali[2], Nathan G. Brady[2], Barry D. Bruce[2,3]

[1] Department of Chemistry, University of Tennessee, Knoxville 37996, USA.
[2] Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville 37996, USA.
[3] Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville 37996, USA.

Styrene-maleic acid (SMA) and diisobutylene-maleic acid (DIBMA) copolymers have been shown to effectively extract membrane proteins while retaining the protein's native conformation by forming nanodiscs containing an annulus of the membrane's native lipids. Recent reports have shown that the esterification of SMA copolymers with various sidechains can drastically alter the efficiency and selectivity of protein extraction. However, much remains unknown on how and why the extent of SMA functionalization and identity of the sidechain alters the protein solubilization process. To probe these relationships, we investigated how a systematic series of monoesterified SMA copolymers bearing hydrophobic alkoxy ethoxylate sidechains of increasing lengths impacts the extraction of trimeric Photosystem I from membranes of the cyanobacterium Thermosynechococcus elongatus. We found that as the length and hydrophobicity of the sidechain, as well as the degree of functionalization, increase Photosystem I is more efficiently extracted. Finally, we demonstrated that the method of copolymer synthesis, solubilization, and storage employed may dramatically impact the structure of the copolymer, and consequently, the efficiency of the protein extraction process. Lastly, we will describe our efforts to develop an alternative copolymer platform combining the benefits of SMA and DIBMA copolymers.

ST-4

Small angle neutron scattering and lipidomic analysis of a native, trimeric PSI-SMALP from a thermophilic cyanobacteria

Nathan G. Brady[1,a], Shuo Qian[2,3], Jon Nguyen[1,b], Hugh M. O'Neill[2], and Barry D. Bruce[1,4,5]

[1] Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996, USA.
[2] Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA.

[3] The Second Target Station Project, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA.
[4] Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN 37996, USA.
[5] Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA.
[a] NGB Current Address: Center for Integrated Nanotechnologies, Sandia National Laboratories, Albuquerque, NM, 87185, USA.
[b] JN Current Address: School of Molecular Sciences, Arizona State University, Tempe, Arizona, 85,287, USA.

The use of styrene-maleic acid copolymers (SMAs) to produce membrane protein-containing nanodiscs without the initial detergent isolation has gained significant interest over the last decade. We have previously shown that a Photosystem I SMALP from the thermophilic cyanobacterium, Thermosynechococcus elongatus (PSI-SMALP), has much more rapid energy transfer and charge separation in vitro than detergent isolated PSI complexes. In this study, we have utilized small-angle neutron scattering (SANS) to better understand the geometry of these SMALPs. These techniques allow us to investigate the size and shape of these particles in their fully solvated state. Further, the particle's proteolipid core and detergent shell or copolymer belt can be interrogated separately using contrast variation, a capability unique to SANS. Here we report the dimensions of the Thermosynechococcus elongatus PSI-SMALP containing a PSI trimer. At ~1.5 MDa, PSI-SMALP is the largest SMALP to be isolated; our lipidomic analysis indicates it contains ~1300 lipids/per trimeric particle, >40-fold more than the PSI-DDM particle and > 100 fold more than identified in the 1JB0 crystal structure. Interestingly, the lipid composition to the PSI trimer in the PSI-SMALP differs significantly from bulk thylakoid composition, being enriched ~50 % in the anionic sulfolipid, SQDG. Finally, utilizing the contrast match point for the SMA 1440 copolymer, we also can observe the ~1 nm SMA copolymer belt surrounding this SMALP for the first time, consistent with most models of SMA organization.

ST-5

Polyethylene glycol-modified DNA-based nanodiscs for incorporation and characterization of membrane proteins

Soumya Chandrasekhar[1,5], Christopher Maffeo[2,3], Aleksei Aksimentiev[2,3,4] and Thorsten-Lars Schmidt[5]

[1]Advanced Materials and Liquid Crystal Institute, Kent State University, OH 44240, USA
[2]Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[3]Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[4]Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[5]Department of Physics, Kent State University, OH 44240, USA

Membrane proteins (MPs) are central to life processes, constitute about a third of all human proteins and perform vital cellular functions across an otherwise impermeable membrane. Several bilayer mimetics have been developed in order to simulate a native surrounding to understand the structure-activity relationship of membrane-associated proteins. However, they suffer from inherent drawbacks associated with lack of control over size and disruption of native protein structures. Hence, alternate tools for the creation of custom-sized nanodiscs would be necessary to achieve the required resolution to study the structure and function of membrane proteins.
Our lab created DNA-based nanodiscs where we modified DNA with hydrophobic alkyl groups in order to enhance interactions with phospholipids. Soon, we found that DNA extensively modified with alkyl groups no longer hybridized with complementary nucleobases. In the current study, we constructed custom-sized DNA minicircles consisting of amphiphilic PEG molecules in order to interact efficiently with a phospholipid bilayer to create a lipid-DNA nanodisc. This method does not influence DNA hybridization and was shown to efficiently recruit lipids depending on the length and number of PEG chains used. All-atom molecular dynamics simulations show that DNA predominantly interacts with lipid headgroups and the PEG chains hold the bilayer stably within the DNA-ring. Next, we will perform protein reconstitution and characterization studies which will aid in discovery of underlying molecular mechanisms of MP function.

ST-6

Membrane extraction in native lipid nanodiscs reveals dynamic regulation of Cdc42 complexes during cell polarization

Lars N. Deutz[1] , Daniel J. Dickinson[1]

[1]Department of Molecular Biosciences, University of Texas at Austin, Austin, TX 78712, USA.

Proper development requires the effective localization of cell components to enable differences in cell fate. This process is regulated by Par complex proteins which direct downstream cell polarization behaviors. Par complexes are localized to the membrane through either the scaffolding protein Par3 or the small GTPase Cdc42. We have developed a single-molecule assay for studying protein interactions in the Par complex and other signaling pathways. This assay, termed single-cell single-molecule pull-down, offers the ability to monitor the binding affinity and interaction kinetics of protein complexes after cell lysis. Although this approach is powerful, it has been difficult to apply to Cdc42 because of its reliance on an intact plasma membrane for function. To enable the study of Cdc42 complexes, we use maleic-acid copolymers which rapidly encapsulate the membrane into native lipid nanodiscs. To verify the capability of these polymers to rapidly solubilize membranes, we lyse single *C. elegans* zygotes and visualize membrane protein dissolution. We further show rapid nanodisc formation in cell lysate via negative-stain EM. We then measure the resulting nanodisc sizes with or without the presence of divalent cations, which have been shown to modulate nanodisc size. Importantly, nanodisc-forming solubilization of Cdc42-bound protein complexes has allowed us to detect Cdc42/Par6 complexes and reveal their regulation at different stages of development. We conclude that nanodisc-based solubilization of membrane may serve as an important improvement to kinetic single-molecule methods by allowing us to capture complexes in their membrane-bound states which more accurately reflect their *in vivo* biochemical environment.

ST-7

Exploring inter-protein energy transfer in the photosynthetic antenna network of purple bacteria with nanodiscs

Olivia C. Fiebig[1], Dihao Wang[1], Dvir Harris[1], Hila Toporik[2,3], Yi Ji[1], Chern Chuang[1], Muath Nairat[1], Ashley L. Tong[1], John I. Ogren[1], Stephanie M. Hart[1], Jianshu Cao[1], James N. Sturgis[4], Yuval Mazor[2,3], and Gabriela S. Schlau-Cohen[1]

[1]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
[2]School of Molecular Sciences, Arizona State University, Tempe, AZ 85281, USA.
[3]Biodesign Center for Applied Structural Discovery, Arizona State University, Tempe AZ 85281, USA.
[4]LISM UMR 7255, CNRS and Aix-Marseille University, 31 Chemin Joseph Aiguier, Marseille Cedex 9 13402, France.

Photosynthetic purple bacteria harvest sunlight through a network of antenna proteins arranged in the intracytoplasmic membrane with almost 100% quantum efficiency: nearly every photon is ultimately captured through charge separation. Energy transfer through this network, however, has been difficult to resolve because of the heterogeneous arrangement of the antenna proteins within the membrane. In this work (Wang, Fiebig, Harris *et al*., *PNAS*, 120 (28), 2023), we use nanodiscs to embed pairs of two variants of the most common antenna protein from purple bacteria, light-harvesting complex 2 (LH2), to reproduce the antenna network in a controlled manner. By varying nanodisc diameter, we are able to reproduce the range of distances found *in vivo*. Using a combination of ultrafast transient absorption spectroscopy, cryogenic electron microscopy, and quantum dynamics simulations, we resolve the inter-protein energy transfer timescale as a function of distance. We find that the closest possible and most common distance found *in vivo*, 25 Å, results in an energy transfer timescale of 5.7 ps, while larger distances of 28-31 Å result in timescales of 10-14 ps. Simulations show that the tightly-packed protein pairs at 25 Å increase total energy transport distances by ~15%. Overall, our work introduces nanodiscs as a platform to study membrane organization and complex energy transfer events in a controlled fashion and shows that energy transfer in the photosynthetic membrane is dominated by tightly-packed protein pairs which maximize transport distance.

ST-8
Membrane-active-polymers (MAPs): A platform to preserve membrane proteins in their local interactome

Snehasish Ghosh[1, 2]; Caroline Brown[1, 2]; Moitrayee Bhattacharyya[3]; Kallol Gupta[1, 2]

[1]Nanobiology Institute, Yale University, West Haven, Connecticut; [2]Yale School of Medicine, Department of Cell Biology, New Haven, CT; [3]Yale School of Medicine, Department of Pharmacology, New Haven, CT;

Macromolecular organizations of membrane proteins (MP) in cell membranes are fundamental to cellular responses. Although detergents have revolutionized MP biology, they failed to maintain the lipid organization associated with MP. Moreover, they are often detrimental to physiologically relevant interactions. Alternatively, SMA class of polymers allow extraction and solubilization of MPs while maintaining native membrane environment by forming small lipid nanodiscs. Currently, nanodisc-forming SMAs are limiting their efficacy in extracting most of the MPs due to their small ND sizes.

To address this, reversible addition−fragmentation chain transfer (RAFT) was used to synthesize a diverse membrane active polymer (MAP) library varying four parameters: hydrophobic moiety, hydrophilic moiety, their ratios, and molecular weights with high-precision. We characterized the pull-down MAP discs using a variety of analytical tools including gel electrophoresis, size exclusion chromatography, transmission electron microscopy, dynamic light scattering, spectroscopy. Spectroscopy and size exclusion chromatography reveal the ratio of fully folded-stable protein in MAP discs (which would be used for downstream analysis) to aggregated, unstable protein whereas dynamic light scattering, and transmission electron microscopy show the disc diameter. Interfacing our high-throughput screening platform with negative stain EM analysis and fluorescence-based assays we have demonstrated that our MAP library can form tunable homogenous native-nanodiscs ranging between 8-20nm in diameter. Hence, these MAP-discs provide a platform for structural and biochemical studies of MPs, directly in their native membrane environment while maintaining the local molecular interactome.

ST-9
Investigation into the basis of protein-lipid interactions for ATP-Binding Cassette (ABC) transporters, using novel polymer-based solubilisation methods.

Olivia Hawkins[1], Alan Goddard[1] and Alice Rothnie[1]

[1] College of Health and Life Sciences, Aston University, Birmingham, Aston Street, B4 7EJ, UK.

ATP Binding Cassette (ABC) transporters are a superfamily of membrane proteins involved in the active transport of substrates across the membrane. *Bacillus subtilis* multi-drug resistance ABC transporter (BmrA) is a member of this group that is linked to multi-drug resistance in bacteria. Its structure is well characterised, but functional study remains problematic due to the difficulty in maintaining structure and function once removed from the membrane. Applying polymer-based solubilisation methods, the native phospholipid bilayer is retained providing a unique ability to study protein-lipid interactions and mechanisms of action. However, Styrene Maleic Acid (SMA) produces nanodiscs that display a high sensitivity to divalent cations – problematic for ABC transporters due to the requirement of magnesium for ATPase activity. A variety of polymers including SMA, Diisobutylene Maleic Acid (DIBMA) and Acrylic Acid Styrene (AASTY) have been tested, with the aim of identifying optimal polymers for functional study of BmrA. Protein yield and purity were assessed, alongside nanodisc sensitivity to Mg^{2+}. ATPase assays were also conducted as a measure of function. While SMA2000 gives the highest protein yield and purity so far, some AASTY polymers produce comparable results and exhibit greater Mg^{2+} tolerance. Protein activity is shown in detergent solubilised BmrA but is yet to be identified in SMA nanodiscs. Investigations into lipids solubilised within nanodiscs have also been carried out via thin layer chromatography - with the aim of identifying lipid classes and polymer specificity – and BmrA has also been reconstituted into proteoliposomes to study the effect of lipid composition on protein functionality.

ST-10

Structural organization of the *Physcomitrium patens* cellulose synthase 5 (PpCesA5) homotrimeric assembly

Lynnicia Massenburg[1], Alexis Williams[2], Sung Hyun Cho[3], Carol Bator[3], Hugh O'Neill[4], and B. Tracy Nixon[1]

[1]Department of Biochemistry and Molecular Biology, Pennsylvania State University, State College, PA, 16802, USA.
[2]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA.
[3]Cryo-Electron Microscopy Facility, Huck Institutes of the Life Sciences, Pennsylvania State University, State College, PA 16802, USA.
[4]Center for Structural Molecular Biology, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA.

