MDH CURE Start Up Project: DRUG DESIGN

An Introduction to Chemical Thinking: Through the Lens of Antimalarial Drug Design:

Paragraph description of the science/background for this CURE:

The research theme at the heart of this CURE is a perennial problem facing drug development- how can you achieve specificity for a pathogen target when host (Human) homologs exist. Malaria, caused by the pathogen Plasmodium falciparum (Pfalciparum) is an excellent example. Both pathogen and host depend on Malate Dehydrogenase as a key part of their energy metabolism. Pfalciparum Malate Dehydrogenase is tetrameric while the human homologs are dimeric- do the oligomeric differences allow specific targeting of inhibitors to preferentially inhibit the Pfalciparum enzyme. Although Malate Dehydrogenases in general show overall tertiary structure similarities there are some regions of sequence difference between Pfalciparum and Human forms of mDH that, as we have shown, may lead to the existence of unique cryptic allosteric sites that could be targeted for allosteric drug design. Similarly, subtle differences in the active site regions could be exploited for orthosteric drug design. In addition to ligand specificity, bioavailability issues involving exploring the Physicochemical Design Space of potential "lead" compounds for future drug design are also incorporated.

Relevant Literature that support this science: (Malate Dehydrogenase specific references are available in the password protected version of this document.

"Allosterism and Drug Discovery" Bell, E & Bell J., Burger's Medicinal Chemistry, Drug Discovery and Development, Eighth Edition. Volume 2, pages 163-240, 2021. Publisher- Wiley

Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings Christopher A. Lipinski", Franc0 I,ombardo, Beryl W. Dominy, Paul J. Feeney, Advanced Drug Delivery Reviews 23 (1997) 3-25, doi: 10.1016/s0169-409x(00)00129-0. PMID: 11259830

3-5 **Learning goals for this CURE**:

1. Students will appreciate what a good research project entails and will develop approaches to develop a novel hypothesis that makes predictions that can be tested experimentally, and present a proposal for their project. Rubric 1
2. Students will learn how to design and execute experiments to test their hypothesis, will learn appropriate data analysis approaches and will appreciate the importance of accurate documentation of their work and reproducibility of their experiments. Rubric 2
3. Students will learn to develop a description of their research project in written, poster or a slide presentation suitable for verbal presentation. Rubric 3

Research question for this CURE:

1. To develop ideas for potential lead compounds that can distinguish between pathogen and host homologs of a potential drug target
2. To understand the target structure-function relationships that underpin orthosteric or allosteric inhibitors of the target.
3. To initiate potential approaches for optimizing the potential of such lead compounds to increase their suitability as candidates for potential future drug design

2-3 Sample hypotheses students could come up with for this CURE:

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Introductory lectures have defined types of approaches to drug development and can be broken down to two basic approaches: structure based drug design or a screening potential candidates approach. Students can select one of these approaches or the approach can be set by the instructor.

Students are led through Hypothesis and Proposal Preparation using the rule of threes approach:

Typically student hypotheses hone in on some unique aspect of theP falci MDH protein structure (established by Clustal Analysis and computational analysis), or some unique aspect of an actual or a potential ligand depending on the overall approach they choose to take (structure based ligand design or screening approach)

How to Measure what you need to measure?

This again stems from the third **"Something"** in the first panel and usually involves measuring some aspect of structure or function, and there are usually several ways to measure a given aspect of structure or function briefly outlined below.

Wet Lab Techniques to show aspects of Structure:

- Secondary Structure
  - CD Spectroscopy
  - IR Spectroscopy
  - NMR Spectroscopy
- Tertiary Structure
  - Fluorescence Spectroscopy
  - UV Spectroscopy
  - NMR Spectroscopy
  - Chemical Modification Approaches
- Quaternary Structure
  - Size Exclusion Chromatography
  - Dynamic Light Scattering
  - Native PAGE
  - CrossLinking & SDS PAGE
- Stability
  - Global
    - Chemical or thermal Denaturation (with DLS, CD or Fluorescence- with tDenaturation can follow using simple kinetics or FbTSA)
  - Local
    - HDEx with Mass Spectrometry

Wet Lab Techniques to show aspects of Function:

- Catalytic Activity
  - Initial Rate Measurements
  - Pre-SteadyState Measurements
    - Either can be combined with
    - Isotope Effect Measurements
- Ligand Binding
  - Specificity
    - Compare K_D for different ligands
  - Affinity
    - Equilibrium Dialysis
    - Spectroscopy Techniques
    - Protection Techniques
      - (vs Chemical Modification, or FbTSA)
  - Stoichiometry
    - Equilibrium Dialysis
    - Spectroscopy Techniques
  - Sidechain pKa Determination
    - NMR
    - Chemical Modification Approaches

Computational Approaches to Explore Aspects of Structure and Function

Molecular Dynamics:	Local Motion & Response to Ligand Binding
HawkDock:	Local Interactions in Protein-Protein Complexes
SwissDock:	Ligand Binding Sites and Affinities
H++:	Sidechain pKa Values from structure
Deep ΔΔG:	Impact of Mutations on global stability from structure
HINT:	NonCovalent Interactions involved in structure or ligand-protein complexes
MolView:	Construction of Small Molecules, Calculation of Molecular Properties

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CURE format (modular, semester, or either): Either

Ideal group size for this CURE: Groups of 2-3 students

Ideal course/level for this CURE (chem, bio, biochem, interdisciplinary; first year, middle years, capstone): (list as many as are possibilities)

First Year Chemistry or Biology, middle years, upper level

Teaching Resources Available:

Templates for student activities for each of the 9 essential elements of research incorporated into the CURE

Rubrics to Guide & Assess Student Performance

Mol and Mol2 files of 3,4, 5 and 6 carbon ligand analogs for use in Computational Experiments

Pdb files of Human Cytosolic MDH Dimer, Human Mitochondrial MDH-Dimer and Plasmodium falciparum MDH Tetramer suitable for computational experiments

Week by week lab activities for a modular and/or semester long version of this CURE (include protocols that need to be linked to each week): (Full details in Password Protected Version)

The CURE starts with discussions of the target enzyme, Malate Dehydrogenase and what it does in both pathogen and host and introduces some basic ideas of both orthosteric and allosteric drugs. Students then decide which approach they want to pursue, do background reading into Malaria, start to pose questions of what they need to know or be able to do to uniquely target the pathogen MDH. They start some bioinformatics and protein visualization approaches and develop ideas for compounds they would like to screen (may include aspects of high throughput screening, screening extracts of natural products eg herbal extracts etc), making a hypothesis and developing their research proposal. They screen potential "drug-like" molecules based on known or potential orthosteric ligands using enzyme inhibition kinetics, and explore potential cryptic allosteric sites computationally all the time using pathogen target and human homologs. They explore Lipinsky rule of 5 properties both experimentally, determining logP, or structure-activity relationship properties computationally.

Instrumentation/equipment/key reagents needed for this CURE , Uv-vis Spectrophotometer

Equipment for acid-base titrations, pH Meter, Balance, Water Bath, Stir Plate

Protein (WT and/or specific mutant), organism:

Plasmids needed can be obtained from Addgene:

Plasmodium falciparum, Human Mitochondrial, Human Cytosolic

Clone Data Sheets:

Plasmodium falciparum

Human Cytosolic

Human Mitochondrial

MW(subunit/biological)/pI/ ε_{280} , extinction coefficient (280 nm: calculated using ProtParam.) of protein (WT and/or specific mutant):

Plasmodium falciparum: **MWt**: 35,715/142,860, **pI**(theoretical): 6.89 ε_{280} 0.375 $mL.mg^{-1}.cm^{-1}$

Human Mitochondrial: **MWt**: 34,806/69612, **pI**(theoretical): 8.33 ε_{280} 0.257 $mL.mg^{-1}.cm^{-1}$

Human Cytosolic:**MWt**: 39,749/79,498, **pI**(theoretical): 7.14 ε_{280} 0.853 $mL.mg^{-1}.cm^{-1}$

PDB ID for the WT version of this protein (if there are multiple options, specify differences between them):

Plasmodium falciparum: 5.nfr.pdb

Human Cytosolic: 7rm9.pdb

Human Mitochondrial: 2dfd.pdb

Available Resources for structural analysis and computational approaches: Biologically relevant pdb files

Plasmodium falciparum Tetramer

Human Cytosolic Dimer

Human Mitochondrial Dimer

Landmarked .pse files for use with the project - see clone datasheets for descriptions

Plasmodium falciparum

Human Cytosolic

Human Mitochondrial: