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Re-visiting lactate dehydrogenase from a different dimension: a model bioinformatics study for wrestling

Levent Cavas[1]*, Onder Daglioglu[2] Bulent Cavas[3]

[1]Department of Chemistry, Faculty of Science, Dokuz Eylül University, 35390, İzmir, Türkiye
[2]Department of Physical Education and Sports, Faculty of Sport Sciences, Gaziantep University, 27310, Gaziantep, Türkiye
[3]Department of Science Teaching, Faculty of Education, Dokuz Eylül University, 35380, Buca, İzmir, Türkiye

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Corresponding Author
Tel.: +90 232 301 8701
E-mail: levent.cavas@deu.edu.tr

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Abstract

Sports bioinformatics is of great importance in the understanding of sports performance from different perspectives. Accumulated bio-sequences in databases provide considerable contributions to compare proteins in different organisms. In Kingdom of Animalia, some animals have experienced evolution for excellent athletic performances in nature. The present paper exhibits a model *in silico* approach for the evaluation of sports performance by comparing lactate dehydrogenases (LDH) in humans (*Homo sapiens)* and saltwater crocodiles (*Crocodylus porosus)*. The results show that a high sequence similarity is observed between the LDHs from *H. sapiens* and *C. porosus* with minor modifications. The stability and grand averages of hydrophobicity index values for studied LDHs were found as 24.79–25.18 and -0.006 –0.020 in *H. sapiens* and *C. porosus*, respectively. In conclusion, the identification of amino acid modifications in important enzymes of specific animals that are related to sports physiology are lessons we learn from from nature, which can open a new gate for the development of sports performance and talent selection.

Introduction

Lactate dehydrogenase (LDH, EC:1.1.1.27) is an important housekeeping 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 nicotinamide adenine dinucleotide (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 & Voet, 2004). After the completion of the genome project in 2000, biosequence based data has increased in 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 which is based on rapid rotation about the longitudinal axis of the body (Fish et al., 2007). Similarly, in wrestling, the athletes should also exhibit enormous performance in a limited time such as 5 min (Yard & 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 & Comstock, 2008). This technique is very similar to the hunting move of the crocodiles since they have to grab their prey and then spin very fast. These events are partly anaerobic and lactate dehydrogenase is of great

importance (Baldwin et al., 1995; Bennett et al., 1985; Owerkowich & Baudinette, 2008). 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 saltwater crocodile *Crocodylus porosus* (hereafter *C. porosus*) at molecular levels, LDH 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 bioinformatics in wrestling.

Materials and Methods

FASTA formats of the human (*H. sapiens*) and crocodile (*C. 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 Omega (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; Studer et al., 2021; Waterhouse et al., 2018). The outline of the study is shown in Figure 1. In bioinformatics, FASTA formats are obtained after isolation and sequencing experiments. 3-D structures of the sequences can be modelled by various tools such as SwissModel. The superposition of the sequences exhibits similarities and also differences of the proteins compared. The superposition of the sequences exhibits similarities and also differences of the models.

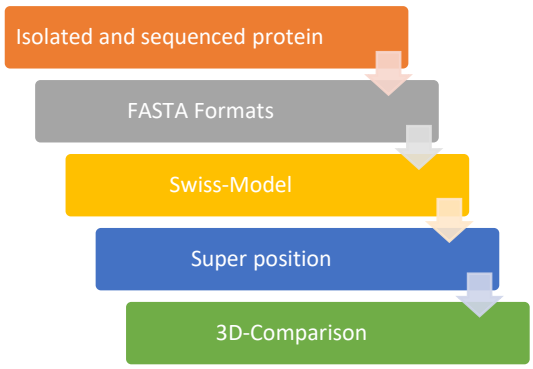

Figure 1. The architecture of the study.

Results and Discussion

Amino acid numbers and percentages of human and crocodile LDHs obtained from the protparam tool are depicted in Table 1. Leu and Val have been found as 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.

Table 1. Amino Acid Numbers and Percentages in the Lactate Dehydrogenases from Homo sapiens and Crocodylus porosus

Amino acids	*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

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 *H. sapiens* and *C. porosus* LDHs are given in Table 2. There are three amino acids difference between the total number of negatively charged residues (Asp + Glu) and the total number of positively charged residues (Arg + Lys) in *H. sapiens* LDH, the difference between these amino acids is only one in *C. porosus* LDH.