Plant membrane proteins called cellulose synthases (CesAs) make cellulose, the most abundant plant polymer in the world found in plant cell walls. The cryo-EM structure of poplar PttCesA8 trimer has revealed structural insights, but an evolutionary context of CesA structure in early plant species is needed. The aim of my research proposal is to understand the role of early plant CesA trimer oligomerization in functional cellulose synthesis and assembly. To approach this aim, I seek to characterize the structure of an early plant moss *Physcomitrium patens* cellulose synthase in detergent and nanodiscs using cryo-Electron Microscopy (cryo-EM). This structural analysis will provide information on residue-level oligomer interactions and global conformation of moss CesA trimers. A preliminary PpCesA5 trimer cryo-EM structure was determined using the cryo-EM capabilities at the Center for Nanophase Materials Sciences (CNMS) at Oak Ridge National Laboratory. Our current PpCesA5 cryo-EM structure was determined to be 2.96 Å with further collaborative efforts in cryo-EM data imaging and analysis. Using cryo-EM, we were able to isolate a growing glucan chain in PpCesA5 translocation channel. Extra density was found in-between the PpCesA5 trimeric interface that may play a role in trimer stabilization. Lastly, weak electron density was seen for transmembrane helix 7 suggesting helix flexibility. Future work involves an evolutionary structure comparison with poplar PttCesA8 with a previously published cryo-EM structure. This work reveals the importance of structural techniques such as cryo-EM for rational design of biofuels and new biomaterials.

ST-11
Enhancing Solubilization and Stability of Membrane Proteins for High-Throughput Antibody Screening Using Polymer Nanodiscs.

Shakiba Nikfarjam[1], Megan Shelby[1], Steven A. Hoang-Phou[1], Chao Liu[1], Ted Laurence1, and Matthew A. Coleman[1]

[1] Division of Biology and Biotechnology, Lawrence Livermore National Laboratory, Livermore, CA, USA.

The limited solubility of the SARS-CoV-2 Spike protein receptor binding domain (Spike RBD) protein presents a challenge in producing sufficient quantities for antibody screening. This reduced solubility is primarily attributed to the challenges in achieving proper folding when expressing RBD in prokaryotic systems, while mammalian expression systems yield lower protein production. In this study, we propose an innovative approach to enhance the stability and solubility of Spike RBD utilizing nanodiscs. We leverage bacterial cell-free protein synthesis systems and incorporate synthetic nanodiscs composed of phospholipids and synthetic polymers. Polymer nanodisc constituents can be directly added to cell free reactions or added near the end of protein expression. Specific concentrations of polymers will impact the reaction's productivity and needs to be optimized. During purification, additional lipid and polymer are critical for nanodisc formation with the membrane protein. The synergistic approach we are developing offers multiple advantages. Firstly, it enables the direct solubilization and stabilization of large quantities of RBD produced from bacterial lysate. Additionally, it extends the shelf-life of the protein and facilitates scalable production. Our method not only addresses the solubility issue but also opens new possibilities for high-throughput production of antigens for screenings in antibody discovery research. By enhancing the production and solubility of Spike RBD, we aim to expedite the antibody screening process, providing a valuable tool in the fight against infectious diseases. This innovative approach holds promise for accelerating research and development efforts, ultimately leading to more effective therapeutic solutions in the field of antibody discovery.

ST-12
The effect of lipid saturation on the structure of styrene maleic acid lipid nanoparticles (SMALP)

Evelyn Okorafor [1], Emma Gordon[1], and Gary Lorigan[1,]

[1]Chemistry and Biochemistry Department, Miami University, Oxford, Ohio 45056, USA.

Since the membrane is not two-dimensional, it is important to know the molecular events that occur in the deep dimensions of membrane lipids. The interaction of coexisting membrane domains can be studied in terms of hydrophobicity, alkyl chain order, and fluidity. Studying these membrane properties is essential to understand membrane functions like lateral movement of membrane proteins and lipids. Styrene Maleic Acid (SMA) copolymer has been known to be a suitable membrane mimic for the study of membrane proteins and lipids. Also, Continuous Wave-Electron Paramagnetic Resonance Spectroscopy (CW-EPR) is a relevant instrument for characterizing membrane domains without separation. In this study, phospholipids with varying degrees of saturation (DOPC, POPC, DMPC, and interestingly DSPC) were used to make hybrid vesicles with spin-labeled lipids of varying depths (5, 12, and 16-doxyl pc) to act as membrane probes and we further titrated them with two SMA polymers having different hydrophobic tail lengths. Dynamic light scattering (DLS) was used to confirm the formation of SMALPs while CW-EPR was utilized to characterize the dynamics of the hybrid vesicles incorporated in the SMALP system. Analysis of the resulting line shape indicates that the hydrophobic tail of SMA, the degree of lipid saturation, and the length of the phospholipids affect the membrane fluidity and alkyl chain ordering as well as the interaction between the lipids. Longer chains, fully saturated lipids and SMA samples exhibited more rigidity in movement, less fluidity and better ordering of the alkyl chain in the membrane.

ST-13
Polymer-based lipid-nanodiscs for NMR studies

Thirupathi Ravula,[1], Ayyalusamy Ramamoorthy[2]

[1]National Magnetic Resonance Facility at Madison, University of Wisconsin, Madison, WI, 53706, USA.

[2] National High Magnetic Field Laboratory and Florida State University Tallahassee, FL, 32310, USA.

Membrane proteins are crucial in cellular functions and disease pathology. However, studying these proteins in high resolution is challenging due to solubilization and reconstitution issues. To overcome this, membrane mimetics like liposomes and nanodiscs have been developed to emulate the native cellular environment. Among these, polymer-based nanodiscs provide a detergent-free solubilization, preserving the protein's native lipid environment. In this presentation, I'll discuss the design, synthesis, and application of polymer nanodiscs, emphasizing their magnetic alignment for NMR experiments. I will also introduce the newly developed inulin-based polymer nanodiscs. Our results showcase the novel use of lyotropic liquid crystalline polymer macro-nanodiscs (>20 nm) in solid-state NMR studies on membrane proteins. This innovative approach also serves as an alignment medium for measuring RDCs with high-resolution NMR. I will also discuss the application of various NMR techniques, including ^{31}P, ^{14}N, and TROSY-HSQC. The main advantages of these macro-nanodiscs lie in their easy preparation, robust stability against pH fluctuations and divalent metals, and broad application in studying molecules like proteins and RNA.

ST-14

A Comparison of the Effect of SMA Derivatives on the Structural Topology and Dynamics of two Bacteriophage Peptides.

Nancy C. Rotich, Evelyn Okorafor, Dominik Konkolewicz, and Gary A. Lorigan.

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056

To preserve a natural setting for membrane proteins, it is imperative to explore and cultivate suitable membrane mimetics. This involves emulating the properties of lipid bilayers, particularly within the hydrophobic core milieu. Membrane mimetics can assume diverse forms, such as micelles, bicelles, liposomes, and nanodiscs. Polymers, exemplified by styrene-maleic acid (SMA), offer a means to solubilize membrane proteins without resorting to detergents. It is widely recognized that various membrane mimetics yield distinct structural and dynamic configurations in membrane proteins. Of particular significance in this study is the styrene-maleic acid copolymer (SMA) and its derivatives, which are known for their ability to generate lipid nanoparticles. The development of SMA derivatives has demonstrated that utilizing SMA derivatives with the same charge as the target protein preserves the protein's structural and dynamic attributes when compared to other bilayer mimetics.
This study delved into the impact of different charges on SMA derivatives, specifically focusing on their influence on two positively charged bacteriophage lytic peptides. Positively charged, neutral, and negatively charged SMA derivatives interactions with pinholin S^{21} and the phage-encoded cationic antimicrobial peptide gp28 lipid vesicles were assessed. The characterization of these interactions was accomplished using techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), and continuous wave electron paramagnetic resonance (CW-EPR).

ST-15

Cholesterol inhibits oncogenic EphA2 assembly & activation

Ryan J Schuck [1], Alyssa Ward[1], Robert J Pyron[1], Nick Trybala[2], Amita R Sahoo[3], Matthias Buck[3], Joshua A Baccile[2], Ioannis Sgouralis[4], Rajan Lamichhane[1], and Francisco N Barrera[1]

[1]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37916, USA.
[2]Department of Chemistry, University of Tennessee, Knoxville, TN 37916, USA.
[3]Department of Physiology and Biophysics, Case Western University, School of Medicine, Cleveland, OH 44106, USA.
[4]Department of Mathematics, University of Tennessee, Knoxville, TN 37916, USA.

The EphA2 receptor acts as a tumor suppressor through binding of Ephrin-A ligands leading to receptor oligomerization, tyrosine autophosphorylation and kinase activity. Alternatively, EphA2 can promote tumor progression in the absence of ligand after phosphorylation of serine 897 (Ser897) by kinases. To prevent oncogenic EphA2 signaling, we hypothesized specific membrane properties, such as cholesterol content, may keep EphA2 oligomerization, activation and signaling under control. Cholesterol exerts a variety of direct and indirect effects on membrane proteins. Such interactions have the ability to affect protein structure, activity, localization within the cell and available interacting partners. To address how cholesterol impacts the self-assembly of EphA2, we developed a single-molecule photobleaching method, SiMPull-POP. Using SiMPull-POP, we found that cholesterol inhibits EphA2 self-assembly. Furthermore, cholesterol blocked EphA2 oncogenic activity by preventing phosphorylation of Ser897. Such changes in EphA2 oncogenic activity are accompanied by increased cAMP/PKA signaling. Our data suggests that cholesterol safeguards oncogenic assembly and activation of EphA2 via maintaining homeostasis of the cAMP/PKA signaling axis.

ST-16

Vinyl Ether and Maleic Anhydride Copolymerization Forming Lipid Nano Discs to Study Membrane Protein

Muhammad Zeeshan Shah[1], Nancy Rotich[1], Evelyn Okorafor[1], Quinton Henoch[1], Godfred Prempeh[1], Julia Kilbey[1], Gabby Demidovich[1], Jeremy Eapen[1], Rick Page[1], Gary Lorigan[1], Dominik Konkolewicz[1]

[1]Chemistry and Biochemistry Department, Miami University, Oxford, Ohio 45056, USA.

Studying and understanding the structure of membrane protein is an important topic in bioscience. Styrene-Maleic anhydride copolymers are well known for facilitating lipid discs formation to provide a native like environment. Amphiphilic behavior of copolymer is the key to the development of this system. The absorption of UV light in styrene ring at 280nm is observed in this system, whereas protein also absorbs at this wavelength. Replacing hydrophobic styrene ring in copolymer can be the possible solution to this problem. In this study, Styrene was replaced with butyl vinyl ether and kinetics of polymerization were investigated. To investigate the copolymerization of butyl vinyl ether and maleic anhydride (BVEMA) a kinetic study was performed. It was observed that changing mole ratios of BVE and MAn effects the weight averages of polymers, dispersity, and average time. A series

of reactions was performed changing mole ratios of polymers from 100% to 0% with respect to each other. It was observed that 66:50 ratio for BVE:MAn can provide maximum conversion of both monomers. After that, optimized ratio of MAn, BVE and dodecyl vinyl ether (DVE) copolymer was achieved to incorporate comparable hydrophobicity in the copolymer. A thin layer of 3:1 POPC and POPG was used to interact with polymers following the removal of end group and hydrolysis. DLS and TEM images show formation of lipid discs.

ST-17

Synergies of nanoparticles and cell-free expression for the cryo-EM analysis of GPCRs and their nanotransfer into living cells.

Simon Umbach[1], Zoe Köck[1], Roman Levin[1], Kilian Schnelle[2,3], Margherita Persechino[4], Hannes Schihada[4], Dovile Januliene[2,3], Kristian Parey[2,3], Steffen Pockes[5], Peter Kolb[4], Volker Dötsch[1], Arne Möller[2,3], Daniel Hilger[4], Frank Bernhard[1]

[1]Centre for Biomolecular Magnetic Resonance, Institute for Biophysical Chemistry, Goethe-University of Frankfurt/Main, Germany
[2] Department of Biology/Chemistry, Structural Biology section, University of Osnabrück, Germany
[3]Center of Cellular Nanoanalytic Osnabrück (CellNanOs), University of Osnabrück, Germany
[4]Department of Pharmaceutical Chemistry, University of Marburg, Germany
[5]Institute of Pharmacy, University of Regensburg, Germany

We present new strategies of implementing tailored nanoparticles for the structural and functional characterization of G protein coupled receptors (GPCRs). The GPCRs are co-translationally inserted into preformed nanoparticles by using a semi-defined, open accessible and customized cell-free expression system. Size, membrane composition, concentration and insertion strategies of nanoparticles are adjusted to each individual GPCR in order to obtain optimal yield and quality. By adding agonists and cognate G protein heterotrimer directly into the cell-free reaction, we demonstrate the preparative scale production of GPCR/G protein/nanodisc complexes in few mL reaction volumes within 24 hours. We thus could solve the first high resolution cryo-EM structure of the full-length human histamine 2 receptor in active conformation and bound to the Gs heterotrimer. Structural comparison with related GPCRs in combination with structure-guided functional experiments revealed important molecular insights into its selectivity for ligand binding and G protein coupling. We further established nanodiscs as efficient transfer vector for cell-free synthesized GPCRs into living cells. In addition to agonist-dependent internalization and phosphorylation of the transferred GPCRs, we demonstrate a new approach to analyze GPCR dimerization by simultaneously performing *in vitro* and *in vivo* assays with aliquots of identical GPCR samples. We explore the potential of the nanotransfer strategy by analyzing specific interactions of nanotransferred histamine and free fatty acid receptors with endogenously expressed GPCRs in HEK293 cells. We propose the nanotransfer as alternative strategy to analyze the impact of protein modifications, lipid environment or differential ligands in GPCR activation and oligomerization.

