What to do with a fluorophore

Intracellular localization (ER, Golgi, PM, nuclear, lysosome, MT, actin, ...)

Dynamic processes (protein synthesis, trafficking, turnover, DNA replication, cytoskeletal remodeling, membrane potential, enzyme activity, endocytosis, exocytosis, membrane fluidity)

Cell signaling (calcium, cyclic AMP/GMP, IP3, reactive oxygen species, pH, ...)

Cell integrity (live, dead, apoptotic)

What to look for in a fluorophore

Fluorescence Spectrum

Brightness
~ extinction coeff (10^4 - $3x10^5$) x quantum yield (0.05 - 0.99)

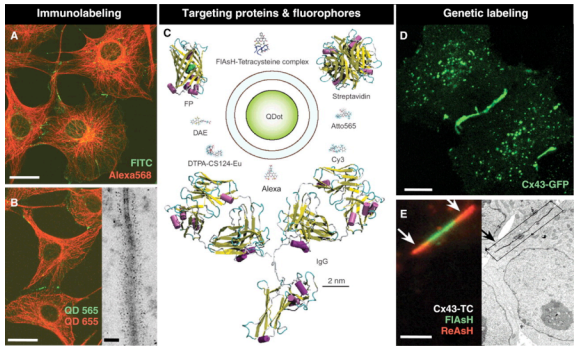
Stability (Photobleaching)

Sensitivity to Environment (pH, metal ions, quenching agents)

Toxicity

Reactivity

Solubility`



B. N. G. Giepmans et al., Science 312, 217 -224 (2006)

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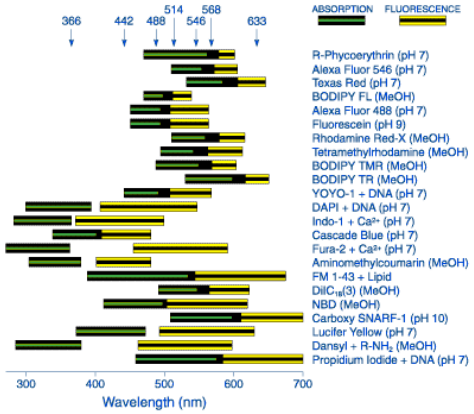
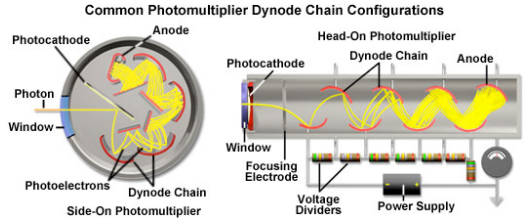
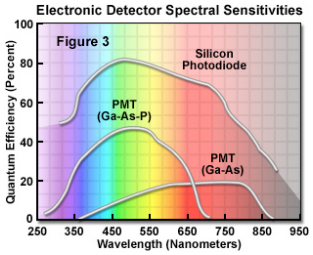
Types of fluorochromes

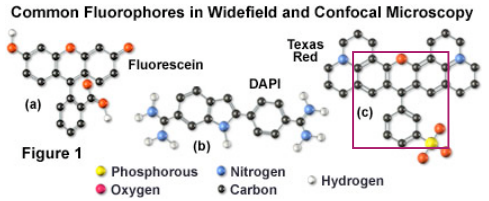
1. organic molecules (polyphenolic)
   natural & synthetic
2. metal chelates (lanthanides)
3. semiconductor crystals (Q-dots)
4. fluorescent proteins
   natural & engineered
5. expressible affinity reagents

Fluorochromes



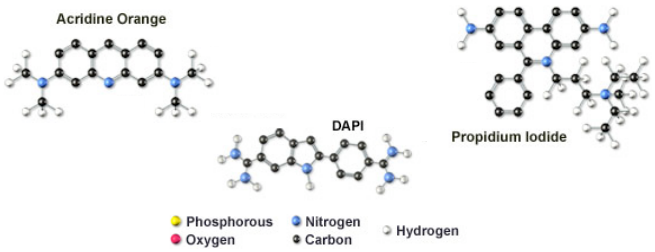






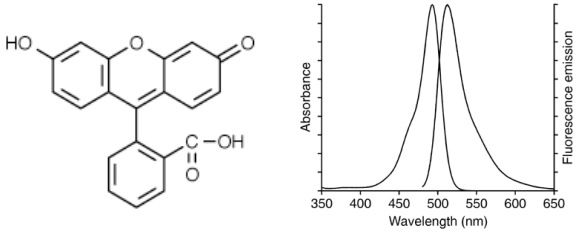
- Fluorescein & derivatives remain most commonly used dyes
- Fluorescein & Rhodamine dyes are xanthene derivatives
- DAPI (4',6-diamidino-2-phenylindole) binds outside DNA helix to A-T rich regions

