GELH Application Submitted: 1/31/2013 2:45:29 PM

Applicant Name : Long, Adam

Class: Junior

Expected Graduation: Spring 2015

Individual or group: Individual

ABSTRACT Protein-protein interactions (PPI) play critical roles in a large number of biological processes, meaning that their dysregulation can lead to diseases such as Alzheimer's, diabetes, and various forms of cancer. Laboratory techniques that allow for the manipulation of proteins are highly useful as they can reveal how PPIs function to control protein activity. Several techniques exist that make it possible to cause the association of proteins. However, there are fewer tools that trigger their dissociation, an equally important mode of interaction. This summer, pending funding, we plan to develop a genetically encodable linker that undergoes photo-cleavage and dissociation upon irradiation with light.

PROPOSAL See Emailed Document (contains image). Herein is the text-only version:

Highly regulated networks of sensing and signaling proteins, in which information is often propagated via protein associations, control the majority of biological processes. Protein-protein interactions (PPIs) are well recognized as one of the main routes of increasing the functional diversity of proteins. However, PPIs are inherently complex and subtle differences in their association can dictate their biological activity, thus making them difficult to study. Herein, we propose the development of a minimally disruptive and widely applicable tool to study fleeting and complex PPIs. Our tool will provide insight into the normal and abnormal functions of PPIs while focusing on identifying potential targets for therapeutic intervention.

In order to characterize interactions between proteins most researchers use non-covalent, small molecule-mediated interactions that require monitoring and manipulating the protein-protein equilibrium of association and dissociation. Non-covalent protein-protein studies thus introduce confounding factors such as chemicals and equilibrium states that can inadvertently introduce artifacts to the sample or complicate the analysis. We propose a generalized method for protein or protein-protein studies via covalent ligation by making the process light-activated, which will remove those concerns and afford us greater control on the timing and location of activation.

To generate this tool, we will re-engineer a photo-responsive AsLOV2 domain from Avena sativa phototropin I via directed evolution into a photo-cleavable and dissociable construct. The newly evolved protein will respond to a specific wavelength of light by first self-cleaving at the backbone and then dissociating into two non-interacting units with temporal and spatial control. The use of this genetically encodable linker between proteins will initially induce a covalently "forced" protein-protein association. The sample can be analyzed at the pre-irradiation stage to delineate the effect of the PPIs. Upon the

irradiation of the sample, the dissociation of the linker will rapidly release the two protein halves due to the intein activity and the immediate cellular response to this exquisitely precise dissociation can then be monitored at real time.

In order to develop this tool, we will use molecular cloning to insert the photoactive AsLOV2 domain in between two “split” intein halves, the N- and C- termini of a complete intein. In this way, intein activation and subsequent excision will be predicated on blue-light activating AsLOV2 and dimerizing the separated sequences. The protein segments to be studied will be introduced using similar molecular cloning techniques into the flanking sequences on either side of the intein portions, denoted “exteins” due to their expression in the protein following intein splicing and ligation of the protein segments. This photocleavage and ligation reaction serves to link previously separate N- and C- termini of a single protein or two discrete proteins together.

So far, we have made initial progress to create the AsLOV2-intein gene complex. We have isolated AsLOV2 and confirmed it retains functionality by monitoring its blue light-activated absorbance shift, and optimized photoactivation distances for a blue-light rig assembly. The C-termini has also been cloned into the AsLOV2 plasmid. Thus, what remains is finalizing the construct and fine-tuning the photocleavage process via directed evolution and error-prone PCR to establish a library of mutant variants, followed by expression in bacterial cells until light-induced proliferation is observed. Mutant variants that do not properly connect the protein segments, which in this case will be the antibiotic resistance gene for Kanamycin (KanR), will be eliminated and only the mutants that express the KanR gene will remain under blue-light activating conditions. When this is accomplished, the KanR gene segments can be replaced with other proteins that have physiological relevance and their interactions can be observed in a controlled manner. The work will be completed by the end of the summer. We envision that a novel methodology which allows for the rapid covalent disassembly of two proteins using a genetically encodable linker with spatial and temporal control will pave the way to the interrogation of previously intractable biological questions.

The timeline for the project’s completion is as follows:

• Weeks 1-3: Perform the molecular cloning of the initial plasmid for the construction of the AsLOV2 protein with a split intein. Confirm that the construct is correct via DNA sequencing and expression in bacterial systems.

• Weeks 4-5: Analyze the switching mechanism of the construct with varying light sources. Bacteria containing the correct plasmid will be grown on plates and exposed to various light wavelengths that correspond to AsLOV2 triggers. Colonies that display red fluorescence will be analyzed further.

• Weeks 6-9: Install two new split proteins around the central AsLOV2-intein domains for barnase and kanamycin resistance. These novel genes will allow us to readily screen for colonies that have the protein with the desired function.

• Week 10: Pick colonies that survive the conditions related to barnase/kanamycin expression. These mutants will be sequenced to identify points of mutation and varied further to generate improved protein variants.

DELIVERABLE Presentation at the 2013 GELH Symposium and a poster at the 2014 Undergraduate Research Symposium

BUDGET REQUEST $4,000

BUDGET DESCRTIPTION 10-week stipend for 40 hours/week of research

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Activity Start Date: June 2013

Activity End Date: August 2013

Location of Activity: Lehigh University in the laboratory of Dr. Marcos Pires

Mentor: Marcos PiresDr.
Lehigh University
Lehigh University
Mudd 695
6 East Packer Avenue
Bethlehem, PA 18015

Letter of support requested? Yes

Additional information for committee to consider:

I have completed the following courses and their associated labs:

Organic Chemistry I & II

Biochemistry I

Biology Core I & II (for Biology Core II I completed the more advanced, HHMI-sponsored Phage Genetics laboratory)

I hope to obtain a bachelor's of science degree in both molecular biology and behavioral neuroscience by May 2015 and to follow up my undergraduate studies by pursuing an MD/PhD or MD towards a career in biomedical research and clinical practice. Thus, the opportunity to develop this molecular construct would not only further scientific research in general, but would also help me choose between possible career paths. Furthermore, the experience would greatly enhance my overall preparedness for graduate school, thus putting me in a strong position to be admitted to a top program upon graduation. The GELH Scholarship will be critical component of my development as a researcher and a critical

thinker. While I have worked on parts of this project during the semester, it will take a full-time laboratory effort to make substantial progress. At the end of the summer, I look forward to sharing my exciting findings with the rest of the Lehigh University community upon completion of the proposed work. The GELH Scholarship will provide me with the funds to pursue this exciting project, make important scientific discoveries, and enhance my overall experience as a Lehigh University undergraduate researcher.

Submitted to other funding sources? No

Is IR approval required? No