ST-18

Single-Molecule Visualization of Human A_{2A} Adenosine Receptor Activation by a G Protein and Constitutively Activating Mutations

Shushu Wei[1], Niloofar Gopal Pour[2], Sriram Tiruvadi-Krishnan[1], Arka Prabha Ray[2], Naveen Thakur[2], Matthew T. Eddy[2], and Rajan Lamichhane[1]

[1]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, 1311 Cumberland Avenue, Knoxville, TN 37996, USA
[2]Department of Chemistry, University of Florida, 126 Sisler Hall, Gainesville, FL 32611, USA.

G protein-coupled receptors (GPCRs) are integral membrane proteins involved in a wide range of cellular signaling processes. The structural and dynamic information is important in understanding the activation mechanism and essential for designing new drugs. As a prototypical class A GPCR, the human A_{2A} adenosine receptor (A_{2A}AR) plays a significant role in the development of neurodegenerative and coronary diseases. We utilized single-molecule total internal reflection fluorescence (TIRF) imaging to visualize the dynamic process of constitutively activating mutations (CAM)-mediated activation of A_{2A}AR. In a native-like MSP1D1 nanodisc environment, we observed an increased active-state population in the absence of agonists for the CAMs R291Q and I92N compared to the wild type. Importantly, activating mutations increased the population of an intermediate state crucial for receptor activation, notably distinct from the addition of a partner G protein. On the other hand, the constitutively inactive mutant, D52N, exhibits an increased inactive population in all conditions. Quantitative analysis of activation kinetics showed that CAMs increased the frequency of transitions to the intermediate state, whereas the sodium-insensitive mutation D52N pushed the equilibrium away from the intermediate state to an inactive conformation. Significantly, transitions among intensity states follow a consistent and reversible sequential order, corroborating our earlier research. These findings suggest that changes in GPCR function caused by mutations may be predicted based on their impact on intermediate state formation. This provides a new framework for designing receptors with altered functions or therapies targeting intermediate states.

P O S T E R S :

P-1

PSI-SMALPs: Isolation, characterization, and simulation using novel SMA co-polymers

Fidaa Ali[1], Cameron E. Workman[2], Micholas D. Smith[3,4], Barry D. Bruce[1,4]*

1. Genome Science and Technology, University of Tennessee, Knoxville, Tennessee, United States.
2. Department of Chemistry, University of Tennessee, Knoxville 37996-1600, Tennessee, United States
4. UT/ORNL Center for Molecular Biophysics, Oak Ridge National Laboratory, Oak Ridge, TN, USA
5. Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville
37996-1939, Tennessee, United States.

In Cyanobacteria, It was discovered that the tetrameric arrangements of Photosystem I (PSI) had a greater level of excitation-energy quenching, probably, non-photochemical quenching, which could enhance the photo-protection under excessive light irradiation. The role of PSI tetramers, in terms of structure, physiology, and evolution, is still under investigation. There has been considerable interest in using styrene-maleic acid copolymers (SMAs) to make membrane protein-containing nanodiscs without isolating them initially with detergents. Capturing PSI with a larger annulus of boundary lipids may alter the exciton and electron kinetics of PSI. We are interested in characterizing the oligomeric form of PSI from two thermophilic cyanobacteria, Chroococcidiopsis sp TS-821 and Thermosynechococcus elongatus in their native membrane environment using styrene maleic acid and other alkane-containing copolymers using different methods including BN-PAGE, SDGC, 77K fluorescence and single-molecule and lipidomic analysis. Moreover, The organizing principles for membrane proteins and the characterization of lipid-protein interactions could be investigated by different experimental approaches such as electron crystallization, X-ray crystallography, mass spectrometry or fluorescence techniques. Although some of these approaches are quantitative, they do not provide great spatial resolution and instead only capture strong interactions. Computational methods, such molecular dynamics simulations, may offer such details and have been extensively employed to explore lipid-protein interaction. Coarse-grained molecular dynamics (CG-MD) models are particularly useful for simulating reversible binding and unbinding processes and identifying lipids with both strong and weak binding properties. Using CG-MD simulations, we are capable of investigating the dynamics of the PSI-lipid membrane at temporal scales in the range of tens of microseconds (60μs), which enables investigating lipid-protein interactions at the molecular scale and aim to show how these interactions may affect the energetics of the binding of PSI monomers.

P-2

Novel amphiphilic terpolymers for the detergent-free isolation of molecular drug targets

Elaine Barnard[1], Gestél.C Kuyler[1], and Bert Klumperman[1]

[1] Department of Chemistry and Polymer Science, Division of Polymer Science, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

Membrane proteins (MPs) represent major molecular targets in drug discovery, thus, preserving their structural integrity is paramount for accurate therapeutic design. Recently, amphiphilic polymers have revolutionised MP extraction through the detergent-free disruption of the cell membrane. This innovative approach stabilises MPs and their annular phospholipids in nano-scale discs, allowing for their analysis in a native-like environment while retaining their original physiological properties.Our research, conducted at Nanosene in collaboration with Stellenbosch University's Klumperman Research Group, focuses on the development of novel amphiphilic polymers designed for membrane protein isolation. Leveraging controlled polymerization techniques, we have systematically designed polymers with predetermined structural and chemical characteristics, ensuring narrow molecular weight distributions. Through the partial modification of available comonomer moieties within the base copolymer, poly(styrene-alt-maleic anhydride) (SMAnh), we have synthesized a series of terpolymers with varying hydrophobic/hydrophilic balance. These novel terpolymers have shown improved efficiency for solubilising synthetic, bacterial and mammalian membrane systems. Through our research we have shown the crucial role that the polymers hydrophobicity plays in membrane solubilisation.

P-3

Capturing membrane snapshots: A quantitative proteome-wide guide for high-throughput spatially-resolved extraction of membrane proteins for structural/functional studies on native membranes

Caroline Brown[1,2], Snehasish Ghosh[1,2], Rachel McAllister[1,2,3], Jeff Coleman[1,2], Eric Sun[1], Hong Zheng[2], Shailesh Kumar[3], Aniruddha Panda[1,2], Jim Rothman[1,2], Moitrayee Bhattacharyya[3], Kallol Gupta[1,2]

1. Nanobiology Institute, Yale University, West Haven, Connecticut, United States
2. Department of Cell Biology, Yale School of Medicine, New Haven, CT, United States
3. Department of Pharmacology, Yale School of Medicine, New Haven, CT, United States
4. Cancer Biology Institute, Yale University, West Haven, CT, United States

The local membrane environment plays a paramount role in regulating the biology of membrane proteins (MP). Standard detergent-based MP-solubilization strategies disrupt the native bilayer-environment, leading to a fundamental gap in our understanding of how nanoscale molecular organization of MPs regulates their ability to elicit cellular responses. Addressing this, we have developed a proteome-wide quantitative guide for high-throughput, spatially-resolved extraction of MPs into tunable native nanodiscs of 8-20nm diameter. This enables downstream structural/functional studies of target MPs directly from native membranes. Combining existing MAPs with in-house chain-controlled polymers, we developed a high-throughput, fluorescence-based assay for rapid screening of synthesized MAPs against target cells quantitatively evaluating native-nanodisc (MAPdisc) forming capability. Next, we combine MAP extraction with label-free quantitative proteomics to generate a high-throughput, proteome-wide quantitative guide to inform the most optimal conditions for spatially-resolved extraction of a target, endogenously expressed MP into MAPdiscs. Applied to

HEK293 cells, this culminated in a library of MAP-extraction conditions for >2000 unique MPs. We have further packaged this resource into a WebApp where users search for their target MP or multi-MP complex systems to ascertain optimized conditions for native extraction into MAPdiscs. We used this library to discern optimal solubilization conditions for several multipass MPs to yield high-purity target MP-containing MAPdiscs. We further couple this rapid MAP-extraction with our recently developed single-molecule Native-Nanobleach platform, and a range of orthogonal approaches to demonstrate high-throughput extraction and subsequent detection of the molecular organization of endogenously expressed MPs.

P-4

Some assembly required: structural analysis of the *Helicobacter pylori* Cag type IV secretion system

Kaeli Bryant[1], Jacquelyn Roberts[2], Sirena Tran[1], Chiamaka Okoye[1], Arwen Frick-Cheng[2], Melanie Ohi[2,4], Timothy Cover[1,3,5]

[1]Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN
[2]Life Sciences Institute, University of Michigan, Ann Arbor, MI
[3]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN
[4]Department of Cell and Developmental Biology, University of Michigan School of Medicine, Ann Arbor, MI
[5]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN

Helicobacter pylori colonization of the stomach is the strongest known risk factor for gastric cancer. The *H. pylori cag* pathogenicity island encodes the Cag type IV secretion system (T4SS), which has a key role in pathogenesis. We previously analyzed the Cag T4SS outer membrane core complex (OMCC) by cryo-EM and showed that it contains five protein components—CagY, CagX, CagT, CagM, and Cag3. CagT is a VirB7 homolog required for assembly in other T4SSs, and CagM is an *H. pylori*-specific T4SS component. In this study, we sought to define how CagT and CagM each contribute to OMCC formation. We used immunoprecipitation methods to isolate the OMCC from a strain containing an intact Cag T4SS and mutant strains lacking an OMCC component (Δ*cagT* or Δ*cagM*). Complexes were visualized by cryo-EM and the proteins present in the purification were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Cryo-EM analyses of the Δ*cagT* and Δ*cagM* OMCCs both showed structures that contain a well-defined periplasmic ring with 17-fold symmetry and a poorly organized outer membrane cap. This contrasts with the wild-type OMCC, which has a 17-fold-symmetric periplasmic ring and a 14-fold-symmetric outer membrane cap. These data indicate that both CagT and CagM have key roles in assembly of the outer membrane cap but are not required for assembly of the periplasmic ring.

P-5

The Length of SMA Copolymers Influence the Extraction of Photosystem I from Thylakoids

Grayson B. Cobb [1], Godwin Ochola[2], Cameron E. Workman[3], Brian K. Long[2], and Barry D. Bruce[1,4]

[1]*Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee at Knoxville, Knoxville, TN*
[2]*Department of Chemistry, University of Tennessee at Knoxville, Knoxville, TN*
[3]*Eastman Chemical Company, Kingsport, TN*
[4]*Department of Chemical and Biomolecular Engineering, University of Tennessee at Knoxville, Knoxville, TN*

Styrene-maleic acid copolymers (SMAs) effectively extract membrane proteins by forming nano-discs that preserve the native lipid environment of the membrane protein. Previous research has shown that SMA polymers with extended side chains yield an increased solubilization efficiency (S.E.) of membrane-bound proteins. The mechanism through which SMA polymers extract membrane proteins stochastically remains unknown. In this study, we analyzed the effects of three standardized SMA polymers with varying molecular weights and polymer dispersity index (PDI) on the protein extraction efficiency of trimeric Photosystem I (PSI) in the thylakoid membranes of cyanobacterium *Thermosynechococcus elongatus* (Te). The PDI of these polymers was altered by mixing the three different lengths of SMA polymers (MN= 2.6, 8.6, & 20.0 kDa) at varying ratios to create a synthetic mixture, which were investigated either by 1) combining the polymers before solubilization; 2) by a stepwise addition during solubilization. The highest yield of solubilized PSI was achieved by adding the medium molecular weight SMA polymer alone. Interestingly, the sequence of polymer addition appears to play a critical role in how PSI is extracted, suggesting divergent roles of polymers with different lengths. SMA polymer mixtures synthesized through a step addition yielded higher S.E. than mixtures synthesized before solubilization. Further research may determine how the mechanism of membrane protein extraction is affected by the sequential addition of SMA polymers at varying molecular weights.

P-6

Interaction between the human A_{2A} adenosine receptor and G protein in lipid-nanodiscs

Alyssa Gonneville[1], Asmita Chaudhary[1], Larissa Silva[2], Matthew T. Eddy[2], Rajan Lamichhane[1]

[1]Department of Biochemistry & Cellular and Molecular Biology, College of Arts and Sciences, University of Tennessee, 1311 Cumberland Avenue, Knoxville, TN 37996, USA
[2]Department of Chemistry, College of Liberal Arts and Sciences, University of Florida, 126 Sisler Hall, Gainesville, FL 32611, USA.

G-protein coupled receptors (GPCRs) are the largest and most diverse class of membrane proteins in eukaryotic cells that play critical roles in signal transduction. GPCRs bind many ligands, causing the receptor to undergo a conformational change, activating a signaling cascade within the cell. The human A_{2A} adenosine receptor (A_{2A}AR) is a class A GPCR involved in many physiological functions and human diseases. Lipids have been shown to affect the activity levels of many membrane proteins. It is important to understand how particular lipids affect the activity level of these types of GPRCs because of their role in the signal transduction process. Currently, it has been shown that anionic phospholipids prime A_{2A}AR to form complexes with the G-protein, but the dynamics of these interactions have not been studied. We utilized lipid nanodiscs to purify A_{2A}AR in native-like lipid environments and used single-molecule fluorescence resonance energy transfer (FRET) to observe how A_{2A}AR interacts with G-proteins. This information will help us understand

the mechanism of receptor activation that could be used for future pharmaceutical drug development.

P-7
DNA-APols Nanodiscs: towards better uniformity and stability

Sarvinaz Hajiyeva [1,2], Soumya Chandrasekhar[1,3], Gestél Kuyler[4], Bert Klumperman[4,] Thorsten-Lars Schmidt[1*]

[1]Physics Department, Kent State University, Ohio 44242, USA. Email: tschmi21@kent.edu
[2]School of Biomedical Science, Kent State University, Ohio 44242, USA.
[3]Advanced Materials and Liquid Crystal Institute, Kent State University, Ohio 44242, USA.
[4]Chemistry and Polymer Science Department, Stellenbosch University, Stellenbosch 7600, South Africa.

