M1D8:
Image TDP43-localization experiment and complete data analysis

1. Prelab discussion
2. Complete statistical analysis
3. Data summary prep!

Finishing up Mod 1!

- Research talk comments to be returned early next week 😔
- Data summary submitted via Stellar
  - Draft due Saturday, March 12 by 10 pm
  - Revision due Sunday, March 20 by 10 pm
- Blog post submitted via Slack #spring-2022-blog
  - Due Monday, March 14 by 10 pm
- Notebook submitted via Stellar
  - Due Friday, March 4 by 10 pm
  - Submit M1D2 for 'detailed' grading
  - Entire notebook will be reviewed for 'completion' grading

How will you interpret your data?

- Variance = measures the variability from the average or mean

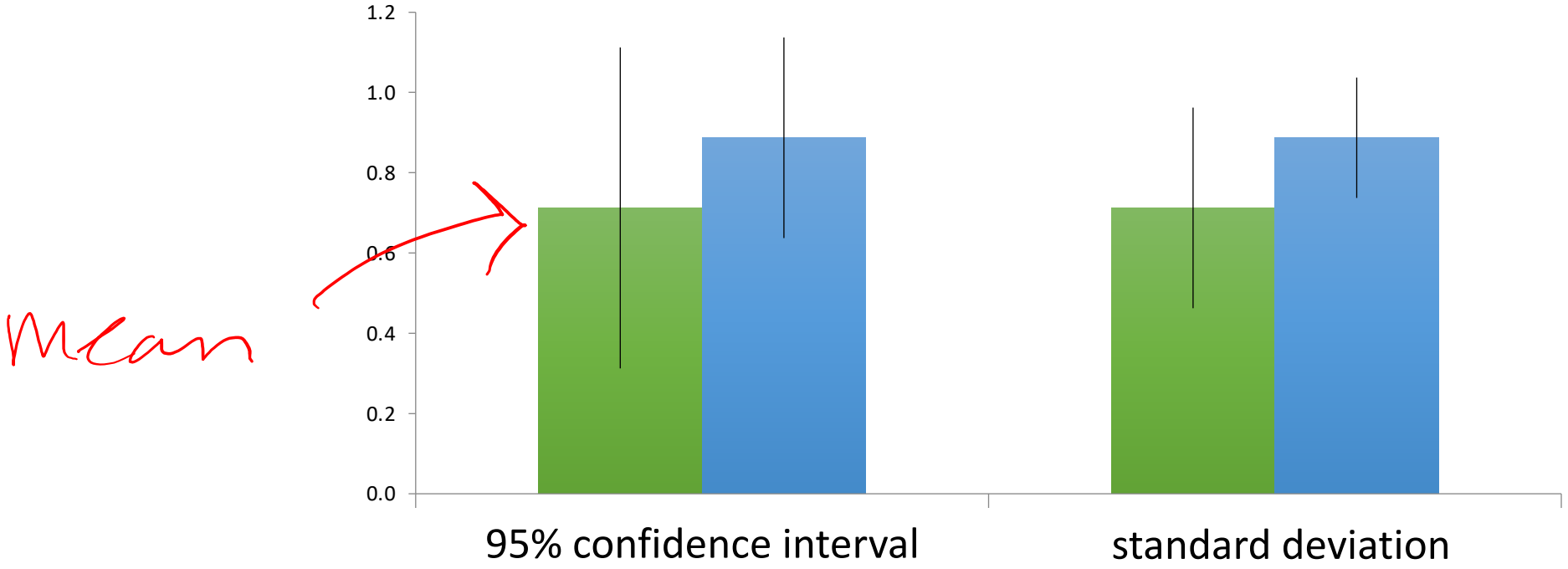
SPREAD OF THE DATA

- Significance = quantifies the likelihood of a result occurring by chance

ODDS OF THE RESULTS

Confidence intervals show variance in data

- At 95% confidence interval, there is a 95% chance that the true mean is within the defined range
- Error bars used to represent variance



Calculating confidence interval in Excel

= CONFIDENCE(confidence level, standard dev., size)

- Confidence level:

  TYPICAL IS 95 % ⇒ 0.05

- Standard deviation:

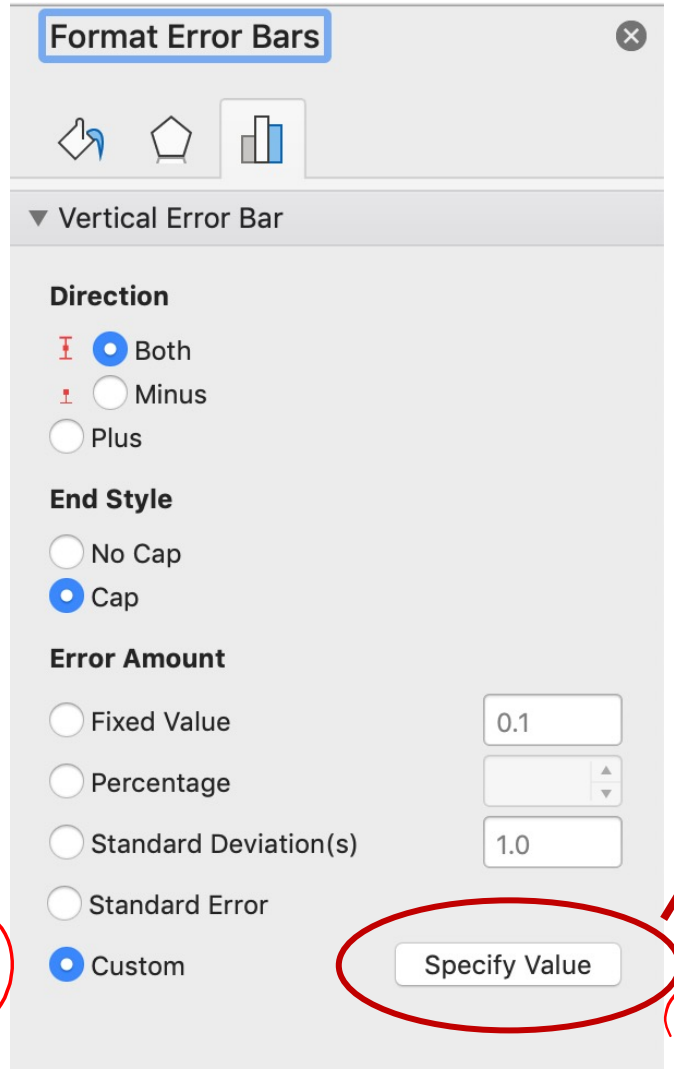
  WITH CI BUILDS IN CAVEAT OF SMALL N

- Size:

  = N OF EXPERIMENT

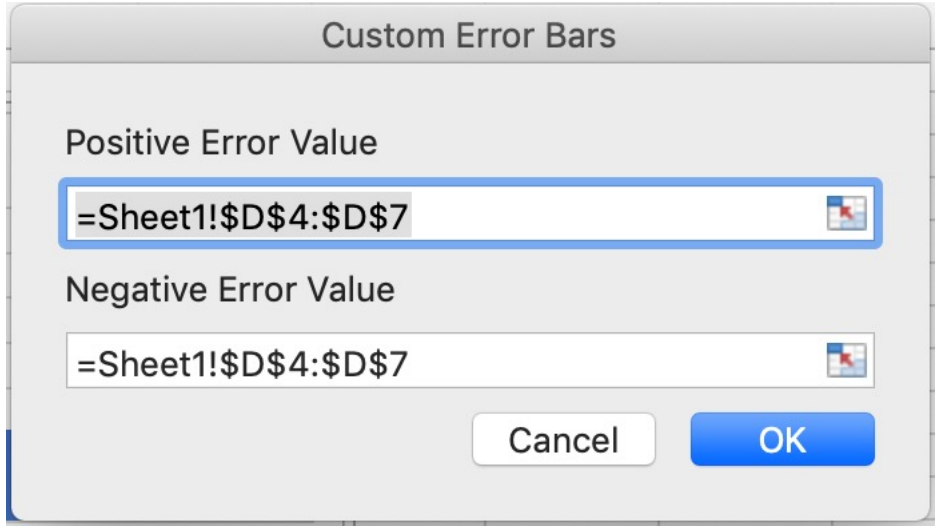
How do you customize error bars in Excel?

