

M2D1: Examine SMM data collected using TDP43 protein

1. Prelab
2. Walk through SMM analysis
3. Examine chemical structure of hits
4. Discuss journal article

Office Hours:

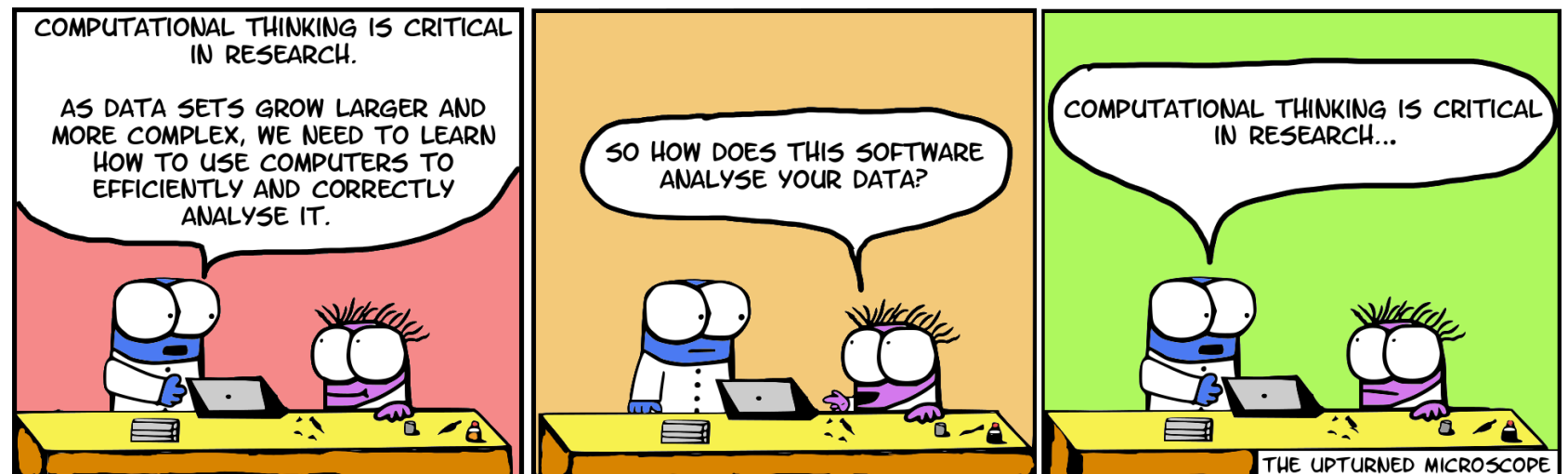
Monday: 1-2pm @ 16-319 and Zoom [Becky](#)
3-5pm @ 16-317 and Zoom [Noreen](#)

Tuesday: 10-11am @ 1-390 [Becky & Jamie](#)

Thursday: 10-11am @ 1-390 [Noreen & Jamie](#)

*After lecture by request

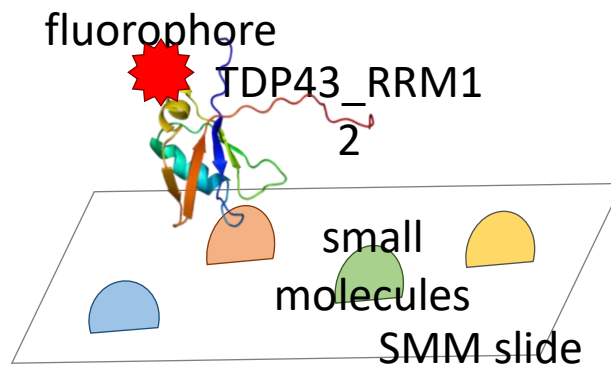
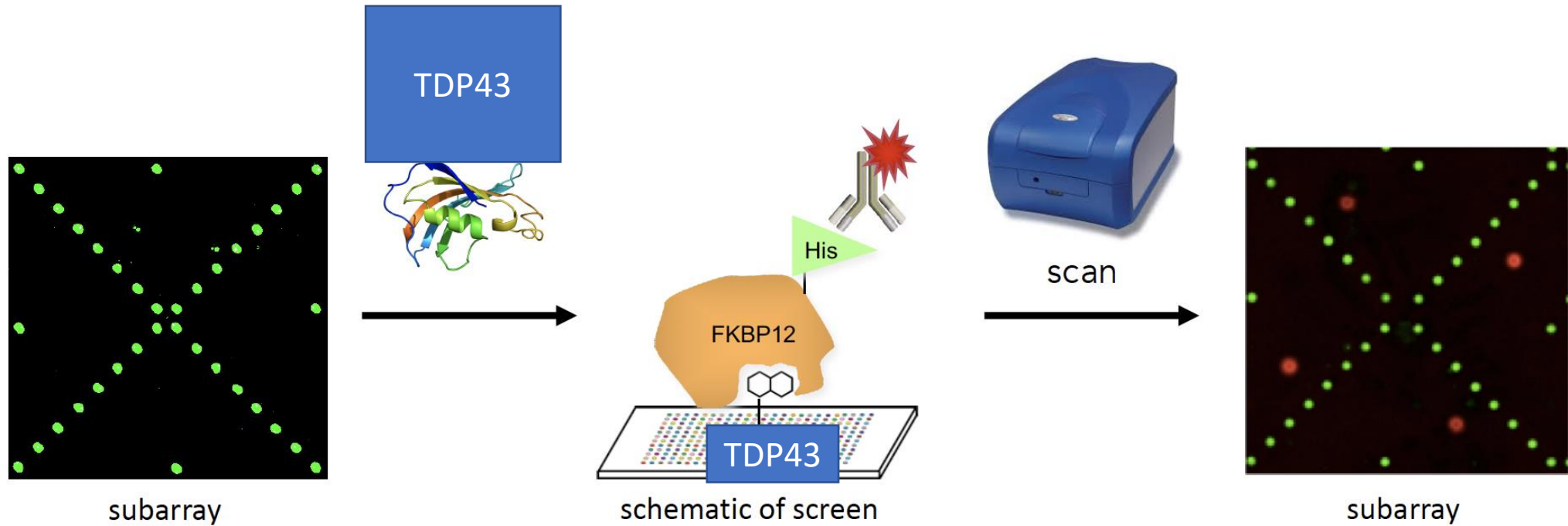
*Also available by appointment



