MDH CURE Start Up Project: ACTIVE SITE & LOOP
Exploring the role of the Active Site and Dynamics in specificity & Catalysis in MDH

Description of the science/background for this CURE: The research theme at the heart of this CURE is a perennial debate over the roles that protein dynamics can play in enzyme function. Do protein dynamics play a direct role in hydride transfer reactions as has been proposed or in the electrostatic pre-organization of the active site . What role do protein dynamics play in conformational selection processes that may be involved in promiscuous substrate utilization. Many enzymes have Histidine-Aspartate pairs involved in catalysis and Malate Dehydrogenases universally contain a His-Asp diad involved in proton abstraction/donation from/to malate/oxaloacetate. The role of loop residues and active site second sphere residues in catalysis and substrate specificity is understudied.. Evolutionary relationships between Malate Dehydrogenases and Lactate Dehydrogenases. Adaptation of enzymes to extreme conditions includes roles of dynamics and second sphere residues in structure function relationships.

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

Dalziel K. Dynamic aspects of enzyme specificity. Philos Trans R Soc Lond B Biol Sci. 1975 Nov 6;272(915):109-22. doi: 10.1098/rstb.1975.0074. PMID: 1807.

De Luca V, Mandrich L. Enzyme Promiscuous Activity: How to Define it and its Evolutionary Aspects. Protein Pept Lett. 2020;27(5):400-410. doi: 10.2174/0929866527666191223141205. PMID: 31868141.

Weikl, T.R. and Paul, F. (2014), Conformational selection in protein binding and function. Protein Science, 23: 1508-1518. https://doi.org/10.1002/pro.2539

Andrew J. Adamczyka, Jie Caoa, Shina C. L. Kamerlinb,1, and Arieh Warshel. Catalysis by dihydrofolate reductase and other enzymes arises from electrostatic preorganization, not conformational motions ,1www.pnas.org/cgi/doi/10.1073/pnas.1111252108

Li L, Luo M, Ghanem M, Taylor EA, Schramm VL. Second-sphere amino acids contribute to transition-state structure in bovine purine nucleoside phosphorylase. Biochemistry. 2008 Feb 26;47(8):2577-83. doi: 10.1021/bi7021365. PMID: 18281958. PNAS | August 23, 2011 | vol. 108 | no. 34 | 14115–14120

3-5 Learning goals for this CURE:

1. Students will appreciate that a good research project entails nine essential elements of research and will develop a novel hypothesis that makes predictions that can be tested experimentally, and present a proposal for their project. **Rubric 1 (link)**
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 (Link)**
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 (Link)**

Research question for this CURE:

1. Can you explore the role of loop dynamics in the catalytic steps of the reaction?
2. Can you understand the structure-function relationships of the protein dynamics that underpin substrate specificity?
3. Can you propose and initiate potential strategies for altering the specificity of the enzyme

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for biotech purposes?

Hypothesis possibilities:
Students are led through Hypothesis and Proposal Preparation using the rule of threes approach: (see password protected area version)

Typically student hypotheses hone in on some unique aspect of the loop region(established by Clustal Analysis and computational analysis), or some unique aspect of so called "second sphere residues in the active site region of the protein.

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

Week by week lab activities for a modular and/or semester long version of this CURE
Modular: (Templates for student guidance, rubrics and class powerpoints are available in the password protected Faculty only area.

PyMOL or other rendering site worksheet/assignment, Site Directed Mutagenesis (if not using existing mutants), protein expression and purification, enzyme assay, possible structural assay

Instrumentation/equipment/key reagents needed for this CURE: Thermocycler (if doing SDM), incubator for expression (room temp or 37°C), centrifuge (min 10K x g; optimal speed 30Kxg), 2 ml IMAC beads and column/batch chromatography– 500 ml culture will produce >2-5 mg of purified protein, spectrophotometer capable of 340 nm UV measurements. One per group for real time assays or stop time assay using plate reader.

Bacterial Expression: Both human Mitochondrial and Cytoplasmic MDH clones express well after a 3-4 hour 37°C or an overnight 20°C IPTG induction in BL21(DE3) cells. A 500 ml culture should yield between 10 and

20 mg of purified protein. Both mitochondrial and cytosolic MDH are protein is stable but should be diluted to 1.2 mg/ml (determined by Bradford) for short term storage. Failure to do so will result in aggregation/precipitation. Long term storage should follow instructions on MDH Storage (link provided). The Plasmodium falciparum MDH clone gives Modest protein expression at 37°C 1mm IPTG for 3-4 hour induction pET28a (Novagen) is a low copy plasmid (~40) and will not give high yields of DNA preps. Kan Resistant. Do not freeze thaw purified protein. Purification easily performed in column or batch format. This is a high yield – range from 35-90 mg of purified protein per 1000 ml. Dilute after purification to ~0.9 mg/ml or aggregation/precipitation will occur overnight. Stable at 4°C for 4 weeks dialyzed against (10 mM K phosphate, 0.1 mM EDTA, pH 8.0). Long term storage in glycerol > 6 weeks. Recommended -20 to -80°C (10-20% Glycerol, 50 mM NaCl, 10 mM K phosphate, pH 8.0).

Plasmids needed and where to obtain them: Adgene or Ellis Bell

MW/pI/ extinction coefficient (280nm) of protein (WT and/or specific mutant): *Additional detailed information can be found in MDH Members page (link provided).*

hMDH1V3	hMDH2
- 352 aa (386 with His tag), - Monomer MW: 40,601 Da, - Extinction Coefficient (280 nm predicted based from sequence) 35,410 M^{-1} cm^{-1} - 280 nm Absorbance, 0.1% 0.87 - pI 6.83 - Charge at pH 7.0 (-1.36)	- 314 aa (324 with His tag), - Monomer MW: 34,806 Da, - Extinction Coefficient (280 nm predicted based from sequence) 8,940 M^{-1} cm^{-1} - 280 nm Absorbance, 0.1% 0.26 - pI. 7.11 - Charge at pH 7.0 (1.08)

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 $mL.mg^{-1}.cm^{-1}$

PDB ID:: 7RM9.pdb (human cytoplasmic MDH) 2DFD.pdb (human Mitochondrial MDH), 5NFR.pdb (Plasmodium falciparum MDH). Annotated .pse files for each are available in the password protected Faculty only area