1



2

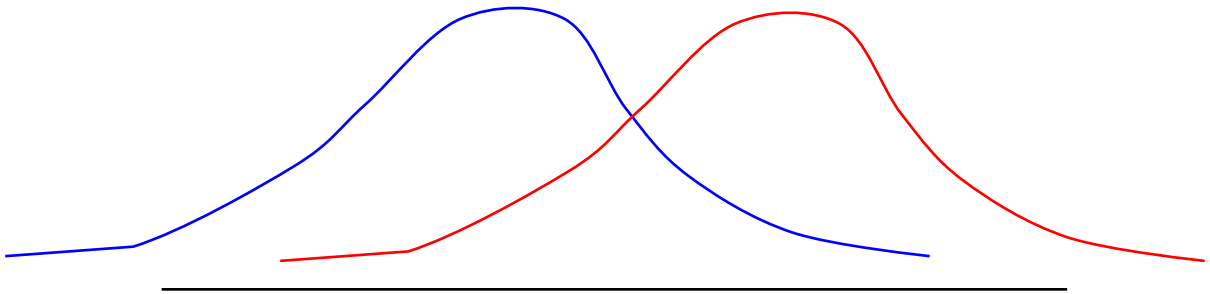
3



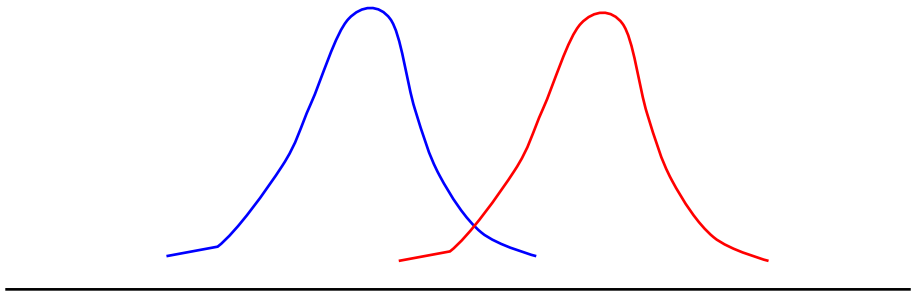
Enter value calculated for confidence level as custom error bars

Student's *t*-test determines if populations are significantly different

- Assume data follows *t*-distribution
- At $p < 0.05$, there is less than a 5% chance that populations are the same (95% chance that populations are different)
- Examines signal (means):noise (variance) ratio



high noise



low noise

Calculating Student's *t* in Excel

P = TTEST (array1,array2,2,3)

- Arrays:

  POPULATIONS TO BE COMPARED

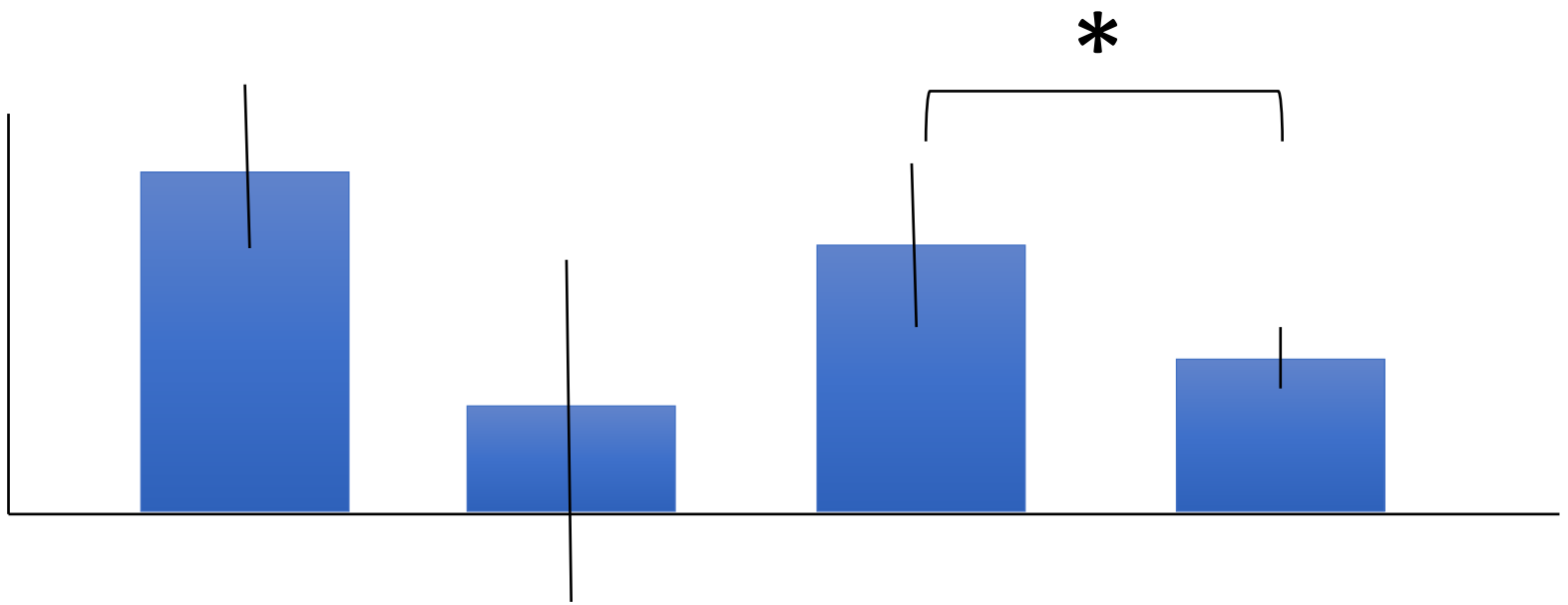
- 2 = two-tailed test:

  NOT ASSIGNING DIRECTIONALITY

- 3 = population variances not assumed:

  NOT ASSUMING DISTRIBUTION

How will you statistics in your data analysis?



- Student's t-test can only be used to compare two populations

- What if data are not significant? Almost significant?

0.051

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…

Aggregation

What was tested?

How was the effect measured?

Advantages?

Disadvantages?

Localization

What was tested?

How was the effect measured?

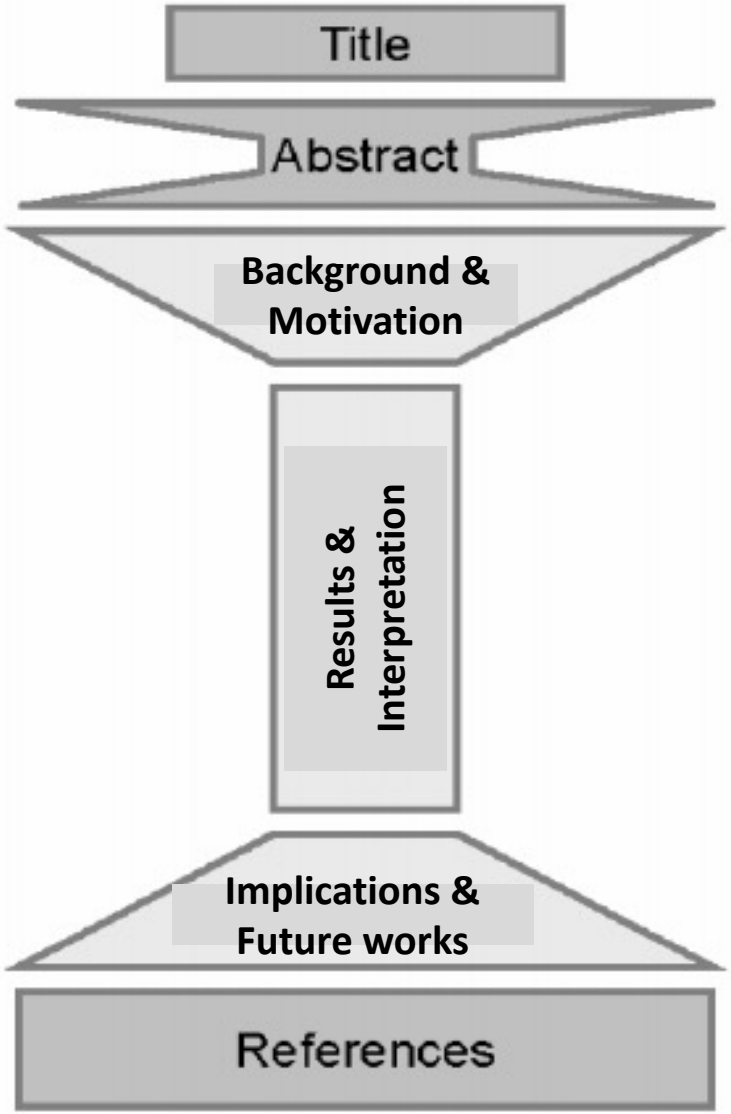
Advantages?

