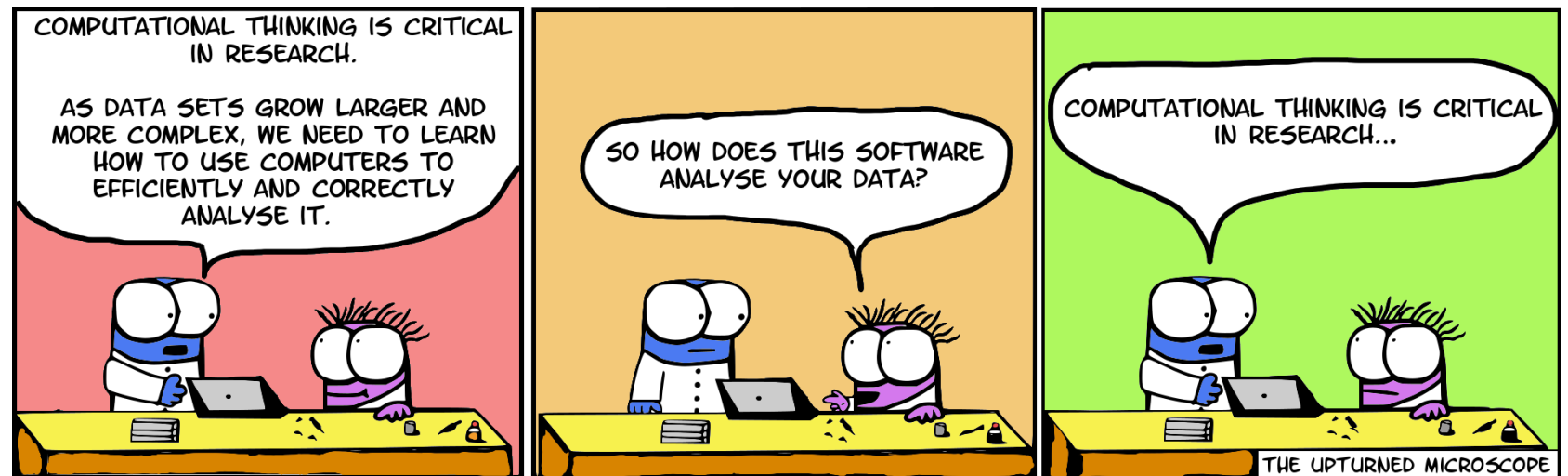


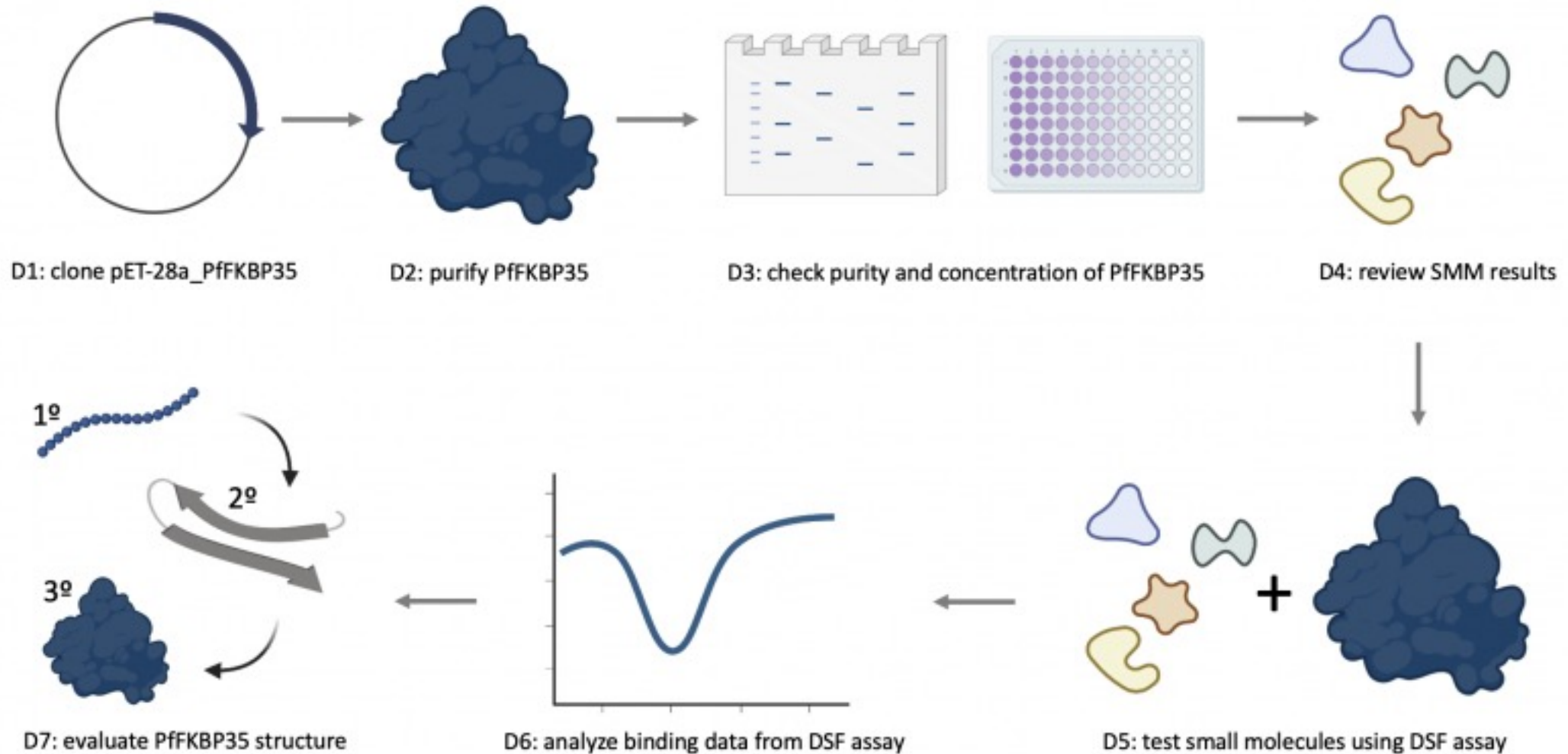
# M2D4: Review small molecule microarray (SMM) technology and data analysis

1. Comm Lab
2. Quiz
3. Prelab
4. Walk through SMM



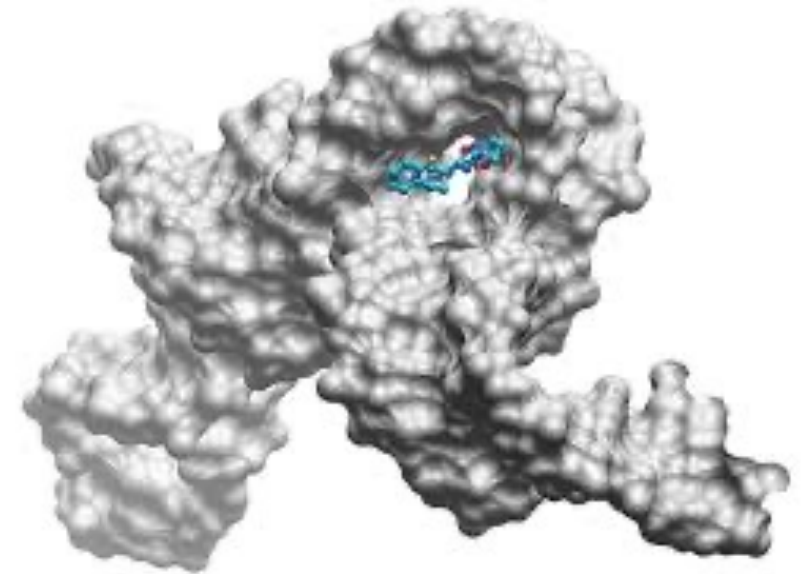
# Overview of M2: drug discovery

Research goal: Test small molecules for binding to the *Plasmodium falciparum* FKBP35 protein using a functional assay.

