Wrapping Up Module 1

Data Summary Assignment

March 7, 2023

Module 1 experimental path from Wiki

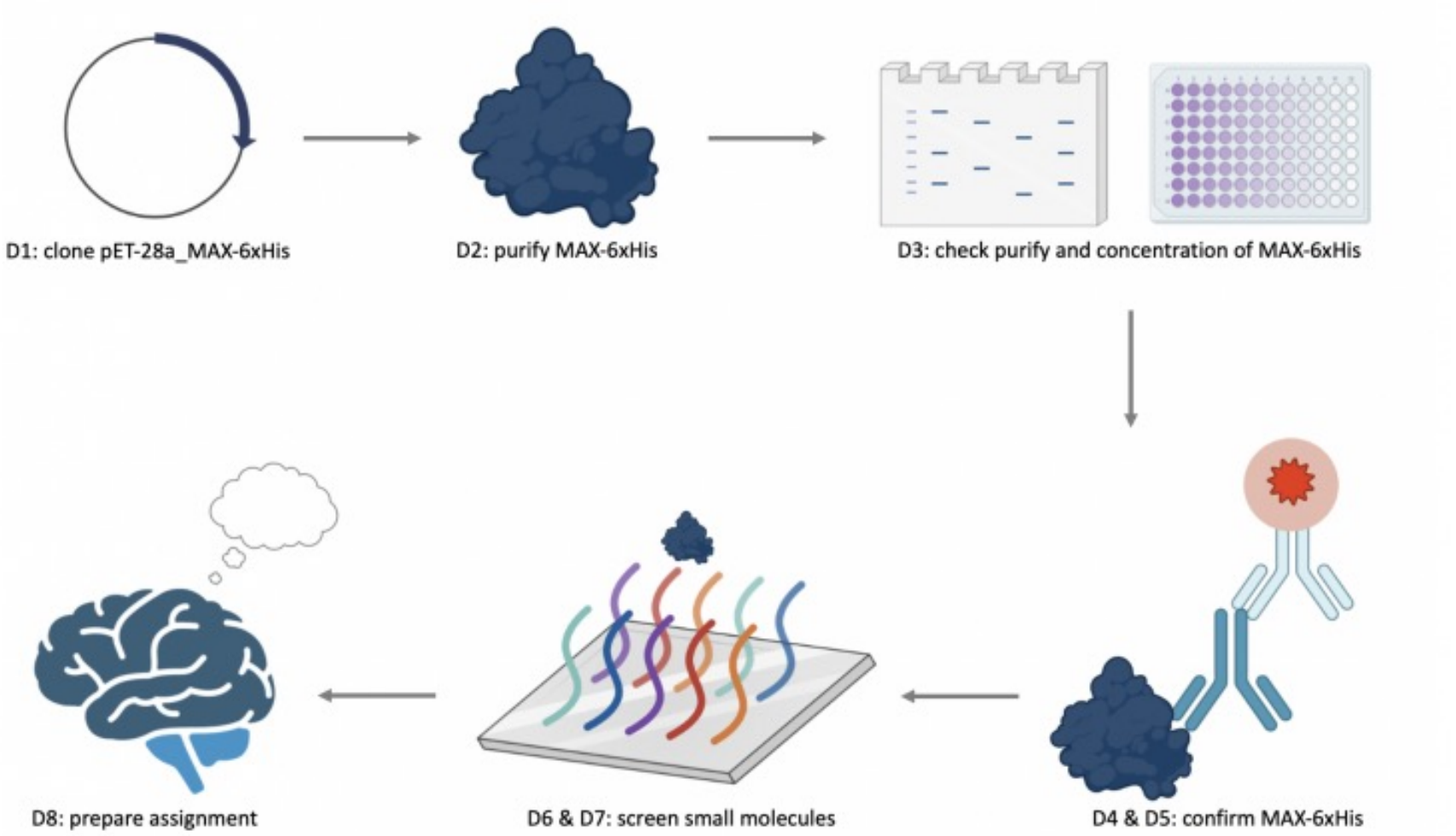
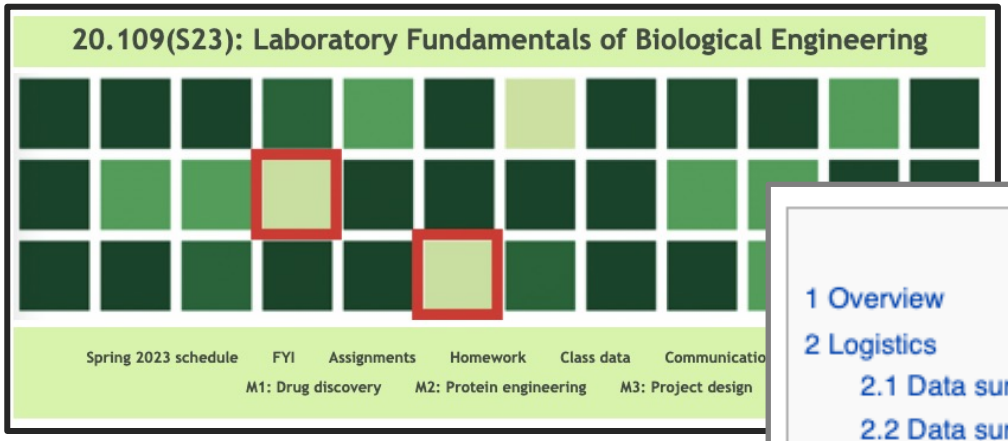


