Research

Computational Structural Analysis and Interaction Network Profiling of Lipases: Implications for Biomedical and Therapeutic Applications

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Abstract

Lipases are widely used enzymes that facilitate the process of breaking down triglycerides to glycerol and fatty acids and are involved in different processes of lipid metabolism, drug metabolism, the diagnosis of diseases, and several industrial processes. In this paper, a detailed computational work has been conducted to examine the structural determinants of the stability of organic solvents in lipases. An eighty-two-lipase dataset was obtained in the Protein Data Bank and assessed by amino acid composition profiling, stabilizing structural interactions, and calculation of physicochemical properties. The parameters such as the hydrophobic interactions, salt bridges, hydrogen bonds, and packing density were determined to determine factors that contributed to the stability of the solvents. The analysis of protein-protein interaction with the STRING database also revealed the functional association of the lipases in the different species (including Homo sapiens, Pan troglodytes, and Mus musculus). This study has limitations, however, in terms of its being based on computational analysis and the small number of experimentally characterized solvent-stable lipases that can be compared. Thus, experimental validation by mutagenesis, soluble stability experiments with enzymes, and rational protein engineering strategies should be undertaken in future efforts to improve solvent tolerance level depending on the structural characteristics observed. The results can inform the structural aspects of lipase stability and can be used in the future in the fields of biomedical studies, drug development, and enzyme engineering.

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1. Introduction

The family of enzymes that promote the hydrolysis of triglycerides to free fatty acids and glycerol are called the lipases (triacylglycerol hydrolases) **(Javed *et al.* 2018)**. Lipases have numerous large-scale applications in most industrial and biotechnological applications, including food production, pharmaceutical production, biodiesel production, laundry detergent formulation, and organic production owing to their remarkable catalytic versatility **(Chandra *et al.* 2020)**. Unlike most enzymes, which cannot be utilized under anything but an aqueous environment, lipases are allowed a unique chance to not only be able to catalyze anything in an aqueous environment, but also organic solvents. This property makes them particularly handy in the industry where the reaction is likely to occur in non-aqueous conditions **(Sharma and Kanwar 2014)**. However, preservation of enzymes in organic solvents is a critical issue and when subjected to organic solvents there is a possibility of perturbation of the structure, loss of activity and denaturation of the proteins. Enzymatic lipases that resist organic solvents have therefore been given much attention due to their resistance to harsh chemical environments regarding structural integrity and catalytic activity **(Kumar *et al.* 2016)**. The enzymes are also able to work in polar and nonpolar organic solvents and this has drastically increased their application in industrial biocatalysis. Organic solvents, used in synthetic chemistry, generally enhance the solubility of substrates, change reaction equilibria and allow the formation of hydrophobic compounds to be possible. Thus, enzymes that can be stored at this temperature and the activity of which is preserved are of significant interest in large-scale work of industries **(Chen *et al.* 2024)**. Microbial lipases, especially, have proven to be highly sought-after industrial biocatalysts due to their high catalytic efficiency, extensive substrate selectivity, and flexibility to the various environmental conditions. Bacteria and fungi have been known to produce a large number of lipases that have diverse biochemical and structural characteristics **(Ali *et al.* 2023)**. Some of these include microbial lipases that exhibit extraordinary levels of stability in organic solvents, and they are therefore promising in industrial use. It is thus necessary to understand the structural aspects that make this solvent stable in order to enhance the performance of the enzymes and direct the protein engineering processes **(Cheng and Nian 2023; Vardar-Yel *et al.* 2024)**.

