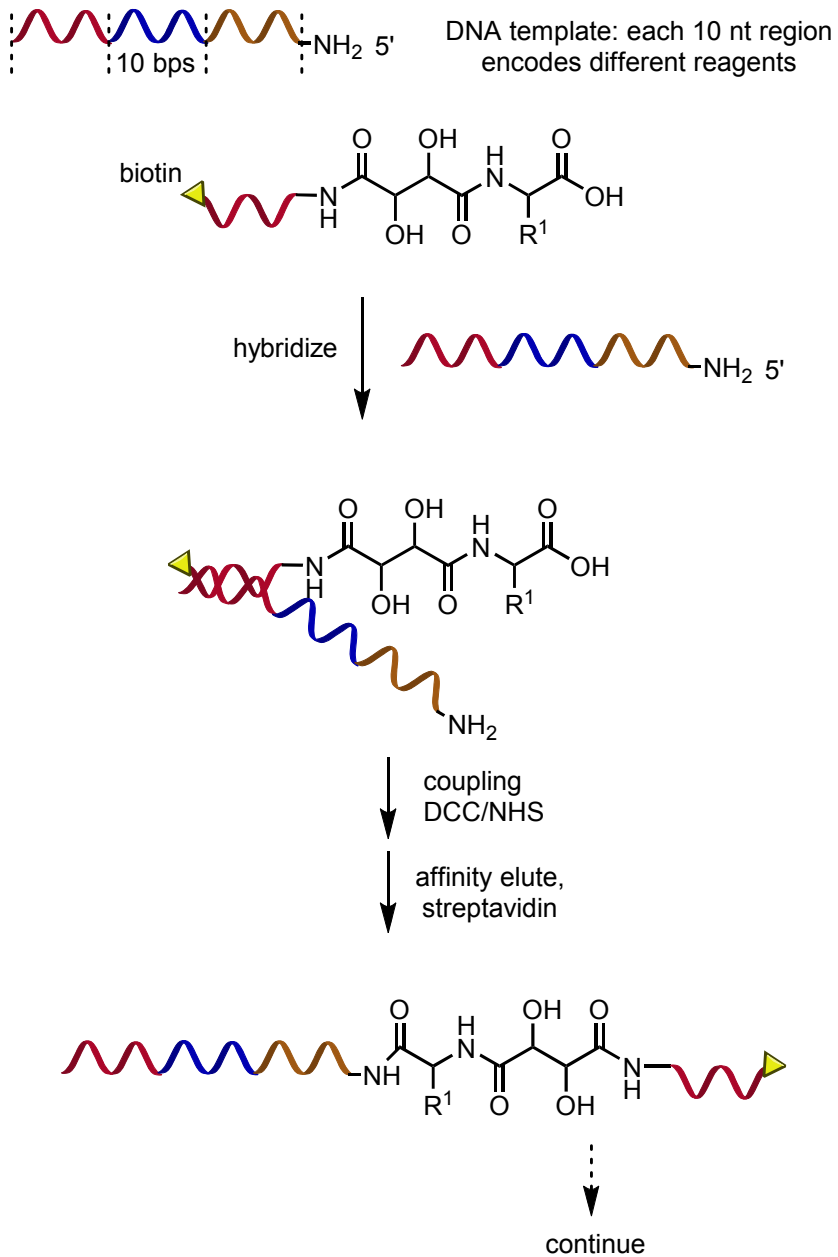


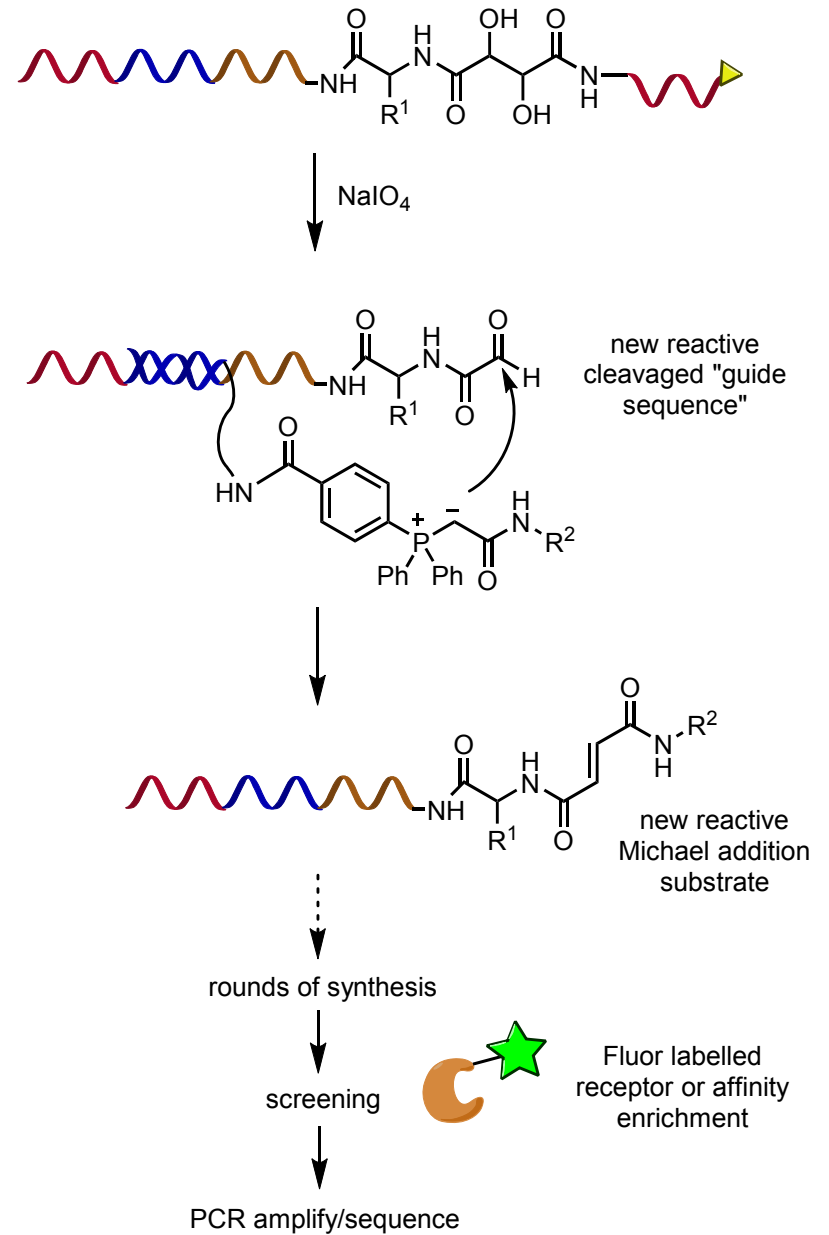
11.24.14 - Lecture - DNA template synthesis and biological probes

DNA Templated Synthesis



DNA Template Synthesis

continue

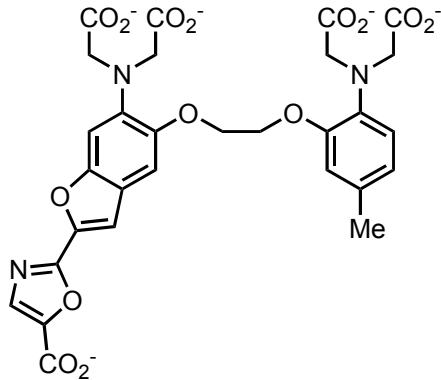


11.24.14 - Lecture - DNA template synthesis and biological probes

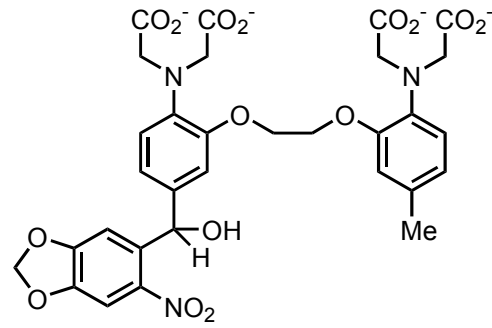
Biological Probes

- Ca^{2+} ~ important signaling messenger in the cell [Ca^{2+}]
- Develop Ca^{2+} sensors for live cell imaging
- Based on Ca^{2+} chelator, in which fluorescence λ or Φ_F is sensitive to [Ca^{2+}]