Common Dyes for Nucleic Acids

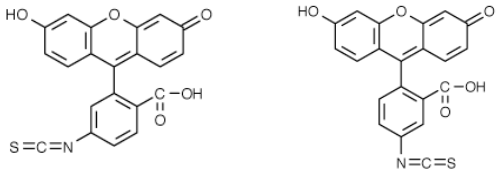


Acridine Orange (em 530 nm DNA; 640 nm RNA)
Propidium Iodide (ex 536 nm; em 617 nm)
DAPI (~ ex 351 nm; em ~ 460 nm)
Hoechst Dyes [33258, etc]

Fluorescein Fluorochromes



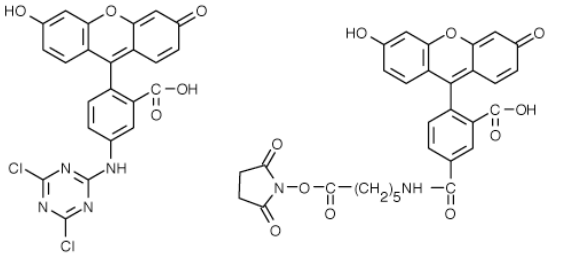
FITC Isomers



fluorescein-6-isothiocyanate (FITC 'Isomer II')

fluorescein-5-isothiocyanate (FITC 'Isomer I')

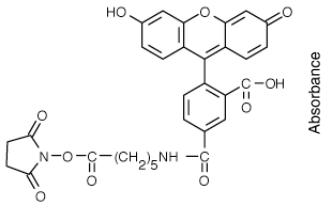
FITC Reagents

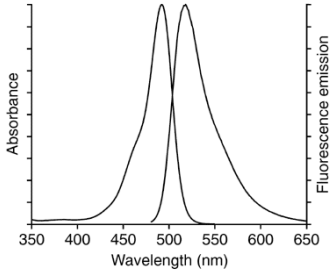


5-(4,6-dichlorotriazinyl)aminofluorescein (5-DTAF)

6-(fluorescein-5-carboxamido) hexanoic acid, succinimidyl ester (5-SFX)

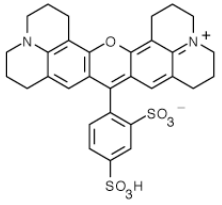
FITC Reagents



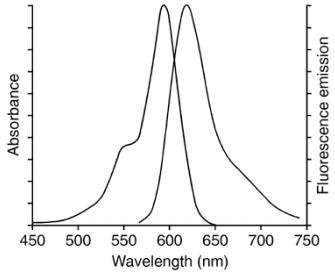


6-(fluorescein-5-carboxamido) hexanoic acid,
succinimidyl ester (5-SFX)

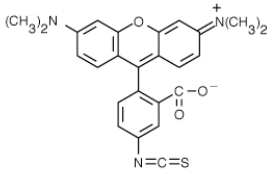
Rhodamine Fluorophores



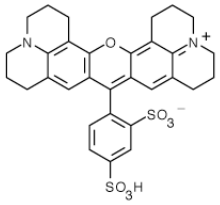
Sulforhodamine 101
(Texas Red)



Rhodamine Fluorophores

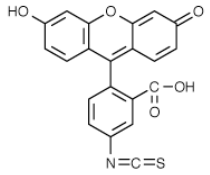


tetramethylrhodamine-5-isothiocyanate
(5-TRITC; G isomer)

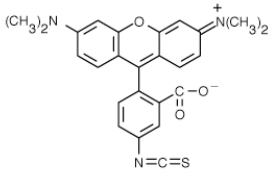


Sulforhodamine 101
(Texas Red)

FITC / TRITC

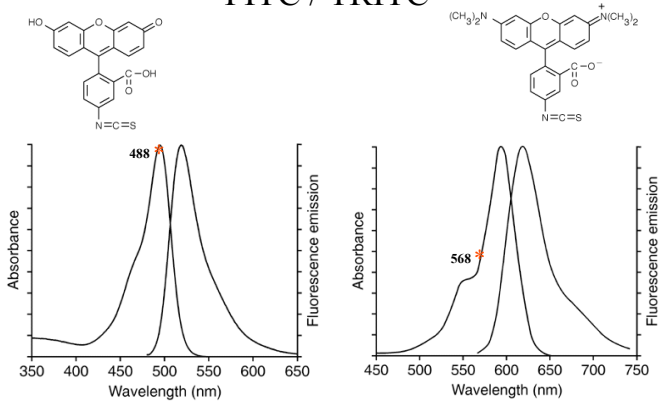


fluorescein-5-isothiocyanate
(FITC 'Isomer I')



tetramethylrhodamine-5-isothiocyanate
(5-TRITC; G isomer)