Some of these factors are protein composition, intramolecular interaction, accessibility to solvents, and packing structure, which affect protein stability in organic solvents**(Arakawa 2018; Pace *et al.* 2004)**. Stabilization of interactions including hydrophobic contacts, ionic interactions, hydrogen bonds, aromatic interactions, and disulphide bridges, among others, helps in maintaining the three-dimensional structure of proteins **(Borrelli and Trono 2015; Soni 2022)**. Amino acid composition may also affect stability of enzymes, as changes in structural flexibility, formation of hydrophobic core, and electrostatic interactions may be altered. Thus, the study of these structural parameters can give valuable information about the mechanism of solvent tolerance of enzymes **(Errami *et al.* 2003; Panja *et al.* 2020)**. The development of structural biology and bioinformatics has facilitated the study of protein structures and protein-protein interactions on a large scale by accessing publicly available databases like the Protein Data Bank **(Berman *et al.* 2000; Gabanyi *et al.* 2011; Kouranov *et al.* 2006; Standley *et al.* 2008)**. Computational methods can now be used to study stabilizing interactions, compute structural descriptors, and build protein-protein interaction networks to gain a deeper insight into the functional role played by enzymes **(Grassmann *et al.* 2024)**. Also, interaction databases, such as STRING database, aid in the exploration of functional associations between proteins of various organisms. This study has entailed a computational analysis of organic solvent-stable lipases in a comprehensive manner using structural data obtained through experimentally determined protein structures **(Mieres-Perez *et al.* 2025; Szklarczyk *et al.* 2021)**.

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This work aimed at finding out the amino acid composition of solvent-stable lipases, the structural interactions that stabilize these lipases the determination of the structural parameters of surface area, volume, and solvation energy, and the development of a protein-protein interaction network to find out the functional relationship (Sraphet and Javadi 2022). This study will combine a compositional analysis, structural characterization, and interaction network analysis to give a more in-depth understanding of the molecular factors that allow lipases to be stable and active in organic solvents. These results could be useful in the logical design and engineering of strong lipases to be used in industries and biotechnological applications.

2. Materials and Method

2.1. Dataset Preparation

Lipase protein structural data were acquired by accessing the Protein Data Bank (PDB), which is a well-known library of experimentally solved three-dimensional structures of biological macromolecules. In 2026, there are estimated to be about 250359 protein structures in the PDB, which is a valuable resource to carry out structural bioinformatics analyses (Burley *et al.* 2022). A search strategy based on a keyword was used to retrieve lipase structures in the database, and at the beginning 278 lipase entries were obtained (Godoy *et al.* 2022). In order to make the dataset reliable and relevant in the structural analysis, sequential filtering criteria were used (Fischer and Pleiss 2003). To begin with, we have selected only those proteins whose crystal structure is of high resolution, i.e., a structural resolution between 1 Å-3 Å. Structures covering this resolution range are said to be appropriate to precise atomic-level investigation of intramolecular interactions and structural parameters (Masica *et al.* 2010; Pilla *et al.* 2017; Szymczyna *et al.* 2009). Similar sequences were reduced in the dataset through limiting similarity (Wlodawer *et al.* 2008). Protein sequences with a sequence identity over 80 percent were filtered out to prevent statistical bias that would result due to overly homologous proteins. The sequence identity filtering was used to make sure that the dataset is representative of a wide range of lipase proteins and not close variants (Libbrecht *et al.* 2018). Also, during the curation of the datasets, structural classification and mutation status were also put into consideration (Homeyer *et al.* 2022). The incomplete folds of the lipase structures or highly engineered mutations were avoided so as to concentrate on proteins that depict the original structural characteristics with regard to solubility in organic solvents (Kamal *et al.* 2013). A final dataset of 82 organic solvent-stable lipases was then obtained after the application of the given criteria (Chakravorty *et al.* 2012). The analysis of the amino acid composition of the curated dataset was then done to obtain the frequency distribution of individual residues (Thompson and Pickard 2024). The frequency of each amino acid residue was computed in all sequences of the dataset. This composition study gives information about preferences in residues that can cause protein stability, structural packing, and solvent tolerance in lipases (Anashkina *et al.* 2021).

2.2. Computation of Structural Features

The tertiary structure of proteins constitutes the most important information about the physicochemical forces that mediate protein folding and stability (Diaz-Villanueva *et al.* 2015; Masson and Lushchekina 2022). A number of stabilizing intramolecular interactions were calculated in order to examine structural determinants that could lead to the stability of lipases when present in organic solvents (Qing *et al.* 2022). The interactions analyzed in the present study included disulphide bonds (covalent linkage between cysteine residues), hydrophobic interactions (aggregation of nonpolar residues (protein core)), ionic interactions (salt bridges) between oppositely charged residues, and hydrogen bonds (keep secondary and tertiary structural elements stable); aromatic-aromatic interactions (between aromatic side chains) and aromatic-sulphur interactions (between aromatic residues and sulphur-containing residues) and cation-pi interactions (between the measurements of these interactions) can be used to identify structural characteristics that can promote the thermodynamic stability and solvent tolerance of lipases. Hydrogen bonds were identified using a donor–acceptor distance cutoff of 3.5 Å and appropriate angle constraints; ionic interactions were identified between oppositely charged residues within a distance cutoff of 6.0 Å; hydrophobic interactions were defined between non-polar side chains within 5.0 Å; aromatic–aromatic interactions were identified between aromatic ring centroids within 7.0 Å; and disulfide bonds were identified between sulfur atoms of cysteine residues within 2.2 Å (Kilgore and Raines 2018; Singh *et al.* 2024).

