

Carl Zeiss Microscopy

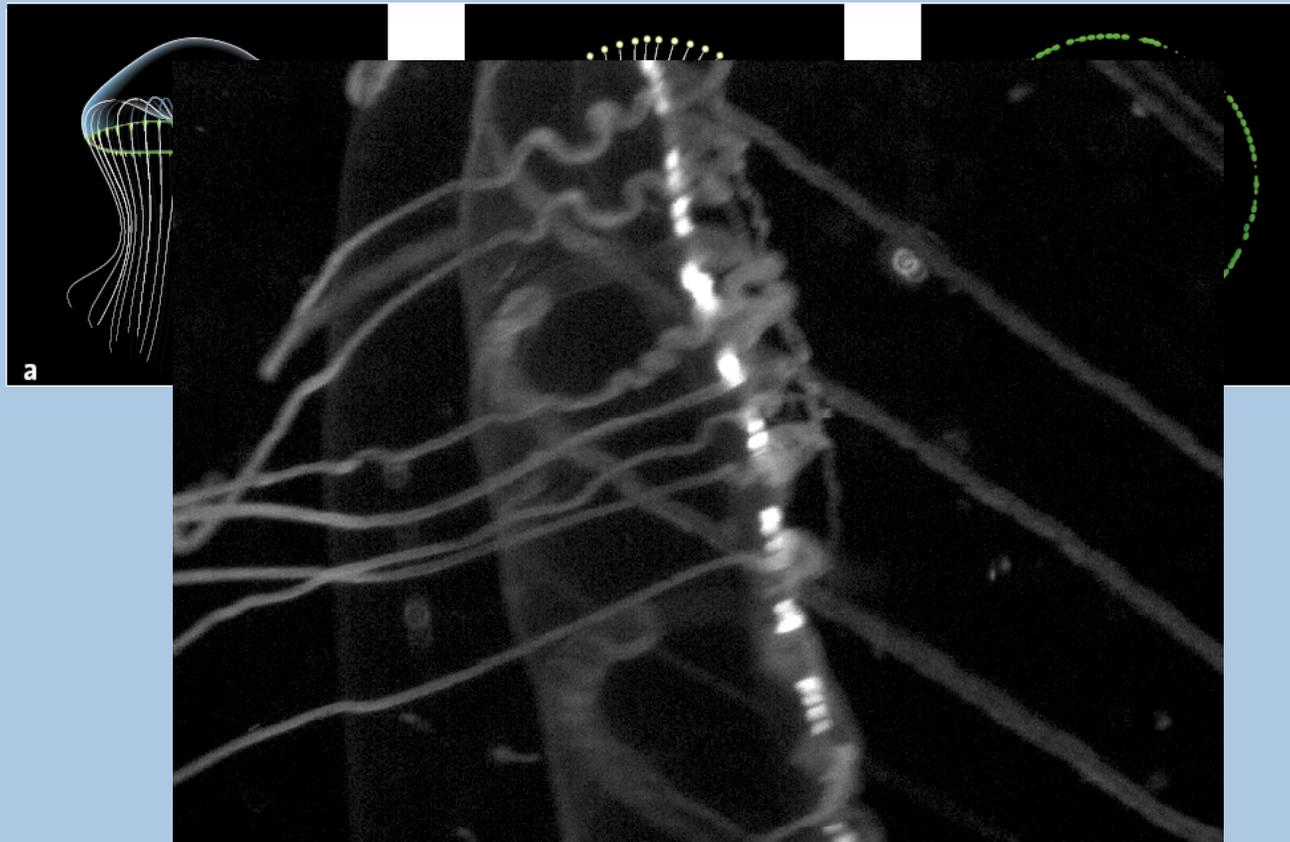


Fluorescent Proteins

Scott Olenych, PhD
solenych@zeiss.com



Aequorea Victoria, Green Fluorescent Protein: The protein that started it all



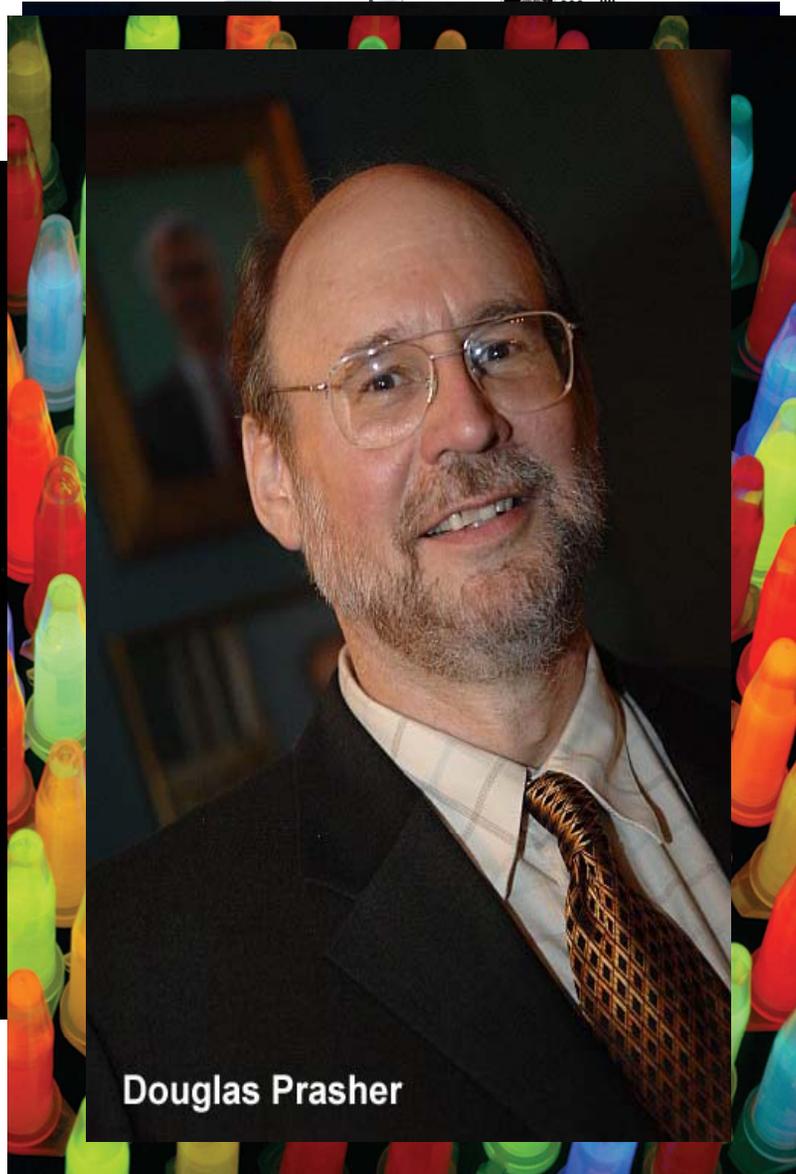
Fluorescent Protein Timeline



Roger Tsien

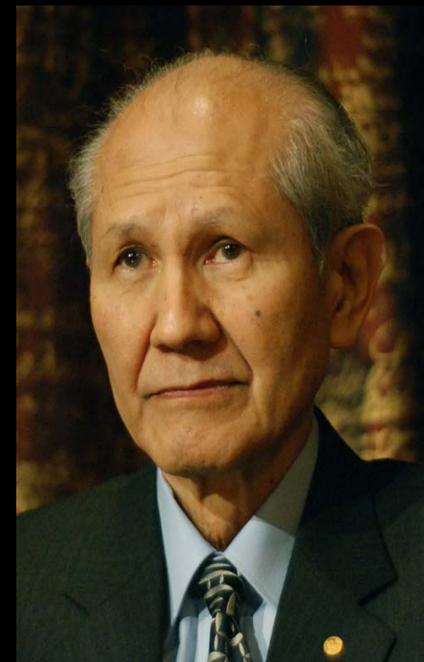


Martin Chalfie



Douglas Prasher

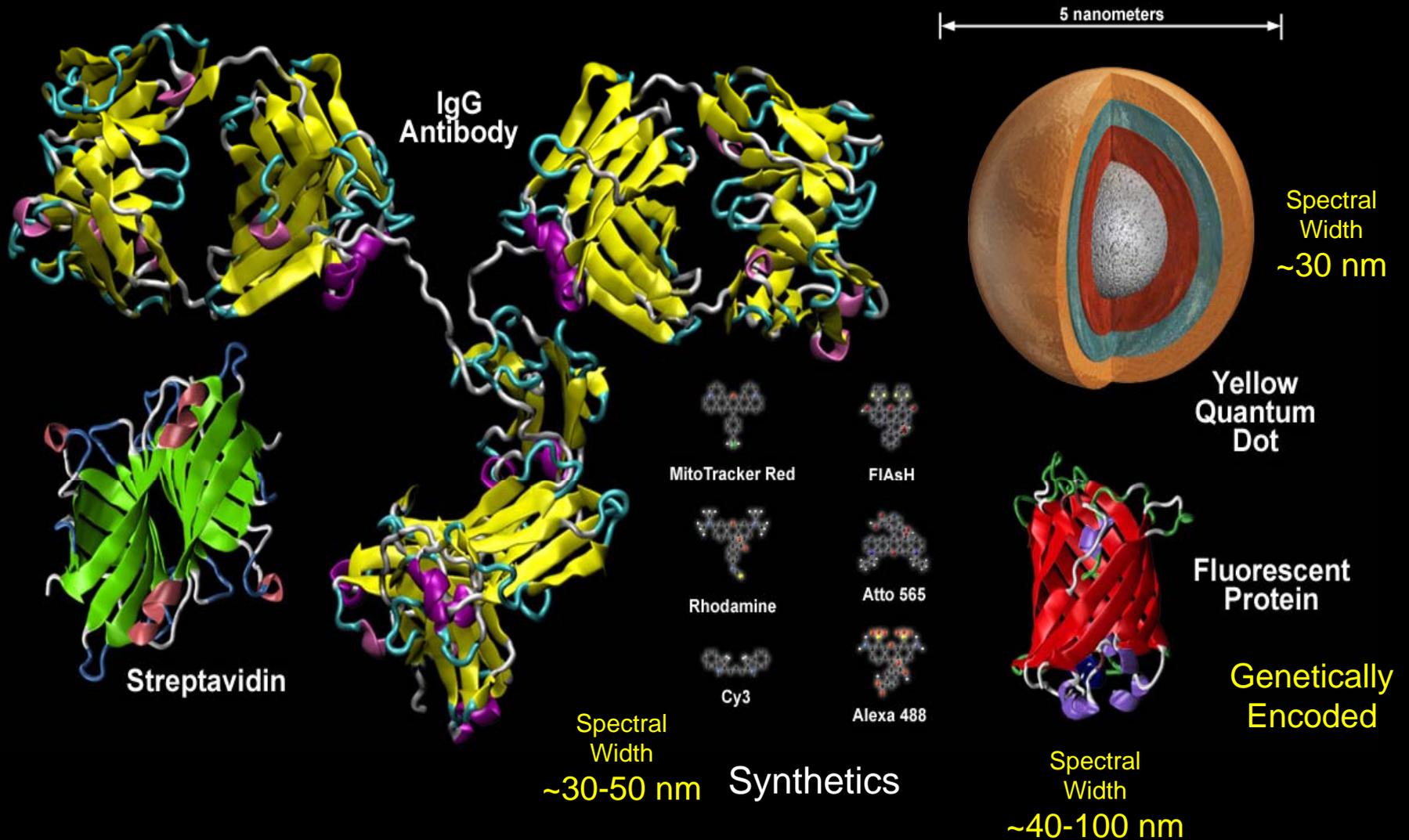
Roger Tsien, Osamu Shimomura &
Martin Chalfie win Nobel Prize



Osamu Shimomura

Day and Davidson
Chem. Soc. Rev.
38: 2887 (2009)

Fluorophores for Live-Cell Imaging, Superresolution & FRET



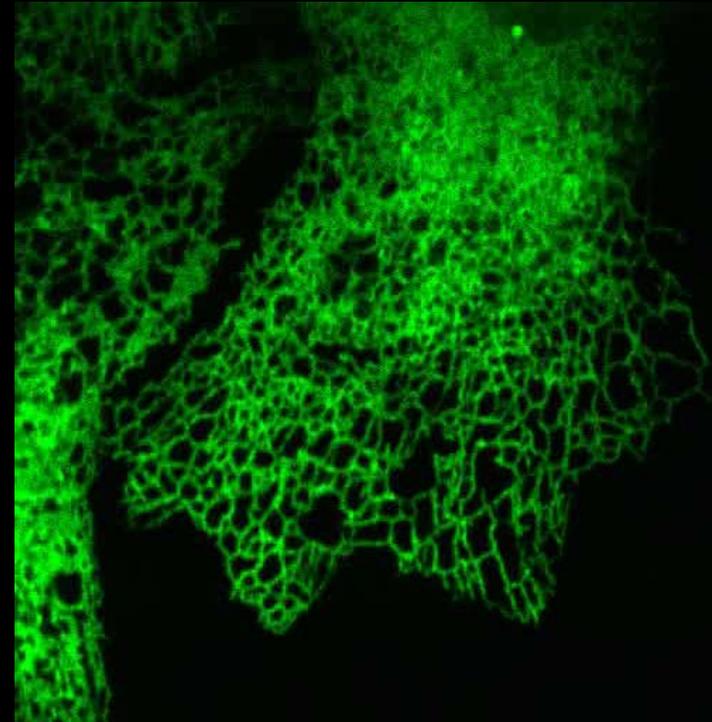
Live-Cell Imaging Requires **High Specificity**, **Low Background Signal**, and **Photostability**
Fluorophore **photophysical & photochemical** transitions critical for breaking diffraction barrier

