





**Key: MDH2 v1 without transit tag is used for comparison (>sp|P40926|25-338).**

— Shown and predicted MDH-CS interaction sites (*Wu et al, Bulutoglu et al*) Dotted line indicates many potential points of interaction.

— Predicted interaction MDH-CS sites (Cluspro & ZDock Provost Lab) Needs to be refined without Sigma Lys and use same local and Xwalk to further define model

Nucleotide (NAD<sup>+</sup>/NADH) binding 11-18, 40-44, 129-131 (MDH1aa)

Substrate (OAA) binding (highly conserved sites) 92-93, 95, 98, 105, 157-164 (MDH1 aa)

Flexible loop 87-105

K MS determined lysing crosslinks with CS in (bovine tissue) and recomb porcine mMDH

Not showing Porcine K161, K179, K191, K255, & K272 from sigma cross-link Bulutoglu et. al. as these do not support best model of interaction.

X possible ionic bridges from Elcock et al 1996.

Active site/Catalytic site(D/R)

Active site/Catalytic site(Base)

X – active site residues

Binding site

Interface (MDH1 subunits)

Interface region I 18-29, Interface region II 49- 63, Interface region III 160 – 178,

Interface region IVa 226 – 289, Interface region IVb 290 - 302

Bold Ser (S), Thr (T) & Tyr (Y) indicate shown phosphorylation sites of MDH1 or MDH2

**Mito – MDH2: K161(185), K283 (301), K277(307) and K314 (parent using Zhao's nomenclature that included the precursor N tail) are also acetylated (MDH2).** Double Underlined K in grey indicates predicted/shown K crosslinks and sites of acetylation.

**Cyto MDH1 acetylation K118, 121, 298\* this number may be off – doesn't align with this MDH1 sequence**

Arg 240 and 248 are also shown to be methylated – losing dimer structure and inhibiting activity. Need to nail down which one before putting on this map.

SMAYAGAR – is phosphorylated to promote Warburg in MDH2 there is no matching site in MDH1.

▼ Arrow indicates S or T to D phosphomimic mutation prepared

Boxed aa indicates deletion of MDH2 aa and insertion of MDH1 aa (swap) or MDH1 mimic deletion

**Mutations planned or prepared:** Mutants available for biochem lab in addition to mito, cyto and wg MDH wild-type

Mutation Description	Formal Mutation Name (xx/yy) w-transit/wo-transit peptide	AKA	Dock Model if available
Delete mito HTP insert PMM from cyto MDH1	hMDH2v1 HTP60/36_del PMM ins	hMDH2 HTP-PMM	ds1
Delete 5 aa from mito insert 10 aa from cyto	hMDH2v1 AADLS65-70/41-46_del LDGVLMEQLD ins	hMDH2 41-46	ds2
Delete 4 aa from mito to match cyto sequence	hMDH2v1 VFKK155-158/131-134_del	hMDH2 131-134	ds3
Delete 5 aa from mito to match cyto sequence	hMDH2v1 AGSATL 244-249/220-225_del	hMDH2 220-225	ds4
Unique to mito phosphomimic	hMDH2v1 S69/45D	hMDH2 S45D	none
Unique to mito phosphomimic	hMDH2v1 S109/85D	hMDH2 S85D	none
Unique to mito phosphomimic	hMDH2v1 S246/222D	hMDH2 S222D	none
Unique to mito phosphomimic	hMDH2v1 S248/224D	hMDH2 S224D	none