

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

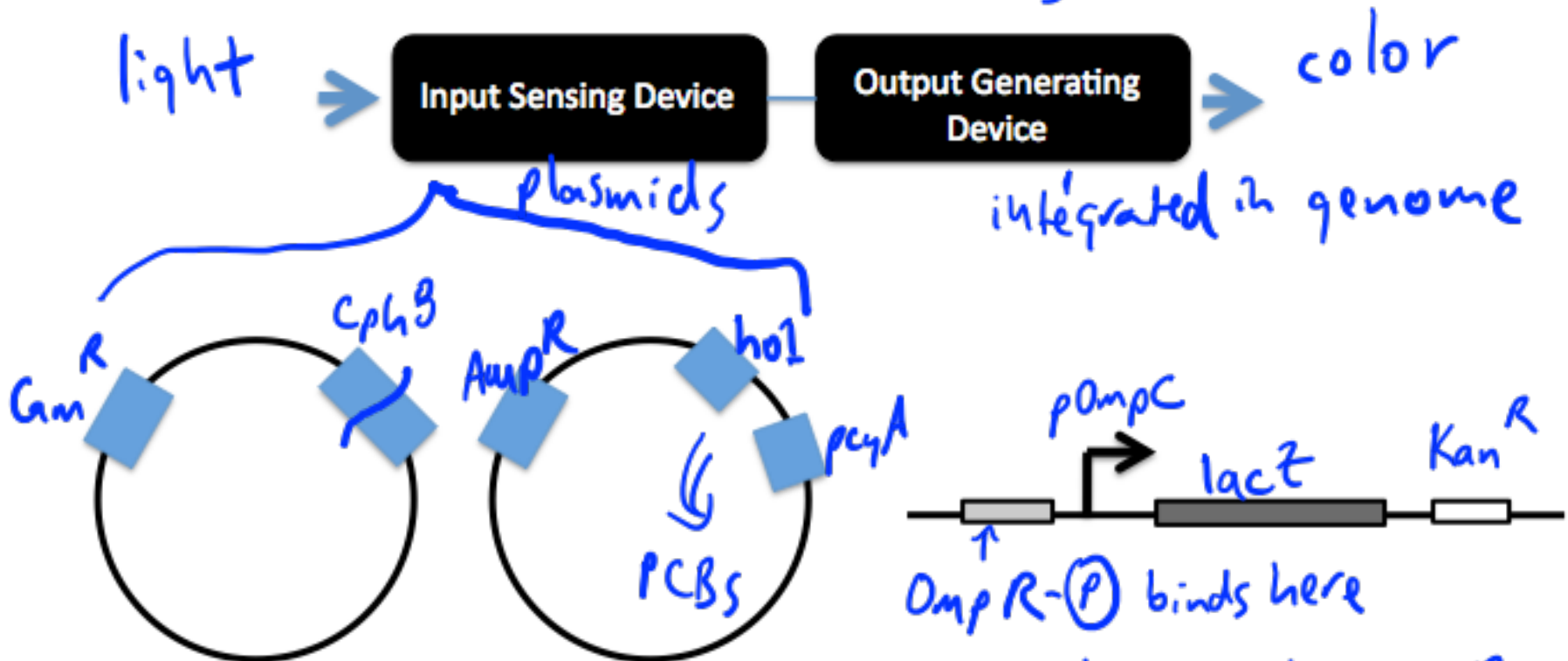
Announcements

- Wrapping up Mod 1
 - summary due Sat 5 pm
 - blog post due Sun 5 pm
- Module 2 oral assessment
 - journal club presentations
 - W 10/22 on 2CSignaling, **F 10/31** on SynthBio
 - thoughts about limiting per-day sign-up or not?
 - please choose paper by M2D3
- Module 2 written assessment
 - draft: Mon 11/10; revision: Wed 11/26
 - can improve up to 1 full letter grade on revision
 - see assignment description and conversational rubric

Recap BP components

Fill in boxes with a partner...