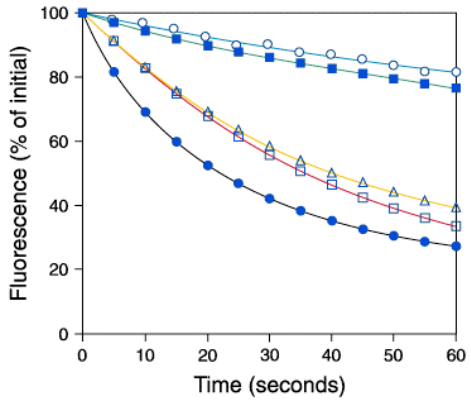
FITC / TRITC



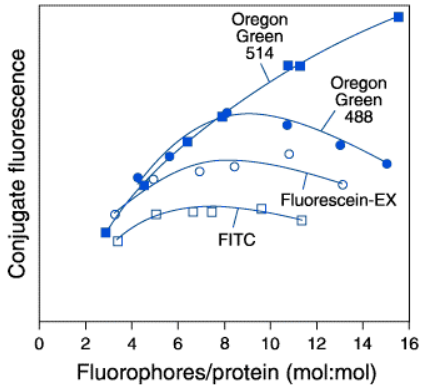
fluorescein-5-isothiocyanate (FITC 'Isomer I')

tetramethylrhodamine-5-isothiocyanate (5-TRITC; G isomer)

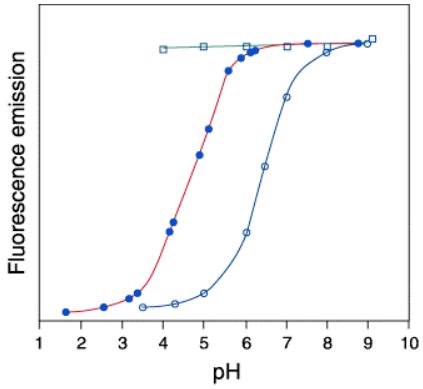
What's wrong with FITC ?



What's wrong with FITC ?



What's wrong with FITC ?



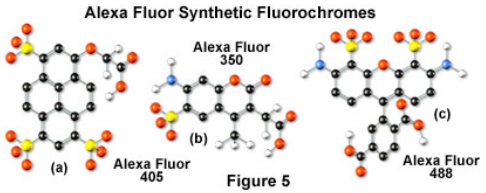
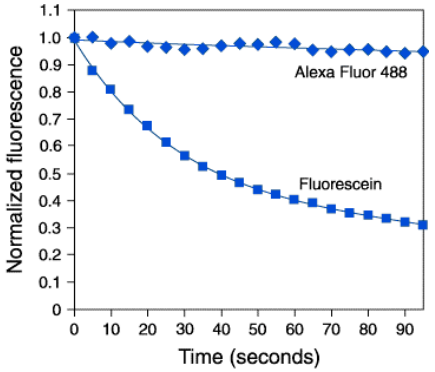


Figure 5

Alexa dyes are sulfonated forms of many “classic’ organic fluors
Greatly increased photostability
Improved water solubility
Low pK of sulfonates reduces sensitivity to pH changes near neutrality
They even taste better (only a rumor; don’t try at home)

