WiSys TECHNOLOGY FOUNDATION

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Monomeric Fluorescent Protein-Ligand Complexes with Strong Fluorescence in the Far-Red Region

Background

Fluorescent proteins can be found today in most molecular biology laboratories. Since the discovery of Green Fluorescent Protein (GFP) in the early 1960s, fluorescent molecules have revolutionized many areas of biology. In addition, with the advent of engineered variants possessing increased versatility and sensitivity, their utility as molecular probes (amongst a host of other uses) continues to increase in biological research.

Despite recent advances however and as with any technology, existing fluorescent proteins have inherent limitations and there remains a need for further development of new molecules that possess improved functionality and extended utility.

Technology

Research from the University of Wisconsin-Washington County in collaboration with the Institute for Stem Cell Biology and Regenerative Medicine in India, has resulted in the development of monomeric variants of the naturally occurring Sandercyanin Fluorescent Protein (SFP) using site-directed mutagenesis. This work has stemmed from earlier research focused on development of the tetrameric form of SFP, a biliverdin-binding lipocalin protein originally isolated from the mucus of the blue walleye fish, *Sander vitreus*. Monomeric variants of SFP (mSFPs) have been found to possess the same non-covalent, bili-binding characteristics of the tetramer but are one-quarter the size (~18.6kDa) and do not oligomerize. They are therefore anticipated to be more useful in a host of biotechnology applications.

Like the tetrameric form, the mSFPs have a large stokes shift (375nm/675nm) and fluoresce in the far-red or near infrared region, which is advantageous for a wide range of applications including investigation of protein-protein interactions, spatial and temporal gene expression, assessing cell biology distribution and mobility, studying protein activity and protein interactions in vivo, as well as cancer research, immunology, and stem cell research and sub-cellular localization. In addition, the newly developed mSFP's far-red fluorescence is particularly advantageous for in vivo, deep-tissue imaging.

Research and Development Status and Commercialization Needs

A suite of monomeric variants have been developed and tested with the crystal structures of various mutants in complex with biliverdin confirmed. Further work is currently being undertaken to optimize the quantum yield (e.g. brightness) of fluorescence as well as develop expression systems for this novel protein in yeast, insect and mammalian cells.

Applications and Key Benefits

- New monomeric variants are small in size, ~18.6kDa and are stable;
    - Monomers are highly crystallizable and do not lose color after storage for months;
    - Fluorescence is observed over a wide range of pH and at high temperature;
- mSFPs do not oligomerize, making them useful as fluorescent protein tags when fused other proteins;
- mSFPs exhibit large stokes shift (375nm/675nm), enabling improved detectability as excitation and emission photons are more easily distinguished in a sample;
- The monomer's non-covalent ligand binding results in replenishment of fluorescence following photo-bleaching;
    - mSFP's ability to 'turn on' when required and when photo-bleached by adding additional biliverdin provides significant advantages over conventional fluorescent proteins;

- Monomer's possess ultra-long quenching time (e.g. fluorescence intensity does not attenuate/reduce following continuous exposure to UV or red light making them useful in live-cell fluorescent imaging where long exposure is required;
- Potential to be used as a replacement for quantum dots;
    - With further development, mSFPs could be genetically expressed for imaging of specific tissues;
- Utility as a direct replacement of Green Fluorescent Protein or similar molecules for confocal microscopy, flow cytometry, fluorescence microscopy and other optical-based methods;
- Useful for deep-tissue imaging, medical imaging and biosensors.
- Useful for detection of protein-protein interaction in Fluorescence Resonance Energy Transfer (FRET) experiments due to large energy difference in excitation providing for a clearer signal if used for instance in combination with a Cy5-based dye.

Intellectual Property

A U.S. Provisional Patent is pending for this technology. WiSys holds rights to a further issued US patent (9,383,366) covering the tetrameric form of SFP and its use as a molecular biology tool. For more information, please contact Jennifer Cook at jennifer@wisys.org or by phone at 608-316-4131.

Publications

Ghosh, S.; Yu, C.; Ferraro, D.; Sudha, S.; Kumar Pal, S.; Schaefer, W.F.; Gibson, D.T.; and S. Ramaswamy. 2016. Blue Protein with Red Fluorescence. *PNAS*, *(in print)*.