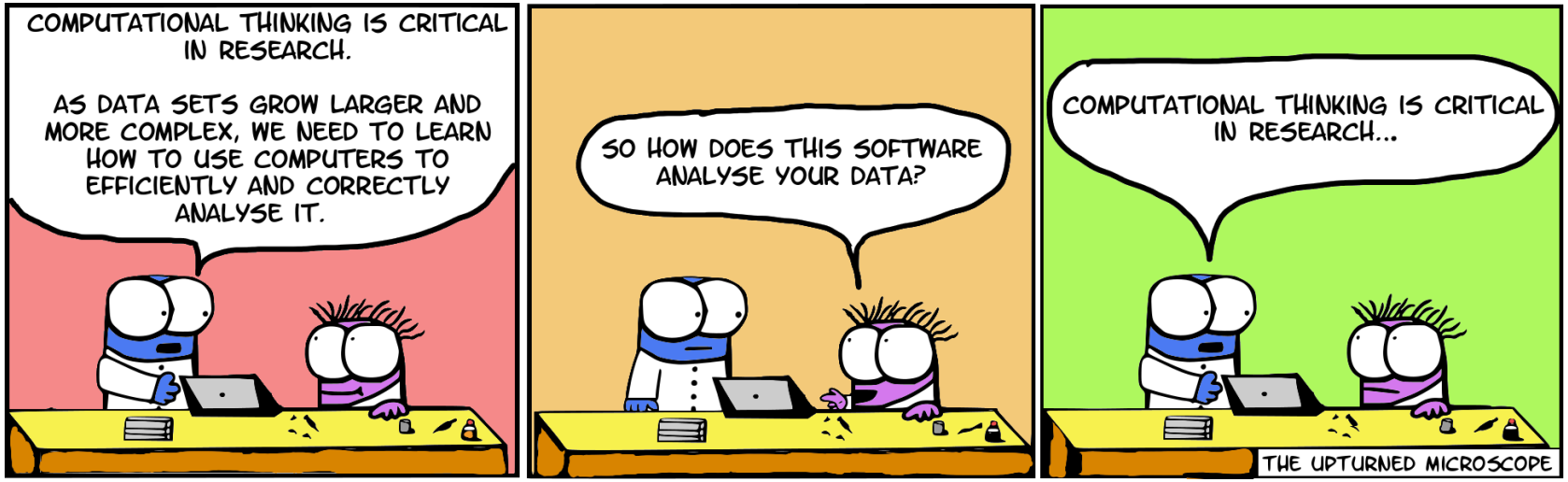
M2D7: Organize results and incorporate class data

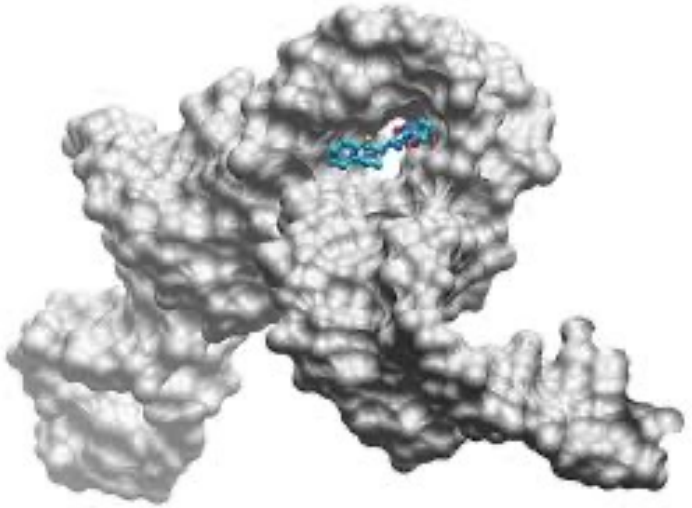
1. Quiz
2. Prelab-- walk through SMM
3. Research Article discussion



Selecting small molecules to test for potential therapeutics

Rationale design

- Our small molecules
  - Modifications to a known binder (FK506)
  - Take advantage of what is already known about protein-ligand interactions
  - Gives potential for focused hypothesis to test