DNA-encircled bilayers provide the opportunity for improved size control and sites for the functionalization of nanodiscs. However, this approach still requires the use of detergents for the reconstitution of lipids/membrane proteins. Some amphiphilic polymers (SMA polymers and CyclAPols) are known for their capability to solubilize lipids and membrane proteins without detergents. We plan to combine the features of DNA-minicircles and amphiphilic polymers by conjugating oligonucleotides to the various polymers of amphiphilic nature (including SMA polymers). The resulting nanodiscs are expected to have a narrow size distribution, high complex stability, ease of functionalization and solubilization of lipids/membrane proteins without detergents.

P-8
Single-molecule analysis reveals that a glucagon-bound extracellular domain of the glucagon receptor is dynamic.

Gillian D. Hertslet, Ting Liu, Susmita Khanal, and Rajan Lamichhane

Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, 1311 Cumberland Ave., Knoxville, TN 37932, USA

G Protein-coupled receptors (GPCRs) are integral membrane proteins involved in a wide range of cellular signaling processes. Structural and dynamic information is vital to understanding the activation mechanism of these receptors. Despite the availability of high-resolution structures at different conformational states, the dynamics of those states at the molecular level are poorly understood. We used total internal reflection fluorescence (TIRF) imaging to investigate the conformational dynamics of the Glucagon Receptor (GCGR), a class B family GPCR that controls glucose homeostasis. The GCGR was expressed and purified from the human embryonic kidney 293T (HEK293T) cells and studied at a single molecule level. Fluorescence Resonance Energy Transfer (FRET) was used to observe extracellular domain (ECD) dynamics of the donor and acceptor fluorophore labeled GCGR molecules. We observed that for apo-GCGR, the ECD is dynamic and spends more time in a closed conformation. In the presence of glucagon, the ECD is wide open and shows more dynamic behavior than apo-GCGR, a finding not previously reported. These results suggest that both apo-GCGR and glucagon-bound GCGRs showed ligand-dependent reversible opening and closing of the ECD with respect to the seven-transmembrane (7TM) domain. Demonstrating a molecular approach to visualizing the dynamics and mechanisms behind GPCR activation provides an important foundation for understanding conformational changes surrounding GPCR activation and is critical for developing new therapeutics.

P-9
Purification of ATP Synthase from *Heliobacterium modesticaldum* using Diisobutylene/Maleic Acid (DIBMA)

Ruby Huynh[1], Petra Fromme[1,2], Jay-How Yang[2], Joseph Goyer[2]

[1]School of Molecular Sciences, Arizona State University, Tempe, AZ 85287, USA
[2]Center of Applied Structural Discovery, Biodesign Institute, Tempe, AZ 85287, USA

The purification of ATP synthase, a vital enzyme involved in cellular energy production, has posed significant challenges, particularly when attempting to isolate it from extremophilic microorganisms like *H. modesticaldum*. This study presents an innovative approach to overcome these challenges by employing Diisobutylene/Maleic Acid (DIBMA) as a potent solubilization and purification agent. Our research showcases the successful partial extraction and purification of ATP synthase complexes from Heliobacteria's membranes (HF_1F_0), preserving their structural integrity and functional activity. This achievement was confirmed through various analytical techniques, including SDS-PAGE and Native-PAGE. Cryogenic electron microscopy (Cryo-EM) will later be used to determine the structure of the purified HF_1F_0.

P-10
Conformational Dynamics of Glucagon Receptor Transmembrane Domain Revealed by Single-Molecule Fluorescence Microscopy

Susmita Khanal [1], Sriram Tiruvadi-Krishnan [1], Rajan Lamichhane [1]
[1]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, 1311 Cumberland Ave., Knoxville, TN 37932, USA

G Protein-coupled receptors (GPCRs) are integral membrane proteins that mediate cellular responses to most hormones, metabolites, cytokines, and neurotransmitters. Representing the most prominent family of proteins with approximately 800 members in humans, GPCRs are the target for more than 40% of current pharmaceutical drugs. The structural and dynamic information is vital to understanding the activation mechanism of these receptors. Despite the availability of high-resolution structures of GPCRs at different conformational states, the dynamics of those conformational states at the molecular level are missing. We used total internal reflection fluorescence (TIRF) imaging to investigate the conformational dynamics of the Glucagon Receptor (GCGR), a class B family GPCR involved in glucose homeostasis. We expressed GCGR in mammalian cells, extracted and purified them in the native environment using Styrene Maleic Acid Lipid Particles (SMALPs), and analyzed them at a single molecule level. We used Single-molecule Förster Resonance Energy Transfer (smFRET) to observe the conformational dynamics of the donor and acceptor fluorophore-labeled GCGR molecules in SMALPs. We observed slow and

reversible conformational fluctuation of transmembrane domain VI of GCGR. The addition of a saturated concentration of full agonist glucagon altered the dynamics towards the lower FRET states, thus activating the receptor. We also observed various intermediate states before finally reaching the active states. These results provide new insight into the structural dynamics of glucagon receptor and receptor activation, which might be helpful for diabetes drug design in the future.

P-11
Large supramolecular SMALPs from chloroplast thylakoids: native PAGE, proteomics, and TEM analysis

Olena Korotych [1], P. Chotewutmontri [2, 3], Lucy Gao [4], Matthew Huff [5], Margaret Staton [5], Julian Whitelegge [4], and Barry Bruce [1, 6]

[1] *Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee at Knoxville*
[2] *Graduate School of Genome Science and Technology, University of Tennessee at Knoxville, Knoxville, TN 37996*
[3] *Institute of Molecular Biology, University of Oregon, Eugene, OR 97403*
[4] *Pasarow Mass Spectrometry Laboratory, Geffen School of Medicine, University of California, Los Angeles, CA, USA.*
[5] *Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA*
[6] *Department of Chemical and Biomolecule Engineering, University of Tennessee at Knoxville*

Chloroplast thylakoid membranes constitute one of the extensively studied biomembranes, housing light-harvesting complexes, reaction centers, electron transport complexes, and ATP synthases*. In addition, there are many other protein complexes involved in biogenesis and ion homeostasis.* Despite the depth of research in this area, the characterization of these complexes has primarily relied on detergent solubilization and conventional separation techniques. Recently, we *began to utilize* styrene-maleic acid (SMA) copolymers for the isolation of large supramolecular complexes from pea chloroplasts. *This work utilized the top two SMA copolymers (SMA 1440 and XIRAN 30010) with the highest efficacy in solubilization of galactolipid-rich thylakoid membranes.* Building upon our initial work, we have now expanded our investigation to characterize these styrene-maleic acid lipid particles (SMALPs) using transmission electron microscopy (TEM), proteomics, native PAGE, and nanoparticle tracking analysis (NTA). Our research introduces a novel approach for studying chloroplast thylakoid complexes, shedding light on their native organization and paving the way for deeper structural insights into these critical biomembranes. Using native PAGE we uncovered the presence of at least seven distinct thylakoid supramolecular complexes, exhibiting apparent molecular masses spanning from 120 kDa to 870 kDa. The availability of the fully sequenced genome of *Pisum sativum* has significantly enriched our proteomic analysis capabilities. *The proteomic analysis indicates that these distinct complexes are enriched in well-known membrane proteins (PSII, LHC2, b_6/f, PSI, and ATP synthase) and* previously undiscovered proteins. SMA solubilization removes the need to use conventional detergents for membrane protein extraction, suggesting that these large protein-containing SMALPs may capture the native organizational intricacies of thylakoid complexes in pea chloroplasts. Our future investigations will focus on employing a cutting-edge cryo-EM technique to unravel the structural intricacies underlying the assembly of these newly discovered supramolecular complexes.

P-12
Breaking Barriers: extracting respiratory supramolecular protein complexes with SMA copolymers.

[1]**Spencer Moore**, [1]Mahipal S. Rao, [1]Olena Korotych, and [1,2]Barry D. Bruce

[1] Biochemistry and Cellular and Molecular Biology Department, University of Tennessee, Knoxville
[2] Chemical and Biomolecular Engineering, University of Tennessee, Knoxville

Membrane proteins are vital to the function of a cell and play a role in several critical biological processes, including ion transport, signal transduction, and electron transport. A prime example is the mitochondrial membrane protein complexes I-IV, which are part of the respiratory electron transport chain (ETC) and are arranged into a large supramolecular complex known as respirasome. Electrons from NADH and $FADH_2$ are transferred from one complex to another through the respiratory ETC, establishing a proton gradient across the intramitochondrial membrane. The oxidation of electron carriers is coupled with ATP synthesis. This process, called oxidative phosphorylation, is responsible for recycling ADP back to ATP. Before the complexes can be studied in detail, they must be isolated from the inner mitochondrial membrane. Traditionally, scientists have employed detergents to isolate membrane protein complexes from the lipid bilayers as detergent micelles. However, recent discovery indicates that the lipids attached to the protein play an essential role in its structure and function, and detergents have been found to remove native lipids during protein extraction. This has led to the development of another isolation method that utilizes styrene-*co*-maleic acid (SMA) copolymers that extract the membrane protein and its native lipids and encase them in a polymer layer called SMALPs. Herein, we used the top five commercially available SMA copolymers (SMA 1440, SMA PRO, SMA 2625, SMA 17352, and XIRAN 30010) to extract the supramolecular complexes from bovine heart mitochondrial membranes. The solubilization efficacy of different SMA modifications was determined based on total protein content. Protein-containing SMALPs were characterized using electrophoretic techniques (SDS-PAGE and native PAGE) and mass photometry.

P-13
Investigating lipid-lipid and lipid-protein interactions with bicelles and nanodiscs

Matthew Necelis[1], Valentina Corradi[2], Peter Tieleman[2], Linda Columbus[1]

[1] Chemistry Department, University of Virginia, Charlottesville, Virginia, USA
[2] Biocomputing, University of Calgary, Calgary, Alberta, Canada

Membrane proteins are solvated by and interact with lipids. The bacterial membrane environment varies in composition between bacteria and a single bacterial membrane is composed of many different lipids. The molecular details of the interactions between lipids and membrane proteins within this variation is not well understood. One hypothesis is that membrane proteins interact selectively with the lipid environment, as specific lipid species can be enriched or

depleted in the vicinity of the protein. We aim to experimentally test the hypothesis that membrane proteins interact and preferentially organize their surrounding lipids. In order to identify and quantify these lipid – membrane protein interactions, two approaches are being developed. First, to identify preferential organization, this research aims to use styrene-maleic anhydride (SMA) copolymer nanodiscs. Most common approaches to isolate membrane proteins involves the use of detergents to solubilize and stabilize membrane proteins, but this process removes the locally associated lipids. To overcome these limitations, SMAs and other copolymers, which preserve annular lipids, will be used to establish a fundamental understanding of the lipid-protein interactions, and assess the impact these interactions have on the organizational effect membrane protein's have on their local lipid environment. Secondly, this project aims to use membrane mimetic bicelles to understand lipid-detergent self-assembly using small-angle X-ray scattering (SAXS). Bicelles have emerged as a promising tool for studying membrane lipid bilayer properties and interactions with detergents. By utilizing SAXS to study the lipid-detergent self-assembly, this research project will provide valuable insights into the fundamental mechanisms of lipid-lipid interactions and the key determinates that dictate it. We believe that the overall impact of this segregation lies within the detergent's ability to stabilize the hydrophobic tails of the lipids. Here, we determine the segregation with detergents of varying hydrophobic areas to systematically elude key determinants in this interaction. Overall, this research project's multipronged approach, including the use of SMALPs and bicelles, will provide a comprehensive understanding of lipid-lipid and lipid-protein interactions, ultimately contributing to a better understanding of membrane-bound processes and potentially advancing the development of novel therapeutic interventions.

P14
Advancing Amphiphilic Copolymer-Mediated Membrane Protein Solubilization

Godwin Ochola,[1] Cameron Workman,[1] Grayson Cobb,[2] Barry D. Bruce,[2] and Brian Long[1]

[1]Department of Chemistry, The University of Tennessee, Knoxville, TN 37996, USA.
[2]Department of Biochemistry & Cellular and Molecular Biology, The University of Tennessee, Knoxville, TN 37996, USA.

Analyzing and manipulating membrane proteins is challenging due to the complex environment in which they reside, their hydrophobicity, and their susceptibility to denaturation upon exposure to common detergents. To address this issue, amphiphilic copolymers have become crucial research tools for membrane protein solubilization and study. Recently, α-olefin-*co*-maleic acid copolymers (αMAs) and esterified styrene-*co*-maleic acid (SMA) copolymers bearing alkoxy ethoxylates have been shown to selectively extract trimeric Photosystem I (PSI) from cyanobacterial membranes more efficiently than previously known copolymer systems (e.g., diisobutylene-*co*-maleic acid (DIBMA) and non-esterified SMAs). Herein, we will describe our ongoing efforts to develop new amphiphilic copolymer analogues to advance this area of research. More specifically, we will describe the synthesis of esterified αMAs and αMA analogues that combine the benefits of our prior copolymer systems. We hypothesize that the incorporation of esterified, or side chain modified, polar side groups will enhance membrane protein selectivity, solubilization efficiency, stability, and function retention. We anticipate that these αMA copolymers, and analogues thereof, will mark a dramatic advance in the study of membrane protein solubilization and structure due to adaptability and tunability of these copolymer systems.

P-15
Usage of Styrene Maleic acid co-polymers (SMALPs) to isolate TOC-TIC complex in its Active & Native Form.

Sree Kavya Penneru[1], Nilabh Saksena[1], Barry D. Bruce[1]

[1]Biochemistry and Cellular & Molecular Biology, University of Tennessee, Knoxville, United States.