Alexa Dyes



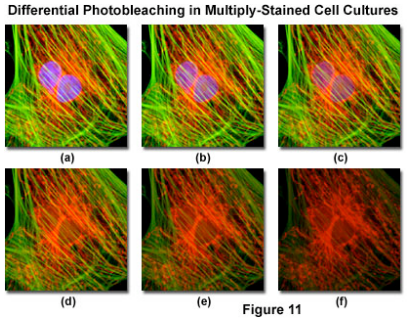


Figure 11

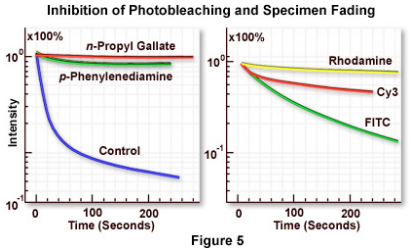
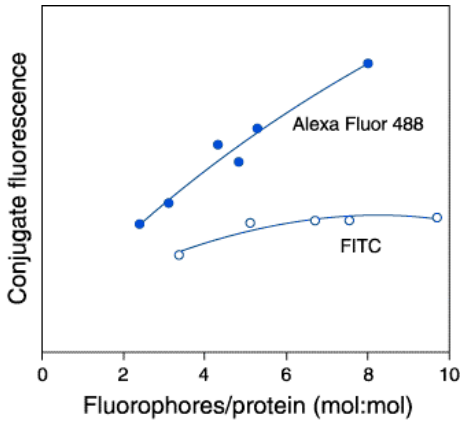
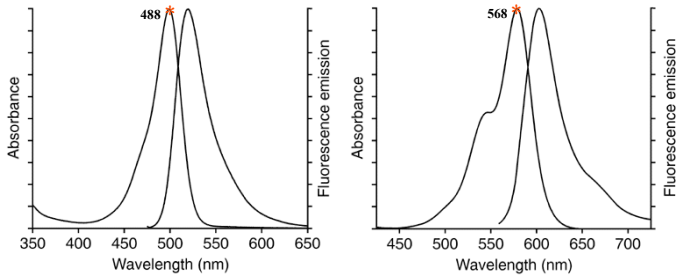


Figure 5

Alexa Dyes

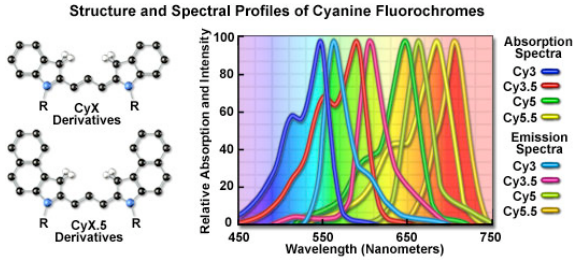


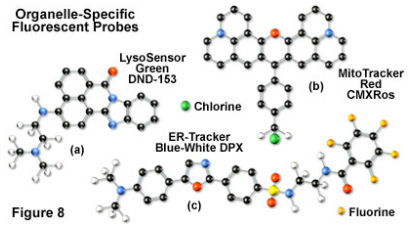
Alexa Dyes



Alexa 488

Alexa 568





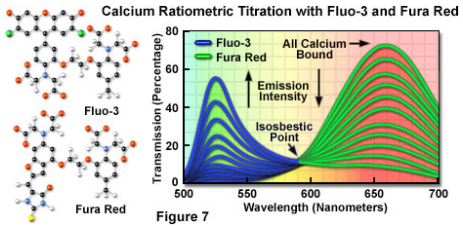
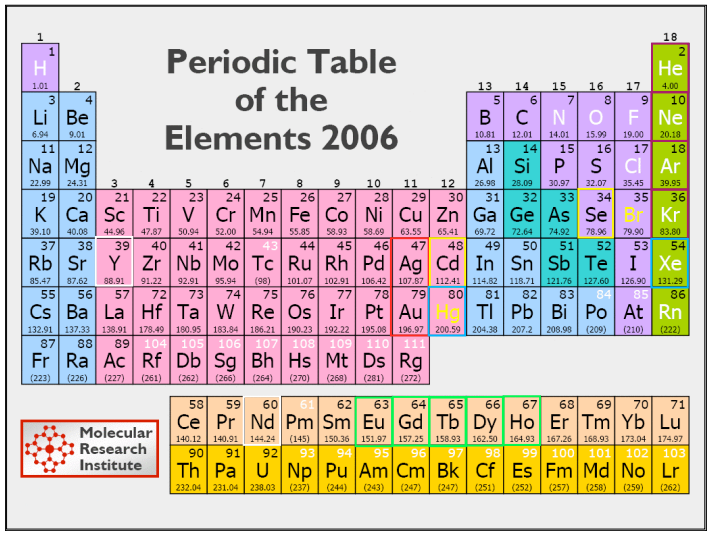


Figure 7

Types of fluorochromes

1. organic molecules (polyphenolic)
   natural & synthetic
2. metal chelates (lanthanides)
3. semiconductor crystals (Q-dots)
4. fluorescent proteins
   natural & engineered
5. expressible affinity reagents



