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COMPUTATIONAL EXPLORATION OF THE INTERACTIONS BETWEEN BUFFALO SPERM-DERIVED POLYUNSATURATED FATTY ACIDS AND ANTIFREEZE PROTEINS

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Abstract

The cryopreservation of buffalo sperm remains difficult because of the sensitivity of polyunsaturated fatty acids (PUFAs) in the sperm membrane to oxidative destruction. PUFAs, including docosahexaenoic acid (DHA), linoleic acid, eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA), are essential for membrane fluidity, structural integrity, and sperm function. The peroxidation of these bacteria under freezing conditions reduces membrane stability, resulting in decreased motility and fertility. Antifreeze proteins (AFPs), which are known for their distinct ice-binding and ice-growth inhibition capabilities, represent a viable method not only for preventing ice crystal formation but also for interacting with and stabilizing lipid components. The molecular interactions between selected AFPs and important buffalo sperm PUFAs were evaluated in this study via molecular docking and PM6 semiempirical energy optimization. Eight AFP structures from the Type II and Type III classes were docked with the four target PUFAs. The findings demonstrated that all fatty acids had favourable binding energies, with DHA demonstrating the strongest and most stable associations across numerous AFPs, notably with Type III variations such as 1KDE and 1HG7. The contact mechanisms included hydrophobic alignment and hydrogen bonding, indicating structural complementarity between AFP surfaces and PUFA chains. These findings indicate that AFPs may have a dual cryoprotective effect, alleviating oxidative stress while also strengthening the integrity of PUFA-rich sperm membranes. The discovered molecular affinity provides a mechanism for incorporating AFPs into enhanced cryopreservation methods. By understanding the nature of PUFA-AFP interactions, this study helps in the design of physiologically informed strategies aimed at increasing postthaw sperm survival and reproductive results in buffalo and possibly other species.

INTRODUCTION

Some organisms, such as fish, insects, plants and soil bacteria, contain ice-binding proteins called antifreeze proteins, which inhibit ice growth in organisms living at very cold temperatures (Białkowska *et al.*, 2020). In this way, these proteins support the ability of organisms to live at freezing temperatures. Antifreeze proteins (AFPs) are ice-binding proteins that prevent water from freezing by inhibiting ice crystal growth. AFPs, which are found in various species, including fish, plants, and microorganisms, enable organisms to survive at subzero temperatures. The distribution of these genes across species is thought to result from convergent evolution or horizontal gene transfer (Chi Fai Cheung *et al.*, 2017). AFPs have potential applications in food processing, cryopreservation, and anti-icing material development, offering benefits

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from the frost resistance of the organisms that produce them. This review provides an overview of the current understanding of AFPs, including their interactions and mechanisms (Eskandari *et al.,* 2020). Type II and Type III AFPs are structurally diverse and have varying ice-binding affinities. Type III AFPs have a globular β-rich architecture and amphipathic surfaces, allowing for nonice-related interactions, including lipid binding. This work investigated their potential interactions with biologically relevant PUFAs found in buffalo sperm membranes, which are extremely vulnerable to lipid peroxidation during freezing (Evans *et al*., 2012; Campbell, 2010).

There are different types of AFPs extracted from different species. AFPs of different types have different ranges of molecular weights because of differences in sequence in different fishes. Their range of molecular weights is 3.3 kDa to 4.5 kDa. AFP I is rich in alanine at approximately 60 mole% extracted from northern winter flounder *(Pleuronectes arnericarzus)*. AFP II is similar to C-type lectins extracted from sea ravens *(Hemitripterus arnericanus)* (Kristiansen, 2020).

Fishes living in polar oceans have type I antifreeze proteins in their body fluids. This protein has a helical structure that is rich in alanine. There are two forms of these proteins on the basis of their origin. One belongs to the winter flounder and has a sequence of HPLC6 and repeating units of 11 residues along with the starting residue of threonine. Another form belongs to the sculpins, which do not contain repeating units of 11 residues. However, some amino acids are present in its structure, which disrupt the helix, resulting in helix breakers in the N-terminal region. Two proteins, HPLC6 and HPLC8, are present in the winter flounder. These proteins are different from each other in two residues. One is residue 22, and the other is residue 26 (DeVries, 2020).

Nonglycoprotein proteins are extracted from many species, such as sea ravens, smelts and Atlantic herring. These proteins are also called nonglycoproteins and significantly take part in thermal hysteresis. These proteins have disulfide bonds present in cysteines, and these bonds stabilize their structure. Two sets of structures are present in tertiary proteins: one contains two β chains, and the other contains two chains of α-helices with a molecular mass of approximately 11 to 24 kDa (Vidanarachchi *et al.,* 2014; Eskandari *et al.,* 2020).

Bufflao (*Bubalus bubalis*) belongs to the Bovidae family. According to the Food and Agricultural Organization (FAO) report of 2004, the population of buffalo is almost 170 million. Mostly, they are found in Asian rivers. The buffaloes are located in five major regions. These are the Central Indian, Murrah, South Indian, Uttar Pradesh and Gujarat breeds. Bufflao, which produces more milk, is referred to as Nili–Ravi buffalo from the Murrah group (Minervino *et al.,* 2020). In buffalo sperm, the most important component is phospholipids. Its components include sinogomyelin (8.0 ± 1.9), phosphatidylethanolamine (22.9 ± 1.6), phosphatidyl inositol (2.5 ± 0.4), phosphatidyl choline (66.0 ± 3.5), diphosphatidyl glycerol (5.0 ± 0.9), lysophosphatidylcholine, phosphatidyl serine (3.5 ± 0.5), and phosphatidyl glycerol (4.7 ± 0.6) (Andrabi, 2009).

Lipid phase transition during the cryopreservation of sperm involves leakage of potassium ions from the plasma membrane of the sperm. This phase transition causes damage to sperm during freezing at very cold temperatures. PUFAs are present in large amounts in buffalo sperm, along with saturated fatty acids such as stearic acid. Among PUFAs, the main PUFA is docohexanoic acid (22:6). Among aldehydes, palmitalaldehyde (16:0) is the main aldehyde (Dalal *et al.,* 2018; Andrabi, 2009).

