Re-visiting of Lactate Dehydrogenase From a Different Dimension: a Model Bioinformatics Study for Wrestling

Levent Çavaş (levent.cavas@deu.edu.tr)
Dokuz Eylul University: Dokuz Eylul Universitesi https://orcid.org/0000-0003-2136-6928
Onder Daglioglu
Gaziantep University: Gaziantep Universitesi
Bulent Cavas
Dokuz Eylul University: Dokuz Eylul Universitesi

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Abstract

Sports informatics is of great importance in the understanding of sportive performance from different dimensions. Accumulated bio-sequences in the databases provide big contributions to compare proteins in different organisms. In Phylum Animalia, some animals have experienced evolution for excellent athletic performances in nature. The present paper exhibits a model *in silico* approach for evaluation of sports performance by comparing lactate dehydrogenases (LDH) in *Homo sapiens* and *Crocodylus porosus*. The results show that although a high sequence similarity is observed between the LDHs from *H.sapiens* and *C.porosus*, these enzymes also present important modifications which could be used in sport science such as talent selection in wrestling. In conclusion, the identification of amino acid modifications in important enzymes of specific animals, lessons from nature, related to sports physiology can open a new gate for the development of athletic performance.

1. Introduction

Lactate dehydrogenase (LDH, EC:1.1.1.27) is an important house-keeping enzyme in human metabolism. The main role of the enzyme is to catalyse the oxidation-reduction reaction between pyruvate and lactate. In anaerobic conditions, glycolysis should be continued in performance requiring activities. However, depleted NAD+ levels must be replenished in muscle cells. LDH catalyses the conversion of pyruvate into lactate. In this reaction, while pyruvate is reduced into lactate, $NADH+H^+$ is oxidised into NAD^+. The formed NAD^+ provides glycolysis to continue in human metabolism under anaerobic conditions (Voet and Voet, 2004). After completion of the genome project in 2000, biosequence based data has increased in the various data banks such as Uniprot and PDB (Berman et al., 2000; The Uniprot Consortium, 2021). Comparison of the data from different organisms may provide important information in different areas. As an example, the sport “wrestling” resembles many natural events in nature. The crocodiles should grab their prey very fast and then they should show an excellent performance within minutes. Similarly, in wrestling, the athletes should also exhibit enormous performance in a limited time such as 5 min (Yard and Comstock, 2008). The leg lace technique is an important technical move found in all positions of freestyle wrestling. For wrestlers, this technique is important for their performance in competitions (Yard and Comstock, 2008). This technique is very similar to the move of the crocodiles since they have to grab their prey and then they have to spin very fast. These events are partly anaerobic and lactate dehydrogenase is of great importance. This is the aim why LDH is selected to be investigated in this model study.

In order to compare these events in *Homo sapiens* and *Crocodylus porosus* at molecular levels, lactate dehydrogenase was selected to be investigated. The sequence-based properties from crocodile and human-originated LDHs were compared by using bioinformatics tools in the present study. This model paper is the first scientific study on the use of sports informatics in wrestling.

2. Materials And Methods

FASTA formats of the human (Homo sapiens) and crocodile (Crocodylus porosus) LDHs were retrieved from uniprot.org (The Uniprot Consortium, 2021). The accession numbers of the human and crocodile LDHs in uniprot.org are P00338 and A0A7M4G2G2, respectively. The protein parameters such as amino acid composition both number and percentage, pI values, the total number of negatively charged residues (Asp + Glu) and the total number of positively charged residues (Arg + Lys), estimated half-life, Instability index, aliphatic index, grand average of hydropathicity values were computed by using protparam tool developed by Gasteiger et al (2005). Multiple sequence analysis was carried out by Clustal O (1.2.4 version) (Sievers et al., 2011). 3D models of the LDHs were studied by using Swiss-Model (Bertoni et al., 2017;Bienert et al., 2017; Studer et al., 2020; Waterhouse et al., 2018; Studer et al., 2021). The architecture of the study was shown in Figure 1. After isolation of the protein, their FASTA sequences are uploaded to various databanks. 3-dimensional structures are modeled by Swiss-Model. Superposition of the sequences exhibits similarities and also differences of the models.

3. Result And Discussion

Amino acid numbers and percentages of human and crocodile LDHs obtained from the protparam tool were depicted in Table 1. Leu and Val have been found as the amino acids in both species in terms of max number and percentage. This could be explained by the high hydrophobic nature of the enzyme, especially in the inner sides of the enzymes. From the results, no irregular amino acids were detected in both human and crocodile LDHs. The percentages of Cys and Trp in human and crocodile LDHs were found to be 1.5% and 1.7%, respectively. These results show that disulphide bridges are not common compared to other proteinic structures. Even if Cys is not at the minimum level in crocodile LDH, the percentage is very close to the min value (1.9%). The total number of negatively charged residues (Asp + Glu), the total number of positively charged residues (Arg + Lys), estimated half-life, Instability index, aliphatic index, grand average of hydropathicity values in Human and Crocodile LDHs were given in Table 2. There are 3 amino acids difference between the total number of negatively charged residues (Asp + Glu) and the total number of positively charged residues (Arg + Lys) in human LDH, the difference between these amino acids is only 1 in crocodile LDH. Regarding enzyme stability of LDHs from *Homo sapiens* and *Crocodylus porosus*, it is almost the same since they have the same values. Instability indexes of the studied LDHs were found as 24.79 and 25.18 in *Homo sapiens* and *Crocodylus porosus*, respectively. These results exhibit that both enzymes are very stable, and they are resistant to being degraded. Grand averages of hydrophobicity index values in human and crocodile LDHs were found to be very different. They are -0.006 and 0.020, respectively. Grand averages of hydrophobicity index values in bioinformatics are used to estimate the hydrophobicity value of a polypeptide sequence. Positive and negative values are explained with the adjectives “hydrophobic” and “hydrophilic” peptides. Based on this definition, it is said that although human LDH is a hydrophobic polypeptide, crocodile LDH is a hydrophobic polypeptide. Pairwise sequence comparison of human and crocodile LDHs was depicted in Table 3. From the result, it could be said that the amino acid sequences of human and crocodile LDHs were very similar. However, there are some differences among the sequences as follows. First, the symbols are important to understand the pairwise sequence alignment. The symbols “*”, “:” and “.” show