SMM workflow

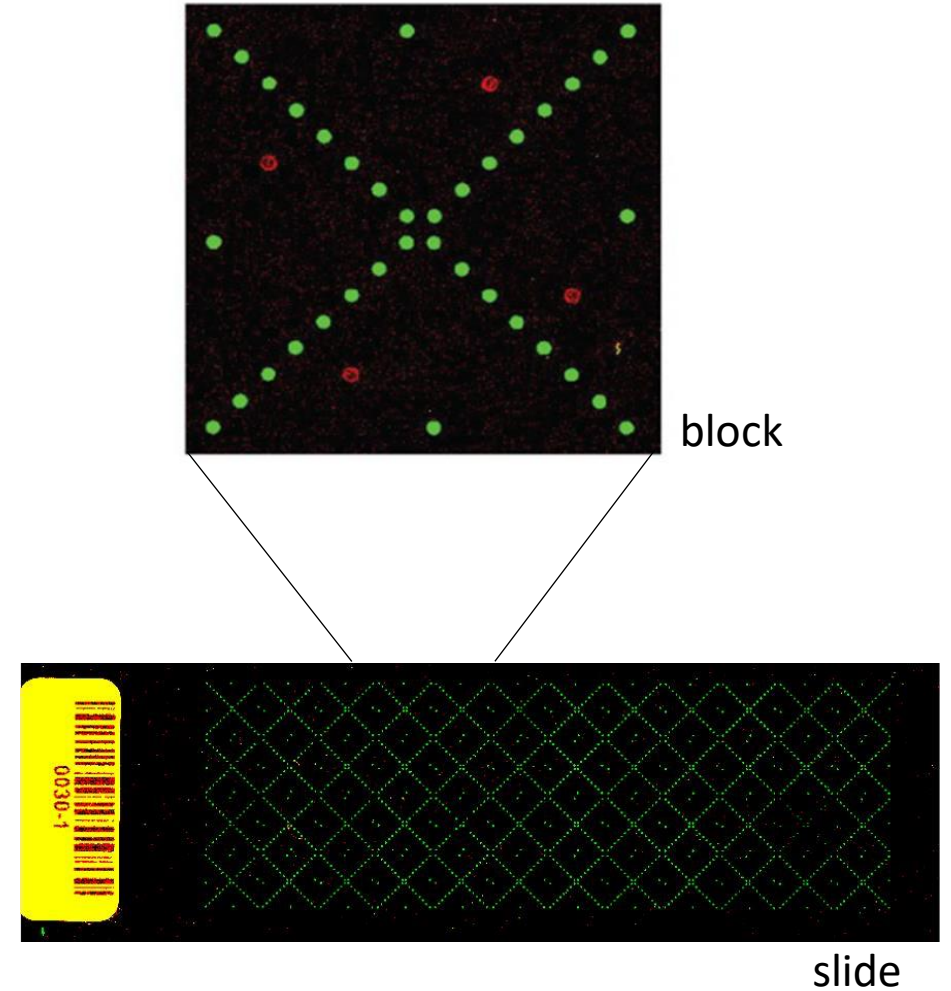
SMM Screen

Data Acquisition



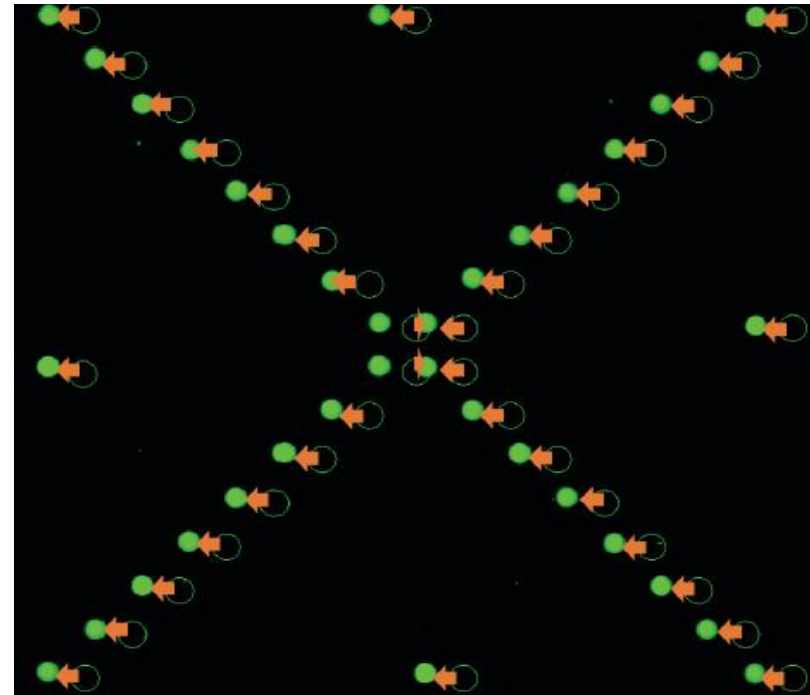
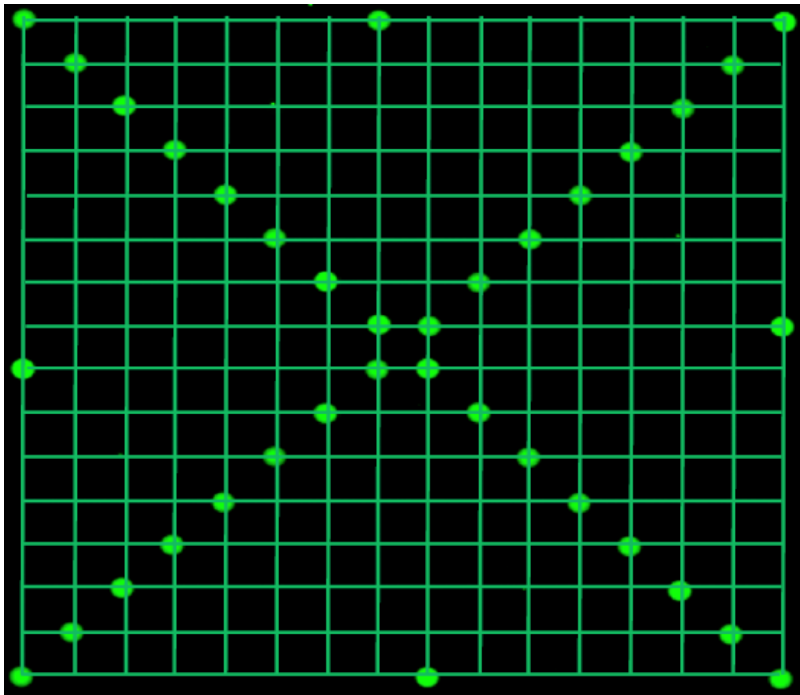
Workflow for SMM data analysis

