ENGINEERING A LIGHT ACTIVATED CASPASE-3 FOR CELL BIOLOGY RESEARCH AND CANCER TREATMENT

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Molecular Biology
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EPSCoR Summer Fellowship Research Project

- Goals
  - Create an infrared light-activated caspase-3
  - Optimize it
- Significance
  - Study development and diseases in model organisms
  - Treatment of diseases through gene therapy

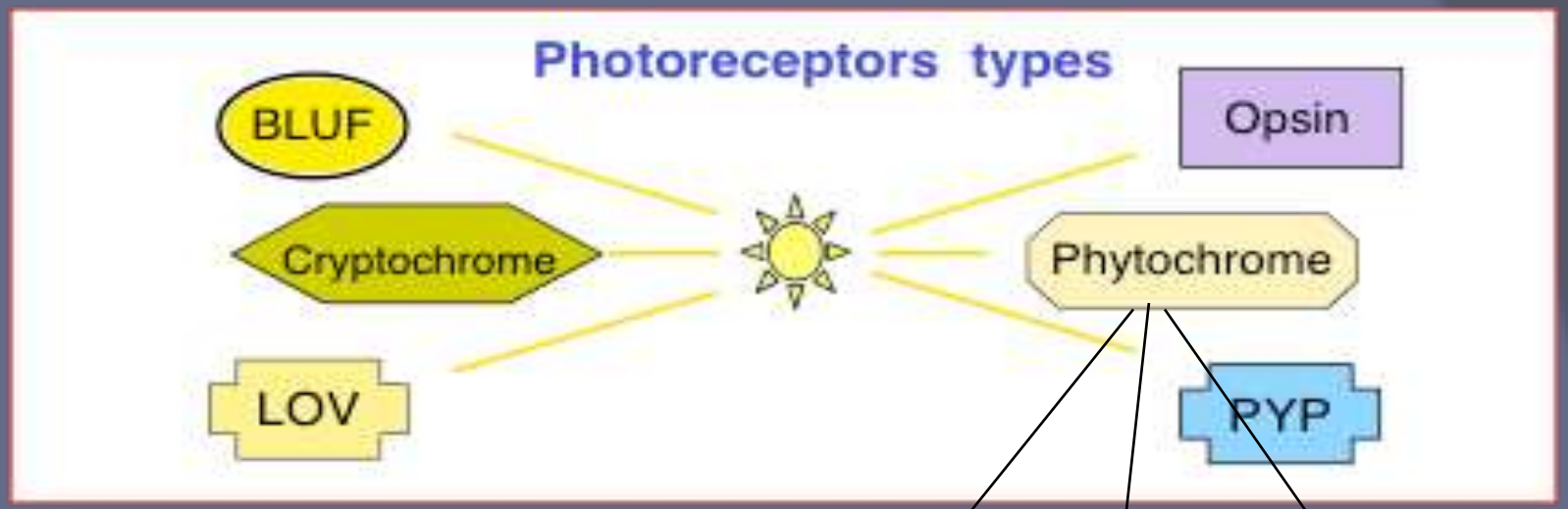
Optogenetics

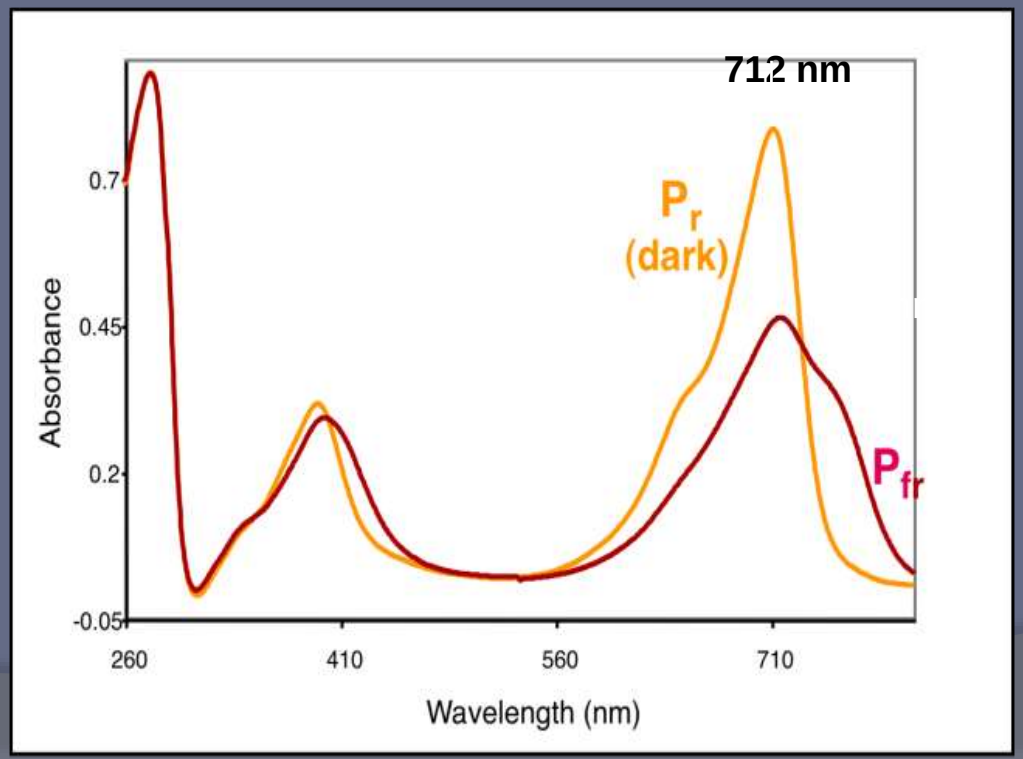
Optogenetics = use of genetically encoded photoactivated proteins to regulate biological processes *in vivo*

