- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Recap of BP components
  - Signaling details of BP system
  - Introduction to TinkerCell
  - Today in Lab (M2D2)
Announcements

• Draft slides for presentation
  – due M1D3 at midnight (midnight after lab Fri)
  – data section (4-6 slides) and summary (1 slide) only
  – 10% of final grade
  – everybody hands this in

• Final presentation
  – all slides due M1D4 or M1D8 at 11 am (day of presentation)
  – oral communication concentration only

• Written work
  – full report (written comm. concentration), or
  – results and discussion only (oral comm. concentration)
  – due Monday, 11.12.12

• Read assignment descriptions for guidance
• Bevin giving CEHS seminar this Friday
Recap BP components

Fill in boxes with a partner...

- light
- z plasmids
- $\text{Cam}^R$
- $\text{Ch}^2$
- $\text{Env}^2$
- $\text{Amp}^R$
- $\text{Pol}^A$
- $\lambda\text{ac}^2$
- $\text{Kan}^R$

$\text{Gph}^B = \text{Gph}^1 / \text{Env}^2$

$\text{apo-Gph}^1 + \text{PCB} = \text{holo-Gph}^1$

$\text{OmpR} \text{ binds here}$

$\text{OmpR} \Delta \text{Env}^2$

$\Delta \text{Env}^2$

$\text{Amp}^R$ relies on native $\text{OmpR}$
Where are we going?

- What’s the purpose of our initial experiments in both solid and liquid culture?
  
  define original system properties

- What will we do next (overall expt’l goal)?
  
  improve dynamic range/contrast (by genetic screen)
How is the signal processed?

1. phosphorylation
2. time...
3. dephosphorylation

- light
- dark

- on color if substrate (plates: 5-9 AL)
- original state
Chemistry of phospho-aa

Phosphohistidine vs Phosphotyrosine

1. Acid-labile --- Stable
2. 

\( \text{His12} \)

\( \text{Phosphohistidine} \)

\( \text{Phosphotyrosine} \)

\( \text{1) unstable to nucleophilic attack} \)

Slide from N. Kuldell
Chemistry of phosphorelay system

Slide from N. Kuldell
Thermodynamics of phosphorelay

What is a “high-energy” bond? 
Classic e.q. \( ATP + H_2O \rightarrow ADP + Pi \)
\( \text{P-O} \) energy release on breaking

Typical \( \Delta G^\circ \) of hydrolysis
-6.5 to -9.5 kcal/mol

Which one is phosphoester, which is phosphoramidate?
\( \text{P-O} \) \( \text{P-N} \) (H₂O)

TinkerCell modeling program

• Two major utilities for biological systems
  • 1: Visualizing networks
    – GUI to combine enzymes, promoters, etc.
  • 2: Simulating and perturbing networks
    – ODE-based modeling
    – initial concentrations, rate constants, etc.
• Consider assumptions and reliability in modeling vs. experiments
Today in Lab: M2D2

• Observe/take pics of solid media from last time
• Prepare bacterial photograph
• Test liquid cultures from last time
  – β-gal assay (lyse cells, etc.)
  – Expected results: $[\beta\text{-gal}]_{\text{light}} < [\beta\text{-gal}]_{\text{dark}}$
• TinkerCell
  – Draw network
  – Simulate changes to k’s, etc. (finish next time)
• Atissa will give talk about talks @ 4 pm