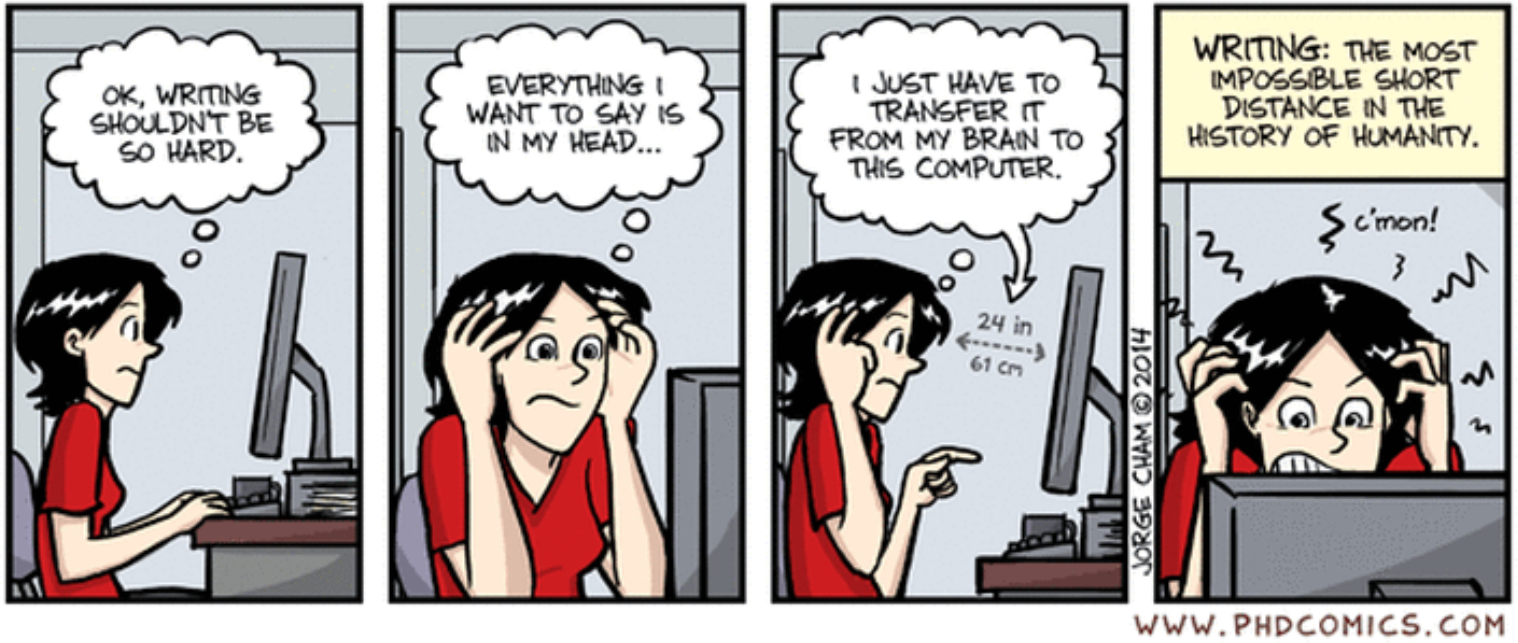
M1D7: Analyze ligand titration curves

1. Prelab discussion

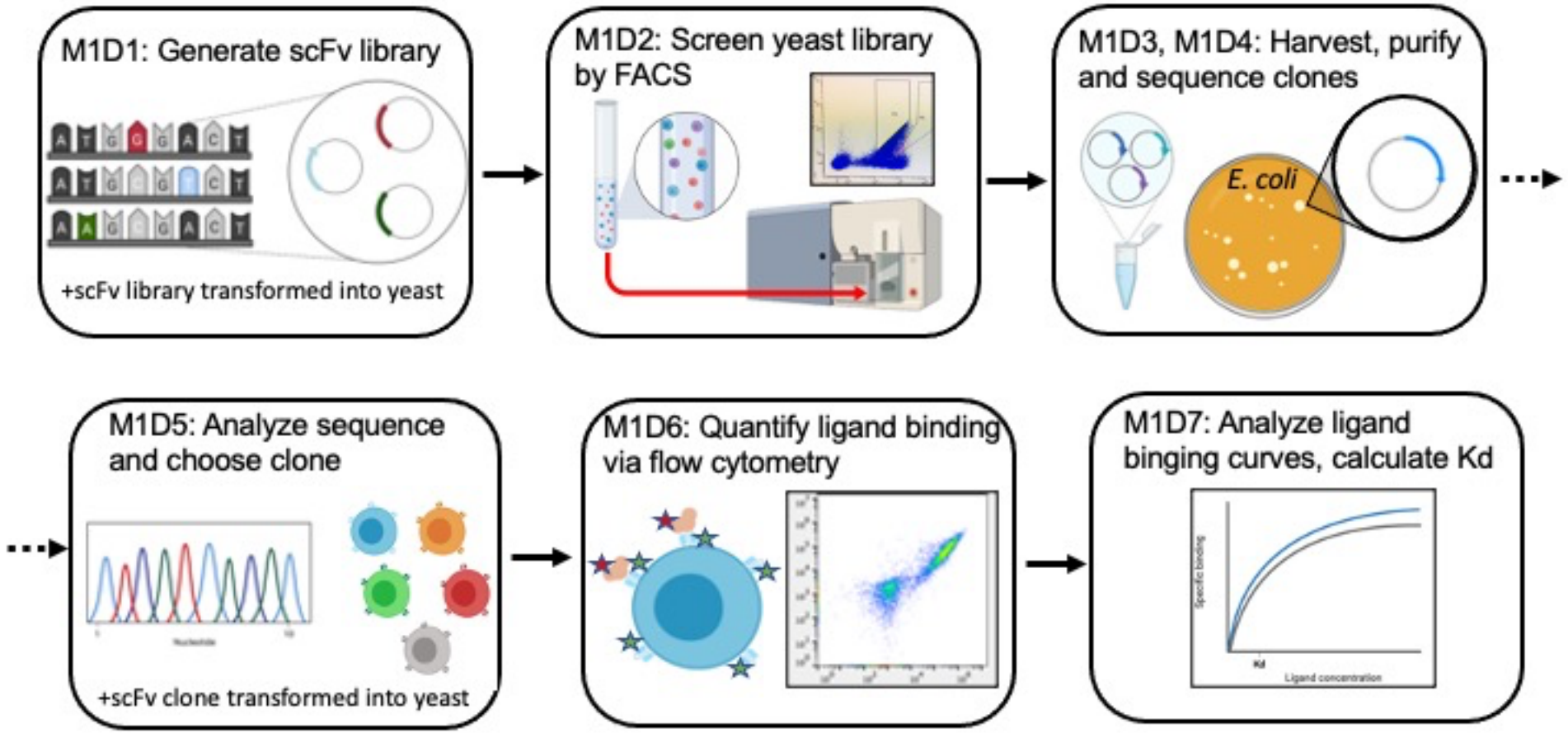
2. Determine K_d for clone:ligand binding

3. Quiz



Overview of M1: antibody engineering

Research goal: Identify and characterize an antibody fragment (scFv) that shows improved binding to the antigen, lysozyme.

