2017 Awardee Examples

Sojung Kim

Penn State University

Discipline: *Biochemistry and Molecular Biology*

AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

Abstract Title: Synthesis of a Novel Small Molecule Inhibitor of D14-Type Strigolactone Receptors

Strigolactones are a class of plant hormones that can regulate shoot branching and stimulate germination of parasitic Striga hermonthica seeds. Since strigolactone signaling affects the growth of devastating parasitic weeds, plant height, and overall plant architecture, finding an effective way to regulate strigolactone receptors and inhibit strigolactone perception could lead to novel pathways to improving crop yields and controlling agricultural pests. Recently, the Itami Group discovered a novel small molecule inhibitor, named as DL1, which binds to the DWARF14 (D14) receptor and inhibits further strigolactone hydrolysis and signaling. This study was derived from their recent discovery, and had two specific aims: 1) to investigate the structure-activity relationship (SAR) of DL1, and 2) based on the SAR, design a new, stronger inhibitor. To research the effect of the adamantyl moiety on inhibitor efficacy, derivatives of the original DL1 molecule with various carbon scaffolds such as cubane, cyclohexane, and benzene in place of the adamantyl were synthesized. Derivatives of DL1 with different hydrocarbon branches on the indole moiety were also synthesized. The binding activity and dosedependent hydrolysis inhibition efficacy of all derivatives were studied through a competition bioassay using Yoshimulactone Green, a molecule that fluoresces when hydrolyzed by uninhibited D14 receptors. Preliminary bioassay results revealed that replacing the adamantyl moiety with a simple aromatic group yielded a surprisingly strong inhibitor, which disputed the original idea that the polycyclic cage nature of the adamantyl moiety would play a key role in inhibition activity. Based on these results, new families of derivatives, like those having ortho, meta, para, and disubstituted phenyl moieties, were systematically designed, synthesized, tested, and modified to create more effective inhibitors. The structures of these new inhibitors were characterized with ^{1}H NMR and X-ray crystallography. This systematic approach ultimately led to the discovery of a new potent inhibitor of D14-mediated strigolactone hydrolysis that is nearly 4.5x more effective than the original DL1 molecule. The IC_{50} value (half-maximum inhibitory concentration) of the new compound is 0.29 micromolar while that of DL1 is 1.3 micromolar. This new small molecule inhibitor has great potential as an agrochemical to control strigolactone signaling in crops, particularly because DL1 and its derivatives enhance shoot branching in crops such as Arabidopsis thaliana and rice.

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AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

2017 Awardee Examples

Olga Vafaeva

Hunter College (CUNY)

Discipline: *Neuroscience*

AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

Abstract Title: Adrenergic Signaling Mediates Synapse Elimination in Developing Central Nervous System by Induction of Astrocyte-Derived Interleukin-33

Neuronal synapse development and refinement is critical to normal brain function. Brain glial cells, including astrocytes and microglia, can sense and produce signals that control synaptic development. We recently found that astrocytes express an immune molecule, Interleukin 33 (IL-33), that regulates microglial synapse engulfment in developing central nervous system (CNS). However, the signals that induce IL-33 expression in astrocytes are unknown. Our lab has found that IL-33 expressing astrocytes highly express neurotransmitter receptors, particularly adrenergic receptors which are specific for norepinephrine (NE) in CNS. In addition, IL-33 expression increased during neuronal circuit formation in thalamus and spinal cord, which led us to hypothesize that IL-33 expression is regulated by neuron-derived signals, particularly norepinephrine. To test this, we cultured thalamic astrocytes of IL-33 reporter mice, treated them with different concentrations of norepinephrine, and measured IL-33 expression by immunocytochemistry and quantified the number of IL-33 expressing cells. We observed a significant increase in both IL-33 expression and in the number of cells expressing IL-33 in a dose dependent manner after norepinephrine treatment. In future work, we plan to develop a co-culture model of astrocytes and adrenergic neurons derived from cervical ganglia to test whether neuron-derived NE affects IL-33 expression. Our data indicate that NE is a positive regulator of IL-33 expression in cultured astrocytes, suggesting that neuron-derived cues may regulate astrocyte functional maturation, and adjust the microglial synapse engulfment to match synaptic load.