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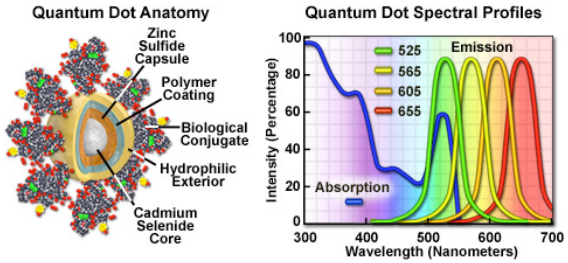

Fluorescence emission peak increases with size
Can activate all colors with same excitation band
Brightness increases with shorter wavelength excitation
ZnS coating increases photostability (huge !!)
Polymer coating is necessary to improve hydrophilicity
Disadvantage = size (10 - 15 nm)

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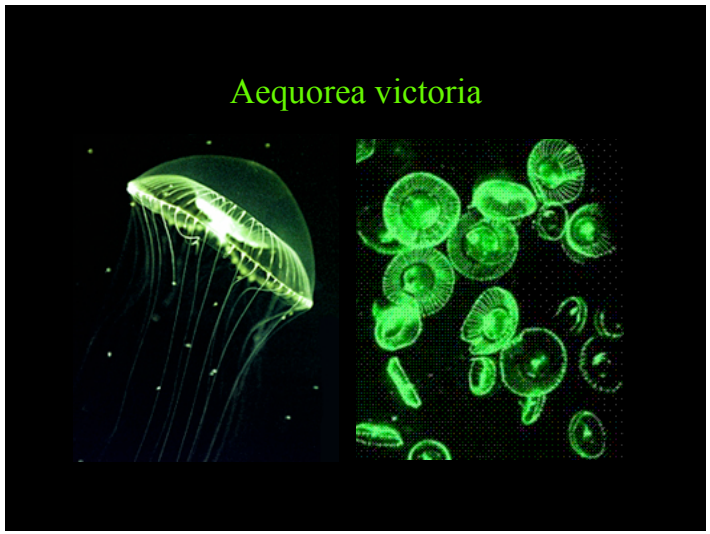
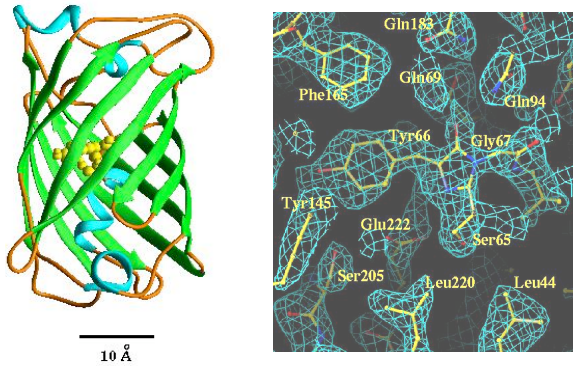
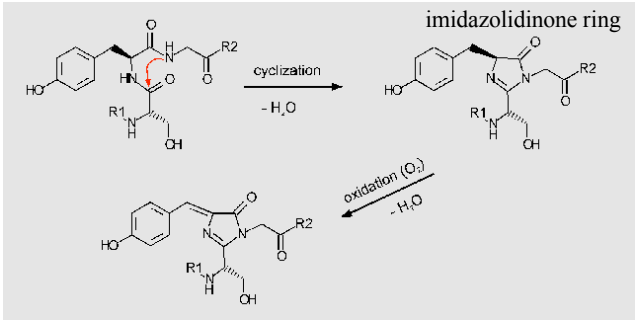


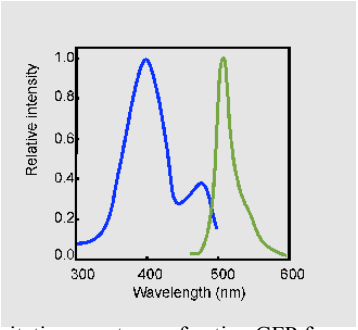
Green Fluorescent Protein
Aequorea Victoria



