M2D6: Analyze expression data and prepare metal uptake experiment

- Prelab

- Perform metal uptake experiment and prepare samples for ICP-OES

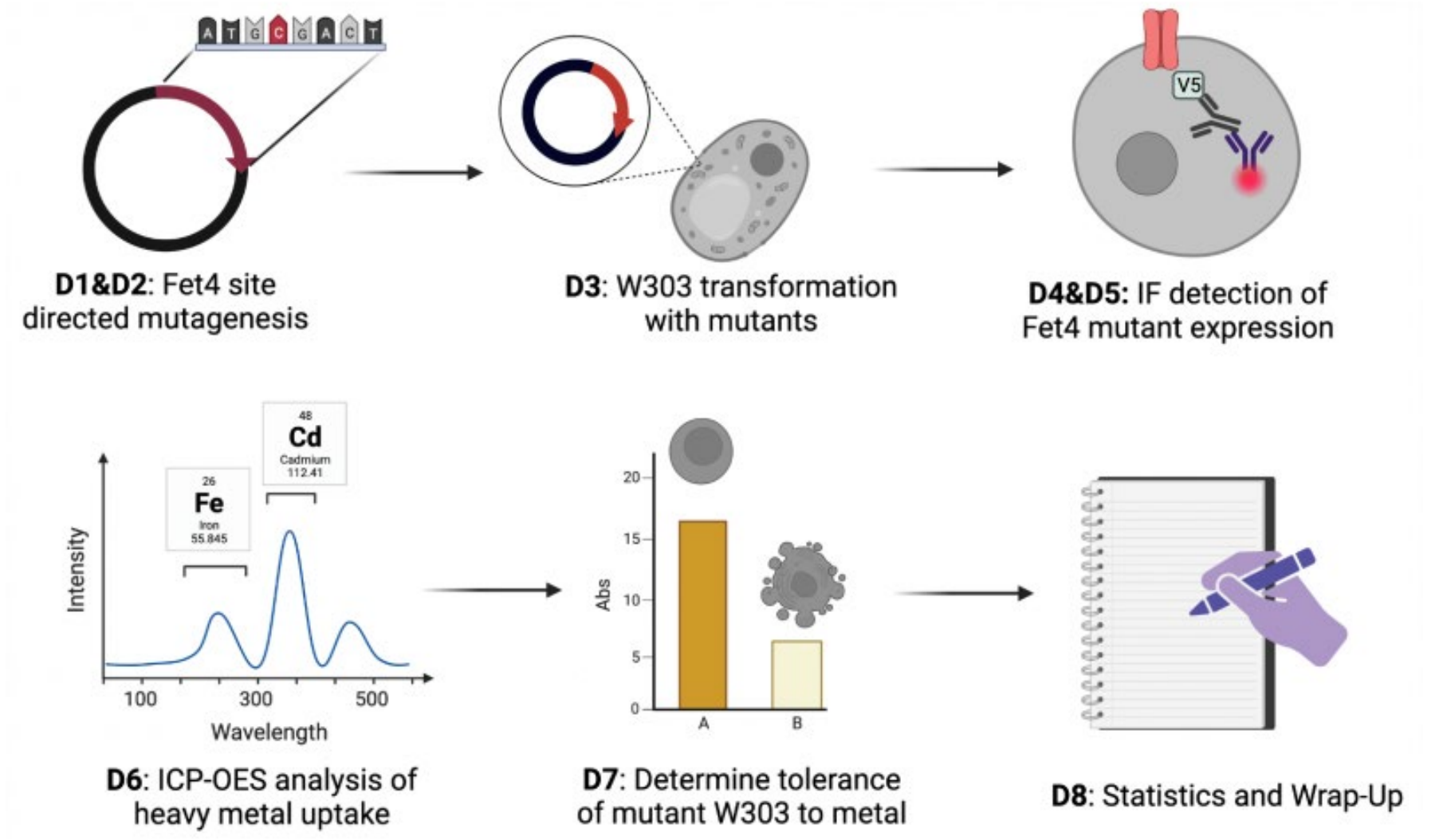# Overview of Mod 2 experiments

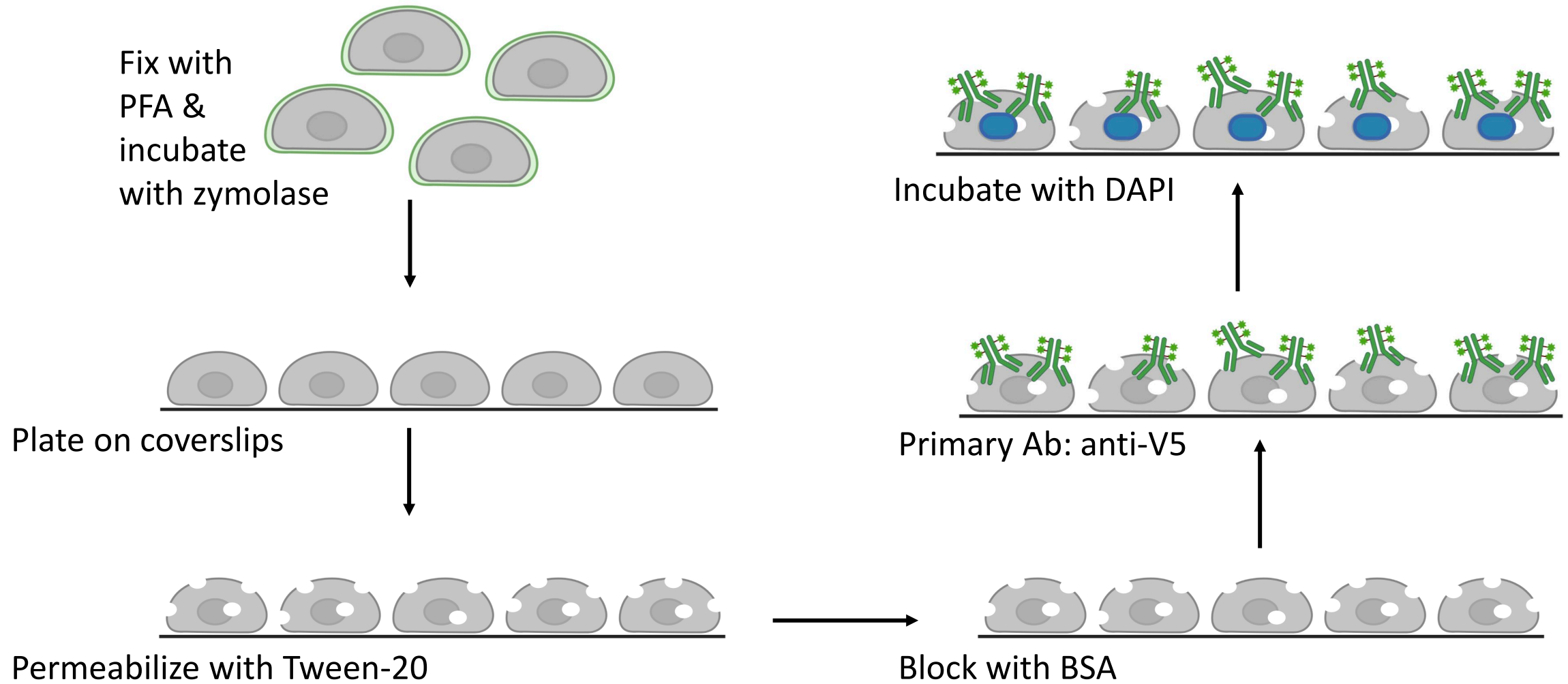Last lab:

Perform IF

This lab:
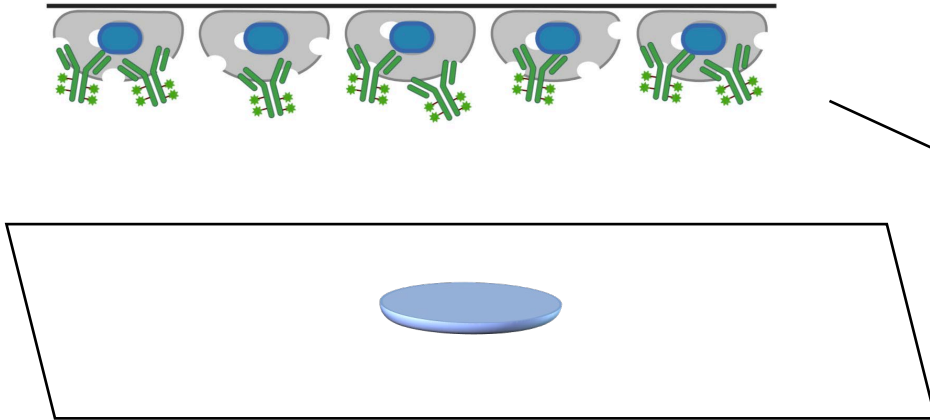
Analyze Images

Metal Uptake Experiment

Next lab:

Analyze ICP-OES data

D1&D2: Fet4 site directed mutagenesis

D3: W303 transformation with mutants

D4&D5: IF detection of Fet4 mutant expression

D6: ICP-OES analysis of heavy metal uptake

D7: Determine tolerance of mutant W303 to metal

D8: Statistics and Wrap-Up

Using immunofluorescence (IF) in yeast: steps in protocol

Fix with PFA & incubate with zymolase

Plate on coverslips

Permeabilize with Tween-20

Block with BSA

Primary Ab: anti-V5

Incubate with DAPI

Finish IF by mounting coverslips on slides

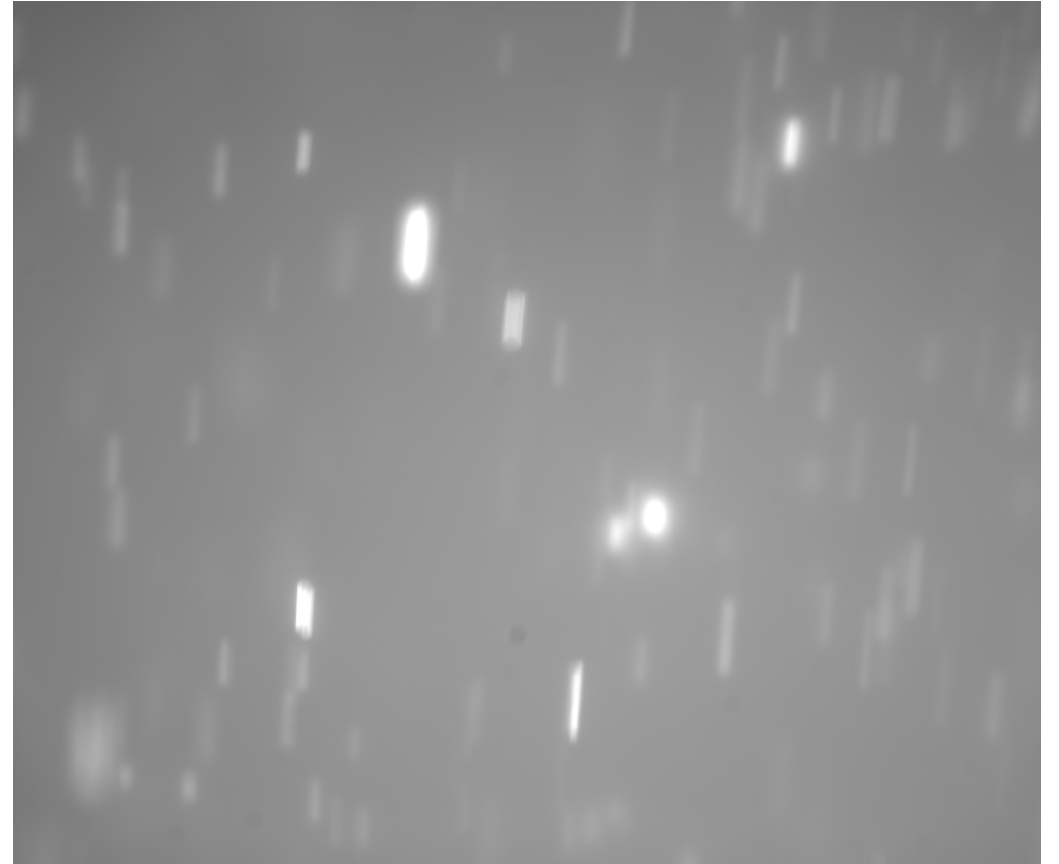Mount coverslip on glass slide
with mounting media
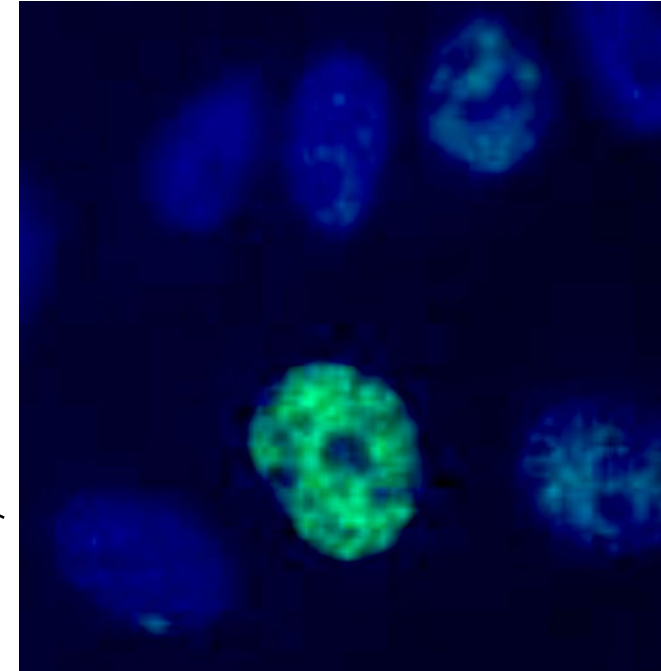
Blue= DAPI
Green= antibody staining

