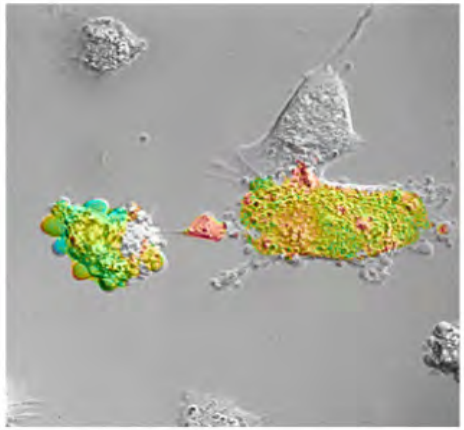
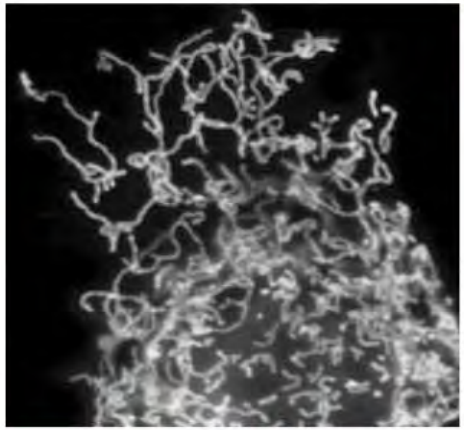
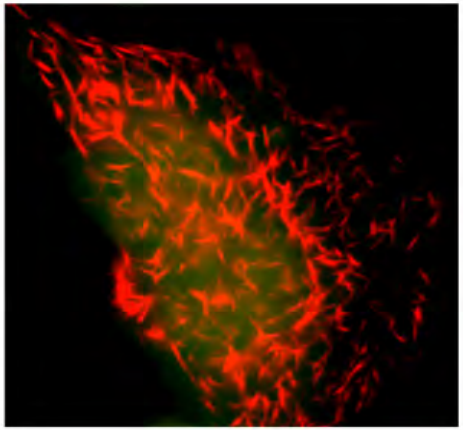
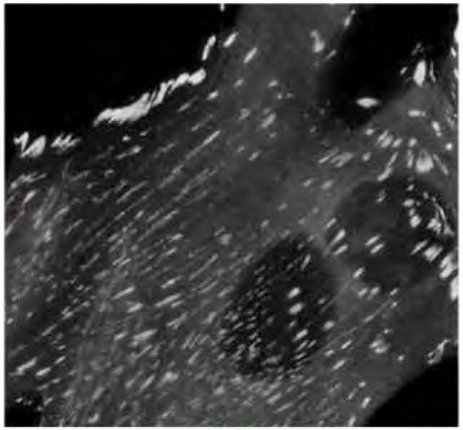
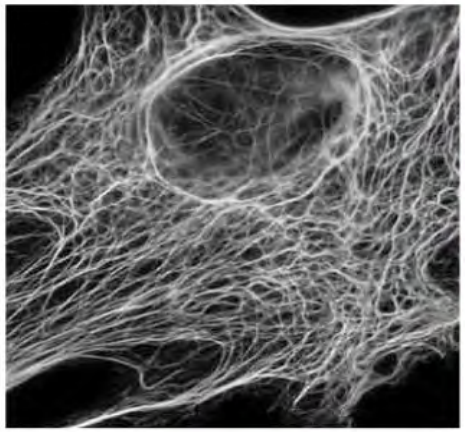
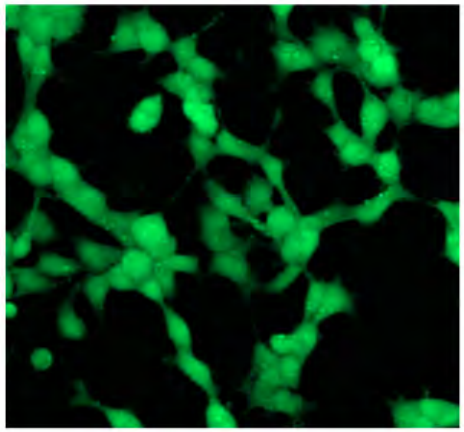
