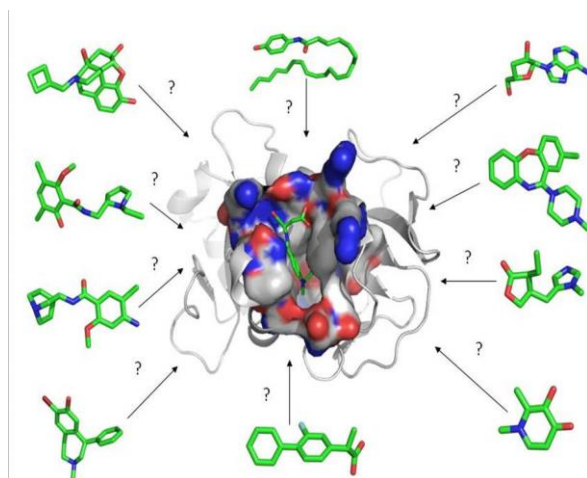




Virtual Drug Screening (VDS) Stream

14th Class – Fall 13



Freshman Research Initiative (FRI)

Dr. Jon Robertus - PI (principal investigator)

Dr. Josh Beckham – RE (research educator)



FRESHMAN RESEARCH INITIATIVE

COLLEGE OF NATURAL SCIENCES

Journal Club –

Arabidopsis thaliana		Danio reiro		Mus musculus		Caenorhabditis elegans		Gallus gallus	
SERENA Z	ANITA Y	ANTONIO G	JACQUELINE E	SEO K	KEVIN E		MELISSA H	KATHERINE	KATHERINE Y
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 Y	HYUN-YOUNG L	KEELY Y	RAMIRO R	VICTORIA G	Madeline J
KAVYA K	FENG G	D'ONDRIA F	GAUTAM Y	DANIEL D	JENSEN G	JAMES T	NICOLET F	NICOLE Y	Jesus De La O
GRANT T		JASON P							
						Allyssa 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

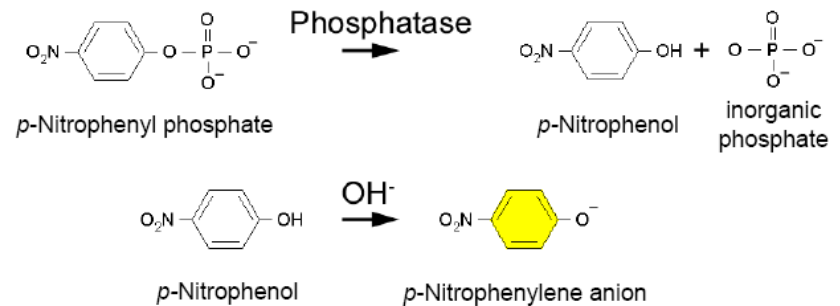
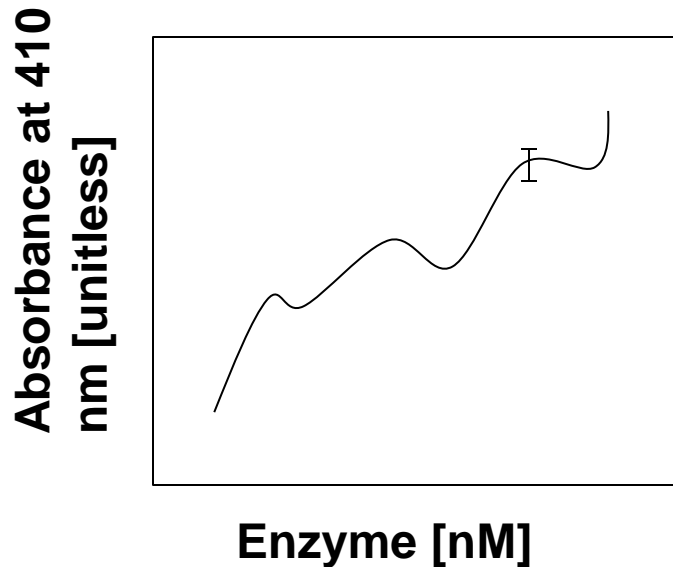


