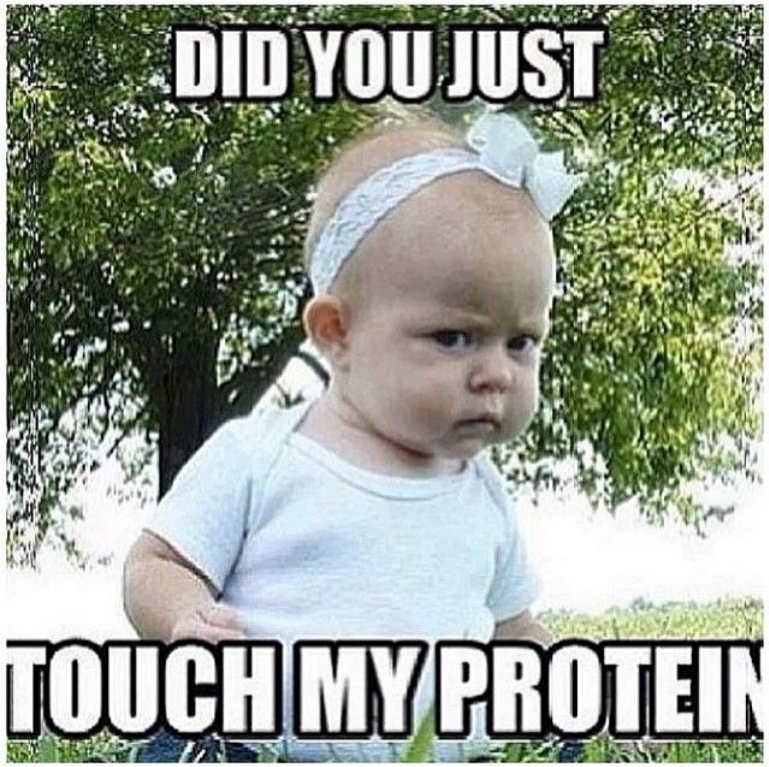
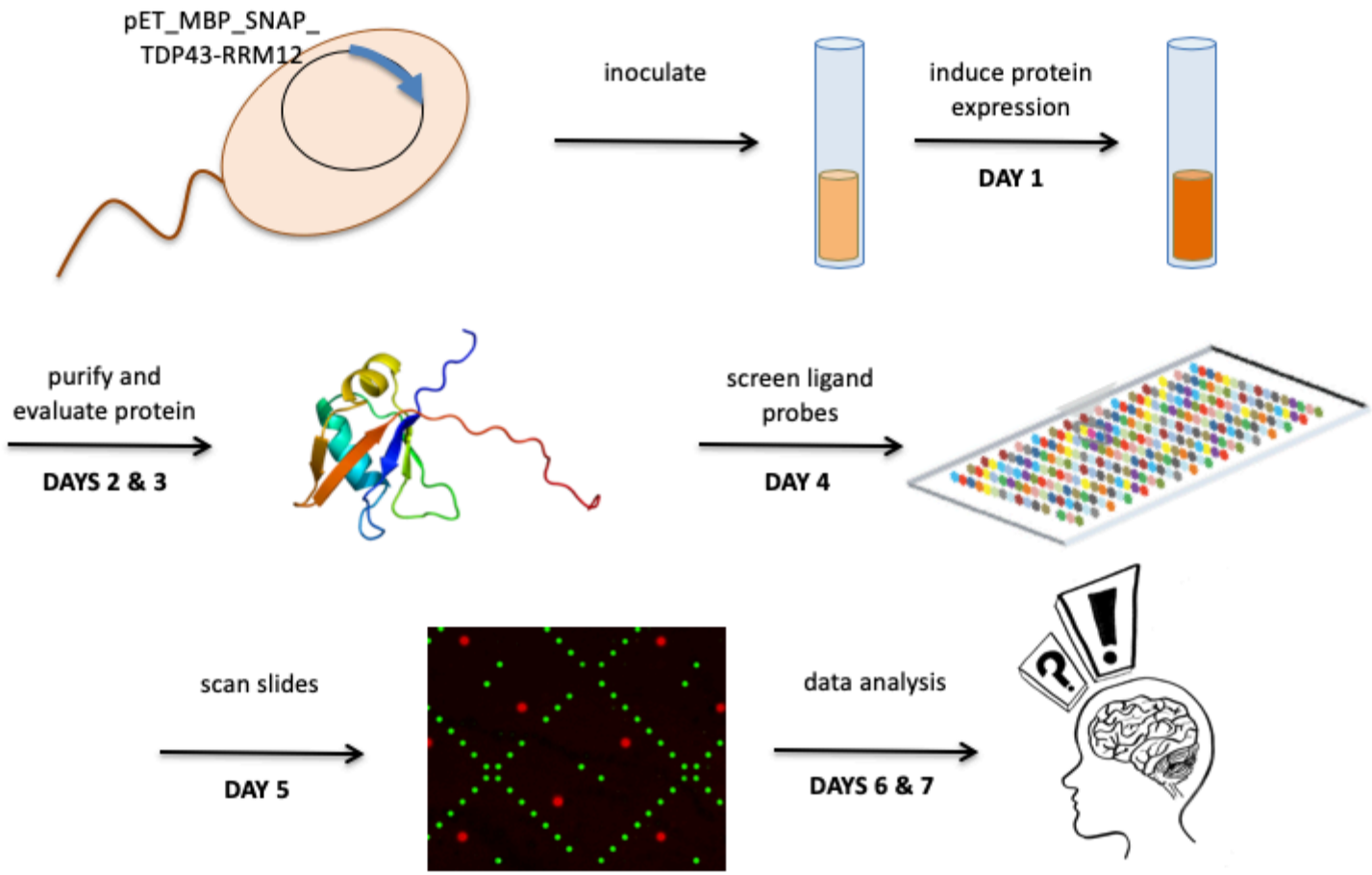
- Lecture starts at 11:05a, absent after 11:15a
- Comm Lab meetings must be scheduled >24 hr in advance!

