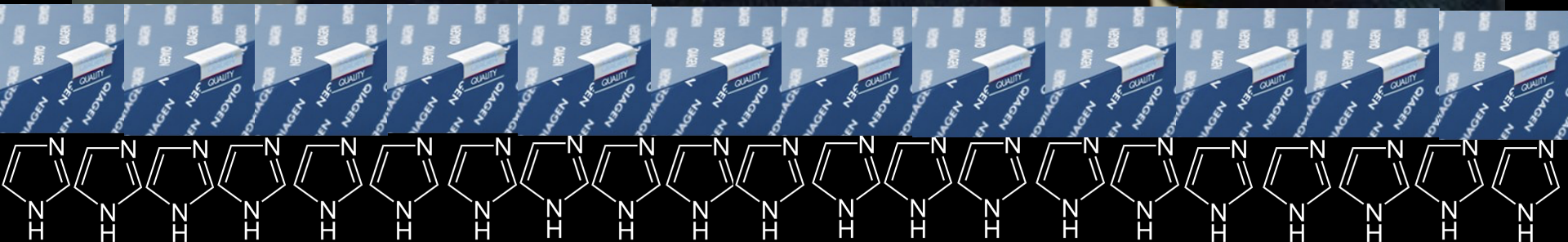


L3 – Small Molecule Microarrays

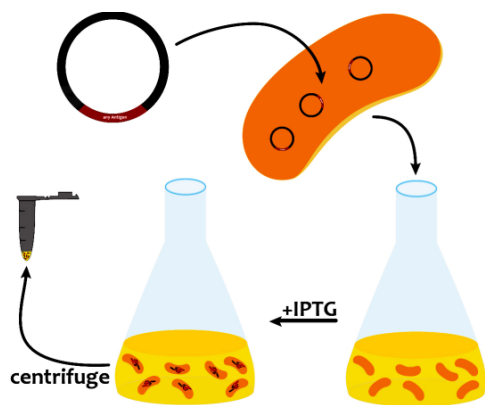
February 23, 2017

**I DON'T KNOW WHO YOU ARE OR WHY
YOU TOOK MY PROTEIN**

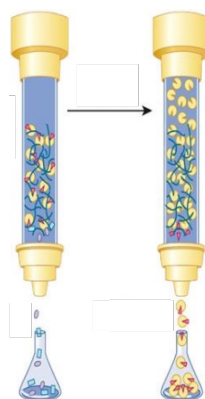
**BUT I WILL FIND YOU, AND I WILL
KILL YOU**



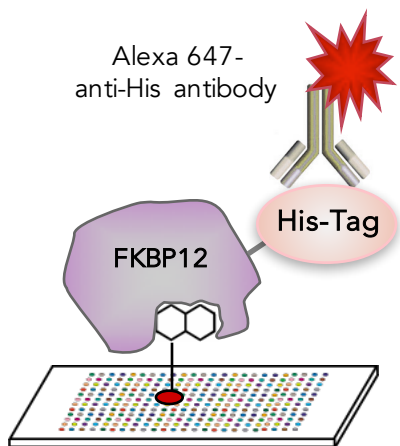
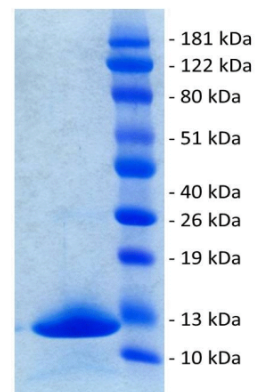
Our path to probe discovery



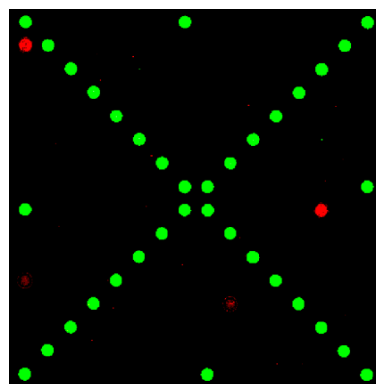
overexpress FKBP12
lab day 1



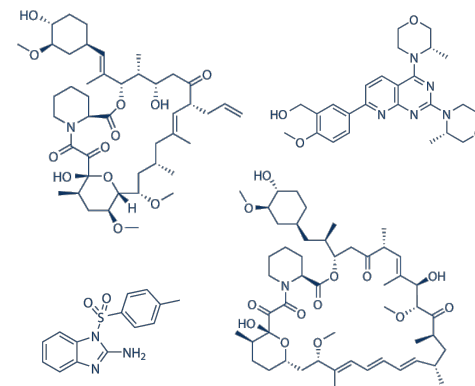
purify and characterize FKBP12
lab days 2 and 3



SMM screen
lab day 4



scan images and analyze data
lab days 5 and 6



compare hit lists for teams
lab day 7

The view from 2000

Diabetes (type 2)



< 100 Mendelian disease genes

*12 common disease genetic variants
(outside of HLA locus)*

PPAR γ

2000

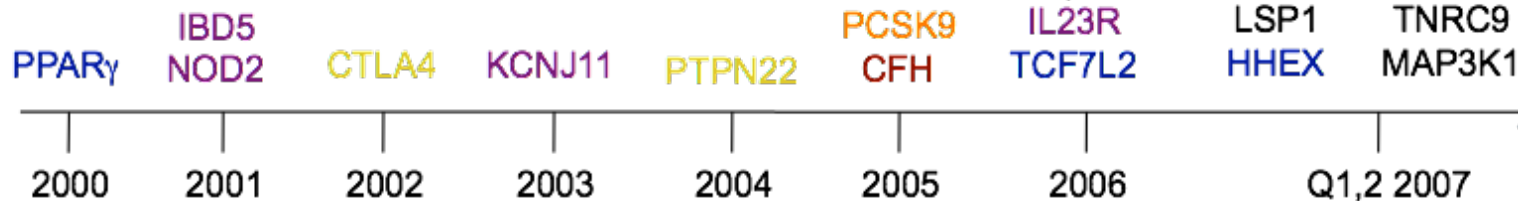
~ 15 years on from the Human Genome Project

- Asthma
- Atrial fibrillation
- Breast cancer
- Crohn's disease
- Diabetes (type 1)
- Diabetes (type 2)
- Hypercholesterolemia
- Lupus
- Macular degeneration
- Myocardial infarction
- Obesity
- Prostate cancer
- Others...

- CDKN2B/A 8q24 #2
- CDKAL1 SLC30A8
- 8q24 #3
- ORMDL3
- 8q24 #4
- 4q25
- 8q24 #5
- TCF2
- 8q24 #6
- TCF2
- ATG16L1
- GCKR
- 5p13
- FTO
- 10q21
- CDKN2B/A
- IRGM
- C12orf30
- NKX2-3
- ERBB3
- IL12B
- KIAA0350
- 3p21
- CD226
- 1q24
- 16p13
- PCSK9
- PTPN2
- PTPN2
- IFIH1
- SH2B3
- LOC387715
- 8q24
- FGFR2
- IRF5
- IL23R
- TNRC9
- PCSK9
- IL23R
- LSP1
- TNRC9
- CFH
- TCF7L2
- HHEX
- MAP3K1



> 2000 loci affecting > 200 common diseases



~ 15 years on from the Human Genome Project

- Asthma
- Atrial fibrillation
- Breast cancer
- Crohn's disease
- Diabetes (type 1)
- Diabetes (type 2)
- Hypercholesterolemia
- Lupus
- Macular degeneration
- Myocardial infarction
- Obesity
- Prostate cancer
- Others...

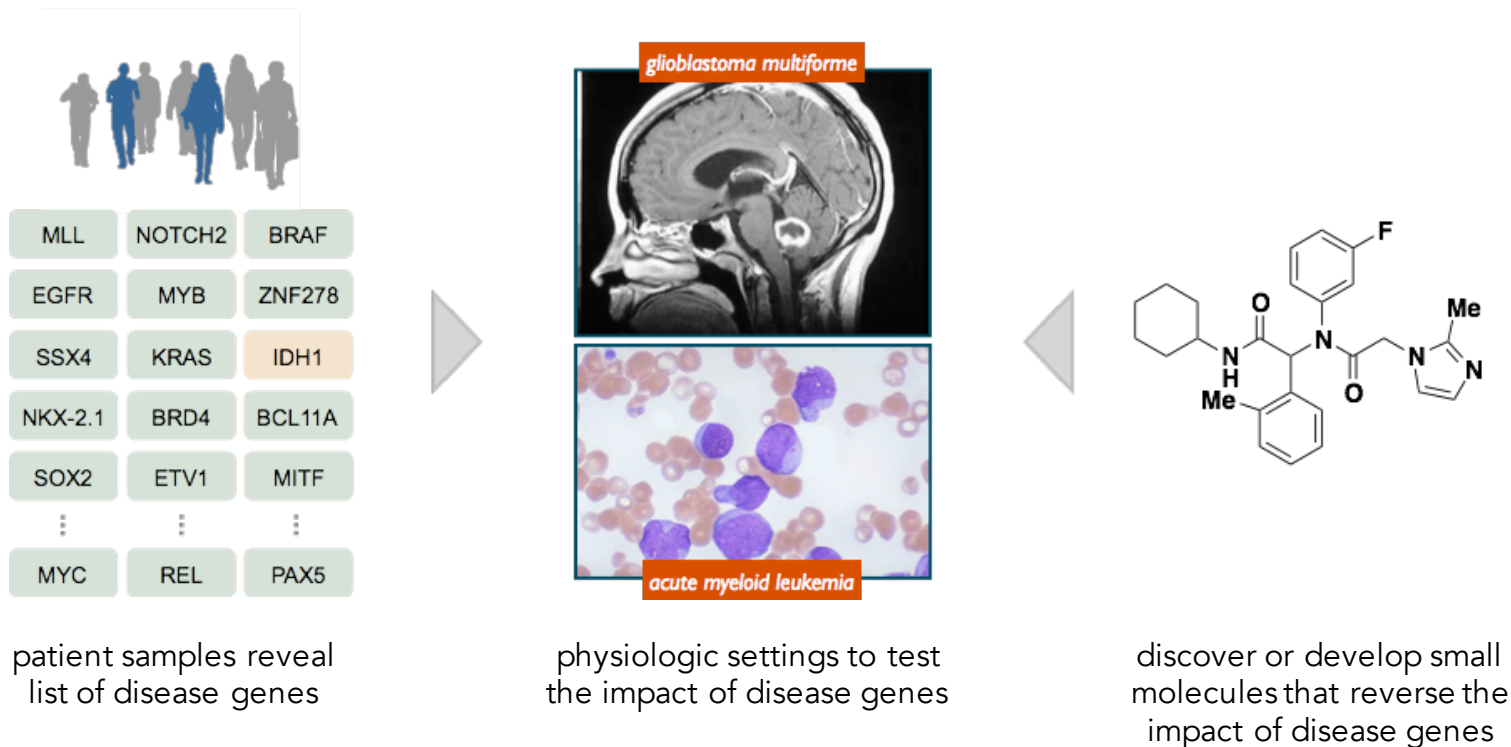


> 2000 loci affecting > 200 common diseases



- | | | | | | | | | |
|---------------|--------------|-------|--------|--------|----------------------|-------------------------|----------------------|--------------------------|
| PPAR γ | IBD5
NOD2 | CTLA4 | KCNJ11 | PTPN22 | IRF5
PCSK9
CFH | 8q24
IL23R
TCF7L2 | 8q24
LSP1
HHEX | FGFR2
TNRC9
MAP3K1 |
| 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | Q1,2 2007 | 2017 |

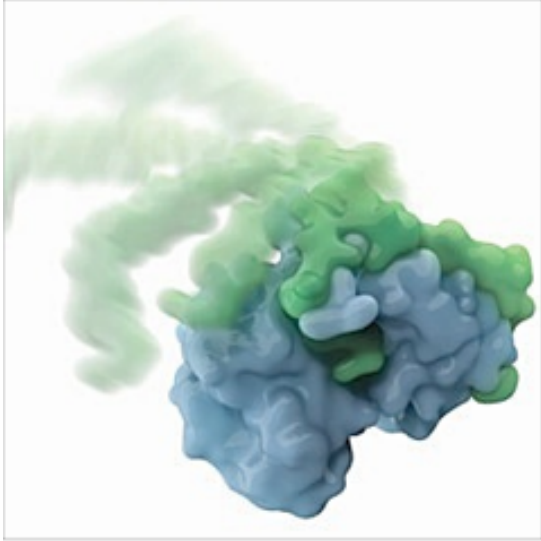
Chemical probes of disease biology



Approach: use small molecules to test emerging concepts in human disease in physiologically relevant settings

Output: validated small-molecule probe to facilitate human clinical development or diagnostic applications