FRESHMAN RESEARCH INITIATIVE

COLLEGE OF NATURAL SCIENCES

Enzyme Assays with YopH

- Purpose?
 - Vary enzyme amount (XY graph)

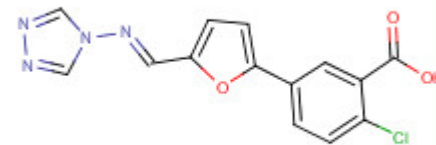


Overview of phosphatase activity assay. SAP hydrolyses *p*-nitrophenyl phosphate to (colorless) *p*-nitrophenol. Deprotonation of the phenolic group gives rise to the yellow colored *p*-nitrophenylene anion. The concentration of this anion in solution is determined by measuring the absorbance at 405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

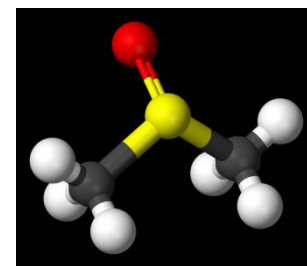


Compounds

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



A generic compound



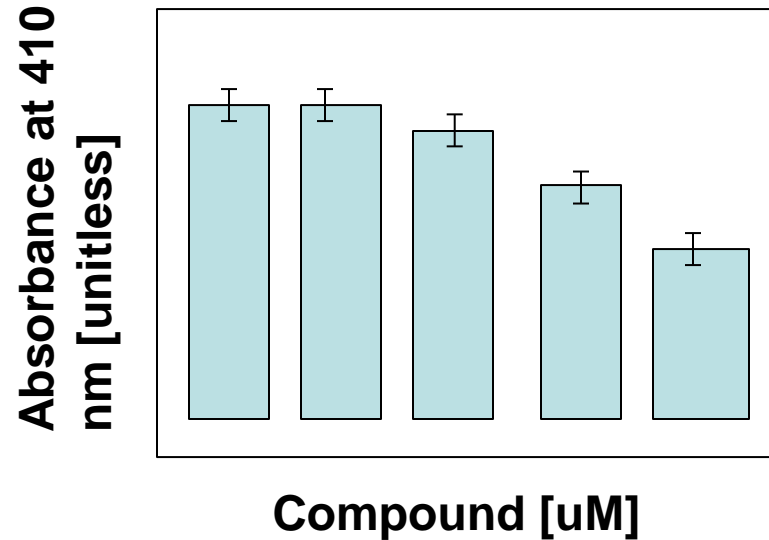
DMSO (dimethylsulfoxide)



Amber colored vials to reduce light damage to compounds



Inhibition Assays with YopH



Bad figure caption

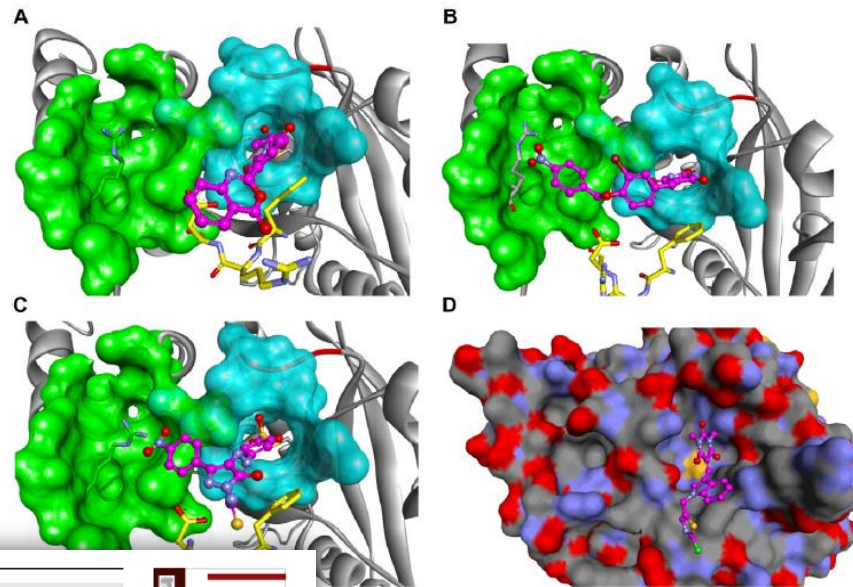
Fig 1: This is a picture of my assay with the drug

Good figure caption!

