

- Announcements

j.club D4 or D7

- Lab Quiz

↓
1 week!

- Pre-lab Lecture

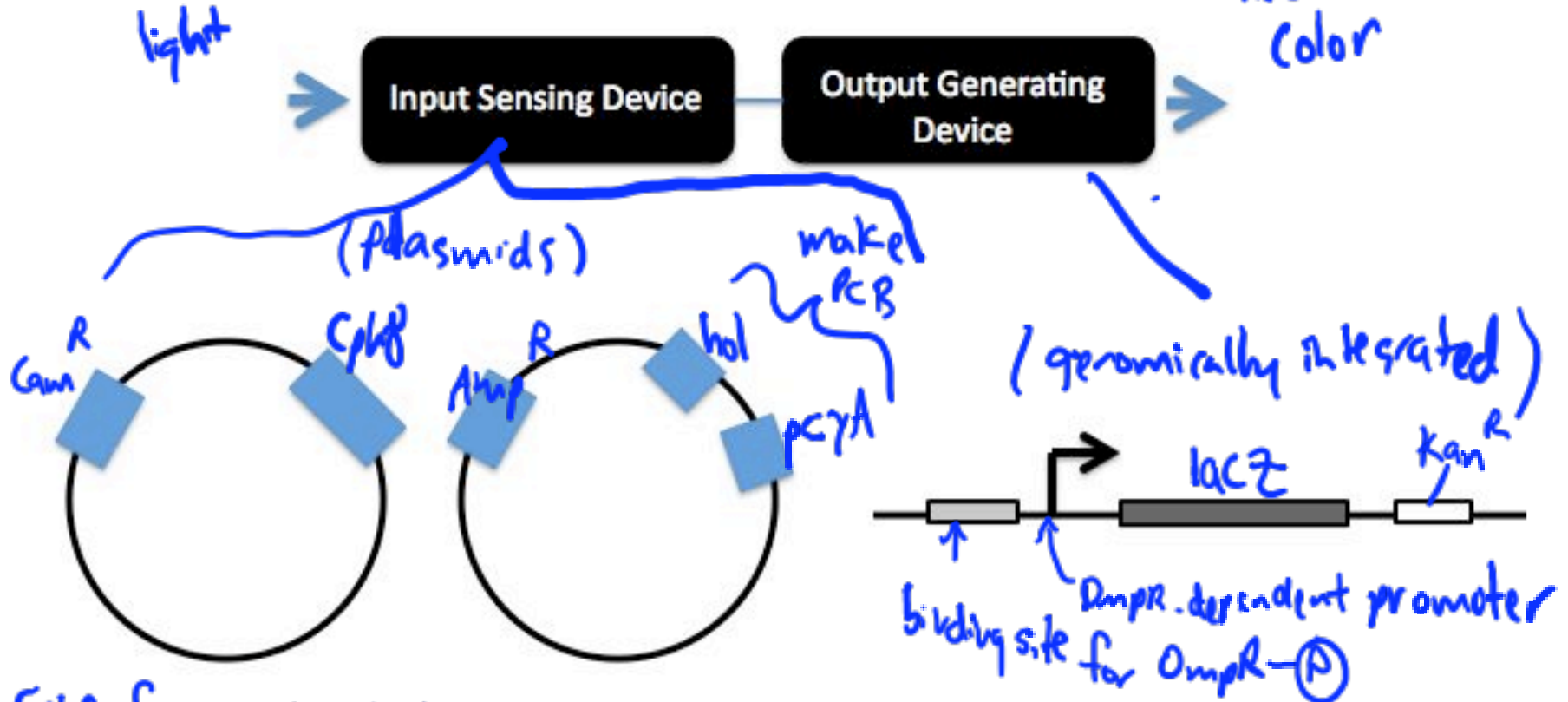
*
Otl by appt. (M/T off.)

- ❖ 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...

