

定点突变D-乳酸脱氢酶生产高光学纯羧酸

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

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Contents

Introduction to D-lactate dehydrogenase

Sequence analysis of D-lactate dehydrogenase

Structure analysis of D-lactate dehydrogenase

Experimental findings

Contents

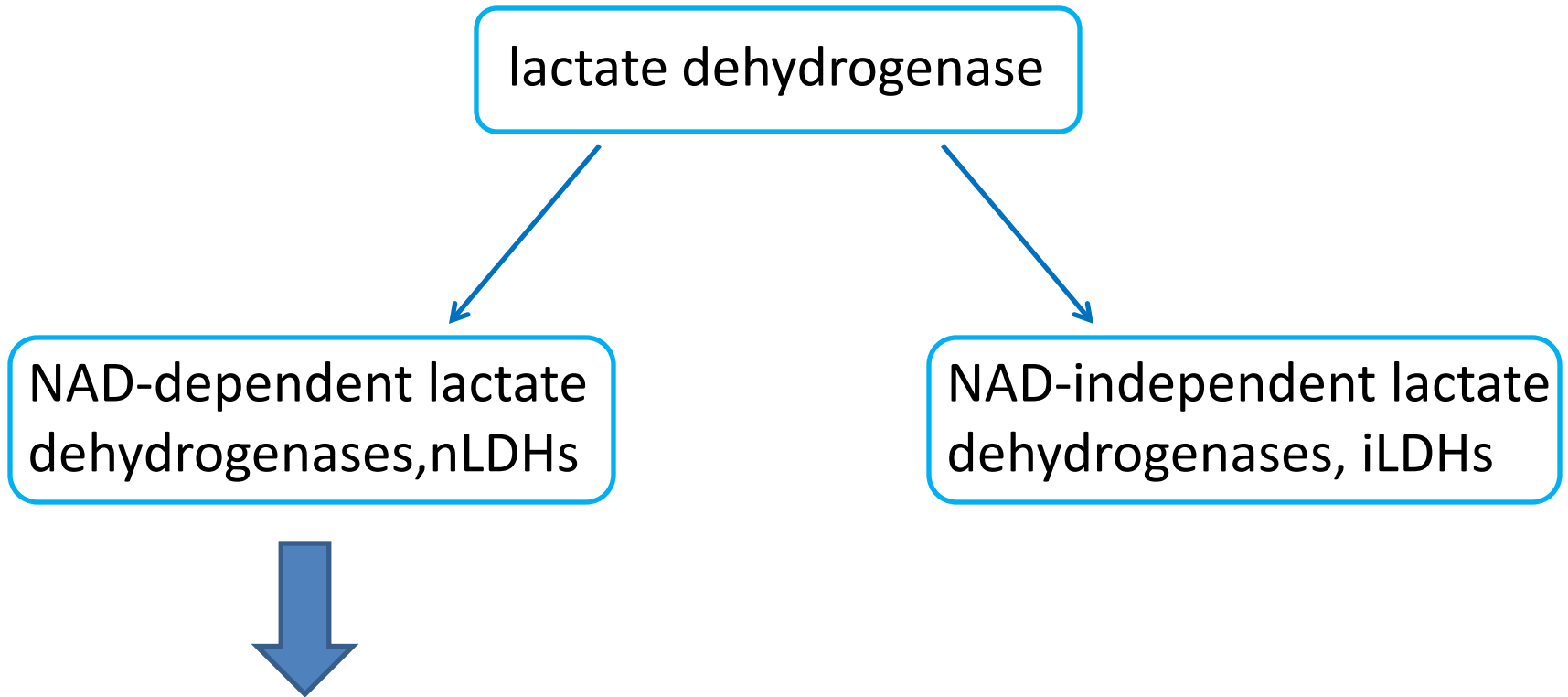
Introduction to D-lactate dehydrogenase

Sequence analysis of D-lactate dehydrogenase

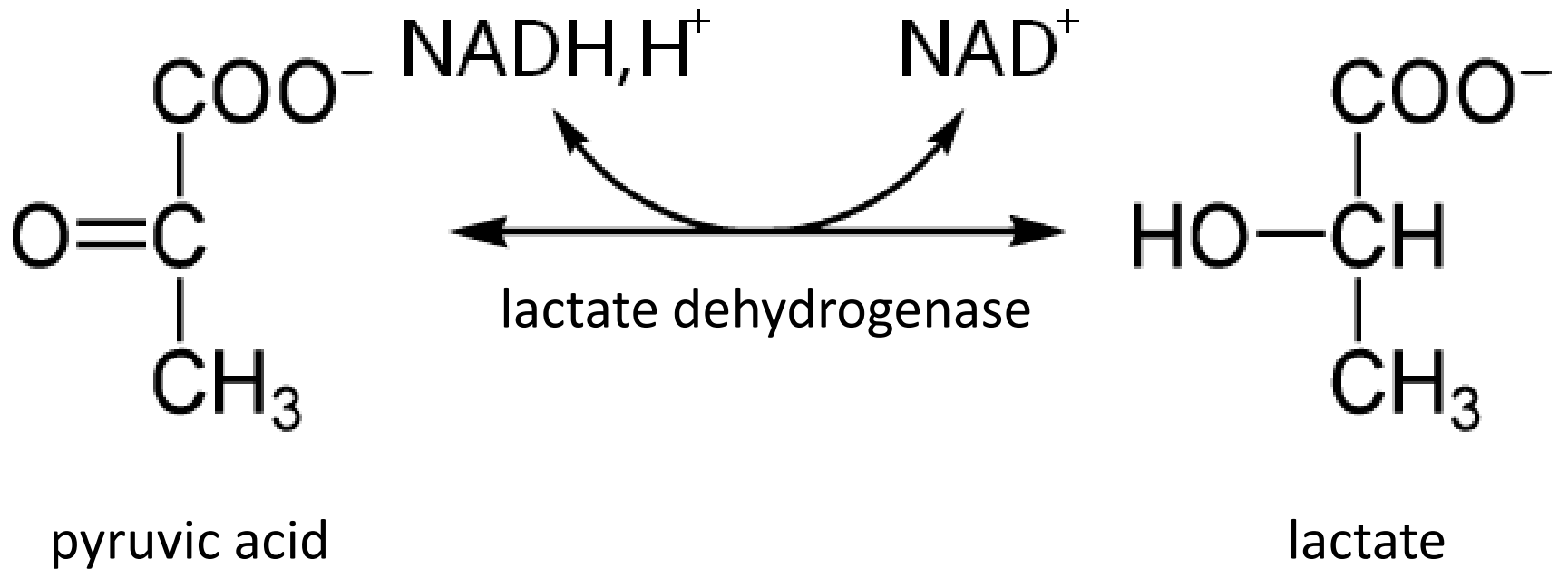
Structure analysis of D-lactate dehydrogenase