M1D4:
Perform SMM with TDP43 protein

1. Prelab discussion
2. Prepare protein
3. Add protein to SMM slide

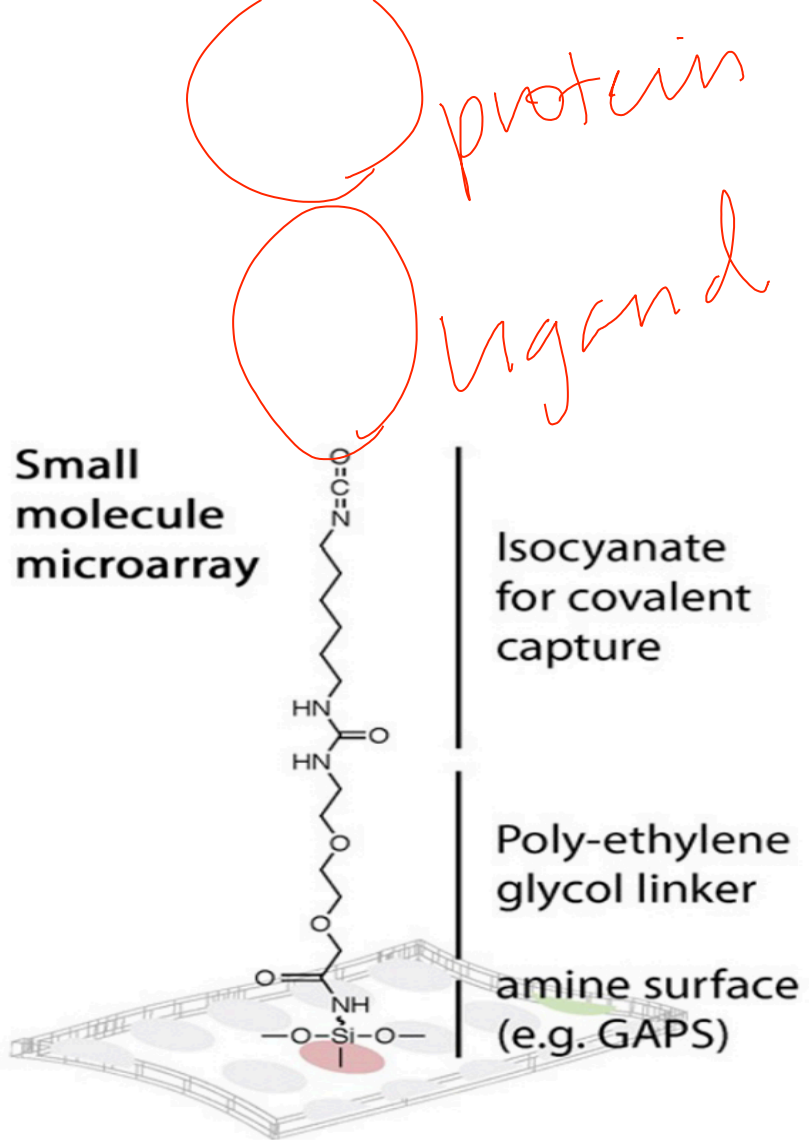


Overview of Mod1 experiments



SMM slide preparation

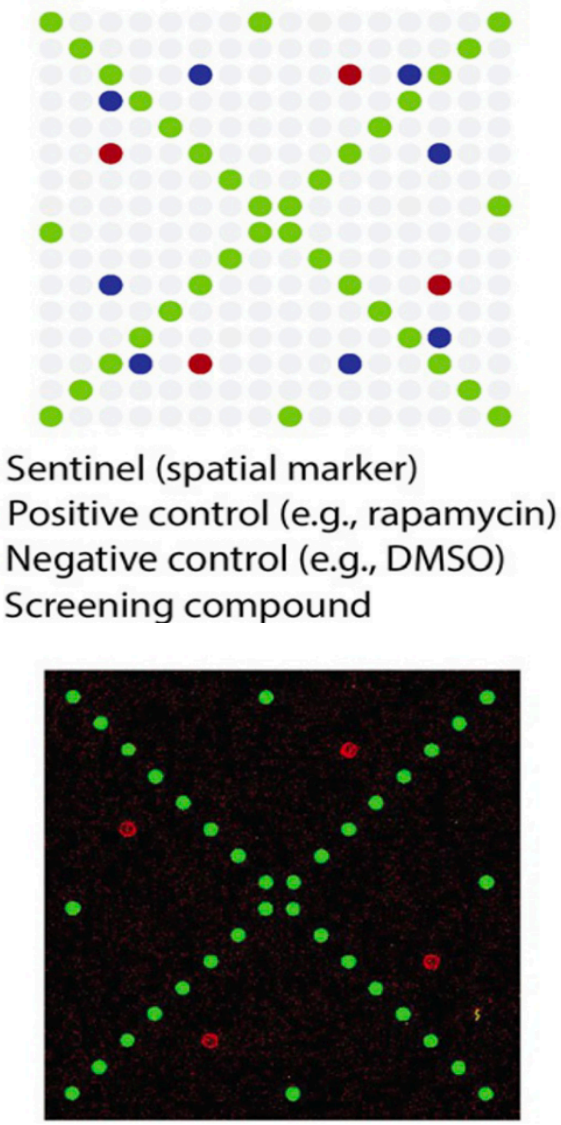
- Gamma-aminopropylsilane (GAPS) slide coated with polyethylene glycol (PEG) spacer
- PEG coupled to 1,6-diisocyanatohexane to generate isocyanate-functionalized slide
- Isocyanate able to react with nucleophilic functional groups



Leifer et al. Methods in Enzymology. 2018.