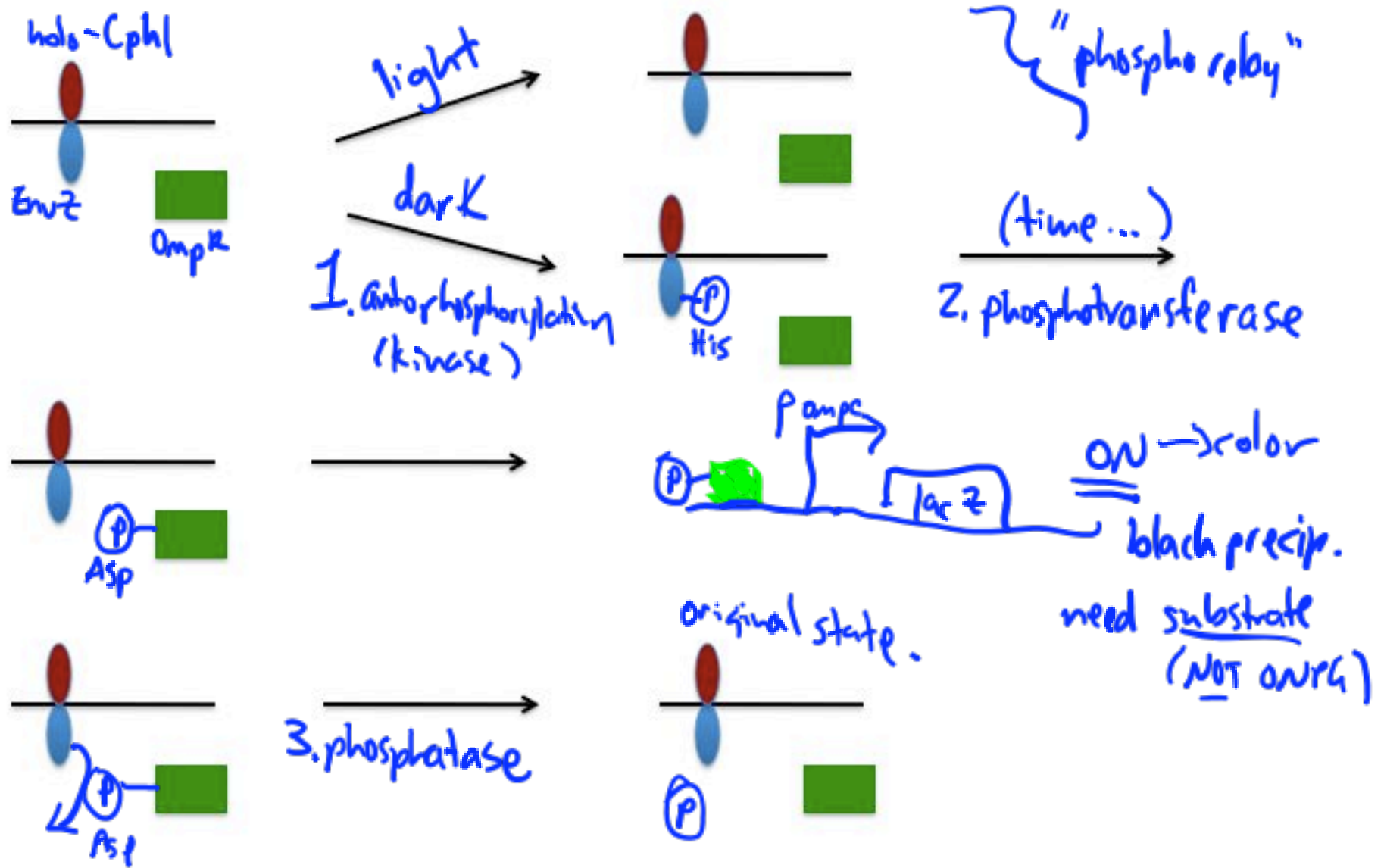
Exploiting C_{ph1} → $EnvZ - OmpR$ sensor-response pair → $lacZ$ Color