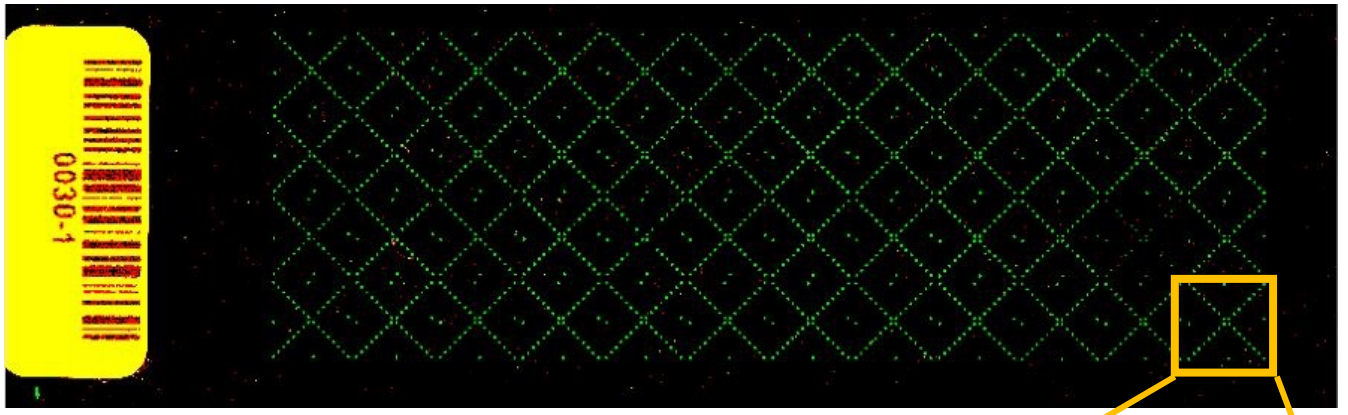
Unbiased screen

- High throughput assays can test tens of thousands of ligands in one assay
  - Allows unbiased exploration of potential therapeutics
  - Allows examination of targets with limited information

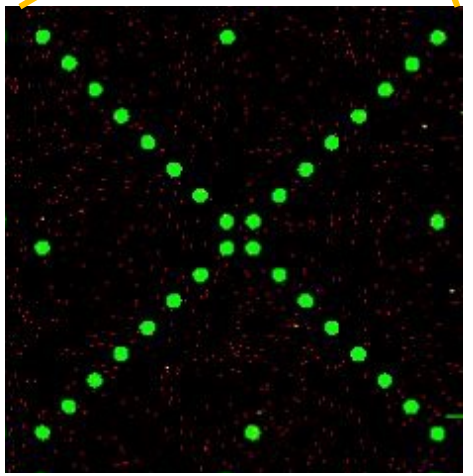
Small molecule microarray (SMM)

- Each slide contains ~12,000 spots
  - ~4,200 small molecules / ligands (in duplicate = ~8,400)
  - Fluorescein sentinel spots
  - DMSO negative control spots
    - Each slide has several blocks
    - Each block has sentinel spots which are landmarks
    - Rest of dots are small molecules and controls