Experimental findings

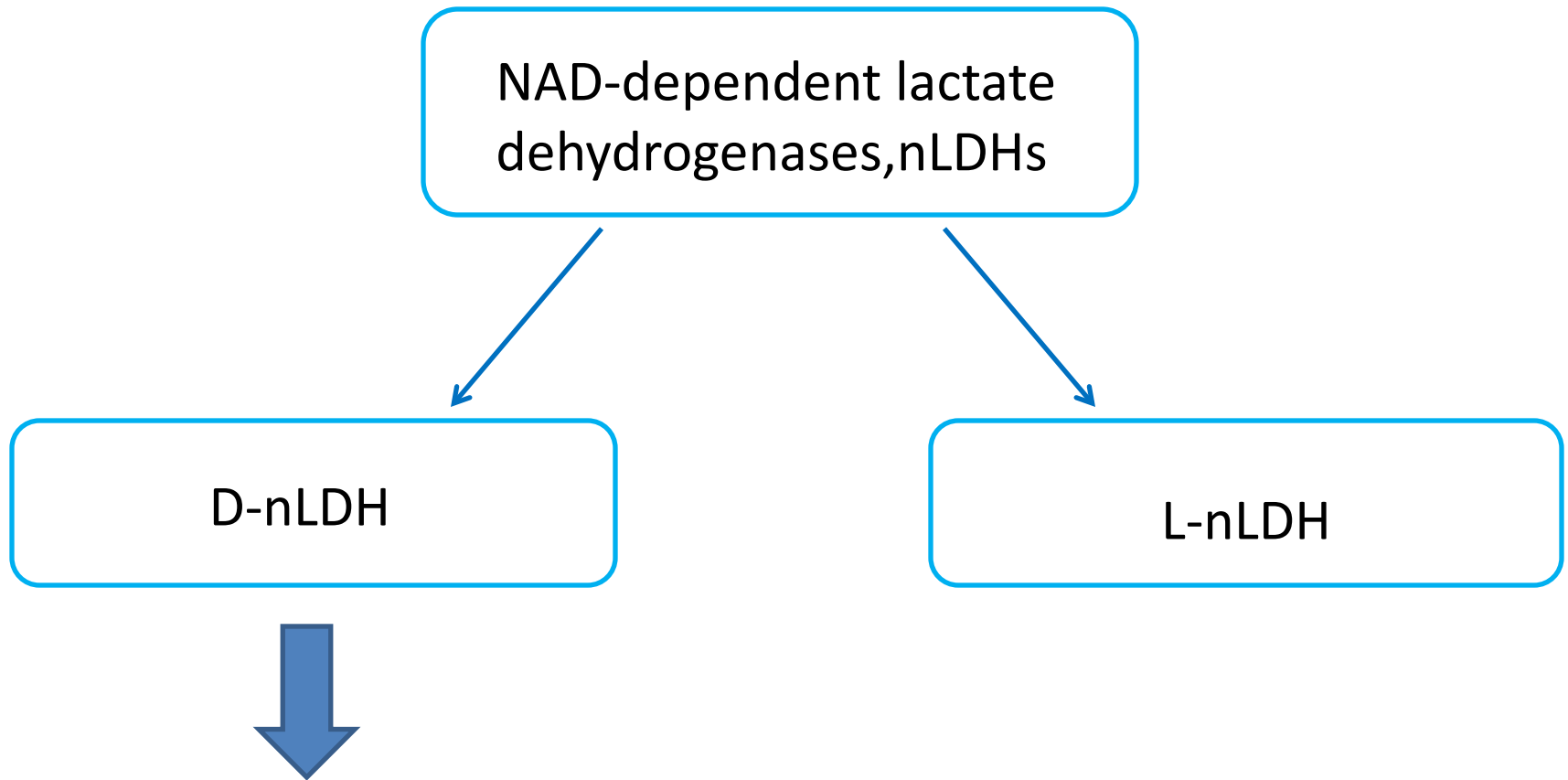
Introduction to D-lactate dehydrogenase



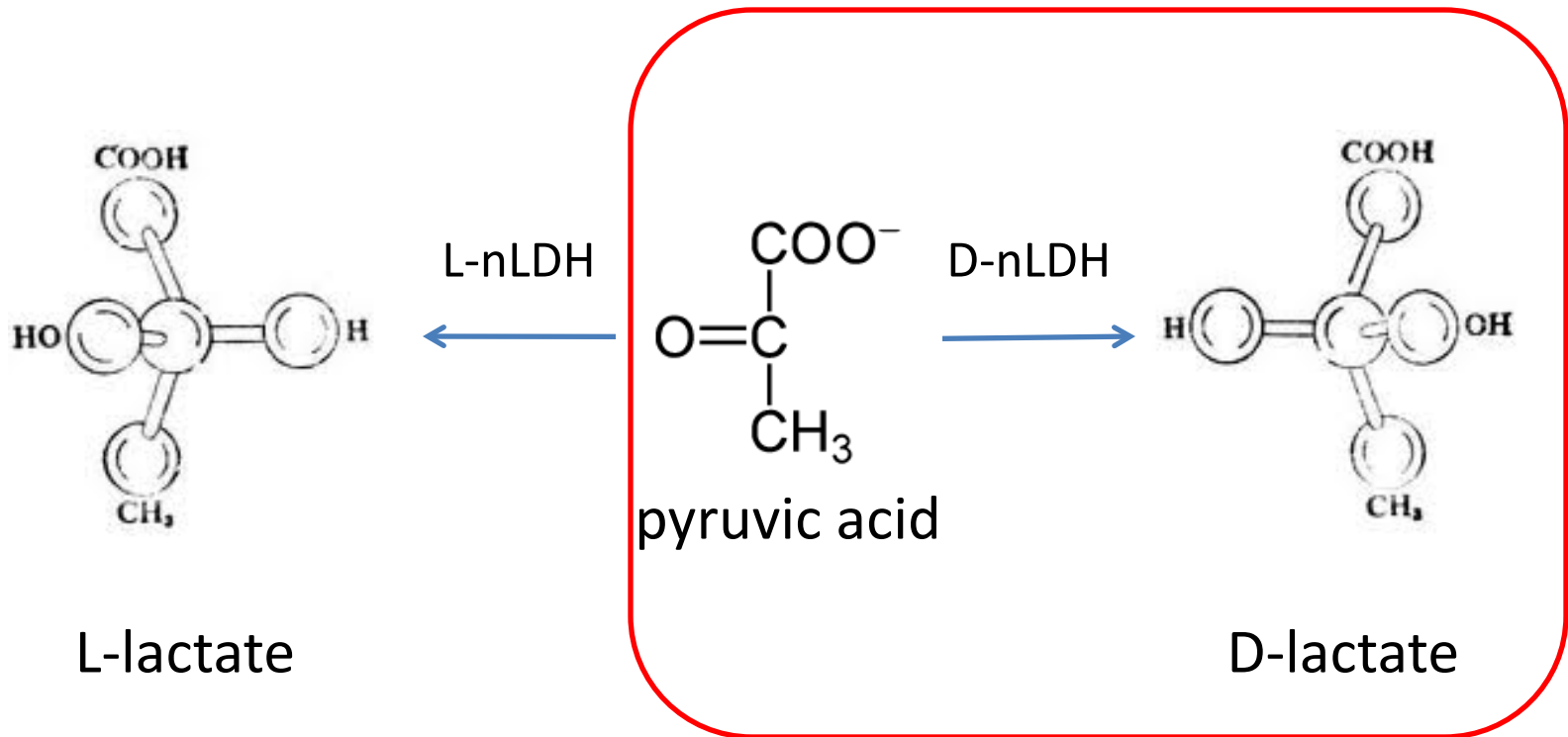
Introduction to D-lactate dehydrogenase



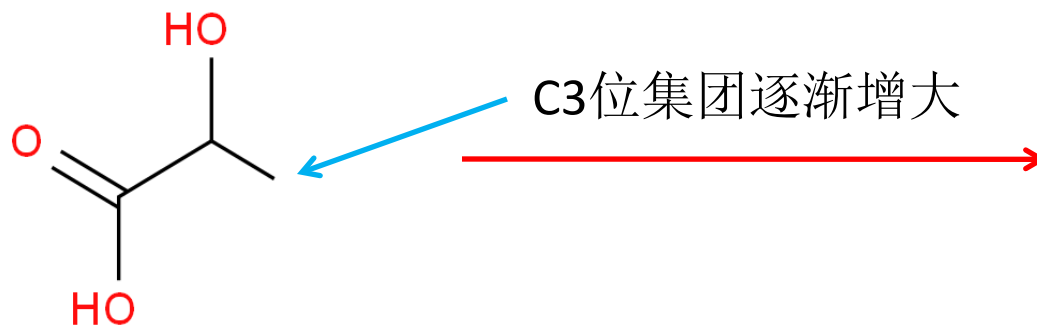
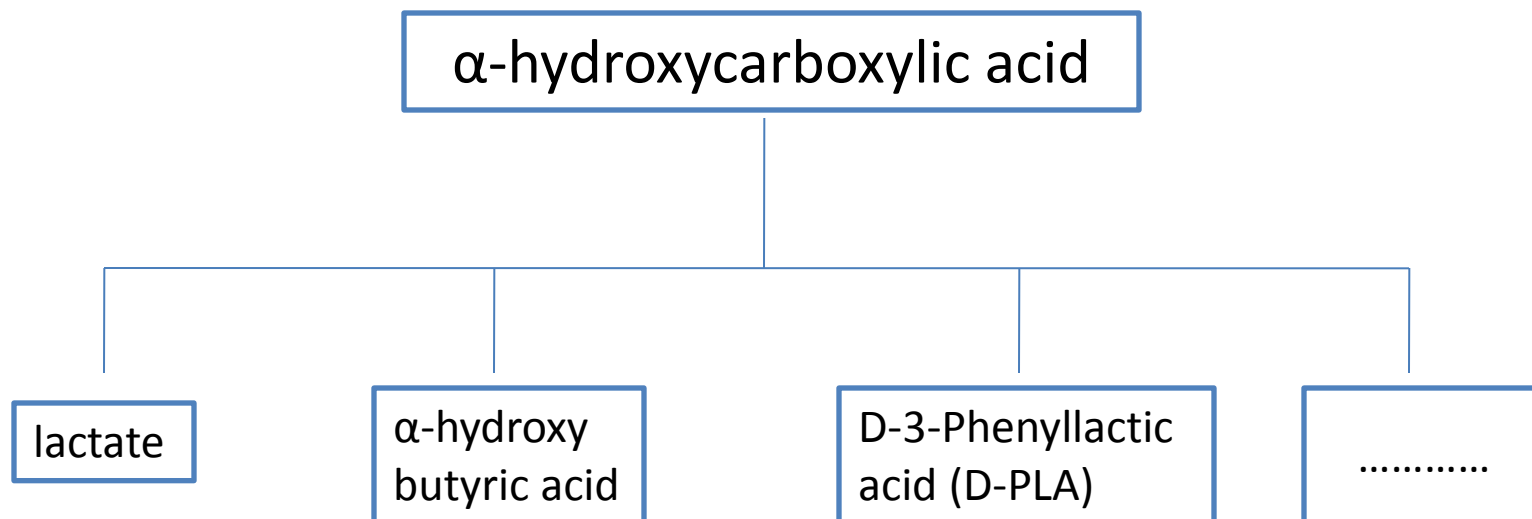
Introduction to D-lactate dehydrogenase



