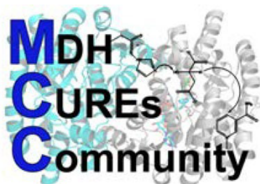

MDH CURE Start Up Project: Allosteric Regulation

Understanding the role of Non Covalent Interactions in Allosteric Regulation of MDH

Description of the science/background for this CURE:

Many oligomeric enzymes show non canonical homotropic enzyme kinetic or ligand binding behaviour that is important in regulation of their activity, however the simple existence of such non canonical behaviour is not sufficient to involve allosteric regulation of subunit cooperativity as there are many other explanations possible. Likewise, heterotropic non covalent regulation may be allosteric but not involve subunit cooperativity or interactions which involves the transmission of information from one subunit to another in an oligomeric structure via conformational changes. Pioneering work by Bell and Dalziel established a variety of ways to detect such changes and combined with modern computational approaches paved the way to explore the mechanism of such subunit interactions. Various Malate Dehydrogenases show a variety of non-canonical kinetic or ligand binding data and a reciprocating subunit model for both catalytic steps and regulation has been proposed but not substantiated.

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

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

Ellis Bell, Proteins & Enzymes, Chapters 12 (Conformational Changes), 16 (Deviations from Linear Kinetics) & 21(Allosteric Models of Enzyme Regulation) , Ellis Bell.

Bell JE, Dalziel K. A conformational transition of the oligomer of glutamate dehydrogenase induced by half-saturation with NAD + or NADP + . Biochim Biophys Acta. 1973 May 5;309(1):237-42. doi: 10.1016/0005-2744(73)90336-7. PMID: 4145351.

Sinha S, Tam B, Wang SM. Applications of Molecular Dynamics Simulation in Protein Study. Membranes (Basel). 2022 Aug 29;12(9):844. doi: 10.3390/membranes12090844. PMID: 36135863; PMCID: PMC9505860.

M. Karplus and J. Kuriyan., **Molecular dynamics and protein function., May 3, 2005,** 102 (19) 6679-6685 https://doi.org/10.1073/pnas.040893010

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)**
4.

Research questions for this CURE

1. Can you develop a way to demonstrate that non canonical behaviour in malate dehydrogenase is due to subunit interactions

2. Can you understand the target structure-function relationships that underpin potential allosteric interactions in malate dehydrogenase?
3. Can you propose and initiate potential strategies to identify the triggers, information relay path and elicitors of intersubunit communication in malate dehydrogenase?

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 subunit interface structure (established by comparative Hawkdock Analysis), or some key aspects of the ligand binding site depending on the overall question they choose to study.

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; middle years, capstone):

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

Watermelon Glyoxysomal MDH:

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

Ignicoccus Islandicus MDH:

In reality almost any MDH available can be used in this project area- the above are those that have been most studied to date.

Pdb files available:

PDB ID: 1smk.pdb (**Watermelon Glyoxysomal** MDH). Additional pdb files for use in Computational studies, and Annotated .pse files for each are available in the password protected Faculty only area

PDB ID:*:* 5nfr.pdb (Plasmodium falciparum MDH). Additional pdb files for use in Computational studies, and Annotated .pse files for each are available in the password protected Faculty only area)

PDP ID: 6qss.pdb (**Ignicoccus Islandicus** MDH). Additional pdb files for use in Computational studies, and Annotated .pse files for each are available in the password protected Faculty only area