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ABRCMS 2018 INDIANAPOLIS, INDIANA

2017 Awardee Examples

Olubunmi Fariyike

Columbia University

Discipline: *Engineering, Physics and Mathematics*

AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

Abstract Title: Designing Environmental Sensors for Living Biomaterials

We aim to design environmental sensors to be integrated into a living biomaterial made from plant refuse. This structural composite can be shipped flat across the world and grown from local resources at a fraction of the cost of the transport of conventional materials to provide affordable, biologically-adaptive, and environmentally-conscious refuge worldwide.

We hypothesize that the we can utilize the promoters of yeast strain S. cerevisiae combined with a fluorescent readout to design these sensors. Due to the well-elucidated yeast genome, previous literature points to different repair pathways that include promoters whose protein transcriptions are sensitive to each of the environmental stimuli of interest. For this project, we aim to design sensors for UV toxicity, heavy metal poisoning, and heat stress, and, to do so, we used the promoters of RNR3, HUG1, and HSP104, respectively. We used Gibson Assembly to fuse each of these promoter sequences into a core acceptor plasmid directly upstream of a red fluorescent mCherry readout and a terminator sequence from the CYC1 gene. We then transformed each plasmid construct into a laboratory yeast strain that had already undergone CRISPR with a promoter associated with glycolysis fused with a green fluorescent readout. The green fluorescence served as a model for basal cellular protein transcription and the red fluorescence served as a model for the transcription as driven by our new construct. This allows us to standardize the two fluorescence values by optical density (OD) and then compare the red fluorescence per cell to the green fluorescence per cell to ascertain the specificity of our construct to its respective stimulus.

So far, we have tested 2 of the 3 sensors we have designed. For the heat stress sensor, we chose to incubate cultures at 25, 30, 37, and 42 degrees, measuring fluorescence and OD every hour. We used the optimal growth temperature of 30 degrees as our control. For the UV toxicity sensor, we exposed samples to direct outdoor sunlight for 30 minutes, 1 hour, and 2 hours, using a sample that received no exposure as our control and taking the same measurements post-exposure every 30 minutes. For the heat stress sensor, we noted a 2.5 times increase in protein expression after 8 hours at 42 degrees and after 12 hours at 37 degrees. For the UV toxicity sensor, we discovered that the UV irradiance provided by ordinary sunlight was not intense enough to drive a marked response with this promoter.

Though we saw promising results for the heat stress sensor, we plan on assaying constructs with 6 other heat-sensitive promoters to ensure we have designed the sensor with the highest specificity. Additionally, although the UV sensor construct did not yield significant results, cells did show trends of lower growth at longer exposures. Therefore, we are currently researching other promoters associated with the DNA damage repair pathway that may yield better results.

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We hypothesize that the we can utilize the promoters of yeast strain S. cerevisiae combined with a fluorescent readout to design these sensors. Due to the well-elucidated yeast genome, previous literature points to different repair pathways that include promoters whose protein transcriptions are sensitive to each of the environmental stimuli of interest. For this project, we aim to design sensors for UV toxicity, heavy metal poisoning, and heat stress, and, to do so, we used the promoters of RNR3, HUG1, and HSP104, respectively. We used Gibson Assembly t each of these promoter sequences into a core acceptor plasmid directly upstream of a red fluorescent mCherry reado d a terminator sequence from the CYC1 gene. We then transformed each plasmid constru R with a promoter associated with glycolysis fused with a g el for basal cellular protein transcription and the red fluore ew construct. This allows us to standardize the two fluoresc escence per cell to the green fluorescence per cell to ascertain the specificity of our construct to its respective stimulus.

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AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

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Abstract must contain a brief statement of the experimental methods/methodology used

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So far, we have tested 2 of the 3 sensors we have designed. For the heat stress sensor, we chose to incubate cultures at 25, 30, 37, and 42 degrees, measuring fluorescence and OD every hour. We used the optimal growth temperature of 30 degrees as our control. For the UV toxicity sensor, we exposed samples to direct outdoor sunlight for 30 minutes, 1 hour, and 2 hours, using a sample that received no exposure as our control and taking the same measurements post-exposure every 30 minutes. For the heat stress sensor, we noted a 2.5 times increase in protein expression after 8 hours at 42 degrees and after 12 hours at 37 degrees. For the UV toxicity sensor, we discovered that the UV irradiance provided by ordinary sunlight was not intense enough to drive a marked response with this promoter.

Though we saw promising results for the heat stress sensor, we plan on assaying constructs with 6 other heat-sensitive promoters to ensure we have designed the sensor with the highest specificity. Additionally, although the UV sensor construct did not yield significant results, cells did show trends of lower growth at longer exposures. Therefore, we are currently researching other promoters associated with the DNA damage repair pathway that may yield better results.

