EnLIGHTened Therapeutics: Engineering Light-Activated Proteins for Optogenetic Applications

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Background: Traditional chemical-based treatments for disease are non-specific and cause undesirable side effects. These non-target effects can be avoided by site-specific, targeted therapy and by controlling the ‘when’ and ‘where’ of treatment. Light has high spatial-temporal resolution, which eliminates non-target effects. Optogenetics is a technique that uses light to regulate genes that control a variety of biological processes. Optogenetics uses Near Infrared Window (NIRW) light because of its ability to penetrate through layers of tissue. Aim: The objective of the project was to design and optimize a light-responsive system that produces cyclic guanosine monophosphate (cGMP) in a light-dependent manner. cGMP is a second messenger that regulates important biological functions in humans, including heart rate. Methods: The system was created by cloning bacterial phytochrome to human guanylate cyclase with via an amino acid linker. Two extended versions of the original RAELAE linker, with the sequences RAELERKE (M4) and RAELAERKE (M5) were also tested. NNK mutagenesis was used to randomly mutate the leucine (L) and alanine (A) amino acids in the M5 linker. The constructs were transformed into E. coli by electroporation. LacZ blue/white screening was used to evaluate cGMP production. Results: The light-responsive system that produces cGMP was successfully constructed. The systems with the M4 and M5 linkers both demonstrated constitutive activity. More importantly, the systems with the NNK mutagenesis linkers both exhibited activity in response to NIRW-light. Conclusion: These light-activated constructs can be used for future testing in cultured cells and animal models for potential use in human optogenetic applications.

Awards Won:

Patent and Trademark Office Society: Second Award of $500