light *versus* **drugs**

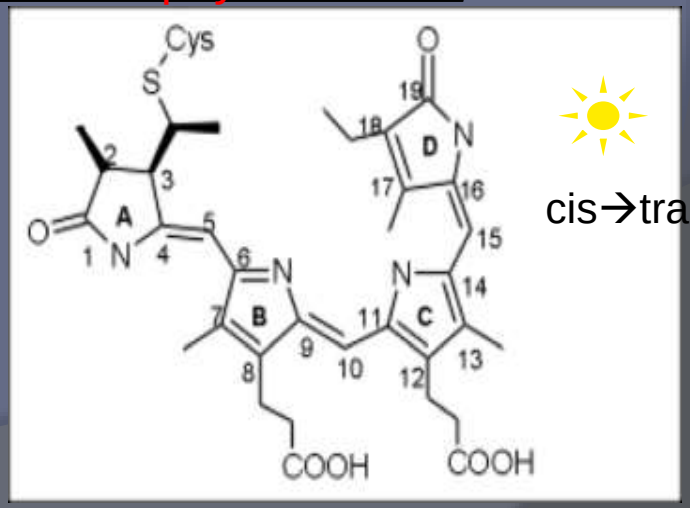
- Few, if any side effects
- Spatial precision (single cells)
- Temporal control
- Reversibility

Photoreceptor modules for optogenetics



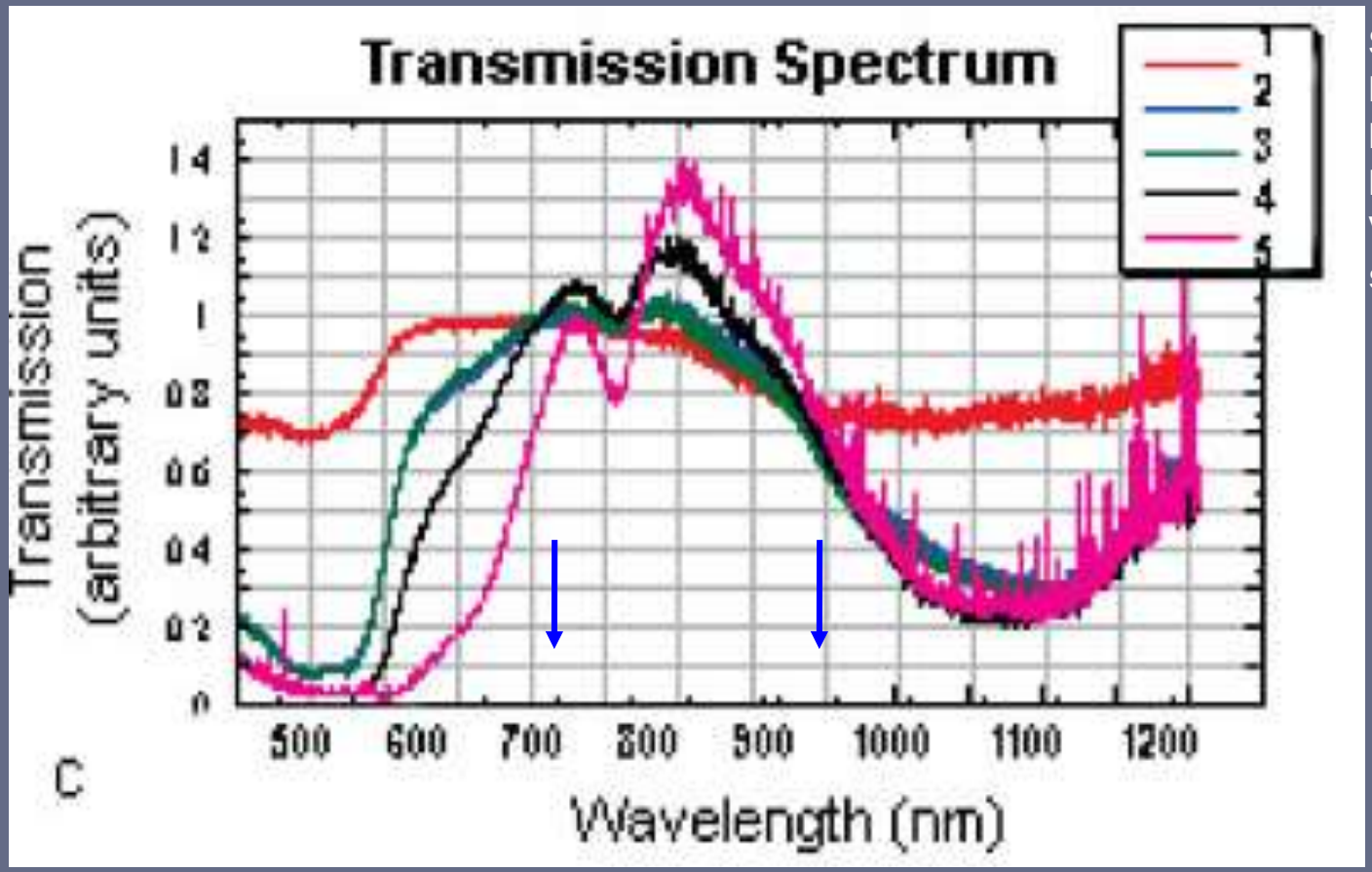


Bacteriophytochrome



cis→trans

Why bacteriophytochrome optogenetics?



