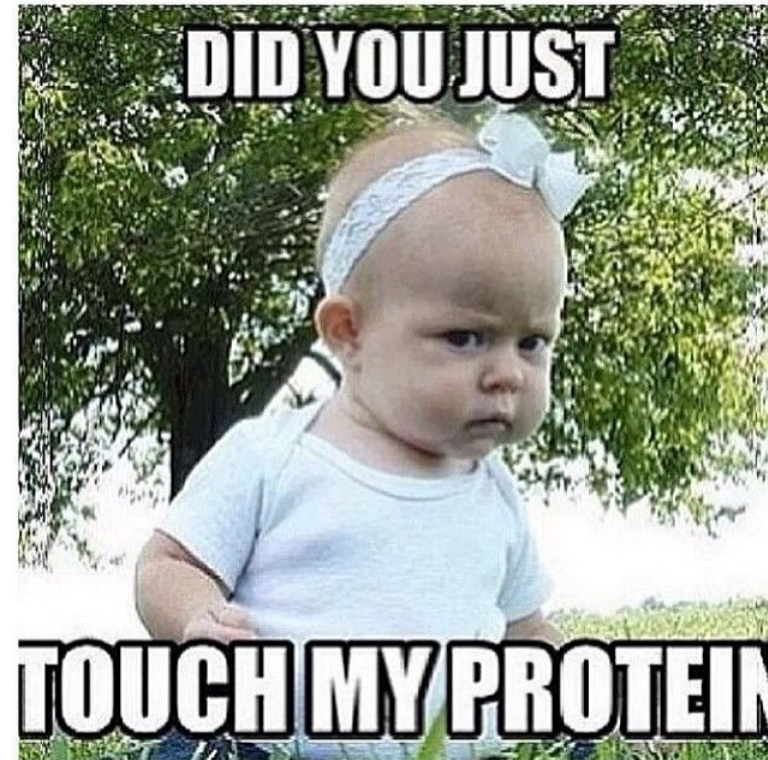


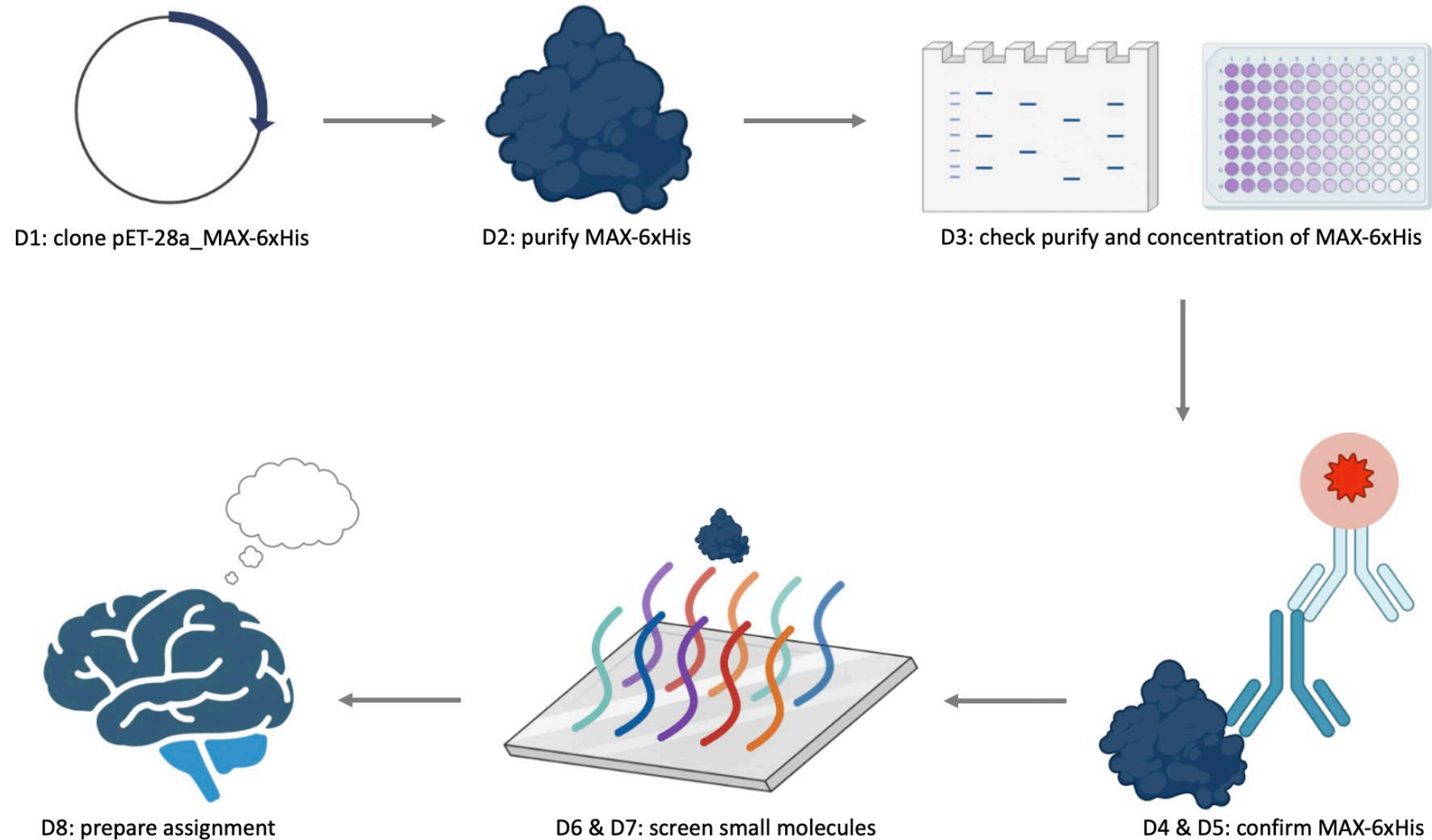
M1D6:

Prepare and scan small molecule microarray (SMM) slides

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

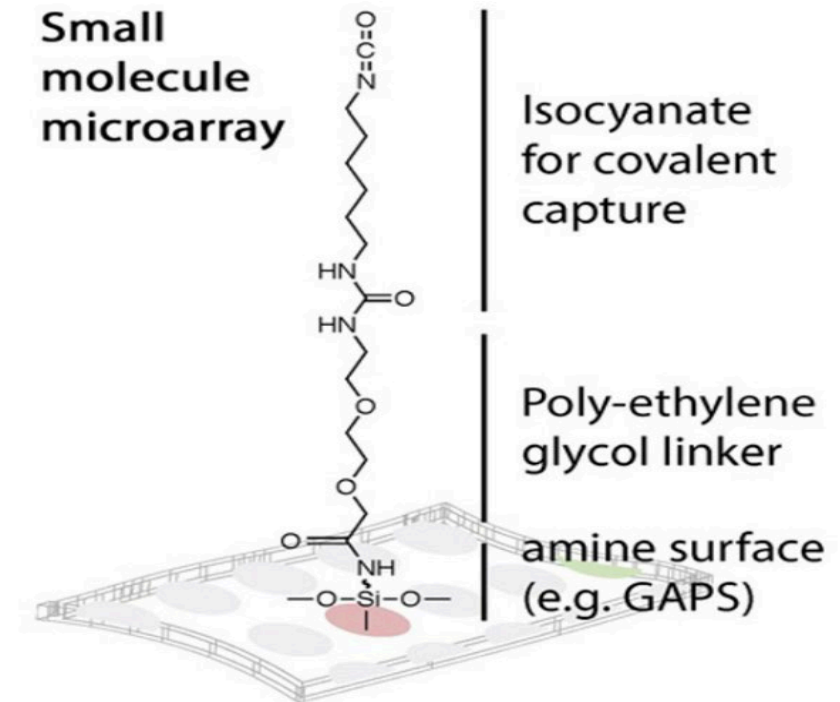


Overview of Mod 1 experiments:



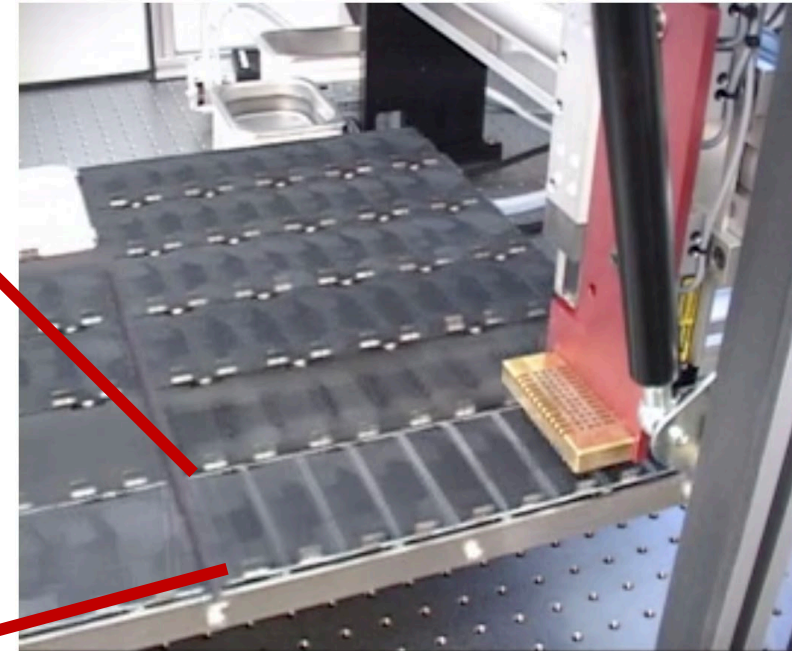
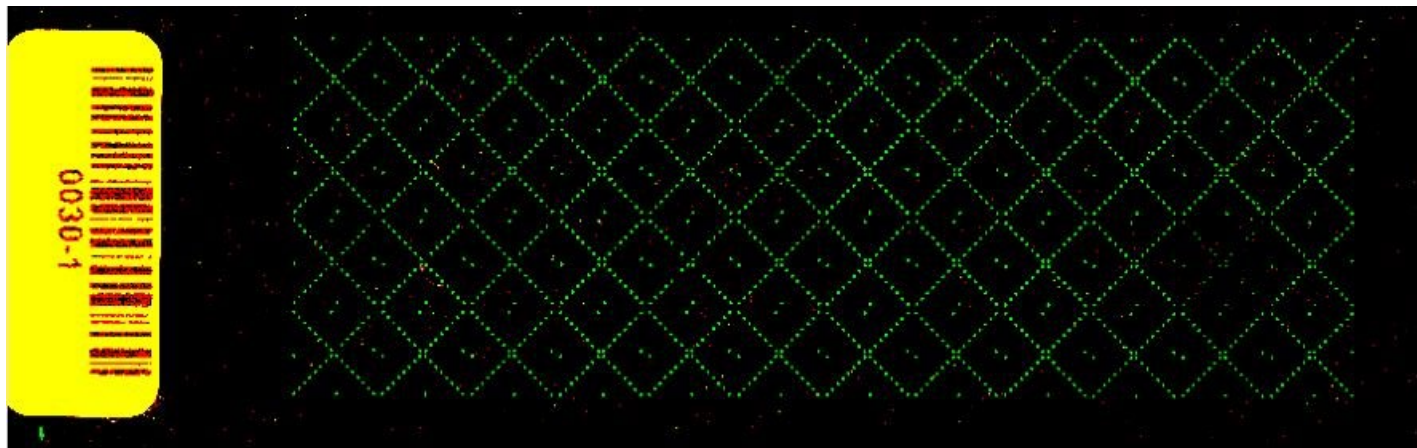
How are SMM slides prepared?

- 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



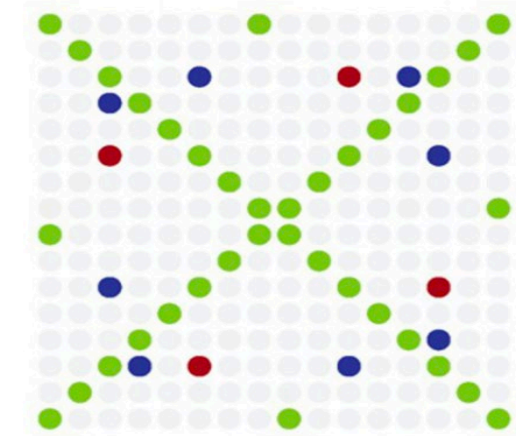
How are SMM slides printed?

