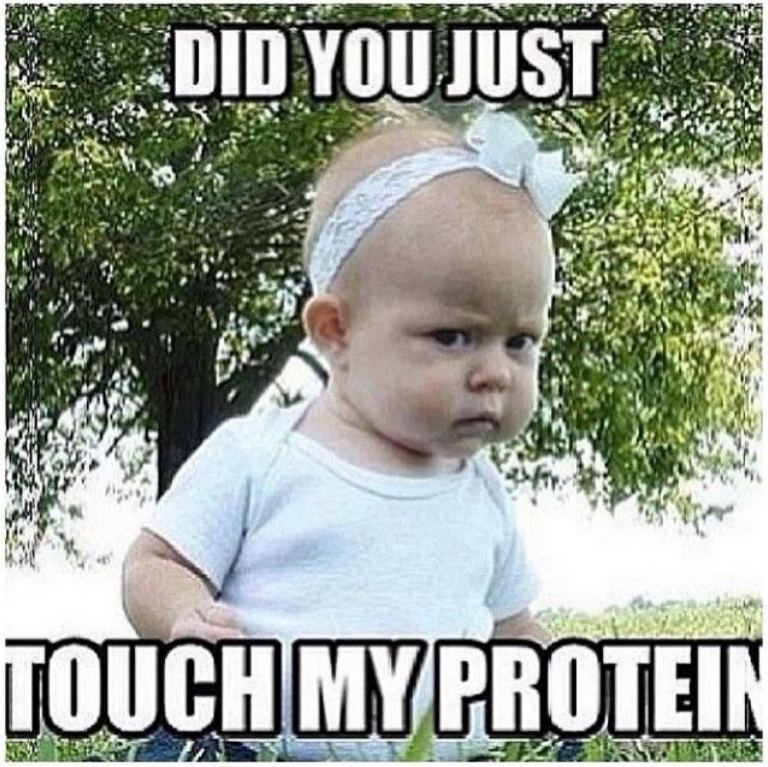
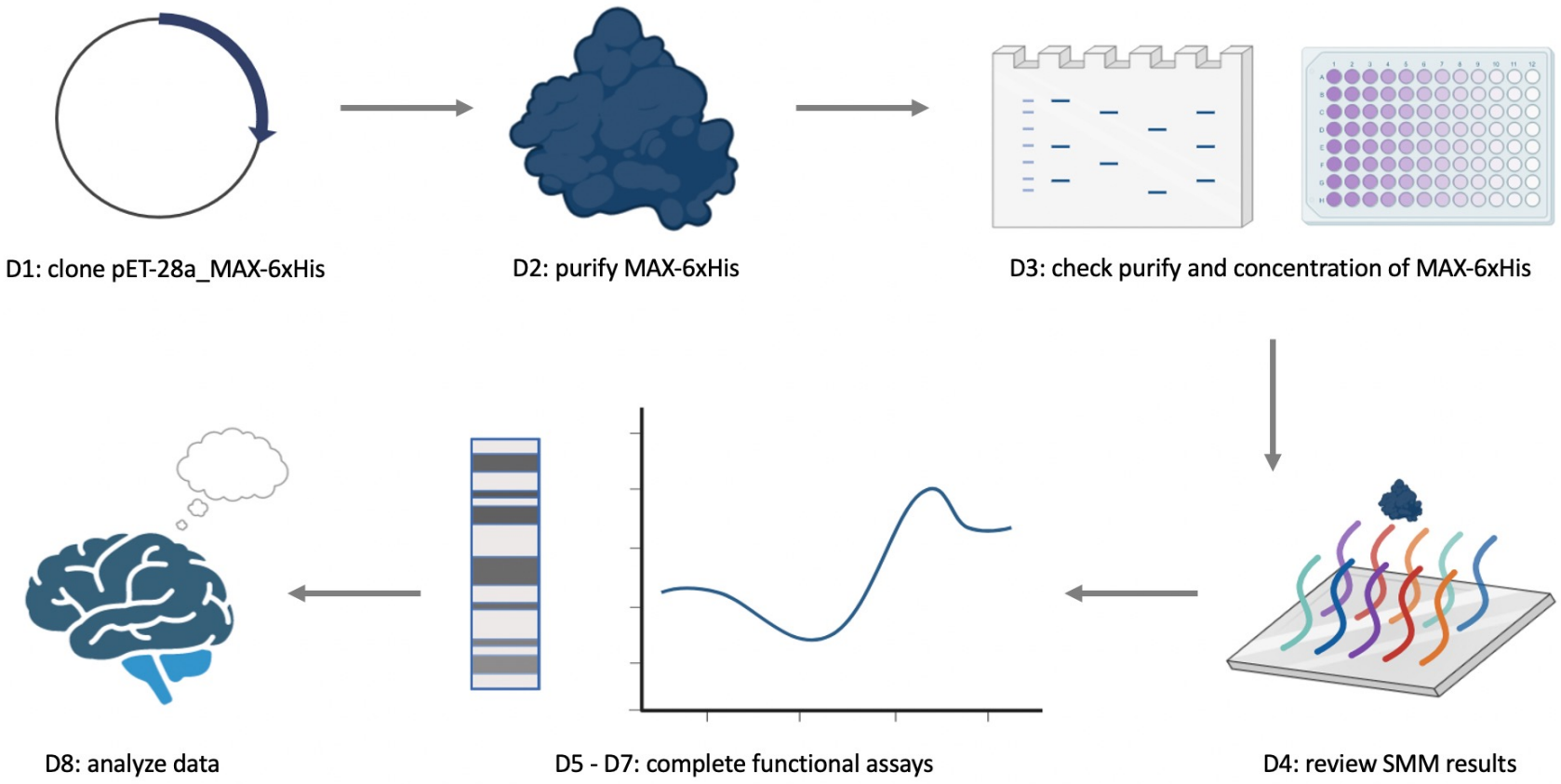
M1D4: Review results of small molecule microarray (SMM) screen

