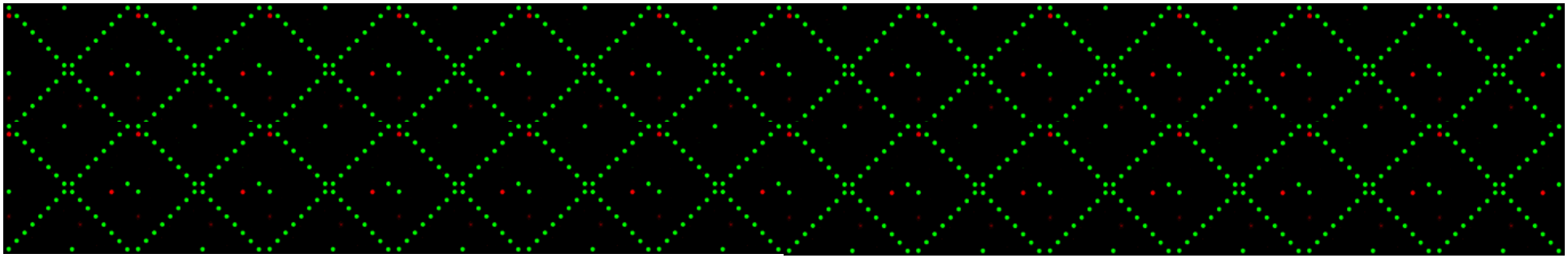
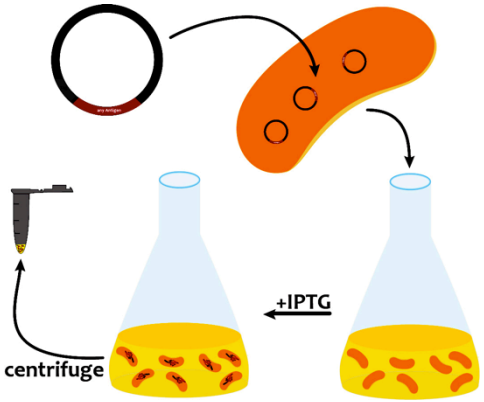


