

M2D1:

Testing an engineered biological system

10/07/14

Lab business

- Welcome Yongjin Park!
 - For Evernote access: yjpark352@gmail.com
- DNA engineering abstract and data summary
 - Due Friday, October 10 by 5pm
 - Office Hours:
 - Shannon – W 7-8:30p (Simmons Hall, Rm 528),
R 7-8:30p (Rm 56-302), F 10-11a (16-429b)
 - Noreen – W 1-3p (16-429b)
 - Agi – T 4-5p (16-319), R 4-5:30p (16-319)

Mod 2: Bacterial photography system

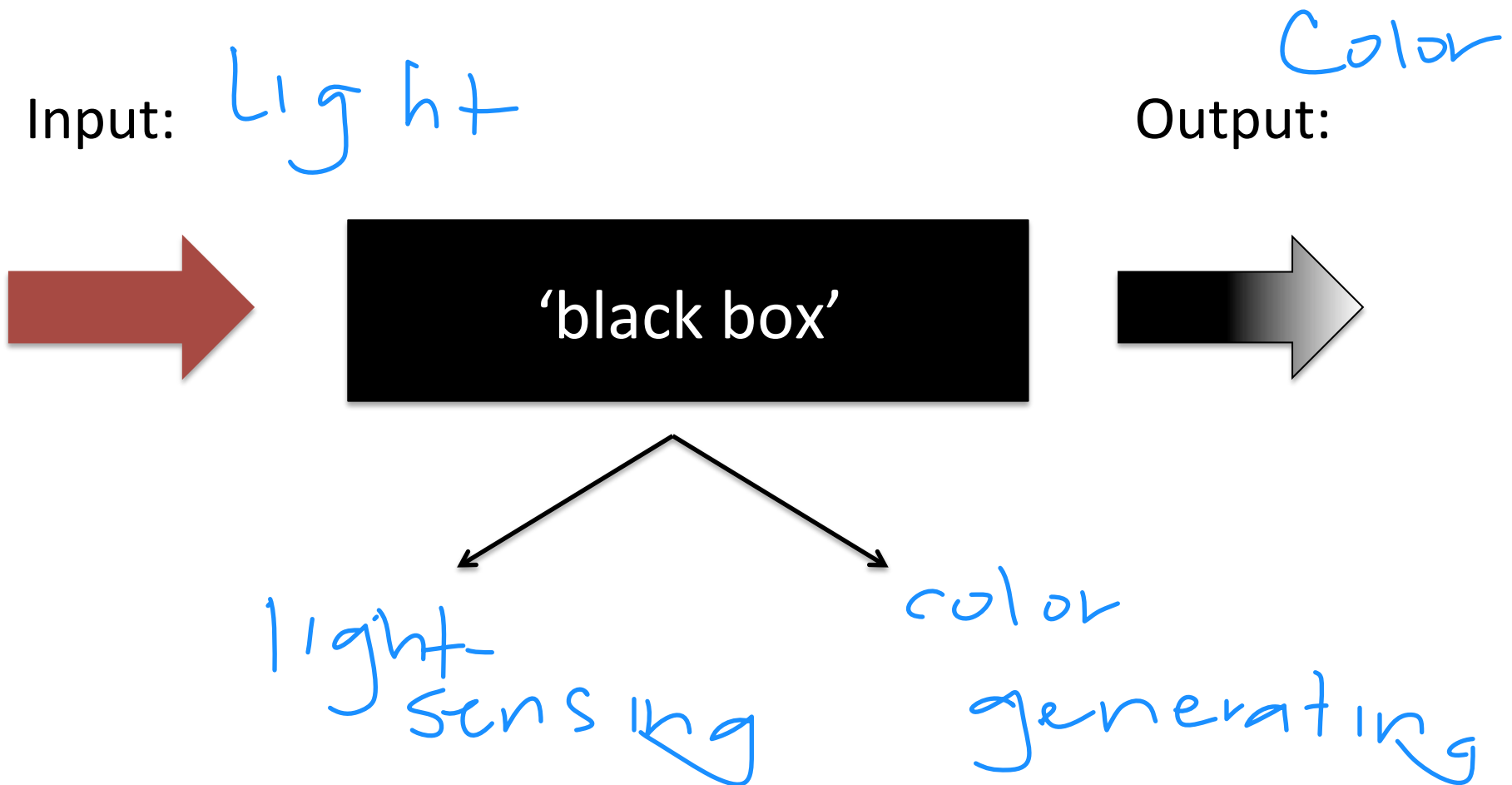
Buddy

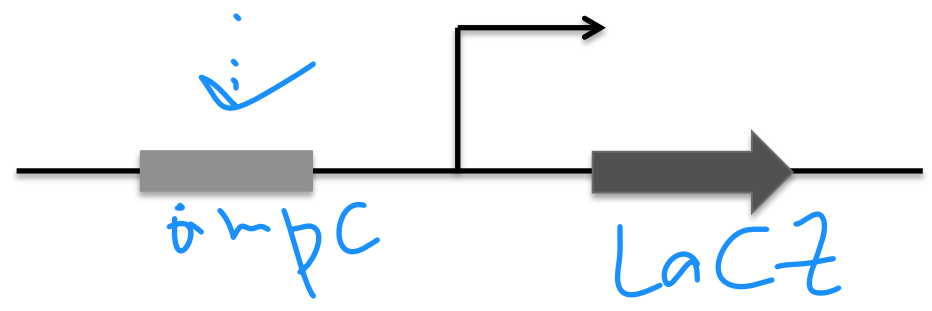
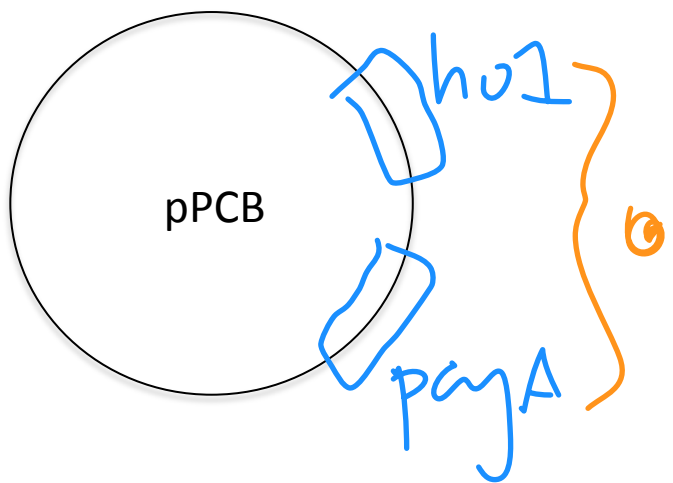
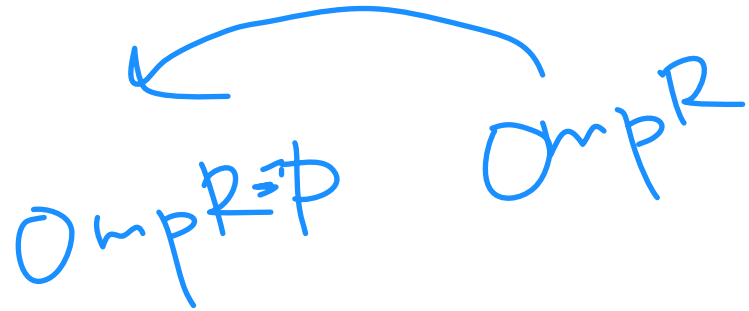
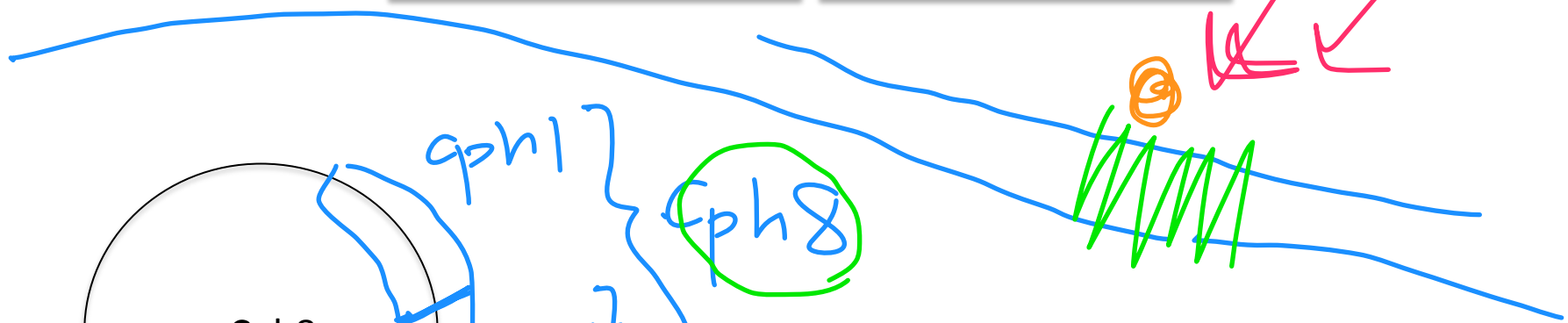


“What manner of sorcery is this?”