Plastids are ubiquitous organelles in plant cells. One subtype, the chloroplast is not only involved in photosynthesis but also in many other essential processes of metabolism including amino acid biosynthesis, and lipid biosynthesis. Although the plastid is a semi-autonomous organelle like the mitochondria, its genome is small and only encodes <125 proteins. It is now clear that most of proteins involved in various processes in chloroplast, including the photosystem complexes in thylakoid membranes, are encoded by the nuclear DNA and translated in the cytoplasm. It is estimated that ~3600 different proteins are imported into the chloroplast via a post-translational, translocation mechanism across the outer and inner envelope membrane of the chloroplast. Despite progress in identifying the individual components of the TOC (Translocon of the Outer Chloroplast envelope) and TIC (Translocon of the Inner Chloroplast envelope) complexes, both the structure and function of these subunits is poorly understood. The complexes forming the translocon are dynamic and their activation depends on the presence of a transit peptide. The peptide is designed to bind but not enter the translocon due to its charged residues with a dual tag. We use a novel peptide to bring the subunits together, and then isolate them with Styrene Maleic Acid copolymers (SMAs). The particles isolated are characterized by Native PAGE and immunoblotting to confirm the presence of specific subunits of the complex. Isolated complexes will be used to perform single-particle, cryo-EM analysis imaging in future to elucidate TOC-TIC organization and its atomistic structure.

P-16
DNA Peptidiscs

Praneetha Sundar Prakash [1], Soumya Chandrasekhar[2], and Thorsten-Lars Schmidt[3*]

[1]Department of Biomedical Sciences, Kent State University, Kent, OH 44240, USA.
[2]Advanced Materials and Liquid Crystal Institute, Kent State University, Kent, OH 44240, USA.
[3] Department of Physics, Kent State University, Kent, OH 44240, USA.
*Email: tschmi21@kent.edu

Native membrane mimetic systems such as nanodiscs are key for characterization of membrane proteins as they help preserve the folding and environment in which they are present. We have developed a novel DNA nanotechnology-based nanodisc system which is made up of customizable single-stranded DNA minicircles hybridized to several complementary short DNA strands. These nanodiscs can be

rendered amphiphilic either through electrostatic binding to poly-lysine modified 4F peptides or through chemical conjugation of the 4F peptides to the short oligonucleotides. Preliminary studies have shown that these poly-lysine tail modified 4F peptides can electrostatically bind to double stranded DNA minicircles and are also capable of solubilizing lipid suspensions in the absence of detergents. However, the solubilization behavior was found to be dependent on the length of the poly-lysine tail. On the other hand, the conjugate-based rings can form nanodiscs through the conventional detergent removal process. Future studies will focus on the capabilities of incorporation of membrane proteins in these nanodiscs.

P-17

Capture of peripheral, non-integral membrane proteins using mild detergent/copolymer treatments: application to pigment-protein complexes from cyanobacterial thylakoids

[1]Dylan Pruitt, [1]Mahipal Rao, [2]Fidaa Ali, [1,2,3], [4]Julian Whitelegge, [123]Barry D. Bruce

[1]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee at Knoxville

[2]Program in Genome Science and Technology, ORNL/University of Tennessee at Knoxville

[3]Department in Microbiology, University of Tennessee at Knoxville

[4]Pasarow Mass Spectrometry Laboratory, University of California at Los Angeles

Peripheral membrane proteins can be removed with chaotropic agents and alkaline washes. However, some proteins with post-translational lipid modifications are not as readily removed with this mild condition. We have explored using a selection of nonionic detergents including maltopyranosides, glucopyranosides, and glucamines at concentrations well below the levels used for solubilization transmembrane proteins. The goal is to find conditions that can selectively solubilize peripheral membrane proteins due to their relatively weak attachment to the membrane compared to integral membrane proteins with large hydrophobic transmembrane domains. We have had very promising results with maltopyranosides that can release a carotenoid-rich protein fraction from cyanobacterial thylakoids while solubilizing very little chlorophyll-containing photosystems (PSI or PSII) that have many transmembrane segments. However, despite this success, even mild detergents may have significant limitations since they may disrupt the native association with cofactors/prosthetic groups with these proteins. We are now exploring a selection of SMA and related copolymers that may overcome this limitation due to their fundamentally different solubilization mechanism, most notably characterized by their ability to solubilize membrane proteins within their native lipid environment. Here, we use detergent and polymer solubilization to investigate a carotenoid binding protein (CBP) peripherally associated with the membrane via post-translational acylation. Solubilized CBP was purified by sucrose density gradient ultracentrifugation and ion exchange chromatography, and analyzed by BN-PAGE, SDS-PAGE, and mass spectrometry (MS). Additionally, liquid chromatography-mass spectrometry (LC-MS) was used to analyze the associated carotenoid species. Further analysis of styrene maleic acid (SMA) solubilized membrane fractions containing the CBP would allow for structural determination using cryo-EM and/or X-ray diffraction (XRD). Finally, with the identification of submits of this novel complex, we can investigate the environmental effects such as high light and other stress that may induce expression of this complex and shed light into the mechanism of its association with the photosynthetic apparatus within the thylakoid membrane.

P-18

Characterization and mechanistic study of polymer-membrane nanoparticle formation by neutron scattering

Shuo Qian

Oak Ridge National Laboratory, Oak Ridge, TN 37831 USA.

The remarkable effectiveness and heightened bioactivity of lipid nanoparticle formed by some amphipathic polymers and native membranes have prompted the exploration and development of additional formulations. A comprehensive understanding of these polymers' interaction with membranes, including lipids and proteins, will greatly enhance their applications and further improvement. Neutron scattering, with its non-destructive and contrast variation capability, provides an excellent tool for studying different components of a nanoparticle selectively in solutions. Here, some of our recent Small-Angle Neutron Scattering (SANS) studies will be presented as examples of leveraging neutron contrast matching with the intrinsic contrast of different biomolecules and/or the deuterium-labeling induced contrast. The first example is the trimeric Photosystem I SMALP measured at different neutron contract, which showed ~1nm SMA 1440 copolymer belt surrounding the lipid particle [*Brady, N. … Bruce, B. BBA-Bioenergetics 2022, 1863 p148596*]. The second example is the SANS structural study of DIBMA copolymer under various concentrations and ionic strengths. Remarkably, DIBMA exhibits a neutron contrast akin to typical lipids, rendering it an ideal candidate for contrast matching experiment with deuterium-labelled membrane proteins [*Guo, R. … Qian, S. ACS Appl. Bio Mater. 2021, 4, p4760*]. Furthermore, I will also present the latest progress in understanding how DIBMA progressively fragmented membranes to form well-defined monodisperse nanoparticles with SANS and deuterium-labelled membranes.

P-19

SMA Copolymer Isolation of Functional Respirasome Subcomplexes: A Comparative Study

[1]Mahipal S. Rao, [1]Olena Korotych, [1]Spencer Moore, and [1,2]Barry D. Bruce

[1]Biochemistry and Cellular and Molecular Biology Department, University of Tennessee, Knoxville

[2]Chemical and Biomolecular Engineering, University of Tennessee, Knoxville

The respirasome is a large supramolecular ensemble of the electron transfer complexes I-V in the mitochondrial inner membrane. Electrons from NADH and $FADH_2$ are transferred sequentially through a series of respiratory chain complexes to produce ATP through oxidative phosphorylation. The lipid microenvironment around protein complexes is considered essential for stabilizing and enabling the function of these membrane proteins. Extensive research has focused on isolating and characterizing membrane protein complexes I-V using detergents. However, a significant limitation of detergent-based isolation is the loss of the native lipids, which are necessary to preserve the functional activity of membrane proteins. Studying proteins in their native lipid environment provides a more accurate understanding of their function

and regulatory mechanisms. Styrene maleic acid (SMA) copolymers have gained recognition as a powerful and versatile tool for isolating membrane protein complexes with their native lipids. In this study, we evaluated five commercial SMA copolymers (SMA 1440, SMA PRO, SMA 2625, SMA 17352, and XIRAN 30010) for the isolation of functional respirasome from the bovine heart mitochondrial membranes. The copolymer's ability to solubilize respiratory complexes I-V was determined by immunoblotting using monoclonal antibodies specific to each complex, and the results were compared to dodecyl maltoside (DDM) control. Additionally, the redox cofactors were characterized using reduced minus oxidized difference spectroscopy. The results suggest that different SMAs can selectively disrupt the respirasome while preserving different supramolecular ensembles. The functional activity of these different SMALPs was examined using the Seahorse XF Analyzer to determine the oxygen consumption rate (OCR) and sensitivity to substrates and inhibitors. The ability to isolate native-like respiratory SMALPs will be further characterized using native electrophoresis, mass photometry, SAXS, and lipidomics. Gaining insights into the behavior of the respirasome within its native-like surroundings will enhance our ability to address respirasome dysfunction. This dysfunction has been associated with various human conditions, including heart and liver diseases, developmental delays, and neurodegenerative disorders like Alzheimer's and Parkinson's diseases.

P20
SMA derivatives differentially solubilize EGFR and EphA2

Jennifer A Rybak[1], Dibyayan Maity[2], Cameron E. Workman[3], Brian K Long[3], Francisco N Barrera[2]

[1]Department of Genome Sciences and Technology, University of Tennessee, 1311 Cumberland Avenue, Knoxville, TN 37996, USA
[2]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996, USA
[3]Department of Chemistry, University of Tennessee, Knoxville, TN 37996, USA

The development of synthetic amphipathic copolymers for efficient protein and lipid solubilization from native membrane environments was a massive advance for the field. The two most commonly used copolymers, SMA and DIBMA, have been thoroughly studied for their differences in nanodisc size, pH dependency, divalent cation compatibility, solubilization efficiencies, and more. Additionally, many labs have begun investigating derivatives of these polymers, systematically determining how small changes can have large effects on each of these properties. Despite these efforts, there is no clear way to determine the best copolymer for a given protein. Our lab has recently begun investigating interactions between the receptor tyrosine kinases EGFR and EphA2. In this work, we determine how recently published SMA derivatives differentially co-solubilize EGFR and EphA2, with the end goal of finding a copolymer that can be used for coimmunoprecipitation studies. We were intrigued to find that even within the same family of proteins, there were differences in solubilization efficiencies.

P-21
A combined membrane memetic and cell-free expression approach to preparing membrane proteins for structural studies.

Megan L. Shelby,[1] Shakiba Nikfarjam,1 Adam Laurence,[1] Steven A. Hoang-Phou,[1] Brent Segelke,[1] and Matthew A. Coleman[1,2]

[1]Lawrence Livermore National Laboratory, Livermore, CA, 94550
[2]University of California Davis, School of Medicine, Radiation Oncology, Sacramento, CA, 95817

Understanding membrane protein structure and function demands developing novel means of membrane protein expression, solubilization, and purification that preserves native conformation. Using membrane mimetics, especially apolipoprotein-based lipid nanodiscs, with cell-free expression, we have demonstrated expression of solubilized, stable, full-length membrane proteins which are not amenable to detergent solubilization and purification. Cell-free reaction conditions can be modified to control nanoparticle size, membrane protein oligomerization state, and monodispersity of membrane proteins. This adaptable production platform facilitates membrane protein structural and functional studies, using X-ray solution scattering, atomic force microscopy, or electron microcopy for example, as well as other applications requiring a native fold, such as subunit vaccine development. As an initial step towards integrating polymer-based lipid nanodisc technologies with cell-free membrane protein expression, we have screened conditions for styrene maleic anhydride co-polymer solubilization during and after cell free expression in the presence of lipids. Under some solubilization conditions (e.g., choice of co-polymer, polymer concentrations, and lipid mass ratios) polymer effects on expression yields were minimal and the solubilities of membrane proteins initially expressed in proteoliposomes were enhanced. This demonstrates the feasibility of one-pot, SMALP solubilized, membrane protein expression and nanodisc assembly via cell free expression.

P-22
Kinetic analysis of the activation process of the human A_{2A} adenosine receptor in the lipid-nanodiscs

Sriram Tiruvadi-Krishnan[1], Shushu Wei[1], Niloofar Gopal Pour[2], Arka Prabha Ray[2], Naveen Thakur[2], Matthew T. Eddy[2], Rajan Lamichhane[1]

[1]Department of Biochemistry & Cellular and Molecular Biology, College of Arts and Sciences, University of Tennessee, 1311 Cumberland Avenue, Knoxville, TN 37996, USA
[2]Department of Chemistry, College of Liberal Arts and Sciences, University of Florida, 126 Sisler Hall, Gainesville, FL 32611, USA.

The human A_{2A} adenosine receptor (A_{2A}AR) is a prototypical class A G protein-coupled receptor (GPCR). The constitutively activating mutations known as CAMs change cell signaling activity and alter the efficacy of drugs that control the signaling. They are prevalent in numerous diseases with limited treatment options, but the structural basis for how CAMs modify the GPCR activity remains unknown. We employed single-molecule total internal reflection fluorescence (smTIRF) imaging to visualize the dynamics of the activation mechanism of A_{2A}AR and its CAM variants embedded in the lipid nanodiscs. In the CAM variants, we observed an increased active-state population even in the absence of agonist stimulation and antagonist-bound receptors. From the data compiled from individual fluorescence emission trajectories (n>100), we observed that CAMs significantly

increased the population of an intermediate state that may be crucial for receptor activation. Quantitative analysis of activation kinetics confirmed that CAMs increased the frequency of transitions to the intermediate state. In contrast, a mutation that decreases sodium sensitivity increases transitions away from the intermediate state to an inactive conformation. Our results emphasize the significance of characterizing the intermediate state, which seems to control the activation of A_{2A}AR. These observations may aid us in predicting the functional changes caused by mutations based on whether they increase or decrease the intermediate state population. Overall, our work offers a new framework to engineer GPCR with altered functions and discover drugs explicitly targeting the intermediate state.