This is what your data look like

V5 Fluorescence

1) Is this what you expected your data to look like?

2) If not, what do you think happened?

3) What conclusions can you make about your experiment?

4) If this is unexpected, can we continue on with this module?

This is what your data look like

V5 Fluorescence

1) Is this what you expected your data to look like?

2) If not, what do you think happened?

3) What conclusions can you make about your experiment?

4) If this is unexpected, can we continue on with this module?

5) Given that your IF data is not analyzable, you will not be responsible for covering the IF in the final report

6) Instead, today you will look at my pilot data to practice analysis using ImageJ
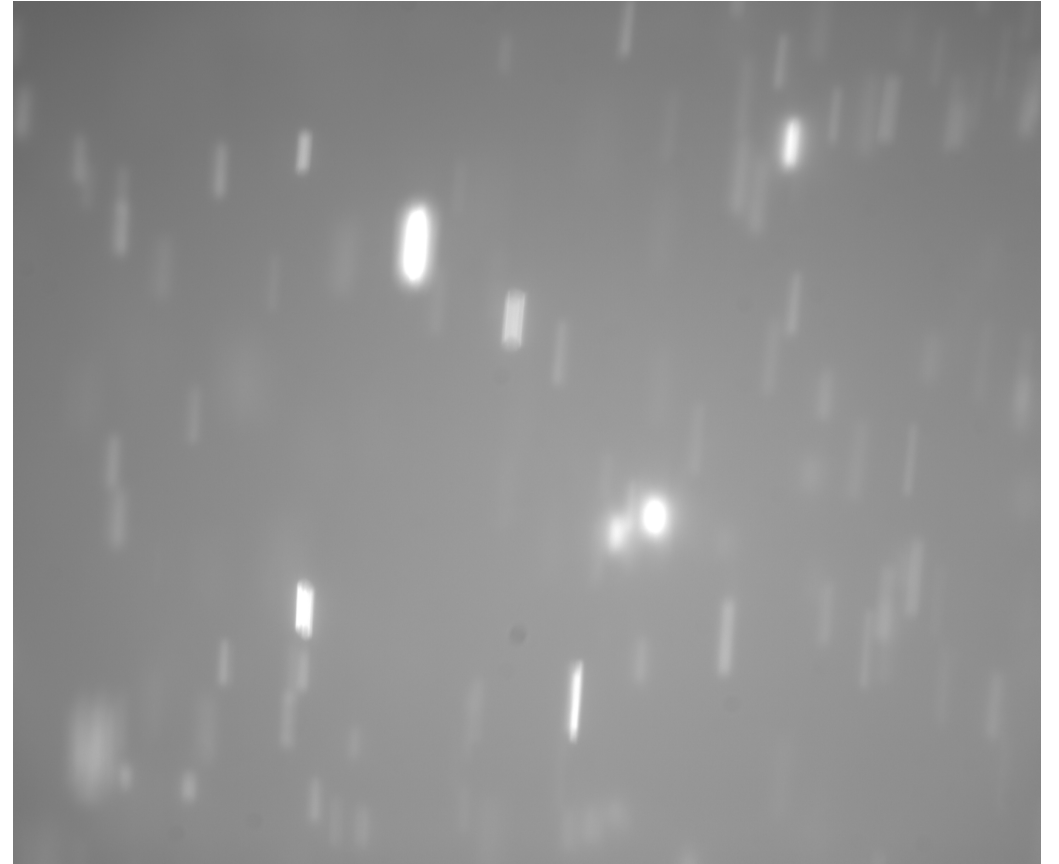
Uptake experiment overview