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!

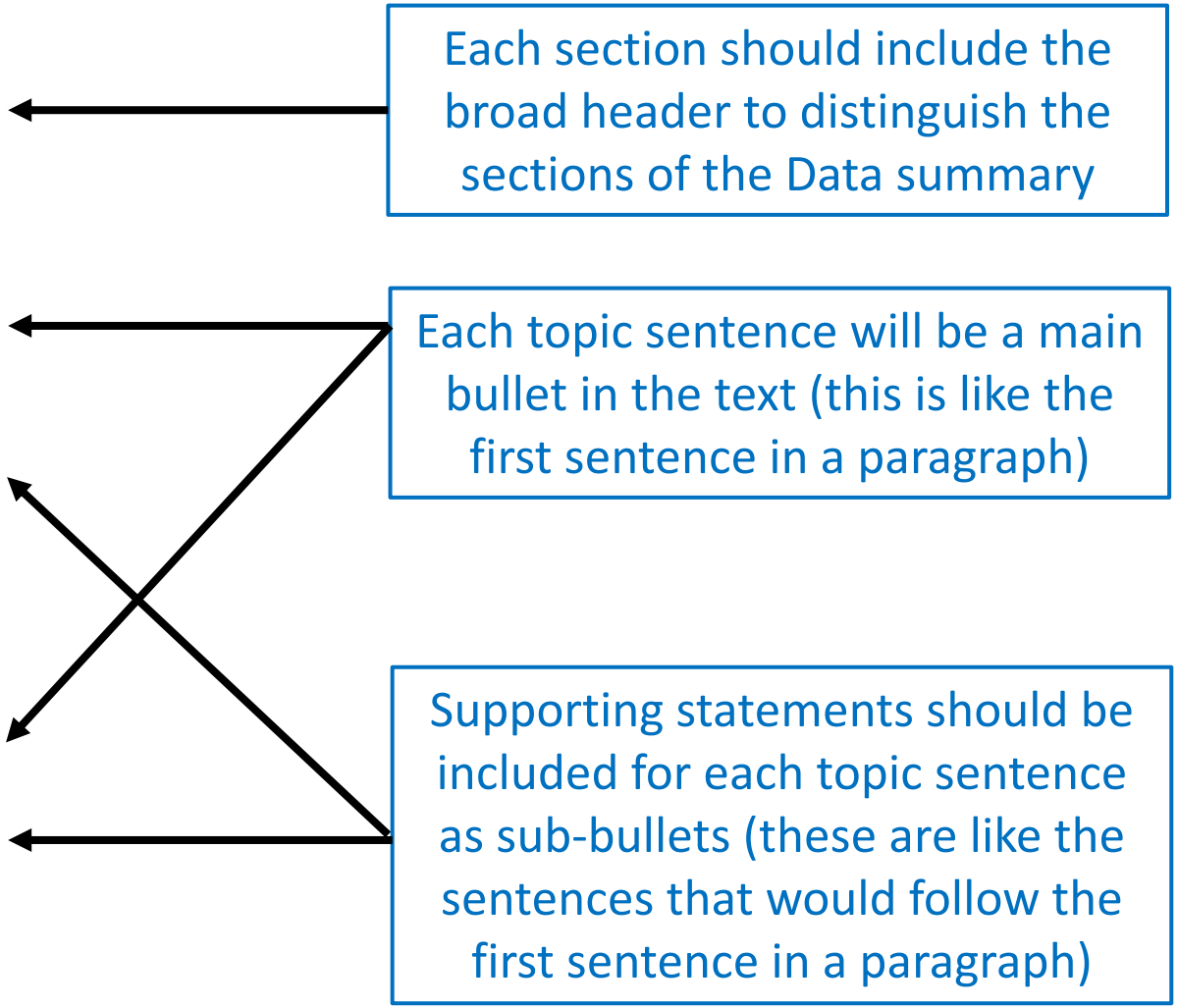
Review of Background & Motivation section…