Green Fluorescent Protein

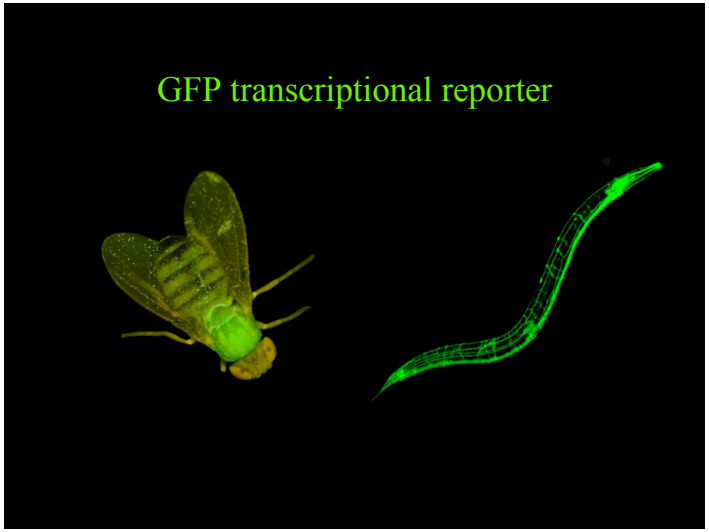


Green Fluorescent Protein

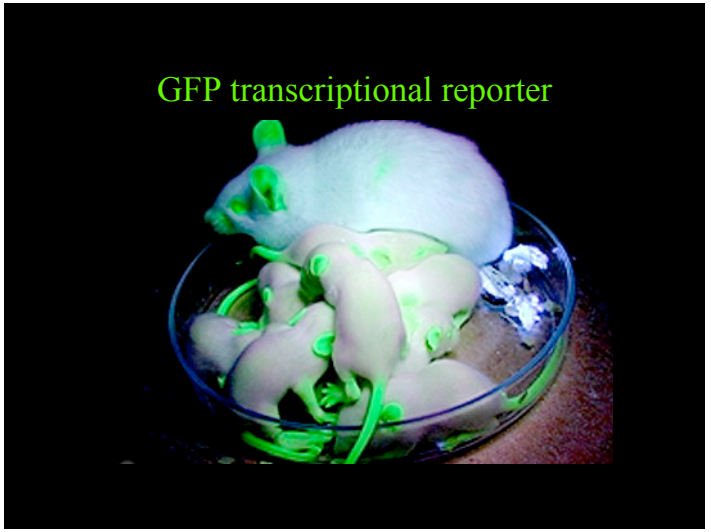


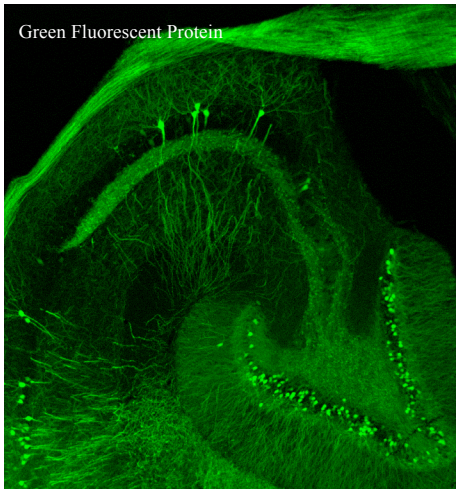
The excitation spectrum of native GFP from *A. victoria* (blue) has two excitation maxima at 395 nm and at 470 nm. The fluorescence emission spectrum (green) has a peak at 509 nm and a shoulder at 540 nm.

GFP transcriptional reporter



GFP transcriptional reporter





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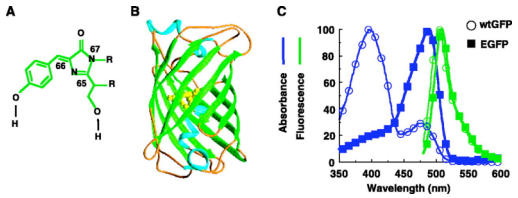

J. Lippincott-Schwartz et al., Science 300, 87 -91 (2003)

Published by AAAS

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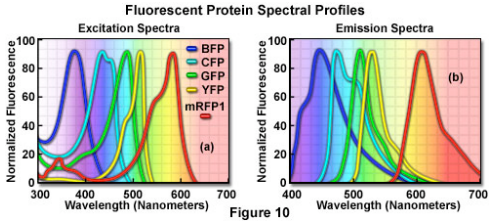
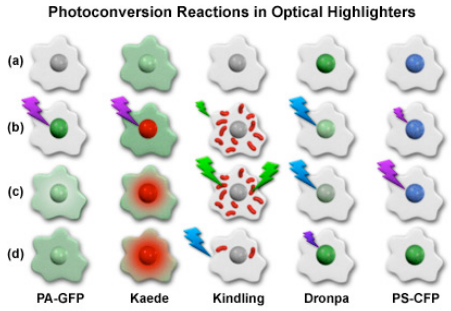
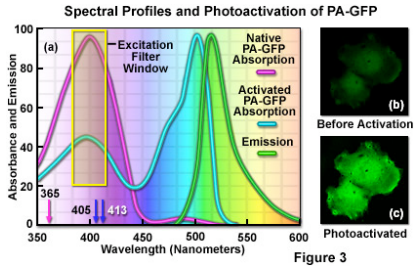
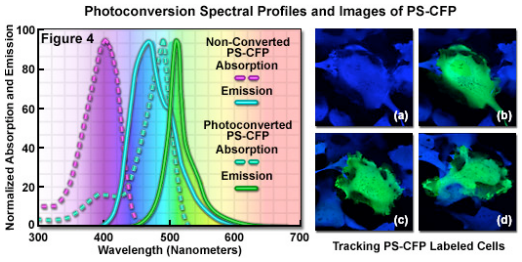