FURA2 (by Tsien's lab): Ca^{2+} sensor

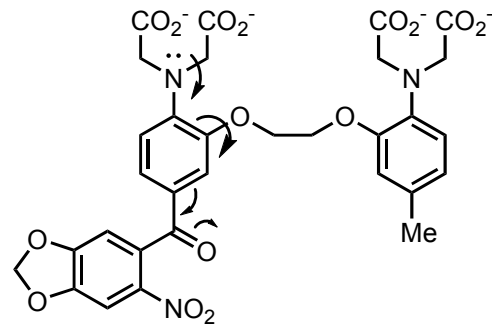


Ca^{2+} binding leads to shift in λ from 385 nm to 350 nm
 $K_d \sim 135$ nM
 (close to physiological cellular Ca^{2+} concentration)



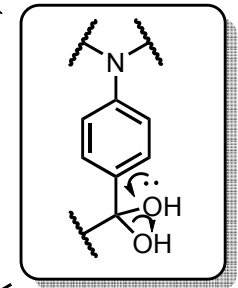
$K_d \sim 150$ nM

$h\nu$ 3000 s $^{-1}$ release rate



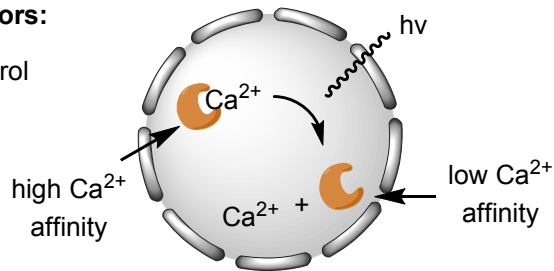
generate vinylogous amide - N lone pair less available to chelate Ca^{2+}

$K_d \sim 6.5$ μM

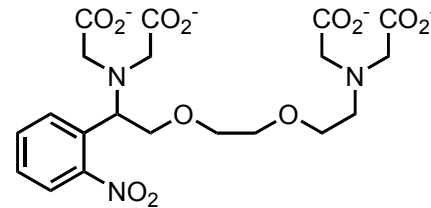
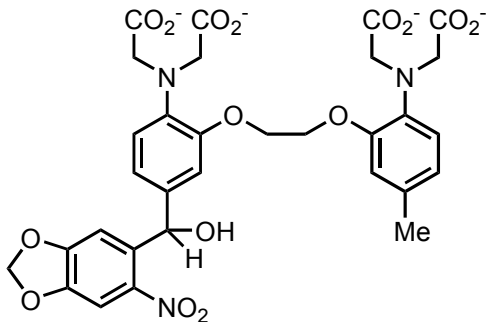


Photocaged Ca^{2+} chelators:

Release of Ca^{2+} with control over spatial and temporal distribution of Ca^{2+} in cell



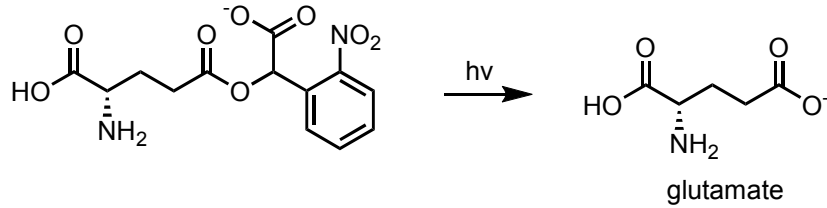
NITR-5



$h\nu$ leads to a 12,500-fold decrease in affinity for [Ca^{2+}]

11.24.14 - Lecture - DNA template synthesis and biological probes

CNB-caged L-glutamic acid



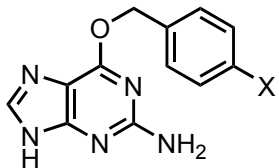
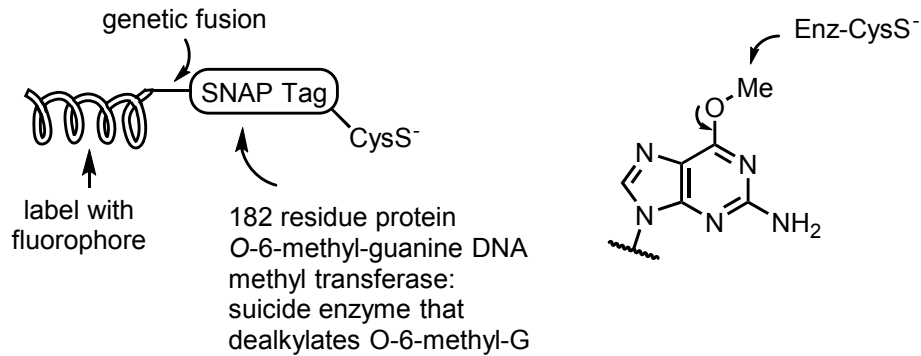
Imaging agents for proteins