'Undruggable' targets are aplenty



disordered proteins

e.g. amyloids, transcription factors, enzymes



*DNA binding proteins
protein-protein interactors*

e.g. transcription factors,
extracellular growth factors,
scaffold proteins

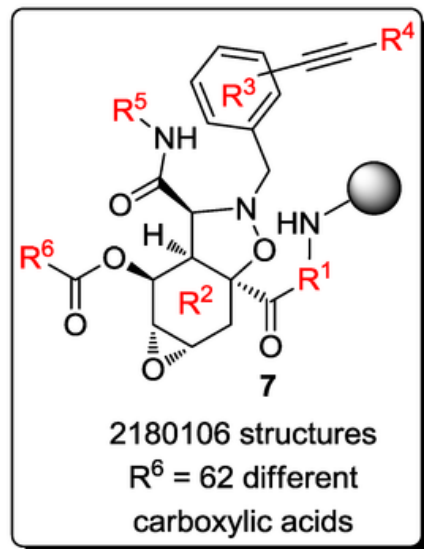


*integral membrane
proteins*

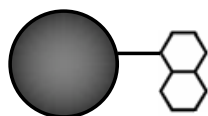
e.g. cell adhesion proteins,
enzymes, receptors

1998 – 'on-bead' binding assays

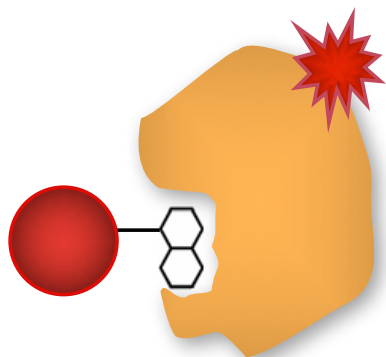
Library = 2.18M on
90 μm Tentagel beads



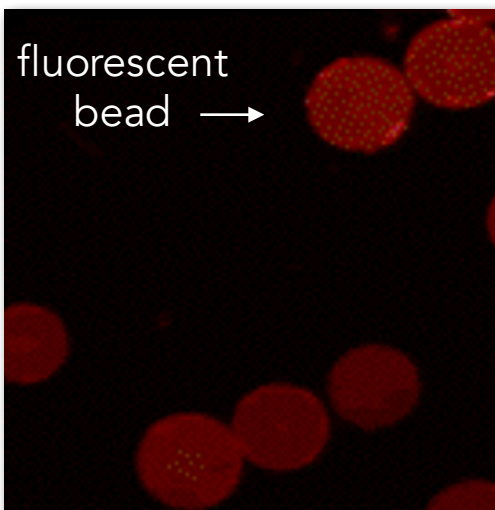
'Gradbot'
Angela



no
binding



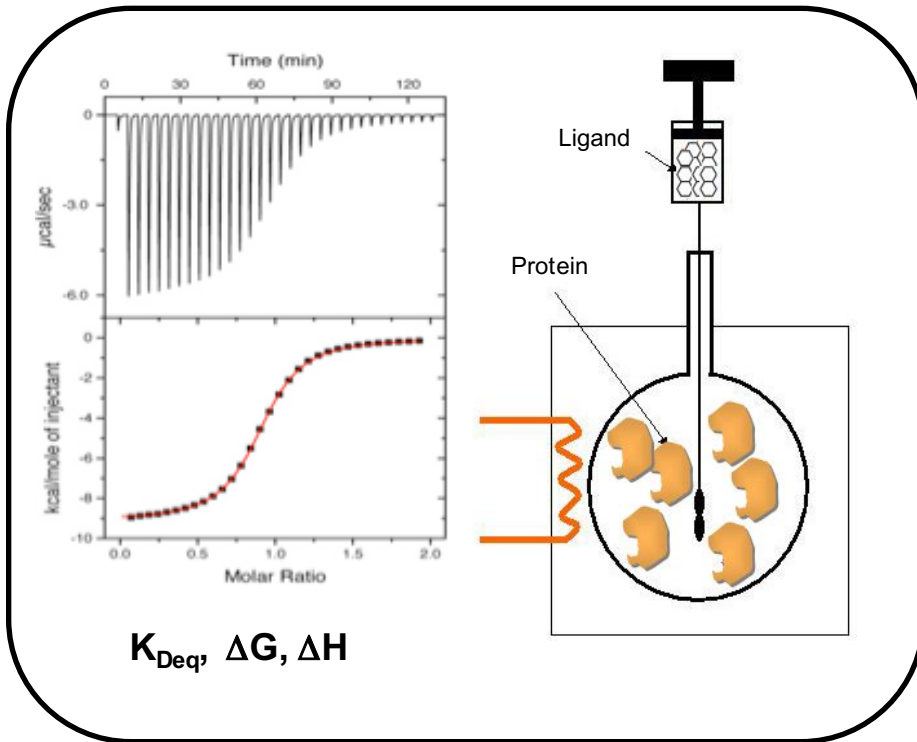
assay
positive



fluorescent
bead \rightarrow

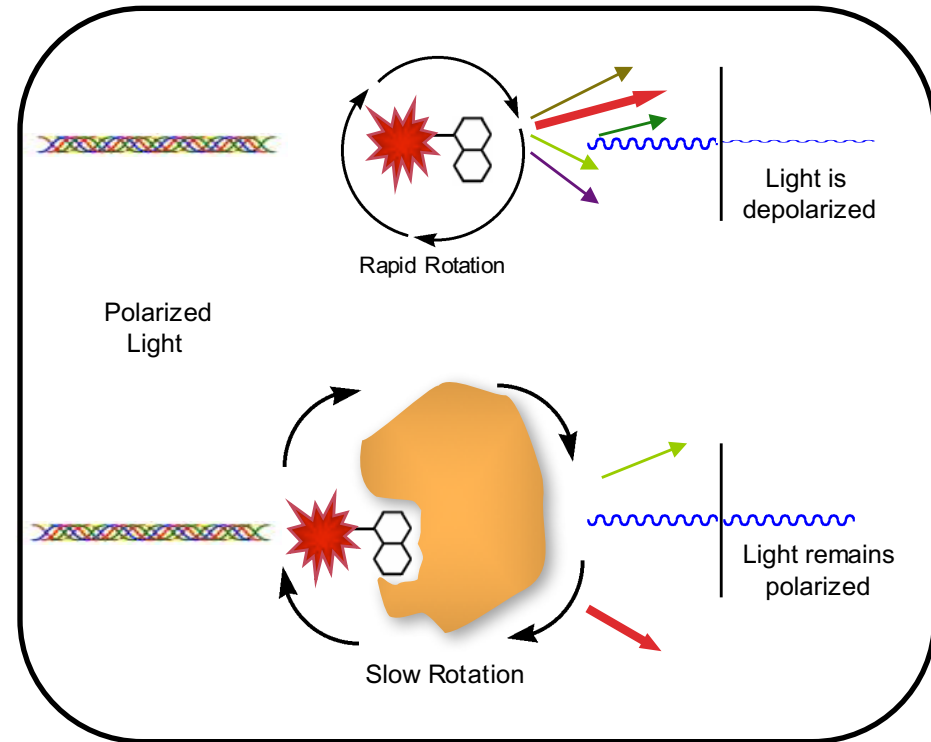
rhodamine dye
540/625 nm

1998 - other binding assay formats



isothermal titration calorimetry

measure changes in temperature upon binding



fluorescence polarization

measure changes in rate of rotation upon binding

Spatially addressable systems

Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray

Mark Schena,* Dari Shalon,*† Ronald W. Davis,
Patrick O. Brown‡

A high-capacity system was developed to monitor the expression of many genes in parallel. Microarrays prepared by high-speed robotic printing of complementary DNAs on glass were used for quantitative expression measurements of the corresponding genes. Because of the small format and high density of the arrays, hybridization volumes of 2 microliters could be used that enabled detection of rare transcripts in probe mixtures derived from 2 micrograms of total cellular messenger RNA. Differential expression measurements of 45 *Arabidopsis* genes were made by means of simultaneous, two-color fluorescence hybridization.

SCIENCE • VOL. 270 • 20 OCTOBER 1995

Pat Brown, Stanford

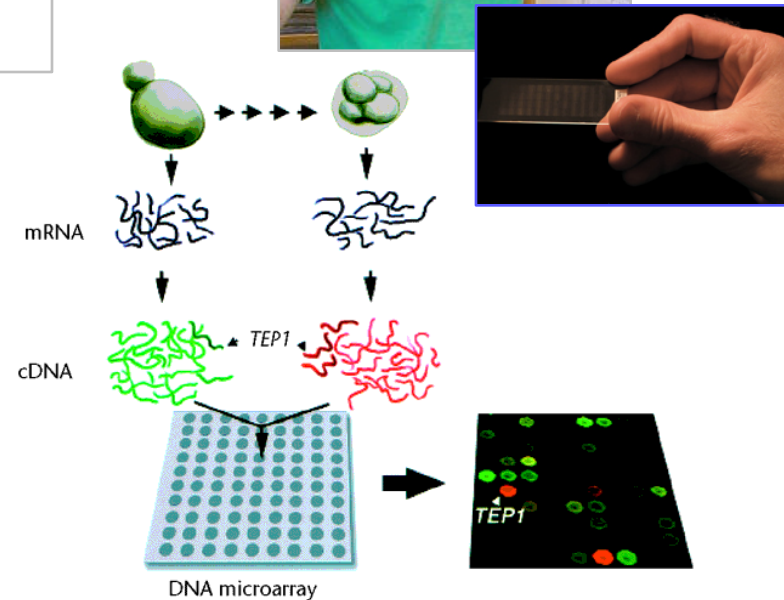


Exploring the new world of the genome with DNA microarrays

Patrick O. Brown^{1,3} & David Botstein²

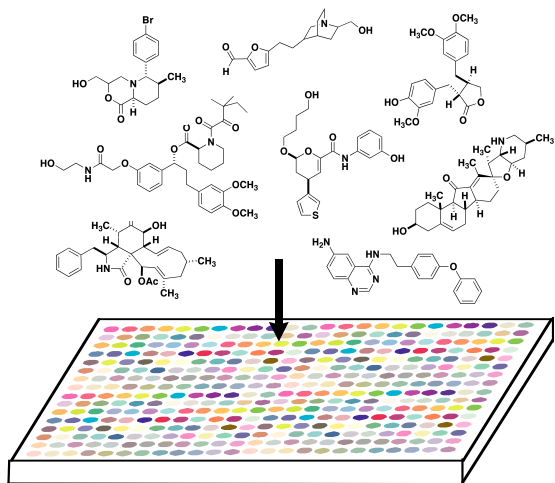
Departments of ¹Biochemistry and ²Genetics, and the ³Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California 94305, USA. e-mail: pbrown@cmgm.stanford.edu

Thousands of genes are being discovered for the first time by sequencing the genomes of model organisms, an exhilarating reminder that much of the natural world remains to be explored at the molecular level. DNA microarrays provide a natural vehicle for this exploration. The model organisms are the first for which comprehensive genome-wide surveys of gene expression patterns or function are possible. The results can be viewed as maps that reflect the order and logic of the genetic program, rather than the physical order of genes on chromosomes. Exploration of the genome using DNA microarrays and other genome-scale technologies should narrow the gap in our knowledge of gene function and molecular biology between the currently-favoured model organisms and other species.

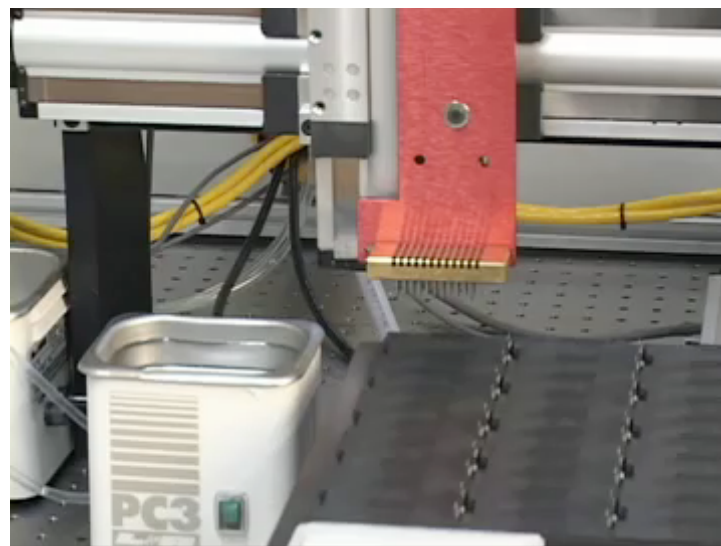


follow changes in gene expression during yeast sporulation

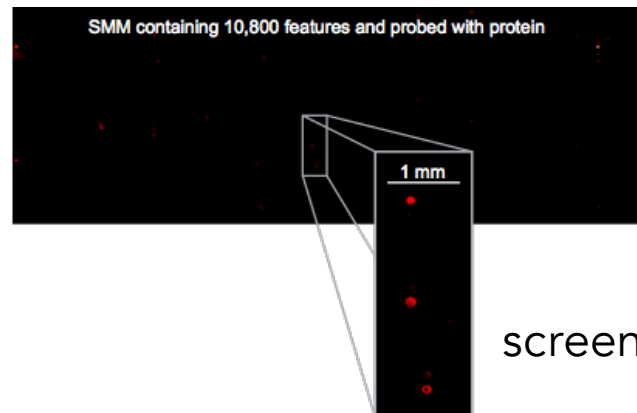
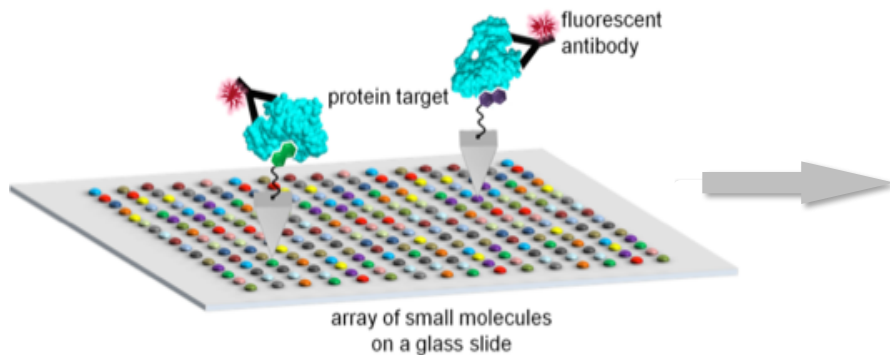
Small Molecule Microarrays (SMMs)



