Announcements

Lab Quiz

Pre-lab Lecture
  - Recap of BP components
  - Signaling details of BP system
  - Today in Lab (Mod 2 Day 2)
Recap BP components

Fill in boxes with a partner...

Input Sensing Device → Output Generating Device

- Exploding EnvZ-OmpR sensor response pair
- Light
- lacZ color
- (Plasmids)
- Make PCB
- Genomically integrated
- lacZ
- DmpR dependent promoter
- Binding site for DmpR
- Rely on native DmpR to need AQWZ, ΔlacZ strain

Cph8 fusion in ΔCph1 and EnvZ
apo-Cph1 + PCB; holo-Cph1 (functional)
How is the signal processed?

1. Photosynthetic activity
   - Light
   - Dark
   - Light
   - Dark

2. Phosphorylation
   - ATP
   - Phosphorylated
   - Phosphatase

3. Phosphate transferase
   - ATP
   - Phosphorylated
   - Phosphatase

4. "Phospho relay"
   - (Time...)
   - Phosphotransferase

Original state:
- Need substrate (not ATP)
- Black precipitate
- Color

Diagram:
- Enzyme-Cphl
- Biot
- AmpR
- Asp
- Phosphorylated
- Phosphatase
- Phosphotransferase
- Phosphorylated
- Phosphatase
Chemistry of phospho-aa

**Phosphohistidine**
- Acid-labile

**Phosphotyrosine**
- Rel. acid-stable

Slide from N. Kuldell
Chemistry of phosphorelay system

Slide from N. Kuldell
Thermodynamics of phosphorelay

What is a “high-energy” bond?

\[ \text{classic eq. } \text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{PPi} \]

\[ \Delta G < 0 \Rightarrow \text{spontaneous} \]

Typical \( \Delta G^\circ \) of hydrolysis:

-6.5 to -9.5 kcal/mol

-12 to -14 kcal/mol

\[ \left\{ \text{hydrolysis more spontaneous} \right\} \quad \text{\( \Rightarrow \) P-N less stable} \]

Which one is phosphoester, which is phosphoramidate?

\[ \text{P-O (Asp)} \]

\[ \text{P-N (His)} \]

How can we tune the output?

Think with your partner about...

• How to make plates lighter in the light
  1. auto
  2. transfer
  3. off

• How to make plates darker in the dark
  1. ↑
  2. ↑
  3. ↓

* is in our library

* mutants have specific residues targeted

X make more basic → side effects

method; open screen mutants
Today in Lab

- Observe/take pics of solid media from last time
- Prepare bacterial photograph
  - Considerations for preparing mask: X-gradations (just B&W) resolution
  - Condensation of the original
- Test liquid cultures from last time
  - β-gal assay (lyse cells, etc.)
  - Expected results: $\left[ \beta\text{-gal} \right]_{\text{light}} < \left[ \beta\text{-gal} \right]_{\text{dark}}$
- Atissa will give talk about talks @ 3:30 pm