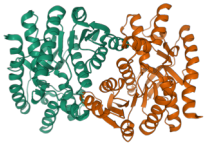
Protein-Protein Interactions

OR *how do proteins interact, why do they interact, and why do I care?*



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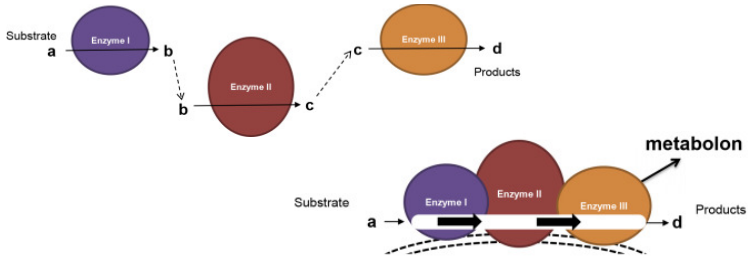
University of San Diego

Who is doing what to who?

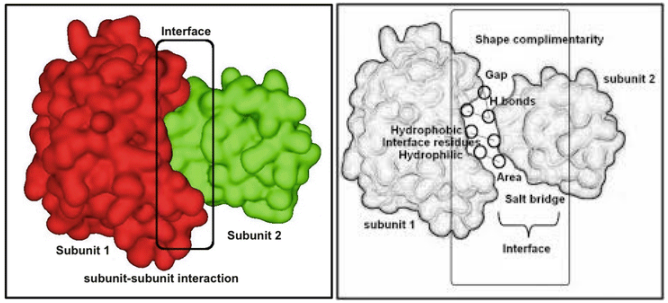
We study how proteins interact with other proteins

- Helps us understand how SOME proteins "hand off" their metabolites



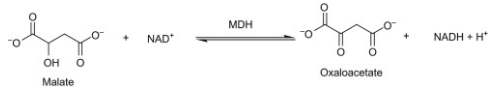
So what – protein interactions?

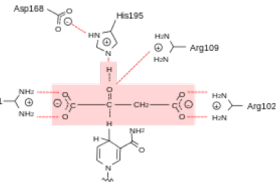
- Knowing where proteins interact is important
- What drives these interactions? Non-covalent bonds!!! Intermolecular forces!!!

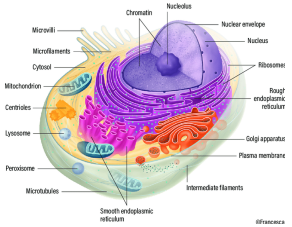


Malate Dehydrogenase (MDH)

- Enzyme catalyzing the redox reaction of malate and oxaloacetate using the cofactor NAD^+ / NADH

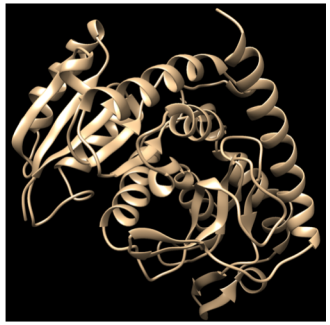




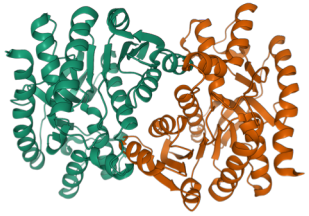


- Found in all organisms, bugs, plants and mammalian
- Different copies of the same gene code for the same protein (with some differences) in mitochondria, cytoplasm, and other parts of the cell (called isoforms or isozyme) with different regulation-function
- Mitochondrial MDH (aka MDH2) is involved in the Krebs cycle

Malate Dehydrogenase



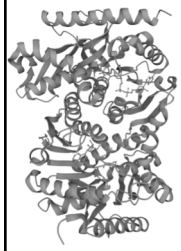
- MDH is a homodimer – two identical protein chains that come together to form the actual protein
- Two binding/active sites
- Human Cytosolic MDH structure is based on pig cyto MDH >90% homology



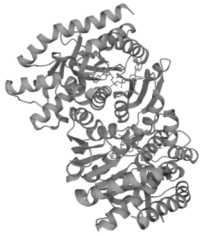
Human cytosolic vs mitochondrial MDH

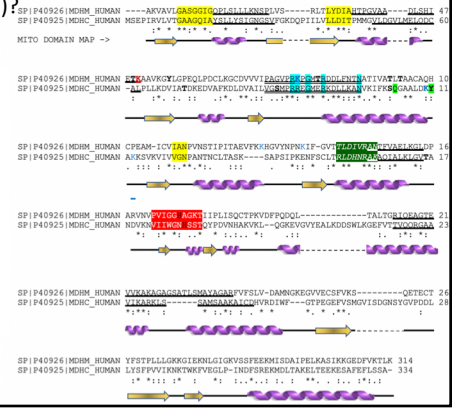
- What are isozymes (aka isoenzymes)?

