M1D4:
Screen chemical library for FKBP12 binders

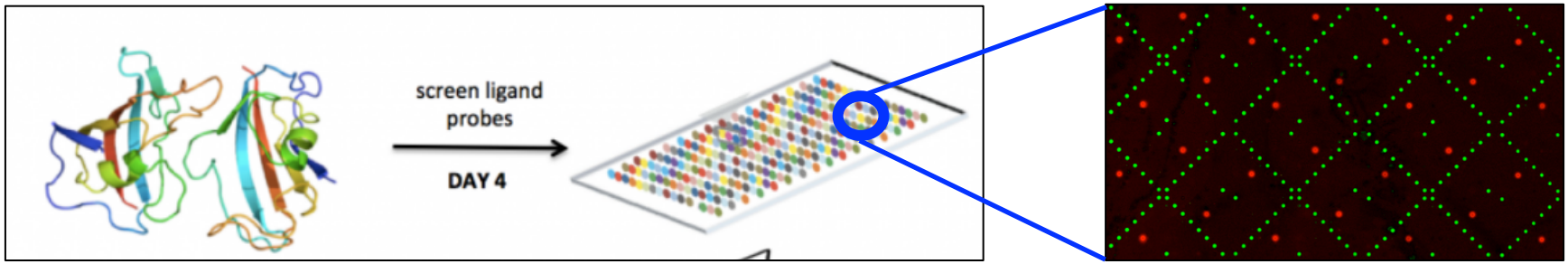
using "a collaborator's pure FKBP12"

02/23/2017

Start with Part 1 protocols…

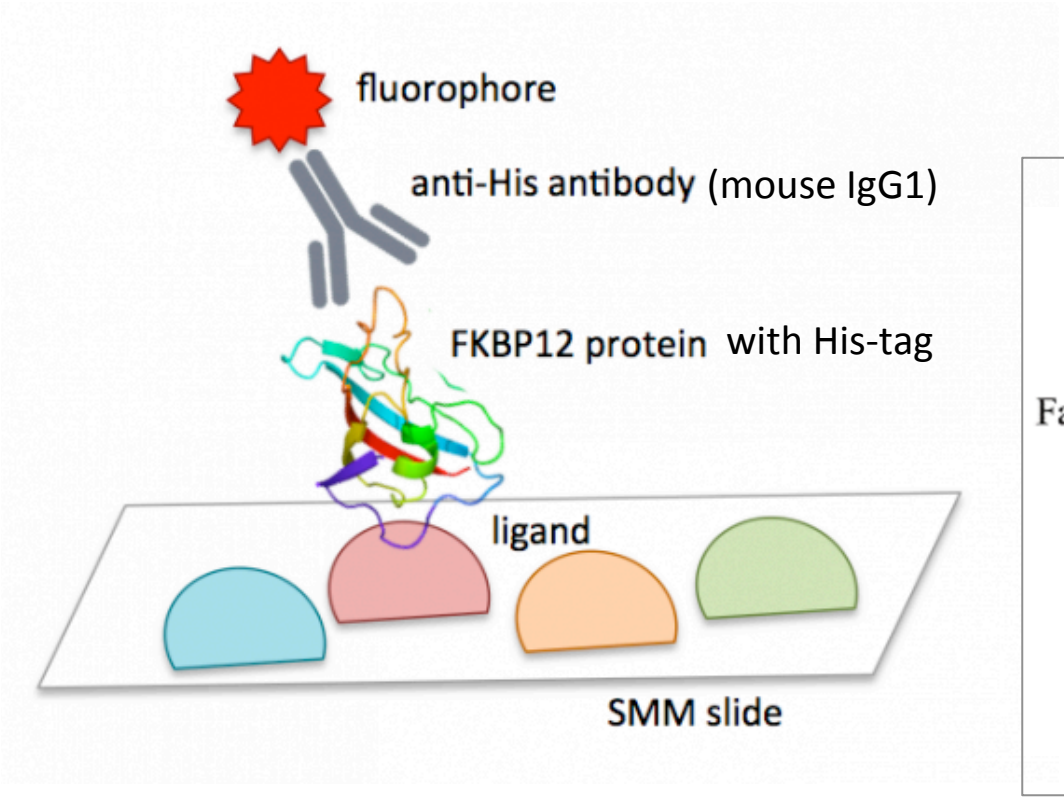
stock concentration = 50 ug/mL

- Prepare 7 mL of pure FKBP12 diluted to 0.5 µg/mL
- Incubate FKBP12 with SMM
  - 12,000 spots
  - ~ 4,200 small molecules (x2)
  - 4 x 48 positive control spots: rapamycin
  - "X" pattern of fluorescein spots

