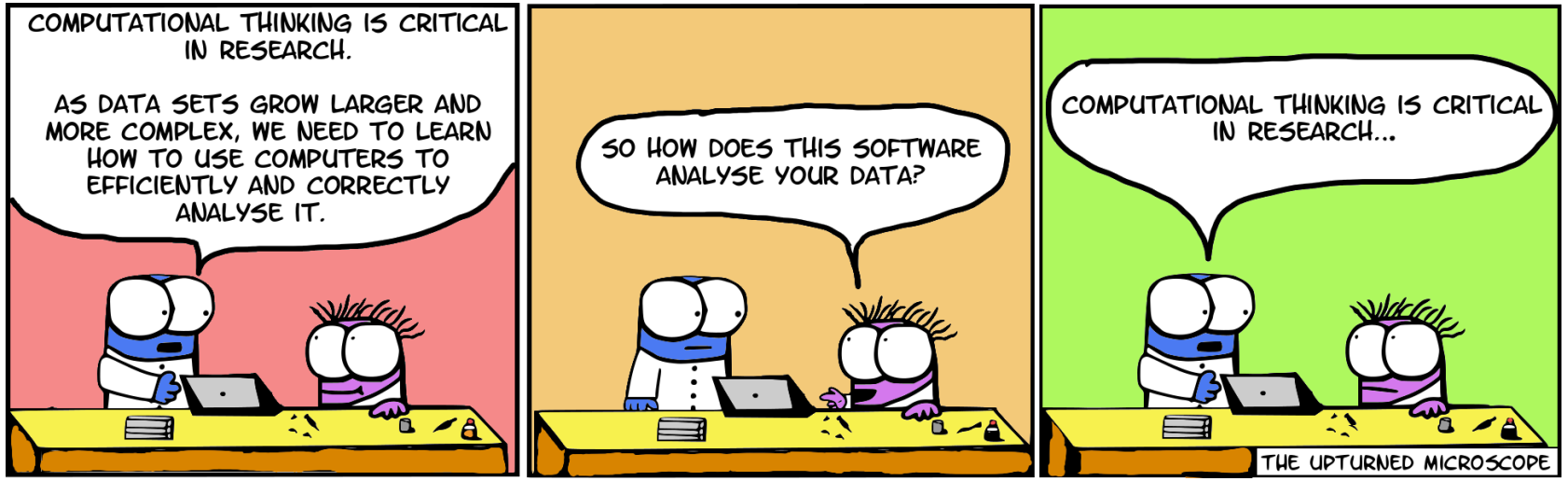
M2D1: Examine SMM data collected using TDP43 protein

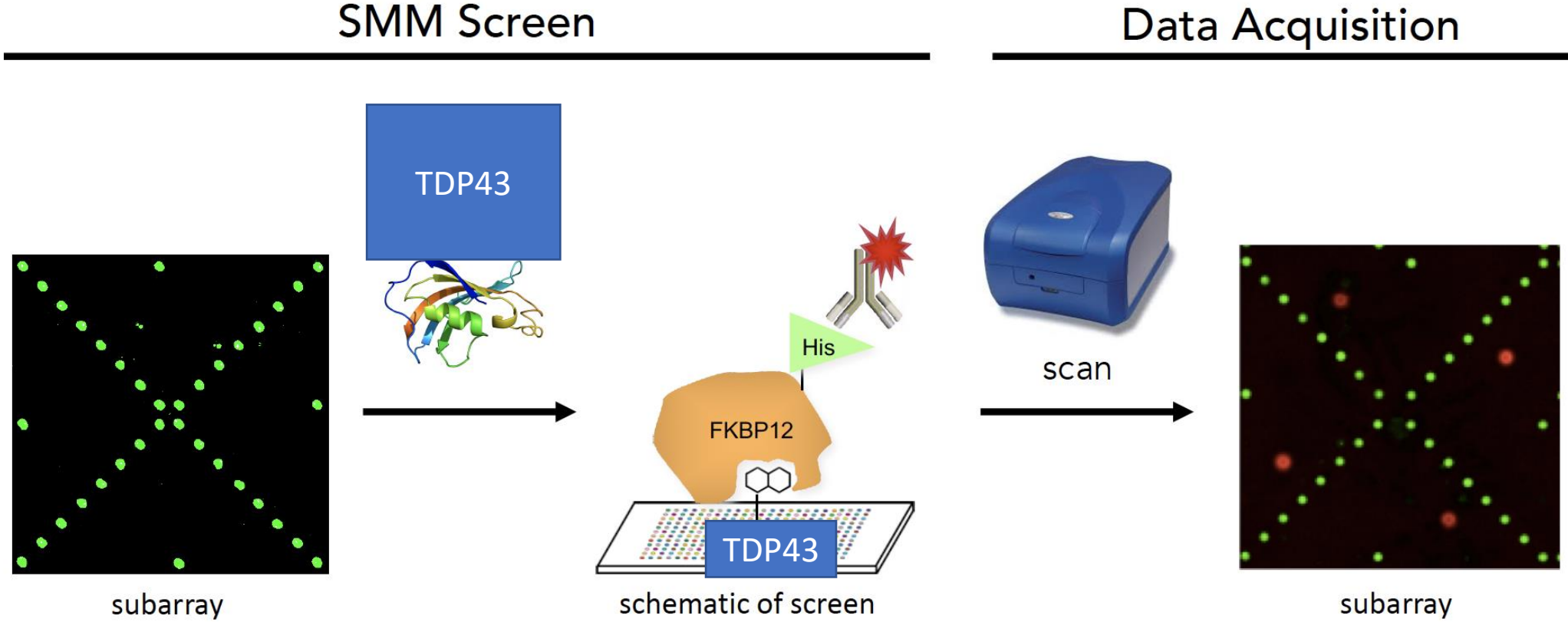
1. Prelab
2. Walk through SMM analysis
3. Examine chemical structure of hits
4. Discuss journal article

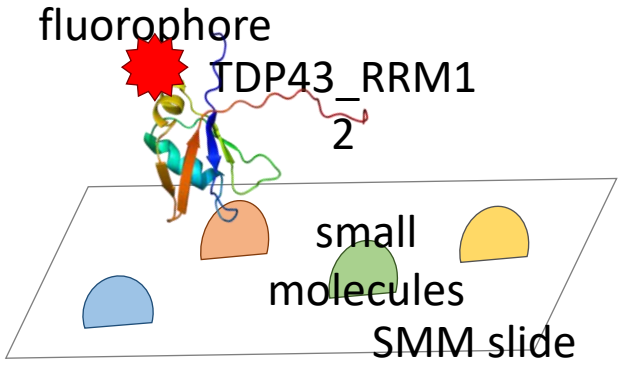
Office Hours:
Monday: 1-2pm @ 16-319 and Zoom Becky
3-5pm @ 16-317 and Zoom Noreen
Tuesday: 10-11am @ 1-390 Becky & Jamie
Thursday: 10-11am @ 1-390 Noreen & Jamie