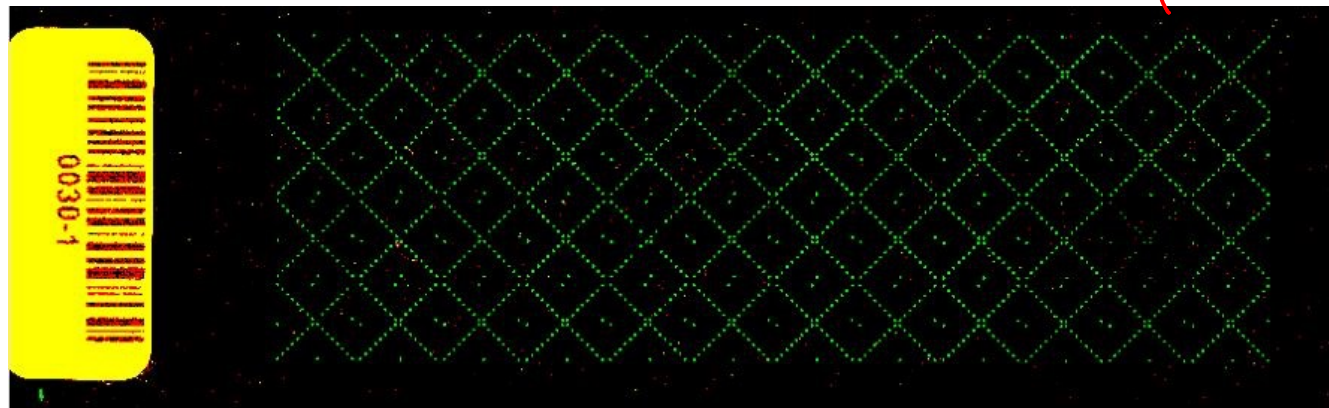
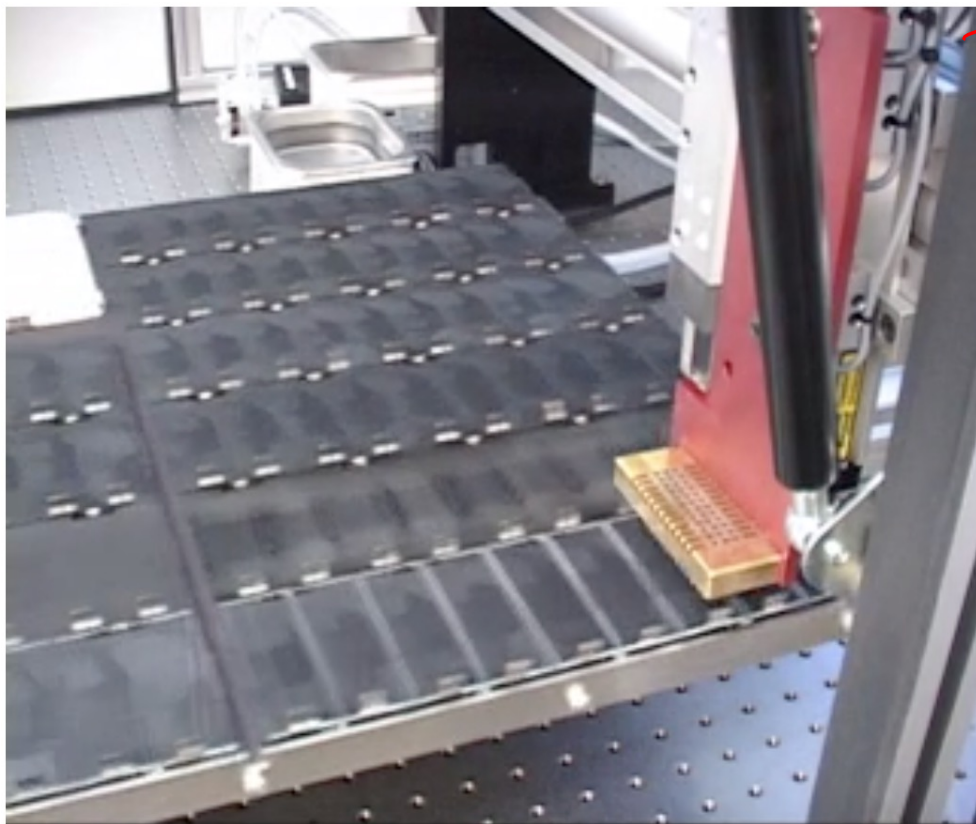


# Why are we discussing this approach?

- High throughput assays are useful in screening potential therapeutic targets
  - Allows unbiased exploration of potential therapeutics
  - Allows examination of targets with limited information
- Small molecules
  - Mw < 500 Da
  - Natural or synthetic
  - Frequently comprised of Carbon/Nitrogen/Oxygen
- Our small molecule library is based on FK506 (known binder)
  - Could also use the SMM to screen a broad library of compounds to cast a wide net



# Small Molecule Microarray (SMM)



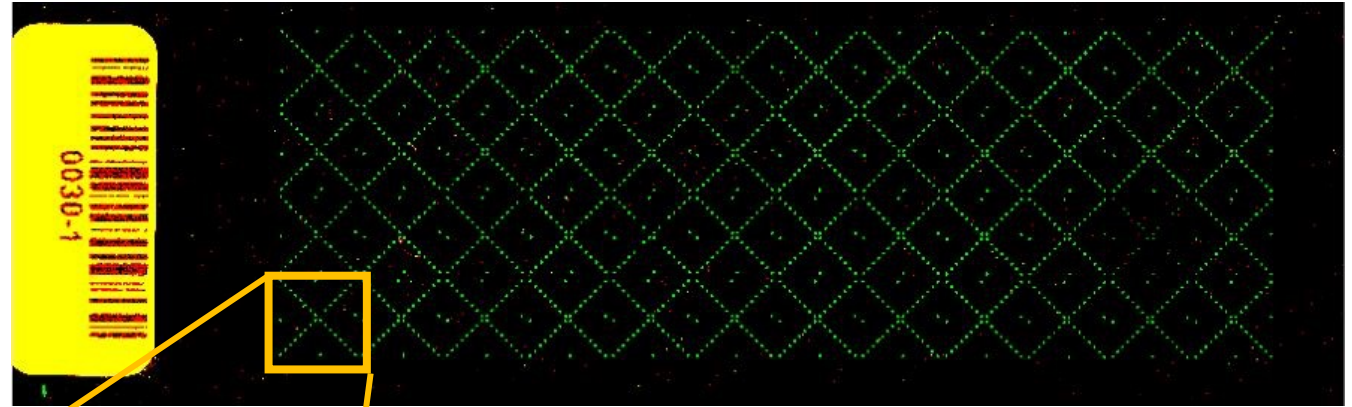
- Each slide contains ~12,000 spots
- ~4,200 small molecules / ligands (in duplicate = ~8,400)
- Fluorescein sentinel spots
- DMSO negative control spots