Regarding enzyme stability of LDHs from *H. sapiens* and *C. 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 *H. sapiens* and *C. porosus*, respectively.

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

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

These results exhibit that both enzymes are very stable, and they are resistant to being degraded easily. 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 (Gasteiger et al., 2005). 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 hydrophilic polypeptide, crocodile LDH is a hydrophobic polypeptide. This could have been due to the amino acid sequence difference of both species. A pairwise sequence comparison of human and crocodile LDHs were depicted in Figure 2.

From the result, it could be said that the amino acid sequences of human and crocodile LDHs are very similar. However, there are some differences among the sequences as follows. First, the symbols are important to understand in Figure 2.

Figure 2. Pairwise sequence comparison of human and crocodile LDHs via Clustal omega (1.2.4).

The symbols "*", ":" and "." show the same amino acids, amino acids with very similar physicochemical properties, and similar physicochemical properties, respectively. Glu and Asp can be given as examples of amino acids with very similar physicochemical properties, Ile and Val are also examples of amino acids with similar physicochemical properties since both are hydrophobic. However, their structures (Ile and Val) are not very same compared to 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. The binding site for NAD^+ in human LDH are at the 99th, the positions between 29-57th and 138th positions. The positions at the 106th, 138th, 169th, and 248th are reported for the substrate binding site. His at the position of 193 is located at the active site and it plays as role for proton acceptor. When we compare this pattern with that of crocodile LDH, it is the same. The nucleotide-binding domain of the LDH in humans is very important (Read et al., 2001). The pairwise sequence alignment in our study clearly shows that this residue in crocodile LDH is also conserved. Two mutageneses are reported for human LDH and they are at the position of 56th and 99th. Asp at the position of 56th is reported for the wild-type human LDH. If it is substituted with Ala, this change abolishes interaction with MP31 which is a micropeptide and it limits lactate-pyruvate conversion in mitochondria (Huang et al., 2021). Since lactate-pyruvate conversion is of paramount importance in both anaerobic and aerobic exercises, this substitution should be noted for further investigations. 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 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. As Bienvenut et al (2012) mentioned that the modifications at N-terminal peptides are of great importance for the crucial processes of the proteins such as activity, stability, and subcellular locations. Inasmuch as there is a clear difference between the initial sequences of the human LDH compared to the

sequence of crocodile LDH, the initial regions may have importance in the enzymatic activity. However, there is a great need for more structural investigation to reveal the importance of the initial sequences. 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. The hydroxyl residue of Tyr is generally important in enzymatic activities through phosphorylation. Since there is no phosphorylation residue in the 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 (Bian et al., 2014; Huang et al., 2021; 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 *C. porosus* 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 structure (ribbon model) of the lactate dehydrogenase is shown in Figure 3.

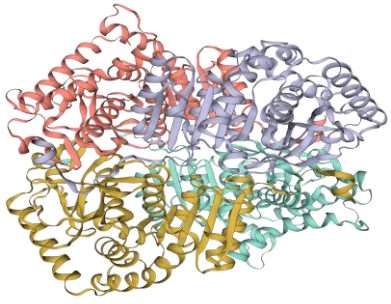

Figure 3. Modelling of the lactate dehydrogenase from *C. porosus* via Swiss Model (Waterhouse et al., 2018).

The sequence identity percentage was found as 88.48%. The plot related to the local quality estimation versus residue number was drawn in Figure 4.

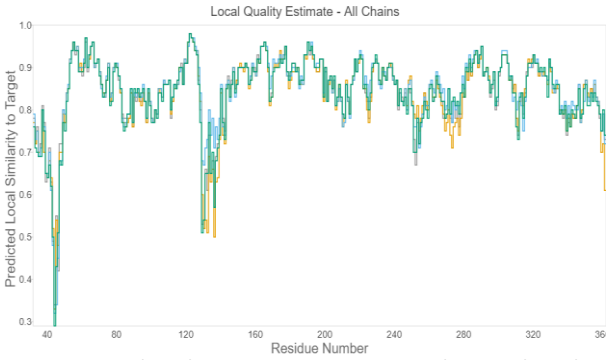

Figure 4. Local quality estimation versus residue number plot.