the same amino acids, amino acids with very similar physicochemical properties, and similar physicochemical properties. Glu and Asp can be given as examples of the amino acids with very similar physicochemical properties, Ile and Val are also examples for the amino acids with similar physicochemical properties since both are hydrophobic. However, their structures are not very same compared to the amino acids such as Glu and Asp. If there is no symbol in the pairwise sequence comparison, it could be explained with the deletion or insertion. Binding sites in P00338 (human LDH) for NAD, substrate, NAD, substrate, substrate, proton acceptor and substrate were given as 99, 106, 138, 169, 193 and 248, respectively (Read et al., 2001). These amino acids are Arg, Arg, Asn, Asn, Arg and Thr, respectively. When we compare this pattern with the pattern in crocodile LDH, the pattern is the same. The nucleotide-binding domain of the LDH in humans is (Read et al., 2001), pairwise sequence alignment clearly shows that this residue in crocodile LDH is also conserved. Two mutagenesis are reported for human LDH and they are at the position of 56 and 99. Asp at the position of 56 is reported for the wild-type human LDH. If it is substituted with Ala, this change abolishes interaction with MP31 (Huang et al., 2021). Similarly, if the amino acid at the position of 99 (Arg) is substituted with Ala, this case also resulted in decreased interaction with MP31 (Huang et al., 2021). When these regions (Asp and Arg) are checked in crocodile LDH, it is seen that these regions are also conserved. Amino acid modifications are also reported in the uniprot.org records for P00338 (human LDH). The positions with the modifications are 2 (N-acetylalanine), 5 (N6-acetyllysine), 5 (N6-succinyllysine), 10 (Phosphotyrosine), 14 (N6-acetyllysine), 18 (Phosphothreonine), 57 (N6-acetyllysine), 57 (Glycyl lysine isopeptide (Lys-Gly) (interchain with G-Cter in SUMO2)), 81 (N6-acetyllysine), 118 (N6-acetyllysine), 118 (N6-succinyllysine), 126 (N6-acetyllysine), 224 (N6-acetyllysine), 232 (N6-acetyllysine), 239 (Phosphotyrosine), 243 (N6-acetyllysine), 309 (Phosphothreonine), 310 (Phosphoserine), 318 (N6-acetyllysine), 318 (N6-succinyllysine) and 322 (Phosphothreonine). In the light of these modifications, we wanted to compare these regions in crocodile LDH. When we revisit the pairwise sequence alignment, N-terminal regions are different in both LDHs. The position-18 is Thr in human LDH and the relevant modification is phosphorylation. On the other hand, this residue is substituted with His in crocodile LDH and it is not possible to observe phosphorylation in this residue. The high similarity starts at the position of 20 of the human LDH compared to the sequence of crocodile LDH. The second amino acid in human LDH is Ala and it is reported with acetylation (Bienvenut et al., 2012; Gauci et al., 2009). Since this amino acid does not exist in crocodile LDH, this modification may be of importance. It is very interesting to note that even if the initial sequences are different, the amino acid position-5 is conserved in both species and the succinylation and acetylation of lysine are also possible in crocodile LDH (Choudhary et al., 2009). The position of 10 in human LDH is very also important to be compared with crocodile LDH due to amino acid differences in this region (Mayya et al., 2009). It is Tyr in human and it is His in crocodile LDH. Hydroxyl residue of Tyr is generally important in enzymatic activities through phosphorylation. Since there is no phosphorylation residue in His of crocodile LDH, this region should also be noted for the enzymatic activity of LDH. Although initial sequences are seen differently in both enzymes, Lys residue is conserved in both species and this region (position 14 in human LDH) is mentioned with acetylation in Uniprot.org (Choudhary et al., 2009). The position of 57 in a human LDH is Lys and it is mentioned that it is modified with acetylation (Choudhary et al., 2009). This region is conserved in crocodile LDH and similar

