Research Tools


# GFAbs – GFP-Based Biosensors with the Binding Properties of Antibodies

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**The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing an expression library of fluorescent biosensors capable of detecting and isolating peptides, small molecules, nucleic acids or other molecules of interest.**

## Overview

Antibodies are used widely in biological and medical research to bind to and help isolate molecules of interest. However, the reduced stability and production yields of antibody fragments frequently utilized in *in vitro* platforms has led to attempts to create alternative binding scaffolds.

By mutagenizing solvent-exposed loop regions or inserting diverse loop repertoires into non-antibody protein scaffolds, specific binding attributes can be conferred to proteins with desirable properties, such as high stability and production titers. Using these techniques, alternative scaffolds have been developed to bind to targets with antibody-like affinity.

Green fluorescent protein (GFP) is another potential scaffold. As a binding scaffold, GFP would have two advantages over other alternative scaffolds. First, by combining binding attributes with the intrinsic fluorescence of GFP, the proteins could act as single step detection reagents in applications such as fluorescence-based ELISAs, flow cytometry or intracellular targeting/trafficking in live cells. Second, GFP fluorescence requires that the protein is properly folded, providing an *in situ* metric for folding fidelity. However, attempts to create robust, fluorescent GFP-based binding proteins with multiple binding loops that act together to form a cooperative binding interface have not been successful thus far.

## The Invention

UW-Madison researchers have developed a GFP-based scaffold that maintains its fluorescence properties in the presence of two inserted binding loops. The scaffold is capable of accepting a diverse loop repertoire from which fluorescent binding proteins could be isolated.

Inserting multiple loops into the scaffold yields fluorescent biosensors known as GFAbs. The researchers have developed expression libraries consisting of multiple fluorescent biosensors, which are capable of detecting and isolating antigens or other molecules of interest, to provide a resource for identifying binding ligands.

## Applications

- Single step detection applications, including fluorescence-based ELISAs, flow cytometry and real-time intracellular trafficking/targeting in live cells
- Replacements for antibodies as traditional protein detection elements or therapeutic targeting reagents
- High throughput screening to identify binding ligands

## Key Benefits

- GFAbs offer an inexpensive alternative to antibodies.
- Biosensors enable one-step detection of the binding event because of their intrinsic fluorescence.
- Multiple loops form a cooperative binding interface.
- Scaffold maintains fluorescence in the presence of multiple inserted loops.
- Other fluorescent proteins may be used in addition to GFP.

## Additional Information

**For More Information About the Inventors**

- Eric Shusta

**Publications**

- Pavoor T.V., Cho Y.K. and Shusta E.V. 2009. Development of GFP-Based Biosensors Possessing the Binding Properties of Antibodies. PNAS. 106, 11895-11900.

**Tech Fields**

- Research Tools : Genomics & proteomics

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854