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Fluorescent proteins illuminate cell biology

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The ability to unravel the fine details of biological functions has advanced remarkably. Green fluorescent protein (GFP) isolated from the jellyfish *Aequorea victoria* and GFP-like fluorescent proteins from other animals have had an important role in the technical innovations that have driven these advances. Inspired by the emergence of numerous spectral variants of fluorescent proteins in the past decade, an increasing number of researchers have been awaiting the development of a tool that enables the direct visualization of biological function. Although the most common application of fluorescent proteins is the imaging of gene expression and protein dynamics, biosensors have also been created using fluorescent proteins to image the concentrations of ions and small molecules, enzyme activity, protein post-translational modification and changes in protein conformation. In addition, an emerging property of some fluorescent proteins is that their fluorescence can be photo-modulated by illumination at specific wavelengths, which enables individual cells, organelles and proteins to be highlighted with high spatiotemporal resolution. This poster provides a broad perspective of the fluorescent proteins now available and their potential applications.

Optical properties of bright fluorescent proteins

Protein	Peak ex./em.	EC	QY	EC × QY vs EGFP
Monomers				
mTagBFP	399/456	52	0.63	0.98
EBFP2	383/448	32	0.56	0.53
mTurquoise	433/475	30	0.84	0.75
mTFP1	462/492	64	0.85	1.6
mWasabi	493/509	70	0.80	1.7
sfGFP	488/507	83	0.65	1.6
EGFP	488/507	56	0.60	1.0
YPet	517/530	104	0.77	2.4
Venus	515/528	92	0.57	1.6
mOrange2	549/565	58	0.60	1.0
TagRFP-T	555/584	81	0.41	0.99
mKate2	588/633	63	0.40	0.74
mCherry	587/610	72	0.22	0.47
mNeptune	600/650	67	0.20	0.40
IFP1.4*	684/708	92	0.07	0.19
Dimers				
RFP611	559/611	116 × 2	0.45	1.6 × 2
(td)Tomato	554/581	69 × 2	0.69	1.4 × 2
(td)Katushka	588/ 633	66 × 2	0.37	0.73 × 2
E2-Crimson	611/646	126 × 4	0.23	0.86 × 4

*requires biliverdin cofactor

Bright monomers

Monomeric fluorescent proteins can be used to follow protein localization and abundance, which in turn can report on processes such as membrane lipid production, cell shape changes and organelle assembly and movement. They can also be used as components of single-chain or multiprotein FRET reporters and as simple cell fillers.

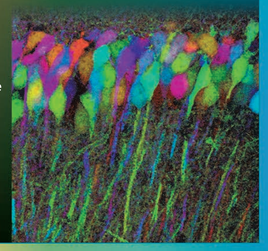
The fluorescent protein spectrum

Infrared	Far-red	Red	Orange	Yellow	Green	Teal	Cyan	Blue
IFP1.4	mNeptune	mKate2 mCherry	mOrange2 TagRFP-T	YPet Venus	mWasabi sfGFP EGFP	mTFP1	mTurquoise	mTagBFP EBFP2

Bright multimers

Certain multimeric fluorescent proteins are brighter than available monomeric fluorescent proteins of the same colour and so may be useful for reporting gene expression and marking cells, and may be preferred when it is crucial to maximize signal/noise. Typical applications are cell filling and tracking. Tandem dimer (td) versions allow protein fusions.

Combinations of multimers and monomers were used to generate 'Brainbow' mice (right)[3].

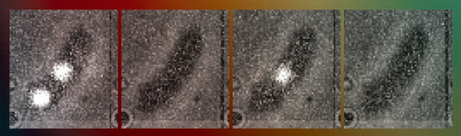


Optical properties of selected bright photoswitchable proteins

Protein	Switch state	Peak ex./em.	EC	QY	EC × QY vs EGFP
Reversible					
EYFP	Pre	514/528	83	0.61	1.5
	Post	405/528	ND	ND	ND
Dronpa	Pre	503/518	95	0.85	2.4
	Post	388/518	28	0.02	0.02
Padron	Pre	503/522	ND	ND	0.01
	Post	505/522	43	0.64	0.82
mTFP0.7	Pre	453/488	60	0.50	0.89
	Post	376/ND	ND	ND	ND
Irreversible					
PA-GFP	Pre	400/515	21	0.13	0.08
	Post	504/517	17	0.79	0.40
PS-CFP2	Pre	400/468	43	0.20	0.26
	Post	490/511	47	0.23	0.32
PATagRFP	Pre	351/ND	ND	ND	ND
	Post	562/595	66	0.38	0.75
Kaede	Pre	508/518	98 × 4	0.88	2.6 × 4
	Post	572/580	60 × 4	0.33	0.59 × 4
mKikGR	Pre	505/515	49	0.69	1.0
	Post	580/591	28	0.63	0.53
Dendra2	Pre	490/507	45	0.50	0.68
	Post	553/573	35	0.55	0.58
mEos2	Pre	506/519	56	0.84	1.4
	Post	573/584	46	0.66	0.90