modification is most likely to be observed in crocodile LDH. Glycyllysine isopeptide (Lys-Gly) interchain with G-Cter in SUMO2 was reported by Hendriks et al (2017). The position of 81 in both species is the same and it is Lys. Acetylation is reported in this residue (Henriks et al., 2017). The latter explanation is also valid for the position of 118, 126, 224, 232, 243 and 318 (Choudhary et al., 2009). The position of 239 in both species is the same and it is Tyr. Phosphorylation is mentioned in Uniprot.org for this position (Huang et al., 2021; Bian et al., 2014; Zhou et al., 2013). The last modification residue is positioned at 322. When amino acids are compared for this position, Thr is found for both enzymes. Modelling of the lactate dehydrogenase from Crocodylus porosus via was carried out through the Swiss Model (Waterhouse et al., 2018). The Swiss Model template 5nqb.1.A (Rabbit Muscle L-lactate dehydrogenase in complex with malonate) was selected for modelling (Alam et al., 2017) and the model of the lactate dehydrogenase was shown in Figure 2. The sequence identity percentage was found as 88.48%. The local quality estimation versus residue number was drawn in Figure 3. Qmean Z-Scores as QMean, CβQMEANDisco Global values were found as 0.86 and 0.84, respectively (Waterhouse et al., 2018; Studer et al., 2021). Normalised QMEAN4 Score versus residue number plot was shown in Figure 4. The template 5nqb.1.A was selected since it does not contain any ligand and also it has a homo-tetramer structure. Moreover, the method for the modelling was X-ray and 1.58 A. When scientific literature was examined, generally lactate dehydrogenase is used in sports science to evaluate athletic performance. Here we review some of the lactate dehydrogenase-based papers. Hoff et al (2016) investigated the brains of the hooded seal (*Cystophora cristata*), the ferret (*Mustela putorius furo*) and mouse (*Mus musculus*) to provide evidence of whether these animals have enhanced cerebral capacity for anaerobic energy production. The study revealed significant differences in the mRNA, protein expression of lactate dehydrogenase (LDHA and LDHB) and the LDH activity in the ferret brain compared to the other two animals. The research did not observe significant differences in the LDHA and LDHB sequences. The results also show that the high hypoxia tolerance of seals for anaerobic energy production cannot be explained by the seal brain's enriched capacity. In addition to the above, the study addressed that the hooded seal's cerebral tolerance to hypoxia may be partially affected by the different LDH isoenzymes. The study conducted by Barranco et al (2017) investigated some enzymes (creatine kinase (CK), LDH and aspartate aminotransferase (AST)) results in saliva to see the impact of intensive sports training (Futsal) on eleven young males. After Futsal training, while dramatic increases are found in CK, LDH and AST in serum samples, significant increases are determined for CK and LDH in saliva. There was no change in saliva AST after the intensive training. The study highlighted that changes in CK and LDH in saliva can be used as a potential indicator to determine muscle injuries and stress. In a study comparing the CK and LDH concentrations of 20 men while doing resistance training, it was reported that serious muscle damage could be caused if 1 minute of rest intervals was applied (Rodrigues et al., 2010). Rumley et al. (1985) focused on the CK and the LDH isoenzymes in serum. The study consisted of 35-50 years aged men who did marathon training for 30 weeks. It was determined that marathon training did not have a significant effect on muscle CK and LDH release. However, it has been mentioned that isoenzyme distribution changes occur in muscle during endurance training. Similar scientific reports can be found in sport science-based literature. However, the enzymatic activity of LDH or its concentrations are measured to estimate lactate levels or muscle injuries in the athletes in these investigations. As can be seen from

this paper, there are plenty of amino acids modifications and also variants that could affect enzyme activities. Observation of significantly elevated activities in the athletes could be associated with individual differences in the LDHs. From this point, it is highly suggested to isolate the LDH from the elite athletes. The results within this paper can be used to compare with the sequence of the isolated enzymes. Swiss-Model clearly provides big contribution on the understanding of 3-Dimensional structures of the enzymes studied. As can be seen from Figure 5, not only 3 dimensional structure but also different characteristics such as polarity, amino acid sequence similarities, sizes, charges can also be shown on the 3-dimensional structures. Any modification on the enzyme structure can also be understood from these images (Fgure 5). Observation of different modifications in the amino acid sequences of the elite athletes may open a new route of scientific investigations in the sport sciences. Obtaining important amino acid modifications in elite athletes (Olympic and World Champions) may be used as important biomarkers in talent selection. The results mentioned in this paper can be used to compare the amino acid sequences from Olympic and World Champions. A sample figure is also drawn to explain the latter (Figure 6). Two sequences can be compared by superposition in Swiss-Model and this could give important idea to the sport scientists about the enzymes in elite athletes. Moreover, the methodology mentioned in this paper could also be extended for other sport disciplines.

Table 1. Amino acid numbers and percentages in the lactate dehydrogenases from *Homo sapiens* and *Crocodylus porosus.*

	H.sapiens		*C.porosus*	
	#	*%*	*#*	*%*
A	*18*	*5.4*	*20*	*5.5*
B	*0*	*0.0*	*0*	*0.0*
C	*5*	*1.5*	*7*	*1.9*
D	*18*	*5.4*	*20*	*5.5*
E	*18*	*5.4*	*21*	*5.8*
F	*7*	*2.1*	*9*	*2.5*
G	*26*	*7.8*	*27*	*7.4*
H	*7*	*2.1*	*19*	*5.2*
I	*23*	*6.9*	*23*	*6.3*
K	*28*	*8.4*	*29*	*8.0*
L	*38*	*11.4*	*37*	*10.2*
M	*9*	*2.7*	*11*	*3.0*
N	*15*	*4.5*	*11*	*3.0*
O	*0*	*0.0*	*0*	*0.0*
P	*11*	*3.3*	*10*	*2.8*
Q	*12*	*3.6*	*9*	*2.5*
R	*11*	*3.3*	*11*	*3.0*
S	*24*	*7.2*	*27*	*7.4*
T	*14*	*4.2*	*15*	*4.1*
U	*0*	*0.0*	*0*	*0.0*
V	*34*	*10.2*	*42*	*11.6*
W	*6*	*1.8*	*6*	*1.7*
X	*0*	*0.0*	*0*	*0.0*
Y	*8*	*2.4*	*9*	*2.5*
Z	*0*	*0.0*	*0*	*0.0*