compound stock solutions



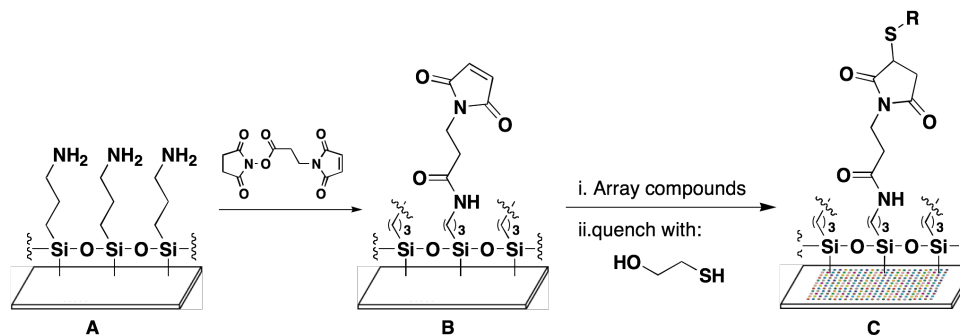
SMM manufacture and screening



screened SMM

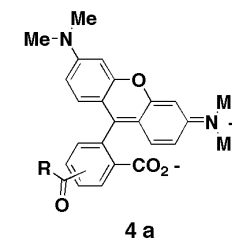
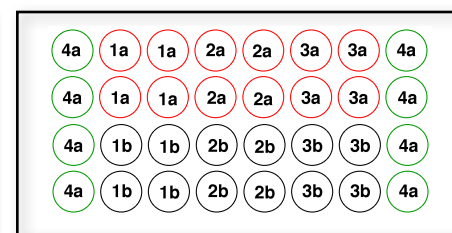
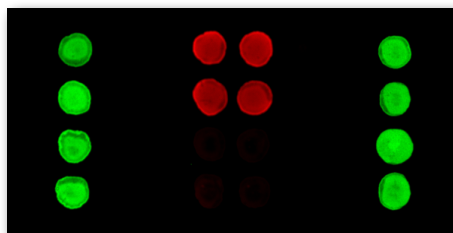
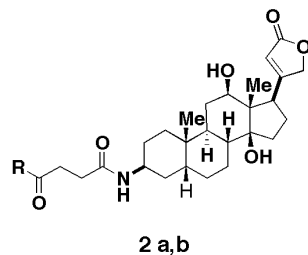
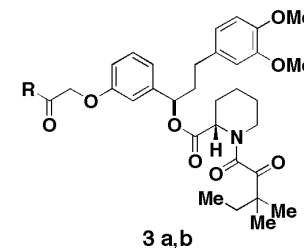
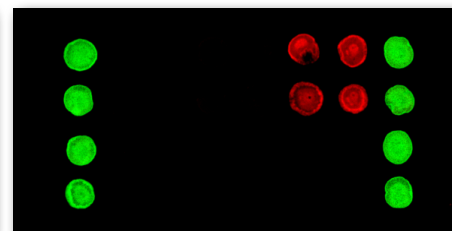
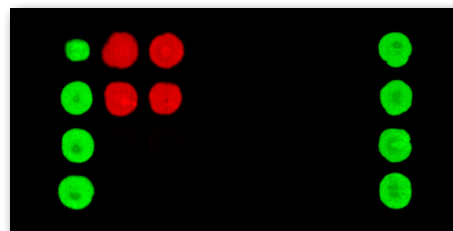
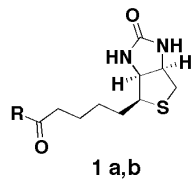
Proof-of-concept experiments for SMMs

detecting known protein-ligand interactions

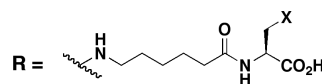


Streptavidin

FKBP12



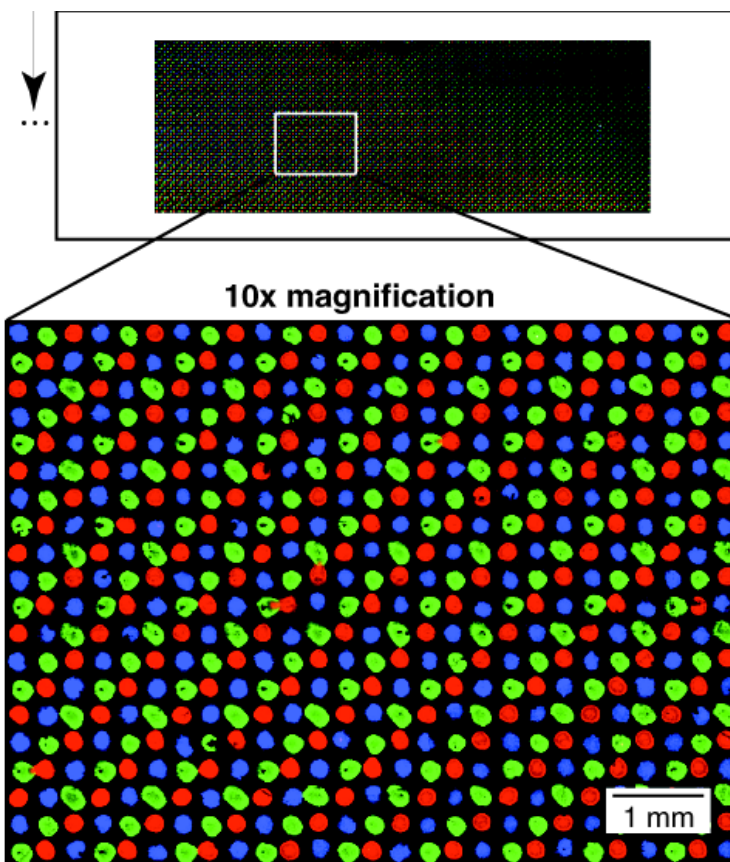
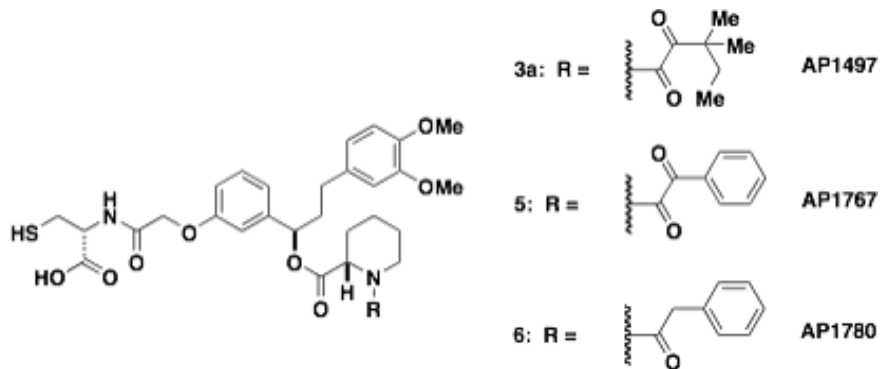
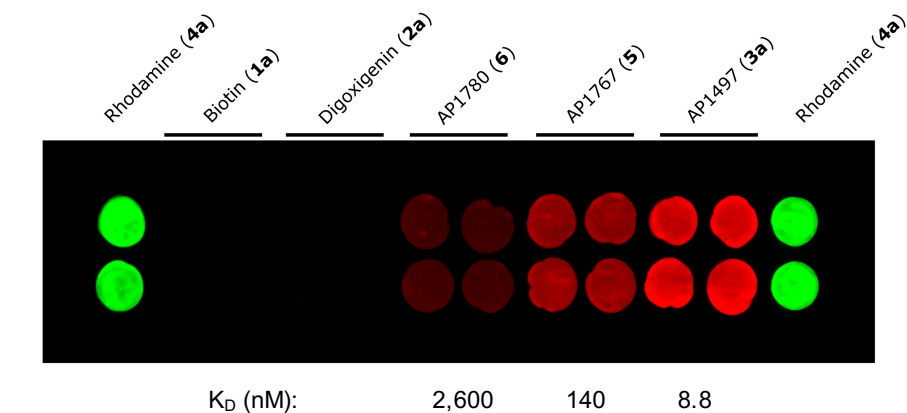
Anti-Digoxin mAb



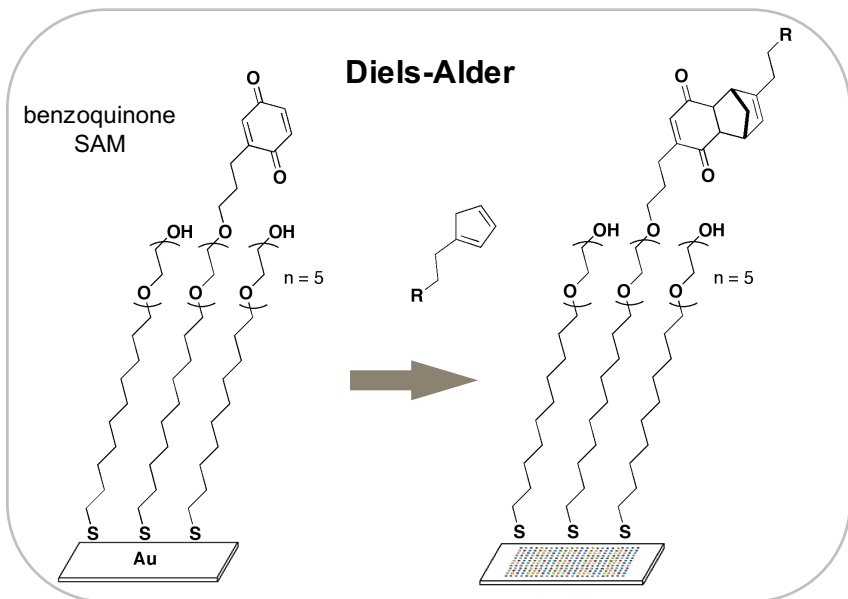
a: X = SH b: X = H

Proof-of-concept experiments for SMMs

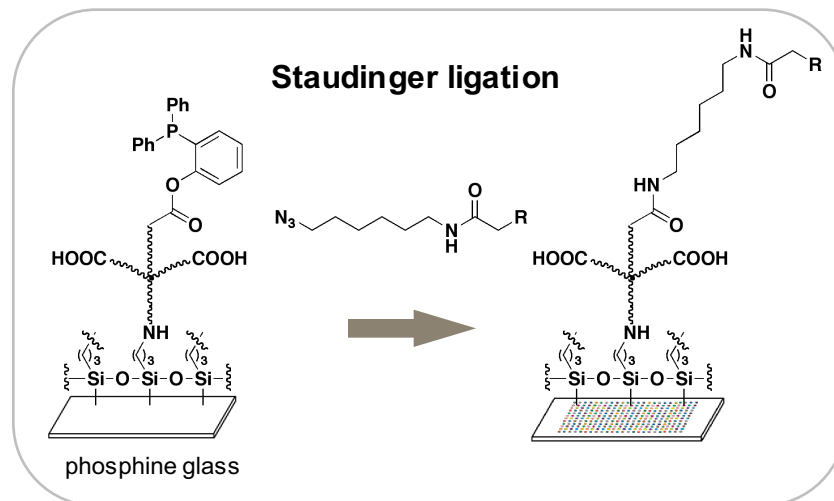
evaluating affinities and multiplexed formats



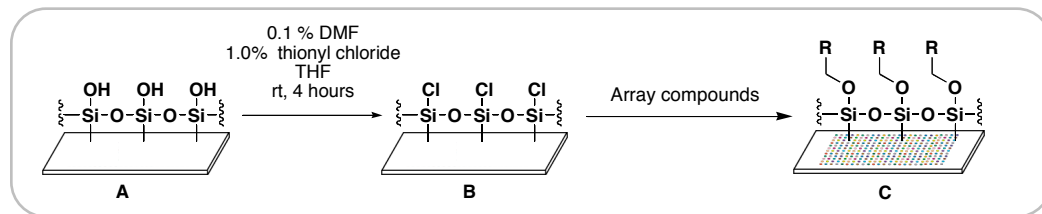
Capture chemistries for making SMMs



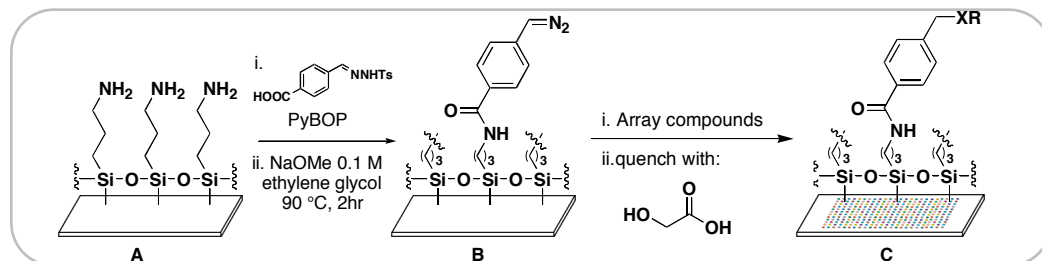
Houseman, B.T., Mrksich, M. *Chem. Biol.* 9, 443-454, 2002



