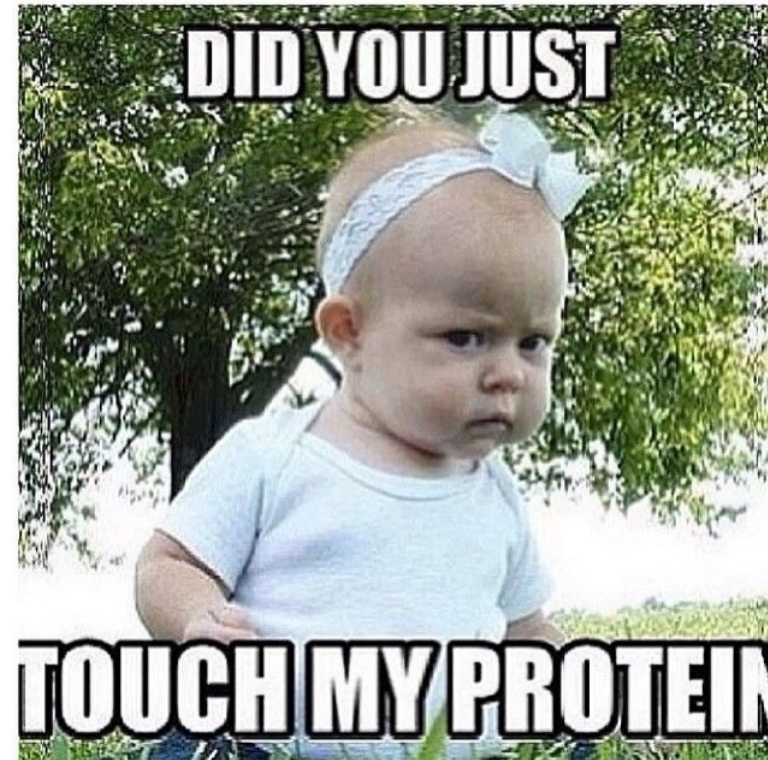


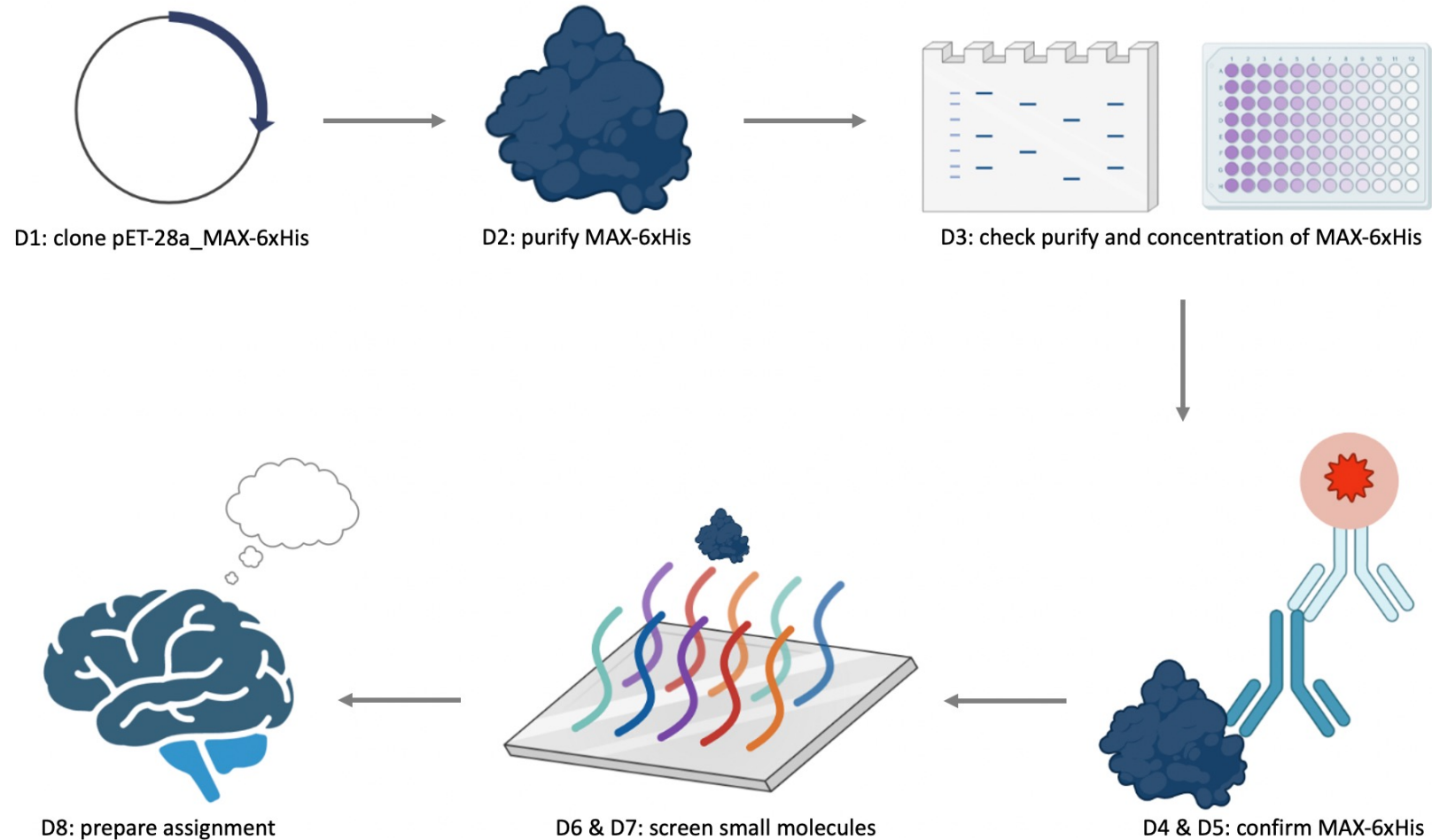
M1D6:

Prepare and scan small molecule microarray (SMM) slides

1. Prelab discussion
2. Complete western blot experiment
3. Incubate MAX-6xHis with SMM slides

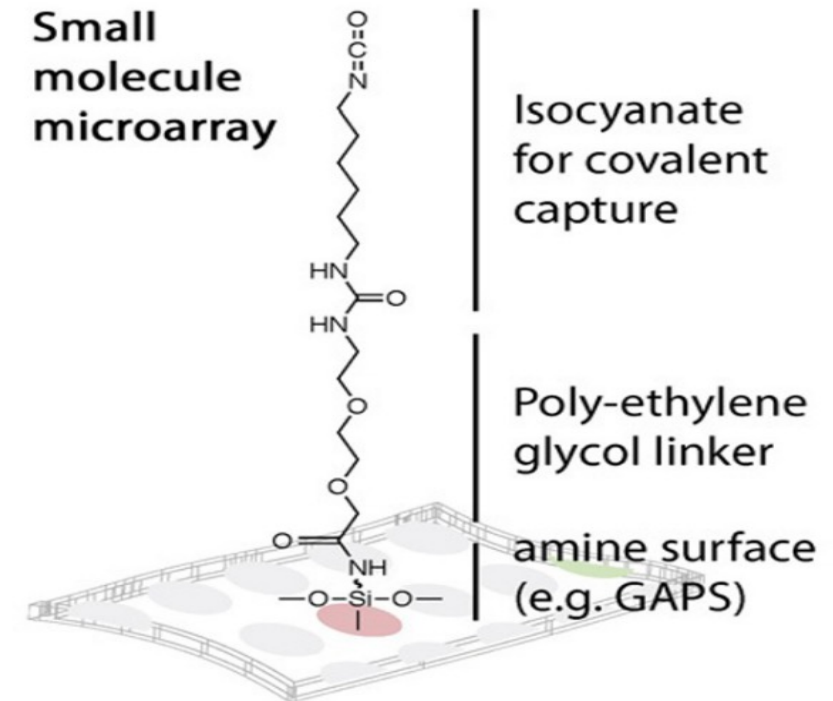


Overview of Mod 1 experiments:



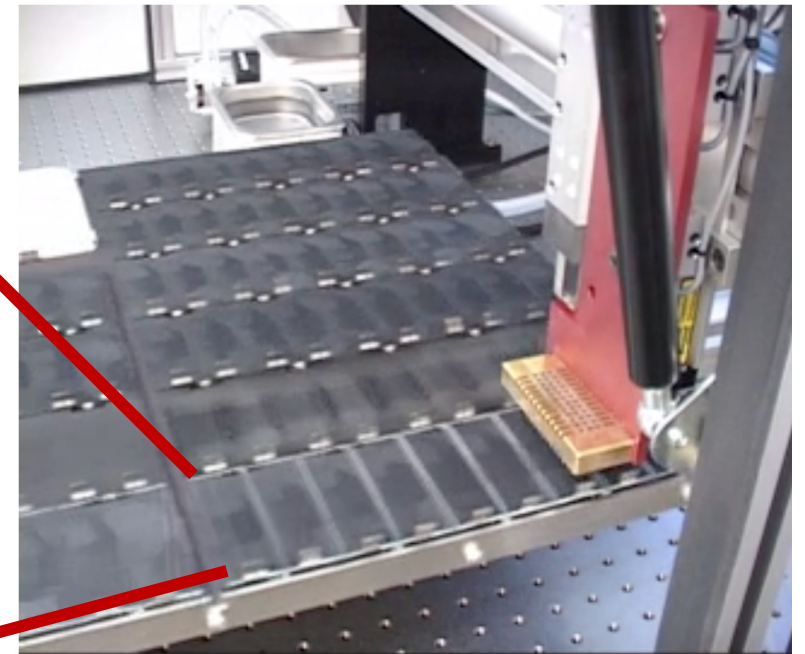
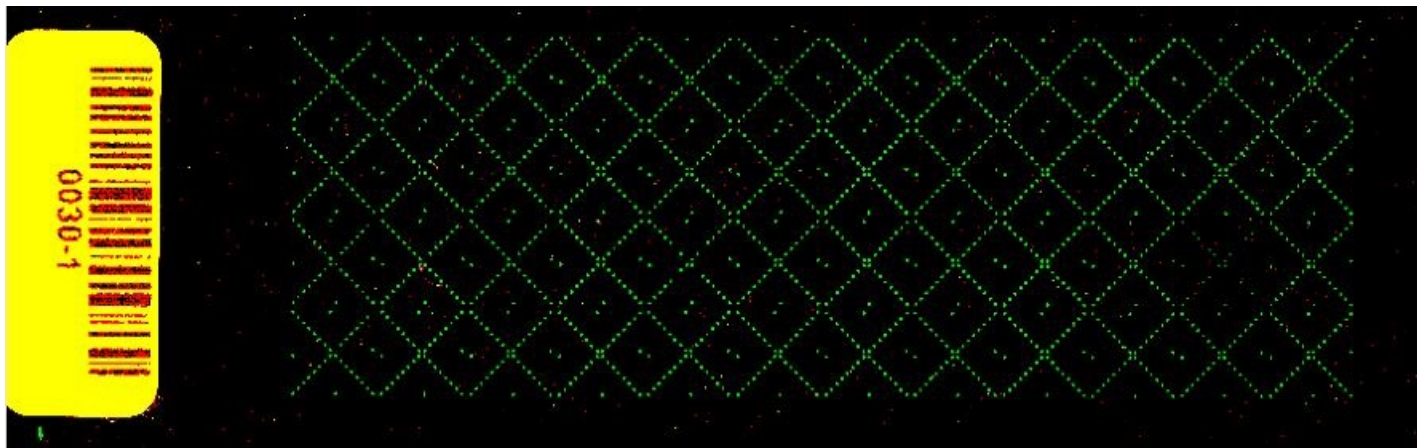
How are SMM slides prepared?