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

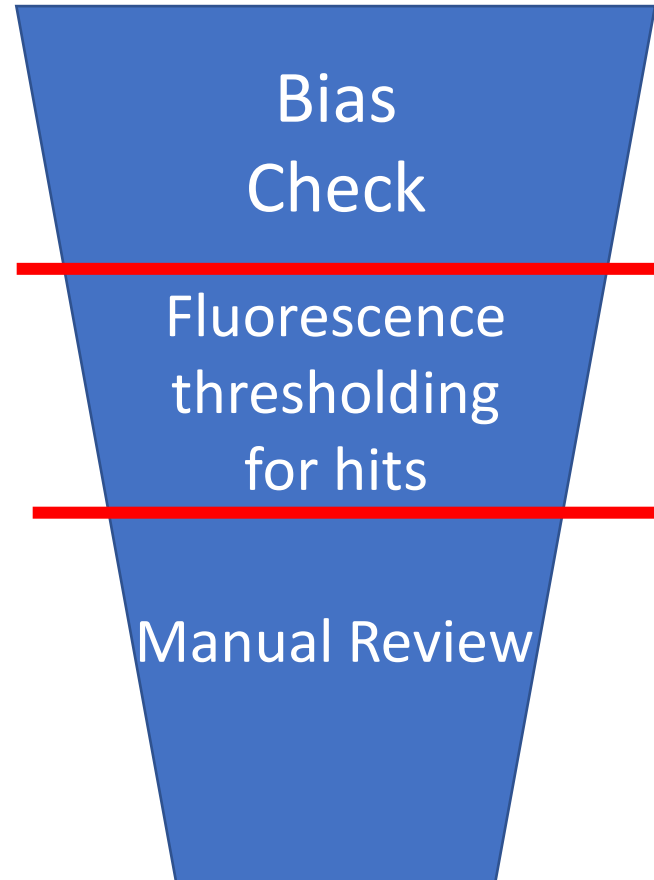


Align SMM using sentinel spots

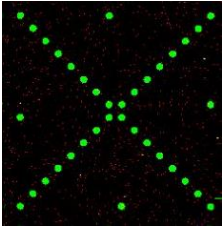
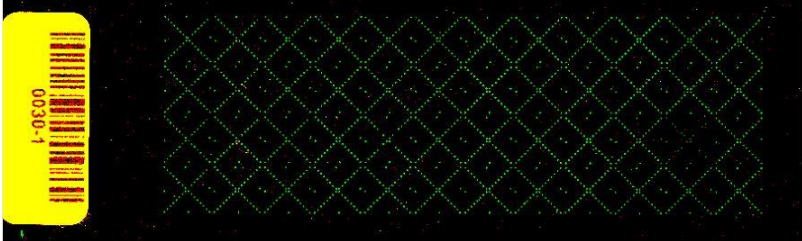
- Slides are printed in block patterns (16 rows x 16 columns)
- Each ligand spot is identifiable via intersecting lines between sentinels



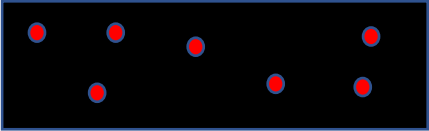
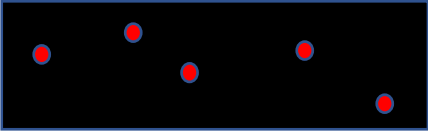
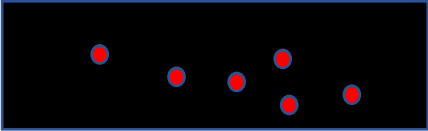
Refining your hits



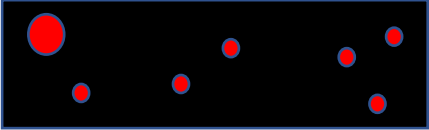
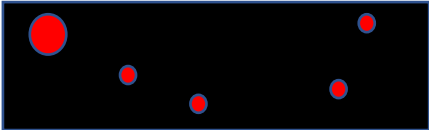
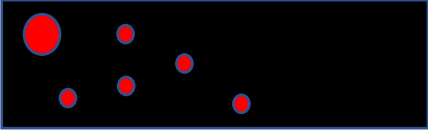
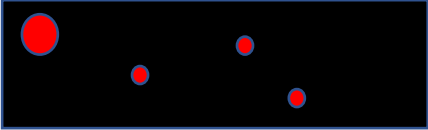
What might bias look like?



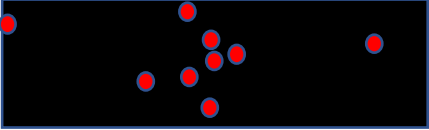
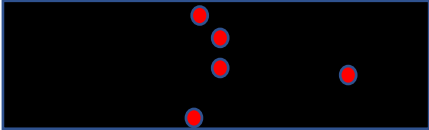
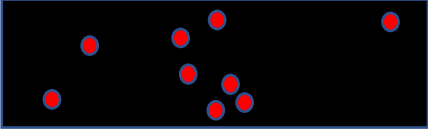
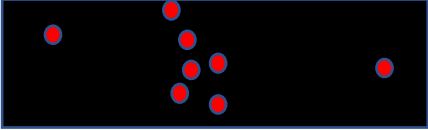
1) Bias across slides