Table 2. Protein parameters of the lactate dehydrogenases from *Homo sapiens* and *Crocodylus porosus* *(*mammalian reticulocytes, in vitro).*

Protein Parameters	*Homo sapiens*	*Crocodylus porosus*
Extinction coefficient (M^{-1} cm^{-1})	45170	46785
Estimated half-life*	30	30
Instability index	24.79	25.18
Aliphatic Index	106.78	103.53
Grand average of hydropathicity	-0.006	0.020

Table 3. Pairwise sequence comparison of human and crocodile LDHs via Clustal omega (1.2.4).

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CLUSTAL O(1.2.4) multiple sequence alignment


sp|P00338|LDHA_HUMAN                 ----------------------------MATLKDQLIYNLLKE-EQTPQNKITVVGVG        29
tr|A0A7M4G2G2|A0A7M4G2G2_CROPO       MLYVDICRHFITSQIPEDKLHCALNTFQLYTMSVKEHLIHNVHKEEHGHAHNKITVVGVG        60
                                                                ::*::**:*: ** .   :*********

sp|P00338|LDHA_HUMAN                 AVGMACAISILMKDLADELALVDVIEDKLKGEMMDLQHGSLFLRTPKIVSGKDYNVTANS        89
tr|A0A7M4G2G2|A0A7M4G2G2_CROPO       AVGMACAISILMKDLADELALVDVVEDKLRGEMLDLQHGSLFLKTPKIVSGKDYSVTANS        120
                                     ************************:****:***:*********:**********.*****

sp|P00338|LDHA_HUMAN                 KLVIITAGARQQEGESRLNLVQRNVNIFKFIIPNVVKYSPNCKLLIVSNPVDILTYVAWK        149
tr|A0A7M4G2G2|A0A7M4G2G2_CROPO       KLVIITAGARQQEGESRLNLVQRNVNIFKFIIPSVVKHSPDCKLLVVSNPVDILTYVAWK        180
                                     ********************************.***:**:****:**************

sp|P00338|LDHA_HUMAN                 ISGFPKNRVIGSGCNLDSARFRYLMGERLGVHPLSCHGWVLGEHGDSSVPVWSGMNVAGV        209
tr|A0A7M4G2G2|A0A7M4G2G2_CROPO       ISGFPKHRVIGSGCNLDSARFRYLMGEKLGVHSLSCHGWIVGEHGDSSVPVWSGVNVAGV        240
                                     ******:********************:**** ******::*************:*****

sp|P00338|LDHA_HUMAN                 SLKTLHPDLGTDKDKEQWKEVHKQVVESAYEVIKLKGYTSWAIGLSVADLAESIMKNLRR        269
tr|A0A7M4G2G2|A0A7M4G2G2_CROPO       SLKALHPELGTDADKEHWKEVHKQVVDSAYEVIKLKGYTSWAIGLSVADLAETVMKNLRR        300
                                     ***:***:**** ***:*********:************************::******

sp|P00338|LDHA_HUMAN                 VHPVSTMIKGLYGIKDDVFLSVPCILGQNGISDLVKVTLTSEEEARLKKSADTLWGIQKE        329
tr|A0A7M4G2G2|A0A7M4G2G2_CROPO       VHPISTMVKGMYGIKDDVFLSVPCVLGYHGITDVVMMTLKSEEEEKLRKSADTLWGIQKE        360
                                     ***:***:**:*************:** :**:*:* :**.**** :*:************

sp|P00338|LDHA_HUMAN                 LQF 332
tr|A0A7M4G2G2|A0A7M4G2G2_CROPO       LQF 363
                                     ***
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4. Conclusion

Comparison of LDHs in human and crocodiles by using the in silico tools clearly show that bioinformatics may have a potential application area in sports science. The crocodiles have -long years- been experienced evolution for better physical performance for their survival in nature. Therefore, the sequence similarities, differences and also important modifications could be used in talent selection. The lessons learned from nature may open a new gate in sports science. To get the full picture, more enzymes

and also genes from different animals with different adaptations may be used in bioinformatics analysis in sport. In conclusion, sports informatics is waiting to be explored.

Declarations

Statements and Declarations

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Levent Cavas, Onder Daglioglu and Bulent Cavas. The first draft of the manuscript was written by Levent Cavas and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