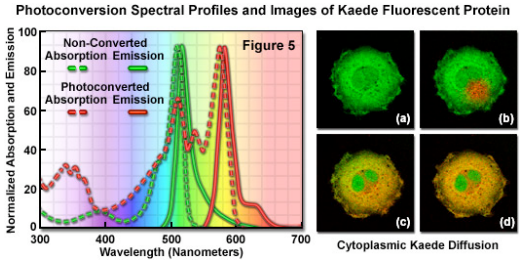
Figure 10



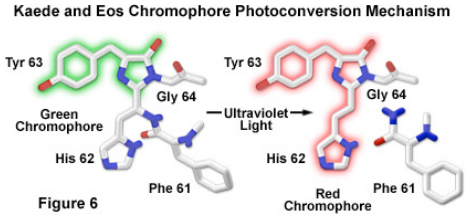




Photoactivation @ 405 nm
5 x decrease in cyan fluorescence
300 x increase in green fluorescence



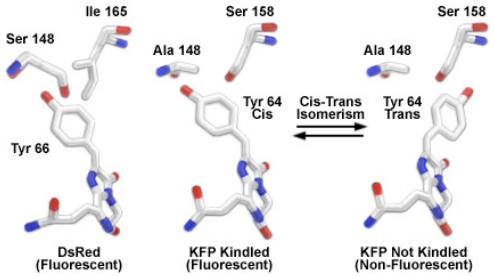
Trachyphyllia geoffroyi **(brain coral)**
Green --> shift discovered accidentally
Photoactivation @ 380 - 400 nm
results in em 518 nm --> 582 nm



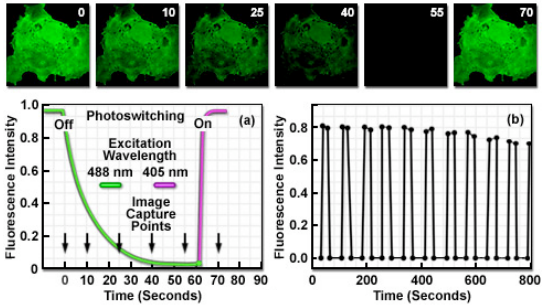
Tracking Organelle Movements with Kindling Fluorescent Protein

Anemonia sulcata
low intensity ex 543 nm --> transient red fluorescence
high intensity @ 543 nm --> permanent red fluorescence

DsRed and Kindling Fluorescent Protein Chromophores

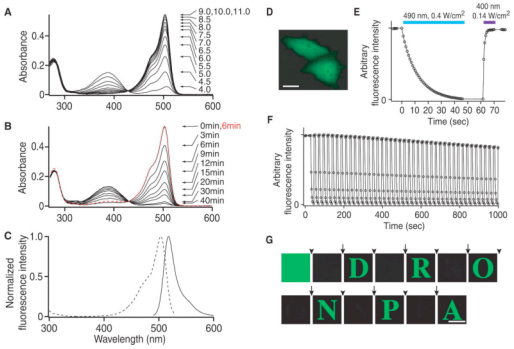


Reversible Photoswitching of Dronpa Fluorescent Protein



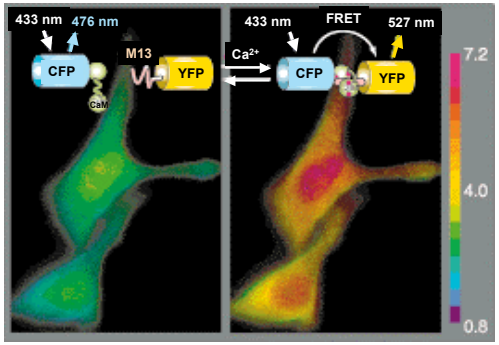
Pectiniidae

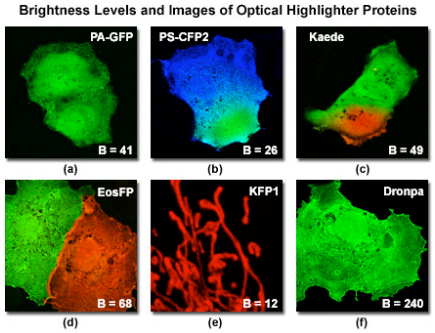
Fig. 1. Photochromic properties of Dronpa (22Gm3)