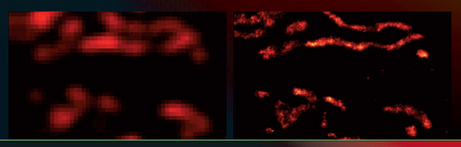
Reversibly photoswitchable proteins

Certain fluorescent proteins undergo changes in the conformation of the chromophore on illumination, resulting in increased or decreased brightness with spontaneous reversal. In some cases, a distinct absorbance peak is formed, allowing acceleration of reversal by light at the new wavelength. These proteins are useful as probes in single-molecule-based superresolution microscopy, as shown below with a fusion of EYFP to the bacterial actin homologue MreB[4].



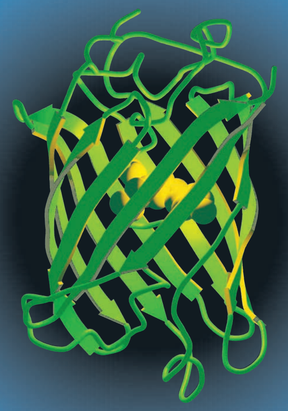
Irreversibly photoswitchable proteins

Certain fluorescent proteins undergo covalent changes in structure on illumination, resulting in a brightening at a specific wavelength. Applications include connectivity tracing, protein diffusion and superresolution microscopy, as shown below with PA-GFP targeted to mitochondria and imaged with standard light microscopy (left) and a single-molecule-based superresolution technique (right)[5].



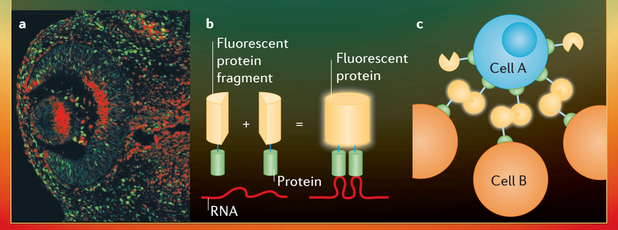
Basic features of fluorescent proteins

Fluorescent proteins related to *A. victoria* GFP[1] are monomeric or homomultimeric polypeptides of 224–231 amino acids[2] that form an 11-strand β-barrel (diameter 2.4 nm; length 4.0 nm) with a central α-helix. The chromophores of these fluorescent proteins are formed from a X-(H/Y/W)-G tripeptide in an autocatalytic reaction that requires O_2 and generates H_2O_2. Unrelated domains that bind to exogenous chromophores and function as fluorescent proteins also exist.



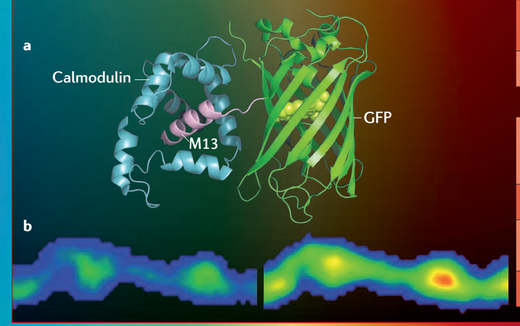
Multipart sensors

Beyond simple sensors of protein localization or abundance, complex reporter systems can be constructed by using fluorescent proteins of various colours to tag multiple proteins simultaneously. For example, Fucci reporters use fluorescent protein fusions to proteins that show cell cycle-dependent proteolysis to mark cells in G1 phase in red and cells in S, G2 or M phase in green (below; a)[6]. A specific RNA tag can be visualized by the simultaneous recruitment of weakly complementing fluorescent protein fragments fused to sequence-specific RNA-binding proteins (below; b)[7]. Spontaneously complementing GFP fragments expressed in different cells reveal cell–cell interactions (below; c)[8].



Chromophore-modulating sensors

In chromophore-modulating sensors, a noncovalent structural change influences the fluorescence of a single fluorescent protein domain. In pH and redox sensors, sensing is carried out directly by the chromophore or by amino acids of the fluorescent protein β-barrel domain. Other sensors rely on the propagation of a conformational change from a sensing domain to a fused fluorescent protein domain, as with the VSFP sensors of voltage[9] and G-CaMP sensors of Ca^{2+} (REF. 10) (below; a). In most cases analysed, fluorescence changes are due to changes in chromophore protonation. Exocytosis of synaptic vesicles can be analysed with a lumenal pHluorin sensor (below; b)[11].