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Contents

Introduction to D-lactate dehydrogenase

Sequence analysis of D-lactate dehydrogenase

Structure analysis of D-lactate dehydrogenase

Experimental findings

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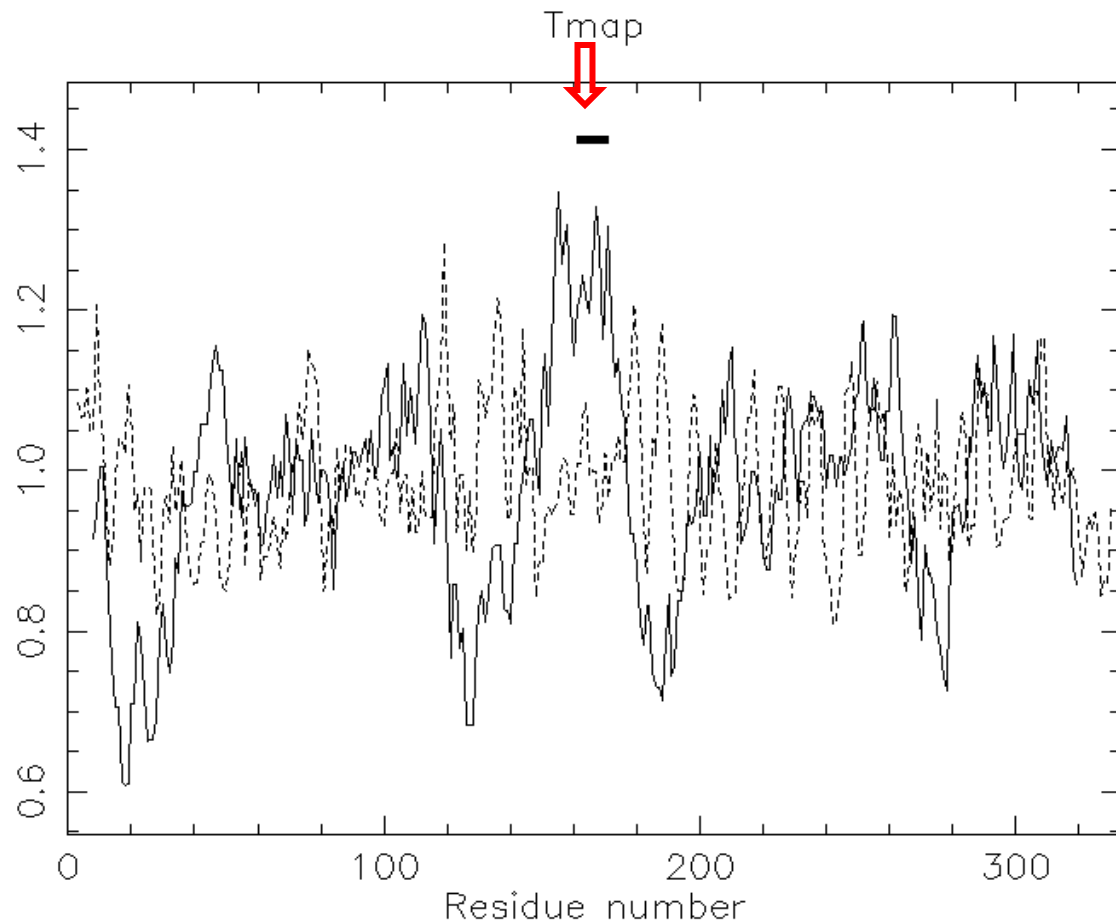


Protein name:	D-lactate dehydrogenase(D-LDH)
Gene name:	ldhA
Sequence length:	333AA
Sequence similarities:	belongs to the D-isomer specific 2-hydroxyacid dehydrogenase family
Ligand:	NAD
molecular function:	D-lactate dehydrogenase activity NAD binding