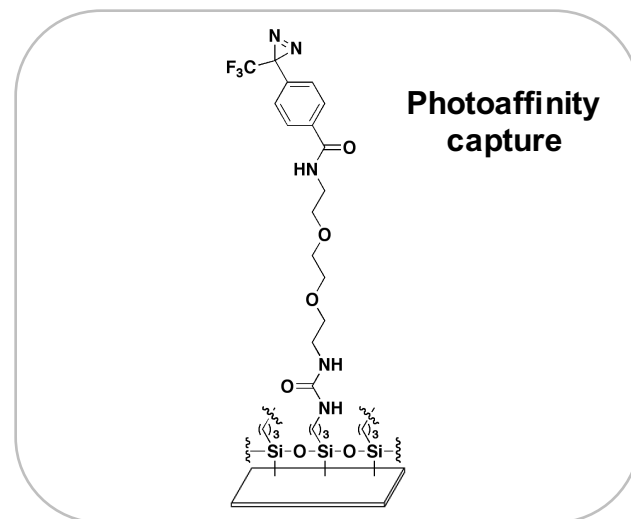
Köhn et al., *Angew. Chem. Int. Ed.* 42, 5830-5834, 2003



Hergenrother et al., *J. Am. Chem. Soc.* 122, 7849-7850, 1999

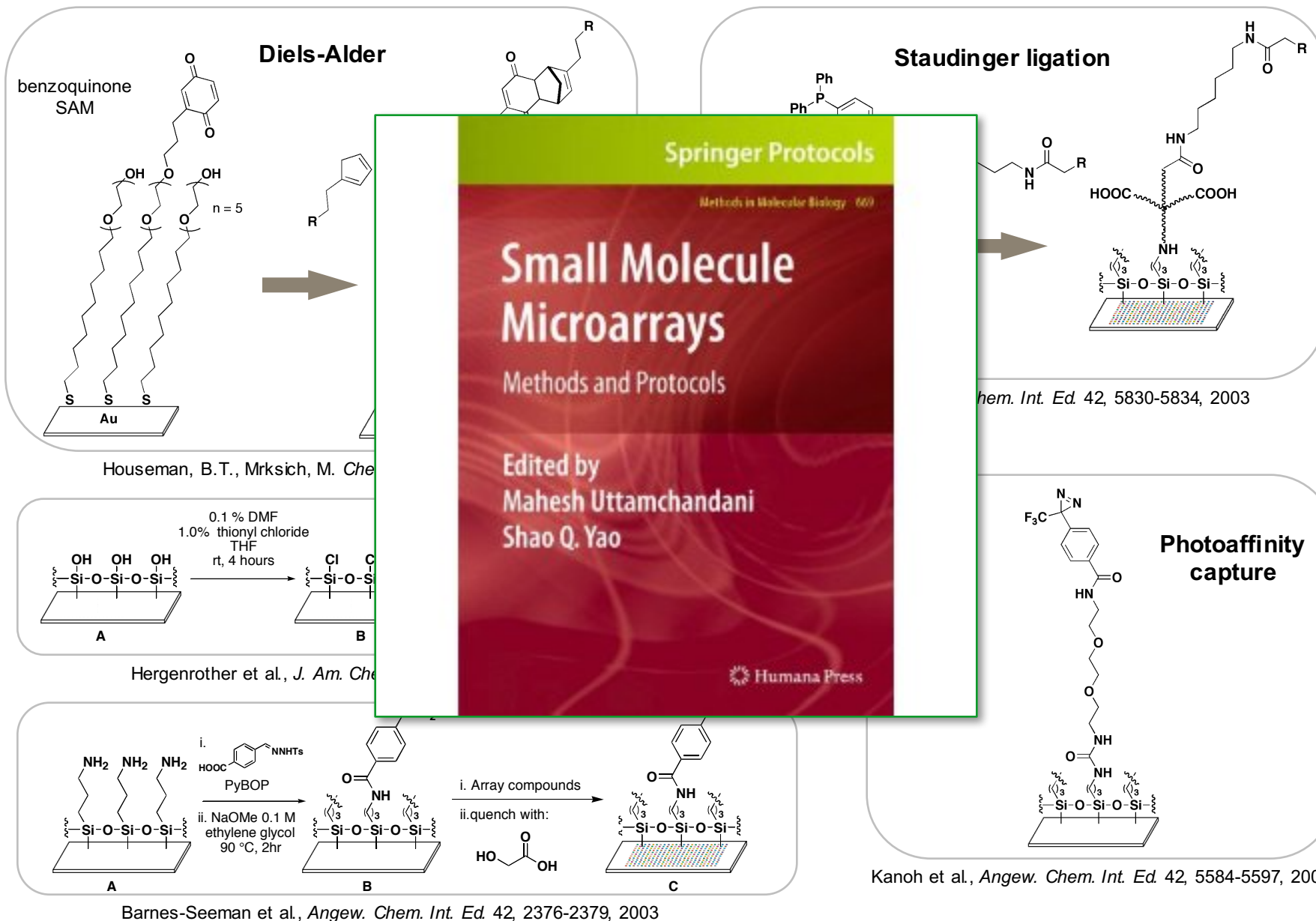


Barnes-Seeman et al., *Angew. Chem. Int. Ed.* 42, 2376-2379, 2003

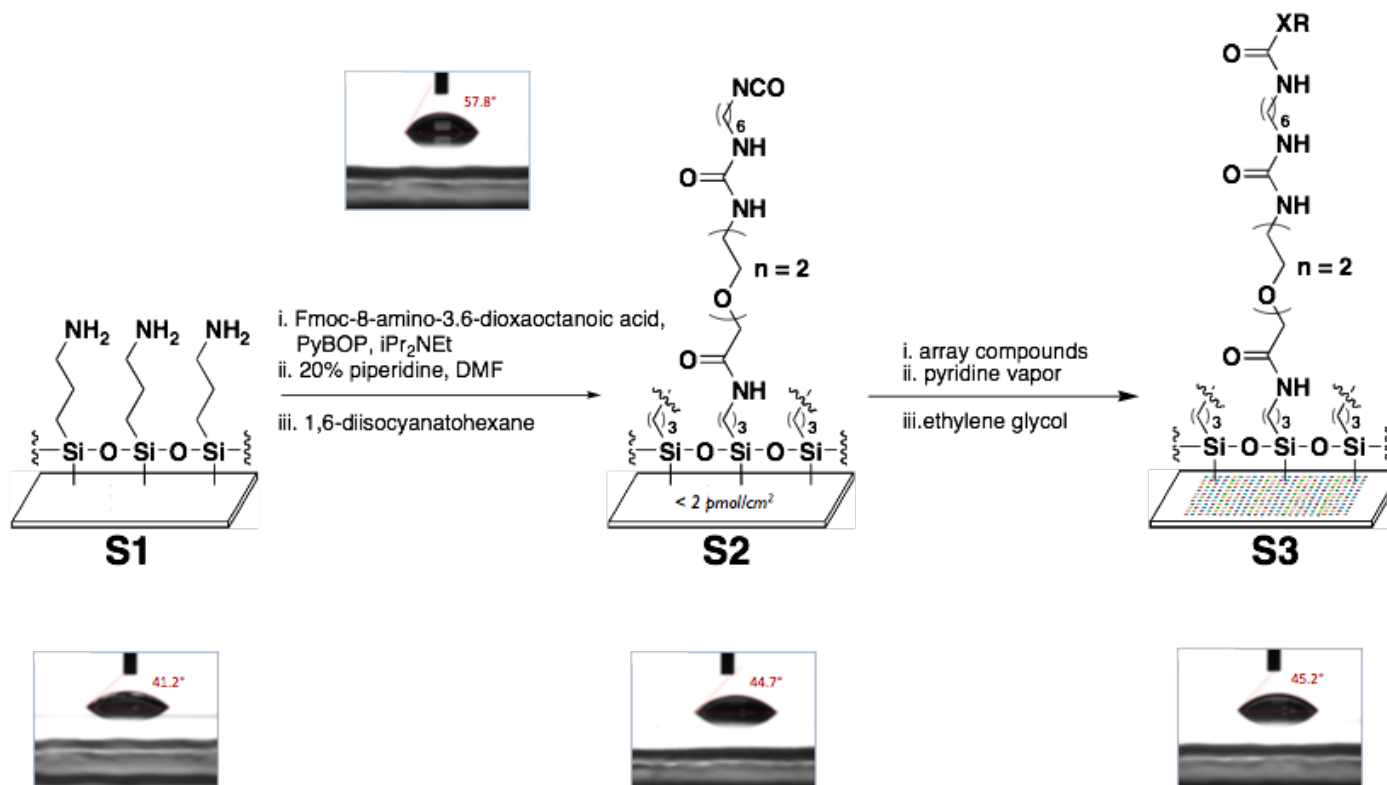


Kanoh et al., *Angew. Chem. Int. Ed.* 42, 5584-5597, 2003

Capture chemistries for making SMMs



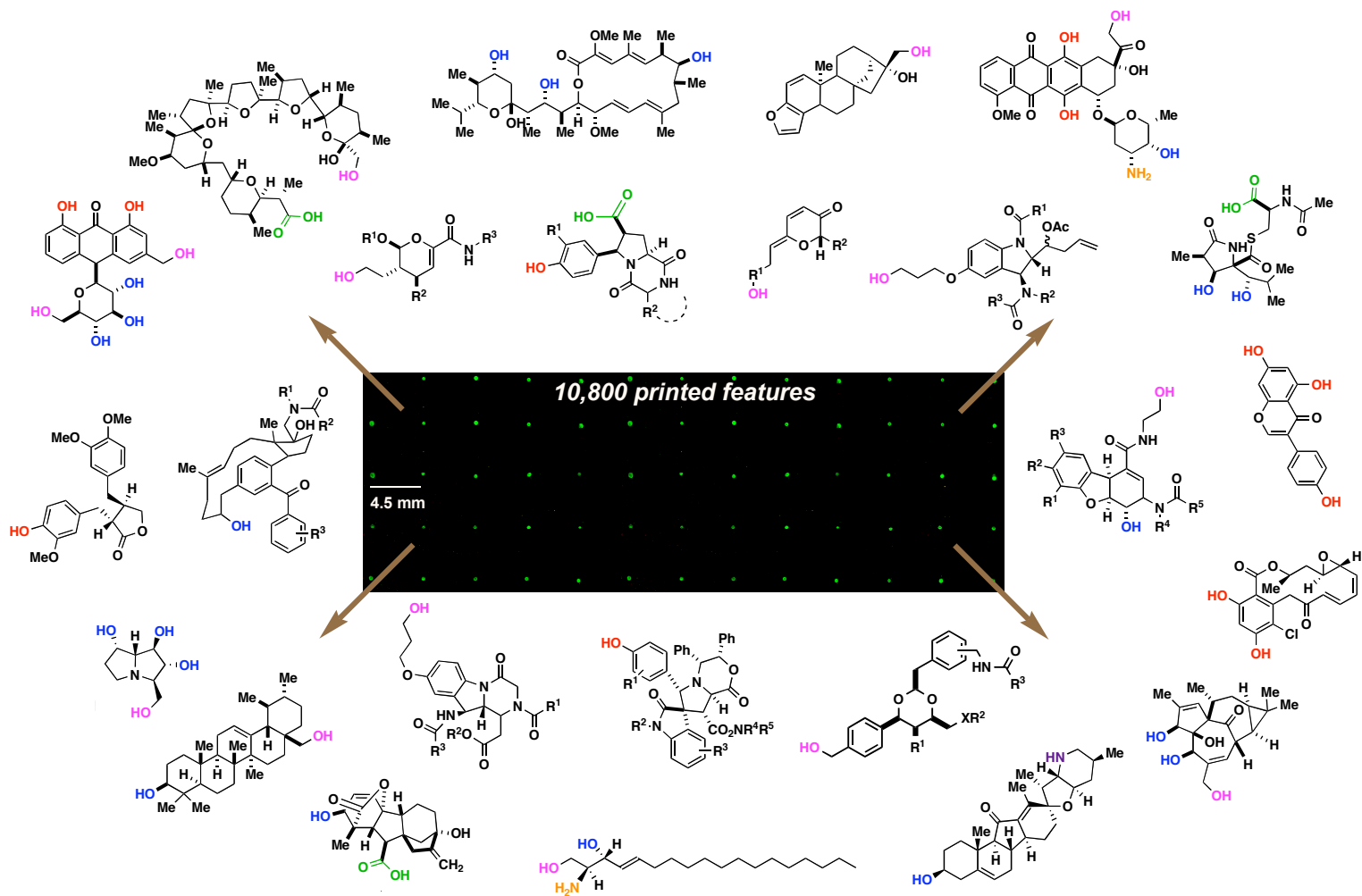
Capture chemistries for making SMMs



Bradner, J. E., McPherson, O. M., Mazitschek, R. M., Barnes-Seeman, D., Shen, J. P., Dhaliwal, J., Stevenson, K., Duffner, J. L., Park, S. B., Nghiem, P. T., Schreiber, S. L., Koehler, A. N., *Chem Biol*, 13, 493-504 (2006)