Materials and Methods

Eight antifreeze proteins (AFPs) representing both Type II and Type III classes were selected for molecular docking. Their three-dimensional structures were retrieved in PDB format from the RCSB Protein Data Bank. The selected AFPs included PDB IDs: **1KDE, 1HG7, 1B7J, 1UCS, 4UR6, 2AFP, 2PY2,** and **1WFA,** corresponding to various cold-adapted species such as *Zoarces americanus* and winter flounder.

AutoDock 4 version 1.5.6

In AutoDock4, the structures that we used were proteins. The protein in our work is an antifreeze protein taken in the form of a protein data bank (PDB) file obtained from the RCS Bank. After saving protein in pdb, this file is opened in autodock in the following way: Open the autodock tools version 1.5.6,

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click the file and then click on the read molecule, i.e., the protein in the pdb file that is saved. Then, the algorithm clicks on the edit option, which has many options, such as bonds, deletions, atoms, charges, etc.,, and follows the steps in sequence. For example, click on option hydrogen- Add hydrogen (polar only). Then, kollman charges are added by clicking the charge option. Click on delete water molecules. Click on atoms to assign AD4-type atoms. Our protein is then prepared and saved in a pdb file by clicking on the file and writing the PDB.

Ligand preparation

The ligand is then prepared in the same way. Our ligand, which is obtained from PubChem, is saved in the form of an SDF file. Since autodock reads only those files that are in PDB, the ligand file is converted into a PDB file via open babble software. Now, click the option file, read the molecule, i.e., take the ligand PDB file, and then repeat all options as discussed above. Only one change occurs here, that is, add compute gasteiger charges instead of kollman charges. Finally, the ligand is saved in PDBQT by clicking on the file menu and the open output option.

AutoGird

After both proteins and ligands are prepared, the next step is to run AutoGird via the following steps: Move to the brown color menu bar and click on the Gird option and further click on the macromolecule, choose the protein and save it. Click on set map types on the Grid menu, choose the ligand, and select the ligand. Then, the algorithm clicks on the grid box, makes the file and saves it. Next, we move on the output and save it in the .gpf file. Now, we move on the docking menu, click on the macromolecule and then open the protein in pdbqt format by clicking on the set rigid file name.

By further clicking on the docking menu, the ligand can be selected by clicking on the chosen ligand. Now, in the docking menu, click on the output, further clicking on the Lamarckian GA and saving the file in the form of .dpf. To run AutoGird, the program path name autogird4 is selected. Now, the parameter filename gird.gpf is selected. These two files are saved in the file directory that is folder C. After that, they click on the launch to run autogird. To run autodock, the following steps are performed: Select the program path name as autodock4.exe, which is saved in file directory C. Select the parameter filename as dock.dpf and then click on the launch. After running autodock, a dlg file is prepared.

Docking analysis

Now, we have to analyse the docking by performing the following steps: move on the Analyse menu, click on docking and select the dlg file. The macromolecules are further clicked on, and the protein is selected. Now, select the conformation in the Analyse menu. In conformation, click-on play is ranked by energy. Finally, the & button is clicked on. In the same section, we click on set play options and build hydrogen bonds. Click on show information, view the interaction energy and write complex, save in file.pdb.

Postdocking visualization was conducted via PyMOL 2.5, and the interaction energy was further evaluated via PM6 semiempirical optimization via Gaussian 09.

PyMOL Software

It was installed at https://pymol.en.softonic.com/. It is used to visualize protein structure, ligands and complexes and was prepared from Autodock4. In PyMOL, Click on file menu, move on an open bar from where a protein, our ligand or complex in pdb was taken. Our structure is visualized on the PyMOL interface. On the right side of the PyMOL interface, four options are shown on the bar. These are Action, Hide, Show, Label and Color. After the structure is prepared to use all these options, the resulting structure is saved by moving on the file menu and clicking on the export image as PNG.

Gaussian 09 software

This software is used to calculate the energies of different structures, form different models of complex structures and optimize them, etc. In our work, the complex is prepared from AutoDock Vina in the form of a pdb file. In the guassian view, this file is opened, the view menu is clicked, all options are selected, the file menu is moved, and the file is saved in the form of a guassian input file.

Proteins used in the study

IKDE is a type III antifreeze protein that is found in the North Atlantic Ocean. It is an isoform of the

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HPLC12 mutant. IMSI is an antifreeze glycoprotein. IUCS is a globular and type III antifreeze protein. 4UR6 is a type III antifreeze protein found in Zoarces viviparous (ZvAFP6). IB7J is also a type III antifreeze protein with a globular structure. 2SPG is a type III antifreeze protein that belongs to the isoform HPLC 12 T15S and is extracted from Zoarces americanus, which is a type of ocean pout fish (Figure 1a-1e). 2PY2 is a type II antifreeze protein that contains six chains and has 136 residues. IWFAs, referred to as winter flounder antifreeze proteins, live at 4°C. It has two chains with 38 residues. 2AFP is a type II antifreeze protein that belongs to the lectin family. Because its structure in solution resembles that of that family. It has one chain with 129 residues. 1HG7 is a type III antifreeze protein with an HPLC12 structure extracted from ocean pout fishes. It has a chain with 66 residues (Figure 2a-2e).

Ligands used in the study

Ligands used in our study were obtained from PubChem. These ligands are essentially fatty acids and phospholipids present in buffalo spermatozoa. Ligands such as Docohexanoic Acid have a structure that is deduced from PubChem, *i.e.,* PubChem CID 445580, and this structure contains six double bonds, which are cis to each other. Its molecular formula is $C_{22}H_{32}O_2$. The ligand linoleic acid is a polyunsaturated fatty acid, PubChem CID 5280450, which has two double bonds. Its molecular formula is $C_{18}H_{32}O_2$, whereas lysophosphatidylcholine is a phospholipid with PubChem CID 5311264. Its molecular formula is $C_{10}H_{22}NO_7P$. Eicosapentanoic acid is a polyunsaturated fatty acid containing six double bonds. Its structure is obtained from PubChem CID 446284. Its molecular formula is $C_{20}H_{30}O_2$, whereas docosapentanoic acid contains five double bonds; thus, it is a polyunsaturated fatty acid with PubChem CID 5497182. Its molecular formula is $C_{22}H_{34}O_2$ (Figure 3a-3e).

