Finishing up Mod 1!

- Research talk comments to be returned early next week
- Data summary submitted via Canvas
  - Draft due Wednesday, October 11th by 10 pm
  - Revision due Saturday, October 21st by 10 pm
  - Check email for Office hours days / times!
- Blog post submitted via Slack #fall-2023-blog
  - Due Thursday, October 12th by 10 pm
- Notebook submitted via Canvas
  - Due Friday, October 6th (TR section) or Saturday, October 7th (WF section) by 10 pm
  - Submit M1D4 for 'detailed' grading
  - Entire notebook will be reviewed for 'completion' grading

Let’s review our Mod 1 project goals…

What is our overall goal / question in this project?

How are we addressing the goal / answering the question?

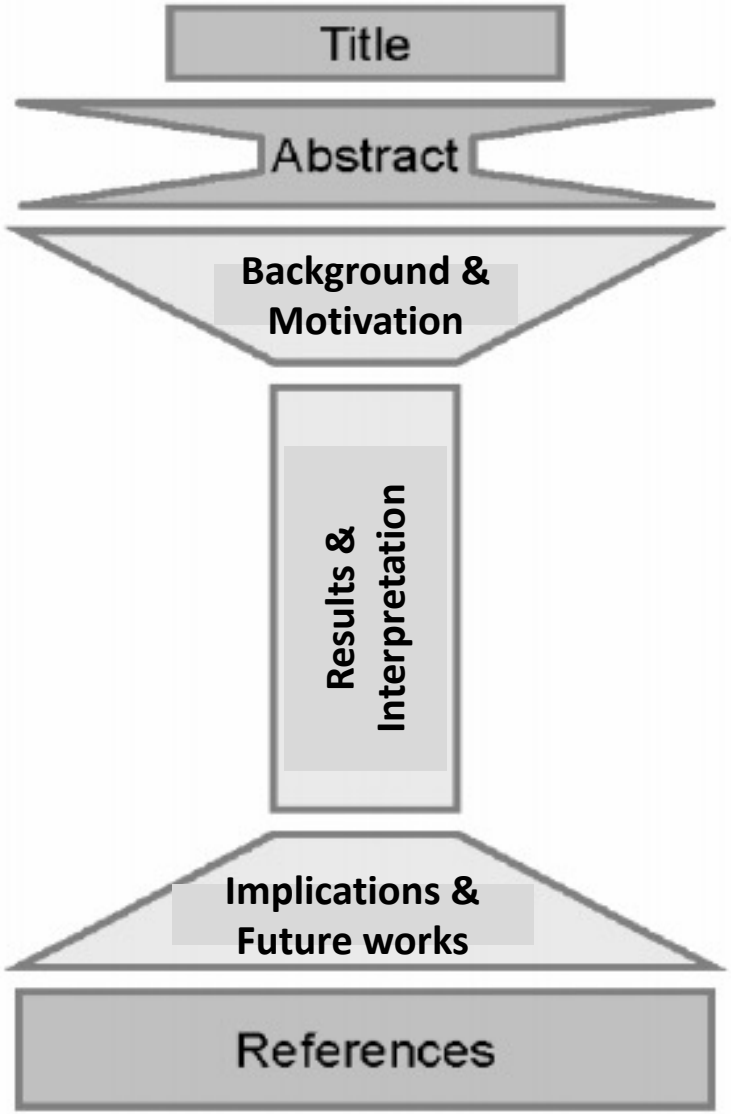
Let’s review our Mod 1 experiments…

gamma-H2AX	CometChip
What was tested?	What was tested?
How was the effect measured?	How was the effect measured?
Advantages?	Advantages?
Disadvantages?	Disadvantages?

Getting started on the Data summary

- Title: take-home message / conclusion
- Abstract: paragraph, NOT bullet points

- Background & Motivation (include citations)
  - ~ 2 slides
- Results & Interpretation
  - 4-5 slides
- Implications & Future works (include citations)
  - ~2 slides
- References



Data summary structure / logistics

- To be submitted as a powerpoint file!
  - Change page settings such that 'slides' are portrait and 8.5" x 11"

- Title and Abstract can be included on the same slide

- 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!

