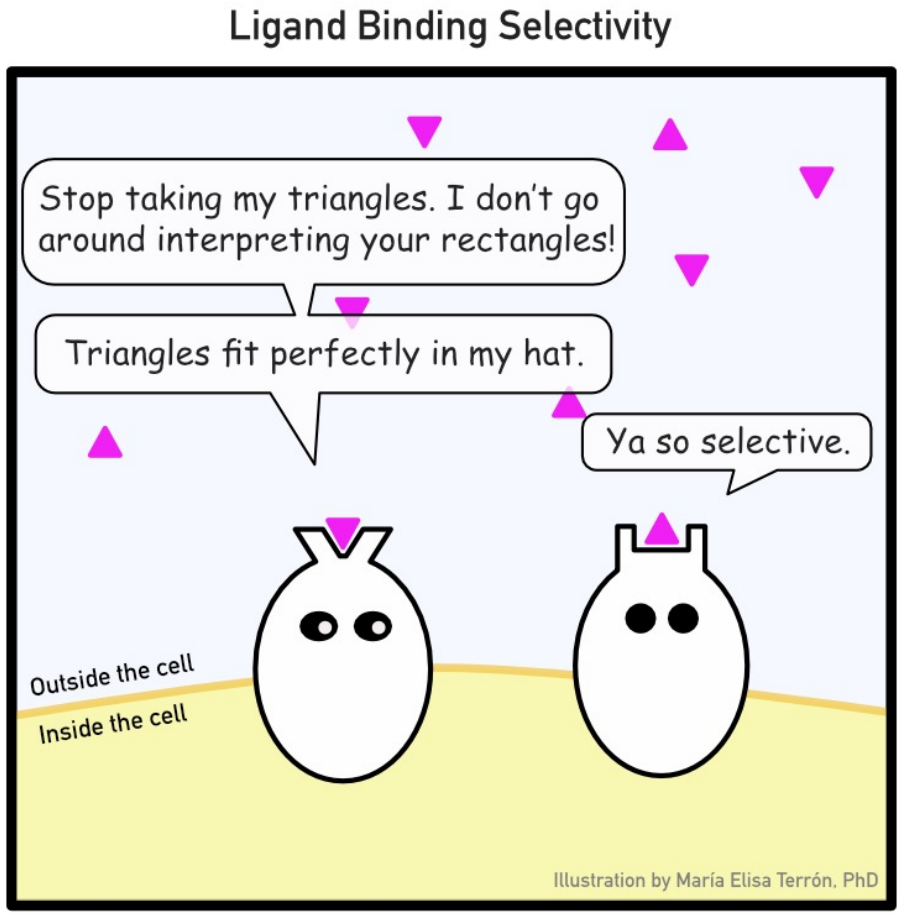
M2D3: Prepare small molecule microarray (SMM) slides with purified protein

