Leave-One-Out GFP Biosensors: In vivo library screening through bacterial two-hybrid interactions

by

Nicholas R. Talbot

An Abstract of a Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the degree of

MASTER OF SCIENCE

Major Subject: BIOLOGY

The original of the complete thesis is on file
In the Rensselaer Polytechnic Institute Library

Approved:

Professor Christopher Bystroff, Thesis Adviser

Rensselaer Polytechnic Institute
Troy, New York

April 2011
(For Graduation May 2011)

ABSTRACT

Of the thousands of pharmaceuticals, drugs, and other designed chemicals produced by bioengineering companies each year, a recent trend towards rational design has become apparent. This trend is not without reason as molecules tailored for a specific reaction tend to have a higher level of efficacy and a lower potential for side effects; but serious design difficulties preventing wide development of these rational compounds endure. Our understanding of the forces regulating protein design remains incomplete, and the resultant combinatorial design possibilities stress even the largest computational and experimental methods.

Biological systems, through evolution of structure and function, provide an insight into possible methods to overcome this difficulty in de-novo design. By basing design on trends in biologically observed conformations or by utilizing largely parallel biological systems for screening of protein designs, it may be possible to circumvent our lack of complete understanding in relation to protein biophysics.

Our efforts in this vein seek to provide a biologically relevant screening methodology for designed protein libraries as they relate to a specific biosensor: Leave-One-Out Green Fluorescent Protein (LOO-GFP). In this work, we show the appropriate fusion of both LOO7-GFP molecules with the N-terminal domain of the □ subunit of RNA Polymerase as well as the fusion of GFP Strand 7 (s7) to the N-terminal (activator) domain of □-bacteriophage repressor (λcI). Through protein expression and complementation methods, we show that these fusion products are effectively expressed in vivo and available for complementation. Finally, we demonstrate positive binding of these fusion elements in vivo through an increase in transcriptional activation of both HIS3 (which allows histidine biosynthesis) and aadA (which provides streptomycin resistance via adenylyltransferase activity).

Through the synthesis of a bacterial two-hybrid screening methodology with the flexible detection profile of LOO-GFP, we seek to enable a system which, through recursive cycles of library generation and selection, has the capability to produce an array of biosensors tailored through a rapid computational design process.