Skin
Loose connective tissue
Dense connective tissue
Muscle
Vertebral column and spinal cord

Kimberly et al. 2005, Lasers in surgery and medicine 36:171-185

(i) Near-IR light penetrates animal tissues much deeper than visible light

(ii) Near-IR light harmless

(iii) The chromophore is biliverdin, the first product of natural heme breakdown

(iv) Phytochromes can be instantly turned “off” (i.e. photoinactivated)

(v) Fluorescent phytochromes have been used for whole-body imaging in mice

Why Caspase-3?

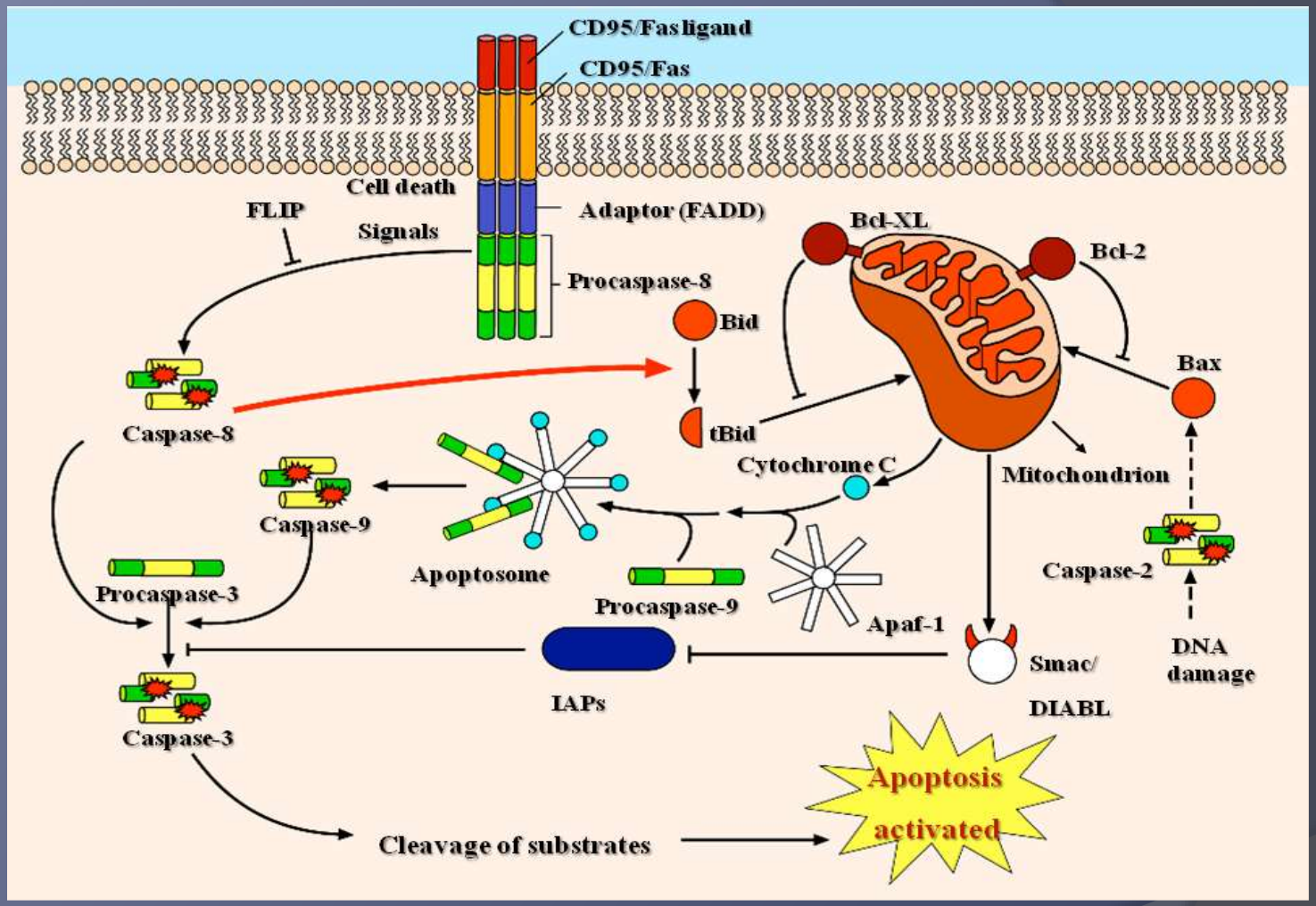


Figure 1. Apoptosis activation sequence. Signals cause a cascade of caspases. Caspase-3 is the final effector caspase that causes the cell to execute apoptosis.
Figure by Faris Q Alenzi.

