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Topic: Fluorescent Proteins

Fluorescent proteins: biological paint

Paint is a material, too. Particles such as quantum dots have nanoscale optical properties that give them color. Paint materials are usually inert: there are no complex dynamic changes that give them their color. However, some biological molecules can light up with “living colors.”

Green fluorescent protein comes from jellyfish.

Red fluorescent protein comes from coral

Reporter Systems (www.clontech.com) has commercialized green fluorescent proteins and its variants. Some naturally occurring fluorescent proteins include

Ptiloscarus GFP from the sea pen, Renilla RFP from the sea pansy, DsRED from anthozoa, and a range of other fluorescent proteins from bacteria, fungi, insects, squid, corals, fish, crustaceans, plankton, and other organisms! The highest concentrations of fluorescent organisms is in the band of the equator and the tropics, especially in coral reef. Unfortunately, coral reefs are endangered by the impacts of human activity.

Green Fluorescent Protein

Green fluorescent protein was originally isolated from *Aequorea Victoria* jellyfish from Puget Sound, WA. The protein was purified in 1974 by Movin and in 1979 by Ward and Cormier. The first cloning of a wild-type GFP gene was achieved in 1992 by Prasher at Woods Hole Marine Biology Lab, not far from here. Variants began to emerge in 1996 thanks to the work of Cormack, Tseun, and others. After 18 years of efforts to clone the wild-type gene, it took relatively little time to develop a variety of mutants. This often happens in science – activity greatly increases after the first steps are achieved.

Why do jellyfish contain GFP? We don't know exactly what function it serves.

Where does the light come from to stimulate GFP? Calcium ions activate the cleavage of the photoprotein aequorin, which releases chemical bond energy in the form of high-frequency blue light. GFP captures this light and converts it to lower-frequency, visible green light.

The structure of GFP was featured on the cover of *Nature Biotechnology* on October 10th, 1996 (Volume 14, Number 10). The protein is sometimes called “paint in a can” because the fluorescent region is enclosed in a “can” of beta sheets. GFP contains 238 amino acids, roughly 50% of which have beta-sheet secondary structures, and 5% of which are alpha helices. The sheets fold into a beta-barrel, with the chromophore shielded from solvents and oxygen in the core. The protein is very stable, with a melting point above 65°C, and forms a dimer at higher concentrations.

The chromophore appears to be self-catalytic, requiring proper folding of the entire structure, with the chromophore encased in a beta-barrel composed by residues 7 through 229, and requiring molecular oxygen. GFP is extremely resistant to photobleaching.

Electron density maps for GFP show the cyclization of the tripeptide “SYG” from the primary sequence of GFP. The covalent bond formed between serine, tyrosine, and glycine, looks somewhat like the five-membered ring on the side group of histidine. If tyrosine binds to tryptophan or histidine instead, it can produce a blue or a cyan color. A rainbow of colors have been produced by engineering of enhanced fluorescent proteins. The red protein did not come from an alteration of GFP, but instead was derived from coral RFP. In addition, GFP was improved to be brighter.

Brightness can be calculated as the product of the extinction coefficient and the fluorescence quantum yield. The extinction coefficient quantifies the energy fraction taken from a beam of radiation in terms of a geometric cross-section:

$$\text{Absorbance} = \text{extinction coefficient} * \text{path length} * \text{concentration}$$
$$\text{Fluorescence quantum yield} = \# \text{ photons emitted} / \# \text{ photons absorbed}$$

Wild-type GFP has an extinction coefficient of 9,500 1/cm/M, compared to 55,000 1/cm/M in enhanced GFP, and the quantum yield of wild-type is 0.8, compared to 0.6 in enhanced GFP. The overall effect is a 4.3-fold increase in brightness with enhanced GFP.

Enhanced fluorescent vectors can now be purchased commercially! The availability of the protein has revolutionized biological research. This species-independent protein can be transfected to detectable levels in living cells and tissues. Without any need to cofactors, substrates, or additional gene products, the protein can be incorporated into a genetic sequence to produce inheritable color in an organism, minimally perturbing the state of the cell. We've made green mice, fish, plants, etc. – and even fish that are half green and half red! Some companies are even trying to make green “designer pets,” which has caused controversy. Combinations of proteins can be used as reporters of gene delivery and as probes for localization and expression level of fusion proteins. The distribution of proteins can be observed in real time, and can be coupled to specific cell functions.

Fluorescent labeling of cells enables researchers to sort cells using FACS: Fluorescence Activated Cell Sorter. Fluorescently labeled antibodies can be attached to cells with specific markers, or cells can simply express the GFP gene, to be sorted at a rate of 10,000 cells per second.

Red Fluorescent Protein

This protein was first isolated from *Discosoma Coral* (Matz, 1999). Slow maturation and strong oligomerization were achieved by Baird and Tsein in 2000. There is currently a need for faster-folding, higher-expressing, brighter RFP molecules for intracellular studies.

MIT alum Beau Pelle dedicated his Ph.D. work here to RFP. Improving quantum yield without causing spectral overlap with GFP was tricky: one approach was a genetic selection in which libraries of $\sim 10^8$ genes were produced, and the individual genes were packaged in retroviruses, infected into target cells, and selected for brightness using FACS. Cycles of this form of screening can identify genes that produce stronger color than the wild-type RFP. One trick to finding improved genes for RFP is to use DNA polymerases that make many mistakes in replication or transcription, potentially mutating the gene to make a brighter RFP.

The brightest mutant was 30 times brighter than wild-type RFP. The proteins folded faster and produced higher stable expression peaks. Amazingly, the FACS selection acted like billions of years of natural selection to select for the best red fluorescent protein!

Phycobiliproteins

Not homologous to GFP, these fluorescent proteins can be found in cyanobacteria and eukaryotic algae. Covalently linked tetrapyrrole groups collect light and, through FRET, transfer energy to a pair of chlorophyll molecules located in the photosynthetic reaction center. The proteins have evolved to maximize absorption and fluorescence, and to minimize quenching from internal energy transfer or external changes in pH, ionic strength, or other conditions. The proteins are relatively stable and highly soluble, but understanding of these proteins is still in early stages!