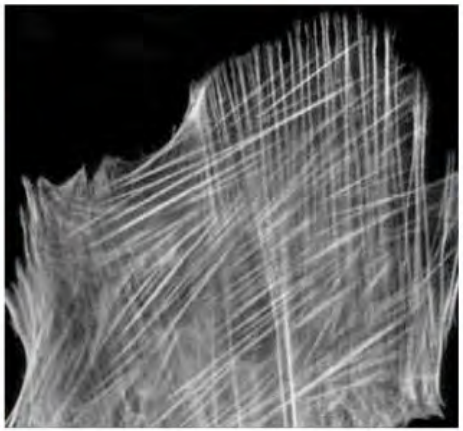
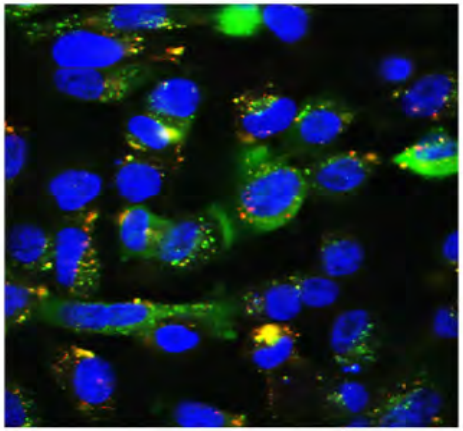
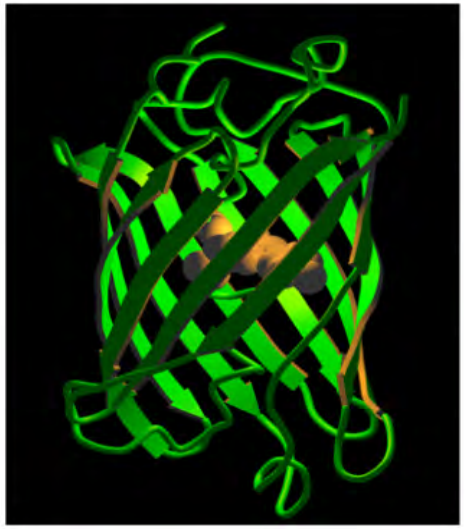
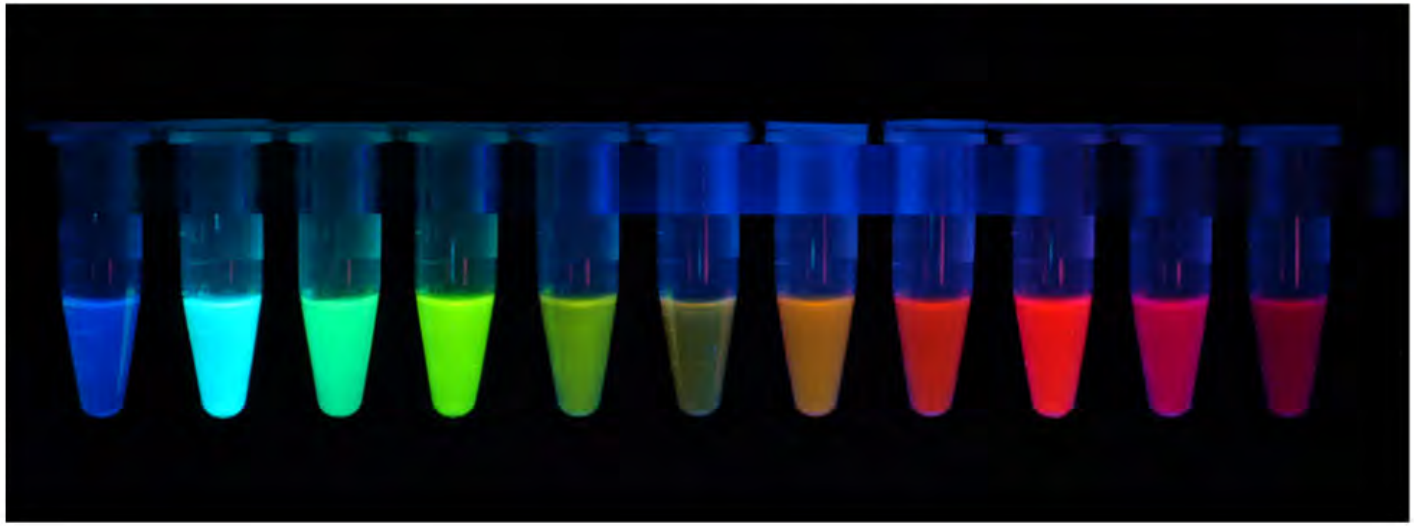
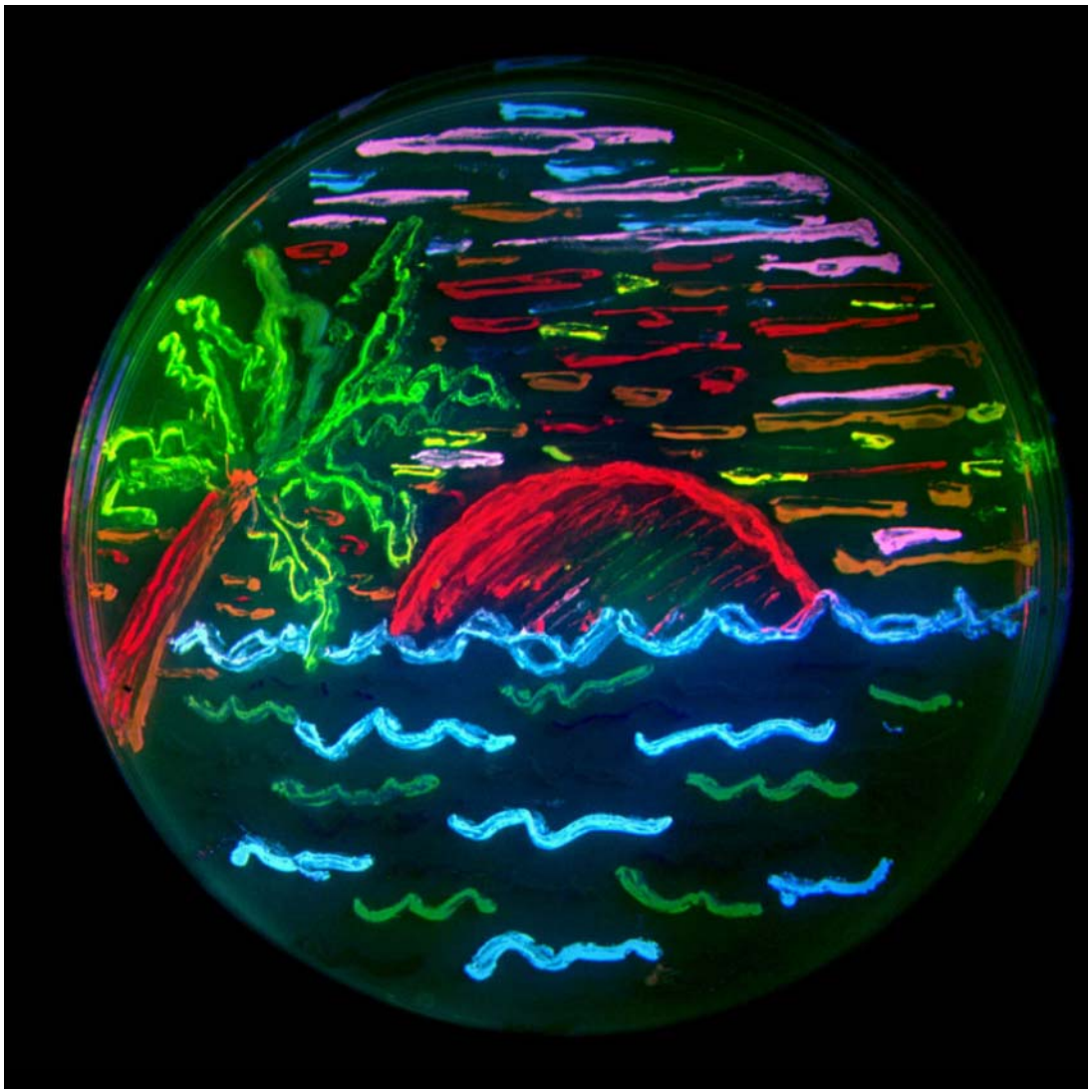
Immunofluorescence and Fluorescent Proteins