Cytosolic



Mitochondrial

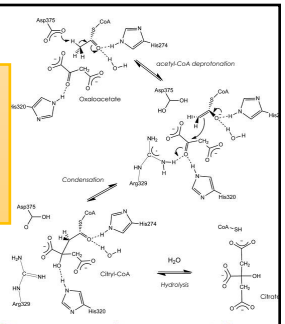




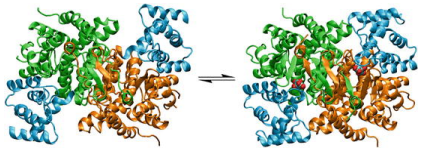
Citrate Synthase

The enzyme citrate synthase exists in nearly all living cells and stands as a pace-making enzyme in the first step of the citric acid cycle.

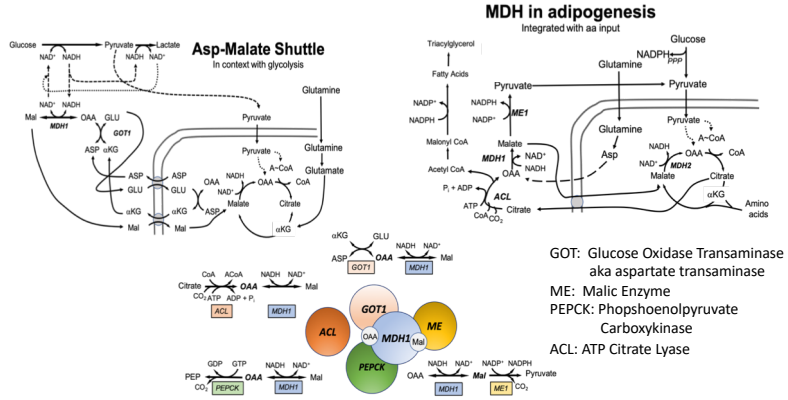
- Citrate synthase is localized within eukaryotic cells in the mitochondrial matrix, but is encoded by nuclear DNA rather than mitochondrial DNA
- CS catalyzes the condensation of acetate from acetyl CoA and oxaloacetate to form citrate



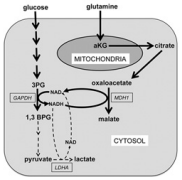
- Dimer
- Mostly helical
- First step of TCA
- Highly exergonic reaction
- Significant structural shifts to a closed form when bound to either substrate



MDH in metabolism / metabolon

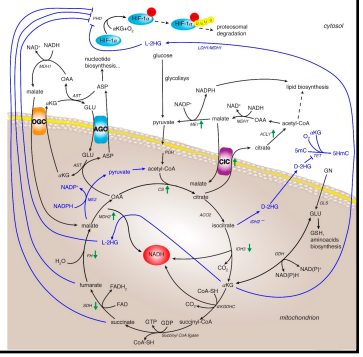


GOT: Glucose Oxidase Transaminase
aka aspartate transaminase
ME: Malic Enzyme
PEPCK: Phopshoenolpyruvate Carboxykinase
ACL: ATP Citrate Lyase

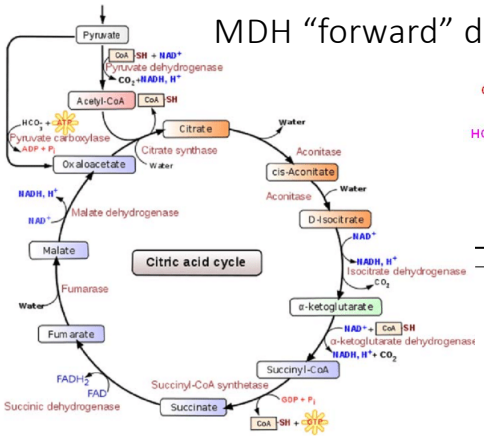


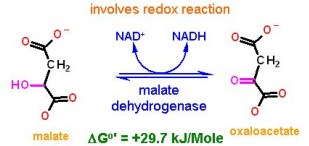
Malate Dehydrogenase: Regulation, Metabolism and Disease - Why do we care?