Caspase-3

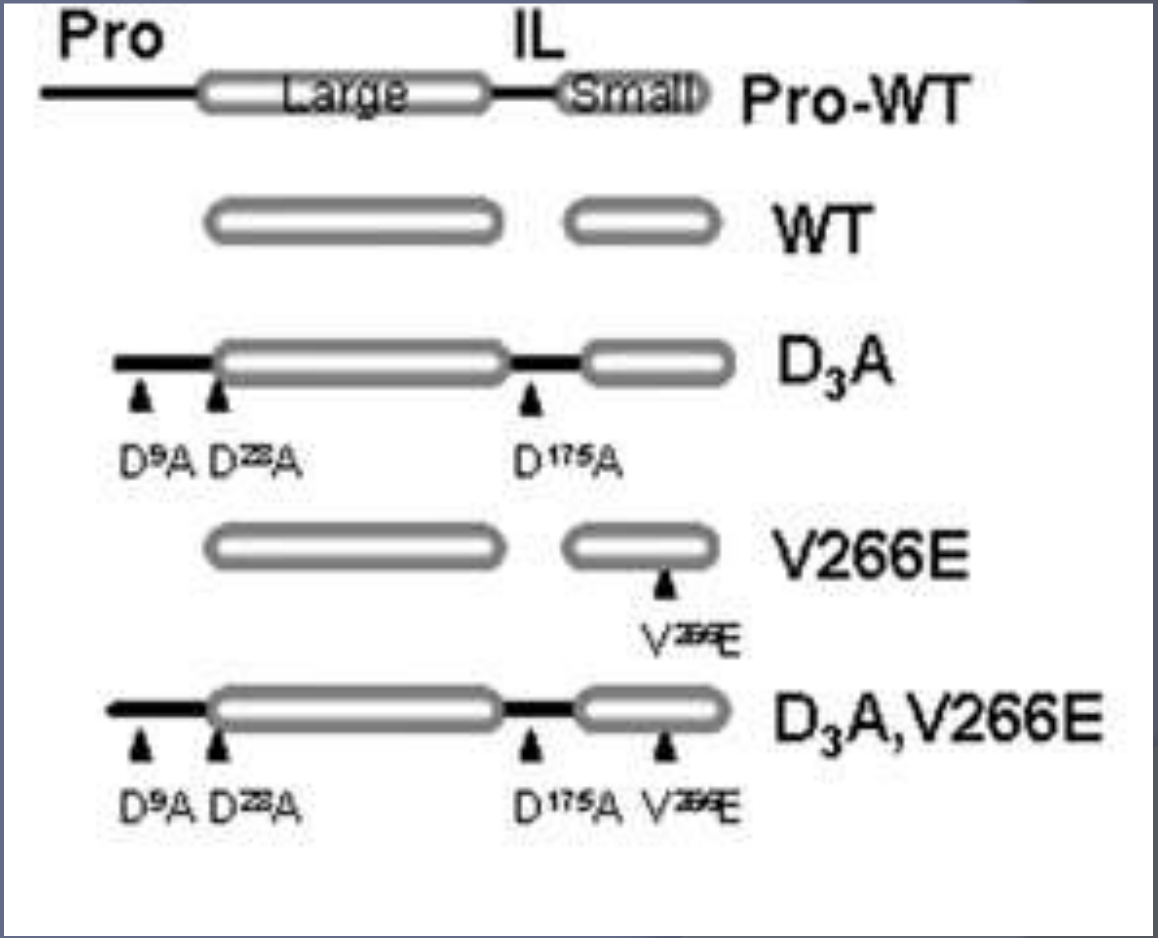
Inactive WT

Active WT

Uncleavable
Constitutively Inactive

Constitutively Inactive

Mutant Procaspase-3
Activated by
Homodimerization



Creating a Light-Activated Caspase-3

