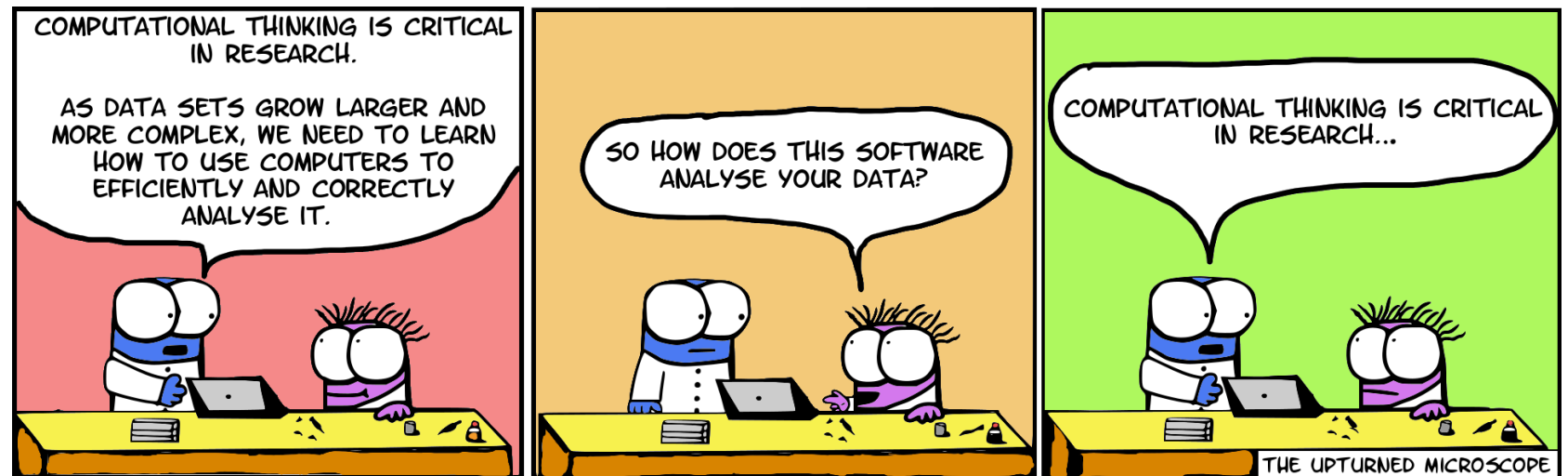


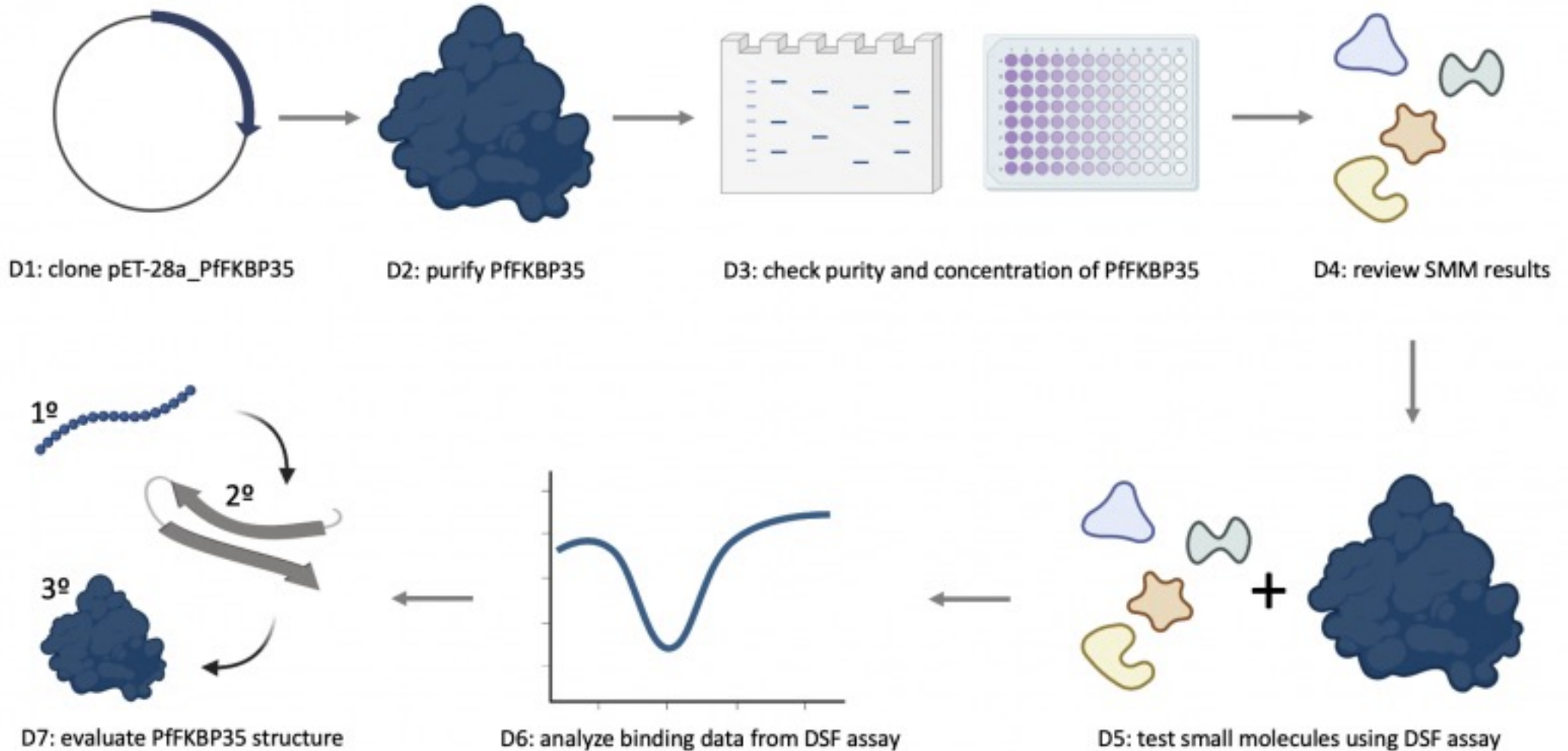
M2D4: Review small molecule microarray (SMM) technology and data analysis