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

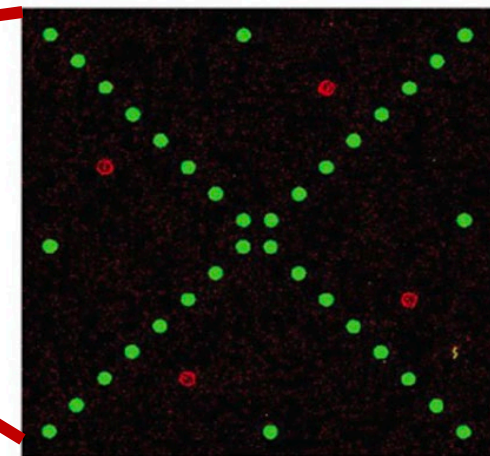
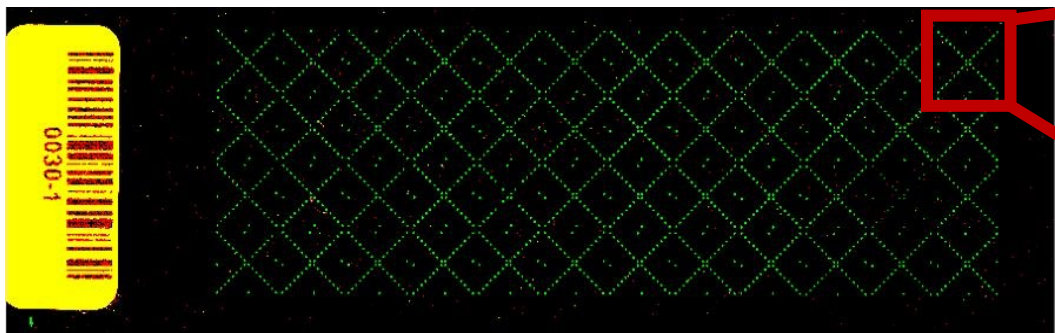


SMM slide layout

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



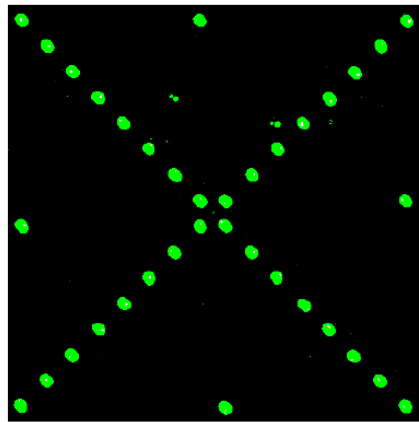
- Sentinel (spatial marker)
- Positive control (e.g., rapamycin)
- Negative control (e.g., DMSO)
- Screening compound



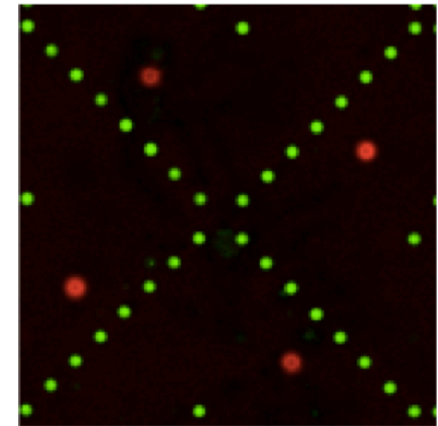
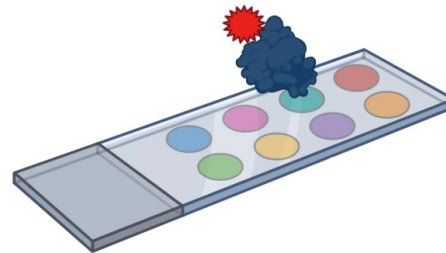
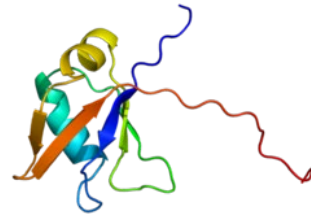
Workflow for SMM experiment

