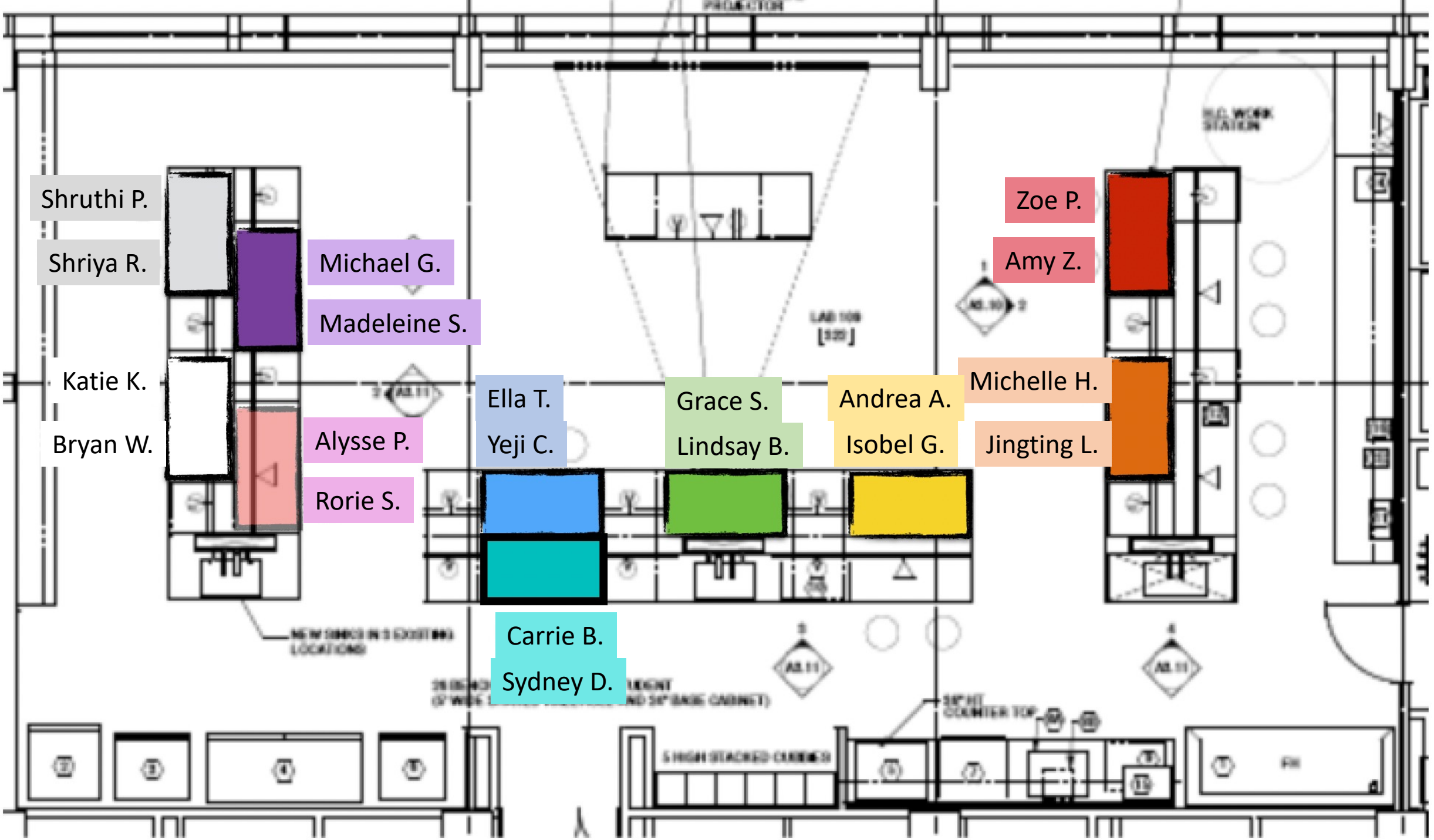
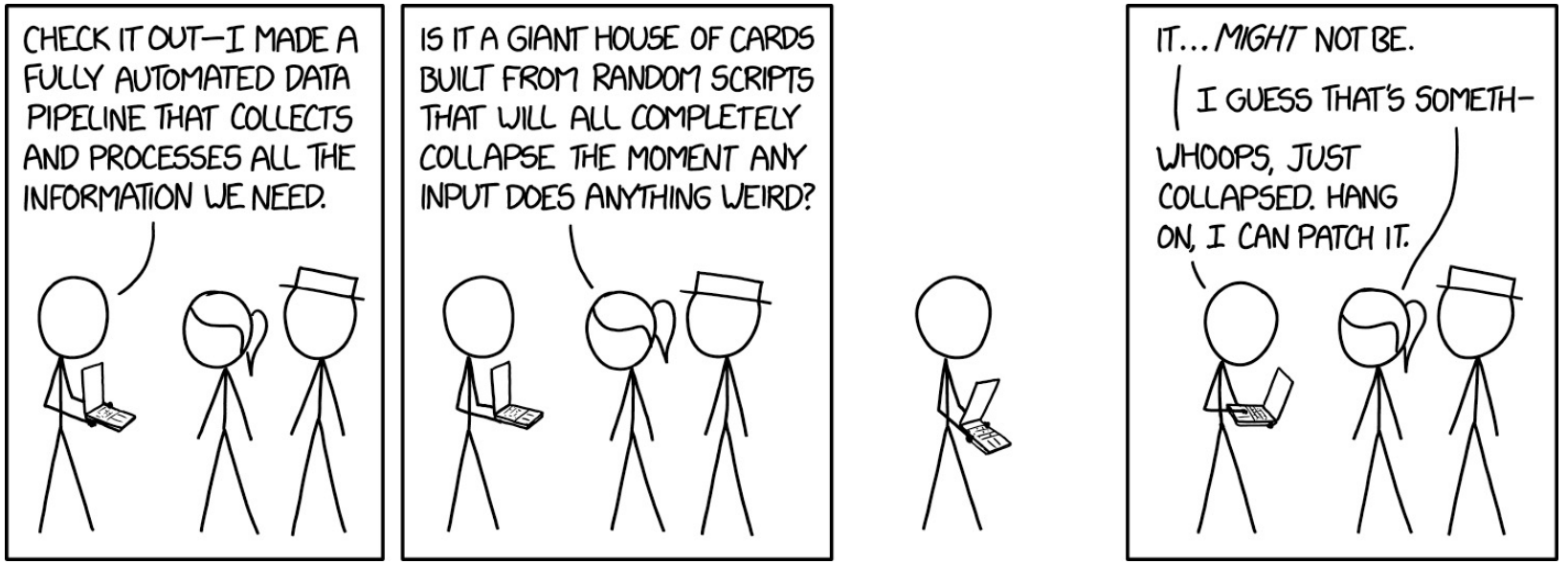