SMM slide layout

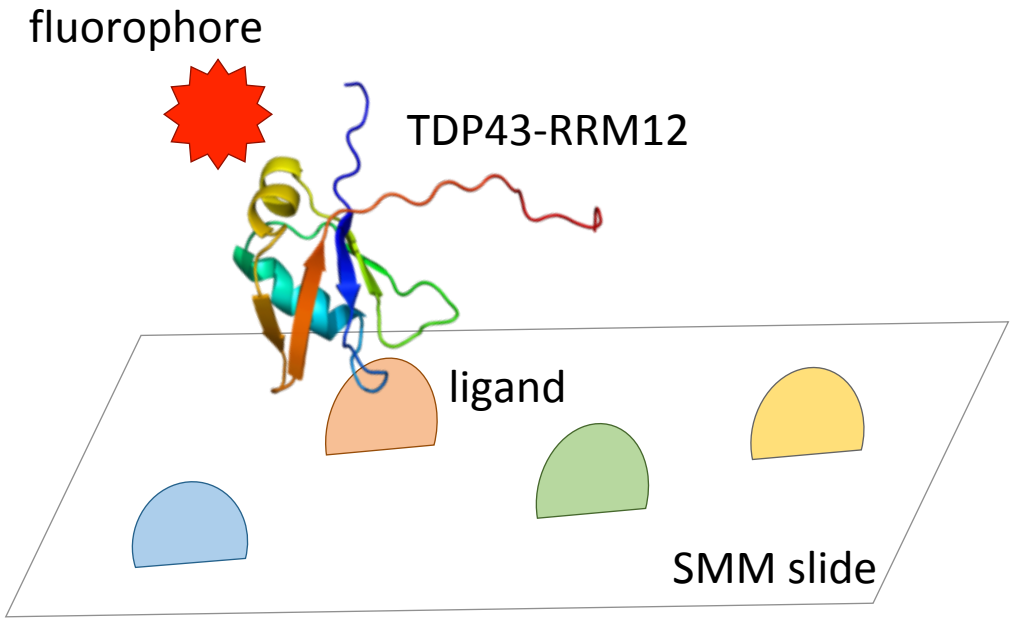
- Sentinel spots used for alignment during imaging / data analysis
- Control spots used to validate results
  - Negative control = DMSO
  - Positive control = none!



Leifer et al. Methods in Enzymology. 2018.

How will we screen for ligands that bind TDP43-RRM12?

- Each team will use 2 slides for the SMM screen
- Each slide contains ~12,000 spots
  - ~4,200 small molecules / ligands (in duplicate = ~8,400)
  - Fluorescein sentinel spots
  - DMSO negative control spots



For today…

- Use downtime to review / begin homework due M1D5!

For M1D5…

- Design figure and write results / discussion text for experiments completed on M1D3
- Draft methods section for protocols completed on M1D1– M1D3

Notes on results / discussion text…

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?

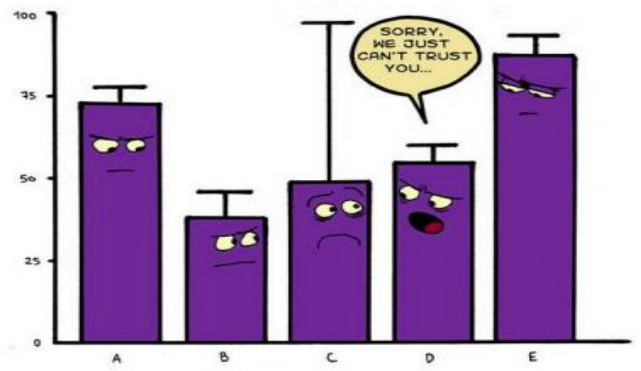


Figure X: Title.
Caption.

Sub-section header.

- Bulleted text
- that answers
- the results / discussion
- prompts!

Notes on methods section…

Include enough information to replicate the experiment

- Cite manufacturer for supplies / equipment (Company)
- Be concise and clear in your description

Use subsections with descriptive titles

- Put in logical order, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in full sentences and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write concentrations (when known) rather than volumes

Eliminate 20.109 specific language and obvious details

- Example “labeled Row A, Row B…”
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say “completed by teaching faculty.”

How can you improve this example?

What DNA?

Which?

DNA was cut to check insert. Enzymes were used for single and double

1X TAE buffer

digest then run on gel made by adding 1 g of agar to 100 mL of ~~water~~.

Conditions!

Concentration

Gel was imaged on a gel box.

DNA Stain

How can you improve this example?

What DNA? How much?