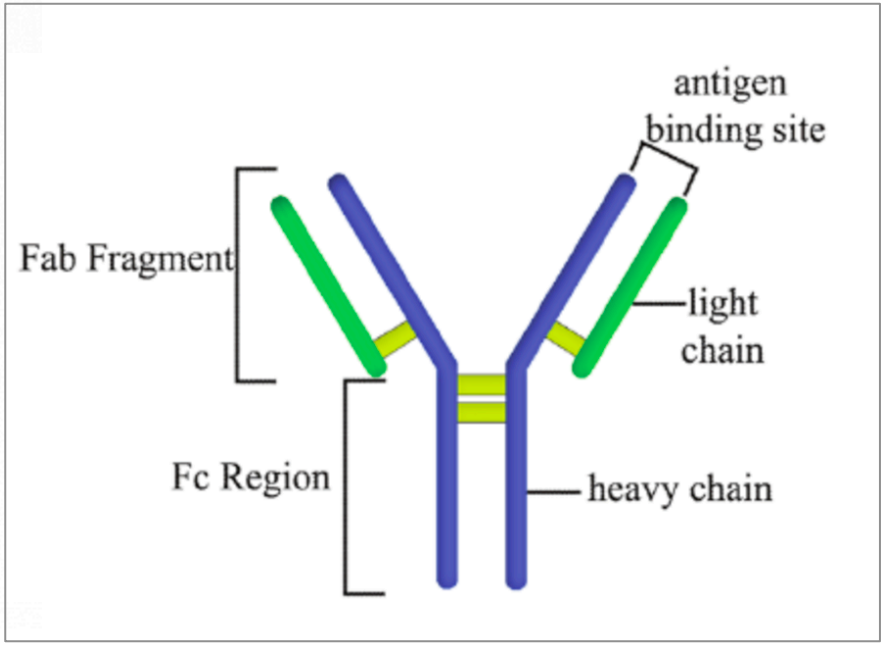


- Prelab during first 1-hour incubation
- Add (anti-His) primary antibody

Using immunofluorescence: to detect FKBP12-ligand binding

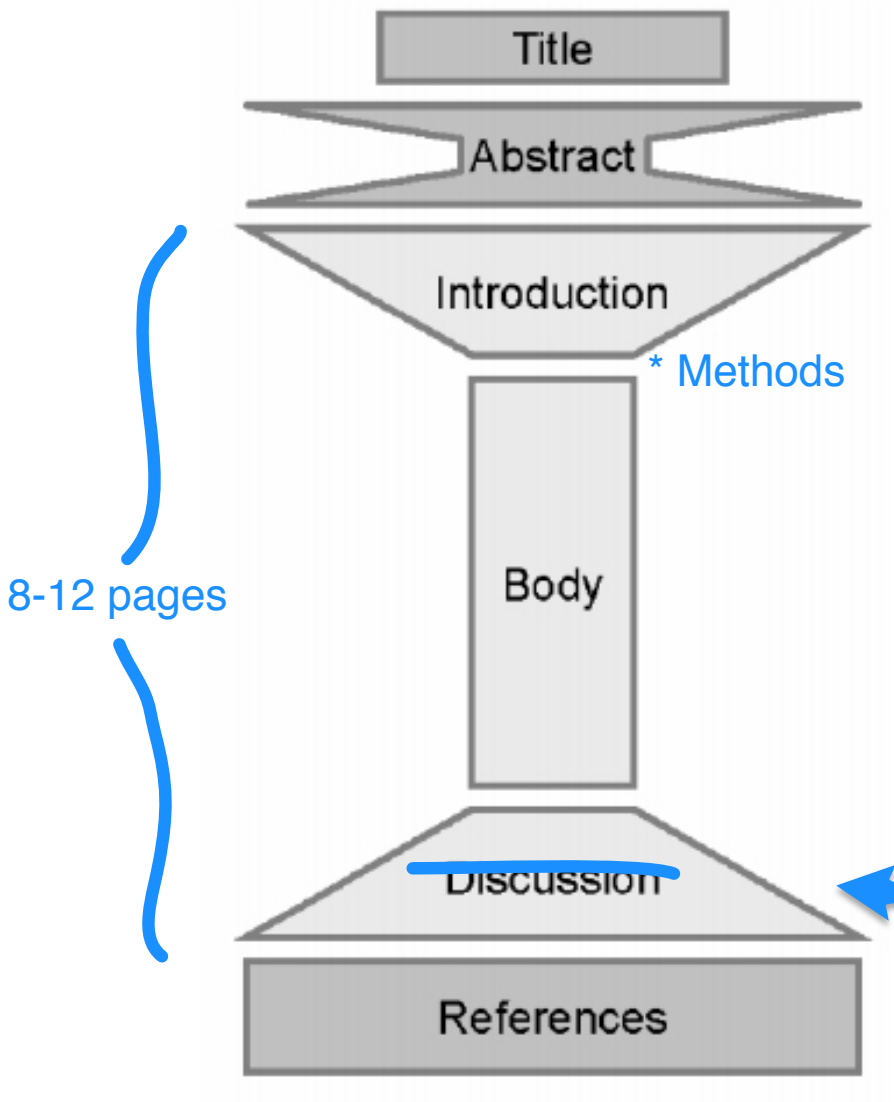


Alexa Fluor 647
- excitation ~ 647 nm
- emission ~ 665 nm



draft due March 13

Your M1 Data Summary



Title: take-home message

Abstract: the only page *not* in bullet points

In bullet points:
Introduction: background and motivation

Results and
interpretation / discussion
bundled together

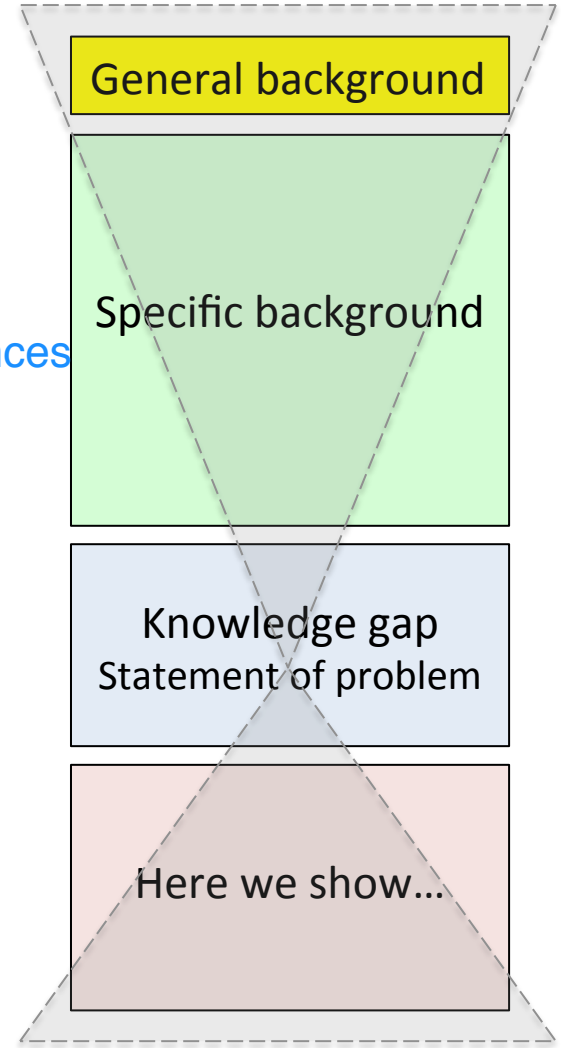
transitions from one page to the next

Implications and future work

References (*not* in bullet points)

What goes into an introduction?

add
a lot of
references



- Your research is anchored in a general topic that your audience cares about.
  - focus on outsiders
  - include references
- All information connects your project with the general topic.
  - minimum essential information
  - accurately represents the field
  - correctly referenced, give credit
- The question you address is clearly articulated, connected to the background, and appears meaningful.
  - give evidence of incompleteness of current understanding, of value of investigation
  - CLEARLY state your hypothesis
- A preview of your findings and their implications fills the demonstrated gap.