1. Comm Lab workshop
2. Review results of SMM screen
3. Choose small molecules for secondary assays

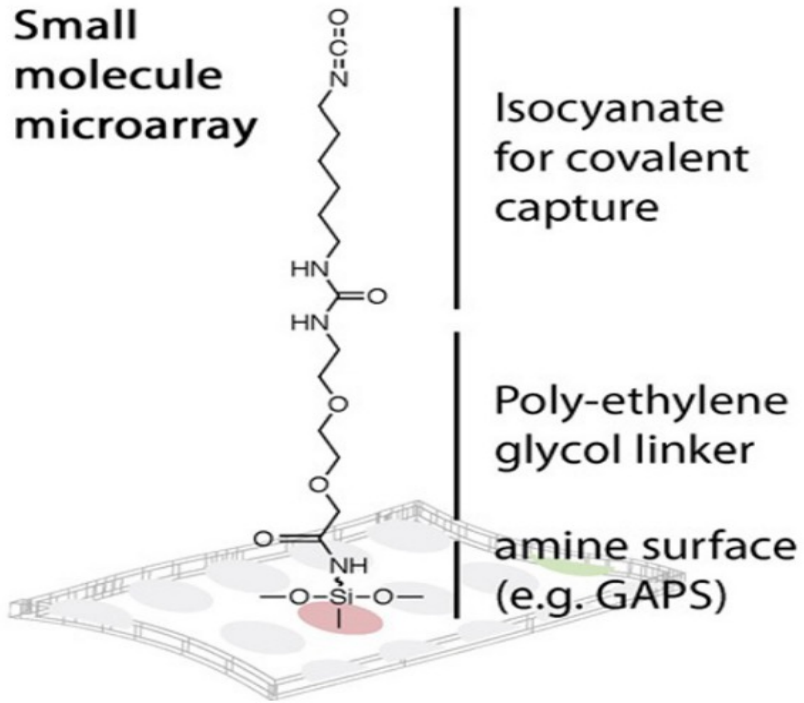


Overview of Mod 1 experiments:



How are SMM slides prepared?

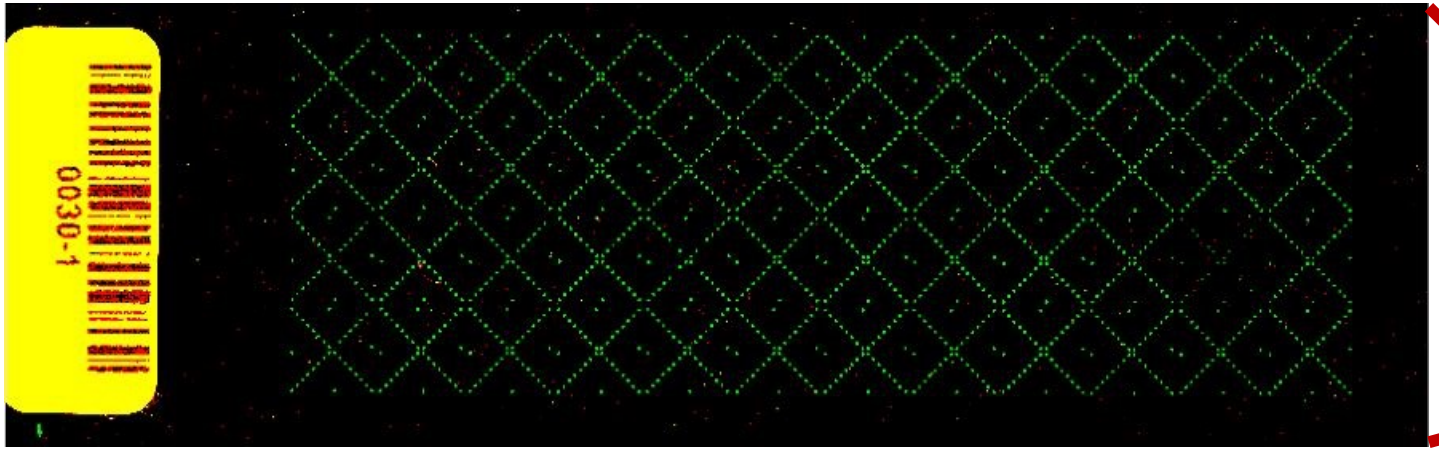
- Gamma-aminopropylsilane (GAPS) slide coated with polyethylene glycol (PEG) spacer
- PEG coupled to 1,6-diisocyanatohexane to generate isocyanate-functionalized slide
- Isocyanate able to react with nucleophilic functional groups