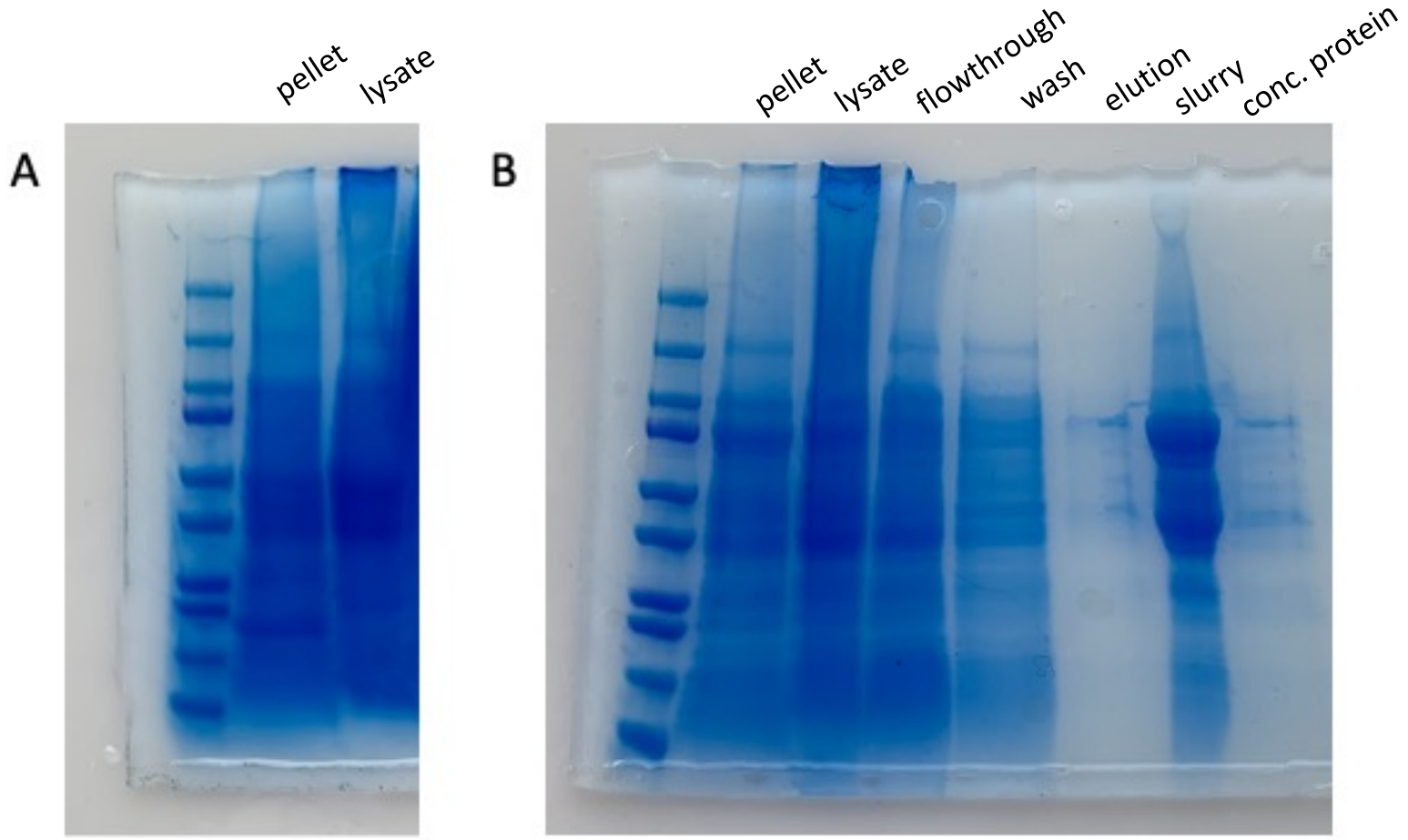
1. Comm Lab workshop
2. Prelab discussion
3. Begin SMM screen



Review of Coomassie gel

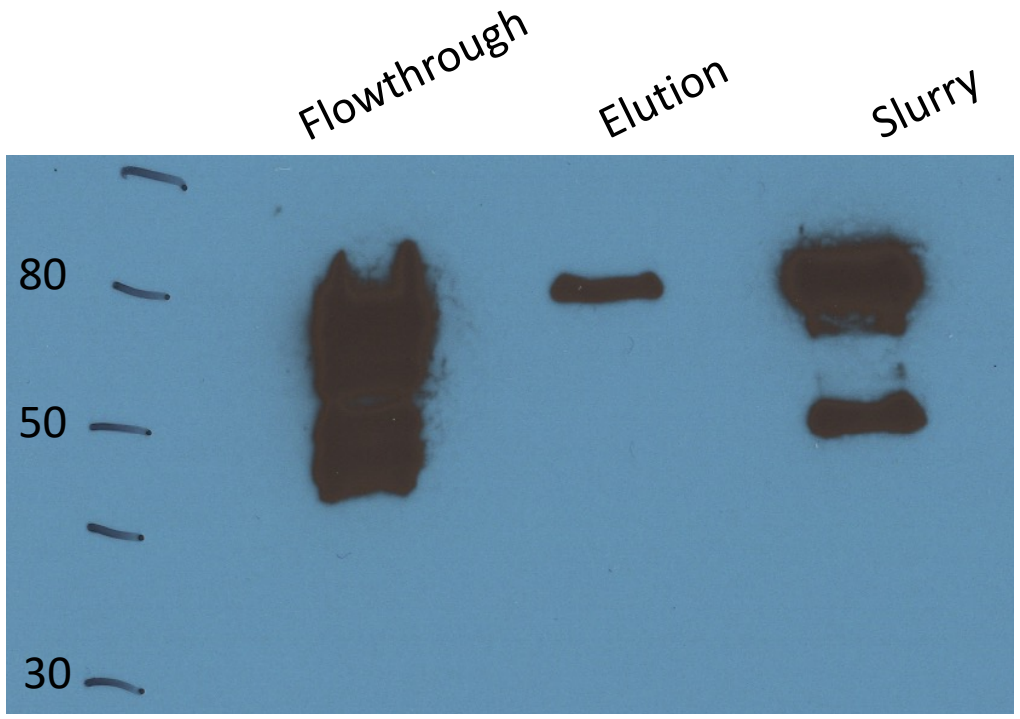
Each lane of the gel should be explained in the results