Sequence analysis of D-lactate dehydrogenase

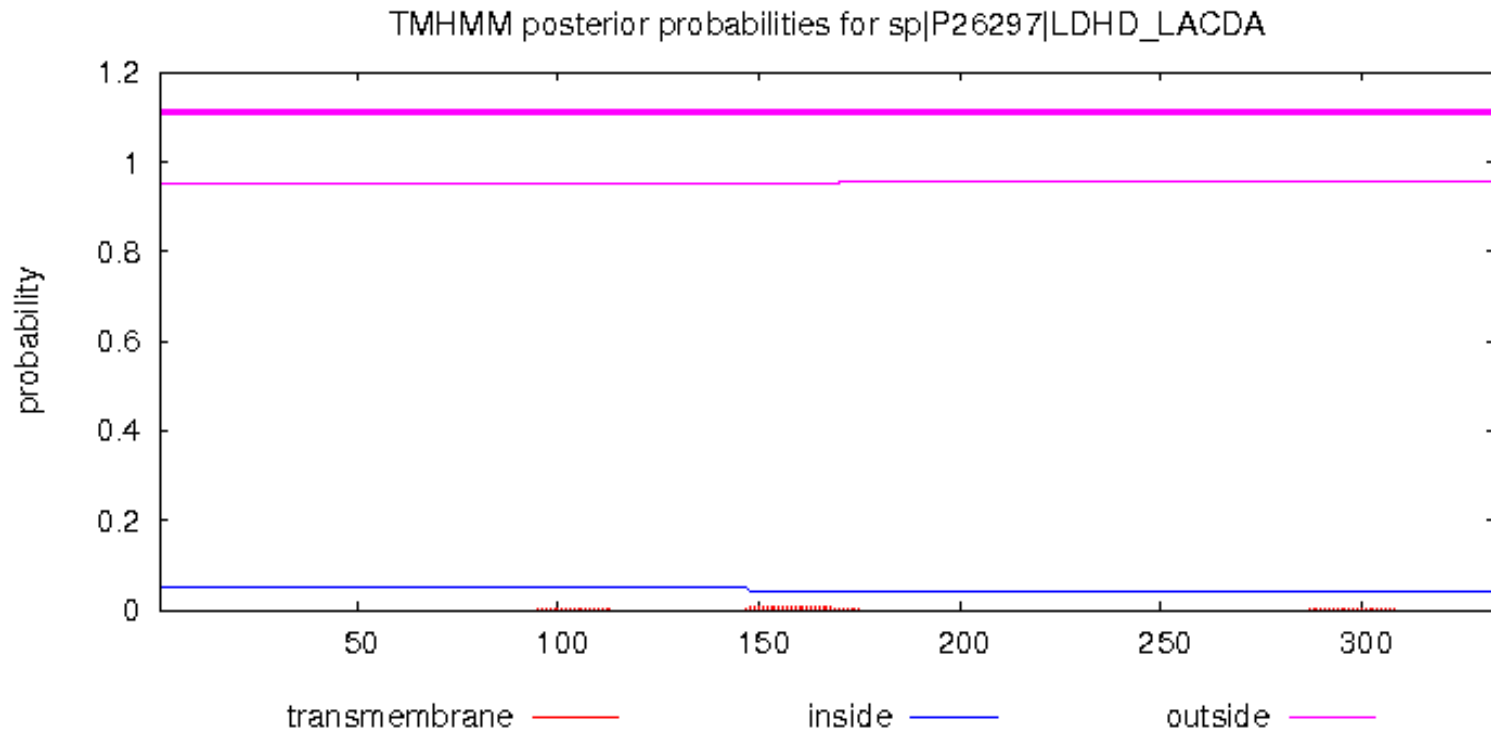
Transmembrane tendency analysis

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
```

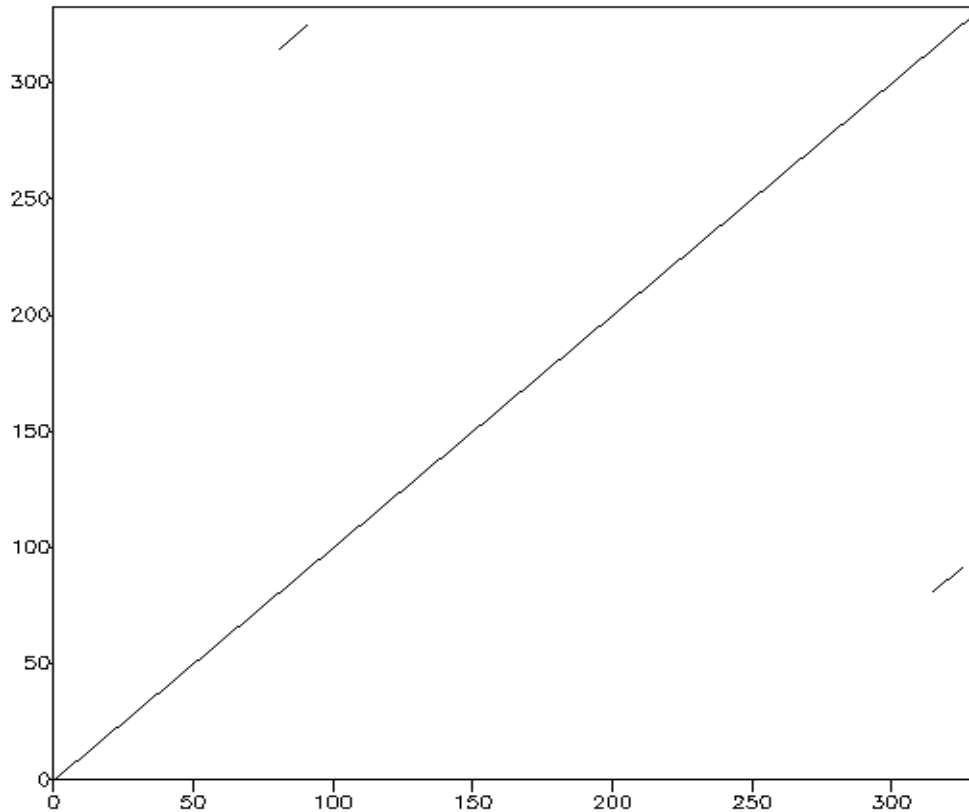


Sequence analysis of D-lactate dehydrogenase

Repeated sequence analysis

WebLab—dotmatcher(v6.0.1)

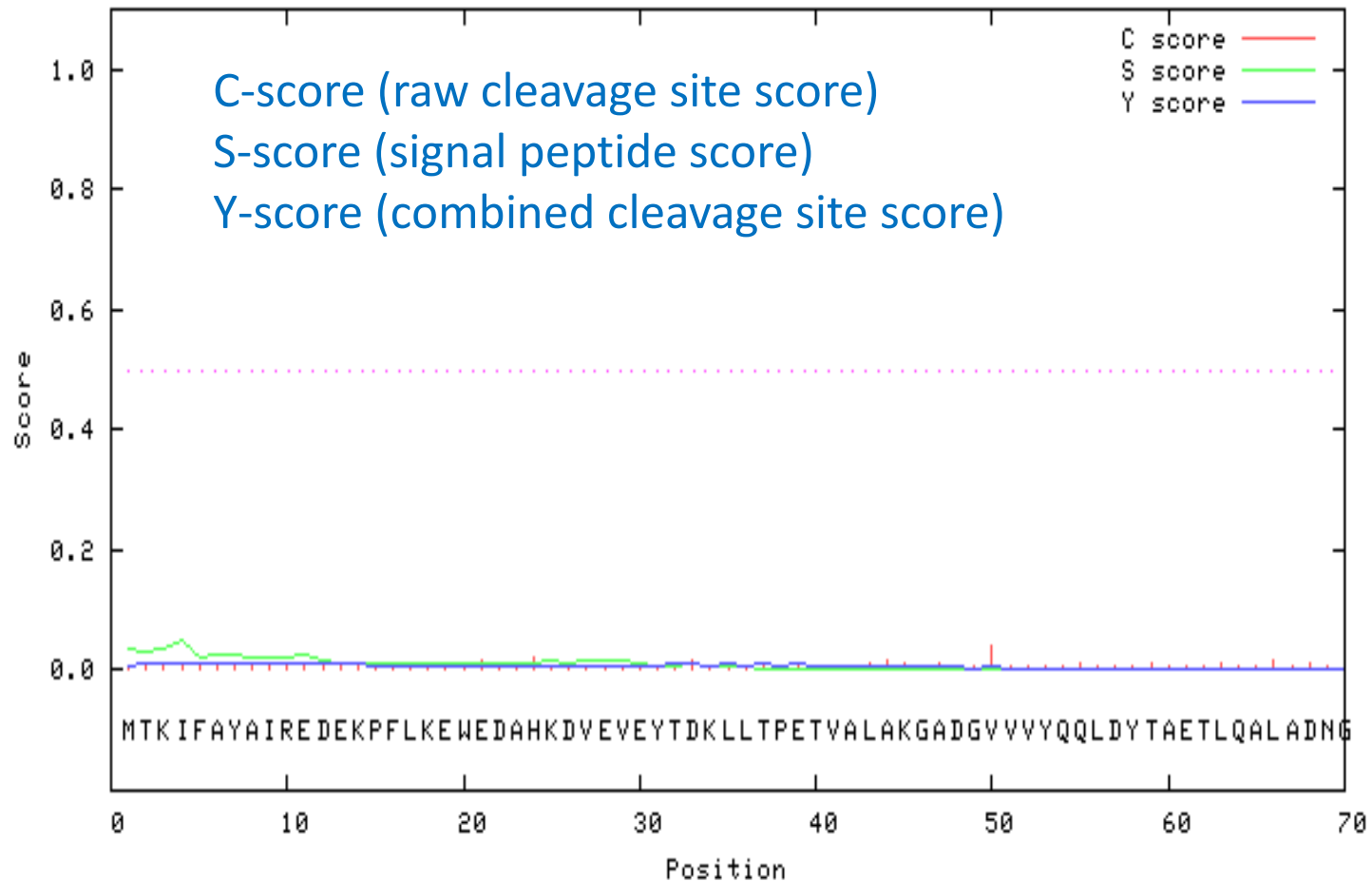
Dotmatcher: raw::751847 vs raw::751848
(windowsize = 10, threshold = 23.00 15/06/14)



Sequence analysis of D-lactate dehydrogenase

Signal peptide prediction

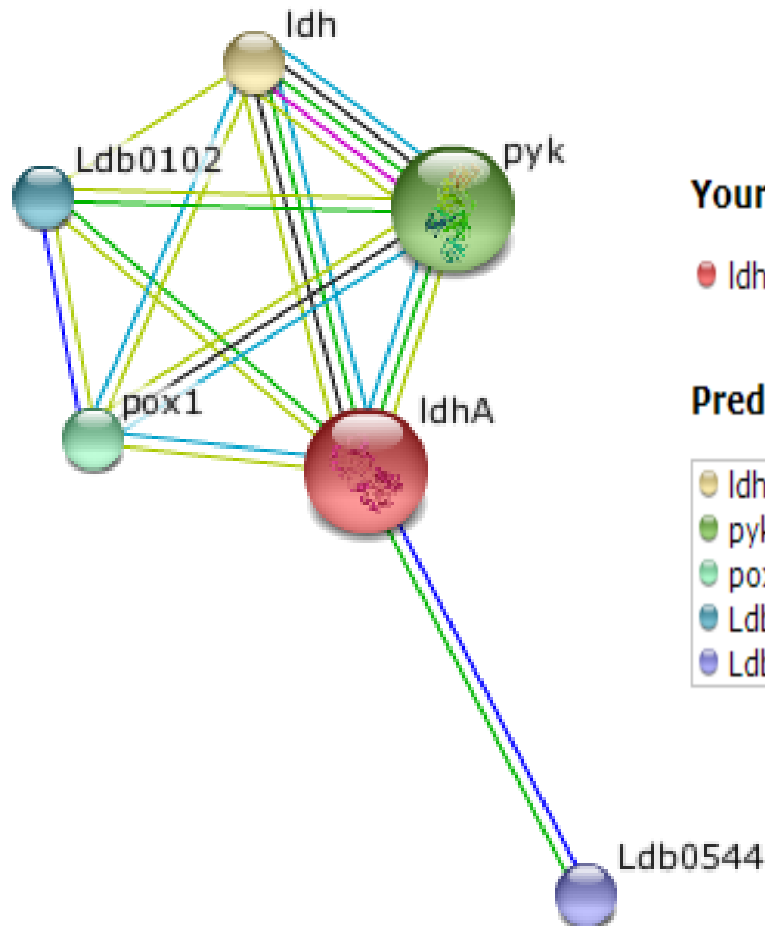
SignalP



Sequence analysis of D-lactate dehydrogenase

Interaction proteins

STRING



Your Input:

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

Predicted Functional Partners:

	Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
