

定点突变D-乳酸脱氢酶生产高光学纯羟酸

Highly stereoselective biosynthesis of (R)- α -hydroxy carboxylic acids through rationally re-designed mutation of D-lactate dehydrogenase

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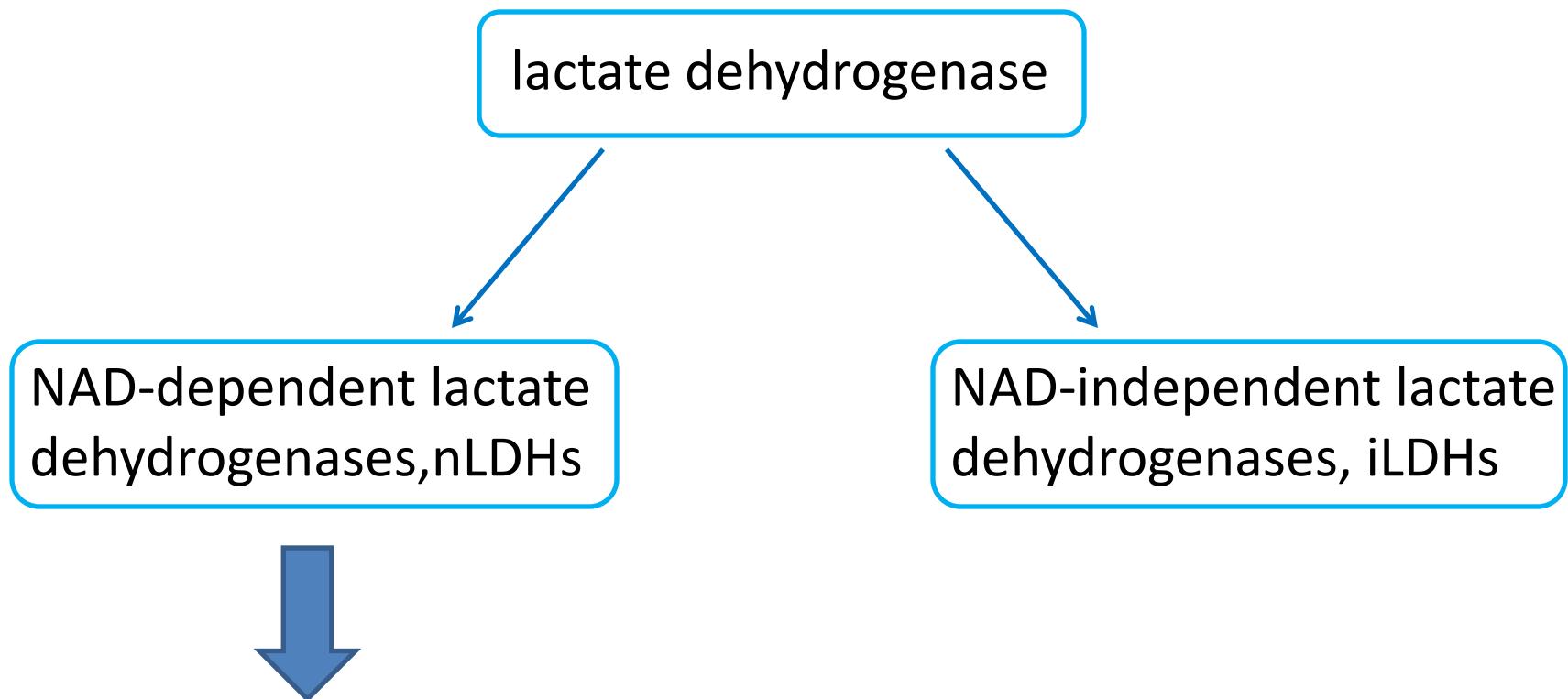
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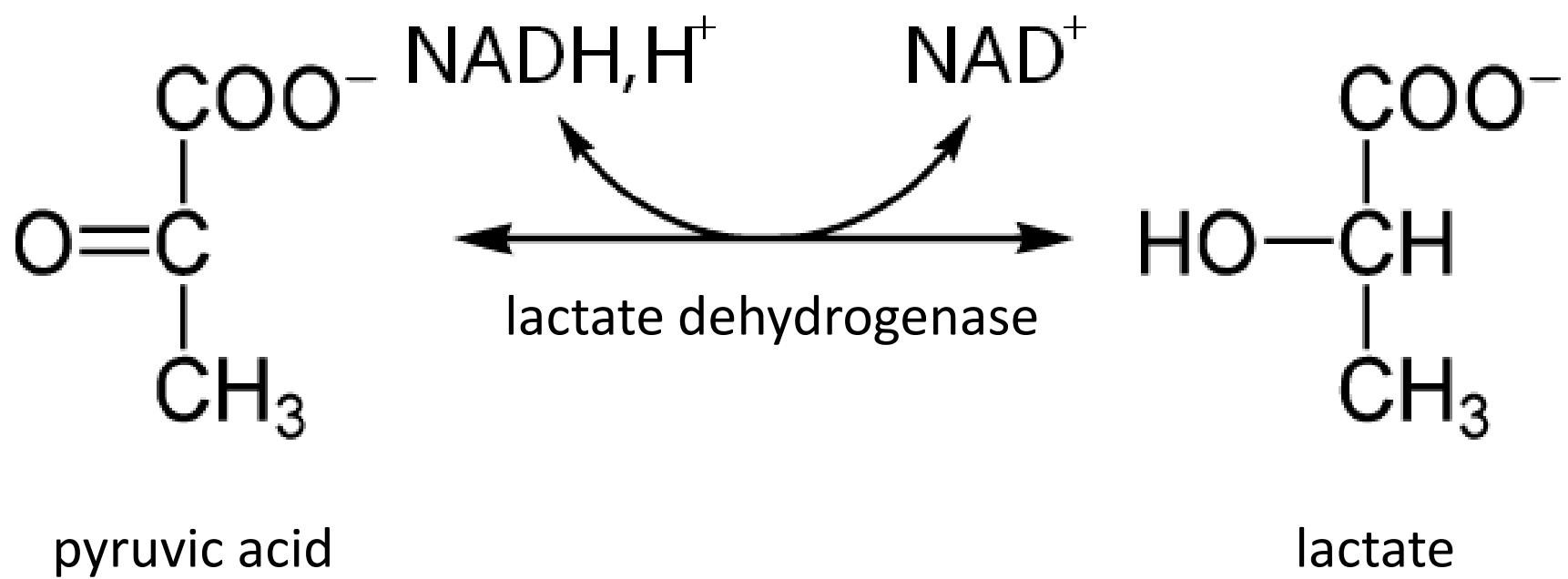