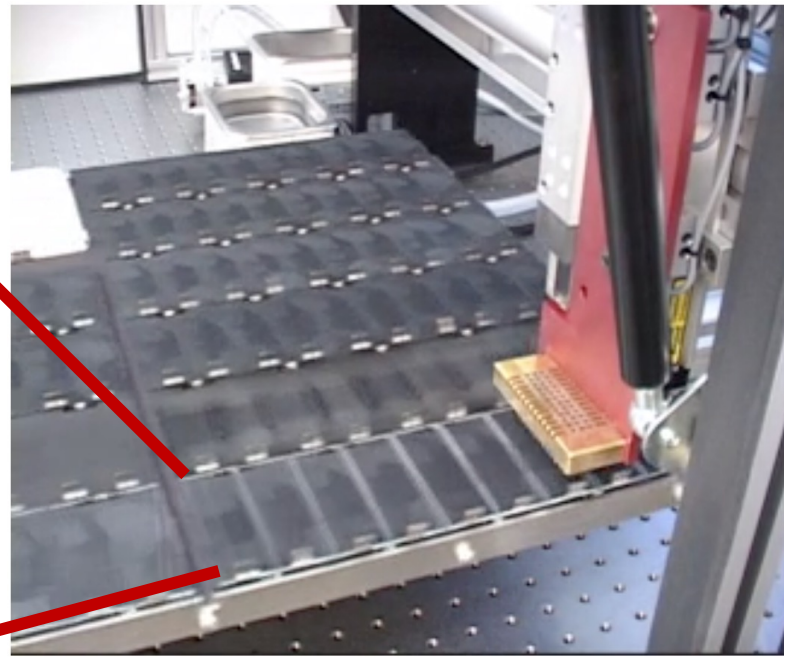


Leifer et al. Methods in Enzymology. 2018.

How are SMM slides printed?

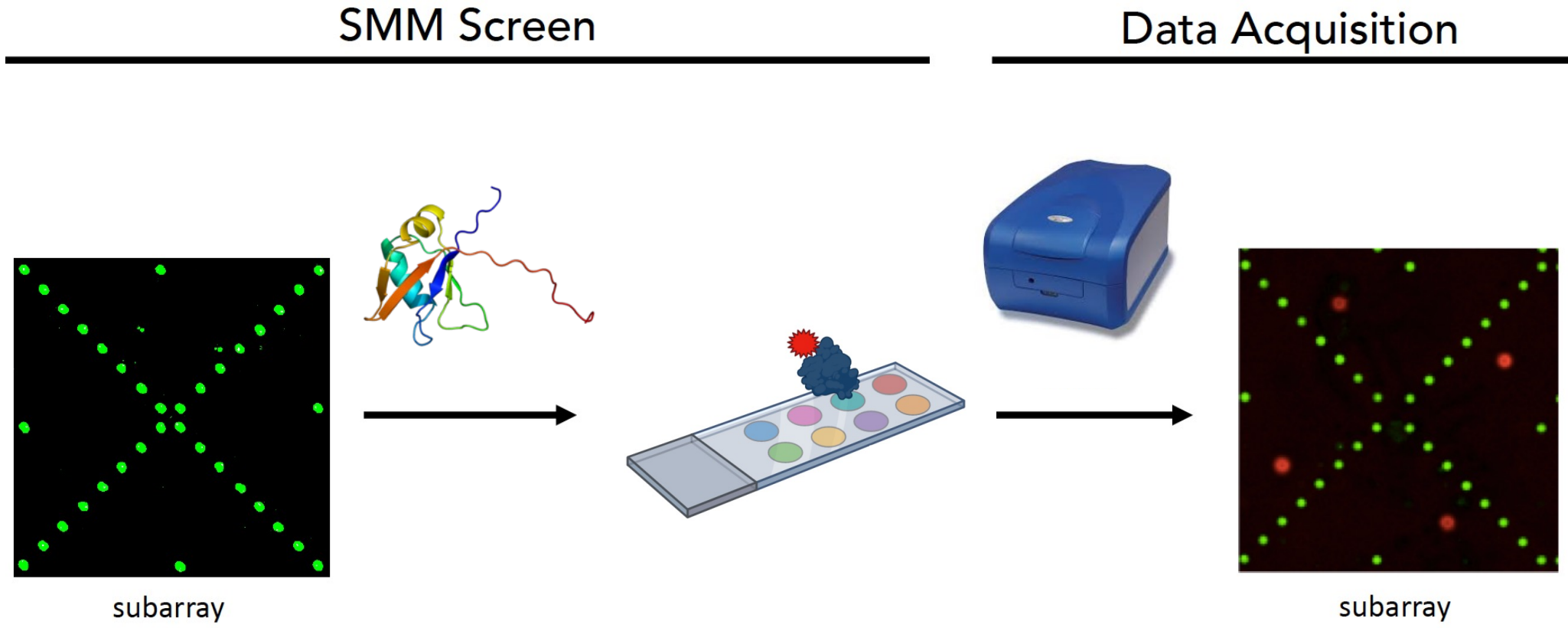
- Each slide contains ~12,000 spots
  - ~4,200 small molecules / ligands (printed in duplicate = ~8,400)
  - Fluorescein sentinel spots
  - DMSO negative control spots





Koehler Lab 2014 - Small-molecule Microarrays from Koehler Lab on Vimeo.