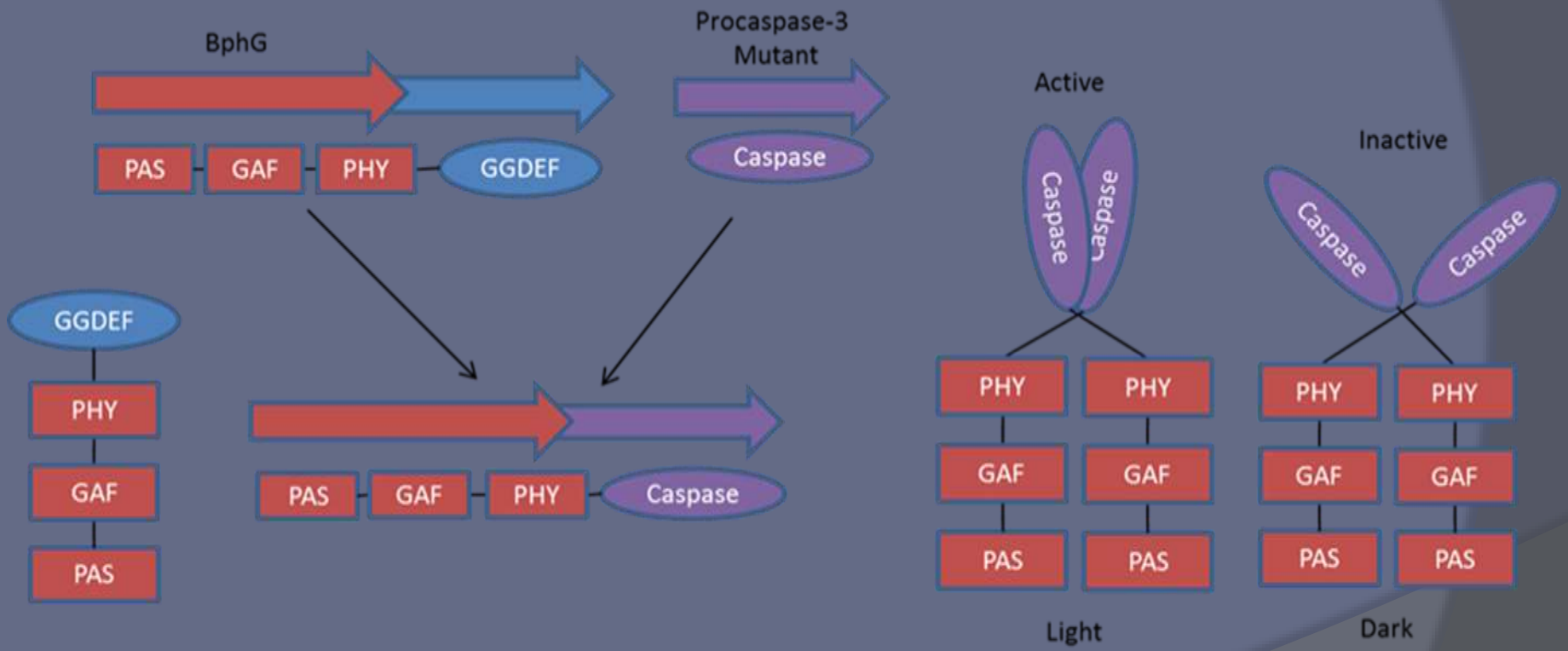
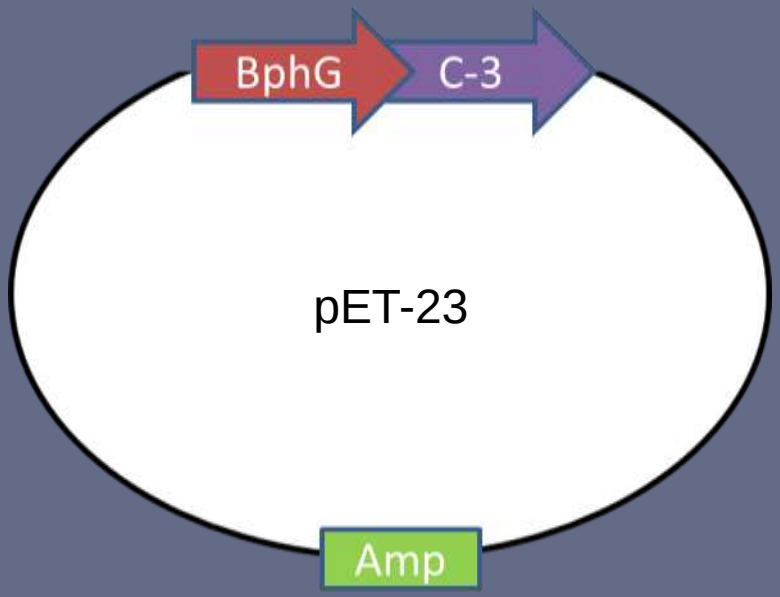


Figure 2. Bph photoreceptor is fused to the caspase-3 mutant. Light causes a conformational change activating the caspase.
Figure by Tricia Jensen

