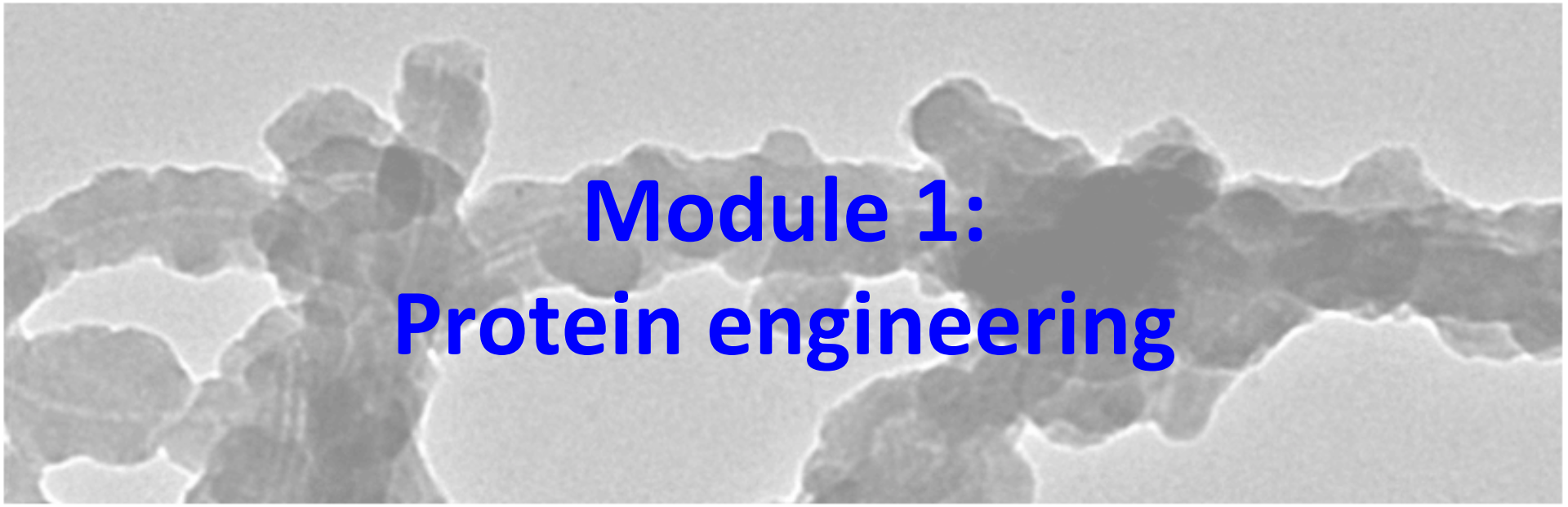


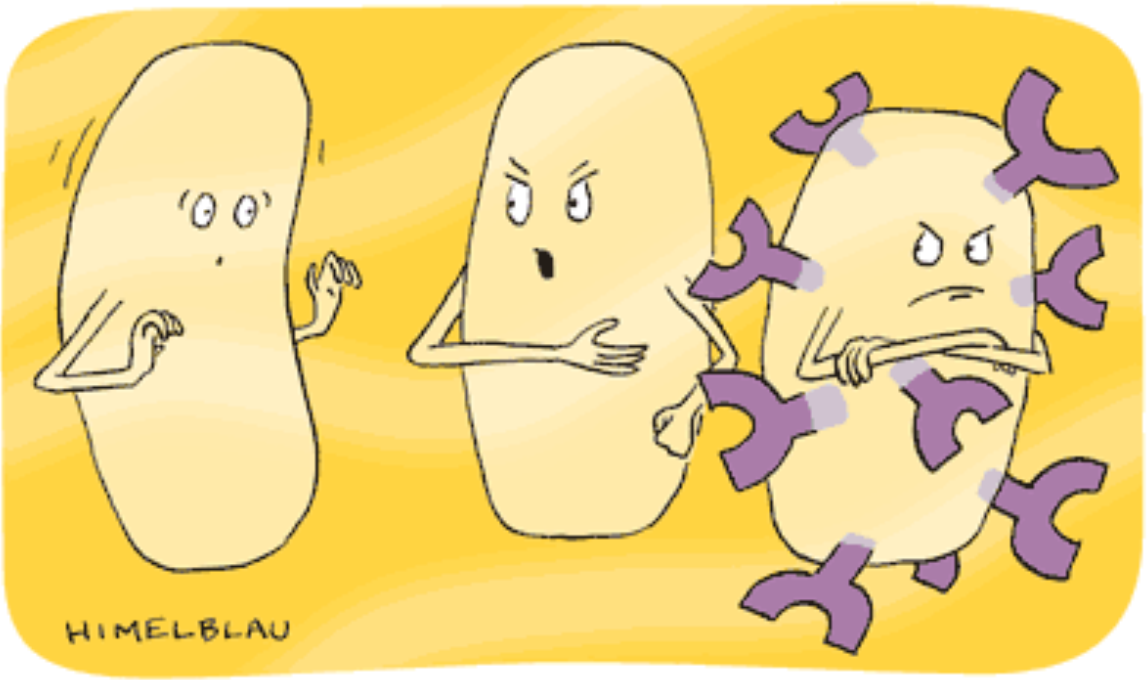
# Module 1:
# Protein engineering

I Module review

II Protein engineering summary

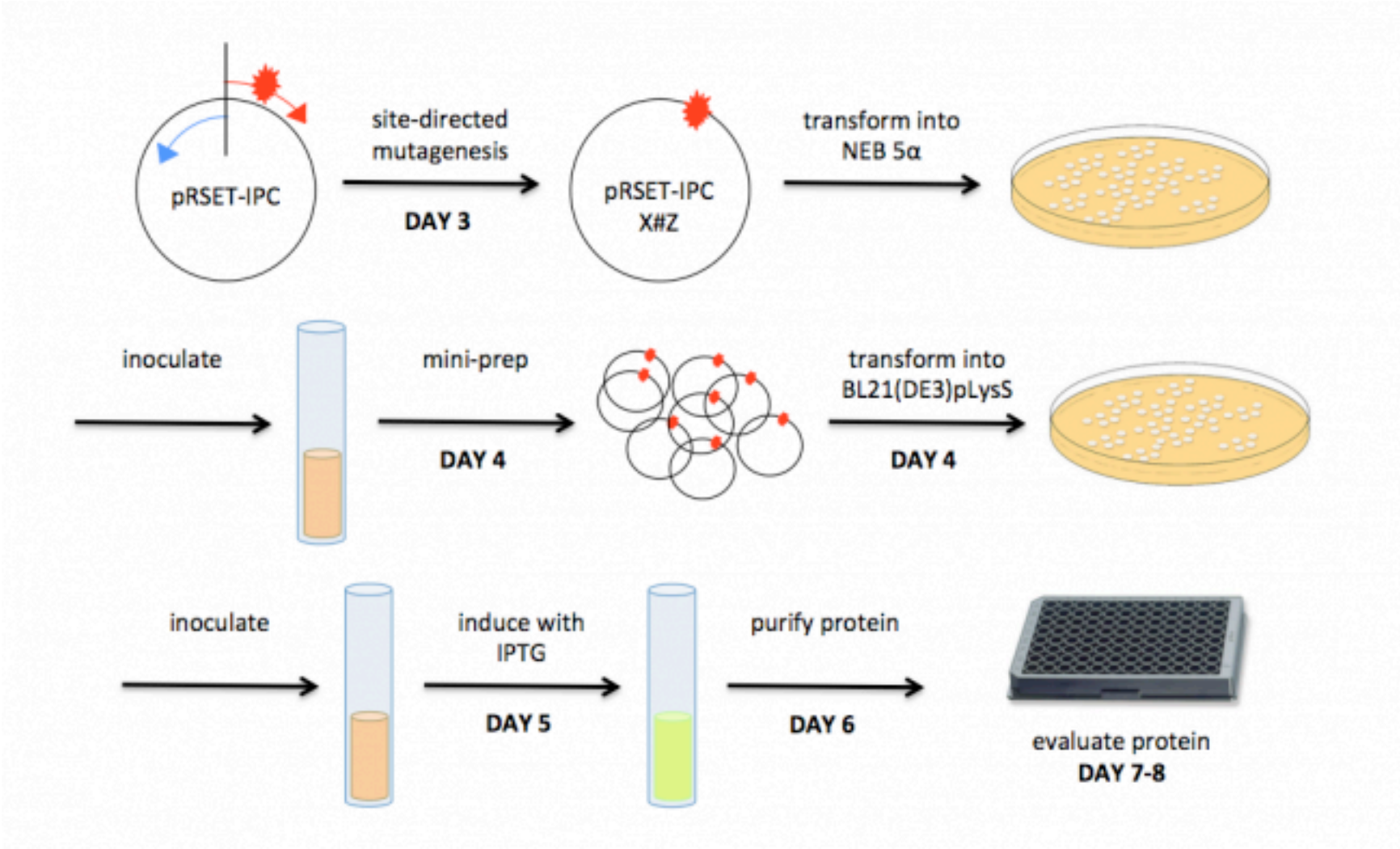
III Protein engineering mini-presentation