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2017 Awardee Examples

Eden Ramirez

Schoolcraft College

Discipline: *Physiology*

AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

Abstract Title: Role of Obstructive Sleep Apnea in Nocturnal Heart Blocks

Since nocturnal heart blocks (NHB) have been known to lead to sudden cardiac death, the purpose of this study is to examine if patients with obstructive sleep apnea (OSA), have increased NHB and if there is a correlation of detected NHB to specific sleep cycles. Past studies have shown that human patients with NHB mostly had occurrences during the rapid-eye movement (REM) stage of sleep. A preliminary study of human subjects (n=40) conducted in our lab also suggested NHB appear more frequently in patients with severe sleep apnea [defined as Apnea-Hypopnea Index (AHI) of greater than 30]. Furthermore, a rat study (n=4) conducted in our lab indicated NHB are exclusively found during the REM stage of the sleep/wake cycle. This study was designed to expand our preliminary study to more human patients with OSA. Polysomnographic data was used to determine sleep wake cycles, electrocardiogram (ECG) was monitored to determine electrical signals of the heart, and movement of thoracic/abdominal cavities was recorded to monitor air intake. Utilizing a sophisticated Graphic User Interface (GUI) system developed in our lab, all signals (sleep stage, air intake, RR intervals, and ECG) were viewed simultaneously. Using data from the Sleep Heart Health study (SHHS), 360 OSA patients with varied AHI were examined. From these patients, 2 of 120 patients with AHI ≤ 4, 1 of 120 patients with AHI between 5 and 30, and 4 of 120 patients AHI ≥ 30 had confirmed NHB. The results confirmed that patients with severe OSA had more cases of NHB. Additionally, unlike the animal study, human patients showed NHB events in different sleep/wake cycles. In fact, during this study, human patient had more NHB events during the non-REM phase of sleep. This study demonstrates that NHB is more prevalent in patients with severe sleep apnea and NHB is found in both REM as well as Non-REM sleep states in OSA patients.

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AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

2017 Awardee Examples

Kristina Correa

Stanford University

Discipline: *Cancer Biology*

AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

Abstract Title: Characterization of Sialic Acid-Containing Glycans in Myc-Driven Cancer Models

Up to 70% of cancers are characterized by deregulation of Myc, a transcription factor that regulates numerous cell proliferation genes. Burkitt Lymphoma, T-cell acute lymphoblastic leukemia (T-ALL), renal cell carcinoma, and ovarian carcinoma are cancers associated with amplifications in Myc expression. Myc has been demonstrated to impact cell surface glycosylation, and, as established previously by the Bertozzi Lab, expression on tumor cells of glycans containing sialic acid (sialoglycans) can inhibit the anti-cancer immune response by engaging immune cell sialic acid immunoglobulin-like lectins (Siglecs). Thus, hypersialylation is possibly involved in promoting cancer's immune evasive phenotype. However, there is little research characterizing how oncogenes promote production of sialoglycans. Understanding sialoglycan synthesis and immunomodulatory action could be valuable to the field of cancer biology in facilitating the discovery of novel immunotherapy targets. We postulated that Myc expression promotes production of sialoglycans. Toward this end, we have the following two specific aims: 1) to quantify sialic acid expression at varying Myc expression levels and 2) to identify enzymes in the sialoglycan biosynthesis pathway regulated by Myc. Experiments utilized mouse T-ALL and human Burkitt lymphoma cell lines in which Myc expression may be titrated by doxycycline administration. Preliminary RNA sequencing data suggest that Myc directly regulates expression of genes in the sialic acid biosynthetic pathway. We performed flow cytometry after staining Burkitt lymphoma cells with recombinant Siglec-7 (found on natural killer cells) and Siglec-9 (found on myeloid cells); the resulting data indicated Siglec-7 and Siglec-9 ligands decreased 24 hours following reduction of Myc levels. Likewise, recovery of sialoglycans that bind Siglec-7 and Siglec-9 24 hours following treatment with sialidase, which cleaves sialic acids, is mitigated by decreased Myc expression. Binding of sialoglycans on cancer cells to these recombinant Siglec probes suggests potential inhibitory interactions between Burkitt lymphoma cells and immune cells, supporting a link between Myc expression and immune system inhibition. Through qPCR experiments, we identified 4 sialyltransferases (ST3GAL2, ST3GAL4, ST6GALNAC2, and ST8SIA4), which transfer sialic acid to nascent glycans, in T-ALL cells with transcript levels reduced by over 90% following Myc suppression. Future studies are directed at developing CRISPR knockout T-ALL cell lines of the sialyltransferases identified and performing immune function assays to determine how sialoglycans modulate the anti-cancer immune response. Consistent with our initial hypothesis, we conclude that Myc expression affects both production of sialoglycans and cell surface sialic acid composition in a manner that may facilitate immune evasion.