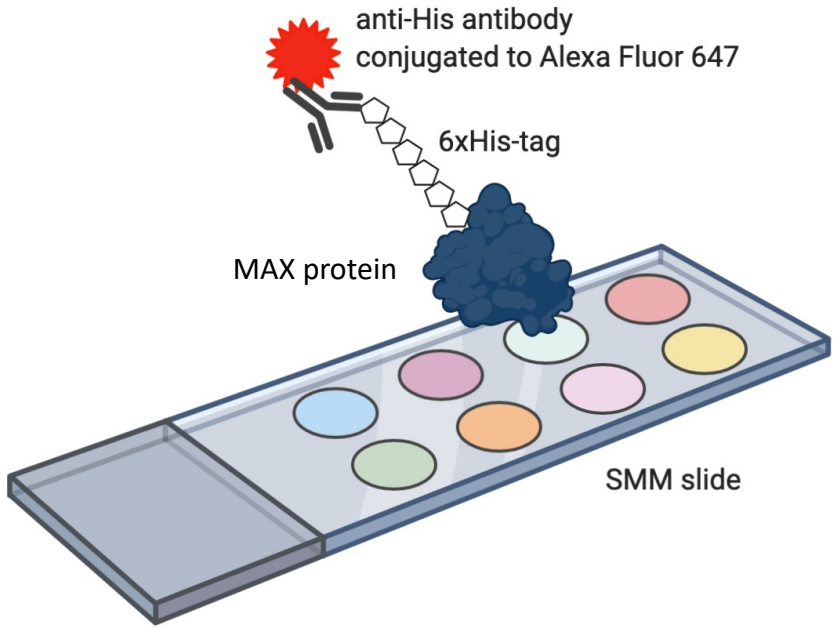
Workflow for SMM experiment



Modified from B. Leifer & S. Doyle

How did we screen for small molecules that bind MAX-6xHis?

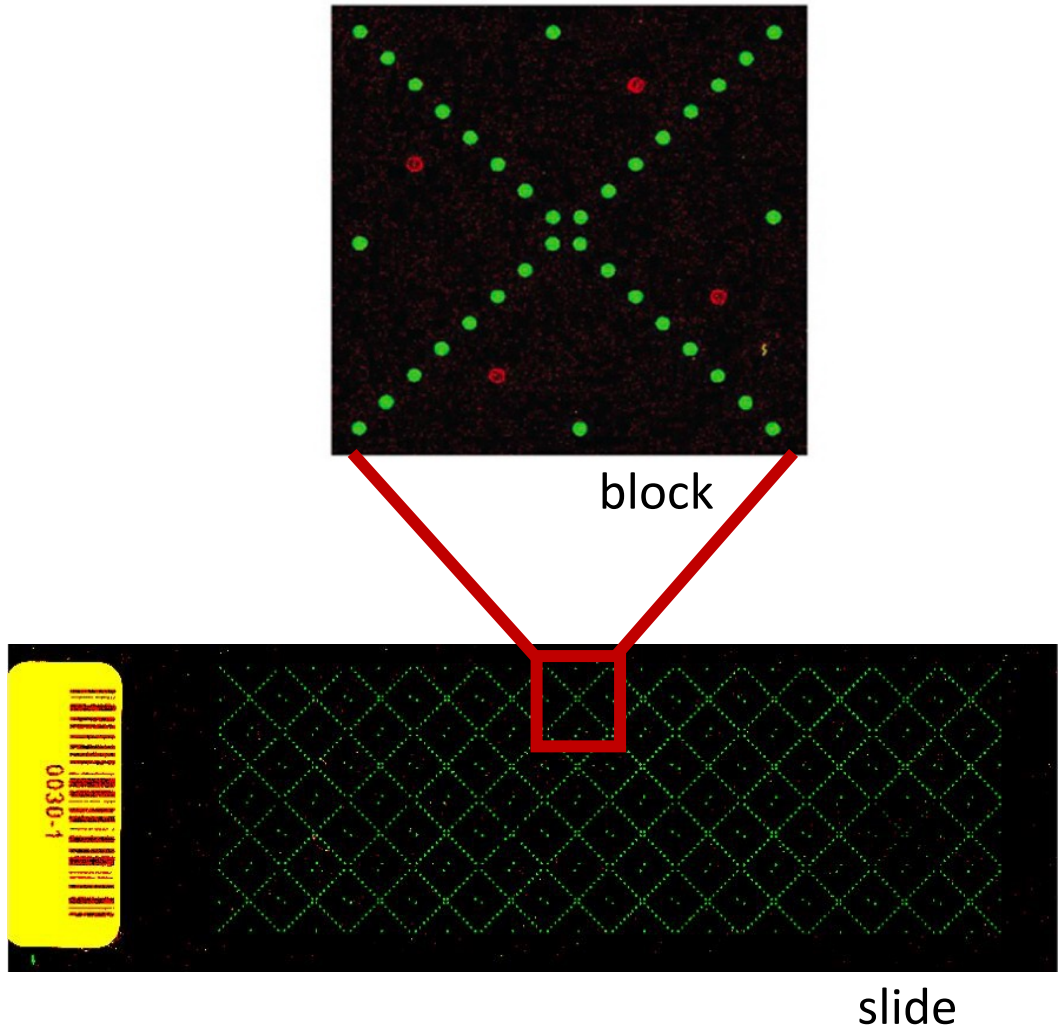
1. Incubate SMM slide with MAX-6xHis protein
2. Wash away excess protein
3. Incubate SMM slide with AlexaFlour 647 anti-His antibody



Is it problematic if proteins other than MAX-6xHis are present?

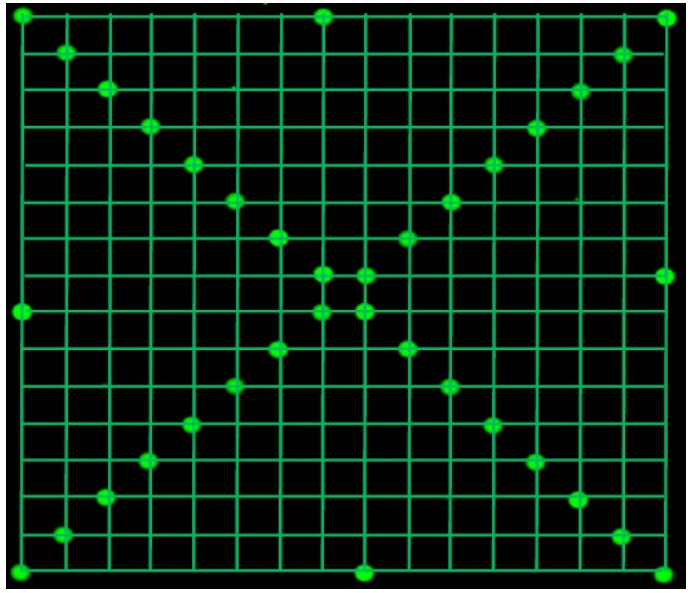
How will you analyze the SMM results?

