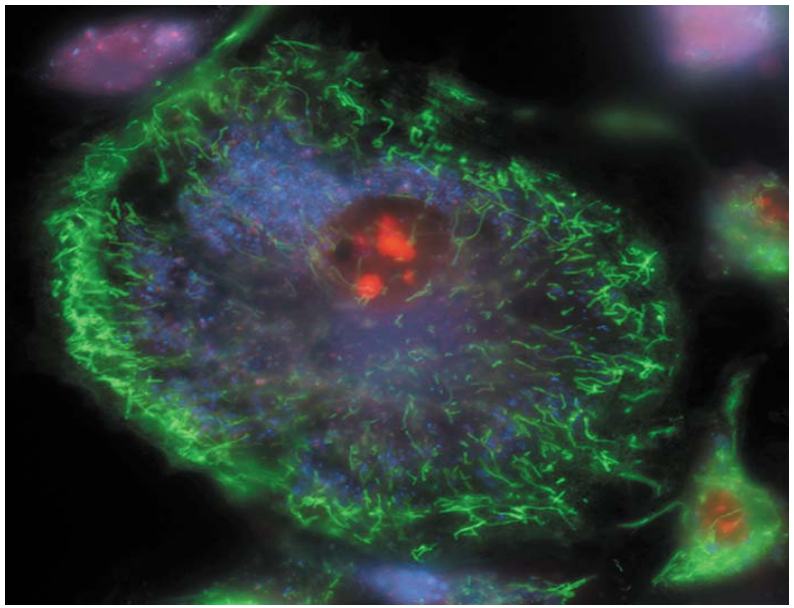
Visualizing Molecules in Living Cells: Fluorescent Tools

Small Molecule Dyes



MitoTracker® Green
http://www.lifetechnologies.com/us/en/home/life-science/cell-analysis/cell-structure/mitochondria.html

- Many small molecules are available that enter live cells, are fluorescent, and stain specific cellular structures
- Hoechst—DNA/nucleus
  - Any dna stain can be toxic over long time periods
  - Also require near UV excitation
- Mitotracker ®--mitochondria
  - Available in many colors
- Various other molecules are available—see Invitrogen/Molecular Probes
  - Microtubules—taxol
  - Cytoplasm—CellTracker™
  - Endoplasmic Reticulum—ER-Tracker™
  - Various stages of endosomal transport
  - Golgi—ceramides
  - Nucleus—SYTO®
  - Plasma Membrane—Wheat Germ Agglutinin

- **Biosensors**
  - created by attaching one or more fluorescent proteins (in most cases) to a target protein or peptide that is sensitive to its biochemical environment

