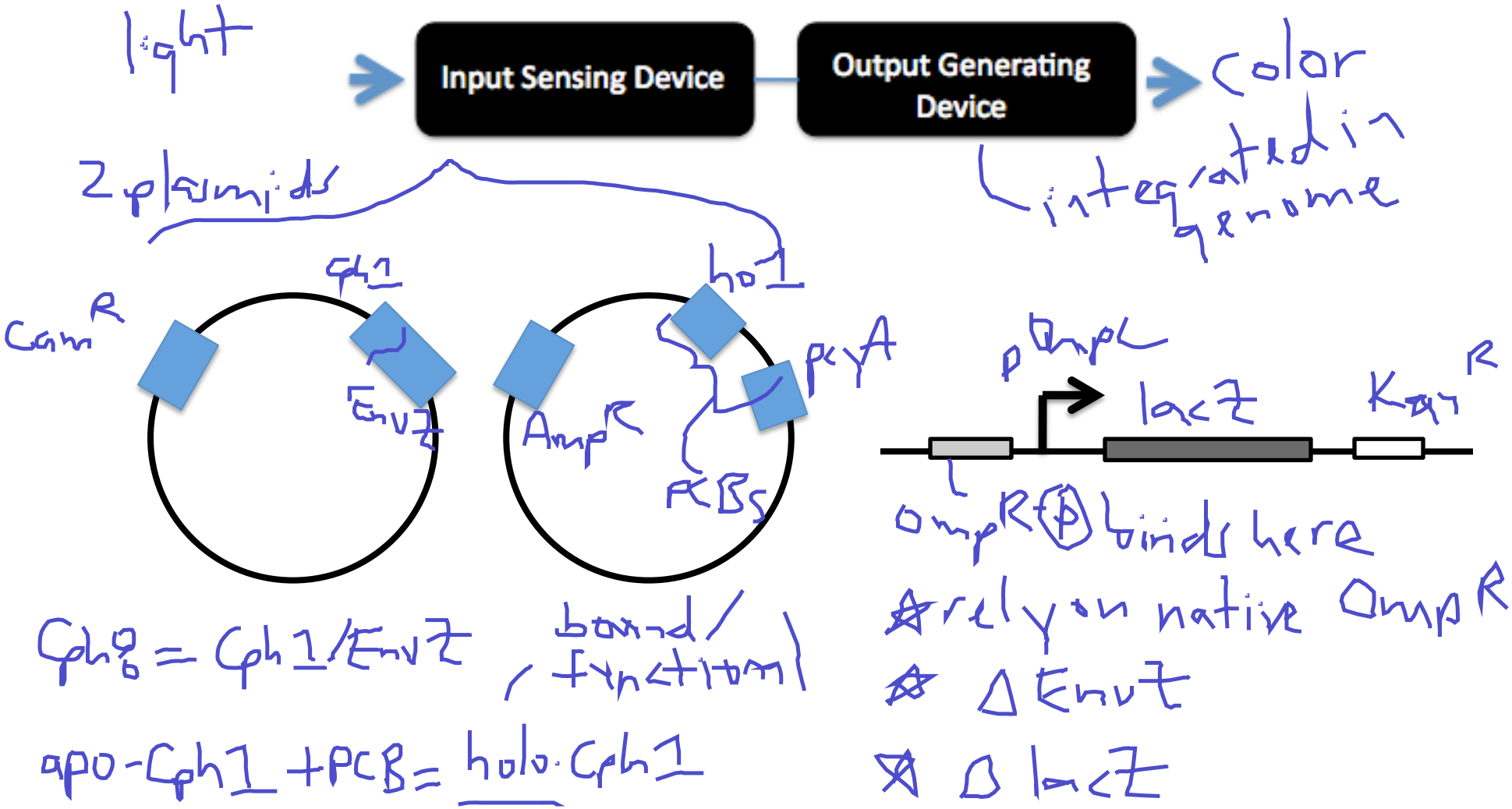
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - ❖ Recap of BP components
  - ❖ Signaling details of BP system
  - ❖ Introduction to TinkerCell
  - ❖ Today in Lab (M2D2)

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~~ 1pm (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…



Where are we/going?