- Cancer tissues use MDH and GOT to support additional metabolic burden via glutamine or lactate
- MDH expression and activity increases in cancer cells
- Some cancer tissues have mutations in the MDH gene
- Protein interactions seem to change in larger hypoxic tumors
- Neuro disorders: Both Alzheimer's and ALS have changed MDH function
- Many MDH1 residues are phosphorylated without known function



MDH "forward" direction

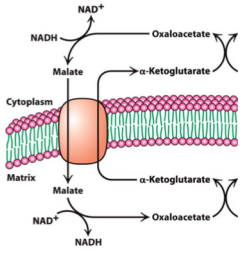




Standard free energy changes in the citric acid cycle

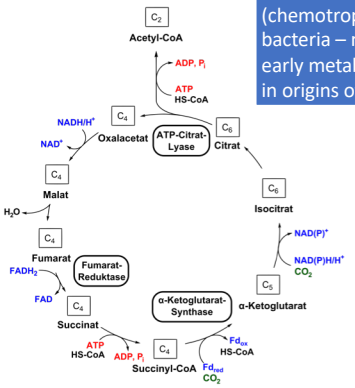
Reaction	Enzyme	ΔG°'	ΔG'
1	Citrate synthase	-31.5	Negative
2	Aconitase	~5	~0
3	Isocitrate dh	-21	Negative
4	α-KG dh	-33	Negative
5	Succinyl-CoA synthase	-20.1	~0
6	Succinate dh	+6	~0
7	Fumarase	-3.4	~0
8	Malate dh	+29.7	~0

The "spontaneous direction"!

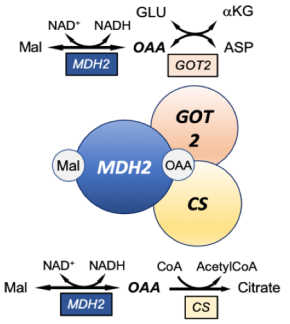


NADH Shuttle

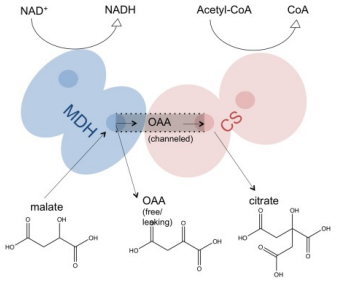
Reverse Krebs Cycle: way to produce CO2 (chemotrophs) in some bacteria – may be an early metabolic pathway in origins of life…



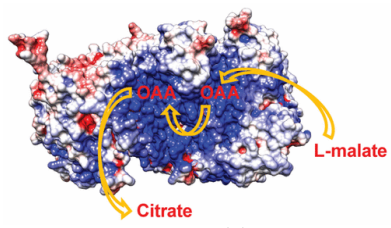
Potential Interactions – shared metabolites



MDH and CS



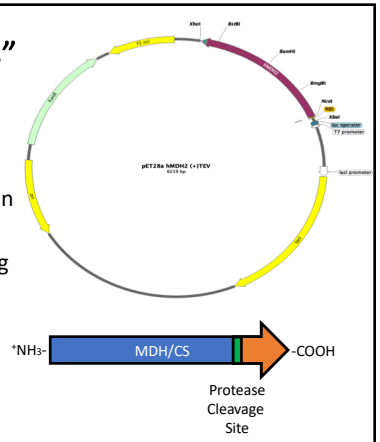
Protein Science 2018 27(2): 463-471



ACS CHEM BIOL 2016 11(10): 2847-2853

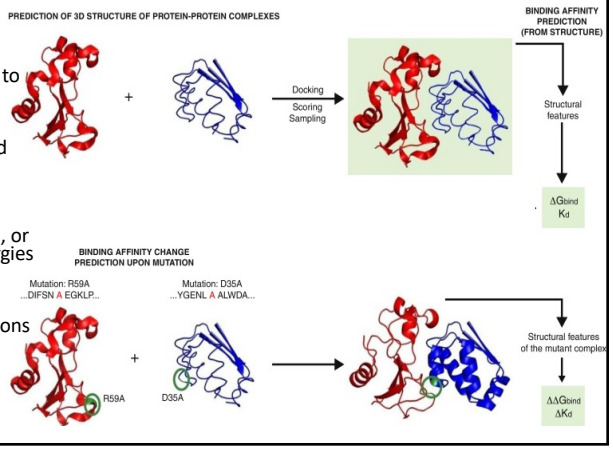
"Recombinant MDH and CS"