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 identify false positives

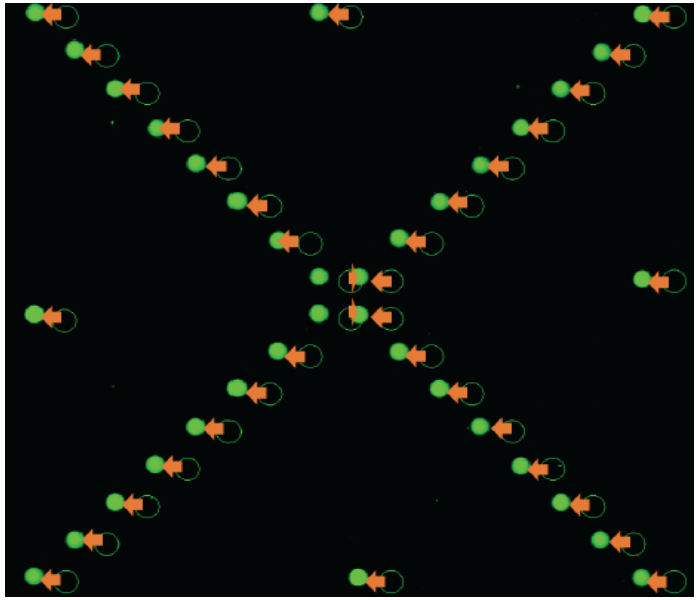


Sentinel spots are used to align the slides

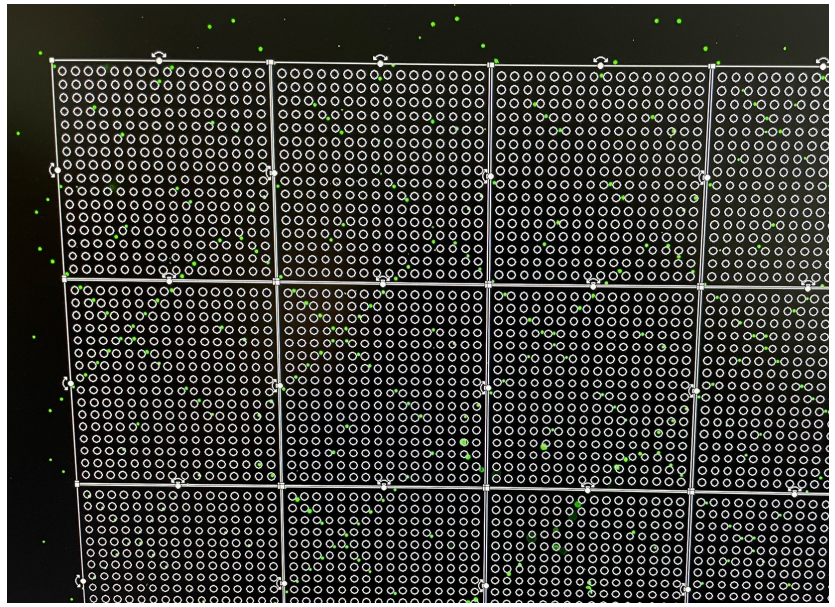
- Slides printed in blocks (16 rows X 16 columns)
- Each small molecule is identifiable via intersecting lines from sentinels



in theory



in ideal situation

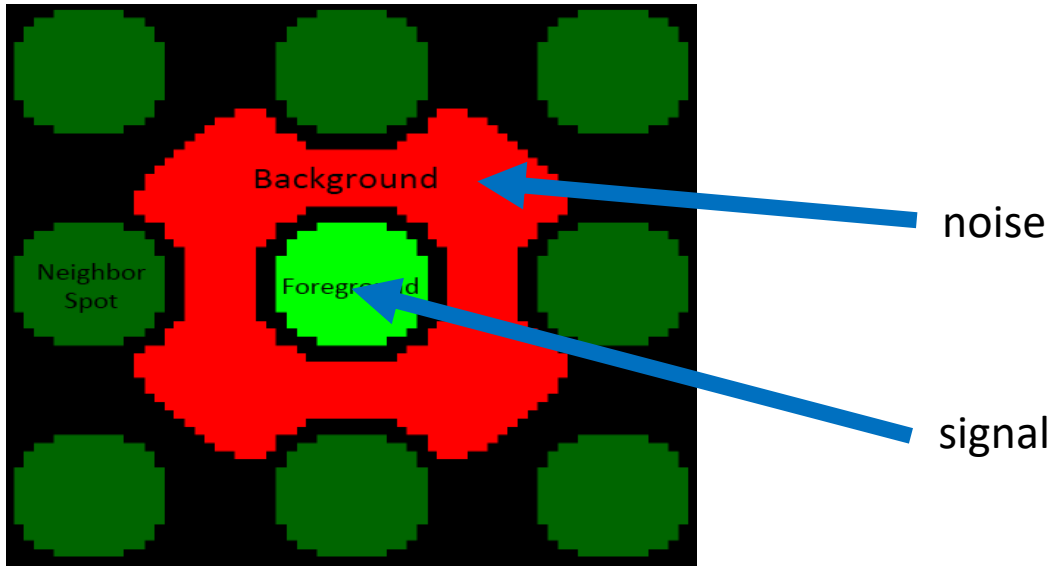


in practice

Fluorescence is quantified to identify ‘hits’