*After lecture by request
*Also available by appointment



SMM workflow

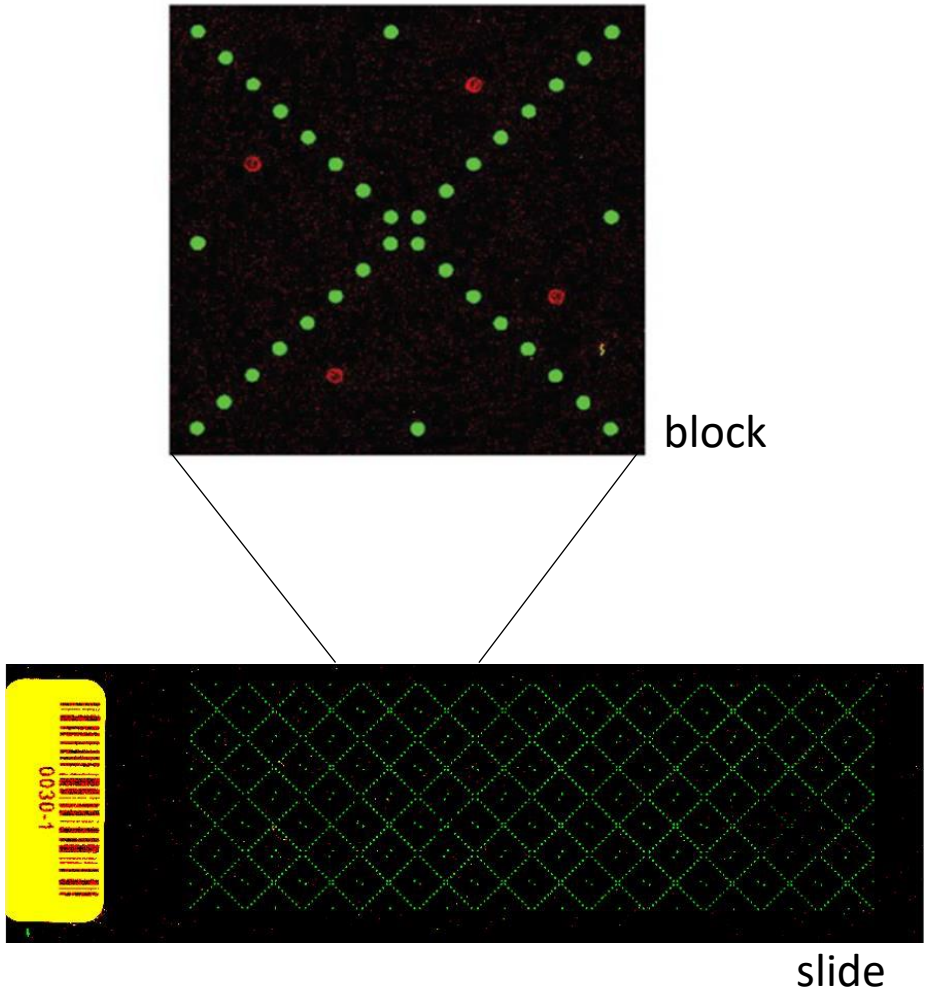




B. Leifer, S. Doyle

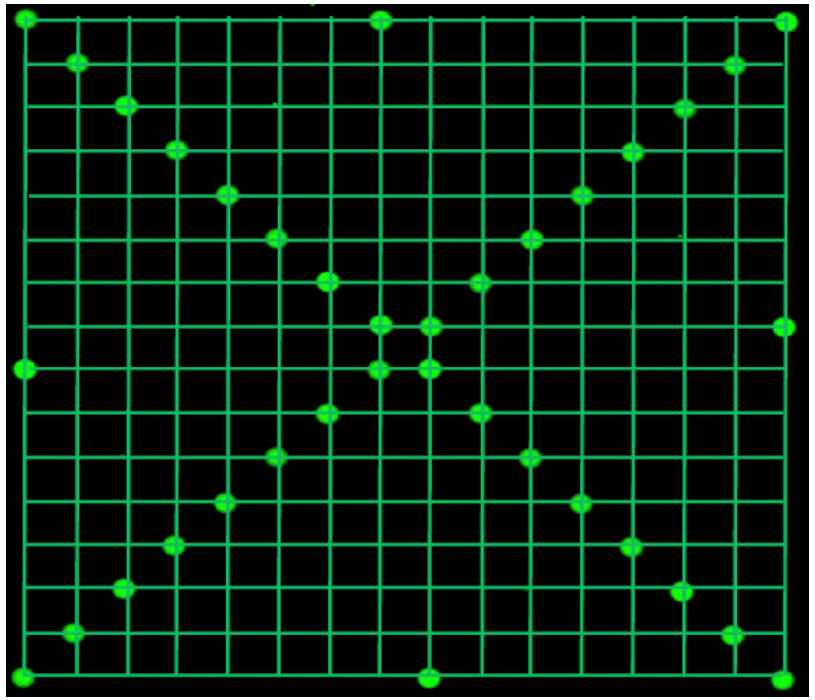
Workflow for SMM data analysis

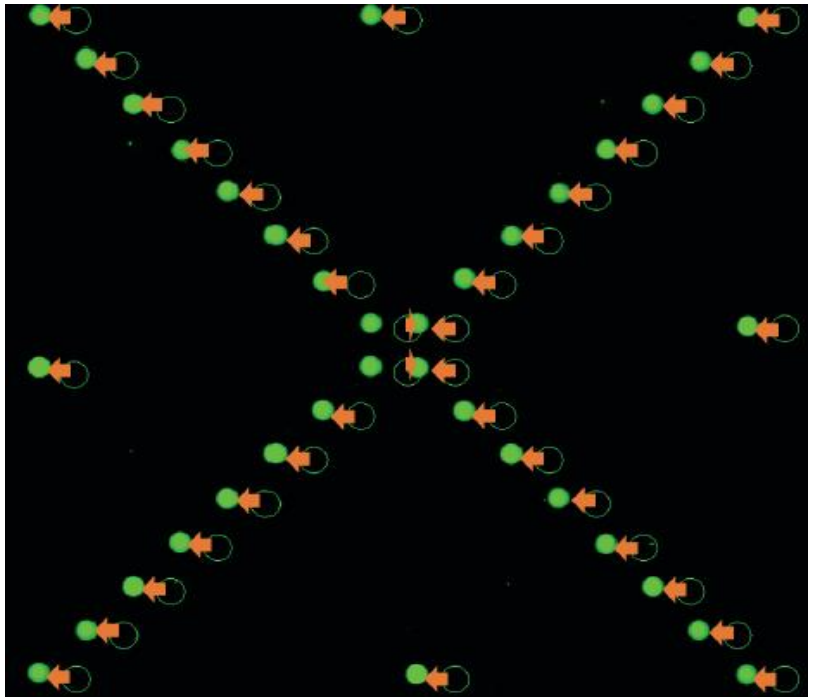
1. Align spots using fluorescence on 532 nm channel (sentinel spots)
2. Quantify fluorescence on 635 nm channel
3. Identify ‘hits’ with improbably high fluorescence