2.3. Surface Accessibility and Residue Depth Analysis

Besides the study of the interacting regions, structural descriptors associated with the exposure of the residues were also computed. The degree of solvent exposure in the protein structure was measured by calculating the Accessible Surface Area (ASA) of the residues (Lins *et al.* 2003; Pan *et al.* 2020; Tien *et al.* 2013). ASA can give us information about the partitioning of surface and buried residues, which can have an effect on protein solubility and solvent interactions (Savojardo *et al.* 2020; Zhou and Zhou 2004). The other structural parameter that was analysed in this study was the depth of residue, which is measured as the distance of the residue to the surface of the protein (Tan *et al.* 2013; Tan *et al.* 2011). Deep residues in the protein core are normally involved in structural packing and stability, whereas surface residues may be involved in protein-protein interactions or environmental adaptability (Ma *et al.* 2003).

2.4. Voronoi Volume and Solvation Energy Calculations

In order to refine the descriptions of structural packing and energetic contributions to stability, Voronoi volume and solvation energy were estimated on each lipase structure. Voronoi volume characterizes three-dimensional space segmentation of atoms in the protein structure and gives information on the atomic packing density and compactness of the structure (Niazi 2025). The computations were made with the help of the VLDP server, which is based on the techniques of Voronoi tessellation to calculate the atomic volumes and packing features. Computations of solvation energies were conducted using the CHARMM molecular modeling program, which is an estimation of the energetic contribution of solvent interactions to protein stability. All protein structures used in this study were retrieved from the Protein Data Bank and prepared prior to analysis by removing ligands, ions, and crystallographic water molecules where necessary (Esque *et al.* 2013). Missing residues were checked and structures were inspected for structural integrity. The prepared structures were then subjected to energy minimization using the CHARMM force field to remove steric clashes and optimize geometry before solvation energy calculations. The minimized structures were subsequently used as input for VLDP server analysis and CHARMM-based solvation energy calculations using default parameters unless otherwise specified (Ait Lahcen *et al.* 2026). Solvation energy is one of the parameters that are believed to play a significant role in the study of protein folding and stability as well as solvent adaptation, especially when considering enzymes that are functional even when non-aqueous conditions are maintained (Rosgen *et al.* 2005).

2.5. Construction of Protein–Protein Interaction Networks

Protein-protein interaction (PPI) networks were built with the help of the STRING database, and the aim of studying possible functional connections between lipases and other proteins is to create the connection between the protein and other proteins based on the existing experimental evidence, methods of computational prediction, and literature mining

(Safari-Alighiarloo *et al.* 2014). The interaction network was constructed using a medium to high confidence score threshold (e.g., 0.7), and interaction sources included experimental data, curated databases, co-expression, gene neighborhood, gene fusion, co-occurrence, and text mining. Lipase database networks of three representative organisms (*Homo sapiens*, human; Pan troglodytes, chimpanzee; and Mus musculus, mouse) were chosen because of their evolutionary relationship and relevance to human systems (Coradetti *et al.* 2018). A comparative study between these species allows discovering the presence of conserved functional interactions and biological pathways of lipases. Associations between proteins in the STRING database are quantified with a probabilistic confidence score that combines the evidence of many different sources, such as experimental data, co-expression of genes, genomic context, and biologist-curated databases. These scores of confidences were employed in the current research to measure and assess the interactions between lipases and their protein associates. The obtained PPI networks give us a clue about the functional topography of lipases and can point out possible interaction partners and biological pathways in which the enzymes can be involved (Safari-Alighiarloo *et al.* 2014).

