DATE: Day 18 Month 5 Year 2018

SUMMARY of
2017 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Probing G protein-coupled receptor (GPCR) structural dynamics using selective aromatic labels
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	Affiliation	**Harvard Medical School**
	Present Title	**Assistant Professor; Indiana University**
Research Collaborator (Host PI)		Yohei Miyanoiri

Summary

G protein-coupled receptors (GPCRs) are the largest signaling protein superfamily – regulating diverse processes from vision, smell and taste to immune, neurologic and reproductive functions. Residing at cellular membranes they are the primary means of translating extracellular stimuli into intracellular responses. Nonetheless, the molecular details of signal transduction remain obscure despite an explosion of new GPCR crystal structures.

Crystallization has been impossible without additional modifications and very high-affinity ligands that force receptors into a subset of nearly indistinguishable conformations – regardless of the presumed activation state. Yet, in contrast to other proteins, GPCRs are highly dynamic and possess multiple active states rather than a binary, 'on or off', mode. Nuclear magnetic resonances (NMR) spectroscopy remains the only technique capable of quantifying protein dynamics at atomic resolution, but requires isotope labeling (e.g. ^{2}H, ^{13}C, ^{15}N). Proteins recombinantly produced in bacteria are amenable to the multitude of isotopic labeling schemes; however, overexpression of membrane proteins results in non-functioning, insoluble aggregates. In 2013, directed evolution of neurotensin receptor 1 (NTR1) made possible large-scale prokaryotic expression of functional receptors, but NTR1's membrane environment greatly restricting traditional NMR methods.

Cell-free protein synthesis presents a unique solution as selectively-labeled amino acids can be polymerized, in a H_2O-based reaction, without scrambling from biosynthetic pathways. E. coli-derived cell-free systems are the most efficient, but previous attempts at GPCR expression failed. We hypothesized that our evolved NTR1 construct would be more amenable to E. coli-based cell-free methods. In collaboration with Prof. Yohei Miyanoiri, we pursued cell-free protein synthesis of NTR1 for NMR-based structural dynamics studies. Our initial structural characterization at IPR did not produce meaningful results as the protein yields were too low to be detected. We maintain our hypothesis that receptors evolved to express in E. coli are far more likely to yield properly-folded constructs in vitro. Using a mixed detergent-lipid micelle system, our preliminary studies of the evolved NTR1 construct produce high yields (> 2 mg/1 ml reaction) of folded receptor. We are currently supplementing cell-free reactions to improve the yield of functional receptor. We anticipate our method will be generally applicable to other membrane protein targets.

***Deadline: May 18, 2018**

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