Shruthi P.
Shriya R.
Michael G.
Madeleine S.
Katie K.
Bryan W.
Alysse P.
Rorie S.
Ella T.
Yeji C.
Grace S.
Lindsay B.
Andrea A.
Isobel G.
Carrie B.
Sydney D.
Zoe P.
Amy Z.
Michelle H.
Jingting L.

M1D1: Review small molecule microarray (SMM) technology

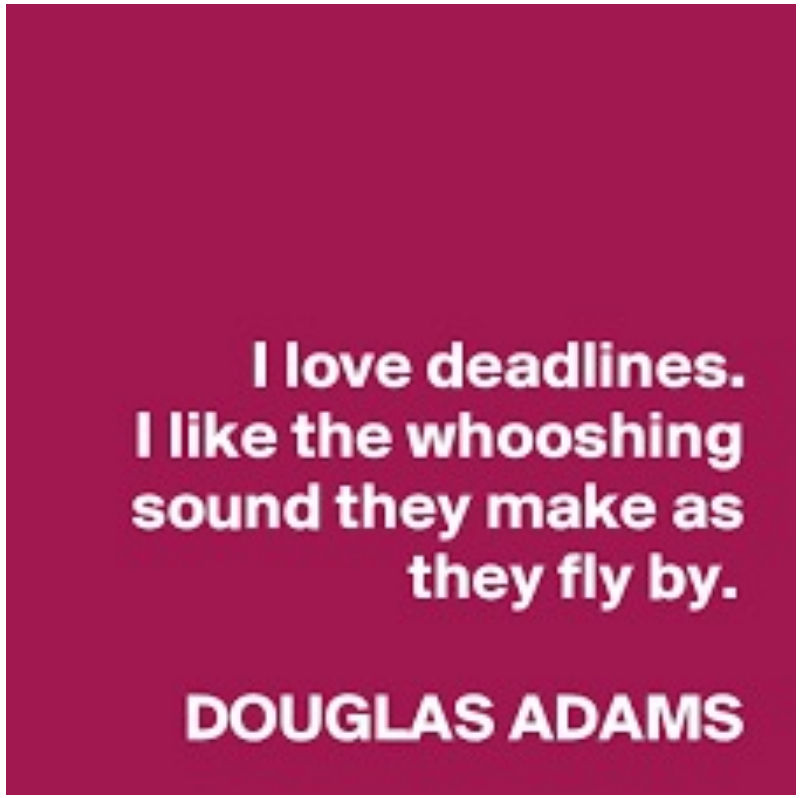
- Orientation quiz!
- Prelab discussion
- Walk through SMM procedure