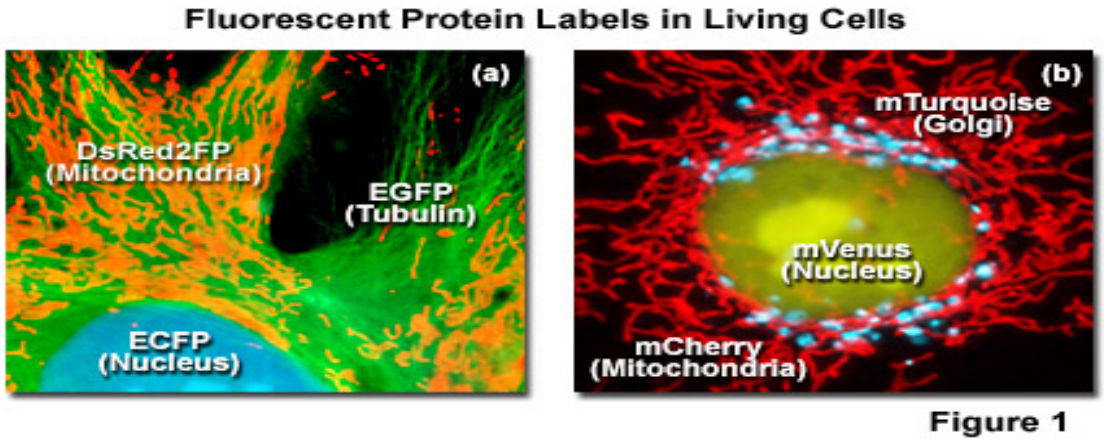


Figure 1

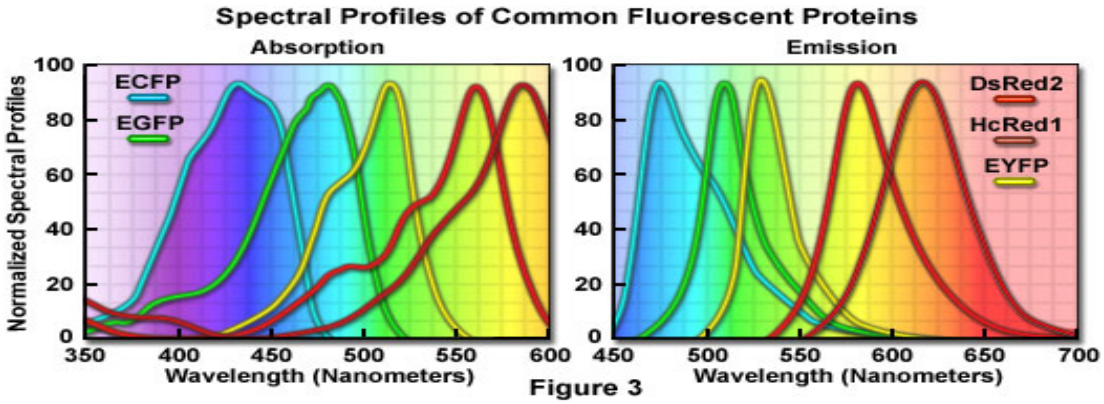
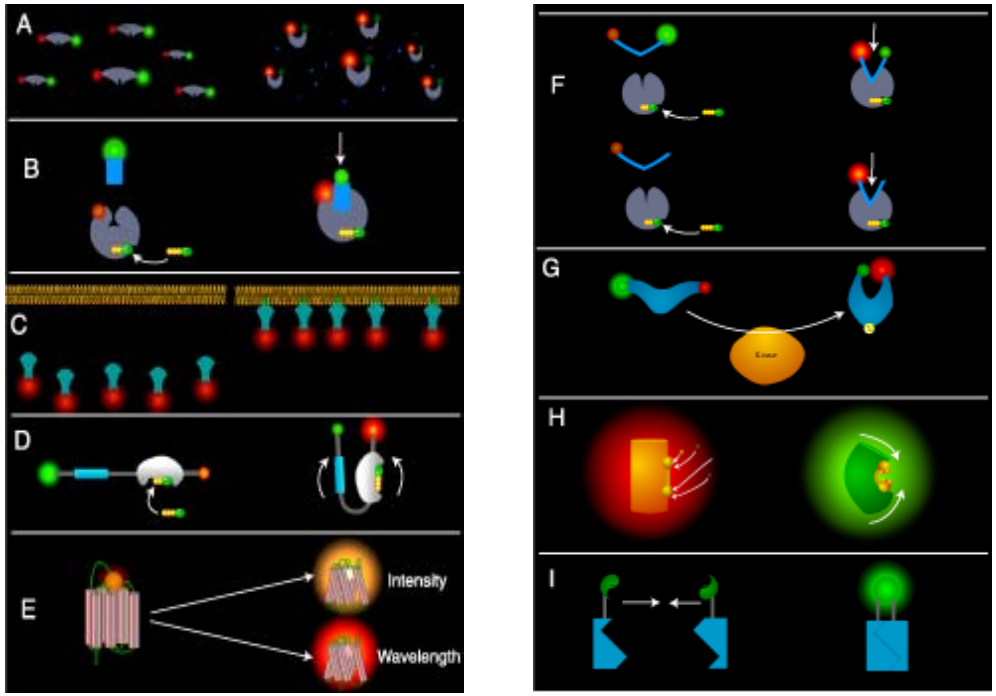


Figure 3

http://www.microscopyu.com/articles/livecellimaging/fpintro.html

- Fluorescent proteins can be targeted to various cellular locations by including defined peptides on their C or N termini
- Original FP was GFP—Nobel in 2008
- There are now a huge number of fluorescent proteins
  - Reviews Shaner et al., 2005 Nat Methods; Dean and Palmer, Nat Chem Biol 2014
  - Good website
    - http://nic.ucsf.edu/FPvisualization/
  - Some are photoswitchable and photoactivatable