Qmean Z-Scores as QMean, CβQMEANDisco Global values were found as 0.86 and 0.84, respectively (Studer et al., 2021; Waterhouse et al., 2018). Normalised QMEAN4 Score, which is composed of four statistical potential terms and shows the quality of the model, versus the residue number plot was shown in Figure 5.

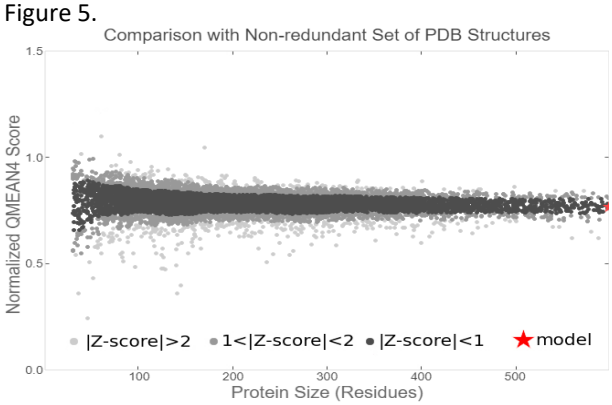

Figure 5. Normalised QMEAN4 Score versus residue number plot.

Since the values in Figure 5 are considered to be high, the model is acceptable. 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 the resolution is 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 the 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 researchers 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. Inaddition 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 one 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 muscles during endurance training. Similar scientific reports can be found in sports 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 acid 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 a big contribution to the understanding the 3-Dimensional structures of the enzymes studied.

As can be seen from Figure 6, not only 3-dimensional structures but also different characteristics such as polarity, amino acid sequence similarities, sizes, and charges can also be shown on the 3-D structures. Any modification on the enzyme structure can also be interpreted from these images (Figure 6).

Observation of different modifications in the amino acid sequences of elite athletes may open a new route of scientific investigations in the sports 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 of Olympic and World Champions. A sample figure is also drawn to explain the latter (Figure 7).

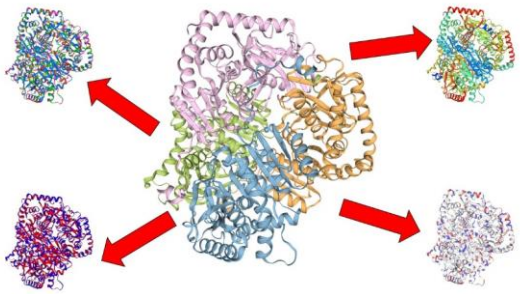

Figure 6. Four different drawings of the lactate dehydrogenase in Swiss-Model based on the different characteristics. Left-upper: Clustal, Left-down: size, Right-upper: rainbow, Right-down: charged amino acids.

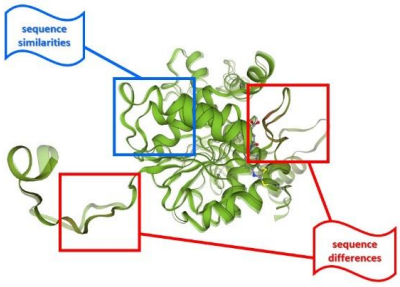

Figure 7. Superposition of two different lactate dehydrogenase structures. Sequence differences and similarities are shown within the figure.

Two sequences can be compared by superposition in Swiss-Model and this could give important ideas to other sports scientists about the enzymes in elite athletes. Moreover, the methodology mentioned in this paper could also be extended to other sports disciplines.

Conclusion

Comparison of LDHs in humans and crocodiles by using the *in silico* tools show that bioinformatics may have a potential application area in sports science. Possible modifications and/or mutations in side chains of amino acids may alter the enzymatic activity. In this route, bioinformatics may provide a great contribution to sports biochemistry and physiology by analysing sequence of the enzymes which are important in athletic performance. The animals such as crocodiles have-long years-experienced evolution for better physical performance for their survival in nature. Therefore, the sequence similarities, differences, and also important modifications of the selected animals could be used in talent selection. Moreover, understanding the modifications at amino acid sequences in elite athletes may also contribute to the latter. 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 sports. In conclusion, sports bioinformatics is waiting to be explored: Let's start for the ideas from other sports disciplines.