3/1/16

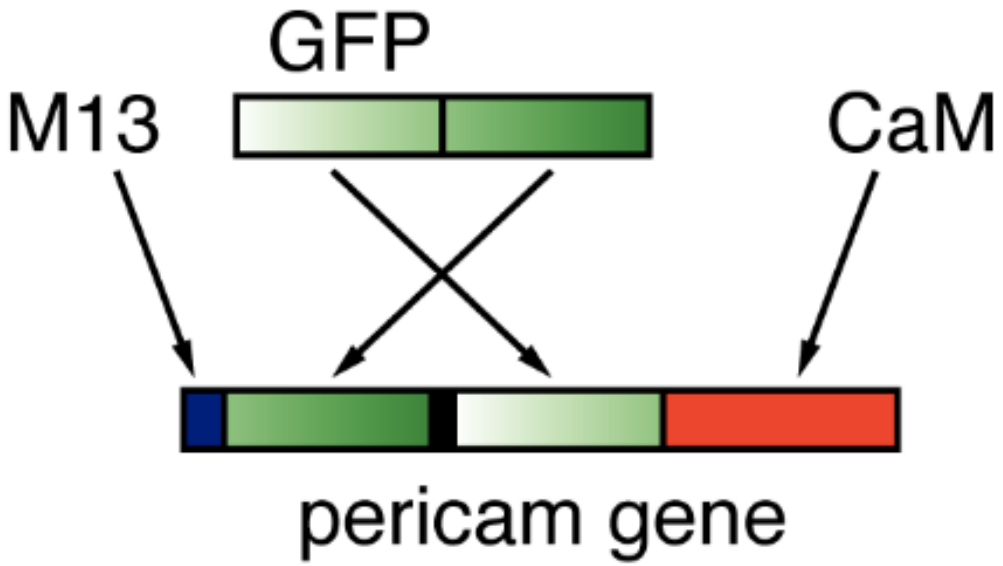



"Don't pick it up," I say, and he says, "It's just a *plasmid*, what harm could it do?" Well just look at him now....God knows *what* protein he's expressing!