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2017 Awardee Examples

José Valentin-Lopez

University of Puerto Rico, Cayey

Discipline: *Cancer Biology*

AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

Abstract Title: SGK-1-mediated ATP Generation: a Novel Metabolic Pathway that Supports the Survival of ECM-detached Cells

Successful metastasis requires cancer cells to overcome both anoikis - caspase-dependent cell death triggered by extracellular matrix (ECM) detachment - and ECM-detachment-induced metabolic defects that compromise cell survival. While studies have begun to elucidate signal transduction cascades responsible for anoikis evasion, less is known about the precise signals cancer cells use to overcome ECM-detachment-induced metabolic deficiencies. Previously, it was discovered that oncogenic Ras utilizes a PI(3)K/SGK-1 signaling cascade in order to promote glucose-mediated ATP generation and survival of ECM-detached cells. We have expanded on these studies and found that SGK-1 signaling is required in a variety of cell types and oncogenic backgrounds (during ECM-detachment) for glucose-derived ATP production (through regulating GLUT1 levels and localization) and anchorage-independent growth. Intriguingly, ATP generation instead requires flux through the pentose phosphate pathway (PPP). PPP-mediated ATP generation occurs independently of NADPH generation and regulation of oxidative stress. This was concluded after performing lentiviral transduction on different cell lines and running ATP, Glucose Uptake, Soft Agar and Reactive Oxygen Species Assays while these were growing in detached conditions. Overall, this study represents a novel metabolic pathway downstream of SGK-1 that is highly conserved across multiple epithelial cancer cell lines during ECM detachment. Our data suggest that SGK-1 may act as a master regulator of glucose metabolism and energy production during ECM-detachment that may be amenable to novel targeted therapies aimed at eliminating ECM-detached cancer cells through disruption of metabolism.

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Abstract must contain a conclusion that explains how the work contributes to the hypothesis, objective or statement of problem

2017 Awardee Examples

Jesus Valencia

St. Mary's University (TX)

Discipline: *Biochemistry and Molecular Biology*

AMERICAN SOCIETY FOR MICROBIOLOGY

ABRCMS 2018 INDIANAPOLIS, INDIANA

Abstract Title: Synthesis and Crystallization Trials of New Delhi Metallo β-Lactamase

β-Lactam antibiotics are a class of broad spectrum antibiotics that are used to treat illnesses such as urinary tract infections and meningitis. Although β-Lactam antibiotics are an effective method of terminating bacteria, bacteria have become resistant due the use of the New Delhi Metallo-β-Lactamase (NDM-1). NDM-1 is a class B β-Lactamase with a binuclear zinc center that renders β-Lactam antibiotics useless via hydrolysis. Since NDM-1 has the ability to hydrolyze nearly all β-Lactam antibiotics, it poses a great threat to the world. In order to fully understand the hydrolysis of β-lactam antibiotics by NDM-1, the protein will be crystallized and its structure will be determined in its static form and in various intermediate states on the path to hydrolysis leading to product release. NDM-1 was successfully cloned in pNIC28-BSA4 by Gibson Assembly. The construct was then transformed in *E. Coli* (D5α) and the recombinant DNA was then purified and transformed in a protein expression cell line of *E. Coli* (BL21). NDM-1 was expressed and then purified by affinity chromatography using a Ni-column, followed by a TEV Digest subtractive immobilized metal affinity chromatography to remove the recombinant 6X-histidine tag and size exclusion chromatography. NDM-1 was set for crystallization trials and is currently under incubation. The crystals generated can then be optimized as microcrystals for use at an X-ray free electron laser to obtain data sets that can be processed to determine protein structures leading to the generation of a molecular movie of NDM-1 catalyzing the hydrolysis of β-lactam antibiotics. The molecular movie would provide an understanding of the NDM-1's relevant conformations and the time frames in which they occur, ultimately leading to the design of a new generation of antibiotics.

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