Poster 1 – Tuba Marjan, Taimoor H. Qazi

Affiliations: Weldon School of Biomedical Engineering, Purdue University

Hydrogel platform with tunable biochemical properties to study cell-matrix interactions

Skeletal muscle degenerative conditions pose a significant challenge in tissue repair, largely due to a limited understanding of how various extracellular matrix (ECM) properties influence cell behavior. The ECM is a complex network of various proteins, glycans, and other biomolecules and is fundamental in providing structural support and biochemical signals that direct cell behavior and function. In diseased or injured muscle, the ECM undergoes fibrotic remodeling leading to changes in biochemical composition, yet how these altered biochemical signals influence cell behavior is unknown and challenging to study in vitro. Here, we describe a tunable hydrogel platform based on norbornene-modified hyaluronic acid (Nor-HA) that undergoes light-mediated crosslinking and allows functionalization with thiolated biomolecules. As one example, we modify Nor-HA with fibronectin-derived cell adhesive peptide containing the arginine-glycine-aspartic acid (RGD) sequence, which promotes integrin-mediated cell adhesion, an important step in cell attachment, spreading, and signaling. The presence of thiol groups on the peptide allowed its chemical conjugation to Nor-HA. When seeded with C2C12 myoblasts, widely used in skeletal muscle tissue engineering studies, the hydrogels supported cell adhesion, proliferation, and differentiation into multinucleated myotubes as confirmed by fluorescent staining for F-actin, nuclei, and myosin heavy chain. Future studies will study the impact of including peptides that mimic other proteins overexpressed in injured muscle, ultimately enabling us to elucidate the role of cell-matrix interactions on muscle repair and regeneration.

Poster 2 – Shu Li, Anika Jain, Daisuke Kihara

Affiliations: Department of Biological Sciences, Purdue University

Ligand modeling into Cryo-EM maps

A Deep Learning method is used to predict ligand densities in Cryo-EM maps. This prediction is then analyzed for generalizability using unique ligand clustering methods that can be used to assess if the deep learning model can be utilized for predicting ligand densities it has not been trained on. Further, after predicting the ligand densities the ligand can be modeled into the predicted densities using a pipeline that combined cross-correlation based docking and cross-correlation analysis to rank them.

Poster 3 – Alexander. Baena[1-3], D. Marshall Porterfield[1,2]

Affiliations: [1]Department of Agricultural & Biological Engineering, Purdue University, [2]Elevated BioAstronautics, Cape Girardeau, MO, [3]Monte Jongas, Bogota, Colombia

Novel Sporocarp Production System using Engineered Biophysical Elements Without Solid Substrates

Novel biotechnological approaches are needed to enhance renewable food production and waste recycling capabilities in resource-constrained environments. Edible mushrooms possess nutritious profiles and represent emerging opportunities to develop circular bioproduction by transforming waste organic materials into food and high-value products. However, traditional mushroom cultivation methods utilizing solid substrates have technical limitations like 1) uneven nutrient diffusion, 2) constant sterilization needs, 3) numerous intermediate steps that are energy and time-demanding, and 4) waste production from bags and jars. This work introduces an

innovative hydroponic fungal cultivation system called Mycoponics™ using bioengineered ceramic materials. This liquid-nutrient-based technique optimizes fungal metabolism and facilitates precise nutrient delivery through biophysically engineered ceramic growth elements, overcoming the limitations of traditional solid substrates. To validate Mycoponics™, we have developed different geometries of the biophysical ceramic growth elements (i.e. mycoponic tubes) and formulated novel living nutrient solutions for experiments using various commercial fungal strains and growth conditions. Sporocarp (i.e. mushroom) production is achieved through different nutrient solutions engineered for the nutritional requirements of each fungal strain. Infrared imaging and microscopy demonstrated consistent, robust mycelial development compared to traditional methods, even when the nutrient solution is not sterilized likewise allowing mycelium access to circulating nutrients without substrate penetration showed longer constant production, avoiding nutrient depletion seen in conventional cakes. Synthesis of high-value products like mycoleather was explored, showing uniform, high-quality material production. By avoiding bulky substrates and exploring new growth surfaces, Mycoponics™ represents a solution for food security, sustainable biotechnology, and bioregenerative life support systems on Earth.

Poster 4 – Leon F Laskowski[1,2], Isaac J. Fisher[1], Elisabeth E. Garland-Kuntz[1], Kennedy Outlaw[1], Faith McCauley[1], Ketaki A. Mahurkar[1,2], Angeline M. Lyon[1,3]

Affiliations: [1]Department of Chemistry, [2]Purdue University Interdisciplinary Life Sciences Program, [3]Department of Biological Science, Purdue University

Determine the mechanism by which the D630Y mutation constitutively activates PLCbeta4 Authors

Uveal melanoma (UM) is the most prevalent malignant intraocular tumor with a 1-year survival rate <15%. 90% of all UM cases are driven by a constitutively activating mutation (Q209X) in Gaq/11 activating two distinct signaling pathways: PLCbeta4-PKC-ERK and Trio-RhoA-YAP. The latter is the focus of drug discovery efforts, with minimal success. Furthermore, these efforts fail to benefit UM patients wherein the cancer is caused by a constitutively activating mutation in PLCbeta4 (D630Y) independent of Galphaq/11 stimulation. Introduction of the D-to-Y mutation at the equivalent position in other PLC enzymes also results in constitutive activity, suggesting a conserved function of this position. Previous functional characterization failed to identify why this mutation results in maximal lipase activation. Thus, to determine how D630Y activates PLCbeta4, I am determining the structures of PLCbeta4 and PLCbeta4-D630Y using Cryo-EM and use functional assays to test whether the D-to-Y mutation alters autoinhibitory interactions through intramolecular interactions.

Poster 5 – Shukun Wang[1], Clinton Gabel[1], Romana Siddique[1], Thomas Klose[1], and Leifu Chang[1,2]

Affiliations: [1]Department of Biological Sciences, Purdue University, [2]Purdue Institute for Cancer Research, Purdue University

Molecular mechanism for Tn7-like transposon recruitment by a type I-B CRISPR effector
Tn7-like transposons have co-opted CRISPR-Cas systems to facilitate the movement of their own DNA. These CRISPR-associated transposons (CASTs) are promising tools for programmable gene knock-in. A key feature of CASTs is their ability to recruit Tn7-like transposons to nuclease-deficient CRISPR effectors. However, how Tn7-like transposons are recruited by diverse CRISPR effectors remains poorly understood. Here, we present the cryo-EM structure of a recruitment complex comprising the Cascade complex, TniQ, TnsC, and the target DNA in the type I-B CAST

from Peltigera membranacea cyanobiont 210A. Target DNA recognition by Cascade induces conformational changes in Cas6 and primes TniQ recruitment through its C-terminal domain. The N-terminal domain of TniQ is bound to the seam region of the TnsC spiral heptamer. Our findings provide insight into the diverse mechanisms for the recruitment of Tn7-like transposons to CRISPR effectors and will aid in the development of CASTs as gene knock-in tools.