Results

To study the interaction between proteins and ligands, AutoDock4 software was used. Protein pdb files obtained from the RCSB protein data bank. Ligands are obtained from PubChem and saved in a sdf file. Since ligands are used in pdb format in docking, converting the sdf file into pdb files via open babble software is compulsory (Figure 4).

The binding affinities (kcal/mol) of the five PUFA-AFP complexes revealed that lower (more negative) values indicate stronger binding. Docosahexaenoic acid (DHA) had the highest binding affinity for the AFPs 1KDE (−5.9 kcal/mol) and 1HG7 (−5.4 kcal/mol), suggesting persistent connections via hydrophobic contacts and hydrogen bonding. Eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) exhibited favourable binding to 1KDE and 3VN3, respectively. Linoleic acid had the poorest interaction of all the PUFAs studied. These findings corroborate the structural complementarity of long-chain PUFAs and Type III AFPs, suggesting their potential involvement in lipid stabilization during cryopreservation (Table 1).

Dockohexanoic acid

Now, we use a new ligand, docohexanoic acid, which is docked with protein 2py2. The structure of the ligand was obtained from PubChem and analysed via PyMOL. Molecular docking analysis demonstrating the binding interaction of docosahexaenoic acid with the antifreeze protein's active site. The ligand is represented in stick form, fitting snuggly into the electrostatically defined binding pocket. The connection is stabilized by hydrophobic interactions and possibly hydrogen bonding, resulting in high affinity and compatibility with the protein active site design (Figure 4a).

Docosahexaenoic acid (DHA) interacts with the antifreeze protein, as visualized via molecular docking. The electrostatic potential determines the color of the protein surface, which includes positively charged (blue), negatively charged (red), and hydrophobic (green/yellow) areas. The DHA molecule, depicted in a zigzag shape, covers the binding groove, implying significant hydrophobic interactions along the fatty acid chain. The ligand orientation results in significant surface complementarity, which contributes to sustained and energetically favourable binding (Figure 4b).

The results illustrate a molecular docking model that shows the interaction between a ligand (in green) and the receptor's active site, which is represented by a surface with electrostatic potential fluctuations. The surface colouring shows electrostatic charge

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distributions, with red representing negative potential, blue representing positive potential, and yellow/white representing neutral potential. The visualization shows the ligand's binding conformation as well as the receptor's structural topography, allowing for better investigation of binding affinity and interaction specificity (Figure 4c).

Linoleic acid

Figure 5a depicts the docking interaction of the Winter Flounder Antifreeze Protein (1WFA) with linoleic acid. The receptor surface is coloured according to electrostatic potential: blue represents positive potential, red represents negative potential, and yellow/green represents neutral regions. The ligand linoleic acid (in green) is displayed in a binding position inside the receptor's active region, highlighting unique interactions. This docking visualization helps us comprehend the molecular connections and potential binding affinity between the protein and the fatty acid.

The graphic depicts the docking studies between Type II antifreeze protein (2PY2) and linoleic acid. The receptor's molecular surface is coloured according to electrostatic potential: blue for positive regions, red for negative regions, and green for neutral regions. The linoleic acid molecule (green) is shown bound at the receptor's active site, demonstrating the binding conformation and contact sites. This analysis revealed crucial residues and binding sites that are important for understanding protein–ligand interaction dynamics (Figure 5b).

Molecular docking was used to determine the interaction between the type III antifreeze protein (PDB ID: 1B7J) and linoleic acid. The docking stance depicts linoleic acid (green sticks) occupying a hydrophobic groove on the protein surface, which is coloured by electrostatic potential. The contact is stabilized by van der Waals forces and a possible hydrogen bond (yellow dotted line), indicating a high binding affinity that could facilitate functional lipid–protein interactions (Figure 5c).

The docking of linoleic acid with the Type III Antifreeze Protein (PDB ID: 1HG7) shows that the ligand (green sticks) fills a shallow hydrophobic cleft on the protein surface, which is coloured by electrostatic potential. The binding appears to be predominantly mediated by hydrophobic interactions, as seen by the nonpolar surface patches around the ligand. This interaction shows that linoleic acid has a potential affinity for the protein, which may influence its structural or functional behaviour in lipid-associated settings (Figure 5d).

The docking study of linoleic acid with the type III antifreeze protein from the North Atlantic Ocean pool (PDB ID: 1KDE) revealed that the ligand (green sticks) was contained within a mainly hydrophobic pocket on the protein surface. The surface is coloured according to electrostatic potential, with red representing negative regions, blue representing positive regions, and yellow/white representing hydrophobic areas. This orientation supports strong hydrophobic interactions and potential hydrogen bonding at the polar head of linoleic acid, which would allow sustained binding within the protein's active groove (Figure 5e).

Discussion

This study involves a thorough in silico analysis of the molecular interactions between key buffalo sperm-derived polyunsaturated fatty acids (PUFAs) and selected antifreeze proteins (AFPs), with the goal of elucidating novel cryoprotective mechanisms for buffalo sperm cryopreservation. The findings show that certain AFPs, particularly Type III variants, have substantial and specific binding affinities for long-chain PUFAs, most notably docosahexaenoic acid (DHA), implying a promising synergistic role in stabilizing sperm plasma membranes during freezing.

Docosahexaenoic acid (DHA): The most promising ligand

DHA consistently had the highest binding affinities among the evaluated AFPs. The docking scores of −5.9 kcal/mol for AFP 1KDE and −5.4 kcal/mol for 1HG7 indicate stable and energetically favourable contact. Pose analysis revealed substantial hydrophobic interactions with residues, including ILE42, THR49, and THR50, as well as hydrogen bonding with GLY46 and GLY47. The electrostatic surface maps show that DHA is lodged within hydrophobic structures or grooves, which are ideal settings for stabilizing long hydrocarbon chains. Given the crucial biological involvement of DHA in sperm membrane fluidity, ion transport, and mitochondrial function, AFP stabilization may

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minimize lipid peroxidation under cryogenic stress, hence increasing postthaw motility and fertility. Furthermore, the carboxylic acid head group of DHA appears to have consistent polar interactions with positively charged or polar amino acids on AFP surfaces from reactive oxygen species (ROS) produced during cryopreservation. Similar studies were previously conducted by Xu *et al.* (2022) and Alpha *et al.* (2023).