Image generated using BioRender.

Mod1 Assignment – Data Summary



Contents [hide]

The course Wiki sums up the assignment details – *scope, formatting, length, etc.*

Module 1 Data 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

The figure format is similar to a scientific journal article, but the traditional *Results and Discussion* sections are to be condensed into succinct bullet form accompanying each figure

you will complete the assignment with your lab partner

Data Summary Due Dates

Draft data summary – due Saturday, March 18th by 10 PM

Submissions read by instructional staff with feedback and a preliminary grade, which can be improved in a revised report

Comments provided by March 22nd

Data summary revision – due Monday March 27th by 10 PM

Instructions on how to incorporate changes are posted on the Wiki

Data Summary for Module 1

format and content guidelines – see Wiki

Create your report as a series of PowerPoint slides. This will allow you to create figures that are representative of those found in the literature (*i.e.* sized appropriately with sub-panels if necessary). See example of appropriate format here.

Format details

- **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**: 4-6 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.

Data Summary 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.

Please review Title and Abstract slides from
BE Comm Lab workshop

Background and Motivation

suggested topics or figures

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

Topic: Introduce and discuss the utility of small-molecule microarrays (SMMs) to find putative ligands

Topic: Introduce and discuss the roles of MAX in biology and/or disease

What are Max's roles?
Why is it an interesting protein from a therapeutic perspective?
Which functions of MAX would you like to perturb?

Topic: Discuss your experimental goal

Schematic: Experimental approach - clear and simple summary of your strategy (something similar in scope to Wiki figure)