~~EnvZ~~
CphB = sensing OmpR = response regulator



CphB = Cph1/EnvZ
Cph1 + PCB = functional (holo-)Cph1

- * rely on native OmpR
- * ΔEnvZ
- * ΔlacZ

Where are we/going?

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

define original system properties

Solid → contrast

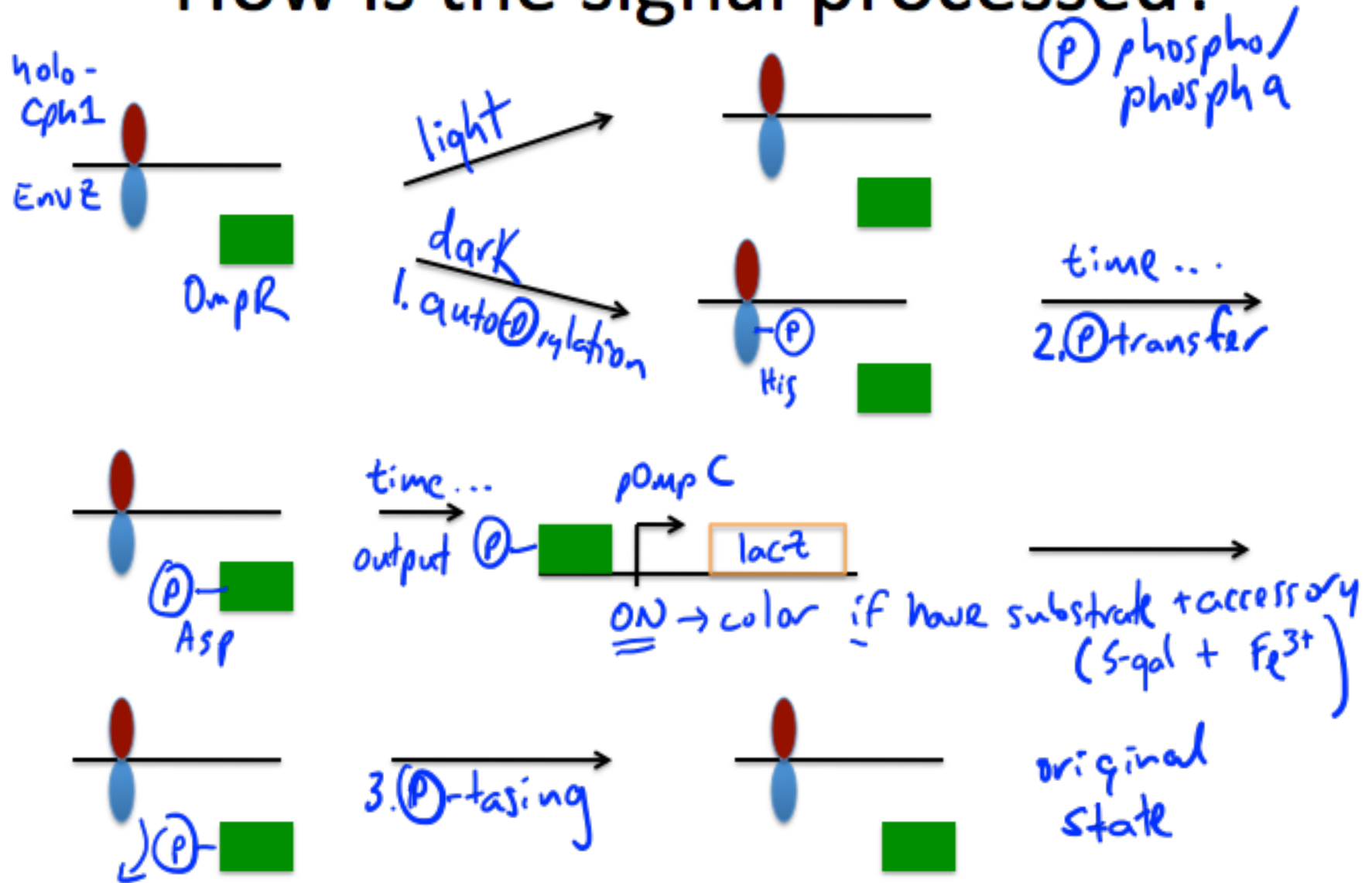
liquid → quantifying

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

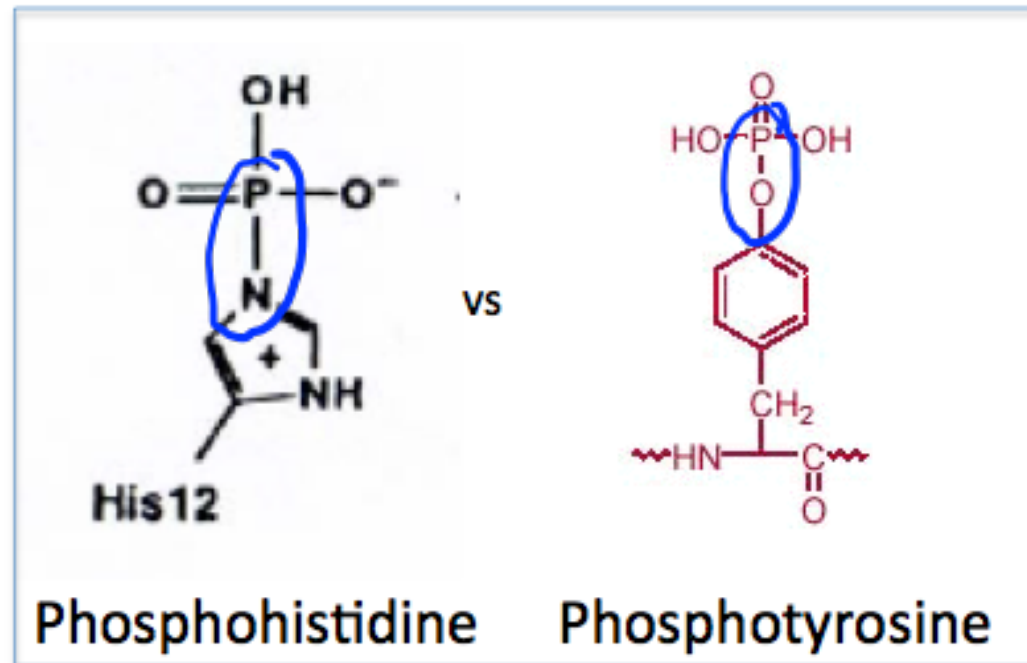
improve dynamic range / contrast

(by genetic screen)

How is the signal processed?

