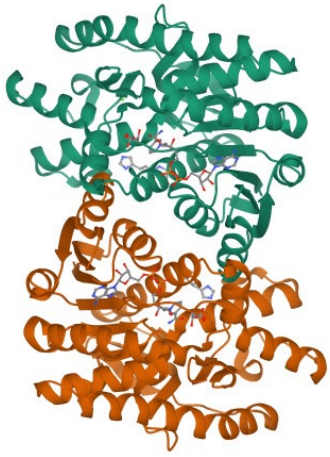
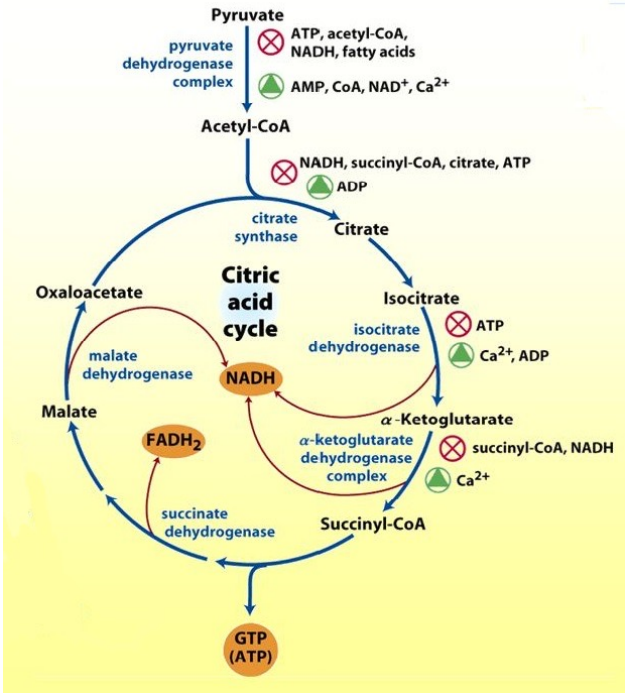
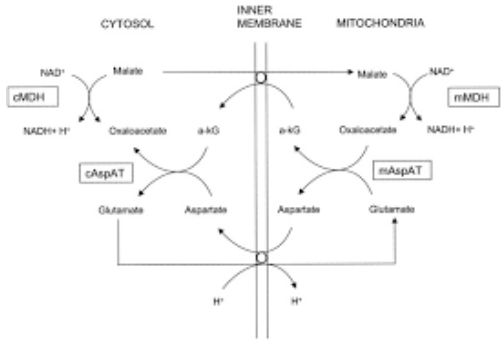
Phosphorylation of MDH CURE project

- Mass spectrometry has identified several experimentally determined phosphorylation sites for both MDH isoforms. Additionally, various online phosphoprediction tools have predicted several more potential phosphorylation sites.
- The dysregulation of protein kinases, which control many metabolic enzymes and regulators, is implicated in various disorders, including cancer and metabolic syndrome.
- Surprisingly, there is a scarcity of studies investigating the impact of MDH phosphorylation, making it an intriguing area for students to explore.
- Phosphorylation of serine, threonine, and tyrosine residues in proteins modifies their polarity, charge, hydrogen bonding, and other non-covalent interactions, making projects focused on this subject an exciting application of structure-function relationships in a CURE.