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



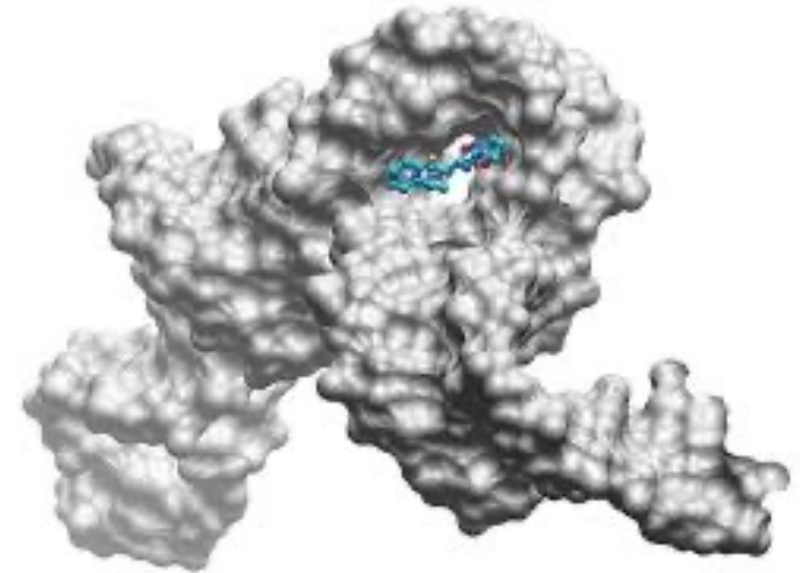
Overview of M2: drug discovery

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

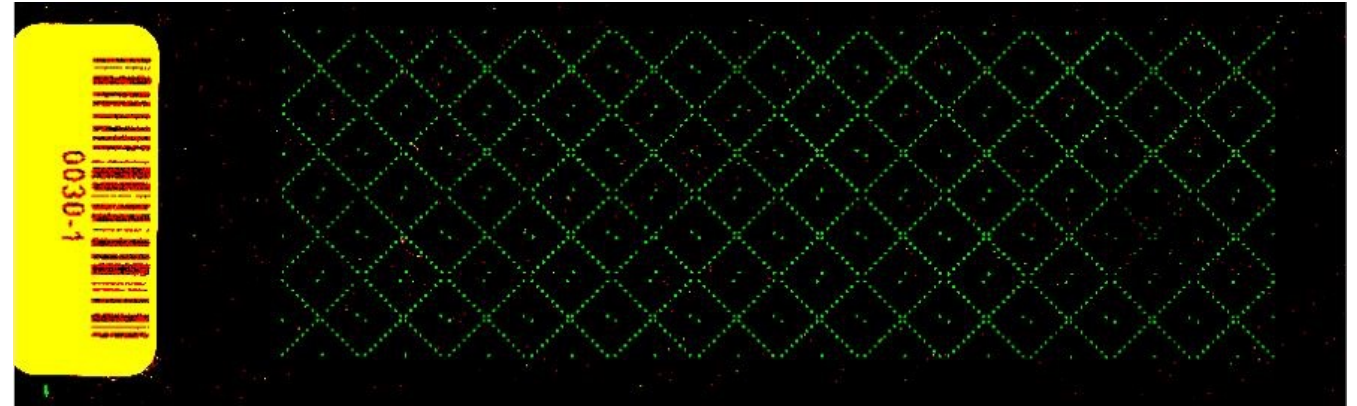


Why are we discussing this approach?