- Recombinant? Human gene for MDH or CS cloned into "expression vector" for bacterial expression.
- Added 6X His tag on N or C-term of protein for ease of detection and purification
- Added protease cleavage site between Tag and MDH/CS to help purify using the His Tag of either MDH or CS not both…
- Culture bacteria in large volume, collect bacterial cells and break open cells to isolate (purify) MDH or CS



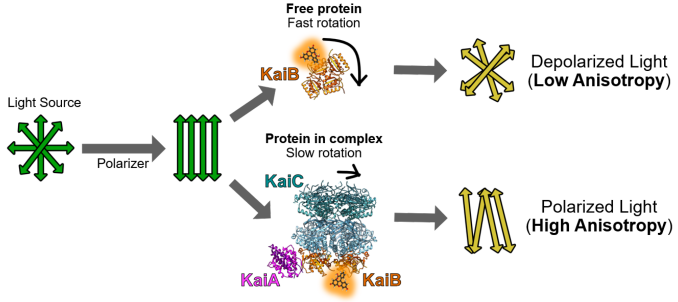
Docking

- So what does that have to do with us?
- We don't know how and where most proteins interact!
- Need to predict, model, or calculate based on energies
- Then we can see what happens when a mutations occurs or a drug binds…

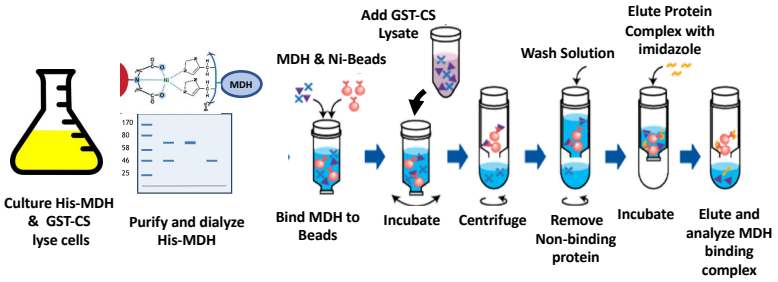