Slide

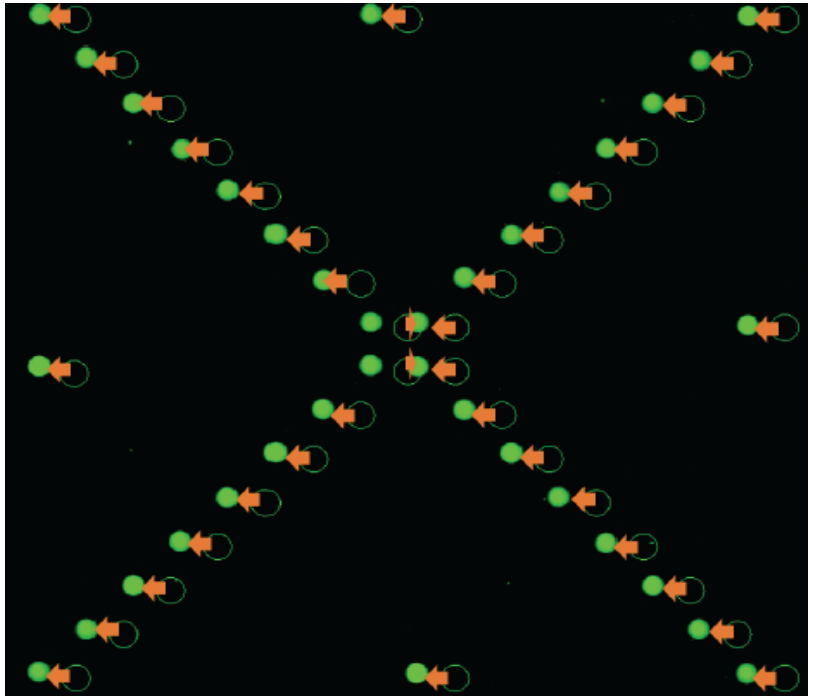


Block

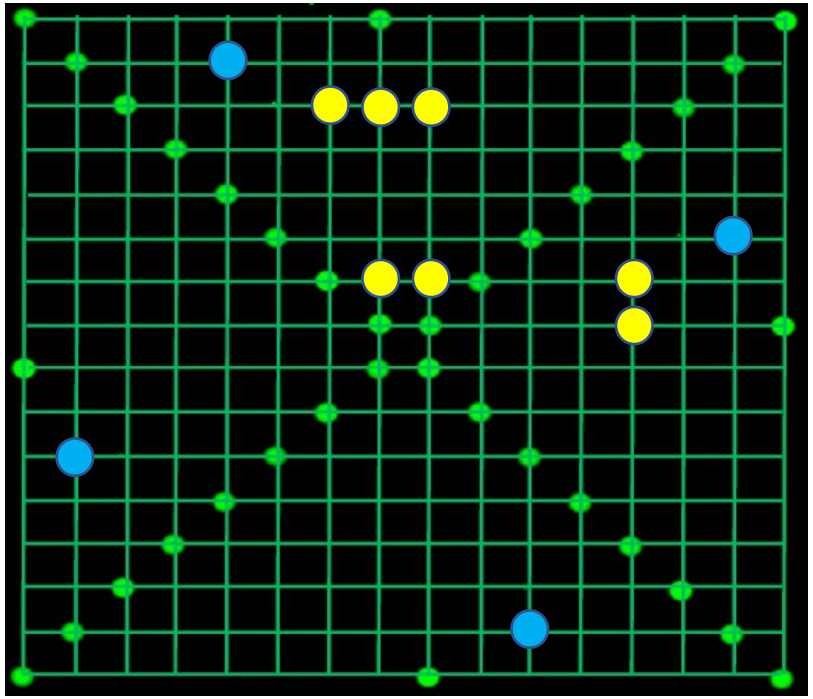


Green= sentinel spots (fluorescein dye)

Computational map of small molecule location can be overlaid on slide blocks using sentinel spots



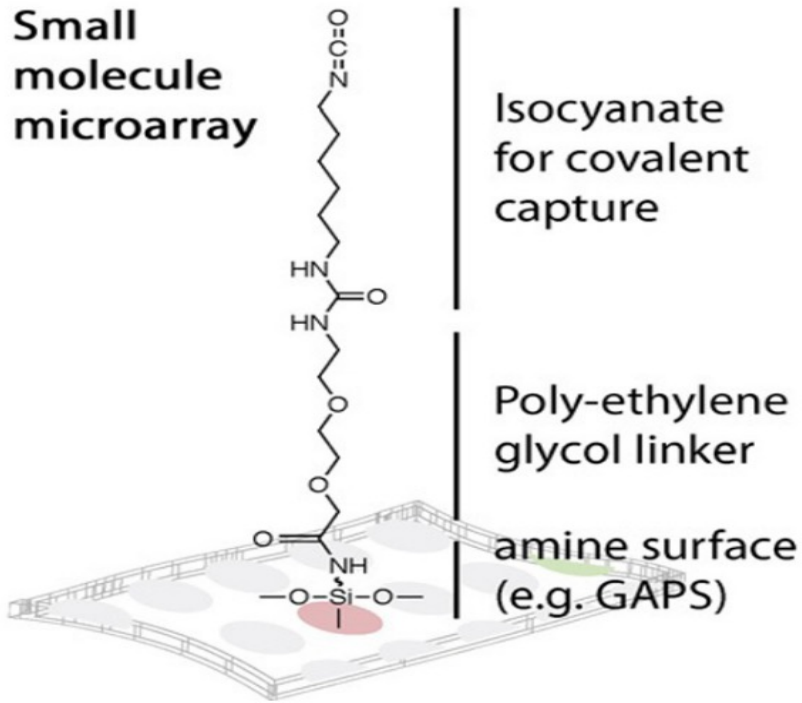
Green= sentinel spots
(fluorescein dye)