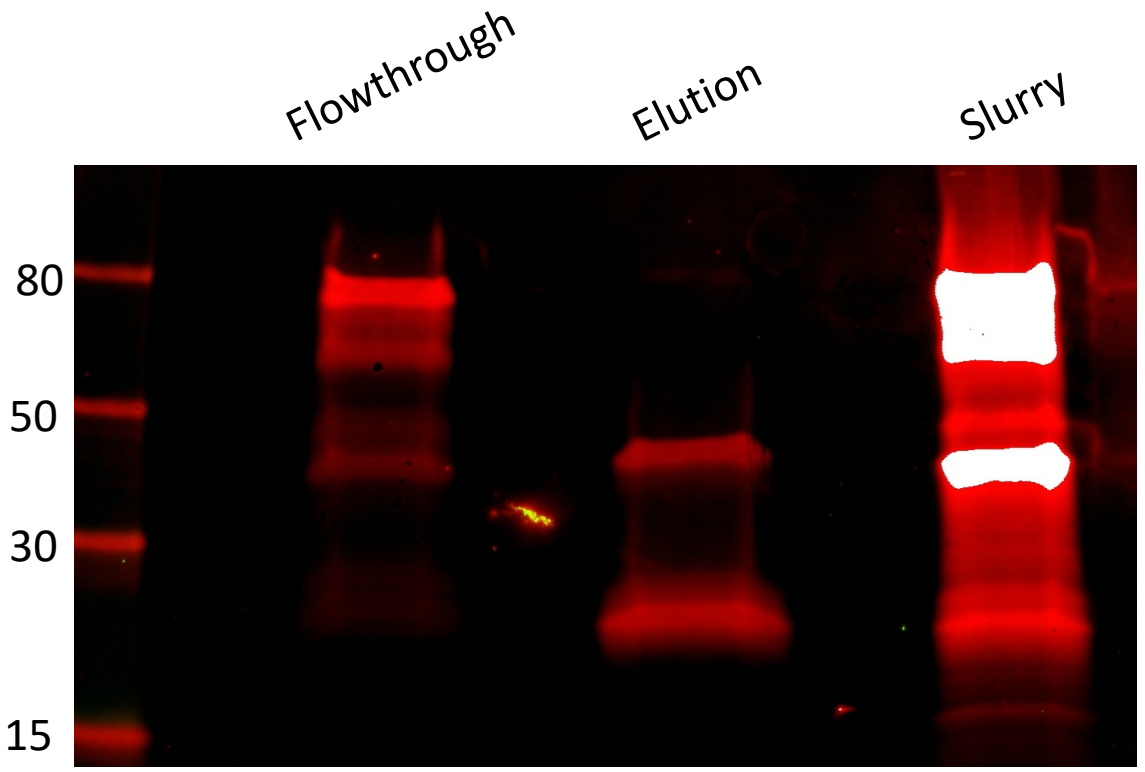
- What bands are expected? Do you see the bands you expected?
- Do you see any unexpected bands?
- What do the bands tell you about the purity of your protein?
- What does might this tell you about the protein concentration calculated in the next step?



What additional information could we gather about our protein sample?

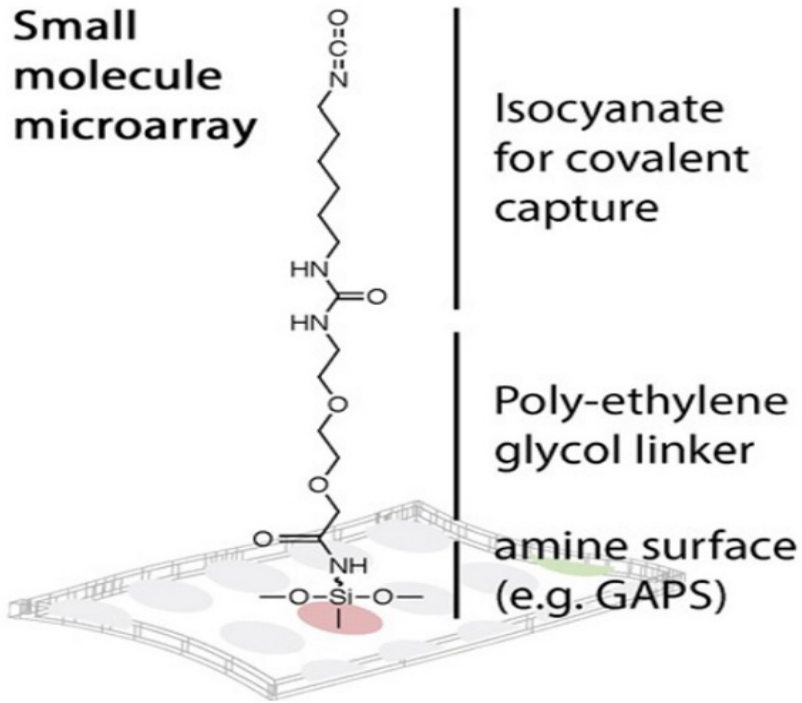
- How could we differentiate between aggregated 647-TDP43_RRM12 and His-MBP-TDP43_RRM12?