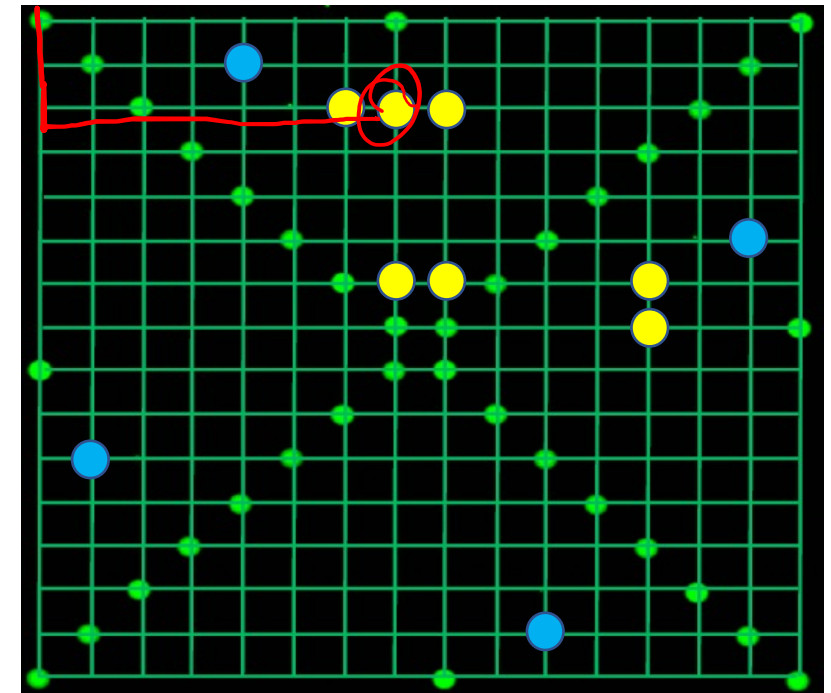
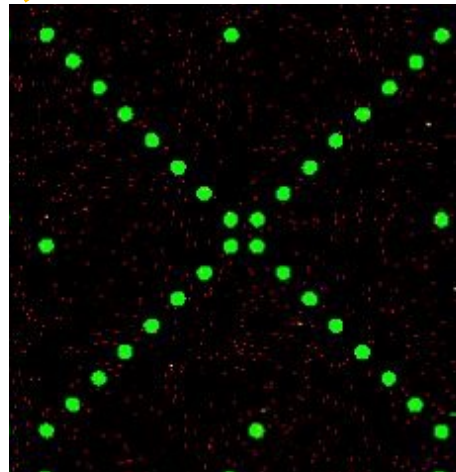
# Guide to the SMM slide

- 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

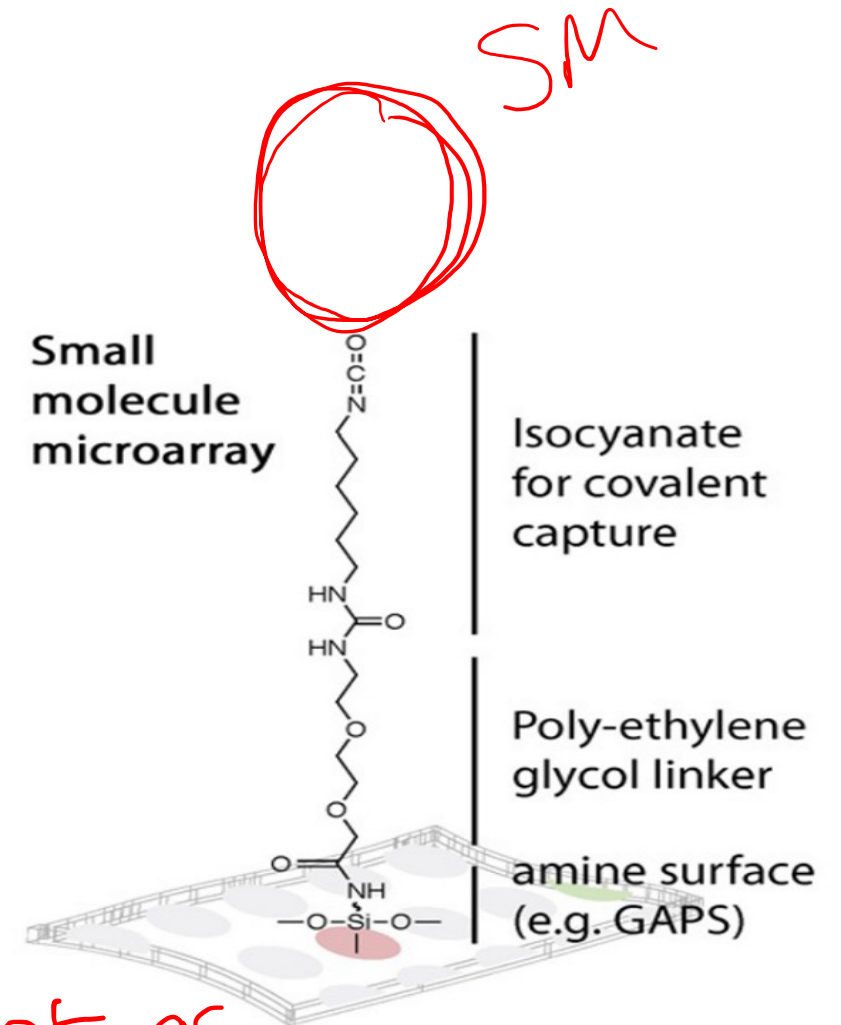


Green= sentinel spots  
(fluorescein dye)

Blue= DMSO  
Yellow= SM

# SMM slide preparation

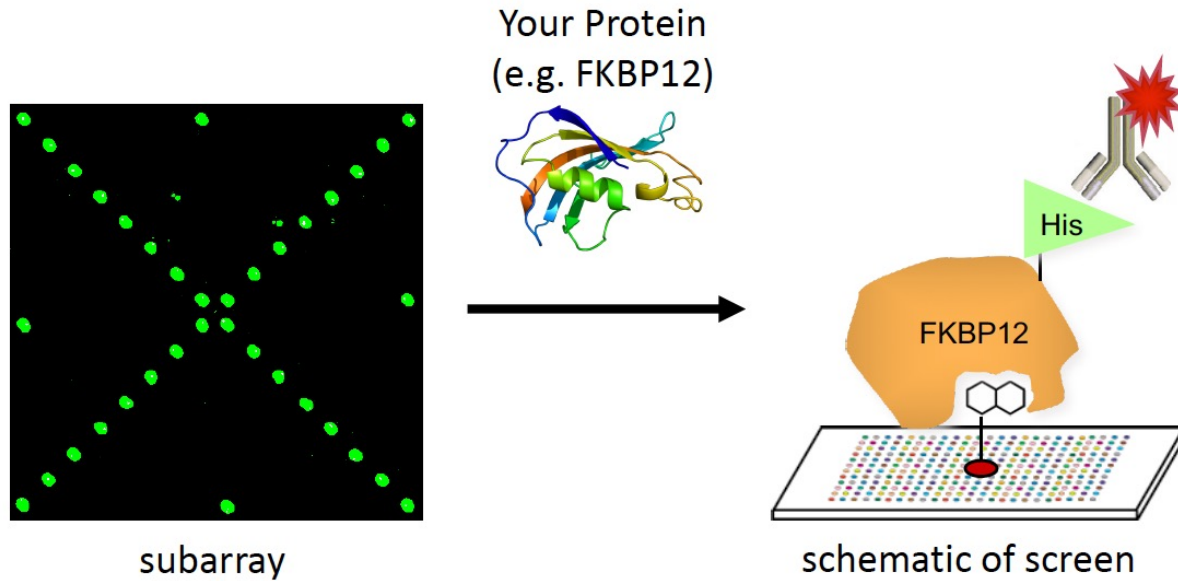
- Gamma-aminopropylsilane (GAPS) coated slide with polyethylene glycol (PEG) spacer
- PEG coupled to 1,6-diisocyanatohexane to generate isocyanate-functionalized slide
- Isocyanate able to react with nucleophilic functional groups



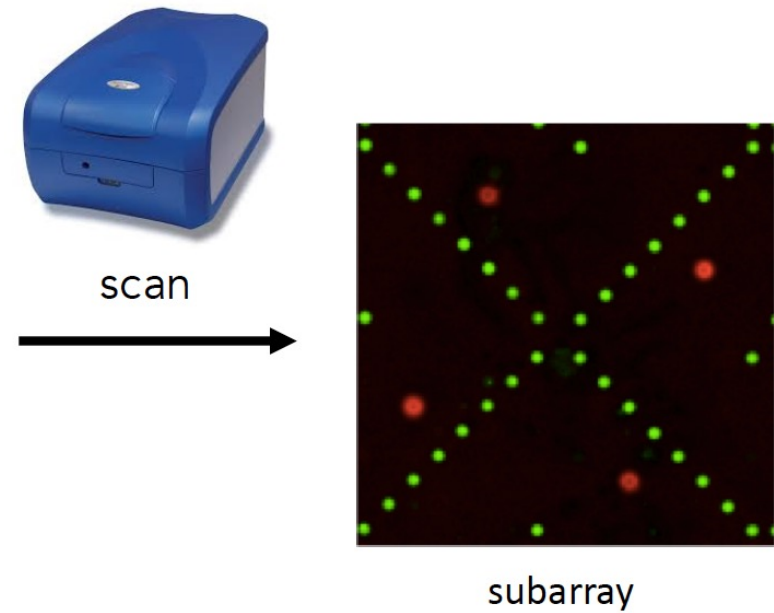
→ covalent bond not as  
strong bond / ~~not~~ specific

# SMM workflow

## SMM Screen

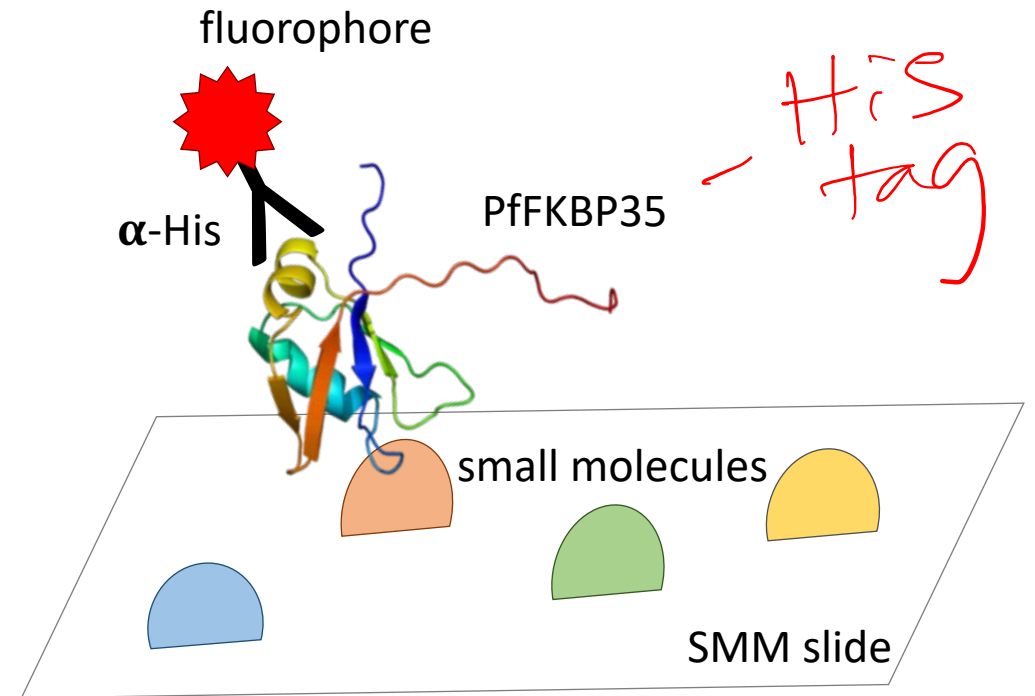


## Data Acquisition



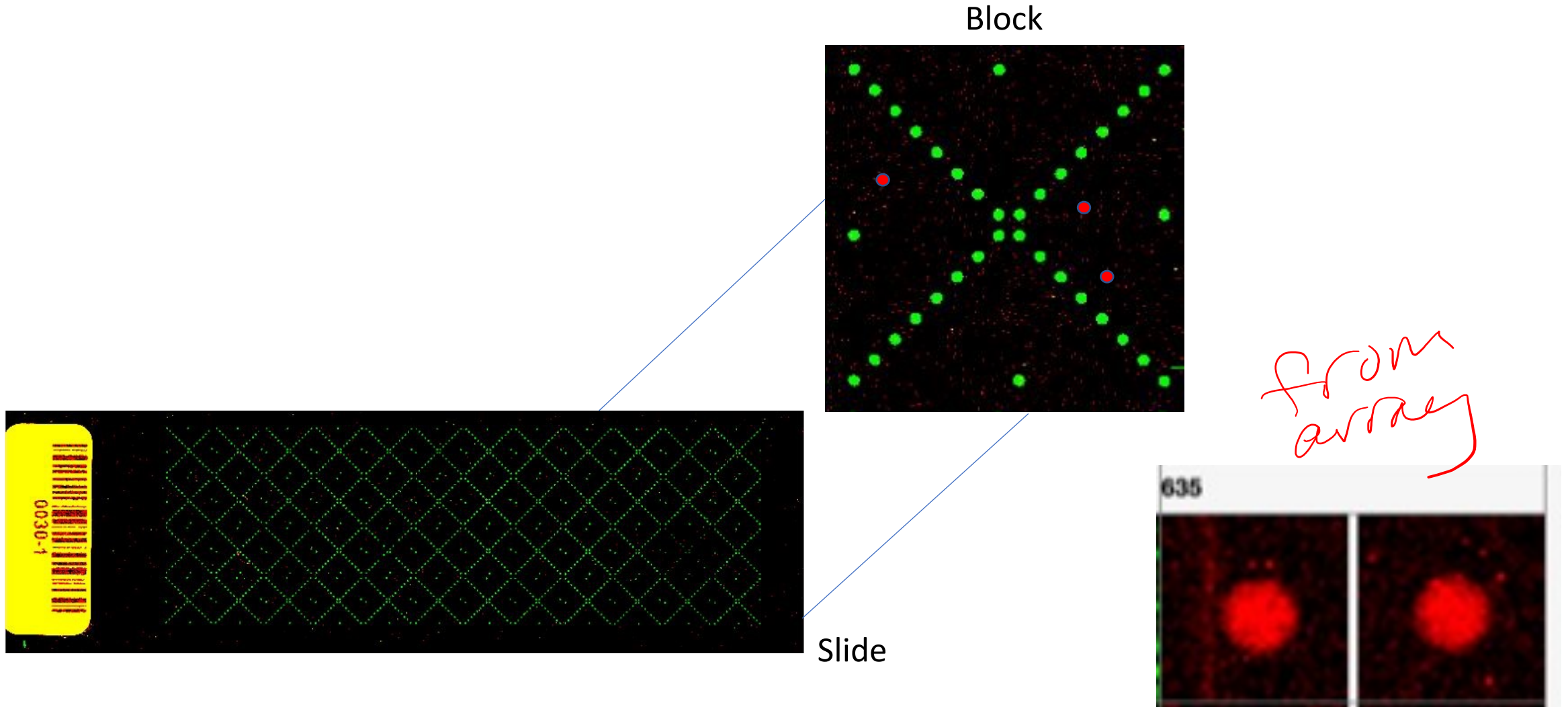
# How would we screen for ligands that bind PfFKBP35?