Blue= DMSO
Yellow= SM

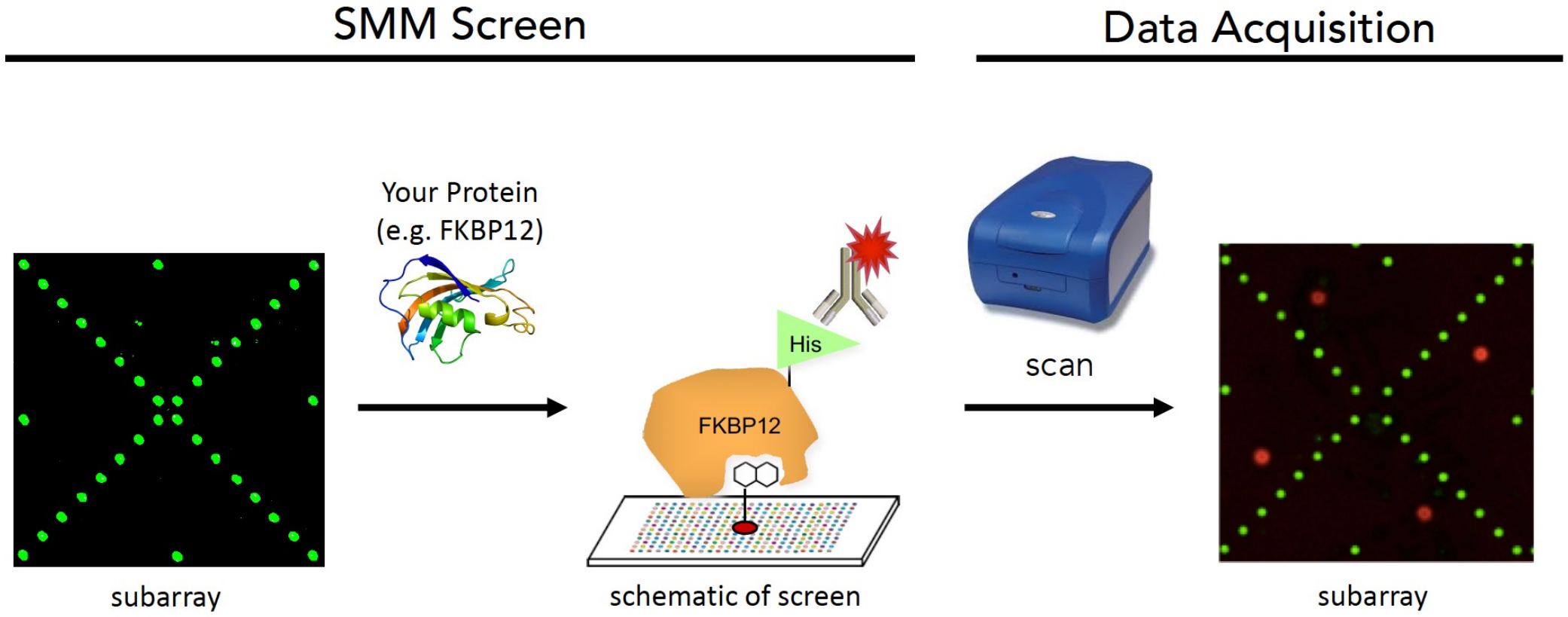
SMM slide preparation

- 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



Leifer et al. Methods in Enzymology. 2018.