- 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

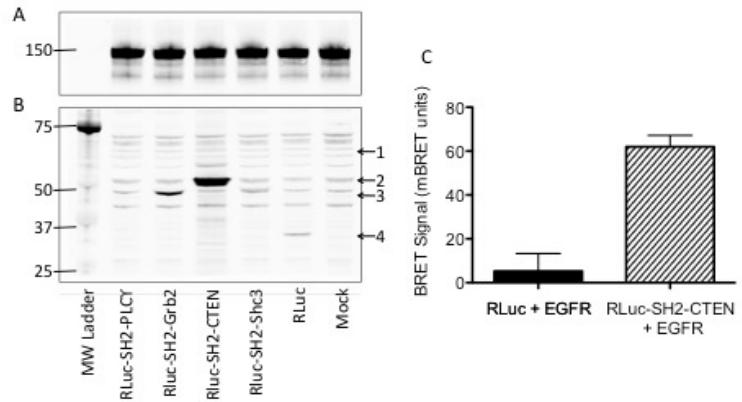


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies.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

What figures will you include in the Data Summary?

1. OVERVIEW SCHEMATIC *OPTIONAL*
2. EXP SCHEMATIC
3. SDS-PAGE & BCA
4. AGGREGATION
5. LOCALIZATION

How will you incorporate class data?

- Must show data from your experiments!

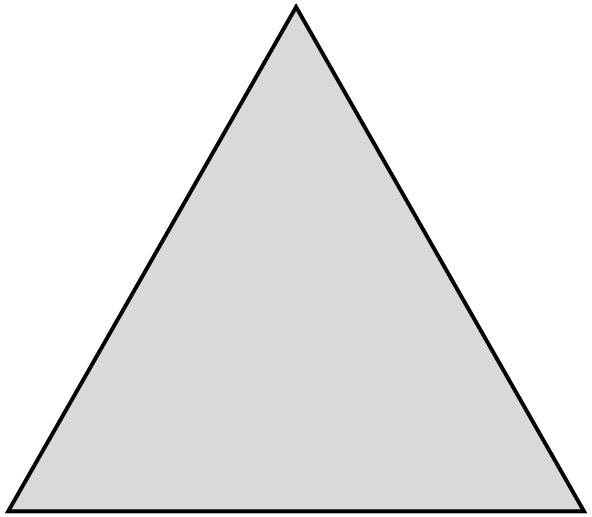
- Should include data from one other team for aggregation assay
  - Can use data for same small molecule to show reproducibility
  - Can use data for another small molecule to show treatment effect

- Can include data from more than one team *OPTIONAL*
  - Should be purposeful
  - Should be thoroughly explained / incorporated into the narrative

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
  - 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

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?

Implications and Future Work: potential topics [edit]

- ***Topic***: Did this experimental approach result in a scFv clone with an increased or decreased Kd?
  - If no, provide a putative explanation. If yes, how can you further test this approach?
- ***Topic***: Did the characterization of the scFv clones add new knowledge to what's known about the antibody-antigen interaction?
- ***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, broaden the sample set with optimized screen conditions, or provide further characterization of clones.
- ***Topic***: How might this approach be improved?
- ***Topic***: Discuss a novel way this approach can be used in research, medicine or industry.
- ***Topic***: What are the broader implications of this experiment and approach?
  - Don't overreach. Suggest impact within the field of antibody engineering.

Ideas for Future works:

- What are some next steps?

- What are some broader possibilities?

For today…

- Work through M1 data analysis with your laboratory partner
  - Schedule time to meet / prepare Data summary!!
- Image TDP43-localization experiment
- Submit completed notebook entry for M1D2 tomorrow by 10 pm

For M2D1…

- Review project overview and M2D1 introduction