Type III AFPs exhibit stronger and more specific binding

Comparative docking data demonstrate that Type III AFPs (e.g., 1KDE and 1HG7) consistently outperform Type II AFPs (e.g., 2AFP) and tiny antifreeze glycoprotein variants (3VN3) in terms of binding affinity and specificity. Type III AFPs are globular proteins with β-strands and amphipathic surfaces, which may allow them to interact with aqueous ice crystals and lipophilic ligands such as polyunsaturated fatty acids (PUFAs). Type II AFPs, which are lectin-like and calcium dependent, exhibited weaker or inconsistent binding (e.g., DHA binding to 2AFP was −4.6 kcal/mol), perhaps due to poorly developed hydrophobic binding clefts. Our results closely align with the studies of Kumari *et al.* (2020), Graham *et al.* (2021) and Zhang *et al.* (2024).

EPA, linoleic acid, and DPA: Moderate affinity and physiological relevance

The remaining examined PUFAs, eicosapentaenoic acid (EPA), linoleic acid, and docosapentaenoic acid (DPA), had moderate but still favourable binding affinities. For example, EPA has a −5.3 kcal/mol interaction with 1KDE, with hydrophobic interactions including ILE42 and THR49, whereas DPA interacts well with 3VN3 (−5.5 kcal/mol) and 1KDE (Wang *et al.*, 2024). These interactions, while significantly weaker than those of DHA, indicate the potential for noncovalent stabilization via van der Waals forces and occasional hydrogen bonding (Ahmmed *et al.*, 2020; Murakami *et al.*, 2023).

Functional implications for sperm cryopreservation

The dual interaction capabilities of AFPs, which include adhering to ice surfaces to hinder recrystallization and partnering with lipophilic molecules to stabilize cell membranes, may greatly increase buffalo spermatozoa cryosurvival. The sperm membrane is particularly high in PUFAs, which are susceptible to lipid peroxidation, resulting in membrane rupture, mitochondrial dysfunction, and DNA fragmentation during freeze–thaw cycles (Upadhyay *et al.*, 2021). In silico research supports the concept that AFPs can encapsulate or stabilize PUFAs, hence preserving the structural integrity of the sperm plasma membrane (Venkatesh *et al.*, 2024; Selvaraju *et al.*, 2023; Khan *et al.*, 2021).

Conclusion

This study provides a new mechanistic understanding of how AFPs, particularly Type III AFPs, may aid in lipid membrane protection during cryopreservation by interacting directly with physiologically relevant buffalo sperm PUFAs. The strong, specific, and hydrophobic interactions with DHA and EPA point to a lipid-stabilizing effect in addition to ice recrystallization inhibition. These findings pave the way for the creation of next-generation cryoprotective media supplemented with customized AFPs and PUFAs, potentially improving buffalo sperm preservation techniques and outcomes in artificial insemination and germplasm conservation.

Table 1: Molecular docking results showing binding affinities and key interactions between buffalo sperm-derived polyunsaturated fatty acids (PUFAs) and selected antifreeze proteins (AFPs).

Name	Protein (PDB)	Binding energy (kcal/mol	Key Interactions	H-bonds
DHA	1KDE	-5.9	ILE42, THR49	2
DHA	1HG7	-5.3	GLY46, GLY47	1
EPA	1KDE	-5.5	THR49	1
DPA	3VN3	-5.4	ILE44, LEU53	1
Linoleic acid	1B7J	-4.8	THR49	1

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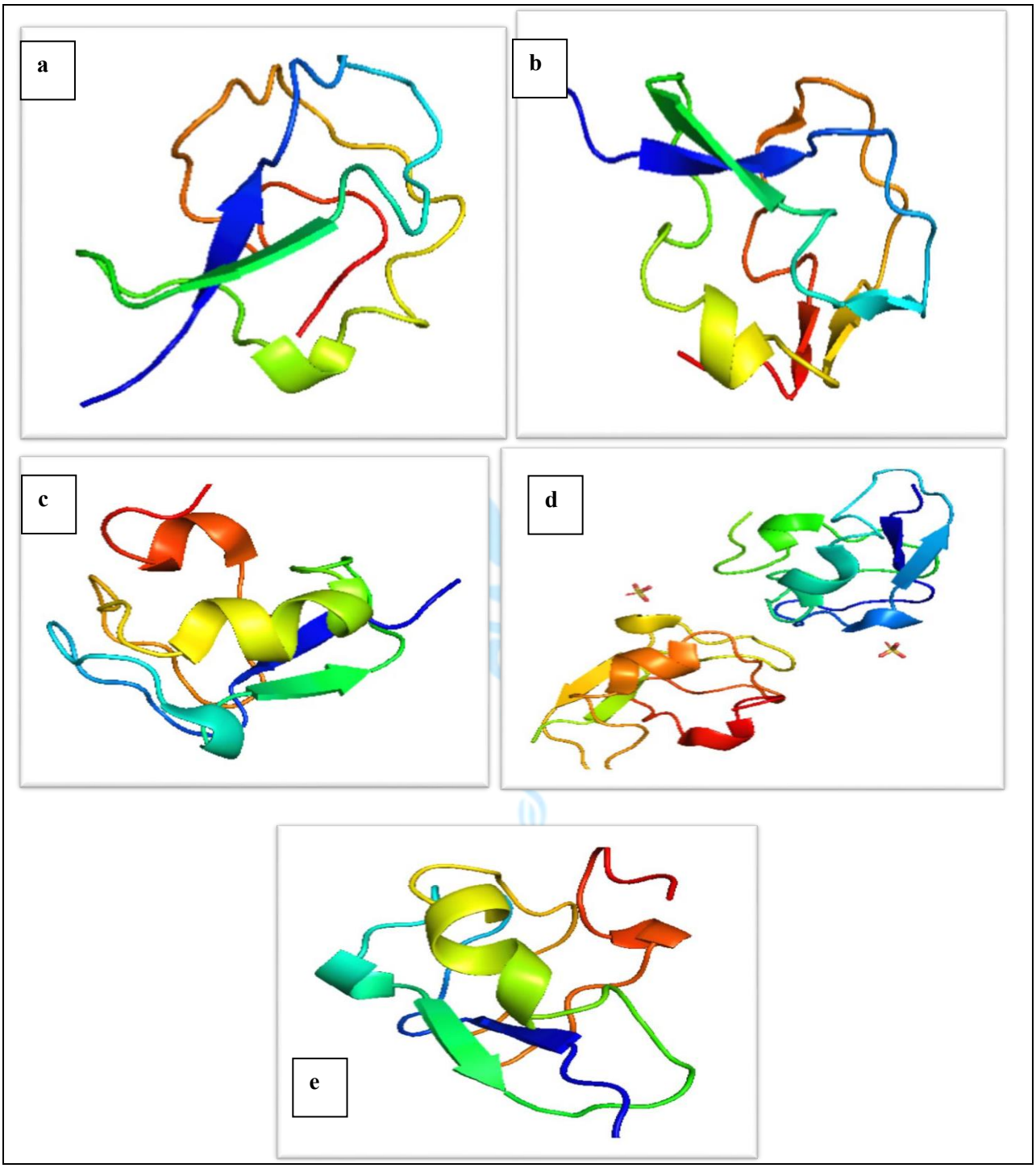