R. Ando et al., Science 306, 1370 -1373 (2004)

Published by AAAS





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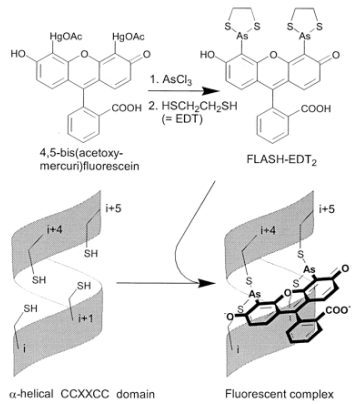
   natural & engineered
5. expressible affinity reagents



FlAsH = Fluorescein Arsenical Hairpen

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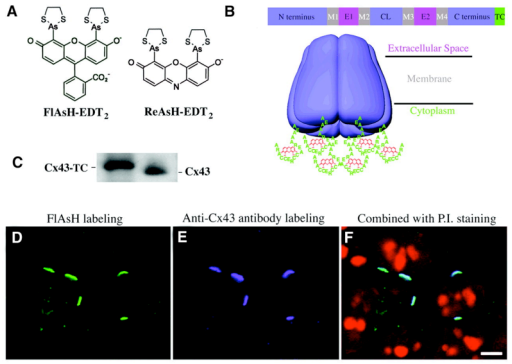

G. Gaietta et al., Science 296, 503 -507 (2002)

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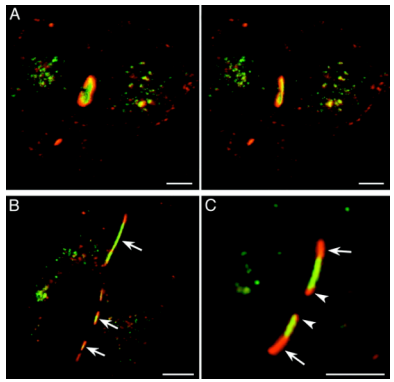
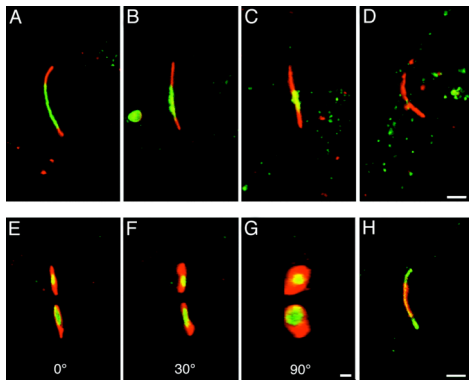


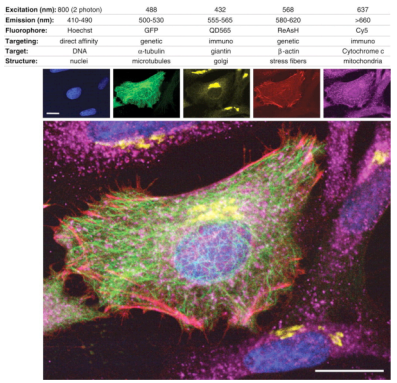
G. Gaietta et al., Science 296, 503 -507 (2002)

No Caption Found



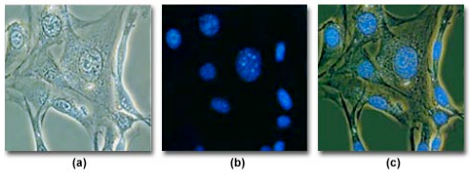
G. Gaietta et al., Science 296, 503 -507 (2002)

Fig. 3. Parallel application of targeting methods and fluorophores



B. N. G. Giepmans et al., Science 312, 217 -224 (2006)

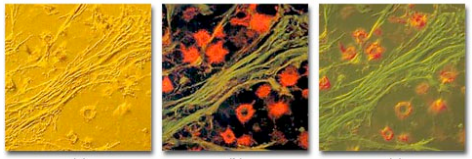
Combined Use of Phase Contrast and Fluorescence Illumination



(a) (b) (c)

Figure 3

Combined Use of DIC and Fluorescence Illumination



(a) (b) (c)

Figure 4

http://www.olympusconfocal.com/java/confocalsimulator/index.html