Results and Interpretation

suggested topics or figures

Protein purification

Schematic: Experimental design

Topic: MAX protein purification

Figure: Image of polyacrylamide gel

Figure: Graph or table displaying cell protein concentration

Small-Molecule Microarray Screen

Schematic: Experimental approach

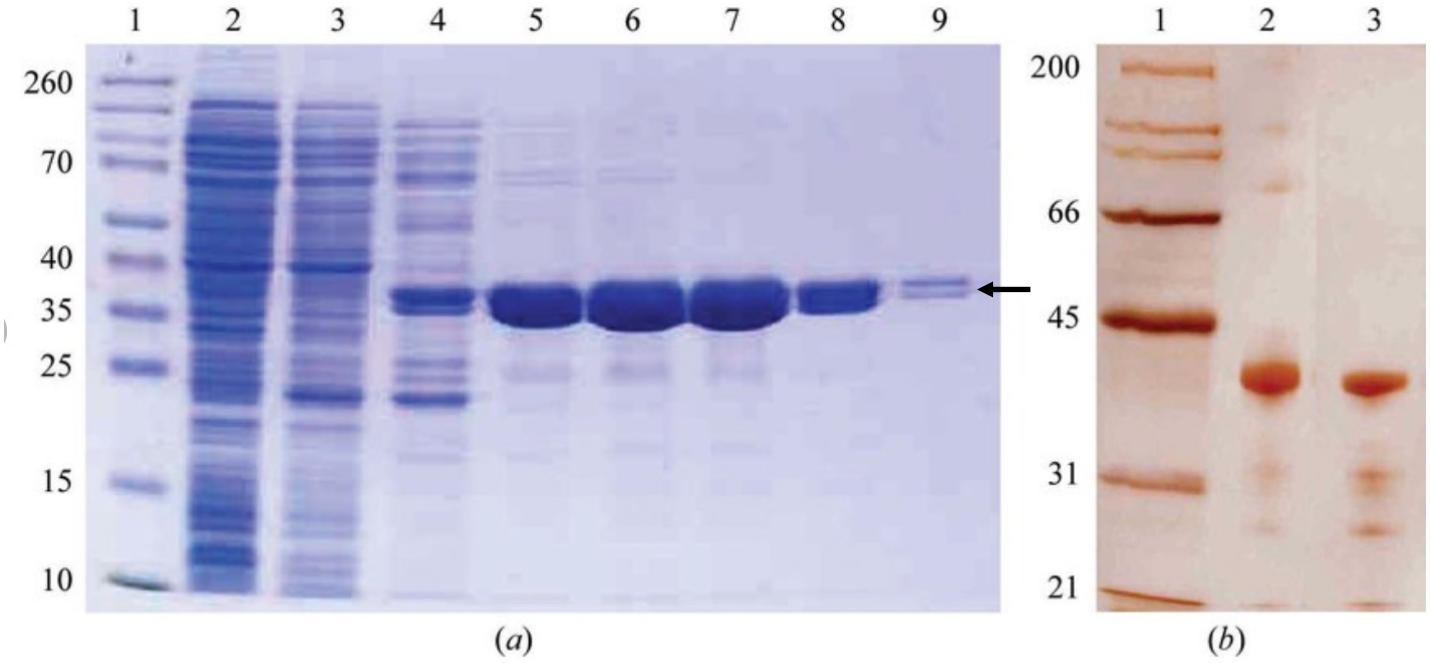
Topic: Identification of positive hits

Figure: Graph or table comparing hit metrics

Topic: Chemical structure comparison (useful to include identifier)

Figure: Images of positive hits (individual spots that correspond to hits)

Nice example of a figure: Please include captions, labels



SDS-PAGE analyses of TrxB protein and crystals. (a) 15% SDS-PAGE gel stained with Coomassie Brilliant Blue R-250 following the isolation of S. coelicolor TrxB. Lane 1, molecular-weight standards (Fermentas; labelled in kDa); lane 2, cell extract loaded onto the Ni column; lane 3, flowthrough from the Ni column; lane 4, fraction washed from column by 60 mM imidazole; lanes 5-9, fractions eluted from Ni column by elution buffer (20 mM Tris-HCl pH 7.9, 0.5 M NaCl) supplemented with 0.1, 0.2, 0.3 and 1 M imidazole, respectively. Fractions 5-8 were pooled and used for crystallization. (b) 15% SDS-PAGE silver-stained gel of TrxB crystal. Lane 1, molecular-weight standards (Bio-Rad; labelled in kDa); lane 2, dissolved crystal of TrxB; lane 3, protein sample of TrxB used for crystallization experiments.

Crystallization and diffraction analysis of thioredoxin reductase from Streptomyces coelicolor

August 2011 · Acta Crystallographica Section F Structural Biology and Crystallization Communications 67(Pt 8):917-21

DOI:10.1107/S1744309111021385

Source · PubMed

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.

