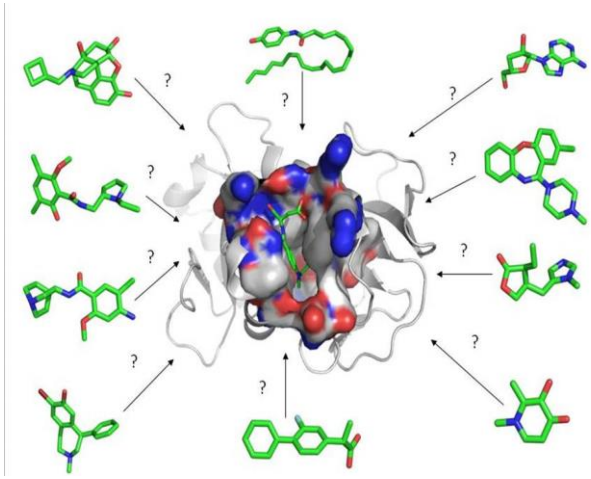
Virtual Drug Screening (VDS) Stream

14th Class – Fall 13



Freshman Research Initiative (FRI)

Dr. Jon Robertus - PI (principal investigator)

Dr. Josh Beckham – RE (research educator)

Journal Club –

Arabidopsis thaliana		Danio reiro		Mus musculus		Caenorhabditis elegans		Gallus gallus	
SERENA Z	ANITA V	ANTONIO G	JACQUELINE E	SEO K	KEVIN E		MELISSA H	KATHERINE	KATHERINE V
WILLIAM E	JESSICA N	ADITI S	JULIA C	PRIYA P	BRANDY C	Mohammad	Shelby F-G	MANUEL Z	IMRAN Z
				CAROLINE C		OSCAR V		Jiaqi Z	

Arabidopsis thaliana		Danio reiro		Mus musculus		Caenorhabditis elegans		Gallus gallus	
BRENDAN C	MARIANNA U	ARIEL C	GRACE T	ASHLEE W	HYUN-YOUNG L	KEELY W	RAMIRO R	VICTORIA G	Madeline J
KAVYA K	FENG G	D'ONDRIA F	GAUTAM W	DANIEL D	JENSEN G	JAMES T	NICOLET F	NICOLE W	Jesus De La O
GRANT T		JASON P							
						Alyssa K			

Outline

Early Class

- Dr. B slides
- Journal Club
  - Dax, Qasim
- Research Presentations
  - Dax, Will, Julia
  - Manuel
- Technique Presentations
  - none
- Dr. B slides
- CIS Forms

Late Class

- Dr. B slides
- Journal Club
  - Keely, Grant
- Research Presentations
  - Gautam, Ariel
- Technique Presentations
  - none
- Dr. B slides
- CIS Forms