SMM Screen

Data Acquisition



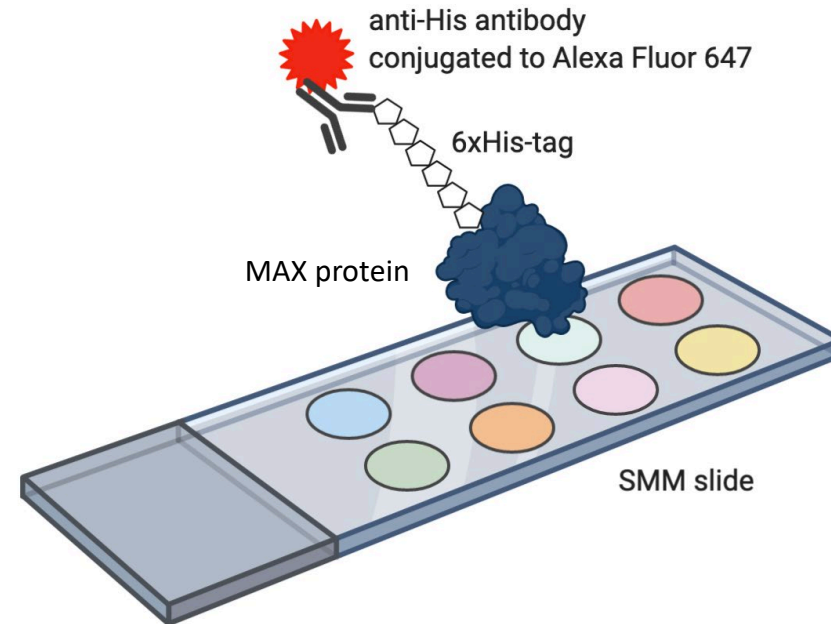
subarray



subarray

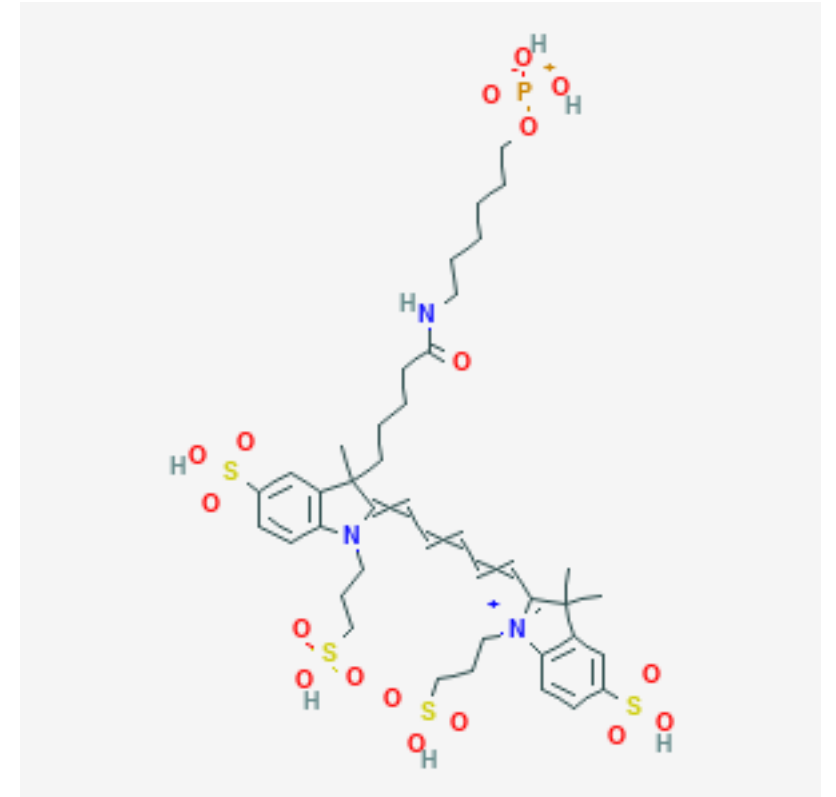
How will we screen for small molecules that bind MAX-6xHIS?