- Fluorescent signal from each small molecule spot is represented by an array of numerical values
  - Intensity of signal at each pixel is quantified
- Signal-to-noise ratio is calculated for each array

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



Significant fluorescence calculated as Z-score

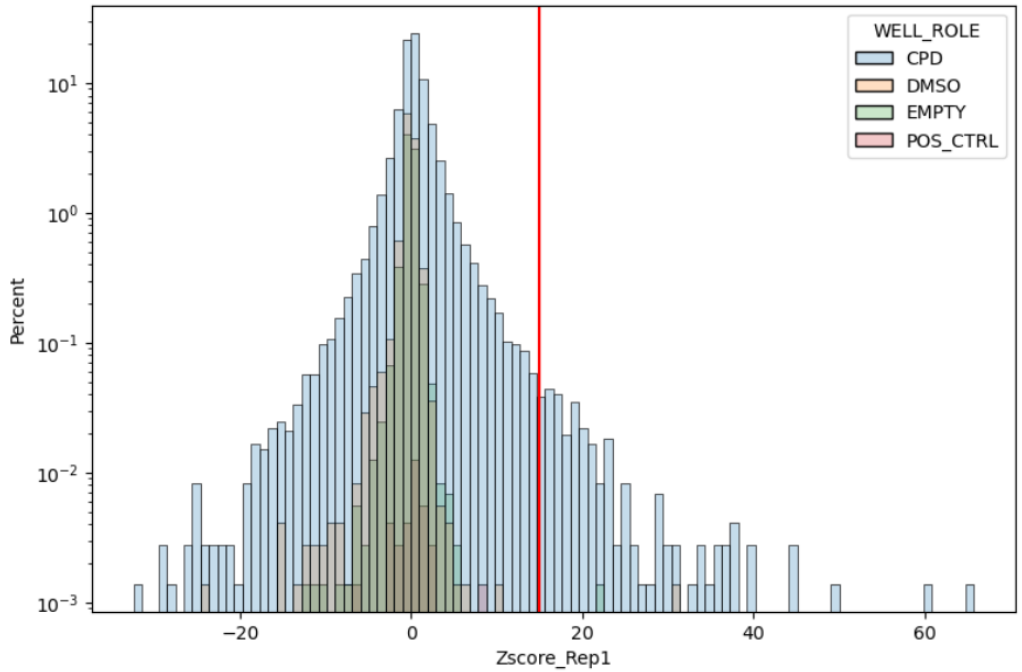
median absolute deviation (MAD)

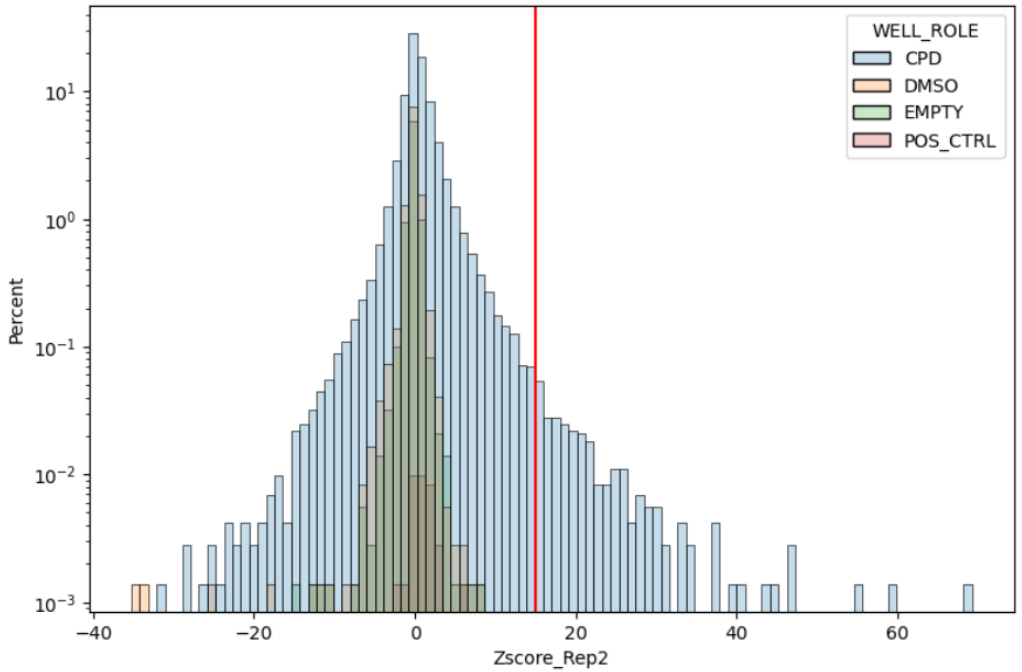
$$\text{Robust Z-score} = \frac{SNR_i - \text{median}(SNR)}{\text{median}(|SNR_i - \text{median}(SNR)|) * 1.48}$$