Poster 6 – Mengxi Chen, Alone Bera, Nicholas Noinaj

Affiliations: Department of Biological Sciences, Purdue University

Unveiling TdfG: a Putative TonB-dependent Transporter in Neisseria gonorrhoeae

TdfG is a putative TonB-dependent transporter that is essential for Neisseria gonorrhoeae to survive in the host cells. It is an iron transporter with unknown binding partner. This poster aims to reveal the structure of TdfG along with its binding protein.

Poster 7 – Lan Chen

Affiliations: Purdue Institute of Drug Discovery

Purdue University, Chemical Genomics Facility

This is a poster to introduce the capability of the Chemical Genomics Facility at the Purdue Institute for Drug Discovery.

Poster 8 – Shivam Mahapatra, Bin Dong, Matthew G. Clark, Mark Carlsen, Karsten J. Mohn, Seohee Ma, Kent Brasseale, Grace Crim, Chi Zhang

Affiliations: Department of Chemistry, Purdue University

Spatiotemporally precise optical manipulation of intracellular molecular activities

Developing an effective clinical solution for any disease begins with understanding its pathogenesis through in-depth biological investigations. This requires systematic decoupling of numerous structure-function correlations with subcellular precision to account for any spatiotemporal heterogeneity. While fluorescence microscopy alongside biochemical assays has facilitated the visualization and identification of various biochemical pathways, they lack real-time control. Herein, we develop a closed-loop optoelectronic control system enabling precise manipulation of biomolecular activities in live cells. Chemical-selective fluorescence signals are used to command lasers that trigger specific chemical processes at desired targets. Using this real-time precision opto-control technology (RPOC), we demonstrate the selective killing of cancer cells in a co-culture, without significant harm to adjacent healthy cells. In conjunction with photoswitchable anti-cancer drugs, RPOC can enhance the efficiency of photodynamic therapy in a tumor microenvironment, while minimizing its side effects. Furthermore, it seamlessly integrates with laser-scanning confocal fluorescence microscopes, facilitating real-time target detection, decision-making, and optical treatment without a priori knowledge of chemical targets. This is especially advantageous for tracking and simultaneously treating targets with highly dynamic signatures such as LDs, mitochondria, lysosomes, tubulin, actin, and other highly mobile proteins. Coupled with suitable photoswitchable inhibitors, we demonstrate subcellular control over cytoskeletal proteins, microtubules, and actins, as well as explore selective interactions of a 405 nm laser with targeted organelles. With its submicron spatial precision and nanosecond response time, RPOC offers unprecedented opportunities to control biochemical processes and drug activities exclusively at desired targets, while minimizing off-target effects.

Poster 9 – Samson Marvellous Oladeji and Herman Sintim

Affiliations: Department of Chemistry, Purdue University

Development of a Mycobacterium smegmatis oligoribonuclease inhibitor

Oligoribonuclease (Orn) is a vital exonuclease involved in RNA turnover and quality control. It targets short oligoribonucleotide sequences and plays a key role in cellular homeostasis. Orn's unique function in bacterial cyclic-di-GMP (c-di-GMP) signaling and RNA degradation make it a potential drug target. A linear dinucleotide fluorescent probe, (etheno)pG, was developed to monitor Orn activity for high throughput screening of potential small molecule inhibitors.

Poster 10 – Badeia Saed[1], NT Ramseier[1], T Perera[1], J Anderson[2], J Burnett[3], H Gunasekara[1], A Burgess[1], H Jing[1], YS Hu[1]

Affiliations: [1]Department of Chemistry, College of Liberal Arts and Sciences, [2]Department of Chemical Engineering, College of Engineering, University of Illinois Chicago, [3]Nikon Instruments Inc, Melville, New York

Imaging Vesicular Dynamics and Intracellular IL-2 in Activated Jurkat T cells

Intercellular communication orchestrated by T cells is tightly regulated through cytokine secretion. While effective therapies for cytokine imbalance are available, they lack the potential to modulate and target multiple proinflammatory cytokines. Direct visualization of vesicular transport and intracellular distribution of cytokines provides valuable insights into the temporal and spatial mechanisms involved in regulation. Using Jurkat E6.1 T cells and interleukin-2 (IL-2) as a model system, we studied vesicular dynamics using single particle tracking and the intracellular distribution of IL-2 in fixed T cells by utilizing superresolution microscopy. Following in vitro activation, increased vesicular dynamics were observed using live cell imaging. Direct stochastic optical reconstruction microscopy (dSTORM) revealed an accumulation of IL-2 nanoclusters into more pronounced, elongated clusters. These observations of accelerated vesicular transport and spatial concatenation of IL-2 clusters may provide insights regarding potential mechanisms for modulating cytokine release. An enhanced understanding of the cytokine release mechanism holds great therapeutic promise for addressing cytokine imbalance.

Poster 11 – Ketaki A. Mahurkar[1,2], Caroline Chandler[3], Elisabeth E. Garland-Kuntz[1], Kaushik Muralidharan[4], Hanna D. King[1,2], Brayden Buschman[4], Vaani Ohri[4], Leon F. Laskowski[1,2], Angeline M. Lyon[1,5]

Affiliations: [1]Department of Chemistry, [2]Purdue University Interdisciplinary Life Sciences Program, [3]Department of Biochemistry, [5]Department of Biological Science, Purdue University; [4]Abigail Wexner Research Institute at Nationwide Children's Hospital

Understanding Ras-Mediated Activation of Phospholipase Ce

Phospholipase C (PLC) enzymes hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2), creating inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 leads to an increase in intracellular calcium, while DAG activates Protein Kinase C, resulting in cell growth, proliferation, and migration. PLC signaling pathways are essential for healthy cell signaling and function, and dysregulation of PLC signaling can lead to the development of pathologies including cancers. PLC epsilon is activated by the small G-protein Ras, a major oncogene and target in cancer research. PLC epsilon has a conserved catalytic core but has unique domains on the N and C terminus. The N-terminal CDC25 acts as a guanine nucleotide exchange factor for other small G-proteins whereas the C-terminus has two Ras association (RA) domains, with the RA2 domain

required for Ras binding. Previous studies identified that Ras expression results in PLC epsilon translocation to the plasma membrane. However, there are critical gaps in our understanding of the mechanism of PLC epsilon activation by Ras. Cell-based assays are used to investigate Ras isoform specific activation of PLC epsilon and determine the domains of PLC epsilon involved in activation. Total internal reflection fluorescence microscopy is used to visualize binding events of individual molecules of PLC epsilon on a model membrane with and without Ras, and structural studies will define the intermolecular interactions between the proteins. This study will provide insights into PLC epsilon and Ras interactions in healthy and cancerous states as well as contribute to the future development of selective small molecule regulators that target the activated PLC epsilon-Ras complex.