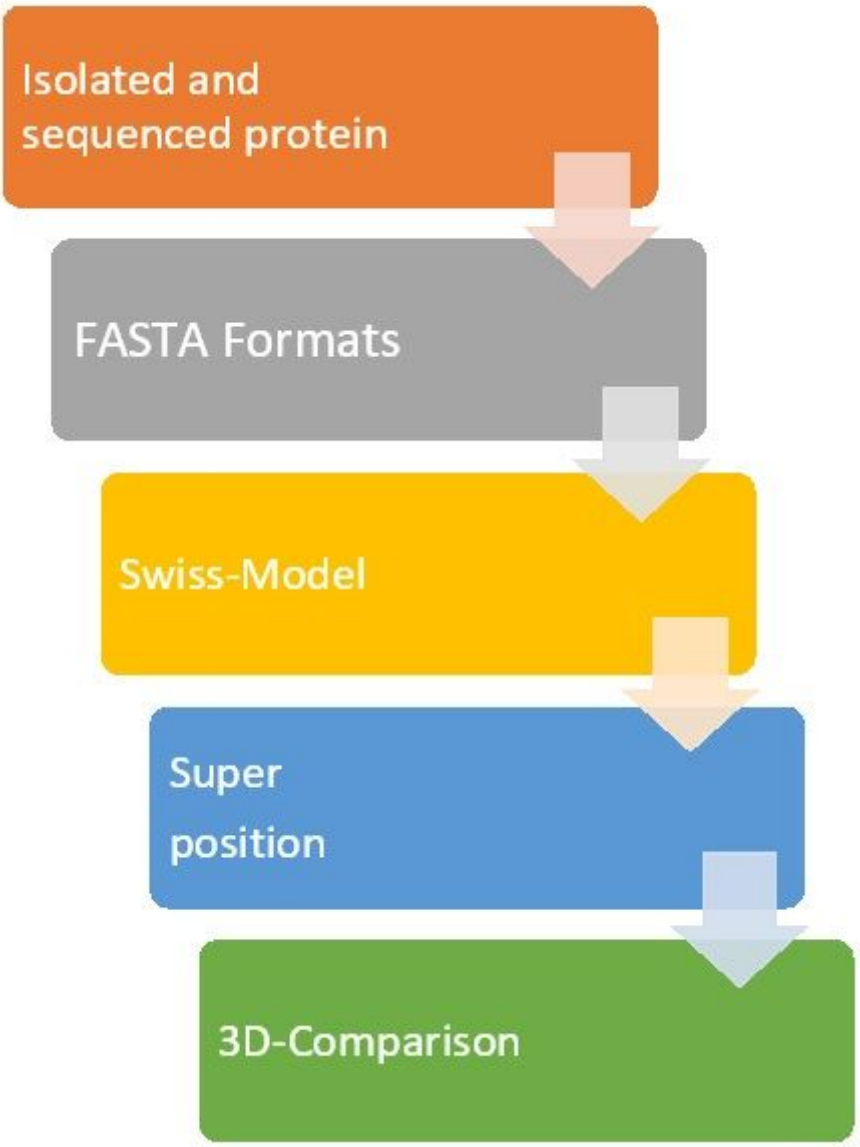

Figure 1

The architecture of the study

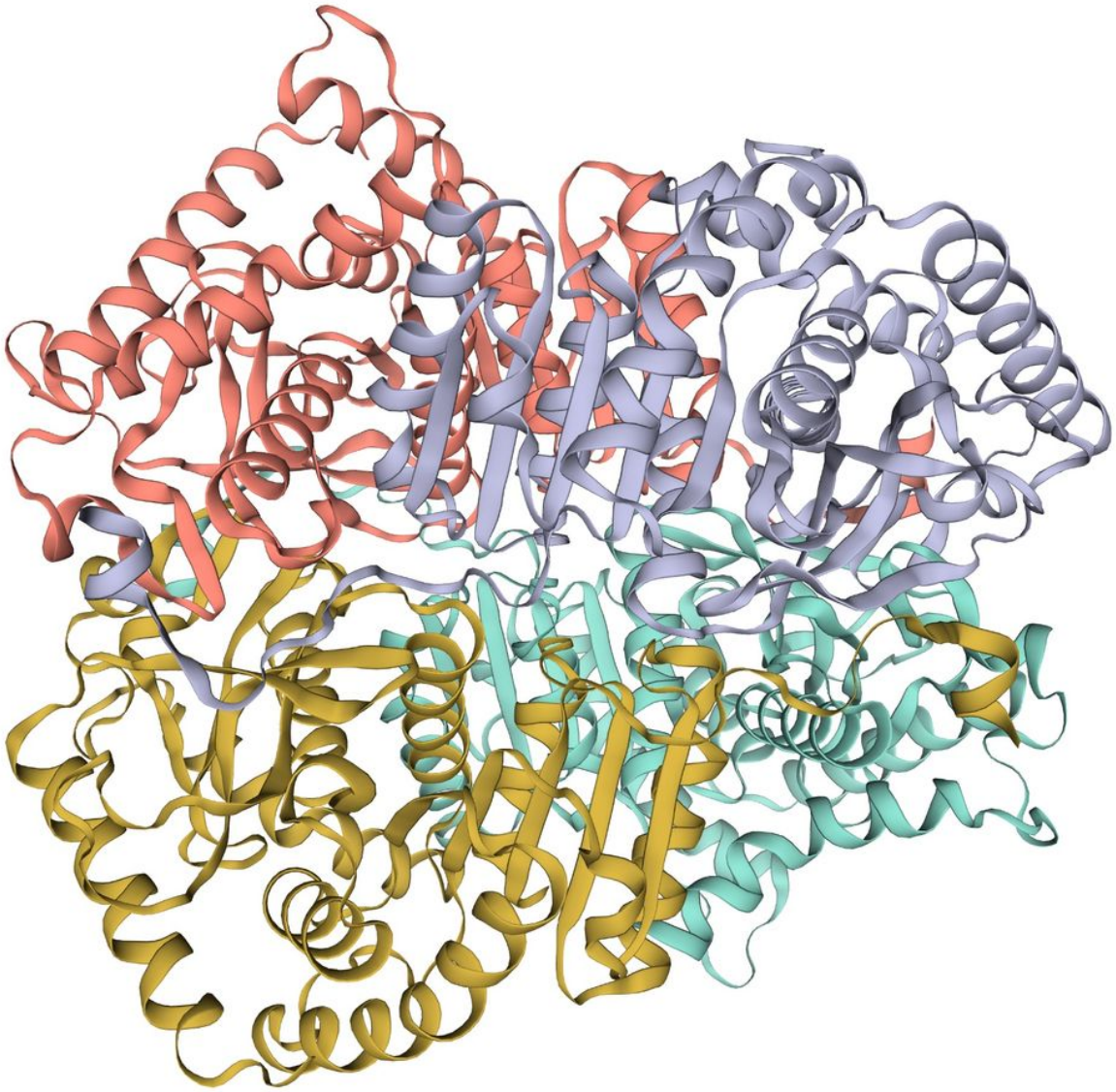

Figure 2

Modelling of the lactate dehydrogenase