- Incubate the SMM slide with 3ml of our purified PfFKBP35
- Wash away unbound protein
- Incubate SMM slide with AlexaFluor 647 anti-His antibody
- Wash away excess antibody
- Store for scanning



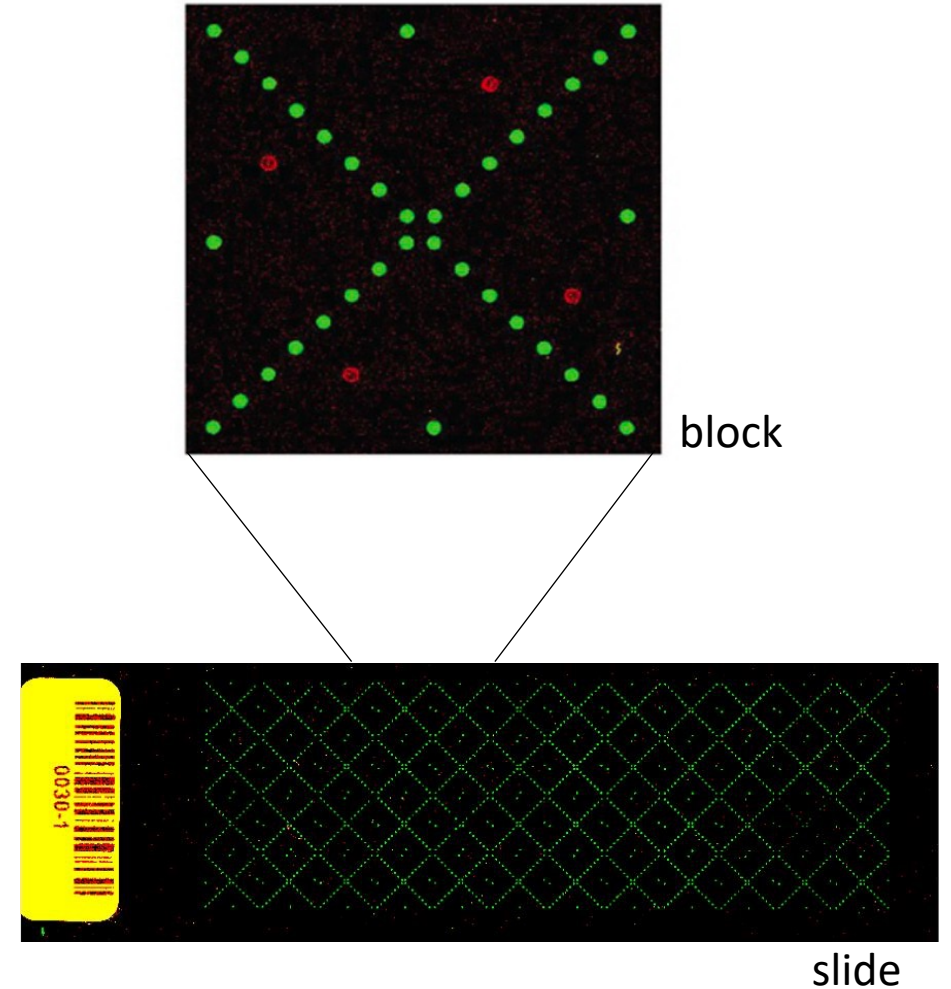


# What do putative binders look like on the SMM slide?



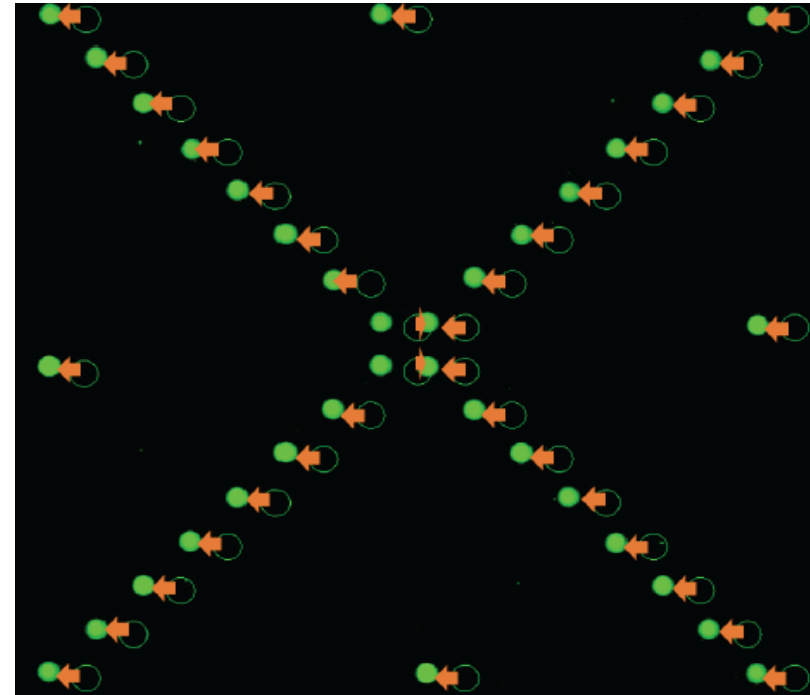
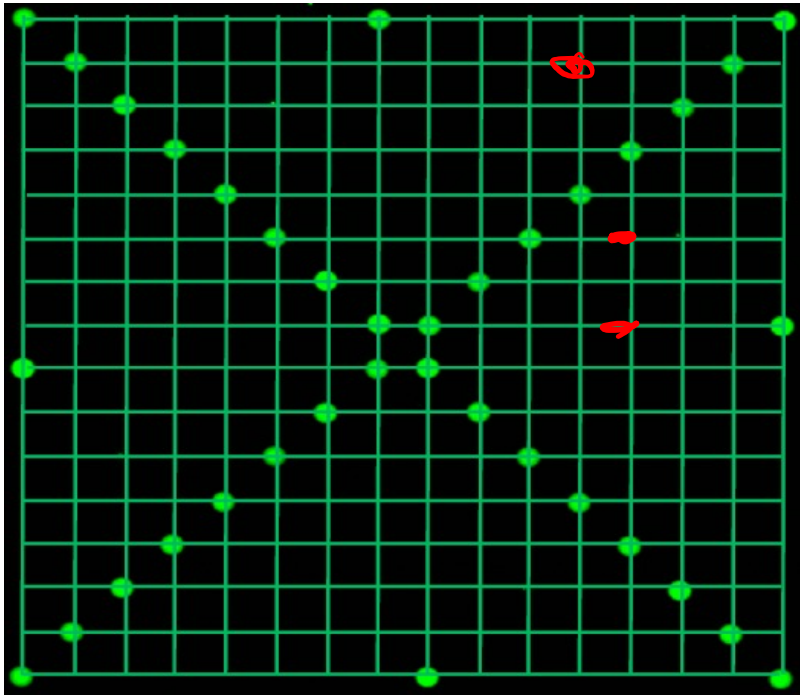
# Workflow for SMM data analysis

1. Align spots using fluorescence on 532 nm channel (~~sentinel spots~~)  
↓
2. Quantify fluorescence on 635 nm channel  
↓
3. Identify 'hits' with improbably high fluorescence  
↓
4. Complete 'by eye' analysis of putative hits to manually remove false positives