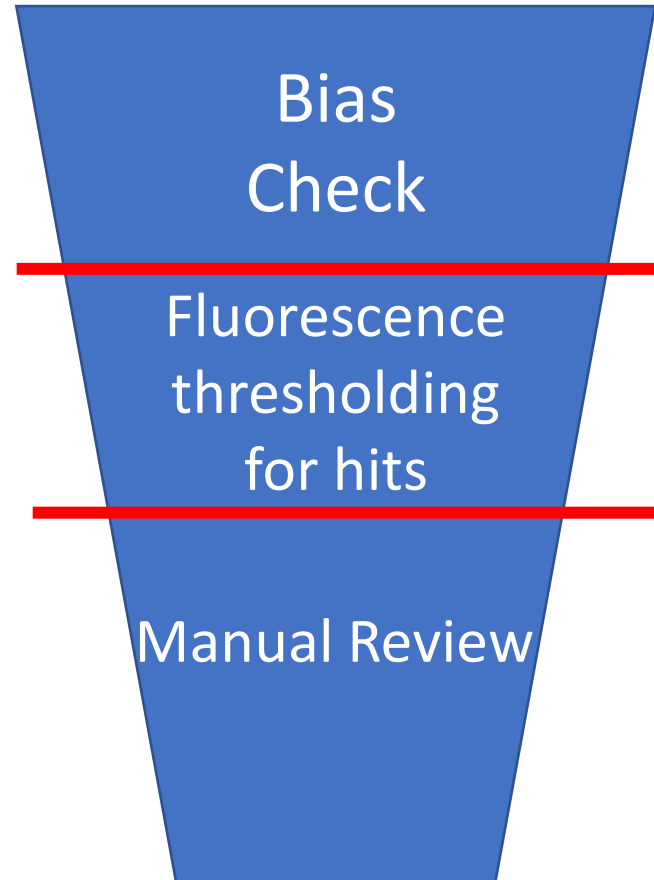
2) Bias within Block



3) Bias within Slide



Refining your hits



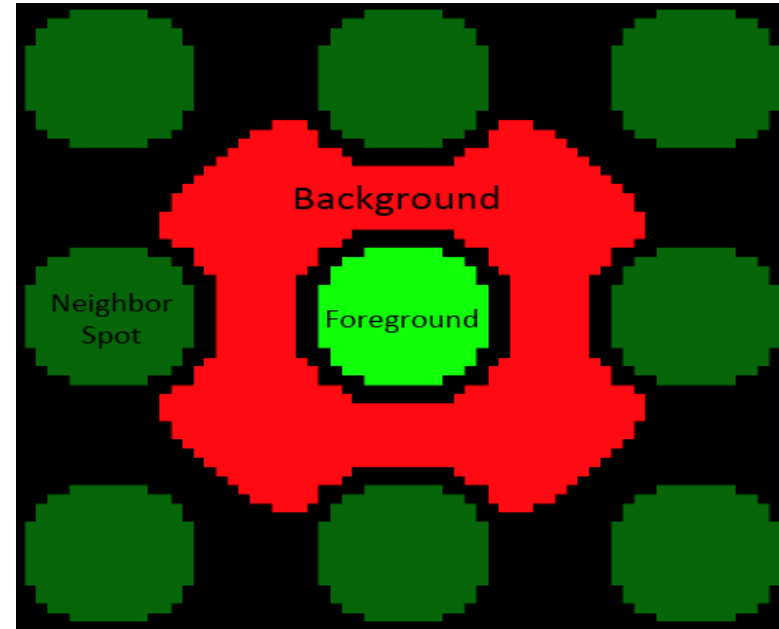
Spots are represented by an array of numerical values

- 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:
 - Where SMM was printed
- Background:
 - Residual ligand