Poster 12 – Yulia Pushkar

Affiliations: Department of Physics, Purdue University

Electronic requirements for low barrier O-O bond formation in Natural and Artificial Photosynthesis

Using the energy of the light, the Photosystem II (PS II) produces oxygen by splitting water into electrons, protons and O2. From the mechanistic perspective the catalytic center is required to store several oxidative equivalents (holes) at ~ +1.23 V redox potential. This can proceed via highly oxidized metal-oxo species such as MnIV=O, FeV=O or RuV=O. In these species large spin density is localized on the oxygen making it effectively a radical. Metal-oxo species can undergo two main reactions: i) directly react with water with the formation of Metal-OOH peroxide or ii) by combining two oxo-species with the formation of the bridging Metal-O-O-Metal peroxide. Later is expected to have significantly lower activation barrier due to the physical nature of the radical coupling. Using time-resolved X-ray emission spectroscopy, a method uniquely sensitive to the electronic structure of the Mn4Ca, we measured the step of the O-O bond formation in PSII (S3 to S0 transition). Spectroscopic changes are consistent with reduction of the Mn in the cluster (compared to the S3 state) detected at ~50 ⌈s and ~500 ⌈s ahead of the reduction of the redox active TyrZ•+. These results indicate the multi-step nature of the O-O bond formation and O2 release by the oxygen evolving complex; O-O bond formation likely occurs prior to the final electron transfer step. This model resolves the kinetic limitations associated with O-O bond formation and suggests an evolutionary adaptation to avoid releasing of harmful peroxide species. Our atomistic model of the S3 state incorporates a MnIV=O fragment of radicaloid character and exhibits antiferromagnetic alignment with opposite spin orientations between two Mn centers and associated oxygens, allowing for a low barrier to O-O bond formation. [1-3] In D2O the lifetime of the early transient state is extended to 500 μsec allowing further spectroscopic and structural analysis.

Poster 13 – Cecon Mahapatra

Affiliations: EVPR, Purdue University

Responsible Conduct of Research (RCR)

Responsible Conduct of Research (RCR) is the practice of scientific investigation with integrity. It involves the awareness and application of established professional norms and ethical principles in the performance of all activities related to scientific research (NIH/ORI). According to the Retraction Watch Database, the majority of retracted scientific articles are linked to detrimental research practices and research misconduct; these may lead to wasted investment and career and reputational damage to those who engage in unethical conduct. RCR education provides the knowledge, skills, and resources needed to conduct science with integrity and prevent misconduct

and detrimental research practices. Purdue is committed to fostering a culture of research integrity and implemented an RCR Standard (S20) that requires all faculty, staff, trainees, graduate and undergraduate students who design and conduct research and/or report and publish research outcomes to complete RCR training. Purdue has also developed RCR training resources, including a template for Lab Expectations – Life Sciences to facilitate researcher involvement in lab based plans for fostering research integrity and creating a safe, ethical, secure and productive research environment.

Poster 14 – Livia Bogdan[1], Kadidia Samassekou[2], Angeline Lyon[1,2]

Affiliations: [1]Department of Biological Sciences, [2]Department of Chemistry, Purdue University

Investigating the GEF activity of Phospholipase Cε

Phospholipase Cε (PLCε)is essential for normal cardiovascular function and its dysregulation can lead to cardiovascular disease, the leading cause of death in the United States. PLCε is activated by direct binding of the small GTPase Rap1A which allows for the hydrolysis of phosphatidylinositol phosphates into inositol phosphates and diacylglycerol. This pathway is required for maximum cardiac contractility, but sustained activation leads to cardiac hypertrophy. PLCε contains a highly conserved catalytic core that is flanked by unique N- and C-terminal regulatory domains. At the N-terminus, the CDC25 domain acts as a guanine nucleotide exchange factor (GEF) for Rap1A. The C-terminus contains two Ras association domains, RA1 and RA2, important for stability and binding active Rap1A, respectively. Guided by our cryo-electron microscopy reconstruction, an AlphaFold2 model, and sequence conservation, we identified extensive interdomain contacts between the CDC25 and PH module, and clusters of positively charged and hydrophobic residues that may be involved in membrane binding. Using site-directed mutagenesis, we mutated residues of interest in the background of full-length PLCε and assessed their effect on basal and Rap1A-stimulated activity using our cell-based functional assays. From this, we determined a group of mutants that were shown to decrease both basal and Rap1A-mediated activity. We are currently investigating the impact of these mutants on PLCε GEF activity for Rap1A through pulldown assays.

Poster 15 – Chang Jiang, Amanda East, Zhengxiang Zhao, Ziyi Jiang, Suritra Bandyopadhyay, Jefferson Chan

Affiliations: Department of Chemistry, College of Chemical Sciences, University of Illinois at Urbana-Champaign

Logic-gated mvGlu Photoacoustic Probe for Companion Diagnostic Applications

Optimizing the precise delivery of cargo, including (companion diagnostic) CDx imaging agents and activatable chemotherapeutics, to cancer cells has always been of great interest. Despite decades of development, FDA-approved imaging CDx is still limited. Thus, developing a new CDx enabling real-time monitoring is highly desirable. Photoacoustic (PA) imaging, known as ‘light in, sound out’, enables deep tissue imaging, making it ideal for CDx. To solve the solubility problem of the aromatic compounds, our group developed the first-generation multivalent glucose targeting moiety (mvGlu) in 2023. Despite this moiety enhances tumor targetability, the permanent linkage via click chemistry makes it undesirable for drugs. The glucose might get caught by organelles or affect the drug’s binding affinity. On the other hand, toxic side effects remain a major concern for anticancer drug development. Take camptothecin (CPT) as an example, it binds

topoisomerase I and DNA complex which results in high toxicity to hematopoietic stem cells. In the present work, we utilize a strategic exploration of logic-gated designs dependent on the interaction with two sequential biomarkers. The first is glutathione (GSH) which is elevated in cancer cells. The second is GLUT1, which is overexpressed in tumors due to their heightened reliance on glucose for energy known as the Warburg effect. By connecting the GLUT1 targeting mvGlu with the GSH cleavable disulfide linker, we can further enhance targetability in both the probe mvGlu-SS-ABCl and the prodrug mvGlu-SS-CPT. The cleavable feature of moiety maximizes the potency as well as biocompatibility while minimizing the off-target effects. The current results show that both the probe and the prodrug have the desired targetability under in vitro and intracellular conditions.