long wavelength fluorophores/chromophores to image or release *in vivo*

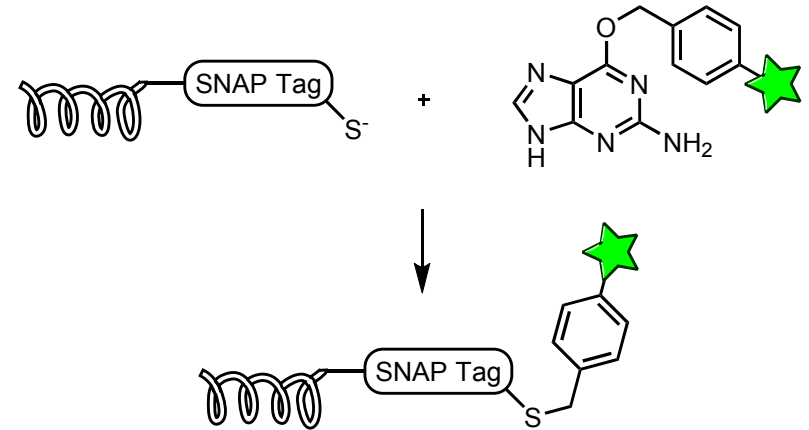
image agents for proteins

1. fixed cells
2. live cells

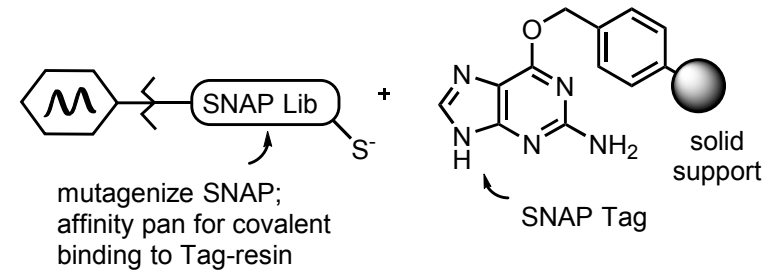
Snap-Tag



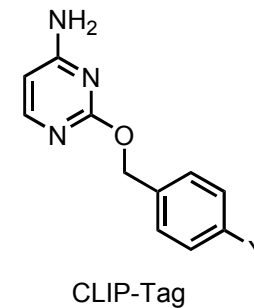
X = Tag, fluorophore, photoaffinity label



