Engineering Bacterial Guanylate Cyclase for Optogenetic Applications

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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 α-helical linker. Because the length of the α-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 α-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.