- 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)

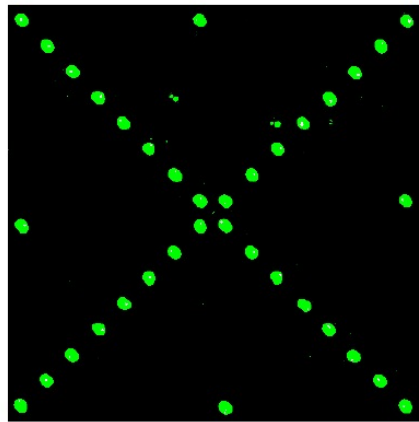


- Each slide contains ~12,000 spots
 - ~4,200 small molecules / ligands (in duplicate = ~8,400)
 - Fluorescein sentinel spots
 - DMSO negative control spots

SMM workflow

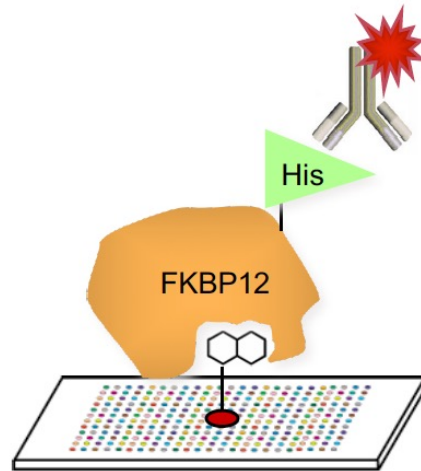
SMM Screen

Data Acquisition



subarray

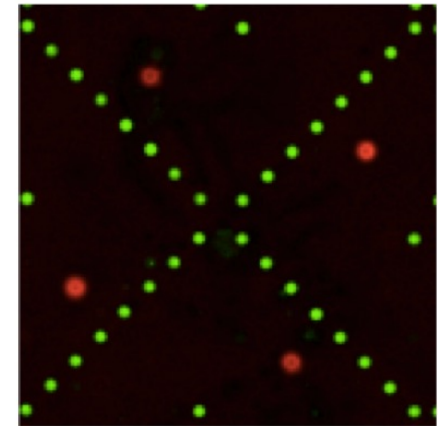
Your Protein
(e.g. FKBP12)