● Idh L-lactate dehydrogenase (307 aa)	●			●	●	●	●	●	0.936
● pyk pyruvate kinase (589 aa)	●				●	●	●	●	0.927
● pox1 pyruvate oxidase (616 aa)	●				●	●	●	●	0.900
● Ldb0102 putative oxidoreductase (463 aa)	●					●	●	●	0.551
● Ldb0544 hypothetical protein (357 aa)	●	●							0.405



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--SGFPKRVIGTGT-SLDSASLR	152
LDHD_LACDA	43	LAKGADGVVVY--QQLDYTAETLQALADNGITKMSLRNVGVDNIDMAKAK	90
Q1G7P4_LACDA	153	VELAKRL-QVPIESVNAWVLGHEGDSSFENFSSAVVNGKPLLDYPGMTEA	201
LDHD_LACDA	91	-ELGFQITNVPVYSPNA--IAEH-----AAIQAARIL----RQDK	123
Q1G7P4_LACDA	202	ALDE-IEAH-----VREKGEIIVKKGATYYGVAMML-----AKIV	236
LDHD_LACDA	124	AMDEKVARHDLRWAPTIGREVRDQVVGVIQTGHIGQVFMQIMEFGAKVI	173
Q1G7P4_LACDA	237	T-AILENNDLALPLSAPLHGEYGIKDEIYLGTLAIINGQG--IS-HVLEL	282