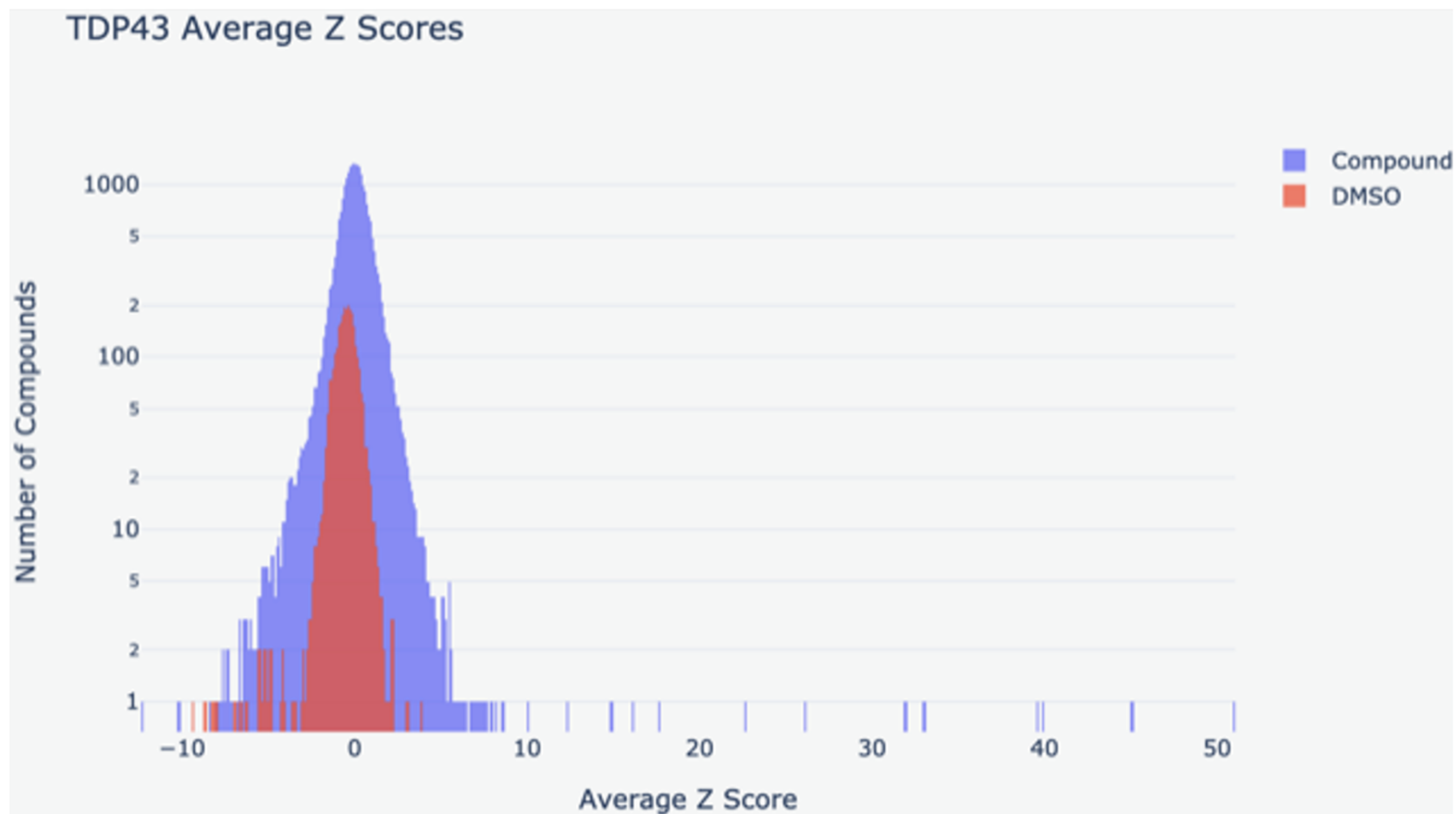


$$\text{Signal-to-noise ratio (SNR)} = \frac{\mu_{\text{foreground}} - \mu_{\text{background}}}{\sigma_{\text{background}}}$$

SNR is then used to calculate the **robust z score**

- How different is the foreground signal from the background?
- Able to plot the distribution of the z scores to give an overview of whole data set

Average Z-score calculated for all compounds



Replicate spots averaged

Each count = unique compound

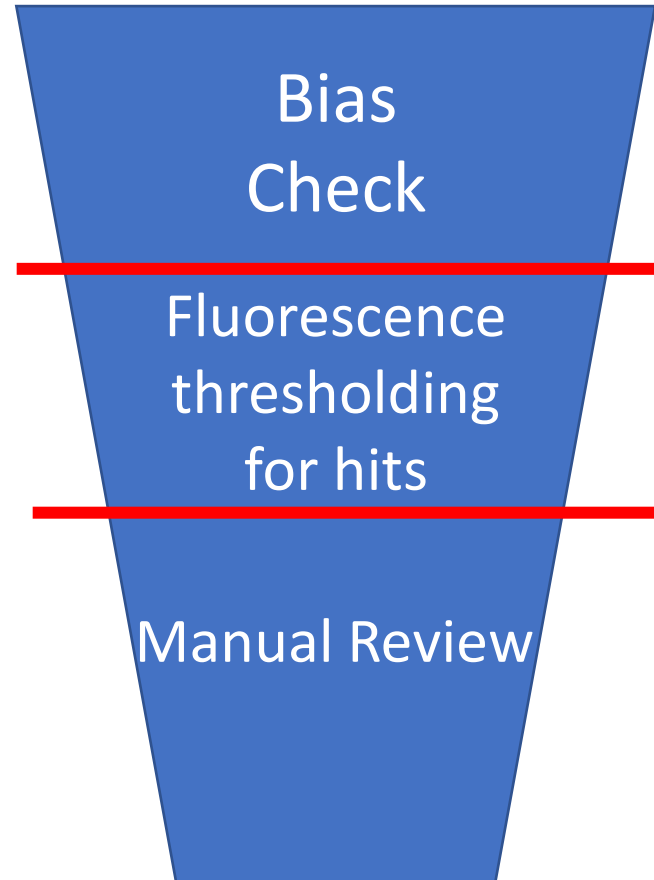