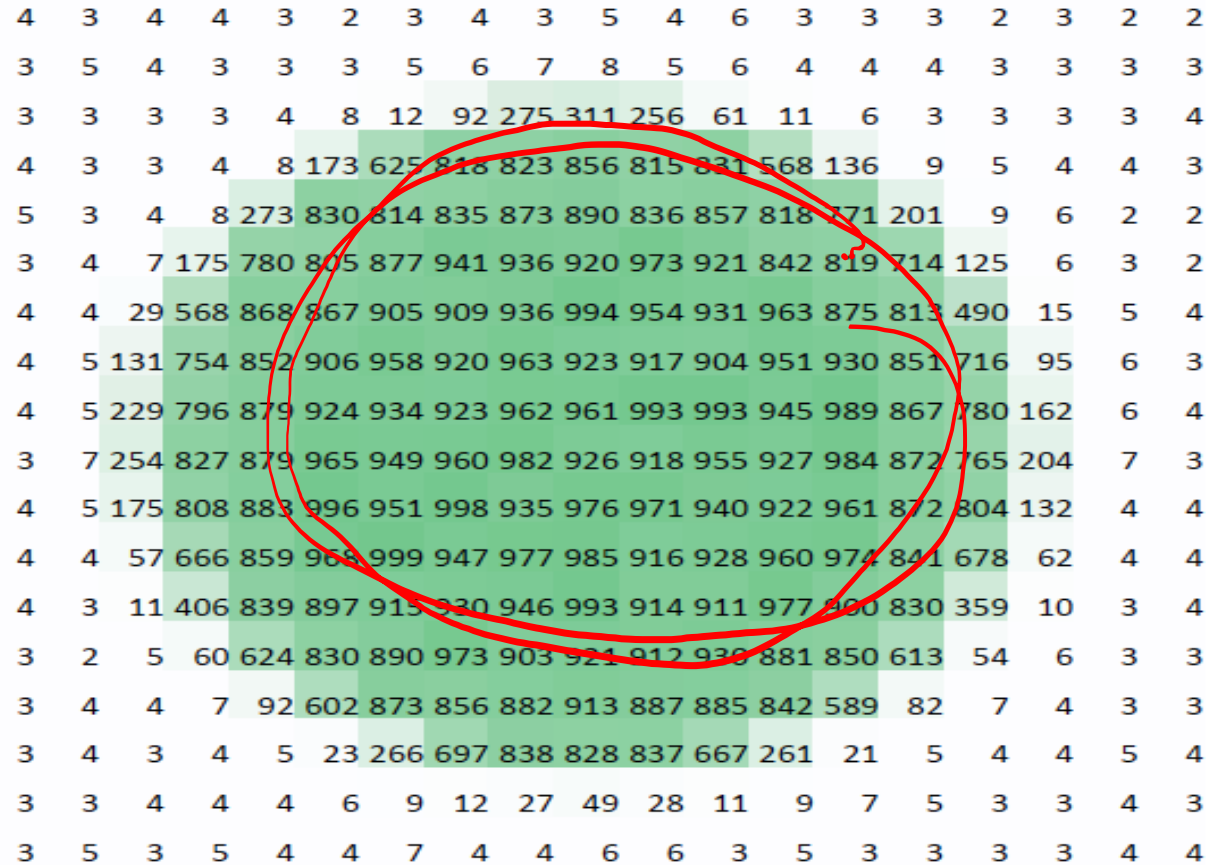
# Align SMM using sentinel spots

- Slides are printed in block patterns (16 rows x 16 columns)
- Each ligand spot is identifiable via intersecting lines between sentinels



# Spots are represented by an array of numerical values

- Each pixel is represented by a number that indicates intensity of the signal
- Computational analysis used to define 'hits'



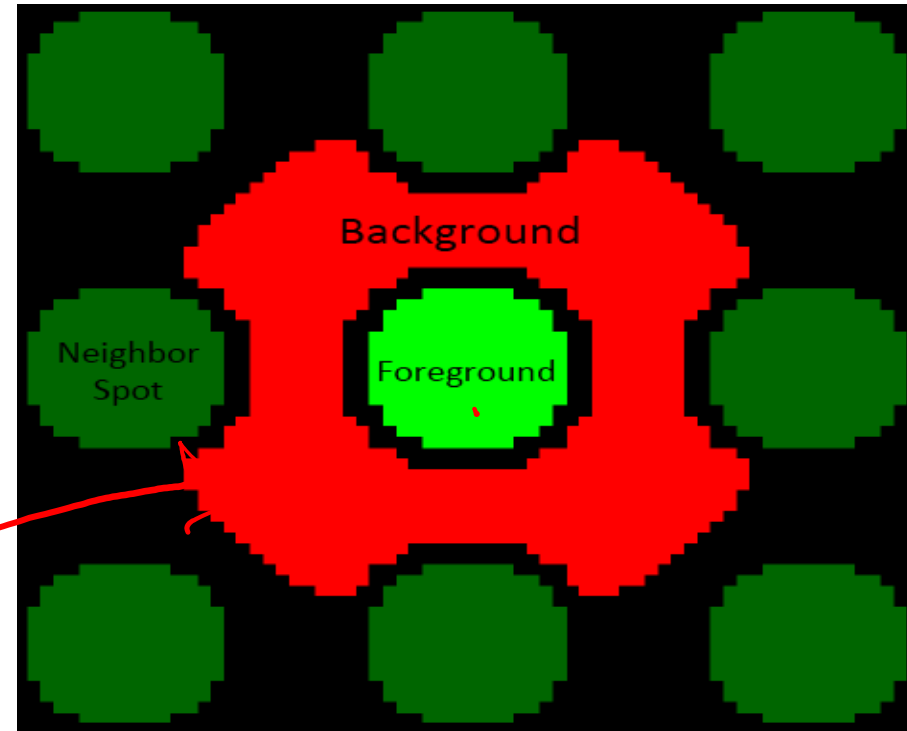
# Fluorescence is quantified to identify hits

- Foreground:

*SM attached*

- Background:

*area immediately around SM*



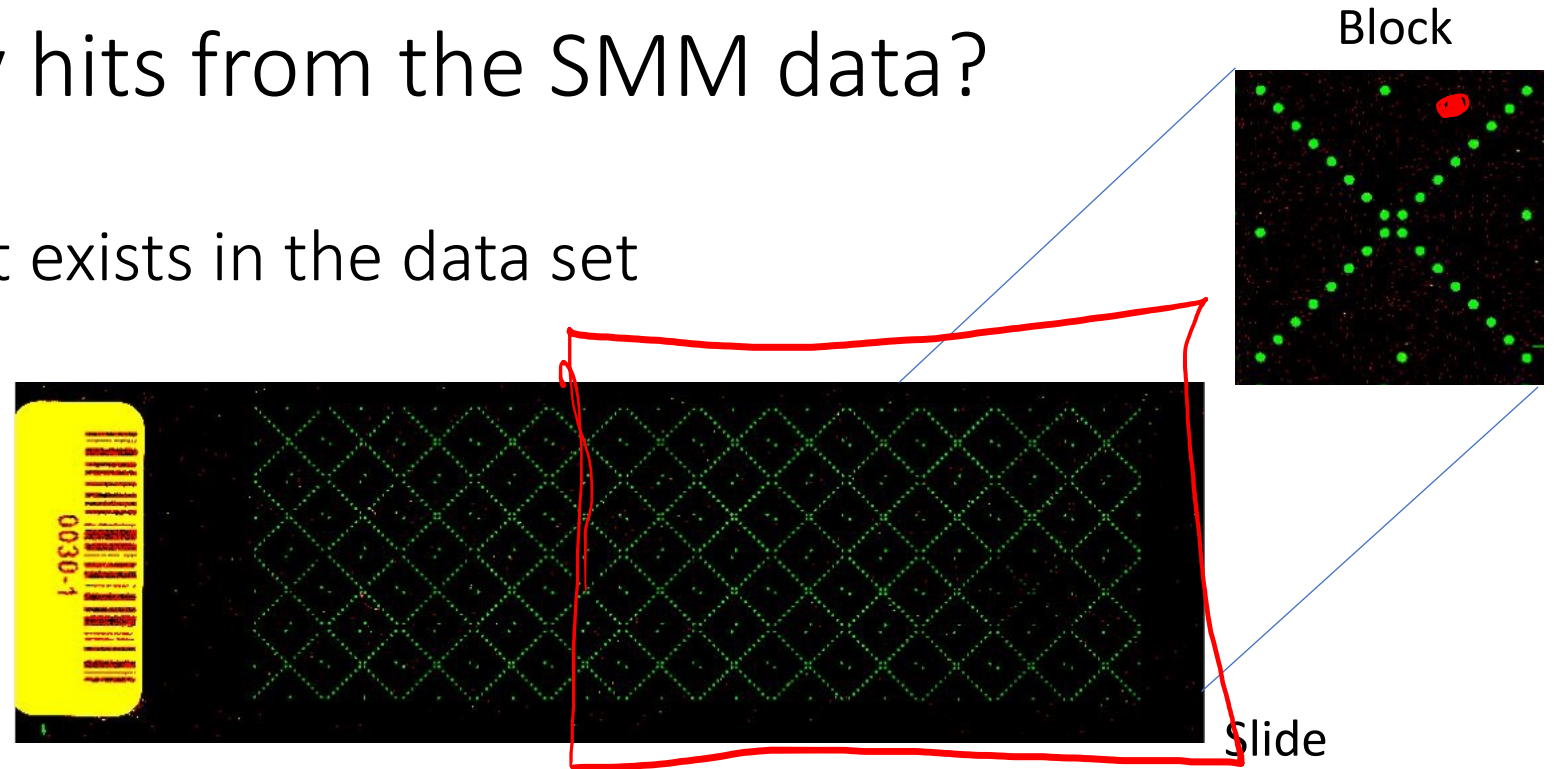
$$\text{Signal-to-noise ratio (SNR)} = \frac{\mu_{\text{foreground}} - \mu_{\text{background}}}{\sigma_{\text{background}}}$$