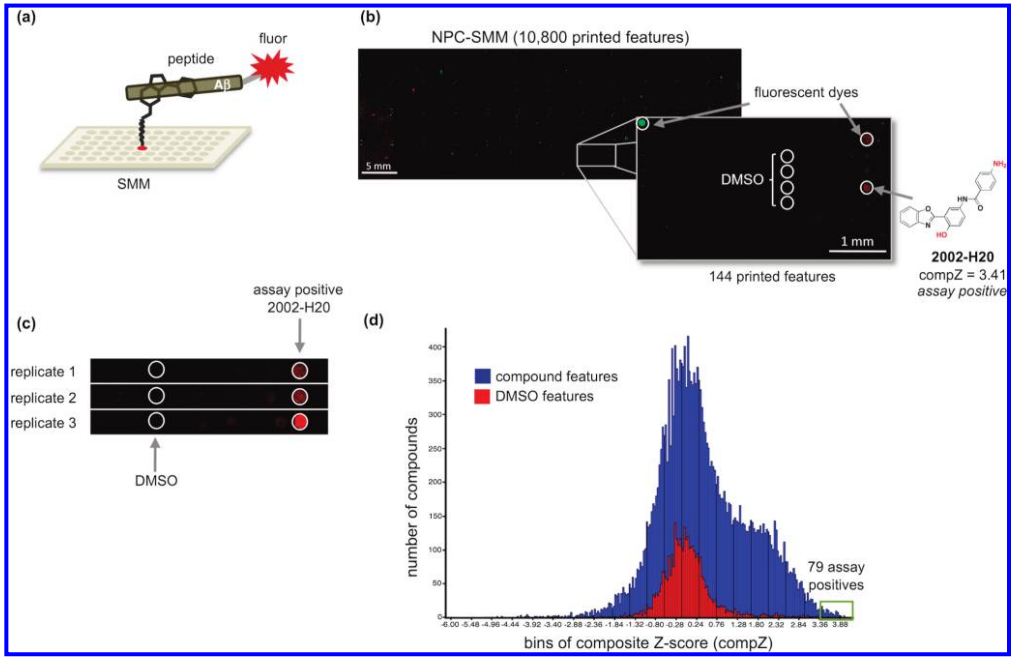
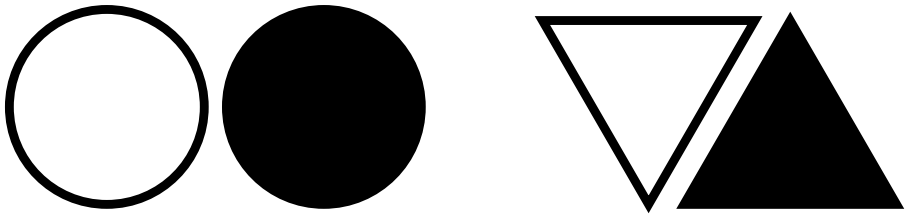


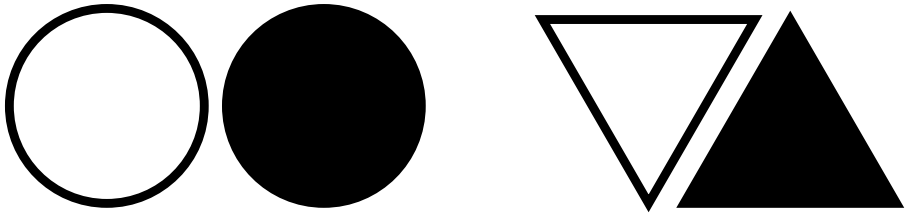
Figure 2. (a) The SMM binding screen. Compounds are covalently attached in an array of spots on the surface of a slide, and probed with fluorescently tagged Aβ peptide. Those compounds that bind Aβ and withstand several washes are revealed as fluorescent spots. (b) Fluorescent read-out of the NPC-SMM 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.