  - light on Methods

The meat of your paper

- Figures and captions
  - Decide on these first
  - Use subpanels
  - Text: limited on figure, explicit in caption
  - reasonable size
  - descriptive title
  - caption purely descriptive of image, factual
  - intro sentence in caption
- Results
  - topic sentence: setup
  - What you did: experiments and expectations, including controls
  - What you found:
  - transition

To help you write Results for M1D5

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?

What goes into the Discussion / Interpretation?

- Interpret
- Put in context: how does this fit with other studies?
- Highlight significance: how might this impact this/other field?
- Discuss controversial or surprising results
-

What goes into Implications & Future Work?

- Describes caveats and suggest remedy
- Conjecture (one layer only!) implications
- Propose future work, identify new questions that arise

- Follow same order as in Figures/Results
- Make sure you come back to big picture introduced in intro
- Don't overreach / overpromise!

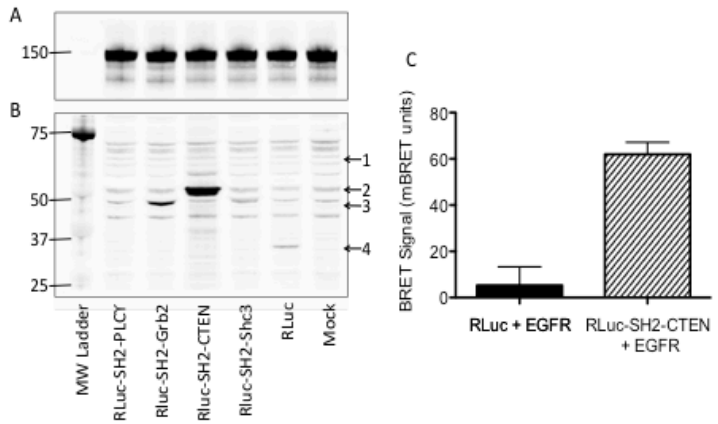


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies.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

Example M1 “Results & Interpretation” slide