4. Complete ‘by eye’ analysis of putative hits to manually remove false positives



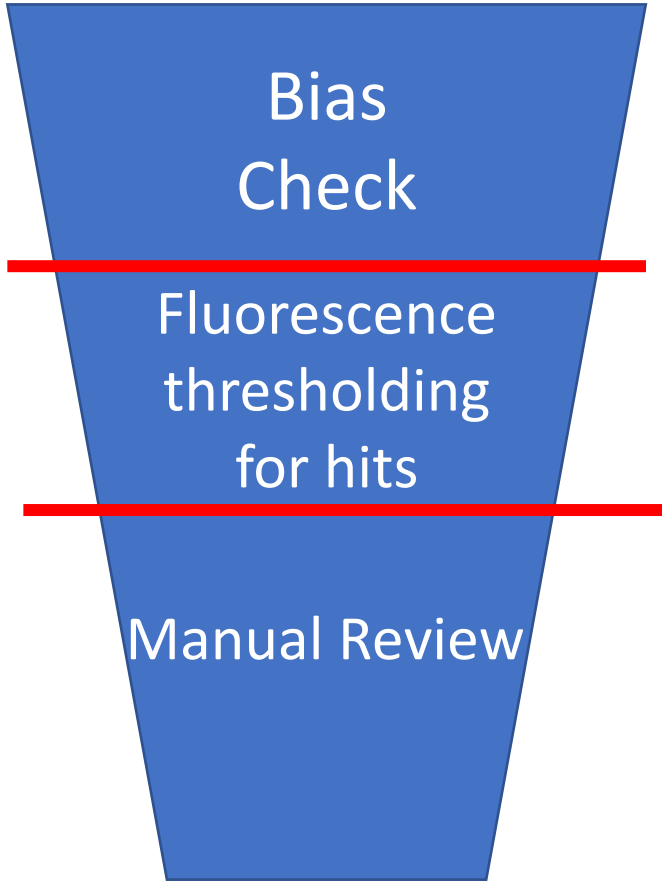
Align SMM using sentinel spots

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

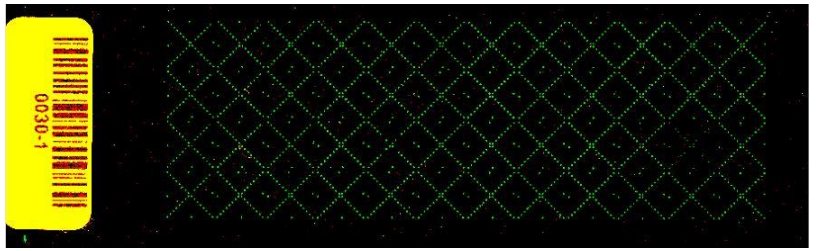


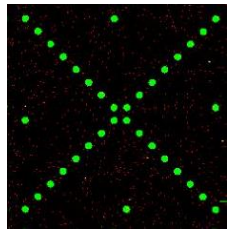


Refining your hits

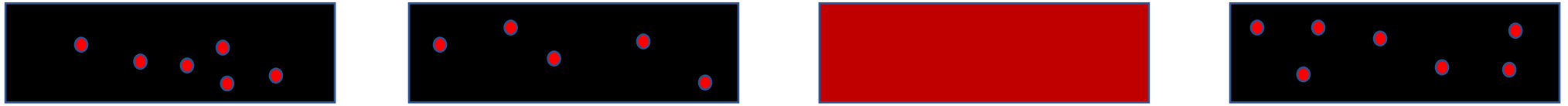


What might bias look like?