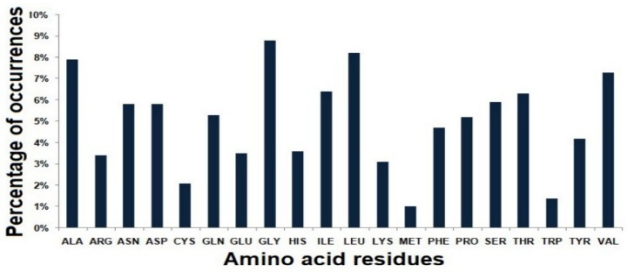

Figure 1. Amino acid composition of organic solvent stable lipases.

3. Results

3.1. Organic Solvent–Stable Lipases: Amino Acid Profile

Amino acid preferences linked to solvent tolerance in the curated database of 82 organic solvent-stable lipases were identified by the amino acid composition of the dataset. The mean value of the theoretical isoelectric point (pI) of the lipases within the dataset was 5.62, which implies that the majority of the enzymes have a slightly acidic nature. The molecular weight of the lipases was average (13,247 Da), as it is within the size range that has been reported to be typical of microbial lipases. Figure 1 summarizes the percentage frequency of the twenty standard amino acids in the dataset. Glycine (8.8%), the highest frequency in the residues, and methionine (1.0%), the lowest frequency in the residues, occurred most frequently and least frequently, respectively. Saturating glycine residues can be significant in providing structural flexibility and conformational versatility that are vital in enzyme stability in non-aqueous conditions. Previous experimental studies have shown that the replacement of glycine by aspartic acid greatly decreases the stability of enzymes, which suggests that glycine plays an important role in enhancing the organic solvent stability of lipases. The structural features identified in this study, including glycine abundance in flexible regions, increased hydrophobic residue content, and improved hydrophobic core packing, provide useful insights for enzyme engineering strategies. Conversely, the methionine residues are less common in solvent-stable lipases that are organic. The functional role of methionine has been reported to control the opening and closing of the lipase lid domain, which regulates the access of substrate to the catalytic active site. The variation in the frequency of methionine and histidine residues has also been observed in comparing aqueous and organic solvent-stable lipases, indicating that compositional differences can cause the variations in solvent tolerance. Further comparison with the amino acid composition of globular proteins showed that there were significant differences in the abundance of some of the residues. Specifically, the presence of glycine residues in solvent-stable lipases is not consistent with the presence of glycine residues in globular protein datasets. Some hydrophobic residues, such as alanine, phenylalanine, isoleucine, and valine, were also found in relatively elevated percentages, which showed that hydrophobic packing interactions were of great significance in structural stability in solvent-exposed conditions. On the other hand, the frequencies of other residues like tryptophan and cysteine were lower than that of methionine. Asparagine, glutamine, arginine, and lysine were found in moderate concentrations and indicated that these charged and polar residues might have taken part in electrostatic interactions and stabilization by the solvent. Combined, the compositional profile suggests that an equal representation of hydrophobic, flexible, and charged residues could be the cause of the increased stability of lipases in organic solvent conditions.

3.2. Solvent-Stable Lipase Structural Features

The Table S1 summarizes the structural characteristics of 82 organic-solvent-stable lipases used in this research. The organic solvents in which these enzymes maintain catalytic stability were rather loosely classified into polar and non-polar solvents. Most of the lipases represented in the dataset are of a microbial origin, mostly bacterial species, with a lesser proportion having a fungal origin. Microbial lipases have been commonly known in industrial applications, especially due to their wide substrate selectivity, catalytic activity, and adjustability to different environmental factors. Despite the fact that lipases can be found in plants, animals, and microorganisms, microbial lipases are the most useful in the industrial sector, biotechnology, pharmaceutical production, and food processing.
Out of the lipases studied, 75 lipases were stable in the polar organic solvents, and only 7 lipases were stable in non-polar solvents. The nonpolar solvent-stable lipases were mainly related to fungal species, with one mostly recognized as *Thermomyces lanuginosus*, known to produce highly robust industrial lipases. The non-polar organic solvents that were related to the lipase stability were lauric acid, decanoic acid, 16-hydroxyhexadecanoic acid, and hexylene glycol. One of them was reported to be 4-nitrobenzaldehyde as a stabilizing solvent. Conversely, lipase was found to be stable in a vast variety of polar solvents, such as tetraethylene glycol, ethylene glycol, triethylene glycol, polyethylene glycol, glycerol, ethanolamine, and a variety of buffer solutions, including HEPES and Tris. Other polar solvents that were found in the dataset were compounds like spermidine, bicine, imidazole, sodium acetate, and polyethylene glycol with different molecular weights.

3.3. Stabilizing Interactions in Lipase Structures

In order to gain more insight into the foundational structure of solvent stability, the stabilizing interaction between each lipase structure was examined by using the Protein Interaction Calculator. The calculated interactions were hydrophobic interactions, disulphide bonds, ionic interactions, aromatic-aromatic interactions, and aromatic-sulphur interactions, and the observations of the interactions in each structure of lipase have been summarized in Table S1. Hydrophobic interactions were one of the most frequent forms of stabilizing forces within the data set, indicating their significance in ensuring structural integrity and core packing. In a variety of lipases, disulphide bridges could be found, but not all lipases that were stable in polar solvents contained disulphide. It has been hypothesized in previous studies that the disulphide bonds can be more affected at the enzyme activity compared to the structural stability, especially when the bonds are at or close to the active site location. It is possible in these cases that disulphide bonds can have a functional part in avoiding the development of undesirable ionic interactions surrounding catalytic residues. Interestingly, the aromatic-sulphur interaction was not observed in some polar solvent-stable lipases, and this indicates that other stabilizing interactions might have balanced the missing ones. These results show that several structural interaction networks all play a role in enzyme stabilization in the solvent environments.

Table 1: Closely related proteins for lipases from different organisms

Similar proteins			Common proteins			Different proteins		
Homo Sapiens	**Chimpanzee**	**Mouse**	**Homo Sapiens**	**Chimpanzee**	**Mouse**	**Homo Sapiens**	**Chimpanzee**	**Mouse**
ABHD12	ALAS1	ALAS1	-	ALAS1	ALAS1	ABHD12	ISPD	ALAS2
MGLL	SPTLC3	ALAS2	-	SPTLC3	SPTLC3	MGLL	ESD	OXCT2B
FAAH	GCAT	SPTLC3	-	SPTLC1	SPTLC1	FAAH	ENOSF1	OXCT2A
ABHD4	SPTLC1	SPTLC1	-	SPTLC2	SPTLC2	ABHD4	PARL	AWAT1
UBC	SPTLC2	SPTLC2	-	OXCT1	OXCT1	UBC	ACSL5	MOGAT2
PHF7	OXCT1	GCAT	-	MOGAT1	MOGAT1	PHF7	ACSL6	DGAT216
ABHD11	ISPD	OXCT1	-	GCAT	GCAT	ABHD11	ACSL4	AWAT2
ABD12B	AP3D1	OXCT2B	-	AP3D1	AP3D1	ABD12B	ACSL1	DGAT2
CNR1	ESD	OXCT2A	-	-	-	CNR1	ACSL3	-
MAPKAPK3	ENOSF1	AP3D1	-	-	-	MAPKAPK3	ACSBG2	-
GPR119	PARL	AWAT1	-	-	-	GPR119	ACSBG1	-
DAGLA	ACSL5	MOGAT2	-	-	-	DAGLA	LOC465973	-
NAPEPLD	ACSL6	DGAT216	-	-	-	NAPEPLD	-	-
DAGLB	ACSL4	AWAT2	-	-	-	DAGLB	-	-
ZDHHC3	ACSL1	DGAT2	-	-	-	ZDHHC3	-	-
GPR18	ACSL3	MOGAT1	-	-	-	GPR18	-	-
NAAA	ACSBG2	-	-	-	-	NAAA	-	-
FAAH2	ACSBG1	-	-	-	-	FAAH2	-	-
FAM108B1	LOC465973	-	-	-	-	FAM108B1	-	-
CCKAR	MOGAT1	-	-	-	-	CCKAR	-	-

3.4. Surface Area, Volume, and Solvation Energy

Surface area and volume of every lipase structure were also estimated to measure the structural packing and the molecular compactness. The mean solvent-accessible surface area of the lipases was 87,762.47 Å², and the mean molecular volume was 37,830.51 Å³. Protein surface area and protein surface volume can also be used as useful measures of atomic packing and structural organization in the three-dimensional protein structure. The mean energy of solvation of the solvents, which were able to stabilize the organic solvent lipases, was estimated to be -7609.82, which means that there was good interaction between the protein and the solvent environment. Solvation energy is the energetic term that is needed to dissolve a solute in a solvent and is believed to be a significant factor in protein folding and stability. Though polar and non-polar solvent-stable lipases were studied in the research, the number of non-polar solvent-stable lipases is relatively small, which restricts the comparison of the studies. The more in-depth comparison of the structural parameters of these two categories will be made possible as more structural data is made accessible in protein databases.