Construct



Screening For Caspase-3 Activation

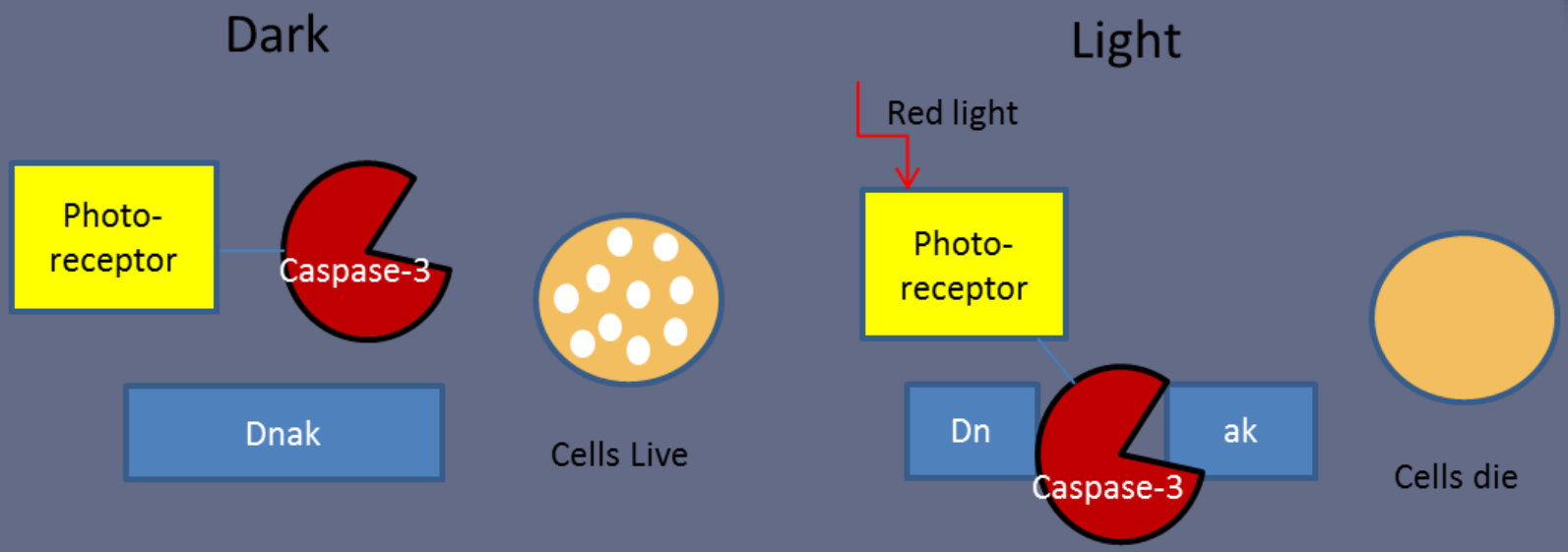
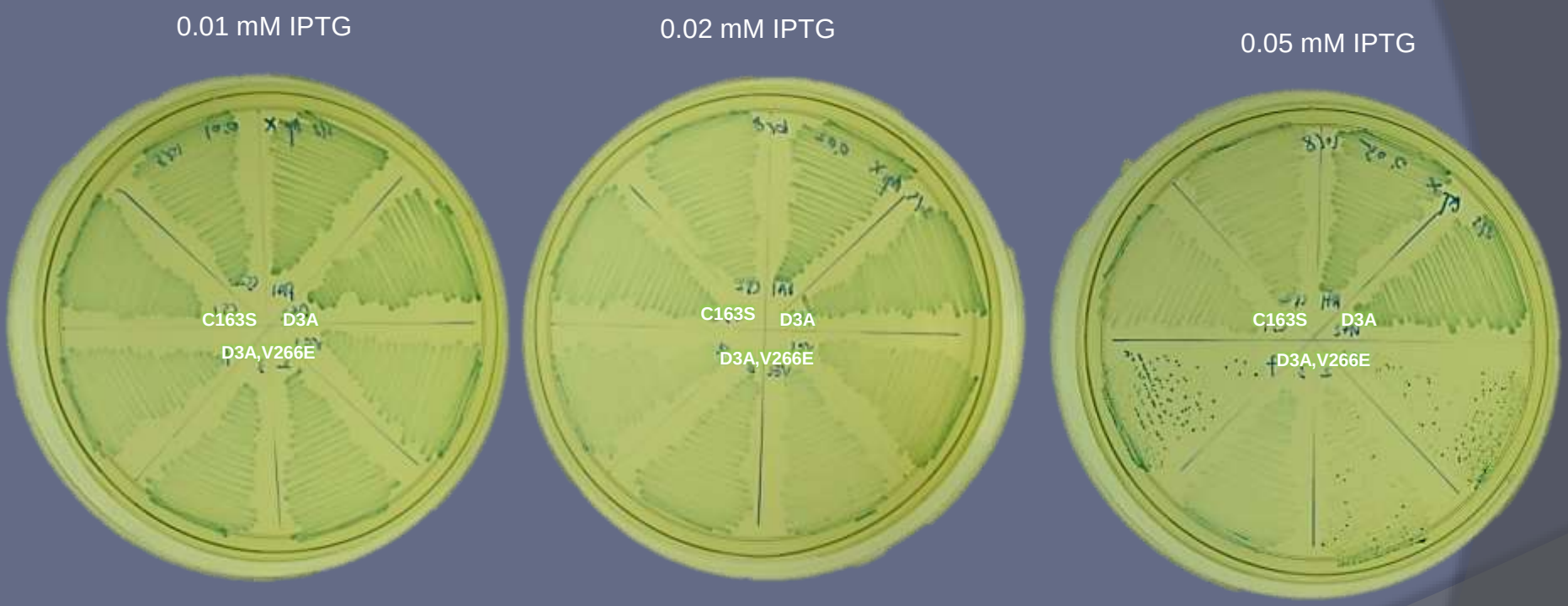


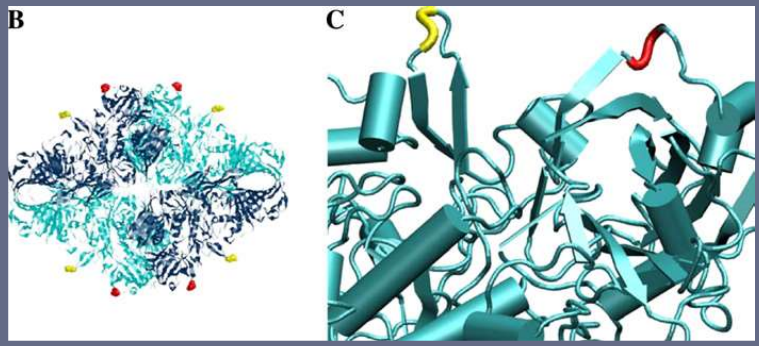
Figure 3. In the dark caspase-3 is inactive and the cells live. In the light caspase-3 is active and the cells die. Figure by Tricia Jensen

Caspase-3 (D3A, V266E) kills *E. coli*

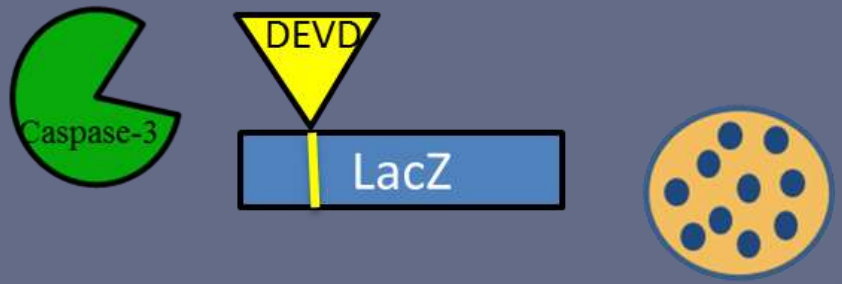


Screening System

- We inserted Caspase-3 DEVD recognition site into the Lac Z.
- Lac Z degrades X Gal medium resulting in a blue color.
- In theory the active caspase-3 should cleave the Lac Z protein resulting in white colony color and cells with inactive caspase-3 should be blue.