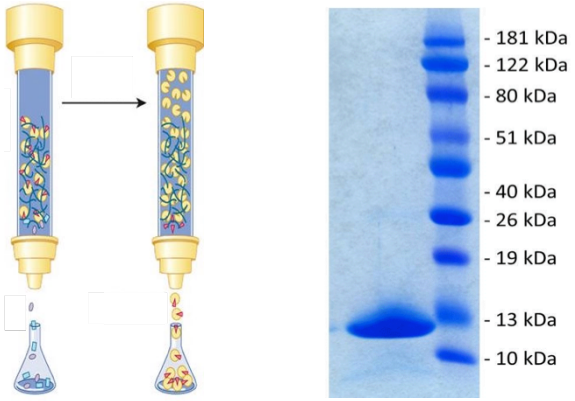
Module 1 wrap up discussion

March 7, 2017

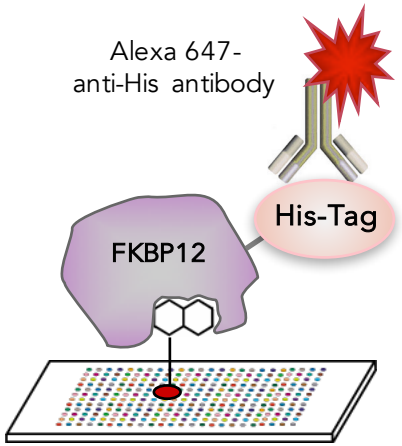
Our path to probe discovery



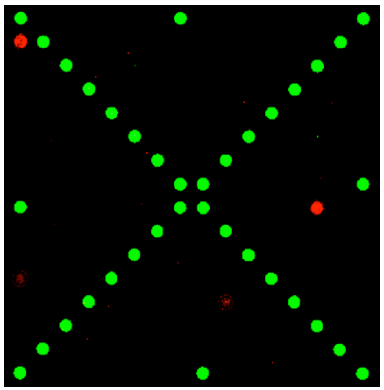
overexpress FKBP12
lab day 1



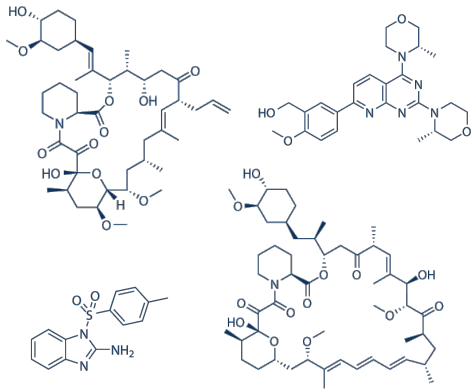
purify and characterize FKBP12
lab days 2 and 3



SMM screen
lab day 4

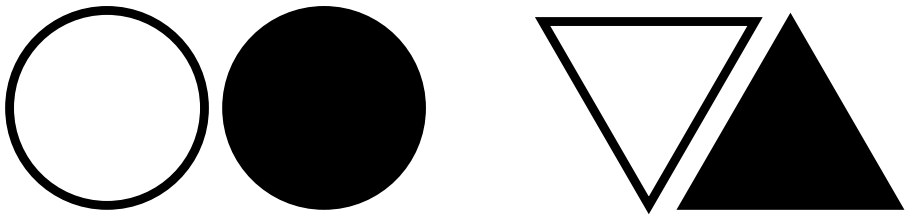


scan images and analyze data
lab days 5 and 6

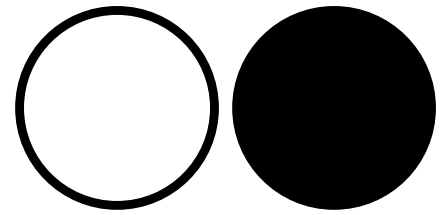


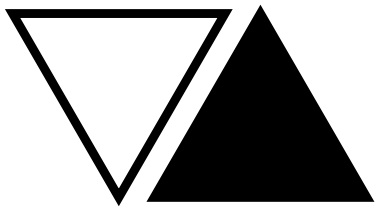
compare hit lists for teams
lab day 7

20.109: ‘By-eye chem-informatics’