xkcd

Mod 1: Major Assignments

- Data summary (15%)
  - In a team
  - Draft due 3/12, final revision due 3/20
  - Format: Bullet points, .PPTX
- Research Talk (5%)
  - Individual, submit video via gmail
  - Due 2/23 by 10pm
- Lab quizzes (5% collectively)
  - Individual (orientation quiz is exception)
- Notebook (5% collectively)
  - Due 3/4 by 10pm, graded by Christine
- Blog (part of 5% Participation)
  - Due 3/14 by 10pm



Mod 1 Background

Overarching focus of Mod1: **Drug discovery!**

- We are studying the effects of small molecules on an “undruggable” target known to play a role in neurodegenerative disease.
  - Can small molecule interactions with our protein provide any biological insight?
  - Do any small molecules provide insight about potential therapeutics for our protein of interest?

Topics we’ll cover today:

- What is TDP-43/ why is it an interesting drug target?
- What kind of drugs will be our focus?
- How did we screen for potential drugs in a previous semester?
- How are you going to follow up on that initial screen?

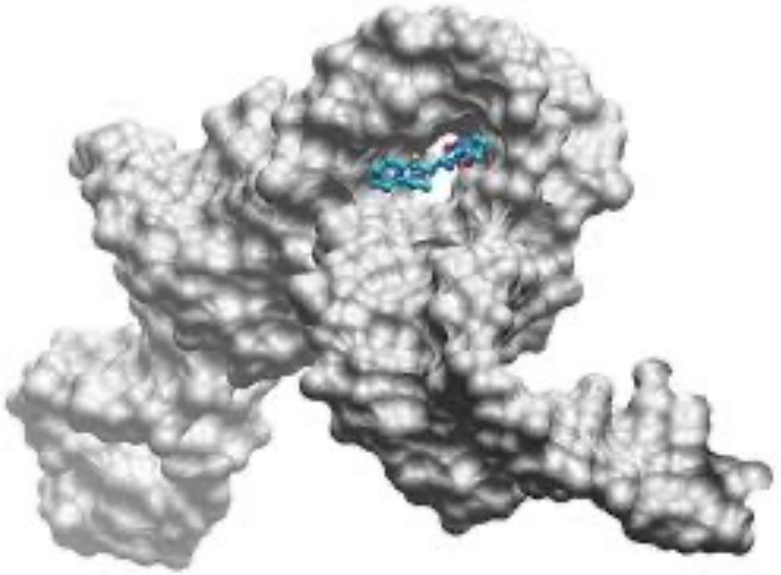
What is our target?

- TAR DNA-binding protein 43 (TDP-43)

- TDP-43 is a DNA- and RNA-binding protein
  - Mainly localized to the nucleus
    - Can become mislocalized to the cytoplasm
  - Can form aggregates and be aberrantly modified
  - Aggregates linked to the development of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia FTD)

- 4 main domains
  - N-terminal & C-terminal domains
  - 2 RNA recognition motifs (RRM1 and RRM2)

What are small molecules?



- Small molecules
  - Mw < 1000 Da
  - Natural or synthetic
  - Frequently comprised of Carbon/Nitrogen/Oxygen

- Why are they interesting probes/theapeutics?
  - Potential to cross membranes and target intracellular molecules
  - Designable/modifiable
  - Numerous possibilities for target interaction