Live-Cell Imaging for Temporal and Spatial Investigations

mKusabira Or – H2B in RK-13 Cells mEYFP – ER in U2OS Cells



Laser Scanning Confocal
Microscopy
30 second Time Lapse Interval

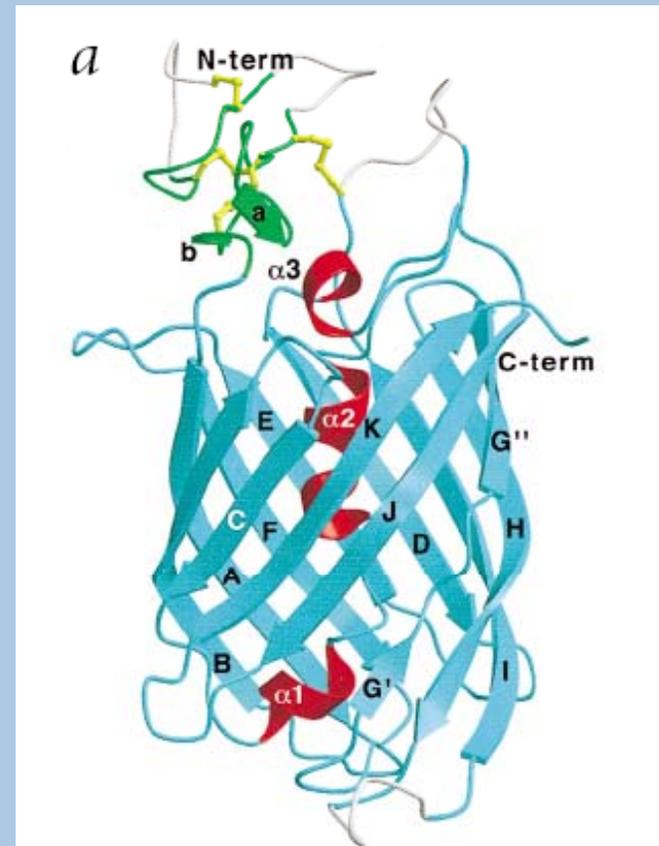


Spinning Disk Confocal
Microscopy
2 Second Time Lapse Interval

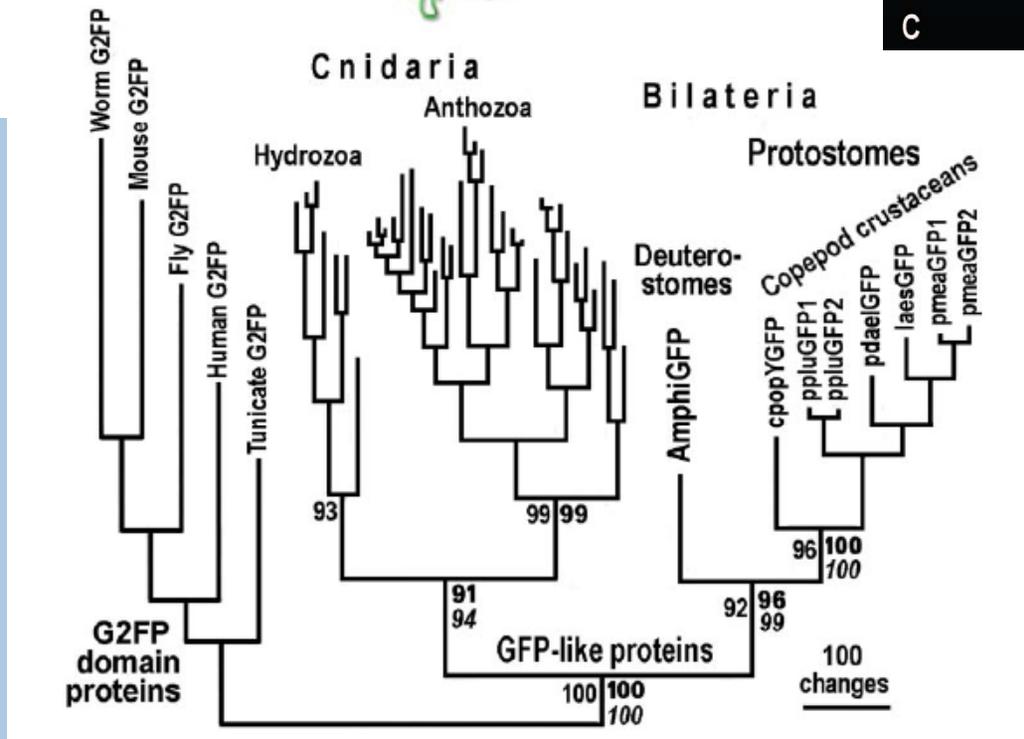
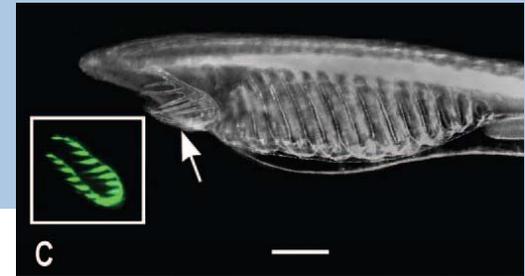
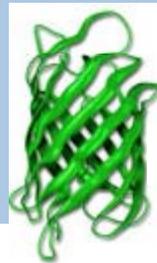
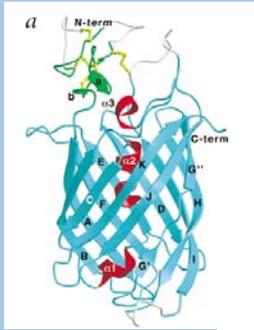
Origin of Fluorescent Proteins: Nidogen/Entactin



- Cellular basement membrane protein
- Found in *C.elegans*, vertebrates and *D. melanogaster*
- G2 Fragment 11 stranded β barrel with a central α helix
- Not fluorescent



Origin of Fluorescent Proteins: The family tree

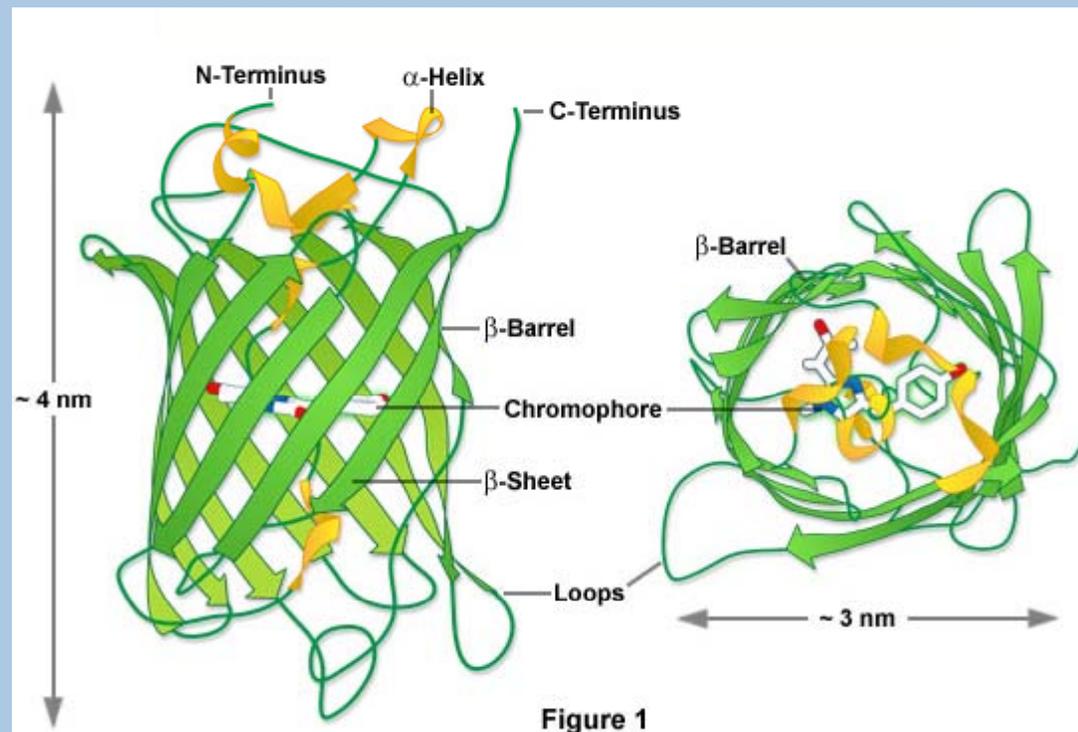


Architecture of *Aequorea victoria* GFP



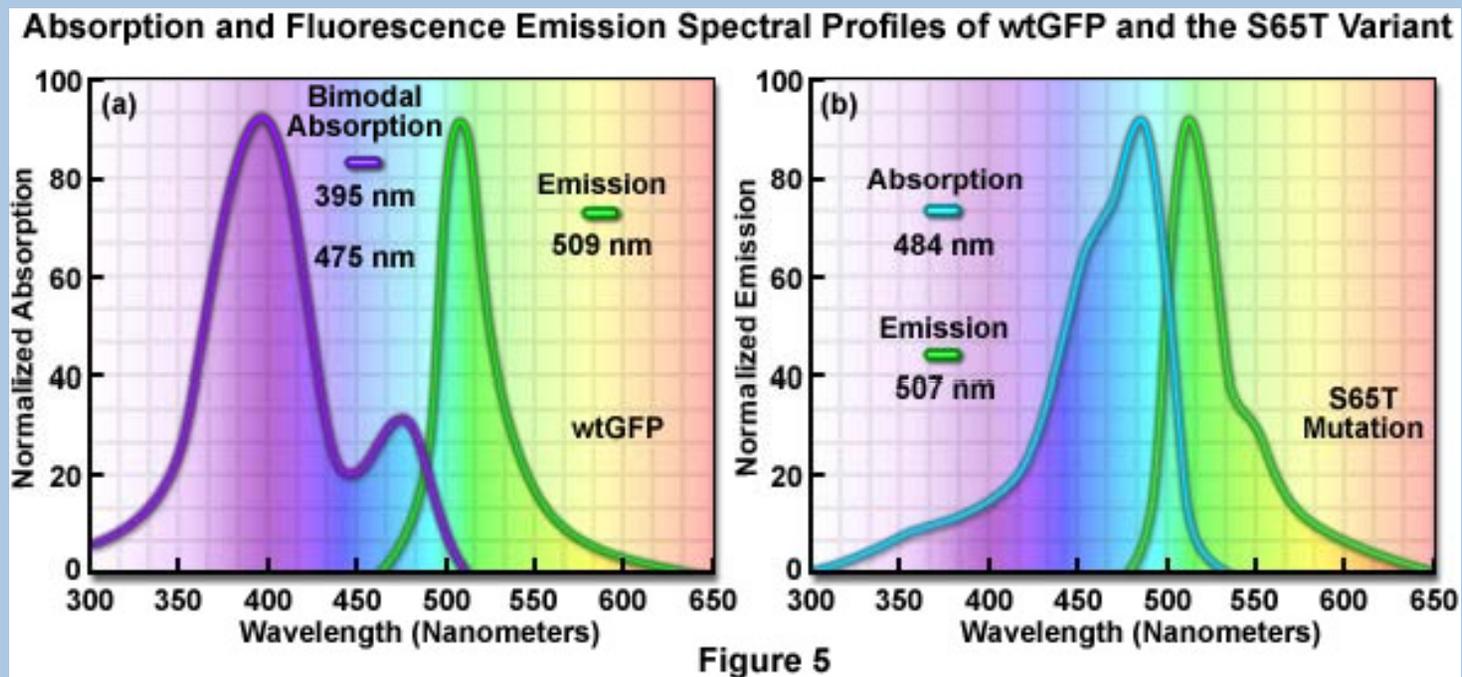
Aequorea victoria GFP
238 Amino Acids

FP Barrel can reduce
FRET Efficiency by
~ 40-60%



GFP Spectral Properties:

wtGFP and EGFP (S65T) and variants



GFP Spectral Properties:

wtGFP and EGFP (S65T) and variants

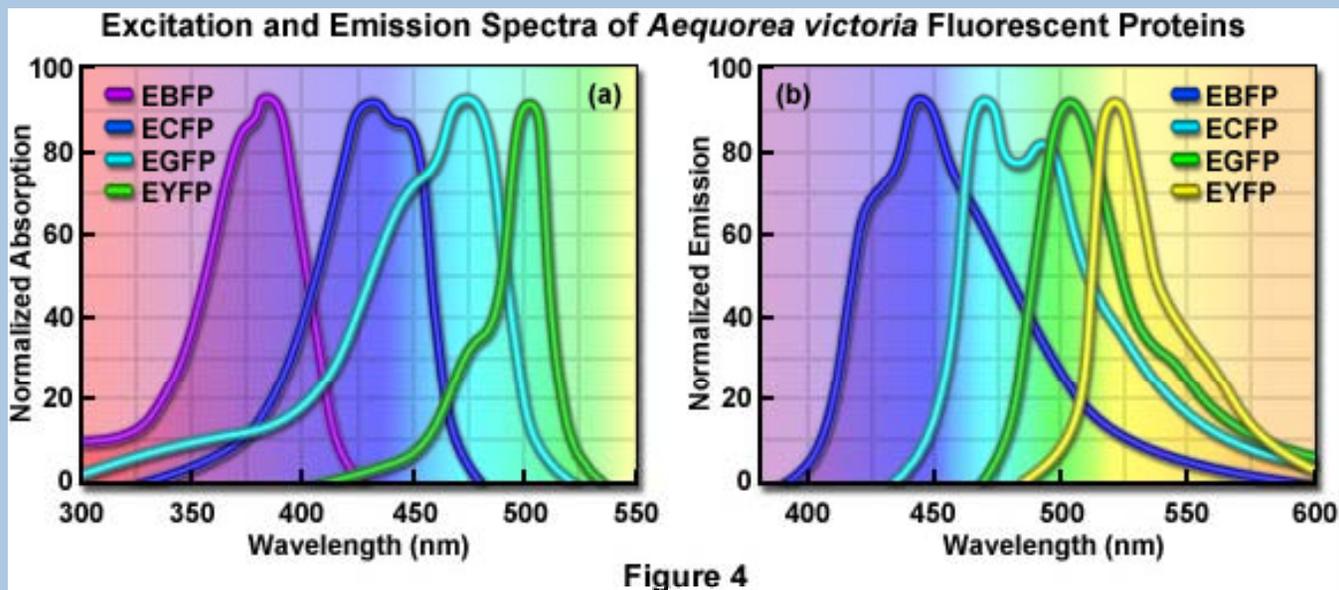
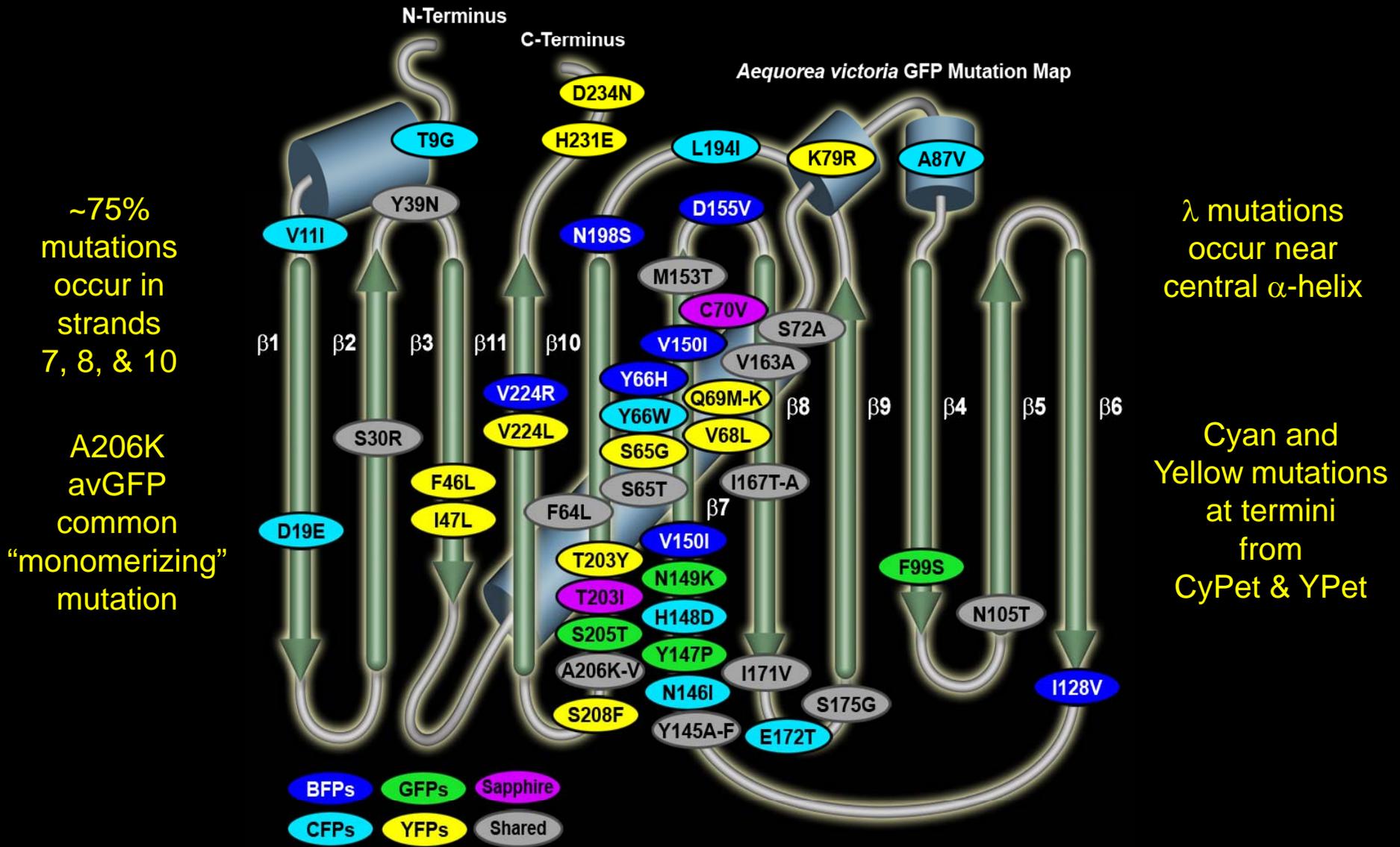