Also can be used for practicing streaking technique…



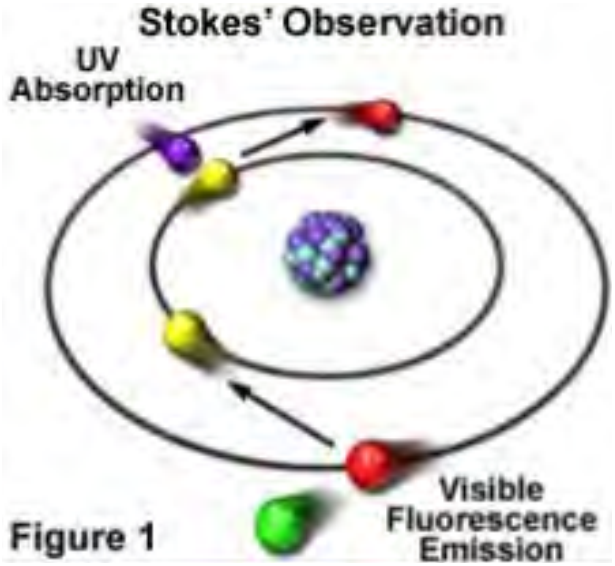


Figure 1

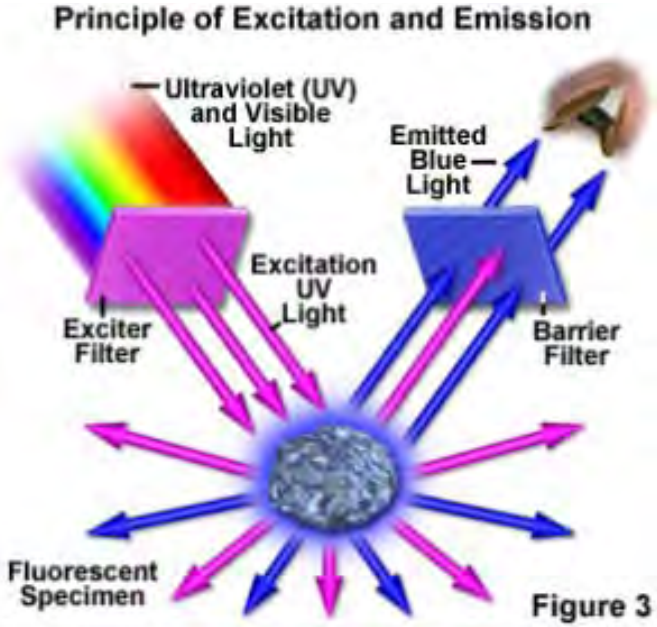


Figure 3

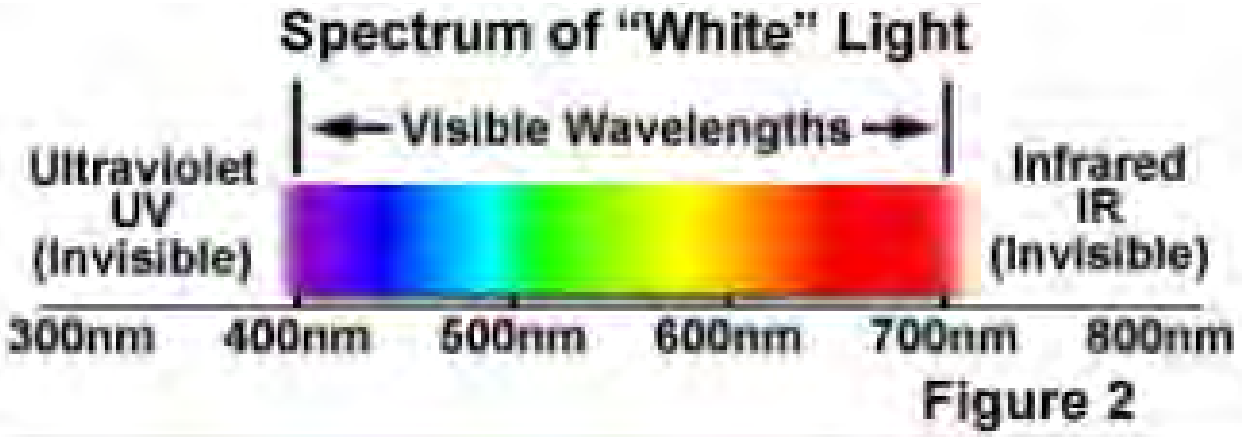
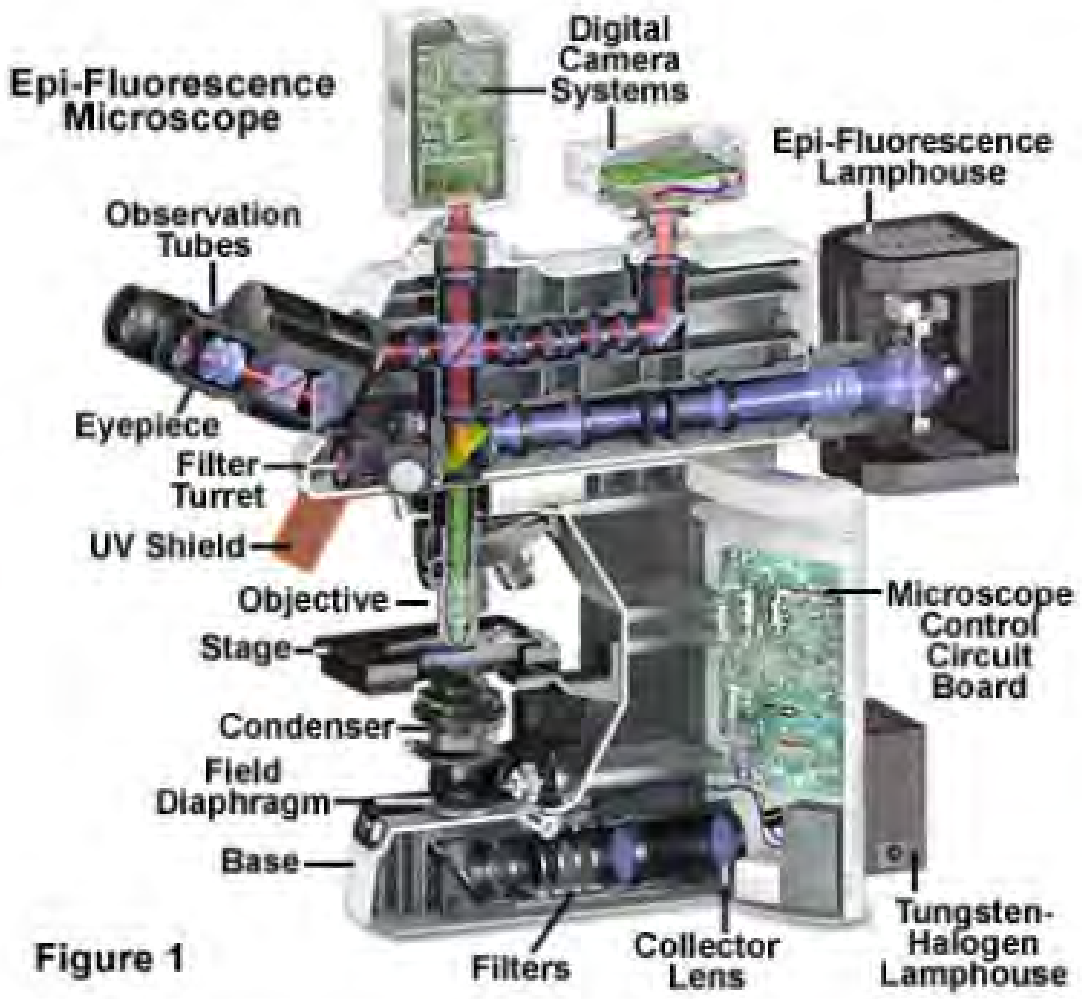


Figure 2

Olympus We site



Fluorescent Microscope (Nikon)

Immunofluorescence
&
Fluorescent Proteins

Applications

May 15, 2008

Immunofluorescence: Principle

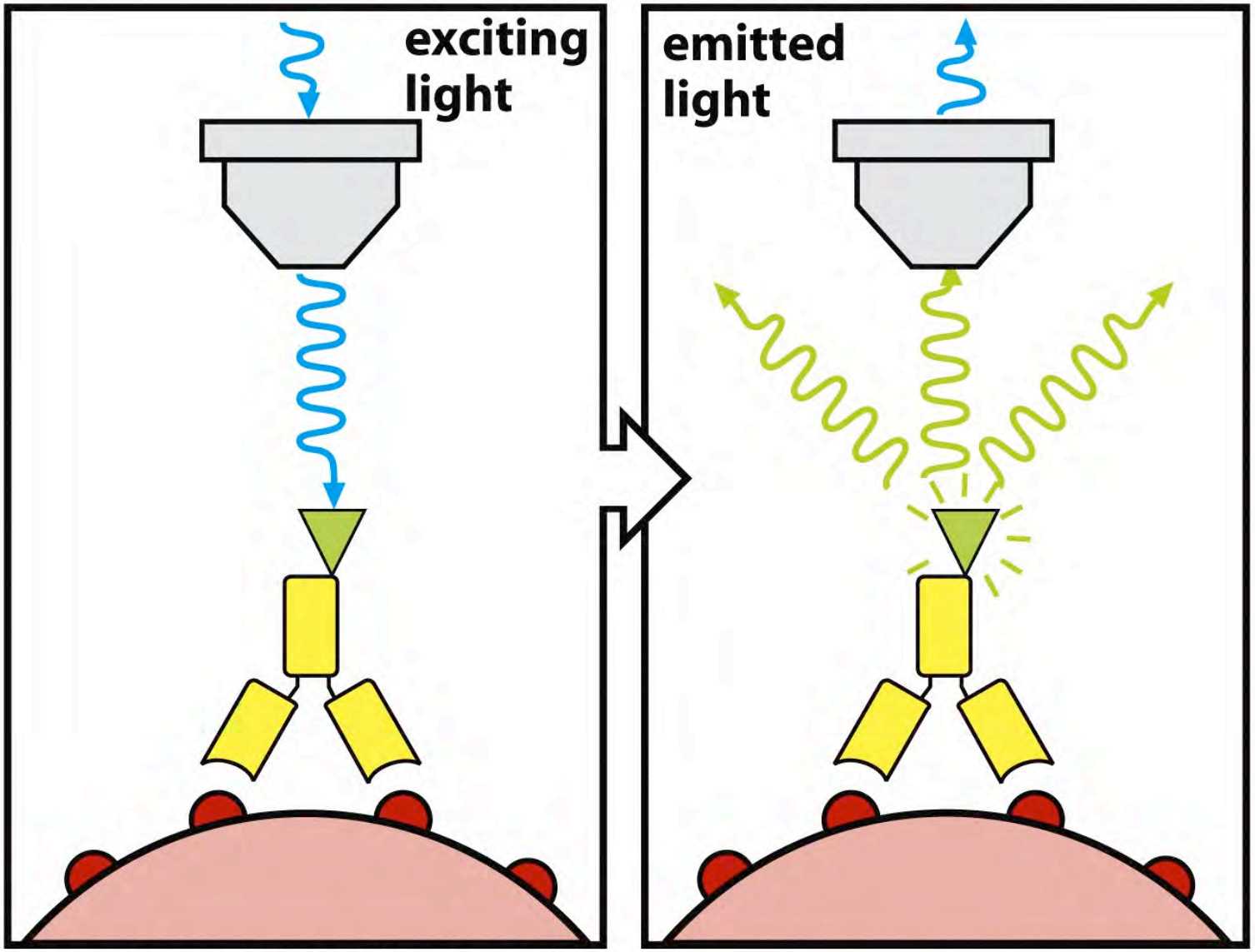


Figure A-18 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Use of a secondary antibody in Immunofluorescence analysis

(a more practical approach)