Peer Reviews of M&Ms

- Due Thursday night
- Peer Review is part of your final report grade

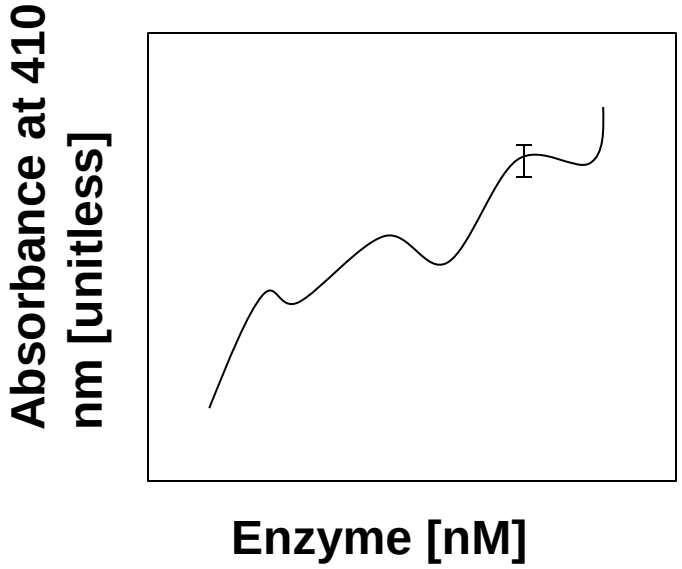
Final Assignments

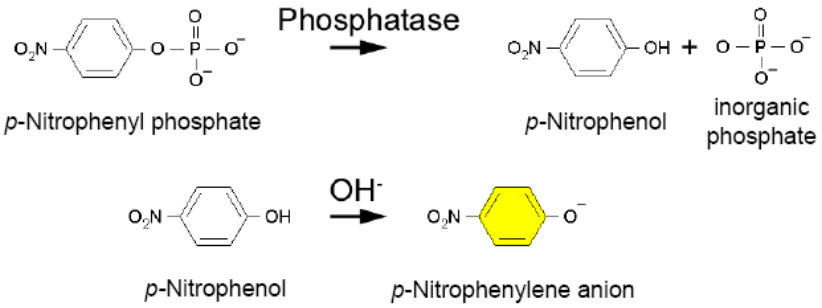
- Final Report Due Saturday December 7th @ 9pm
  - Guidelines will be posted to Google Docs
    - VDSFall13_GuideFinalReportonTarget_VDS_Fall13.doc
    - if you don't have a milestone completed, need to make it clear what you tried to make it work and why it didn't work
  - Wikispaces Due – Sunday night
  - Lab Notebook Due – Monday night
  - Last minute experiments
    - Mentors will be in lab on Saturday

Research Presentations

Enzyme Assays with YopH

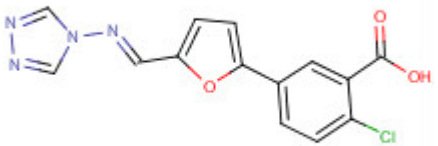
- **Purpose?**
  - **Vary enzyme amount (XY graph)**





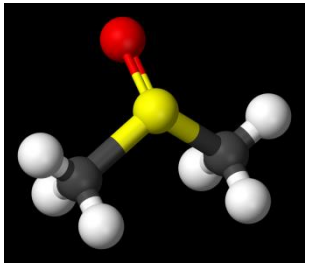
Overview of phosphatase activity assay. SAP hydrolyses *p*-nitrophenyl phosphate to (colorless) *p*-nitrophenol. Deprotonation of the phenolic group gives rise to the yellow colored *p*-nitrophenylene anion. The concentration of this anion in solution is determined by measuring the absorbance at 405 nm and dividing by the extinction coefficient (@pH10.4) of 18.2 $mM^{-1}cm^{-1}$

Compounds



A generic compound

- Stocks are made
  - 50 mM in DMSO (a polar solvent)
    - Water is also a polar solvent, but DMSO can dissolve non-polar compounds (high LogP)
- Solubility determined
  - Already done by mentors

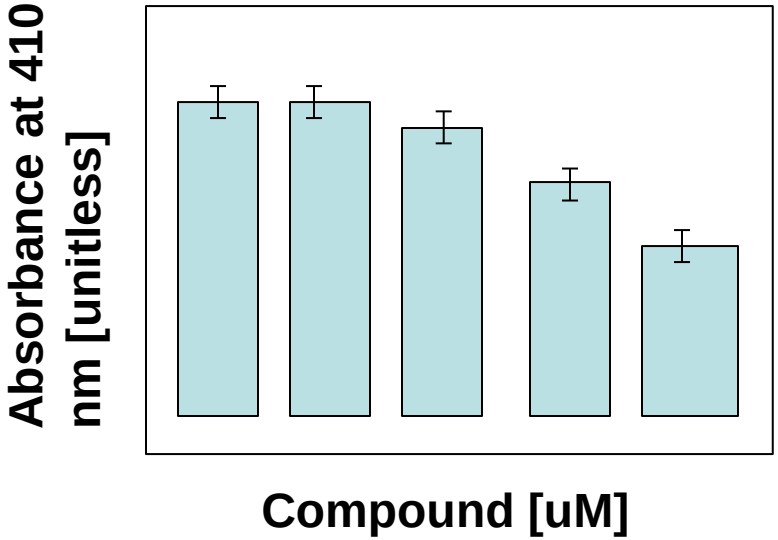


DMSO (dimethylsulfoxide)