Figure 1 (1a-1e): Structure of 1KDE (a) Type III antifreeze protein of Northatlantic ocean pout, taken from pymol. IMSI (b) taken through HPLC12, Structure of IUCS (c), structure of Type III antifreeze protein ZvAFP6 (4UR6) (d). Structure of IBJ7(e)

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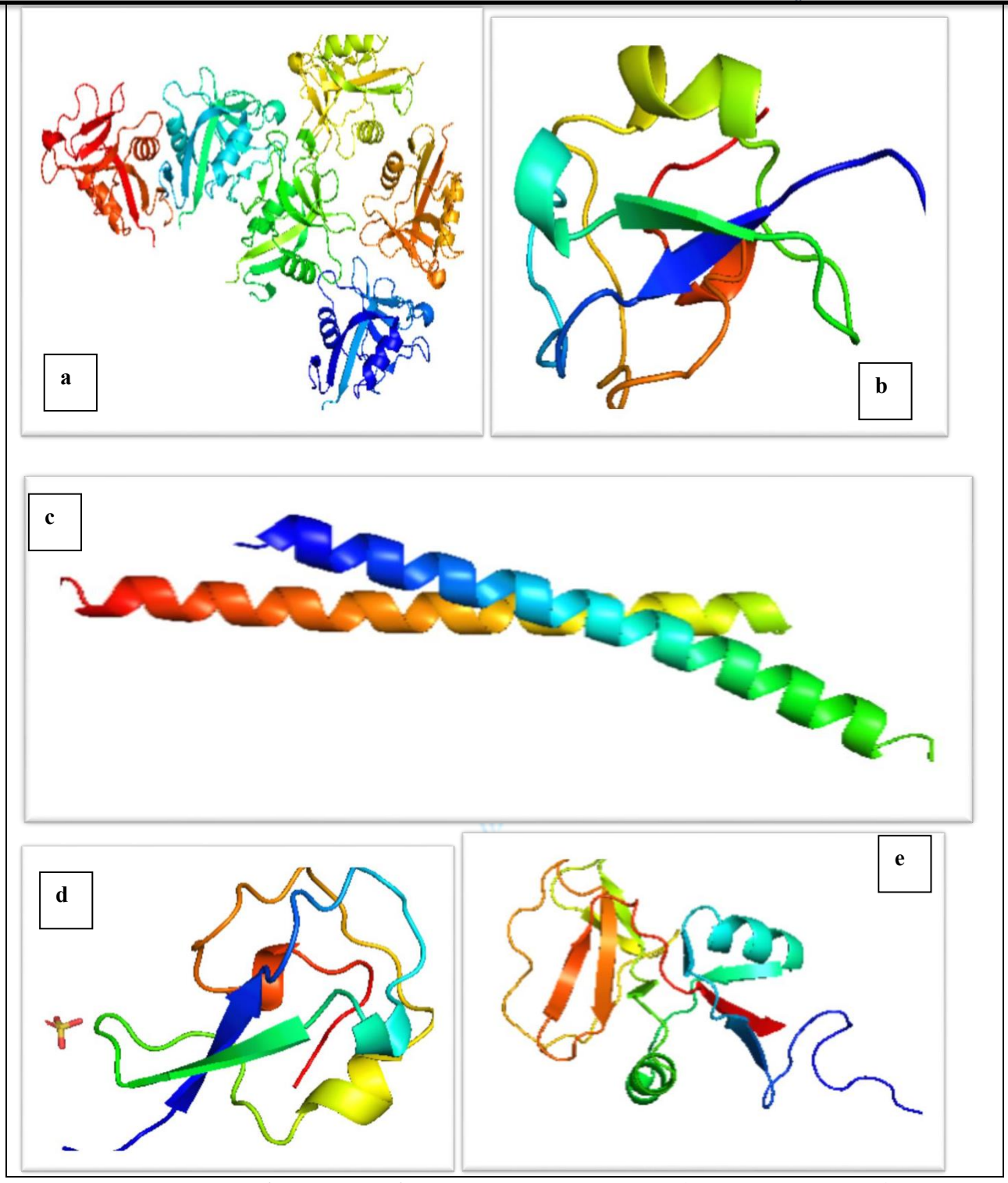

Figure 2 (2a-2e): Structure of Type III antifreeze protein 2SPG (a), 2PY2 (b), 1WFA (c), 2AFP (d), 1HG7 (e)