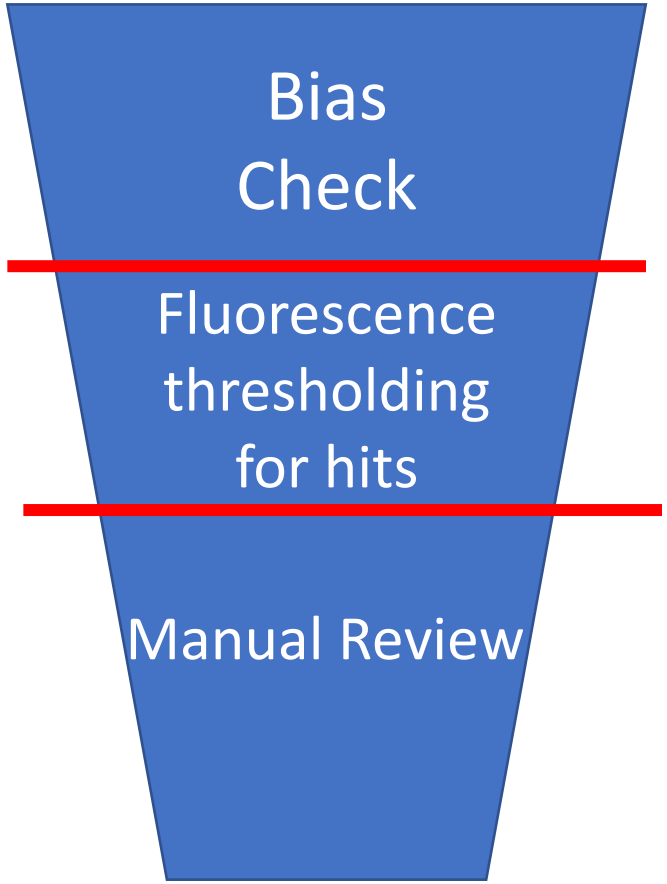
1) Bias across slides



2) Bias within Block

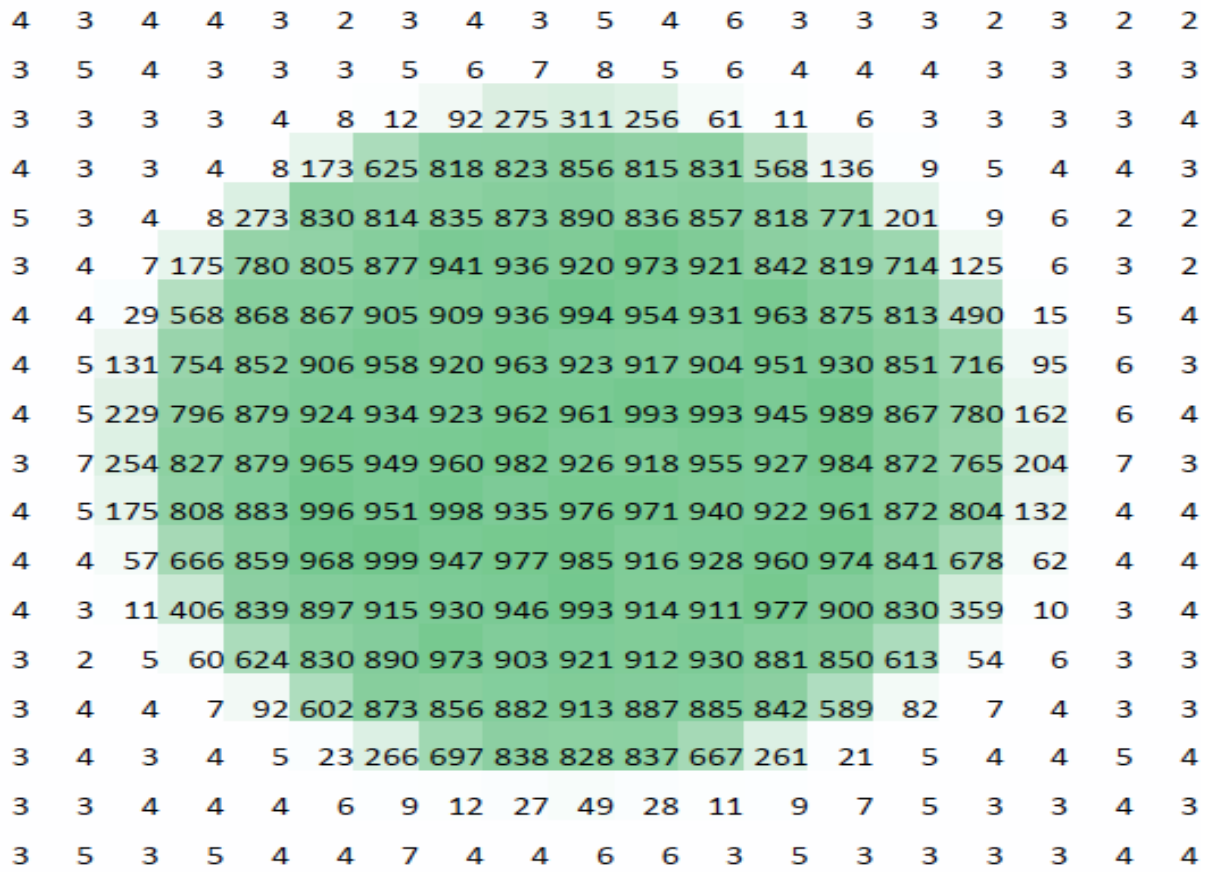
3) Bias within Slide

Refining your hits



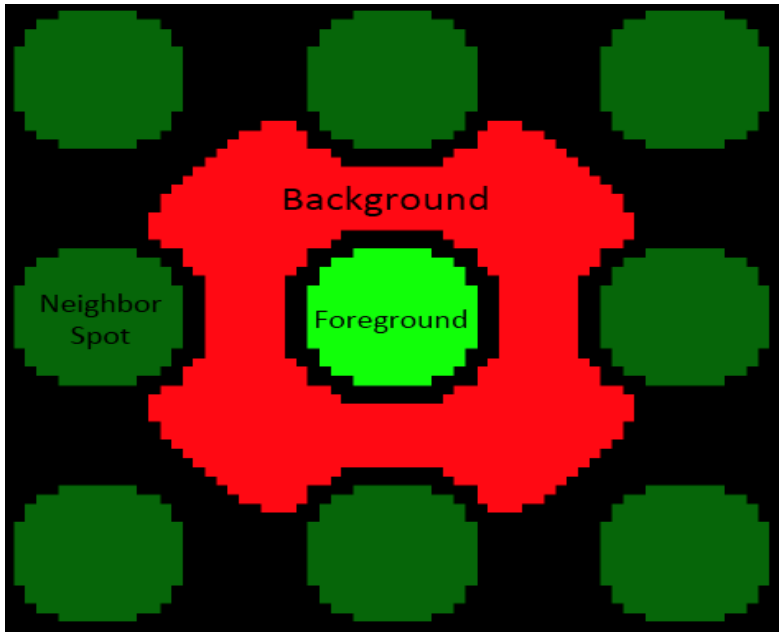
Spots are represented by an array of numerical values