SMM slide preparation

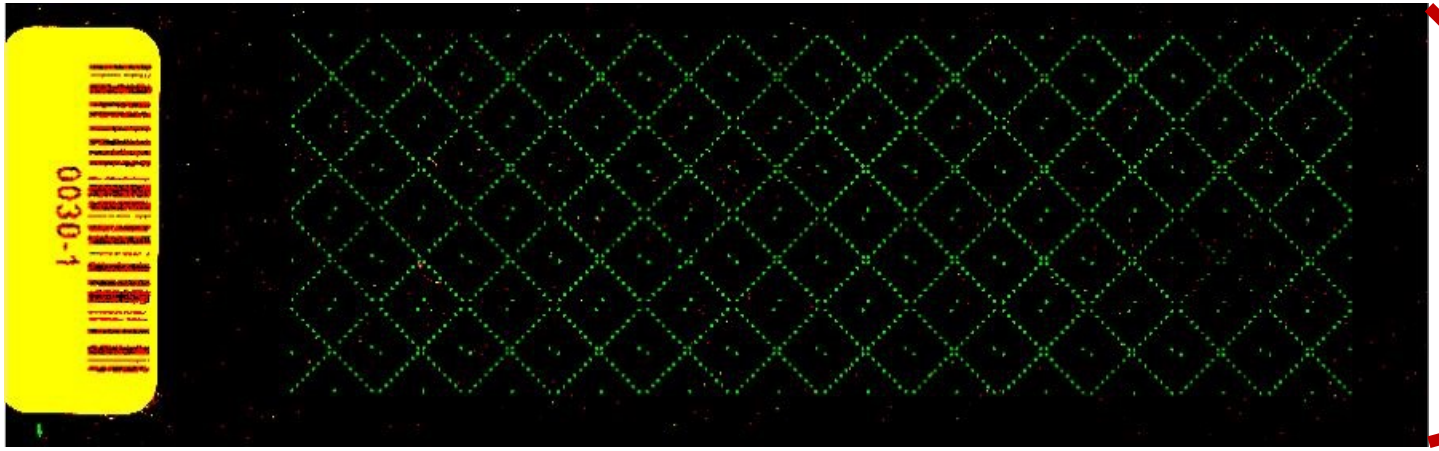
- Gamma-aminopropylsilane (GAPS) slide coated with polyethylene glycol (PEG) spacer
- PEG coupled to 1,6-diisocyanatohexane to generate isocyanate-functionalized slide
- Isocyanate able to react with nucleophilic functional groups