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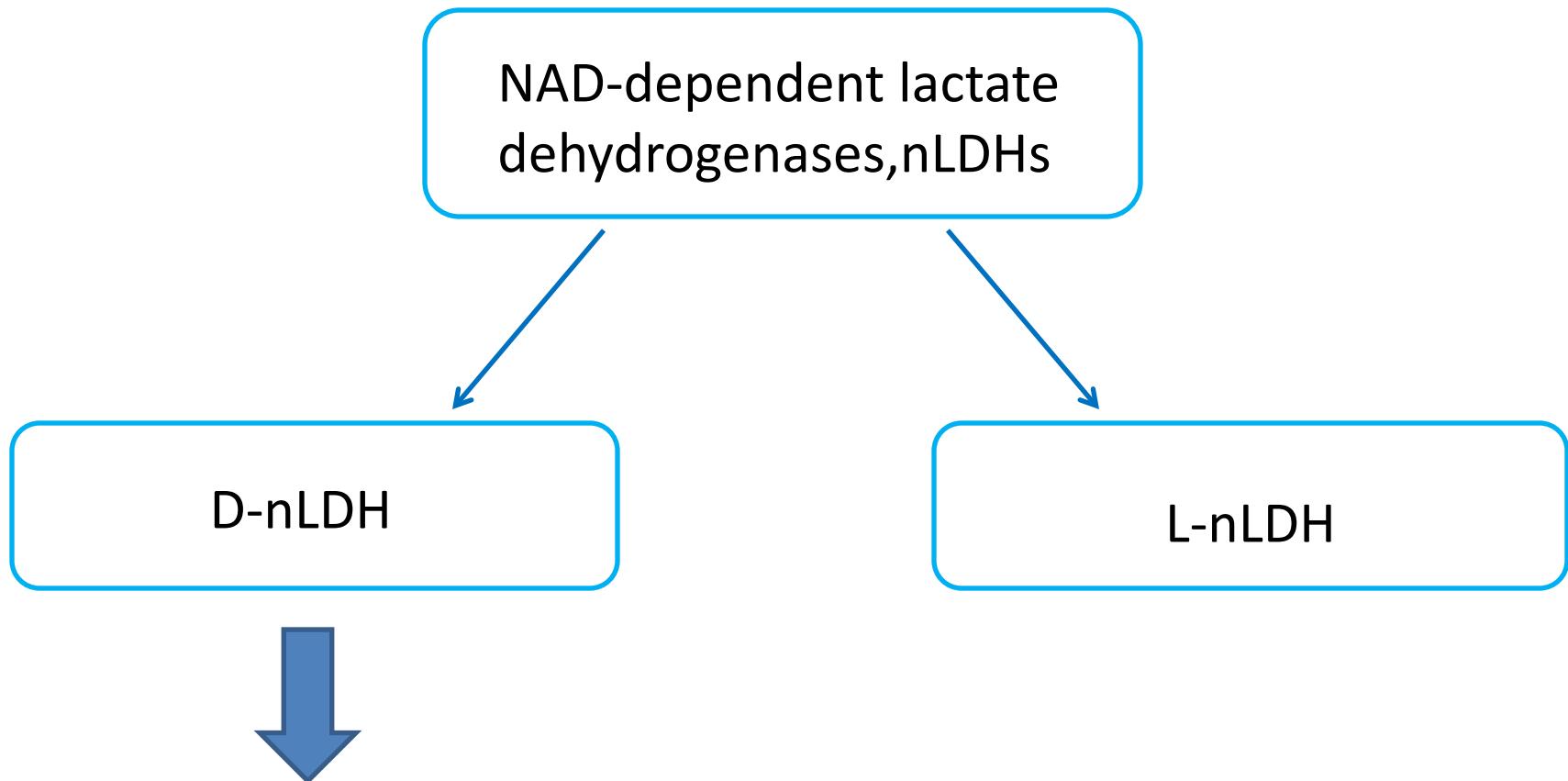
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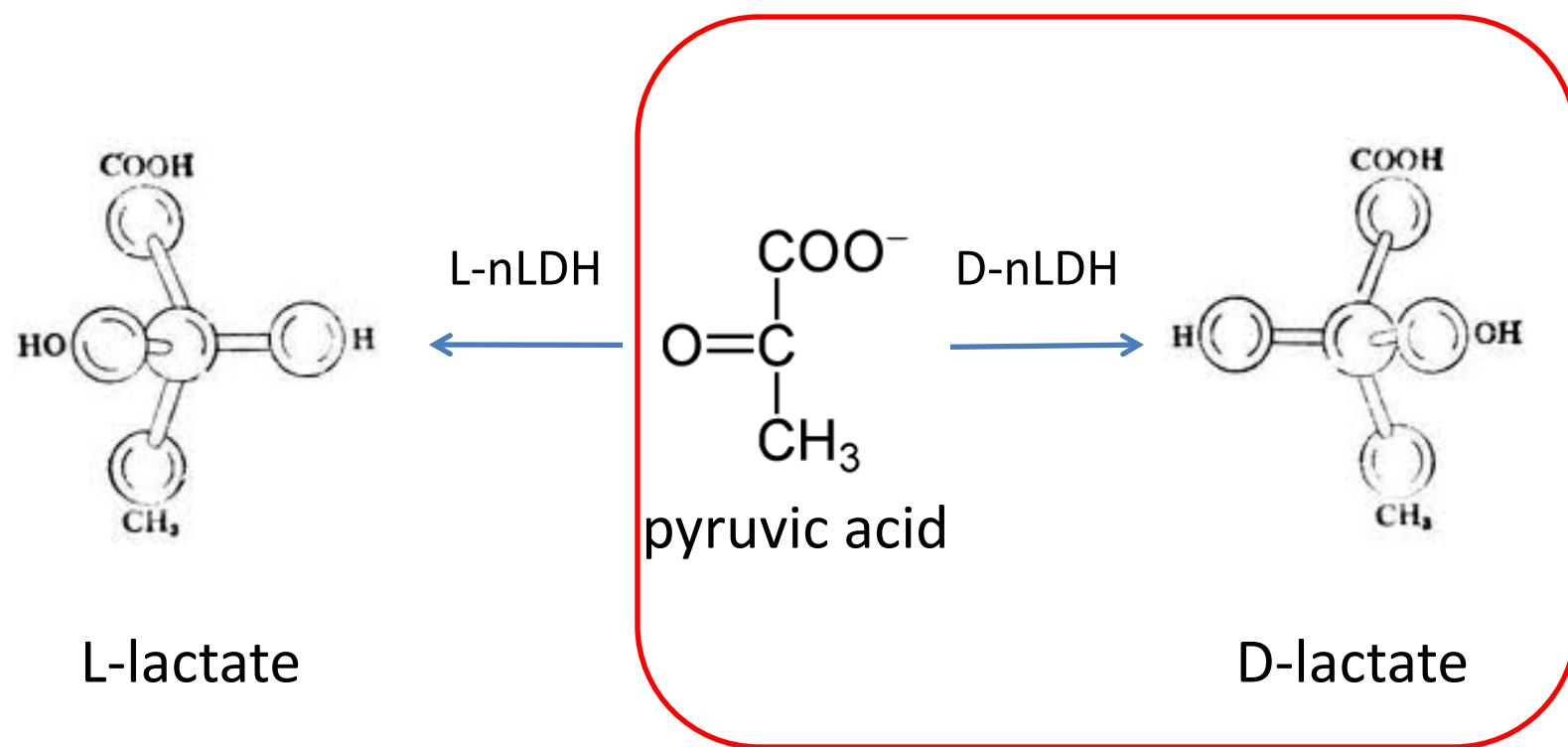
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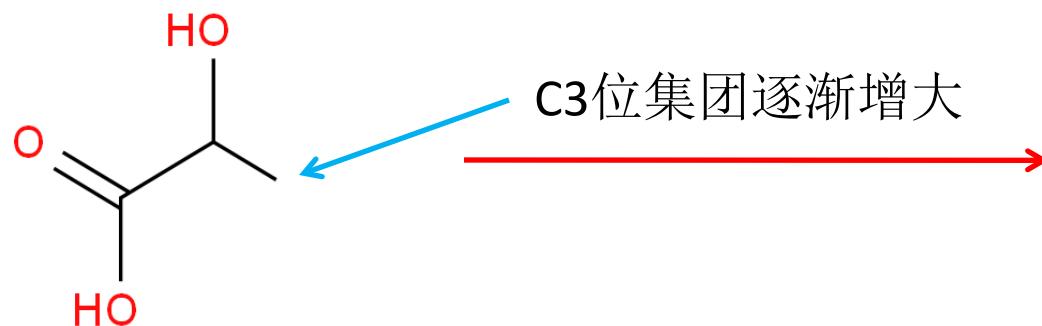
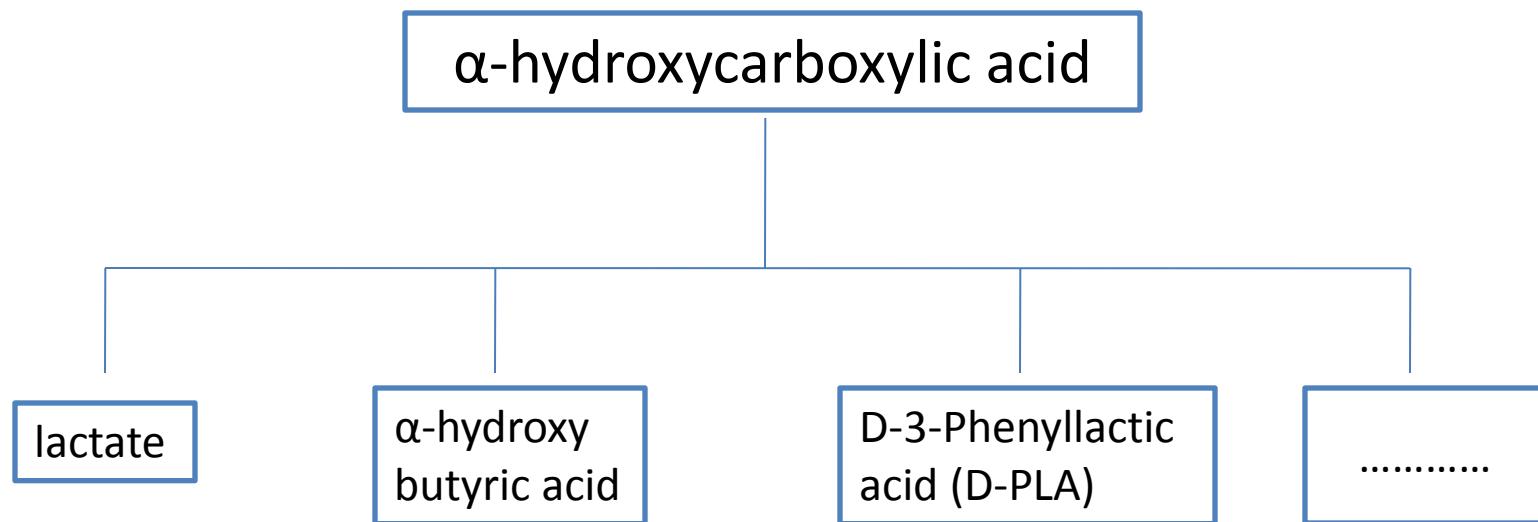
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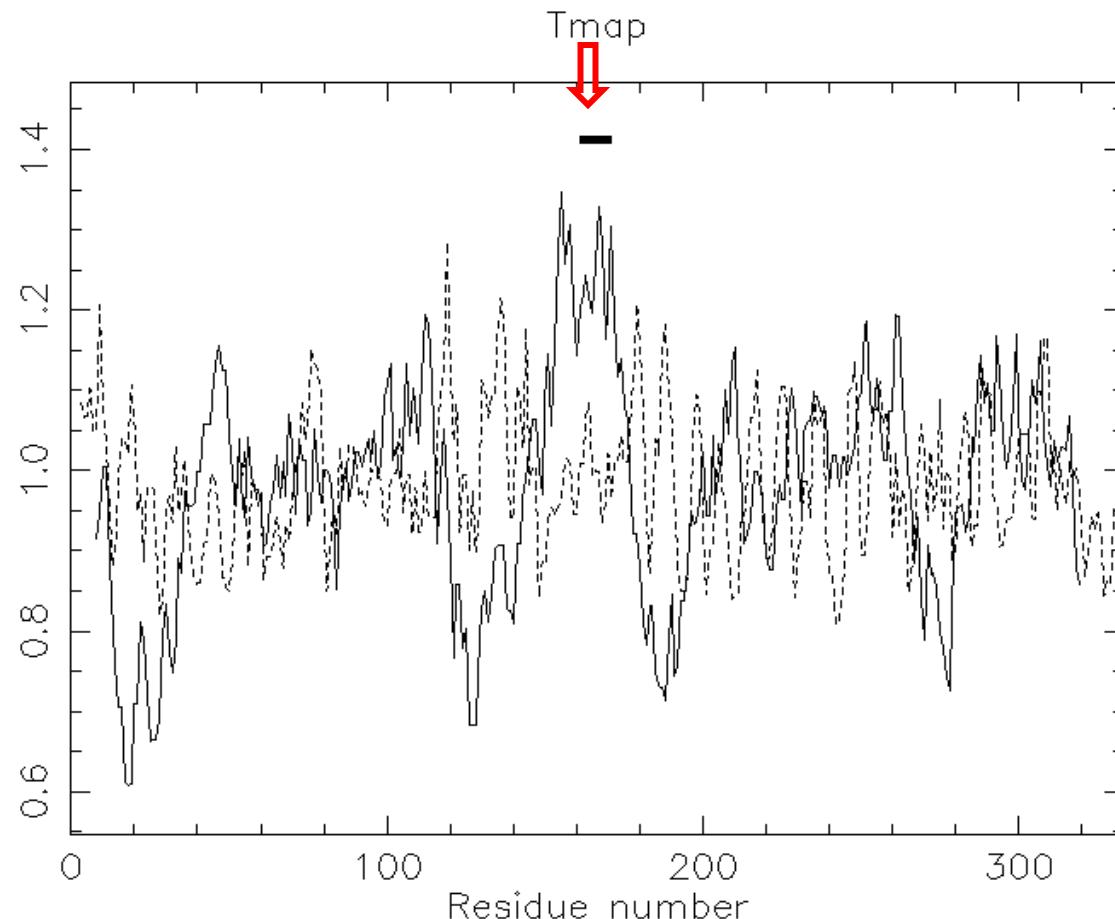