# How do you identify hits from the SMM data?

First, consider bias that exists in the data set

- Across all slides
- Within each block
- Within each slide

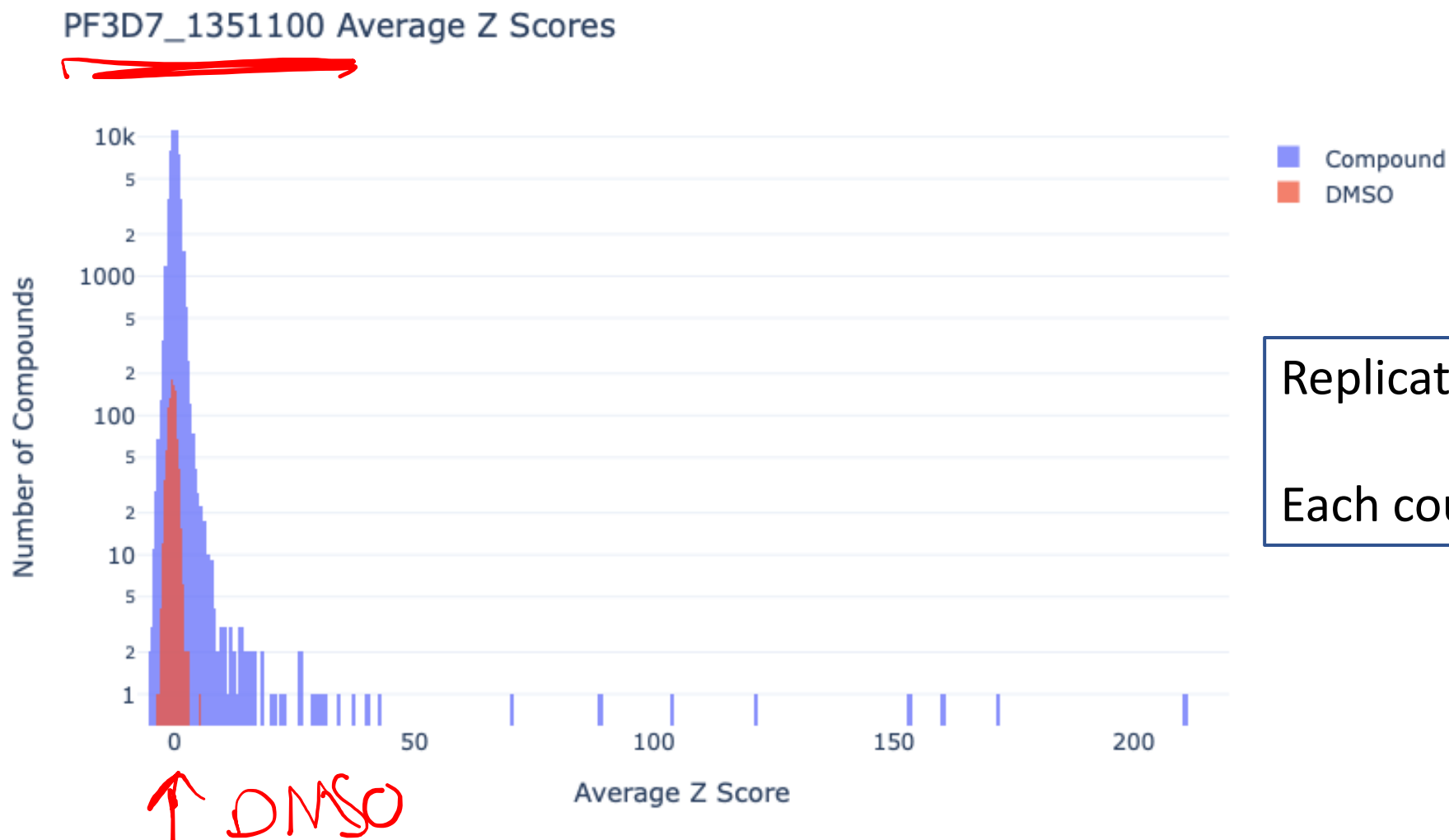


Then, identify hits with significantly higher fluorescence over background

*Z Score*

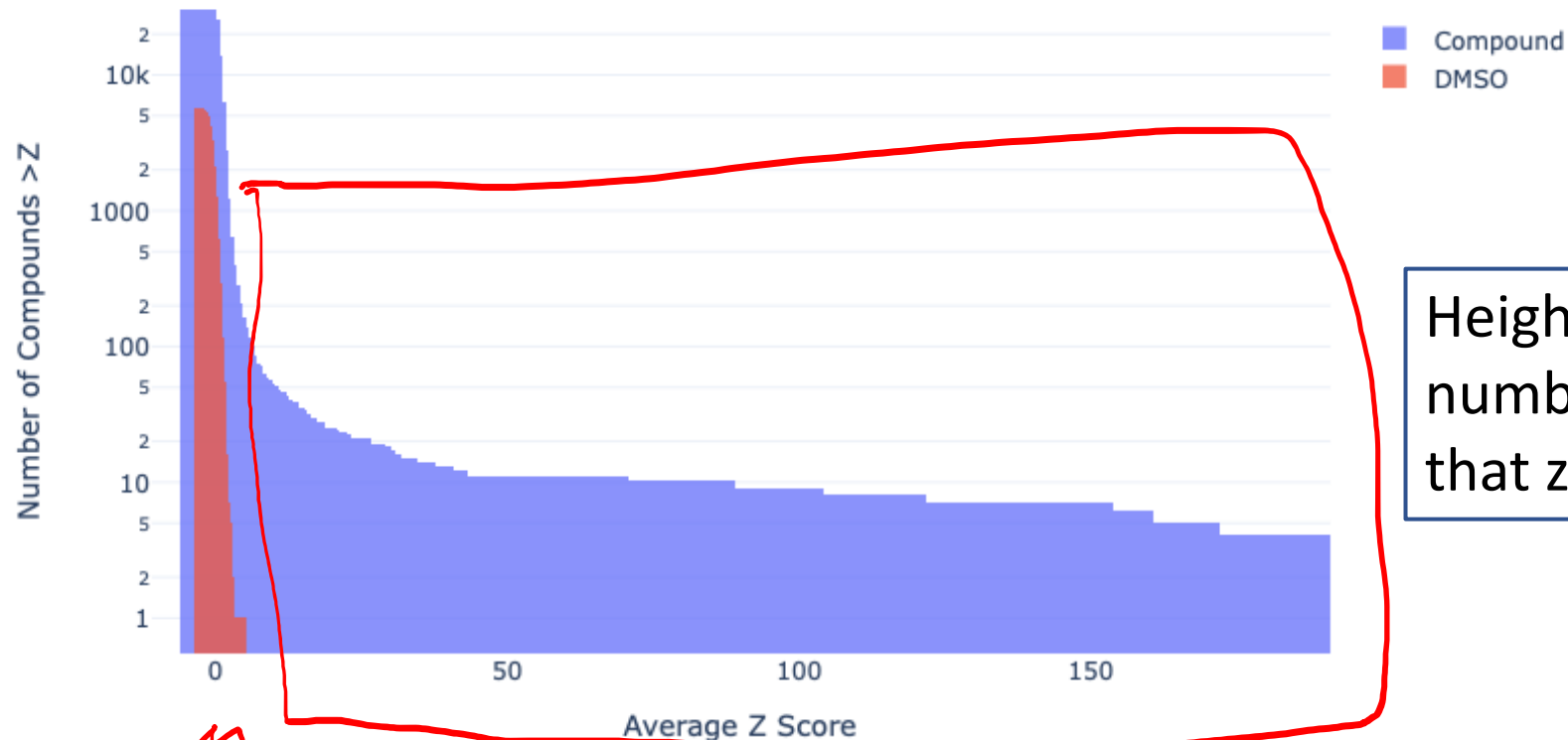
Lastly, manually confirm hits to eliminate false positives