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



Fluorescence is quantified to identify hits

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

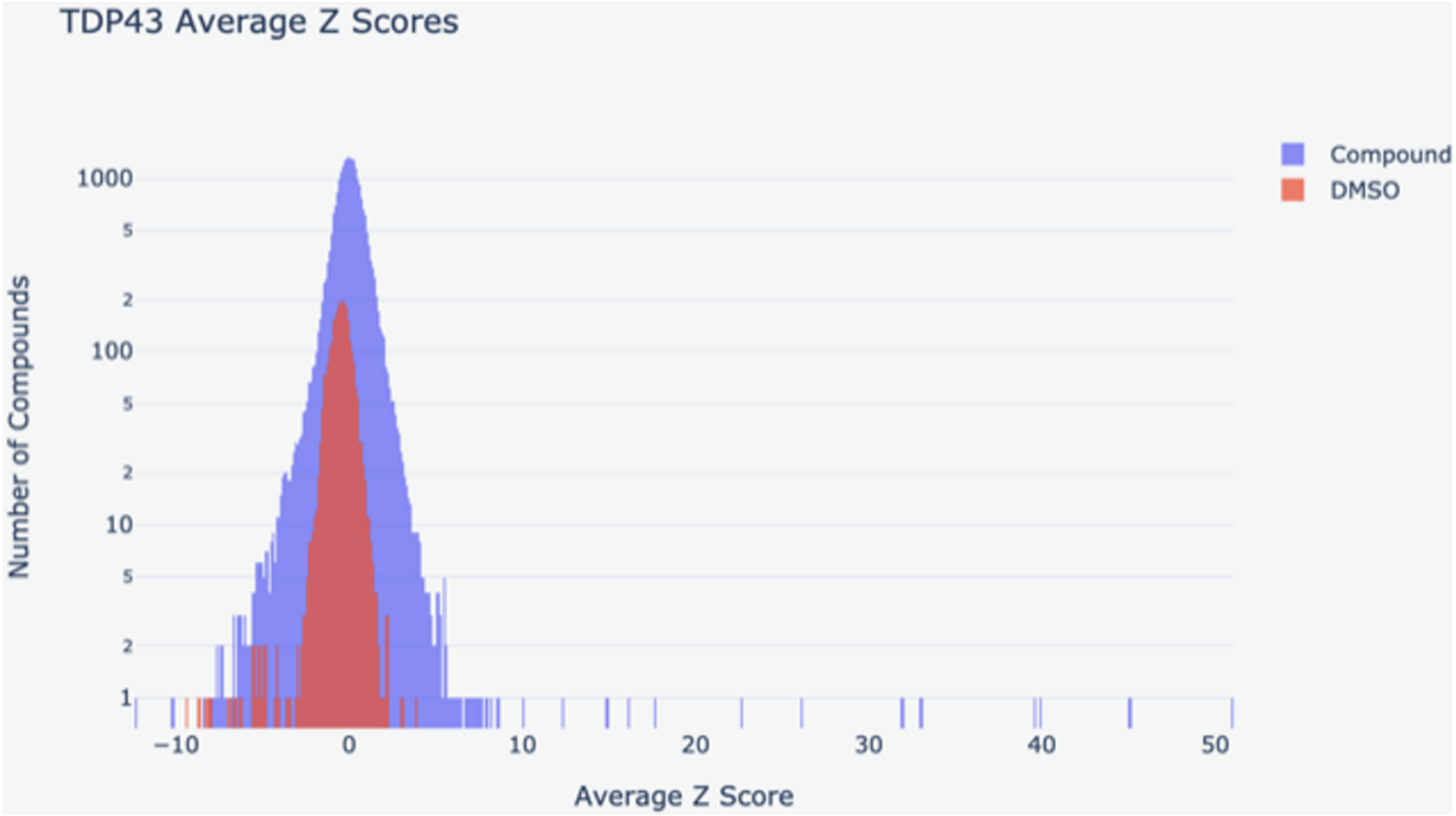


$$\text{Signal-to-noise ratio (SNR)} = \frac{\mu_{foreground} - \mu_{background}}{\sigma_{background}}$$

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

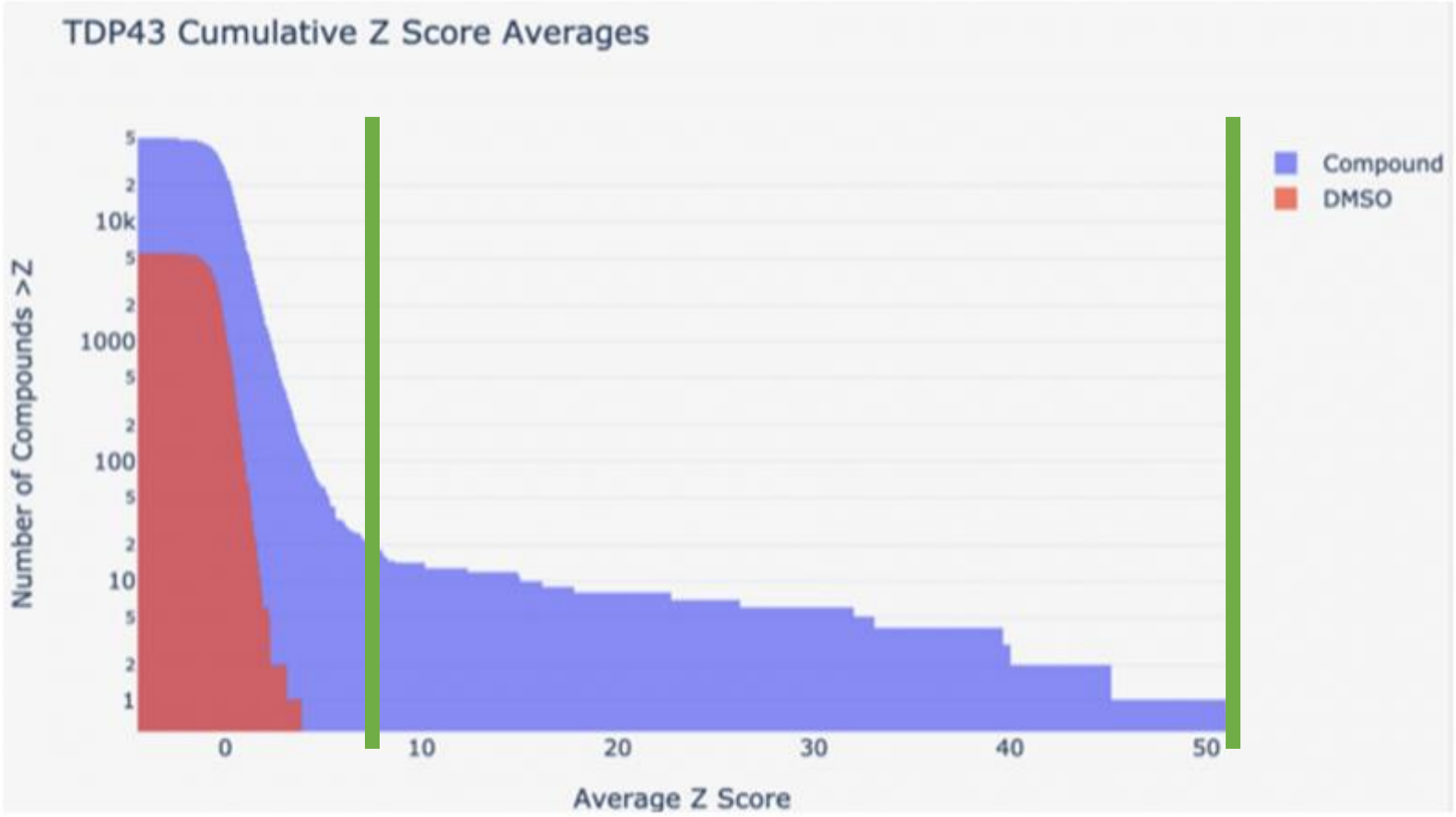
Average Z-score calculated for all compounds



