Engineering Bacterial Guanylate Cyclase for Optogenetic Applications

Nair, Arundathi (School: Laramie High School)

Optogenetics is technique that uses light to regulate biological processes in living cells. It is based on genetically engineered proteins that are activated by light. Due to high spatial-temporal resolution (“when” and “where”), optogenetics has the potential to treat a variety of diseases, without the risk of adverse effects associated with chemical-based drugs. The aim of this project was to design a near-infrared window (NIRW)-responsive synthetic module containing a bacterial phytochrome, connected to the human guanylate cyclase (GC), via an a-helical linker. Because the length of the a-helical linker determines activity, eight linkers of varying length were constructed. Dr BphP_MA was restriction digested to obtain the vector. The GC gene and a-helical linker gene was amplified by PCR and was cloned into the vector via Gibson reaction. The constructs were transformed into E. coli by electroporation. LacZ blue/white screening was used to evaluate cGMP production. The light-responsive system that uses a bacterial phytochrome to activate human GC was successfully constructed. Among the eight fusions, two showed activity in response to NIRW-light. These constructs will be selected for future testing in cultured cells and animal models and have potential to be used in human optogenetic applications.

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