Figure 4

Improved GFPs Through Mutagenesis

Aequorea victoria GFP Mutation Map



Folding mutations occur throughout the sequence

Formation of the EGFP Chromophore



Properties of GFP variants



| Protein (Acronym) | Ex (nm) | Em (nm) | EC ($\times 10^{-3}$) | OY | Photostability | Quaternary Structure | Brightness (% of EGFP) |
|------------------------------------|---------|---------|-------------------------|------|----------------|----------------------|------------------------|
| Blue Fluorescent Proteins | | | | | | | |
| Sirius | 355 | 424 | 15.0 | 0.24 | +++ | Monomer* | 11 |
| Azurite | 384 | 450 | 26.2 | 0.55 | ++ | Monomer* | 43 |
| EBFP | 383 | 445 | 29.0 | 0.31 | + | Monomer* | 27 |
| EBFP2 | 383 | 448 | 32.0 | 0.56 | +++ | Monomer* | 53 |
| Cyan Fluorescent Proteins | | | | | | | |
| ECFP | 439 | 476 | 32.5 | 0.40 | ++ | Monomer* | 39 |
| Cerulean | 433 | 475 | 43.0 | 0.62 | ++ | Monomer* | 79 |
| CyPet | 435 | 477 | 35.0 | 0.51 | ++ | Monomer* | 53 |
| SCFP | 433 | 474 | 30.0 | 0.50 | ++ | Monomer* | 45 |
| Green Fluorescent Proteins | | | | | | | |
| EGFP | 488 | 507 | 56.0 | 0.60 | ++++ | Monomer* | 100 |
| Emerald | 487 | 509 | 57.5 | 0.68 | +++ | Monomer* | 116 |
| Superfolder | 485 | 510 | 83.3 | 0.65 | +++ | Monomer* | 160 |
| T-Sapphire | 399 | 511 | 44.0 | 0.60 | ++ | Monomer* | 79 |
| Yellow Fluorescent Proteins | | | | | | | |
| EYFP | 514 | 527 | 83.4 | 0.61 | ++ | Monomer* | 151 |
| Citrine | 516 | 529 | 77.0 | 0.76 | ++ | Monomer* | 174 |
| Venus | 515 | 528 | 82.2 | 0.57 | ++ | Monomer* | 156 |
| Topaz | 514 | 527 | 94.5 | 0.57 | ++ | Monomer* | 169 |
| YPet | 517 | 530 | 104.0 | 0.77 | ++ | Monomer* | 238 |
| SYFP | 515 | 527 | 101.0 | 0.68 | ++ | Monomer* | 204 |
| mAmetrine | 406 | 526 | 45.0 | 0.58 | + | Monomer | 78 |

High Performance Blue Fluorescent Proteins



Sirius (GFP Derivative)

Emission Max = 424 nm Brightness = 3.6 Photostability ~ 75

Phenylalanine Chromophore



EBFP2 (GFP Derivative)

Emission Max = 448 nm Brightness = 18 Photostability = 55

EBFP + S30R + Y39N + T65S + S72A + N105T + I128V + V150I + D155V +
I171V + N198S + A206V + V224R

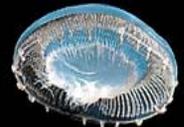


TagBFP2 (*Aequorea macrodactyla* Jellyfish Derivative)

Emission Max = 454 nm Brightness = 44 Photostability ~ 60

LYG Chromophore

All BFPs can be imaged with DAPI Filter sets
Omega QMax Blue Best for BFPs



Cyan and Green Fluorescent Proteins



mTurquoise & mCerulean3 (mCerulean Derivatives)

Emission Max = 475/503 nm Brightness ~ 25 Photostability ~35



mTFP1 – Teal FP (Coral Derivative)

Emission Max = 492 nm Brightness = 54 Photostability = 110



Superfolder GFP (GFP Derivative)

Emission Max = 510 nm Brightness = 54 Photostability = 157



mEmerald (High-Performance EGFP Derivative)

Emission Max = 509 nm Brightness ~ 50 Photostability = 165

EGFP + 4 Folding Mutations (F64L / S65T / S72A / N149K / M153T / I167T)

Cyan proteins require special filter set – Green proteins use FITC

Architecture of the DsRed Variants



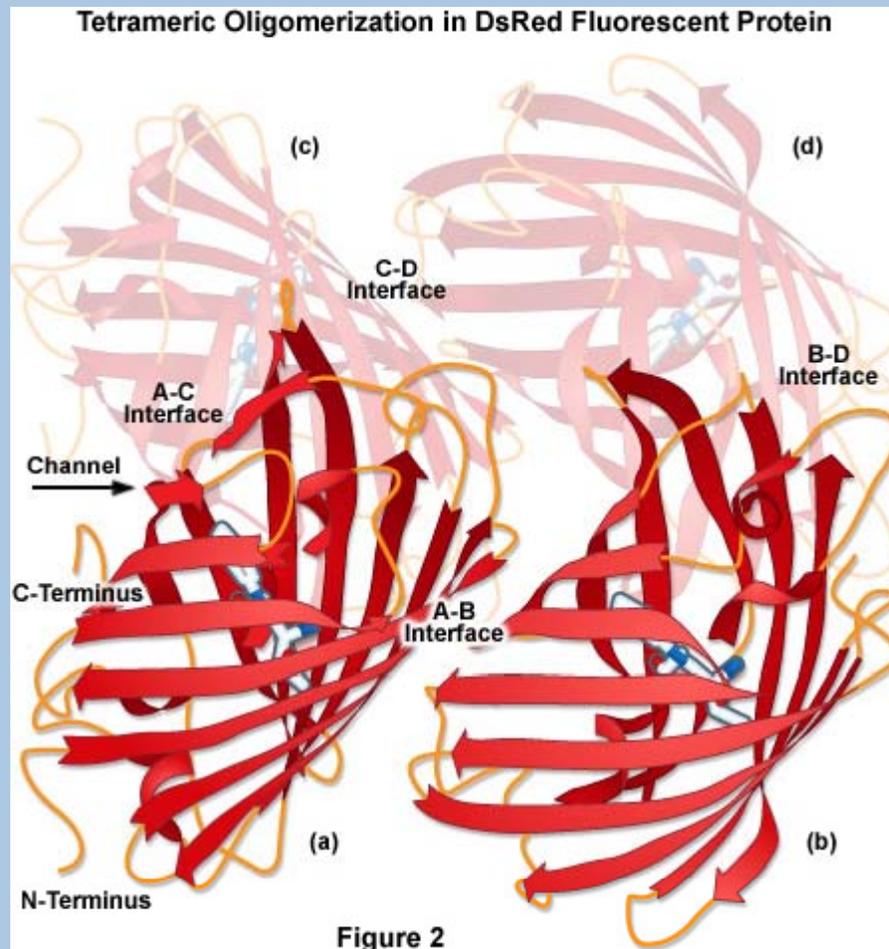
Oligomerization of the DsRed Variants:

An inherent property

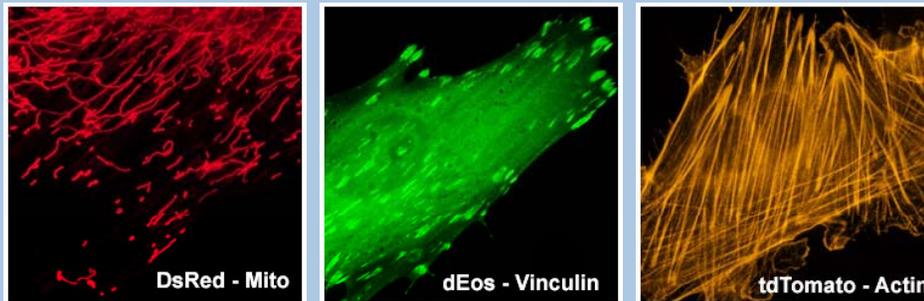


Oligomerization interferes with proper localization in fusions that form biopolymers

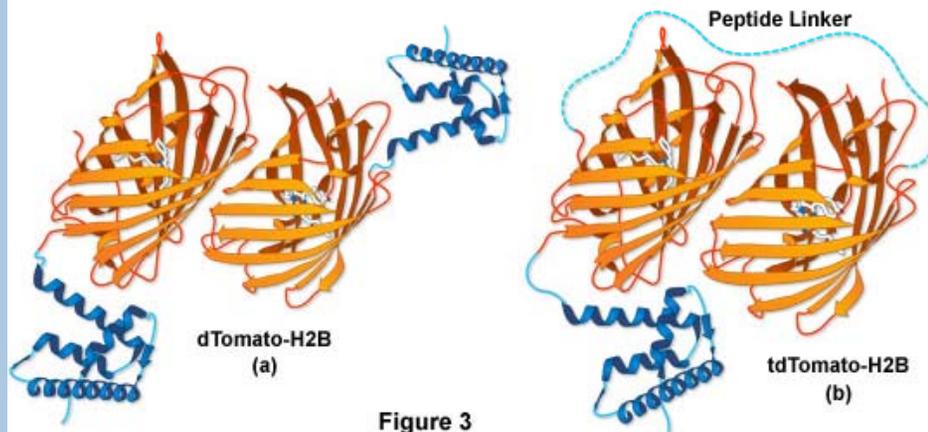
Oligomerization produces aggregation artifacts in live cells



Overcoming Oligomerization of the DsRed Variants



Creating Pseudo-Monomeric Fluorescent Proteins with Tandem Dimers



In some cases, tetramers and dimers don't affect localization

Tandem Dimer may work similar to a monomer but at twice the size

DsRed Derivative Mutation Map



Many red proteins contain “monomerizing” mutations in Strands 6, 8 & 9

mCherry mutations occur near chromophore

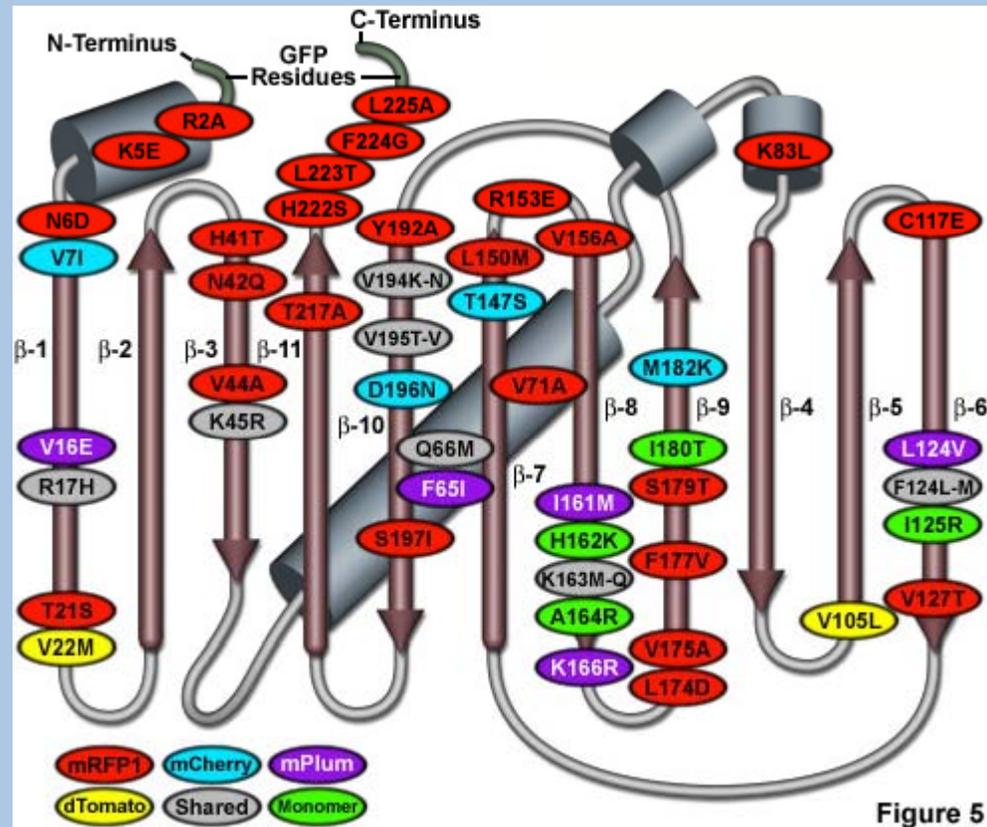


Figure 5

Anthozoa Fluorescent Protein Variants: DsRed Chromophore Formation



Orange and Red Fluorescent Proteins



mKO2 (Kusabira Orange; Coral Derivative)

Emission Max = 565 nm Brightness = 40 Photostability = 100



tdTomato (dsRed Derivative)

Emission Max = 581 nm Brightness = 95 Photostability = 75



mApple (mOrange Derivative; 18 Mutations)

Emission Max = 592 nm Brightness = 37 Photostability ~ 100



mCherry (mRFP1 Derivative)

Emission Max = 610 nm Brightness = 16 Photostability ~ 100

Orange FPs can be imaged with a TRITC or Cy3 filter set
Red FPs require Texas Red or Longpass filter set

Red and Far Red Fluorescent Proteins



mKate and Derivatives (Coral Derivatives)

Emission Max = 635 nm Brightness = 9 (15) Photostability ~ 166

mKate = mTagRFP + R70K - N146S - F177L - H200R



mRuby (eqFP611 Derivative)

Emission Max = 605 nm Brightness = 39 Photostability ~ 100

mRuby = eqFP611 + 28 mutations



mNeptune (mKate Derivative)

Emission Max = 650 nm Brightness = 13 Photostability ~ 150

mNeptune = mKate + 5 mutations

Red FPs are best imaged with a Texas Red or Longpass filter set

The “mFruit” Proteins are Ideal for Live-Cell Imaging

mCherry – Actin fusion expressed in Rabbit Kidney (RK-13) Cells



543 nm; LSCM; 30-second TL; 24 hour observation; 40x Oil

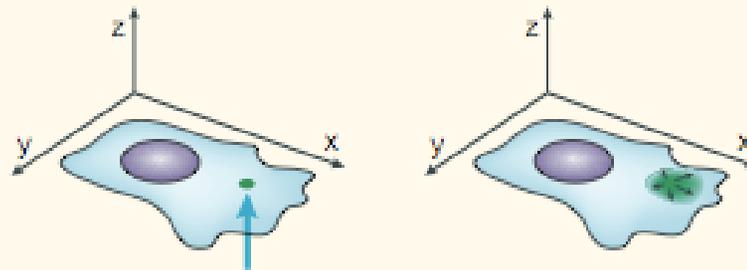
Optical Highlighters



Protein tracking

Parameters determined:

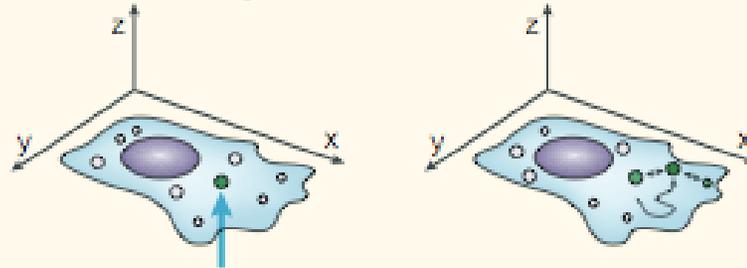
- Movement rate and direction
- Diffusion coefficient
- Mobile and immobile fractions
- Time parameters of compartmental residency and exchange between compartments
- Rate of turnover



Organelle tracking

Parameters determined:

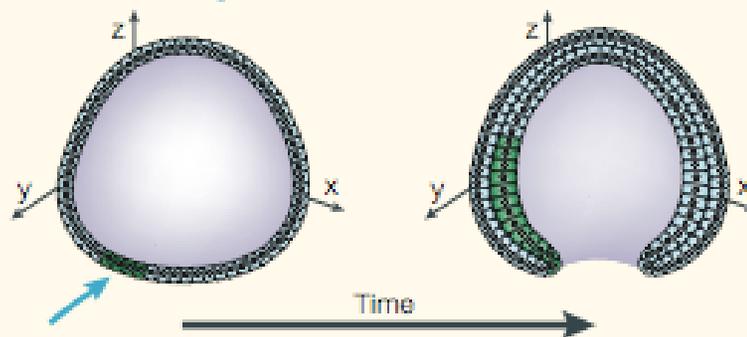
- Movement rate and direction
- Rate of content interchange
- Fission and fusion events



Cell tracking

Parameters determined:

- Movement rate and direction
- Cell localization
- Rate of cell division
- Shape and volume of cells