P-23
Elucidating the molecular targets of bacterial nano-syringes

Calum, Upton[1], Alice, Rothnie[1], and Alan Goddard[1]

[1]Bioscience Department, Health and Life Science, Aston University, Aston Street, Birmingham, B4 7ET

Nano-syringes are naturally found in the bacterial genus *Photorhabdus,* containing toxins to produce a pathogenic effect on insect cells, symbiotically assisting the Nematode, the gut of whom they naturally inhabit. Nano-syringes are a type of extracellular contractile injection system, a perforative biological nanodevice that shares ancestry with one of the most abundant organisms in nature - bacteriophages.
The specific and effective nature of nano-syringe toxin transmission presents an exciting opportunity for site-specific delivery of therapeutic drugs, alleviating the risk of off-target effects.
An essential step in the ability to utilise bacterial nano-syringes as a therapeutic drug delivery vector involves the elucidation of the molecular targets. Where the nano-syringe binds on the cell membrane is unknown. This project involves explicating the binding site, allowing for optimisation of the nano-syringe 'targeting' system, and introducing therapeutic drugs directly to where they are needed most.
Pvc13, the tail fibre protein and binding region of the nano-syringe, has been bound to a *Spodoptera frugiperda* (Sf9) insect cell membranes, and evidence for binding to the polymer nanodiscs DIBMA and Glyco-DIBMA, solubilising Sf9, has been observed. Further work involves detecting Pvc13 binding partners through co-immunoprecipitation pull-down assays and mass photometry contrast measurements for Pvc13-nanodisc complexes.

P-24
SiMPull-POP: A method for quantifying membrane protein oligomerization in native-like membrane environments

Ward, Alyssa[1], Schuck, Ryan[1], Lamichhane, Rajan[1], Sgouralis, Ioannis[2], and Francisco N. Barrera[1]

[1]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville 37916, USA
[2]Department of Mathematics, University of Tennessee, Knoxville 37916, USA

The oligomerization of membrane receptors plays a key role in their activation and consequent downstream cellular activity. However, methods that quantify membrane receptor oligomerization often utilize detergent to solubilize these receptors. This solubilization reagent displaces the native membrane environment surrounding a receptor or complex and subsequently can alter protein conformation and oligomerization. Here we present a modified Single Molecule Pulldown (SiMPull) approach coupled with Total Internal Reflection Fluorescence (TIRF) microscopy to investigate protein oligomerization in native-like membrane environments. To capture a more native-like environment for proteins of interest, we introduce the polymer DIBMA (diisobutylene maleic acid) as the solubilization agent to isolate protein complexes from mammalian cell membrane fractions. To validate this method, termed SiMPull-POP, we quantified the oligomerization of a well-studied ligand-inducible dimer system, the FK506 binding protein (FKBP). To quantify FKBP oligomerization, fluorescently tagged FKBP constructs (FKBP-GFP) were transiently expressed and isolated in membrane fractions from HEK293T cells that were solubilized with DIBMA to form DIBMA lipid particles (DIBMALPs). Constructs were immobilized for imaging on a prism based TIRF microscope system. Photobleaching events were captured and analyzed to generate percent photobleaching step and oligomeric populations. Analysis revealed the expected shift of the construct from a more monomeric to dimeric state upon ligand (AP) addition. Collectively, these results reflect the expected oligomerization behavior of FKBP demonstrating that the SiMPull-POP method quantitatively characterizes protein oligomerization in a native-like membrane environment.

P-25
Deuteration Capabilities of the Center for Structural Molecular Biology at Oak Ridge National Laboratory

Kevin L. Weiss[1], Honghai Zhang[2], Qiu Zhang[1], and Hugh O'Neill[1]
[1]Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA.
[2]Center for Nanophase Material Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA.

Neutron scattering research at the High Flux Isotope Reactor and the Spallation Neutron Source, two world-class user facilities at Oak Ridge National Laboratory (ORNL), offers valuable insight into the structure and dynamics of materials. Due to the remarkable sensitivity of neutrons to hydrogen (H), a ubiquitous element in organic molecules, neutron scattering techniques are especially powerful for studying biological materials. Furthermore, due to the substantial difference in the neutron scattering lengths of H and deuterium (D), efficient production of isotopically labeled biomolecules with varying H/D content can be crucial to the success of a neutron scattering experiment. In support of ORNL's neutron scattering user facilities, biological and chemical deuteration efforts for the efficient preparation of H/D-labeled biomaterials are led by the Center for Structural Molecular Biology (CSMB). In this poster presentation, the technical capabilities and recent developments will be highlighted.

P-26
Biosynthesis of deuterated lipids for structural and biophysical characterization of biomembranes and membrane proteins

Qiu Zhang[1], Hong-Hai Zhang[2], Matthew Keller[3,4], Wellington Leite[1], Shuo Qian[5], Robert Hettich[4], Hugh O'Neill[1]

[1] Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[2] Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[3] UT-ORNL Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, TN 37996, USA
[4] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA
[5] Second Target Station, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

Membrane proteins play crucial roles in many cellular processes, however, studying membrane proteins is challenging because of their complex structure and fragility when isolated from their native environment. One solution is to embed membrane proteins in a membrane-mimic to provide a more native environment to facilitate their characterization. Small-angle neutron scattering (SANS) is an ideal technique to obtain structural information on biomacromolecules under physiologically relevant conditions. With this technique, deuterated phospholipids need be used to suppress their ^{1}H signal in SANS measurements. Currently, there are three ways to obtain deuterated phospholipids; extraction of native lipids from cells produced in deuterated media, chemical synthesis, or semi-synthetic approaches that combine both routes. In this study, we report on producing deuterated phosphatidylethanolamine (PE) by extraction and fractionation from native *Escherichia coli* extracts, and phosphatidylcholine (PC) from an engineered *E. coli* strain. The PC synthase (PCs) pathway was introduced into *E. coli* to produce partially deuterated and perdeuterated PC by feeding deuterated *E. coli* cultures with hydrogenated or deuterated choline chloride. The isolated PC product was confirmed by ^{1}H *Nuclear Magnetic Resonance (*NMR*)* and Liquid Chromatography - Mass Spectrometry (LC-MS) was used to determine the deuteration level of PC produced under different growth conditions. These materials can be used for neutron scattering studies with micelles, bicelles, liposomes, styrene-maleic acid lipid particles (SMALPs), and Membrane Scaffold Protein (MSP)-based lipid nanodiscs to produce a membrane-mimicking environment for studying membrane proteins, and can be used for deuterated lipids for NMR studies as well.

P-27
Unlocking Secrets of the Heart with Surface-Active Polymers

[1]Arba Zhao, [2]Mahipal Singh Rao, [2]Sree Kavya Penneru, [2]Olena Korotych, [2]Spencer Moore, [2,3]Barry D. Bruce

[1] Webb School of Knoxville, Knoxville, TN
[2] Biochemistry and Cellular and Molecular Biology Department, University of Tennessee at Knoxville, Knoxville, TN
[3]Chemical and Biomolecular Engineering Department, University of Tennessee at Knoxville, Knoxville, TN

In recent developments in the field of protein isolation, styrene-maleic acid (SMA) and diisobutylene-maleic acid (DIBMA) copolymers have significantly advanced the study of membrane proteins as it allows researchers to study the proteins in a more native environment. Polymers with surface-active properties, like DIBMA and SMA, offer a potential solution to address drawbacks commonly associated with conventional detergent-based methods. These drawbacks include protein denaturation and the displacement of native lipids, which can impact membrane proteins' structural and functional stability. Compared to SMA polymers, DIBMA polymers have a significant potential for advancing membrane protein research due to their improved properties, such as low sensitivity to divalent cations and pH changes. Heart mitochondrial membranes (HMMs) are well-studied protein-rich membranes that host several multi-subunit protein complexes associated with oxidative phosphorylation. Dysfunction of mitochondrial membrane proteins is related to various diseases, including neurodegenerative disorders, metabolic diseases, and certain types of cancer. Studying these proteins in their native-like environment is crucial for understanding the molecular basis of these conditions and developing potential therapeutic strategies. Herein, we tested a variety of different copolymers (DIBMA-10, DIBMA-12, Sulfo-DIBMA, SMALP-BZ30, Sulfo-SMA, SMALP-BZ40, and SMALP-BZ23) to isolate membrane protein complexes from bovine HMMs. These polymers' efficacy in solubilizing biomembranes was evaluated based on protein content. The extracted complexes were also studied by western-blot, sodium dodecyl sulfate and clear native polyacrylamide gel electrophoresis (WB, SDS- and CN-PAGE) and immunoblot analysis for complexes I-IV. Our results indicate variability in the solubilization efficacy of commercially available surface-active polymers, though all polymer formulations could extract membrane proteins from HMMs efficiently.

SMALP Participant List

Last Name	First Name	Organization	Department	Primary Email	Position
Adetunji	Joy	University Of Tennessee	Biochemistry, Cellular And Molecular Biology	jadetunj@vols.utk.edu	Graduate Student
Agyemang	Eugene	University Of Tennessee, Knoxville	Genome Science & Technology	eagyeman@vols.utk.edu	Graduate Student
Ali	Fidaa	knitme	Genome Science And Technology	fali5@vols.utk.edu	Phd Student
Anton	Jeffrey	UTK	BCMB	janton2@vols.utk.edu	Grad Student
Arcario	Mark	Washington University in Saint Louis	Anesthesiology	mjarcario@wustl.edu	Instructor
Ball	Lauren	Stellenbosch University	Chemistry and Polymer Science	leball@sun.ac.za	PhD Candidate
Banerjee	Rahul	Auburn University	Chemistry And Biochemistry	rzb0120@auburn.edu	Assistant Professor
Bao	Huan	Univeristy Of Virginia		pyn4sg@virginia.edu	
Barnard	Elaine	Stellenbosch University		ebarnard@sun.ac.za	
Barrera	Francisco	University Of Tennessee	Biochemistry & Cellular And Molecular Biology	fbarrera@utk.edu	Associate Professor
Bhattacharyya	Moitrayee (Moi)	Yale University	Pharmacology	moitrayee.bhattacharyya@yale.edu	Assistant Professor
Bishop	Cora	University Of Tennessee	Genome Science And Technology (gst)	cbisho30@vols.utk.edu	Graduate Student
Booth	Paula	King's College, London		paula.booth@kcl.ac.uk	
Brady	Nathan	Sandia National Laboratories		ngbrady@sandia.gov	Postdoctoral Appointee
Brady	Ryan	St Jude Children's Research Hospital	Structural Biology	rbrady@stjude.org	Post-doc
Brown	Caroline	Yale University	Cell Biology	caroline.brown@yale.edu	Graduate Student
Bruce	Barry	University Of Tennessee At Knoxville	BCMB	bbruce@utk.edu	Professor
Bryant	Kaeli	Vanderbilt University	Pathology, Microbiology, And Immunology	kaeli.n.bryant@vanderbilt.edu	Graduate Student
Chandrasekhar	Soumya	Kent State University	MATERIALS SCIENCE	schandr7@kent.edu	Graduate Student
Chaudhary	Asmita	University Of Tennessee	BCMB	achaud11@vols.utk.edu	Graduate Student
Clark	Lindsay	Tectonic Therapeutic	Membrane Protein Biochemistry	lclark@tectonictx.com	Scientist
Cobb	Grayson	University Of Tennessee, Knoxville	Biochemistry & Cellular & Molecular Biology	gcobb3@vols.utk.edu	Undergraduate Research Assistant
Dabney-Smith	Carole	Miami University	Chemistry And Biochemistry	smithac5@miamioh.edu	Ernest H. Volwiler Professor
De Val	Natalia	Thermo Fisher Scientific		natalia.deval@thermofisher.com	
Deutz	Lars	University of Texas at Austin	Molecular Biosciences	larsdeutz@utexas.edu	Undergraduate Researcher
Elswick	Danielle	Thermo Fisher Scientific		danielle.elswick@thermofisher.com	
Eskridge	Shabré	University Of Tennessee, Knoxville	Biochemistry And Molecular Biology	seskridg@vols.utk.edu	Graduate Student
Fiebig	Olivia	Penn State University	Chemistry	ocf5030@psu.edu	Postdoctoral Scholar
Fozo	Elizabeth	University Of Tennessee	Microbiology	efozo@utk.edu	
Ganusova	Elena	University Of Tn	BCMB	eganusov@utk.edu	Phtographer