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References

Alam, M. T., Olin-Sandoval, V., Stincone, A., Keller, M. A., Zelezniak, A., Luisi, B. F., & Ralser, M. (2017). The self-inhibitory nature of metabolic networks and its alleviation through compartmentalization. *Nature Communications*, *8*(1), 1-13. https://doi.org/10.1038/ncomms16018

Baldwin J., Seymour R. S., Webb G. J. W. (1995). Scaling of anaerobic metabolism during exercise in the estuarine crocodile (*Crocodylus porosus*). Comparative Biochemistry and Physiolology A, 112, 285-293. https://doi.org/10.1016/0300-9629(95)00100-X

Barranco, T., Tvarijonaviciute, A., Tecles, F., Carrillo, J. M., Sánchez-Resalt, C., Jimenez-Reyes, P., Rubio M., García-Balletbó M., Cerón J. J., & Cugat, R. (2017). Changes in creatine kinase, lactate dehydrogenase and aspartate aminotransferase in saliva samples after an intense exercise: a pilot study. The *Journal of Sports Medicine and Physical Fitness*, *58*(6), 910-916. https://doi.org/10.23736/S0022-4707.17.07214-0

Bennett A. F., Seymour R. S., Bradford D. F., Webb G. J. W. (1985). Mass-dependence of anaerobic metabolism and acid-base disturbance during activity in the salt-water crocodile, *Crocodylus porosus*. *Journal of Experimental Biology*, *118*, 161-171. https://doi.org/10.1242/jeb.118.1.161

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., & Bourne, P. E. (2000). The protein data bank. *Nucleic Acids Research*, *28*(1), 235-242. https://doi.org/10.1093/nar/28.1.235

Bertoni, M., Kiefer, F., Biasini, M., Bordoli, L., & Schwede, T. (2017). Modeling protein quaternary structure of homo-and hetero-oligomers beyond binary interactions by homology. *Scientific Reports*, *7*(1), 1-15. https://doi.org/10.1038/s41598-017-09654-8

Bian, Y., Song, C., Cheng, K., Dong, M., Wang, F., Huang, J., Sun D., Wang L., Ye M., & Zou, H. (2014). An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. *Journal of Proteomics*, 96, 253-262. https://doi.org/10.1016/j.jprot.2013.11.014

Bienert, S., Waterhouse, A., de Beer, T. A., Tauriello, G., Studer, G., Bordoli, L., & Schwede, T. (2017). The SWISS-MODEL Repository-new features and functionality. *Nucleic Acids Research*, *45*(D1), D313-D319. https://doi.org/10.1093/nar/gkw1132

Bienvenut, W. V., Sumpton, D., Martinez, A., Lilla, S., Espagne, C., Meinnel, T., & Giglione, C. (2012). Comparative large scale characterization of plant versus mammal proteins reveals similar and idiosyncratic N-α-acetylation features. *Molecular & Cellular Proteomics*, *11*(6), M111.015131. https://doi.org/10.1074/mcp.M111.015131

Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehman, M., Walther, T. C., Olsen J. V., & Mann, M. (2009). Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science*, 325(5942), 834-840. https://doi.org/10.1126/science.1175371

Fish FE, Bostic SA, Nicastro AJ, Beneski JT. (2007). Death roll of the alligator: mechanics of twist feeding in water. *The Journal of Experimental Biology*, 210, 2811–2818. https://doi.org/10.1242/jeb.004267.

Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A. Protein Identification and Analysis Tools on the ExPASy Server; (In) John M. Walker (ed): The Proteomics Protocols Handbook, *Humana Press* (2005). pp. 571-607.

Gauci, S., Helbig, A. O., Slijper, M., Krijgsveld, J., Heck, A. J., & Mohammed, S. (2009). Lys-N and trypsin cover complementary parts of the phosphoproteome in a refined SCX-based approach. *Analytical Chemistry*, *81*(11), 4493-4501. https://doi.org/10.1021/ac9004309

Hendriks, I. A., Lyon, D., Young, C., Jensen, L. J., Vertegaal, A. C., & Nielsen, M. L. (2017). Site-specific mapping of the human SUMO proteome reveals co-modification with phosphorylation. *Nature Structural & Molecular Biology*, *24*(3), 325-336. https://doi.org/10.1038/nsmb.3366