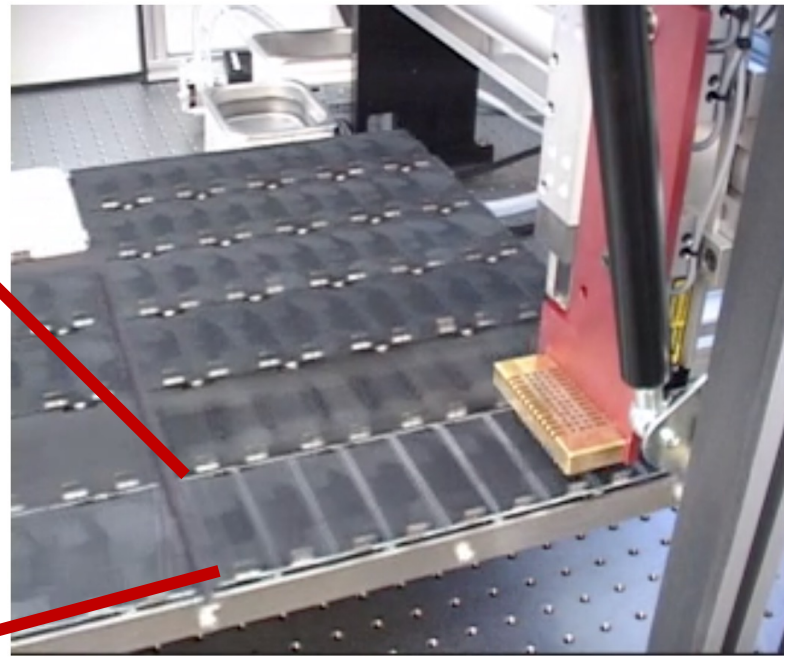


Leifer et al. Methods in Enzymology. 2018.

How are SMM slides printed?

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

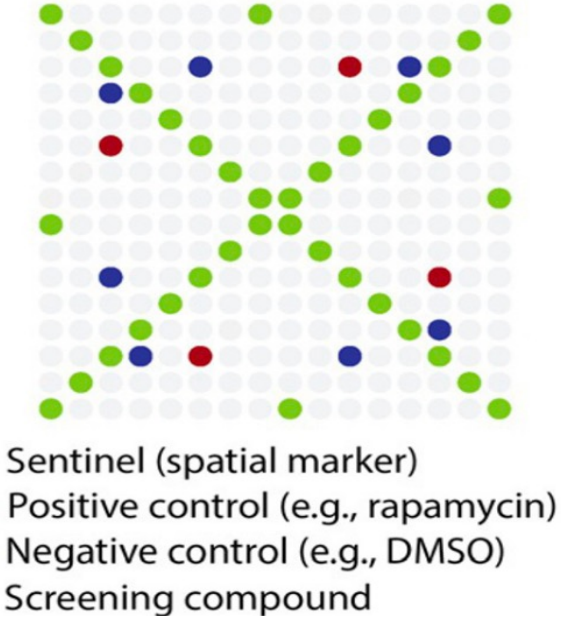


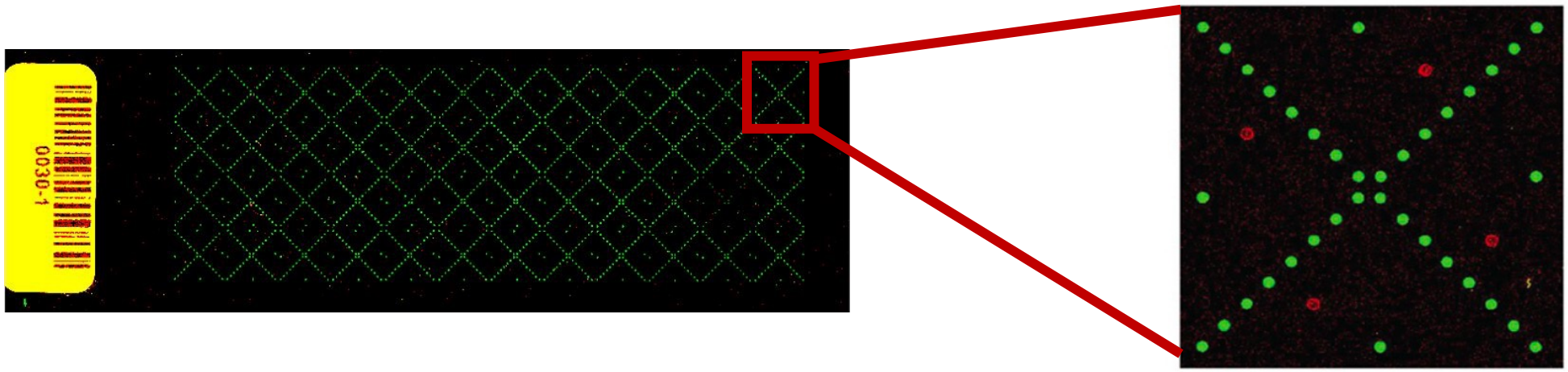


Koehler Lab 2014 - Small-molecule Microarrays from Koehler Lab on Vimeo.