Poster 16 – Jacqueline S. Anderson[1] and Richard J. Kuhn[1,2]

Affiliations: [1]Department of Biological Sciences, [2]Purdue Institute of Inflammation, Immunology and Infectious Disease, Purdue University

Enterovirus D68 VP3 Aspartic Acid 18 is Required for Capsid Assembly and Maturation

Enterovirus D68 (EV-D68) is a respiratory illness implicated in recent outbreaks of acute flaccid myelitis, a neurologic flaccid paralysis like poliomyelitis, yet no therapeutic or vaccine is currently available. During EV-D68 infection, individual structural proteins VP1, VP0, and VP3 are translated and self-assemble into protomers for use in capsid assembly. As capsid assembly progresses, protomers form an unstable immature particle. Immature particles subsequently undergo maturation, a conformational shift initiated by cleavage of precursor protein VP0 in to separate structural proteins VP4 and VP2 producing a stabilized native virion. Based on high VP4 conservation among enteroviruses, we hypothesize that VP4 has critical stabilizing interprotomer interactions, and that alteration of these interactions impair capsid assembly and maturation. Structural analysis identified an interprotomer interaction between VP4 Lysine 33 (K33) and VP3 aspartic acid (D18). VP3 D18 amino acid substitution to glutamic acid (D18E) produces non-infectious virus following RNA transfection. Preliminary cryo-EM imaging showed VP3 D18E can form particles yet VP2 was absent in western blots, suggesting a capsid maturation defect. To delineate the type of particles being assembled, virus purification and discontinuous 10-50% sucrose gradient fractionation was performed. Each fraction was collected and checked for the presence of VP0 and VP2 by western blot and qRT-PCR. In wild type EV-D68, mature particles were present in the 40-45% fractions confirmed by the presence of VP2 and viral genome. In contrast, VP3 D18E VP2 was present in lower density 20-30% fractions, yet RNA was absent suggesting the presence of mature empty capsids. Cryo-EM imaging confirmed the presence of particles in the VP3 D18E 30% sucrose fraction, suggesting that capsid destabilization causes VP3 D18E genome loss following maturation. Further analyses are ongoing, but this work highlights the importance of interprotomer interactions in capsid assembly and maturation mechanisms and reveals important insights into icosahedral particle architecture.

Poster 17 – L-C Choh[1], SK Erramilli[2], Z Li[3], CN Steussy[1], L Chang[1], N Noinaj[1] and CV Stauffacher[1,4]

Affiliations: [1]Department of Biological Sciences, [4] Purdue University Center for Cancer Research, Purdue University; [2]Meso Scale Diagnostics, Rockville, MD; [3]College of Life Sciences, Hubei University, China

Structural Study of a Non-Canonical ABC Importer, the E. coli Ribose ABC Importer

ATP-binding cassette (ABC) importers are essential for nutrient uptakes in prokaryotes. The intake of ribose, an essential carbon source, is aided by the ribose ABC importer complex, RbsABC. As compared to other ABC importers, RbsABC is a non-canonical ABC importer with features of the two main ABC importer classes. Biochemically, RbsABC was predicted to possess a type II transmembrane domain fold, but RbsABC possesses type I ABC importer ATPase activity and relies on a substrate-binding protein that belongs to a substrate-binding protein group that interacting with type I ABC importers. In addition, RbsABC possesses one active and one inactive ATPase site (asymmetric ATP hydrolysis), a stark contrast to other ABC importers that possess two active ATPase sites. According to our X-ray crystallographic data, RbsABC has a unique structure that is significantly different than the prototypes of type I and type II ABC importers. In a further study exploiting cryo-electron microscopy techniques, we have dissected the unilateral transport mechanism of RbsABC. Two hydrophobic amino acid residues were identified that function as the gating residues and occluded the substrate translocation pathway at different ATP hydrolysis/substrate transport stages of RbsABC. Now that we have solved the structure of RbsABC and deciphered its transport mechanism, we would like to propose that RbsABC belongs to a new, distinct group of ABC importers.

Poster 18 – Dorothy DRozario[1], Indranil Arun Mukherjee[1], Susan K. Buchanan[2], and Nicholas Noinaj[1]

Affiliations: [1]Department of Biological Sciences, Purdue University, West Lafayette, Indiana [2]NIDDK, NIH, Bethesda, Maryland

Structural insights into contact-dependent inhibition in bacterial warfare

Contact-dependent inhibition (CDI) is a mechanism in Gram-negative bacteria that inhibits neighboring cell growth through direct contact. It involves specialized systems comprising a toxin, an immunity protein, and a receptor. Upon contact, the producing bacterium delivers the toxin into target cells, where it disrupts essential processes, leading to growth inhibition or cell death. The immunity protein protects the producer from self-intoxication, and specificity is ensured through receptor recognition. CDI provides a competitive advantage to the producer and influences microbial community dynamics. We aim to structurally characterize the CdiA protein implicated in the interaction between inhibitory and target bacteria and to elucidate the mechanism of inhibition. Our focus lies on understanding the molecular interactions of two CDI systems: (i) CdiA EC93 with the BAM complex, and (ii) CdiA EC536 with OmpC/F. We have cloned and purified the target proteins of CdiA, namely the BAM complex, OmpC, and OmpF. The receptor-binding domains (RBD) of CdiA-EC93 and CdiA-EC536 were cloned and expressed in BL21(DE3) cells. Despite both constructs yielding proteins in the form of insoluble inclusion bodies, we successfully refolded and further purified them for forming complexes with CdiA. Our size-exclusion chromatography (SEC) assay and native PAGE results demonstrate that CdiA-EC93 interacts with the BAM complex, while CdiA-EC536 interacts with both OmpC and OmpF, forming stable complexes. We

are currently performing structural studies of these complexes utilizing X-ray crystallography and cryo-EM, which will serve as a foundation for probing the mechanism these CdiA proteins use during bacterial warfare.

Poster 19 – Genki Terashi[1], Tsukasa Nakamura[1] and Daisuke Kihara[1,2]

Affiliations: [1]Department of Biological Sciences, [2]Department of Computer Sciences, Purdue University

DeepMainmast: Integrated Protocol of Protein Structure Modeling for Cryo-EM with Deep Learning and Structure Prediction

In the last few years, an increasing number of protein structures have been determined with cryo-electron microscopy (cryo-EM). Despite the improvements in cryo-EM map resolution, building protein structure models from a cryo-EM map is still challenging, especially when the resolution is worse than 3 Å. To address this problem, we developed an integrated protein structure modeling protocol, DeepMainmast. This protocol employs a de novo protein main-chain tracing method that uses deep learning to identify the positions of Cα atoms and the types of amino acids. The core process of DeepMainmast employs a Vehicle Routing Problem solver and Constraint Problem Solver for main-chain tracing. Moreover, it integrates AlphaFold2 models to enhance accuracy, especially in lower-resolution maps. In many cases, Alphafold2 can make an accurate structure for local structures and domains but not necessarily for the overall protein chains. This integration combines the strengths of direct density tracing from the cry-EM map and structure prediction. Overall, DeepMainmast is a powerful tool that can help researchers overcome the challenges of protein structure modeling from cryo-EM maps. Benchmarking tests demonstrated that DeepMainmast outperforms Alphafold2 and existing methods in accuracy. DeepMainmast is freely available at https://em.kiharalab.org/algorithm/DeepMainMast.