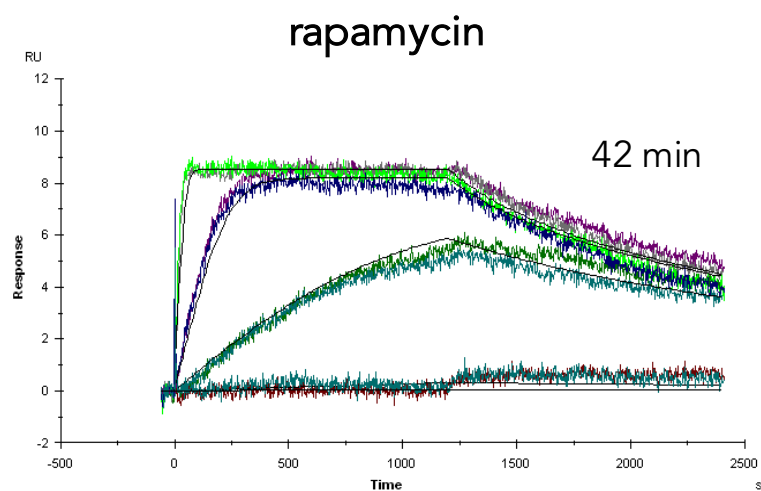
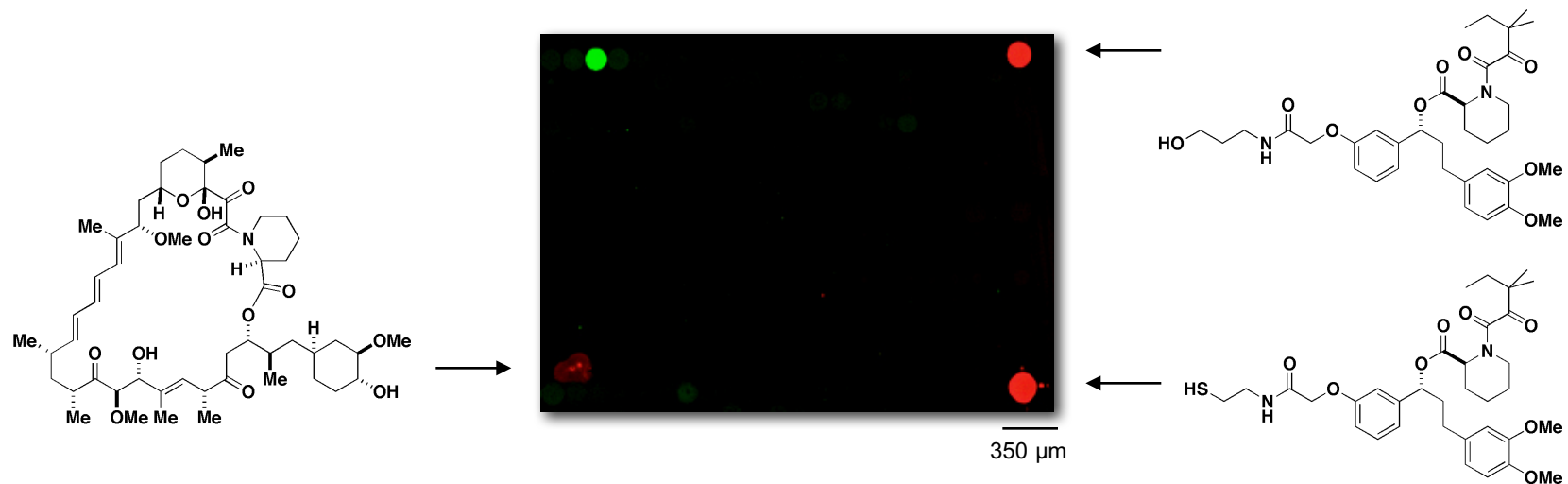
Bradner, J. E., McPherson, O. M., Koehler, A. N., *Nature Protocols*, 1, 2344-2352 (2006)

SMMs contain compounds from a variety of sources

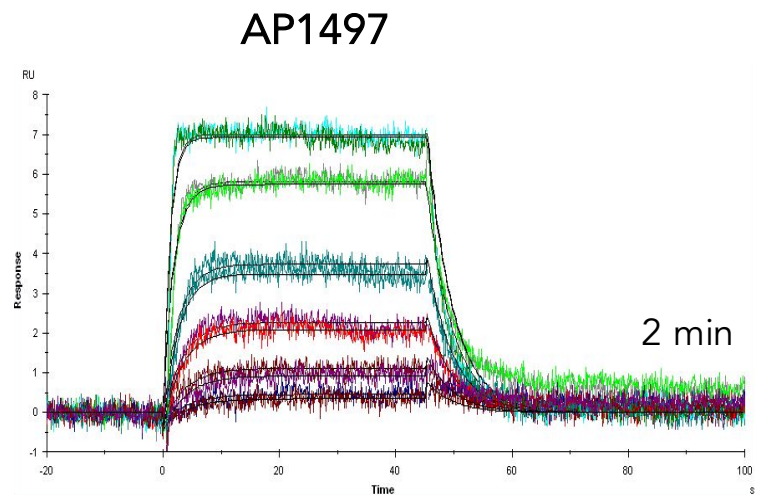


In silico analysis of 400,000 'National Library' for screens:
>75% isocyanate-reactive

Interactions with varying kinetics can be visualized

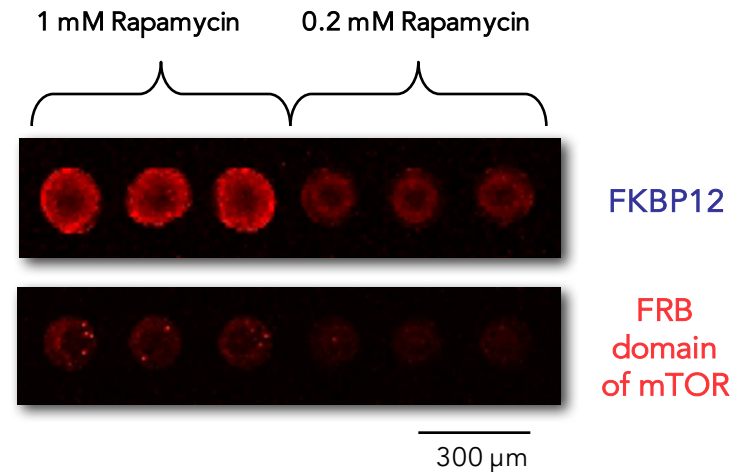
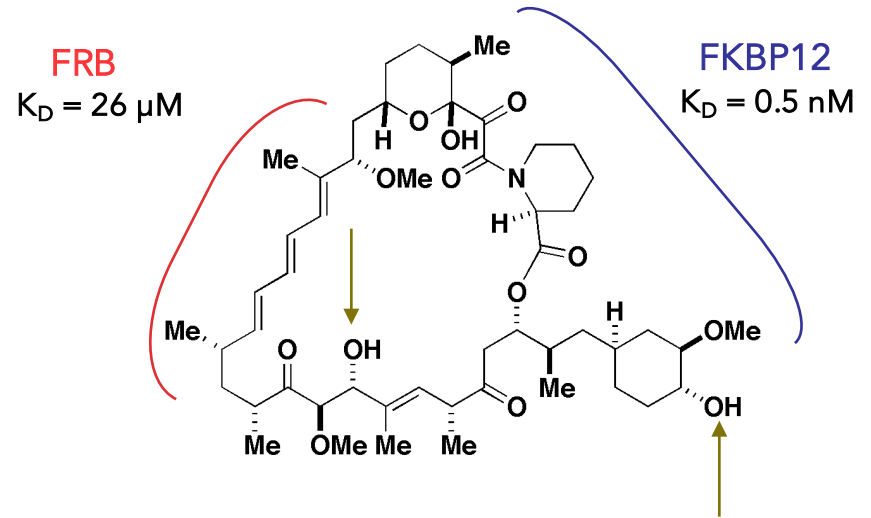
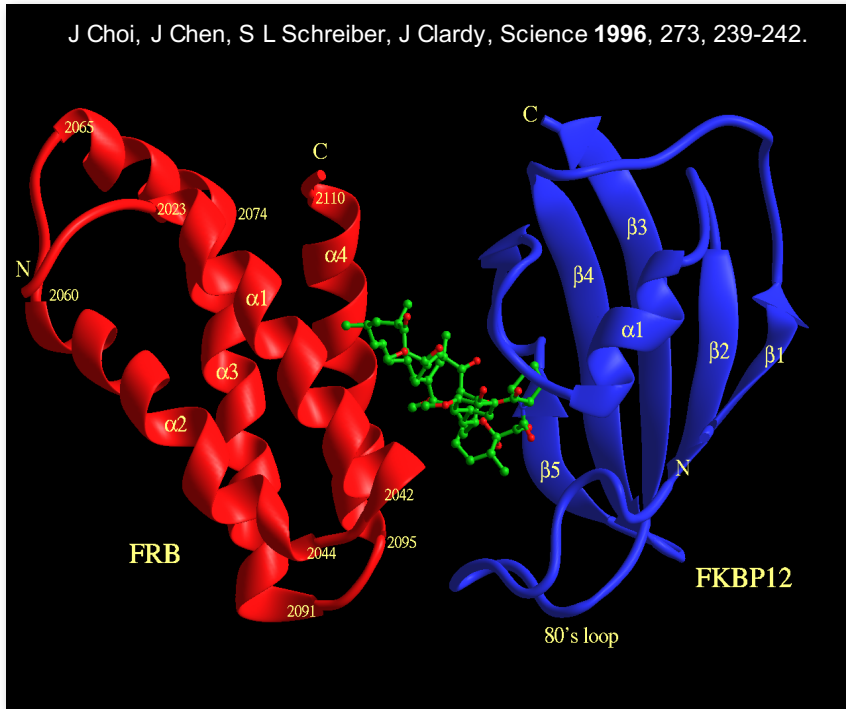


$$K_D = 0.5 \text{ nM}$$
$$k_d = 0.000965 \text{ sec}^{-1}$$



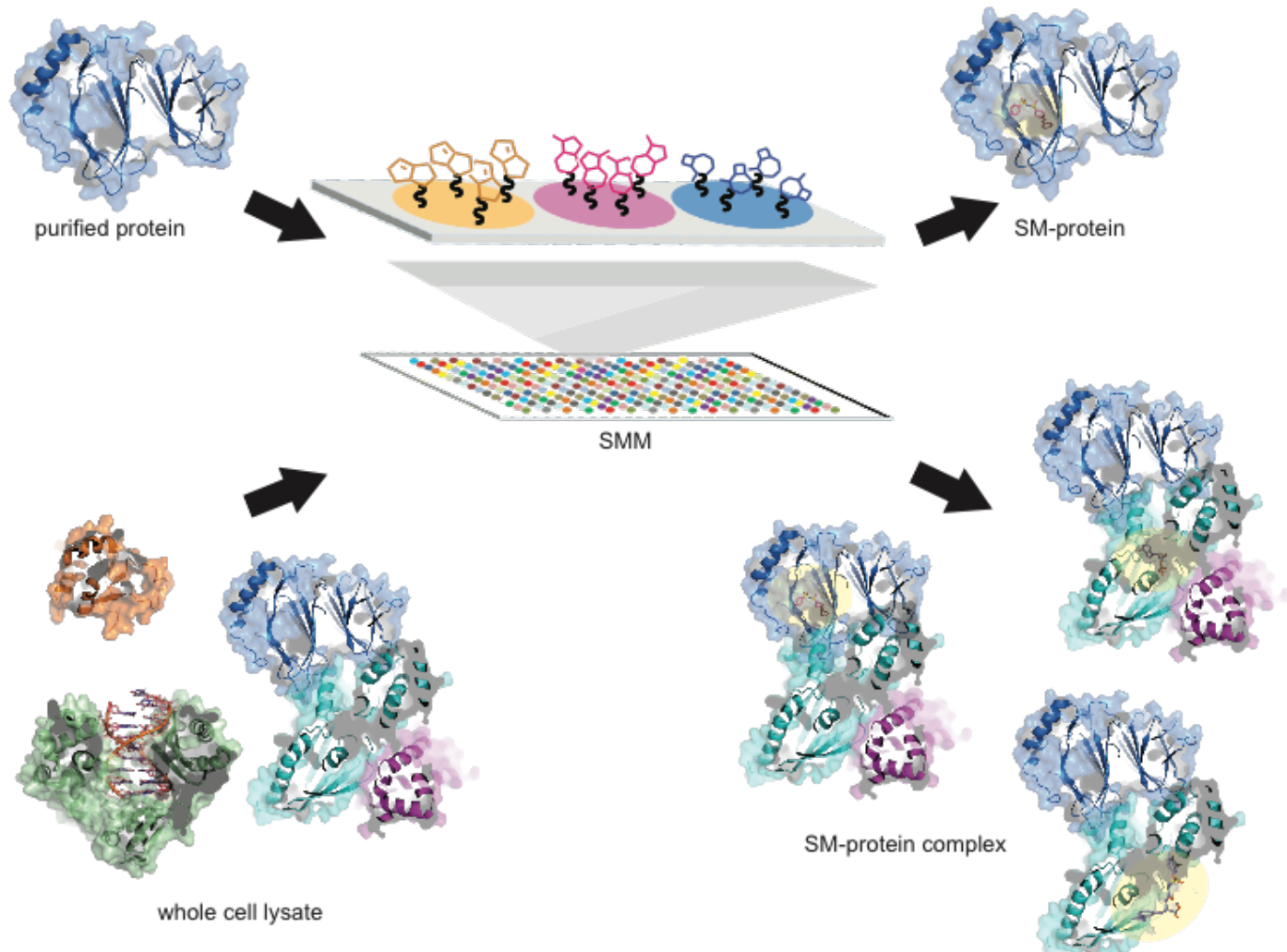
$$K_D = 18 \text{ nM}$$
$$k_d = 0.226 \text{ sec}^{-1}$$