SMM slide layout

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

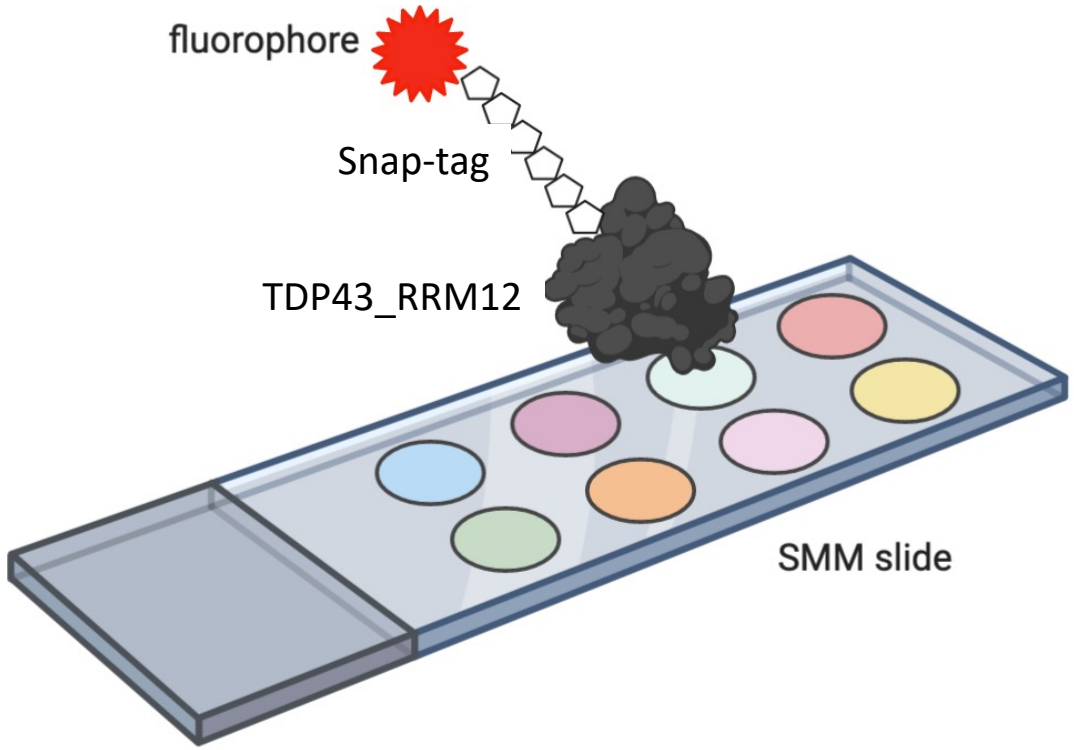




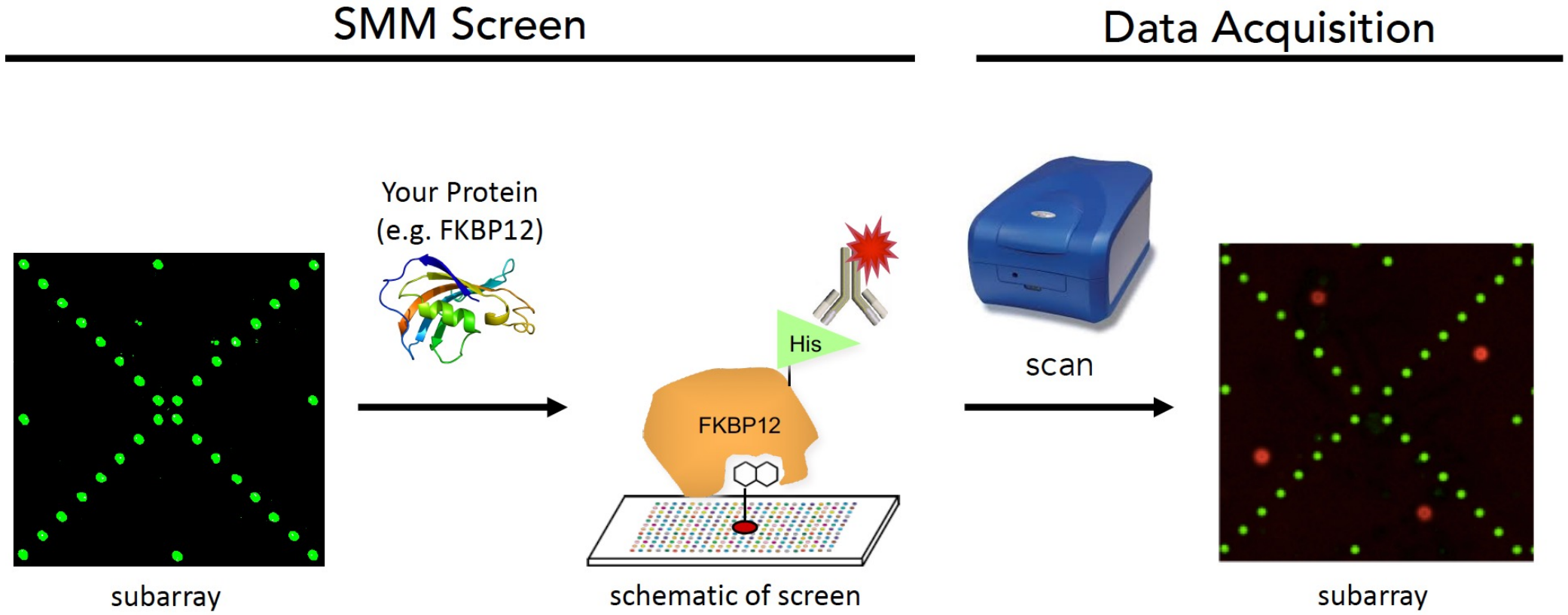
Leifer et al. Methods in Enzymology. 2018.