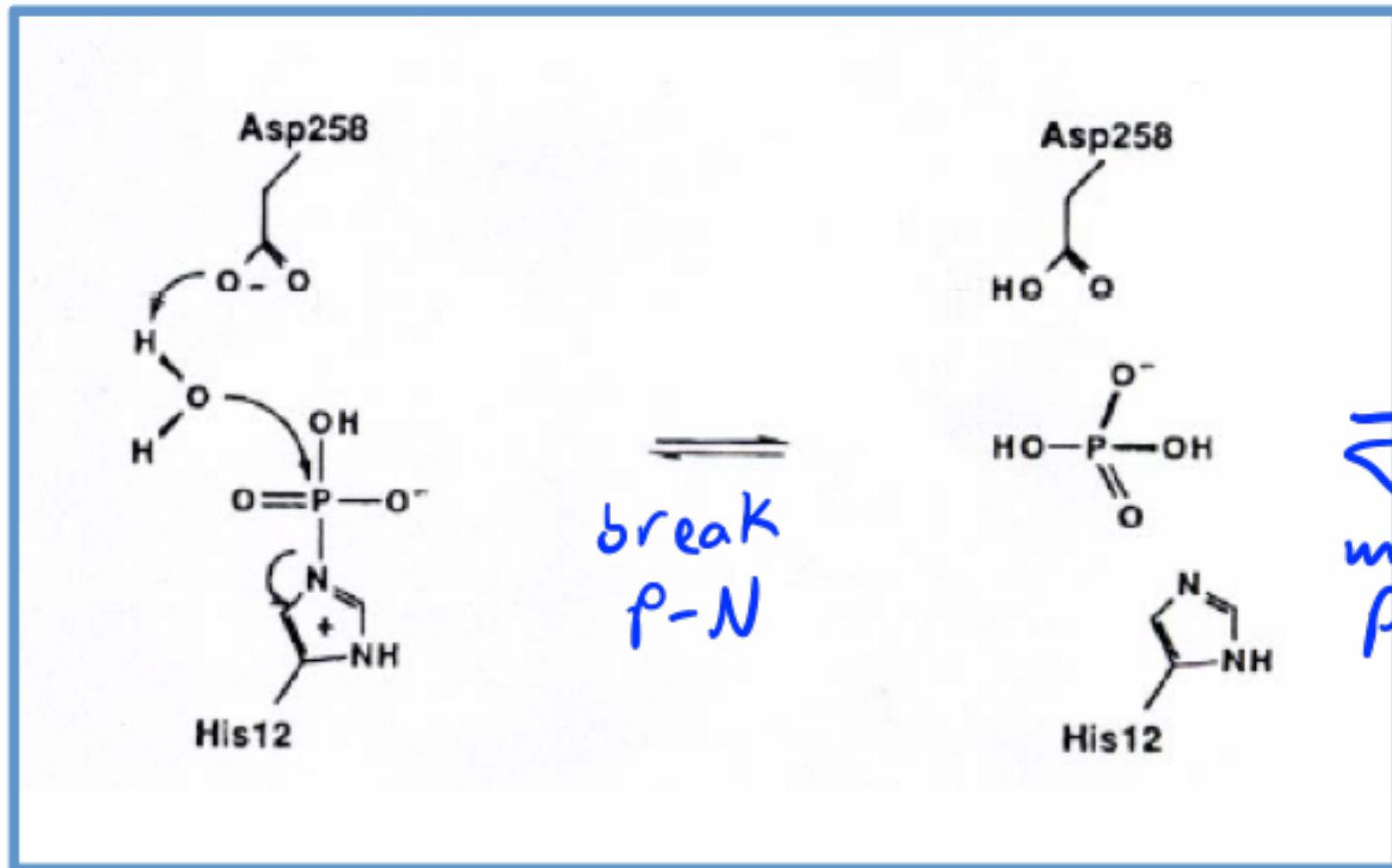


Chemistry of phospho-aa



- (1) acid-labile vs acid-stable
- (2) e⁻-delocalized form is insignificant vs " form plays some role
- ⇓
- ∴ unstable to nucleophilic attack
- Slide from N. Kuldell