# Mod 1 overview

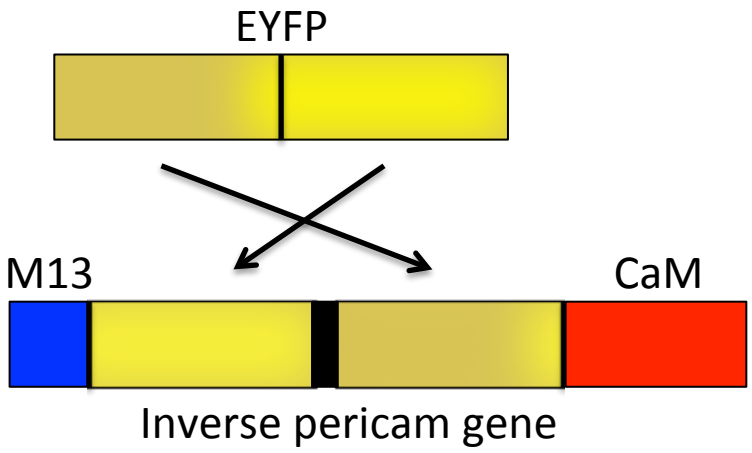


# Pericam engineered to sense [$Ca^{2+}$]

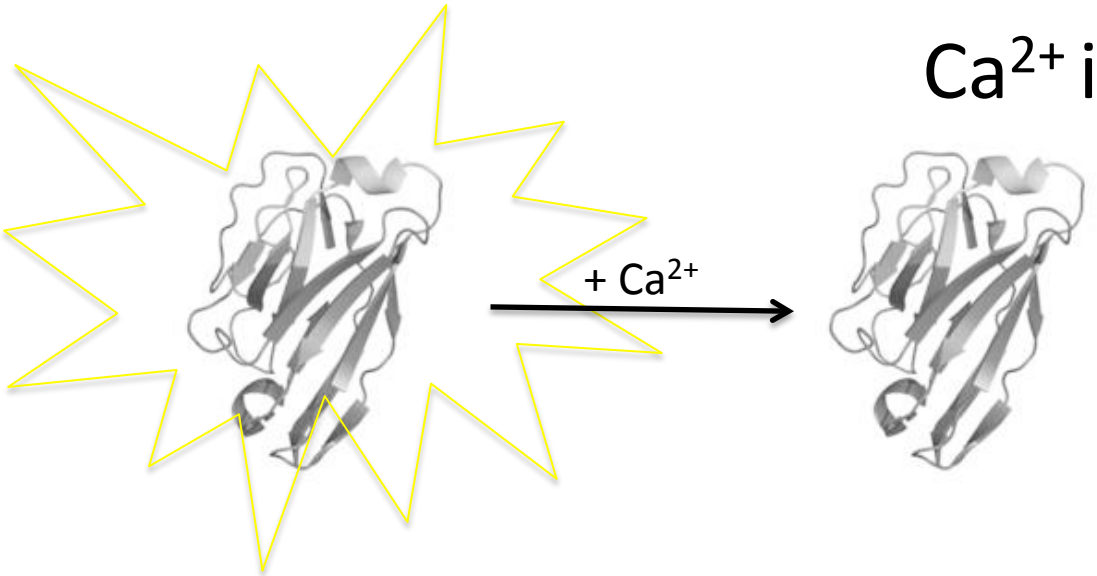



How does inverse pericam differ?