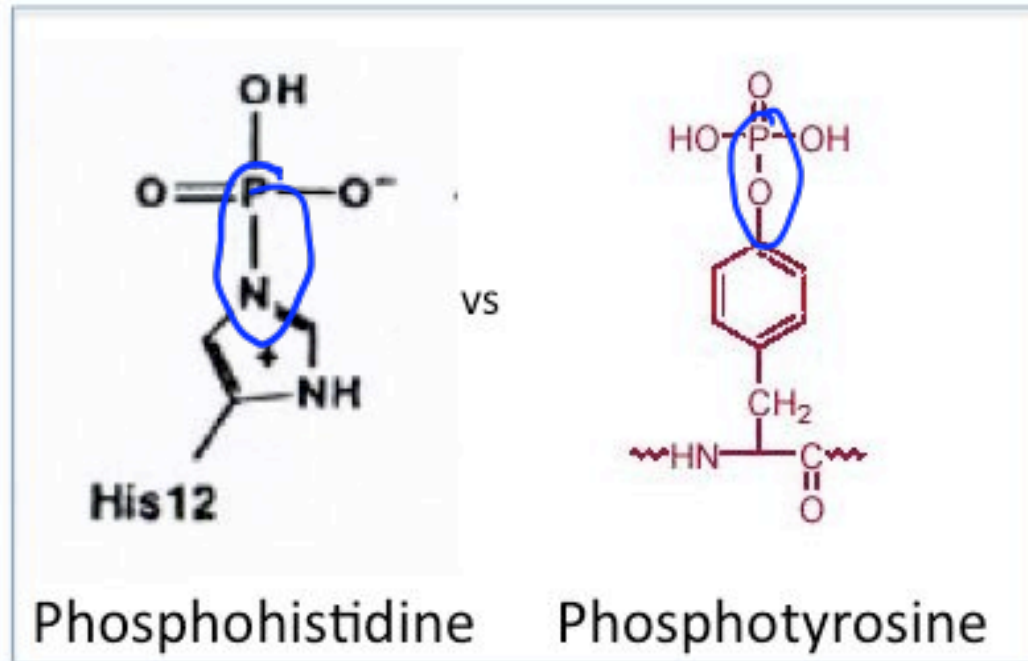
C_{ph8} fusion b/w C_{ph1} and $EnvZ$
 $apo-C_{ph1} + PCB = \text{holo-}C_{ph1}$ (functional)

rely on native $OmpR$
 need $\Delta EnvZ$, $\Delta lacZ$ strain

How is the signal processed?



