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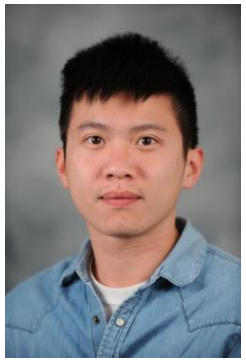
Robert Frederick Smith School of Chemical and Biomolecular Engineering

Spring 2020 Austin Hooey Special Graduate Research Seminar

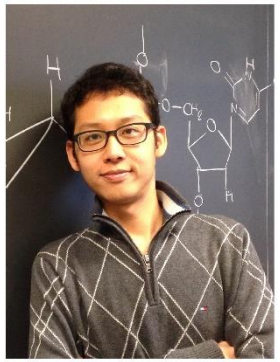


Han-Yuan Liu, Daniel group

Molecularly-Complex Planar Supported Cell Plasma Membranes as Scaffolds for Biotechnology Applications

Membrane proteins are responsible for significant proportion of cellular functions and therefore, implicated in many diseases. For example, more than 60% of therapeutics to date target membrane proteins to alter cellular functions that lead to disease. But membrane proteins alone are not the only actors in these processes. Recent work has shown that the local membrane environment made up of specific lipids surrounding the protein may be critical in regulating their function. However, systematically examining the roles of lipids on protein function is a significant challenge using conventional techniques. Over the past several decades, supported lipid bilayers (SLB) have emerged as a useful model membrane system to investigate these essential protein functions because they can mimic the natural cell membrane environment to assay protein function, while also being compatible with wide range of surface-based analytical techniques to quantitatively analyze the biological outcome. However, at the time that I joined the Daniel group, these platforms were not sophisticated enough to capture membrane proteins in functional states along with the native components of the plasma membrane. My contributions to this field centers around advances in bridging the simple SLB platforms of yesterday to the complexity of live cells today, in a format that allows us to interrogate these species in new ways that provides critical insight into their functions.

In my work, I proposed a novel strategy to take native cell membrane vesicles budded directly from living cell plasma membranes as intermediates to incorporate the native lipids and proteins into SLB platforms, without suffering from the significant downsides of traditional detergent-based reconstitution. This strategy, which I have shown is universal to many cell species from mammals to bacteria to plants, allows preservation of the complexity of the cell plasma membrane and retention of the natural protein structure and functionality. Another critical advance I introduced to this platform was integration of supporting surface that is a transparent, conductive polymeric material that not only supports the membrane to increase its robustness, but also is capable of reporting protein function as well. These polymeric materials can be tuned so their mechanical property mimics native tissues and these materials are biocompatible with cell membranes, while their cushioning ability mitigates the deleterious interaction from commonly used silica-based surfaces. Conducting polymers, owing to their ionic/electronic conductivity, offers a direct means to sense and transduce signals across the membrane and through proteins like ion channels. Due to the merits of the conducting polymer, I developed a biomembrane-based organic electronic device combining the native lipid environment, functional membrane proteins, soft tissue like supporting surfaces, and an electroactive surface that acts as a sensor and reporter. Altogether, with such a device I have demonstrated a variety of biosensing applications including pathogen detection, antibiotic screening, ligand-receptor binding, and, perhaps most significantly, ion channel monitoring. In my most recent works, I focused on studying two important ion channels involved in pain: the ATP-gated P2X family ion channels and the Trek ion channel. These ion channel receptors are important therapeutic targets for multiple pain modalities including neuropathic, inflammatory, and chronic pain states, yet little is understood about how they actually work and furthermore, the role of the membrane in their functions. The device I have built is capable of reading and transducing ion fluxes through these ion channels as it depends on the availability of ATP, lipid binding partners, and exposure to drugs known to block channel function. Given the importance today of development of new drugs for pain management, especially during the ongoing opioid crisis, considerable amount of research focus and development on identifying drug targets and engineering new drug for the treatment of the pain is needed. This new biosensing platform may prove to be a useful new approach for pharmaceutical screening and development, as well as basic science studies of ion channel regulation and biology.



Thapakorn Jaroentomeechai, DeLisa group

Cell-Free Synthetic Glycobiology: Designing and Engineering Glycomolecules Outside of Living Cells

Glycans and glycosylated biomolecules are directly involved in almost every biological process as well as the etiology of most major diseases. Hence, glycoscience knowledge is essential to efforts aimed at addressing fundamental challenges in understanding and improving human health. While much progress has been made, there remains an urgent need for new tools that can overexpress structurally uniform glycans and glycoconjugates in the quantities needed for characterization and that can be used to mechanistically dissect the enzymatic reactions and multi-enzyme assembly lines that promote their construction. To address this technology gap, we develop a collection of cell-free platforms as a simplified and highly modular framework to investigate, prototype, and engineer pathways for glycan biosynthesis and biomolecule glycosylation outside the confines of living cells. First, we engineered a novel cell-free glycoprotein synthesis (CFGpS) system that seamlessly integrates protein biosynthesis with asparagine-linked (N-linked) or serine/threonine-linked (O-linked) protein glycosylation. This technology leveraged a glyco-optimized Escherichia coli strain to source crude extracts that were selectively enriched with glycosylation components. The resulting extracts enabled a one-pot reaction scheme for efficient and site-specific glycosylation of therapeutic proteins including biologically-active human erythropoietin. Next, we applied CFGpS platform to develop technology for in vitro bioconjugate vaccine expression (iVAX) in lysates derived from detoxified, nonpathogenic E. coli. We demonstrated that iVAX synthesized vaccines against Franciscella tularensis subsp. tularensis (type A) strain Schu S4 conferred complete protection in an intranasal mouse model of F. tularensis infection. Finally, we developed a collection of cell-free glycan remodeling modules, enabled by the library of glycosyltransferase enzymes that had been engineered for high-level expression. The glycan remodeling modules are facile and highly modular, providing an efficient platform for rapid biosynthesis of authentic, complex human N-glycans. Together, our cell-free glycosylation system effectively broadens the glycoengineering toolbox and is anticipated to facilitate fundamental understanding in glycoscience and make possible new applications in on-demand biomanufacturing of glycoprotein and glycoconjugate products.

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