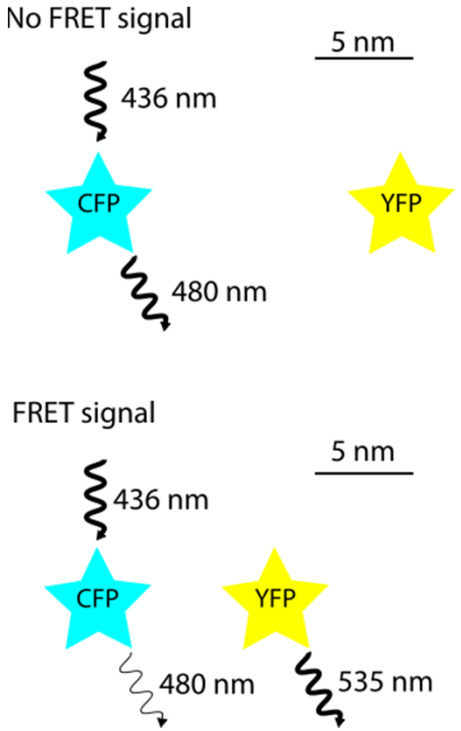
Various Types of Biosensors



F. Gaits, and K. Hahn Sci. STKE 2003;2003:pe3

- Most work based on fluorescence intensity in different locations of the cell, or on FRET between two fluorescent proteins (described more on next slide)

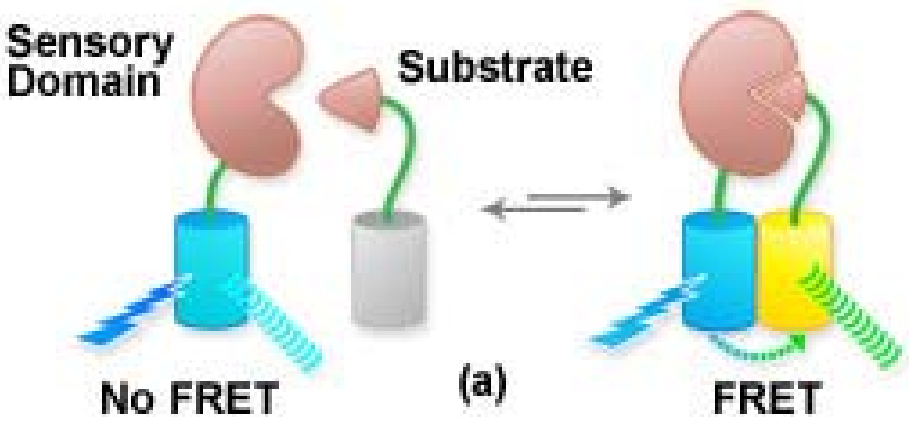
Förster Resonance Energy Transfer (FRET)



http://soft-matter.seas.harvard.edu/index.php/FRET

- A donor fluorophore non-radiatively transfers energy to an acceptor fluorophore
- Highly distance dependent
  - FRET efficiency (defined in a later slide) decays with the 6^{th} power of distance
- Requires spectral overlap between donor emission and acceptor excitation spectra
- Two main ways of measuring FRET
  - Intensity-based/ratiometry
  - Donor fluorescence lifetime
  - In either case, it is important that the donor excitation wavelength has minimal excitation of the acceptor
  - We will cover intensity-based as its more common and easier to implement