Measuring Protein-Protein Interaction

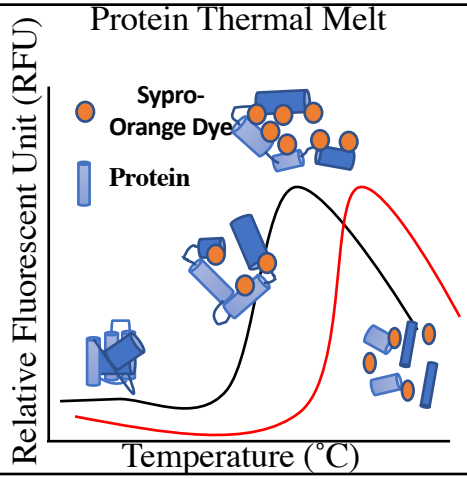
Fluorescence Anisotropy



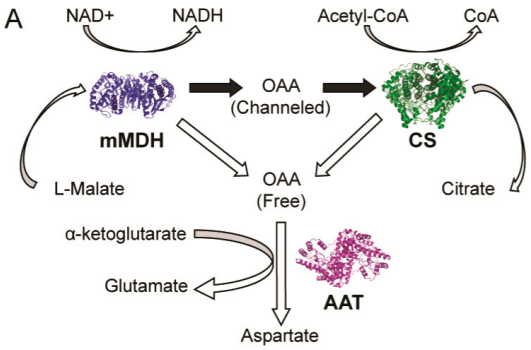
Methods – Pull-Down



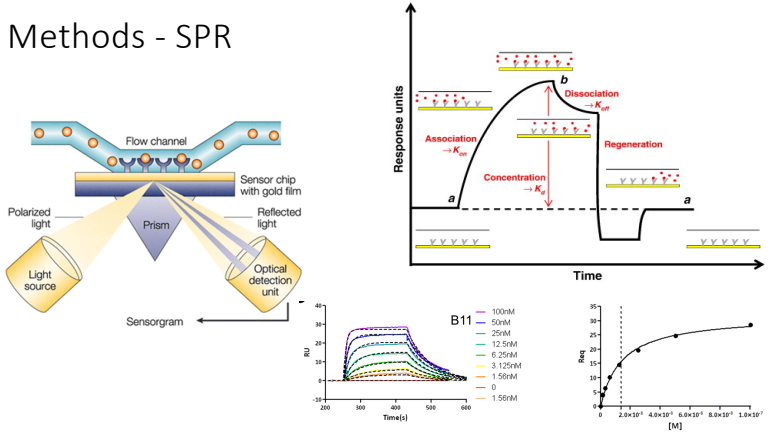
Methods – Thermal Melt



Methods – Kinetic Competition

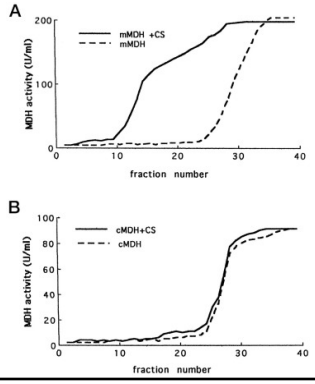


Methods - SPR

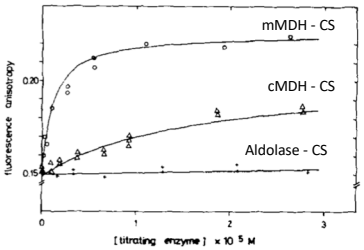


MDH – CS the early days

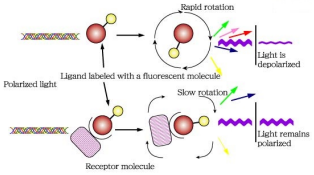
Size Exclusion



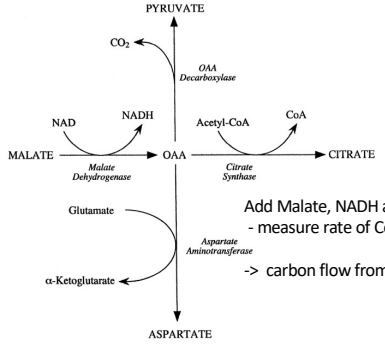
MDH – CS the early days



Fluorescence Anisotropy

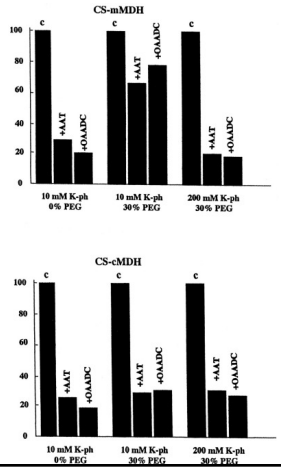


MDH – CS the early days crowding and ionic strength



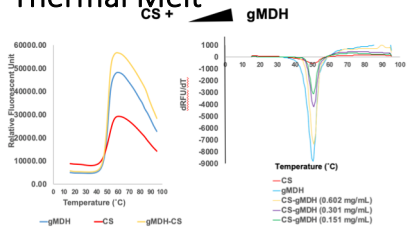
Add Malate, NADH and Acetyl CoA
- measure rate of CoA production