Engineered bacterial photography system

An abstracted overview...

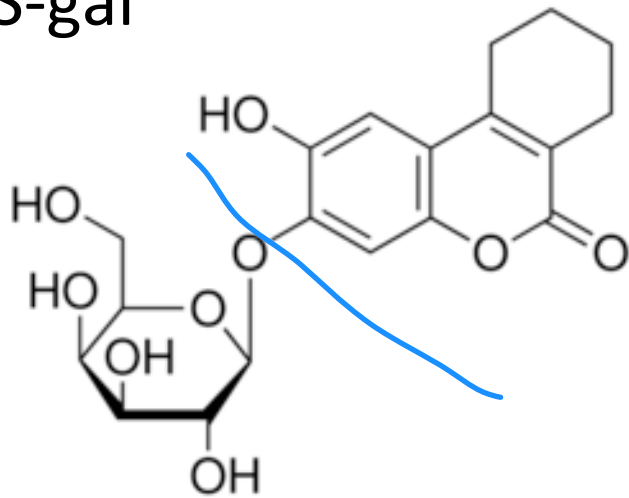




Why does the color change?

- In metabolism, LacZ cleaves lactose

S-gal



galactose

is galactose
glucose

= chelate
ferric ions
⇒ black
precipitate

System details

- Light obstructed:
 - Cph8 is *active*
 - LacZ is *active*
 - Color observed is *black*
- Light unobstructed:
 - Cph8 is *inactive*
 - LacZ is *inactive*
 - Color observed is *whity*

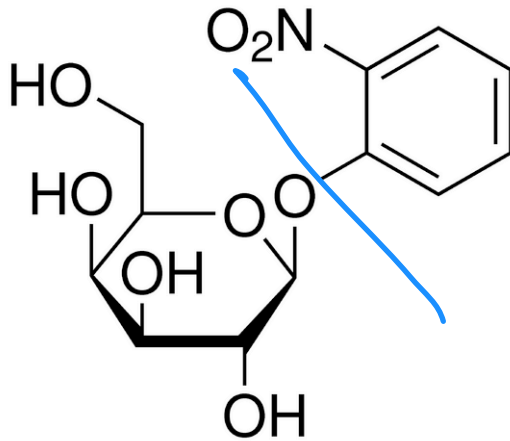


Native vs engineered system

	Native	Engineered
Stimuli	Salt	light
Sensor	EnvZ	Chp8
Regulator	OmpR	OmpR
Response	Porins	color

Testing the system

ONPG

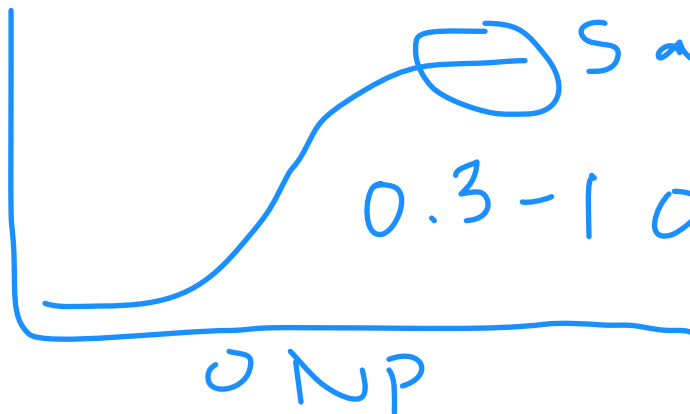


ONP = yellow product

ONP product is proportional to LacZ

galactose

420



β -galactosidase assay

1. Measure OD 600
2. Lyse cells - release LacZ
3. Start reaction - ONPG, time!
4. Stop reaction - Na_2CO_3
5. Eliminate cell debris - 550
6. Measure yellow product - 420
7. Calculate activity

Miller units =
$$1000 * \frac{(Abs_{420} - (1.75 * Abs_{550}))}{(t * v * Abs_{600})}$$

What is your goal?

- Today, you will test the engineered bacterial photography system
 1. Setup 'coli-roid' (NB466)
 2. Inoculate liquid bacterial cultures (NB466)
 3. Practice β -gal assay and calculations (NB5)
- Overall, you will employ a genetic screen to improve the contrast of the existing system!

Module 2 assignment

- Journal club presentations
 - Sign up to present on either M2D4 (October 21) or M2D8 (November 4)
 - List of papers on wiki
 - M2D4 Topic: Two-component systems
 - M2D8 Topic: Synthetic biology
 - See wiki page for assignment details
- Reserve your article by M2D3