from *Crocodylus porosus* via Swiss Model (Waterhouse et al., 2018).

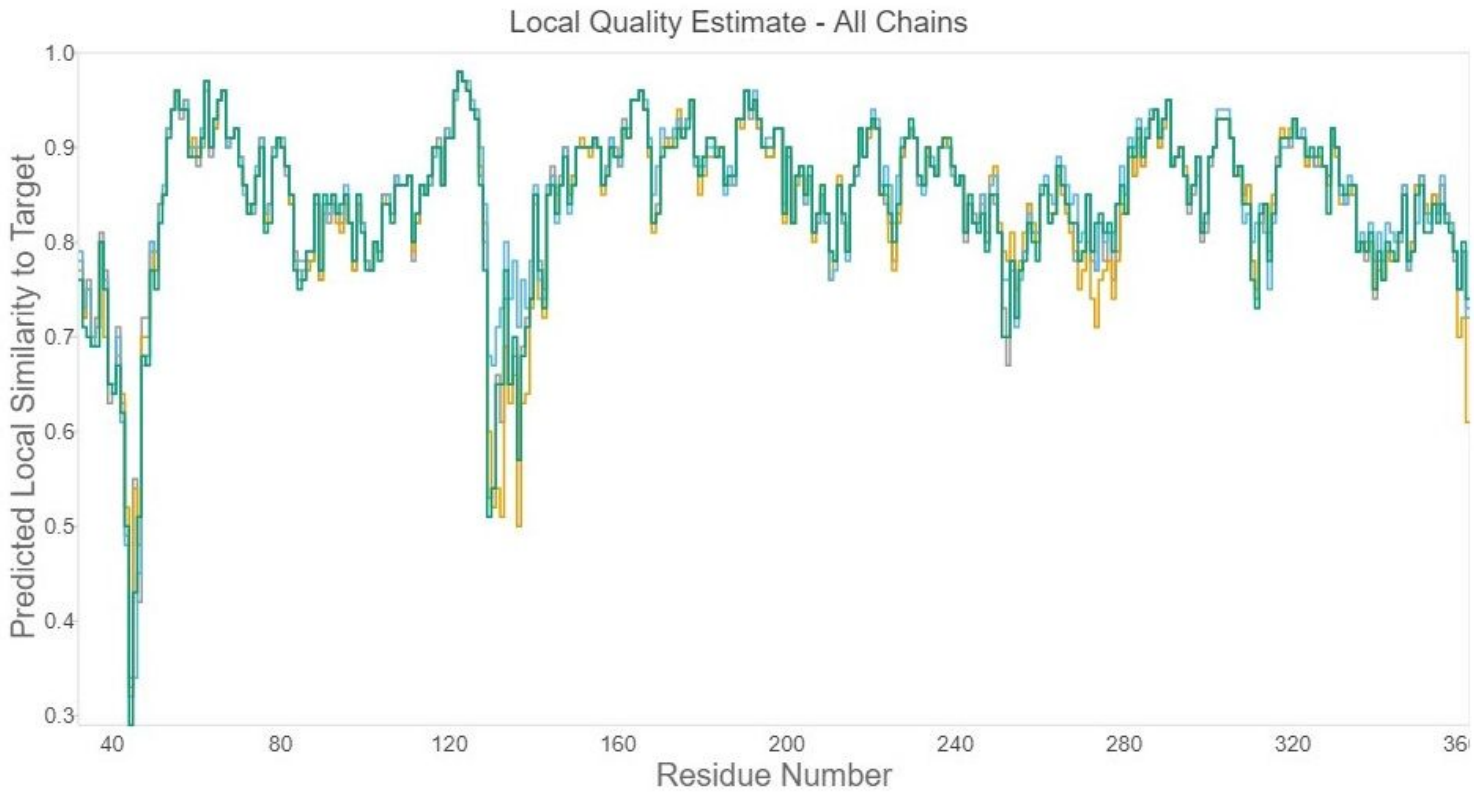

Figure 3

Local quality estimation versus residue number plot.