LDHD_LACDA	174	AYDIFRNPELE-----KKGYYVDSLDDLYKQADVVISLHVDPV	210
Q1G7P4_LACDA	283	P-----LNDSELAKMRASAATIKAT	302
LDHD_LACDA	211	PANVHMINDESIKMKQDVVIVNVS	235

Contents

Introduction to D-lactate dehydrogenase

Sequence analysis of D-lactate dehydrogenase

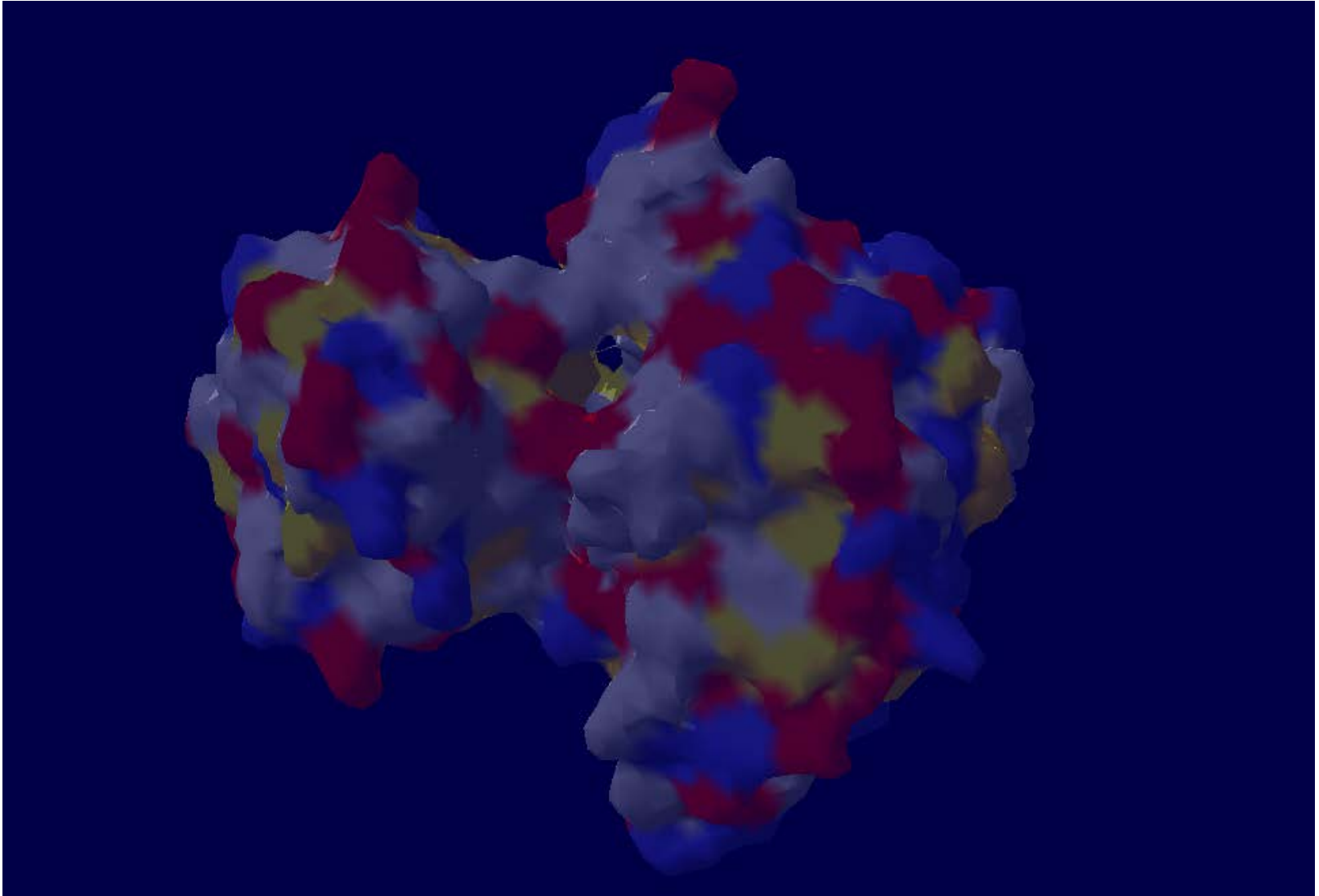
Structure analysis of D-lactate dehydrogenase

Experimental findings

Structure Analysis of D-lactate dehydrogenase







Structure Analysis of D-lactate dehydrogenase

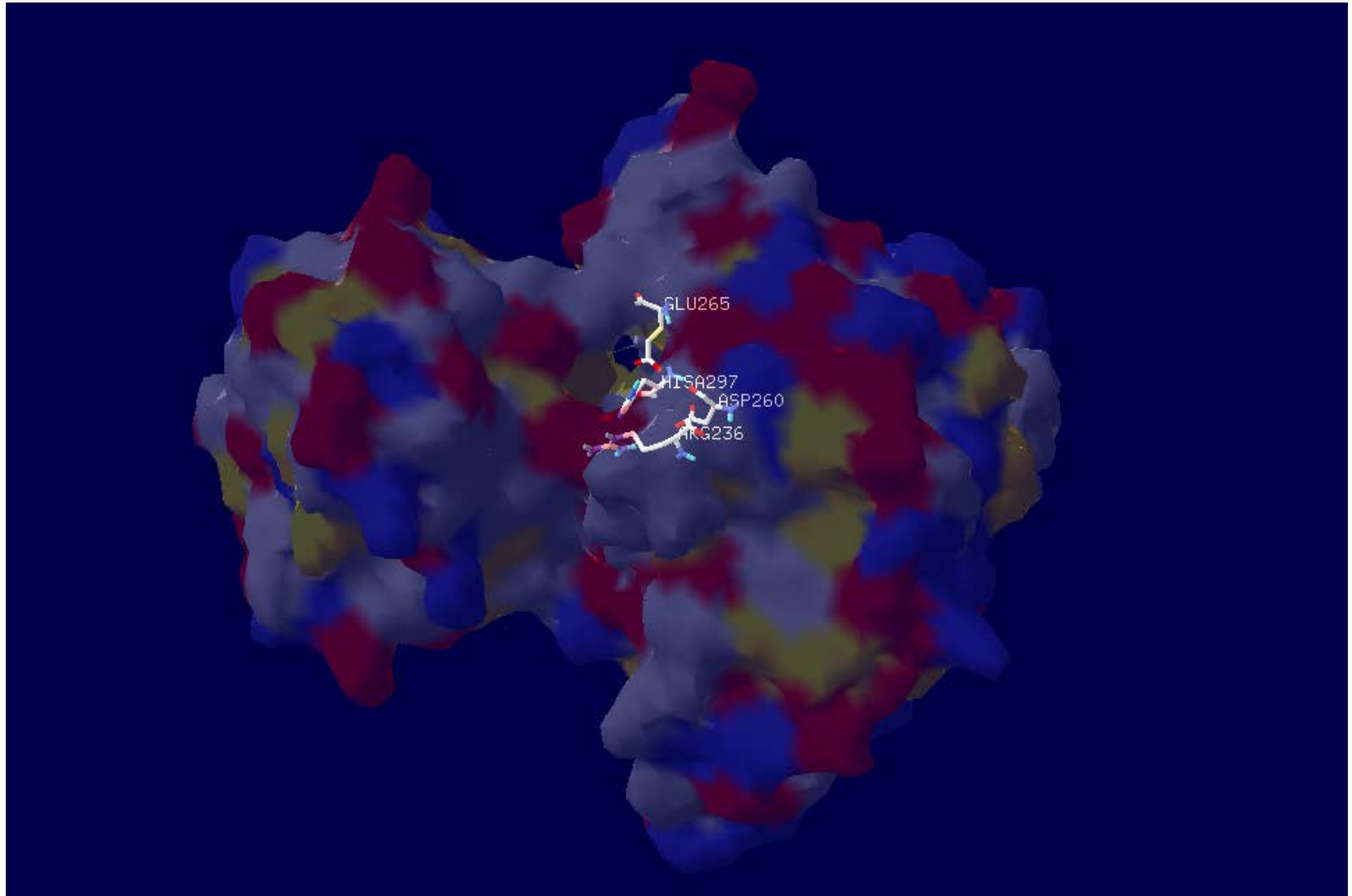


UniProt Sequence Annotation

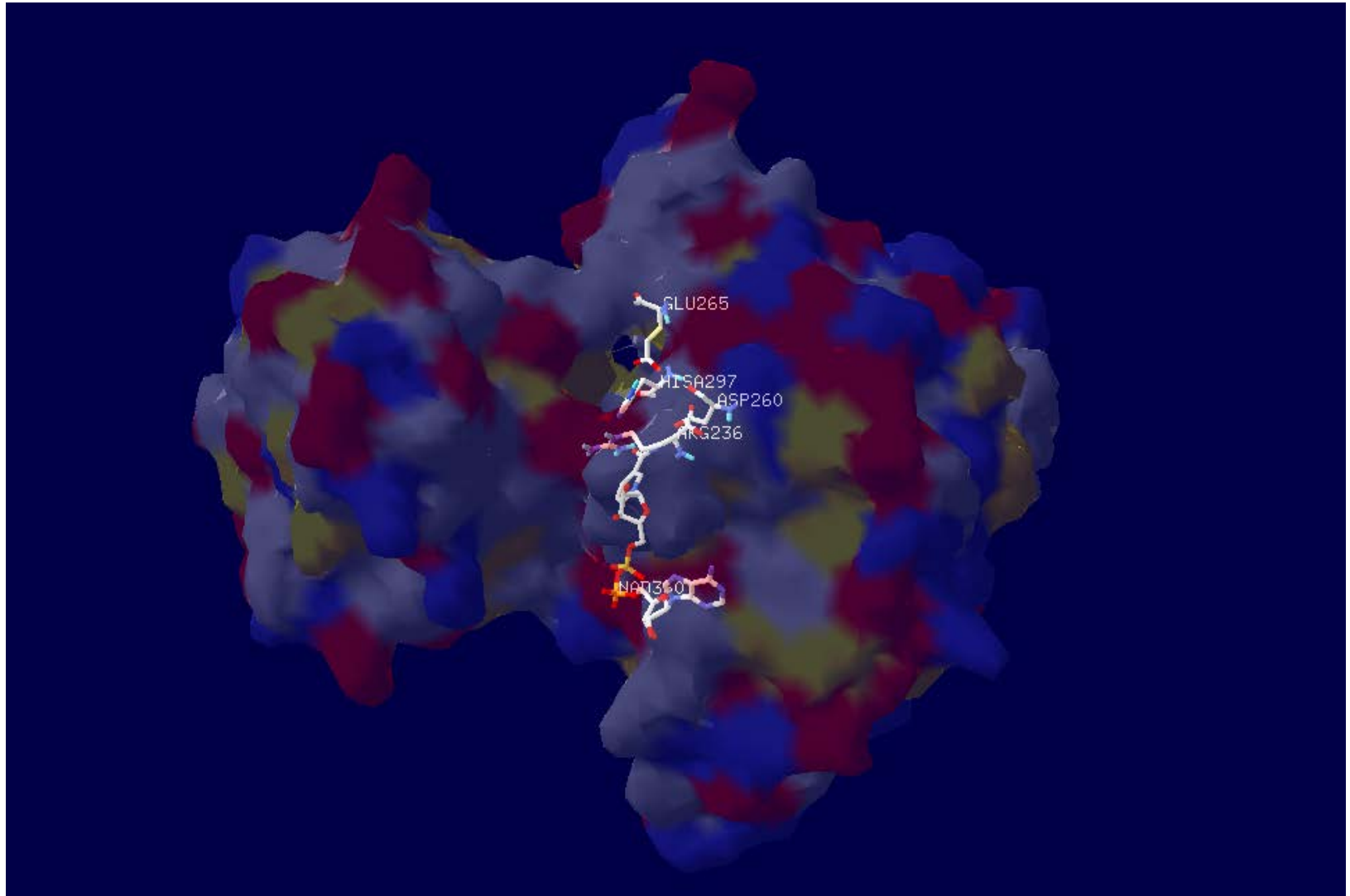
Sites

<input type="checkbox"/>	Active site	236	1		
<input type="checkbox"/>	Active site	265	1		
<input type="checkbox"/>	Active site	297	1	Proton donor	
<input type="checkbox"/>	Binding site	260	1	NAD By similarity	