Hoff, M. L. M., Fabrizius, A., Folkow, L. P., & Burmester, T. (2016). An atypical distribution of lactate dehydrogenase isoenzymes in the hooded seal (Cystophora cristata) brain may reflect a biochemical adaptation to diving. *Journal of Comparative Physiology B*, *186*(3), 373-386. https://doi.org/10.1007/s00360-015-0956-y

Huang, N., Li, F., Zhang, M., Zhou, H., Chen, Z., Ma, X., Yang, L., Wu, X., Zhong, J., Xiao, F., Yang, X., Zhao, K., Li, X., Xia, X., Liu, Z., Gao, S., & Zhang, N. (2021). An Upstream Open Reading Frame in Phosphatase and Tensin Homolog Encodes a Circuit Breaker of Lactate Metabolism. *Cell Metabolism*, *33*(1), 128-144. https://doi.org/10.1016/j.cmet.2020.12.008

Mayya, V., Lundgren, D. H., Hwang, S. I., Rezaul, K., Wu, L., Eng, J. K., Rodionov, V., & Han, D. K. (2009). Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions. *Science Signaling*, *2*(84), ra46-ra46. https://doi.org/10.1126/scisignal.2000007

Owerkowicz, T., Baudinette, R. V. (2008). Exercise training enhances aerobic capacity in juvenile estuarine crocodiles (*Crocodylus porosus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *150*(2), 211-216. https://doi.org/10.1016/j.cbpa.2008.04.594

Read, J. A., Winter, V. J., Eszes, C. M., Sessions, R. B., & Brady, R. L. (2001). Structural basis for altered activity of M- and H-isozyme forms of human lactate dehydrogenase. *Proteins: Structure, Function, and Bioinformatics*, *43*(2), 175-185. https://doi.org/10.1002/10970134(20010501)43:2<175::aid-prot1029>3.0.co;2-#

Rodrigues, B. M., Dantas, E., de Salles, B. F., Miranda, H., Koch, A. J., Willardson, J. M., & Simão, R. (2010). Creatine kinase and lactate dehydrogenase responses after upper-body resistance exercise with different rest intervals. *The Journal of Strength & Conditioning Research*, *24*(6), 1657-1662. https://doi.org/10.1519/JSC.0b013e3181d8e6b1

Rumley, A. G., Pettigrew, A. R., Colgan, M. E., Taylor, R., Grant, S., Manzie, A., Findlay, I., Dargie, H., & Elliott, A. (1985). Serum lactate dehydrogenase and creatine

kinase during marathon training. *British Journal of Sports Medicine*, *19*(3), 152-155. https://doi.org/10.1136/bjsm.19.3.152

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, *7*(1), 539. https://doi.org/10.1038/msb.2011.75

Studer, G., Rempfer, C., Waterhouse, A. M., Gumienny, R., Haas, J., & Schwede, T. (2020). QMEANDisCo-distance constraints applied on model quality estimation. *Bioinformatics*, *36*(6), 1765-1771. https://doi.org/10.1093/bioinformatics/btz828

Studer, G., Tauriello, G., Bienert, S., Biasini, M., Johner, N., & Schwede, T. (2021). ProMod3-A versatile homology modelling toolbox. *PLoS Computational Biology*, *17*(1), e1008667. https://doi.org/10.1371/journal.pcbi.1008667

The Uni Prot Consortium, the universal protein knowledgebase in 2021. *Nucleic Acids Research*, 2021, 49.D1: D480-D489. https://doi.org/10.1093/nar/gkaa1100

Voet D., Voet J. G., (2004). *Biochemistry*, 3rd Edition, John Wiley Sons. USA.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., De Beer, T. A. P., Rempfer, C., Bordoli, L., Lepore, R., & Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research*, 46(W1), W296-W303. https://doi.org/10.1093/nar/gky427

Yard, E. E., & Comstock, R. D. (2008). A comparison of pediatric freestyle and Greco-Roman wrestling injuries sustained during a 2006 US national tournament. *Scandinavian Journal of Medicine & Science in Sports*, *18*(4), 491-497. https://doi.org/10.1111/j.1600-0838.2007.00716.x

Zhou, H., Di Palma, S., Preisinger, C., Peng, M., Polat, A. N., Heck, A. J., & Mohammed, S. (2013). Toward a comprehensive characterization of a human cancer cell phosphoproteome. *Journal of Proteome Research*, *12*(1), 260-271. https://doi.org/10.1021/pr300630k