Poster 20 – Pranav Punuru[1], Daisuke Kihara[1,2]

Affiliations: [1]Department of Biological Sciences, [2]Department of Computer Science, Purdue University,

VIPER: A Conversational Interface for Protein Research and Analysis

VIPER (Virtual Intelligence for Protein Exploration and Research) is a sophisticated conversational interface powered by GPT-4, designed to facilitate protein research and analysis. This platform enables users to query the UniProt database conversationally to fetch detailed protein information, including sequence data and functional annotations. VIPER's capabilities extend to predicting protein structures rapidly using ESMfold, a state-of-the-art protein folding algorithm. Additionally, the platform supports BLAST searches, allowing users to compare sequences and identify homologs across a broad range of species and databases. As a GPT-4 powered tool, VIPER combines natural language processing with an advanced code interpreter and browsing capabilities, breaking down complex scientific information into accessible insights. This enables researchers to conduct sophisticated bioinformatics research and analysis through conversational interactions. VIPER's integration of multiple research tools into a single conversational platform significantly enhances the efficiency and depth of scientific exploration, making it an invaluable resource for advancements in protein science and related fields. Furthermore, VIPER has demonstrated a robust ability to generate detailed function descriptions for proteins with poor annotations in UniProt by leveraging Gene Ontology (GO) terms. It has also

proved extremely helpful in research for CASP16, where it found helpful literature just by providing CASP target information.

Poster 21 – Andreas Langer, Nathan Wallace, Stefanie Kall, Nicholas Van der Werff*

Affiliations: NanoTemper Technologies, GmbH, Munich, Germany
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Optimize your structural biology workflows with stability and affinity insights from NanoTemper

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Poster 22 – Chidinma Ononiwu, Zhenghrui Zhang, and Chittaranjan Das

Affiliations: Department of Chemistry, Purdue University

STRUCTURAL AND MECHANISTIC INSIGHTS INTO DE-ADP-RIBOSYLATION ACTIVITY OF A MACRODOMAIN ENZYME FROM GIARDIA INTESTINALIS

ADP-ribosylation is a reversible post-translational modification (PTM) regulating diverse processes in cells. This PTM involves the attachment of a single or multiple ADP-ribose unit(s) onto various acceptor side chains on target proteins from the nicotinamide adenine dinucleotide (NAD+) donor. ADP-ribosyl hydrolases catalyze the cleavage of glycosidic bonds between ADP-ribose (ADPR) units or between an amino acid residue and the proximal ADPR. Our lab, via bioinformatical analyses have revealed DUF4804 as a class of macrodomain enzymes with distinct selectivity for mono-ADP-ribosylated arginine. Giardia intestinalis, a unicellular eukaryotic parasite, possesses an uncharacterized member in the same macrodomain class referred to here as gMAC1 (Giardia macrodomain 1). Interestingly, this protein also possesses a conserved motif found in the PARG group of ADP-ribosyl hydrolases. In this study, we determined the crystal structures of apo-gMAC1, its ADPR-bound complex and complemented with some biochemical studies in order to gain insights into ADPR binding and residue-level selectivity of the enzyme. Using ADP-ribosylated ubiquitin as a biochemical substrate, we show that gMAC1 can catalyze the hydrolytic removal of ADPR from arginine and this activity is dependent on the conserved catalytic motif in DUF4804 macrodomain members. Co-crystal structure of ADPR-bound enzyme shows a similar mode of ADPR recognition to a previously characterized DUF4804 member called MavL, suggesting the same sort of catalytic mechanism of de-ADP-ribosylation. The mechanism is proposed to be a substrate assisted SN2 reaction, where a water closest to the C1” atom of ADPR, activated by the β-phosphate group, attacks the C1”center to hydrolyze the N–glycosidic bond present in the substrate. These findings offer a better understanding of how this special class of macrodomain enzymes control reversible protein ADP-ribosylation. Future studies will involve further biochemical assays to validate structural findings, probing the basis of arginine selectivity and the function of the PARG motif of gMAC1.

Poster 23 – Javad Baghirov[1], Daisuke Kihara[1,2]

Affiliations: [1]Department of Computer Sciences, [2]Department of Biological Sciences, Purdue University

Macromolecular structure plays fundamental roles in various cellular processes, where the three-dimensional (3D) structure provides critical information to understand molecular mechanisms of their functions. The rapid advances have been witnessed in the macromolecular structure determination using cryo-electron microscopy (cryo-EM), with the exponential growth of deposited structure by cryo-EM. To facilitate the cryo-EM structure determination process, we developed CryoREAD, DeepMainMast for automated DNA/RNA, protein structure modeling, respectively; DAQ for residue-wise local structure quality estimation, and DAQ-Refine for correcting the detected low-quality regions identified by DAQ. All the deployed algorithms have been benchmarked with the state-of-the-art performance and big improvement compared to prior methods. To further provide easy-usage for structural biologists, we provide free EMSuite server online. The web server takes three-dimensional (3D) cryo-EM map and sequence information in fasta format as input, which are then modeled to full atomic structure by CryoREAD(DNA/RNA) and DeepMainMast(protein). The server can also take additional modeled structure as input and achieve structure validation (DAQ) and refinement (DAQ-Refine). The modeled/refined/evaluated structure and its corresponding input cryo-EM map can be visualized online once the running job has finished. In addition to these algorithms, we also offer other cryo-EM structure detection and analysis tools in the server. To the best of our knowledge, our server is the 1st EM server that integrates automated structure modeling, validation, and refinement tools. The EMSuite webserver is available at https://em.kiharalab.org.

Poster 24 – Linda Shen, Erik Sillaste, Michaela Moore, and Nicholas Noinaj

Affiliations: Department of Biological Sciences, Purdue University,

Structural and functional characterization of CyclikEr and Gingiegirl from the bacteriophage Potatosplit

Bacteriophages (phages) are bacteria-infecting viruses that are harmless to humans and present extensive therapeutic potential. However, the majority of phages are undiscovered and most of their protein structures are uncharacterized. This study aimed to characterize the structure of two proteins from the phage PotatoSplit: the structure of CyclikEr and Gingiegirl. After isolating and cloning the genes from PotatoSplit genomic DNA, the two proteins were expressed and purified for structural studies. Preliminary computational biology was performed to study the predicted structure, which aligned well with CD analysis. We have now fully resolved both two structures of the proteins, and its function was estimated from 3D structure alignment with similar proteins that have known functions. Information about the predictability of both computational methods used in this project have also been obtained.

Poster 25 – Shreya Mukherji, Dr. Lauren Ann Metskas

Affiliation: Department of Biological Sciences, Purdue University

Understanding Fusion kinetics of flaviviruses based on their maturation state

Abstract: Flaviviruses are a group of enveloped viruses bearing positive-sense RNA as their genomic material. Commonly known viruses like Dengue (DENV), Zika (ZIKV), Kunjin (KUNV)

and Japanese encephalitis virus (JEV) belong to this group of Flaviviridae family. Even though several structural studies have been done to understand the dynamics of the structural proteins of these viruses, designing an effective vaccine to impart long-term immunity remains enigmatic, to date. The major type of structural proteins present, in these viruses, include envelope (E), pre-membrane (prM), and capsid (C). Amongst these, the envelope (E) and pre-membrane (prM) play a vital role in mediating virus entry and subsequent fusion within the host cell. These viruses assemble into non-infectious immature forms with E and prM glycoproteins forming spikes, decorating the surface of these viruses. As the virions migrate through the trans Golgi network (TGN), it undergoes a huge conformational change in a pH-dependent manner before finally budding out of the cell. Apart from the fully mature and fully immature flaviviruses, some reports have shown the presence of the significant amount of partially mature virions circulating in the system formed as a result of varying degrees of prM cleavage by furin proteases in the cell. The stoichiometric requirement of the number of E glycoprotein and prM to form a mature infectious virion remains to be addressed to date. The goal of my project is to develop a reconstituted in-vitro assay to understand the underpinnings of fusion kinetics depending on varying degrees of flavivirus maturation. It would also help us understand the key factors responsible for mediating the extent of prM cleavage, leading to the formation of mature, immature, and partially mature virus particles. Overall, my project will bridge the knowledge gap in the structure function relationship of E and prM glycoprotein required for Flavivirus maturation and fusion into the host cell.