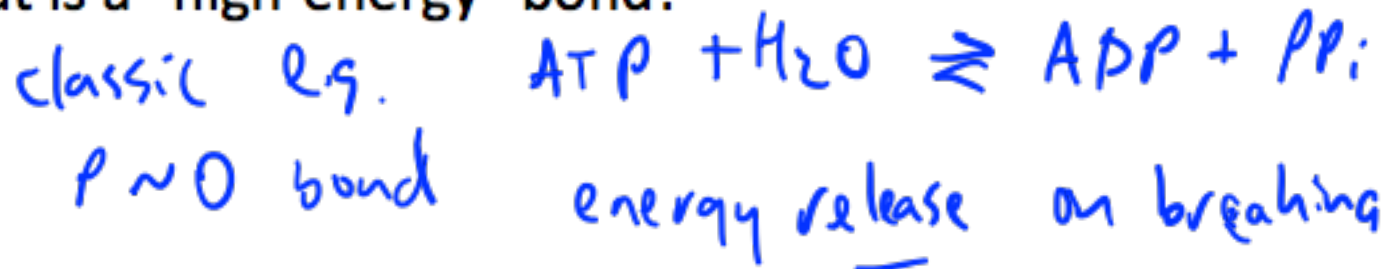
Chemistry of phosphorelay system



Slide from N. Kuldell

Thermodynamics of phosphorelay

What is a "high-energy" bond?



Typical ΔG° of

-6.5 to -9.5 kcal/mol

-12 to -14 kcal/mol

Which one is phosphoester, which is phosphoramidate?

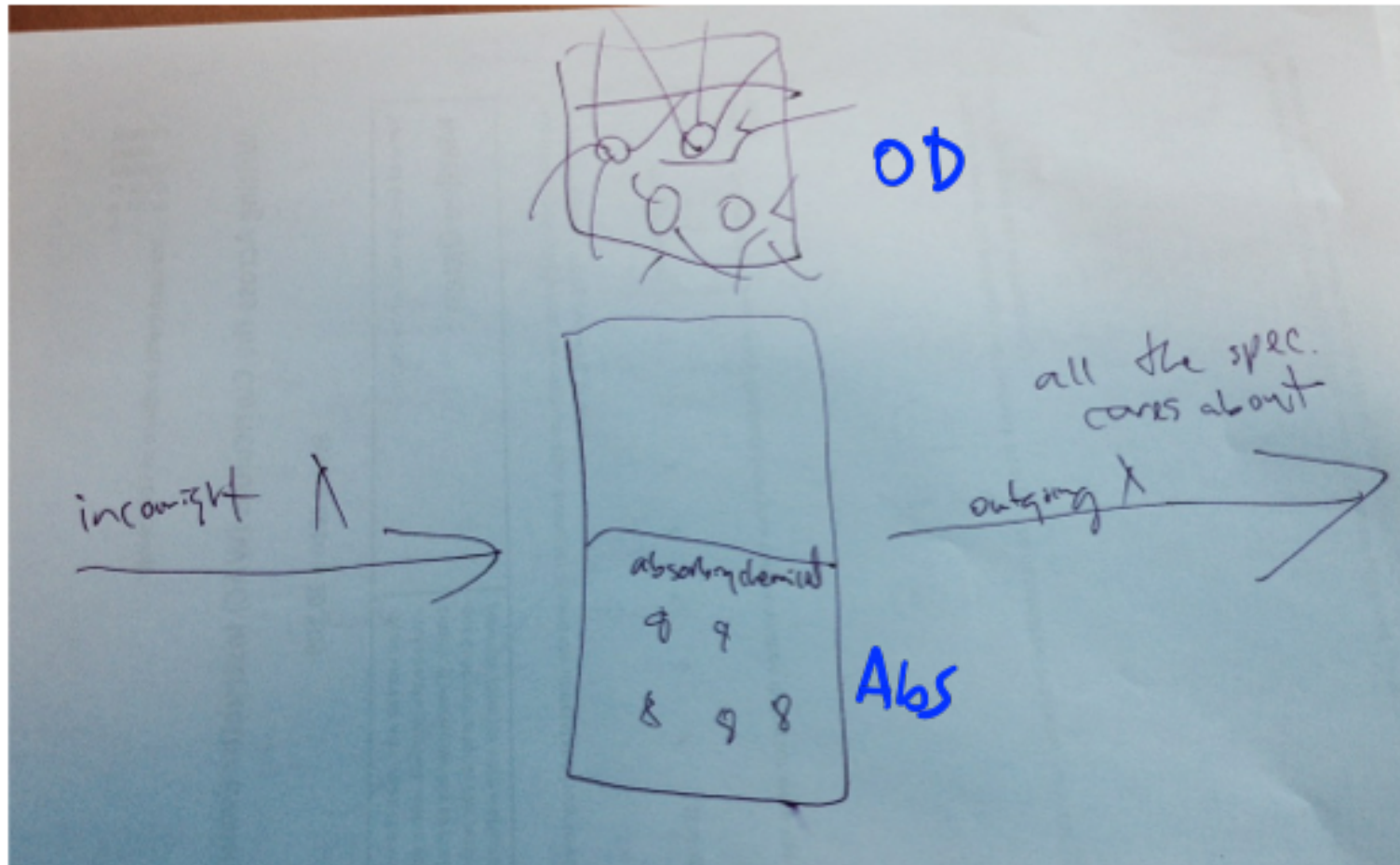
P~N (His)