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



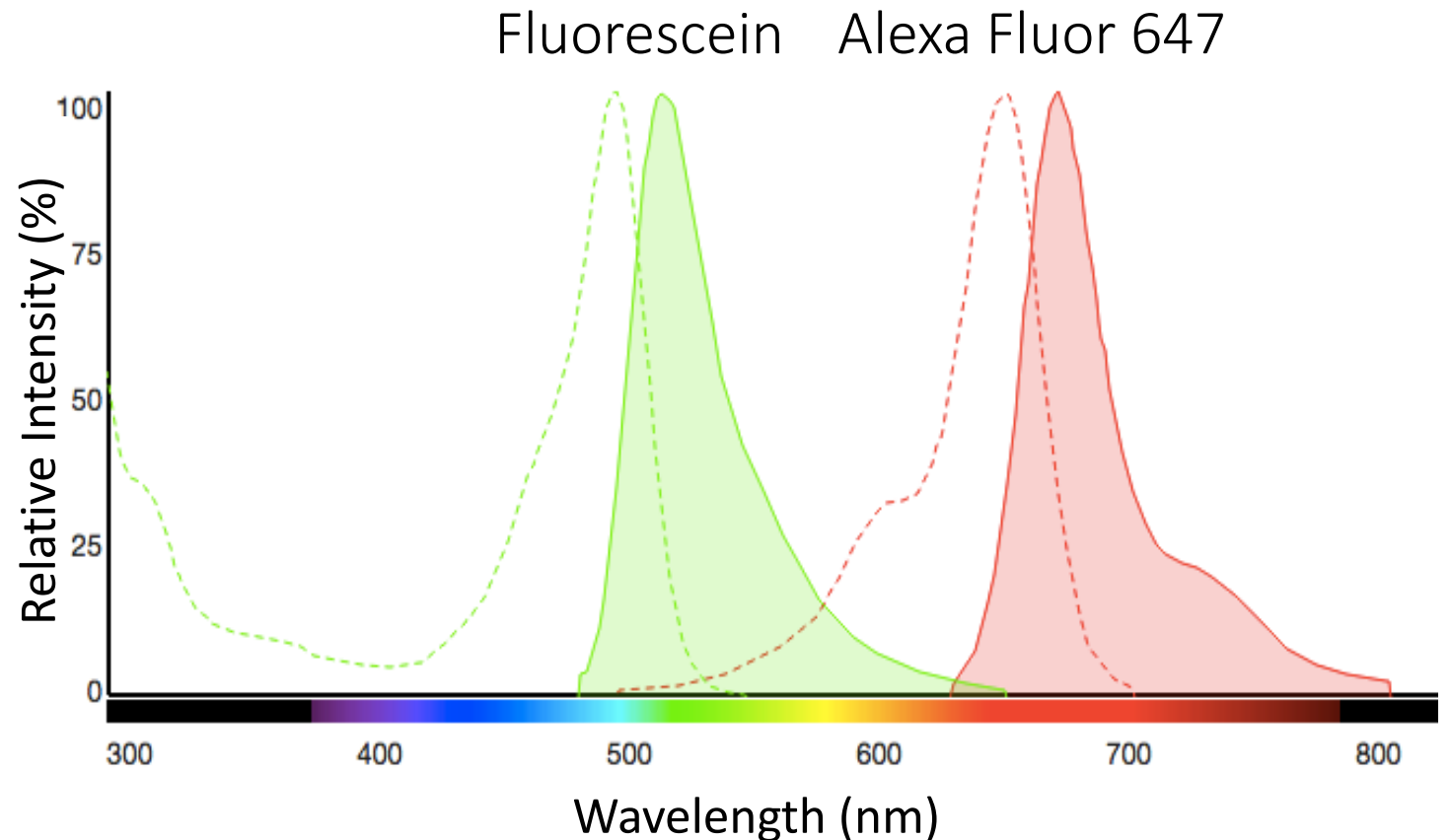
Alexa Fluor 647 used to visualize 'hits'

- 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...

- Set up the SMM

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

THE GREAT BRITISH BAKE OFF



TECHNICAL CHALLENGE
LIST



Notes on experimental schematics

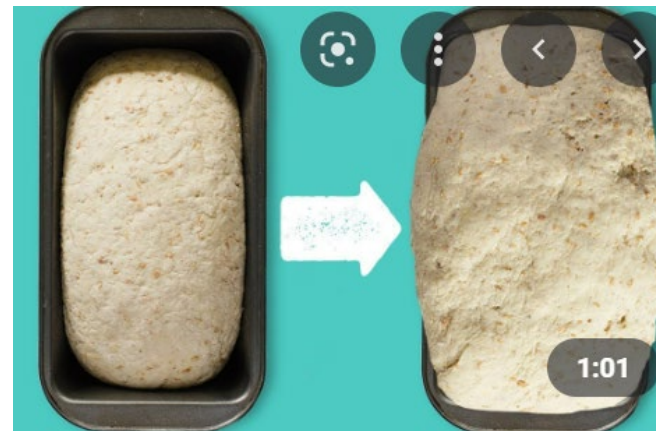
Research Question: What is the ideal flour hydration ratio for the best baguette?



Combine 1000 g All purpose flour, 1 tsp yeast, 1 tsp salt in a large mixing bowl

Add either 300g, 500g or 700g of warm water (37C)

Mix the dough in the mixing bowl, either by hand or using a rubber spatula



Turn the dough over onto a clean work surface and knead by pushing and folding the dough onto itself, creating gluten strands until smooth and elastic

Allow your dough to prove by returning it to the mixing bowl, covering with a wet teacloth at room temperature until the dough has doubled in size

Notes on experimental schematics

Research Question: What is the ideal flour hydration ratio for the best baguette?