1





2

Cytoplasm

Gluconeogenesis

MDH1 PEPCK

malate → OAA → PEP

ASP – Malate Shuttle

MDH1 GOT1

malate → OAA → ASP/αKG

Amino acid metabolism/TCA

GOT1 MDH1 ME1

ASP → OAA → malate → pyruvate

Adipogenesis

ACL MDH1 ME1

Citrate → OAA → malate→pyruvate

Mitochondria

Gluconeogenesis

PC MDH2

pyruvate → OAA → malate

ASP – Malate Shuttle

GOT2 MDH2

ASP → OAA → malate

Amino acid metabolism/TCA

FUM MDH2

fumarate → malate

NADH –NADPH Hydride Transfer

MDH2 PC ME2

malate → OAA → pyruvate → malate

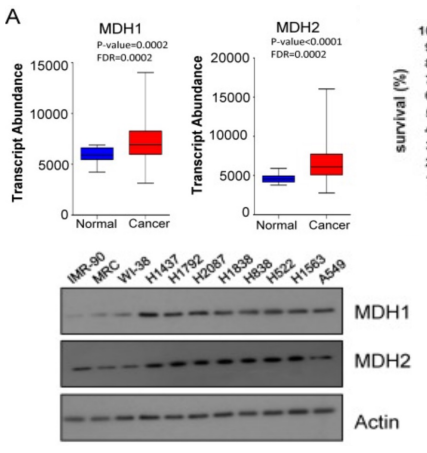
Glutamine

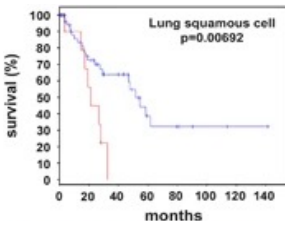
FUM MDH2 GOT2

fumarate → malate → OAA→ASP

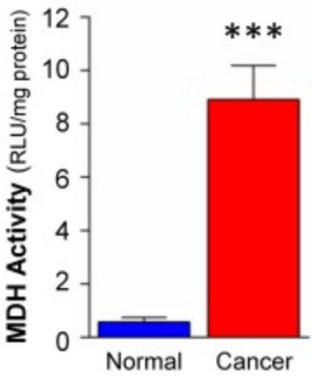
3

MDH and Disease





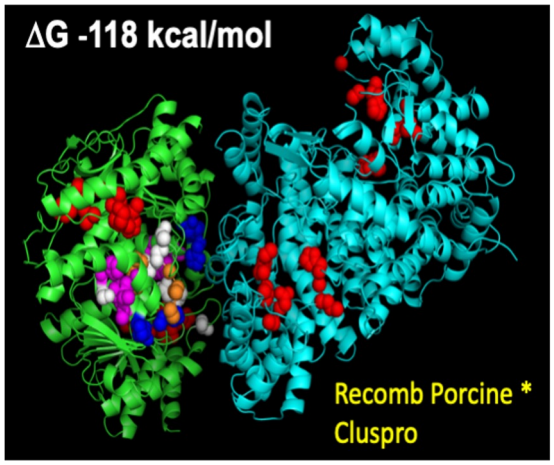
MDH1 is amplified in human cancers and correlates with poor prognosis.

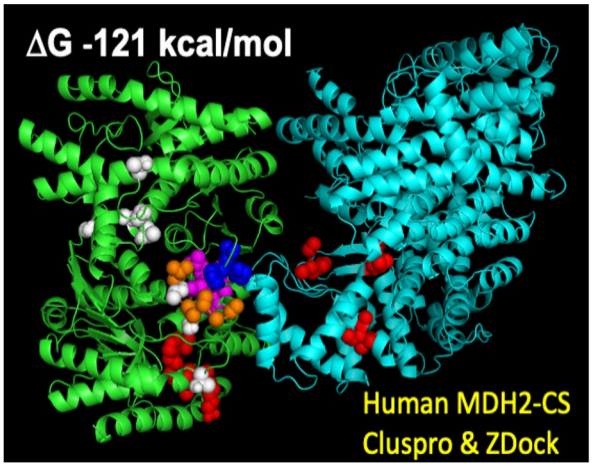


J Cancer ***8****: 2088-2096 (2017) & Oncogene* ***36****, pp 3915–3924 (2017)*

4

hMDH2 interacts with Citrate Synthase





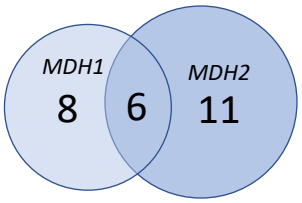
CURE IDEA – Does Phospho-Ser/Thr regulate protein-protein interactions?