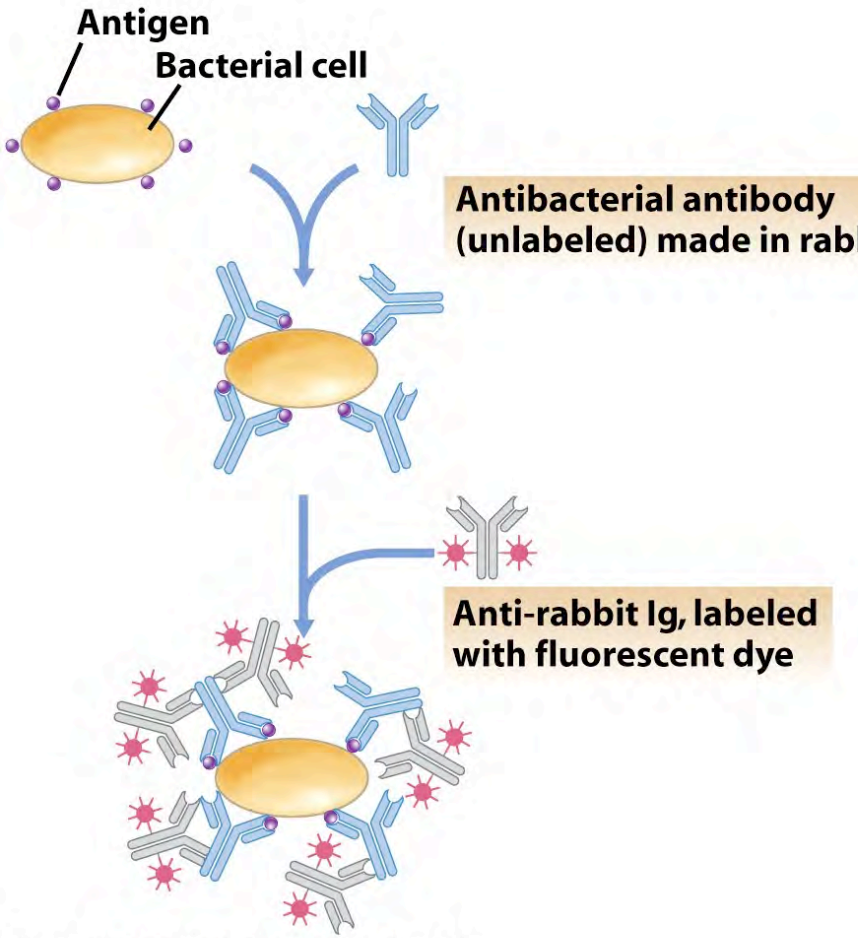


Figure 24-18b Brock Biology of Microorganisms 11/e

Primary Antibody

Secondary Antibody

Immunofluorescence

Excitation and emission wavelengths of some commonly used fluorochromes		
Probe	**Excitation (nm)**	**Emission (nm)**
R-phycoerythrin (PE)	480; 565	578
Fluorescein	495	519
PerCP	490	675
Texas Red	589	615
Rhodamine	550	573

Figure A-17 Immunobiology, 7ed. (© Garland Science 2008)

Immunofluorescence

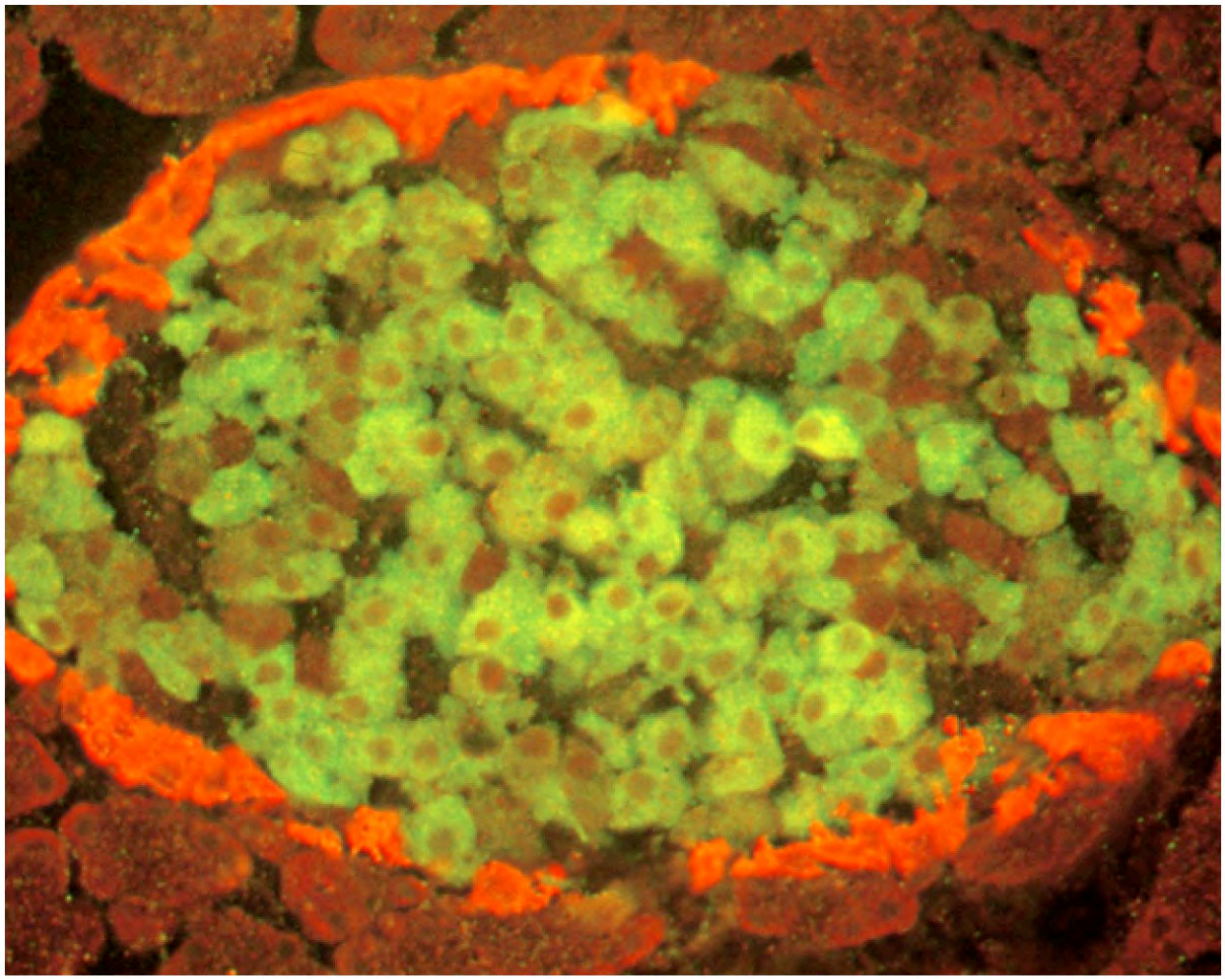
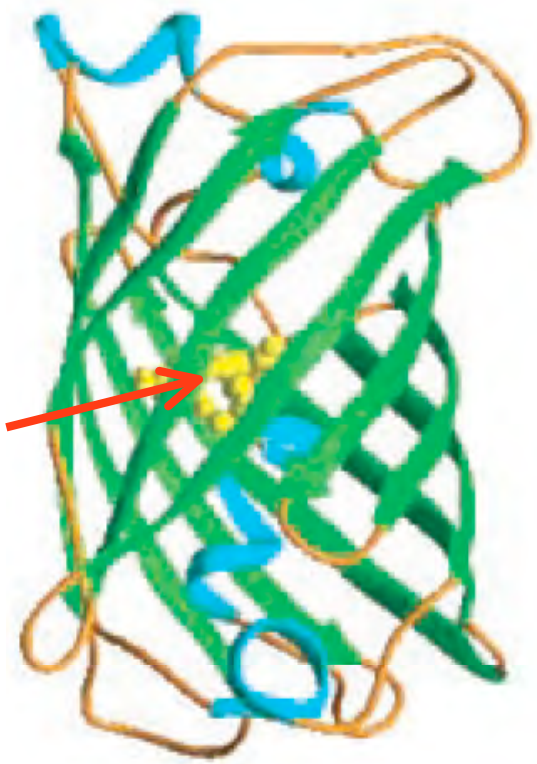


Figure A-18 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Glutamic acid decarboxylase in β cells of pancreatic islets of Langerhans