Fig 1: YopH enzyme inhibition assay with compound 587354. Absorbance at 410 nm after 10 minutes at 37°C was measured with 67 nm enzyme, 5 mM pNPP, 10 mM MgCl₂, 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

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^aNIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, MD 20850, United States

^bLaboratory 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). ^aThe entropy contribution was neglected in the binding free energies calculations using the MM-PBSA method. ^bPromiscuity 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. ^cCID 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



FRESHMAN RESEARCH INITIATIVE

COLLEGE OF NATURAL SCIENCES

Lab Cleanup (This Week)



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

- Put in 1/2 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)

YDS Lab Clean Up		
INSTRUCTIONS: Clean for a minimum of 30 hours. Start by cleaning up all of your old stuff. Once you have done that then move on to do the rest of things in the GENERAL ITEMS section during your 1/2 hour cleaning session. Write your Initials and Date next to the one you completed. Don't use a pen or pencil. Use a blue ballpoint pen or sharpie. Also, enter your time on the YDS sign in holder with the words "Clean Up" - if you need the lab hours made. Save relevant samples if you plan to continue research.		
Start a YOUR STUFF		
4°C Ridges (tilt one and tall white one) Discard poor old plates into biohazard waste especially anything with mold or fungus on it. EXCEPTION: save master plates (D-F) alpha with confirmed good clones or plates used for expression (BL2) Discard old sample tubes (eg 1.7 ml tubes for SDS-PAGE gel samples) Discard old purification samples (eg Flow Through, Wash, elution 1 & 2 that you don't plan to use) Discard spin tube concentrators (unless you plan to use them this month)		
20°C Freezers (upright one and top loader) Discard old samples (PCR tubes, etc.) EXCEPTION: save DNA sequencing verified Mini-Prep or Midi-Prep DNA samples of a good clone in pMC-E14 or pUC19. Give a sample of your verified, positive clone to Dr. El for the Final Plasmid Box. Saved samples should be well labeled: YDS, Date, Initials, what is in the tube, concentration, etc. Use Tough Tag labels on both the top and side of the tubes - duplicate info on each label Clean out good box and remove label - if not continuing research. Organize boxes and tubes.		
80°C Freezers Throw out old vial pullers from protein expression - wash and dry bottles and put in appropriate boxes in our cubby		
GENERAL ITEMS		
#	DATE & INITIALS	YDS Chobby
1		Discard old solutions
2		Discard any solutions with "stuff" growing in them
3		Wash and dry glassware
4		Find marbling tops and sealing caps for protein expression centrifuge bottles and tubes, place centrifuge bottles and tubes in appropriate boxes in cubby
5		Organize solutions and reagents in cubby
6		Add a label on the top of the caps for any solutions in the cubby that doesn't have them - so that they can be easily read from the top
7		
Equipment		
8		Clean out and dry SDS-PAGE gel tags
9		Clean and dry SDS-PAGE glass plates and place in appropriate cardboard boxes
10		Remove & clean rollers on bench top centrifuge in sink, wipe down inside out outside
11		Empty water baths, wipe down inside, refill with fresh water from the tap (not DI water)
12		Clean and organize around the nanopipette - wet nanopipette upper and lower pedestal with water and rub vigorously with Kimwipe to clean
13		Wipe down thermocyclers and clean out wells of thermocycler with Gtips
14		Wipe down top of freezer and refrigerator
15		Fill water carboy

GENERAL ITEMS	
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28	Wipe down thermocyclers and clean out wells of thermocycler with Gtips
29	Wipe down top of freezer and refrigerator
30	Fill water carboy

Final Plasmids

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



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 really 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