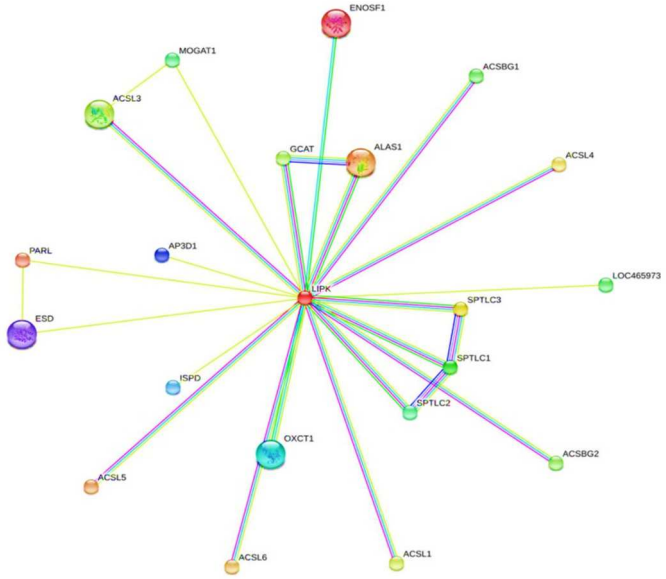

Figure 3. Protein-Protein interactions of lipase with Pan troglodytes.

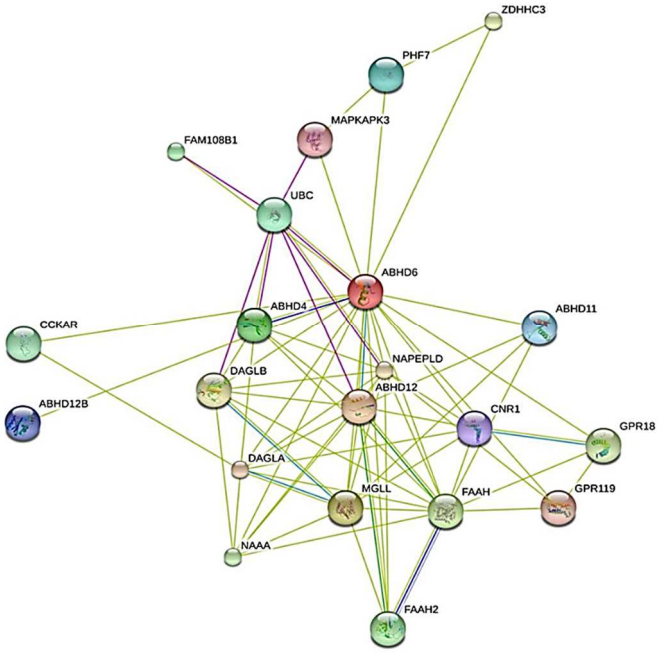

Figure 2. Protein-Protein interactions of lipase in *Homo sapiens*.

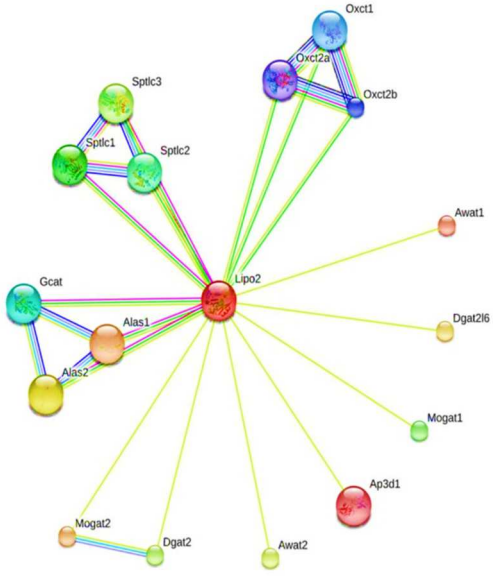

Figure 4. Protein-Protein interactions of lipase with *Mus Musculus*.

