

# Fluorescent Proteins and Tags

## GFP Family

GFP, YFP, CFP, RFP. Ex/Em: 395/509nm (GFP). Nobel Prize 2008.

## Genetic Encoding

Fusion proteins. Minimal perturbation. Live cell compatible.

## Multicolor Imaging

Simultaneous tracking. mCherry, mTurquoise, mVenus.

## Photoswitchable

PALM, STORM superresolution. Dronpa, mEos.

## 1 GFP Family - The Foundation of Fluorescent Imaging

### GFP Family Spectrum



Cyan  
~475nm



Green  
~509nm



Yellow  
~527nm



Red  
~583nm

The Green Fluorescent Protein (GFP) family originated from the jellyfish *Aequorea victoria* and revolutionized cell biology. These proteins spontaneously form a chromophore from their own amino acid residues, eliminating the need for external cofactors. The family has been engineered to span the visible spectrum, providing researchers with a palette of colors for cellular imaging.

### Key Features:

- **Nobel Prize 2008:** Awarded to Osamu Shimomura, Martin Chalfie, and Roger Tsien for the discovery and development of GFP
- **Autocatalytic:** Forms fluorescent chromophore without external enzymes or cofactors
- **Excitation/Emission:** Original GFP - 395nm (UV) & 475nm (blue) / 509nm (green)
- **Variants:** Enhanced GFP (EGFP), superfolder GFP (sfGFP) for improved stability and brightness
- **Beta-barrel structure:** 11-stranded  $\beta$ -barrel protects the chromophore from the environment

Excitation:

UV-Blue (395-488nm)

Emission:

Cyan to Red (475-630nm)

2

## Genetic Encoding - Molecular Tagging Strategy

### Fusion Protein Architecture



**N-terminal or C-terminal fusion**

Fluorescent protein is genetically fused to target protein via peptide linker

Genetic encoding allows fluorescent proteins to be fused directly to proteins of interest through molecular cloning. The DNA sequence encoding the fluorescent protein is inserted into the gene of the target protein, resulting in a single polypeptide chain that carries both functionalities. This approach enables real-time tracking of protein localization, dynamics, and interactions in living cells.

**Advantages & Considerations:**

- **No external addition required:** Once transfected, cells produce tagged proteins autonomously
- **Minimal perturbation:** Small size (27 kDa for GFP) and careful linker design reduce functional disruption
- **Live cell compatible:** Non-toxic, stable expression enables long-term imaging
- **Spatial precision:** Tags can be placed at N-terminus, C-terminus, or internal positions
- **Validation needed:** Must confirm that tagged protein retains normal function and localization
- **Linker design:** Flexible peptide linkers (e.g., GGGS repeats) prevent steric hindrance

**Common Applications:**

- Protein localization and trafficking studies
- Real-time monitoring of protein expression levels
- FRET-based biosensors for detecting protein-protein interactions
- Cell cycle and developmental biology visualization
- Organelle-specific markers (mitochondria, nucleus, ER, etc.)

## Multicolor Imaging - Simultaneous Multi-target Tracking

### Spectral Separation Strategy



mTurquoise

Ex: 434nm  
Em: 474nm



mVenus

Ex: 515nm  
Em: 528nm



mCherry

Ex: 587nm  
Em: 610nm

**Minimal Spectral Overlap = Clear Signal Separation**

Multicolor imaging leverages the spectral diversity of fluorescent proteins to simultaneously visualize multiple cellular components in the same sample. By carefully selecting fluorescent proteins with distinct excitation and emission spectra, researchers can track multiple proteins, organelles, or cellular events in real-time without cross-contamination of signals. This powerful technique has become essential for understanding complex cellular processes involving multiple interacting components.