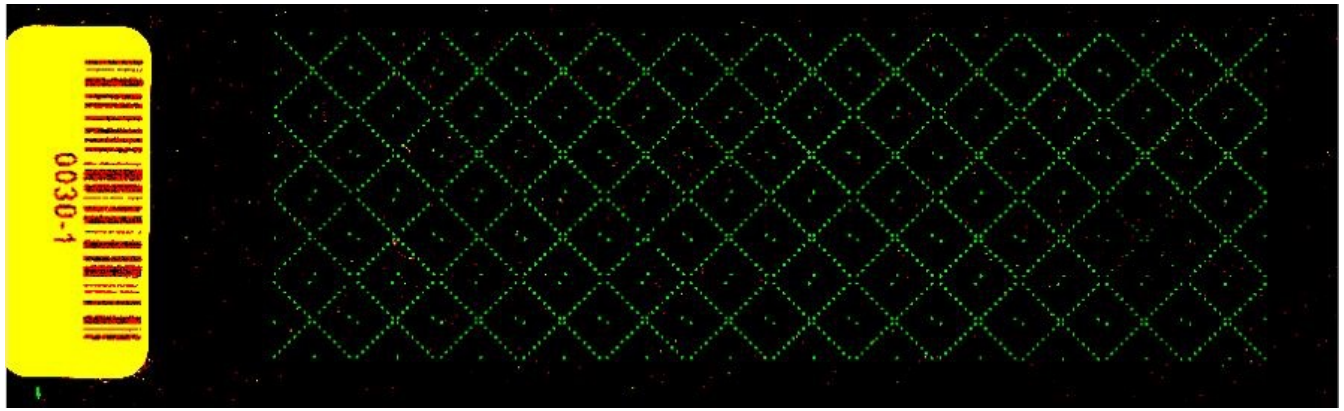
Can small molecules be useful for understanding “undruggable” targets?

How did previous 20.109 students screen for potential small molecule binders for TDP-43?

- Used a high throughput assay, the **small molecule microarray (SMM)**

- High throughput assays like the SMM:
  - Allow unbiased exploration of potential therapeutics
  - Allow examination of targets with limited information
  - Allow for the screening of potentially thousands of putative binders at a time

Small Molecule Microarray (SMM)



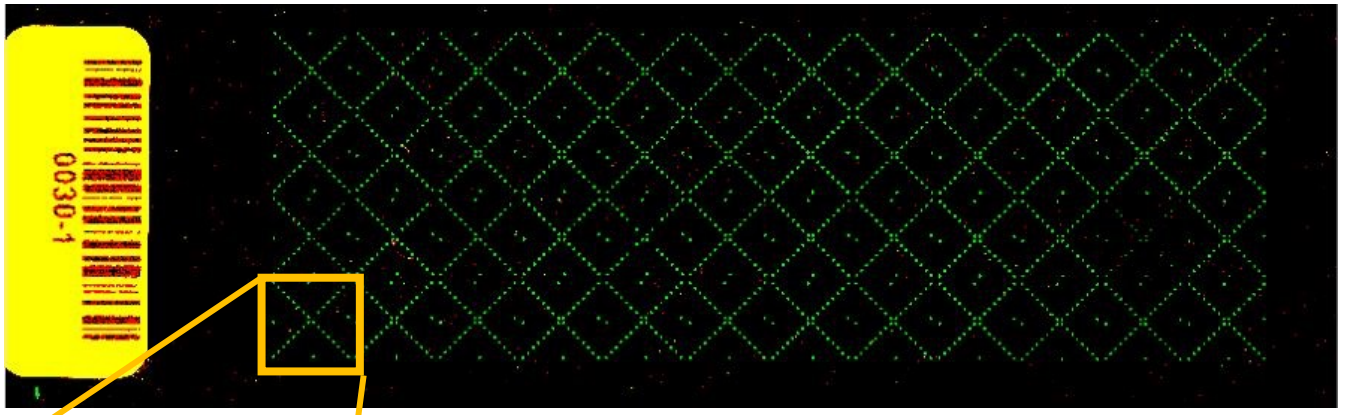
- Each slide contains ~12,000 spots
  - ~4,200 small molecules / ligands (in duplicate = ~8,400)
  - Fluorescein sentinel spots
  - DMSO negative control spots

Koehler Lab 2014 - Small-molecule Microarrays from Koehler Lab on Vimeo.