Glucagon in α cells of pancreatic islets of Langerhans

GFP: The Green Fluorescent Protein

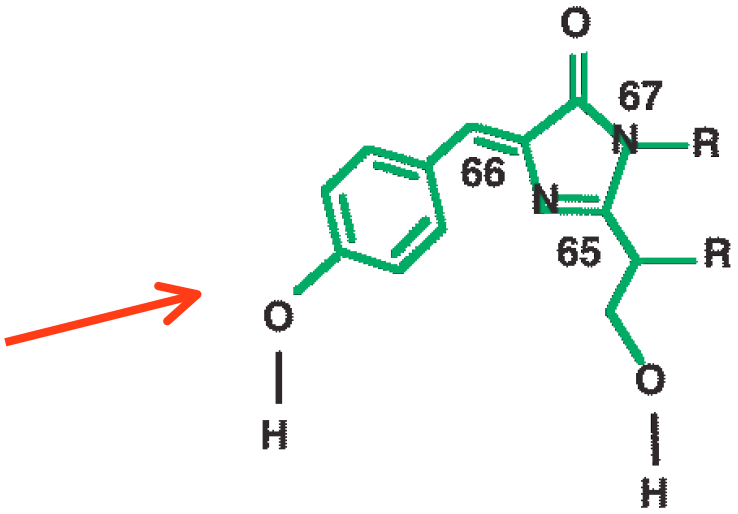


Source: *Aequorea victoria* (jellyfish)

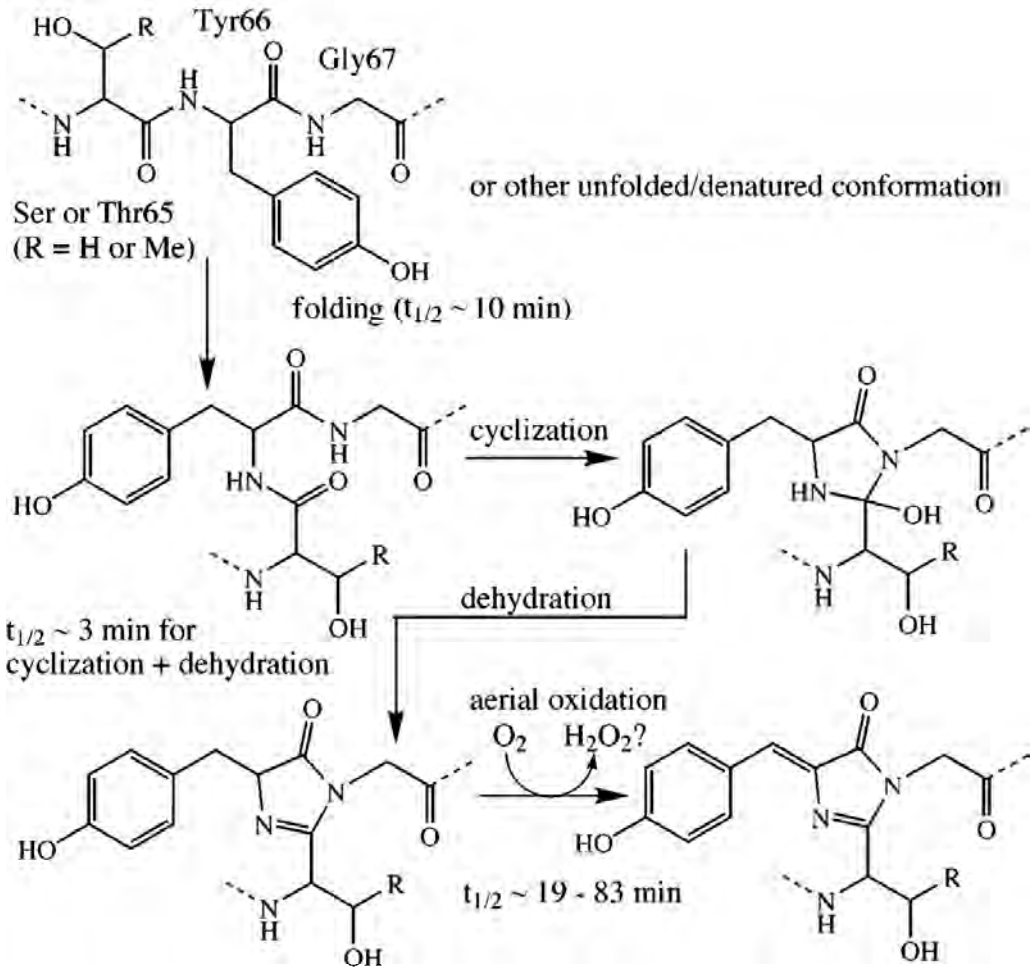
Discovery: Y. Tsien

Structure: 11- β Strand protein containing a fluorophore

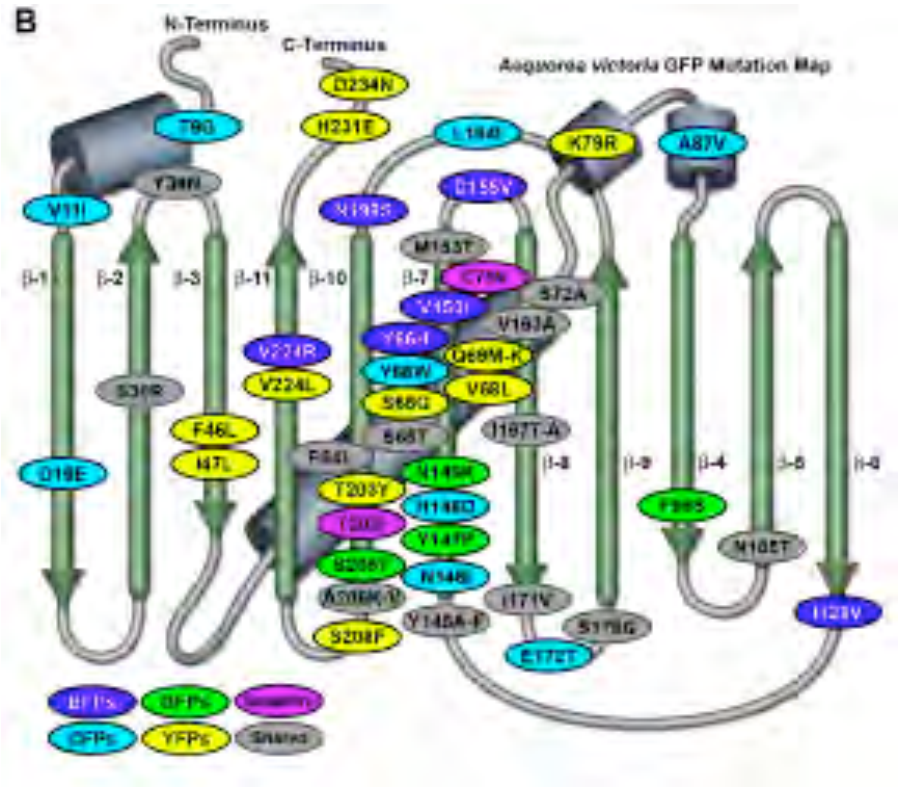
Maturation of active site tripeptide SYG generates the fluorophore



Maturation of the Active Site Tripeptide Generates the Fluorophore



Site-directed mutagenesis of GFP generated proteins with different fluorophores emitting light of different wavelength (color)

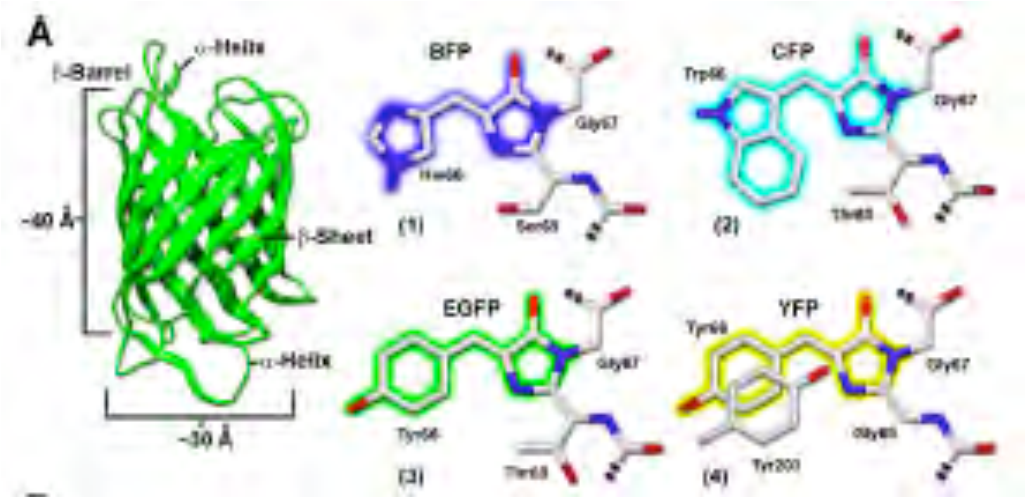


Schematic Summary of Mutations in GFP Variants

(Shaner, N.C. et al. 2007. J. Cell Sci. 120: 4247-4260).

