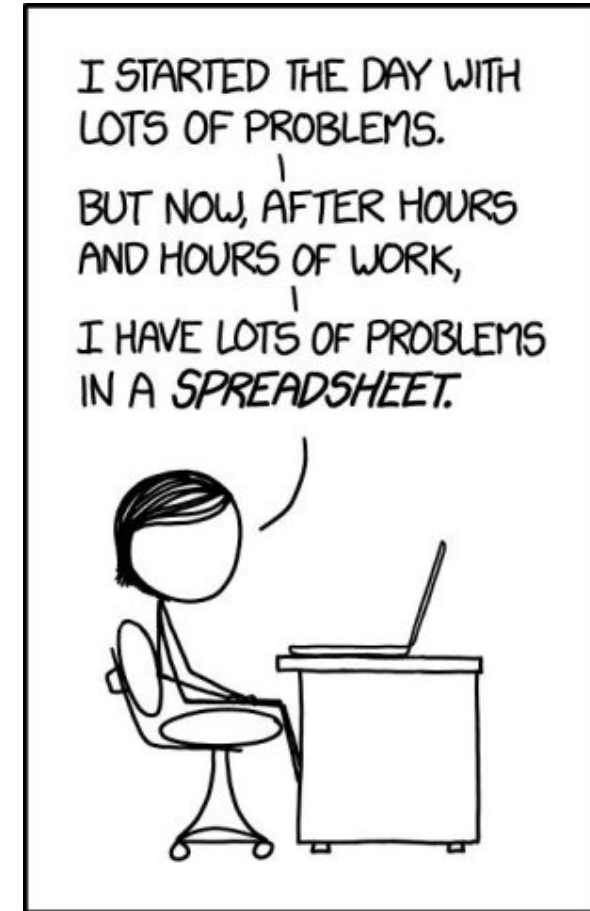


M2D7: Analyze ICP-OES data and examine yeast tolerance to metal

- Prelab
 - Review Mod2 project experiments
- Examine and compile ICP-OES data
- Perform metal tolerance test using Fet4_mutant yeast



Overview of Mod 2 experiments

Last lab:

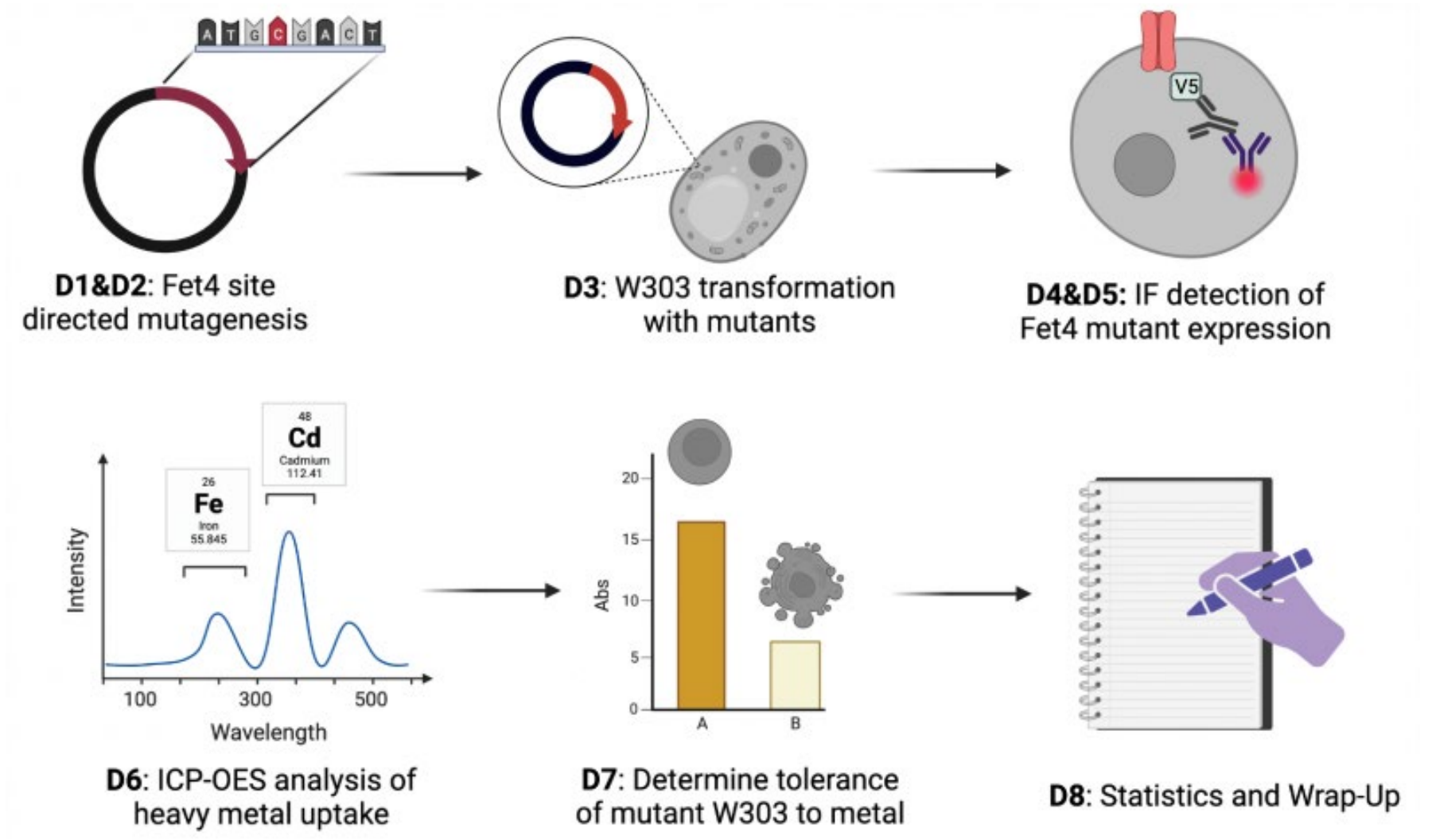
ICP-OES

This lab:

BacTiter - Glo

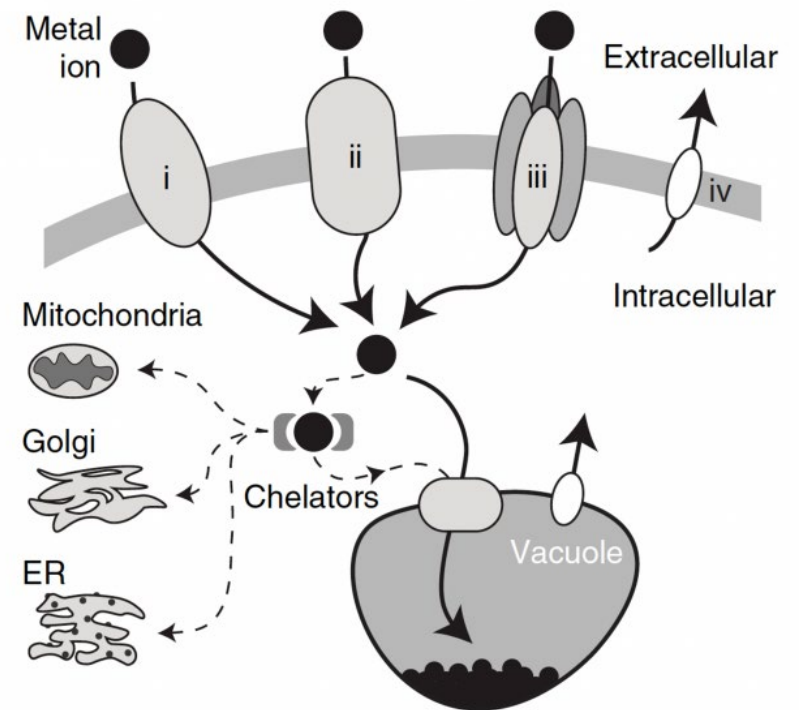
Next lab:

Putting everything together

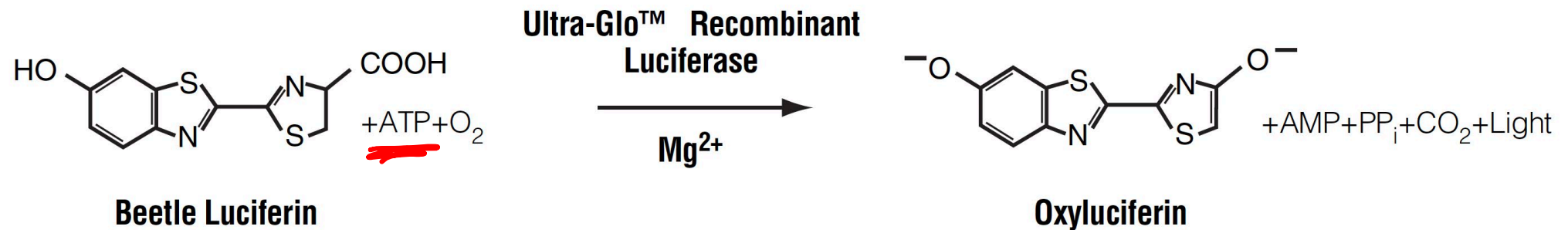


Metal tolerance experiment overview

- Examine OD_{600} for your Fet4_mutant culture
- Dilute your cultures to achieve 8ml of culture at ~ 1.0 OD_{600}
- Split between + cadmium & no cadmium tubes
- Incubate for 2.5 hours
- Recheck OD_{600}
- Add cell suspension and BacTiter-Glo reagent to 96 well plate
- Read luminescence on platereader upstairs



What is the BacTiter-Glo assay measuring?