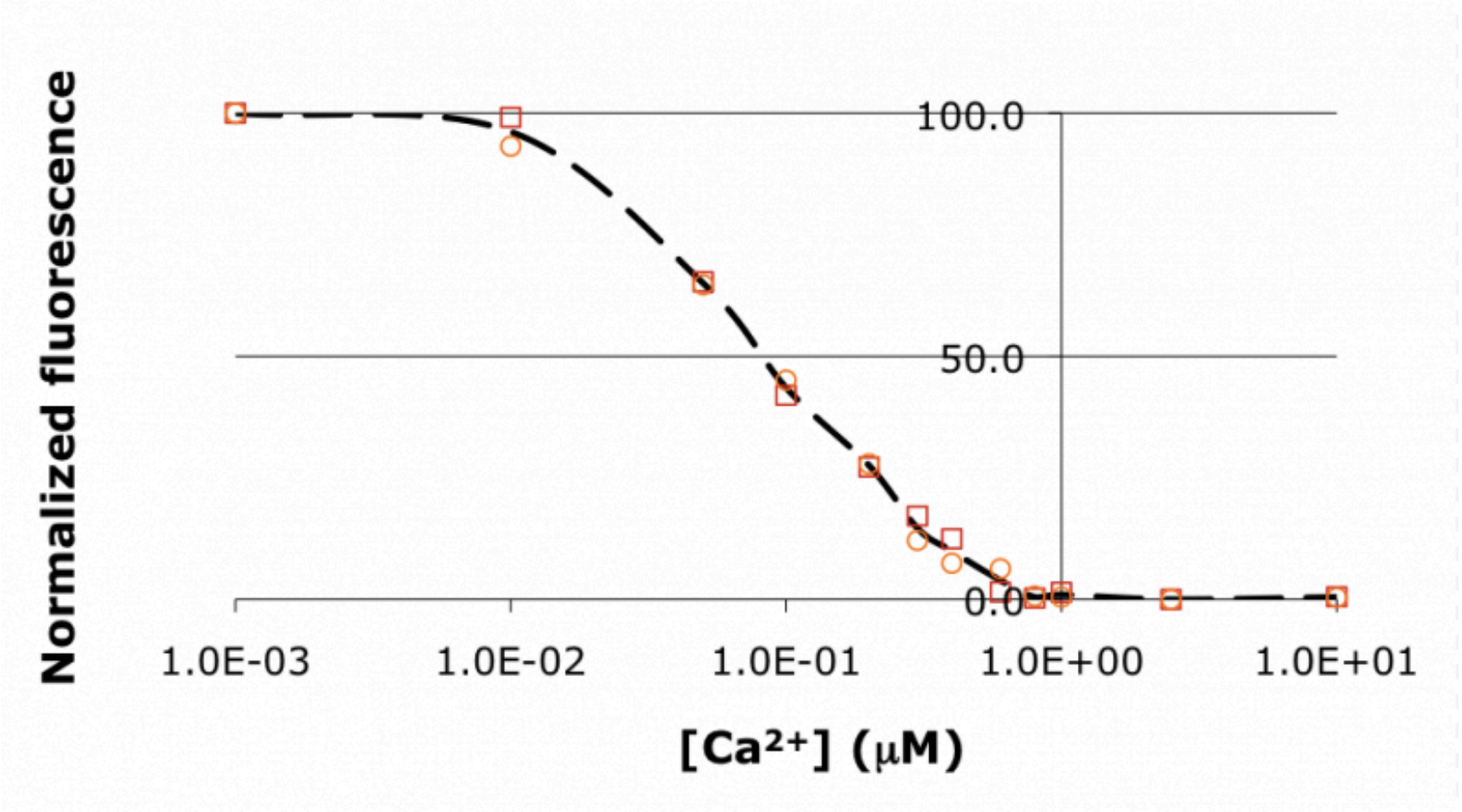
# Inverse pericam (IPC) derived from pericam




- Point mutation (H148T) introduced that reduces fluorescence when $Ca^{2+}$ is present