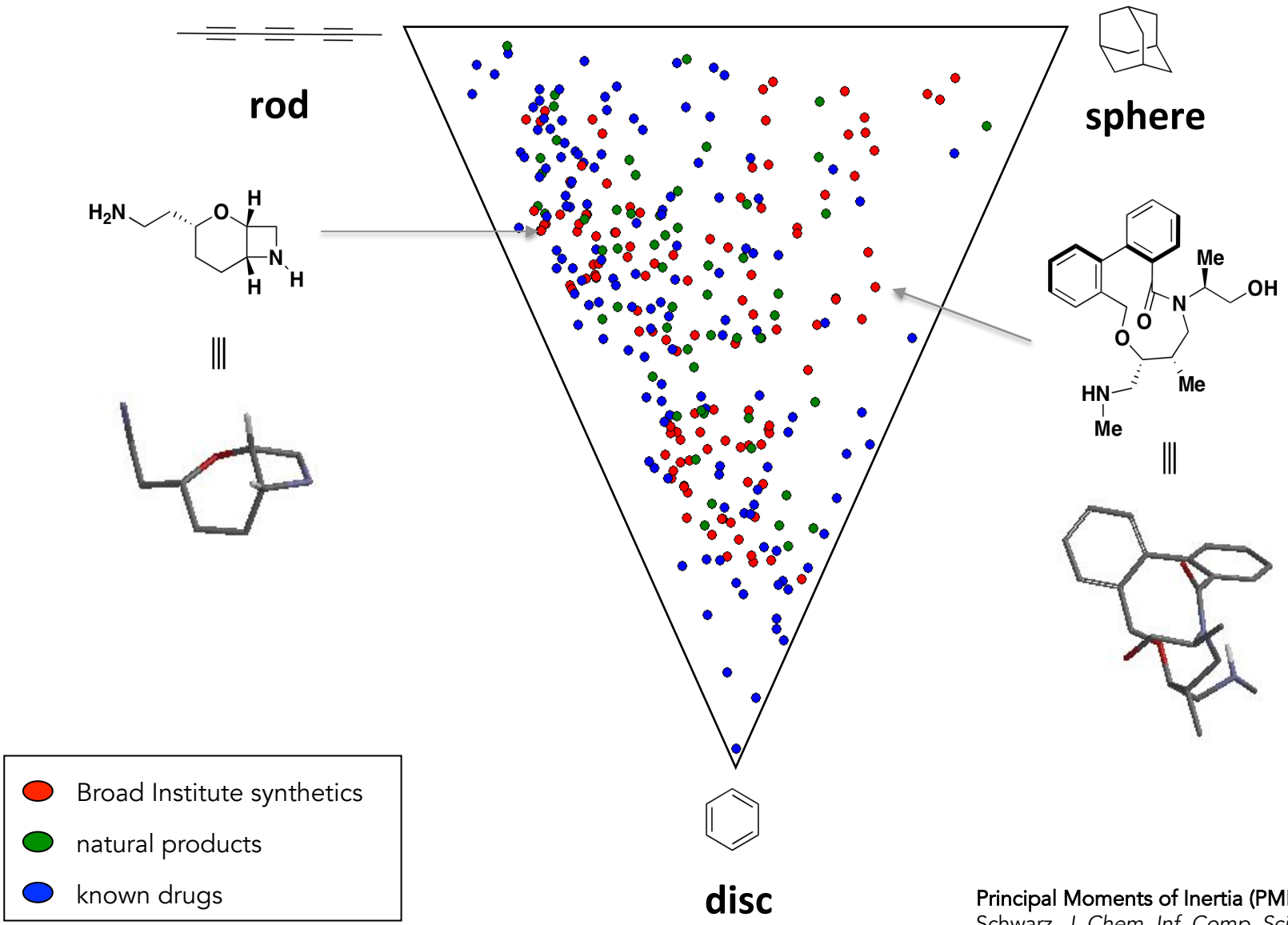
Embedded differences – the domain of computation





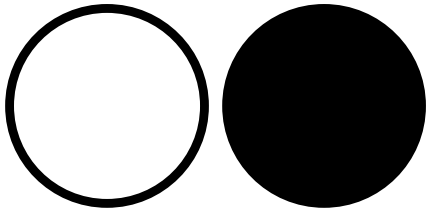
cheminformatics helps probe and drug finders make sense of the tidal wave of information coming from their screens

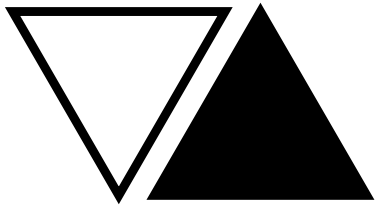
Principal moment of inertia (PMI) plots

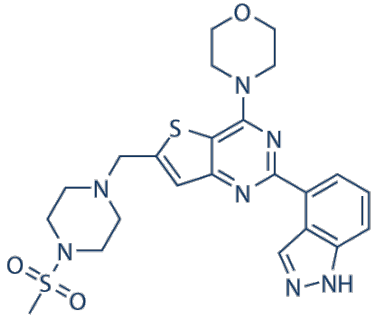


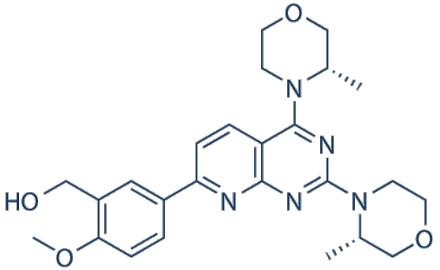
Principal Moments of Inertia (PMI) Plots: Sauer & Schwarz, *J. Chem. Inf. Comp. Sci.* **2003**, *43*, 987-1003