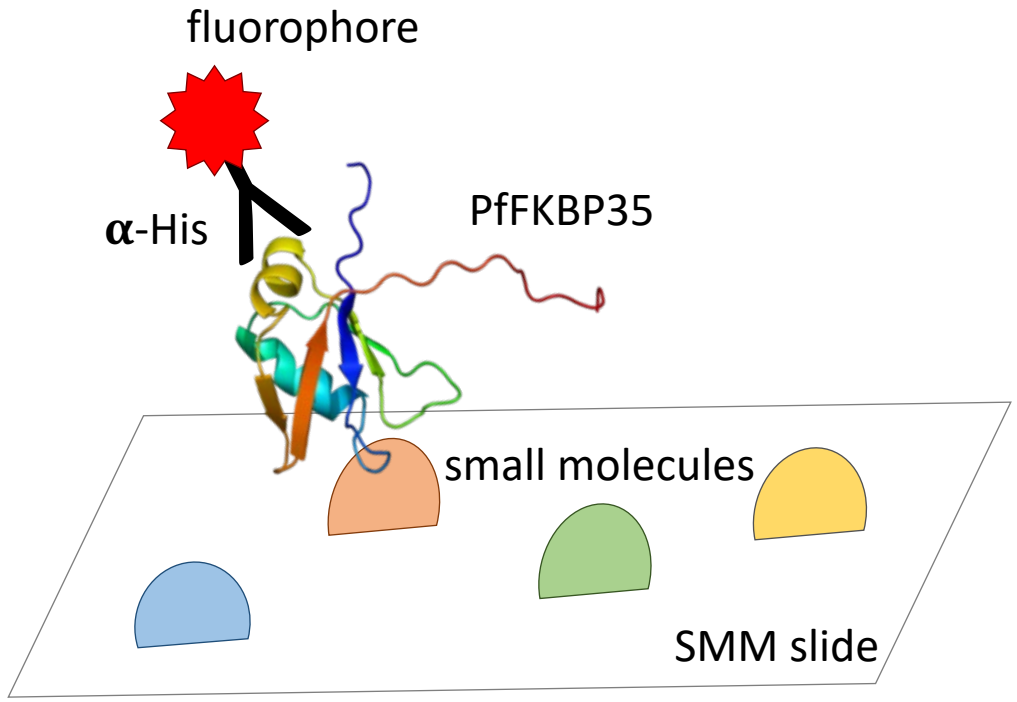
SMM workflow



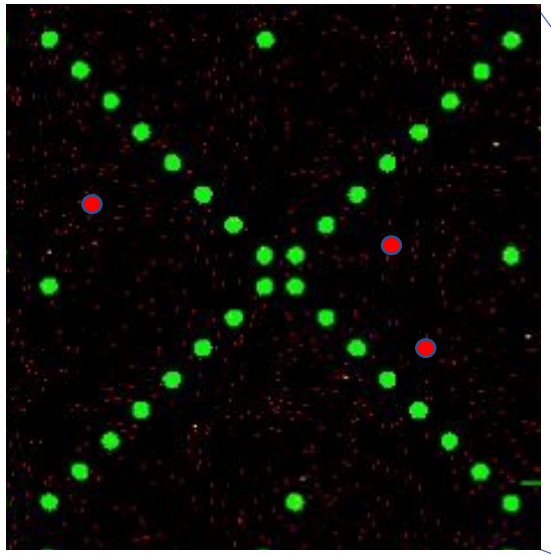
B. Leifer, S. Doyle

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 AlexaFlour 647 anti-His antibody
- Wash away excess antibody
- Scan slide



What do putative binders look like on the SMM slide?