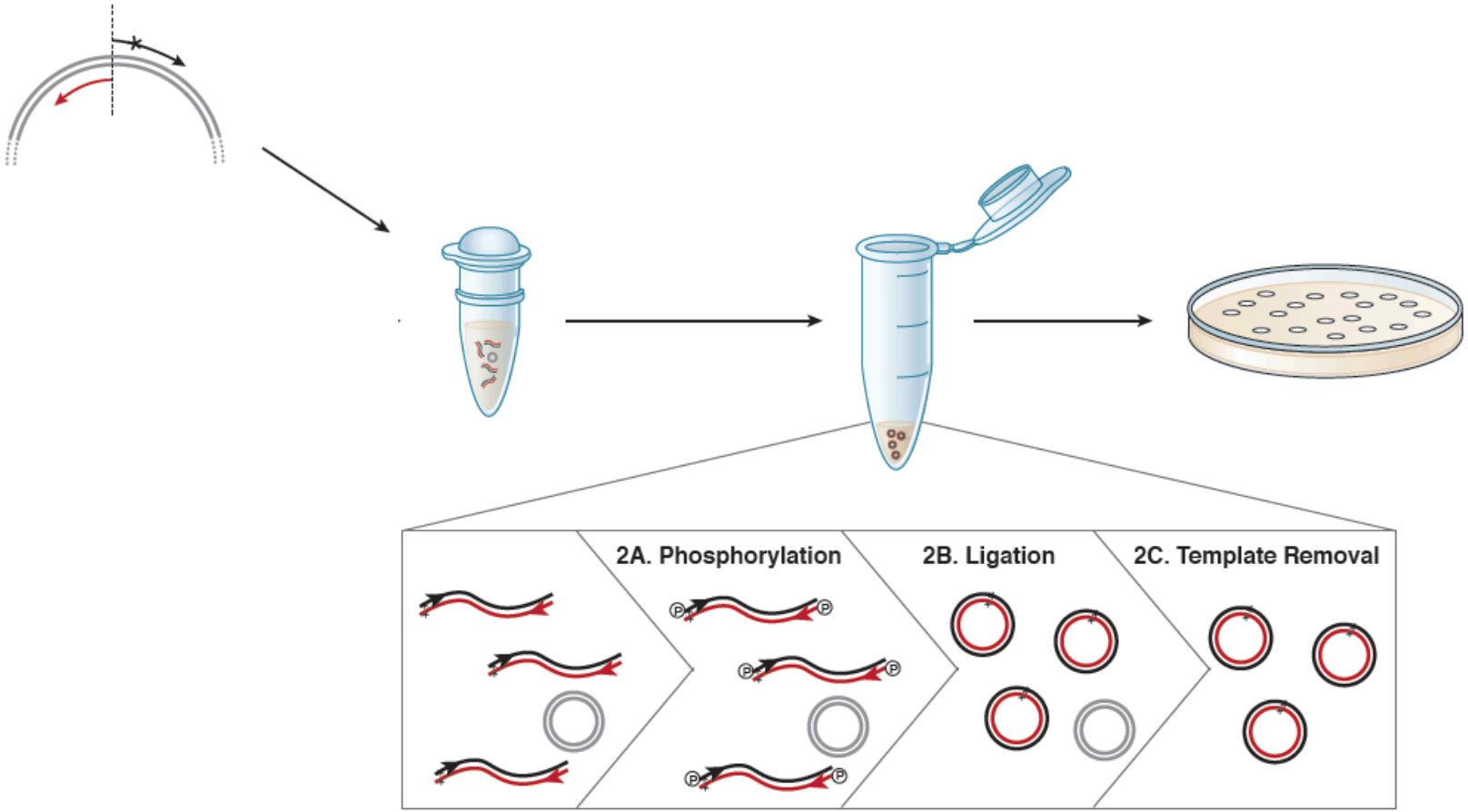


# IPC fluorescence dims when [$Ca^{2+}$] increased



# What is your experimental question?

# Site-directed mutagenesis

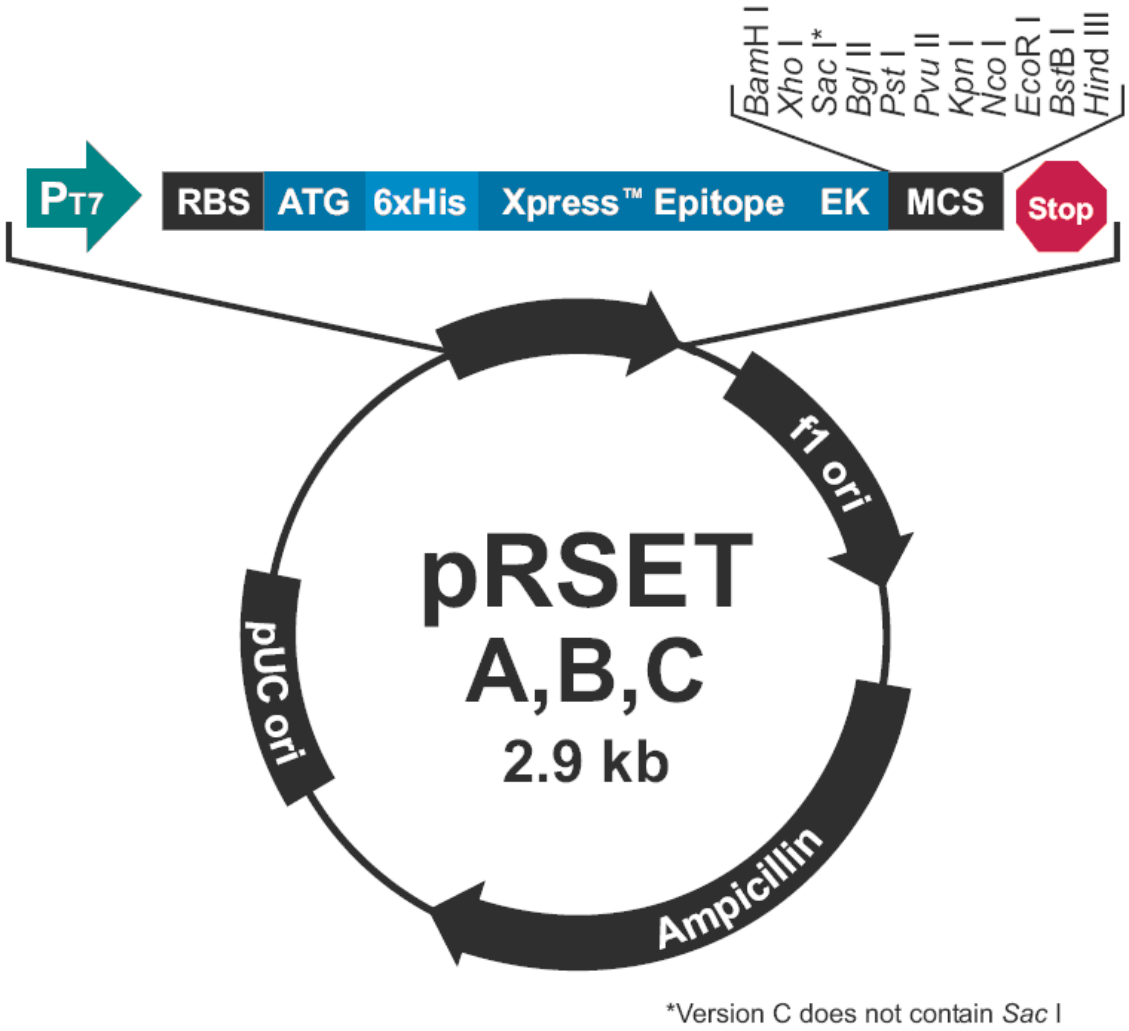