scale factor for the normal distribution

- Robust Z-scores help eliminate influence of outliers

Robust Z-scores used to set threshold for putative binders





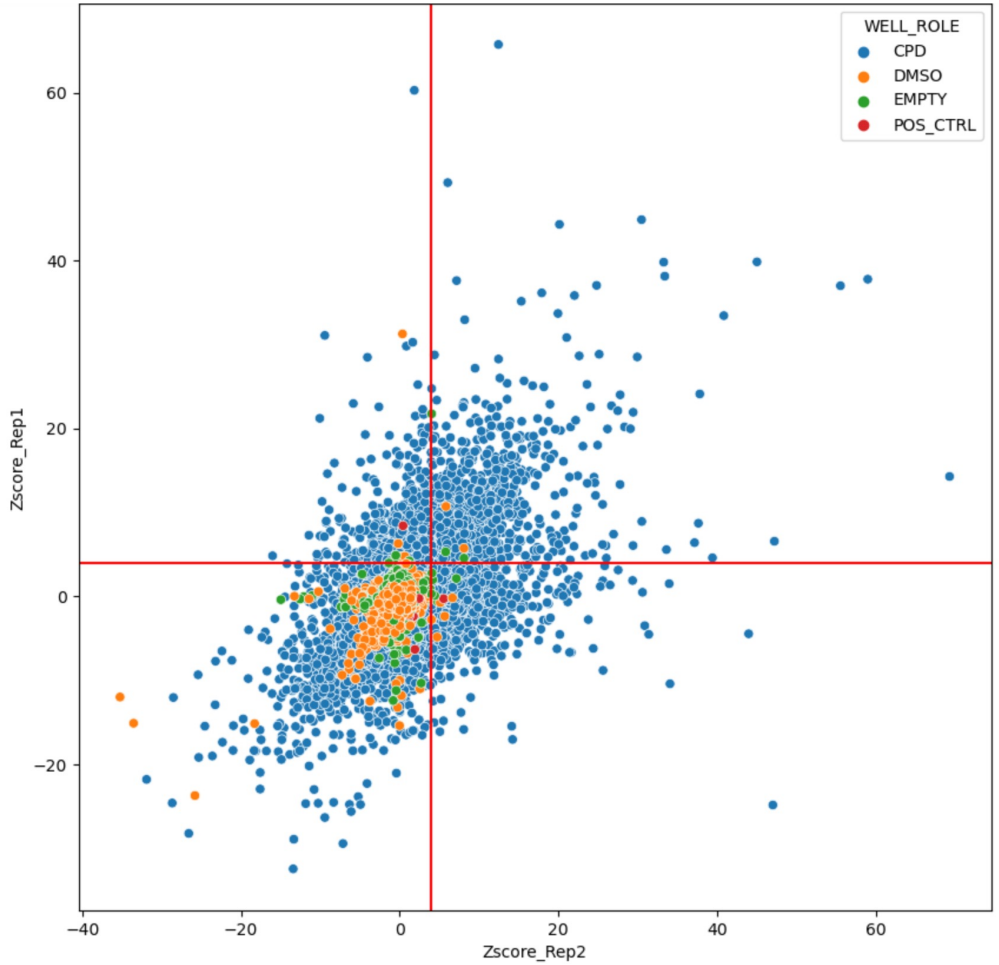
- Why are empty wells clustered at zero?
- Where do you expect to see putative binders?

Robust Z-scores used to compare consistency of replicates

- Linear relationship observed if replicate Z-scores are same

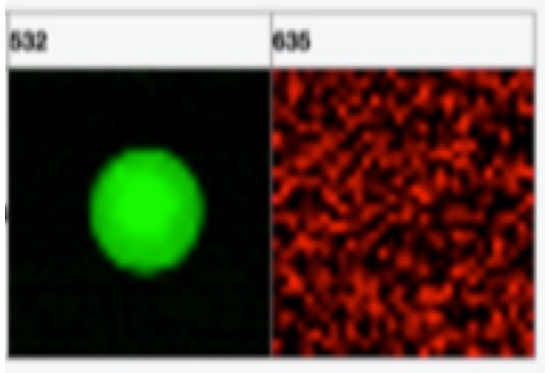
- What does it mean if replicates do not show a linear relationship?

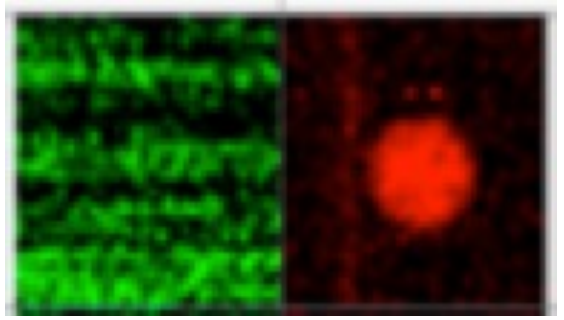
- Where do you expect to see putative binders?



‘By-eye’ analysis used to manually validate hits

Internal_ID	Zscore_Rep1	Zscore_Rep2	Slide_Rep1	Slide_Rep2	Loc1(block, row, col)	Loc2(block, row, col)
KI10167	37.029230	24.797054	50033718.0	50033720.0	2,2,3	2,7,11
KI10451	38.126523	33.380424	50033718.0	50033720.0	42,12,8	42,15,14
KI10796	16.684962	18.340951	50033718.0	50033720.0	45,10,16	45,12,4
KI10776	19.640085	18.046225	50033718.0	50033720.0	41,1,6	41,8,10
KI11103	16.929258	19.408047	50033718.0	50033720.0	42,1,12	42,6,8
KI11145	18.763623	15.206143	50033718.0	50033720.0	8,10,14	8,14,16
KI12064	25.653114	15.651485	50033718.0	50033720.0	41,11,4	41,15,5
KI20071	22.544108	24.043693	50033689.0	50033693.0	26,12,11	26,5,4
KI20165	25.236351	23.586604	50033689.0	50033693.0	4,2,3	4,7,11
KI20173	36.988660	55.511073	50033689.0	50033693.0	4,16,2	4,6,9





How will you choose which small molecules to test using secondary assays?