Fluorophore Structure in GFP Variants

(Shaner, N.C. et al. 2007. J. Cell Sci. 120: 4247-4260)

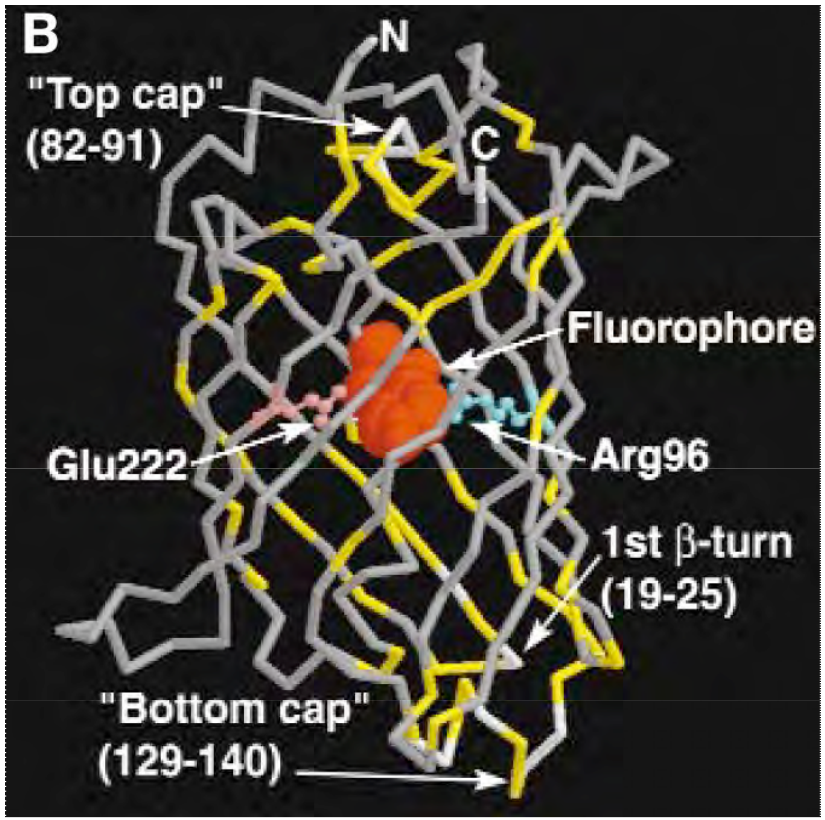


BFP: Blue Fluorescent Protein
CFP: Cyan Fluorescent Protein
YFG: Yellow Fluorescent Protein
EGFP: Enhanced Green Fluorescent Protein

Extended Spectrum Fluorescent Proteins: dsRed

Coral: *Discosoma*

Active site: (N/Q/K)YG



Emission red -> visible light (to 583 nm)

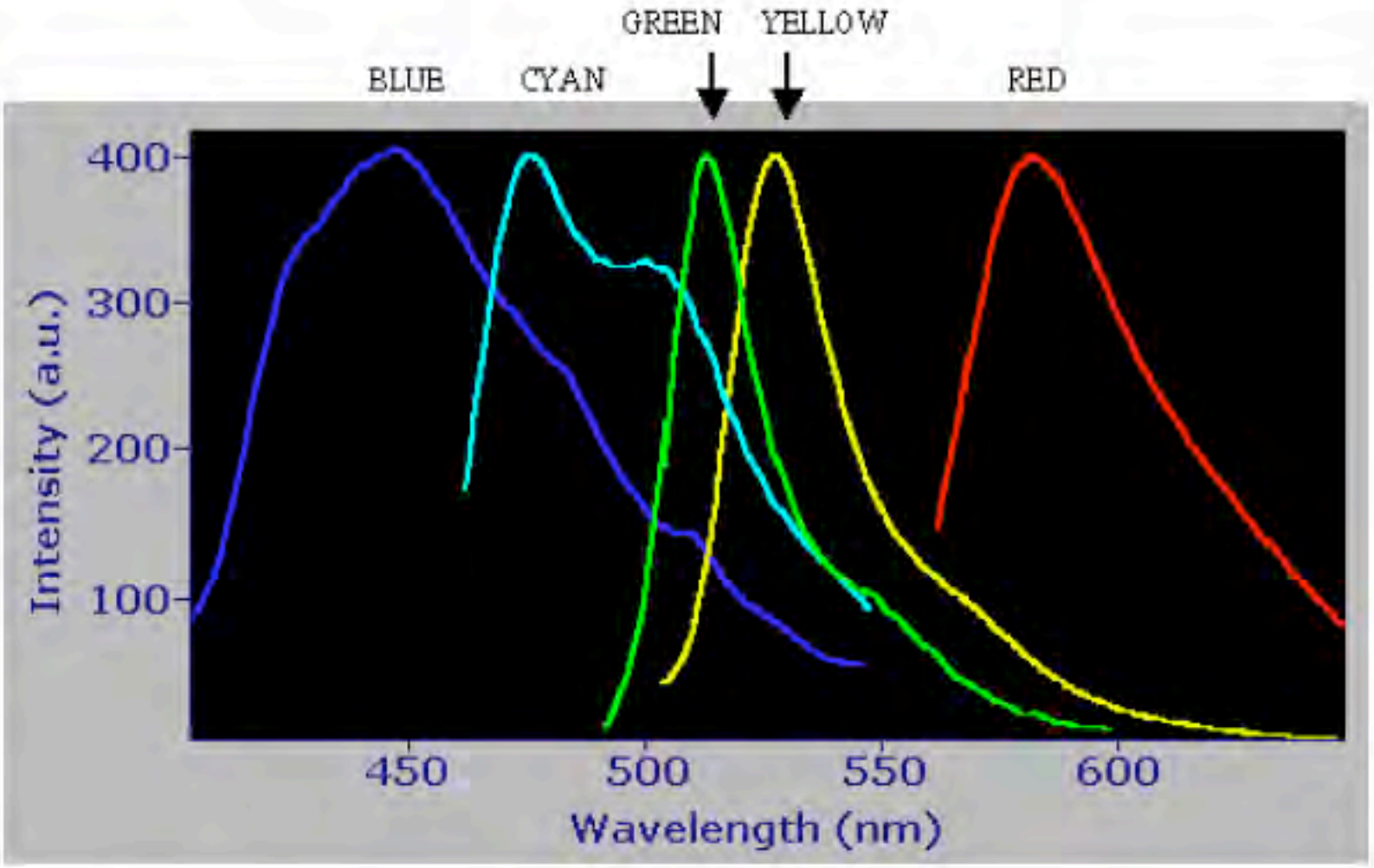
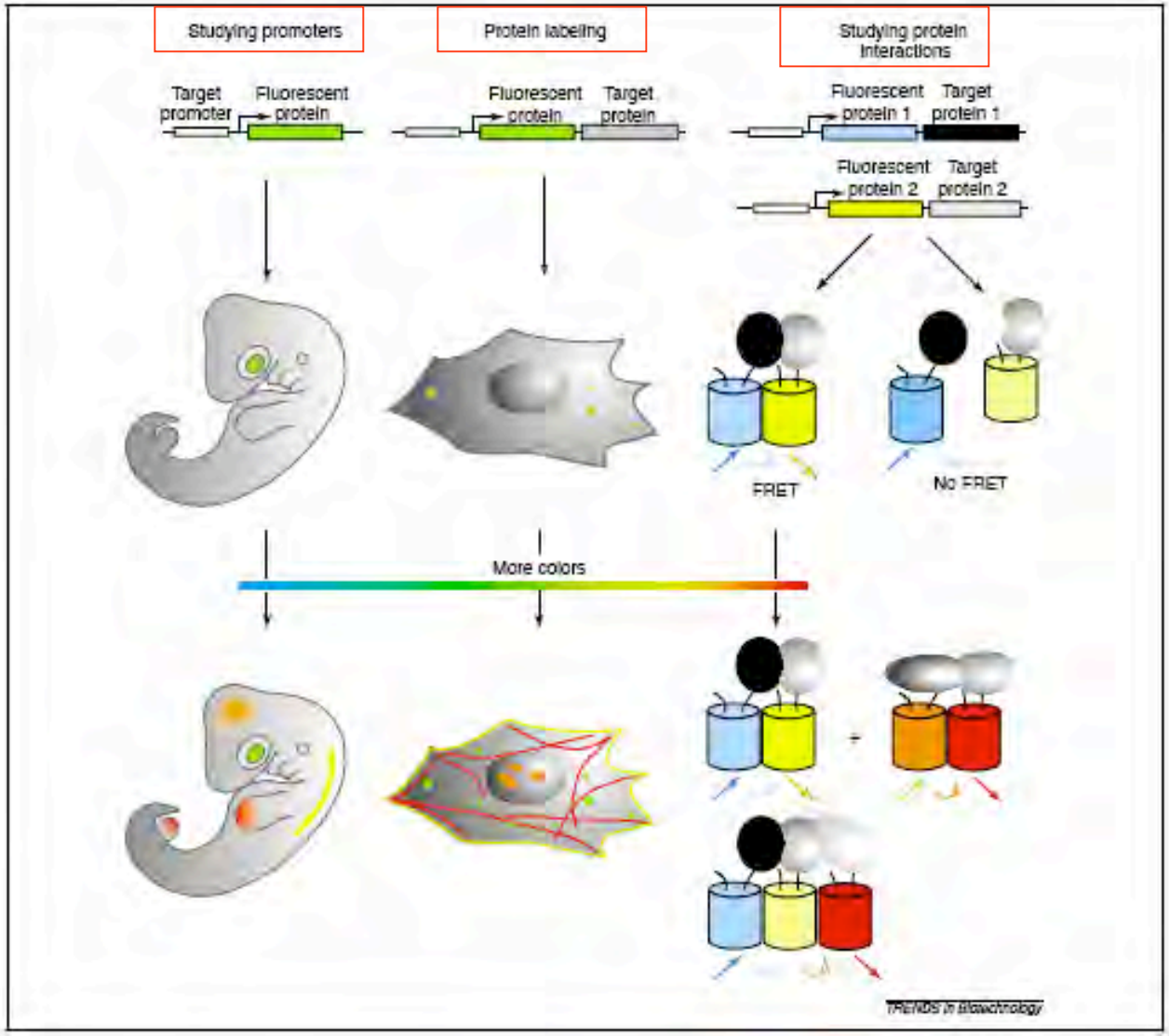