schematic of screen



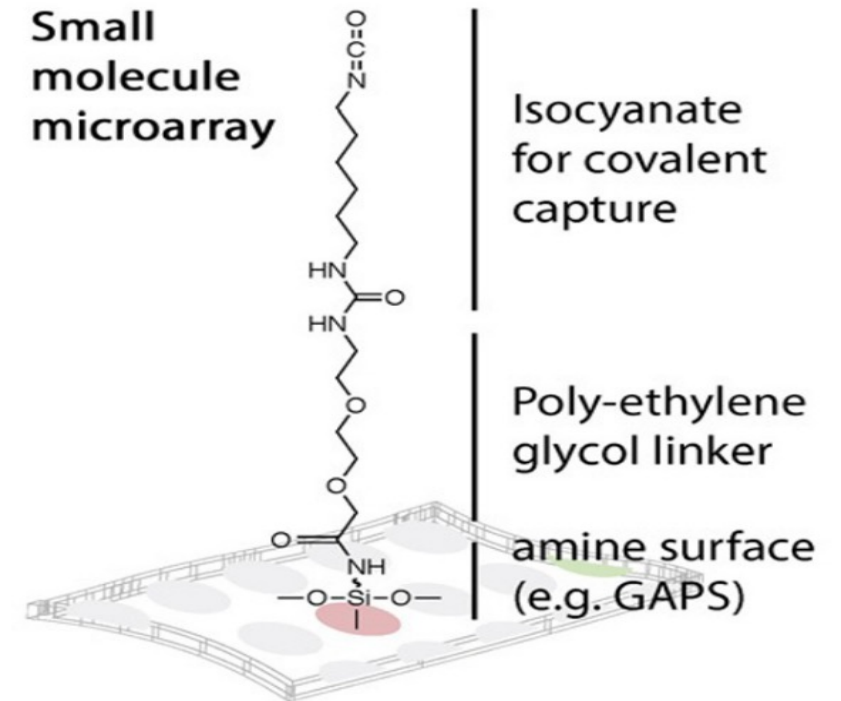
scan



subarray

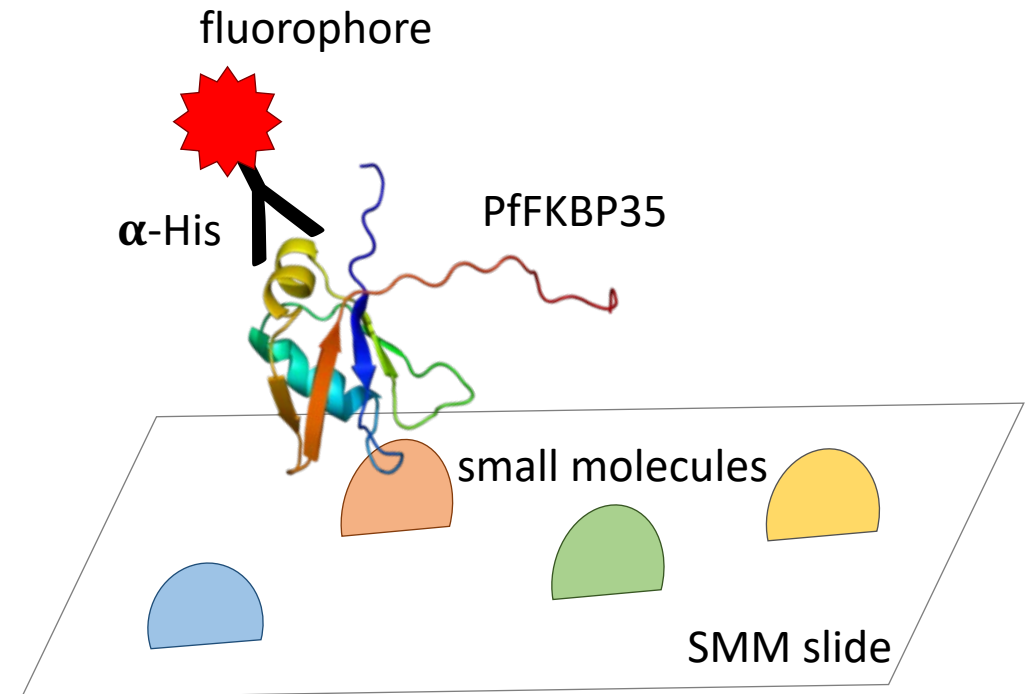
SMM slide preparation

- 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



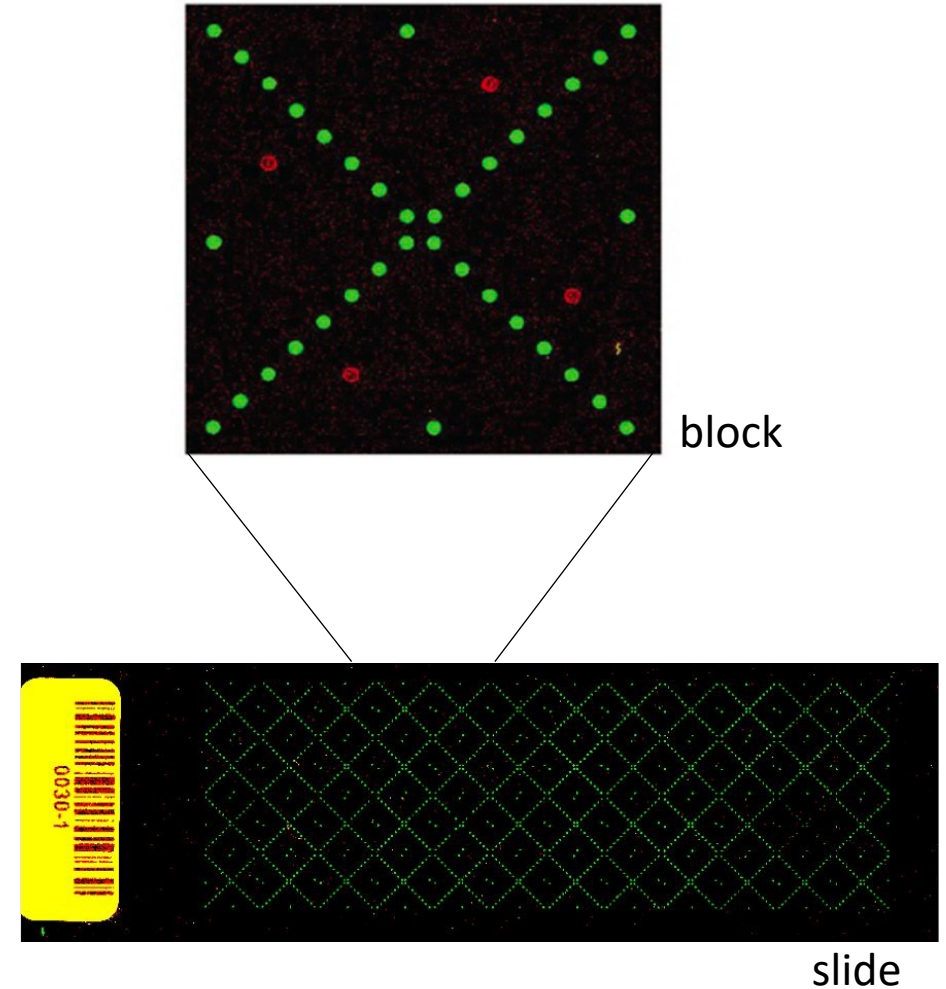
How would we screen for ligands that bind PFKBP35?