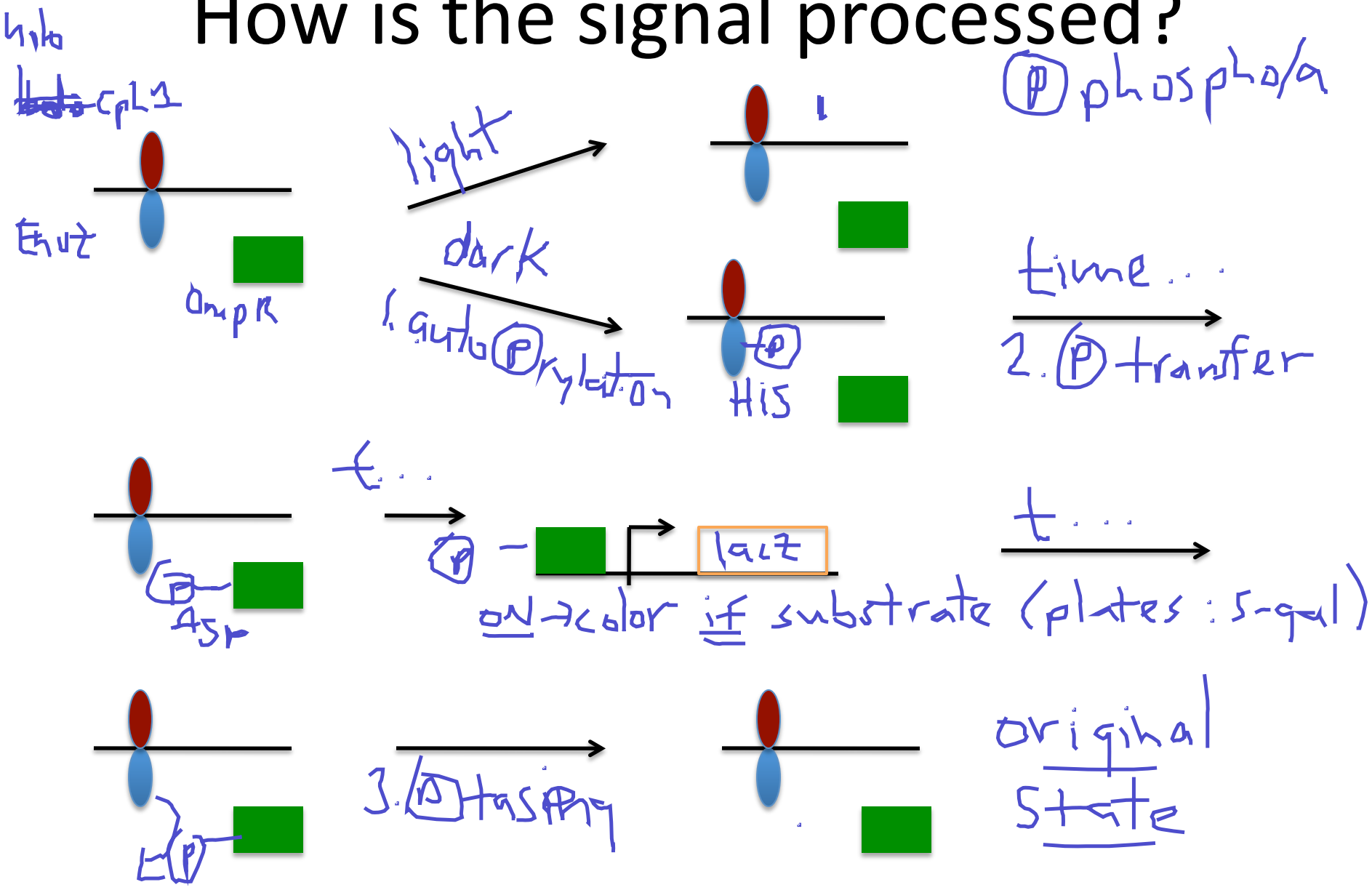
- What's the purpose of our initial experiments in both solid and liquid culture?

define original system properties

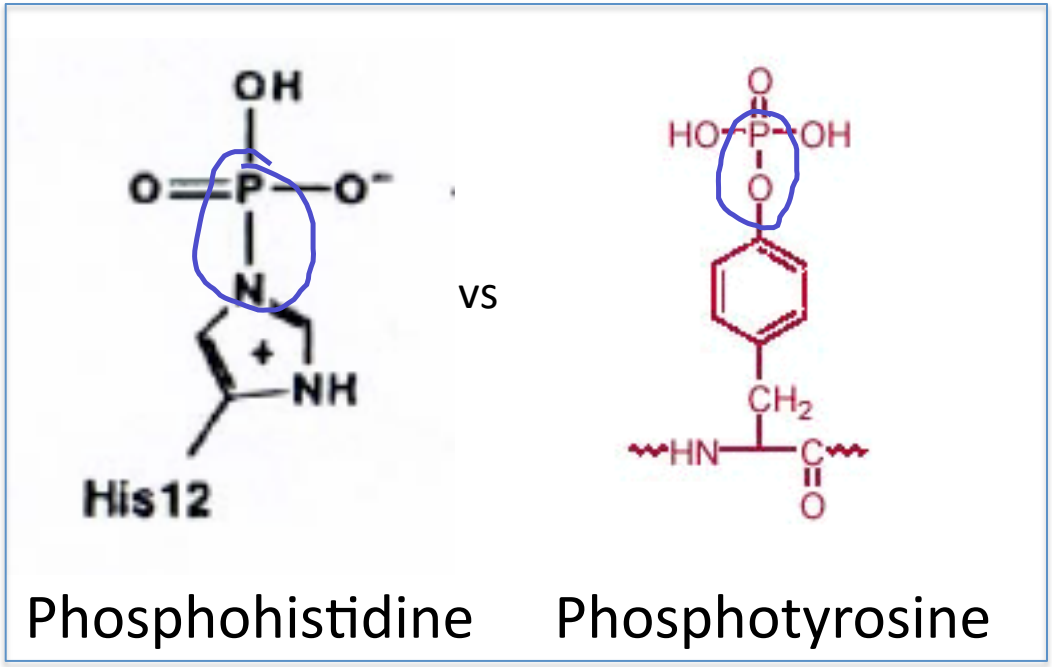
- What will we do next (overall expt'l goal)?

improve dynamic range /contrast
(by genetic screen)

How is the signal processed?