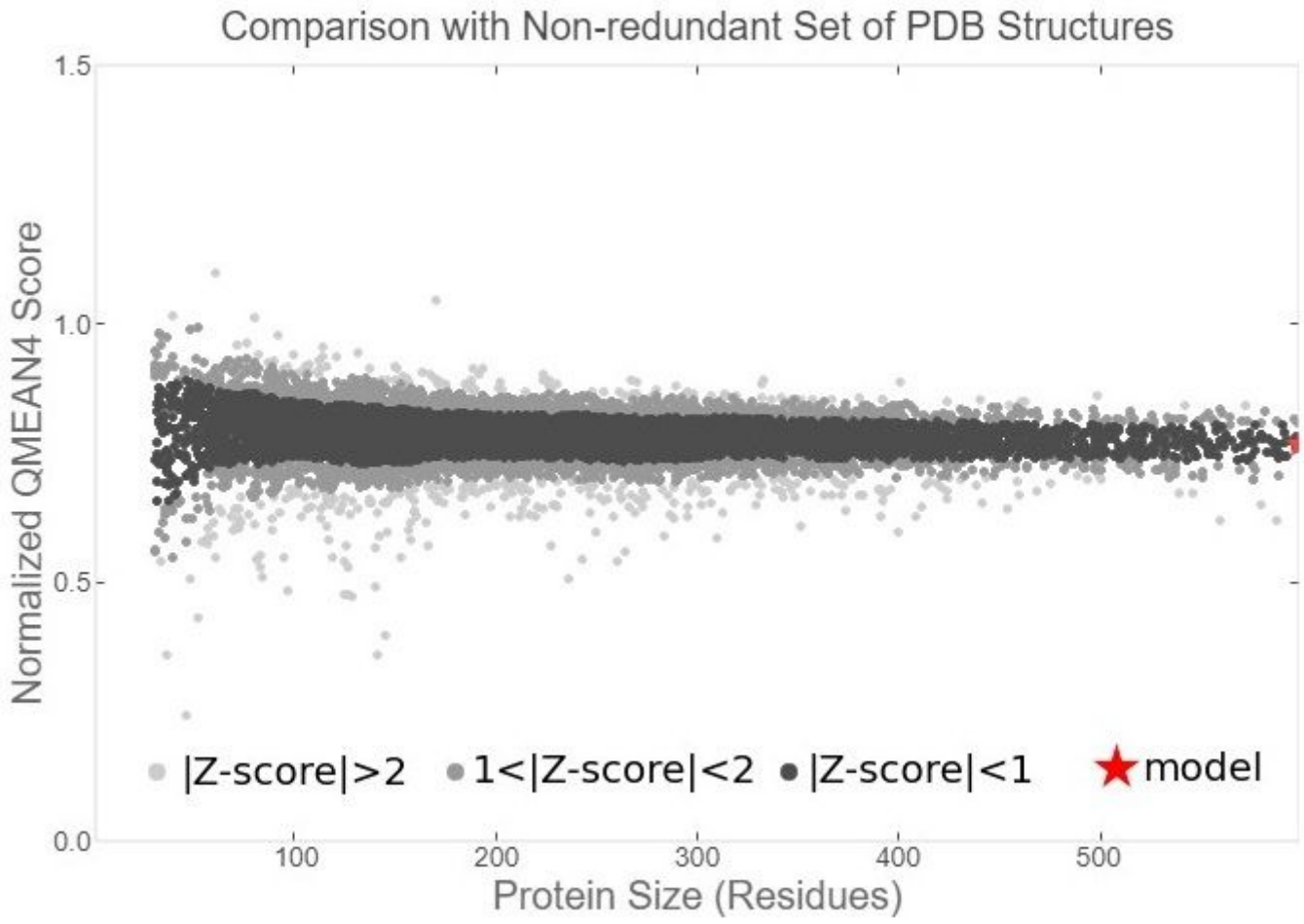

Figure 4

Normalised QMEAN4 Score versus residue number plot.