5

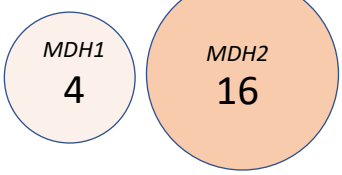
Experimentally Determined PTM

MDH1 = 22, MDH 2 = 38 total

Phospho-Serine

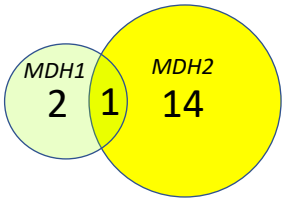


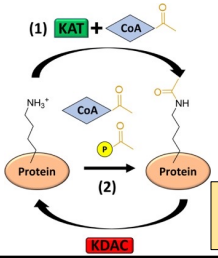
Phospho-Threonine



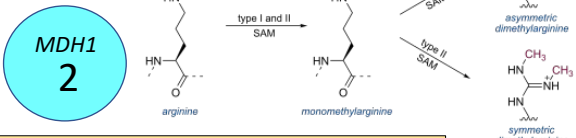
Phospho-Tyrosine

Acetylation



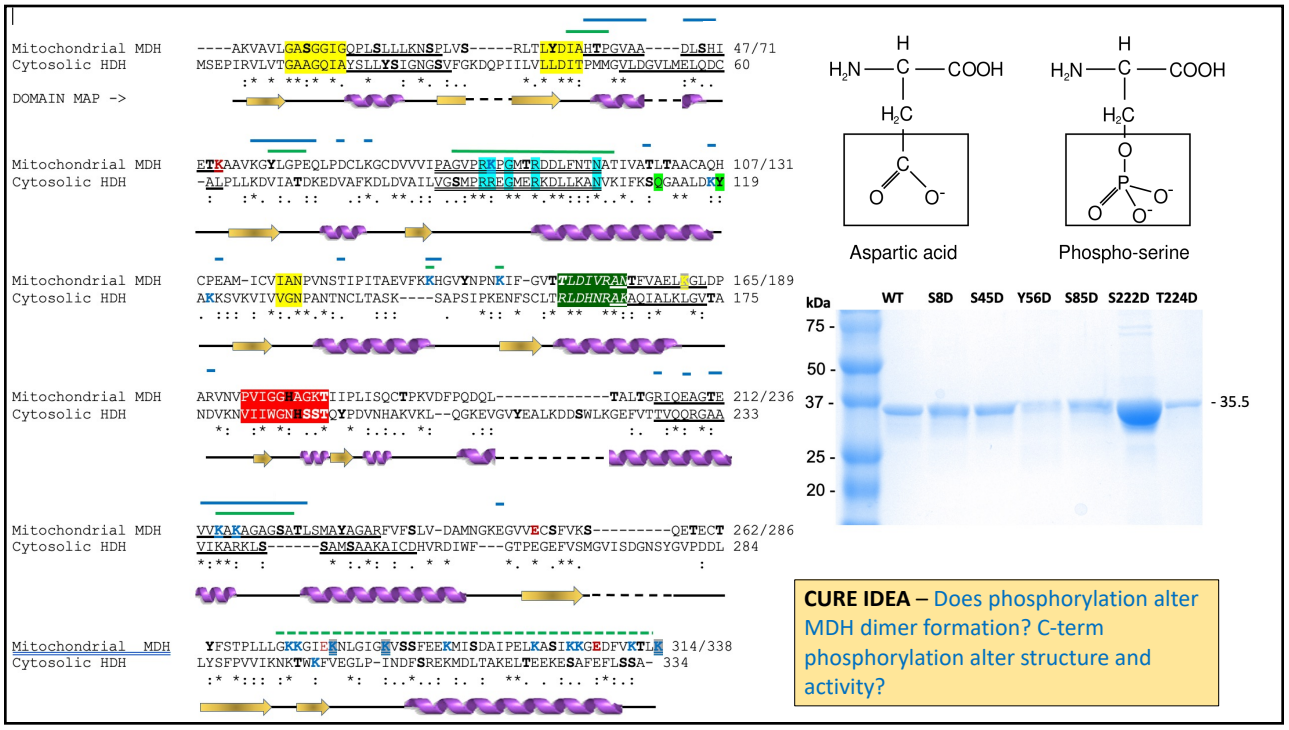


Methylation



CURE IDEA – Sooooo many PTMS… Methylation and Acetylation – wide open!

