2023 ASP Virtual Symposium

Optogenetics and related fields

August 9, 2023

12:00 PM - 4:00 PM Eastern Time

PROGRAM

SCHEDULE-AT-A-GLANCE

2023 ASP Virtual Symposium

Optogenetics and related fields

August 9, 2023 • 12:00 PM - 4:00 PM EST

Time (EST)	Session
12:00 PM	Opening Statements
12:05 PM	**Towards single molecule analysis of signaling circuits in living cells** Klaus Hahn
12:25 PM	**Optical Control of Protein Function through Genetic Code Expansion in Zebrafish** Alexander Deiters
12:45 PM	**Controlling mitochondrial ROS production with light** Andrew P. Wojtovich
1:05 PM	**BREAK**
1:20 PM	**Directed evolution of light-activated proximity labeling enzyme, LOV-Turbo** Joleen Cheah
1:40 PM	**Engineered opsins for subcellular and in vivo optogenetic applications** Ajith Karunaranthe
2:00 PM	**Melanopsin as an Optogenetic Switch for Activating G-protein pathways** Phyllis Robinson
2:20 PM	**BREAK**
2:35 PM	**Upconversion Optogenetics and Beyond** Gang Han
2:55 PM	**Control of biomolecular condensate states using light and chemicals** Chandra Tucker
3:15 PM	**Small but powerful phototriggers for optogenetics** Ivan Dmochowski
3:35 PM	Closing Statements

ABSTRACTS

12:05 PM

Towards single molecule analysis of signaling circuits in living cells

Klaus Hahn

NIH Center for Signaling Dynamics, UNC-Chapel Hill

This talk will focus on new biosensors that can report stretch-induced activation of specific domains within talin and vinculin, proteins that translate mechanical events into morphology signaling (among other functions). We are examining how stretch-activated proteins play a role in guiding the migration of cancer cells from tumors to the vasculature. To understand the flow of information in relevant signaling circuits, we are producing optogenetically controlled vinculin, and exploring new approaches for multiplexed imaging of biosensors together with optogenetic analogs. The talk will emphasize approaches that reduce cellular perturbation and enable analysis of causal relationships between protein activation events.

12:25 PM

Optical Control of Protein Function through Genetic Code Expansion in Zebrafish

Alexander Deiters

Department of Chemistry, University of Pittsburgh

Biological processes, such as signal transduction, gene expression, and cell proliferation, are regulated with high spatial and temporal precision. In order to study and understand these processes, equally precise external control is required. Light is an excellent tool for this purpose, as it can be easily regulated in timing, location, wavelength, and amplitude, thereby enabling control of biological processes with unmatched precision. We are developing optical switches to control protein function through genetic code expansion with unnatural amino acids that can be activated with light. We recently demonstrated that we could add a 21st amino acid to the genetic code of zebrafish using both enzymatic and chemical tRNA acylation. With a focus on photocaged amino acids, we have applied these approaches to the optical control of DNA recombination, gene editing, RNA polymerization, RNA translation, cell signaling, and other essential biological processes in cells and embryos.

12:45 PM

Controlling mitochondrial ROS production with light

Andrew P. Wojtovich

Mitochondria are the metabolic hub of the cell and provide the bulk of cellular energy necessary for survival. Changes in mitochondrial function and reactive oxygen species (ROS) production lead to diverse outcomes ranging from stress signaling to disease. These divergent outcomes are spatially and temporally dependent; when, where and the degree of change in mitochondrial function can influence downstream events. This presentation will highlight the complexity of compartmentalized ROS, the novel tools we use to control mitochondrial function and ROS production, and how site-specific alterations contribute to signaling events and disease.

1:20 PM

Directed evolution of light-activated proximity labeling enzyme, LOV-Turbo

Joleen Cheah

Protein engineering has been used to impart new properties onto proteins beyond their natural functions. In recent years, many optogenetic tools have been developed to regulate protein localization, interactions, and function through light. We incorporated this mode of regulation into proximity labeling, which has been a cornerstone technique for mapping subcellular proteomes, and generated a genetically encoded, single-component proximity labeling tool that is activated by low-power visible light. Our design inserts the light-sensitive LOV domain into an exposed loop of TurboID to allosterically control its labeling activity with light. Further engineering by directed evolution reduced dark-state leak, increased light-state activity, and improved expression level. Additionally, we demonstrate an alternative mode of activation via BRET with a luciferase donor. Overall, our technology provides multiple means to exert precise spatiotemporal control and expands upon the experimental questions that can be explored using proximity labeling.

1:40 PM

Engineered opsins for subcellular and *in vivo* optogenetic applications

Dhanushan Wijayaratna[1], Sithurandi Ubeysinge[1], Waruna Thotamune[1], Filippo Sacchetta[2], Laura Pedraza-González[2], Massimo Oluvucci[2,3]*, Ajith Karunarathne[1]*

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[2]Department of Biotechnology, University of Siena
[3]Department of Chemistry, Bowling Green State University

Monostable opsins need a continuous retinal supply for sustained signaling, making theirs *in vivo* use challenging. However, spectral and signaling properties of bistable melanopsin and lamprey parapinopsin indicate compatibility for *in vivo* signaling control. We show that ultra-low retinal concentrations (in cell culture medium from FBS) are sufficient for functionalizing these opsins, showcasing their potential to function *in vivo* and outside the retina. Enhancing its signaling bandwidth, we also demonstrate that melanopsin activates two major G protein pathways (Gq and Gi/o) with near-similar efficacies. Additionally, we establish the feasibility of engineering mutant melanopsins with exclusive G protein-selectivity. Using *in silico* QM/MM models of squid rhodopsin and human and mouse melanopsins and the Automatic Rhodopsin Modeling (ARM) protocol, we investigated single-residue blue-shift mutations and subsequently engineered several melanopsin mutants that are resistant to red light, however sensitive to blue and green lights. We further show the utility of these novel blue-shifted melanopsins in single-cell and subcellular optogenetics. Similarly, our engineered novel color opsins offer features such as extended plasma membrane life and endomembrane exclusive signaling.

2:00 PM

Melanopsin as an Optogenetic Switch for Activating G-protein pathways

Phyllis R. Robinson

Biological Sciences, University of Maryland Baltimore County (UMBC)

Melanopsin is a fly-like visual pigment that is expressed in a small subset of retinal ganglion cells. It is involved in regulating both non-image forming visual behaviors, such as circadian photoentrainment and the pupillary light reflex, and aspects of image-forming vision. Melanopsin is a light-sensitive G-protein-coupled receptor that is bi-stable, long-lasting, and capable of activating multiple G-proteins families, making it a potential versatile optogenetic switch.

2:35 PM

Upconversion Optogenetics and Beyond

Gang Han

Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School

Chimeric antigen receptor (CAR) T cell-based immunotherapy has demonstrated remarkable curative potential in patients. Nevertheless, safety challenges persist due to limited control over the location and duration of the anti-tumour immune response, leading to cytokine release syndrome and on-target, off-tumour toxicity. In this context, we present the concept of light-switchable CAR (LiCAR) T cells, offering real-time phototunable activation for precise tumour cell killing. By combining surgically removable upconversion nanoparticles, acting as miniature deep-tissue photon transducers with enhanced near-infrared-to-blue upconversion luminescence, LiCAR T cells enable both spatial and temporal control over T cell-mediated anti-tumour therapeutic activity in vivo, significantly mitigating side effects. Our innovative nano-optogenetic immunomodulation platform not only offers a unique approach to understand cellular anti-tumour immunity but also holds promise for the development of personalized precision medicine for delivering targeted anticancer therapy.

Additionally, during the presentation, I will discuss our progress in creating night vision in mice using upconversion nanoparticles. This advancement may have far-reaching implications for both basic research and potential therapeutic applications.

2:55 PM

Control of biomolecular condensate states using light and chemicals

Chandra Tucker, Ph.D.

Department of Pharmacology, University of Colorado School of Medicine

The Tucker lab focuses on developing engineered protein-based tools that enable inducible control of basic molecular and cellular processes, using actuators such as chemicals or light. Advantages of such systems include on-demand temporal resolution, tunability, and precise spatial resolution. In recent work, we developed a new chemical-based platform, BTBolig, for synthetic control of biomolecular condensates, non-membraneous cellular compartments important for cell health and associated with neurodegenerative diseases such as amyotropic lateral sclerosis. Healthy biomolecular condensates can undergo a maturation process, transitioning from dynamic liquid-like states into more rigid solid-like states associated with neurodegenerative diseases. To study this process, we developed a CRY2-based optogenetic method using light to induce condensate maturation. Our work provides new synthetic approaches for exploring complexities of biomolecular condensate biology.

3:15 PM

Small but powerful phototriggers for optogenetics

Ivan J. Dmochowski, Ph.D.

The Dmochowski laboratory at the University of Pennsylvania, Department of Chemistry has advanced photochemistry applications for biomedicine over the past 20 years. Highlights include light-activated oligonucleotides for gene regulation and mRNA capture/transcriptomics, fluorescent general anesthetics for *in vivo* visualization and target identification, ruthenium polypyridyl linkers for multi-visible-wavelength photoactivation and hydrogel formation, and fluoresceinated oligonucleotides for pH sensing during cellular delivery. For this mini-symposium on optogenetics, my talk will focus on different methods for phototriggering conformational-functional changes in oligonucleotides and proteins.