# M1D4:Screen chemical library for FKBP12 binders

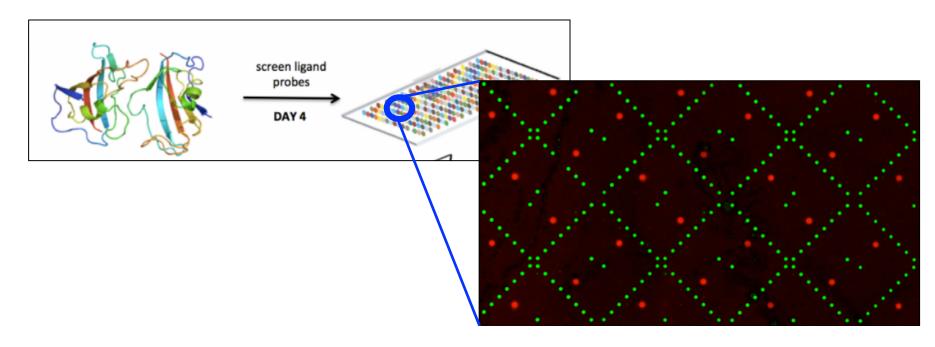
02/24/2017

- 1. Incubate FKBP12 with small molecule microarray (SMM)
- 2. Prelab discussion
- 3. Complete SMM screen and store slides

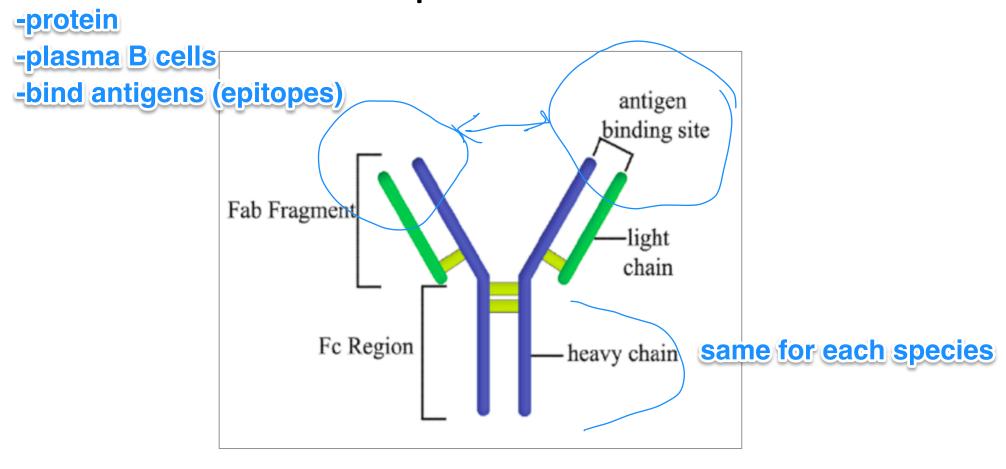


# SMM screen logistics

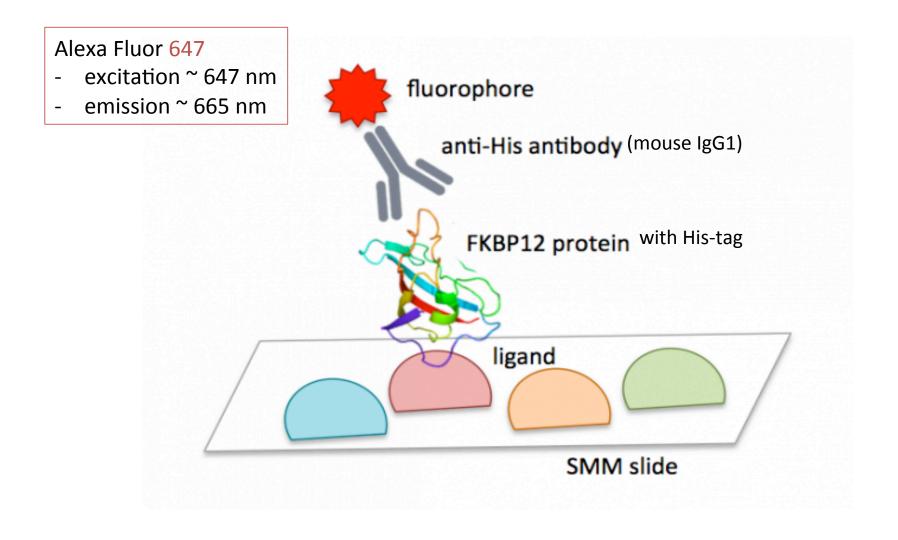
- 1) Incubate FKBP12 with SMM
  - 12,000 spots
  - ~ 4,200 small molecules (x2)
  - 4 x 48 positive control spots: rapamycin
  - "X" pattern of fluorescein spots
- Incubate with fluorescently labeled primary antibody to polyhistidine (6Xhis-FKBP12)