Protein name:	D-lactate dehydrogenase(D-LDH)
Gene name:	ldhA
Sequence length:	333AA
Sequence similarities:	belongs to the D-isomer specific 2-hydroxyacid dehydrogenase family
Ligand:	NAD
molecular function:	D-lactate dehydrogenase activity NAD binding

Sequence analysis of D-lactate dehydrogenase

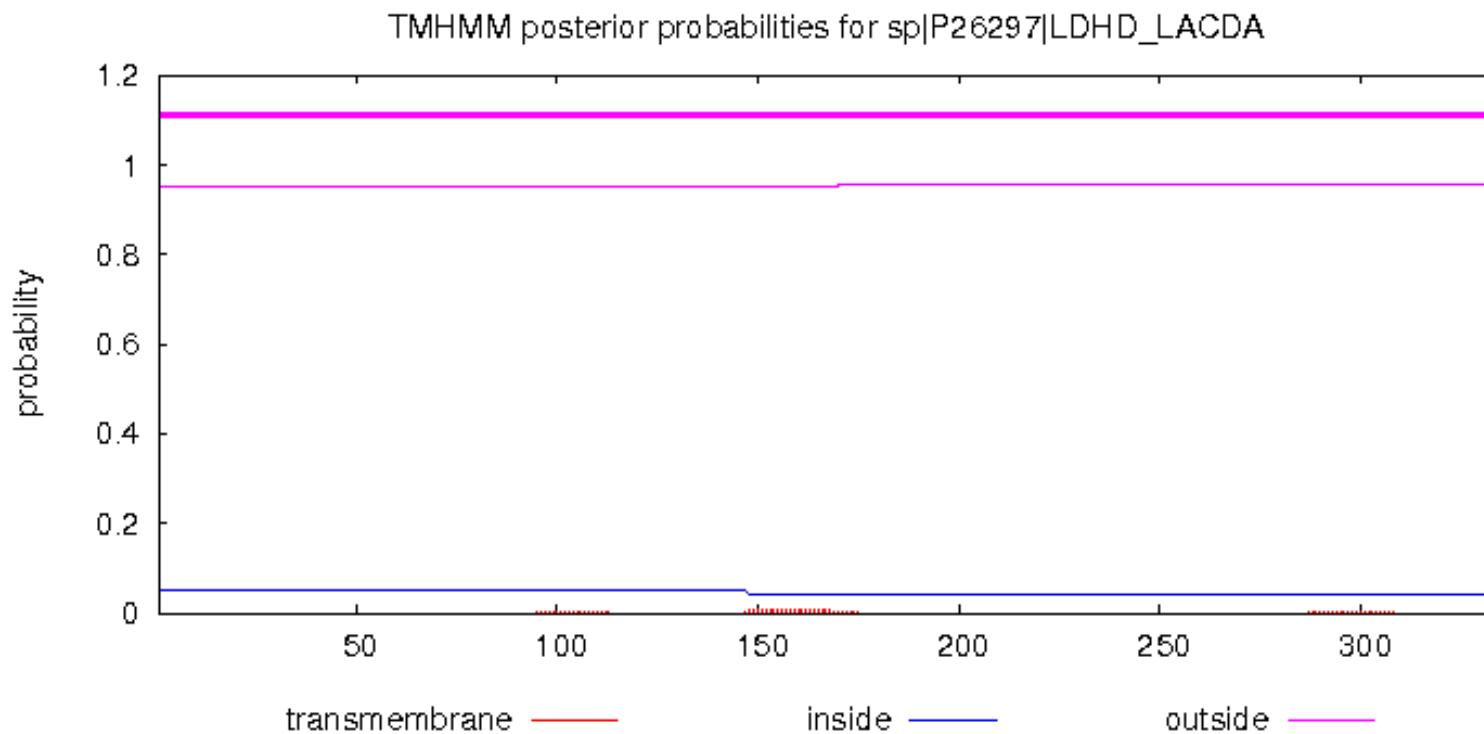
Transmembrane tendency analysis

WebLab—tmap(v.6.0.1)



Sequence analysis of D-lactate dehydrogenase

```
# sp|P26297|LDHD_LACDA Length: 333
# sp|P26297|LDHD_LACDA Number of predicted TMHs: 0
# sp|P26297|LDHD_LACDA Exp number of AAs in TMHs: 0.17501
# sp|P26297|LDHD_LACDA Exp number, first 60 AAs: 0.00015
# sp|P26297|LDHD_LACDA Total prob of N-in: 0.04934
sp|P26297|LDHD_LACDA    TMHMM2.0      outside     1    333
```

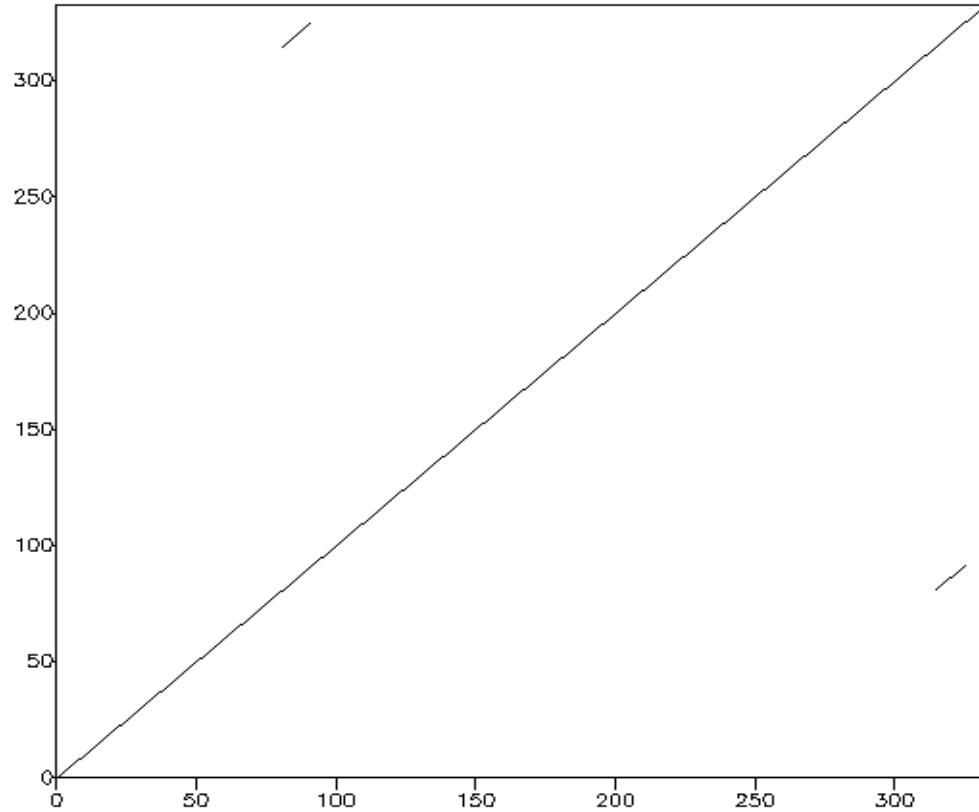


Sequence analysis of D-lactate dehydrogenase

Repeated sequence analysis

WebLab—dotmatcher(v6.0.1)

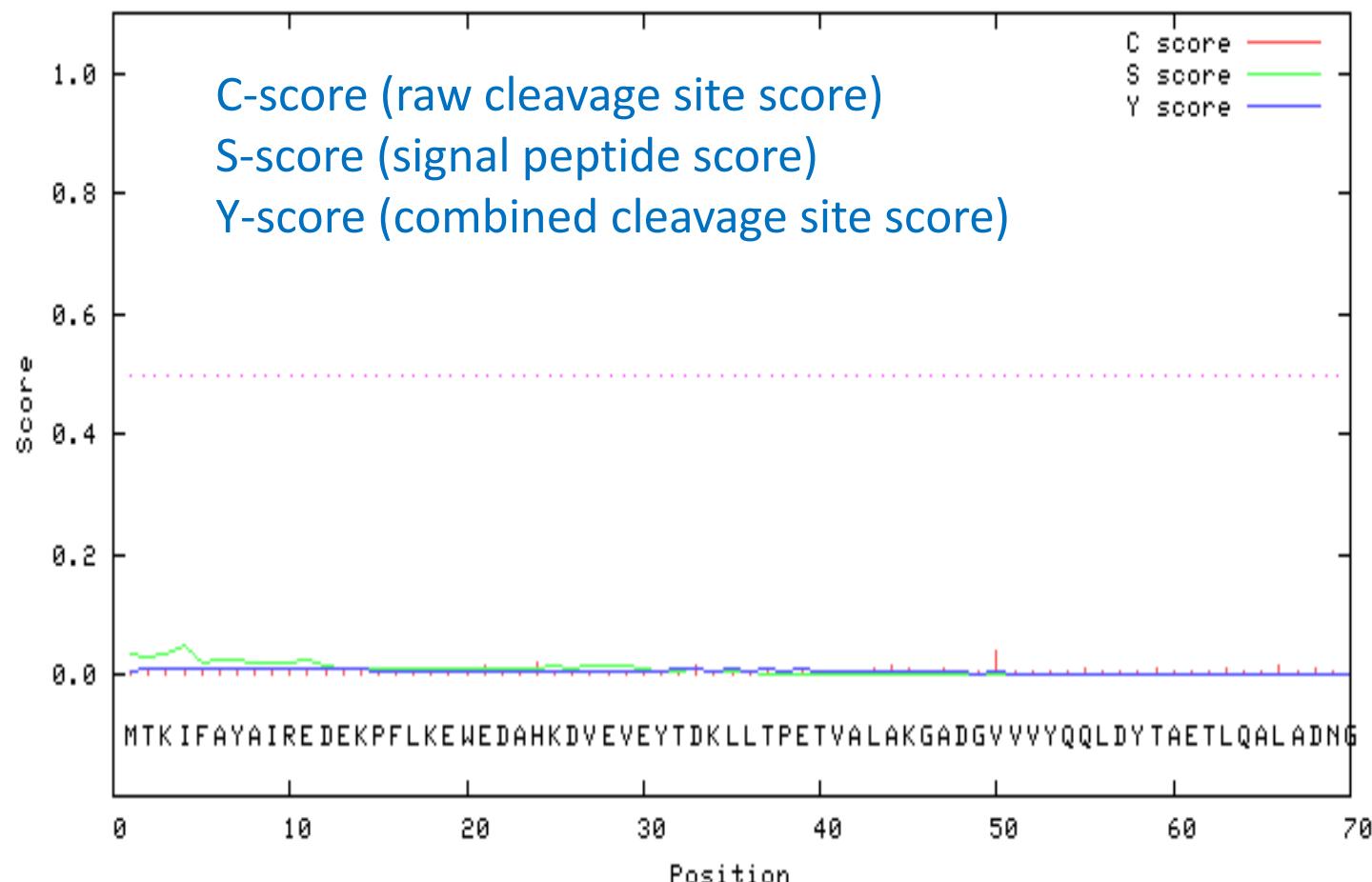
Dotmatcher: raw::751847 vs raw::751848
(windowsize = 10, threshold = 23.00 15/06/14)