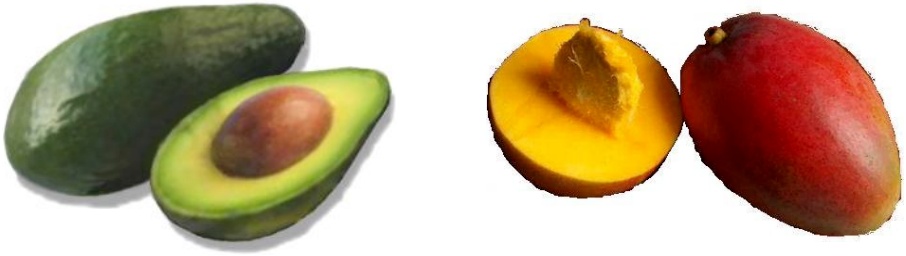
J Am Chem Soc. 2010 Dec 1;132(47):17015-22.
doi: 10.1021/ja107552s.

20.109: ‘By-eye chem-informatics’



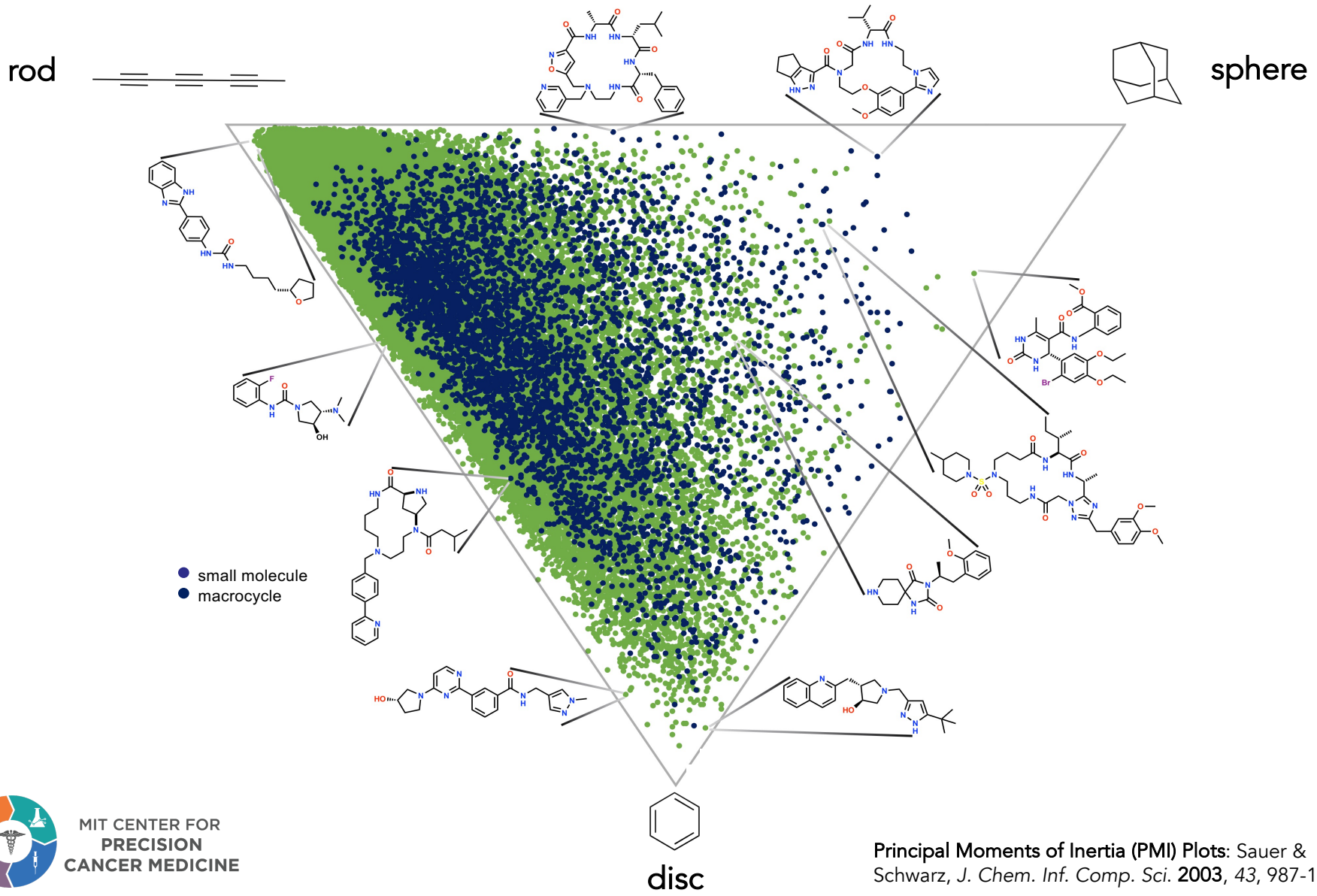
Embedded differences – the domain of computation