Poster 26 –Hanna D. King[1-3], Aron Weldai[2] & Darci J. Trader[4]

Affiliations: [1]Department of Chemistry, [2]Department of Medicinal Chemistry and Molecular Pharmacology, [3]Purdue University Interdisciplinary Life Sciences Program, Purdue University, [4]Department of Pharmaceutical Sciences, University of California- Irvine

Generation of an Antigenic Prodrugs for Monitoring Immunoproteasome Mediated MHC-I Loading

The immunoproteasome (iCP) is a derivative of the standard proteasome (sCP) that occurs under inflammatory conditions. Inflammatory conditions cause the exchange of the sCP (β1, β2, β5) catalytic units for the iCP catalytic units (β1i, β2i, β5i). The difference in the catalytic units allows for the iCP to create antigenic peptides that are capable of loading into immuno-machinery, Major Histocompatibility Complex class I (MHC class I). iCP dysfunction has been associated with cancer, autoimmune, and neurodegenerative diseases. These diseases are also associated with immune dysfunction which related back to the iCP. Therefore, modulating or harnessing the iCP's activity we could combat said diseases. In this project we provide proof of concept that the iCP can be used to cleave a prodrug releasing an antigenic peptide, SIINFEKL, that is loaded and presented by MHC I. Modulating the presentation of antigenic peptides gives the potential to alter the immune response by CD8+ T cells which could be beneficial in disease states.

Poster 27 – Tsukasa Nakamura[1], Xiao Wang[2], Genki Terashi[1], Daisuke Kihara[1,2]

Affiliations: [1]Department of Biological Sciences, [2]Department of Computer Sciences, Purdue University

DAQ-Score Database and DAQ-refine:Deep-learning Based Quality Estimation and Refinement of Cryo-EM Derived Protein Models

It has been noticed that errors occur in the protein structure model building process from cryo-EM maps, probably more frequently than one might think, particularly when the map resolution is not

very high. Thus, establishing quality assessment methods and correction protocols has become a crucial task for biomolecular structure determination with cryo-EM. We have recently developed a quality assessment method to detect protein structural model outliers using machine learning techniques. Our method, DAQ (Deep-learning-based Amino acid-wise model Quality) score, uses deep neural network to capture local density features of amino acids and atoms in proteins and assesses the likelihood that modeled residues are correct (Terashi et al., Nature Methods, 2022). DAQ is also able to detect not only errors in conformations but also shifts in sequence assignment to otherwise correct main-chain conformations, which is often not easy to detect by checking density fitting. We performed a PDB-scale model analysis by applying DAQ to more than 10,000 protein structure models from cryo-EM in PDB. We report the tendency of common errors made in the models through the large-scale analysis. A common type of errors observed include sequence shifts along alpha helices. Model assessment results with DAQ are made available in a database: https://daqdb.kiharalab.org/ (Nakamura et al., Nature Methods, 2023). We also present a protocol, DAQ-refine, for correcting models that are detected to have errors. (Terashi et al., Acta Cryst. D, 2023). After the model-local map assessment with DAQ, the local refinement is performed by a modified procedure of AlphaFold2. Using a trimmed template and trimmed multiple sequence alignment as input of AlphaFold2, the method refines only the regions with errors while leaving other regions intact. All the methods are available at https://kiharalab.org/emsuites/.

Poster 28 – Johanna Bovill[1], Angeline M. Lyon[1,2]

Affiliations: [1]Department of Chemistry, [2]Department of Biological Sciences, Purdue University

Investigating PLCb3 activation by Gaq using Cryo-EM

Phospholipase C (PLC) enzymes regulate numerous processes in the human body. The PLCb subfamily is critical for normal cardiovascular function, and when dysregulated, can cause cardiovascular diseases. Therefore, they are promising therapeutic targets. The best characterized activator of PLCb is the heterotrimeric G protein subunit Gaq, which binds directly to C-terminal regulatory domains within the lipase. High-resolution structures of PLCb, alone and in complex with Gaq, have been determined, but in all cases, known autoinhibitory interactions within PLC® persist. Thus, the structure of an active PLCb or the active Gaq–PLCb complex remains to be determined. To address this gap, we are using cryo-electron microscopy single particle analysis to determine structures of PLCb in complex with Gaq in its fully activated, GTP-bound state, or in its post-GTP hydrolysis conformation. Structure-based hypotheses will be validated through cell-based functional assays. This work will provide crucial new insights into how the Gaq-PLCb complex is activated and deactivated, which can be used to develop new therapeutic strategies for treating cardiovascular disease.

Poster 29 – Nicolas A.C.J. de Cordoba , Mohamed A.A. Elrefaiy, Bailey Raber , Gehan A. Ranepura, Marilyn R. Gunner, Doran I.G.B. Raccah, Lyudmila Slipchenko, and Mike Reppert

Affiliations: Department of Chemistry, Purdue University

Protonation-state Determination and Spectra Manipulation of Charged mutants of Water Soluble Chlorophyll-binding Protein

If photosynthesis was more efficient, solar biofuels would be a more viable green alternative to growing energy demands. One route for increasing photosynthetic efficiency is to reduce the total amount of light absorbed by individual cells while expanding the spectrum of light that the organisms can utilize. In order to rationally design protein pigment complexes that can allow for this, researchers must be able to accurately predict the spectra of modified protein structures. Previously, we demonstrated that mutation-induced frequency shifts for 8 out of 9 single point mutants of the Water Soluble Chlorophyll binding Protein of Lepidium virginicum (LvWSCP) could be predicted using a simple electrostatic model. The only outlier was Q57D, which experimentally displayed a small red shift, rather than the predicted large blue shift. However, the previous simulations assumed that the mutant residue was deprotonated at the experimentally relevant pH. Here we demonstrate by taking spectra at varying pH that both mutant residues in the dimeric interface were protonated during past measurements. Furthermore, we demonstrate that predictable shifts in spectrum can be induced in charged Q57 mutants by modifying the solutions pH.