6


Mitochondrial MDH
Cytosolic HDH
DOMAIN MAP ->
Aspartic acid
Phospho-serine
kDa
WT
S8D
S45D
Y56D
S85D
S222D
T224D
75 -
50 -
37 -
25 -
20 -
- 35.5
CURE IDEA – Does phosphorylation alter MDH dimer formation? C-term phosphorylation alter structure and activity?

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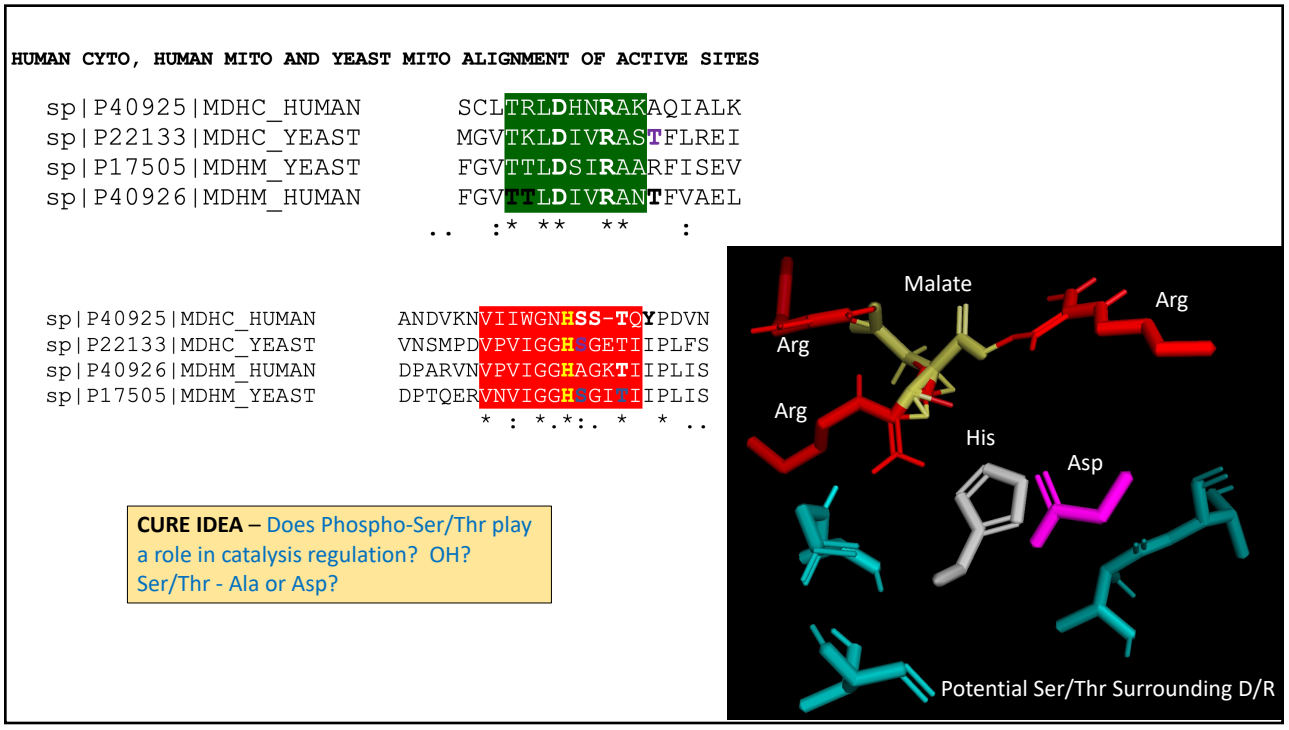
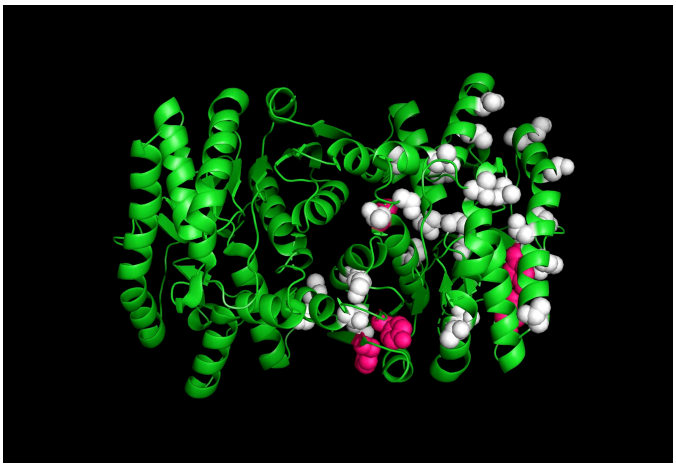