Chemistry of phospho-aa



P-N

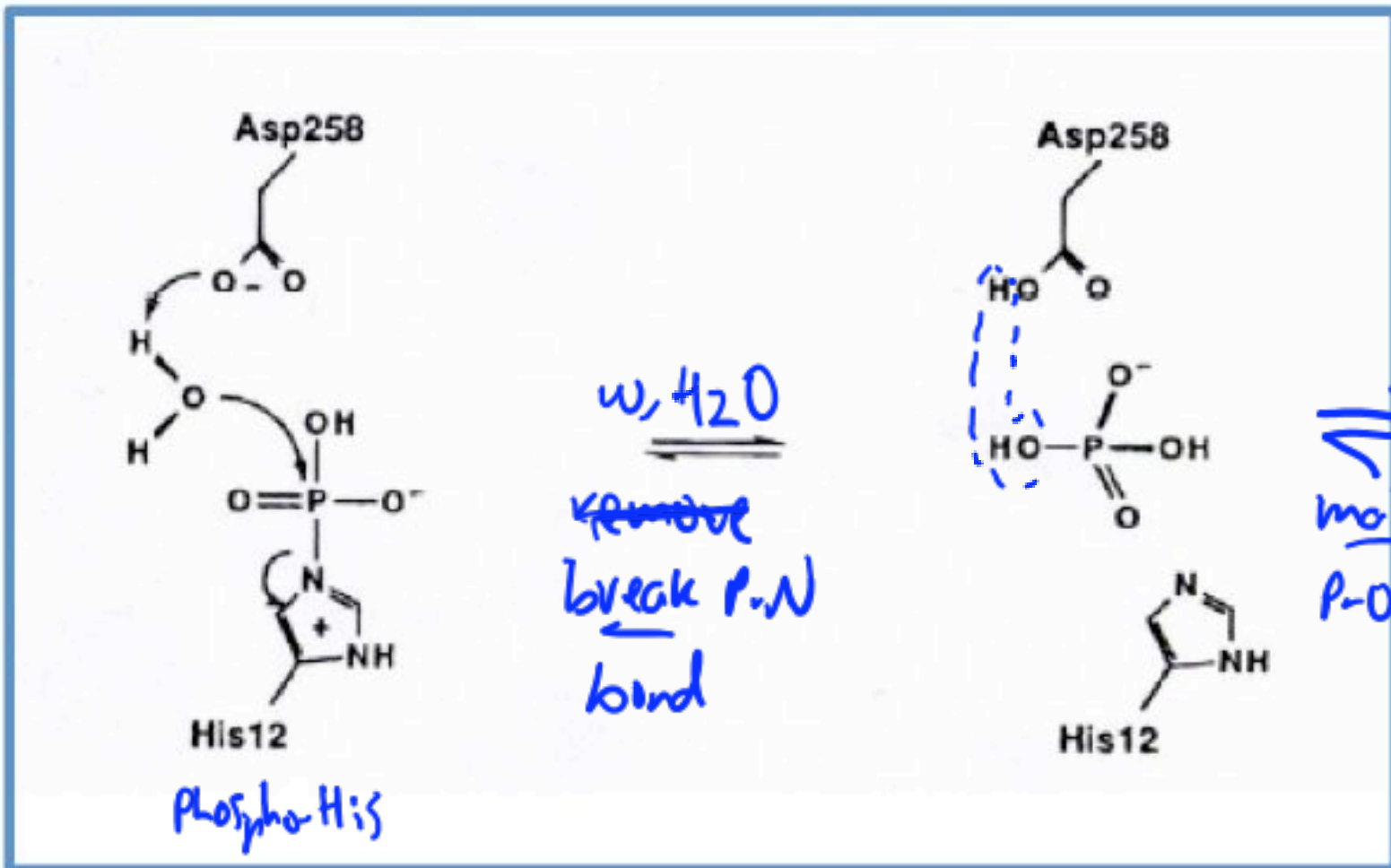
acid-labile

P-O

rel. acid-stable

Slide from N. Kuldell

Chemistry of phosphorelay system



Slide from N. Kuldell

Thermodynamics of phosphorelay

What is a "high-energy" bond?



P=O

$\Delta G < 0 \rightarrow$ spontaneous

Typical ΔG° of hydrolysis

-6.5 to -9.5 kcal/mol

-12 to -14 kcal/mol

[hydrolysis more spont.
∴ P-N less stable]

Which one is phosphoester, which is phosphoramidate?

P-O (Asp)

P-N (His)

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

How can we tune the output?

Think with your partner about...

- How to make plates lighter in the light

1. auto (P) ↓

2. (P) transfer ↓

Xmake more basic → side effects

* 3. (P) off ↑

method; open screen mutants

- How to make plates darker in the dark

1. ↑

* is in our library

* 2. ↑

3. ↓

mutants have specific residues targeted

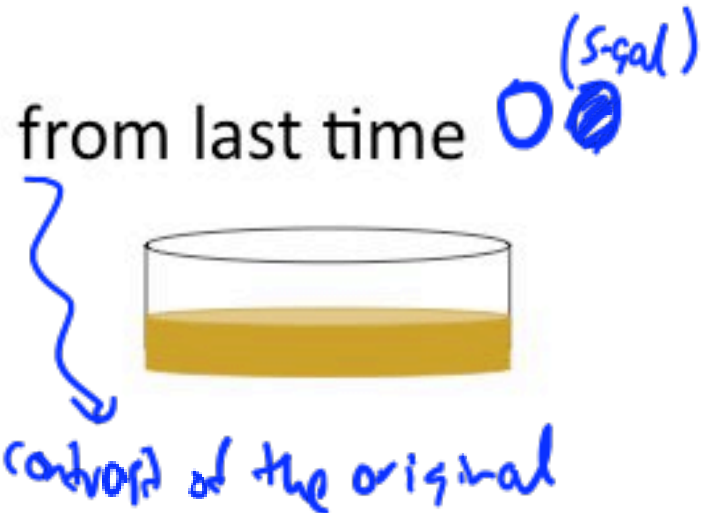
Today in Lab

- Observe/take pics of solid media from last time
- Prepare bacterial photograph

- Considerations for preparing mask

X gradients (just B&W)

resolution



- Test liquid cultures from last time

- β -gal assay (lyse cells, etc.) *ONPG*

- Expected results:

$[\beta\text{-gal}]_{\text{light}} < [\beta\text{-gal}]_{\text{dark}}$



- Atissa will give talk about talks @ 3:30 pm