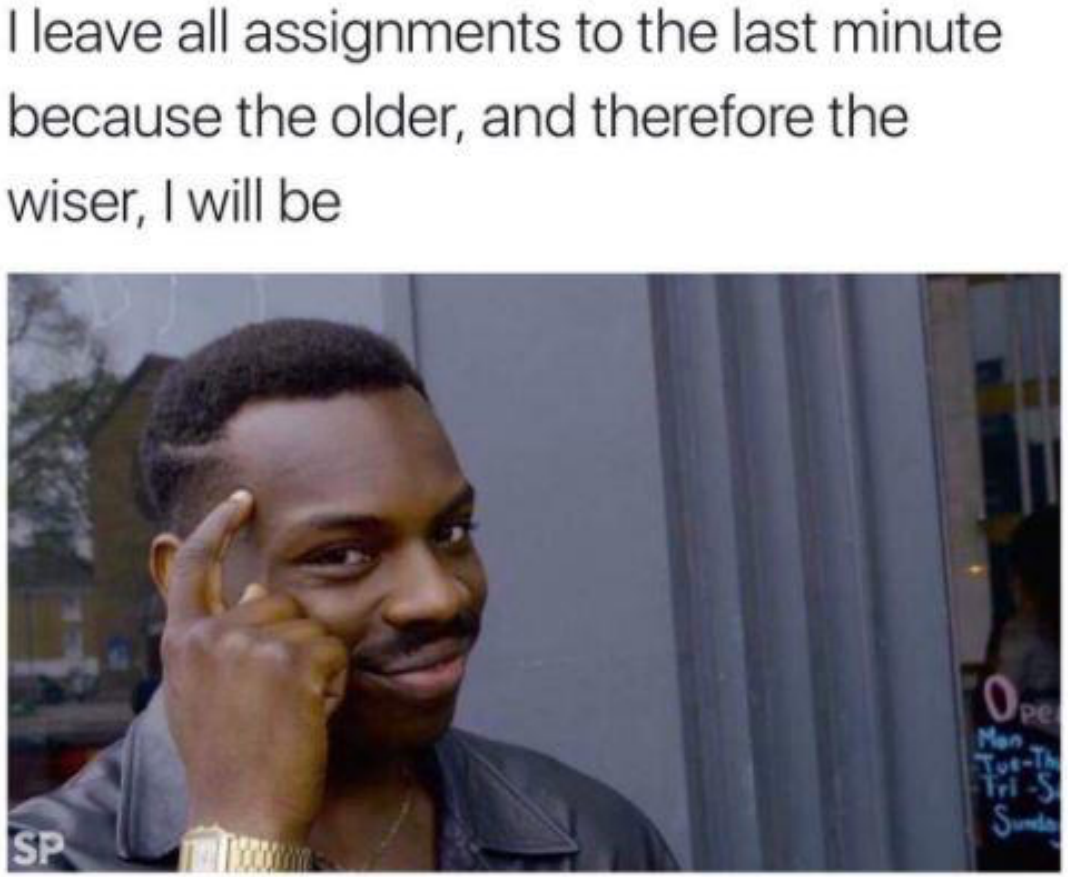


Assignments due for Mod 1

- Data Summary
  - Due Wednesday March 24 by 10pm
  - Revision Due Sunday April 4 by 10pm
  - Submit to Stellar/LMOD

- Mini-presentation
  - Due Sunday March 28 by 10pm
    - Submit to bioeng20.109@gmail.com
- Blog post
  - Due Saturday April 10 by 10pm
  - Submit to Slack

- Notebook
  - Due March 17 by 10pm
  - Submit pdf of M1D5 to Stellar/LMOD



M1D5

Getting started on the Data summary!

Title: take-home message

Abstract: Paragraph, NOT in bullet points!

In bullet points:

Background and Motivation (include citations)