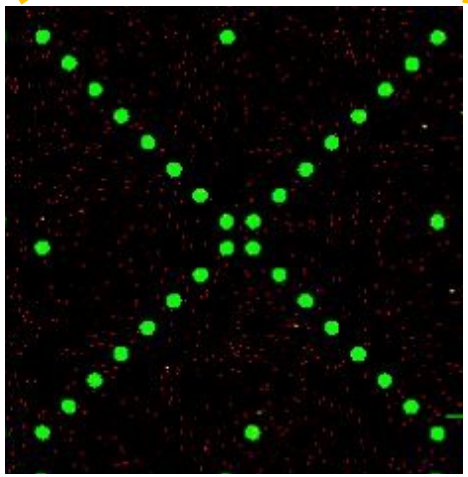
Guide to the SMM slide

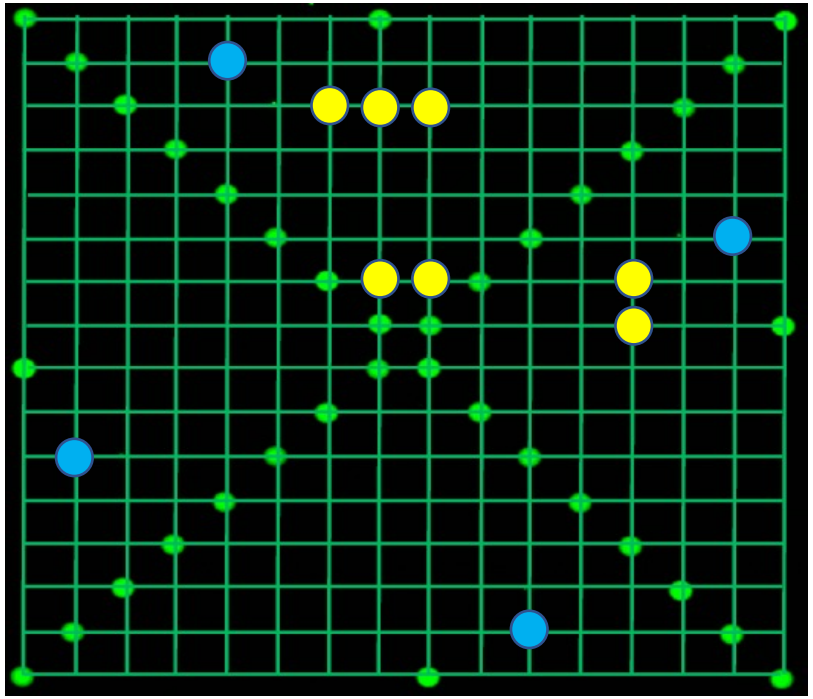
- Each slide has several blocks
- Each block has sentinel spots which are landmarks
- Rest of dots are small molecules and controls
- Can overlay a computational map to identify the location of each small molecule

Slide



Block





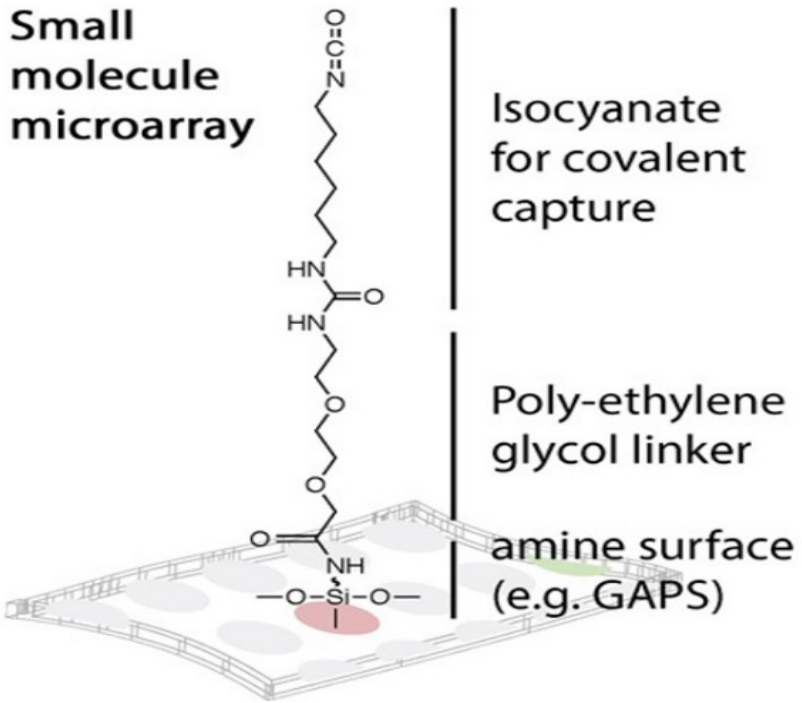
Green= sentinel spots (fluorescein dye)