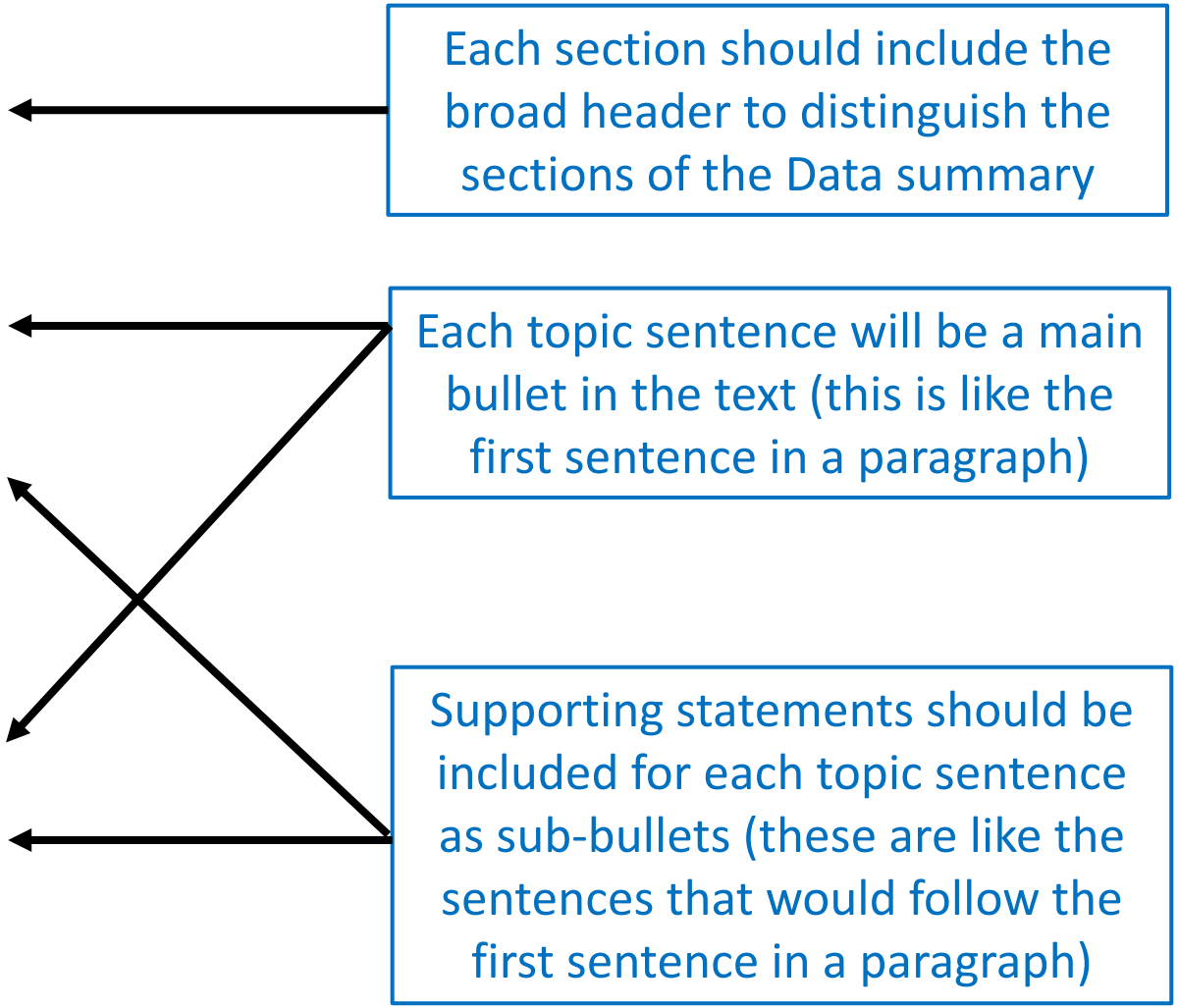
Review of Background & Motivation section…

- Impact statement
  - Why is your research important / useful? Provide context for your project.
- Specific background
  - Introduce topics (pathways, specific technologies, etc)
  - 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…

How should you organize the Background & Motivation section?