phage display to modify enzyme specificity
to take alternative substrates



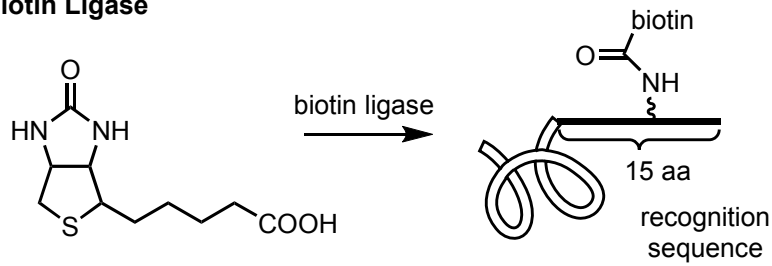
CLIP-Tag: uses O-2-modified cytosine



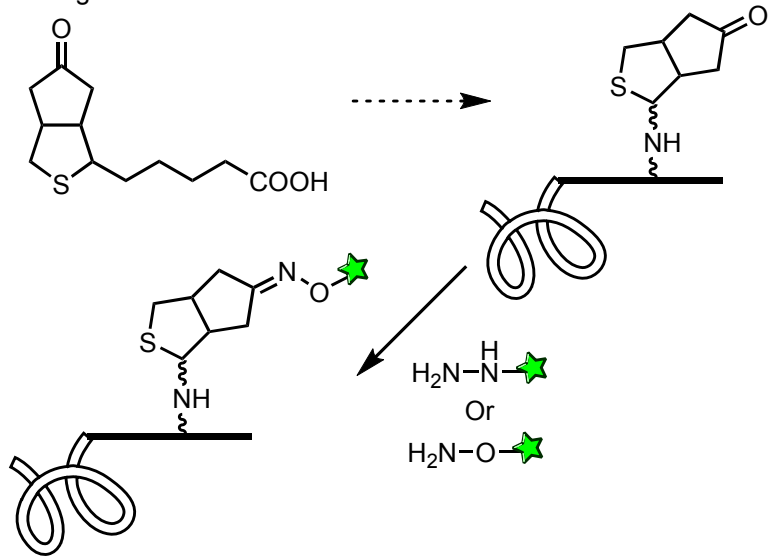
orthogonal substrate specificity to
SNAP Tag

11.24.14 - Lecture - DNA template synthesis and biological probes

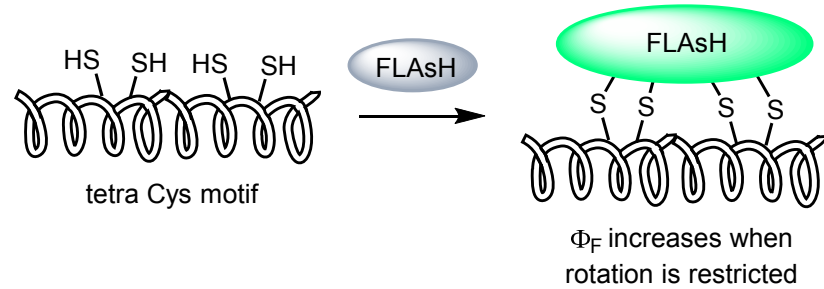
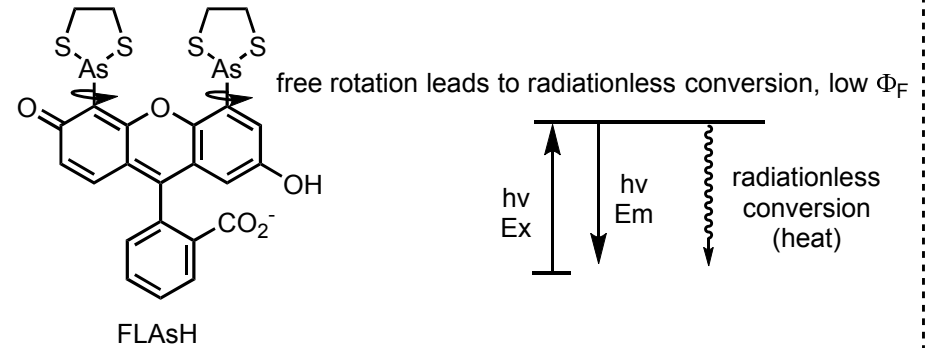
Biotin Ligase



biotin ligase mutant



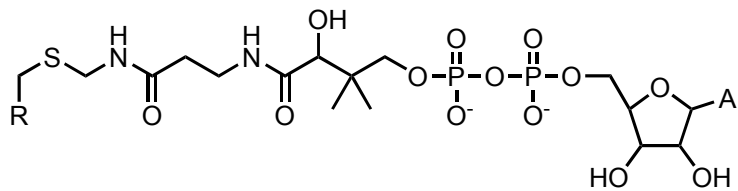
FLAsH



Engineer target 4 Cys in α -helix to react with FLAsH
Downsides: reacts with other thiols ~ some cytotoxicity

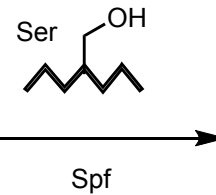
SFP

synthetase phosphopanethienyl transferase (Spf)