How will we screen for small molecules that bind TDP43_RRM12?

- Incubation SMM slide with 647-labeled TDP43_RRM12
- Wash away excess protein
- Store SMM slide for screening



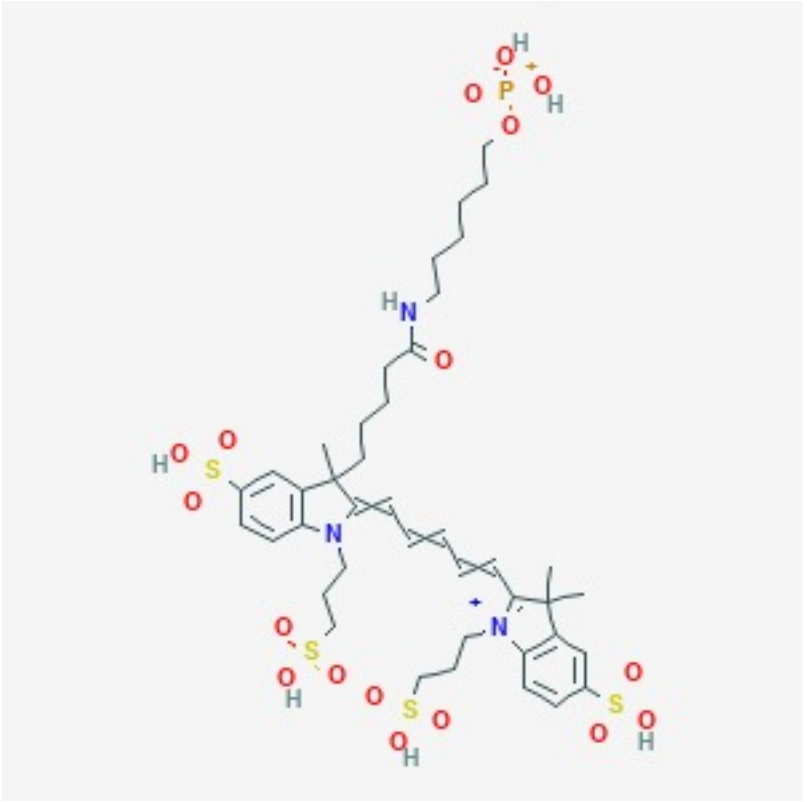
SMM workflow



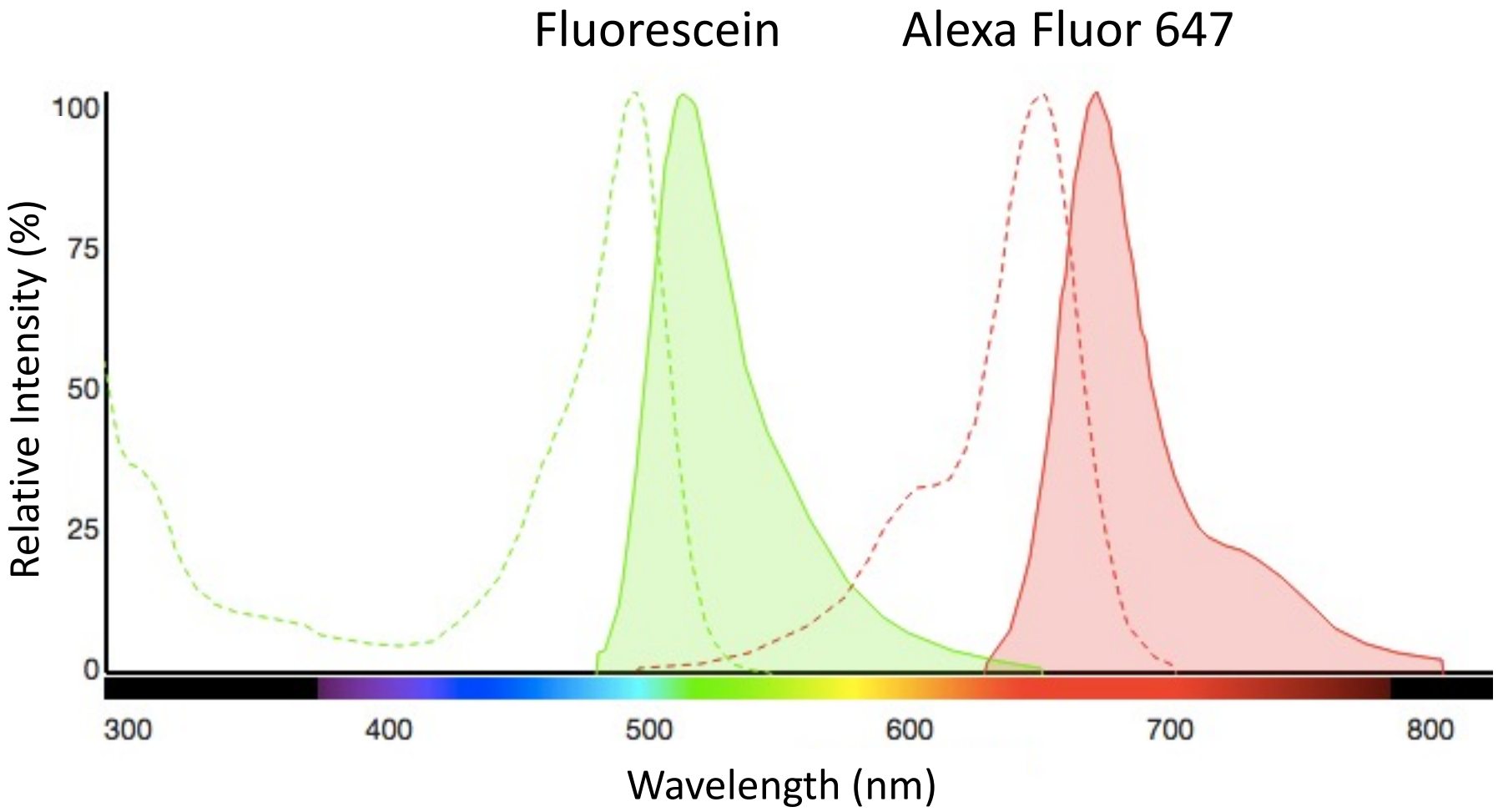
B. Leifer & S. Doyle

Alexa fluor 647 used to visualize ‘hits’

- Associates at high molar ratios without self quenching
  - Enables high sensitivity
- pH-insensitive over a wide molar range
- Has high fluorescence quantum yield and high photostability
  - Allows detection of low-abundance targets
- Remains active after excitation



Can we effectively identify sentinel spots vs TDP43_RRM12 binding to SMs?



For Today

- Work through SMM staining and visualization on wiki
- Review assigned article with lab partner
- Optional: Edit JC homework slide based on Comm Lab information. Due by 10pm
  - Points assigned for turning in the original

For M2D4

- Write a methods section for M2D1-M2D3

Helpful notes for methods…

- The generation and features of TDP43_RRM12 plasmid does not need to be described
- The purification process and tests for purity and concentration should be described
- The SMM scan does not need to be described in detail

Gift methods for SMM scan

- TOPIC SENTENCE. The SMM slides (a gift from the Koehler lab) were imaged using a Genepix 4300 microarray scanner (from Molecular Devices). TDP-43_RRM12 was identified at 635nm and fluorescein sentinel spots were indicated by fluorescence at 532nm. A preview scan was performed to optimize the PMT for the 635 nm emission. The optimal PMT value was used to perform full scans to generate images of the slides.

(Not for inclusion in this homework, but helpful for the future…)

The analysis of images to quantify the intensity and position of the 635nm signal associated with putative small molecule binders was performed using Python code integrated in a Jupyter notebook (a gift from Rob Wilson, Koehler Laboratory). The signal-to-noise ratio (SNR) from each compound was used to calculate the robust Z-scores for each compound averaged over replicates, and the “hits” were identified as compounds with Z-score > 5.