Poster 30 – Jacklyn M. Gallagher, Charles P. Kuntz, & Jonathan P. Schlebach

Towards the Discovery of Small Molecules that Restore the Expression and Function of CTD Variants

Affiliations: Department of Chemistry, Purdue University

Over 100 loss-of-function (LOF) mutations within the SLC6A8 creatine transporter (CT1) are known to cause creatine transporter deficiency (CTD) syndrome. Most of these mutations enhance CT1 misfolding and degradation, and the resulting loss of the transporter protein ultimately compromises creatine uptake within the brain and other organs. Creatine uptake could therefore be restored by small molecule “correctors” that rescue the expression of misfolded variants, the development of which has recently revolutionized the treatment of several other genetic diseases of membrane protein misfolding. Cumulative observations concerning the mechanistic effects of drugs targeting related SLC6 transporters such as the serotonin (SERT) and dopamine (DAT) transporters suggest compounds that selectively bind to their inward-facing (IF) conformation generally enhance their expression and maturation. Based on these considerations, we set out to identify small molecules that selectively bind to the IF conformation of CT1 in order to restore the expression and activity of misfolded CT1 variants. Towards this goal, we developed a virtual screening approach to identify small molecules that selectively bind to the IF conformation of CT1, then profiled their effects on CT1 expression. Of our top 53 candidates, we identify several that alter the expression profile of WT CT1 and a few that enhance its expression at the plasma membrane. We are currently working to determine how these molecules impact CT1 function and how they impact pathogenic CT1 variants. Additionally, based on the structures of our top hits, we are currently searching for second-generation compounds

with increased potency. These results represent an important step toward the development of novel pharmacological chaperones for the treatment of CTD.

Poster 31 – Suritra Bandyopadhyay, Zhenxiang Zhao, Amanda K. East, Rodrigo Tapia Hernandez, Joseph A. Forzano, Benjamin A. Shapiro, Anuj K. Yadav, Chelsea B. Swartchick, and Jefferson Chan*

Affiliations: Department of Chemistry, United States Beckman Institute for Advanced Science and Technology and Cancer Center at Illinois, United States Cancer Center at Illinois, University of Illinois at Urbana–Champaign

Activity-Based Nitric Oxide-Responsive Porphyrin for Site-Selective and Nascent Cancer Ablation

Nitric oxide (NO) generated within the tumor microenvironment is an established driver of cancer progression and metastasis. Recent efforts have focused on leveraging this feature to target cancer through the development of diagnostic imaging agents and activatable chemotherapeutics. In this context, porphyrins represent an extraordinarily promising class of molecules, owing to their demonstrated use within both modalities. However, the remodeling of a standard porphyrin to afford a responsive chemical that can distinguish elevated NO from physiological levels has remained a significant research challenge. In this study, we employed a photoinduced electron transfer strategy to develop a panel of NO-activatable porphyrin photosensitizers (NOxPorfins) augmented with real-time fluorescence monitoring capabilities. The lead compound, NOxPorfin-1, features an o-phenylenediamine trigger that can effectively capture NO (via N_2O_3) to yield a triazole product that exhibits a 7.5-fold enhancement and a 70-fold turn-on response in the singlet oxygen quantum yield and fluorescence signal, respectively. Beyond demonstrating excellent in vitro responsiveness and selectivity toward NO, we showcase the potent photodynamic therapy (PDT) effect of NOxPorfin-1 in murine breast cancer and human non-small cellular lung cancer cells. Further, to highlight the in vivo efficacy, two key studies were executed. First, we utilized NOxPorfin-1 to ablate murine breast tumors in a site-selective manner without causing substantial collateral damage to healthy tissue. Second, we established a nascent human lung cancer model to demonstrate the unprecedented ability of NOxPorfin-1 to halt tumor growth and progression completely. The results of the latter study have tremendous implications for applying PDT to target metastatic lesions.

Poster 32 – Joseph A Forzano, Suritra Bandyopadhyay, Catharine J. Brady, Jefferson K. Chan

Affiliations: Department of Chemistry, University of Illinois at Urbana–Champaign

Development of Aldehyde Dehydrogenase-Activated Photosensitizers for Targeted Photodynamic Therapy

Cancer stem cells (CSCs) are a distinct subgroup of tumor cells contributing to tumor aggressiveness, metastasis, and resistance to conventional therapies. These factors often lead to the formation of treatment-resistant secondary tumors that prove fatal to patients, making the targeting of CSCs a critical yet unmet challenge in cancer therapy. With this in mind, we have introduced the first activatable photodynamic therapy (PDT) agents specifically designed for the selective ablation of CSCs. Our approach leverages the overexpression of aldehyde dehydrogenase 1A1 (ALDH1A1) in CSCs, using differential donor photoinduced electron transfer (d-PeT) to regulate the active and inactive states of the molecule. Prior to activation by ALDH1A1, the photosensitizers produce low levels of singlet oxygen upon irradiation. Post-activation, singlet

oxygen generation is significantly increased, showing up to a three-fold enhancement in quantum yield. A series of photosensitizers has been synthesized to optimize therapeutic outcomes while minimizing off-target toxicity. By leveraging the activity of ALDH1A1 in CSCs, we are able to improve biosafety over non-targeted photosensitizers that suffer from significant damage to healthy tissue adjacent to tumors. Preliminary in vitro studies have allowed us to confirm the light-mediated, ALDH1A1-dependent efficacy of this new platform. To further challenge our photosensitizer, we established an A549 lung cancer model in Nu/J mice to extend our findings to a relevant animal model. Our results are consistent with the light-mediated, ALDH1A1-dependent tumor killing we observed in vitro. Compared to ALDH-inhibitor-treated tumors, we observed a marked decrease in the tumor burden of the uninhibited tumors after PDT treatment.

Poster 33 – Deborah Naa Ayokor Mettle, Lauren Ann Metskas

Affiliations: Department of Biological Science, Purdue University

Elucidating Flavivirus - Host cell interactions in infection using cryo-electron Tomography

Flaviviruses are a major public health concern due to the encephalitic and hemorrhagic diseases they cause globally. These viruses enter cells through endosomal uptake and traverse the endocytic/secretory network hijacking cellular organelles to establish infection. However, the specific virus-host cell interactions facilitating endosomal entry and fusion remain poorly defined. We aim to delineate the structural and morphological dynamics of flavivirus particles during endocytic trafficking using cellular cryo-electron tomography (cryo-ET). Our optimized sample preparation shown here uses extracellular matrix proteins to preserve cellular structure by mimicking aspects of a native cellular environment.