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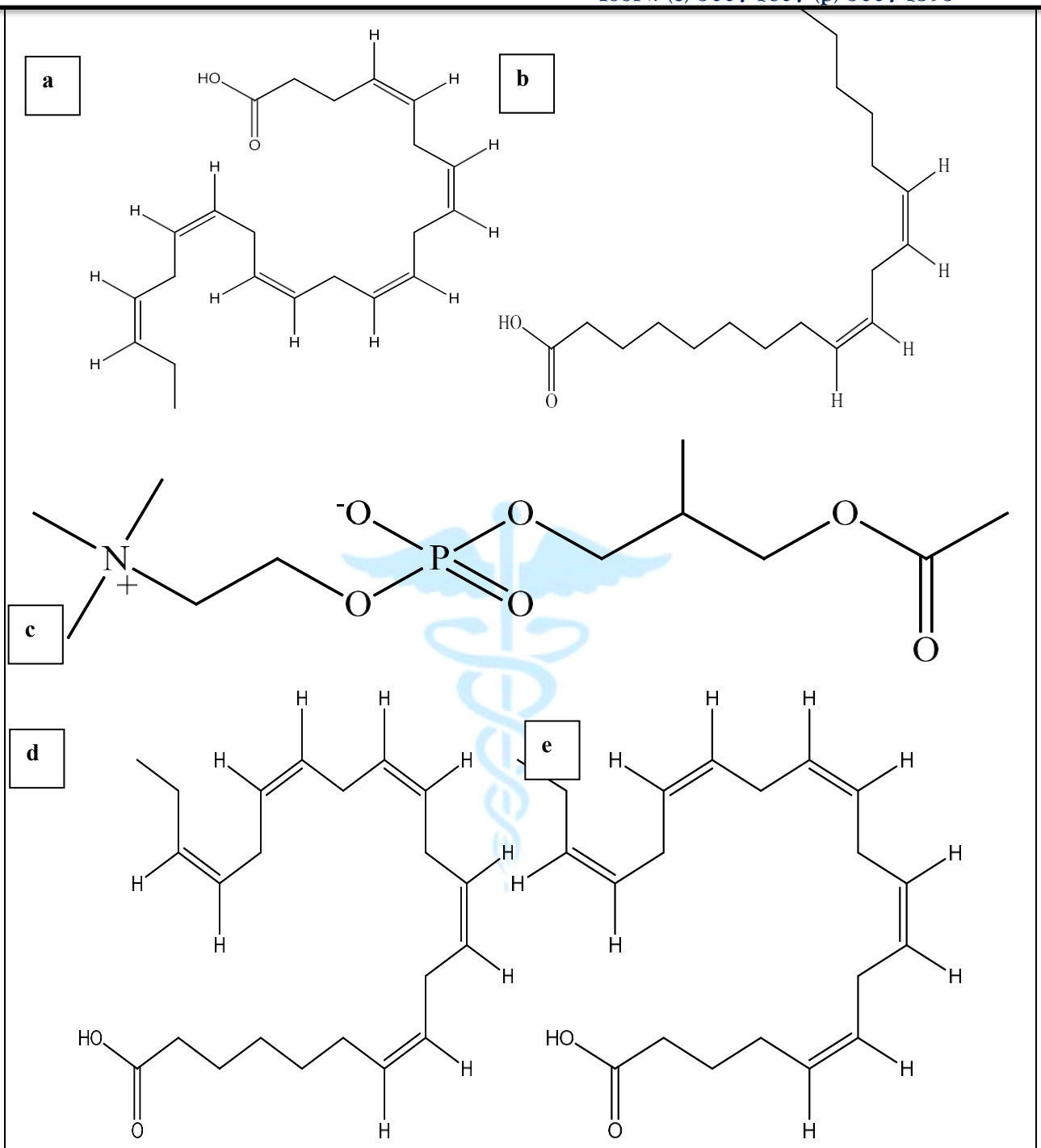

Figure 3 (3a-3e): Structure of Docohexanoic acid (a), Linoleic Acid (b), Lysophohatidylcholine (c), Eicosapentanoic Acid (d), Docosapentanoic (e) Acid by ChemDraw

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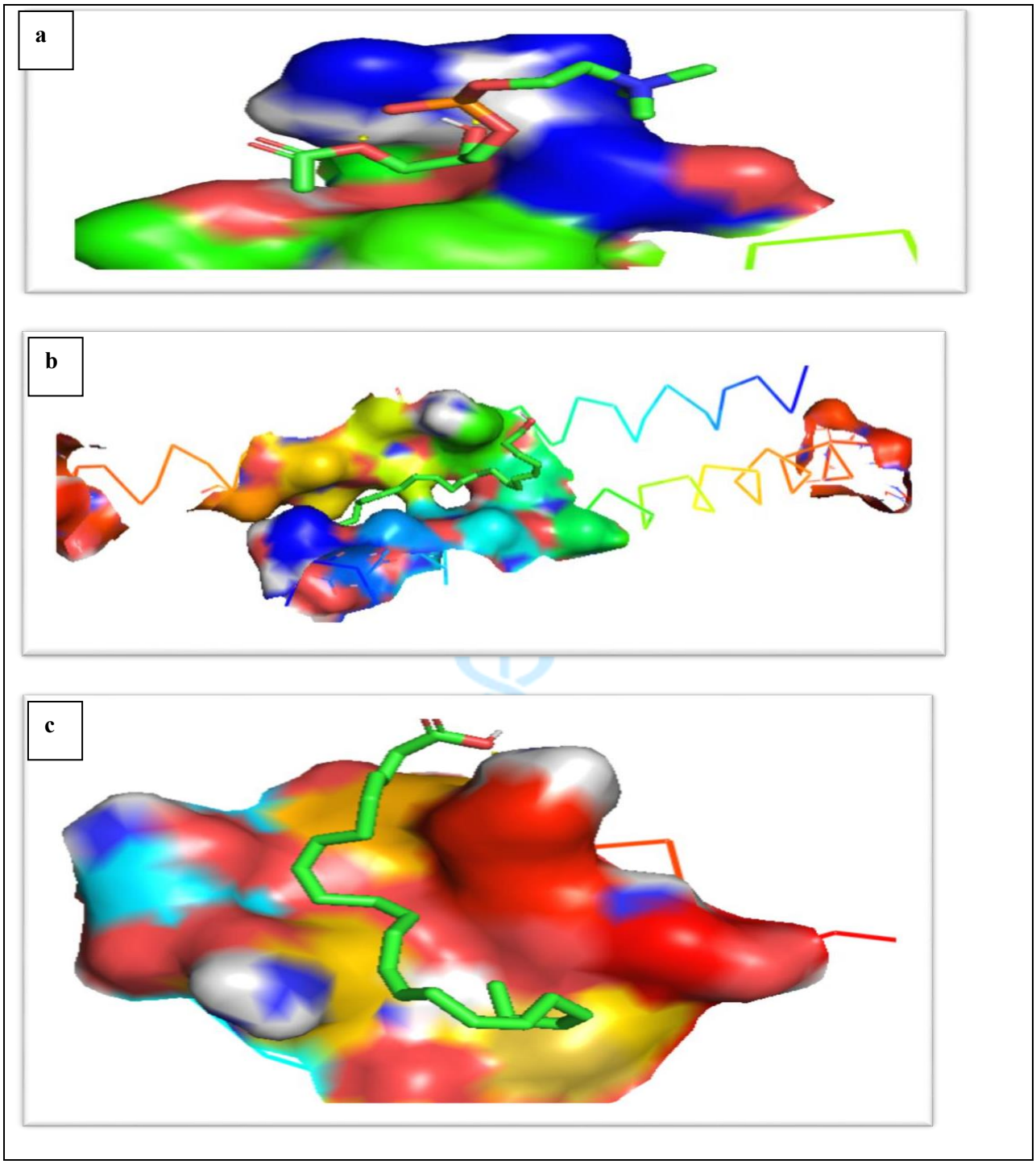