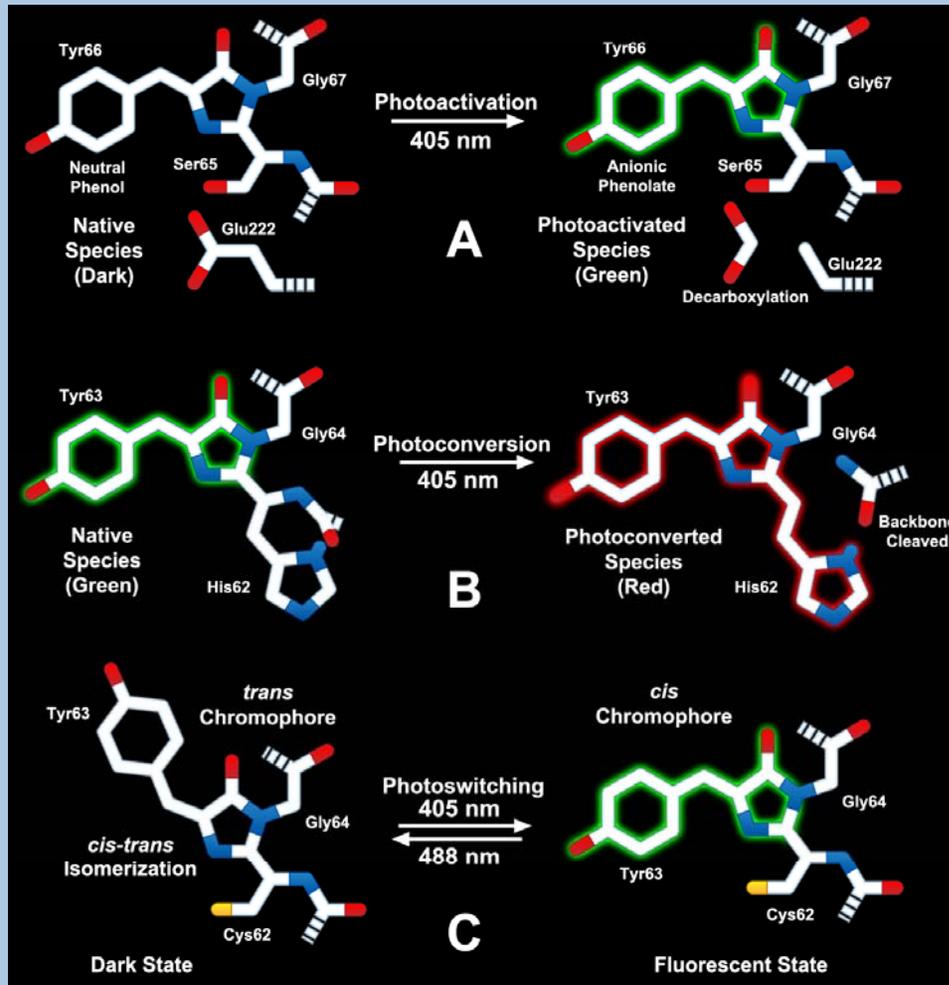


Optical Highlighters



Green to Red Photoconversion

- Kaede – Tetramer
- Eos – Tandem Dimer
- mEos2 – Monomer
- Dendra – Monomer
- KikGR – Tetramer
- mKikGR – Monomer
- mClavGR – Monomer



Photoactivation

- PA-GFP – Monomer
- PS-CFP – Monomer
- PA-mCherry1 – Monomer
- PA-TagRFP – Monomer

Photoswitching

- Dronpa – Monomer
- KFP1 – Tetramer
- mTFP0.7 – Monomer

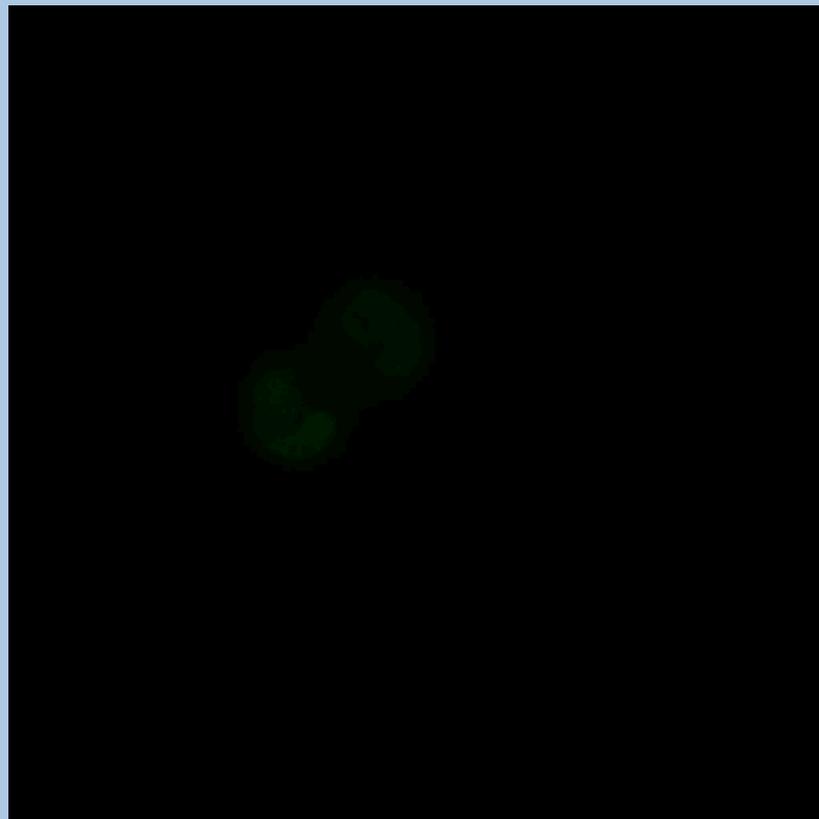
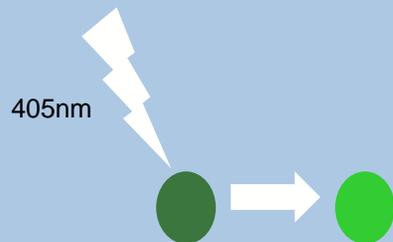
Photoactivatable Fluorescent Proteins: PA-GFP



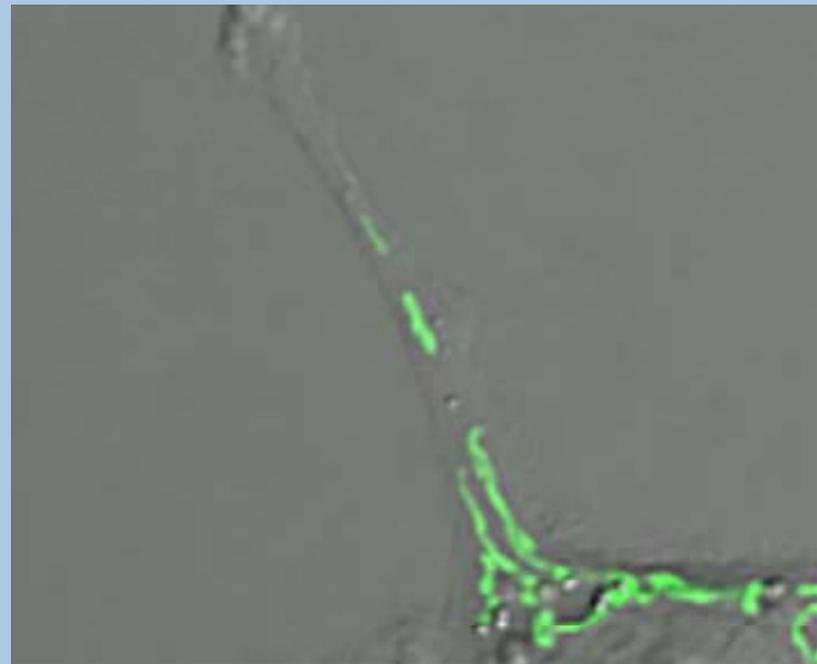
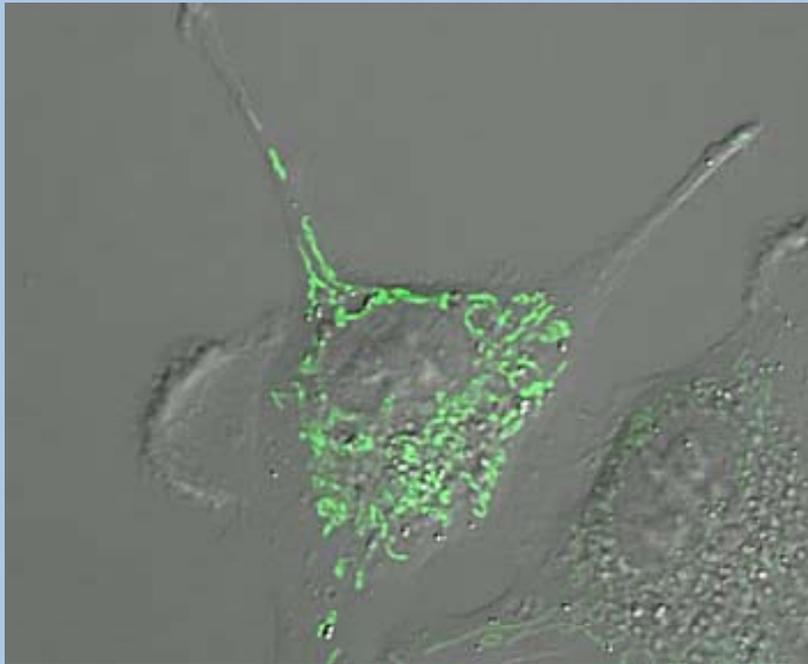
PA-GFP is activated with
405 nm light

Prior to activation, will
fluoresce at 8%
brightness of EGFP
(effectively "off")

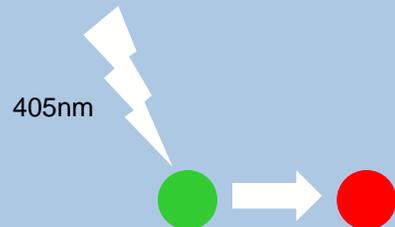
Will fluoresce at 40%
brightness after
activation



Photoconvertible Fluorescent Proteins: tdEos Photoconversion



RK-13 cells expressing tdEos Mitochondria



Photoconvertible Fluorescent Proteins



Photoconversion may be Common



Table 1 | Photoconversion properties

| | Color change | Photoactivation wavelength, power | Fold activation ^a | Residual fluorescence ^b | Change in contrast ^c |
|----------|-------------------|-----------------------------------|----------------------------------|--|---------------------------------|
| Katushka | Red to green | 750 nm, 17.9 mW | 6 ± 2 ^d | 0.22 ± 0.07 (0.30 ± 0.02) ^e | 27 (13) ^e |
| | | 405 nm, 1.4 mW | 7 ± 1 | 0.66 ± 0.03 (0.68 ± 0.03) | 10 (10) |
| | | 561 nm, 0.32 mW | 5 ± 4 | 0.06 ± 0.03 | 82 |
| mKate | Red to green | 750 nm, 17.9 mW | 11 ± 2 | 0.59 ± 0.08 (0.94 ± 0.09) | 19 (12) |
| | | 405 nm, 1.4 mW | 10 ± 3 | 0.41 ± 0.03 (0.8 ± 0.6) | 26 (13) |
| | | 561 nm, 0.32 mW | 2.0 ± 0.3 | 0.07 ± 0.03 | 29 |
| HcRed1 | Red to green | 561 nm, 0.32 mW | 26 ± 18 | 0.22 ± 0.09 | 119 |
| mOrange1 | Orange to far-red | 488 nm, 1.2 mW | 16 ± 4 | 0.10 ± 0.03 | 160 |
| mOrange2 | Orange to far-red | 488 nm, 1.2 mW | 16 ± 5 | 0.10 ± 0.02 | 161 |
| Kaede | Green to red | 405 nm, 0.38 mW | 28 ± 10 (1.8 ± 0.1) ^e | 0.19 ± 0.02 | 148 (10) |
| Dendra2 | Green to red | 405 nm, 0.25 mW | 47 ± 26 (1.2 ± 0.4) | 0.48 ± 0.16 | 97 (2) |

^aFold increase in photoconverted fluorescence. ^bFraction of original fluorescence remaining after photoconversion. ^cThe change in contrast was determined by the increase in photoconverted fluorescence divided by the residual original fluorescence. ^dValues are mean ± s.d. (n = 7). ^eValues in parentheses are for single-wavelength excitation (excitation at 488 nm).

- 8 of 12 proteins tested displayed some photoconversion
- Conversion rate increases supralinearly with laser power

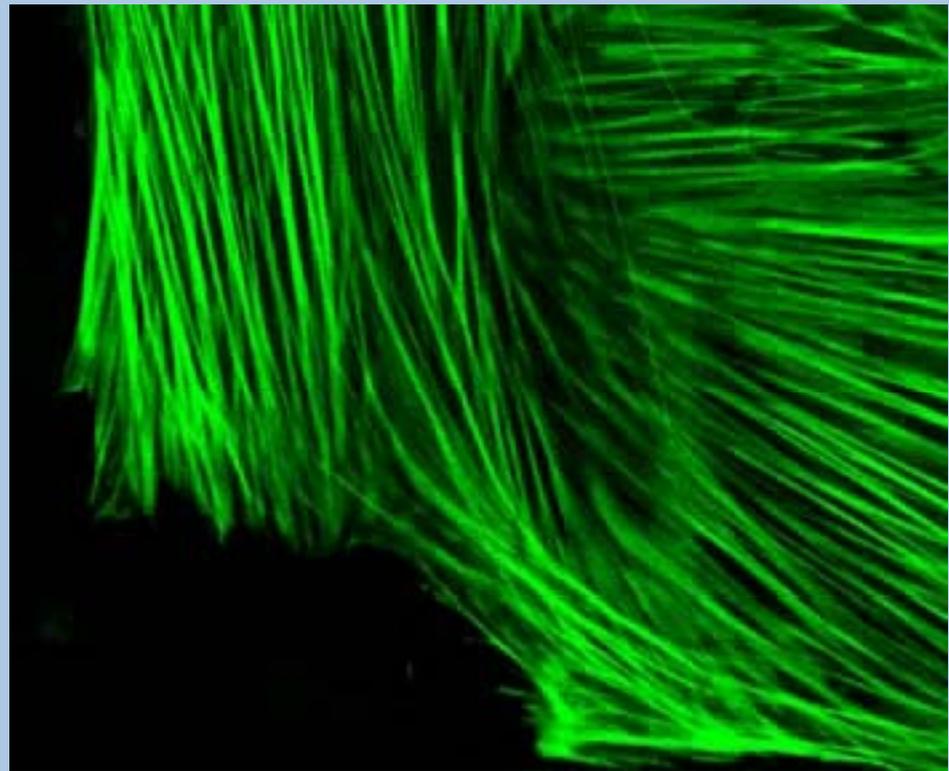
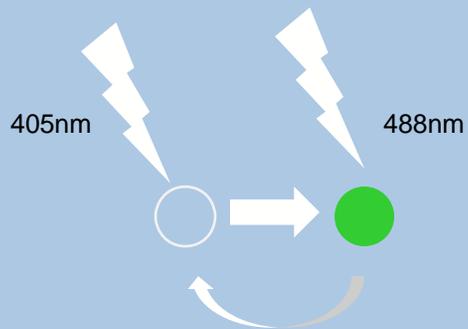