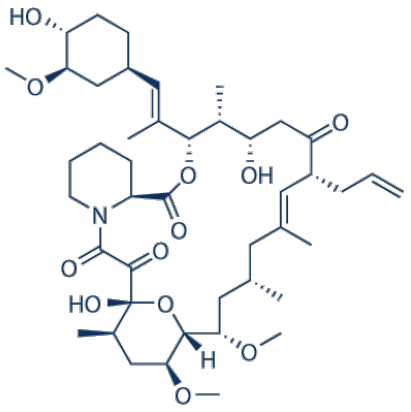
20.109: ‘By-eye chem-informatics’

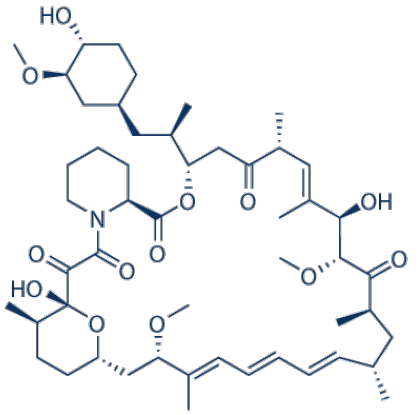




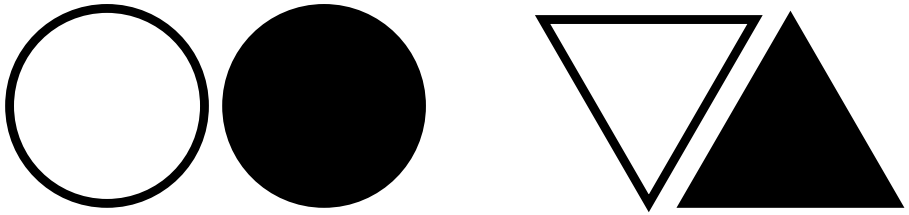


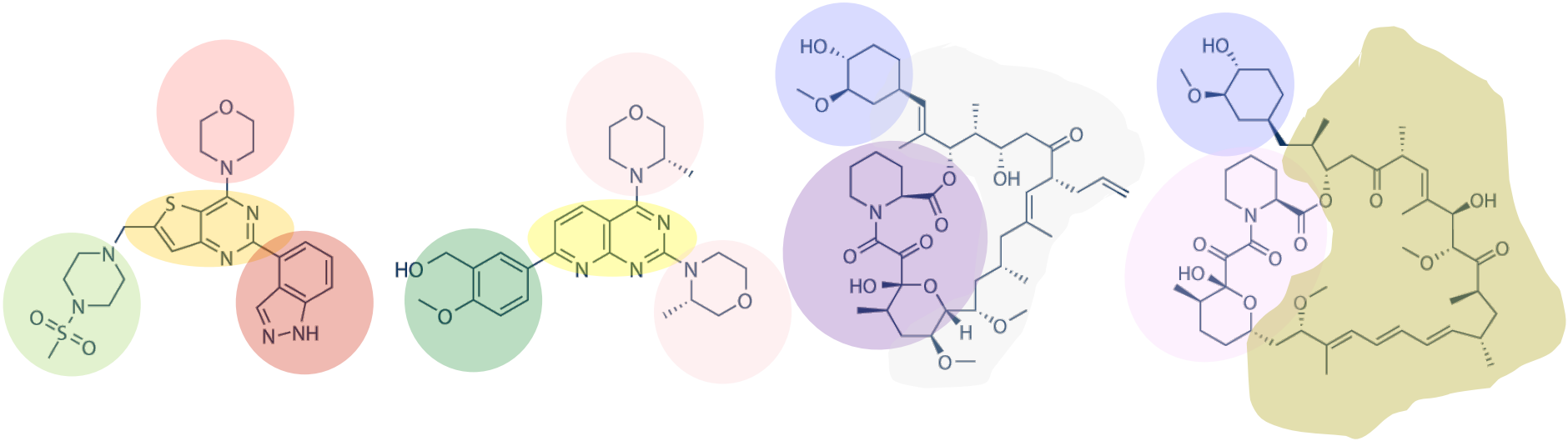






20.109: ‘By-eye chem-informatics’