Combine Flour, yeast, salt & varying volumes of water into a dough and knead until smooth & elastic



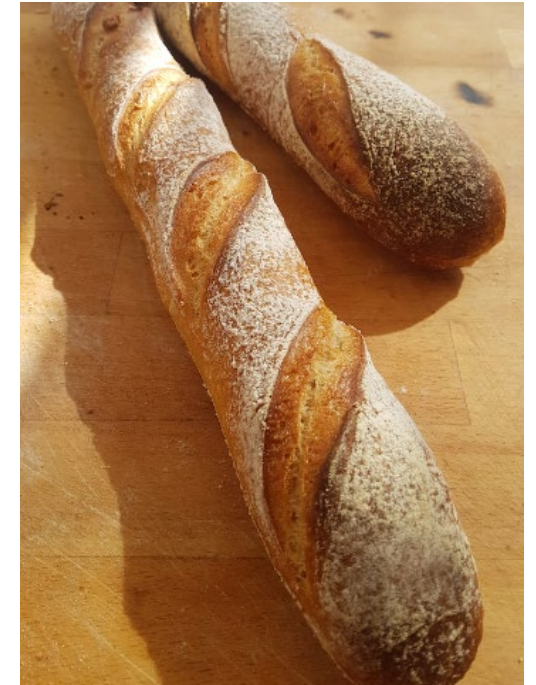
Proof until doubled in size



Deflate the dough and shape into a long rectangle, let proof until doubled in size



Score the dough



Bake until a deep golden brown

Notes on experimental schematics...

How to cross-stitch.



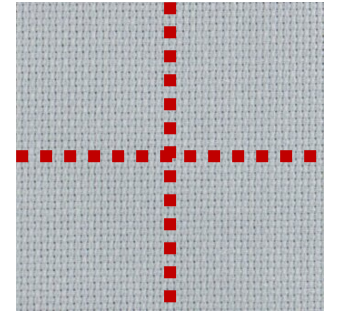
search Etsy for pattern



gather thread colors from my collection



buy needed thread colors from Michael's



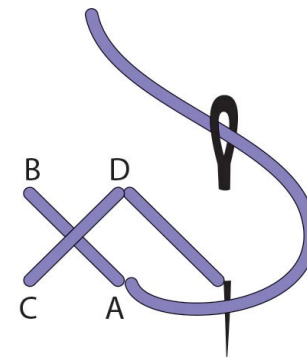
prepare aida fabric by finding the center



complete pattern and frame finished picture!



