Implementing the CURE: Combining Wet-Lab Protein Biochemistry with Computational Analysis to Provide Gains in Student Learning in the Biochemistry Teaching Lab.

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BASIL: Biochemistry Authentic Scientific Inquiry Lab is an undergraduate biochemistry teaching lab consortium at Hope College, Purdue University, Rochester Institute of Technology, Ursinus College, Oral Roberts University, SUNY-Oswego, St. Mary's University, and Cal Poly San Luis Obispo.

Abstract

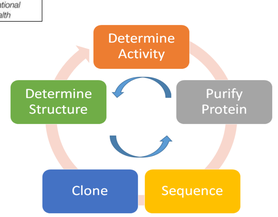
Most undergraduates studying biochemistry and molecular biology get their broadest exposure to wet-lab techniques in protein and nucleic acid chemistry (and, increasingly, computer/visualization) in their upper-level laboratory courses. These tend to be juniors and seniors with well-defined career goals. Some of these students will have already have a research background in a traditional one-to-one (or one-to-few) research mentoring setting, for example a summer research program. This approach has proved effective at increasing student learning and persistence in the sciences. At the same time, extended full-time PI-directed research is limited in the number of students served, and can even present a barrier. To broaden the impact of teaching through research, many practitioners have adopted the CURE, or Course-based Undergraduate Research Experience, approach.

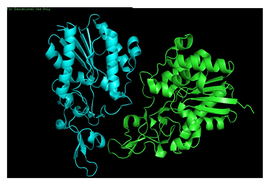
This presentation reports on "BASIL" (Biochemical Authentic Scientific Inquiry Laboratory), a team of faculty who have worked to bring computational and wet-lab protein science to the biochemistry teaching lab. Together, we have developed a protein biochemistry CURE to determine enzymatic function of proteins of unknown activity. This work leverages the results of the Protein Structure Initiative, a fifteen-year NIH-funded effort which concluded in 2015 with the publication and distribution of more than 5000 previously uncharacterized proteins. The great majority of these are "orphans," with high quality structures and pre-cloned expression plasmids available, but no research on their enzymatic function or role in native organisms. The BASIL consortium of undergraduate biochemistry faculty and students seeks to identify functional properties of a subset of these uncharacterized proteins, seeking to unify structure and function relationships.

Materials: Leveraging the NIH PSI

PSI:BIOLOGY — Funded by the National Institutes of Health

DNASU PLASMID REPOSITORY

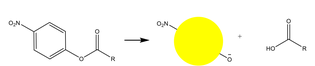




4Q7Q Pymol Structure

We are taking a backwards approach to the normal biochemistry research tasks. Instead of knowing the activity and working towards the structure, we are starting with the structure and trying to determine function.

Wet lab methods: Before work on the unknown proteins began, students were given instruction on basic protein methods including concentration measurement, Michaelis-Menten kinetics, and electrophoresis. For preparation of unknown proteins, BL21 cells were transformed with expression plasmids. Select clones were inoculated into 200 mL Overnight Express™ autoinduction medium with appropriate antibiotic and grown for 18 hours followed by harvesting of cell pellets and lysis. Clarified crude lysates were passed over 1-mL bed volume columns of cobalt-NTA resin (TALON™). His-tagged proteins were eluted with imidazole, which was subsequently removed by passage over PD10 columns. This material was used for activity assays with various colorigenic substrates and analysis by electrophoresis.

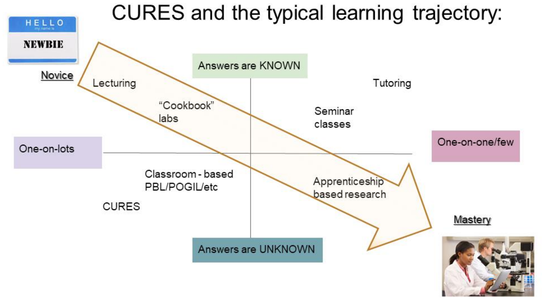


Computational methods: Student instruction began with facilitating installation of PyMol (with ProMol plugin) and PyRX onto students' computers. Where this was not feasible, the suite of software was also installed on college computer lab machines. Student were provided basic tips in viewing protein structures in PyMol as well as given in-class time to explore on their own. Once some familiarity was obtained, students were randomly assigned PDB IDs corresponding to unknown proteins to be "their" protein of interest. Based on the structure and sequence of the protein, students identified known proteins of related sequence (BLAST) and structure (DALI), and used ProMol to predict active site amino acids. Finally, students used PyRX to predict binding interactions with several candidate substrates for the proteins.

Introduction

The pathway from brand-new first year student to mature scientist typically includes a four-year undergraduate trajectory of initiation in "one-on-many" first-year lecture and laboratory coursework. The student who successfully launches on this trajectory then gains increasing access to "one-on-one/few" mentoring, often via apprenticeship style research in a laboratory only after a few years, or even after graduation. Yet it is clear that gains in both content acquisition and self-identification as a practitioner of science happen when the student faces open-ended questions. **Course-based Undergraduate Research Experiences, or CURES**, can accelerate this trajectory for more students, earlier, by offering students open-ended learning on a more widespread basis.

CURES and the typical learning trajectory:

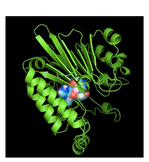


Functions

Protein	Possible Active Site	Possible Protein Family
3DS8	Catalytic triad (His 222, Asp 188, and Ser 102)	Esterase lipase superfamily
3CBW	Unknown	Hydrolase, Transferase, Lysase, Isomerase, or Oxidoreductase
2O14	Catalytic triad (Ser 171, Asp 339, and His 342)	Hydrolase
3H04	Active Site Closely Matched with 2HAD a known haloalkane dehalogenase (Asp 220, Asp 222 and His 248)	Haloalkane dehalogenase
4Q7Q	Catalytic triad (Ser 30, Asp 251, and His 254)	Hydrolase or Lipase
3L1W	Active Site Closely Matched with 1AKO a known exonuclease protein. (Asn 153 and 7 and Asp 215 and 151)	Exonuclease-Endonuclease-Phosphatase Superfamily
4DIU	Catalytic triad (Asp 192, Ser 93, His 222)	Carboxylesterase

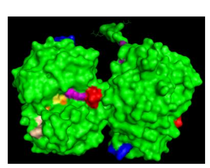
Possible structures and active sights obtained through BLAST results and the molecular visualization Systems Pymol and Promol.

Potential Active Sites

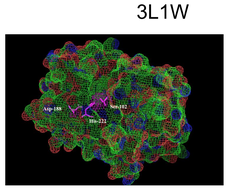


3L1W

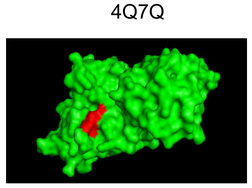
4Q7Q



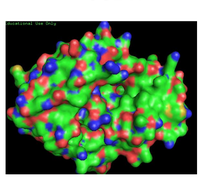
3CBW



3DS8



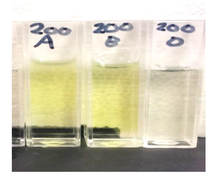
2O14



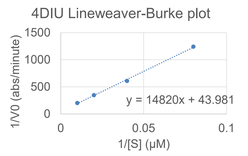
3HO4

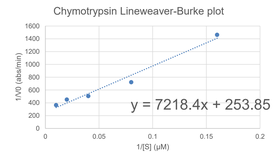
Kinetics

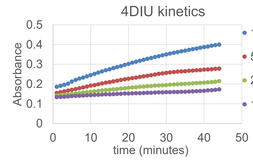
Initially, each expressed product was subjected to end-point assays for para-nitrophenyl ester hydrolysis by combining substrate (200 uM) with crude or purified protein and allowed to incubate at room temperature for 10 minutes and photographed. Some students elected to perform this assay at a range of temperatures. Shown at right is the presumed lipase/esterase 2O14 with PNP-acetate (A), -butyrate (B), and –octanoate (O). Activity decreased dramatically with the longer acyl chain ester.

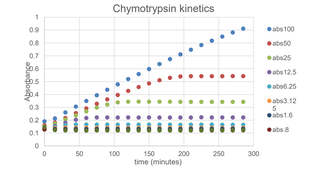


More detailed kinetic studies were performed using Lineweaver-Burke analysis:





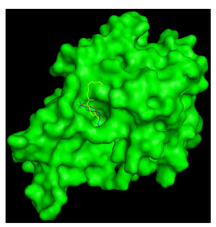


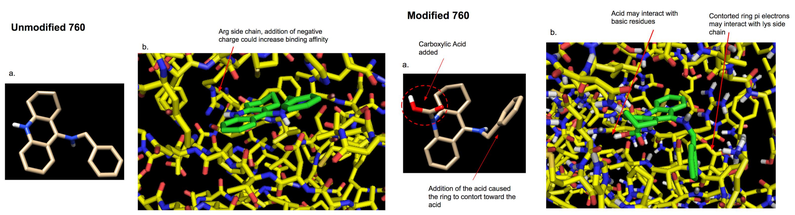


Although Michaelis-Menten kinetics is taught in some detail in typical biochemistry "lecture" coursework, application in a laboratory setting such as this revealed a number of misconceptions, including negative intercept values leading to students reporting $V_{max} > 0$.

Docking studies

As wet biochemistry analysis of the students' purified proteins went on, students were instructed in the use of PyRX to perform Autodock Vina docking studies. Simplified PDB files were prepared by the instructor to remove alternate conformations, nonstandard amino acids (e.g. selenomethione), and thermal anisotropic parameters to render them more amenable to analysis on student computers. Students were provided with an SDF file containing about one hundred potential ligands and, at the same time, were encouraged to design ligand molecules independently using molecule drawing programs such as ChemDraw. The figure at right, above, shows docking results of the acetyl, butryl, and octanoyl esters of para-nitrophenol. The lower figures show one of the better "hits" from the larger library, N-(phenylmethyl)-1,2,3,4-tetrahydroacrdinamine, a presumed acetylcholinesterase inhibitor.





Fit of library ligand to presumed esterase 2O14. Estimated binding affinity was -8.4 kcal/mol

Fit of student-modified library ligand to presumed esterase 2O14. Estimated binding affinity was -8.9 kcal/mol

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