HUMAN CYTO, HUMAN MITO AND YEAST MITO ALIGNMENT OF ACTIVE SITES
sp|P40925|MDHC_HUMAN
sp|P22133|MDHC_YEAST
sp|P17505|MDHM_YEAST
sp|P40926|MDHM_HUMAN
Malate
Arg
Arg
Arg
His
Asp
Potential Ser/Thr Surrounding D/R
CURE IDEA – Does Phospho-Ser/Thr play a role in catalysis regulation? OH? Ser/Thr - Ala or Asp?

8

Human mitochondrial MDH (hMDH2)



Most/ALL? P-Ser/Thr/Tyr are surface accessible – solvent exposed

CURE IDEA – Bioinformatic analysis of impact of phosphorylation – H++/AlphaFold/Docking…

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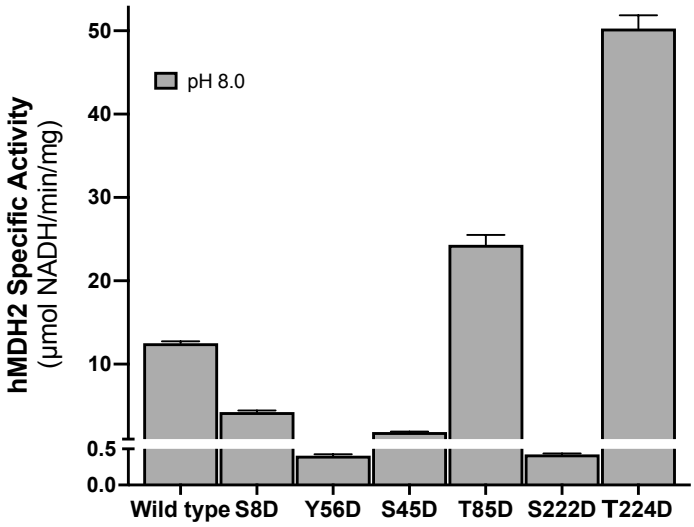
Phosphomimic Impact on hMDH2



S8D: NADH Binding
S45D: MDH Dimer & MDH-CS Interface
Y56D: MDH-CS Interface
T85D: MDH-CS Interface & Mobile Loop
S222D: MDH Dimer & MDH-CS Interface
T224D: MDH Dimer & MDH-CS Interface

CURE IDEA – phosphomimics in regions will alter substrate specificity or other function…
Ser/Thre/Tyr to Ala or Phe?