miscount, remove, and correct stitches



follow the pattern to create a picture using x-stitches

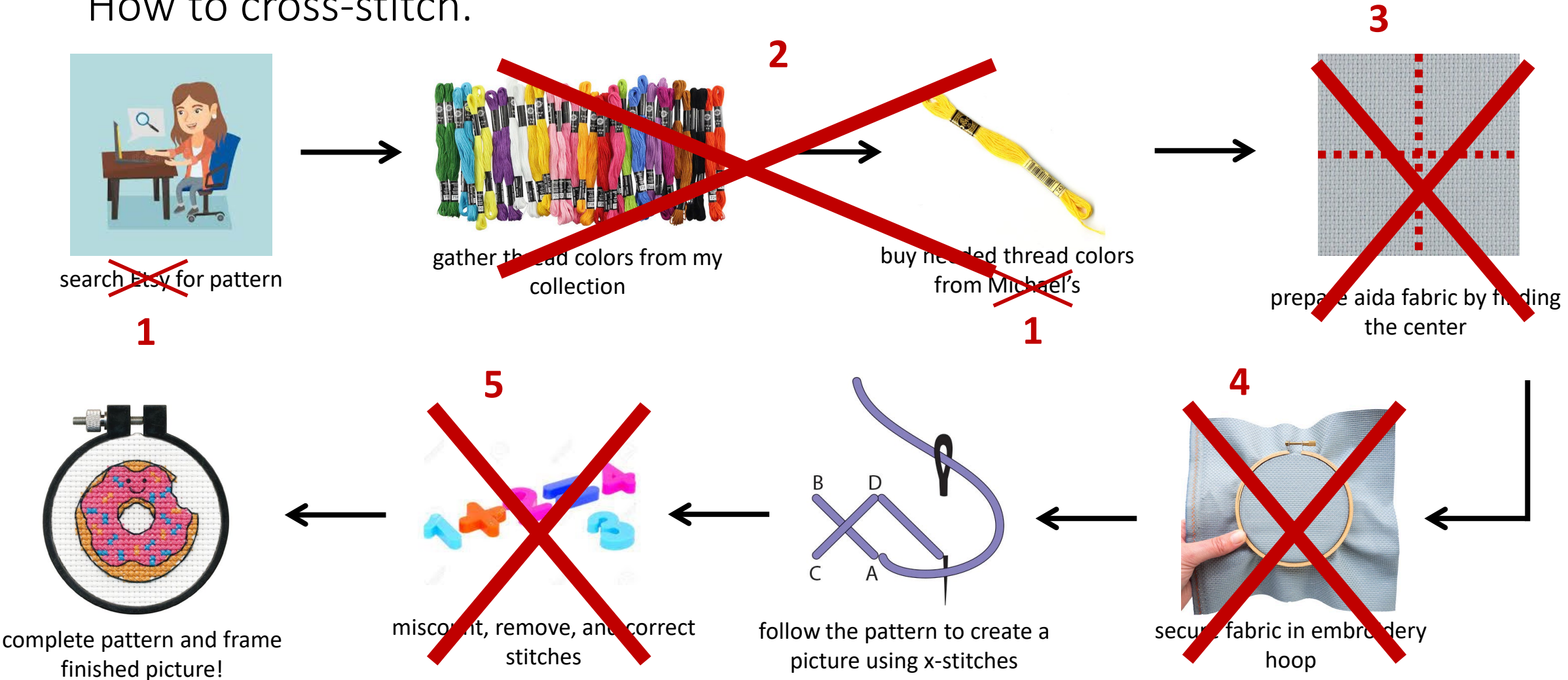


secure fabric in embroidery hoop



Notes on experimental schematics...

How to cross-stitch.



What should be in the Title and Caption?

Title: State what is shown / represented in the schematic

Caption:

- Explain the flow of information using concise / clear language
- Expand on text shown in figure labels to eliminate excess wordiness / clutter from the figure
- Define all abbreviations / jargon / labels / symbols

Notes on experimental schematics...

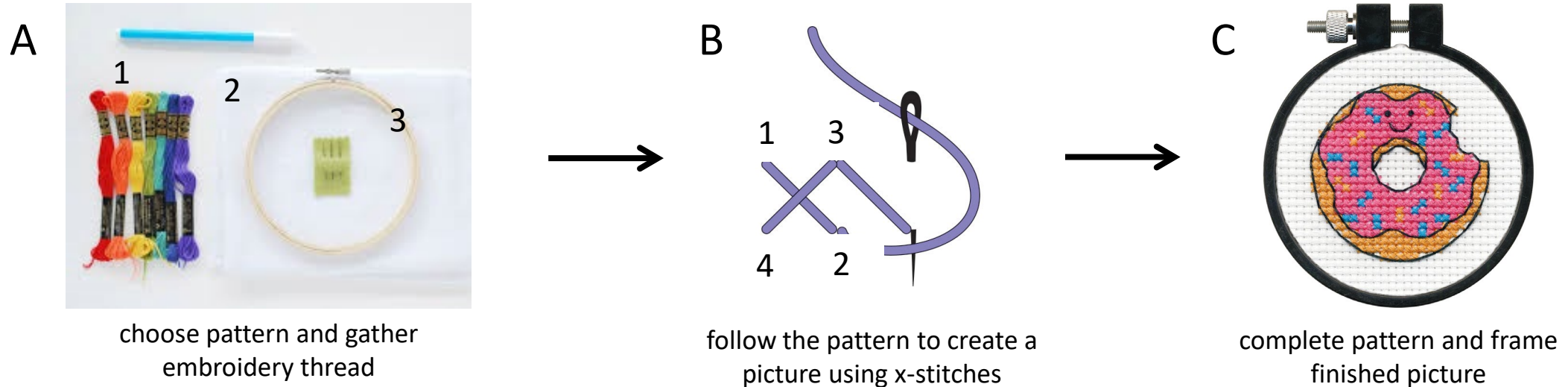


Figure 1: Noreen uses cross-stitching to embroider pictures. Cross-stitching is a method of embroidery that is used to create pictures from x-shaped stitches. (A) The supplies for cross-stitching include: 1. embroidery thread, 2. aida fabric, and 3. a hoop. (B) The x-shaped stitches are completed by using the strokes in the order indicated by the numbers. (C) ...