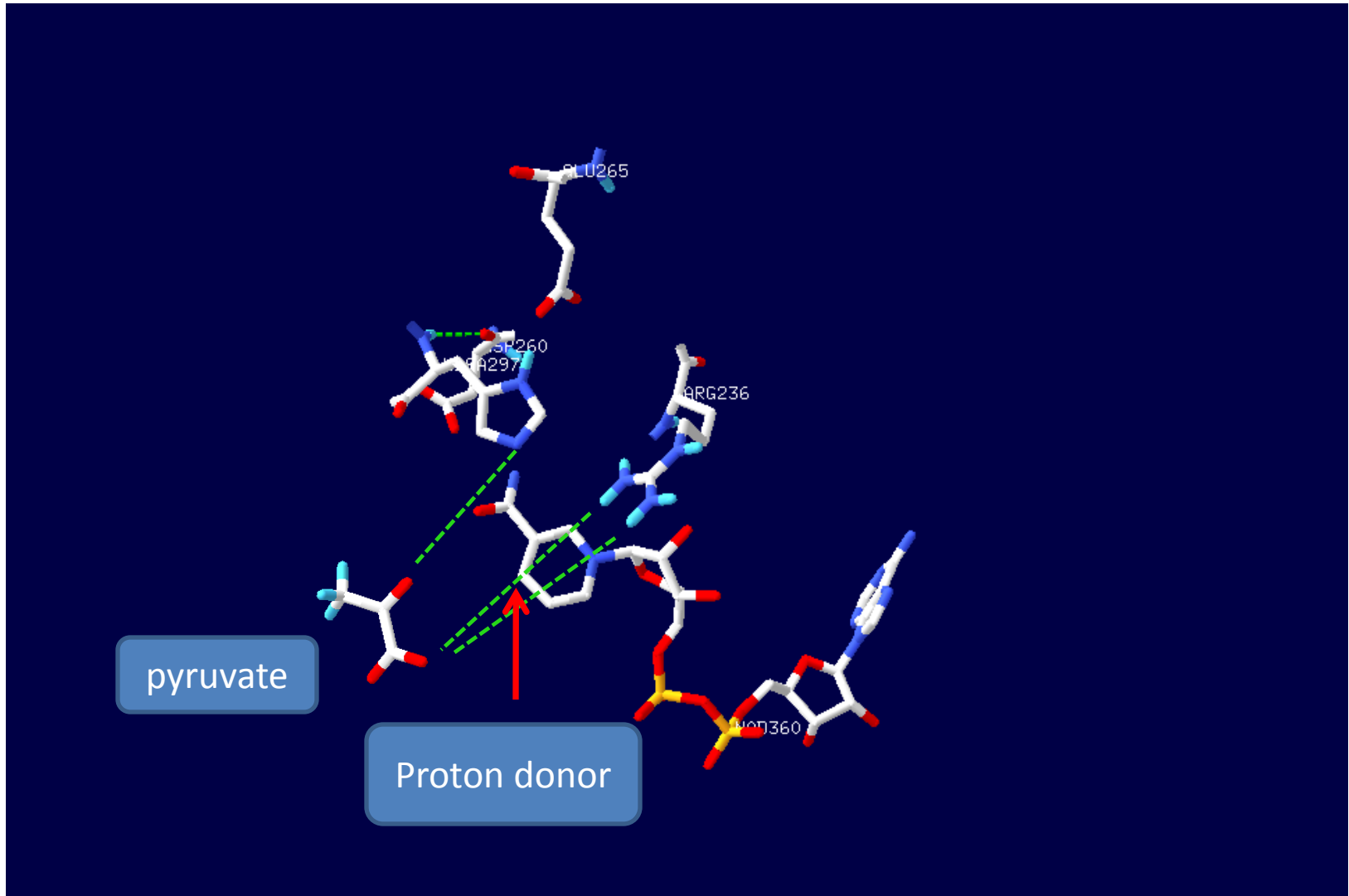
Structure Analysis of D-lactate dehydrogenase



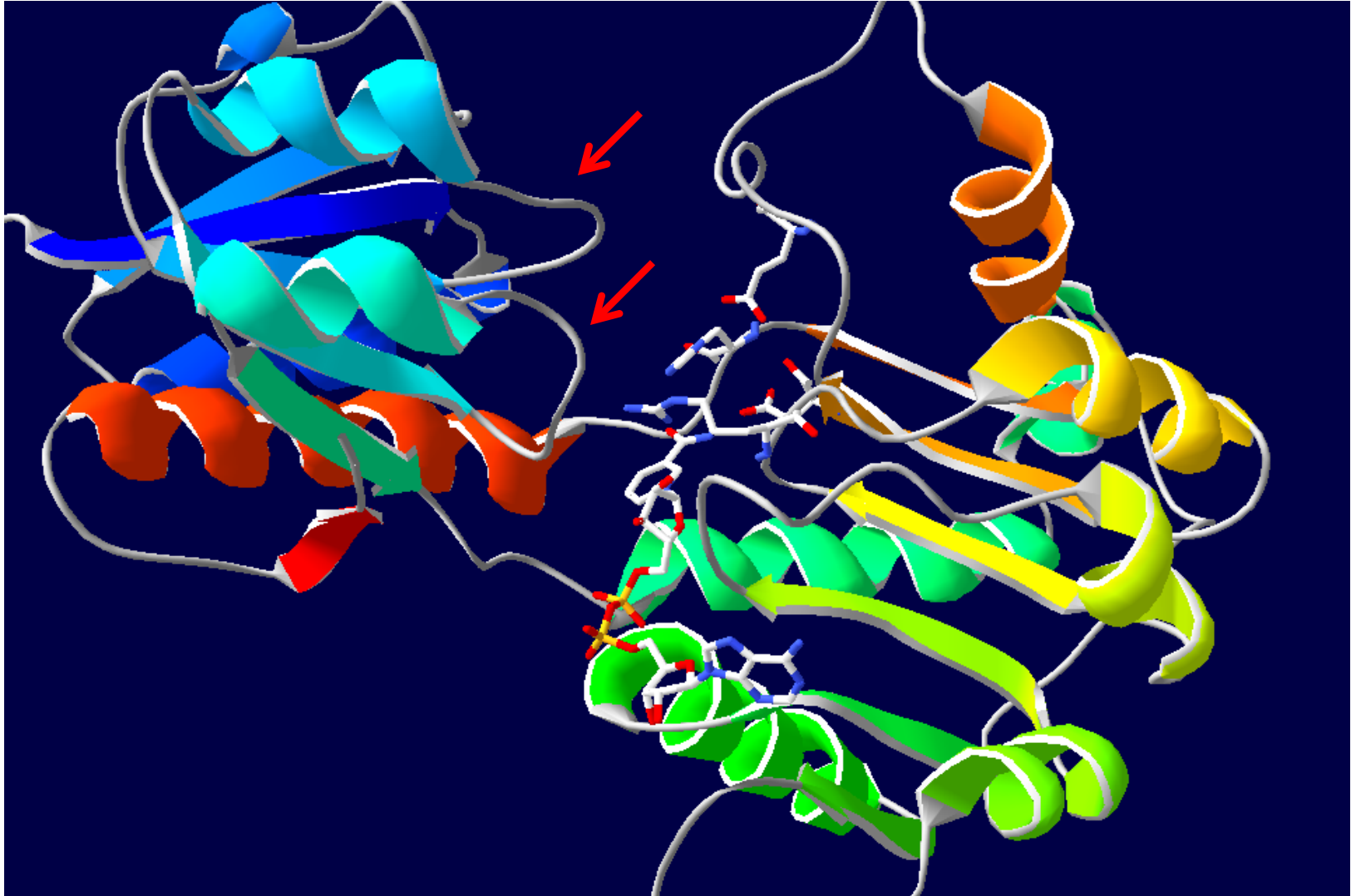
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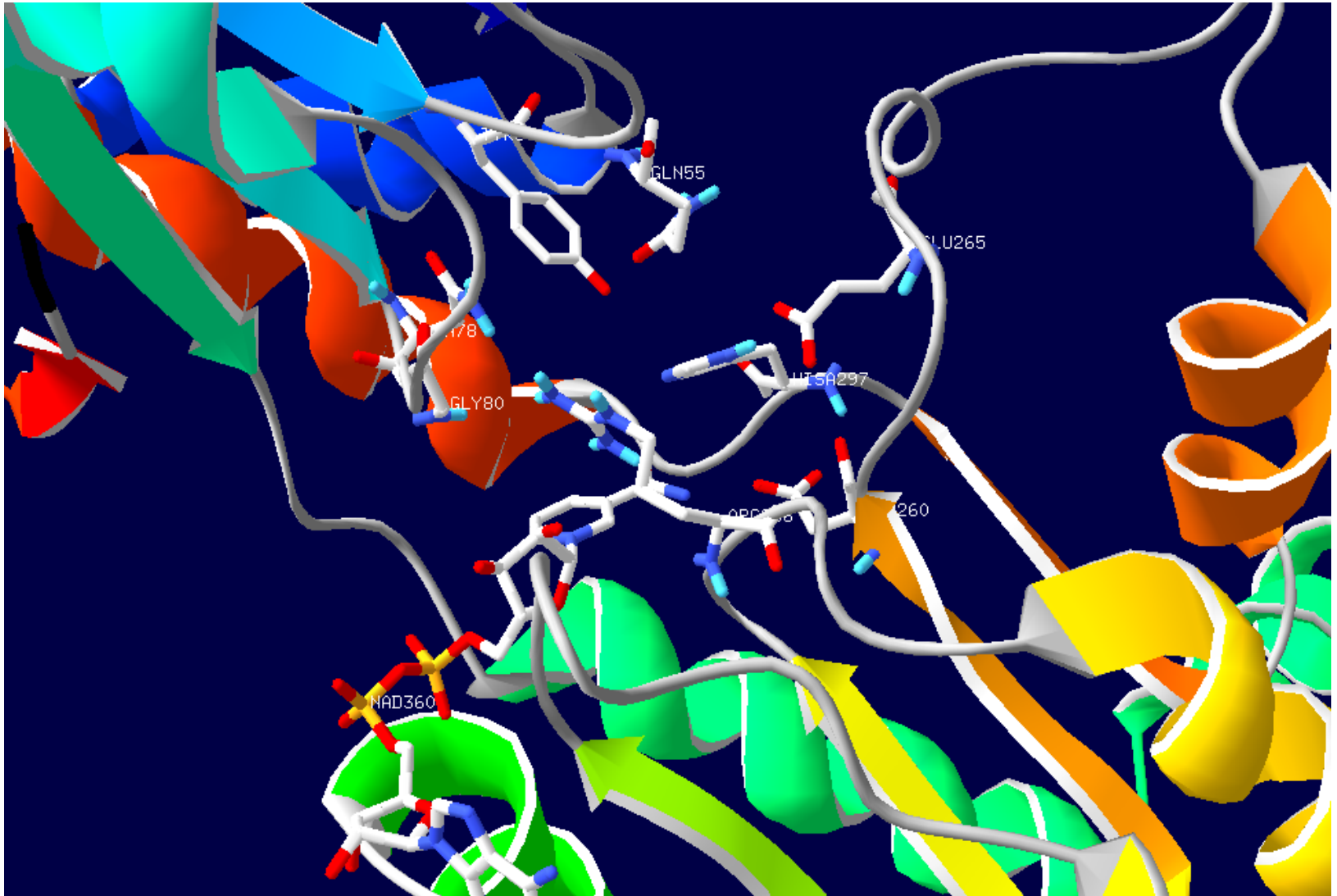
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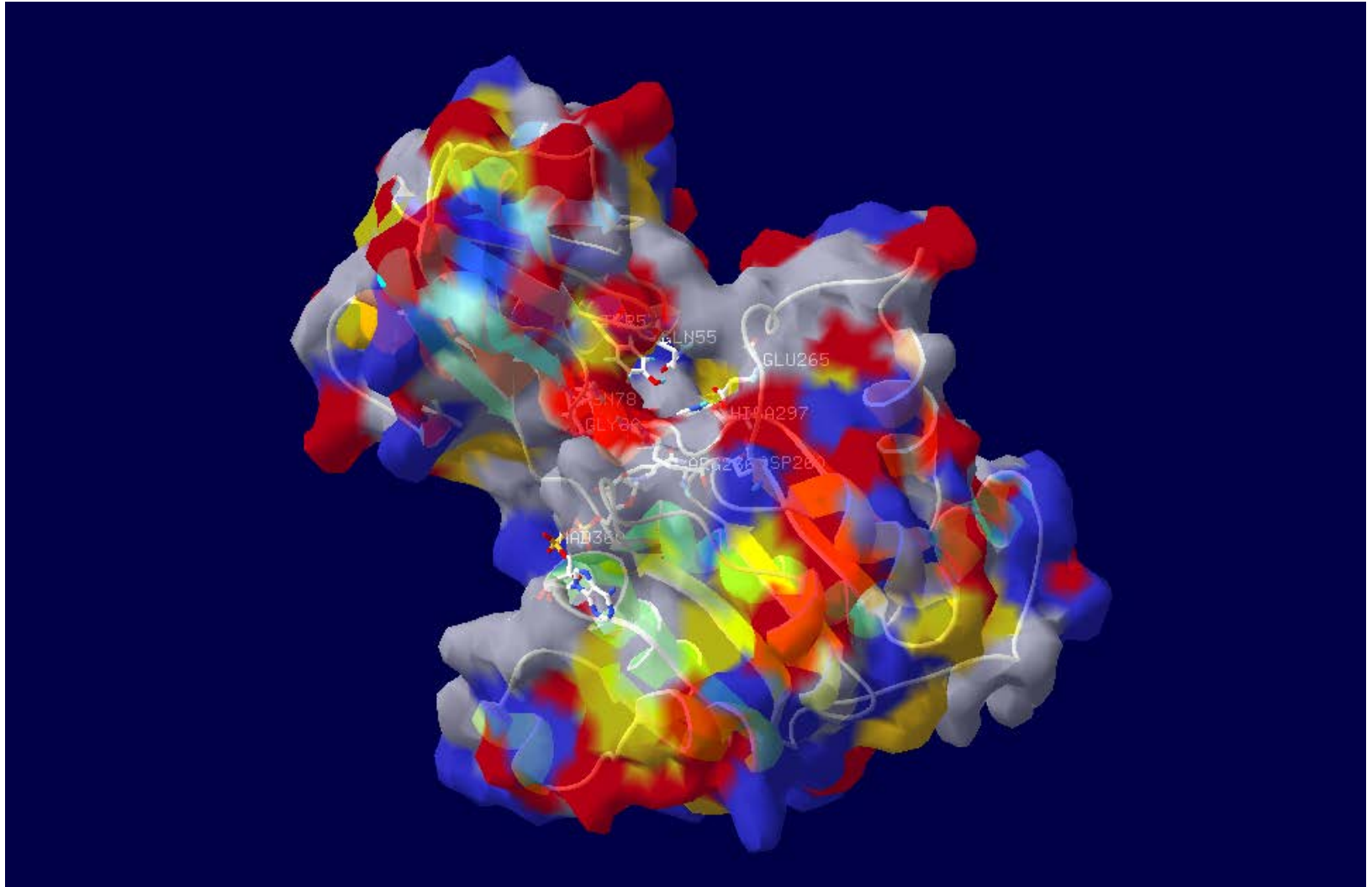
Structure Analysis of D-lactate dehydrogenase



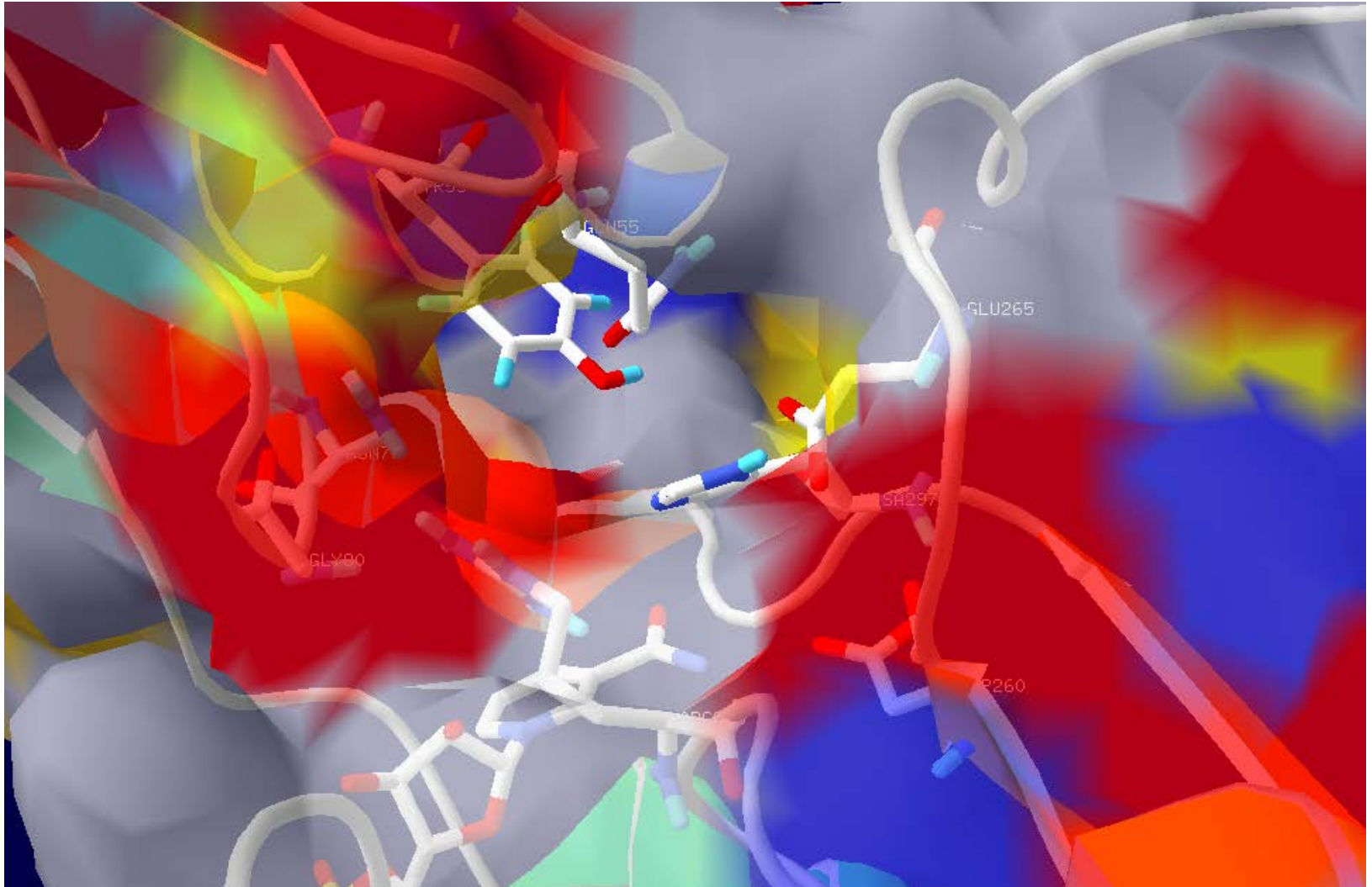
Structure Analysis of D-lactate dehydrogenase



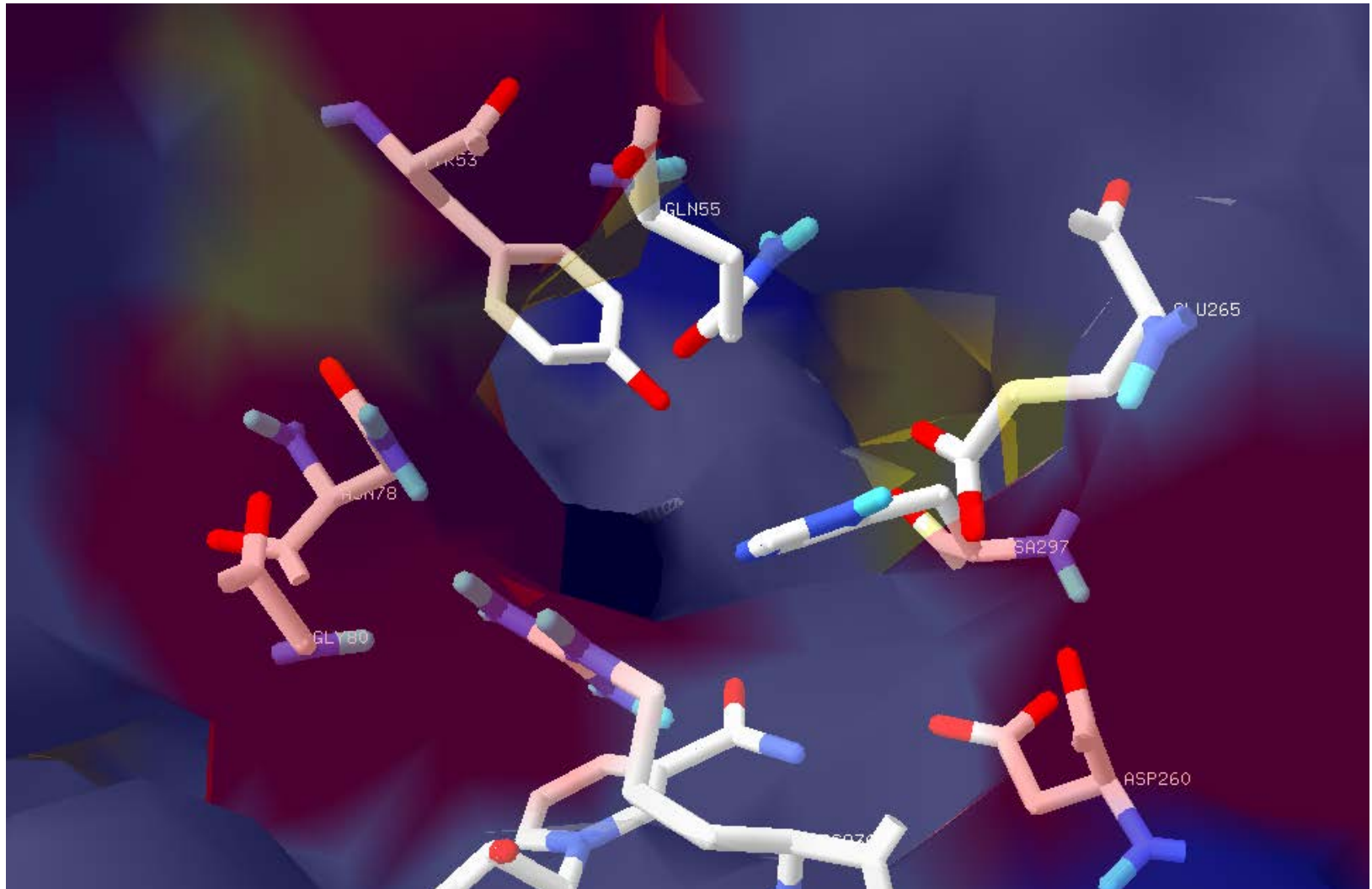
Structure Analysis of D-lactate dehydrogenase



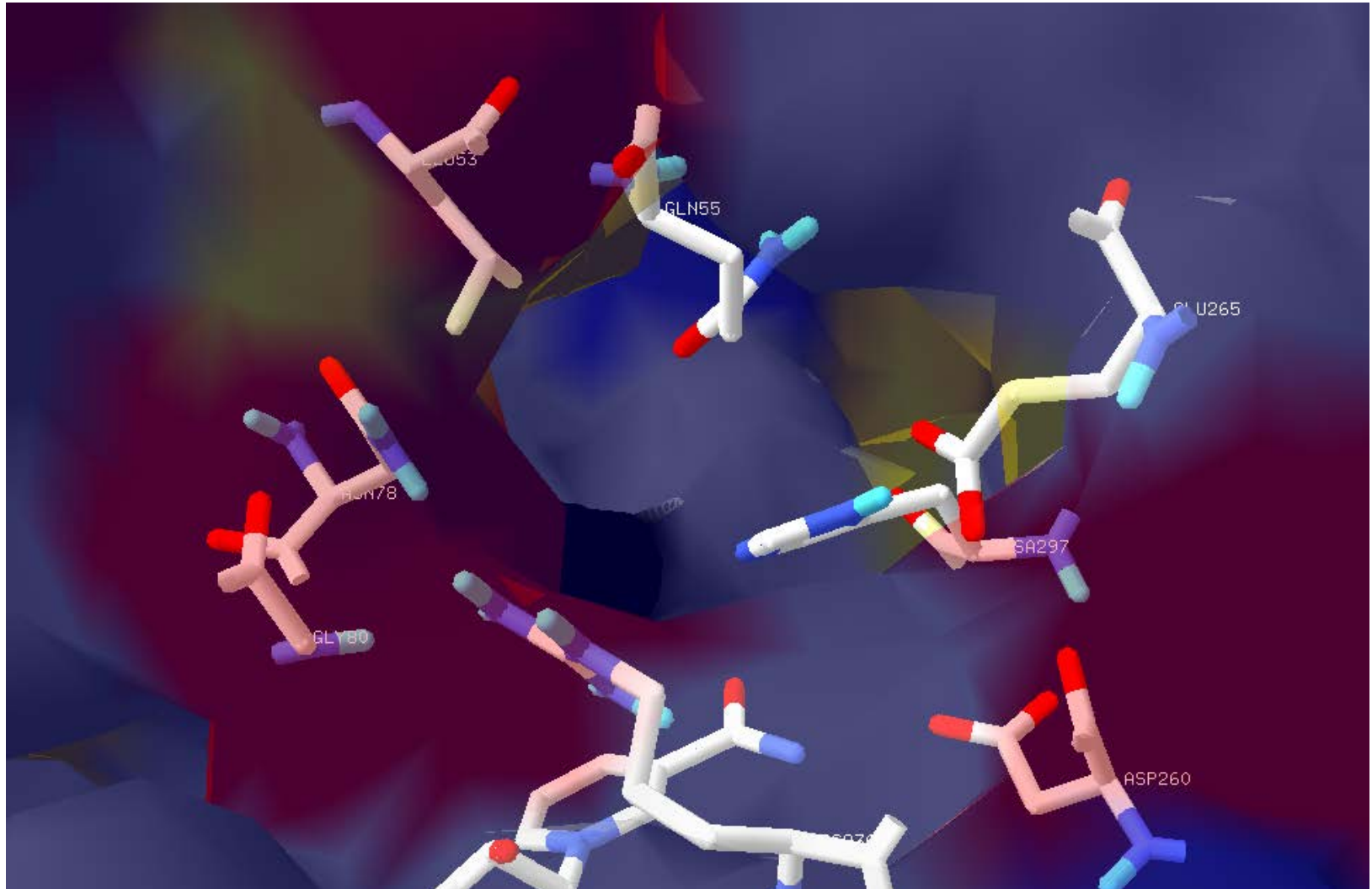
Structure Analysis of D-lactate dehydrogenase



Structure Analysis of D-lactate dehydrogenase



Structure Analysis of D-lactate dehydrogenase



Contents

Introduction to D-lactate dehydrogenase

Sequence analysis of D-lactate dehydrogenase