Nature Methods, 2009,(6), 5,355-360
J Schwartz/Duke

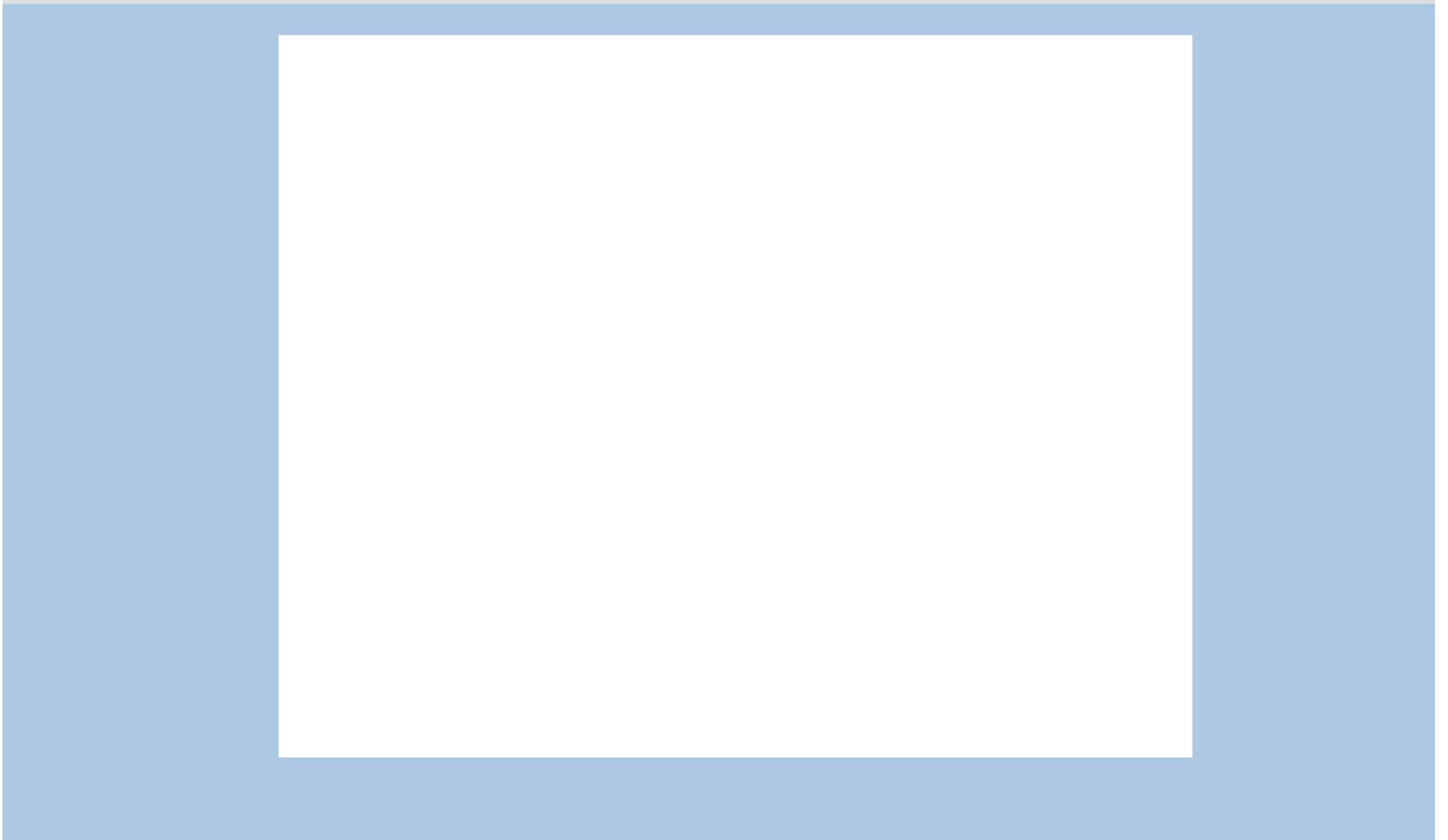
Photoswitching Fluorescent Proteins: Dronpa



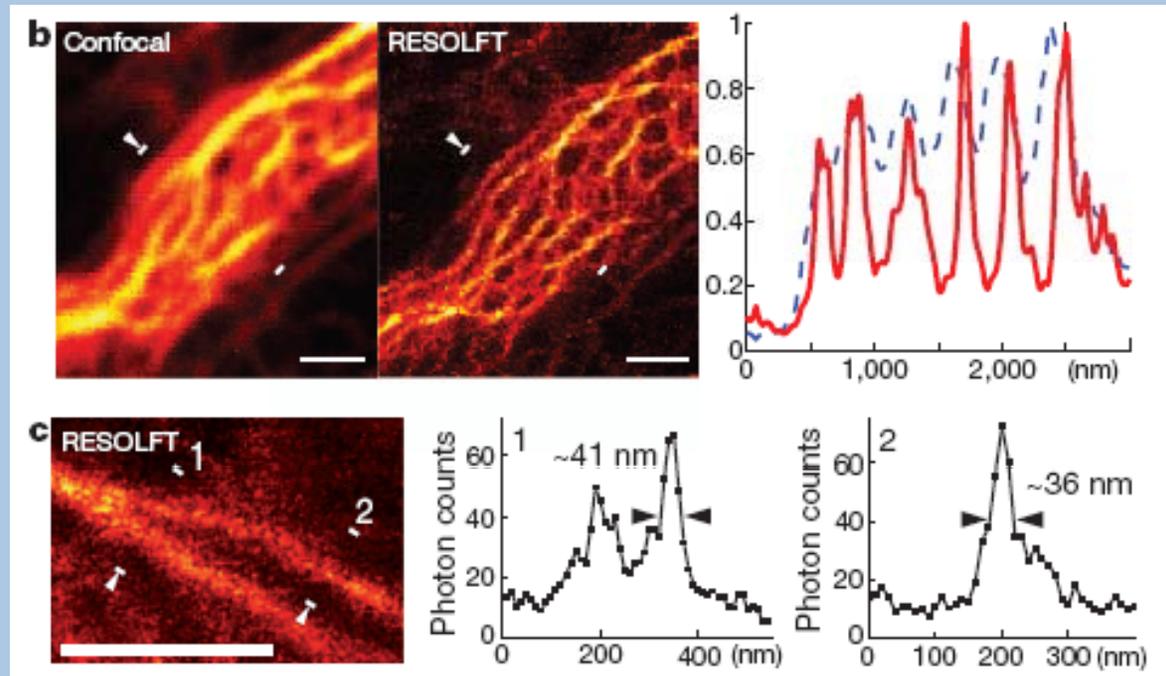
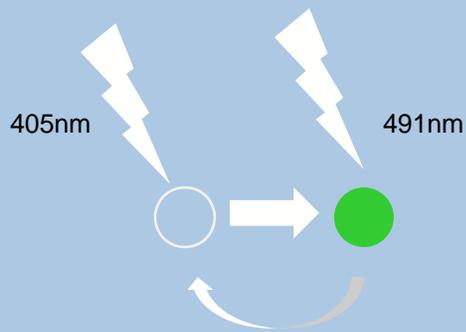
- A7r5 cells expressing Dronpa-Actin
- Capable of hundreds of cycles



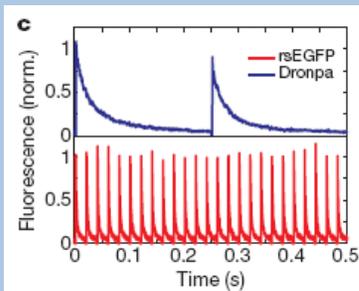
Photoswitching Fluorescent Proteins



Photoswitching Fluorescent Proteins: rsEGFP



ϵ ($M^{-1}cm^{-1}$)
47,000
QY
0.36
~50% GFP



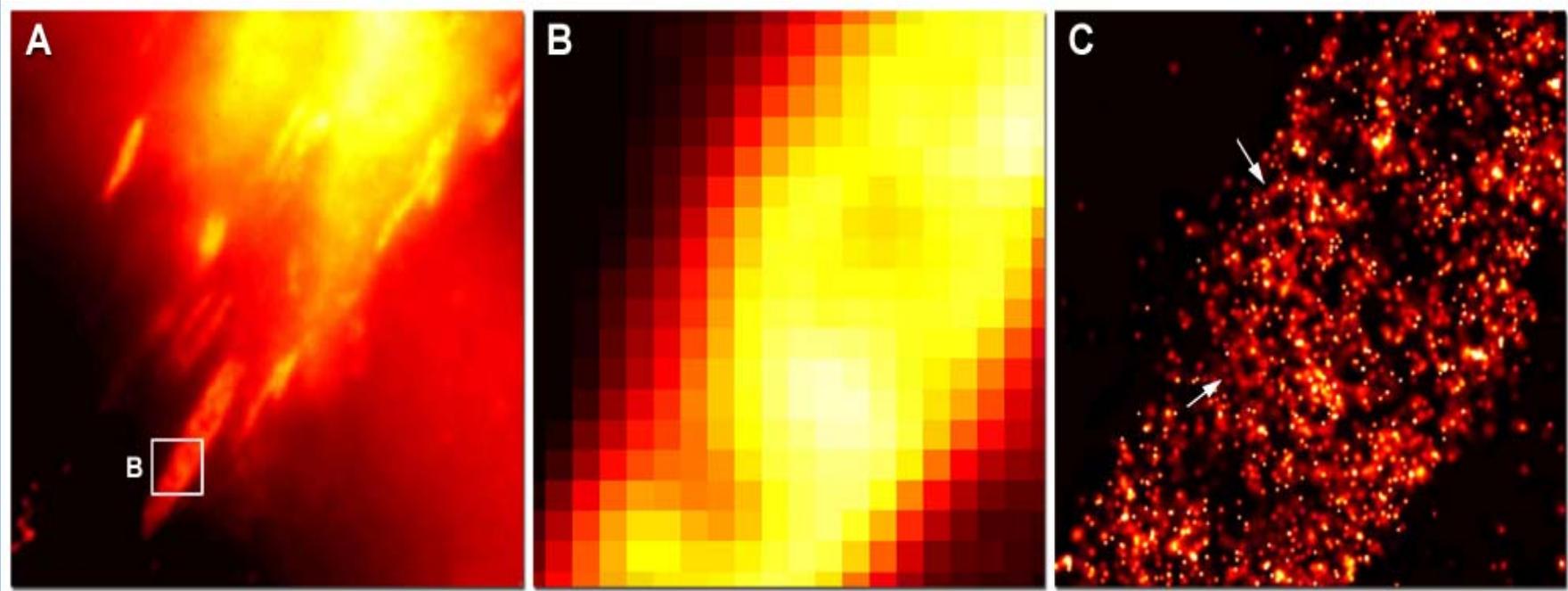
Grotjohann, T *et al*, Nature, 2011

Optical Highlighter Proteins: PALM - Photoactivated Localization Microscopy



Widefield TIRF Images

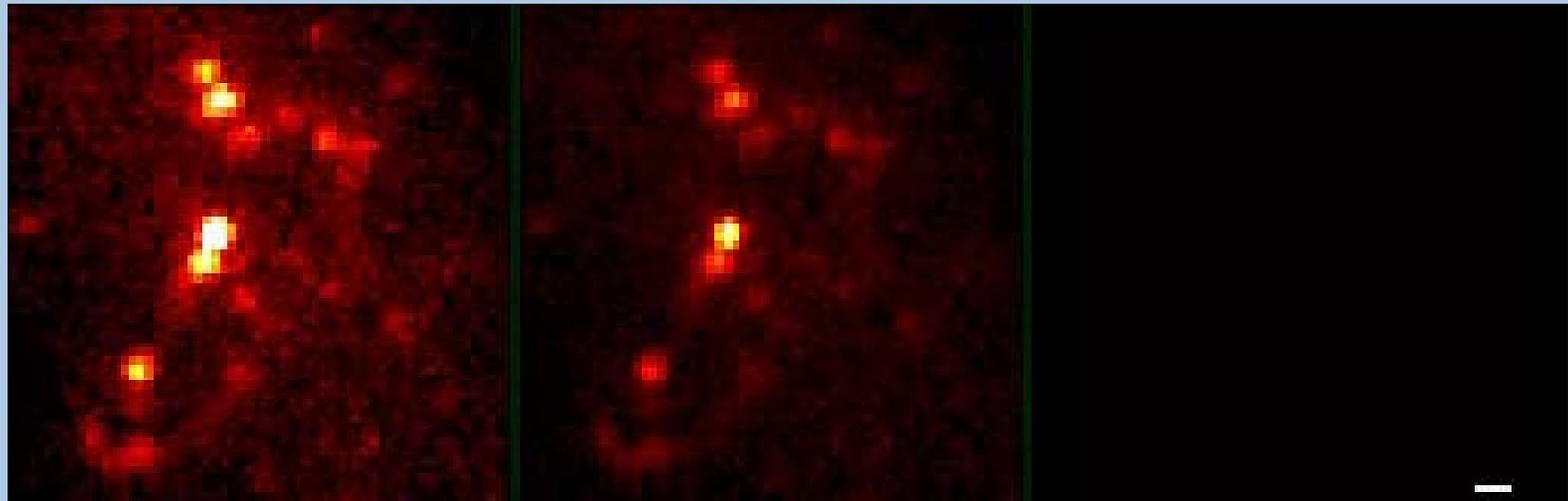
PALM Image



Tandem dimer-Eos-Vinculin in Fox Lung Fibroblast Cells

Optical Highlighter Proteins:

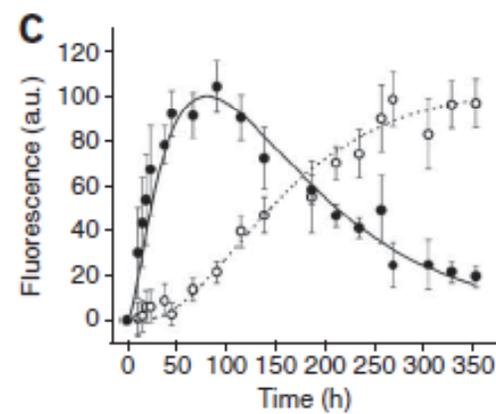
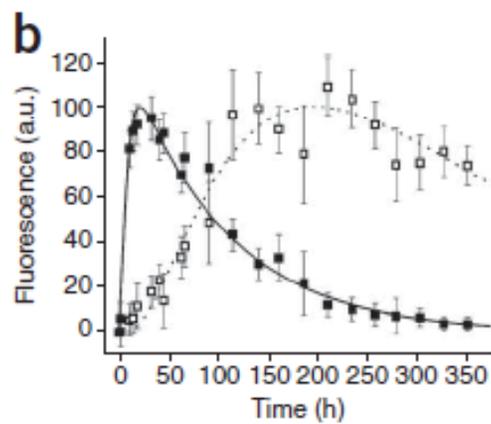
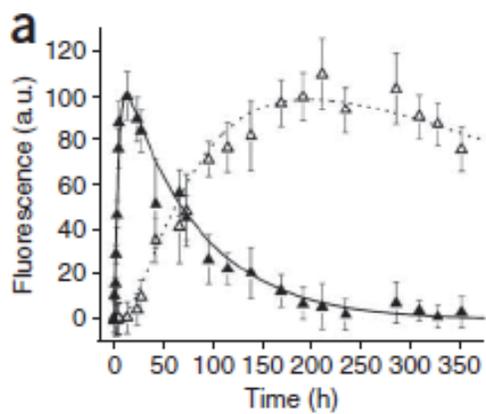
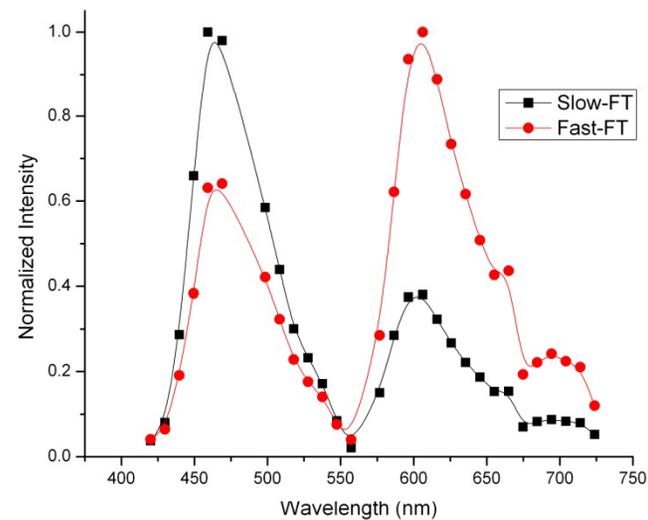
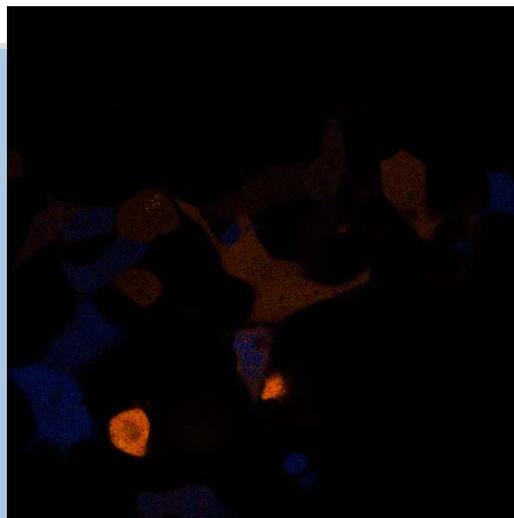
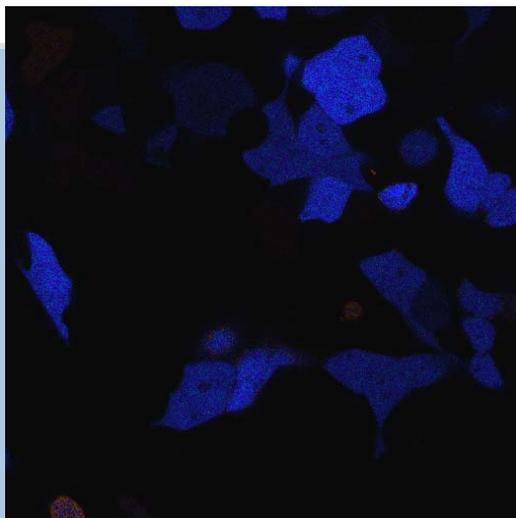
PALM - Photoactivated Localization Microscopy