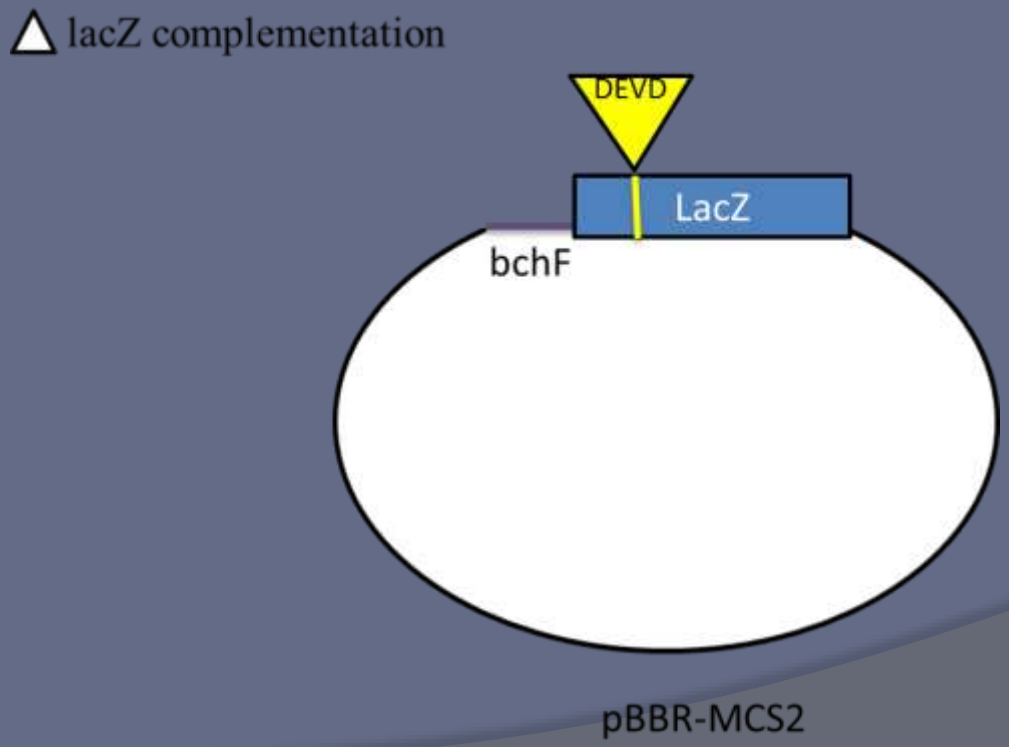
Inactive



Active

Lac Z Plasmid

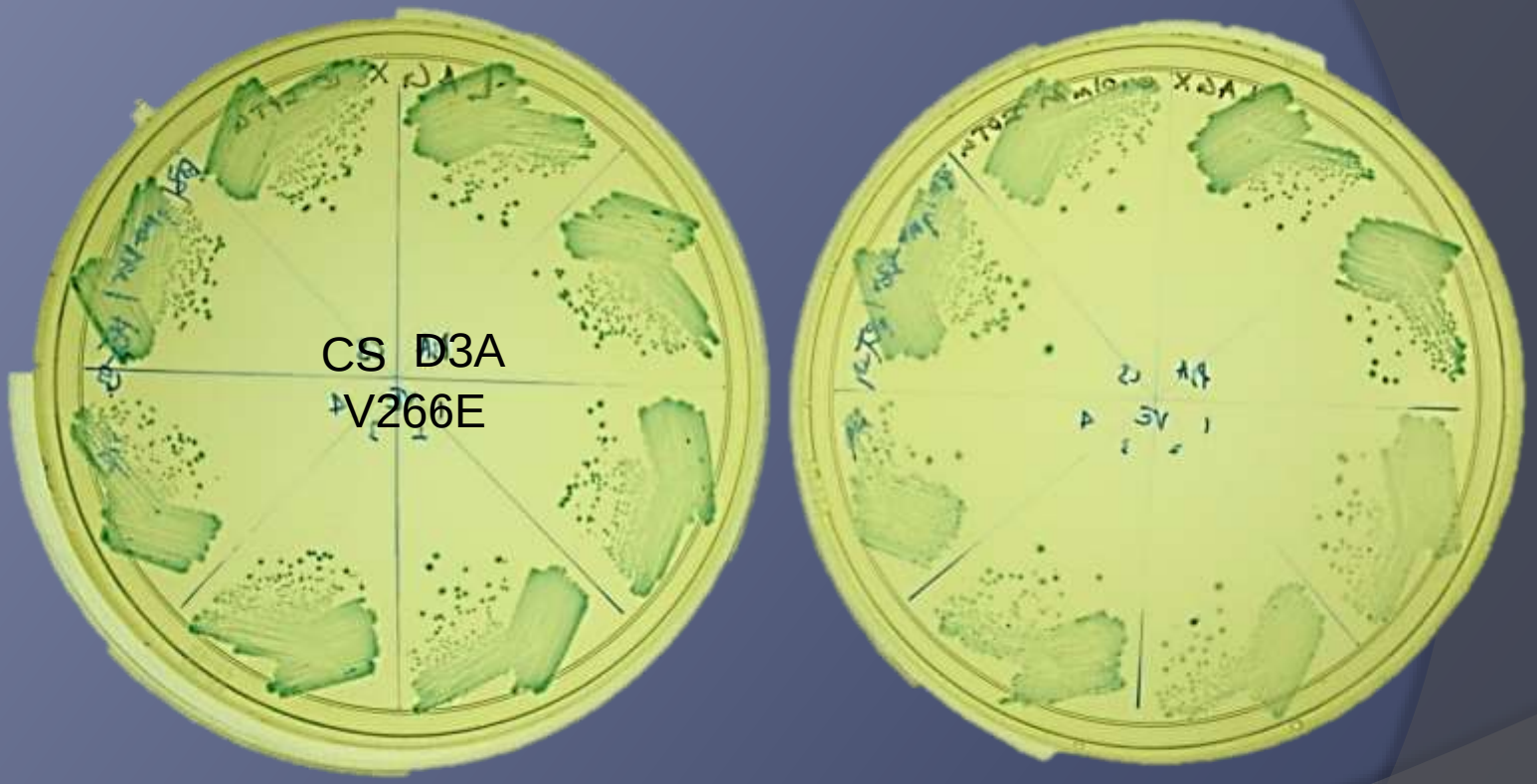
- *E. coli* naturally contain the lac operon.
- We constructed the Lac Z deleted mutant in BL21(DE3).
- Therefore the Lac Z protein with the caspase-3 recognition site contained in our created plasmid will affect screening.