Replicate spots averaged

Each count = unique compound

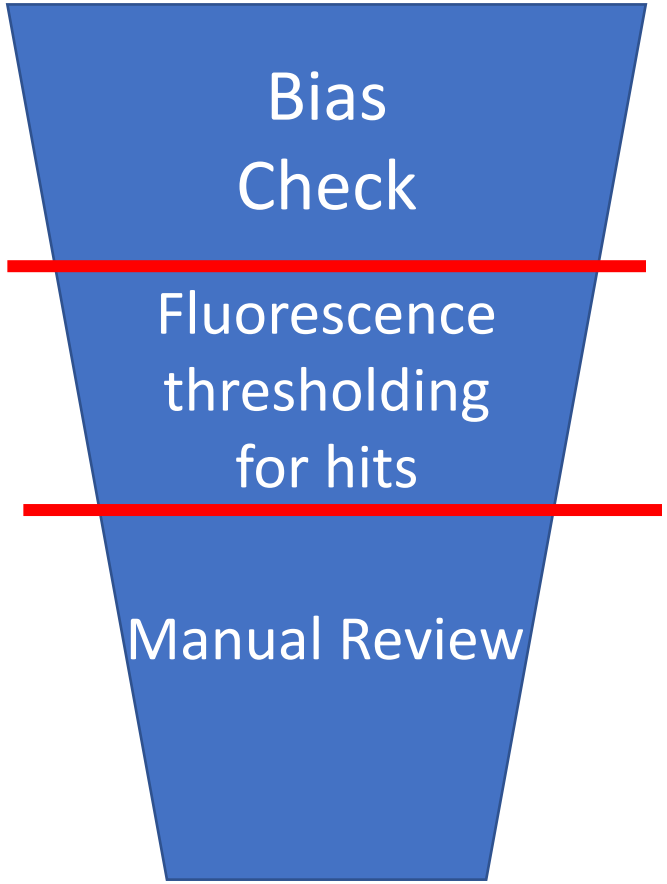
How many compounds have a particular z score?



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

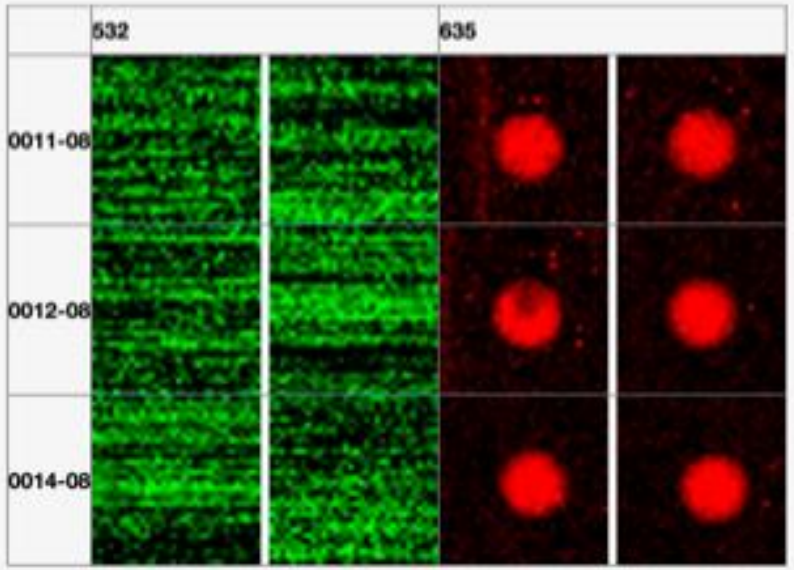
- Useful for setting a threshold to exclude likely non-binders

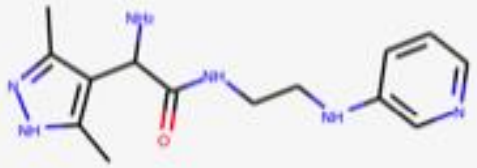
Refining your hits



How do you validate hits manually?

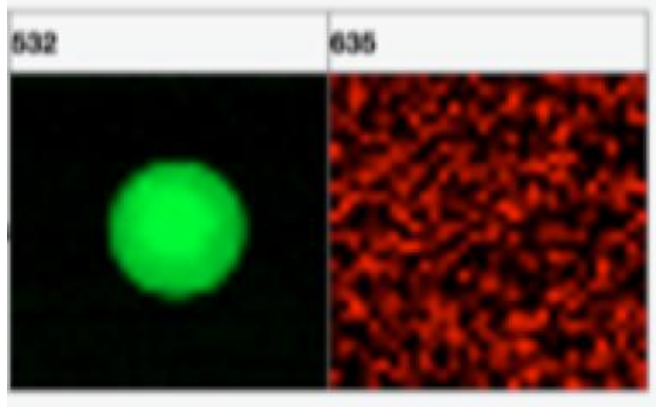
	ID	Robust Z	SMILES	Validated
49592	13:KI0001...	51.03151	C[C@H](C...	-1
42089	11:KI0001...	45.09263	CC1=C(C(...	example
6782	02:KI0001...	39.91118	CCNC(=O...	-1
29108	08:KI0001...	39.59436	C1C(C2=...	-1
44736	12:KI0001...	33.03555	C1CN(C2...	-1
29660	08:KI0001...	31.94118	CC1=NC2...	-1
11360	03:KI0001...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

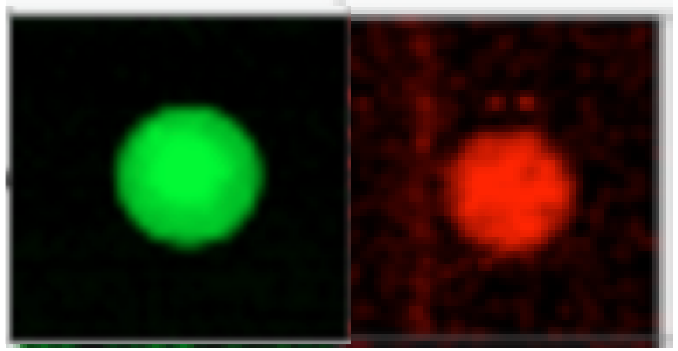




<- Positive Hits

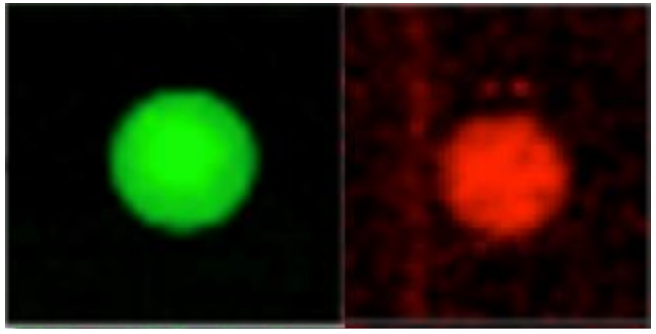
Sentinel Spot





???