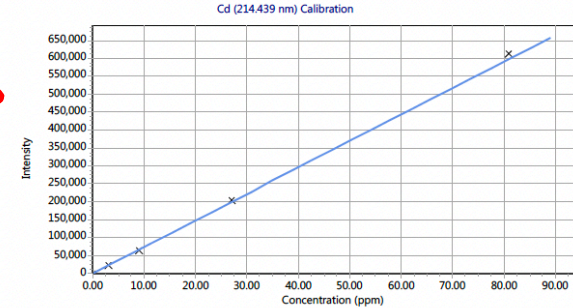


- Generation of bioluminescence
- The luciferase enzyme oxidizes luciferin substrate
 - utilizes Mg²⁺ and ATP
- Light is produced as part of the reaction

Measured **bioluminescence** has a **linear relationship with ATP**
ATP production is indicative of a live, **metabolically active cell**

ICP-OES calibration data (found in pdf)

→ Cd (214.439 nm)
Intensity = 7379.58566373 * Concentration + 13.00393963
Correlation coefficient: 0.99997



→

Standards	Intensity	Method Concentration	Calculated Concentration	% Error
Blank	9.41	0.00	0.00	N/A
Standard 1	22998.83	3.00	3.11	3.83
Standard 2	63918.04	9.00	8.66	3.78
Standard 3	205044.11	27.00	27.78	2.90
Standard 4	614049.08	81.00	83.21	2.73

- each wavelength has a calibration curve established using the known standards we generated
- Standards= 0ppm, 3ppm, 9ppm, 27ppm, 81ppm

ICP-OES sample data (in pdf and csv file)

Sample Name: Sample 17

Date: 4/18/2023 11:22:03 AM

Rack:Tube: 2:22

Weight (g): 1

Volume (mL): 1

Dilution: 1

Analyte Results

Label	Solution Concentration	Unit	SD	%RSD	Intensity	Calculated Concentration
Cd (214.439 nm)	12.58	ppm	0.06	0.49	92819.61	12.58 (ppm)
Cd (219.463 nm)	12.15	ppm	0.56	4.57	97.35	12.15 (ppm)
Cd (223.986 nm)	11.31	ppm	0.75	6.67	63.52	11.31 (ppm)
Cd (226.502 nm)	12.12	ppm	0.07	0.57	175365.66	12.12 (ppm)
Cd (226.742 nm)	10.62	ppm	0.70	6.59	95.29	10.62 (ppm)
Cd (228.802 nm)	12.15	ppm	0.10	0.80	52154.44	12.15 (ppm)
Cd (230.662 nm)	11.68	ppm	0.07	0.63	198.56	11.68 (ppm)
Cd (231.275 nm)	11.81	ppm	0.77	6.55	221.83	11.81 (ppm)

- Each team is a sample
- Each class has a control
 - Untransformed (WT)
 - Fet4 overexpression(Fet4)
- concentration is calculated in parts per million (ppm)
 - based on peak intensity at the listed wavelength and calibration curve

Replicates Concentration

Label	Replicate 1	Replicate 2	Replicate 3	Units
Cd (214.439 nm)	12.51	12.64	12.58	ppm
Cd (219.463 nm)	12.77	11.99	11.69	ppm
Cd (223.986 nm)	12.13	11.14	10.65	ppm
Cd (226.502 nm)	12.12	12.05	12.19	ppm
Cd (226.742 nm)	9.89	11.29	10.68	ppm

Mod2 project review (AKA: what are we doing again?)

- Research goal:
- How does your mutagenesis design fit into this research goal?

Mod2 project review (AKA: what are we doing again?)

- How do your experiments fit into this goal?
 - Sequencing alignment:
 - ICP-OES:
 - BacTiter-Glo assay:

For today:

1. Set up metal tolerance experiment
2. Examine ICP-OES data during incubation
3. Collect data on metal tolerance (last data for the module!)

For M2D8

- Draft an outline of the Research Article discussion using the prompts on the homework section of the wiki and questions you answered for M2D7