- 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



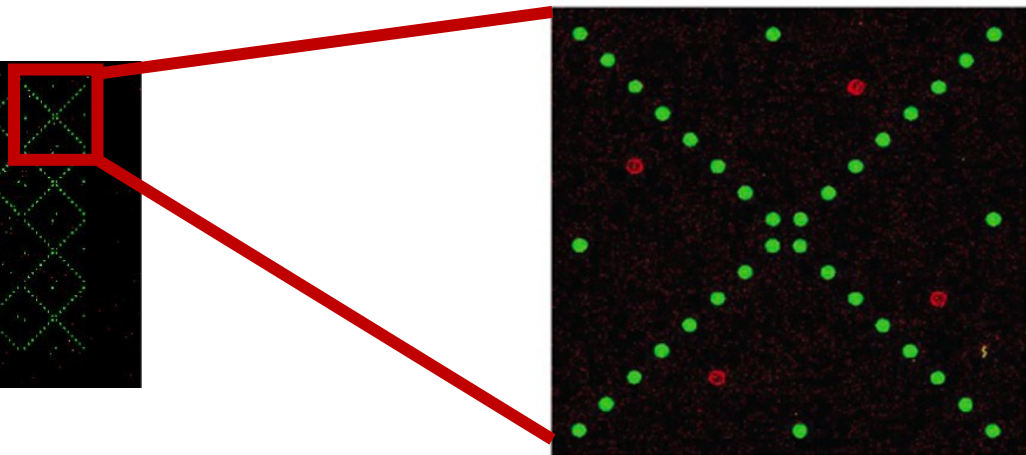
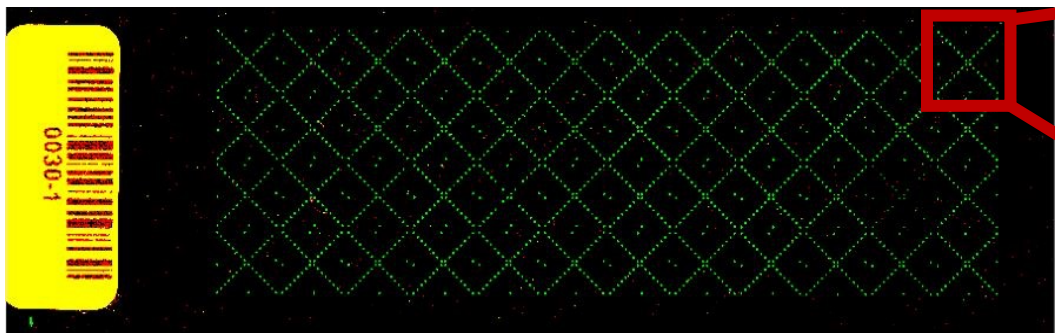
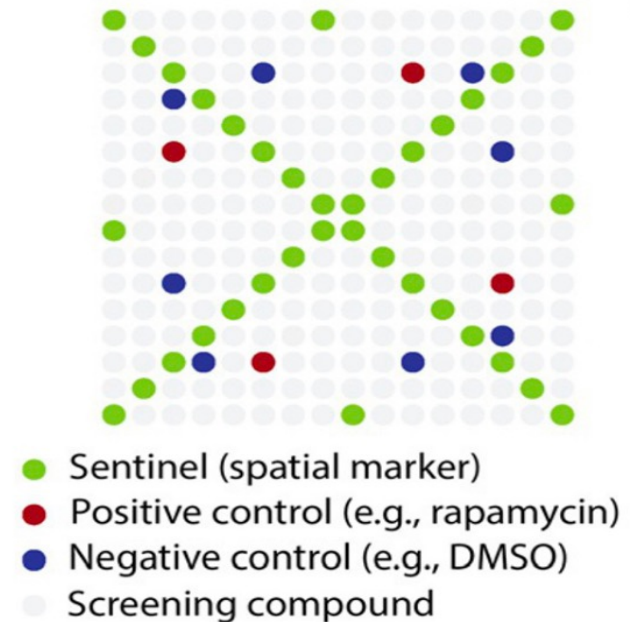
How are SMM slides printed?