Figure 4 (4a-4c): Docking of Type II Antifreeze Protein (2PY2) against Docohexanoic Acid, Docking of Winter flounder Antifreeze protein (1WFA) against Docohexanoic Acid. Docking of Type III Antifreeze Protein of North Atlantic Ocean Pout (1KDE) against Docohexanoic Acid.

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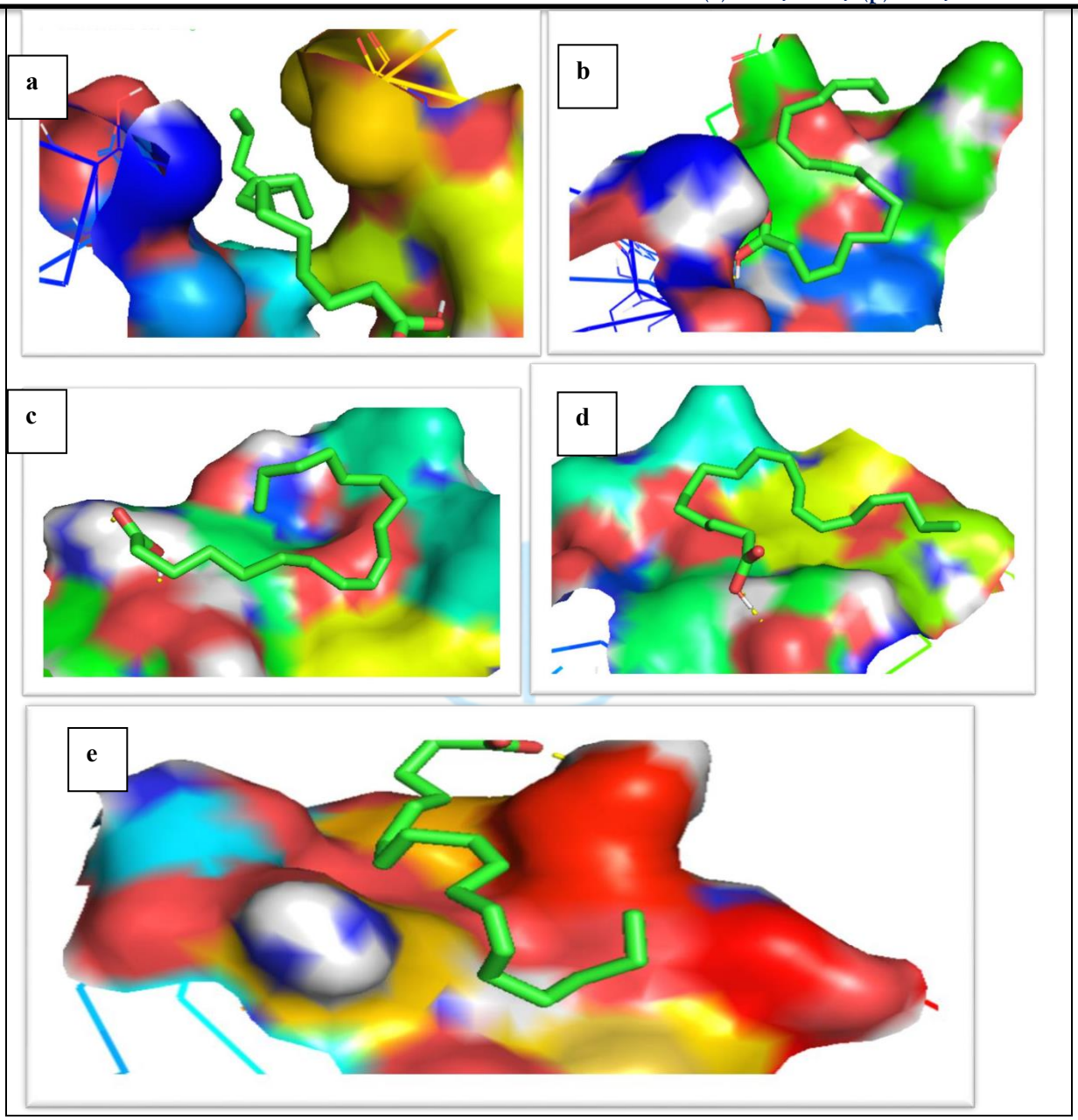

Figure 5 (5a-5e): Docking of Winter flounder Antifreeze protein (1WFA) against Linoleic Acid (a). Docking of Type II Antifreeze Protein (2PY2) against Linoleic Acid (b). Docking of Type III Antifreeze Protein (1B7J) against Linoleic Acid (c). Docking of Type III Antifreeze Potein (1HG7) against Linoleic Acid (d). Docking of Type III Antifreeze Protein of North Atlantic Ocean Pout (1KDE) against Linoleic Acid (e)

Declarations

Ethics approval

The Institutional Ethics and Guideline Committee of the University of Sargodha (Approval No. 128-H33/2019 UOS) has allowed all the protocols used in this experiment. All the experimental methods of this study followed all the appropriate guidance and regulations.

