Multi-color photoactive GFPs from reef corals

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Background

Fluorescent proteins of the green fluorescent protein (GFP) family are encoded by single genes and no other cofactors are needed to form the fluorescent product. By introducing GFP's DNA gene coding into a live cell or attaching it to proteins, fluorescent labeling is achieved and complex biological processes can be studied in live cells or organisms. This has made GFP, and related proteins, the dominant technology for *in vivo* labeling, cellular imaging, drug, gene and protein screening in biomedical research and cell biology. Reef corals have an amazing diversity of multi-colored GFP-type proteins and during our studies of the Great Barrier Reef corals, several novel GFP-like proteins have been identified, with superior optical properties: multi-colored, photostable, with high quantum yields, some of which spontaneously increase fluorescence or are reversibly photoactivated (switched on and off) in response to light and provide a diverse choice of fluorescent labels as imaging or screening tools. Photoactive GFP-type proteins are capable of repeated photoconversion between bright and dim states and in contrast to conventional GFP labeling, they provide ability to track cells or proteins of interest by selective conversion of labeled proteins by brief light pulse and subsequent tracking without the need for constant imaging.

Outcomes:

- Multi-color, genetically encoded fluorescent labels for cellular localization,& gene expression
- Second generation GFPs – photoactivatable GFPs, capable of precise optical targeting, pulse-chase experiments and showing pronounced changes in spectral properties in response to light.
- Diagnostic method for cell, organelle or protein labeling and biosenor technologies

Progress to date.

- Cyan, green, yellow and red (470nm to 635nm) GFP-type proteins were identified, several with photoactive properties (e.g., increasing fluorescence by 70-fold after irradiation);
- Genes for several proteins have been cloned, expressed in bacteria and purified.
- Optical properties have been characterized using confocal, multi-photon, FRET (fluorescence resonance energy transfer) and FLIM (fluorescence lifetime imaging microscopy) methods and spectroscopy *in vivo*, in coral cells, in solution and as dry thin films.

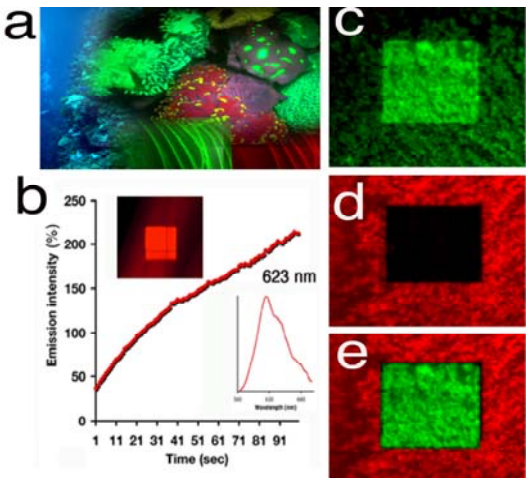

Fig 1. Novel GFP-like proteins from reef corals: (a) fluorescence in corals; (c) photoactive protein, amplifies fluorescence 30-fold in 90 sec of irradiation, emitting at 623 nm; (c-e) Red to green photoconverting protein, exhibiting stable green fluorescence following blue laser irradiation and disappearance of red emissions.

Funding is sought to:

- Construct plasmids, encoding genes of novel GFPs, including far reds, monomers and novel photoactive types for heterologous expression;
- Test their expression in mammalian cultured cell lines and characterize their optical and photochemical properties at different temperatures, pH, etc, and perform mutagenesis studies to improve optical properties of selected proteins if required;
- Perform studies of fusion of selected GFPs to other cellular proteins of interest and identify their cellular localization, trafficking, cell compartment residency and dynamics;
- By combining the use of several photoactivative GFP-type proteins develop advanced fluorescence techniques (FRET, FLIM and multiphoton excitation) for the study of protein dynamics with greater precision.

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