2 slides

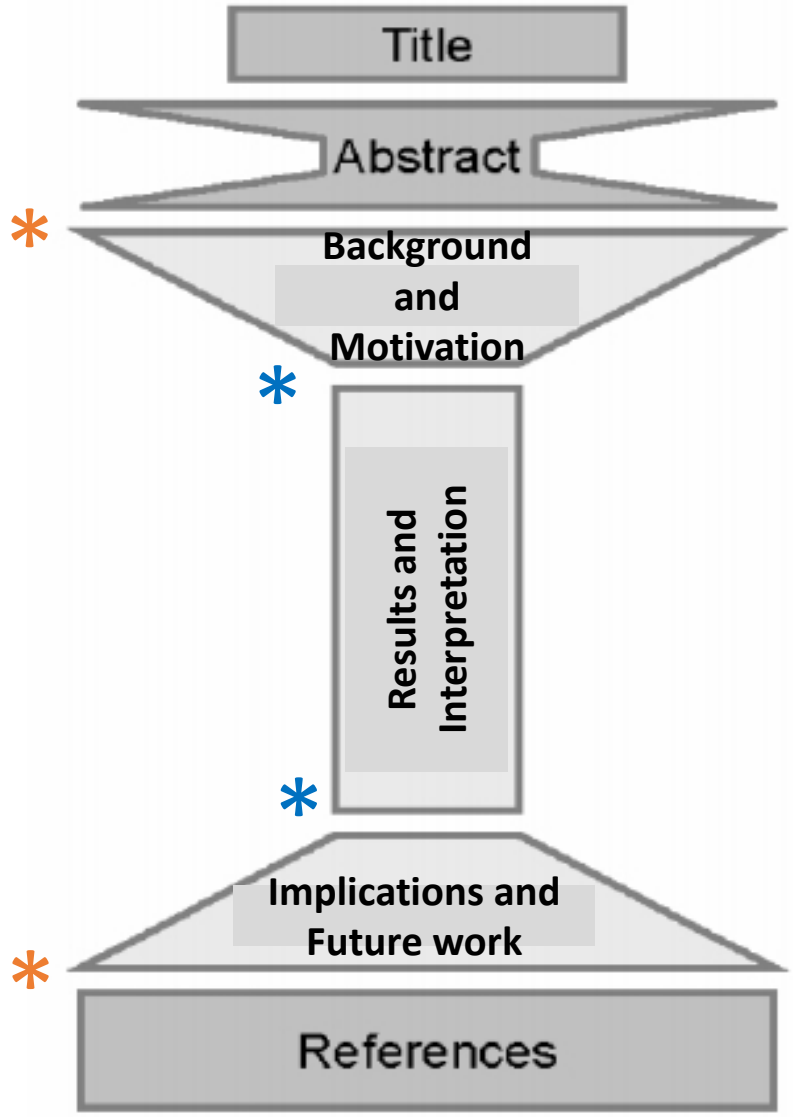
Results and Interpretation

4-5 slides

Implications and Future work (include citations)

1-2 slides

References (see wiki for format suggestions)



Data summary structure / logistics

- To be submitted as a powerpoint file!
  - Change page settings such that 'slides' are portrait and 8.5" x 11"
  - Upload to Stellar (draft due March 24 at 10pm, revision April 4 at 10pm)

- Each figure will be included as a separate Data slide
  - Image should be at the top of the slide with title and caption
  - Results / Interpretation text should be included on same slide
  - Though figures are separated into Data slides, the story should be cohesive between figures!

Review of Background & Motivation section…

- Impact statement
  - General background describing relevant / previous research
- Specific background (e.g. scFvs, Lysozyme)
  - Introduce topics (pathways, specific technologies, etc)
  - Reference overview schematic figure
  - Narrow focus to the specific question addressed in your study
- Knowledge gap / statement of problem
  - State what is unknown
  - Include your research question!
  - What do you propose will be the outcome of your study?
- A brief preview of your findings
  - Here we show…
  - End with broad implications of the study

Structure of Background & Motivation AND Summary/Implications

Background and Motivation

- Topic Sentence
- Supporting information (citation)
- Supporting information (citation)

- Topic Sentence
- Supporting information (citation)

Review of Results & Interpretations section…

- Figures and captions
  - Organize figures logically!
  - Use figure subpanels (label with letters)
  - Limit text on the image, move extra details / explanation to the caption
  - Use appropriately sized images
  - Include description title with take-home message
  - Include introductory sentence at start of caption
- Results and Interpretation (use subheaders)
  - State the goal / intent / purpose of experiment in the first bullet
  - What you did: experiments and expectations, describe controls
  - What you found: quantitatively describe your result, referring to the figure (”Figure 1a shows…”
  - What does this indicate: interpret your results, what does it mean?
  - What does this motivate you to do next: transition to next experiment