Detecting multiple interactions with Rapamycin

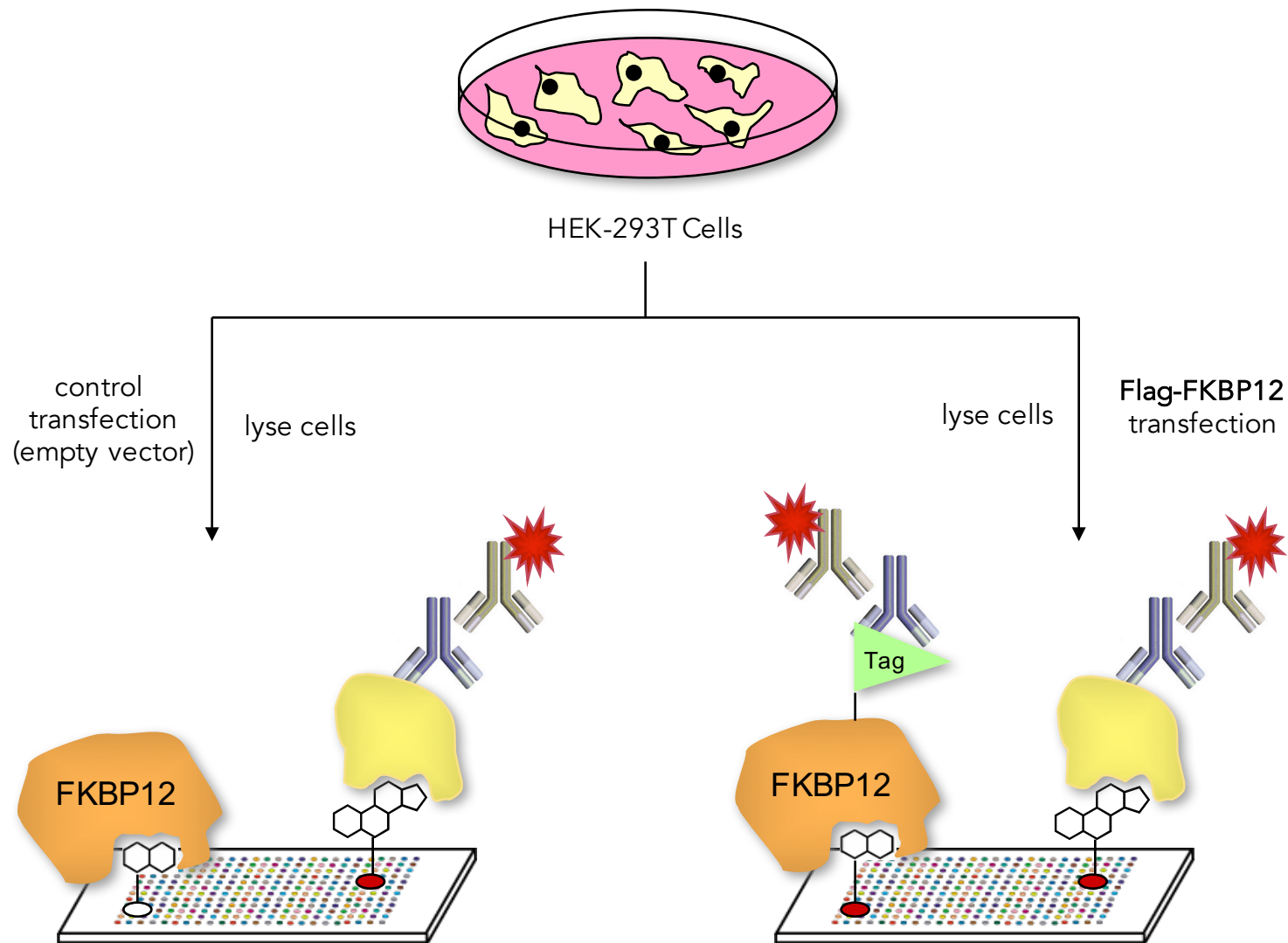


SMMs enable a new type of screen

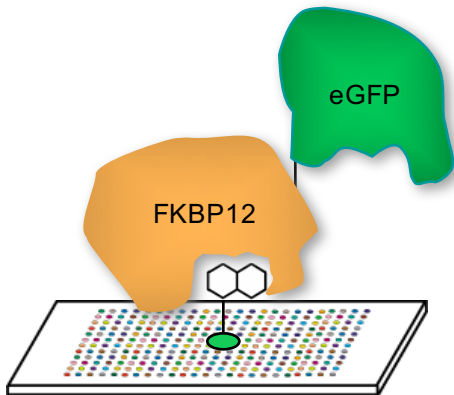
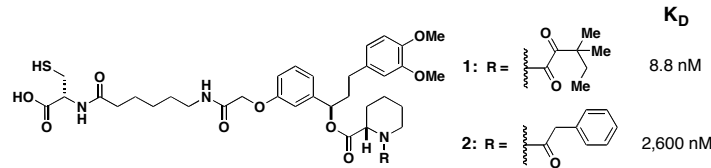
target-directed assays in a native environment



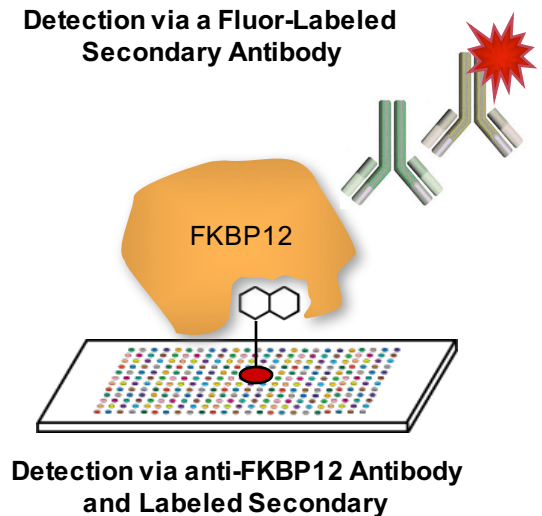
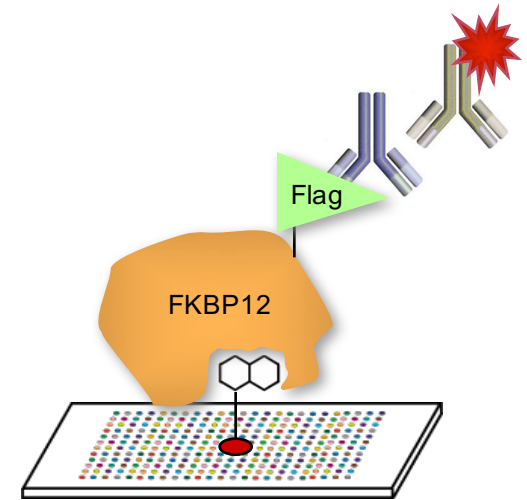
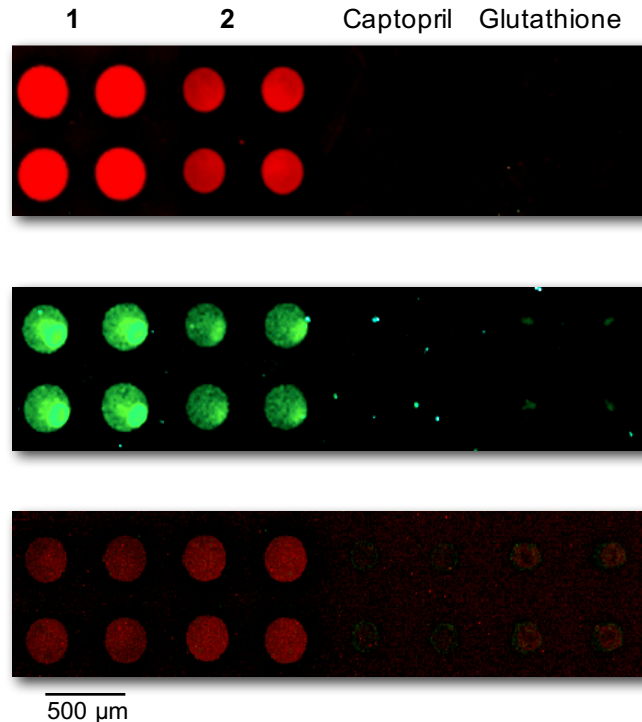
Binding screens involving cell lysates



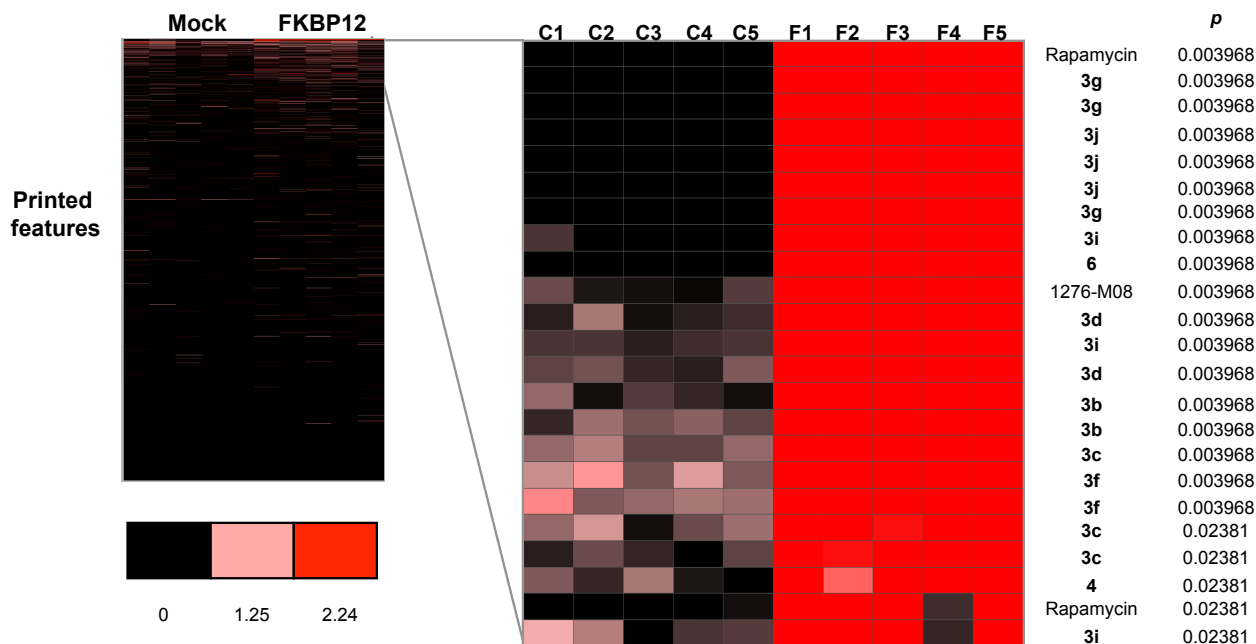
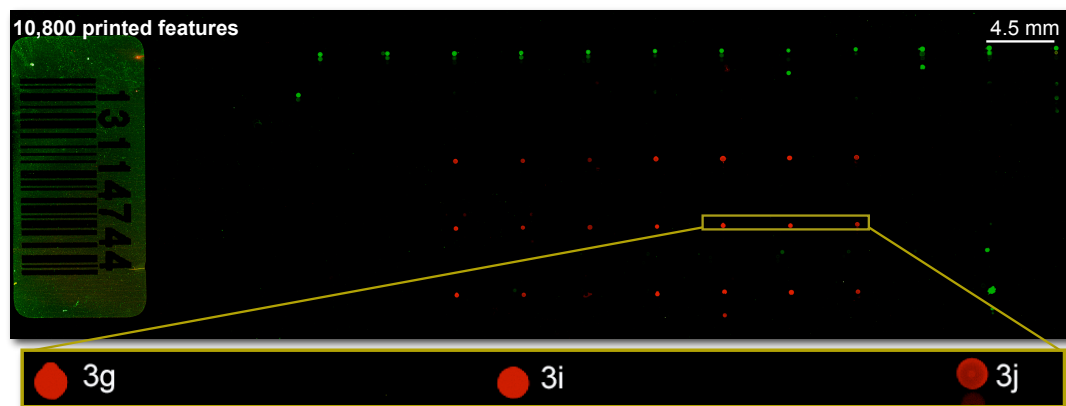
Comparing detection methods using lysates