# Protein induction: host strain

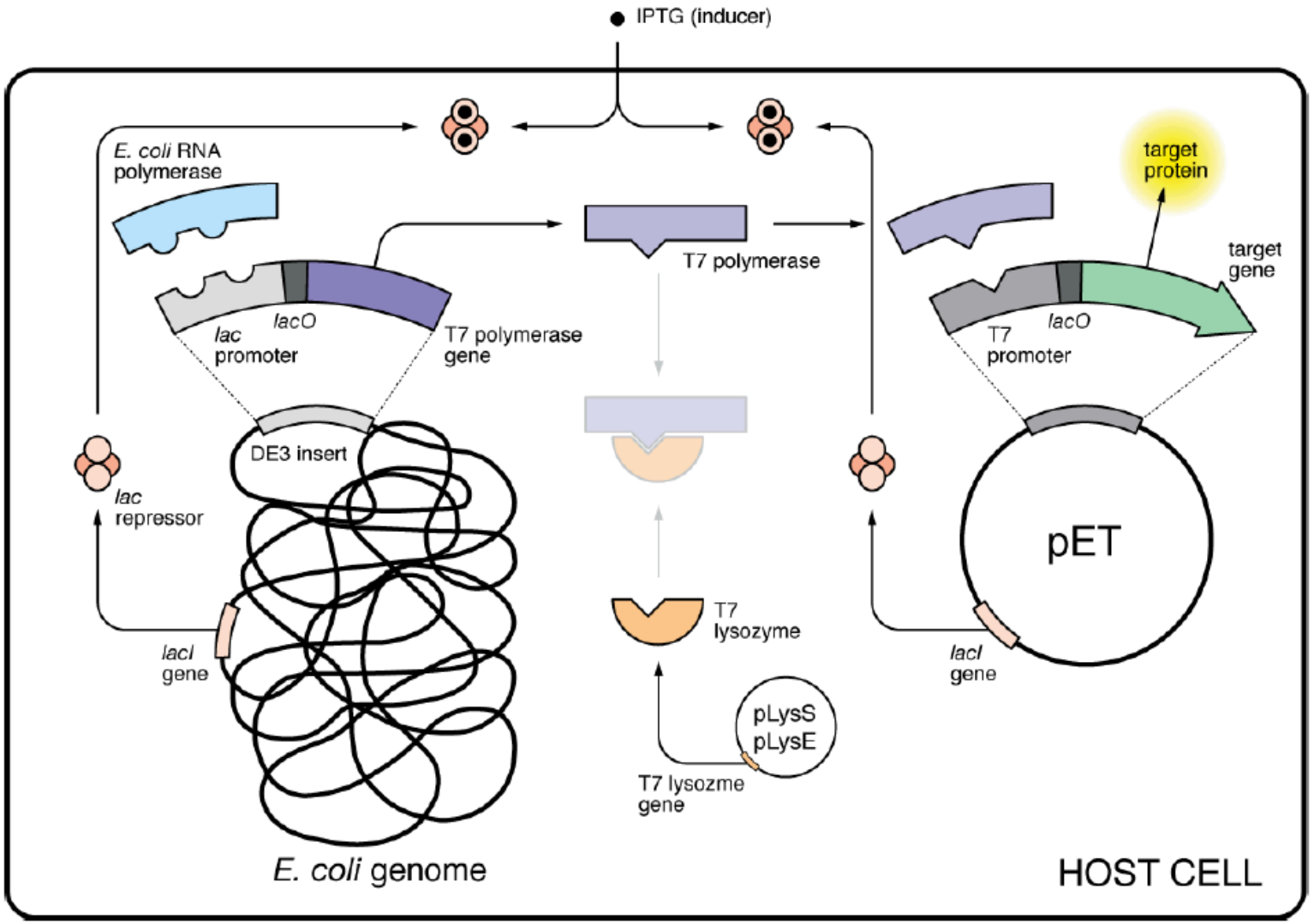
- Growth rate
- Growth conditions
- Genotype

Why is BL21(DE3)pLysS a good host cell for protein expression?