Blue= DMSO

Yellow= SM

SMM slide preparation

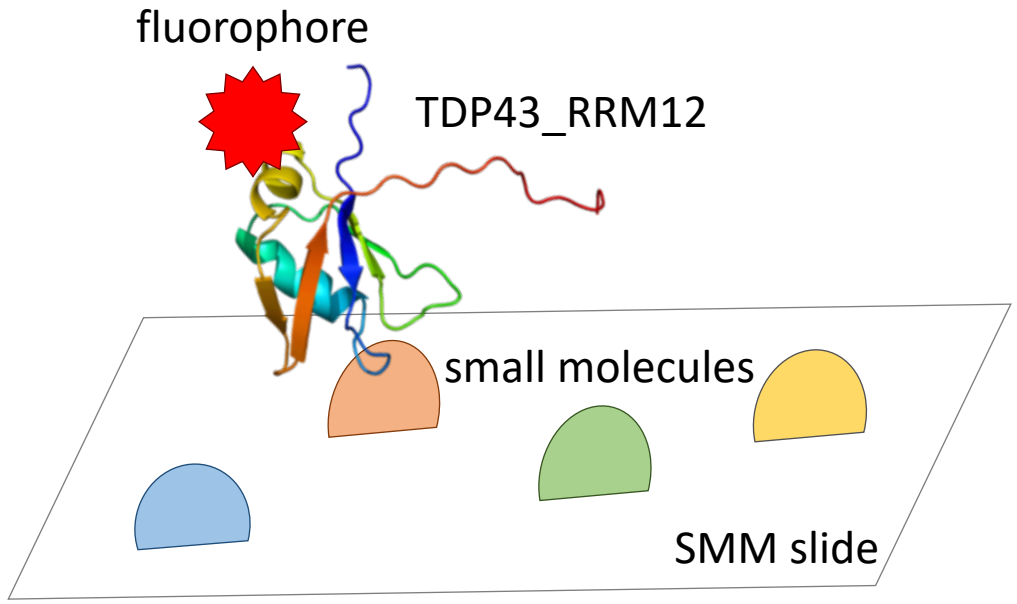
- Gamma-aminopropylsilane (GAPS) slide coated with polyethylene glycol (PEG) spacer
- PEG coupled to 1,6-diisocyanatohexane to generate isocyanate-functionalized slide
- Isocyanate able to react with nucleophilic functional groups



Leifer et al. Methods in Enzymology. 2018.

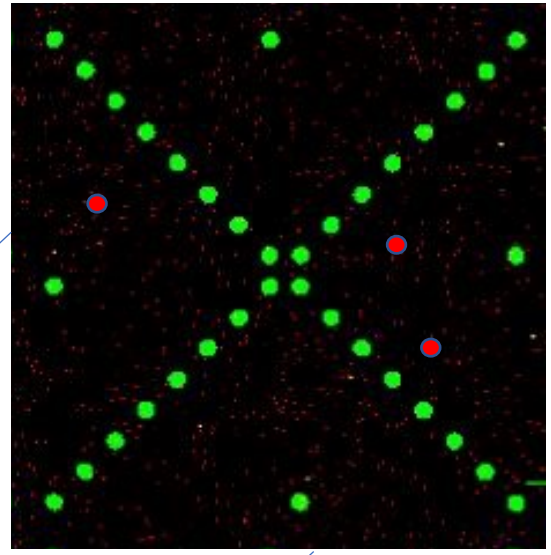
How do we use the SMM to screen for ligands that bind our protein of interest?

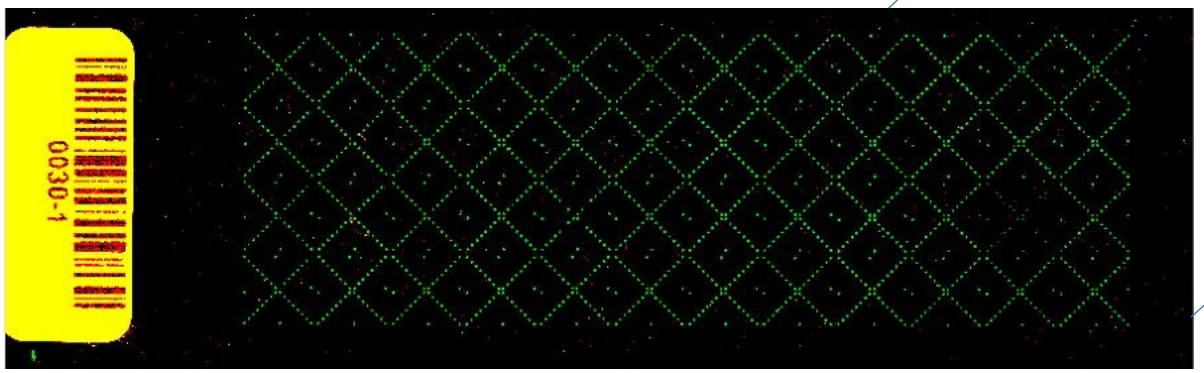
- Create a recombinant protein of the TDP43 RNA binding domains (TDP43_RM12)
  - Label this protein with a Alexa647 fluorophore
- Incubate the SMM slide with our purified and labeled TDP-43_RRM12
- Wash away unbound protein
- Store for scanning