- Incubate the SMM slide with 3ml of our purified PFKBP35
- Wash away unbound protein
- Incubate SMM slide with AlexaFlour 647 anti-His antibody
- Wash away excess antibody
- Store for scanning



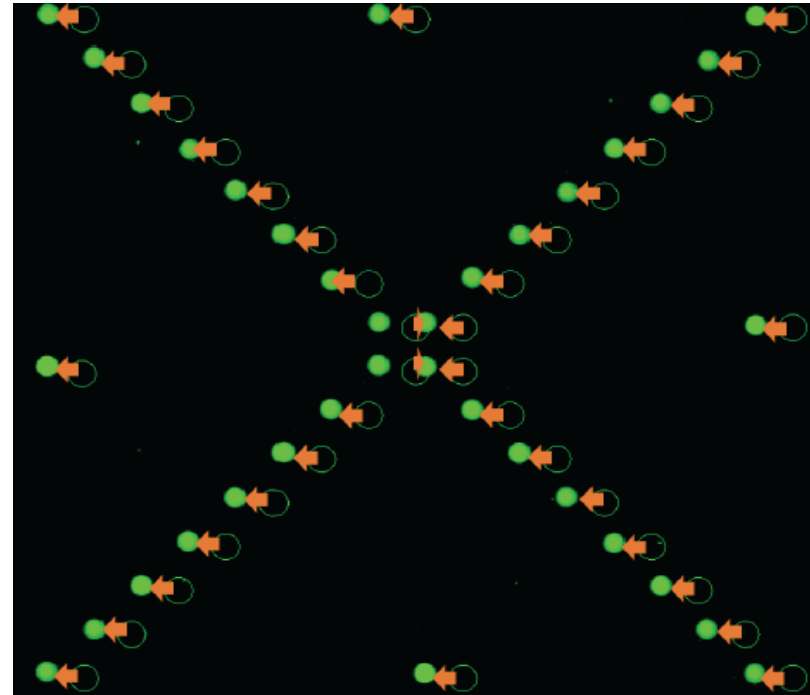
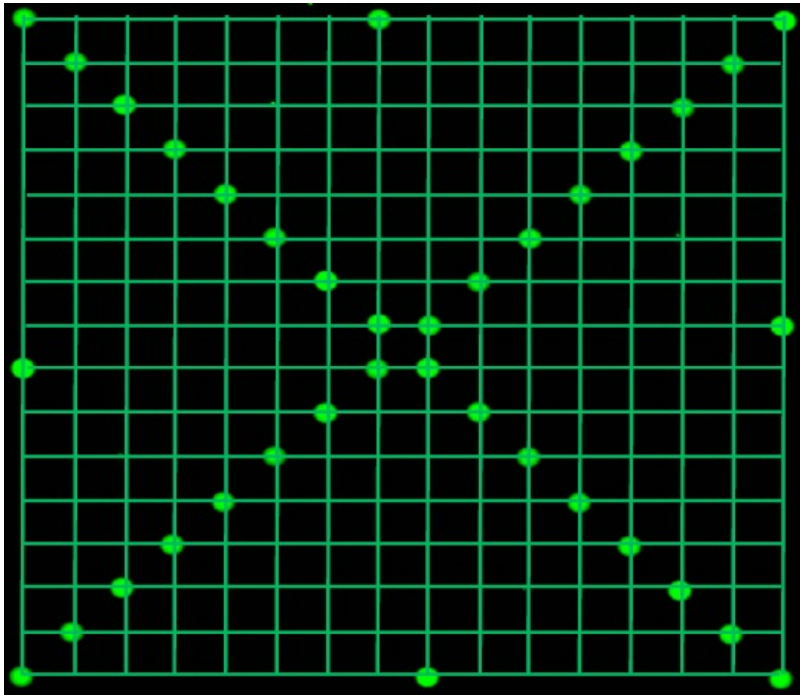
Workflow for SMM data analysis

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



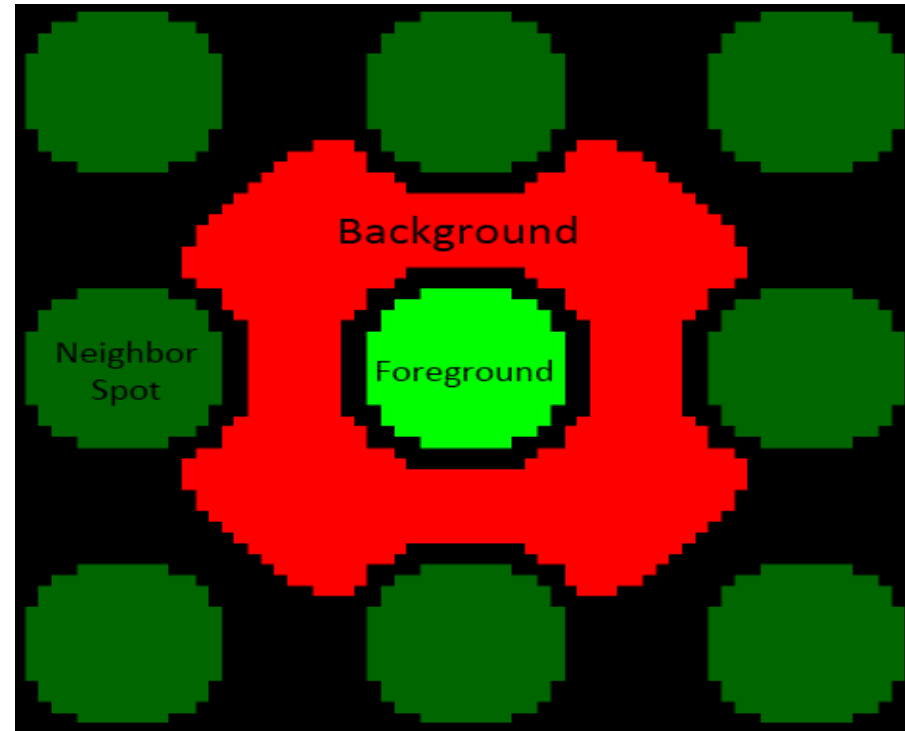
Spots are represented by an array of numerical values