Structure analysis of D-lactate dehydrogenase

Experimental findings

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)	Specific activity (U mg ⁻¹ protein) ^a				e.e. ^b
	Wild-type	Y52L	F299Y	Y52L/F299Y	
1a (CH ₃)	771.4	669.0	1067	70.5	>99.9% R
2a (CH ₂ OH)	545.5	796.3	153.7	73.5	>99.9% R
3a (CH ₃ CH ₂)	52.4	854.1	17.6	207.0	>99.9% R
4a (CH ₃ CH ₂ CH ₂)	2.93	1,121	20.0	738.3	>99.9% R
5a (CH(CH ₃) ₂)	0.08	10.6	0.08	1.01	>99.9% R
6a (C(CH ₃) ₃)	0.06	1.27	0.13	0.06	^c
7a (C ₆ H ₅)	0.05	3.40	0.11	0.11	>99.9% R
8a (C ₆ H ₅ CH ₂)	18.1	1,016	10.4	1,519	>99.9% R
9a (p-OH-C ₆ H ₄ 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

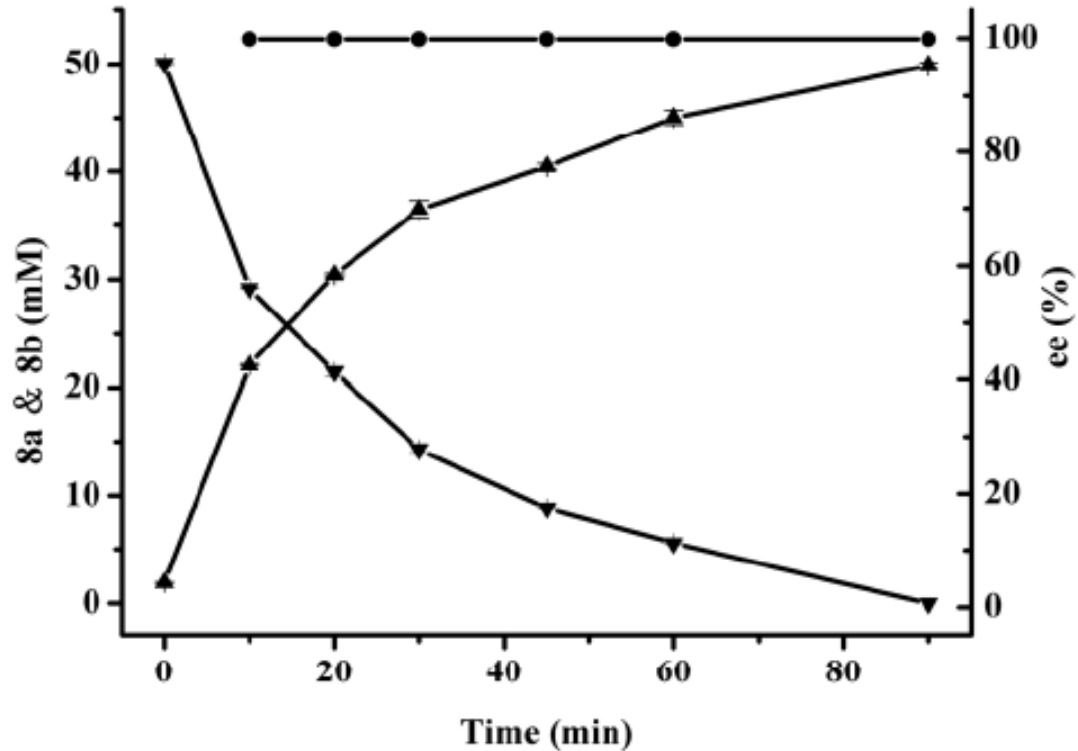
Experimental findings

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

ACCCACGCCAACAAGAGC