How many compounds have a particular z score?



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

- Useful for setting a threshold to exclude likely non-binders

Refining your hits



How do you validate hits manually?

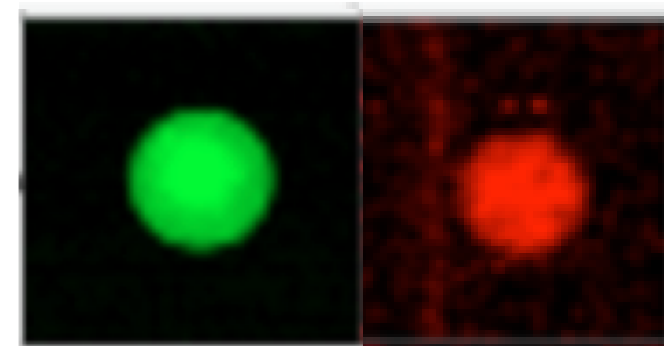
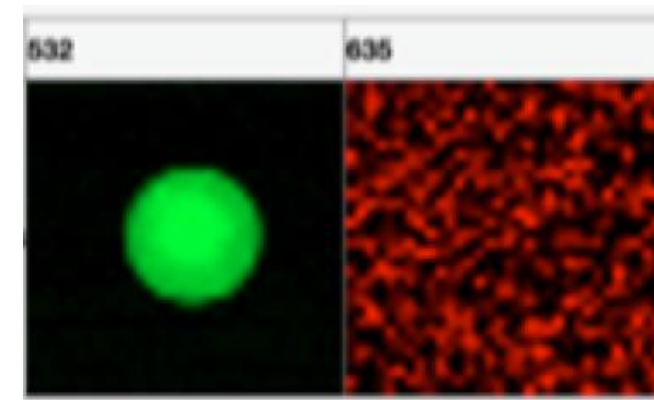
ID	Robust Z	SMILES	Validated
49592	51.03151	C[C@H](C...	-1
42089	45.09263	CC1=C(C(...	example
6782	39.91118	CCNC(=O...	-1
29108	39.59436	C1C(C2=...	-1
44736	33.03555	C1CN(C2...	-1
29660	31.94118	CC1=NC2...	-1
11360	26.13059	C1CN(CC...	-1