3.5. Protein–Protein Interaction Networks of Lipases

Protein-protein interaction (PPI) networks of lipases of organisms close to humans were built to examine possible functional associations of lipases with the help of the STRING database. The organisms that were used in the analysis of interaction were the following: *Homo sapiens* (human), Pan troglodytes (chimpanzee), and Mus musculus (mouse). The interaction networks showed 20 interacting partner proteins of lipases in humans and chimpanzees and 16 interacting partners in mice (**Table 1**). All interacting proteins are expected to have different biological functions in lipid metabolism, signaling, or cell regulation. The comparative study of the interaction networks showed that there were significant differences among the three organisms. The chimpanzee and mouse lipase interaction networks had eight common interacting proteins, thus showing some level of evolutionary preservation between the two species. Conversely, the interaction network of human lipases did not have any overlapping interacting proteins with the other two organisms, indicating organism-specific differences in the functional association of lipase. Besides, 20 interacting proteins were singly related with human lipases, 12 interacting proteins were related with chimpanzee lipases, and 8 interacting proteins were singly related with mouse lipases. These findings suggest that lipase-related interaction networks could be highly diverged at the evolutionary level, which could be associated with dissimilarities in the metabolic control and physiological needs of the species. These protein-protein interaction networks of lipases in humans, chimpanzees, and mice are shown in **Figure 2, 3, and 4**, respectively. The protein–protein interaction (PPI) network analysis provides insight into the biological relevance and functional context of lipases beyond their structural stability. The identified interactions suggest that lipases are functionally associated with proteins involved in lipid metabolism, energy homeostasis, and metabolic regulation pathways across different species. The conservation of interaction partners among *Homo sapiens*, Pan troglodytes, and Mus musculus indicates evolutionary conservation of lipase-related metabolic functions. Therefore, the PPI network analysis supports the biological significance of lipases not only as hydrolytic enzymes but also as components of broader metabolic and regulatory networks, highlighting their importance in physiological and biomedical contexts.

4. Discussion

The compositional and structural analysis of 82 organic solvent-stable lipases reveals several interconnected molecular strategies that underpin enzymatic resilience in non-aqueous environments. These findings carry meaningful implications for both our understanding of protein stability and the rational engineering of industrially relevant biocatalysts. The predominance of glycine residues (8.8%) across the dataset is particularly illuminating. As the smallest and most conformationally flexible amino acid, glycine confers local backbone mobility that may buffer structural perturbations induced by organic solvents. This is consistent with the established understanding that enzymes functioning in non-aqueous media require a delicate balance between rigidity, to preserve catalytic geometry, and flexibility, to accommodate solvent-induced stresses. The observation that substituting glycine with aspartic acid markedly reduces enzyme stability further reinforces its non-redundant structural role. In contrast, the low frequency of methionine (1.0%) is mechanistically meaningful: given methionine's established role in regulating lipase lid domain dynamics, its reduced presence may reflect an evolutionary or functional trade-off wherein solvent-stable lipases minimize conformationally sensitive gating mechanisms that could be disrupted in organic media. The elevated representation of hydrophobic residues, alanine, phenylalanine, isoleucine, and valine, points to enhanced hydrophobic core packing as a central stabilizing strategy. In aqueous environments, hydrophobic burial is driven by the entropic cost of water ordering around nonpolar surfaces. In organic solvents, however, this driving force is altered, making intramolecular hydrophobic interactions increasingly critical for maintaining tertiary structure. The compositional data thus suggest that solvent-stable lipases have evolved, or can be engineered, with denser hydrophobic cores that resist unfolding when the aqueous solvation shell is compromised or displaced. The stabilizing interaction analysis further corroborates this picture. The ubiquity of hydrophobic interactions across structures confirms their primacy in structural integrity, while the variable presence of disulfide bonds introduces an important nuance: these covalent crosslinks may serve a functional rather than purely structural role, potentially shielding catalytic residues from unfavorable ionic microenvironments. The absence of aromatic-sulphur interactions in certain polar solvent-stable lipases suggests that stability is achieved through a combinatorial, redundant network of forces rather than any single dominant interaction, an important insight for enzyme engineering, where introducing one class of stabilizing interaction need not come at the expense of others. Surface area and solvation energy data add a thermodynamic dimension to these observations. The mean solvation energy of approximately -7,610 supports favorable protein-solvent interfacial interactions, suggesting that solvent-stable lipases have surface properties tuned to interact productively with their solvent environments rather than simply resisting them. This is a subtle but important distinction: stability may arise not solely from internal rigidity, but from surface complementarity with the surrounding medium. The dominance of polar solvent-stable lipases (75 of 82) over non-polar solvent-stable ones (7 of 82) likely reflects both biological reality and database sampling bias, as polar organic solvents such as glycerol, polyethylene glycol, and ethylene glycol are far more commonly employed in industrial and pharmaceutical biotransformations. The fungal origin of most non-polar solvent-stable lipases, particularly from *Thermomyces lanuginosus*, underscores the utility of thermophilic and extremophilic organisms as reservoirs of robust biocatalysts. Finally, the protein-protein interaction analysis situates lipases within broader metabolic and regulatory networks. The absence of shared interaction partners between human lipases and those of the other two organisms, despite the conservation seen between chimpanzees and mice, suggests species-specific functional divergence, possibly reflecting adaptations in lipid metabolism suited to distinct physiological demands. Collectively, these findings establish a multi-layered molecular framework for solvent stability and highlight promising avenues for structure-guided lipase engineering.