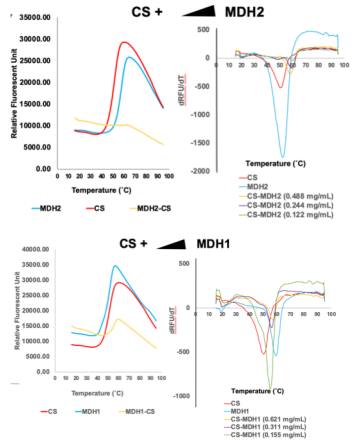
-> carbon flow from MDH to CS

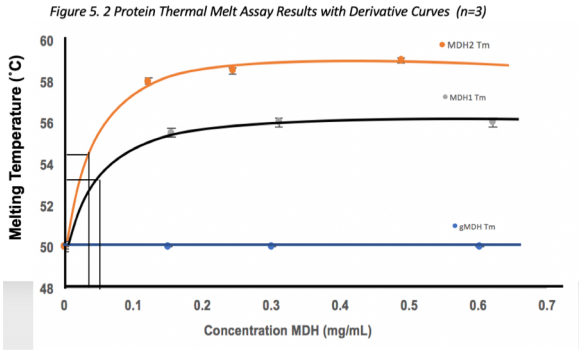


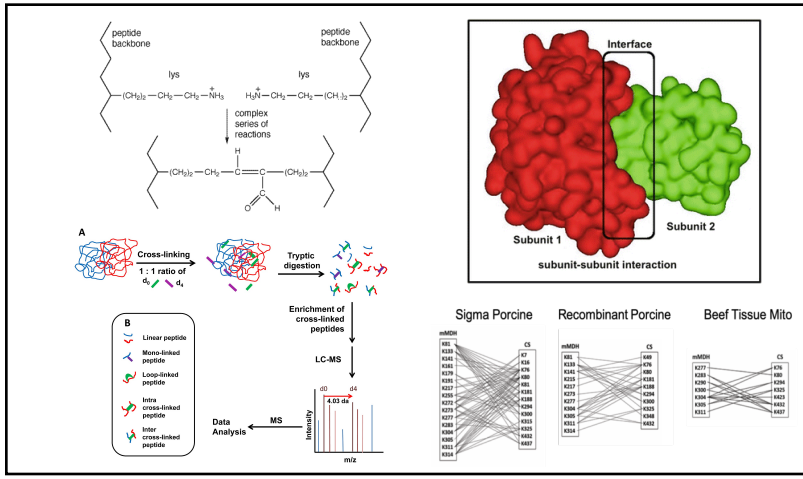
MDH – CS now…

Thermal Melt

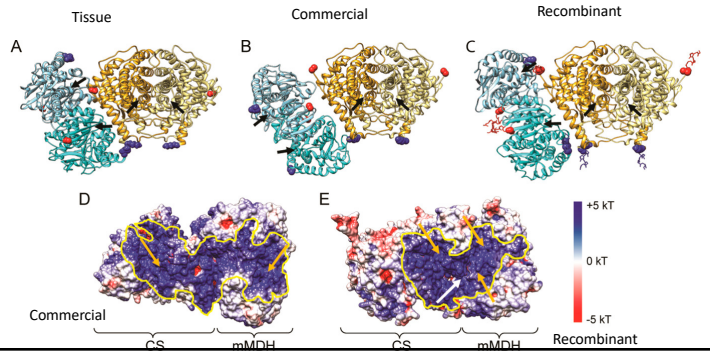








- mMDH and CS active sites are denoted by black arrows. (D) *In vitro* complex formed by commercial enzymes. Surface regions of positive potential and negative potential are colored in blue and red, respectively.
- The electrostatic channeling path for OAA is highlighted by the yellow edge.
- Orange arrows indicate the active sites.



So now what?