Caspase-3 test in B-lacZ- stain

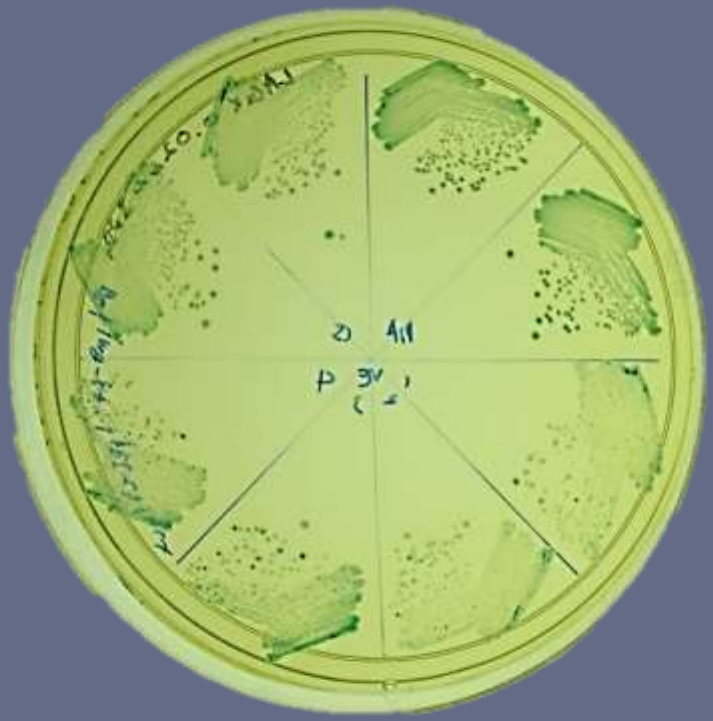
0 mM IPTG

0.01 mM IPTG



pET-CASP3
CS: C163S
D3A: D3A (Procaspase mutant)
D3A, V266E : active procaspase

0.02 mM IPTG



0.05 mM IPTG

Infrared-light activated caspase-3 construction

```
bphC3-1   AIAAEMAQRTSGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
bphC3-2   AIAAEMAQRT------NSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
bphC3-3   AIAAEMAQRT---------KMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
bphC3-4   AIAAEMAQRT----------MDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
bphC3-5   AIAAEMAQRT-----------DYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
bphC3-6   AIAAEMAQRT------------YPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
bphC3-7   AIAAEMAQRT-------------PEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
bphC3-8   AIAAEMAQRT--------------EMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETF
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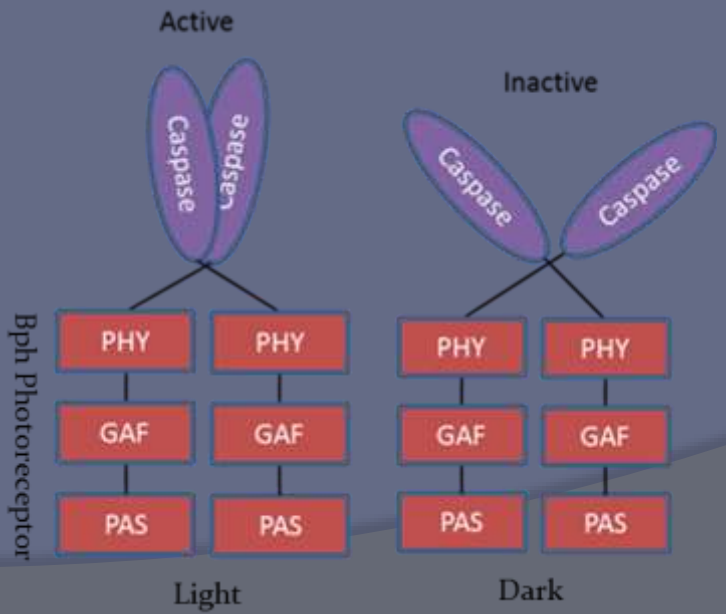

Future Goals

- Create more fusions
- Random mutagenesis
- Once promising:
  - Sequenced
  - Contributors test in mice

Thank You

Special Thanks to:

EPSCoR
Dr. Gomelsky and
Min-Hyung Ryu