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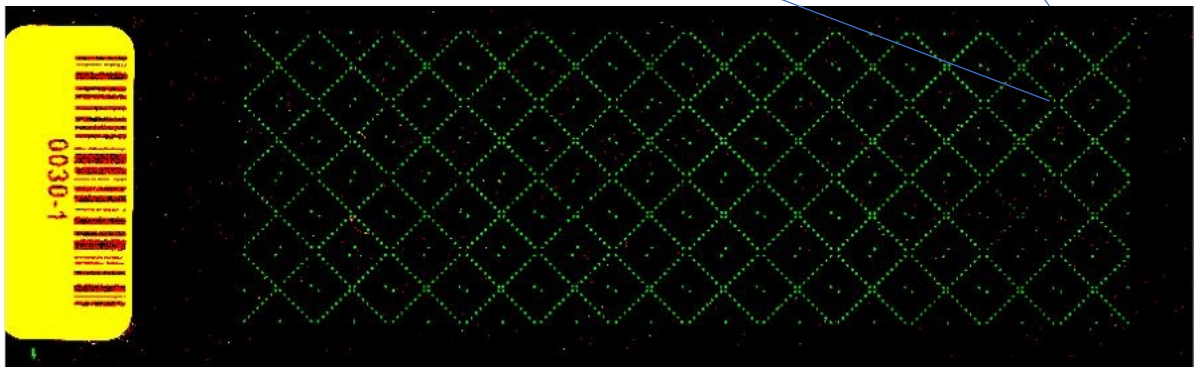
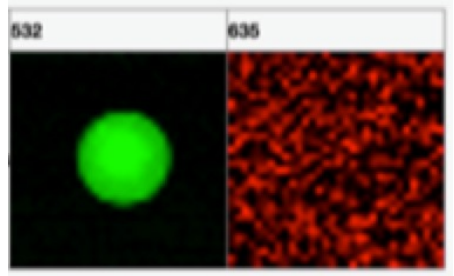
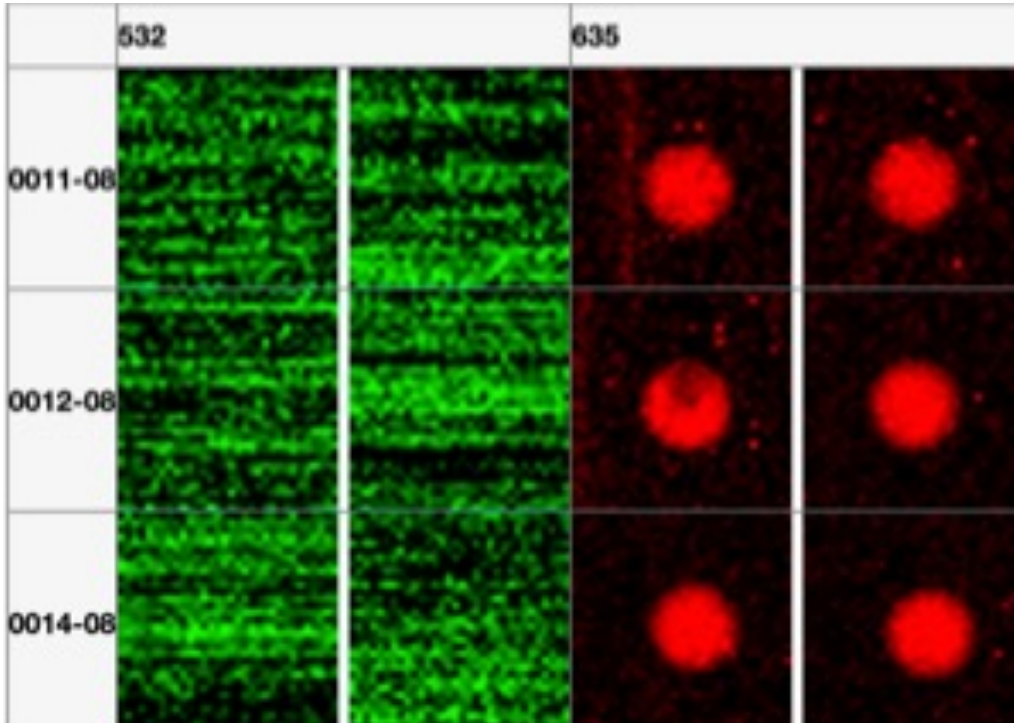


Slide





How does this relate to your project?

This multi-year project has focused on characterizing rationally designed small molecules

- **Previous year**
  - FK506 (our scaffold compound) can bind to both FKBP35 and FKBP12
  - Sequence and predicted structure of both targets are known
  - Used that information to design and test chemical modifications for FK506

- **Your project** uses 4 small molecules selected from that initial screen
  - Follow up with a more detailed characterization for how these SMs bind to our target of interest FKBP35

- Where do we go **next**?
  - Could continue the rational design process to hone our small molecules
  - Could go back to the drawing board and use an unbiased screen to identify new molecules