What is this thing?

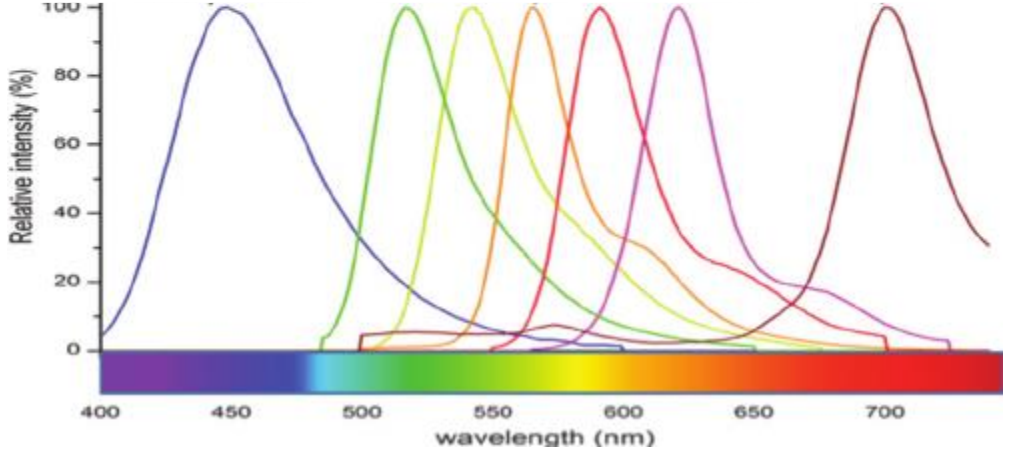


1) Real – your SM maybe is a 532 autofluorescer and the 635 is a real alexa fluor
2) Not real – your SM is an autofluorescer with a broad emissions spectra

Real?

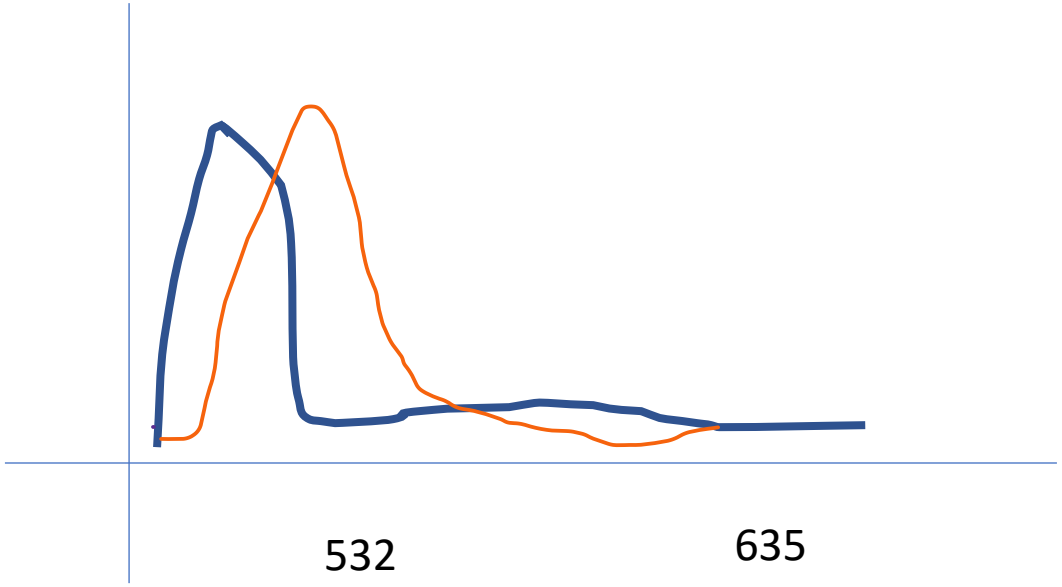
Fake?