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

4	3	4	4	3	2	3	4	3	5	4	6	3	3	3	2	3	2	2
3	5	4	3	3	3	5	6	7	8	5	6	4	4	4	3	3	3	3
3	3	3	3	4	8	12	92	275	311	256	61	11	6	3	3	3	3	4
4	3	3	4	8	173	625	818	823	856	815	831	568	136	9	5	4	4	3
5	3	4	8	273	830	814	835	873	890	836	857	818	771	201	9	6	2	2
3	4	7	175	780	805	877	941	936	920	973	921	842	819	714	125	6	3	2
4	4	29	568	868	867	905	909	936	994	954	931	963	875	813	490	15	5	4
4	5	131	754	852	906	958	920	963	923	917	904	951	930	851	716	95	6	3
4	5	229	796	879	924	934	923	962	961	993	993	945	989	867	780	162	6	4
3	7	254	827	879	965	949	960	982	926	918	955	927	984	872	765	204	7	3
4	5	175	808	883	996	951	998	935	976	971	940	922	961	872	804	132	4	4
4	4	57	666	859	968	999	947	977	985	916	928	960	974	841	678	62	4	4
4	3	11	406	839	897	915	930	946	993	914	911	977	900	830	359	10	3	4
3	2	5	60	624	830	890	973	903	921	912	930	881	850	613	54	6	3	3
3	4	4	7	92	602	873	856	882	913	887	885	842	589	82	7	4	3	3
3	4	3	4	5	23	266	697	838	828	837	667	261	21	5	4	4	5	4
3	3	4	4	4	6	9	12	27	49	28	11	9	7	5	3	3	4	3
3	5	3	5	4	4	7	4	4	6	6	3	5	3	3	3	3	4	4

Fluorescence is quantified to identify hits

- Foreground:
- Background:

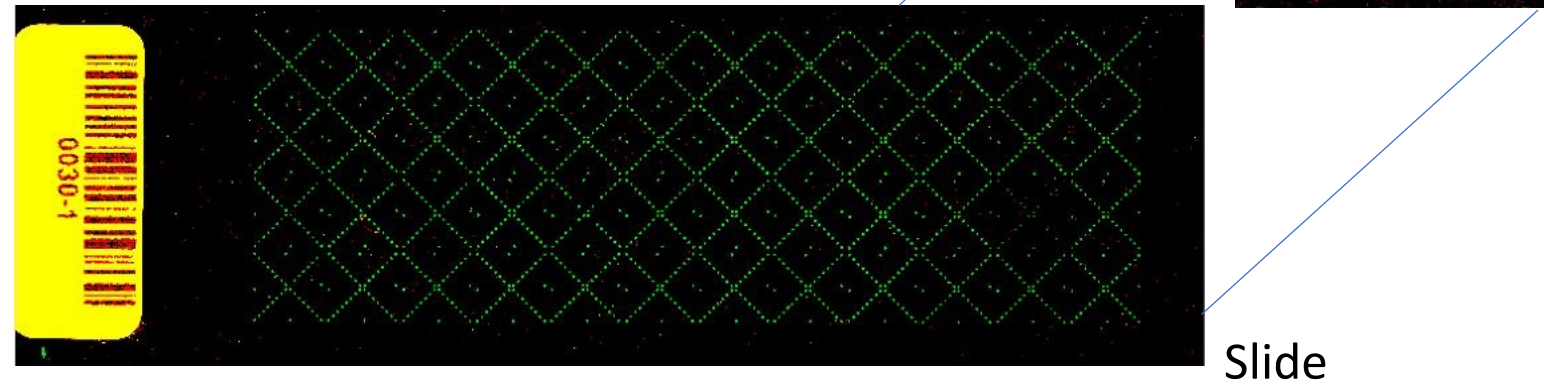


$$\text{Signal-to-noise ratio (SNR)} = \frac{\mu_{\text{foreground}} - \mu_{\text{background}}}{\sigma_{\text{background}}}$$