- Examine OD_{600} for your Fet4_mutant culture

- Dilute your culture to achieve 8ml of culture at ~1.0 OD_{600}

- Spike your yeast culture with 100uM metal

- Incubate for 2.5 hours

- Remove your yeast through centrifugation

- Digest material in media with nitric acid

- Filter the digested media to remove particulates

- Profit
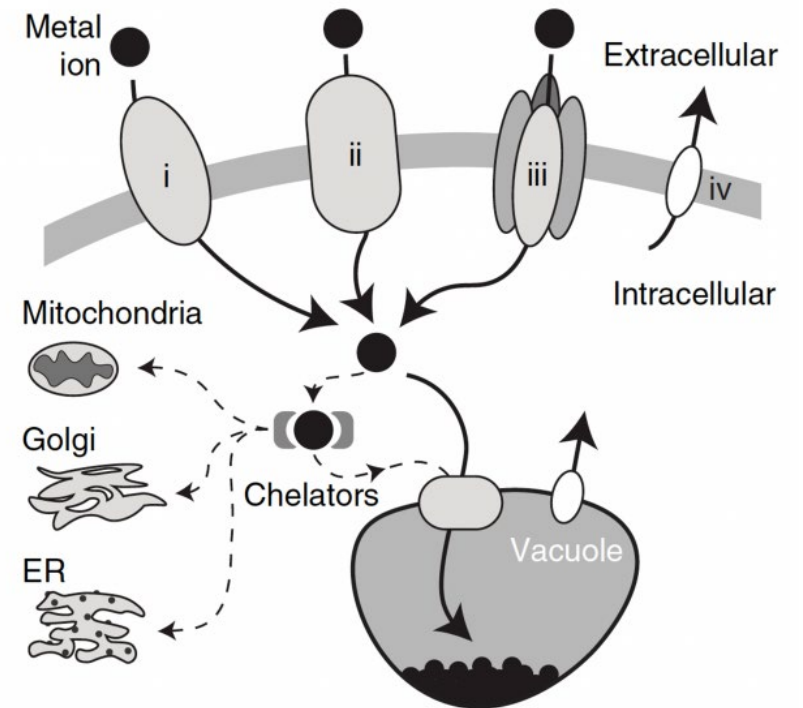
For today:

1. Set up metal uptake experiment

2. Play around with ImageJ as an image tool

3. Work on homework

4. Process metal samples for ICP-OES

For M2D7

1. Work on questions for discussion
   - **First follow up experiment is to determine mutant expression!**
   - Please propose 2 additional experiments for your discussion section
2. Create a research overview schematic
   - Visualizes key components of the project
   - Not an experimental schematic on a larger scale
   - Do NOT use prelab schematic as inspiration – that schematic is more of a teaching tool for 109 than it is a research schematic!