Sequence analysis of D-lactate dehydrogenase

Signal peptide prediction

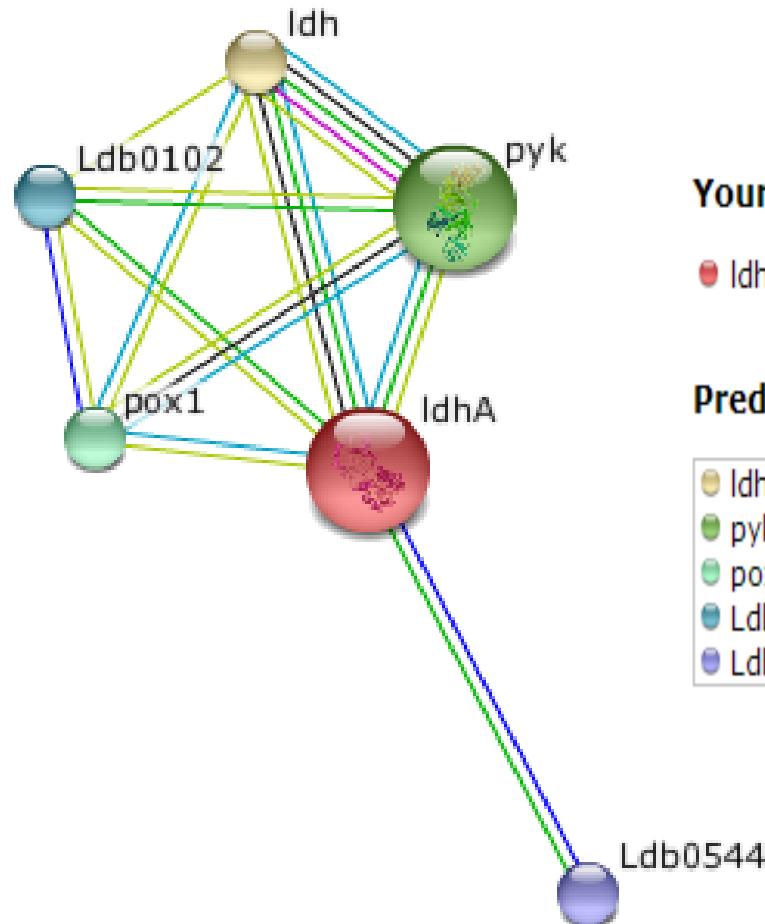
SignalP



Sequence analysis of D-lactate dehydrogenase

Interaction proteins

STRING

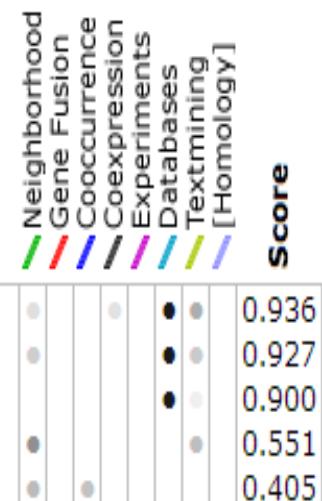


Your Input:

- IdhA D-lactate dehydrogenase (333 aa)
(*Lactobacillus delbrueckii* 11842)

Predicted Functional Partners:

Idh	L-lactate dehydrogenase (307 aa)
pyk	pyruvate kinase (589 aa)
pox1	pyruvate oxidase (616 aa)
Ldb0102	putative oxidoreductase (463 aa)
Ldb0544	hypothetical protein (357 aa)



Sequence analysis of D-lactate dehydrogenase

WebLab—water(v6.0.1)

Pairwise Alignment Result

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
225	69.0	51/225 (22.7%)	85/225 (37.8%)	60/225 (26.7%)

Q1G7P4_LACDA	106	VASGFSGIFVVSANPVDILTTLTQKL--SGFPKKRVIIGTGT-SLDSASLR : : : ::	152
LDHD_LACDA	43	LAKGADGVVVY--QQLDYTAETLQALADNGITKMSLRNVGVVDNIDMAKAK	90
Q1G7P4_LACDA	153	VELAKRL-QVPIESVNAWVLGEHGDSSENFSAVVNGKPLLDYPGMTEA ...: . :: ...:.	201
LDHD_LACDA	91	-ELGFQITNVPVYSPNA--IAEH-----AAIQAARIL---RQDK	123
Q1G7P4_LACDA	202	ALDE-IEAH-----VREKGSEIIVKKGATYYGVAMML-----AKIV : :... : ::	236
LDHD_LACDA	124	AMDEKVVARHDLRWAPTIGREVRDQVVGVIGTGHIGQVFMQIMEGFGAKVI	173
Q1G7P4_LACDA	237	T-AILENNNDLALPLSAPLHGEYGIKDEIYLGLTLAIINGQG--IS-HVLEL: ::	282
LDHD_LACDA	174	AYDIFRNPELE-----KKGYYVDSLDDLYKQADVISLHVPDV	210
Q1G7P4_LACDA	283	P-----LNDSELAKMRAASAATIKAT	302
LDHD_LACDA	211	. : ... : ... : ...	235

Sequence analysis of D-lactate dehydrogenase

Species/Abbrv	Sequence
1. tr KONEF7 KONEF7_9LACO	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKLFGEVMDVYEGEVGVFEDVEG-EEFDA LADLIAA VLVTPHIAFYIIHAVNNMVVAFDNLALLVKGEEAEETRVIVG-
2. sp P26297 LDHD_LACDA_D-1	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKIFGAMDVYEGEVGVFEDVEG-EEFDA LADLIAA VLVTPHIAFYIIHAVNNMVVAFDNLALLVKGEEAEETRVIVG-
3. tr JOMND9 JOMND9_9LACT	-ANDRMINAESIAAMKDGVVIVVNCSIGLID DAVINGLGLGKISGLAIDVYEGEVGVLFEDVEG-EEFDA LADLISRS VLVTPHIAFYIIHAVYEMVQSLSA LDFINGEVPSIAVAVW-
4. tr S4NB46 S4NB46_9LACO	-ENEHMINDDTIAAMKDGVFILNPA CALID DAVINGLGLGKIGGALDLYEVEVGIFEDVEG-EEFDA LALMERS ILVSRIIAFYIIHARNNMVVFAMDAVSLINSG EQLVPL-
5. tr C2EUK0 C2EUK0_9LACO	-DTINMINDDTIAAMKDDGVVLVNVSGALVD DAVVNALDGGKLFGFVMDIVEGEVGVFEDVEG-EEFDA LALLIDRS VLVTPHIAFYIIHAVNNMVVAFDNLALLVKGEEAEETRVIVG-
6. tr B1MXH0 B1MXH0 LEUCK	-GNDQMINAEIIIAAMKDDAVLVNVSGLLVD DAVVAALDGGKLFGFVMDIVEGEVGVFEDVEG-EEFDA LDDDLISRS VLVTPHIAFYIIHARVKEMVQSFDAAVAFAKGEEIASIAVW-
7. tr H1WWN7 H1WWN7 LEUCI	-GNDQMINAEIIIAAMKDDAVLVNVSGLLVD DAVVAALDGGKLFGFVMDIVEGEVGVFEDVEG-EEFDA LDDDLISRS VLVTPHIAFYIIHARVKEMVQSFDAAVAFAKGEEIASIAVW-
8. sp P51011 LDHD LEUMC D-1	-ENIHMLADAIAMKDGVVIMMAAGLMDIDAIIDGLGGKISGFVMDVYEGEVACSMIGLVL-EEFDA LADLIAA VVMIPIIAFYIIHARLEMVQSFDAAVAFAKGEEIASIAVW-
9. tr J4N155 J4N155 OENOE	-ENFHMIDASIAAMKDHVIIIVNDSIGLVD DAVINGLGLGKISAFADVYIEEVGVIFEDVEG-EEFDA LALLIDRS VLLIPHIAFYIIHARVAMVQSLNAGLSFLNGVGRASALIF-
10. tr D3L7P0 D3L7P0 OENOE	-ENIHMINIDESIAAMKDGVVVLVNVAGLVD DAVVAALDGGKVSQMVMDVYEGIGIFEDVEG-EEFDA LDDDLIDRS VLVTPHIAFYIIHARLEMVQSFDAAVAVAIANG ETRNLVIF-
11. tr J4P851 J4P851 OENOE	-ENIHMINIDESIAAMKDGVVVLVNVAGLVD DAVVAALDGGKVSQMVMDVYEGIGIFEDVEG-EEFDA LDDDLIDRS VLVTPHIAFYIIHARLEMVQSFDAAVAVAIANG ETRNLVIF-
12. tr J4N760 J4N760 OENOE	-ENFHMIDASIAAMKDHVIIIVNDSIGLVD DAVINGLGLGKISAFADVYIEEVGVIFEDVEG-EEFDA LALLIDRS VLLIPHIAFYIIHARVAMVQSLNAGLSFLNGVGRASALIF-
13. tr J2ZYF2 J2ZYF2 OENOE	-ENIHMINIDESIAAMKDGVVVLVNVAGLVD DAVVAALDGGKVSQMVMDVYEGIGIFEDVEG-EEFDA LDDDLIDRS VLVTPHIAFYIIHARLEMVQSFDAAVAVAIANG ETRNLVIF-
14. tr S6EK28 S6EK28 LACAI	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKIFGFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLALLIKGEEDSVALDKNF-
15. tr J3EMM8 J3EMM8_9LACO	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKIFGFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLALLIKGEEDSVALDKNF-
16. tr TOP2Y2 TOP2Y2_9LACO	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKIFGFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLALLIKGEEDSVALDKNF-
17. sp P30901 LDHD_LACHE_D	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKIFGFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLALLIKGEEDSVALDKNF-
18. tr I7LDP7 I7LDP7_9LACO	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKVFQFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLALMIGGEEDSVALDKNF-
19. tr C4VP59 C4VP59_9LACO	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKVFQFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLVAMVEGEEETRVIVG-----
20. tr G1UB05 G1UB05 LACJO	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKIFGFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLALMINGGEEDSVALDKNF-
21. tr C4VR47 C4VR47_9LACO	-ANVHMINIDESIAAMKDGVVIVVNCSIGLVDI DAVINGLGLGKIFGFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLALLIKGEEDSVALDKNF-
22. tr G8PDC7 G8PDC7 PEDCP	-DNYHMLDDNAFISMKDGVFVFLNYSIGLIDIAALINGLGLGKIAQGVLDDIVIEEVGVIFEDDEMFLAANLVMIIHAAFYIIHARVNMVVALDNQSLIESGSQSONQVDEG-----
23. tr COYYW1 COYYW1 LACRE	-ENFHMIDDAIAAMKDNVVIVVNCSIGALVD DAVIEGLGLGKIFGFIMDIYIEEVGVIFEDVEG-EEFDA LDDDLIDRS VLVTPHIAFYIIHARVNMVLIAFDNLALLIKGEEDSREADTRVIVG-----
24. tr C5G2Q1 C5G2Q1_9LACO	-ANVHMINDDSIAMKDGVVIVVNCSIGLVDI DAVINGLGLGKVFQFVMDIVEGEVGVFEDVEG-EEFDA LADLIDRS VLVTPHIAFYIIHARVNNMVVAFDNLVAMVEGEEETRVIVG-----