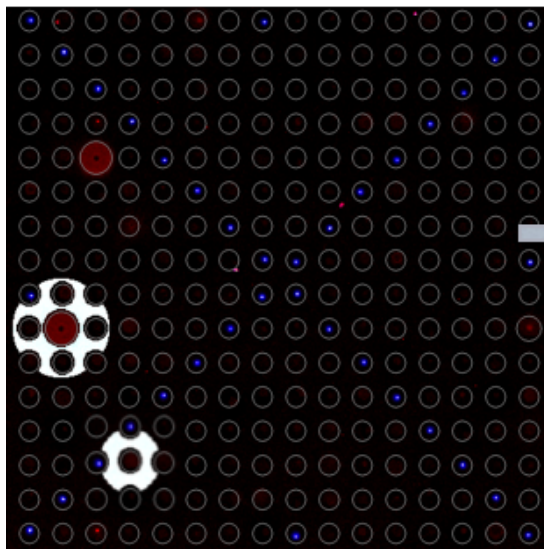
Detection via Green Fluorescent Protein



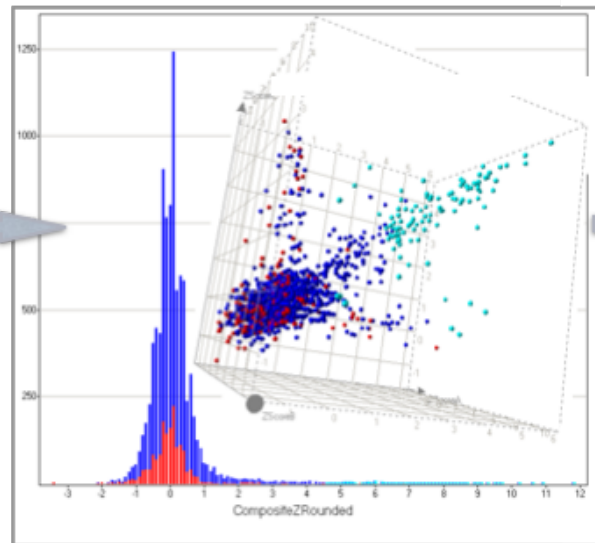
Binding screen using FKBP12 in cell lysates



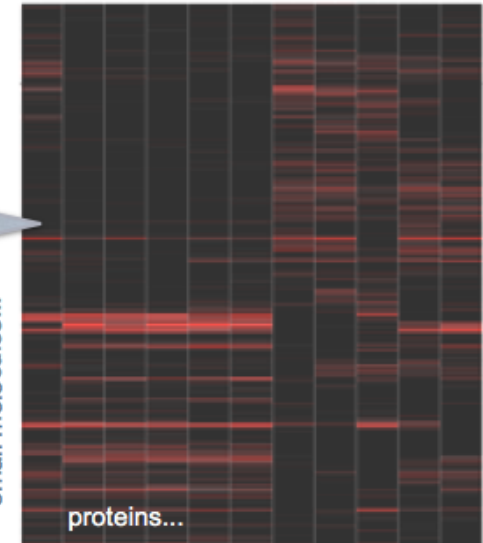
Analysis pipeline – the simple version



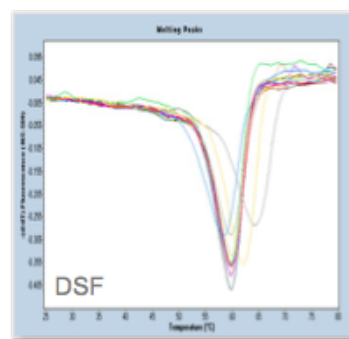
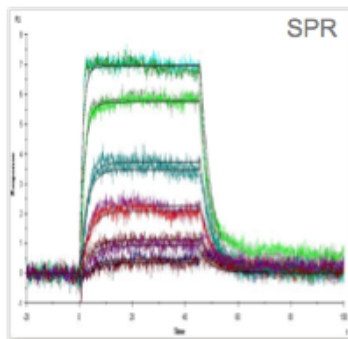
fluorescent features reveal putative interactions



compute composite Z-scores, "hit" calls

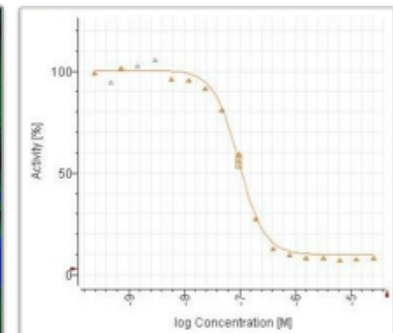
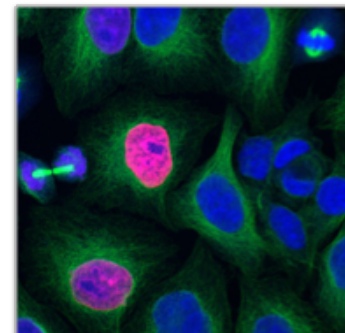


specificity analysis



secondary binding assays

+



functional assays

A community effort

Printed molecules:

Prabhat Arya, Steacie Institute for Molecular Sciences
Aaron Beeler, Boston University
Kay Brummond, University of Pittsburgh
Tom Chang, Utah State University
Young-Tae Chang, Singapore
Jon Clardy, Harvard Medical School
Mike Foley, Broad Institute
Dennis Hall, University of Alberta
Eric Jacobsen, Harvard University
Ohyun Kwon, UCLA
Tim Lewis, Broad Institute
Lisa Marcaurelle, Broad Institute
Ralph Mazitschek, MGH
Andy Myers, Harvard University
Jim Panek, Boston University
Andy Phillips, Yale
John Porco, Boston University
Scott Schaus, Boston University
Karl Scheidt, Northwestern University
Stuart Schreiber, Broad Institute
Matt Shair, Harvard University
Jared Shaw, UC Davis
Derek Tan, Memorial Sloan-Kettering Cancer Center
Junichi Tanaka, University of the Ryukyus
Stefan Werner, University of Pittsburgh
Peter Wipf, University of Pittsburgh
Keith Woerpel, NYU

Biology collaborators

Cris Bragg, MGH
Manoj Duraisingh, Harvard School of Public Health
Benjamin Ebert, Brigham and Women's Hospital
Levi Garraway, Dana-Farber Cancer Institute
Barbara Gilchrest, Boston University Medical School
Laurie Glimcher, Weill Cornell Medical College
Todd Golub, Broad Institute, Dana-Farber Cancer Institute
Isabella Graef, Stanford University
Stephen Haggarty, MGH
Michael Hecht, Princeton University
Peter Howley, Harvard Medical School
Elliott Kieff, Brigham and Women's Hospital
Sam Lee, MGH
Jon Madison, Stanley Center for Psychiatric Research
Anna Mandinova, MGH
Martin Matzuk, Baylor College of Medicine
Karl Münger, Brigham and Women's Hospital
Paul Nghiem, Fred Hutchinson Cancer Center
Stuart Orkin, Dana-Farber Cancer Institute, Children's Hospital
Stephane Richard, McGill University
Stuart Schreiber, Broad Institute
Stan Shaw, MGH
David Spiegel, Yale
David Spring, University of Cambridge
Robert Tjian, UC Berkeley
Jeff Toretsky, Lombardi Comprehensive Cancer Center, Georgetown
Greg Verdine, Harvard University
Warren Zapol, MGH

...

A community effort

Printed molecules:

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Jim Panek, Boston University
Andy Phillips, Yale
John Porco, Boston University
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Stuart Schreiber, Broad Institute
Matt Shair, Harvard University
Jared Shaw, UC Davis
Derek Tan, Memorial Sloan-Kettering Cancer Center
Junichi Tanaka, University of the Ryukyus
Stefan Werner, University of Pittsburgh
Peter Wipf, University of Pittsburgh
Keith Woerpel, NYU

■ SMM positives that score in functional assays

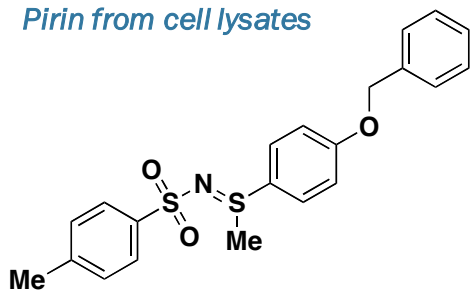
Biology collaborators

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Robert Tjian, UC Berkeley
Jeff Toretsky, Lombardi Comprehensive Cancer Center, Georgetown
Greg Verdine, Harvard University
Warren Zapol, MGH

...

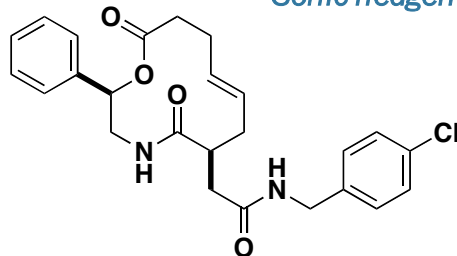
Representative probes discovered by SMMs

Pirin from cell lysates



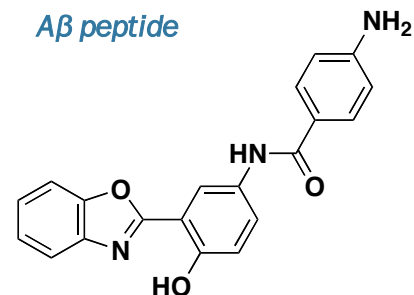
$K_D = 0.6 \mu\text{M}$ (ITC)
 inhibits pirin-Bcl3 interaction in cells
 inhibits melanoma cell migration
 Miyazaki *et al*, ACS Chem Biol 2010

Sonic hedgehog

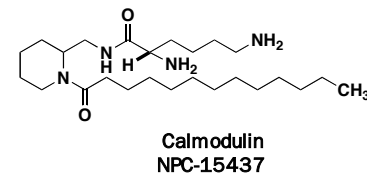
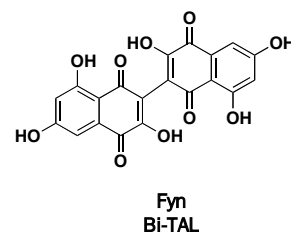
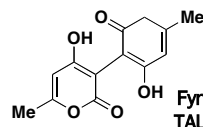
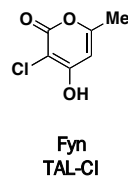
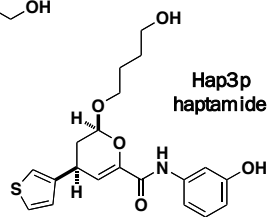
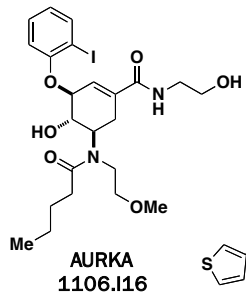
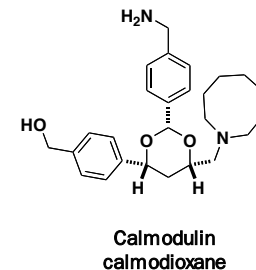
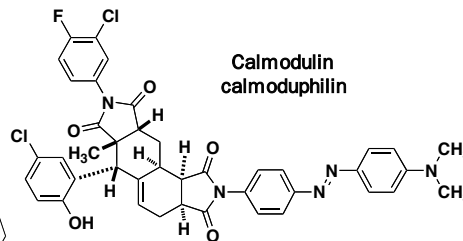
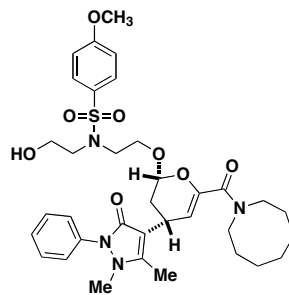
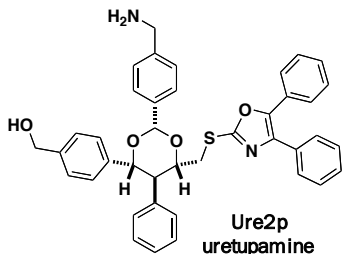
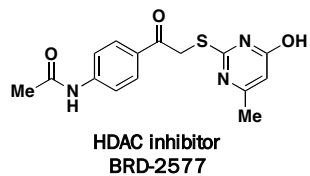


$K_D = 3.1 \mu\text{M}$ (SPR)
 analog of SMM hit that inhibits Shh
 signaling in cells and synthetic skin model
 Stanton *et al*, Nature Chem Biol 2010

A β peptide



K_D A β 40_{mon} ~ 9-17 μM (various methods)
 inhibits A β 42-induced cytotoxicity in PC12
 cells, accelerates fibril formation
 Chen *et al*, J. Am. Chem. Soc. 2010



Public access for SMM data sets

The screenshot shows the ChemBank website with a navigation menu on the left and search options for 'Find Small Molecules' and 'Find Assays'. The 'Find Small Molecules' section includes search methods like 'by substructure', 'by similarity', 'by SMILES', 'using descriptors', 'by assay', 'by function', 'by chemical', and 'by molecule name'. The 'Find Assays' section includes 'small molecule assays', 'biological assays', 'problem assays', and 'by biologist'.