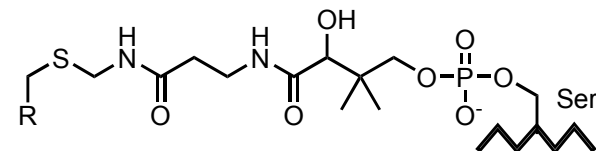


WT and/or mutant enzymes can accept various R groups

11 amino acid tag



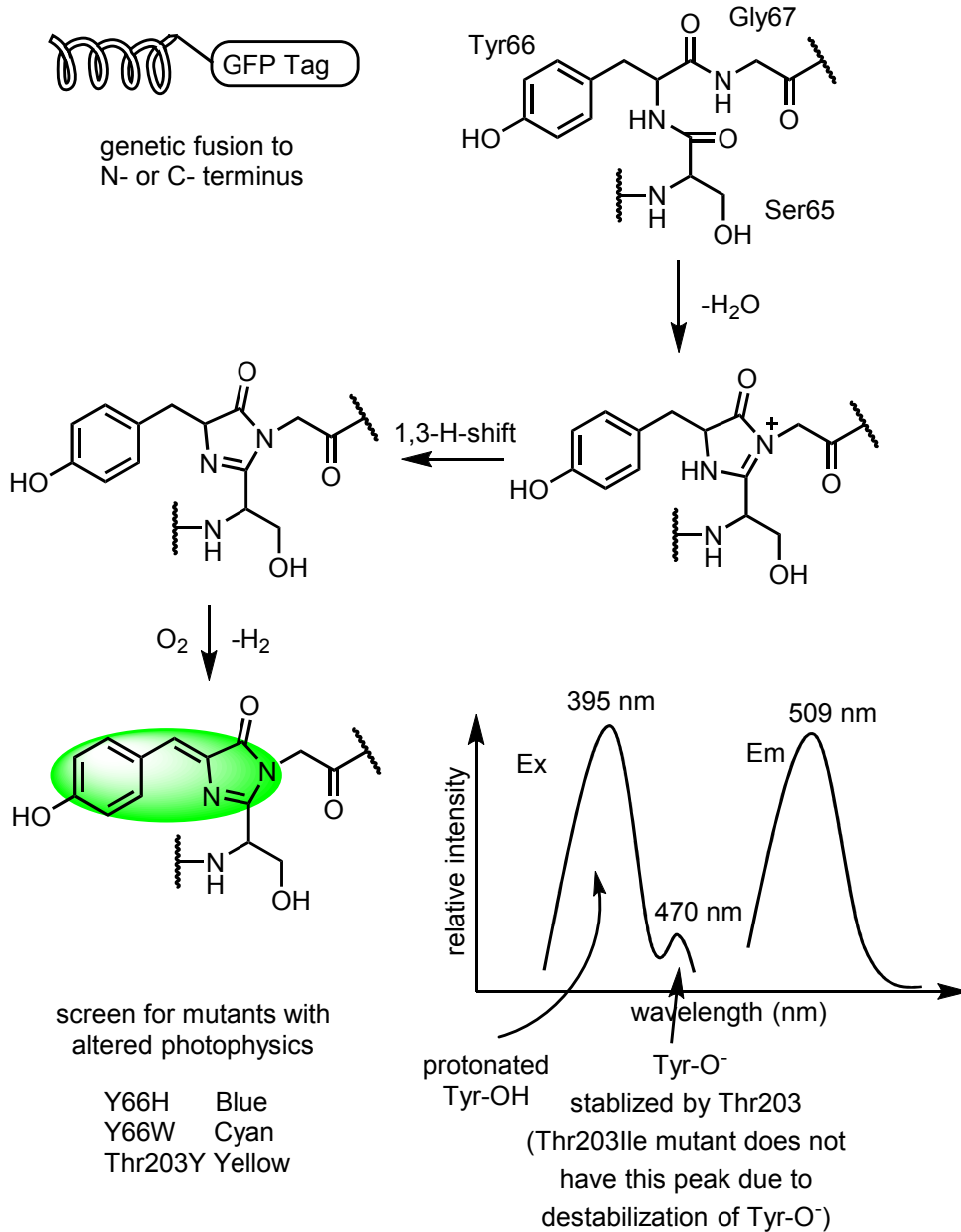
R = fluorophore or other reagent



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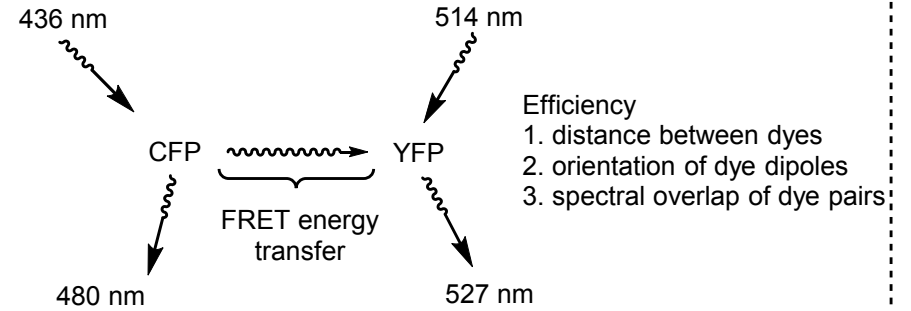
GFP (Green Fluorescent Protein)