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



SMM slide layout

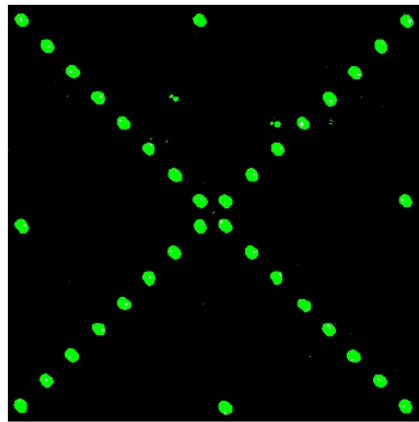
- Sentinel spots used for alignment during imaging / data analysis
- Control spots used to validate results
 - Negative control = DMSO
 - Positive control = MS2



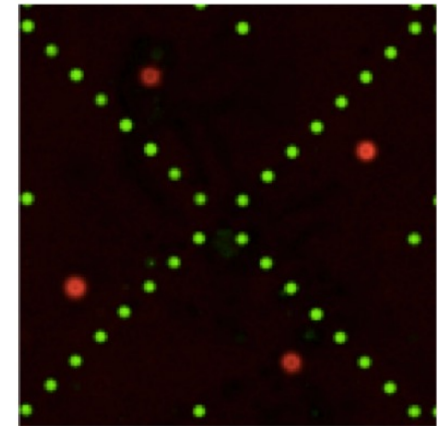
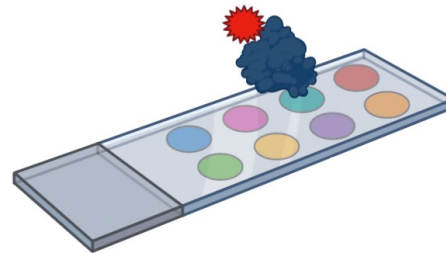
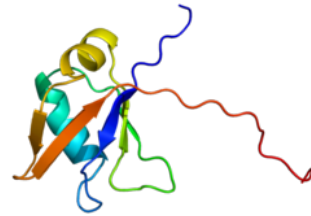
Workflow for SMM experiment