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

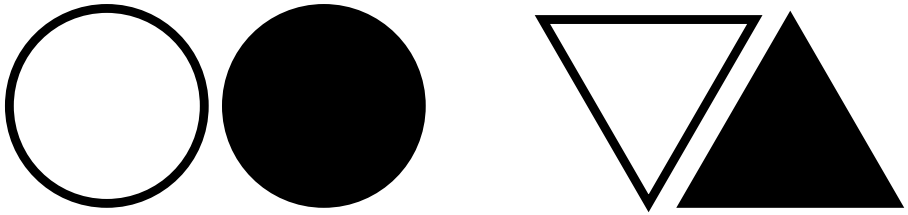
Principal moment of inertia (PMI) plots

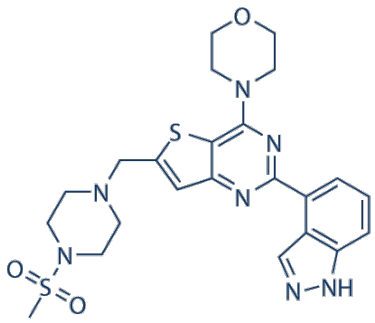


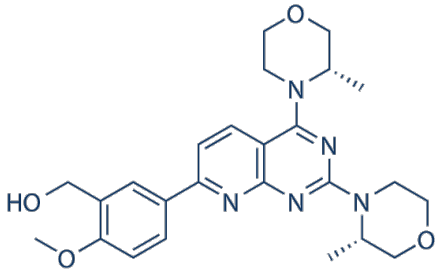
MIT CENTER FOR PRECISION CANCER MEDICINE

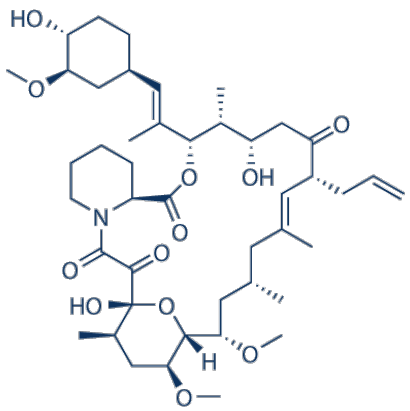
Principal Moments of Inertia (PMI) Plots: Sauer & Schwarz, *J. Chem. Inf. Comp. Sci.* **2003**, *43*, 987-1003

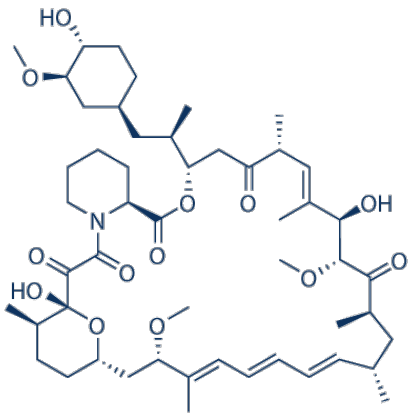
20.109: ‘By-eye chem-informatics’