Green Fluorescent Protein as a biological genetically encoded tag



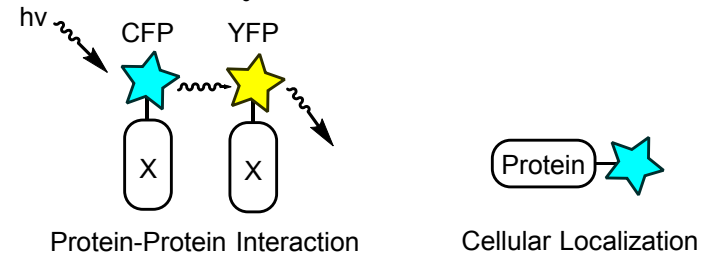
FRET (Förster Resonance Energy Transfer)

distance/orientation

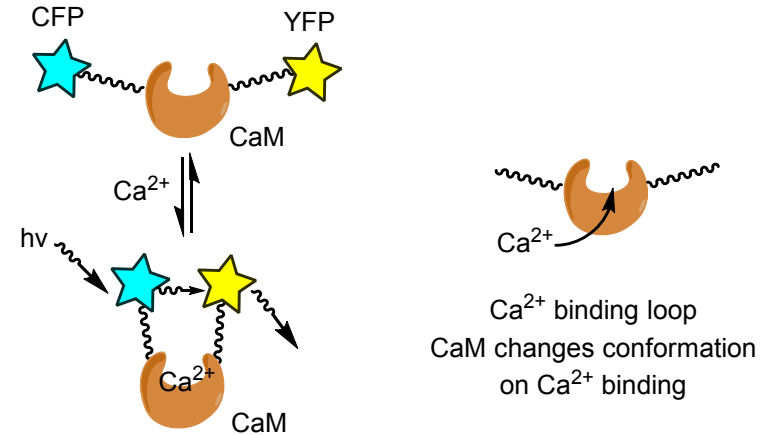


$$\text{FRET efficiency} \sim \frac{1}{1 + \left(\frac{r}{R_0}\right)^6}$$

R_0 is distance of 50% FRET
 R_0 depends on dye pairs

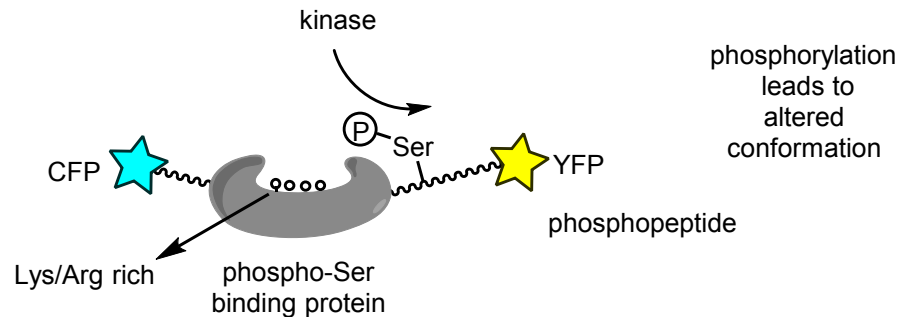


CaM : Ca²⁺ binding protein

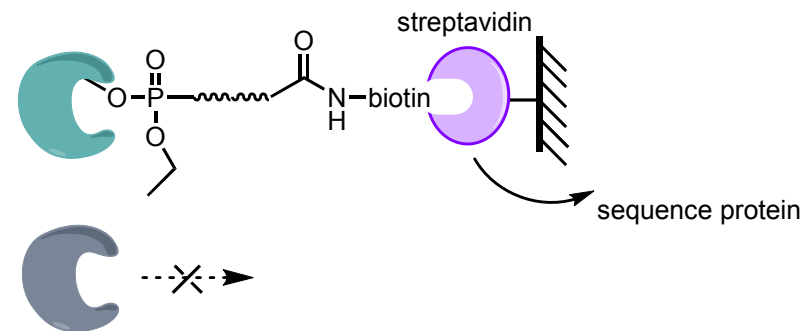