<http://chembank.broad.mit.edu>

DSA-ChemBank: 796,063 curated compounds, 1,963 assays, 149 projects, 16,942,065 well measurements

ChemBank: 528,062 curated compounds, 529 assays, 45 projects, 5,764,724 well measurements

43,651 users
at 8,309
organizations
in 154 countries



The screenshot shows the PubChem website with a navigation menu at the top and search options for 'BioAssay', 'Compound', and 'Substance'. A search bar is present with a 'GO' button and 'Advanced Search' link. Below the search bar, there are statistics: '8 years 100,000,000 substances 200,000,000 bioactivities'. A sidebar on the right lists various tools and services like 'BioActivity Summary', 'Structure Search', and 'Download Facility'. Two news items are visible: 'Nearly 300,000 structures from ChemAxon's chemicalize.org database are now available in PubChem...' and 'More than 8 million structures from the SureChem patent chemistry database are now available in PubChem...'.

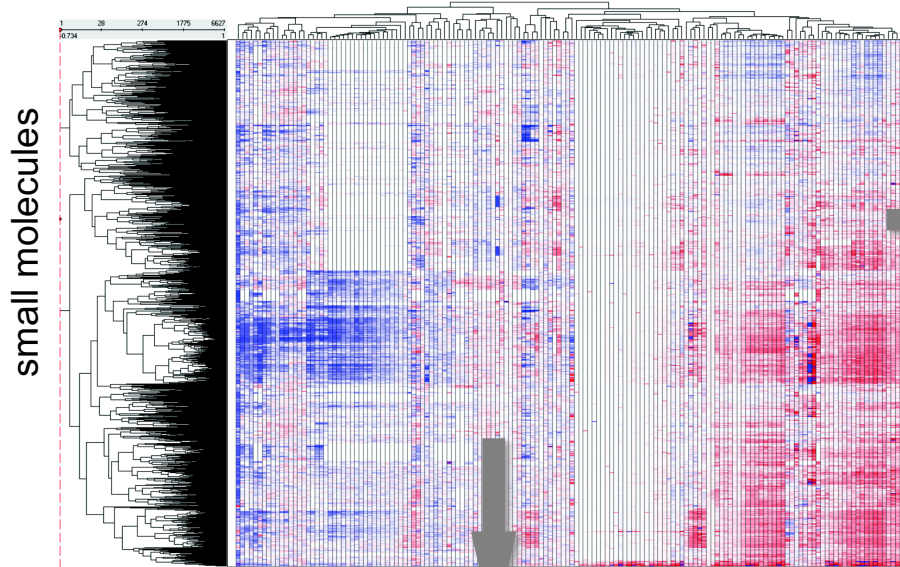
<http://pubchem.ncbi.nlm.nih.gov>



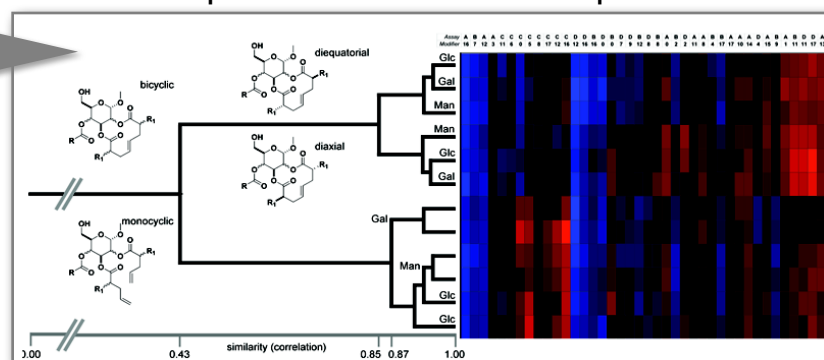
<http://bard.nih.gov/drupal>

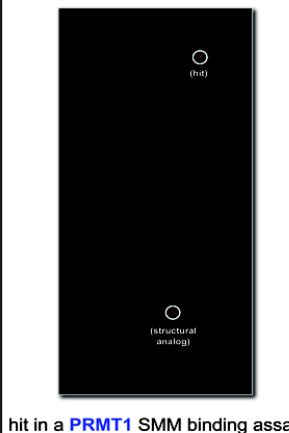
ChemBank: an analytical tool for the community

assays (cell-based, biochemical, binding)



relationship of structure to screen performance





hit in a **PRMT1** SMM binding assay

CHEMBANK

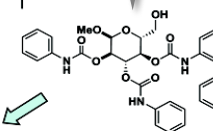
Find Small Molecules

- Search compound collection by substructure (may be searched using SMILES or SMARTS string, or drawn with JME Molecular Editor).
- Search compound collection by similarity to a structure (may be searched with SMILES string, or drawn with JME Molecular Editor).
- Filter compound collection using calculated molecular descriptor values.
- Find compounds scoring as 'hits' in biological assays.
- Find compounds with known biochemical interactions, therapeutic uses, or molecular functions.
- Find compounds made by a particular chemist, or sold by a particular vendor.
- Find compounds with a particular name, or containing a part of a name.

Find Assays

- Find all high-throughput screening (HTS) assays.
- Find all small molecule molecule (SMM) assays.
- Find assays performed by a particular screener.
- Find small molecule assays by screener, assay type, and project description.
- Find proteins using a combination of name, official symbol, UniProt accession, GeneRef, Accession #, Entrez Gene ID, or PDB ID criteria.

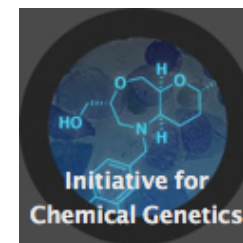
DOS compound



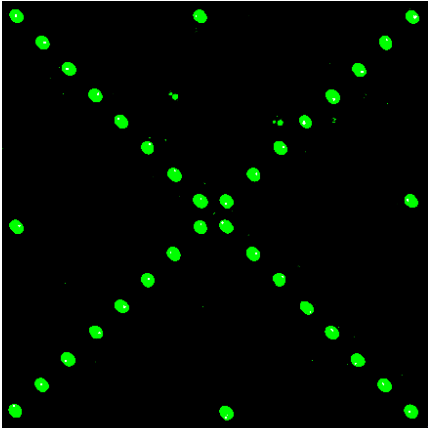
inhibitor of arginine HMT (PRMT1) enzymatic activity

high-signal outlier in a Wnt assay

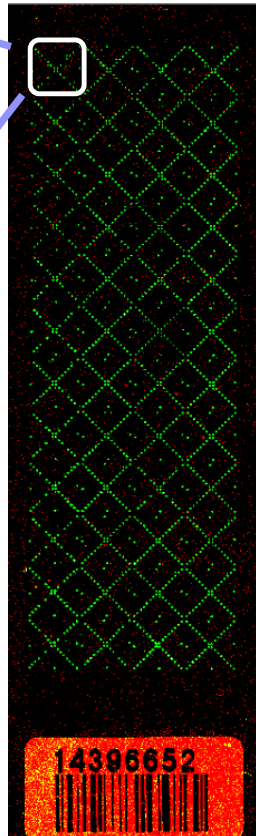
relationships between assays (protein and phenotype)



20.109 FKBP12 screens



subarray with
sentinel pattern for
alignment

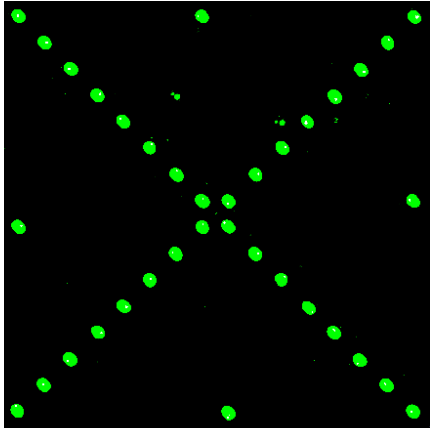


full array with 48
subarrays (4 x 12)

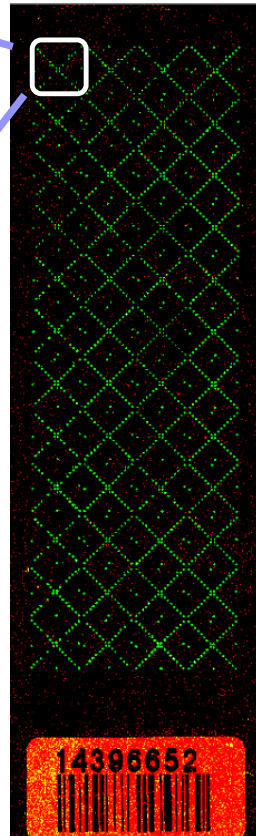


'Gradbot'
Rob

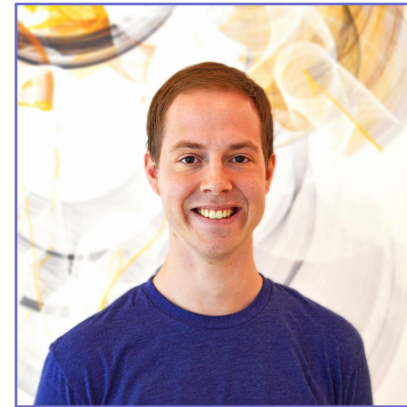
20.109 FKBP12 screens



subarray with
sentinel pattern for
alignment



full array with 48
subarrays (4 x 12)

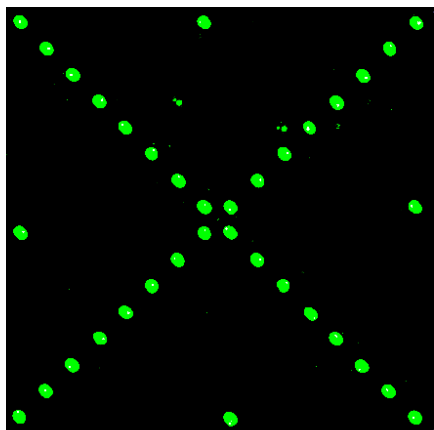


'Gradbot'
Rob

each team screens
5,000 unique
compounds

$16 \times 16 \times 48 = \mathbf{12,288}$
2 replicate slides
4 replicates for each compound

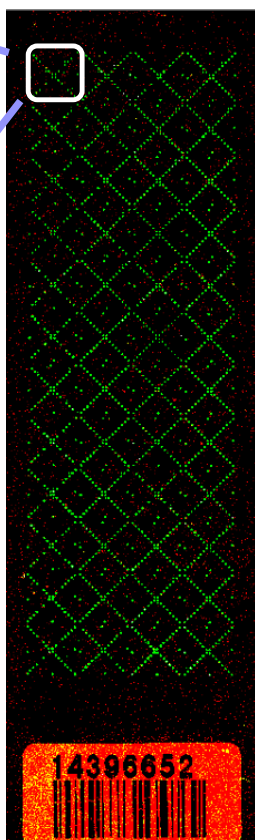
20.109 FKBP12 screens



subarray with sentinel pattern for alignment

each team screens
5,000 unique
compounds

16x16x48 = **12,288**
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4 replicates for each compound

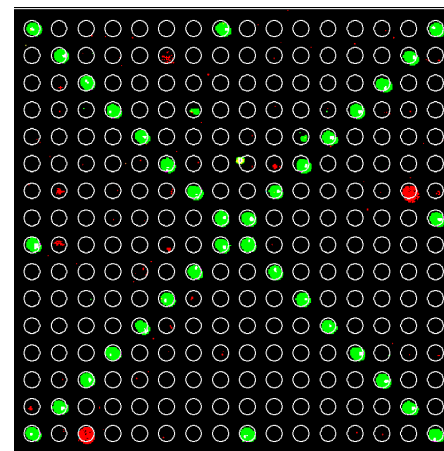


full array with 48
subarrays (4 x 12)

your pure
FKBP12

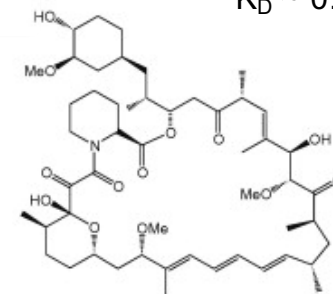


scan



subarray with 'gal file'
(genepix alignment) file
superimposed

$K_D \sim 0.5 \text{ nM}$



rapamycin

Our path to probe discovery - lectures

2/14/17	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
2/16/17	Lecture 2	For the love of proteins: FKBP12 and immunophilins
2/21/17	No Lecture	
2/23/17	Lecture 3	Small-molecule microarrays
2/28/17	Lecture 4	Analyzing SMM data sets (Shelby Doyle)
3/2/17	Lecture 5	Chemical probe stories
3/7/17	Lecture 6	Wrap up discussion: suggestions for how to report your findings