SMM Screen

Data Acquisition



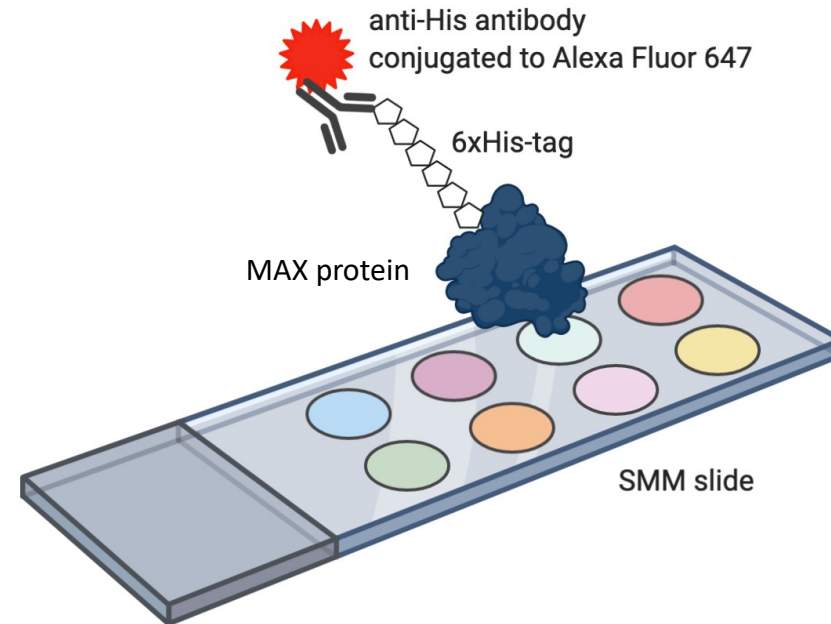
subarray



subarray

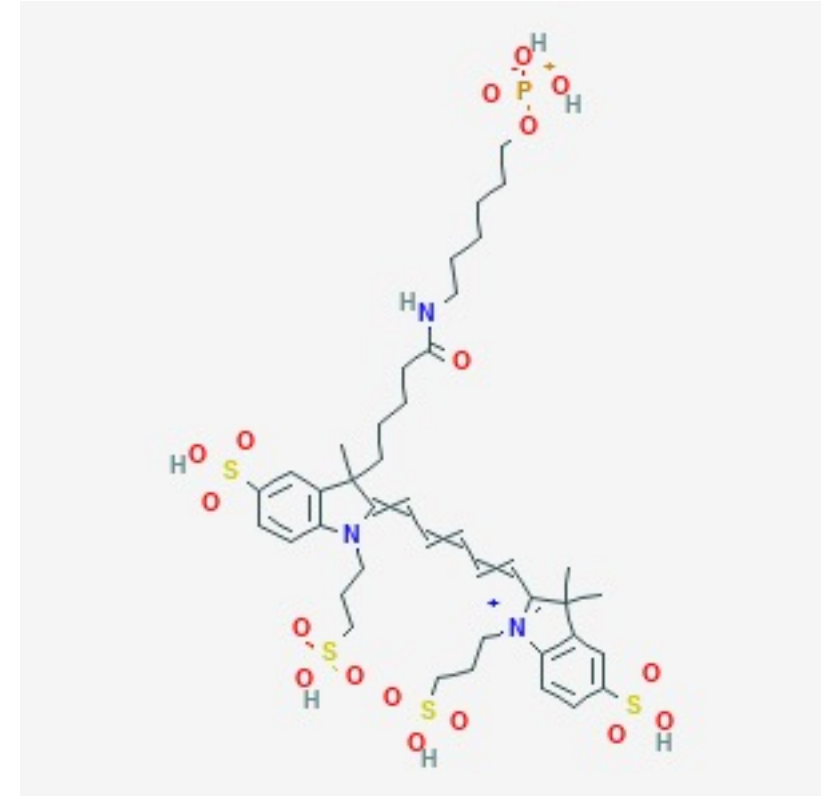
How will we screen for small molecules that bind TDP43-RRM12?

- Incubation SMM slide with protein of interest
- Wash away excess protein
- Store SMM slide for screening