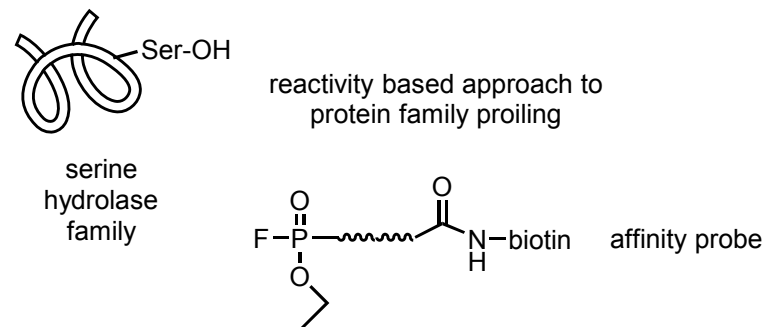


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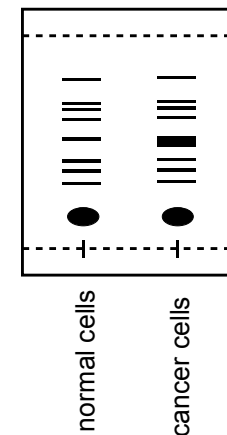
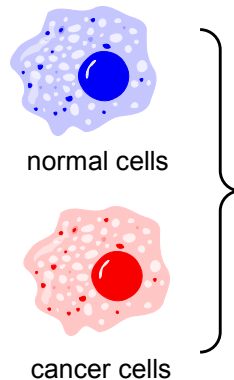
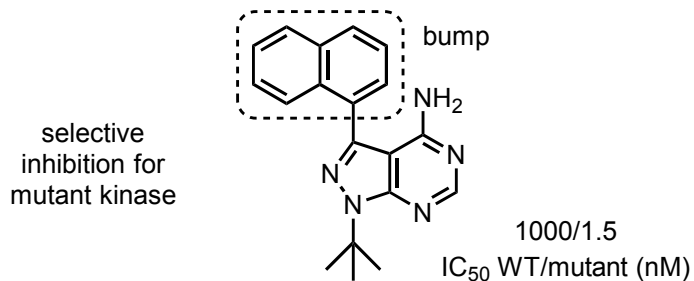
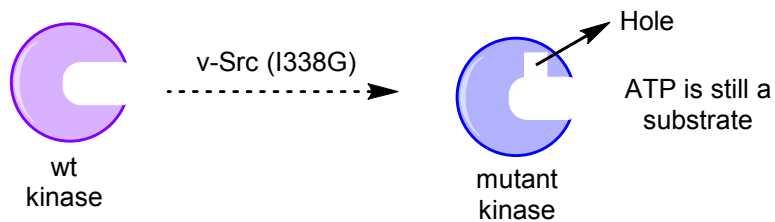
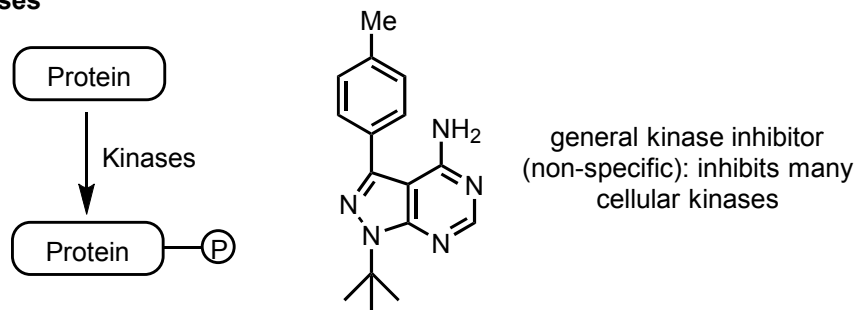
Phospho-Ser Binding Protein



Serine Hydrolase



Kinases



Enzyme families activities associated with disease states, which results in increased protein expression and band intensity.