Report for Module 1

scientific abstract

nature
chemical biology

A small molecule that binds Hedgehog and blocks its signaling in human cells

Benjamin Z Stanton[1,2,7], Lee F Peng[1–3,7], Nicole Maloof[1], Kazuo Nakai[2], Xiang Wang[1], Jay L Duffner[1], Kennedy M Taveras[1], Joel M Hyman[4], Sam W Lee[5], Angela N Koehler[1], James K Chen[4], Julia L Fox[6], Anna Mandinova[5] & Stuart L Schreiber[1,2]

Small-molecule inhibition of extracellular proteins that activate membrane receptors has proven to be extremely challenging. Diversity-oriented synthesis and small-molecule microarrays enabled the discovery of robotnikinin, a small molecule that binds the extracellular Sonic hedgehog (Shh) protein and blocks Shh signaling in cell lines, human primary keratinocytes and a synthetic model of human skin. Shh pathway activity is rescued by small-molecule agonists of Smoothened, which functions immediately downstream of the Shh receptor Patched.

Report for Module 1

project summary

thorough summary of your data and figures with supporting text –

include context so that a **scientifically literate reader** can understand the work and its broader implications

details related to the format and content are on the 20.109 wiki
(RTK example posted)

Report for Module 1

format and content

Layout: Portrait, not landscape.
Font: Arial 14pt for text; Arial 12pt for figure captions.
Text should be written as **bullet points**, not full sentences and paragraphs.

Content details

First page: Title and **Author** information (section/color/names)
Second page: Abstract
Body: 8-12 pages (not including Title and Abstract pages). Recommended section lengths (including both text and figures):

Background and Motivation: 2 slides

Contents of Background and Motivation: The majority of this section will be bulleted text. Include schematic figures when appropriate.

Results and Interpretation: 5-8 slides

Contents of a Results and Interpretation slide: Top half: figure(s) with caption(s). Bottom half: bullet points that present and interpret the data. (Remember that captions should not contain interpretation.)

Figure presentation: In published research figures are rarely a full page in size; rather each plot is usually only 3 inches x 3 inches.

Present you Results and Interpretation such that the figure, caption, and interpretation bullet points **all fit on a single slide**. Remember that when you shrink a figure, you must make sure it remains legible.

Implications and Future Work: 1-2 slides

Contents of Implications and Future Work: This section will be bulleted text.

Background and motivation

suggested topics or figures

Topic: Introduce and discuss the importance of chemical probes in biology and / or research

Topic: Introduce and discuss the utility of small-molecule microarrays (SMMs)