# Antibodies are used as a tool to identify proteins



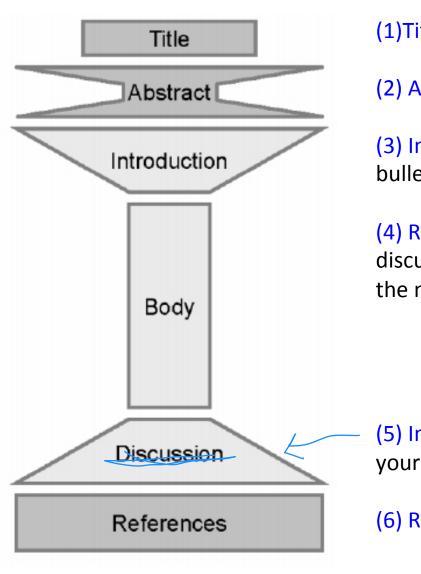
# Using immunofluorescence to detect FKBP12-ligand binding



## M1 major assignments

- Data summary (15%)
  - in teams, on Stellar
  - draft due 03/13, final revision due 03/27
  - bullet points, .PPTX
- Mini-presentation (5%)
  - individual, video via Gmail
  - due 03/18
- Lab quizzes (extra credit on homework grade)
  - M1D3, M1D5, and M1D7
- Notebook (5% total)
  - one day will be collected and graded by Rob on M1D7
- Blog: http://be20109s17.blogspot.com/ (participation: 5% total)
  - by 04/03

# Content of M1 Data Summary



- (1)Title: take-home message
- (2) Abstract: the only page *not* in bullet points
- (3) Introduction: background and motivation, bulleted text
- (4) Results and interpretation: present and discuss the data, <u>transitions</u> from one page to the next, bulleted text

- (5) Implications and future work: tie this back to your motivation, bulleted text
- (6) References (not in bullet points)

### What goes into an background and motivation?

#### General background

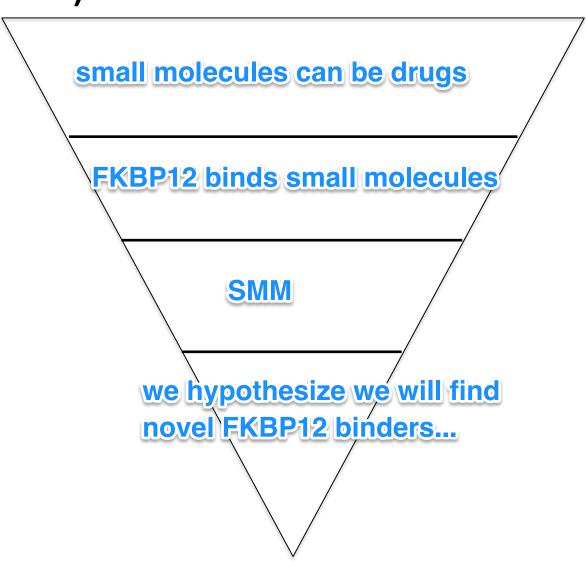
Specific background

Knowledge gap Statement of problem

Here we show...

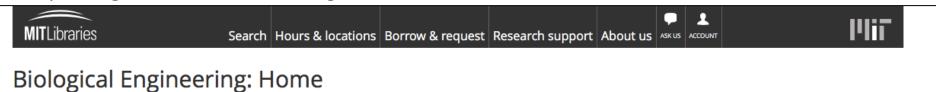
- Your research is anchored in a general topic that your audience cares about.
  - focus on outsiders
- All information connects your project with the general topic.
  - minimum essential information
  - accurately represents the field
  - correctly referenced, give credit
- The question you address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

# Outline of background and motivation (HWM1D5)



# How do you easily format references?

http://libguides.mit.edu/bioleng



### Tools and support: Citation Management, Off campus access, Scholarly Publishing

#### **Manage your Information**

#### Manage your Information -

Contains links to research guides for citation management, managing personal digital content, managing and publishing data, and personal content management tools.

Mendeley - Manage citations and PDFs using a desktop client that syncs with web-based account that supports small group collaboration. Setting up Mendeley is free. The Libraries support Mendeley Institutional Edition which gives MIT users additional web server storage and other features.

Endnote - Widely used application designed to help you to organize citations and create a bibliography. MIT does not offer a site license for EndNote. There's a web version that's free for MIT users through Web of Science.

Zotero - A free, open-source program that can be downloaded as a browser extension for Firefox, Chrome, and Safari or as a standalone program. For many databases and websites, it can tell when a list of books or articles is displayed, so citation information can be saved with just a few clicks:

## To help you write Results for HWM1D5

- 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?

## The meat of your paper

### Figures and captions

- Decide on these first
- Use subpanels (A., B., C.)
- Text: limited on figure, explicit in caption
- reasonable size (1/3 of page)
- descriptive title
- caption purely descriptive of image, factual
- intro sentence in caption

### Results and Implications

- goal/topic/purpose/intro
- What you did: experiments and expectations, including controls
- What you found:
- interpretation of resulttransition/motivation/next step

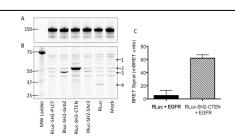


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLC9, GH2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (f) RLuc-SH2-GH2, 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 (on wiki)

# Today in lab:

- Complete FKBP12 + SMM incubation, wash carefully!
- 2. Incubate SMM with His antibody, wash carefully and store at 4°C, protected from light
- Homework due Wednesday, M1D5
  - Background and Motivation
    - 3-7 topic sentences, using the "funnel" outline, bulleted
    - include reference list and reason you chose that reference
  - Results section associated with M1D4 figure
    - address questions in the prompt, bulleted