532 nm and 635nm are not terribly far apart

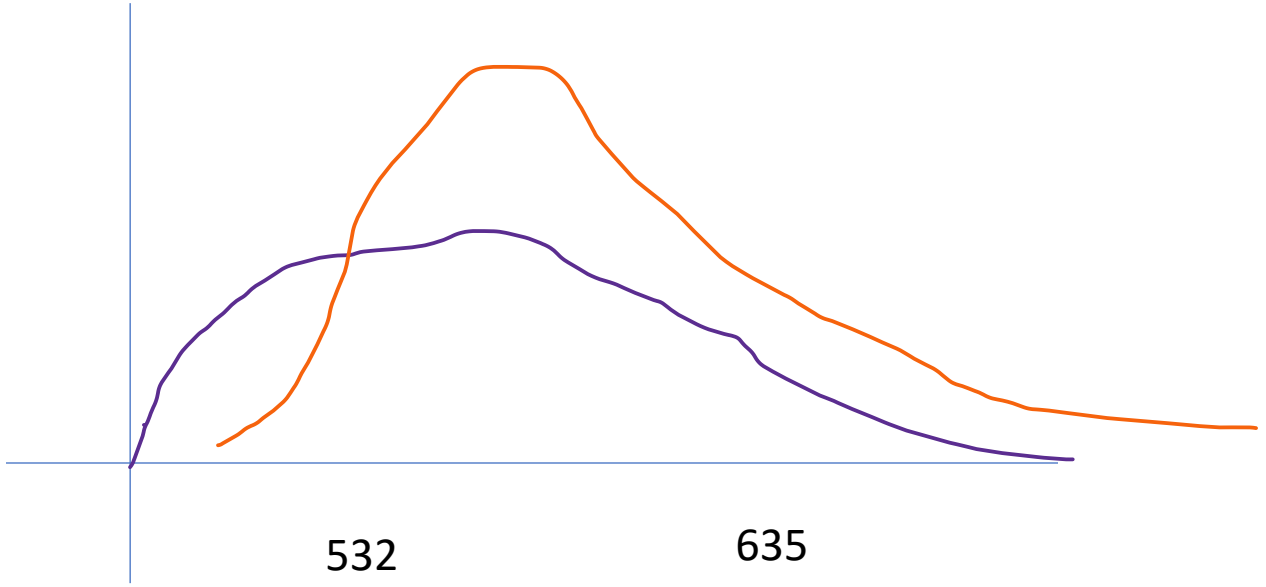


Real: your SM is a 532 fluorescer and 635 signal is from real bound alexa fluor

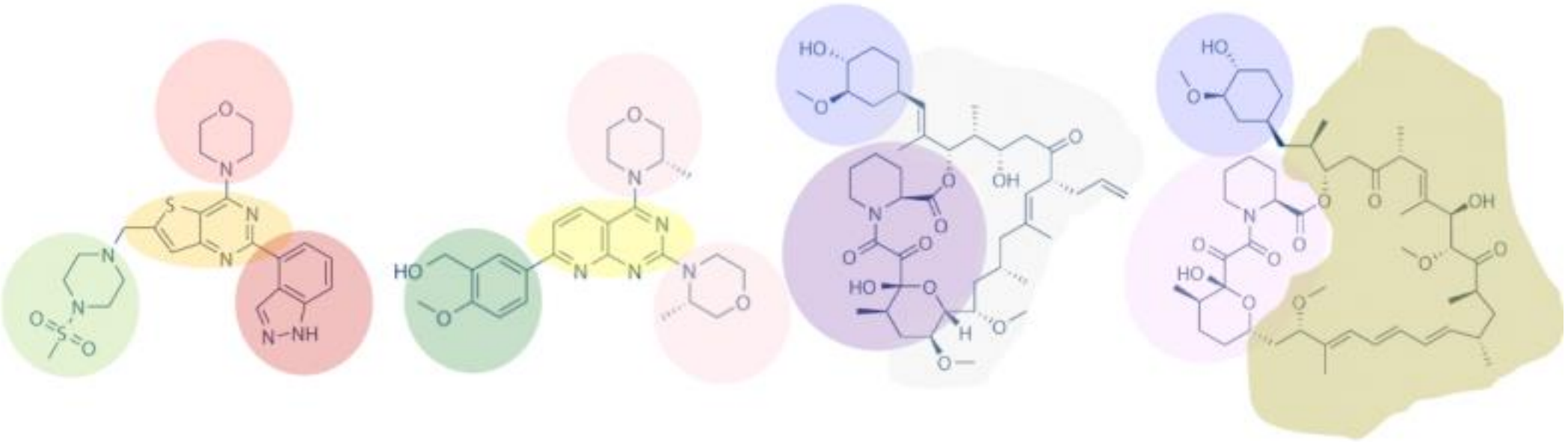
Excitation
Emission



Not real: Your SM has a broad excitation and emissions spectra and is fluorescing in both channels

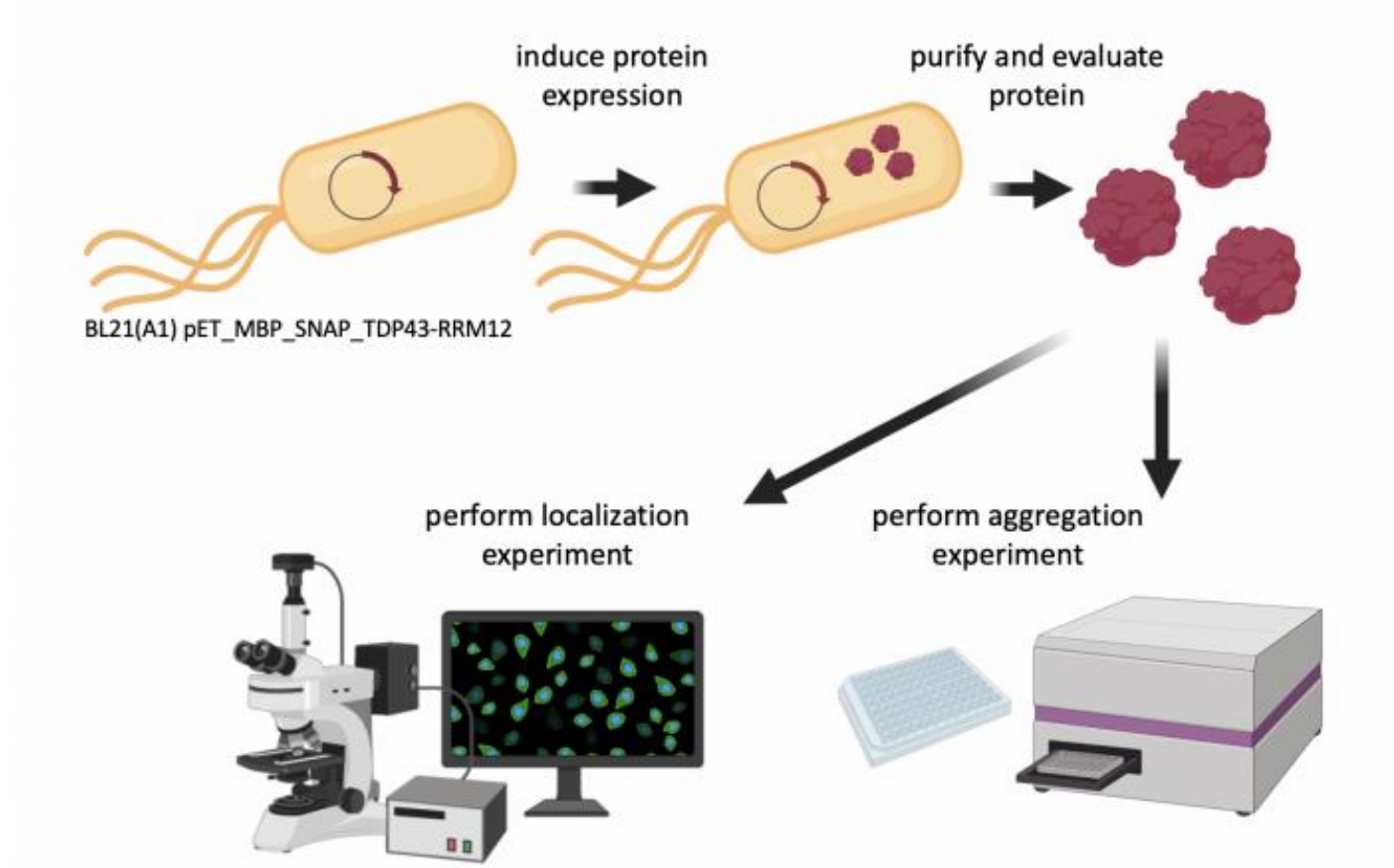


How will you identify common structures?



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 Friday, 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…