GHOSH	SNEHASISH	Yale university	Cell Biology	snehasish.ghosh@yale.edu	Postdoc Associate
Gonneville	Alyssa	University Of Tennessee		agonnevi@vols.utk.edu	
Guo	Youzhong	Commonwealth Virginia		yguo4@vcu.edu	
Gupta	Kallol	Yale University	Department Of Cell Biology	kallol.gupta@yale.edu	Assistant Professor
Hajiyeva	Sarvinaz	Kent State University	Biomedical Sciences	shajiyev@kent.edu	Graduate Student
Hanks	Timothy	Furman University	Chemistry	tim.hanks@furman.edu	Professor And Chair
HARNISH	MICHAEL	University Of Tennessee	BCMB	Mharnish@vols.utk.edu	
Hasan	Md Mahmudul	University Of Tennessee, Knoxville	Genome Science And Technology	mhasan32@vols.utk.edu	Graduate Research Assistant
Hawkins	Olivia	Aston University	HLS	hawkinso@aston.ac.uk	Post-Graduate Student
Heberle	Fred	University Of Tennessee	Chemistry	fheberle@utk.edu	Assistant Professor
Hertslet	Gillian	University Of Tennessee Knoxville	BCMB	vqb784@vols.utk.edu	Undergraduate Student
House	Morgan	University of Tennessee	BCMB	mhouse6@vols.utk.edu	Graduate Student
Huynh	Ruby	Arizona State University		phuynh5@asu.edu	
Joshi	Amit	University Of Tennessee, Knoxville	BCMB	AJOSHI18@UTK.EDU	Assistant Professor
Kaiser	Marie	University of Tennessee	BCMB	mkaiser9@vols.utk.edu	
Kamaradiwela Arachchige	Nimarsha	Miami University	Department Of Chemistry And Biochemistry	kamarans@miamioh.edu	Graduate Student
Keller	Sandro	University Of Graz	Biophysics	sandro.keller@uni-graz.at	
Khan	Zidan	University Of Tennessee Knoxville	Chemistry	zkhan4@vols.utk.edu	Graduate Student
Khanal	Susmita	University of Tennessee, Knoxville	BCMB	skhanal5@vols.utk.edu	Graduate Student
Klumperman	Bert	Stellenbosch University		bklump@sun.ac.za	
Korotych	Olena	University of Tennessee	BCMB	oik@utk.edu	
Kubicek	Jan	Cube Biotech Gmbh	Cube Biotech	Jan.Kubicek@cube-biotech.com	Managing Director
Kumar	Shailesh	Yale University Department of Nanosene |		shailesh.kumar@yale.edu	
Kuyler	Gestél	Stellenbosch/Coventry		gckuyler@sun.ac.za	
Lamichhane	Rajan	University of Tennessee Knoxville	Biochemistry & Cellular and Molecular Biology	rajan@utk.edu	Assistant Professor
Liang	Hongjun	Texas Tech University Health Sciences Center	Cell Physiology And Molecular Biophysics	h.liang@ttuhsc.edu	Professor
Long	Brian	Univ. of Tennessee	Chemistry	Long@utk.edu	Professor
Lynch	Dana	UTK	BCMB	hrt597@vols.utk.edu	Graduate Student
Maharjan	Prabal Singh	THE UNIVERSITY OF TENNESSEE AT	Biochemistry And Cellular And Molecular Biology	pmaharj1@vols.utk.edu	
Maity	Dibyayan	University Of Tennessee	Biochemistry & Cellular And Molecular Biology	dmaity@vols.utk.edu	Graduate Student
Marty	Michael	University of Arizona	Department Of Chemistry And Biochemistry	mtmarty@arizona.edu	Associate Professor

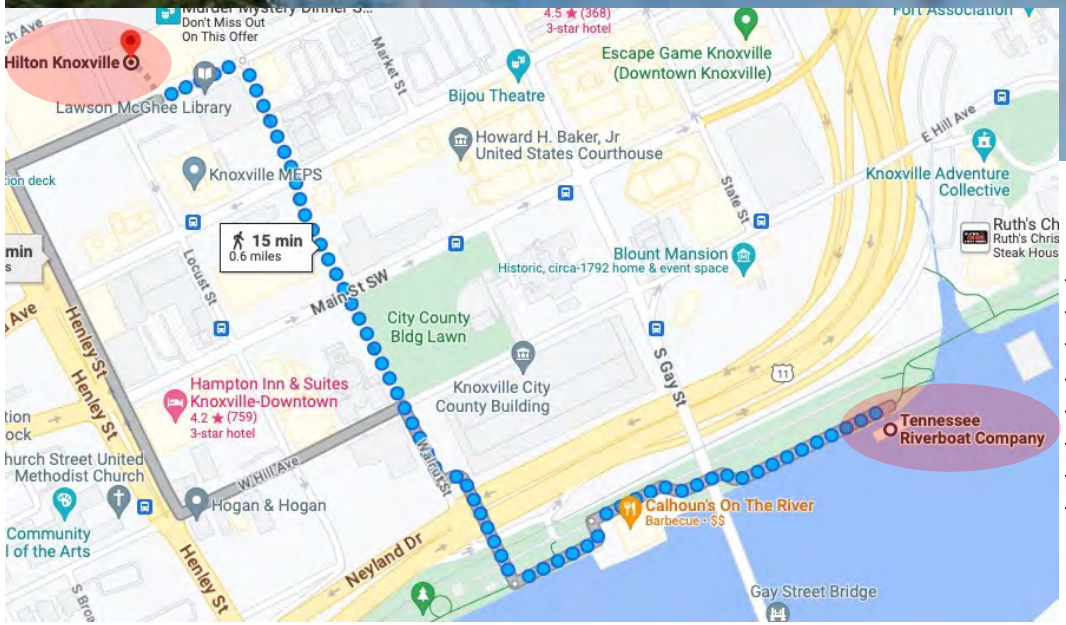
Massenburg	Lynnicia	Pennsylvania State University	Biochemistry, Microbiology And Molecular Biology	lzm225@psu.edu	Phd Candidate
Matthies	Doreen	National Institutes Of Health	Unit On Structural Biology	doreen.matthies@nih.gov	Earl Stadtman Tenure Track Investigator
Mittal	Ritesh	Anatrace Products LLC	R & D	ritesh_mittal@anatrace.com	Vice President
Moore	Spencer	BCMB	Biochemistry & Cellular and Molecular Biology	smoor112@vols.utk.edu	Undergraduate researcher
Mr.	Ryan	University Of Tennessee		rschuck@vols.utk.edu	
Naidoo	Narisa	University Of Tennessee Knoxville	BCMB	nnaidoo@vols.utk.edu	Undergraduate Student
Necelis	Matthew	University Of Virginia		Mrn5ht@virginia.edu	
Nevzorov	Alexander	North Carolina State University		alex_nevzorov@ncsu.edu	
Nicki	Jennifer	Pfizer	Cellular, Genomic, And Protein Sciences	jennifer.nicki@pfizer.com	Senior Scientist
Nikfarjam	Shakiba	Lawrence Livermore National Laboratory		nikfarjam1@llnl.gov	Postdoc Graduate
Ochola	Godwin	University of Tennessee, Knoxville	Chemistry	gochola@vols.utk.edu	Research/Teaching
Okorafor	Evelyn	Miami University	Chemistry And Biochemistry	okorafea@miamioh.edu	Graduate Assistant
O'Neill	Hugh	Oak Ridge National Laboratory		oneillhm@ornl.gov	
Overduin	Michael	University Of Alberta	Biochemistry	overduin@ualberta.ca	Professor
Penneru	Sree Kavya	University Of Tennessee	Biochemistry And Cellular And Molecular Biology	spenneru@vols.utk.edu	Graduate Student
Pruitt	Dylan	University Of Tennessee - Knoxville	BCMB	dpruitt4@vols.utk.edu	Graduate Student
Pyron	Jordan	University Of Tennessee, Knoxville	Genome Science & Technology	rpyron@vols.utk.edu	Graduate Student
Qian	Shuo	Oak Ridge National Laboratory		qians@ornl.gov	
Rao	Mahipal S	University Of Tennessee	BCMB	mrao3@vols.utk.edu	Graduate Student
Ravula	Thirupathi	Nmrfam, University Of Wisconsin	Biochemistry	travula@wisc.edu	scientist
Reynolds	Todd	University of Tennessee Knoxville	Microbiology	treynol6@utk.edu	Professor
Rodriguez Garcia	Maria Teresa	University Of Tennessee, Knoxville	BCMB	mrodrgue@vols.utk.edu	Graduate Student
Rothnie	Alice	Aston University	Biosciences	a.rothnie@aston.ac.uk	Reader
Rotich	Nancy	Miami University	Chemistry and Biochemistry	chepchnr@miamioh.edu	Graduate Student
Russell	Charles	University Of Tennessee, Knoxville	BCMB	xwz976@vols.utk.edu	Graduate Research Assistant
Rybak	Jennifer	University Of Tennessee, Knoxville	BCMB	jschust2@vols.utk.edu	PhD Candidate
Sahoo	Abhishek	University Of Tennessee	Genome Science & Technology	asahoo1@vols.utk.edu	Graduate Student
Saksena	Nilabh	University Of Tennessee	Bcmb	nsaksen1@vols.utk.edu	Undergraduate
Sanders	Charles	Vanderbilt University	Biochemistry	chuck.sanders@vanderbilt.edu	
Schmidt	Thorsten	Kent State University	Physics	tschmi21@kent.edu	Associate Professor
Shah	Muhammad Zeeshan	Miami University	Chemistry And Biochemistry	shahm@miamioh.edu	Phd Candidate

Sharma	Karan	University of Tennessee	Chemistry	ksharma4@vols.utk.edu	Graduate Student
Shelby	Megan	Lawrence Livermore National Lab	Biosciences And Biotechnology	shelby4@llnl.gov	Staff Scientist
Shelby	Sarah	University Of Tennessee	BCMB	sshelby4@utk.edu	
Sligar	Stephen	University Of Illinois	Biochemistry	s-sligar@illinois.edu	Professor
Smith	Madeline	University Of Tennessee, Knoxville	Biochemistry, Cellular, & Molecular Biology	msmit427@vols.utk.edu	Graduate Student
Sundar Prakash	Praneetha	Kent State University	Biomedical Sciences	psundarp@kent.edu	Phd Student
Suresh	Praveena	University Of Tennessee	BCMB	psuresh1@vols.utk.edu	Graduate Student
Tiruvadi Krishnan	Sriram	University of Tennessee, Knoxville	Biochemistry, Cellular And Molecular Biology	stiruvad@utk.edu	Postdoctoral Researcher
Umbach	Simon	Goethe University Frankfurt	Institute for Biophysical Chemistry	umbach@bpc.uni-frankfurt.de	Phd Student
Upton	Calum	Aston University	School Of Bioscience	calum_upton@yahoo.co.uk	Phd Student
Vaithiyalingam	Sivaraja	Vanderbilt University	Biochemistry	sivaraja.vaithiyalingam@vanderbilt.edu	Senior Scientist
Varaich	Mairaj Bibi	University Of Tennessee	BCMB	mvaraich@vols.utk.edu	Graduate Student
Ward	Alyssa	University Of Tennessee, Knoxville	Biochemistry & Cellular And Molecular Biology	award51@vols.utk.edu	Graduate Student
Wei	Shushu	University of Tennessee	BCMB	swei5@vols.utk.edu	
Weiss	Kevin	Oak Ridge National Laboratory		weisskl@ornl.gov	
Williams	Ann	Bartlett-Williams Scientific Resources		justcallann@bartlett-williams.com	
Workman	Cameron	Utk	Chemistry	cworkma3@vols.utk.edu	
Zhang	Qiu	Oak Ridge National Laboratory		zhangq@ornl.gov	
Zhao	Wanxin	Bruce Lab, University Of Tennessee		wzhao29@vols.utk.edu	
Zhou	Yue (Aeric)	University of Tennessee Knoxville	Microbiology	yzhou58@vols.utk.edu	Graduate Research Assistant

Notes:

SMALP-23 CONFERENCE DINNER CRUISE
THE STAR OF KNOXVILLE RIVERBOAT

PRE-REGISTRATION @RECEPTION REQUIRED
SEATING IS LIMITED TO 75



Walking Directions
It is a ~15 min walk
Sailing at 6:00 PM Sharp
Return by 8:30

Hilton Knoxville
501 W Church Ave, Knoxville, TN 37902

-Head northeast on W Church Ave toward Walnut St (253 ft)
-Turn right onto Walnut St (0.2 mi)
-Turn left toward Neyland Greenway (377 ft)
-Turn left onto Neyland Greenway (131 ft)
-Cont. straight to stay on Neyland Greenway (177 ft)
-Turn right to stay on Neyland Greenway (210 ft)
-Slight right to stay on Neyland Greenway
--Destination will be on the right (0.1 mi)

Tennessee Riverboat Company
300 Neyland Dr, Knoxville, TN 37902

Trail Map


TENNESSEE RIVER
IJAMS TRAILHEAD
MCCLURE LANE TRAILHEAD
MEAD'S QUARRY
MEAD'S QUARRY TRAILHEAD
FORKS OF THE RIVER WILDLIFE MGMT AREA
ABERDEEN TRAILHEAD
GRAYSON SUBARU PRESERVE
ROSS MARBLE QUARRY
IJAMS CRAG
MARIE MYERS PARK
IJAMS NATURE CENTER
NORTH
1000 FT

GREENWAYS

CITY OF KNOXVILLE

GREENWAY

City of Knoxville

www.cityofknoxville.org/greenways

Greenways give citizens access to Knoxville's beautiful scenery

Our city is defined by its creek valleys and high ridges. The City's many creeks, rivers and quarries are adjacent to some of the most popular greenway trails. Greenways located next to water create a beautiful synergy. The creeks, rivers and quarries provide scenic places to walk and bicycle. Knoxville greenways bring residents into proximity with our water, which in turn enhances response time to hazards that endanger our water's quality. Water resources touch on the many benefits of greenways: – **Health and Recreation** – The presence of a neighborhood greenway encourages walking for fun and fitness. Regular physical activity is key to health and disease prevention. – **Community Cohesion** – Greenways are a place to walk with neighbors. Multiple neighborhoods that adjoin along the same creek can rally together to lobby for planning of greenways along their creek. – **Alternative Transportation** – Locating greenways in scenic creek valleys is an enticing way to encourage residents to bicycle and walk to work. These creek valleys at the heart of every watershed are the ideal place to locate greenways on land that is prone to flooding and not otherwise suitable for development. – **Economic Prosperity** – Bearden Village Greenway has created an economic boon for Sutherland Avenue. As the greenway was being constructed, West High School received a new landscape for its entrance. New businesses built sidewalks on the north side of Sutherland to improve pedestrian access. – **Natural Resources** – Our watershed's health is protected by park areas owned and monitored by the City and the public. Greenways create vegetative buffers for water runoff coming from roads and parking lots. – **Education Opportunities** – Ijams Nature Center is home to river, creek and quarry greenways for ecological, nature-based education. Soon children will be able to ride their bikes across the South Knoxville bridge—crossing from downtown Knoxville to Ijams. – **Sustainable Development** – Greenways present significant opportunities to guide land use, ensuring that as communities grow, green space and recreation opportunities are preserved. Furthermore, greenways are a redevelopment tool to help "green" brownfields in our urban areas.