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

Lastly, manually confirm hits to eliminate false positives

Average Z-score calculated for all compounds

PF3D7_1351100 Average Z Scores

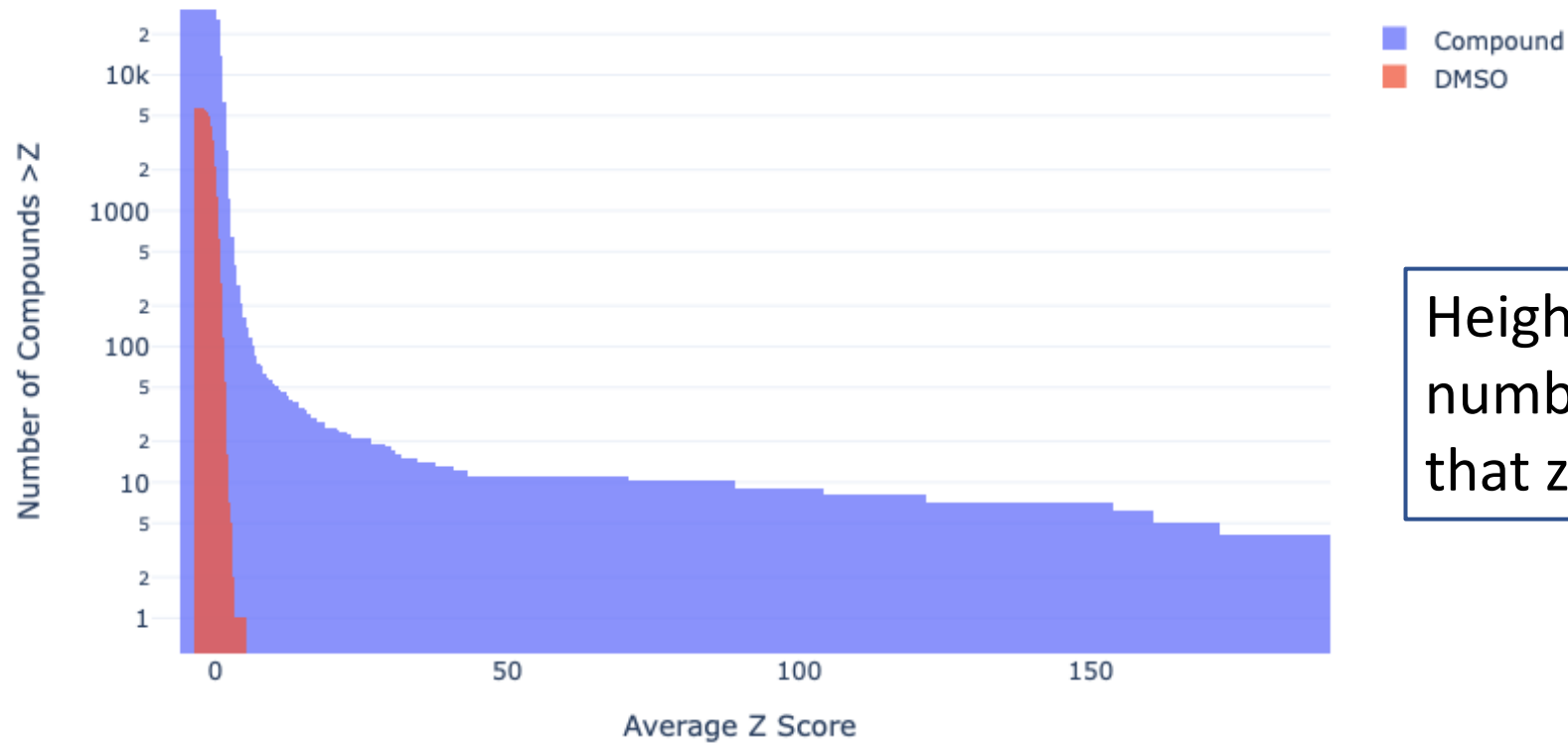


Replicate spots averaged

Each count = unique compound

How do you determine a threshold Z-score?