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

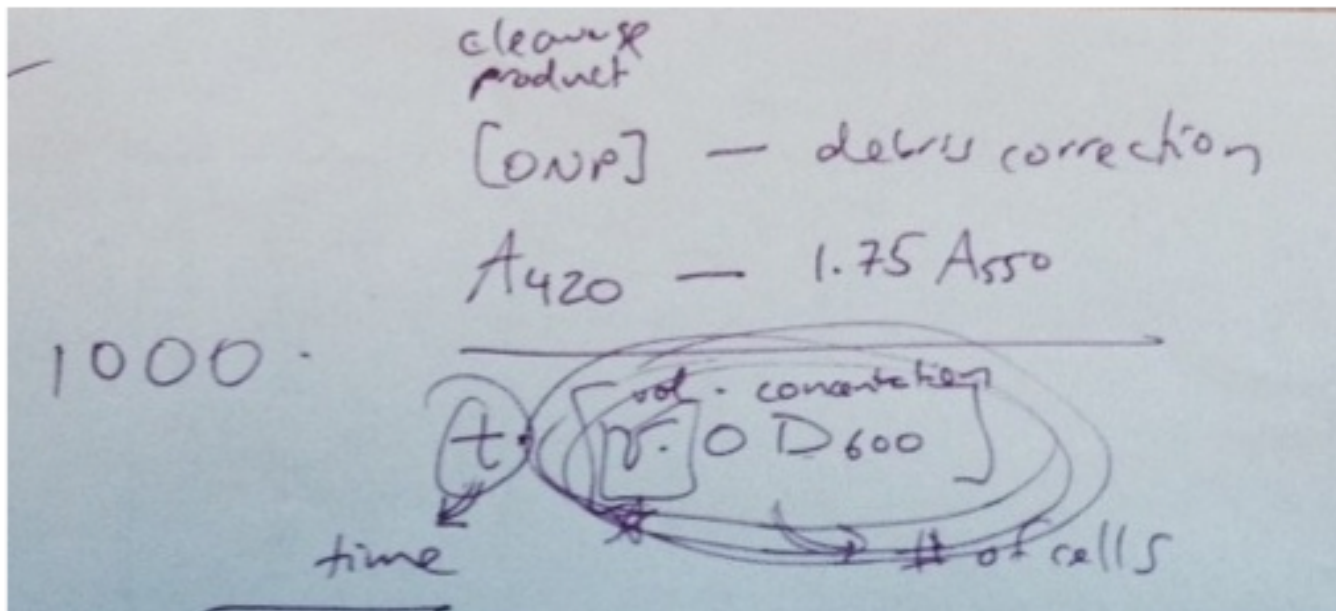
TinkerCell modeling program

- 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

Tidbit 1: OD versus Abs



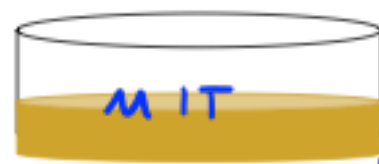
Tidbit 2: Miller equation



activity
per cell · (time)

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



* tips
for
resolution