Figure: Simplified schematic of SMM slide printing

Topic: Introduce and discuss the role of FKBP12

Topic: Discuss your experimental goal

Schematic: Experimental approach

Results and Interpretation

suggested topics or figures

Protein purification

Schematic: Experimental design

Topic: FKBP12 purification

Figure: Image of polyacrylamide gel

Figure: Graph or table displaying cell protein concentration

Ligand screening

Schematic: Experimental approach

Topic: Identification of positive hits

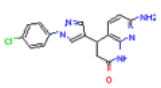
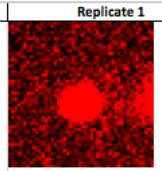
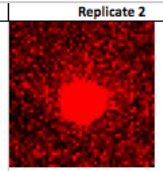
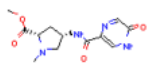
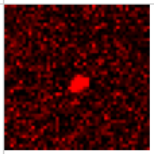
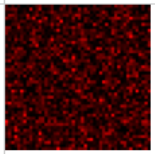
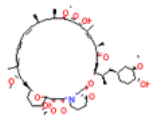
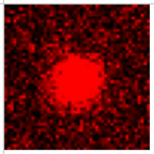
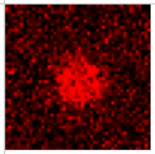
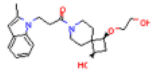
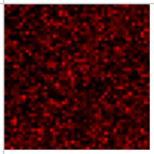
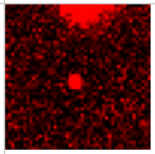
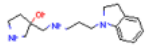
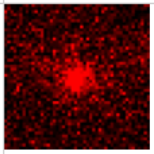
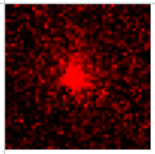
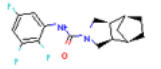
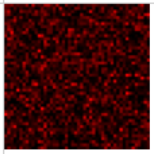
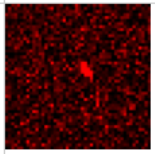
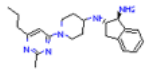
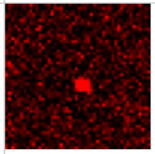
Figure: Image from representative scan

Figure: Graph or table comparing z-scores and p-values

Topic: Chemical structure comparison

Figure: Images of positive hits

slide #14399987

Name	Average SNR 635	StDev SNR 635	Robust Z	CV	Structure	Replicate 1	Replicate 2
-C2)N)C3=C(	11.09194929	6.864722182	146.8931	0.618892			
C(=O)OC)NC	9.279052521	13.17224064	122.9213	1.419567			
C@H](C)C[C	6.022534408	1.746392727	79.86067	0.289976			
N3CCC4(CC	3.761380837	5.202859447	49.96163	1.383231			
C=C21)CCC	2.674391484	0.451015013	35.58846	0.168642			
3[C@H]2CN	2.535966427	3.586650286	33.75808	1.414313			
(CC2)N[C@	2.409495367	3.258417089	32.08577	1.352323			
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Excel files are on the Wiki

Thank you, Rob and Maxine!

J|A|C|S
ARTICLES

Small Molecule Microarrays Enable the Discovery of Compounds That Bind the Alzheimer's Aβ Peptide and Reduce its Cytotoxicity

Jermont Chen,[†,‡] Anne H. Armstrong,[†] Angela N. Koehler,[§] and Michael H. Hecht*[,†]

Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States, and Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02142, United States

Received August 20, 2010; E-mail: hecht@princeton.edu

Abstract: The amyloid-β (Aβ) aggregation pathway is a key target in efforts to discover therapeutics that prevent or delay the onset of Alzheimer's disease. Efforts at rational drug design, however, are hampered by uncertainties about the precise nature of the toxic aggregate. In contrast, high-throughput screening of compound libraries does not require a detailed understanding of the structure of the toxic species, and can provide an unbiased method for the discovery of small molecules that may lead to effective therapeutics. Here, we show that small molecule microarrays (SMMs) represent a particularly promising tool for identifying compounds that bind the Aβ peptide. Microarray slides with thousands of compounds immobilized on their surface were screened for binding to fluorescently labeled Aβ. Seventy-nine compounds were identified by the SMM screen, and then assayed for their ability to inhibit the Aβ-induced killing of PC12 cells. Further experiments focused on exploring the mechanism of rescue for one of these compounds: Electron microscopy and Congo red binding showed that the compound *enhances* fibril formation, and suggest that it may rescue cells by accelerating Aβ aggregation past an early toxic oligomer. These findings demonstrate that the SMM screen for binding to Aβ is effective at identifying compounds that reduce Aβ toxicity, and can reveal potential therapeutic leads without the biases inherent in methods that focus on inhibitors of aggregation.