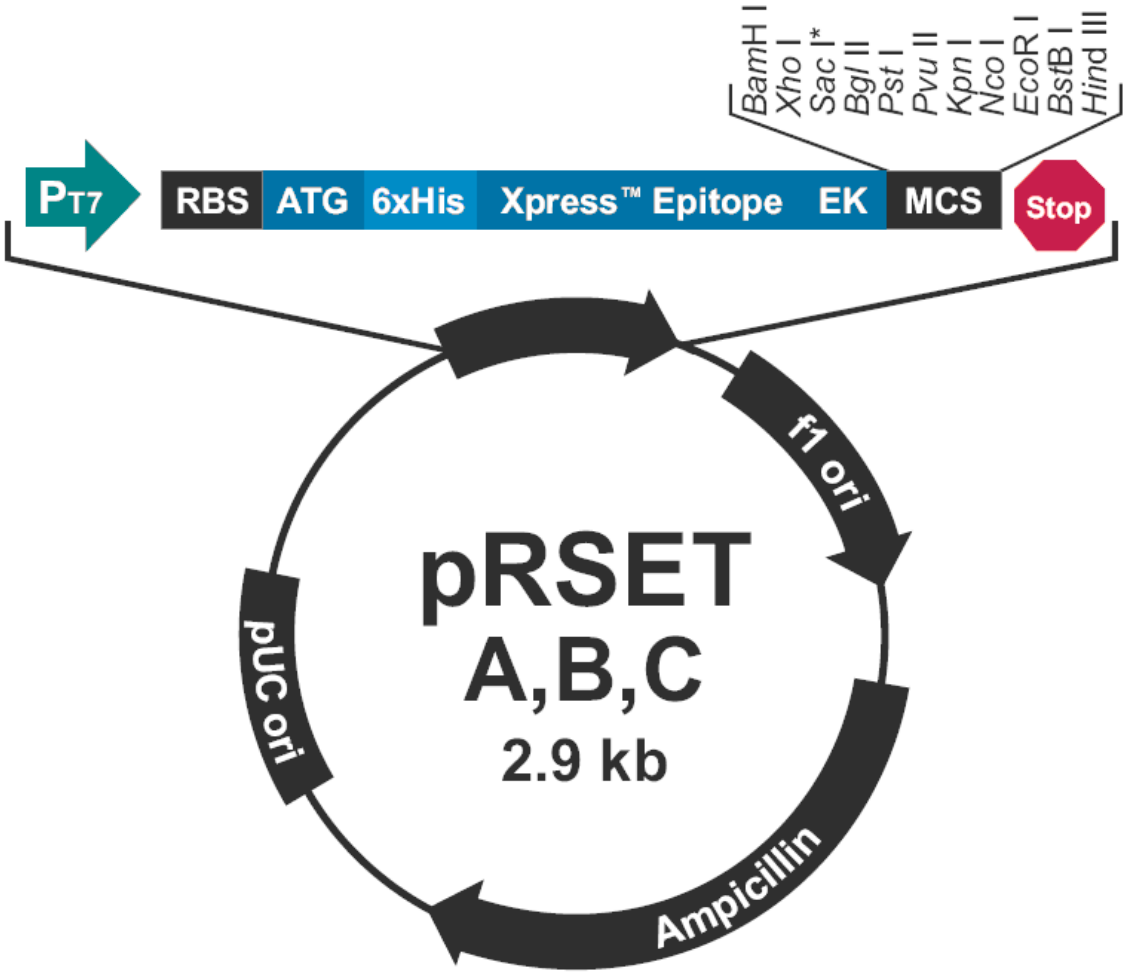
# Protein induction: vector



# Protein induction: control

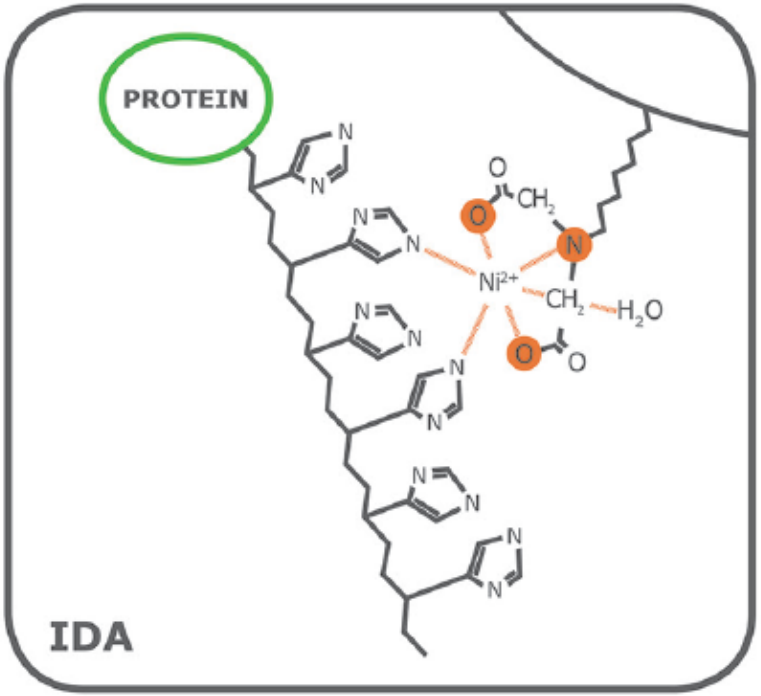


# Protein purification: vector




*Version C does not contain *Sac* I

# Protein purification: method



# How will we communicate our science?

- Protein engineering summary
  - Abbreviated written article that details your experimental results
  - Opportunity for revision
  - Completed with laboratory partner
- Protein engineering mini-presentation
  - Short ‘elevator pitch’ that relays the key results and impact of your project
  - Completed individually

# How will we communicate our science?

- Protein engineering summary
  - Draft submission due: Saturday, March 12th by 5pm
  - Comments returned: Thursday, March 17th
  - Revision due: Monday, March 28th by 5pm

- Guidelines
  - May use landscape rather than portrait
  - Follow ALL other formatting and length restrictions

# Summary: title and abstract

- Title is a statement of the conclusion of your research
- Abstract:

| | |
|---|---|
| General background | Something everyone in your audience cares about. |
| Specific background | Zoom in from General Background to the thing you did. |
| Knowledge gap, Unknown | Question that will be answered by your research. Problem, phenomenon that is not understood. |
| HERE WE SHOW | Conclusion, answer to the Unknown |
| Results | Brief summary of approach + very high-level results. Common pitfall = too much Methods/Results. |
| Implication, Significance | So what? What do your results mean for the thing everyone cares about? Next steps? |