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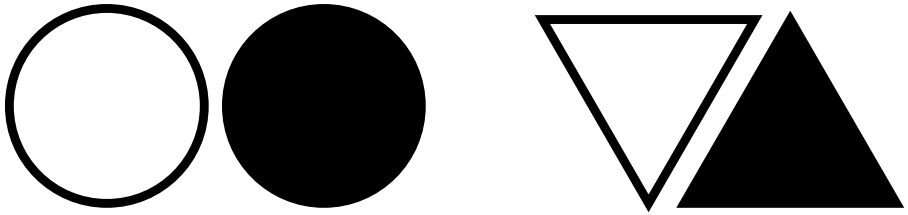
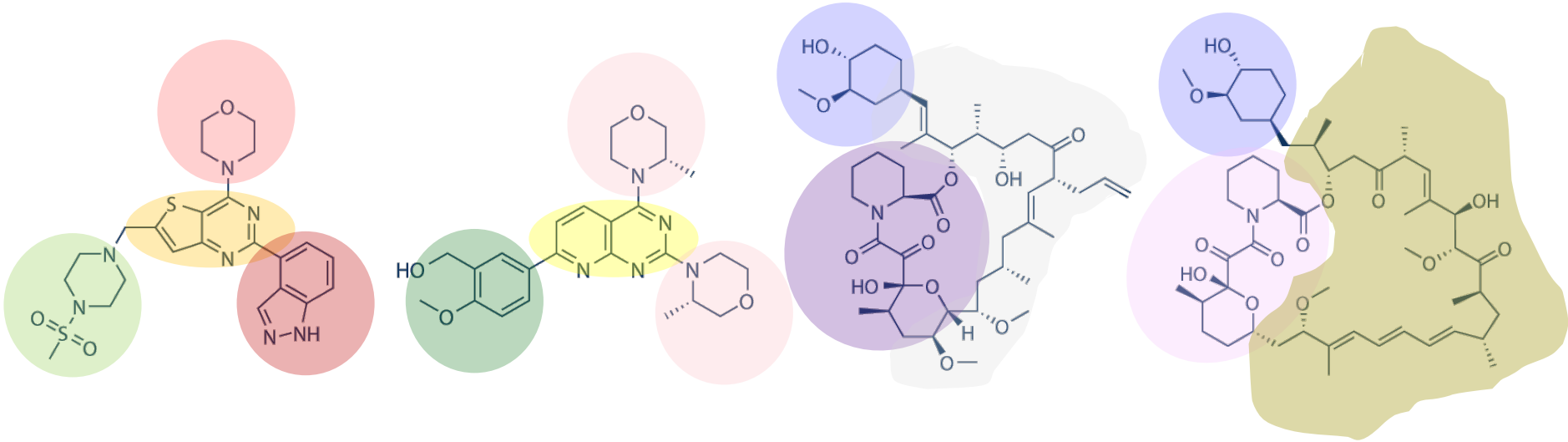




Implications and Future Work

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 positives are binders and measure quantitative affinity values for the protein-ligand interaction?

Topic: How can you use your MAX 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?

A Note on References

Please make References a separate section at the end of the article

suggested format on the wiki:

Pavletich NP, Pabo CO. Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 Å. Science 1991; 252:809-817.

In the body of your report, this article would be cited as follows:

"The crystal structure of the Zif268-DNA complex has been solved (Pavletich 1991)."

If two or more articles can be cited for this finding, then they are listed alphabetically, separated by a comma.