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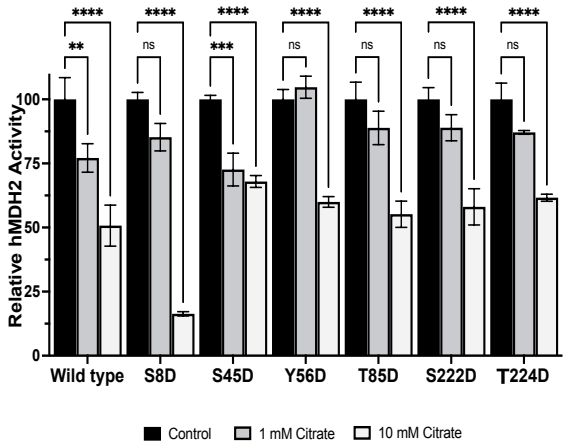
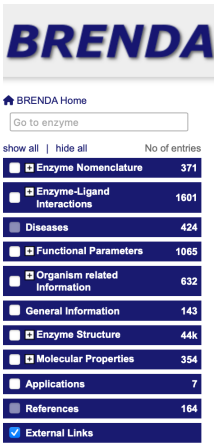
Citrate Inhibition at low concentration is diminished *(Y56D, T85D, S222D & T224D)*



Inhibitors:
Citric acid
ATP/ADP/AMP
a Ketoglutarate
Isocitrate
Malate
ASP

Activators:
Citrate,
Isocitrate
Fumarate



CURE IDEA – Km/Vmax citrate and other inhibitors – ??? Does phosphorylation alter regulation???
use BRENDA and the project page for ideas

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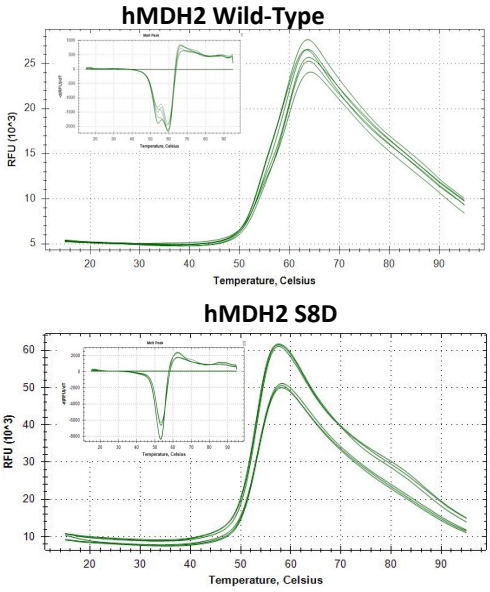
Phosphomimic S8D, S45D & Y56D Destabilize hMDH2



	Avg Tm (°C) *Major*	Avg Tm (°C) *Minor*
Wild-Type	59.35	54.20
S8D	**53.30**	ND
S45D	**47.50**	ND
Y56D	**40.08** **54.13**	ND
T85D	60.00	ND
S222D	**63.50**	54.00
T224D	61.00	53.00

CURE IDEA – heat proteins at diff temp, centrifuge, measure protein and activity.

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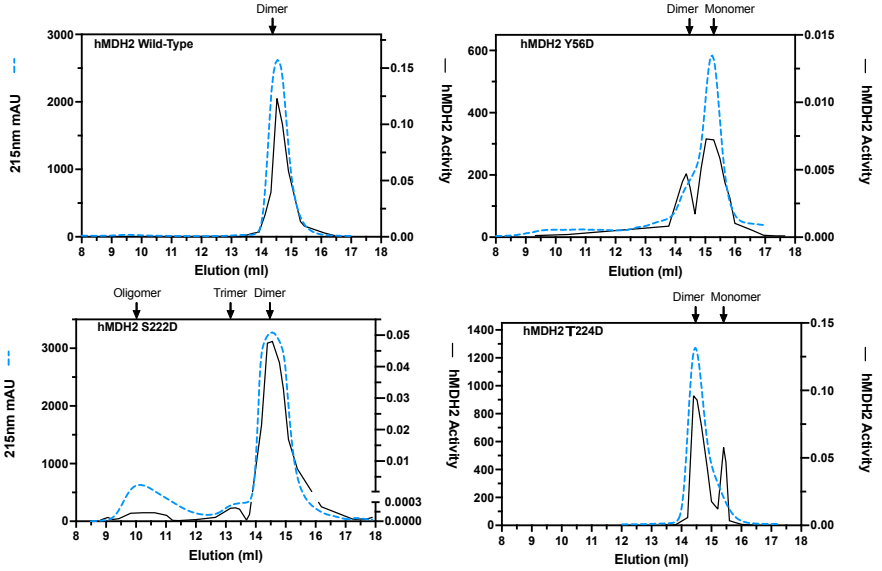
hMDH2 WT, S8D, S45D, and S85D all elute as a dimer