# Average Z-score calculated for all compounds



# How do you determine a threshold Z-score?

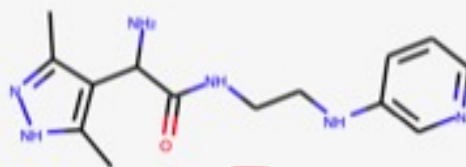
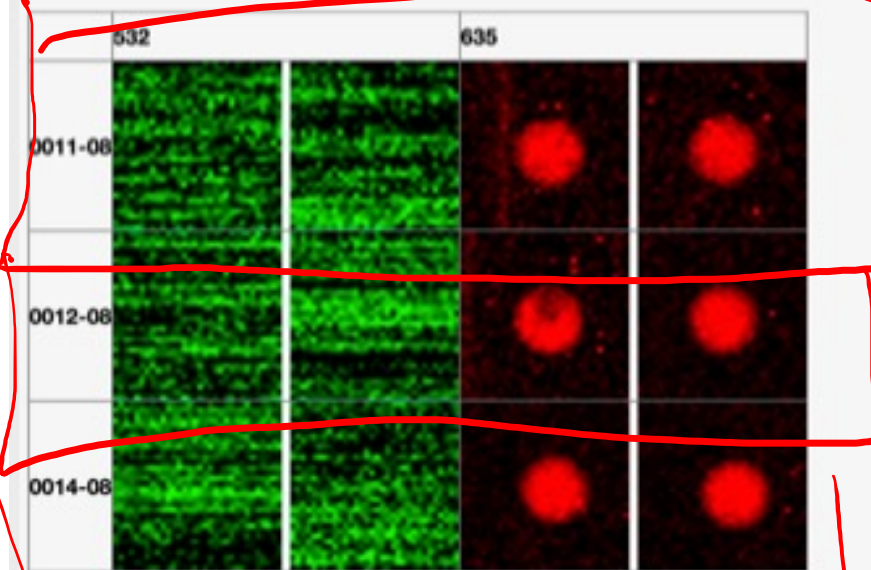
PF3D7\_1351100 Cumulative Z Score Averages



Height at x (average z score) =  
number of compounds (y) with  
that z-score or higher

# How do you validate hits manually?

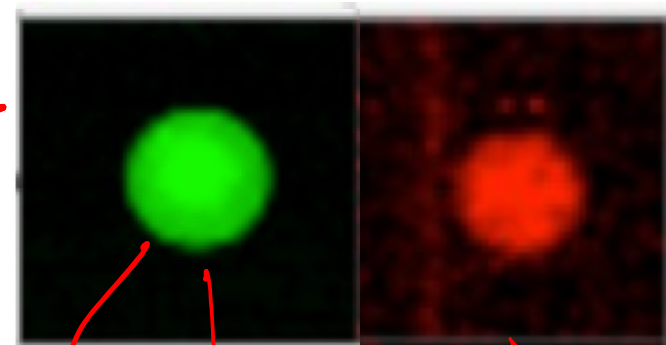
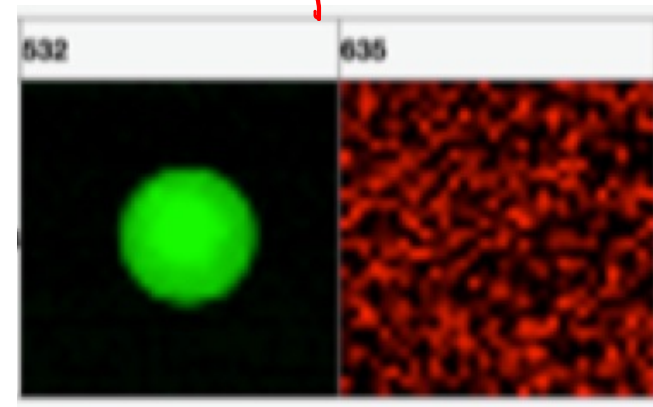
	⌵ ID	⌵ Robust Z ⌵	SMILES	⌵ Validated ⌵	
49592	13:KI0001...	51.03151	C[C@H](C...	-1	
42089	11:KI0001...	45.09263	CC1=C(C{...	example	
6782	02:KI0001...	39.91118	CCNC(=O...	-1	
29108	08:KI0001...	39.59436	C1C(C2=...	-1	
44736	12:KI0001...	33.03555	C1CN(C2...	-1	
29660	08:KI0001...	31.94118	CC1=NC2...	-1	
11360	03:KI0001...	26.13059	C1CN(CC...	-1	



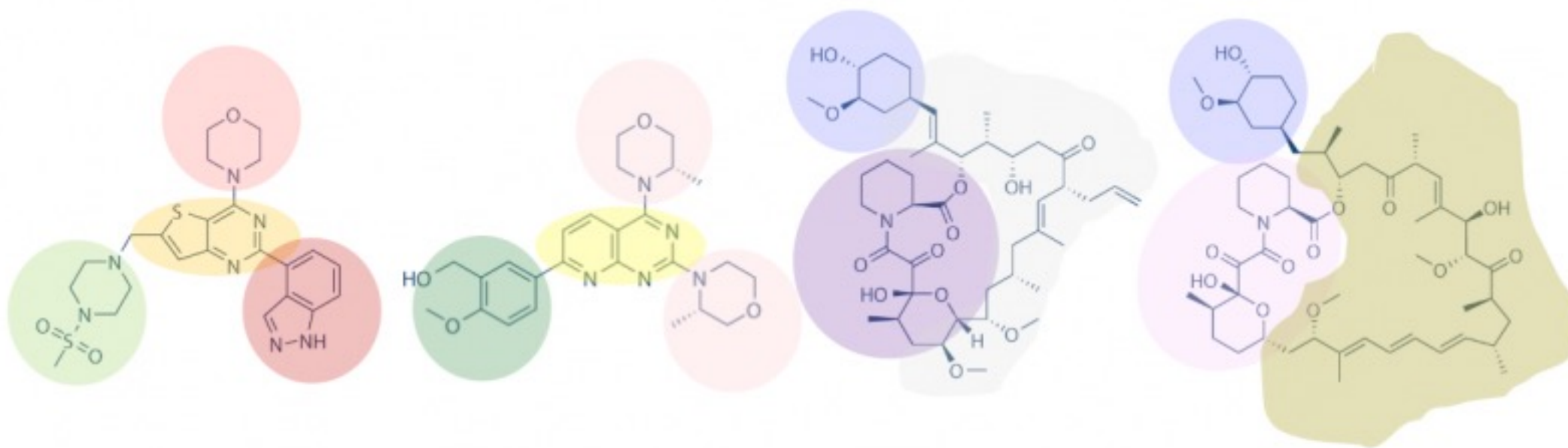
1 Small molecule

SM = autofluorescent protein? bound?

sentinel spot



# Compare chemical structures of identified compounds





# For Today

- Work through SMM procedure
- Evaluate chemical structures of small molecules that will be used in next lab's assay

\*\* If you would like to revise your JP slide and replace your homework, you can do so.  
Turn it in by 10pm tonight \*\*

# For M2D5

- Draft an outline of the introduction for your Research Article
  - Use guidance on the Wiki section for Homework and the Research Article assignment

# Journal Article Presentation Days

## Tues (11/1)

- Abby D
- Isabella
- Kam
- Uzuki
- Vin

## Thurs (11/3)

- Abby L
- Bryan
- Carrie
- Ellie
- Savannah