Figure 3. *Intensity vs emission for the full spectrum of fluorescent proteins.*

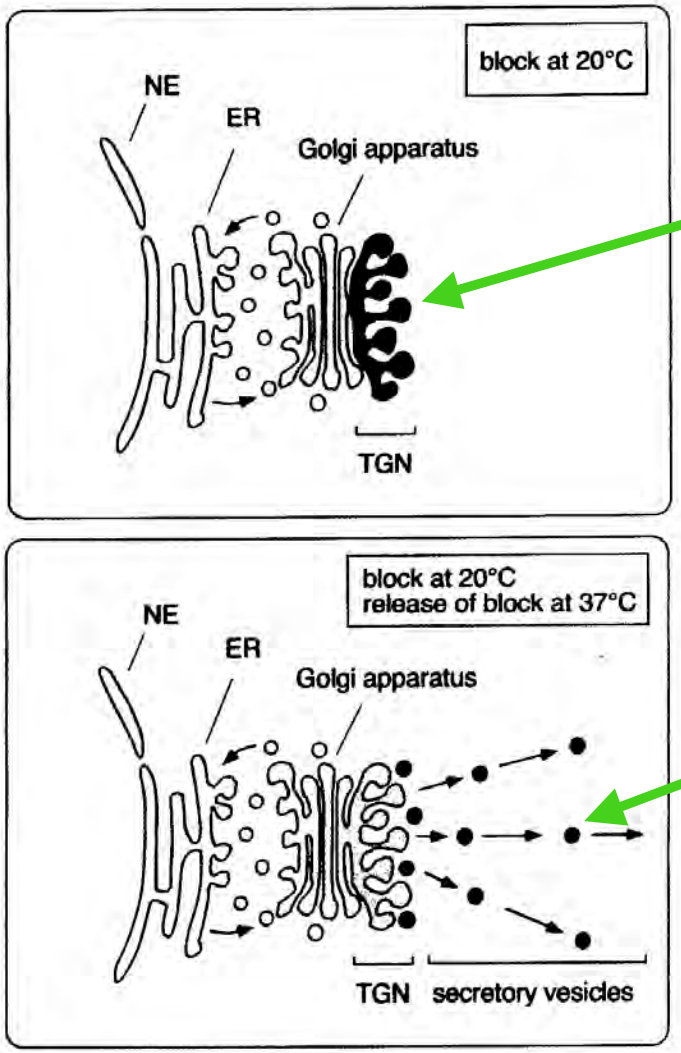
www.varianinc.com/media/sci/apps/app07

Fluorescent Proteins: Applications



Chudakov, D.M. et al. 2005. Trends Biotech. 23: 605-613

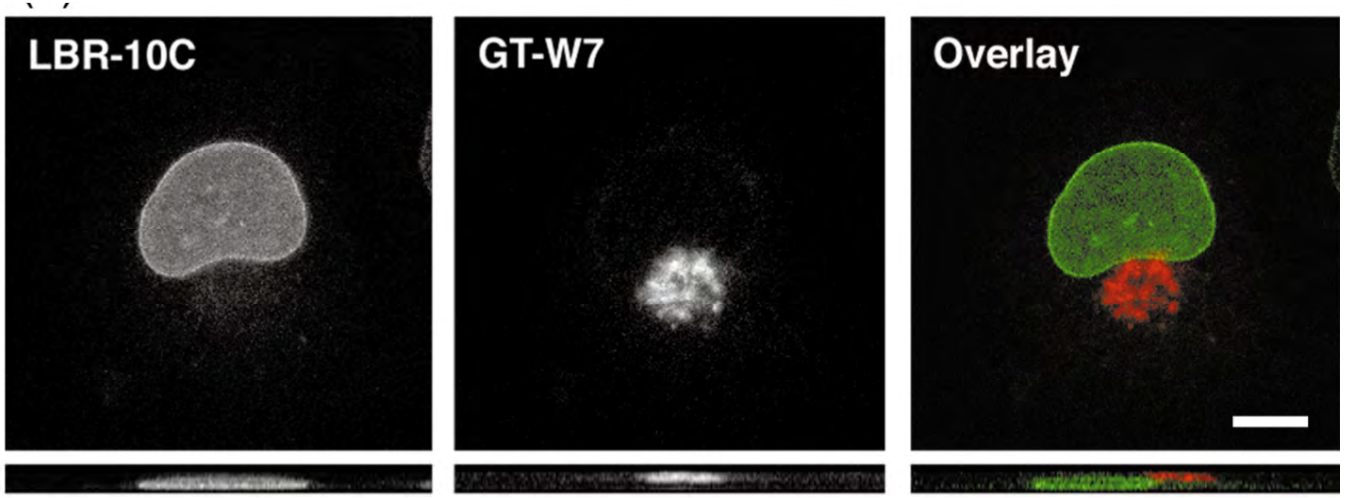
Application of GFP Fusions in Cell Biology



GFP fusion to secreted protein

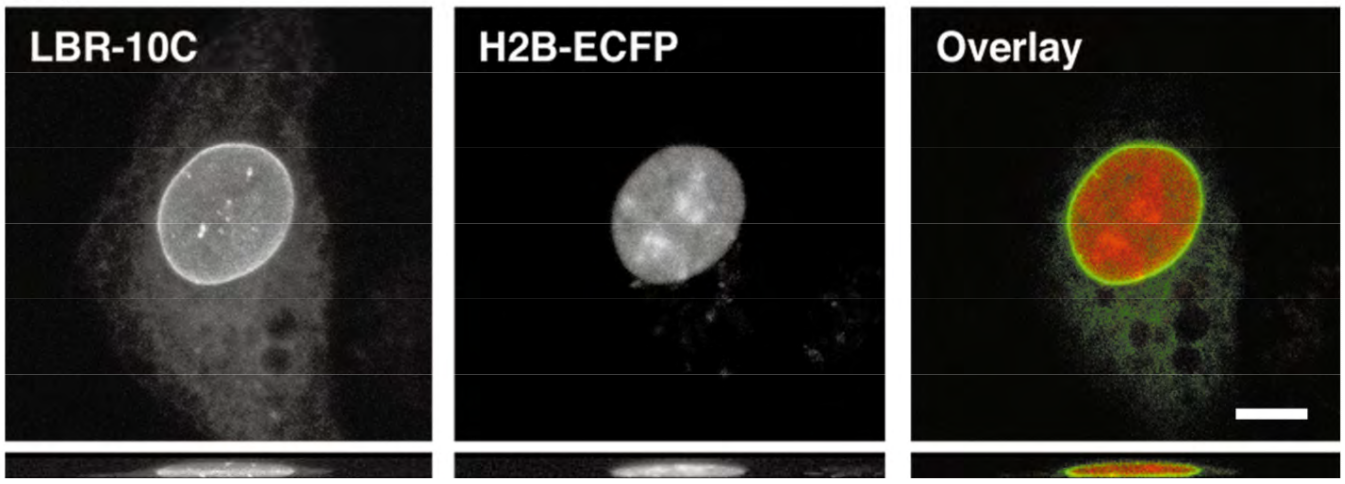
release of GFP-labelled secretory vesicles