- Species/organism uniqueness does this happen everywhere? Is there an evolutionary split in MDH-CS.
- Where are the actual points of contact – show this to be the case
- How do cyto/mito MDH interact differently
- Regulation by PTM (phosphorylation and other)
- Redox/metabolomic intermediate regulation of interaction

```
SP|P40926|MDHM_HUMAN   MLSALARPASAALRRSFST-SAQNNAKVAVLGASGGIGQPLSLLLKNSPLVS-----RLT 54/30
SP|P40925-3|MDHC_HUMAN MRRCSYFPKDVT---VFDKDDKSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILV 57
                       *  .   * ..:    *.. . .:  :* * **:* *.  *   : *. :..      *.

SP|P40926|MDHM_HUMAN   LYDIAHTPGVAA----DLSHIETKAAVKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTR 110/86
SP|P40925-3|MDHC_HUMAN LLDITPMMGVLDGVLMELQDC-ALPLLKDVIATDKEDVAFKDLDVAILVGSMPRREGMER 116
                       * **:   **       :*..  :   :*. :. ::   .:*. **.:: ..:**: ** *

SP|P40926|MDHM_HUMAN   DDLFNTNATIVATLLTAACAQHCPEAM-ICVIANPVNSTIPITAEVFKKHGVYNPNKIF-G 168/144
SP|P40925-3|MDHC_HUMAN KDLLKANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTASK----SAPSIPKENFSC 172
                       .**:::*..*. :  **  ::. ::: : *:.**.*:.   :::      .   *:: *

SP|P40926|MDHM_HUMAN   VTTLDIVRANTFVAELKGLDPARVNVPVIGGHAGKTIIPLISQCTPKVDFPQDQL----- 223/199
SP|P40925-3|MDHC_HUMAN LTRLDHNRAKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKL--QGKEVGVYEA 230
                       :* **  **:: :*   *:    *:  :* *: ..*  * :.:.. *:    .::

SP|P40926|MDHM_HUMAN   --------TALTGRIQEAGTEVVKAKAGAGSATLSMAYAGARFVFSLV-DAMNGKEGVVE 274/250
SP|P40925-3|MDHC_HUMAN LKDDSWLKGEFVTTVQQRGAAVIKARKLS-----SAMSAAKAICDHVRDIWF---GTPE 281
                          :.  :*: *: *:**:  :        * :.*: : . * *      *. *

SP|P40926|MDHM_HUMAN   CSFVKS---------QETECTYFSTPLLLGKKGIEKNLGIGKVSSFEEKMISDAIPELKA 325/301
SP|P40925-3|MDHC_HUMAN GEFVSMGVISDGNSYGVPDDLLYSFPVVIKNKTWKFVEGLP-INDFSREKMDLTAKELTE 340
                        .**.             :   :* *::: :*  :   *:  :..*..: :. :  **.

SP|P40926|MDHM_HUMAN   SIKKGEDFVKTLK 338/314
SP|P40925-3|MDHC_HUMAN EKESAFEFLSSA- 352
                       . :.. :*:.:
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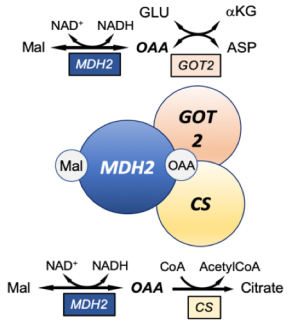
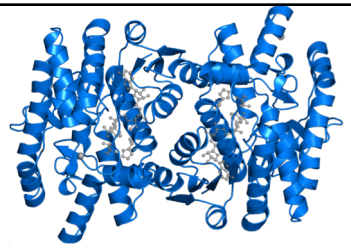
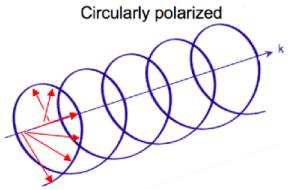
Methods - Docking

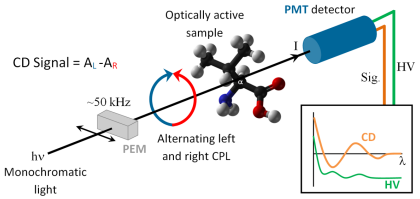




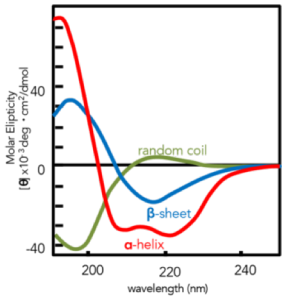
- Find key MDH – CS interactions specific for mMDH vs cMDH to identify interface sites
- Look for regulation of interactions
- Make mutants to show what are the important amino acids for binding



Circular Dichroism Spectroscopy



Express, purify MDH mutants and test to see if mutations altered structure



MDH-CS interactions

- Create a solid interaction detection method
  - Pull down
    - Spin format vs magnetic bead format vs traditional microfuge tube approach
    - Requirement for molecular crowding with wild-type enzyme
    - Determine the best/sensitive /quantitative detection method (gel, enzyme assay, protein assay)
  - Measure impact of mito -> to -> cyto swap (kinetic and interaction)
  - Measure impact of phosphorylation mutants (kinetic and interaction)
- Finish docking to identify current and new potential regulation sites
- Measure kinetic characteristics of mito-cyto MDH swaps and phosphomimics.
- Determine potential kinase(s) responsible for phosphorylation
- Pull down assays, Thermal Melt assays, Fluorescence Anisotropy, SPR, competition assay
  - ✓Look at competition binding
  - ✓Impact of metabolites
  - ✓Impact of phosphorylation