Consent for publication

All subjects gave their "informed consent" for the publication of details within the text ("informed

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consent") to be published in the above Journal and Article. Written "informed consent" was obtained from all authors for the publication of this manuscript.

Availability of data and materials

Data will be available from the on reasonable request

Competing Interest

All authors declare that there are no competing interests.

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REFERENCES

Ahmmed, M.K., Ahmmed, F., Tian, H., Carne, A. and Bekhit, A.E.D., 2020. Marine omega-3 (n-3) phospholipids: A comprehensive review of their properties, sources, bioavailability, and relation to brain health. Comprehensive Reviews in Food Science and Food Safety, 19(1), pp.64-123.

Alba, M.M., Ebright, B., Hua, B., Slarve, I., Zhou, Y., Jia, Y., Louie, S.G. and Stiles, B.L., 2023. Eicosanoids and other oxylipins in liver injury, inflammation and liver cancer development. Frontiers in Physiology, 14, p.1098467.

Białkowska, A., Majewska, E., Olczak, A. and Twarda-Clapa, A., 2020. Ice binding proteins: Diverse biological roles and applications in different types of industry. Biomolecules, 10(2), p.274.

Campbell, W., 2010. Novel sulphur-linked AFGP analogues: Synthesis and evaluation of antifreeze activity (Doctoral dissertation, University of Ottawa (Canada)).

Chi Fai Cheung, R., Bun Ng, T. and Ho Wong, J., 2017. Antifreeze proteins from diverse organisms and their applications: an overview. Current Protein and Peptide Science, 18(3), pp.262-283.

Dalal, J., Kumar, A., Kumar, P., Honparkhe, M., Malik, V.S., Singhal, S., Kaur, S. and Brar, P.S., 2018. Improvement in cryosurvival of buffalo bull (Bubalus bubalis) sperm by altering freezing rate within critical temperature range. Asian Pacific Journal of Reproduction, 7(2), pp.72-78.

DeVries, A.L., 2020. Fish antifreeze proteins. Antifreeze proteins volume 1: Environment, systematics and evolution, pp.85-129.

Eskandari, A., Leow, T.C., Rahman, M.B.A. and Oslan, S.N., 2020. Antifreeze proteins and their practical utilization in industry, medicine, and agriculture. Biomolecules, 10(12), p.1649.

Evans, C.W., Hellman, L., Middleditch, M., Wojnar, J.M., Brimble, M.A. and Devries, A.L., 2012. Synthesis and recycling of antifreeze glycoproteins in polar fishes. Antarctic Science, 24(3), pp.259-268.

Graham, L.A. and Davies, P.L., 2024. Convergent evolution of type I antifreeze proteins from four different progenitors in response to global cooling. BMC Molecular and Cell Biology, 25(1), p.27.

Khan, I.M., Cao, Z., Liu, H., Khan, A., Rahman, S.U., Khan, M.Z., Sathanawongs, A. and Zhang, Y., 2021. Impact of cryopreservation on spermatozoa freeze-thawed traits and relevance OMICS to assess sperm cryo-tolerance in farm animals. Frontiers in Veterinary Science, 8, p.609180.

Kristiansen, E., 2020. Characteristics of Antifreeze Proteins. Antifreeze Proteins Volume 2: Biochemistry, Molecular Biology and Applications, pp.9-41.

Kumari, S., Muthachikavil, A.V., Tiwari, J.K. and Punnathanam, S.N., 2020. Computational study of differences between antifreeze activity of type-III antifreeze protein from ocean pout and its mutant. Langmuir, 36(9), pp.2439-2448.

Minervino, A.H.H., Zava, M., Vecchio, D. and Borghese, A., 2020. Bubalus bubalis: A short story. Frontiers in veterinary science, 7, p.570413.

Frontier in Medical & Health Research

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Murakami, M., Sato, H. and Taketomi, Y., 2023. Modulation of immunity by the secreted phospholipase A2 family. Immunological reviews, 317(1), pp.42-70.

Selvaraju, S., Ramya, L., Swathi, D., Archana, S.S., Lavanya, M., Krishnappa, B., Binsila, B.K., Mahla, A.S., Arangasamy, A., Andonissamy, J. and Kumar, P., 2023. Cryostress induces fragmentation and alters the abundance of sperm transcripts associated with fertilizing competence and reproductive processes in buffalo. Cell and Tissue Research, 393(1), pp.181-199.

Upadhyay, V.R., Ramesh, V., Dewry, R.K., Kumar, G., Raval, K. and Patoliya, P., 2021. Implications of cryopreservation on structural and functional attributes of bovine spermatozoa: An overview. Andrologia, 53(8), p.e14154.

Venkatesh, S., Murugavel, K., Hemalatha, H., Kantharaj, S. and Shalini, G., 2024. PERSPECTIVE: Semen additives for improving frozen-thawed buffalo and cattle semen-a review. CryoLetters, 45(4), pp.194-211.

Vidanarachchi, J.K., Ranadheera, C.S., Wijerathne, T.D., Udayangani, R.M.C., Himali, S.M.C. and Pickova, J., 2014. Applications of seafood by-products in the food industry and human nutrition. Seafood Processing By-Products: Trends and Applications, pp.463-528.

Wang, L., Cheng, C., Yu, X., Guo, L., Wan, X., Xu, J., Xiang, X., Yang, J., Kang, J. and Deng, Q., 2024. Conversion of α-linolenic acid into n-3 long-chain polyunsaturated fatty acids: bioavailability and dietary regulation. Critical Reviews in Food Science and Nutrition, pp.1-33.

Xu, W., Hu, B., Cheng, Y., Guo, Y., Yao, W. and Qian, H., 2022. Material basis research for Echinacea purpurea (L.) Moench against hepatocellular carcinoma in a mouse model through integration of metabonomics and molecular docking. Phytomedicine, 98, p.153948.

Zhang, Y.H., Li, Z., Lu, L., Zeng, T., Chen, L., Li, H., Huang, T. and Cai, Y.D., 2021. Analysis of the sequence characteristics of antifreeze protein. Life, 11(6), p.520.