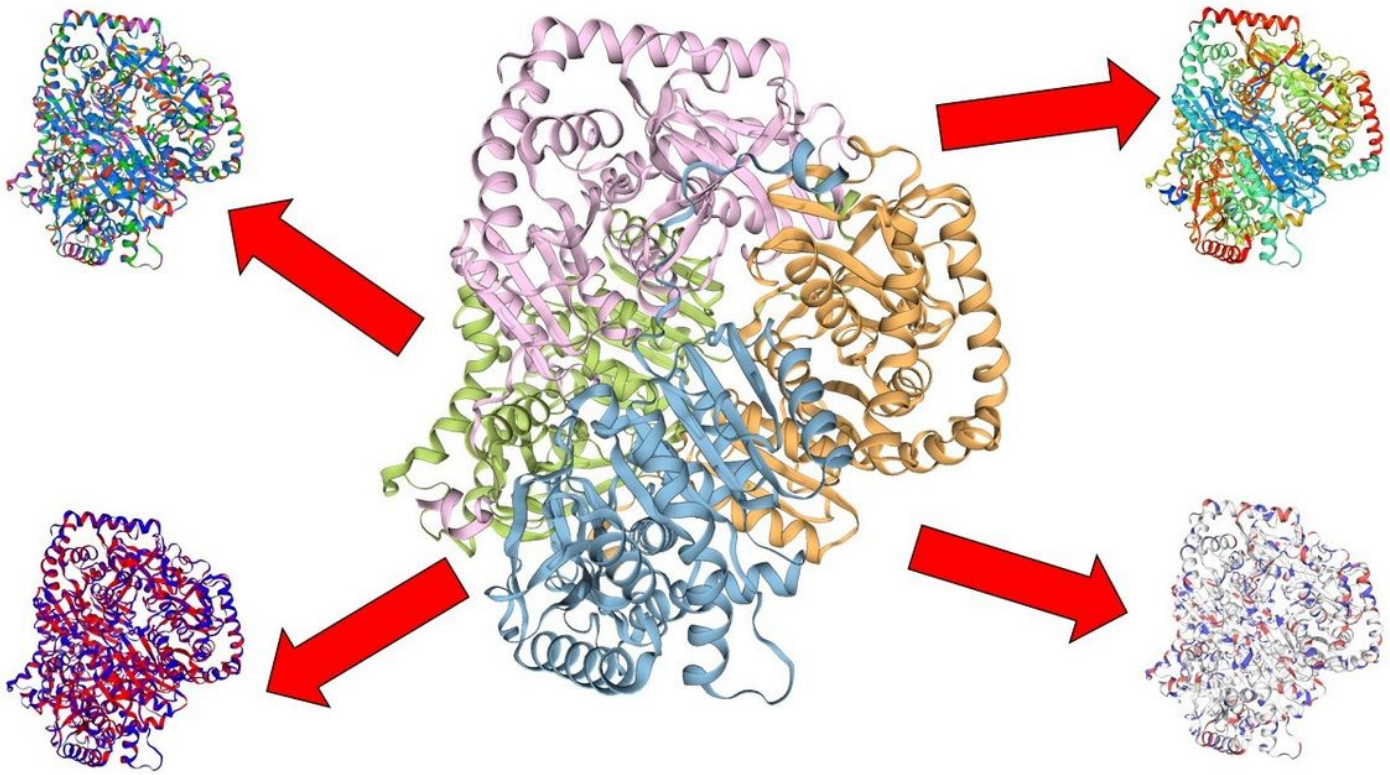

Figure 5

4 different drawings of the lactate dehydrogenase in Swiss-Model based on the different characteristics. Left-upper: Clustal, Left-down: size, Right-upper:rainbow, Right-down: charged amino acids.

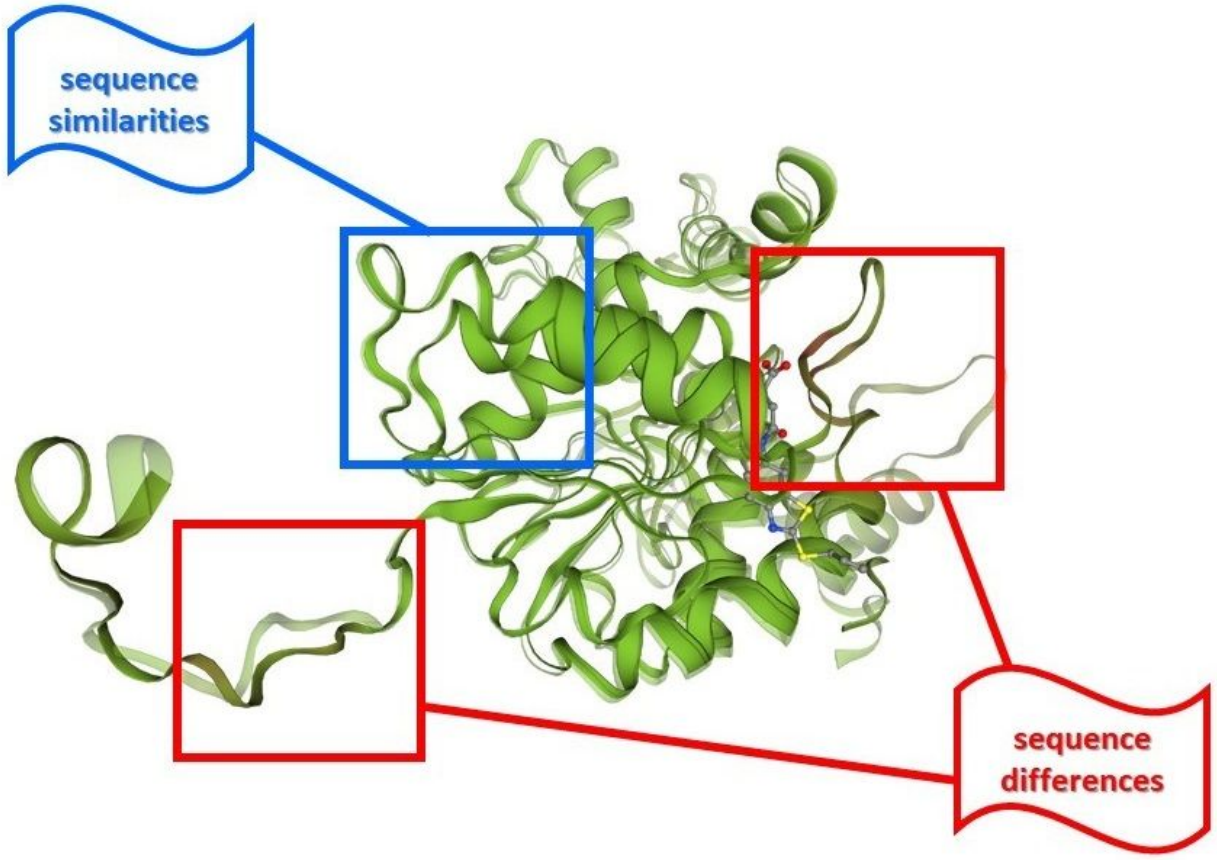

Figure 6

Superposition of two different lactate dehydrogenase structures. Sequence differences and similarities are shown within the figure.