- **<u>Use Working Dilutions</u>**
  - 5 mM in 50% DMSO / 50% water

Amber colored vials to reduce light damage to compounds

Inhibition Assays with YopH



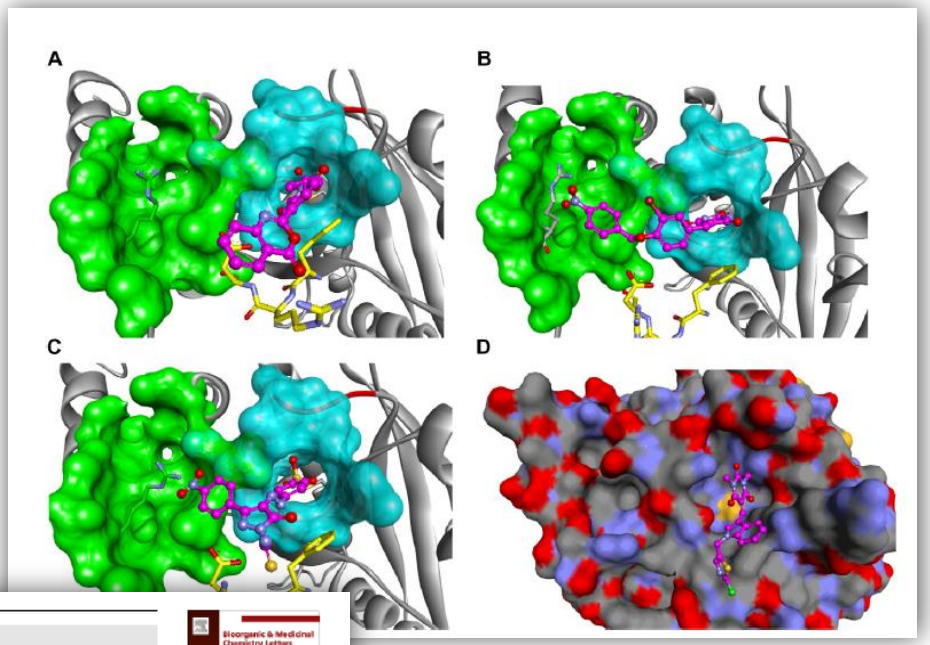
Bad figure caption

Fig 1: This is a picture of my assay with the drug

Good figure caption!

Fig 1: YopH enzyme inhibition assay with compound 587354. Absorbance at 410 nm after 10 minutes at 37^{O}C was measured with 67 nm enzyme, 5 mM pNPP, 10 mM $MgCl_2$, in 80 ul final reaction volume and stopped with 320 ul of NaOH. % DMSO varied from 1, 1, 2, 3, and 5 % respectively. (n=2)

Journal Club



Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

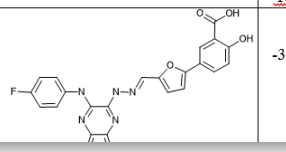
journal homepage: www.elsevier.com/locate/bmcl

Inhibitors of the *Yersinia* protein tyrosine phosphatase through high throughput and virtual screening approaches

Xin Hu [a,†], Milos Vujanac [b,†], Noel Southall [a], C. Erec Stebbins [b,*]

[a] NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, MD 20850, United States
[b] Laboratory of Structural Microbiology, The Rockefeller University, New York, NY 10065, United States

Table S1. Calculated binding free energies of inhibitors bound to protein tyrosine phosphatase YopH (PDB 1PA9), PTP1B (PDB 1AAX) and SptP (PDB 1G4U). [a]The entropy contribution was neglected in the binding free energies calculations using the MM-PBSA method. [b]Promiscuity index (PI) was obtained by inspecting the assays in which the compound (or its analogue) was tested and in how many of those assays it was reported to be active in PubChem. [c]CID number was shown and the analogues of inhibitor is marked with a star.