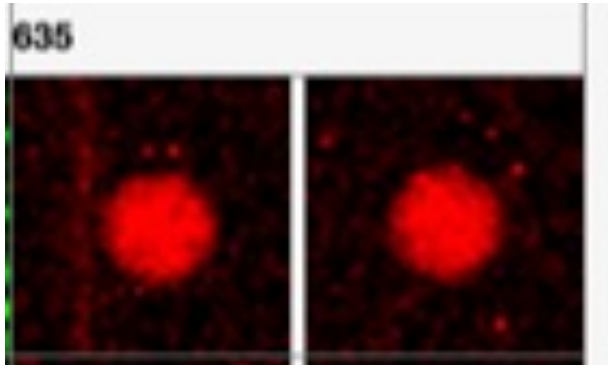
What do putative binders look like on the SMM slide?

Block

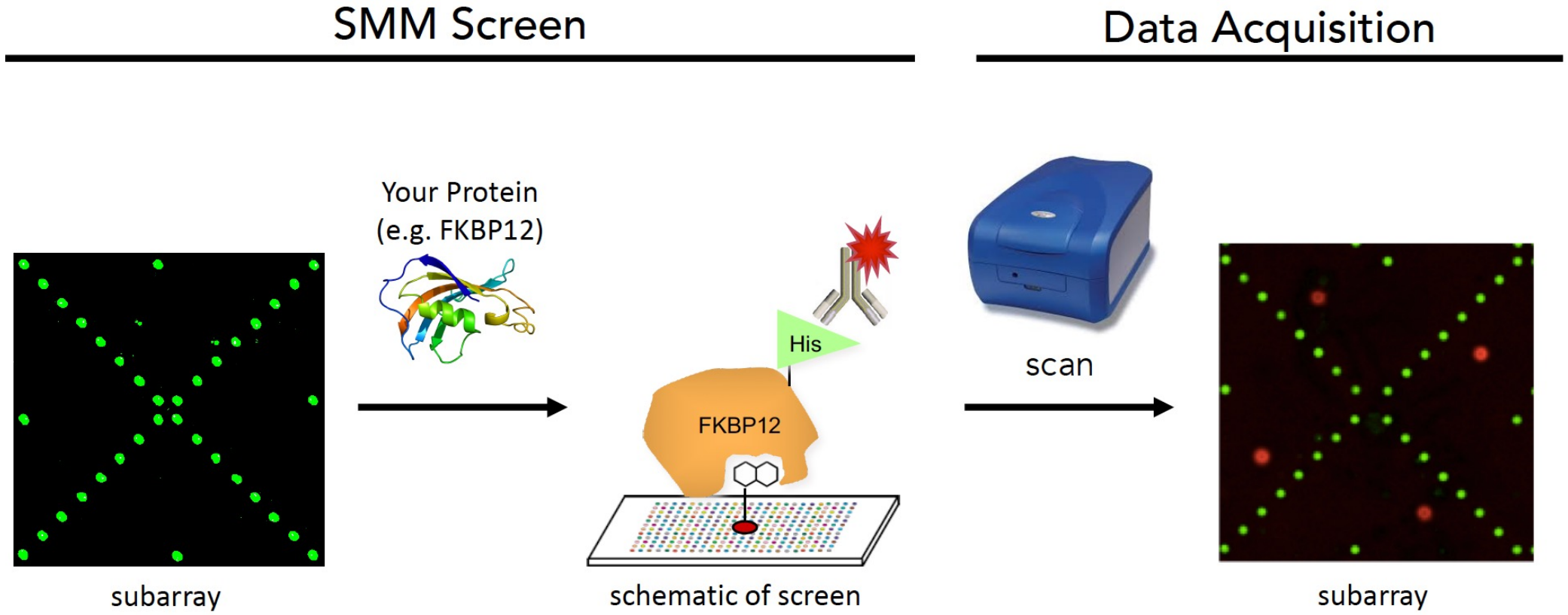




Slide



SMM workflow



B. Leifer, S. Doyle

How do you identify small molecules for further study?