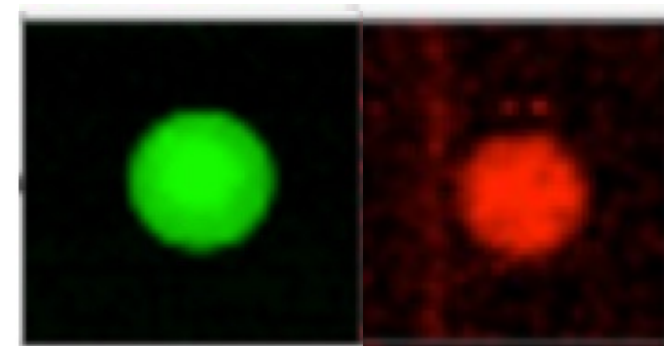
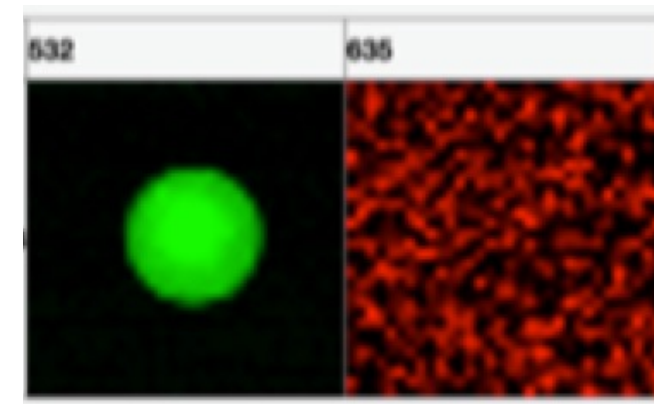
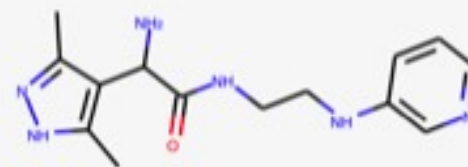
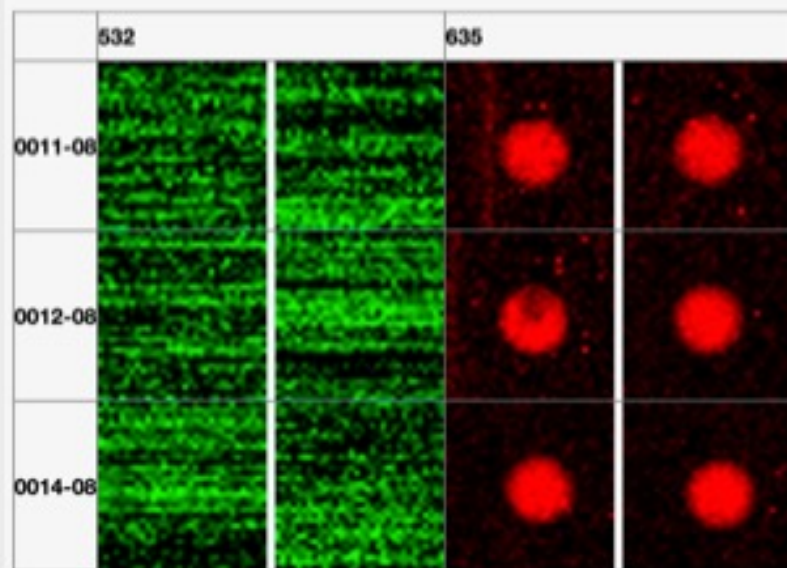
PF3D7_1351100 Cumulative Z Score Averages



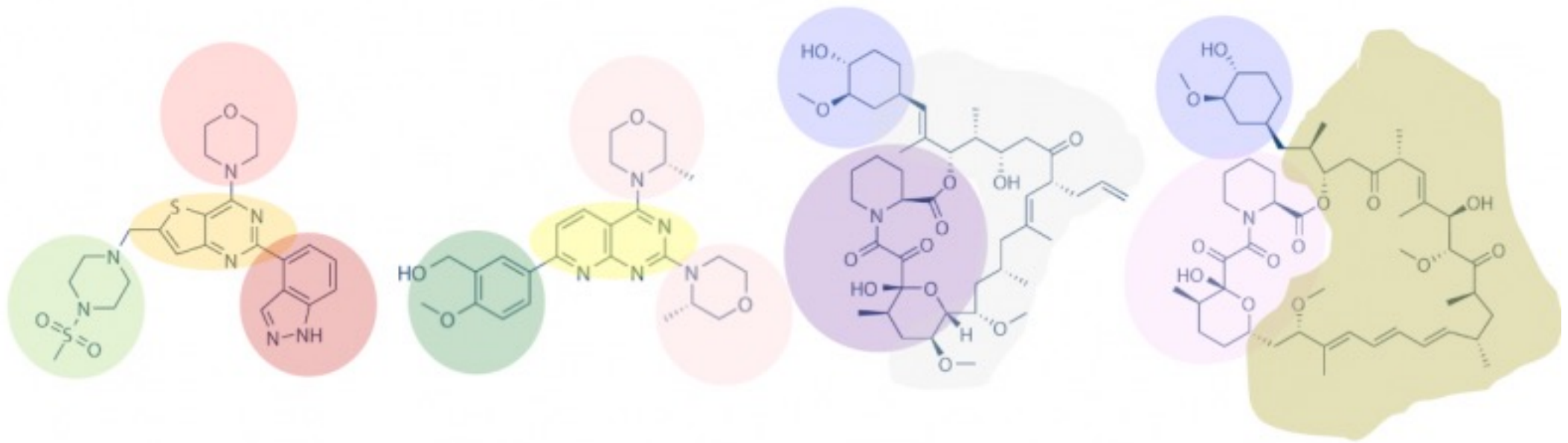
Height at x (average z score) =
number of compounds (y) with
that z-score or higher

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



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

For M2D2

- 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