Office Hours

Simple Questions: koehler@mit.edu

Office Hour Dates/Times (via zoom)

Monday, March 13th, 9-10am

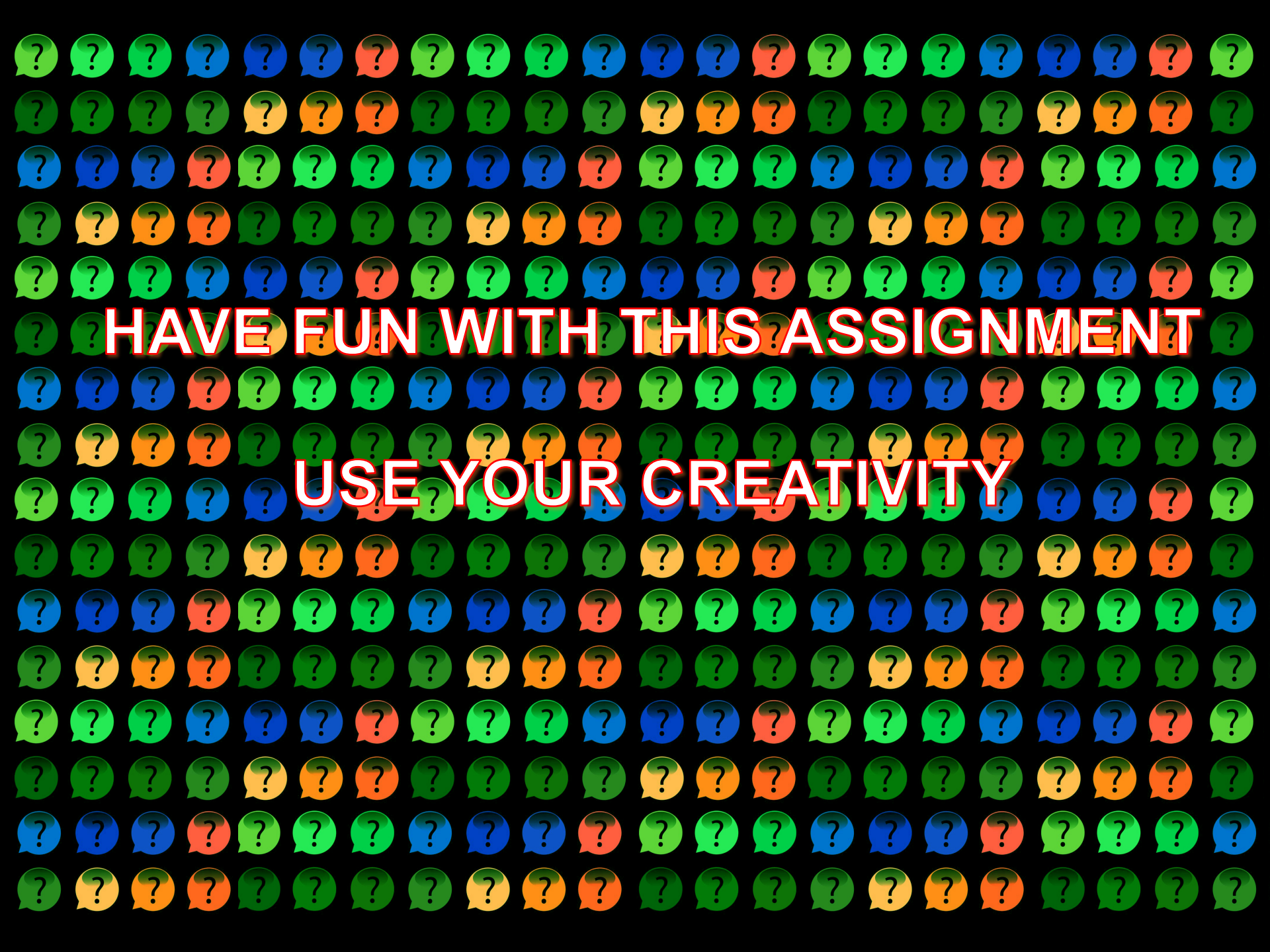
Tuesday, March 14th, 9-10am

Friday, March 17th, 3-4pm

Friday, March 24th, 12-1pm

Join Zoom Meeting
https://mit.zoom.us/j/93057049755

Meeting ID: 930 5704 9755


HAVE FUN WITH THIS ASSIGNMENT
USE YOUR CREATIVITY