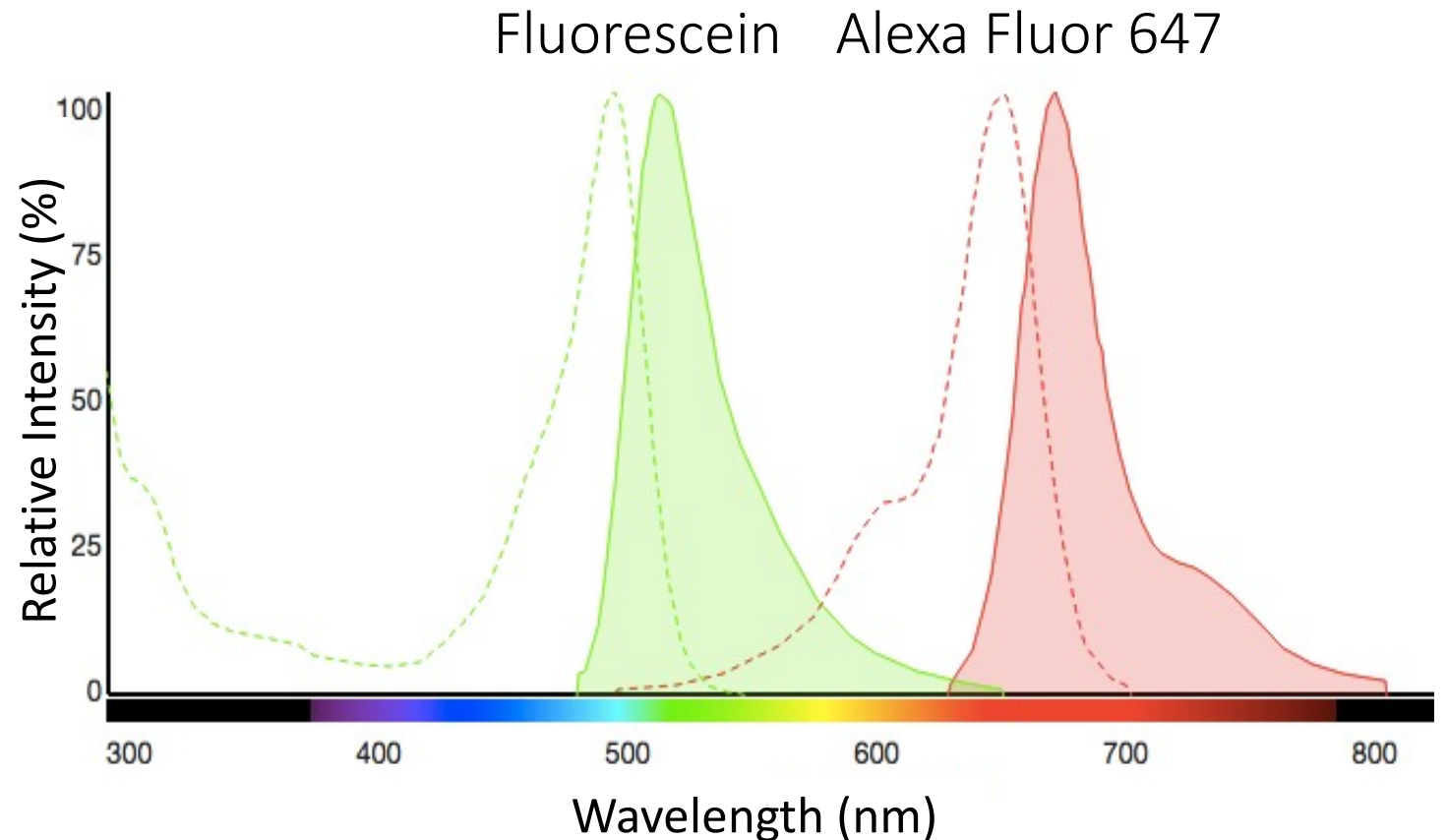
Alexa Fluor 647 used to visualize 'hits'

- 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



Considerations when using two fluorescent signals for data collection

- Can the signals be differentiated?
- Do the emission / excitation overlap?



For today...

- Divide experiments between laboratory partners

For M1D7...

- **With your laboratory partner**, complete data slide for the protein purity and concentration experiments (started on M1D5!)
- Craft experimental schematic for protein purification protocol

Notes on experimental schematics...

How to cross-stitch.



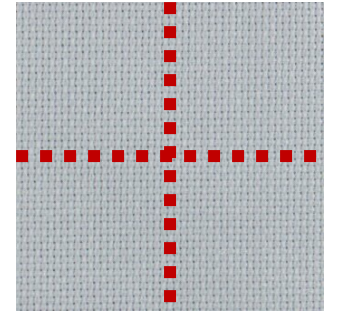
search Etsy for pattern



gather thread colors from my collection



buy needed thread colors from Michael's



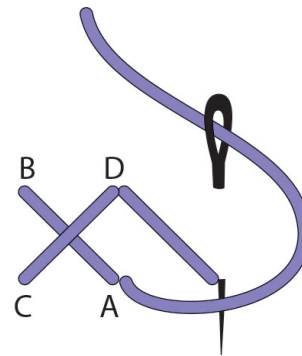
prepare aida fabric by finding the center



complete pattern and frame finished picture!



miscount, remove, and correct stitches



follow the pattern to create a picture using x-stitches



secure fabric in embroidery hoop