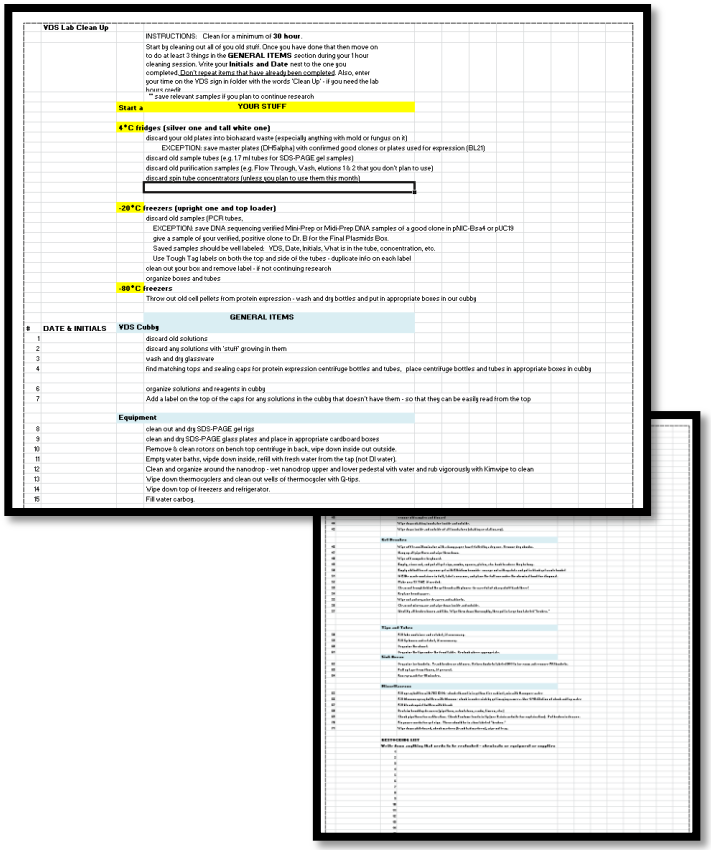
ID	Structure	ΔG[a] YopH	ΔG[a] PTP1B	ΔG[a] SptP	PI[b]	CID[c]
1		-37.00	-30.43	-21.40	NA	6321691

Lab Cleanup (This Week)

- Thursday, Friday, Saturday
  - Sign In on list (paper sheet) on white board

- Put in ½ hour of cleaning
  - Required
    - Part of Lab grade
    - can count as 1/2 hr of lab time

  1. Clean out all your personal un-needed materials
  2. Clean common use areas (pick from the list)



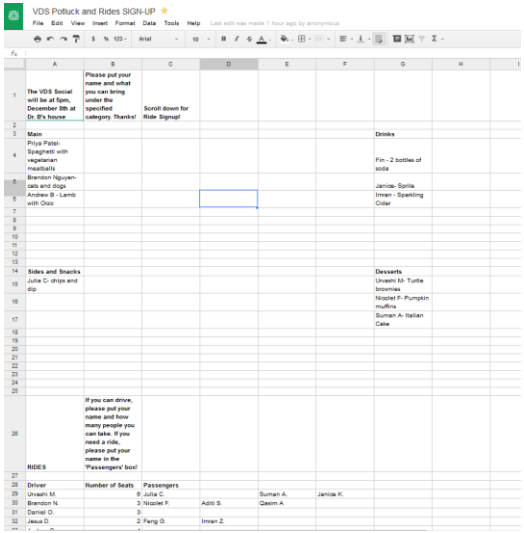
Final Plasmids

- Sample of your verified plasmid to Dr. B for future use in the VDS stream
- 'Final Plasmids' box in the -20^{O}C upright freezer
- Well labeled (Initials, Date, species initials, plasmid name, concentration)
  - e.g. JTB 11/28/13 Yp YopH pNICBsa4 63 ng/ul

End of Semester Party

- Location:
  My house

- Date: Sunday, December 8th
- Time: 5:00 pm



Your success depends on …?

…. Your mother's highest degree attained!

and …

Extracurricular Activities

Advanced Degree

- Everyone in this class should get one!
  - Explore a field, Competitive advantage for jobs
  - Women – if you want to have successful kids !?
- Med/Dental/Vet School
- PharmD
- Nursing

- Graduate School
  - Classes + Research
    - Kind of like VDS!
  - Master's
    - 1-4 yrs
  - Ph.D.
    - If you <u>really</u> like a particular field
    - 6.9 yrs average
    - Paid for in the sciences
      - Tuition and stipend

The End

- It was a pleasure to teach you guys this semester
- You now have a solid foundation for research
  - Best of luck in your future endeavors in the lab!

“Basic research is what I am doing when I don't know what I am doing.”

Wernher von Braun
US (German-born) rocket engineer (1912 - 1977)

CIS forms

- Course-Instructor Survey
  - Don’t fill out if you are TEJAS
  - https://utdirect.utexas.edu/ctl/ecis/

- Put in envelope
- One person to return to office – in BIO Instructional Office (NHB 2.6…)
  - BIO377
  - 51325

End …again