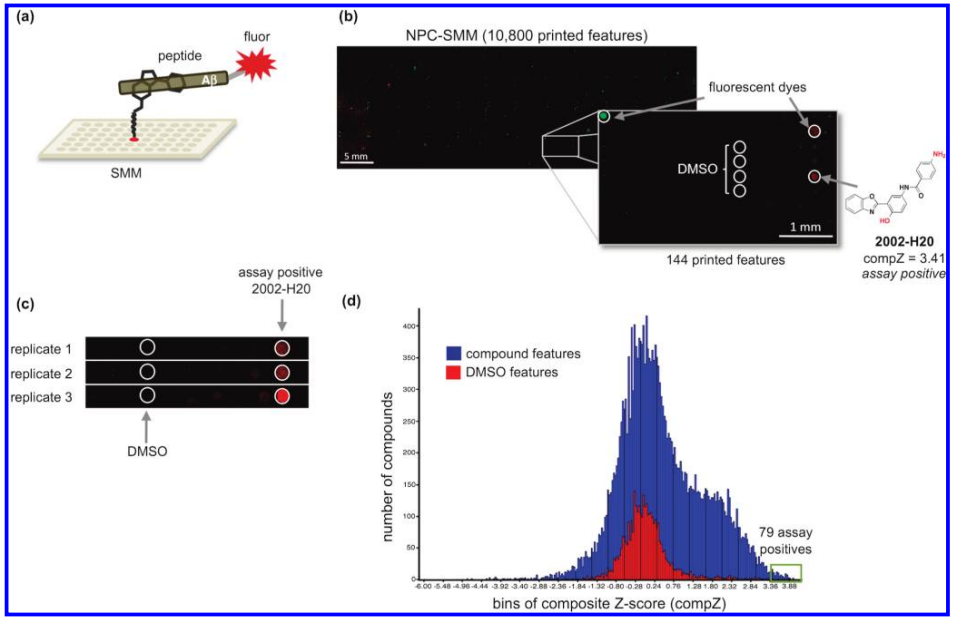


Figure 2. (a) The SMM binding screen. Compounds are covalently attached in an array of spots on the surface of a slide, and probed with fluorescently tagged Aβ peptide. Those compounds that bind Aβ and withstand several washes are revealed as fluorescent spots. (b) Fluorescent read-out of the NPC-SMM slide following incubation with fluorescent Aβ40. Enlargement of a grid section shows compound 2002-H20 binding the peptide (false-colored red) as well as fluorescent dyes used in grid alignment (false-colored green and red) and nonfluorescing DMSO control spots. The structure of 2002-H20 is shown with isocyanate-reactive functional groups colored red to indicate the positions available for attachment to the slide. Because two functional groups (an amine and a phenol) are available for cross-linking, the population displayed on the surface is assumed to include molecules displayed in more than one orientation, with some exposing the amine and others exposing the phenol for interaction with Aβ. (c) Three replicate SMM screens of the NPC compound set show that compound 2002-H20 binds fluorescently labeled Aβ40 reproducibly and consistently. (d) Histogram of the composite *Z*-scores of SMM fluorescence results from 3 replicates of the DIV and NPC slides. Results are divided into 254 bins with compounds shown in blue and DMSO controls in red. The green box surrounds bins for 79 assay positive compounds with composite *Z*-scores $\geq$ 3.4.

J Am Chem Soc. 2010 Dec 1;132(47):17015-22.
doi: 10.1021/ja107552s.

Implications and Future Work

Why is your work impactful and what would you do next?