1. Photoactivate and image PA species single molecules with a high degree of precision
2. Photobleach and repeat step 1 until all molecules are expended
3. Localize single molecule centers and construct super-resolution image

Fluorescent Protein Timers:

Fast-FT, Medium-FT and Slow-FT



Fluorescent Protein Timers:

Fast-FT, Medium-FT and Slow-FT

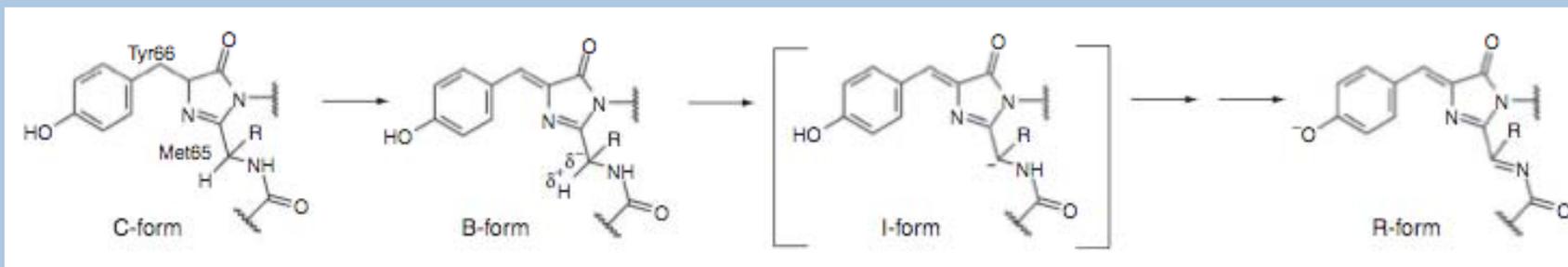


Table 1 Properties of the blue and red forms of the purified FTs

| Protein | | Excitation peak (nm) | Emission peak (nm) | Extinction coefficient ($M^{-1} cm^{-1}$) | Quantum yield | pK_a | Characteristic times (h) | | | |
|-----------|-----------|----------------------|--------------------|---|---------------|--------|--------------------------|-------|-------|-------|
| | | | | | | | 16 °C | 25 °C | 37 °C | 45 °C |
| Fast-FT | Blue form | 403 | 466 | 49,700 | 0.30 | 2.8 | 1.6 | 0.58 | 0.25 | 0.18 |
| | Red form | 583 | 606 | 75,300 | 0.09 | 4.1 | 42 | 18 | 7.1 | 4.2 |
| Medium-FT | Blue form | 401 | 464 | 44,800 | 0.41 | 2.7 | 2.2 | 1.6 | 1.2 | 0.70 |
| | Red form | 579 | 600 | 73,100 | 0.08 | 4.7 | 23 | 8.8 | 3.9 | 2.4 |
| Slow-FT | Blue form | 402 | 465 | 33,400 | 0.35 | 2.6 | 33 | 20 | 9.8 | 7.3 |
| | Red form | 583 | 604 | 84,200 | 0.05 | 4.6 | 108 | 69 | 28 | 17 |

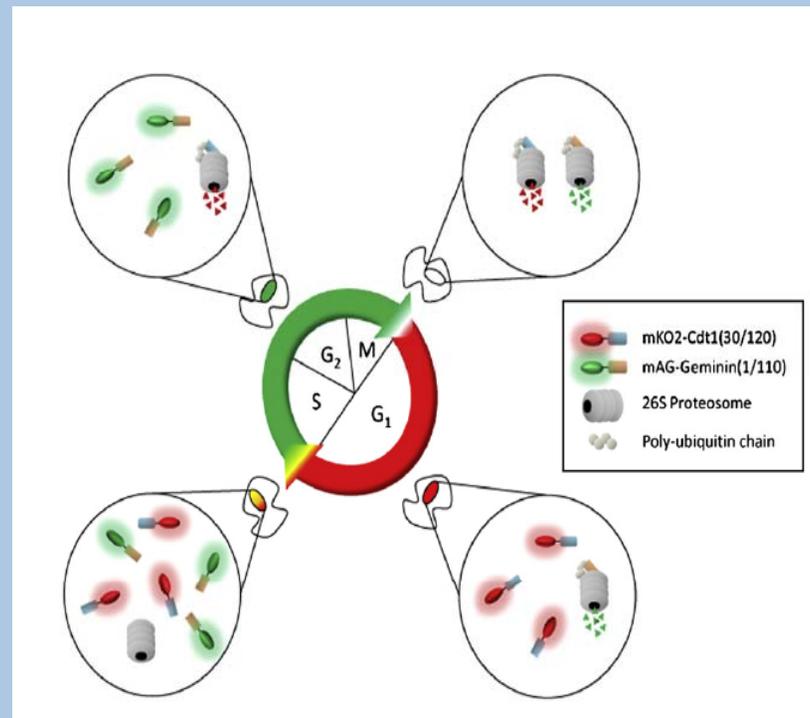
Characteristic times correspond to fluorescence maxima for the blue forms, and to maturation half-times for the red forms (Fig. 2c).

FUCCI:

Fluorescent Ubiquitination Cell Cycle Indicator



- mAzami Green Geminin expressed during S/G₂/M Phases
- mKusabira Orange 2 Cdt1 expressed during G₁ Phase
- Ubiquitination targets proteins for destruction during other cell Cycle phases via the proteasome

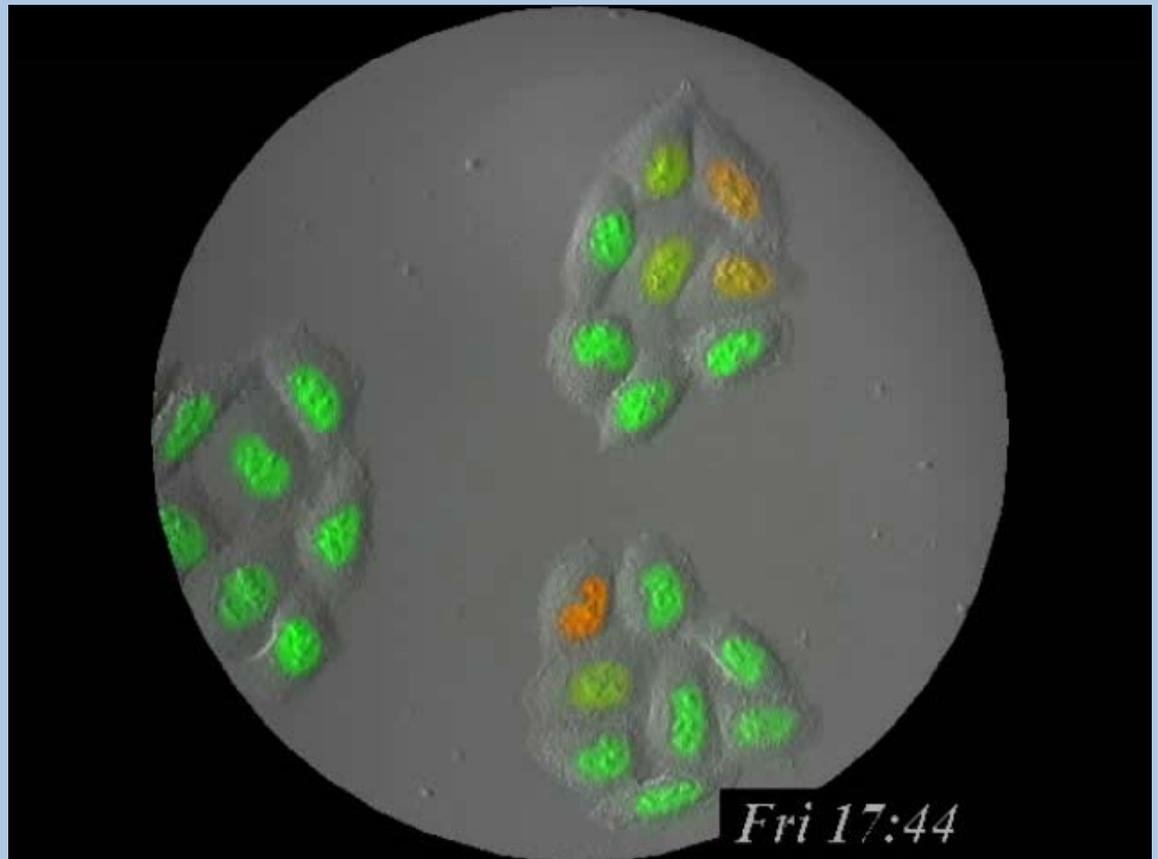


FUCCI:

Fluorescent Ubiquitination Cell Cycle Indicator



- HeLa cells expressing the mAG-Geminin and mKO2 Cdf1 plasmids
- All phases of cell cycle are visible



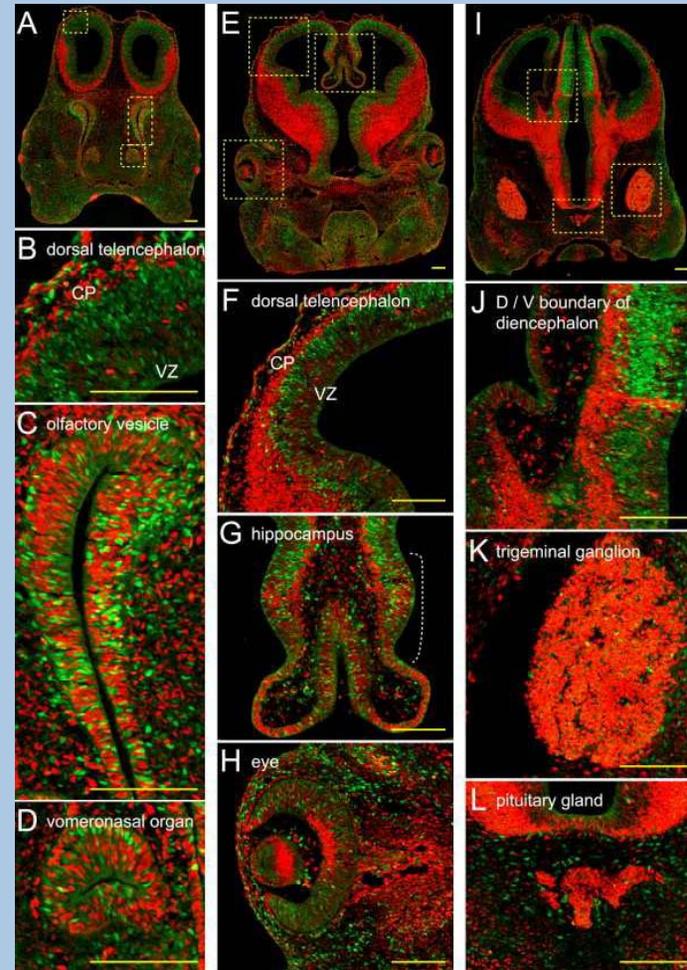
FUCCI:

Fluorescent Ubiquitination Cell Cycle Indicator

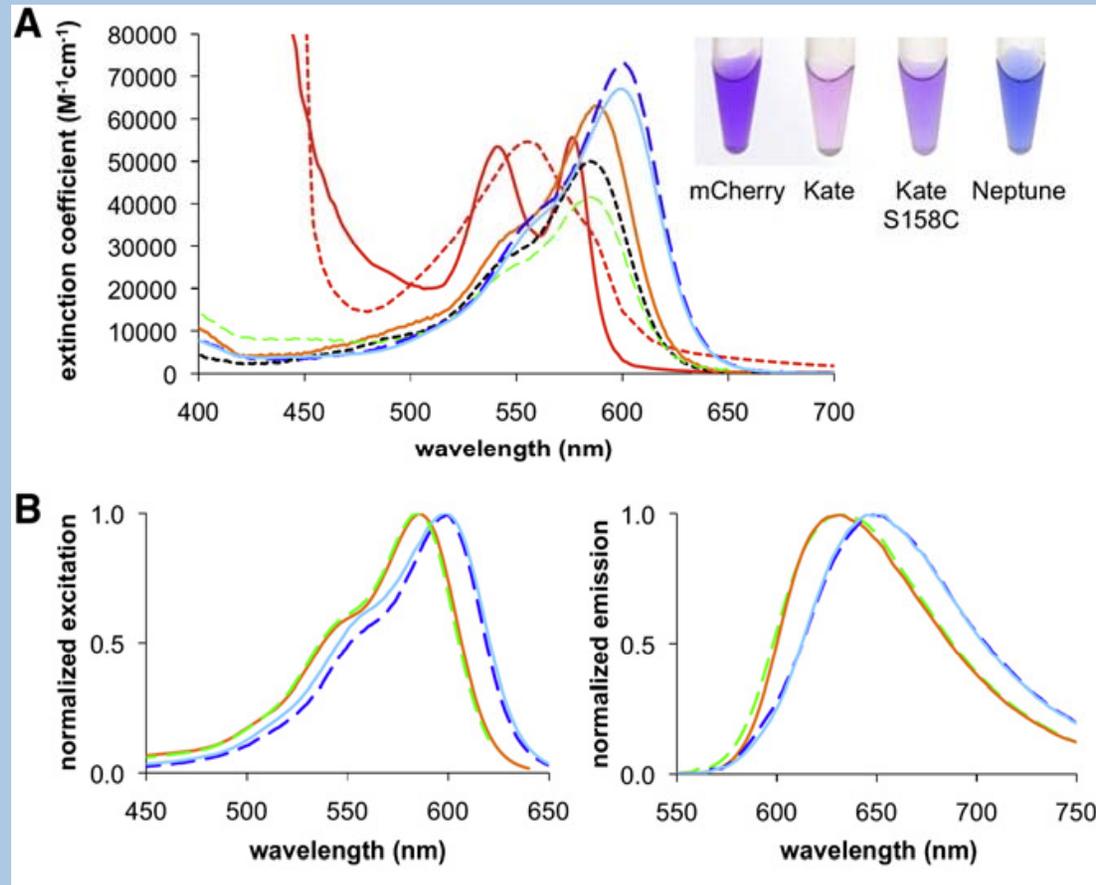


- Transgenic mice embryos expressing FUCCI have balanced red and green expression