5. Conclusion

The present paper includes a structure and composition study of lipases that are stable in organic solvents, which was carried out on a curated protein collection of 82 proteins retrieved according to the Protein Data Bank. The results suggest that amino acid composition is a highly significant determinant that is used to characterize solvent stability. In particular, an equal distribution of Glycine residues implies that the structural adaptability and conformational conformability may be one of the important contributors to the stability of the enzymes in solvents. Conversely, the minor frequency of the appearance of the methionine residues also indicates that there might be few such residues that have functional activities such as the lid-domain dynamics controlling the accessibility of the substrate. Structural analysis also revealed that stabilization of interactions including hydrophobic contacts, ionized interaction, and disulphide bond has a cumulative effect on the structural stability of lipases. The graphical representation of how these interactions change across the dataset evidences the applicability of several stabilizing forces in enabling enzymes to work in a strenuous solvent environment. Also, surface area, volume, and solvation energy studies found that packing of atomic particles, as well as good interactions between solvents, are also important factors that determine stability. Besides, the analysis of protein-protein interaction with the STRING database was revealed to

possess species-species interaction networks in *Homo sapiens*, Pan troglodytes and Mus musculus, where it can be concluded that lipase-related functional pathways diverge. Overall, these findings provide helpful information in the framework of the structural aspects of solvent stability in lipases and may help to develop strong enzymes to apply them in the industrial and biotechnological industries.

6. Disclosure Statements

6.1. Author Contribution

VV: Conceptualization, study design, supervision, data interpretation, manuscript writing and revision. **RS:** Data collection, manuscript drafting, Data curation, literature review, manuscript preparation. All the authors have read and approved the final manuscript.

6.2. Declaration of Generative AI

The authors declare that no generative AI tools were used in the drafting, writing, or editing of the manuscript. All scientific interpretations and conclusions are the author's own.

6.3. Ethics approval (for clinical/animal studies)

This study is entirely based on computational analyses using publicly available datasets and *in silico* methods. No experiments involving human participants, human samples, or animal subjects were conducted. Therefore, ethical approval from an institutional review board or ethics committee was not required. All data utilized in this study were obtained from established public databases and used in accordance with their respective guidelines and policies.

6.4. Informed Consent Statement

Informed consent was not required

6.5. Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

6.6. Acknowledgment

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6.7. Funding Statement

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6.8. Conflicts of Interest

The authors declare that they have no known financial, personal, academic, or other relationships that could inappropriately influence, or be perceived to influence, the work reported in this manuscript. All authors confirm that there are no competing interests to declare.

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6.10. Supplementary Information

The supplementary material is available for this article is available online at https://jomi.aayvu.com/SuppFile/220238/1/.

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