Background and Motivation:

- Topic sentence #1
  - Supporting statement (citation)
  - Supporting statement (citation)
  - Transition sentence
- Topic sentence #2
  - Supporting statement (citation)
  - Transition sentence



Review of Results & Interpretations section…

- Figures and captions
  - Organize figures logically!
  - Use figure subpanels (label with letters to better connect to the text in the caption)
  - Limit text on the image, move extra details / explanation to the caption
  - Use appropriately sized images
  - Include descriptive title that states the 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

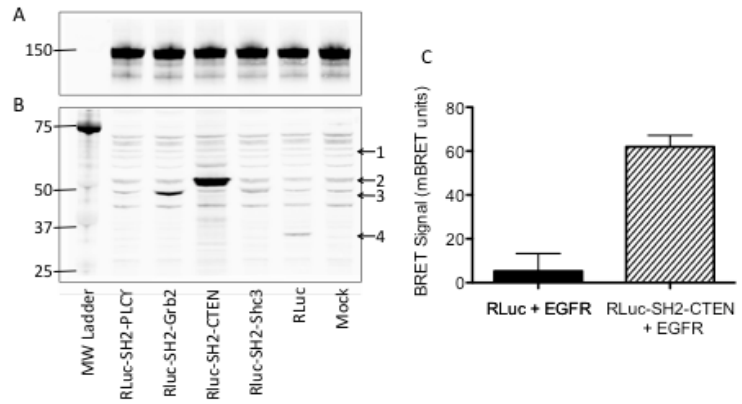


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. 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.

How should you report your results?

RESULT(S)/INTERPRETATION(S): Use the questions below to guide the information you provide in your concise bullets.

- What is the overall goal of your experiment?
- What was your expected result according to your hypothesis?
- What evidence do you have that you result is 'correct' or 'incorrect'?
  - What controls did you include and for what did these control?
  - Did the controls work as expected?
- What was the result?
  - Was the result expected?
- In sum, what do these data suggest or indicate?

What figures will you include in the Data Summary?

1.

2.

3.

4.

5.

Notes on 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
  - **Be critical of your results and discuss caveats of analysis methods**
- 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

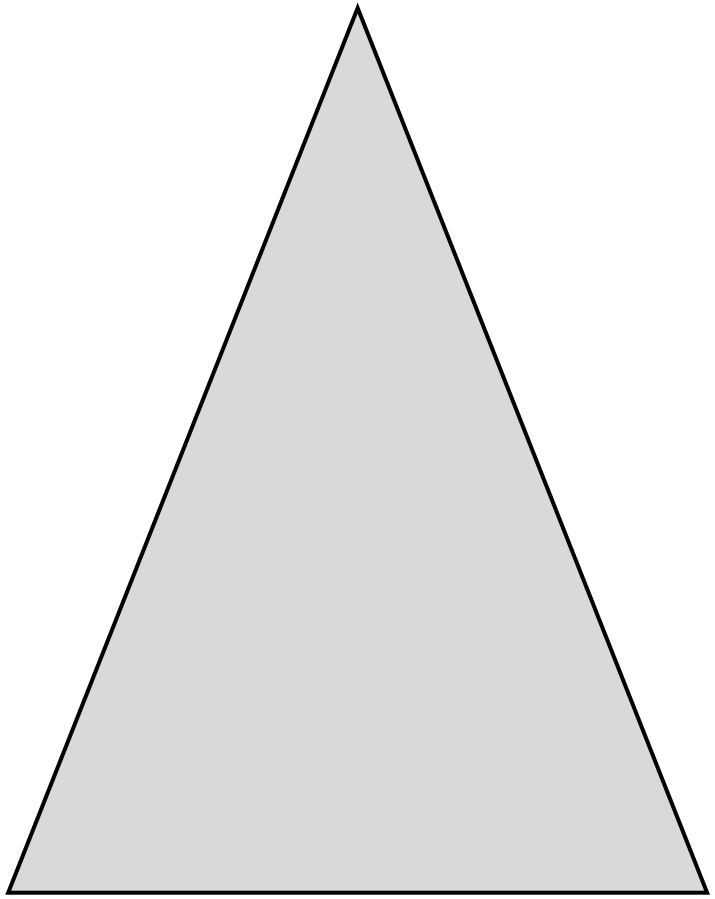
Be critical of your results and discuss caveats of analysis methods

- Do the quantitative results match what you see qualitatively in your raw data images?

- Is there a better way to visualize the data?

- Is there a better way to analyze the data?

How should you conclude your story?



- What are the main findings / conclusions?
- What are the implications of the results?
- How do the results relate to the research question / hypothesis?
- How do the results advance what is known?

Ideas for Future works:

- What are some possible clarifying experiments?

- What are some possible ‘next step’ experiments?