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236

260 265

297

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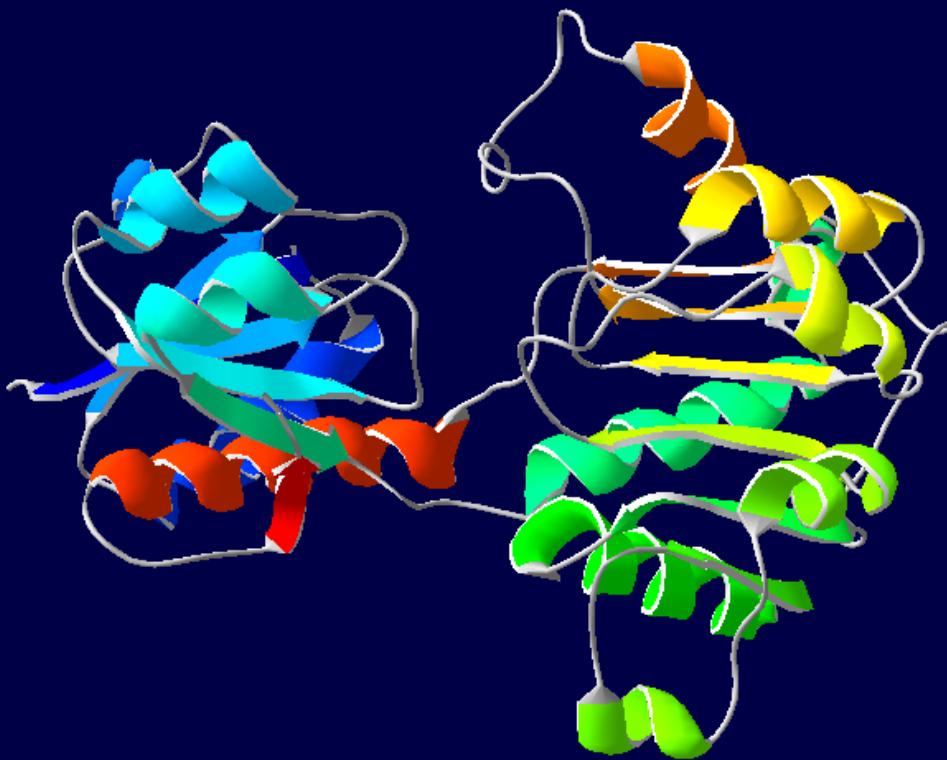
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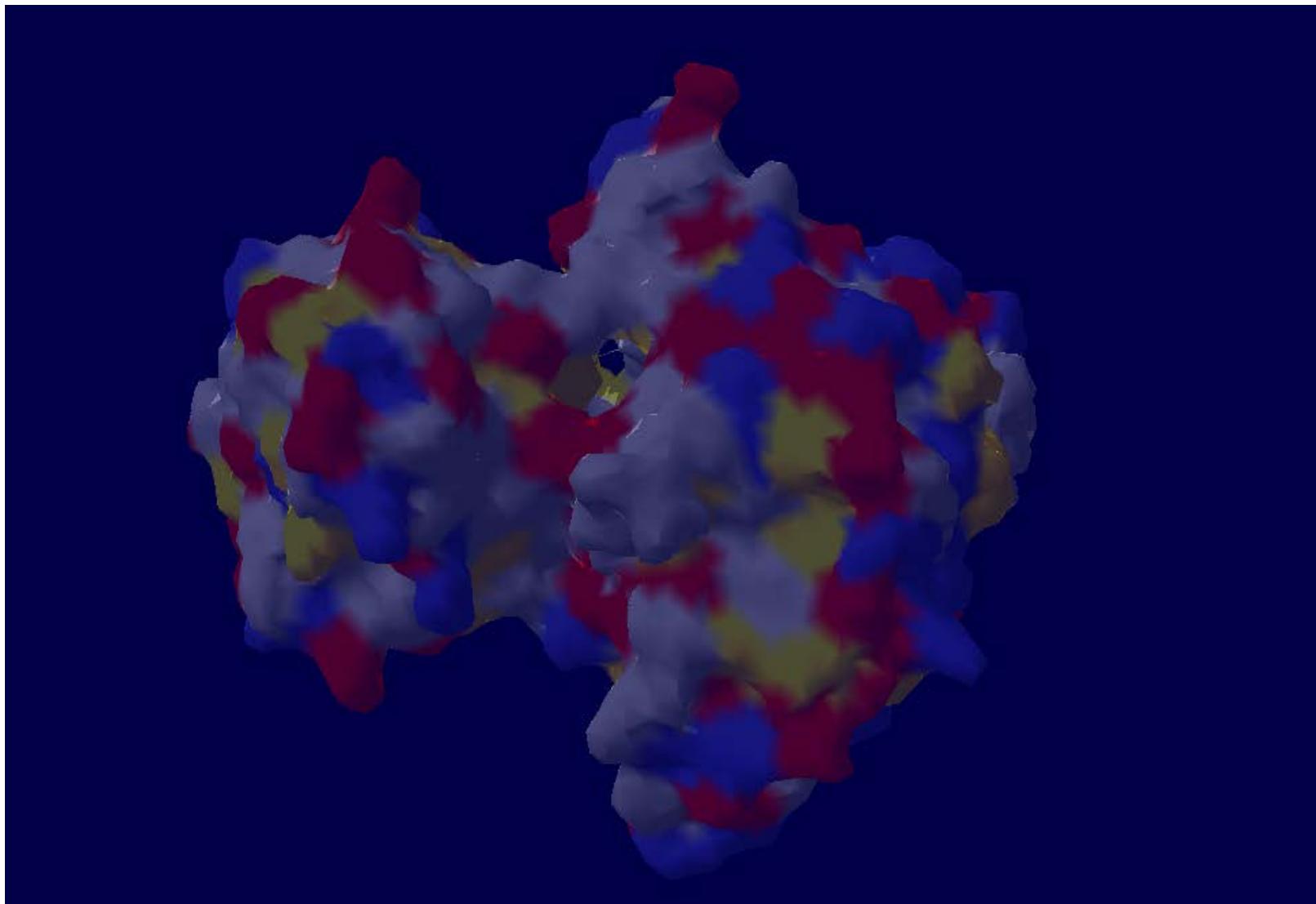
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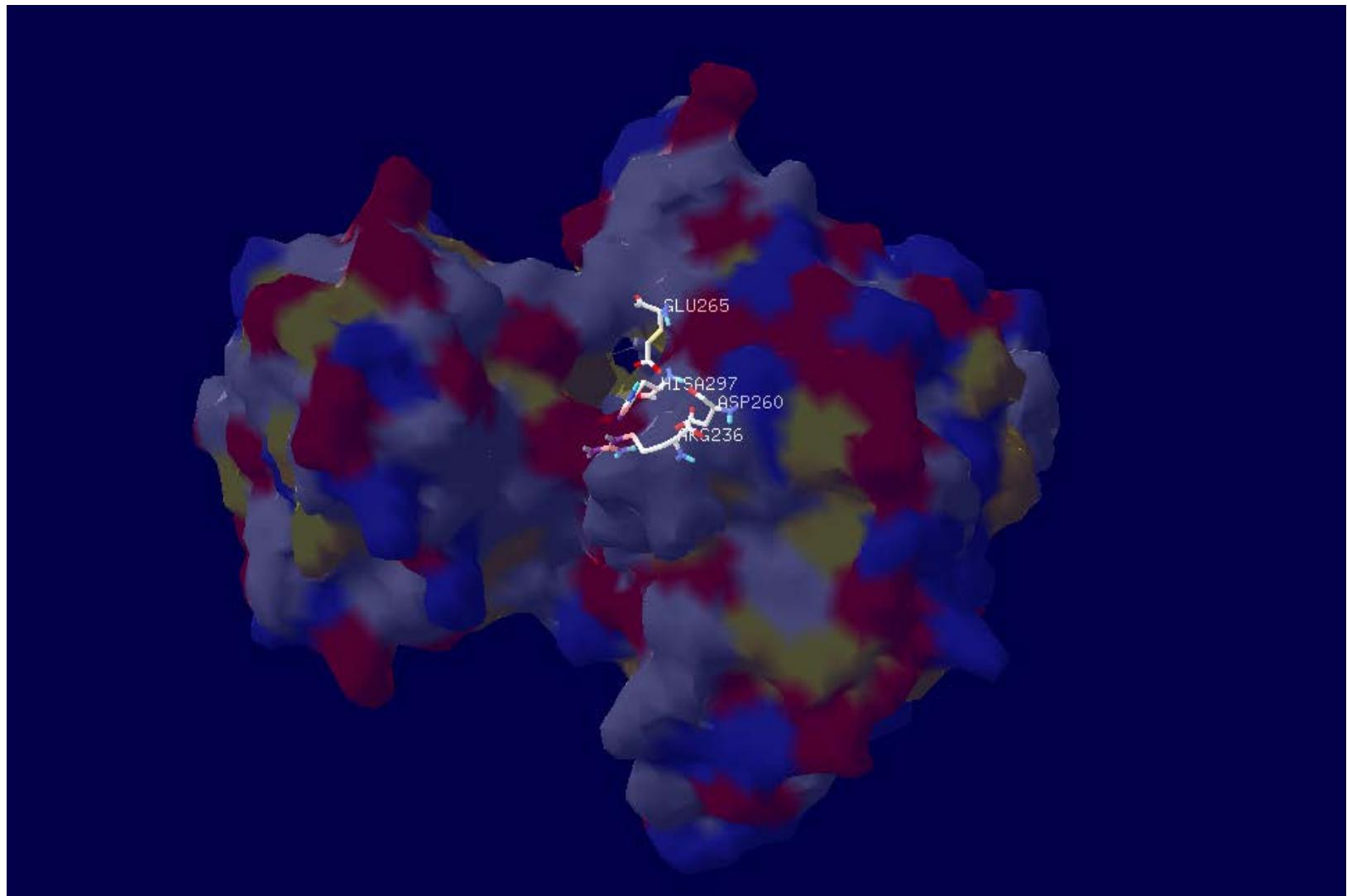


UniProt Sequence Annotation

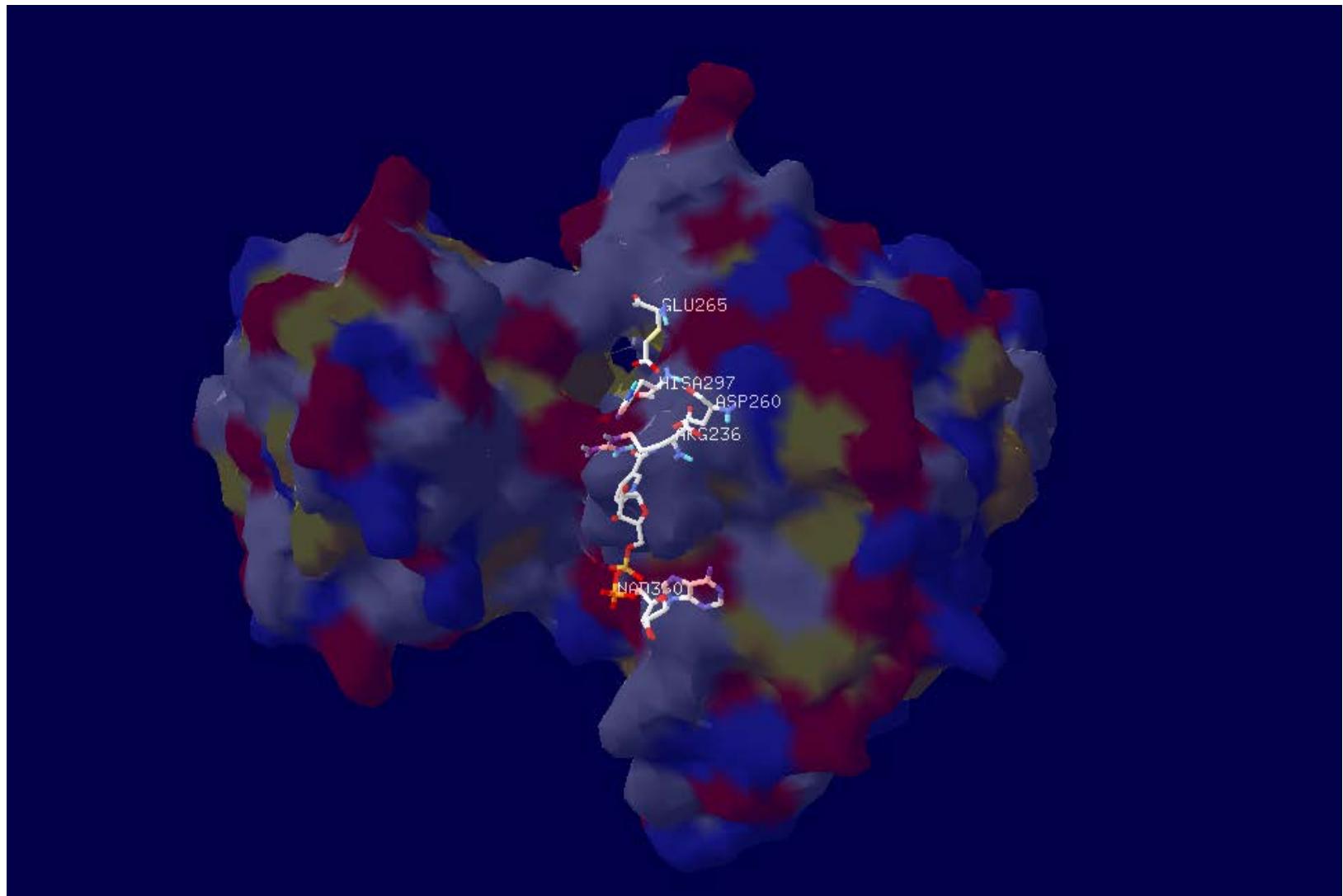
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<input type="checkbox"/>	Binding site	260	1	NAD <small>(By similarity)</small>	