	532	635	532	635
0011-08				
0012-08				
0014-08				

Cc1c(C)nc(C)cc1C(=O)NCCCNc1cccnc1

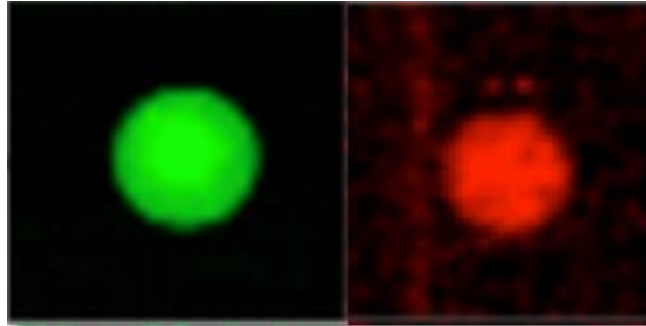
<- Positive Hits

Sentinel Spot



???

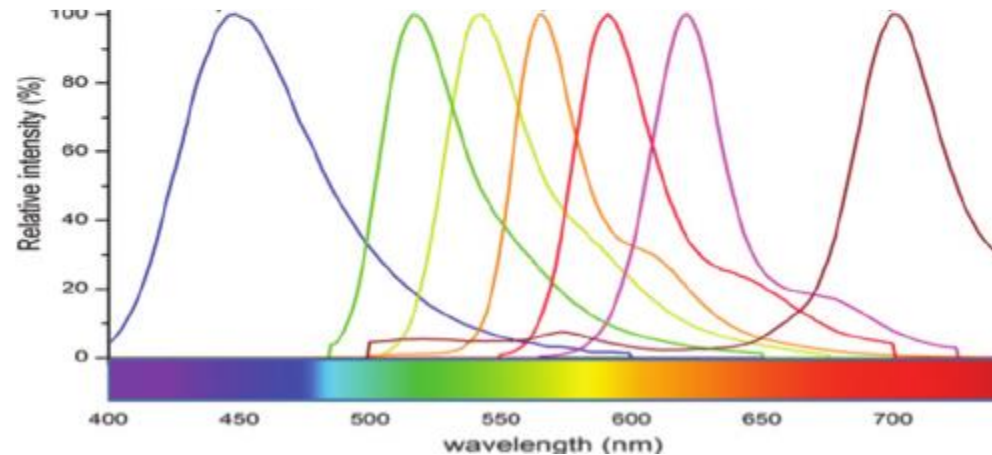
What is this thing?



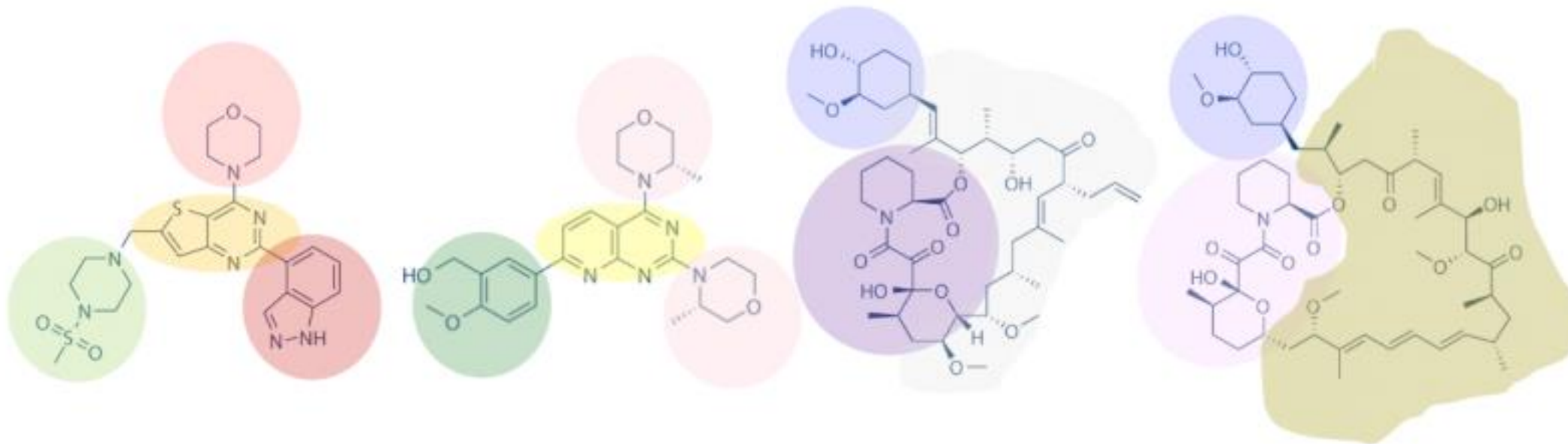
Real?

