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Fluorescent Protein Biosensor for Use in Parkinson's Research

Piper R. Miller, Keelan J. Trull, and Mathew C. Tantama
Department of Chemistry, Purdue University

ABSTRACT

Purinergic signaling is a type of extracellular communication that occurs between cells, mediated by adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine. In Parkinson's Disease, purinergic signaling is disrupted, which contributes to neurodegeneration. In order to monitor this change in cell-to-cell signaling, there is a need for the development of a fluorescent protein (FP) biosensor to study the changes in the concentration of the signaling molecule ATP and its decomposition bioproduct ADP. This summer a genetically encoded ADP sensor that measures changes in ADP concentration was developed. This sensor utilizes Forster Resonance Energy Transfer (FRET) which is a sensing technique that is based on the energy transfer from a donor FP to an acceptor FP. Since this transfer is distance dependent, a change in the sensing domain allows for detection of ADP concentration through changes in fluorescence emission. To develop this FRET based sensor, we are utilizing a cyan-yellow FP pair, as well as a non-fluorescent protein that binds to ADP. Using traditional cloning methods, a small library of ADP sensors from five different versions of both the cyan and yellow proteins was created. This library was screened in *E. coli* cultures using a method developed to optimize an ATP-sensor. The cloning for this sensor has been confirmed and the library is being tested for sensors responsive to changing concentrations of ADP. With confirmation of a responsive sensor, this sensor design will be validated, allowing for further optimization of this biosensor for the study of purinergic signaling and neurodegeneration.

KEYWORDS

FRET, Parkinson's Disease, fluorescent protein, biosensor, ADP