- Strategy: Identify common features present in small molecule hits
- Strategy: Consider the binding metrics from the SMM screen
- Strategy: Research the amino acid residues relevant in Myc:MAX binding
- Strategy: Choose you own adventure ☺

For today…

- Be sure to email which small molecules you want to test to me by 12p on Monday, February 26!
- Submit updated figure homework via email (nllyell@mit.edu) by 10p tonight!!

For M1D5…

- Write title and caption for SDS-PAGE and BCA figure
- Draft outline of script for Research talk

Notes on figure making:

- Title should be conclusive
  - Don’t include what you did, rather include what you found / discovered
- Caption should not include methods details
  - Define abbreviations, symbols, etc.

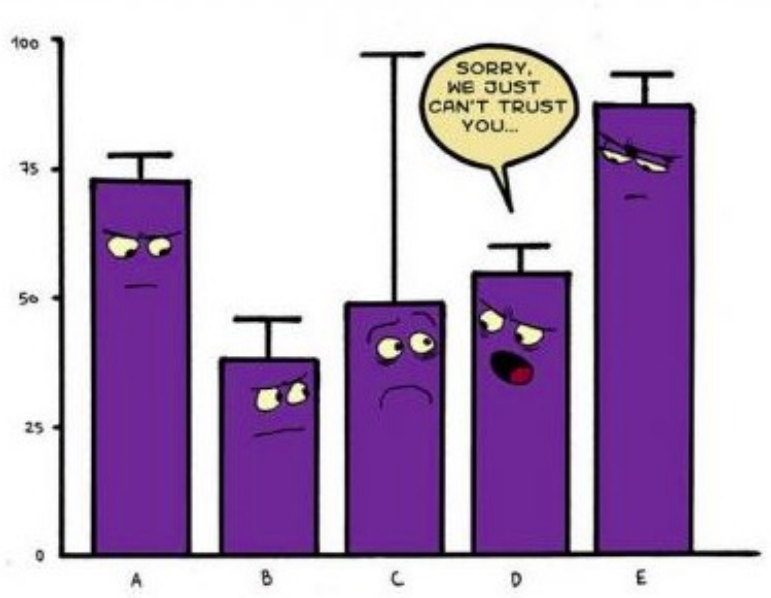


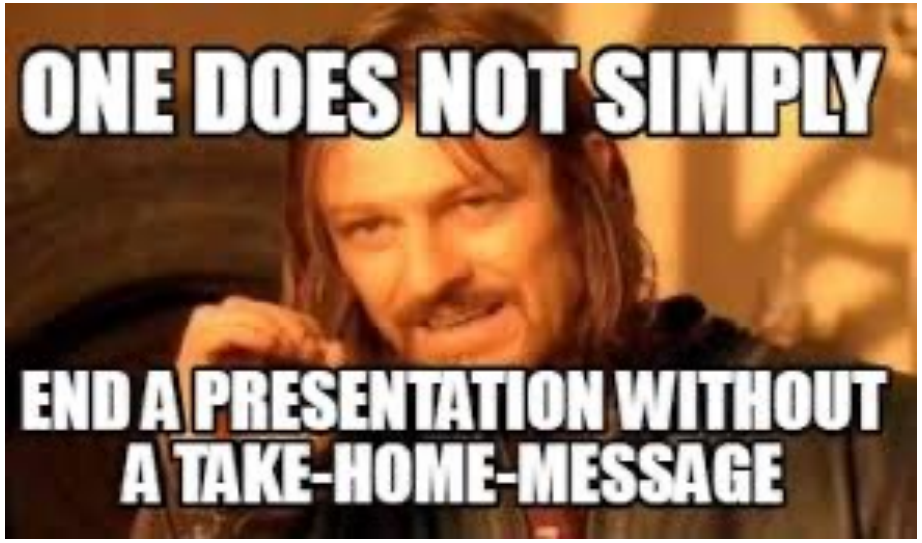
Figure X: Title is the take-home message of the experimental data.
Caption includes all of the details necessary to understand the data presented in the figure…not methods!!

Research talk due Saturday, March 2

- Prepare a video of you verbally discussing your research
  - Use any device or Zoom
  - No visuals / slides
  - Do not edit / splice the video
- Submit to Gmail account!
  - bioeng20.109@gmail.com
  - Remember to follow file name guidelines

Research talk should be 3 min (+/- 15 sec)

- Introduce yourself
- Provide important background information
- Describe key results
  - Briefly describe critical methods used to generate important data
  - Use quantitative descriptions when discussing results
- Highlight the take-home message



What data / results should be included?

- Protein purification
- Protein purity and concentration
- DSF results

Review assignment description on wiki

Category	Elements of a strong presentation	Weight
Introduction	• Introduce yourself and the research • Summarize the background information necessary to understand the research • State the research question	25%
Methods & Data	• Provide ONLY the method information necessary to understand the results • Give complete and concise explanations of the results • Relate the results to the central question	25%
Summary & Conclusions	• Highlight the key finding(s) relevant to the central question / hypothesis	25%
Organization	• Give a logical, easy-to-follow narrative • Include transition statements	15%
Delivery	• Show confidence / enthusiasm and speak clearly • Use appropriate language (technical or informal, as appropriate) • Be mindful of the time limit (3 minutes +/- 15 seconds!)	10%

The Research talk will be graded by Dr. Noreen Lyell with input from Dr. Becky Meyer and Jamie Zhan.