#### Technical Requirements & Benefits:

- **Spectral separation:** Proteins must have minimal overlap in emission spectra (typically  $>30\text{nm}$  apart)
- **Modern variants:** Optimized proteins like mTurquoise2, mVenus, and mCherry offer superior brightness and photostability
- **Simultaneous acquisition:** Multiple fluorescence channels can be imaged concurrently or sequentially
- **Co-localization studies:** Determine if proteins occupy the same cellular location

- **Dynamic interactions:** Track how different proteins move and interact over time
- **3-4 color maximum:** Practical limit due to spectral overlap and detector limitations

### Experimental Examples:

#### Organelle Interactions

Visualize mitochondria-ER contact sites using different colored markers for each organelle

#### Signal Transduction

Track receptor activation and downstream effector translocation simultaneously

#### Cell Cycle Markers

Monitor multiple cell cycle regulators in different colors throughout division

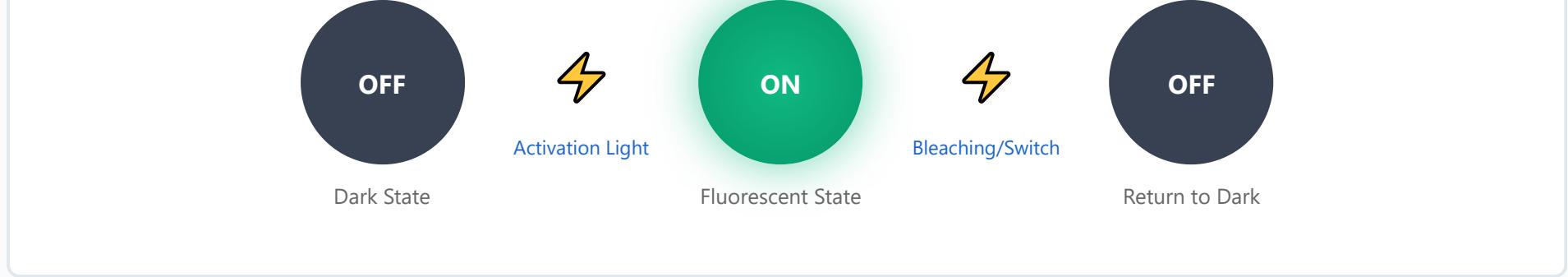
#### Multi-pathogen Studies

Distinguish different bacterial or viral strains within the same host cell

4

## Photoswitchable Proteins - Superresolution Microscopy

### Photoswitching Mechanism



Photoswitchable fluorescent proteins can be toggled between fluorescent and non-fluorescent states using specific wavelengths of light. This unique property forms the basis of superresolution microscopy techniques like PALM (Photoactivated Localization Microscopy) and STORM (Stochastic Optical Reconstruction Microscopy), which overcome the diffraction limit of conventional light microscopy (~200nm) to achieve resolutions of 20-50nm.

### Key Photoswitchable Proteins & Mechanisms:

- **Dronpa:** Reversibly photoswitchable between fluorescent (488nm) and dark states (405nm activation)
- **mEos family:** Photoconvertible from green (516nm) to red (581nm) with 405nm light - irreversible switch
- **PA-GFP:** Photoactivatable - increases fluorescence 100-fold upon 405nm irradiation
- **Dendra2:** Green-to-red photoconversion with improved photostability and brightness
- **Single-molecule detection:** Sparse activation enables precise localization of individual molecules
- **Resolution breakthrough:** Achieves 10-20× better resolution than conventional microscopy

### Superresolution Techniques:

#### PALM (Photoactivated Localization Microscopy)

Uses photoactivatable proteins to sequentially activate, localize, and bleach individual molecules

#### STORM (Stochastic Optical Reconstruction)

Employs photoswitchable probes to create stochastic blinking for superresolution imaging

### Pulse-Chase Experiments

Photoconvert specific protein pools to track their fate separately from newly synthesized proteins

### Subcellular ROI Marking

Selectively photoactivate proteins in specific cellular regions to study local dynamics



### Impact on Cell Biology:

Photoswitchable proteins have revolutionized our ability to visualize cellular structures at the nanoscale level. They enable researchers to resolve previously invisible details such as synaptic vesicle organization, nuclear pore complex architecture, cytoskeletal ultrastructure, and the precise spatial organization of signaling complexes. The 2014 Nobel Prize in Chemistry was awarded to developers of superresolution microscopy techniques that rely on these remarkable proteins.

## Fluorescent Proteins: Illuminating Life at the Molecular Level

From the discovery of GFP in jellyfish to the development of sophisticated photoswitchable variants, fluorescent proteins have transformed our ability to observe living cells. These genetically encoded tools continue to drive innovations in cell biology, neuroscience, developmental biology, and beyond.