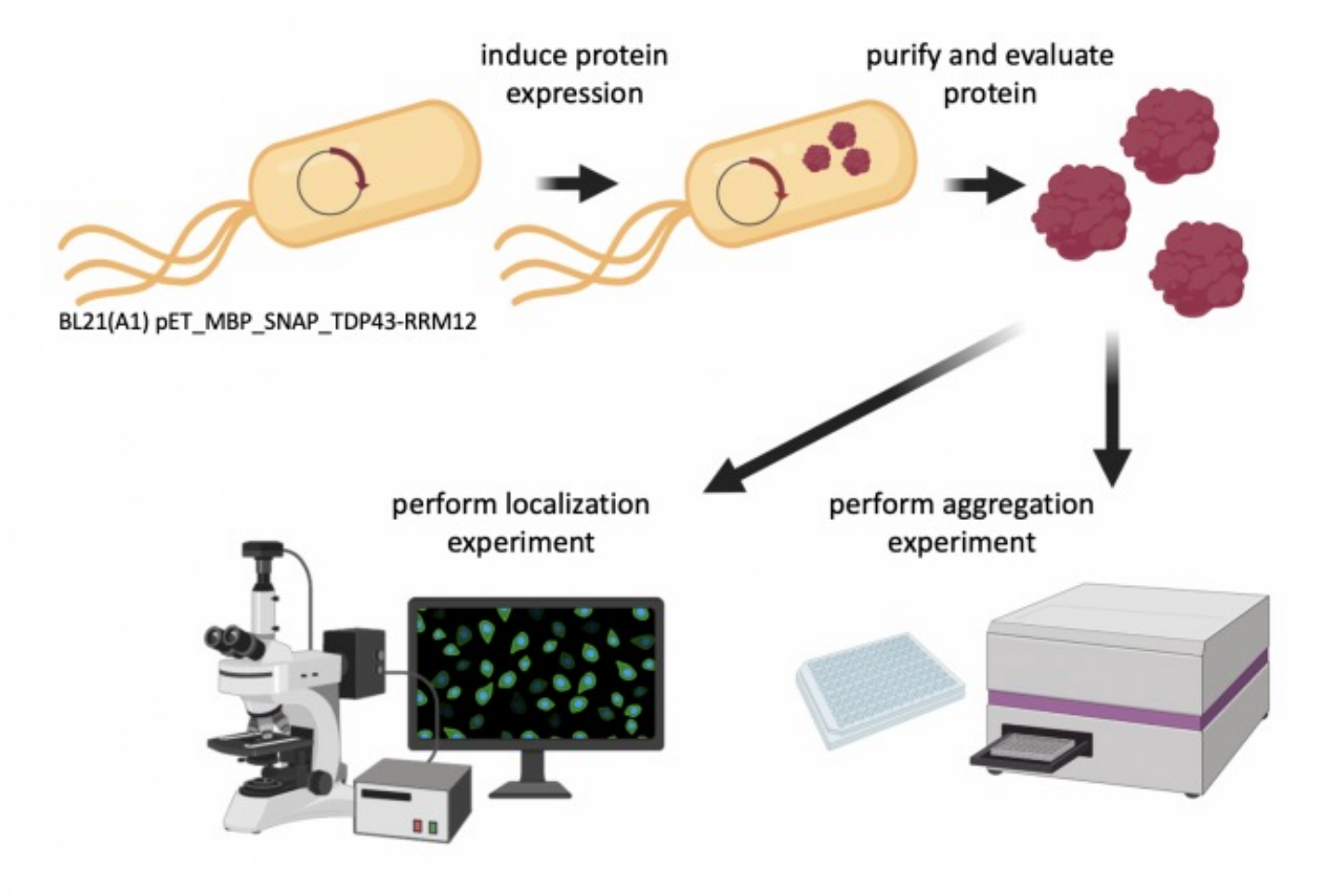
- Next class you will learn about the computational workflow used to analyze the SMM data to determine small molecule hits

- A combination of:
  - Identifying potential signal bias inherent to the production of the slides
  - Identifying a threshold for a strong fluorescent signal
  - Visually validating that fluorescent signal conforms to expected shape

Once we have a group of small molecules that are putative binders to the TDP-43 protein, we will perform follow up assays to assess potential biological impact of association

Overview of Mod1 experiments

Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology



For today…

- Work through SMM on wiki

- Take notes in your Benchling notebook using the template you created
  - Show today's entry to Christine before you leave to receive participation points

For M1D2

- Read the article and guidelines linked on the M1D2 wiki page