Double Labelling with GFP Variants



nucleus/lamin B receptor: green/EYFP

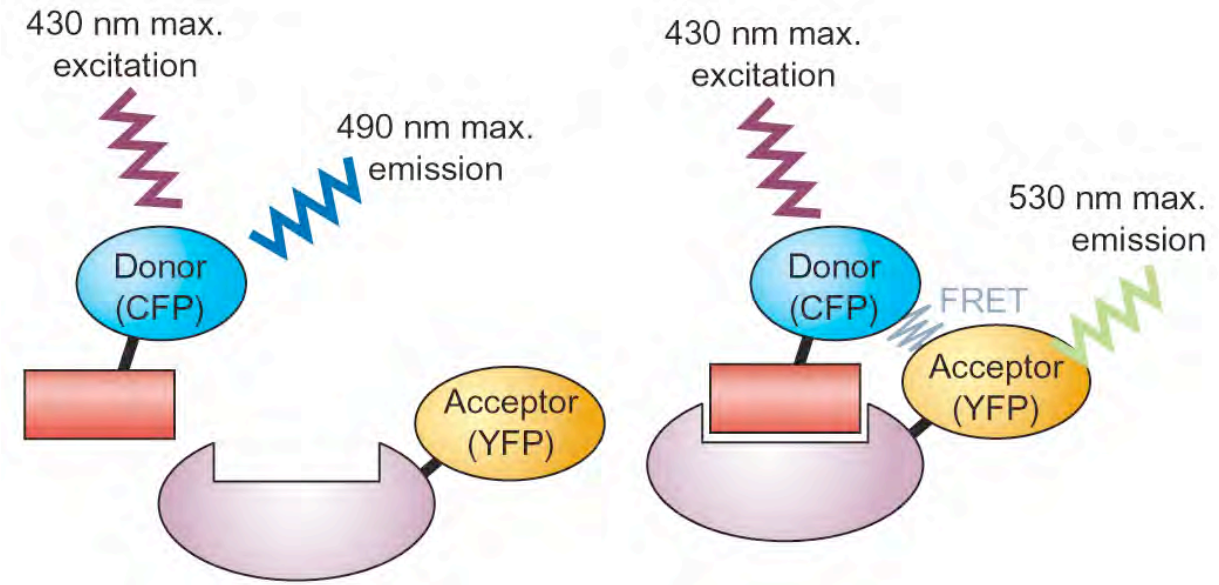
golgi: red/ECFP



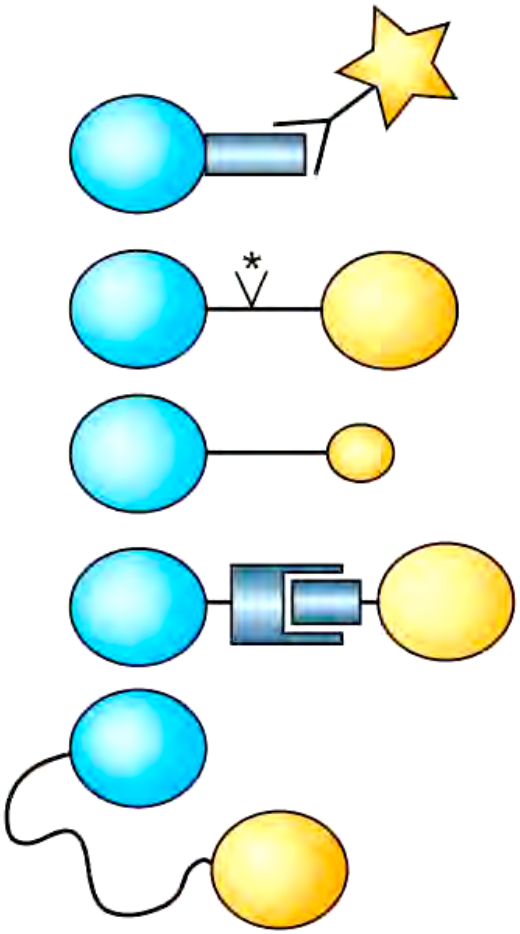
nucleus/lamin B receptor: green/EYFP

chromatin/H2B: red/ECFP

Fluorescence Resonance Energy Transfer (FRET) in the study of protein interaction



Design of Different Types of FRET Experiments



GFP fusion protein and fluorophore-coupled primary antibody undergo FRET[46–48]

Proteolytic cleavage between two fluorescent proteins eliminates FRET[70–72]

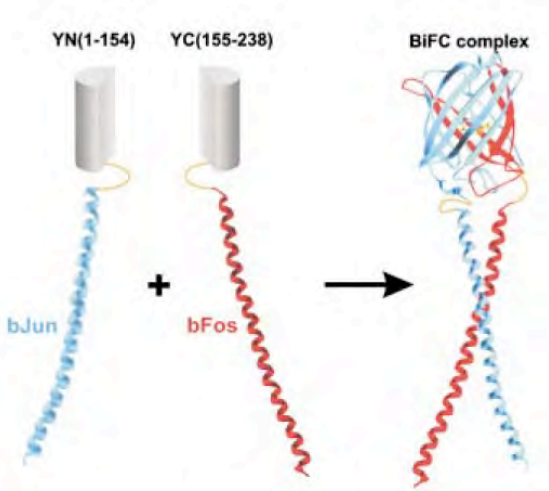
FRET acceptor fluorescent protein is sensitive to chemical environment[62]

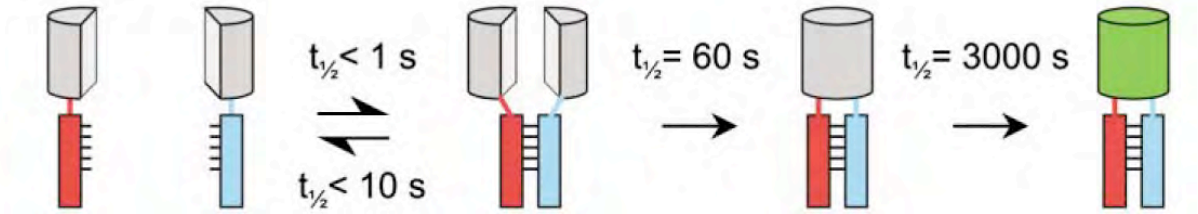
GFP fusion proteins interact and FRET[40,41,73–75]

FRET efficiency varies with linker sequence conformation[39,52,66–69]

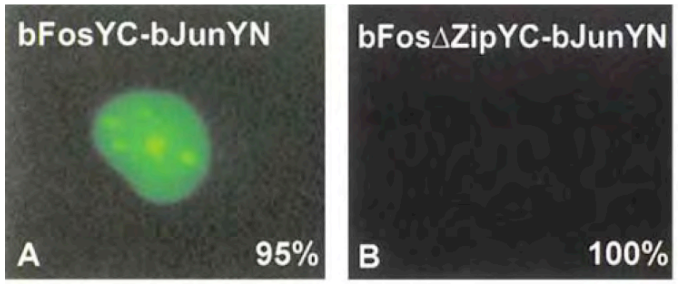
BiFC - Bimolecular Fluorescence Complementation
Split GFP Approach

Leu zipper interaction
in vitro





Fos - Jun interaction
in vivo



Hu et al., 2001