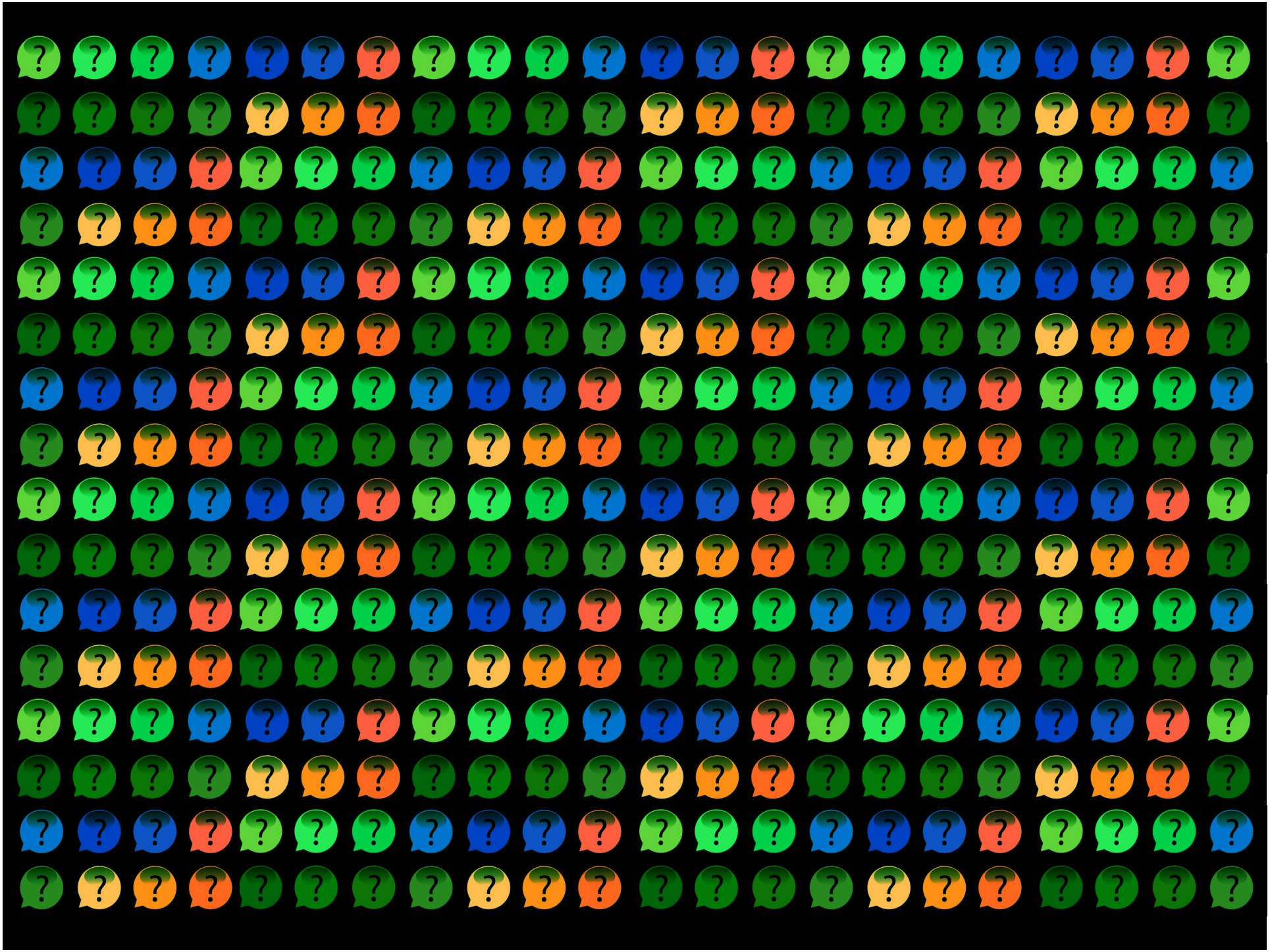
Topic: What is the positive hit rate? Is this consistent with similar research?

Topic: Do your hits share any common chemical structures?

If no, provide a putative explanation.
If yes, how can you further test if this structure is important in binding?

Topic: How might you validate that your SMM positive are binders and measure quantitative affinity values for the protein-ligand interaction?

Topic: How can you use your FKBP12 binders to further research focused on this protein?

Topic: How might this method be improved?

Topic: How might this assay be used in the clinic? in industry?