- Green/Red ratio decreases over time



Imaging with Fluorescent Proteins: Neptune – Optimized for Intravital Imaging



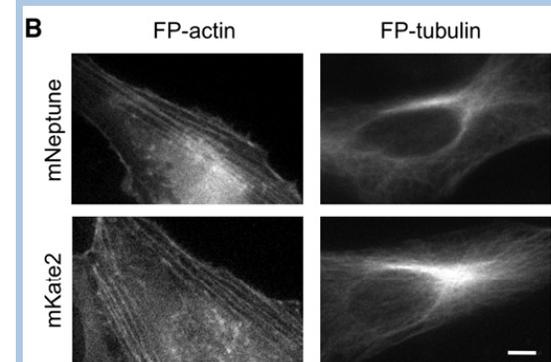
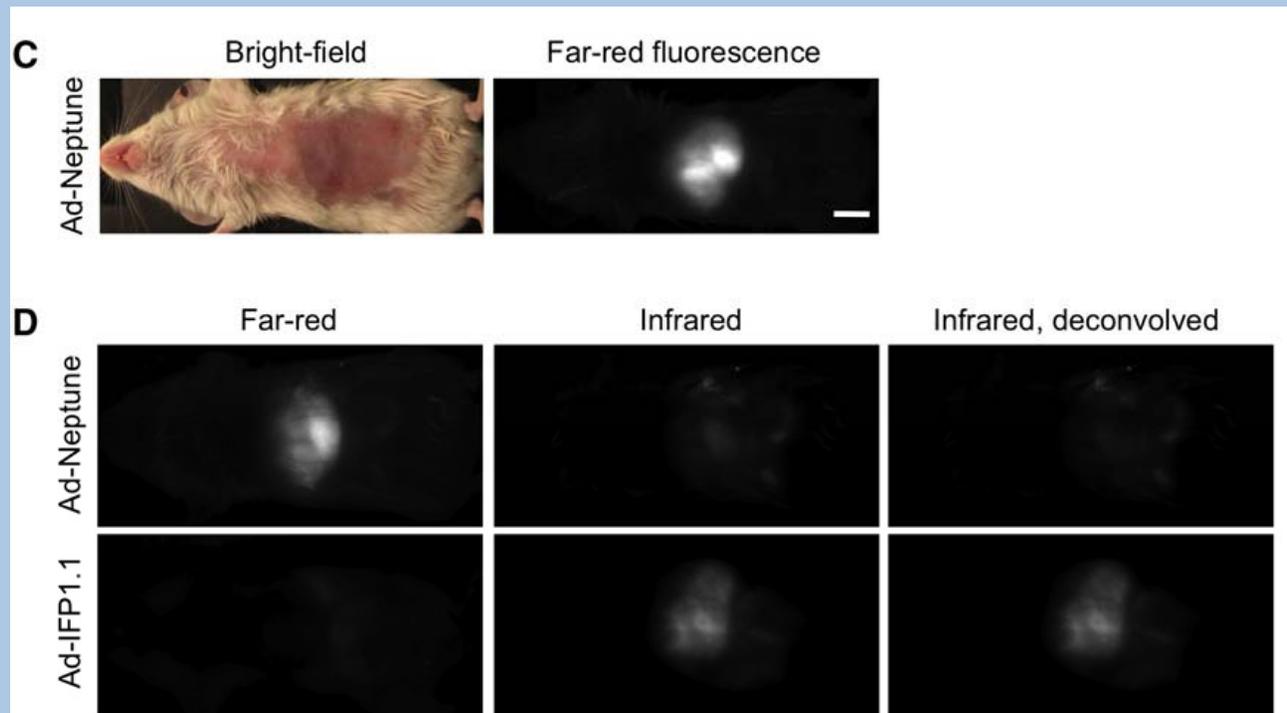
Imaging with Fluorescent Proteins: Neptune – Optimized for Intravital Imaging



Table 1. Characteristics of Far-Red Fluorescent Proteins

| Protein | Excitation Peak ^a | Emission Peak ^a | ϵ^b | ϕ^c | Brightness Excited at Peak ^d | Brightness Excited at 633 nm ^e | Photostability ^f |
|---------------------------------------|------------------------------|----------------------------|---------------------|----------|---|---|-----------------------------|
| dTomato ^g | 554 | 581 | 69,000 | 0.69 | 48 | 0 | 64 |
| mCherry ^g | 587 | 610 | 72,000 | 0.22 | 16 | 0.084 | 68 |
| mPlum ^g | 590 | 649 | 41,000 | 0.10 | 4.1 | 0.26 | 53 |
| mRaspberry ^g | 598 | 625 | 86,000 | 0.15 | 13 | 0.45 | 15 |
| mGrape3 | 608 | 646 | 40,000 ^h | 0.03 | 1.2 | 0.29 | 5 |
| mKate | 585 | 635 | 42,000 | 0.30 | 13 | 0.43 | 82 |
| mKate S158A | 585 | 630 | 75,000 | 0.30 | 23 | 0.48 | 327 |
| mKate2 | 586 | 630 | 50,000 | 0.36 | 18 | 0.42 | ND |
| mKate S158C | 586 | 630 | 63,000 | 0.33 | 21 | 0.56 | 220 ^j |
| mKate M41G S158C | 593 | 648 | 73,000 | 0.22 | 16 | 1.2 | ND |
| Neptune (mKate M41G S61C S158C Y197F) | 600 | 650 | 72,000 | 0.18 | 13 | 1.7 | 185 |
| mNeptune (Neptune M146T) | 600 | 650 | 67,000 | 0.20 | 13 | 1.8 | 160 |

Imaging with Fluorescent Proteins: Neptune – Optimized for Intravital Imaging

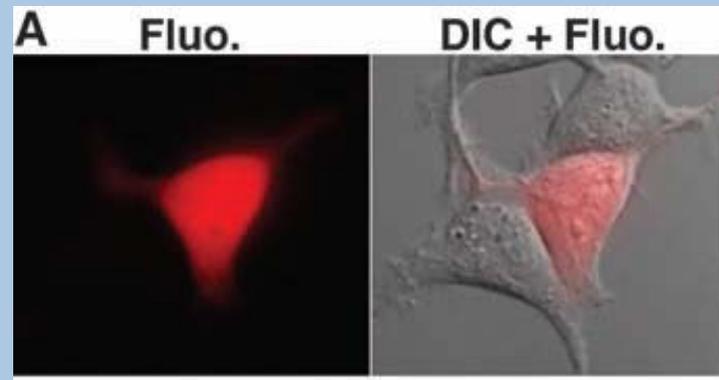
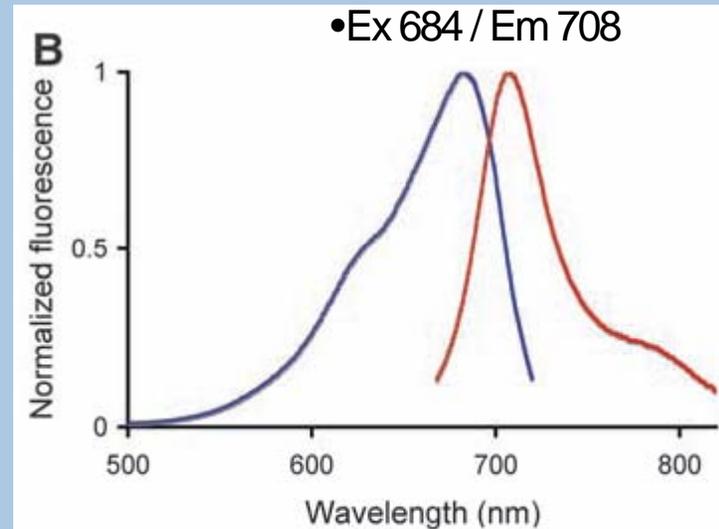


Imaging with Fluorescent Proteins:

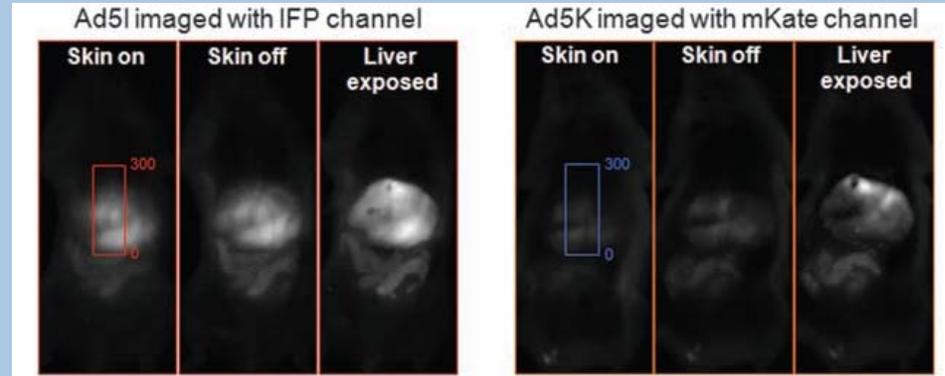
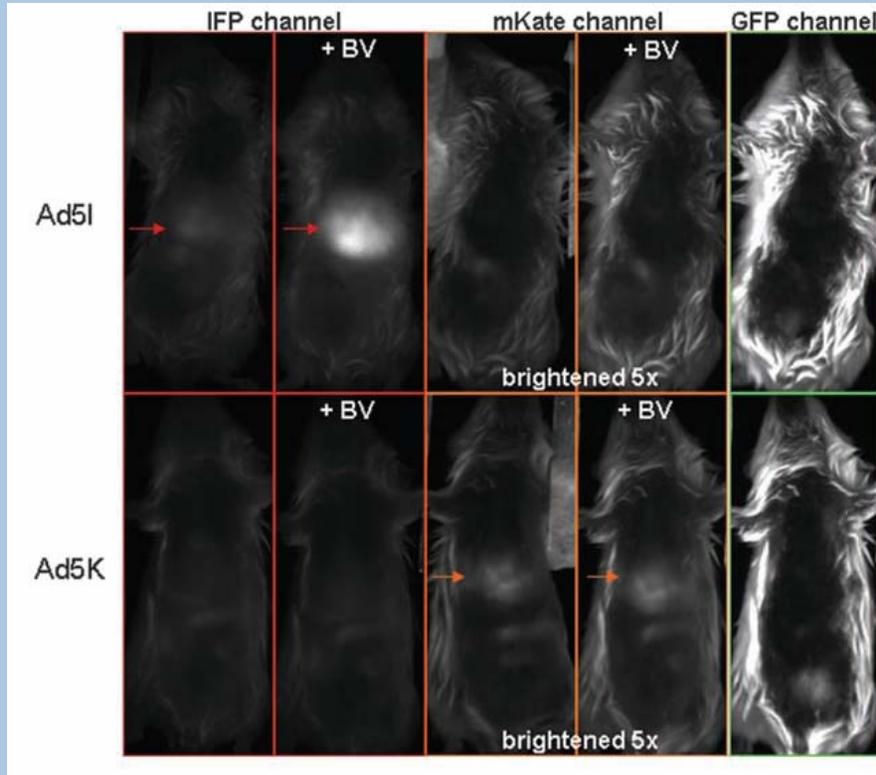
Infrared Fluorescent Proteins – IFP 1.4



- Bacteriophytochrome of *Deinococcus radiodurans*
Biliverdin as chromophore
- More than 1500 bacteriophytochrome like sequences identified



Imaging with Fluorescent Proteins: Infrared Fluorescent Proteins – IFP 1.4



Imaging with Fluorescent Proteins: mCerulean3



| Variant | ϵ ($M^{-1}cm^{-1}$) | Quantum Yield | Brightness | relative to EGFP | Bleaching ($t_{1/2}$) (s) |
|-------------------|--------------------------------|---------------|-------------|------------------|-----------------------------|
| ECFP | 27,000 | 0.30 | 8.1 | 0.25 | |
| mCerulean | 43,000 | 0.48 | 20.64 | 0.65 | 58 |
| mCerulean2 | 47,000 | 0.60 | 28.2 | 0.88 | 25 |
| mCerulean3 | 40,000 | 0.87 | 34.8 | 1.09 | 1,100 |
| EGFP | 55,000 | 0.58 | 31.9 | 1.00 | |
| mVenus | 96,000 | 0.52 | 49.92 | 1.56 | |
| mCherry | 72,000 | 0.22 | 15.84 | 0.50 | |
| TagRFP-T | 81,000 | 0.41 | 33.21 | 1.04 | |
| mPlum | 41,000 | 0.10 | 4.1 | 0.13 | |

- mCerulean3
 - brighter than EGFP
 - 20X more photostable than previous CFPs
 - no reversible photoswitching

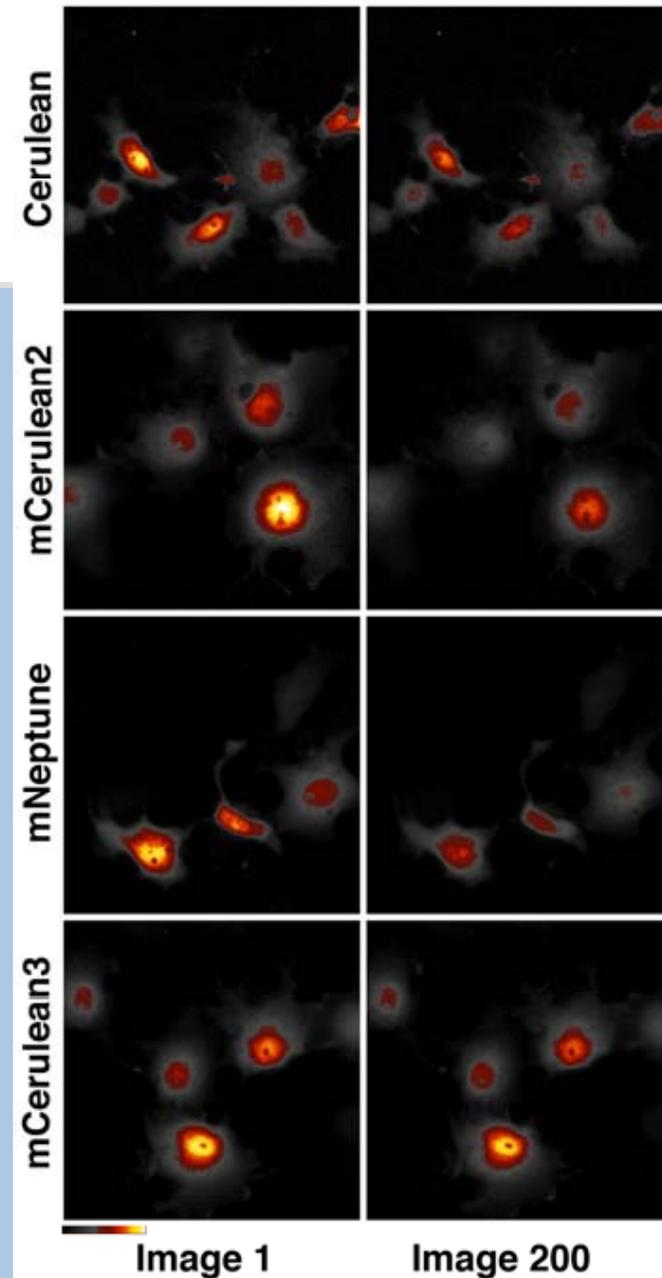
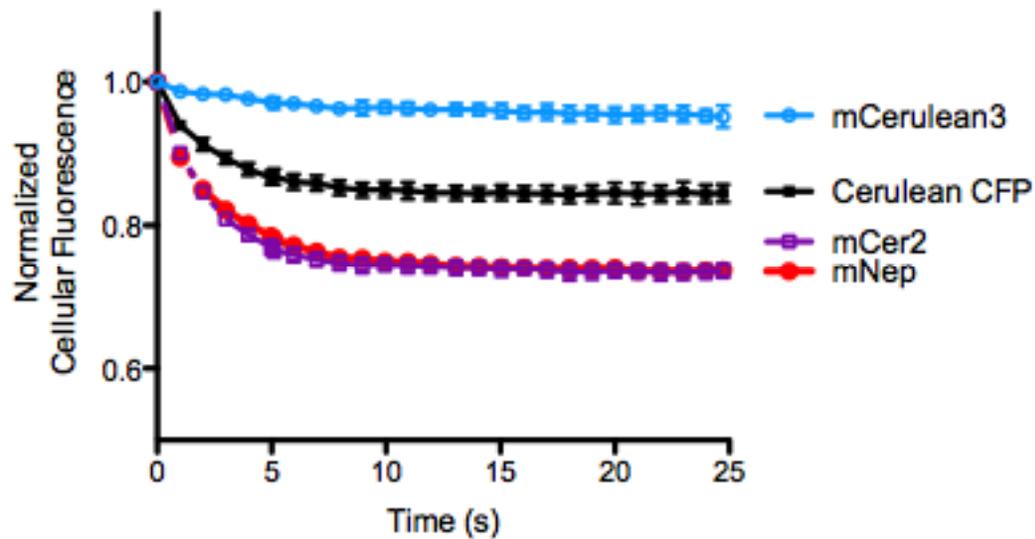
Markwardt, M et al, 2011, PlosONE