Poster 34 – Jiachen Sun[1], Julian Zalejski[1], Seohyeon Song[2], Indira Singaram[1],Ashutosh Sharma[1],Yusi Hu[1], Wen- Ting Lo[3], Philipp Alexander Koch[3], Volker Haucke[3], and Wonhwa Cho[1],*

Affiliations: [1]Department of Chemistry, University of Illinois Chicago; [2]Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, Pohang, 790-784, Korea; [3]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), 13125 Berlin, Germany

PI(3,5)P2 Controls the Signaling Activity of Class I PI3K

3’-Phosphoinositides are ubiquitous cellular lipids that play important regulatory roles in health and disease. Complex cell signaling networks involving 3’-phosphoinositides comprise three families of phosphoinositide 3-kinases (PI3K) but their cellular regulation and interplay are not well understood. Among 3’-phosphoinositides, phosphatidylinositol-3,5-bisphosphate (PI(3,5)P2) is the least understood species in terms of its spatiotemporal dynamics and physiological function. Using direct quantitative imaging of PI(3,5)P2 in live cells we demonstrate that PI(3,5)P2 is generated from a special pool of phosphatidylinositol-3-phosphate (PI(3)P) produced by Class II PI3KC2β in response to growth factor stimulation. This PI(3,5)P2 pool plays a pivotal role in terminating the activity of growth factor-stimulated Class I PI3K, a major driver of cancer and a novel druggable binding pocket to promote cellular growth after a stroke or cardiac episode. Mutations of Class I PI3K inhibit the p85-PI(3,5)P2 interaction and thereby induce sustained activation of Class I PI3K. Our results unravel a hitherto unknown tight regulatory interplay between Class I and II PI3Ks mediated by PI(3,5)P2. This new feedback inhibition mechanism for Class I PI3K may be important for PI3K-mediated growth factor signaling and jumpstarting dormant cells.

Poster 35 – Kavrakova, Simona; Cho, Wonhwa.

Affiliations: Department of Chemistry, University of Illinois at Chicago

Cellular cholesterol modification methodology

Cholesterol homeostasis is a complex process. Cells spend a lot of energy to maintain cholesterol concentrations at specific levels in various locations – like IPM, OPM, ER, Lysosome, etc. Some of the key players that help the cell maintain cholesterol levels are LDLR controlling its uptake, HMG-CoA Reductase part of the rate limiting step in cholesterol biosynthesis, ACAT1 enzyme converting free cholesterol to cholesterol ester, and ABCA1 which flops cholesterol from the inner plasma membrane (IPM) to the outer plasma membrane (OPM) to then be loaded onto HDL for export. The important role that cholesterol plays in maintaining the health of the cell can be illustrated by the fact that its dysregulation and accumulation cause various diseases. For example, the lysosomal storage disease Niemann Pick Disease Type C (NPC) where loss-of-function mutations in the NPC1 protein prevent it from transferring cholesterol out of the lysosome. Furthermore, in Alzheimer's and Parkinson's disease cholesterol metabolism is associated with ER stress, which is a hallmark of neurodegenerative diseases. In hepatocellular carcinoma, which is the end stage of chronic liver disease, cholesterol accumulation in mitochondria causes increased reactive oxygen species generation, impaired oxidative phosphorylation, and contributes to chemotherapy resistance. Finally in certain cancer types, elevated IPM cholesterol levels cause overactivation of signaling pathways, like PI3K/Akt1/mTOR and Wnt/DVL/β-catenin, which are often mutated and activated in cancer. Current techniques for manipulation of cellular cholesterol, such as depletion and repletion using β-Cyclodextrin, statins to inhibit HMG-CoA, and gene silencing or overexpression to affect cholesterol homeostasis, are limited by the time scale in which they occur, or by being non-specific with no direct way to evaluate the process. This work introduces a full suite of tools for direct real-time manipulation of cholesterol in the cell, which provides spatio-temporal control and the ability to manipulate and measure cholesterol in live cells.

Poster 36– Sagarika Taneja, Ziyuan Meng, Reham Hassan, Jon R. Parquette

Affiliations: Department of Chemistry and Biochemistry, The Ohio State University

A pH-Responsive Rhodamine Nanotube Capable of Self-Reporting the Assembly State

Cancer, a global healthcare challenge, demands innovative approaches in drug delivery and intracellular imaging. This research focuses on the development of two pH-responsive self-assembled probes, Rho-KK and Rho-KE, derived from Rhodamine B and incorporating a β-sheet-forming dipeptide motif. The primary objective is to assess the probes' capability to be uptaken by liver cancer HepG2 cells and then to self-report its assembly state (nanotube vs monomer). Rho-KK/Rho-KE can exist either in a monomeric, open-ring state (λmax 580 nm) at low pH (4.1/4.2) or in the assembled nanotubular state which exhibits aggregation-induced emission (λmax 460 nm) at pH> (5.8/6.3). Between pH (4.1-5.8/4.2-6.2) respectively for Rho-KK/Rho-KE, the transition from fluorescent open-ring Rhodamine form to monomeric, non-fluorescent spirolactam form occurs. The probes upon entering the cancer cells would emit fluorescence corresponding to their location within the cells as intracellular organelles are known to have different pH values, from the early (pH 6.3) to the late endosome (pH 5.5) and ultimately the lysosome (pH 4.7). Structural validation involved a comprehensive analysis through UV-Vis, circular dichroism (CD), and fluorescence spectroscopy, complemented by imaging nanotubes

via Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM). Confocal microscopy has been utilized for in vitro cellular imaging, allowing for a correlation between changes in emitted wavelength and the specific cellular compartments. In summary, developed probes have demonstrated the ability to emit wavelength corresponding to the pH-dependent structural state in in vitro cellular imaging.

Poster 37–Amala Phadkule[1] 1, Amit Srivastava[2], and Mike Reppert[1]

Controlled Knockdown of Photosystem I in Synechocystis sp. PCC 6803

Affiliations: [1]Department of Chemistry, Purdue University, [2]University of Jyväskylä, Nanoscience Center, Department of Biological and Environmental Science, 40014 Jyväskylä, Finland

Photosystem II (PSII) contains several chlorophylls – the peripheral chlorophylls harvest energy and pass it towards the reaction center and the ones at the reaction center catalyze electron transfer. Electronic properties of individual chlorophylls are difficult to assign which limits our understanding of their specific functional roles. Such assignments can be made by studies using spectroscopic analysis and site-directed mutagenesis, but characterization of the isolated PSII complex is challenging due to the low stability of the complex outside its native cellular environment. In-cell measurements are a great alternative to undertake these kinds of studies. The overlap with electronic signals from Photosystem I (PSI), these PSII measurements can be made in a PSI-less strain developed by Shen, Boussiba, and Vermaas [Plant Cell, 5, 1853 (1993)], but it has a slow growth rate. We introduce a PSI-knockdown Synechocystis sp. PCC 6803 strains that enables site-directed-mutagenesis of PSII that is easy to grow and characterize by spectroscopic methods. These PSI-knockdown strains are made by replacing the native PsaAB promoter of PSI with a copper repressible PpetJ promoter in Synechocystis sp. PCC 6803. In a copper-deficient environment, PpetJ enables expression of PSI but in the presence of copper, the functioning of PpetJ is altered and hence, the expression of PSI is suppressed. 77K fluorescence measurements show a significant decrease in the PSI signal in the PSI-knockdown strain in the wildtype background in 24 hours after the addition of 0.3µmol CuSO4·5H2O that is the standard concentration of CuSO4·5H2O in BG11 medium. To further reduce PSI intensity higher concentrations of Cu2+prove promising but decreasing cell viability. The immediate goal of the project is to optimize conditions for maximum PSI-suppression without compromising cell viability. The two PSI-knockdown strains seem promising systems to study site-directed-mutagenesis and electronic properties in Synechocystis sp. PCC 6803 PSII using in-cell spectroscopy.