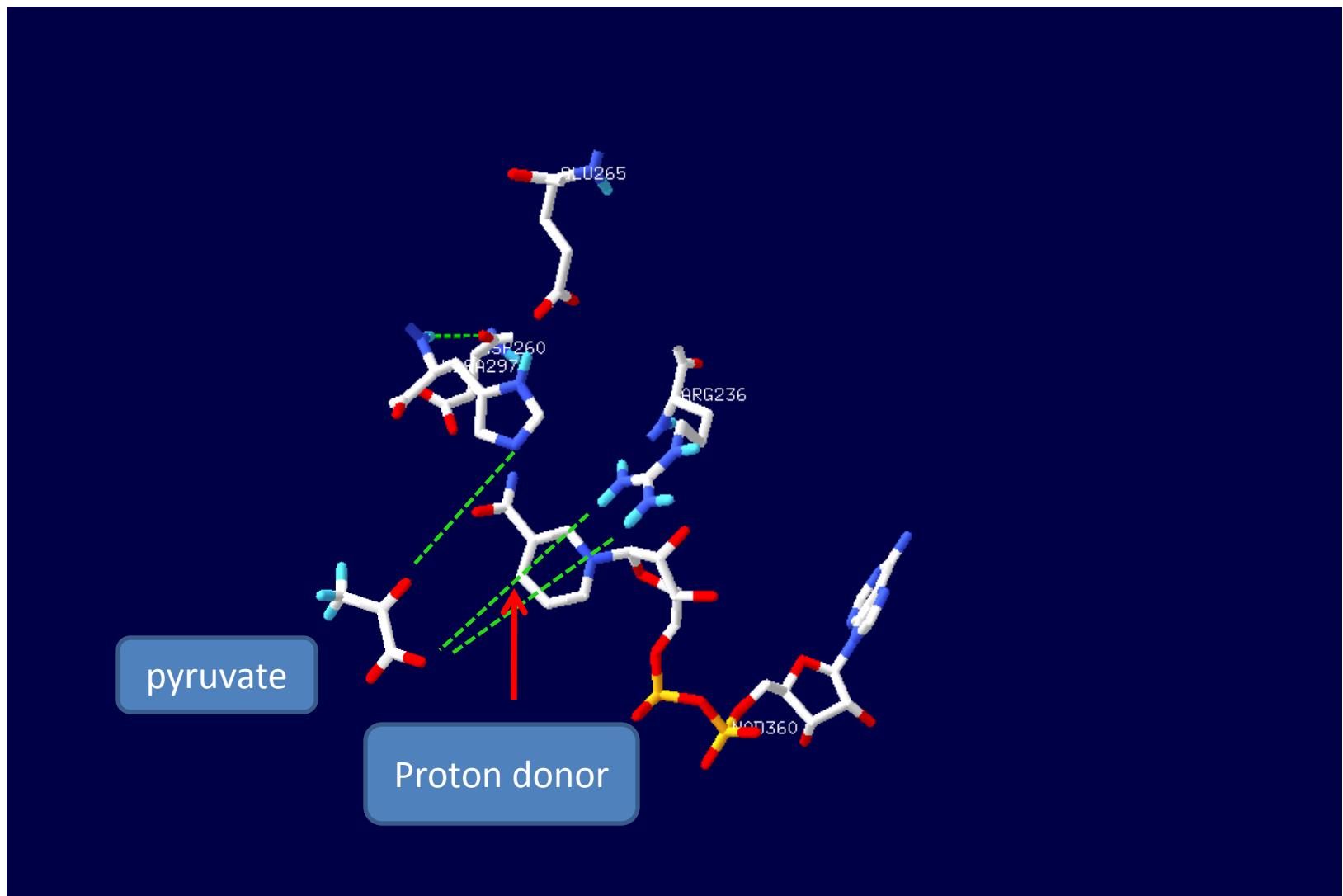
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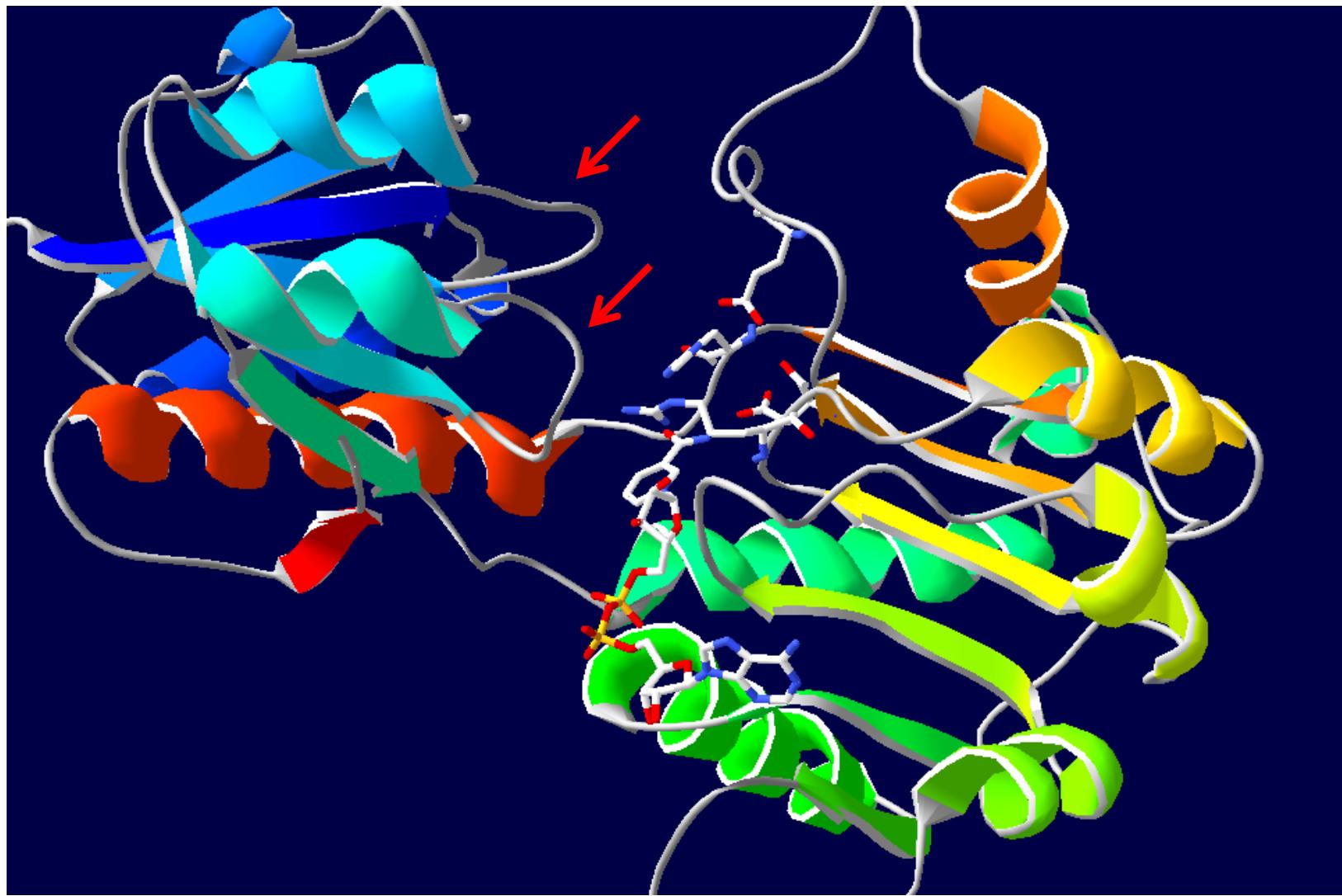
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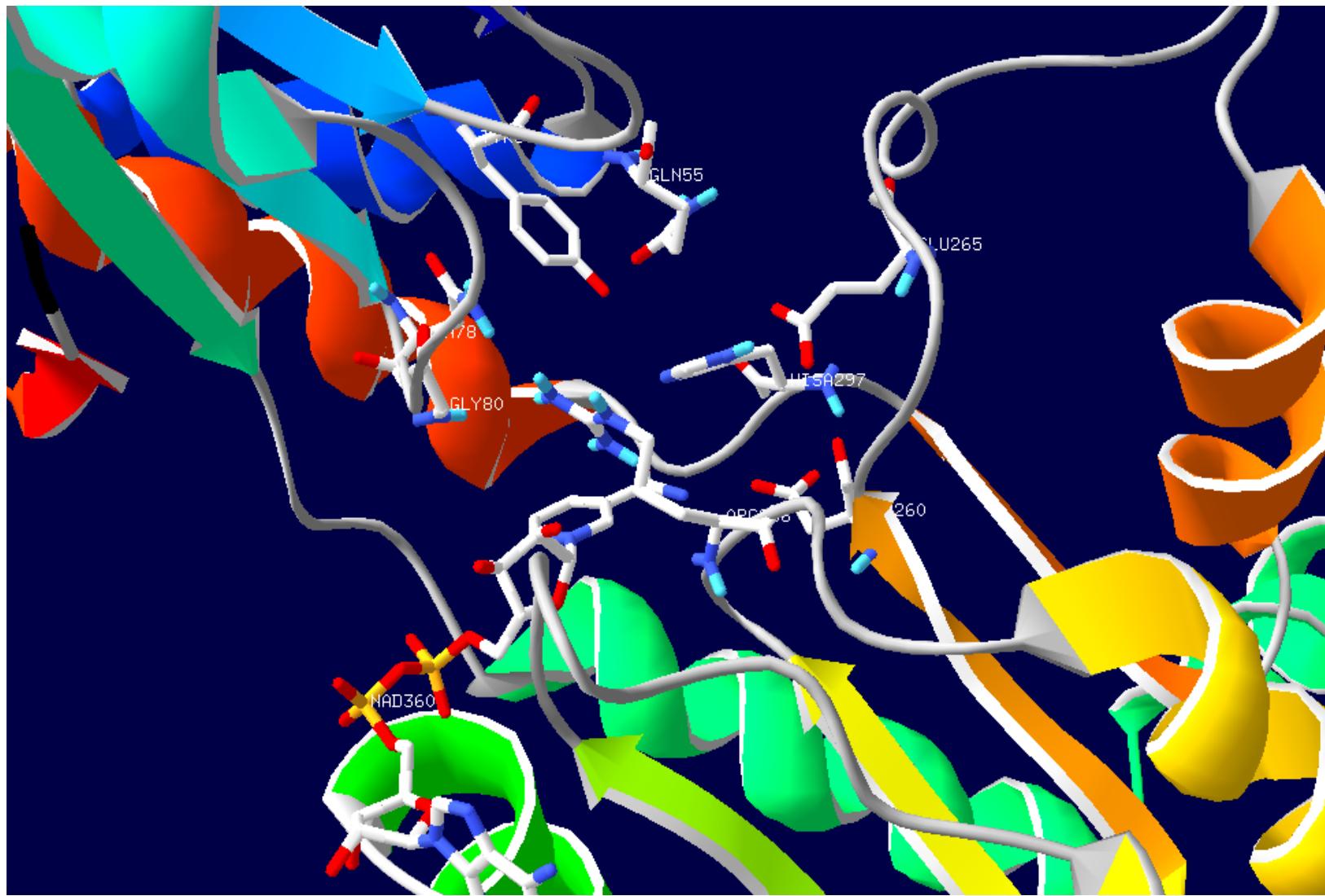
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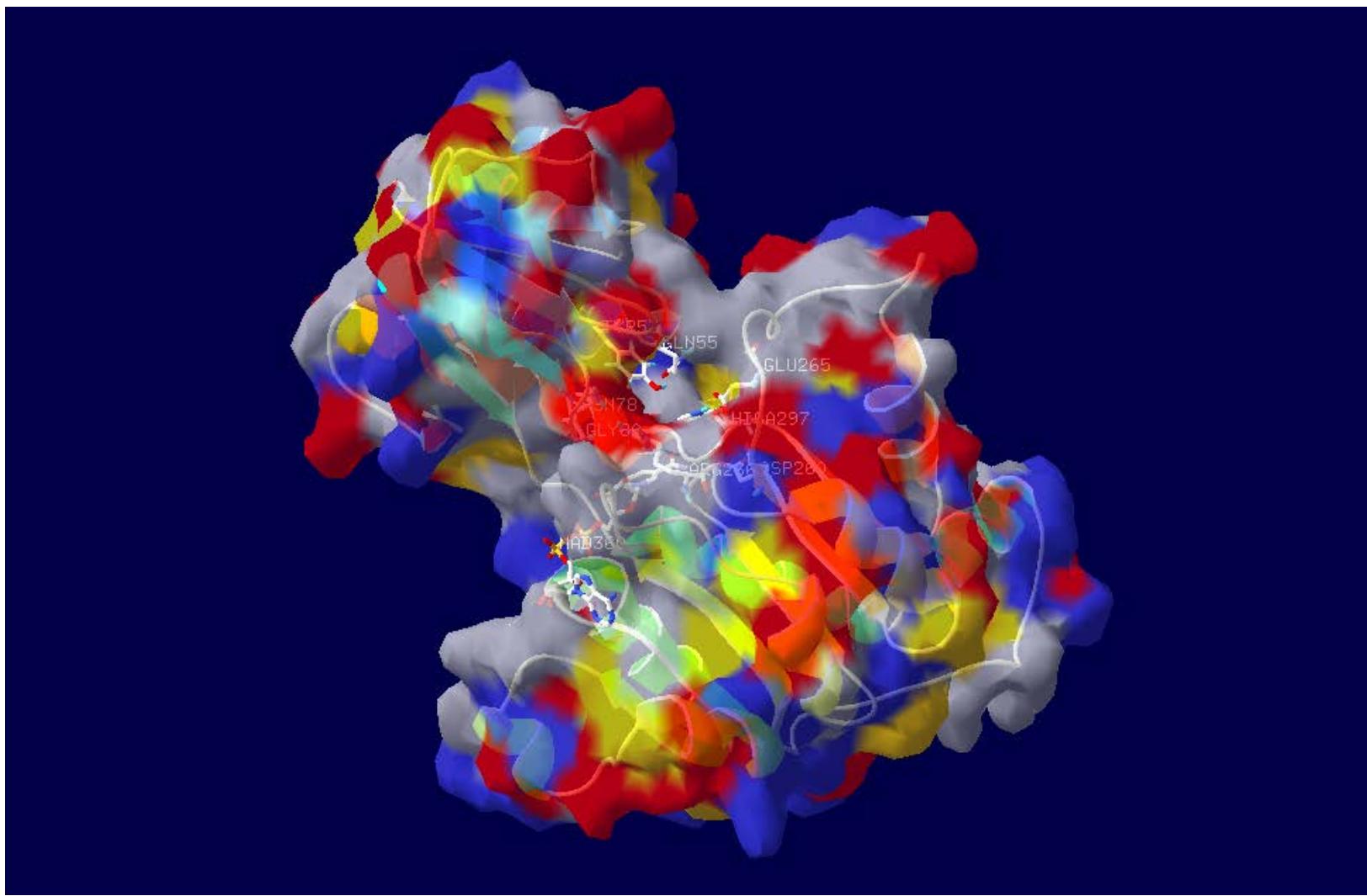