Courtesy of Mark Rizzo,
mrizz001@umaryland.edu

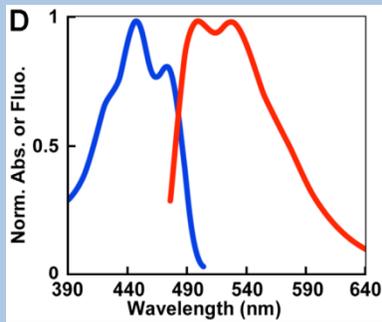
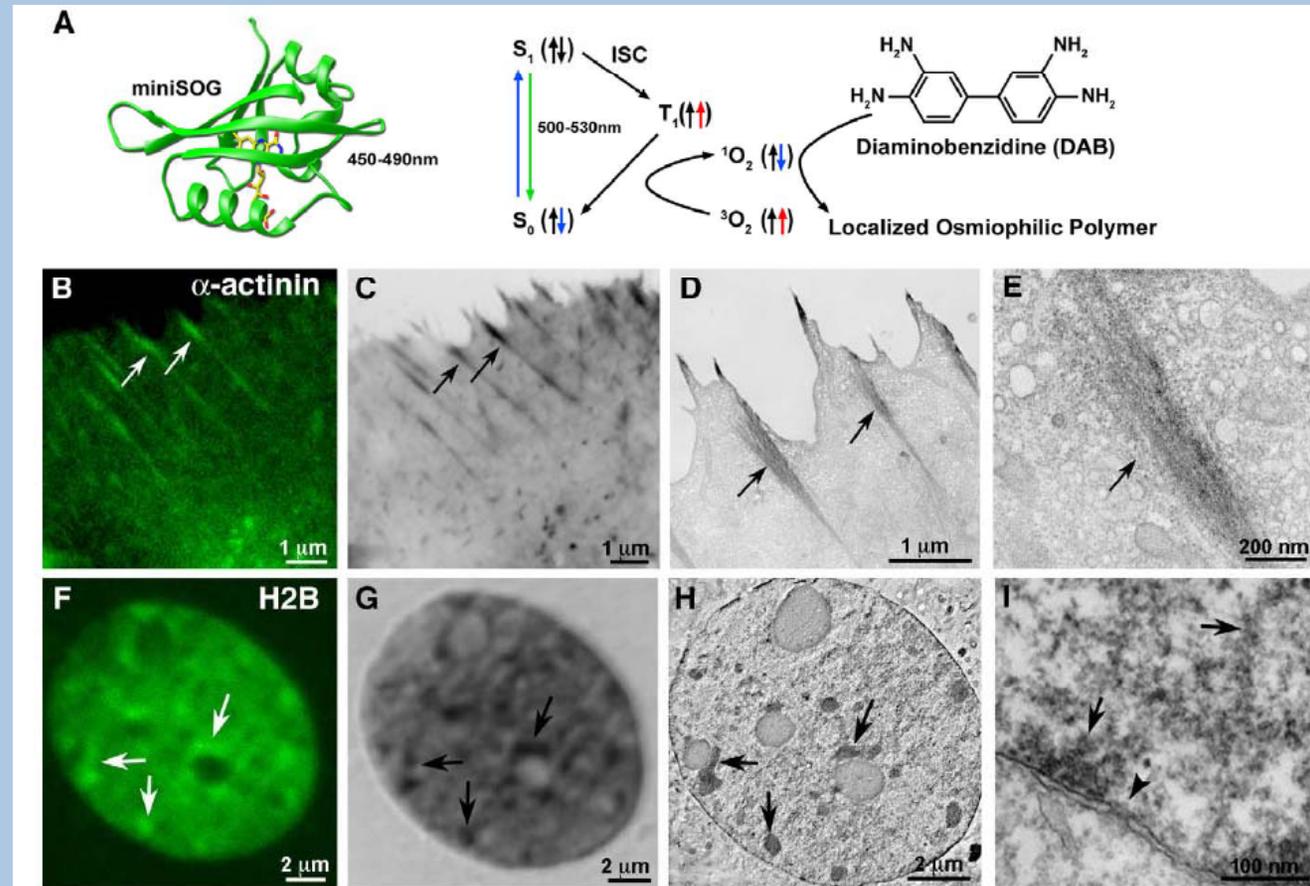
Imaging with Fluorescent Proteins:

mCerulean3 – Performance in Live Cells

- COS7 cells were imaged continuously for 200 frames
- mCerulean3 shows little bleaching in live cells

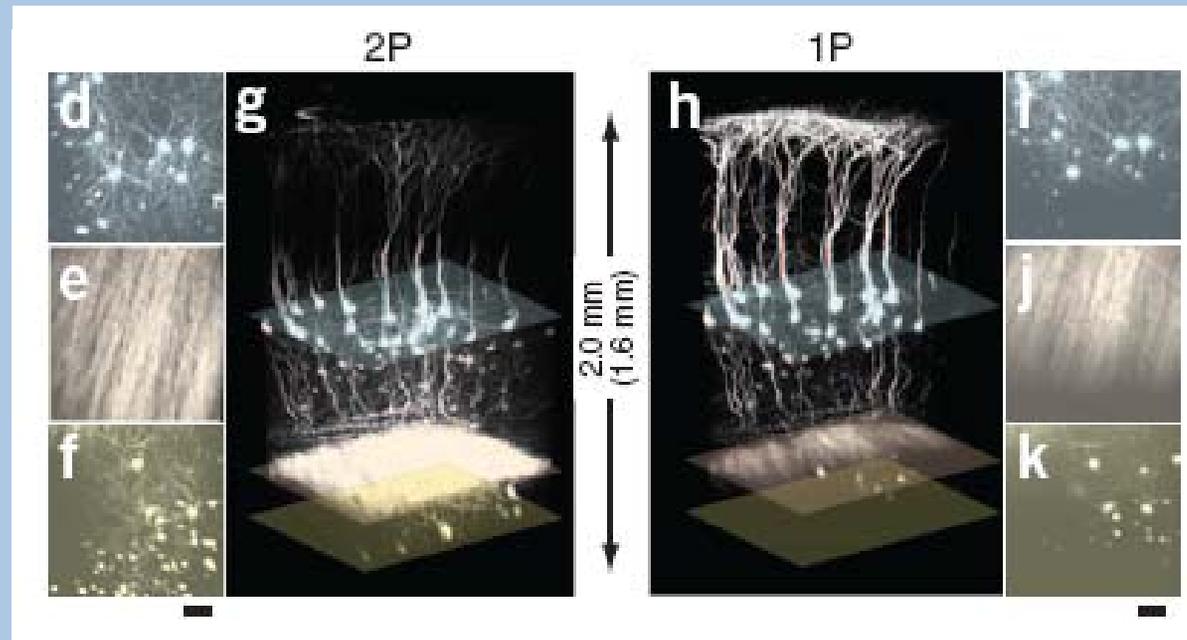


Imaging with Fluorescent Proteins: MiniSOG for Correlative Microscopy



~16% brightness of GFP

Imaging with Fluorescent Proteins: Scale Clearing Tissues



- Scale solution inexpensive mix of Urea, Glycerol and Triton X-100
- Preserves fluorescence of FPs
- Imaging depth limited by working distance!

Popular Fluorescent Proteins

| Protein | Reference | Excitation, nm | Emission, nm | Brightness, % of EGFP |
|----------------|--|----------------|--------------|-----------------------|
| Sirius | Tomosugi et al., Nat. Methods, 2009 | 355 | 424 | 12 |
| Azurite | Mena et al., Nat. Biotechnol., 2006, 24, 1569 | 383 | 447 | 43 |
| EBFP2 | Ai et al., Biochemistry, 2007, 46, 5904 | 383 | 448 | 60 |
| mTagBFP/TagBFP | Chem. Biol. 2008, 15, 1116. www.evrogen.com | 400 | 456 | 105 |
| Cerulean | Rizzo et al., Nat. Biotechnol., 2004, 22, 445 | 433 | 475 | 79 |
| ECFP | www.clontech.com | 439 | 476 | 39 |
| CyPet | Nguyen et al., Nat. Biotechnol., 2005, 23, 355 | 435 | 477 | 53 |
| TagCFP | www.evrogen.com | 458 | 480 | 84 |
| AzamiGreen | www.mblintl.com | 492 | 505 | 121 |
| TagGFP2 | www.evrogen.com | 482 | 505 | 100 |
| EGFP | www.clontech.com | 484 | 507 | 100 |
| Emerald | Cubitt et al., Methods Cell. Biol., 1999, 58, 19 | 487 | 509 | 116 |
| T-Sapphire | Zapata-Hommer et al., BMC Biotechnol., 2003, 3 | 399 | 511 | 78 |
| TagYFP | www.evrogen.com | 508 | 524 | 137 |
| EYFP | www.clontech.com | 514 | 527 | 151 |
| Topaz | Cubitt et al., Methods Cell. Biol., 1999, 58, 19 | 514 | 527 | 169 |
| Venus | Nagai et al., Nat. Biotechnol., 2002, 20, 87 | 515 | 528 | 156 |
| Citrine | Griesbeck et al., J. Biol. Chem., 2001, 276, 29188 | 516 | 529 | 174 |
| YPet | Nguyen et al., Nat. Biotechnol., 2005, 23, 355 | 517 | 530 | 238 |
| mKO | www.mblintl.com | 548 | 559 | 92 |
| mKOk | Tsutsui et al., Nat. Methods, 2008, 5, 683. | 551 | 563 | 200 |

| Protein | Reference | Excitation, nm | Emission, nm | Brightness, % of EGFP |
|--------------------------|--|----------------|--------------|-----------------------|
| mOrange/mOrange2 | Shaner et al., Nat. Biotechnol., 2004, 22, 1524 | 548 | 562 | 146 |
| dTomato (dimer) | Shaner et al., Nat. Biotechnol., 2004, 22, 1524 | 554 | 581 | 142 |
| DsRed2 (tetramer) | www.clontech.com | 558 | 583 | 176 |
| DsRed-Express (tetramer) | www.clontech.com | 555 | 584 | 58 |
| TagRFP/TagRFP-T | Merzlyak et al., Nat. Methods, 2007, 4, 555. www.evrogen.com | 555 | 584 | 146 |
| DsRed-monomer | www.clontech.com | 556 | 586 | 10 |
| mStrawberry | Shaner et al., Nat. Biotechnol., 2004, 22, 1524 | 574 | 596 | 78 |
| mCherry | Shaner et al., Nat. Biotechnol., 2004, 22, 1524 | 587 | 610 | 47 |
| LSS-mKate1 | Piatkevich et al., PNAS, 2010, in press. | 463 | 624 | 10 |
| LSS-mKate2 | Piatkevich et al., PNAS, 2010, in press. | 460 | 605 | 16 |
| mKeima | Kogure et al., Nat. Biotechnol., 2006, 24, 577 | 440 | 620 | 12 |
| mRaspberry | Wang et al., PNAS, 2004, 101, 16745 | 598 | 625 | 37 |
| Katushka2 (dimer) | Shcherbo et al., Nat. Methods, 2007, 4, 741 | 588 | 635 | 67 |
| mKate2 (TagFP635-2) | Shcherbo et al., Nat. Methods, 2007, 4, 741. www.evrogen.com | 588 | 635 | 45 |
| E2-Crimson (tetramer) | Strack et al., Biochemistry, 2009, 48, 8279 | 611 | 646 | 59 |
| mPlum | Wang et al., PNAS, 2004, 101, 16745 | 590 | 649 | 12 |
| mNeptune (dimer!) | Lin et al., Chem. Biol., 2009, 16, 1169 | 600 | 650 | 25 |

Imaging with Fluorescent Proteins:

Fluorescent Protein Summary



FPs considered obsolete:

DsRed variants, mRFP1
EYFP (weak dimer)
ECFP (weak dimer)

Replacement

mCherry, mApple
mCitrine, YPet, mVenus
mCerulean3, mTurquoise

New Colors

Sirius; Azurite; EBFP2; mTagBFP2 (blue)
mTFP1 (teal)
sfGFP; mWasabi (green)
mKusabira Orange (mKO; mKO2 yellow-orange)
TagRFP (orange-red); TagRFP-T
tdTomato (orange-red); mOrange2
mApple; mRuby (red)
mPlum; tdKatushka; mKate; mKate2; mNeptune (deep red)

Web Resources:

Fluorescent Proteins and Microscopy



Molecular Expressions Microscopy Primer: Specialized Microscopy Techniques - Fluorescence - Fluorescent Proteins - Mozilla Firefox

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http://www.molecularexpressions.com/primer/techniques/fluorescence/fluorescentp

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James W. Queen
Queen Microscope
(circa 1870s)

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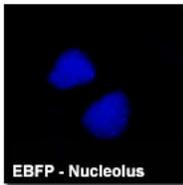
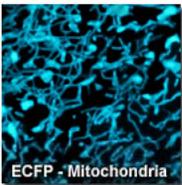
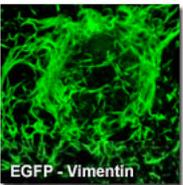
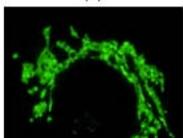
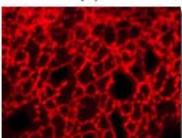
The Galleries:
Photo Gallery

Fluorescence Microscopy and Live-Cell Imaging

Fluorescent Proteins

The discovery and development, over the past decade, of fluorescent proteins from a wide variety of marine organisms has initiated a revolution in the study of cell behavior by providing convenient markers for gene expression and protein targeting in living cells and organisms. The most widely used of these fluorescent proteins, the green fluorescent protein (**GFP**) first isolated from the jellyfish *Aequorea victoria*, can be attached to virtually any protein of interest and still fold into a fluorescent molecule. The resulting GFP fusion product can be used to localize previously uncharacterized proteins or to visualize and track known proteins to further understand cellular events. The use of fluorescent proteins as a minimally invasive tool for studying protein dynamics and function has been stimulated by the engineering of genetic variants with improved brightness, photostability and expression properties (see Figure 1). Cells that express gene products tagged with fluorescent proteins can be imaged with low light intensities over many hours to provide useful information about changes in the steady-state distribution of a protein over time.

Digital Imaging of Localized Fluorescent Protein Chimeras

| | | |
|--|--|---|
|  EBFP - Nucleolus (a) |  ECFP - Mitochondria (b) |  EGFP - Vimentin (c) |
|  |  |  |

Web Resources: Fluorescent Proteins and Microscopy



ZEISS Online Campus | Fluorescent Proteins - Mozilla Firefox

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http://zeiss-campus.magnet.fsu.edu/articles/probes/index.html

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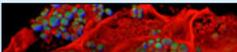
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Product Info



Spinning Disk Microscopy

Featured Gallery



Fluorescent Protein Technology

It took over thirty years, and the advent of recombinant DNA as well as vastly improved molecular biological approaches to see the pioneering work of Osamu Shimomura developed into a useful tool for live-cell imaging by Doug Prasher and Martin Chalfie. Just in the past decade, however, we have witnessed a truly remarkable expansion in the fluorescent protein palette, largely driven by the innovative studies from Roger Tsien's laboratory. Most of the fluorescent proteins that are commonly used today have been modified through mutagenesis to optimize their expression in biological systems. Continued efforts using directed evolution approaches will no doubt improve the spectral characteristics, photostability, maturation time, brightness, acid resistance, and utility of the fluorescent protein tags for cellular imaging.

Review Articles

-  **Introduction to Fluorescent Proteins** - The current thrust of fluorescent protein development strategies is centered on fine-tuning the current palette of blue to yellow variants from jellyfish, while simultaneously developing monomeric fluorescent proteins emitting in the orange to far-red regions of the visible light spectrum.
-  **Fluorescent Proteins Derived from *Aequorea victoria*** - We now have jellyfish proteins that span an 80-nanometer portion visible spectrum from deep blue to yellow-green, providing a wide choice of genetically encoded markers for studies in cell biology.



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