Example for Results slide:

Image **should not** be the entire page

- Only needs to be large enough to be clear / visible

Title **should** be conclusive

- Don't state what you did, rather state what you found (take home message)

Caption **should not** detail the methods or interpret the data

- Define abbreviations, symbols, etc.
- Include details needed to "read" figure

Bullet points **should** present and interpret the data

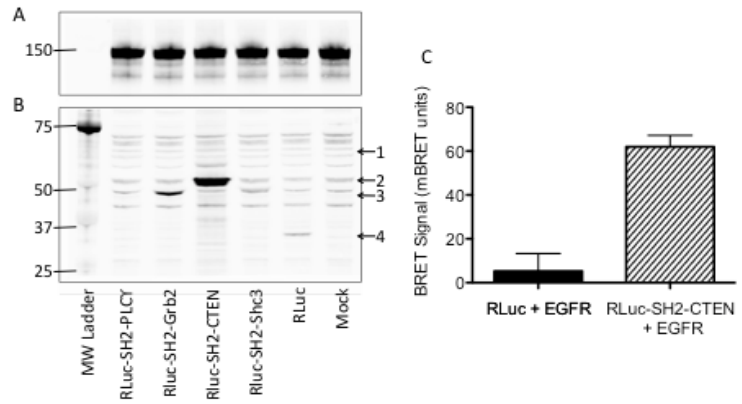


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

BRET system effectively measures EGFR activation:

- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated by correct molecular weight band at 150 kDa (Figure 1A).
- Several protein bands are present in Mock transfection lane suggesting off-target binding of the RLuc antibody (Figure 1B).
- RLuc alone, RLuc-SH2-Grb2, and RLuc-SH2-CTEN were successfully transfected as indicated by correct molecular weight bands (Figure 1B).
- RLuc-SH2-PLCg and RLuc-SH2-Shc3 did not appear by Western blot analysis -- bands different from those in the Mock lane are not identifiable. This outcome could be due to protein expression levels below the detection limit by Western blot or to unsuccessful transfection of cDNA.
- BRET signal increased in cells transfected with Citrine-EGFR and RLuc-SH2-CTEN versus Citrine-EGFR and RLuc alone after EGF stimulation. This difference suggests that the BRET signal is specific for an SH2-EGFR interaction versus randomly localized RLuc.
- In sum, these data suggest that the RLuc-SH2 constructs can be utilized to monitor EGFR phosphorylation, as SH2 domain-EGFR association occurs only at sites of EGFR tyrosine phosphorylation. Next, we determined the dynamic range of the BRET assay.

What figures will you include in the Data Summary?

Background/Motivation OR Results:

1.

Results:

1.
2.
3.
4.

Review of Implications & Future works section…

- Start with ‘here we showed…’
  - Restate major results and broad implications
  - Follow same order as in Figures/Results
- Describe your conclusions from your data
  - If necessary, describe caveats of experiment and suggest improvements
- Identify unknowns and speculate (within reason)
  - Don’t make huge generalizations or overreach
- Propose future experiments, identify new questions that arise
- Come back to the big picture / impact statement topic introduced in background

Mini-presentation logistics

- 3 minutes long! (points deducted for over/under)
  - Introduce your project
  - Give key results
    - Include statement about the methods used to generate those results
  - Give a take home message for the project
- Submit video of just you talking
  - No visuals
  - No edited videos
    - 1 take as though you were speaking to a person

Please submit your completed Mini-presentation **due by Sunday, March 28th at 10 pm** to bioeng20.109@gmail.com, with filename **Name_LabSection_MP.extension** (for example, ImaStudent_TR_MP.mov).

For today…

- Work through M1D7 laboratory exercises with partner
- **Remember to submit notebook check for M1D7**
- Quiz sent at 3:30pm

- **Remember to submit M1D5 notebook entry for full grading by tomorrow 10pm**

For M2D1…

- Review project overview and M2D1 introduction