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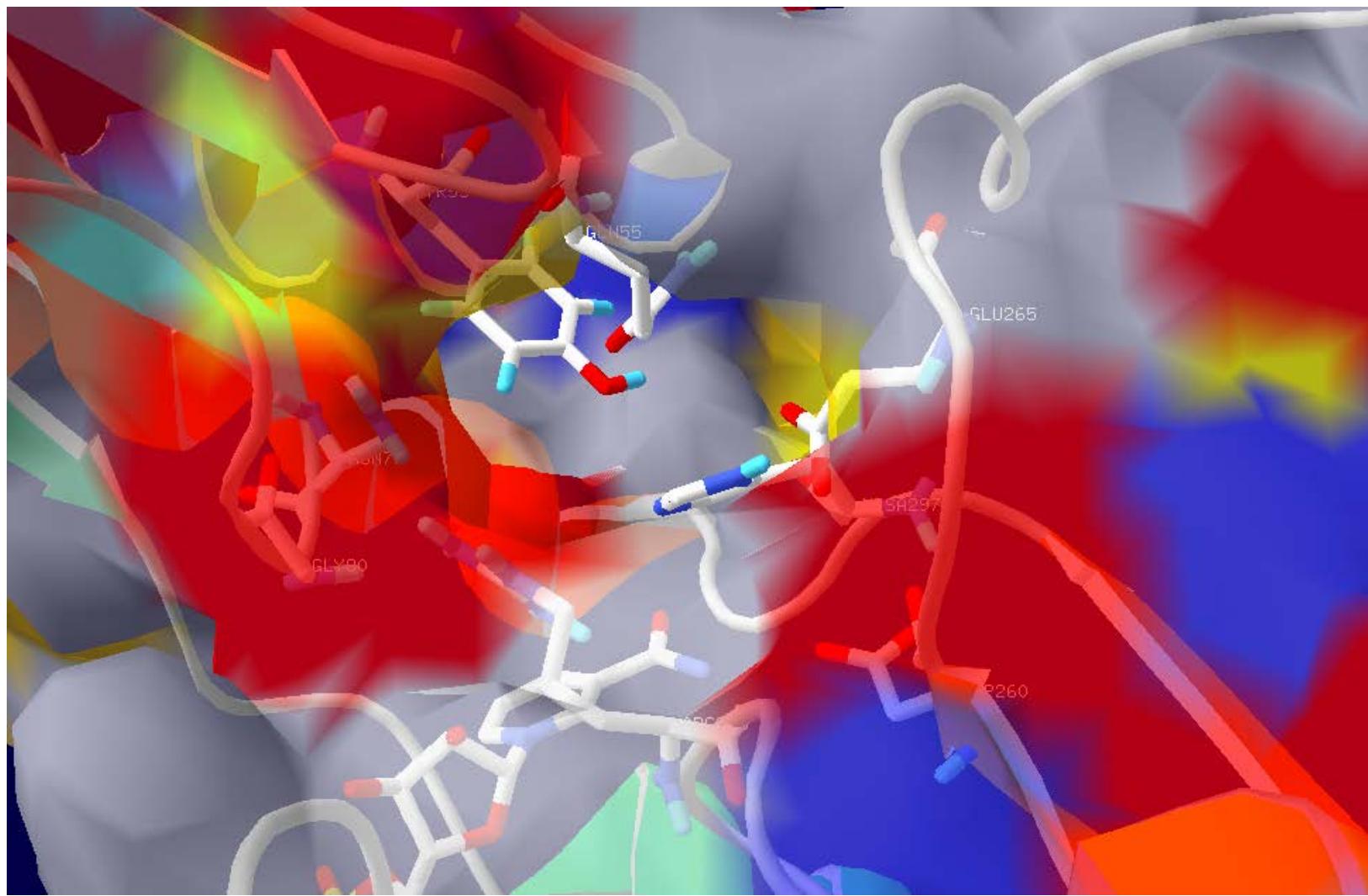
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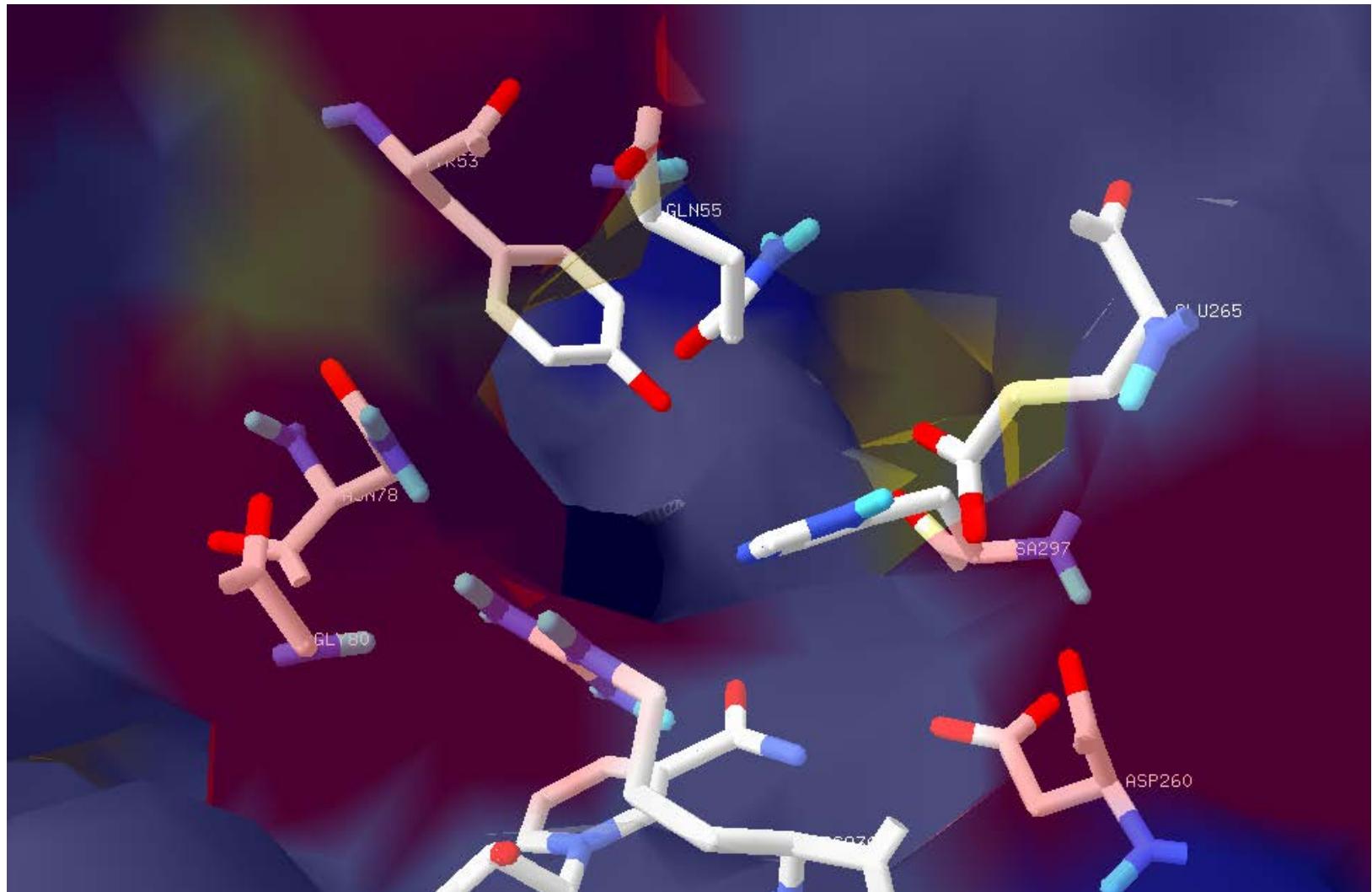
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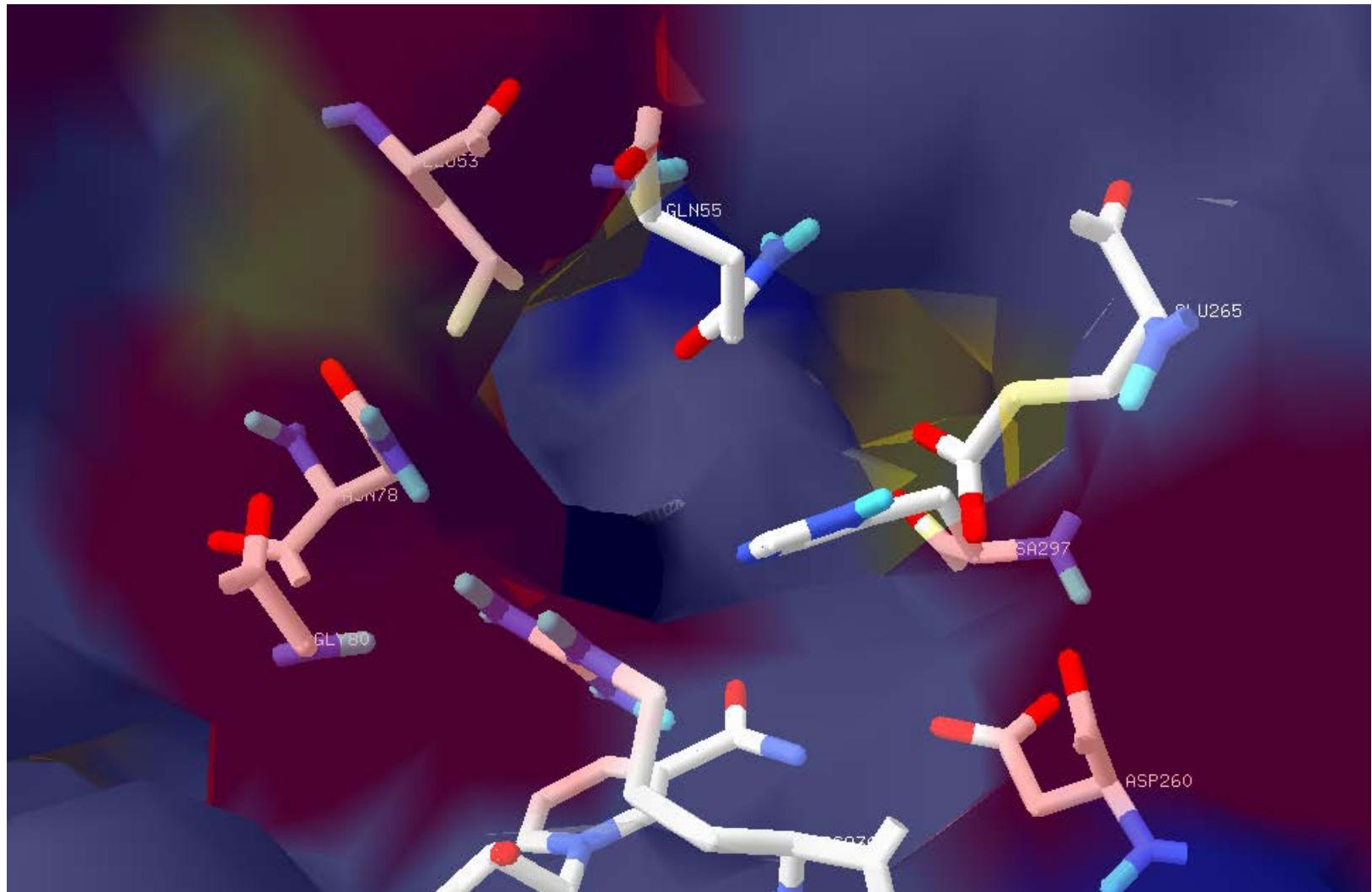
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Experimental findings