Consider more specific language.

What insert?

Which enzymes? From where were the enzymes acquired?

DNA was cut to check insert. Enzymes were used to cut DNA for

Specifically, why was this done?

Redundant

Provide details on how this was done.

Colloquial…use more scientific language. Also, include details.

single and double digests then run on a gel made by adding

What does this mean?

Be mindful of the order of information and of confusing sentence structure.

1 g of agar to 100 mL of water. Gel was imaged on the gel box.

Use the most flexible units / concise description.

What else was needed for imaging?

The?

What would be more informative?

Edited example…

Confirmation digest of pET_MBP_SNAP_TDP43-RRM12

To confirm that TDP43-RRM12 was cloned into pET_MBP_SNAP expression vector, a digest was completed. Restriction enzymes AbcI and DefIII were used to digest X ng of pET_MBP_SNAP_TDP43-RRM12 in single digests (only one enzyme added) and in a double digest (both enzymes added) using Y U / uL of each enzyme and 1X CutSmart buffer (NEB). Digests were incubated at 37C for Z hrs. …

Getting a start on the Data summary!

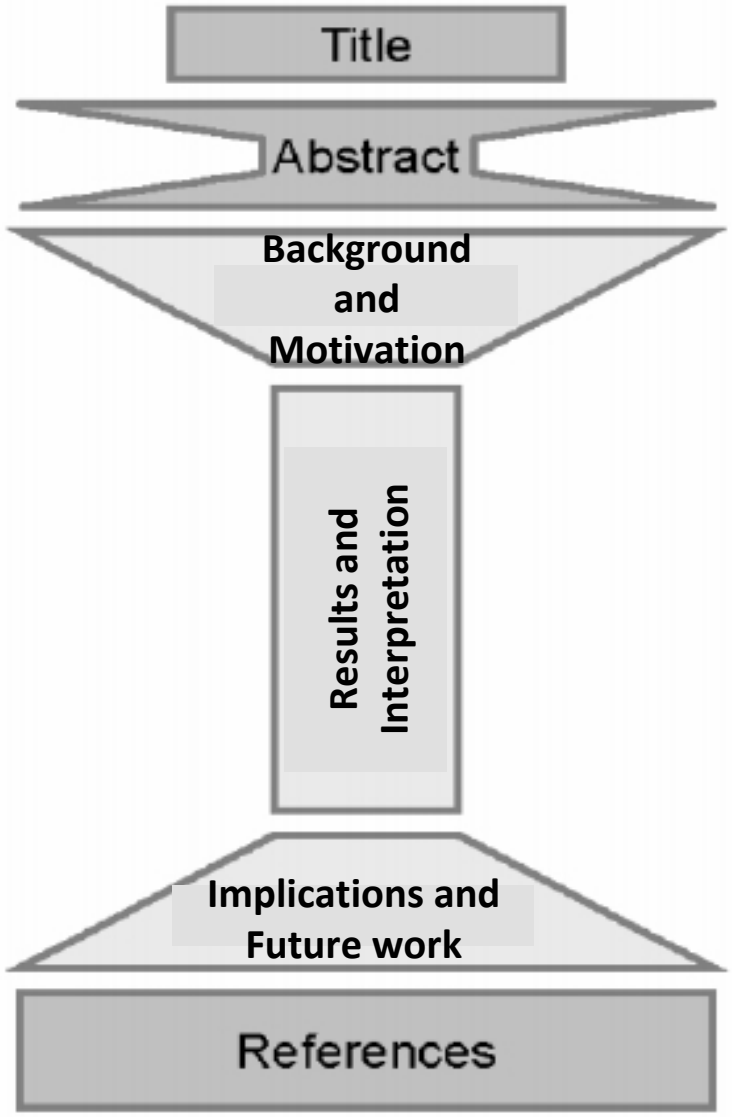
Title: take-home message
Abstract: Paragraph, NOT in bullet points!

In bullet points:
Background and Motivation (include citations)

Results and Interpretation

Implications and Future work (include citations)

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 Mar 8 at 10pm, revision due Mar 22 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!