– **Urban Wilderness and Historic Corridor** The ridges, forests, forts and parks along Knoxville's South Waterfront offer potential for an incredible recreation and historic corridor. The 1,000-plus acre urban wilderness contains three civil war forts, historic settlement sites, parks, trails and recreation amenities all within the heart of the city. The majority of the recreation and historic areas within the corridor are already public lands and parks. The Legacy Parks Foundation is working to add essential properties and connect all of the assets with a system of trails, greenways and sidewalks. – **First Creek Greenway** – The first two non-native settlers in Knoxville, John Adair and James White, chose to live in the environs of First Creek (originally called White's Creek). Today, First Creek links multiple North Knoxville neighborhoods, parks and schools on its route from downtown Knoxville to Fountain City and beyond. – **The First Creek Greenway Plan** – This plan envisions a greenway approximately 10 miles long. It begins at Tommy Schumpert Park and follows Knoxville's historic First Creek under I-640 and I-40, to its confluence on the banks of the Tennessee River. Two portions of the greenway have already been constructed, and the City has received funding for a third segment, which will link Fulton High School to Edgewood Park. The plan will create a "green corridor " of parks and greenways north to south, through the heart of Knoxville. A comparison of the existing Third Creek Greenway and the plans for First Creek Greenway:

First Creek Greenway	Third Creek Greenway
About 10 miles long	**9.25 miles long**
Along the way:	Along the way:
2 high rise towers	1 high rise tower
8 schools	2 schools
18 existing parks	8 existing parks
15 proposed parks	

Greenways encourage the education and the conservation of nature in an urban setting.

Greenways inspire races and other events that attract tourism to our city.

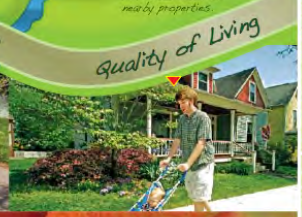
Greenways entice people to be active outdoors.

Commuting opportunities reduce automobile pollution and improve air and water quality.

Greenways serve as a natural buffer against non-point source pollution.

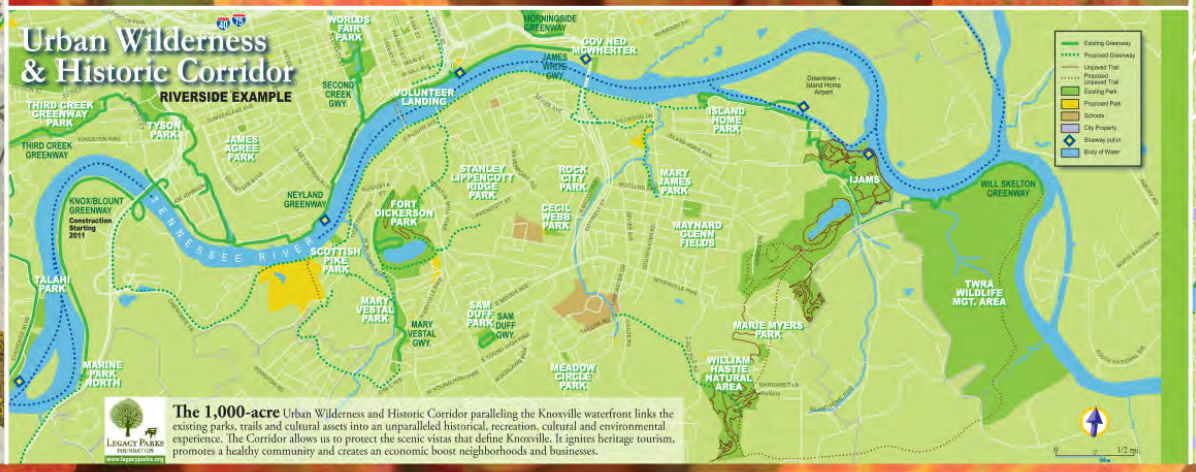
Greenways increase the real estate value of nearby properties.

Conservation of Nature | Tourism | Fitness | Clean Air | Clean Water | Quality of Living



Urban Wilderness & Historic Corridor

RIVERSIDE EXAMPLE



The 1,000-acre Urban Wilderness and Historic Corridor paralleling the Knoxville waterfront links the existing parks, trails and cultural assets into an unparalleled historical, recreation, cultural and environmental experience. The Corridor allows us to protect the scenic vistas that define Knoxville. It ignites heritage tourism, promotes a healthy community and creates an economic boost neighborhoods and businesses.

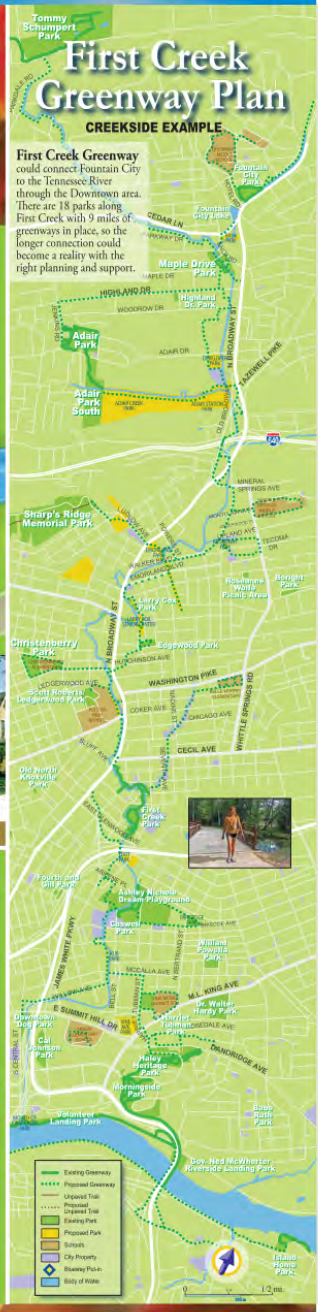
IJAMS NATURE CENTER

Ijams is a post-industrial, natural area of restoration and reclamation efforts. Its 275-acres include extensive nature trails.

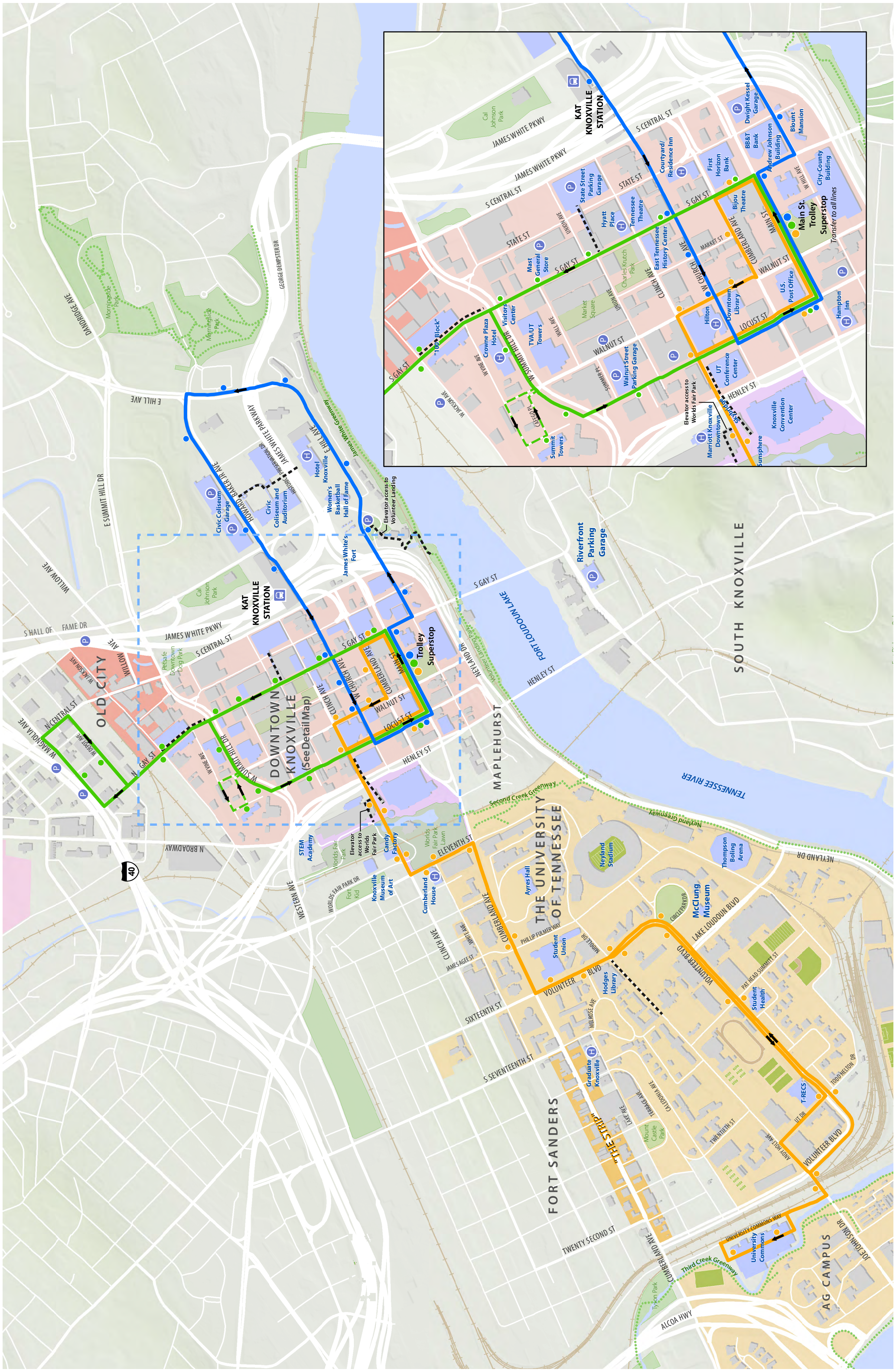
First Creek Greenway Plan

CREEKSIDE EXAMPLE

First Creek Greenway could connect Fountain City to the Tennessee River through the Downtown area. There are 18 parks along First Creek with 9 miles of greenways in place, so the longer connection could become a reality with the right planning and support.



SMALP-23


DOWNTOWN KNOXVILLE
(See Detail Map)
OLD CITY
MAPLEHURST
SOUTH KNOXVILLE
THE UNIVERSITY OF TENNESSEE
FORT SANDERS
AG CAMPUS
KAT KNOXVILLE STATION
Trolley Superstop
Main St. Trolley Superstop
Transfer to all lines
FORT LOUDOUN LAKE
TENNESSEE RIVER
Riverfront Parking Garage
Civic Coliseum Garage
Civic Coliseum and Auditorium
Women's Basketball Hall of Fame
Hotel Knoxville
James White's Fort
Elevator access to Volunteer Landing
Elevator access to Worlds Fair Park
Candy Factory
Knoxville Museum of Art
Cumberland House
STEM Academy
Neyland Stadium
Thompson Boling Arena
McClung Museum
Ayres Hall
Student Union
Hodges Library
Student Health
T-RECS
Graduate Knoxville
"THE STRIP"
University Commons
Knoxville Convention Center
Sunsphere
Marriott Knoxville Downtown
UT Conference Center
Hilton
Downtown Library
U.S. Post Office
Hampton Inn
City-County Building
Blount Mansion
Andrew Johnson Building
BB&T Bank
Dwight Kessel Garage
First Horizon Bank
Bijou Theatre
Courtyard/Residence Inn
Tennessee Theatre
Hyatt Place
East Tennessee History Center
State Street Parking Garage
Mast General Store
Market Square
Charles Krutch Park
TVA/UT Towers
Walnut Street Parking Garage
Visitors Center
Crowne Plaza Hotel
"100 Block"
Summit Towers
Cal Johnson Park
JAMES WHITE PKWY
S CENTRAL ST
STATE ST
S GAY ST
UNION AVE
MARKET ST
W CHURCH AVE
CUMBERLAND AVE
MAIN ST
WALNUT ST
LOCUST ST
HENLEY ST
W SUMMIT HILL DR
CLINCH AVE
WALL AVE
W JACKSON AVE
NEYLAND DR
VOLUNTEER BLVD
LAKE LOUDOUN BLVD
PHILLIP FULMER WAY
SIXTEENTH ST
S SEVENTEENTH ST
TWENTY SECOND ST
ALCOA HWY
ELEVENTH ST
N BROADWAY
WESTERN AVE
E HILL AVE
DANDRIDGE AVE
WILLOW AVE
E SUMMIT HILL DR
N CENTRAL ST
W MAGNOLIA AVE
Second Creek Greenway
Third Creek Greenway
Neyland Greenway
James White Greenway

The Ale Trail

Breweries

1. Abridged Beer Company
2. Albright Grove Brewing Company
3. Alliance Brewing Company
4. Balter Beerworks
5. Blackhorse Pub & Brewery Downtown
6. Crafty Bastard Brewery
7. Crafty Bastard Brewery West
8. Downtown Grill & Brewery
9. Elkmont Exchange
10. Elst Brewing Company
11. Fanatic Brewing Company
12. Hexagon Brewing Company
13. Last Days of Autumn Brewing
14. Next Level Brewing Company
15. Orange Hat Brewing Co.
16. Pretentious Beer Co.
17. Printshop Beer Co.
18. Schulz Brau Brewing Company
19. Smoky Mountain Brewery
20. The Oak Room by Abridged

Breweries On Tap

21. Balter Beerworks
22. Blackberry Farm Brewery
23. Clinch River Brewing
24. Geezers Brewing Co.
25. Gypsy Circus Cider
26. Hi-Wire Brewing Taproom
27. Orange Hat Brewing Co.

VISIT KNOXVILLE TENNESSEE