Table 1 | Specific activity of D-nLDH wild-type and D-nLDH mutants for α -keto carboxylic acids

α -Keto carboxylic acids (R)	Wild-type	Y52L	F299Y	Y52L/F299Y	e.e. ^b
1a (CH_3)	771.4	669.0	1067	70.5	>99.9% R
2a (CH_2OH)	545.5	796.3	153.7	73.5	>99.9% R
3a (CH_3CH_2)	52.4	854.1	17.6	207.0	>99.9% R
4a ($\text{CH}_3\text{CH}_2\text{CH}_2$)	2.93	1,121	20.0	738.3	>99.9% R
5a ($\text{CH}(\text{CH}_3)_2$)	0.08	10.6	0.08	1.01	>99.9% R
6a ($\text{C}(\text{CH}_3)_3$)	0.06	1.27	0.13	0.06	^c
7a (C_6H_5)	0.05	3.40	0.11	0.11	>99.9% R
8a ($\text{C}_6\text{H}_5\text{CH}_2$)	18.1	1,016	10.4	1,519	>99.9% R
9a ($p\text{-OHC}_6\text{H}_4\text{CH}_2$)	0.04	60.5	0.40	62.8	>99.9% R

^aThe enzymes [D-nLDH wild-type, Y52L mutant, F299Y mutant, and Y52L/F299Y double mutant] used for assay were N-terminal His-tagged fusion and purified to apparent homogeneity.

^bThe e.e. values were determined using D-nLDH Y52L mutant. HPLC analysis of the products was performed with a chiral column by using the corresponding racemic α -hydroxy carboxylic acids as authentic standards.

^cLack of authentic standard.

Zhaojuan Zheng et al., 2013

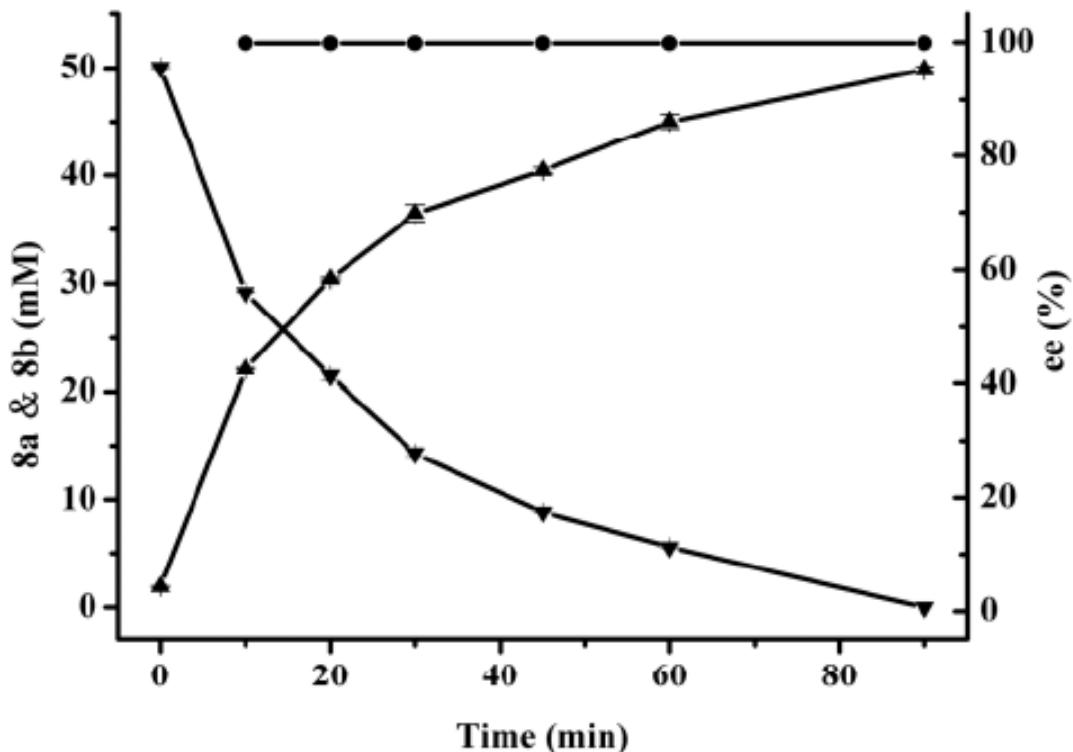
Experimental findings

Table 2 | Kinetic parameters of D-nLDH wild-type and D-nLDH mutants for PPA (8a)

D-nLDH	K_m (mM)	V_{max} (U mg ⁻¹)	k_{cat} (s ⁻¹)	k_{cat}/K_m (M ⁻¹ s ⁻¹)
Wild-type	11.4	17.1	11.3	1.0×10^3
Y52L	0.27	3,049	2,013	7.5×10^6
F299Y	0.32	2.7	1.8	5.7×10^3
Y52L/F299Y	1.4	2,191	1,447	1.0×10^6

Zhaojuan Zheng et al., 2013

Experimental findings



Time course of the production of (*R*)-PLA.
The biocatalyst
was *E. coli* BL21(DE3) harboring pETDuet-*ldhD*^{Y52L}-*fdh*. ▼, PPA, 8a; ▲,
(*R*)-PLA, 8b; ●, ee.

Zhaojuan Zheng et al., 2013

Acknowledgements

Prof.Luo

All my members

Kang Yujian

All the classmates

Thank you

ACCCACGCCAACAAAGAGC