532 nm and 635nm are not terribly far apart

Fake?

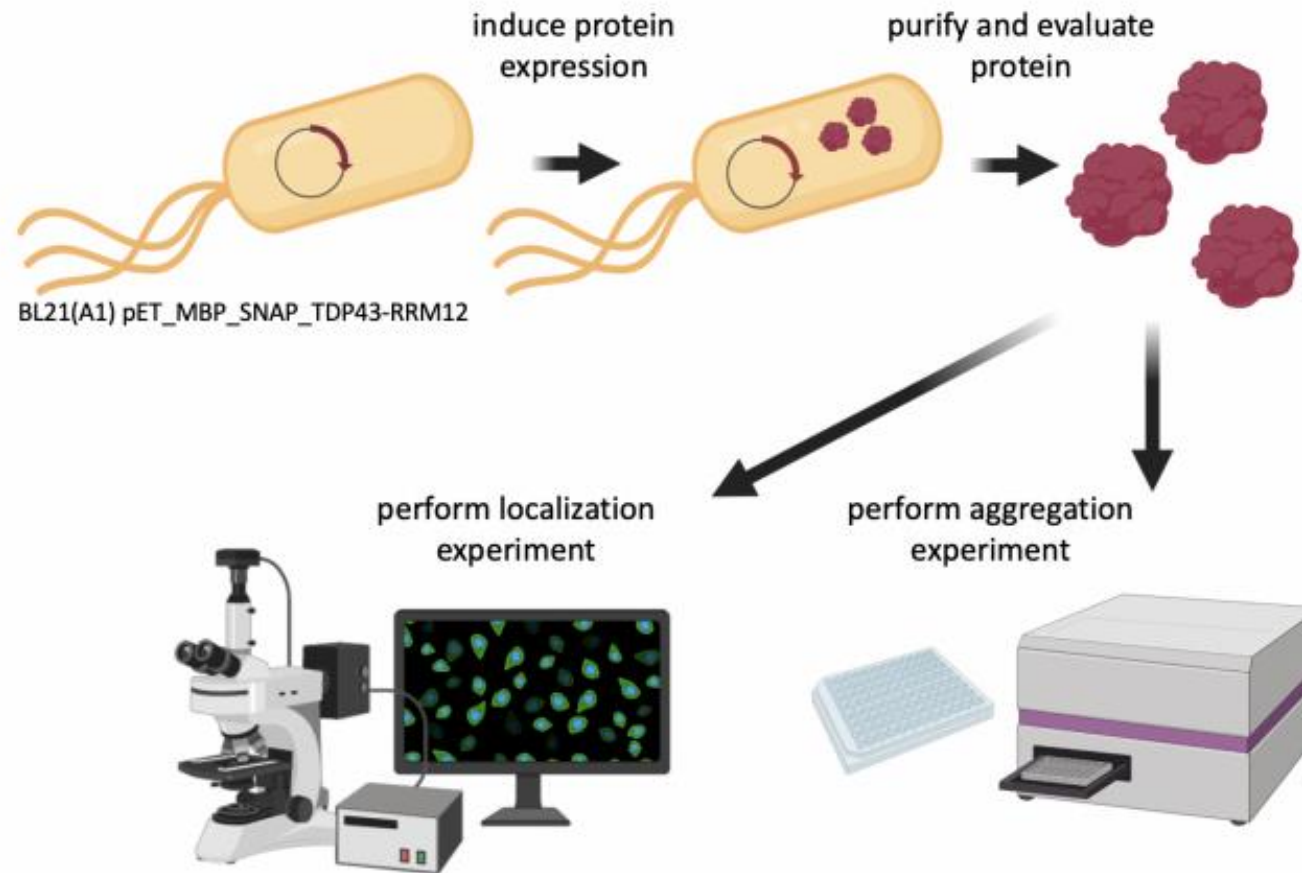


How will you identify common structures?



Overview of Mod1 experiments

Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology



For Today

- Work through SMM analysis procedure
- Evaluate chemical structures of identified hits
- Discuss reading of scientific papers with Noreen
 - Group 1: Blue, White, Purple, Pink
 - Group 2: Green, Yellow, Orange, Red

For M1D3

- Begin thinking about Background and Motivation for Data Summary
 - Submit document answering questions on the Homework section of wiki
 - Due Thursday, Feb. 10 at 1:05pm on Stellar
- Visit Comm Lab by M1D5
 - Can visit to discuss an assignment from any class, a personal statement for an internship application, etc...