CURE IDEA – How does phosphorylation alter the dimer – monomer state of MDH?

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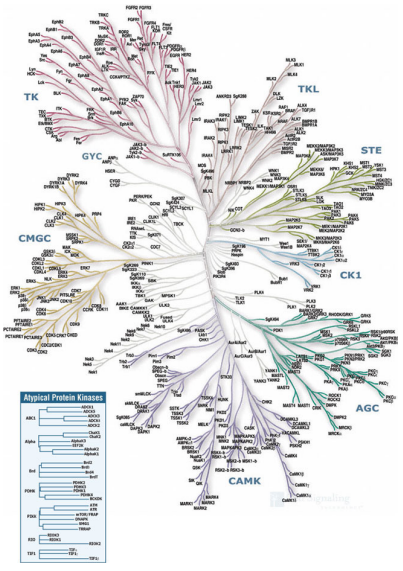
Who is doing what to who????



Kinase/Family	Position
AGC	18, 19, 20, 261
CAMK	19, 224
CK1	47, 210, 290
CMGC	310, 317, 326
PKL	147, 276, 286
RGC	3, 41, 47
STE	18, 20, 248
TKL	20, 69, 246
TK	80, 287, 167
PKA	10, 146, 224, 289, 317
PKC	10, 45, 89, 141, 296
CDC2	19, 89, 146
GSK3	10, 16, 33, 41, 47, 146, 213
CKII	45, 321, 326
Unspecified	45, 123, 135, 192, 222, 277, 309

CURE IDEA – Working on CRSPR K/O and K/I of tagged MDH!!!

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MDH CURE Start Up Project

Human mitochondrial and cytosolic phosphorylation

Week by week lab activities for a modular and/or semester long version of this CURE

- 3-4 weeks on reading, visualization (PyMOL…) computational design and hypothesis development
- 3 weeks for SDM in parallel with…
- 3 weeks for expression, purification and protein assay/westernblot
- 4 weeks for testing hypothesis – enzyme assay, protein stability, SEC, detailed kinetic analysis

Instrumentation/equipment/key reagents needed for this CURE: Thermocycler (if doing SDM), incubator for expression (room temp or 37ºC), 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.

Protein (WT and/or specific mutant), organism: Details on these and other clones can be found in the MDH Members page

Cytosolic (hMDH1) is expressed as several N-terminal splice variant forms.

- hMDH1V3 (human cytosolic MDH splice variant 3) and mutants **S108D, S42D, S236D, S264D, S328D**
- hMDH2 (human mitochondrial MDH) and mutants **S8D, S46D, Y56D, S85D, S222D, S224D**
  - mito MDH numbering does NOT include the mitochondrial targeting peptide.

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MDH CURE Start Up Project

Human mitochondrial and cytosolic phosphorylation

Where to start?

PICK ONE – and a couple of mutations.

Choose a simple enzyme based test – Km/Vmax, heat stability, regulation by an inexpensive inhibitor from Brenda

Hypothesis Development, PyMol-Bioinformatic, Express and Purify, WB/SDS PAGE, enzyme assays

COLLABORATE!

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