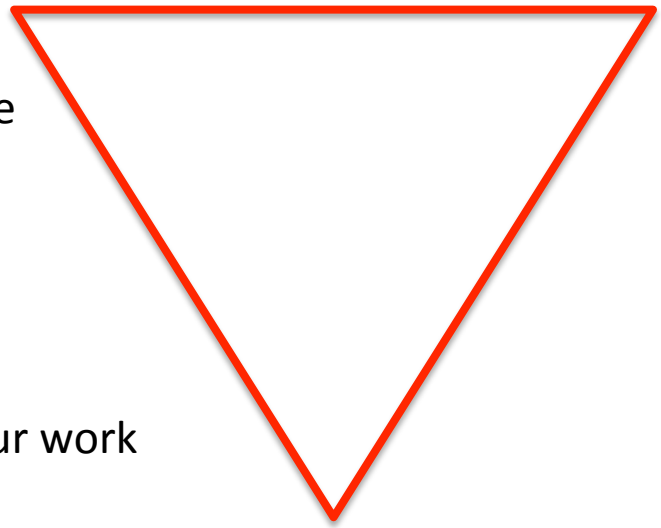
Slide modified from D. Chien, BE Communications Lab Instructor

# Summary: background and motivation

**Background and Motivation: potential topics and figures** [edit]

- *Topic*: Introduce and discuss the importance of calcium signaling.
- *Topic*: Describe the mechanism by which IPC measures calcium concentration.
- **Figure**: IPC conformation with and without bound calcium ions.
- *Topic*: What is your experimental question? How will you answer this question?
- **Schematic**: Experimental approach.
  - You may prepare something similar to the schematic from the lecture notes, but should NOT copy and insert it directly. Be sure your figure is tailored specifically to this assignment and audience. What steps can be cut or added? How can you highlight the key steps?
- *Topic*: Why is it useful to generate new calcium sensors?

Broad in scope



Specific to your work

- Use the ‘funnel’ method to structure topics

# Summary: results and interpretation

**Results and Interpretation: potential topics and figures** [edit]

Figures and topics are listed below according to the two major phases of your experiment. Within each phase, you should look for sub-groupings of interest, rather than treat each piece of data in isolation. In other words, try to both interpret and communicate outcomes holistically.

Keep in mind that you described the detailed methods in a separate homework assignment and it does not need to be included in this report. Therefore, figure captions and/or supporting text should include only the most relevant aspects of the methods, such as the names of the diagnostic enzymes, experimental techniques, or assays.

- Potential topics and figures:
  - System construction: generating and verifying mutant IPC gene
  - Mutant testing: protein production and titration assay

# Guide for results and interpretations

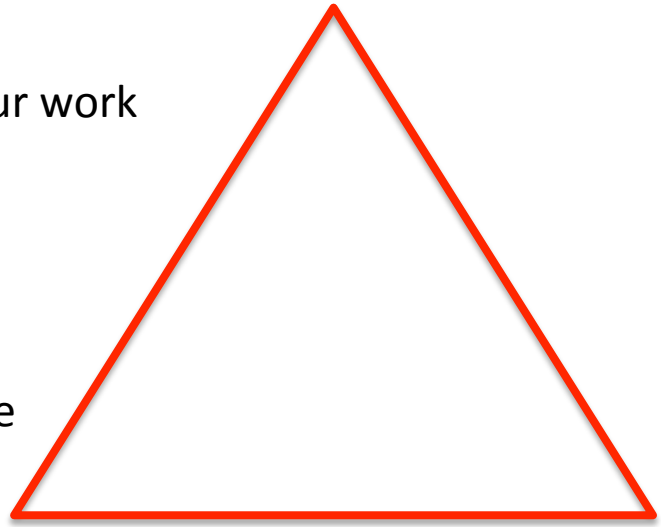
1. What is the overall goal of the experiment?
2. What was your expected result?
   - What are the expected band sizes on your gel?
3. What evidence do you have that your result is correct or incorrect?
   - What controls did you perform and were the results as you expected?
4. What was your result?
5. In sum, what do these data suggest or indicate?
6. What does this motivate you to do next?

# Summary: implications and future work

**Implications and Future Work: potential topics** [edit]

- *Topic*: Did your results match your expectations?
  - If no, provide a putative explanation. If yes, how can you further test if your hypothesis is correct?
- *Topic*: Based on the results, whether they matched your expectations or not, what experiments might you recommend next?
  - Follow-up experiments could distinguish between competing explanations of a given outcome or broaden the sample set for a question you already asked, to give just two examples.
- *Topic*: How might this assay be improved?
- *Topic*: How might this assay be used as a research tool? in the clinic? in industry?

Specific to your work



Broad in scope

- Think back to the background and motivation

# How will we communicate our science?

- Protein engineering mini-presentation
  - Submission due: Tuesday, March 15th or Wednesday, March 16th by 10pm, according to your laboratory section

- Guidelines
  - Follow ALL formatting and length restrictions

# Mini-presentation

## Introduction [edit]

The introduction of your mini-presentation should both introduce your research project and convey the importance of your work in the context of the field. You want the listener to understand why your project is important and give them the information they need to understand your data.

## Results [edit]

Your results should be summarized such that the key finding is clear to your listener. When discussing your results, include details that support your claims. For example, instead of simply stating "The cooperativity increased..." include the actual numerical values, "The cooperativity of WT IPC was A and that of the mutant IPC was B, which supports the hypothesis that the X#Z mutation increases cooperativity."

In addition to stating your results, you should include your interpretations of the data you collected.

If it is necessary, you can include the technique used to obtain your data (e.g. "Using site-directed mutagenesis, we...").

## Conclusions [edit]

The conclusion should put your project into the context of the larger field of research. How is it that your research advances the field?

# In the laboratory…

- Complete SDS-PAGE analysis
- Prepare calcium titration curve
- Perform fluorescence assay