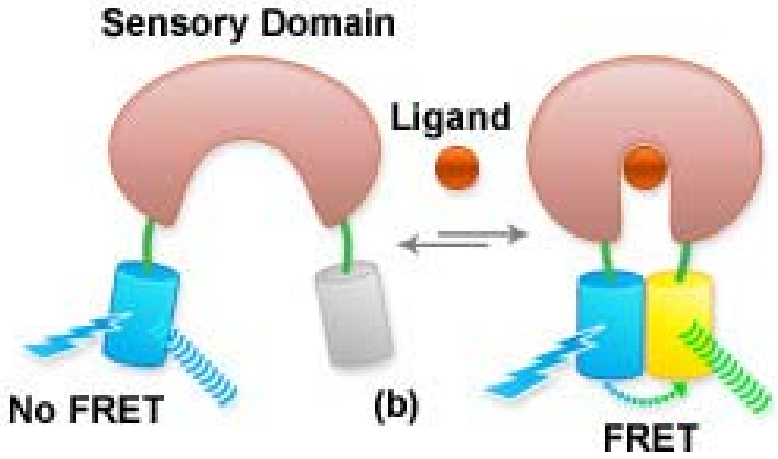
Intermolecular FRET



http://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/spectralfret.html

- One can quantify protein-protein interactions by evaluating FRET between independently tagged proteins
- Independent tagging means the stoichiometry of donor to acceptor is not fixed or known
- Requires a significant number of controls
  - (Ideally) Cells with no fluorophores
  - Cells with the donor alone
  - Cells with the acceptor alone
  - (Ideally) Cells with a positive control for FRET between donor and acceptor (connected with a short linker for example).
  - (Ideally) Cells with a negative control for FRET between donor and acceptor (connected with a very long linker for example).
  - Cells with actual condition of interest
  - Imaging in four channels for intensity-based measures
    - Donor Ex/Donor Em
    - Donor Ex/Acc Em (FRET)
    - Acc Ex/Acc Em
    - Acc Ex/Donor Em (cross talk)
  - Usually in the donor alone sample the FRET channel has significant fluorescence intensity

Intramolecular FRET

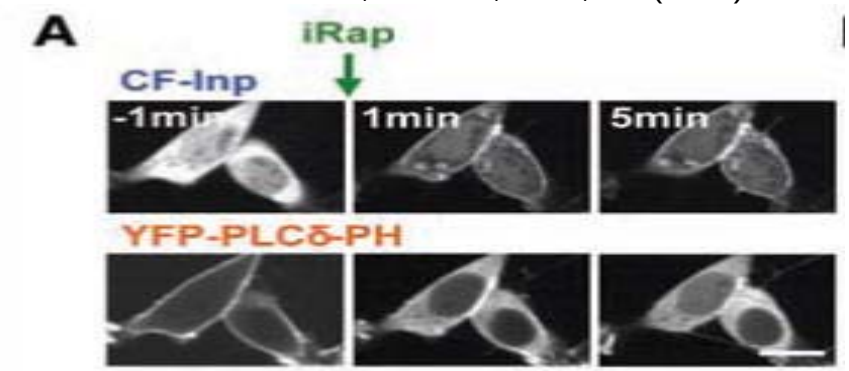


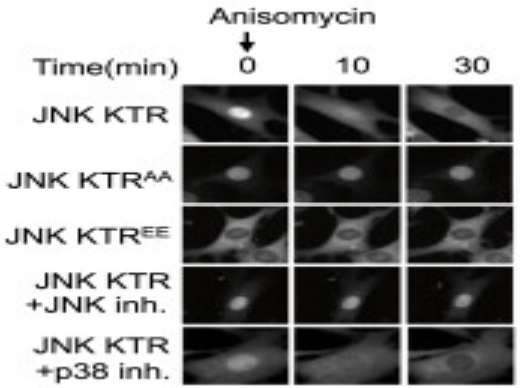
http://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/spectralfret.html

- Variety of probes where both donor and acceptor are within the same molecule at a one-to-one stoichiometry
- The distance between donor and acceptor is affected by some biochemical activity in the cell
  - E.g. cAMP levels, kinase activity
- Because the stoichiometry is fixed, analysis can be simplified
  - Usually two channels are sufficient
    - Donor Ex/Donor Em + Donor Ex/Acc Em
    - OR
    - Donor Ex/Donor Em + Acc Ex/Acc Em
  - Negative controls include probes that are engineered to be non-responsive to the biochemical activity of interest

Translocation Reporters

Heo et al., Science, 2006, 314(5804)





Regot et al., Cell, 2014, 7(157)

- Membrane
  - E.g. phospholipids
- Kinases
  - Based on phosphodependent nuclear translocation
- Single color, but automation normally requires a counterstain