Chemistry of phospho-aa



Phosphohistidine Phosphotyrosine

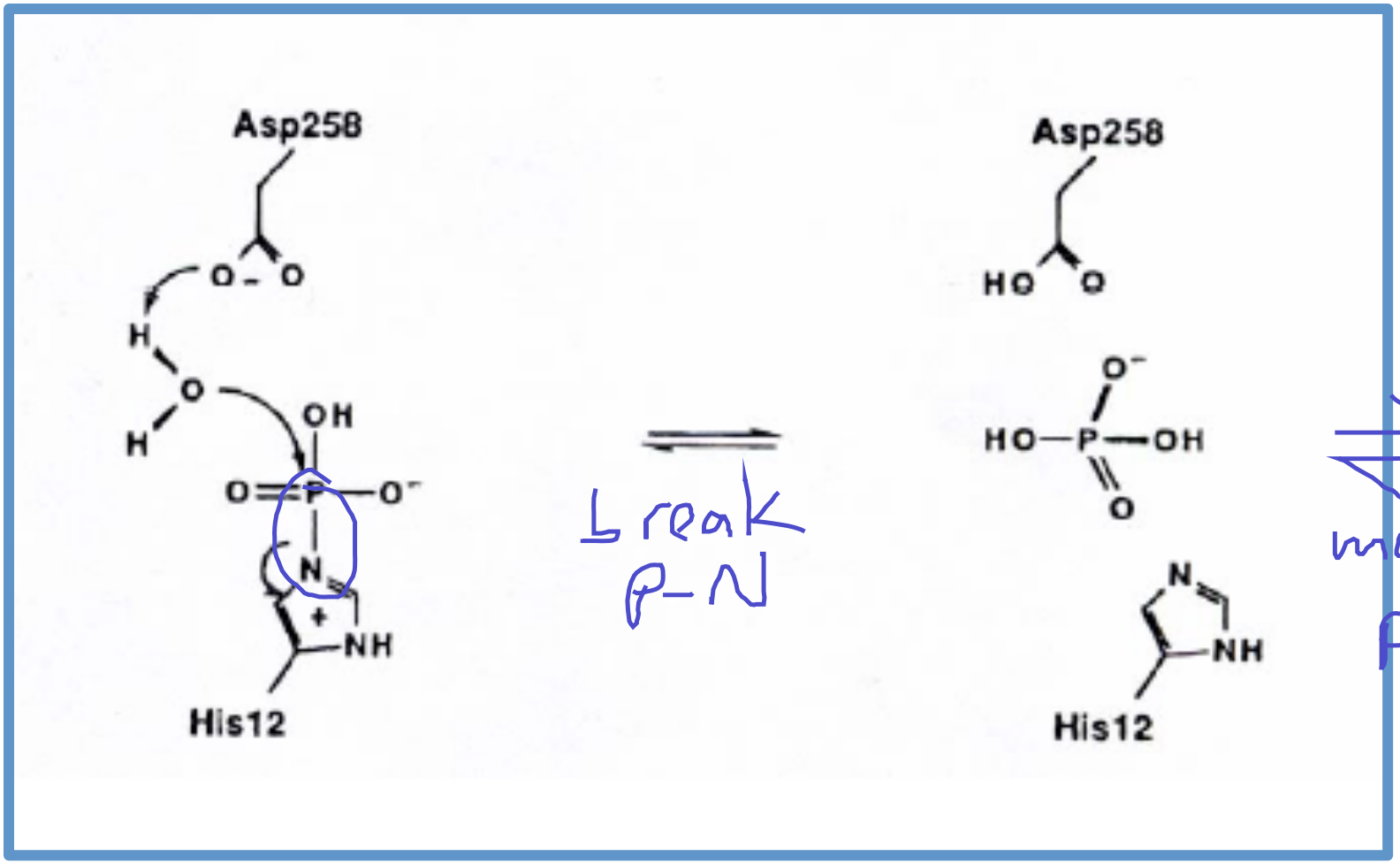
(1) acid-labile " stable

(2) e⁻-delocalized " plays some role
form insignificant

↳ ∴ 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.g. $ATP + H_2O \rightleftharpoons ADP + P_i$

Prod energy release on breaking

Typical ΔG° of hydrolysis

-6.5 to -9.5 kcal/mol

-12 to -14 kcal/mol

Which one is phosphoester, which is phosphoramidate?

P-O

P-N (H-)

ΔG° numbers from review: P.V. Attwood et al., *Amino Acids* **32**:145 (2007). Original research by Stock et al. (1990).

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: $[\beta\text{-gal}]_{light} < [\beta\text{-gal}]_{dark}$
- TinkerCell
  - Draw network
  - Simulate changes to *k*'s, etc. (finish next time)
- Atissa will give talk about talks @ 4 pm