Intensity-based single-chromophore sensors

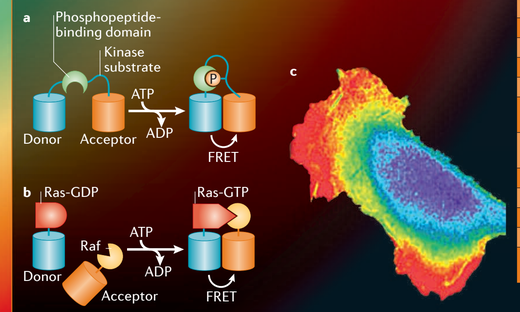
Sensor	Analyte	Min., midpoint and max. input	Peak ex./em.	Max. EC × QY	Max. ΔF/F
VSFP3.1–mOrange2	Membrane voltage	−140 mV, −63 Mv and +60 mV	548/562	ND	0.03
Camgaroo-2	Ca^{2+}	100 nM, 5 μM and 1 mM	506/524	ND	7
Flash-Pericam	Ca^{2+}	1 nM, 700 nM and 1 mM	494/511	17 × 0.2	8
G-CaMP3	Ca^{2+}	100 nM, 700 nM and 10 μM	500/512	ND	12
Superecliptic pHluorin	pH	5.5, 7.1 and 9.5	475/511	ND	15

Ratiometric single-chromophore sensors

Sensor	Analyte	Min., midpoint and max. input	Peak ex. 1/ ex. 2	Peak em.	Max. ΔR/R
Perceval	ATP/ADP ratio	0, 0.5 and 10	490/405	516	1.8
roGFP2	Redox potential	−200 mV, −272 mV and −350 mV	475/400	516	4
HyPer	Hydrogen peroxide	0 nM, ~50 nM and250 nM	500/420	516	3.3

FRET sensors

Sensors for various signals and proteins have been engineered to detect signal-induced conformational changes that alter FRET between fluorescent proteins. For example, phosphorylation can change the distance or orientation of fluorescent proteins fused at the termini, thereby changing FRET (below; a)[12]. Alternatively, two proteins can interact in a signal-dependent manner, increasing FRET between the fluorescent proteins that they are fused to (below; b)[13]. These designs can be generalized to various signals; for example, the approach in part a has been applied to sensing kinases, GTPases (below; c)[14], histone methylation, Ca^{2+} and glutamate.



Selected FRET sensor characteristics

Reporter	Analyte	Measurement	Max. %Δ
AKAR3	PKA	cpVenus/ECFP	35
AktAR	PKB (Akt)	cpVenus/Cerulean	40
CKAR	PKC	Citrine/ECFP	15
DKAR	PKD	ECFP/Citrine	23
EKAR	ERK	Venus/Cerulean	20
Camui	CaMKIIα	Venus/ECFP	60
Picchu	Abl	YFP/CFP	60
Src reporter	SRC	ECFP/Citrine	25
Raichu-Ras	Ras	EYFP/ECFP	100
FRas-F	Ras	EGFP lifetime	-50
Raichu-RHOA	RHOA	ECFP/EYFP	50
RhoA biosensor	RHOA	Citrine/ECFP	100
ATeam	ATP	cpVenus/mseCFP	250
K9 reporter	Histone H3 methylation	Citrine/ECFP	60
K27 reporter	Histone H3 methylation	Citrine/ECFP	29
SuperGluSnFR	Glu	ECFP/Citrine	44
D3cpv	Ca^{2+}	cpVenus/ECFP	510
YC2.60	Ca^{2+}	cpVenus/ECFP	600
TnXXL	Ca^{2+}	Citrine/ECFP	150
Mermaid	Membrane voltage	mUKG/mKOκ	40

Abbreviations

ΔF/F, change in fluorescence from baseline; ΔR/R, change in ratio from baseline; Abl, Abelson kinase; AKAR3, A kinase activity reporter 3; AktAR, Akt activity reporter; CaMKIIα, Ca^{2+}/calmodulin-dependent protein kinase type II subunit-α; CKAR, C kinase activity reporter; DKAR, D kinase activity reporter; EBFP2, enhanced blue fluorescent protein 2; EC, extinction coefficient (in $mM^{-1}cm^{-1}$); EGFP, enhanced green fluorescent protein; EKAR, E kinase activity reporter; em., emission wavelength (in nm); ERK, extracellular signal-regulated kinase; ex., excitation wavelength (in nm); EYFP, enhanced yellow fluorescent protein; FRas-F, FRET sensor of Ras, fast kinetics; FRET, Forster resonance energy transfer; G-CaMP3, GFP–calmodulin Ca^{2+} probe 3; IFP1.4, infrared fluorescent protein 1.4; mTFP, monomeric teal fluorescent protein; mUKG, monomeric umi-kinoko GFP; ND, not done; PA-GFP, photoactivatable GFP; PK, protein kinase; PS-CFP2, photoswitchable cyan fluorescent protein; QY, quantum yield; roGFP2, reduction-oxidation-sensitive GFP 2; RFP, red fluorescent protein; sfGFP, superfolder GFP; VSFP3.1, voltage-sensitive fluorescent protein 3.1; YC2.60, yellow cameleon 2.60.

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