Unlocking the mystery behind the activation phenomenon of T1 lipase: A molecular dynamics simulations approach

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Abstract: The activation of lipases has been postulated to proceed by interfacial activation, temperature switch activation, or aqueous activation. Recently, based on molecular dynamics (MD) simulation experiments, the T1 lipase activation mechanism was proposed to involve aqueous activation in addition to a double-flap mechanism. Because the open conformation structure is still unavailable, it is difficult to validate the proposed theory unambiguously to understand the behavior of the enzyme. In this study, we try to validate the previous reports and uncover the mystery behind the activation process using structural analysis and MD simulations. To investigate the effects of temperature and environmental conditions on the activation process, MD simulations in different solvent environments (water and water-octane interface) and temperatures (20, 50, 70, 80, and 100°C) were performed. Based on the structural analysis of the lipases in the same family of T1 lipase (I.5 lipase family), we proposed that the lid domain comprises $\alpha 6$ and $\alpha 7$ helices connected by a loop, thus forming a helix-loop-helix motif involved in interfacial activation. Throughout the MD simulations experiments, lid displacements were only observed in the water-octane interface, not in the aqueous environment with respect to the temperature effect, suggesting that the activation process is governed by interfacial activation coupled with temperature switch activation. Examining the activation process in detail revealed that the large structural rearrangement of the lid domain was caused by the interaction between the hydrophobic residues of the lid with octane, a nonpolar solvent, and this conformation was found to be thermodynamically favorable.

Keywords: lipase activation; MD simulations; interfacial activation; T1 lipase

Abbreviations: MD, molecular dynamics; PDB, Protein Data Bank; RMSD, root-mean-square deviation.

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Introduction

Lipases (EC 3.1.1.3) are among the most important industrial enzymes that have been extensively studied. Many novel bacterial lipases have been isolated from many different sources, ranging from psychrophiles to extremophiles.¹ As important hydrolases, lipases catalyze the hydrolysis of long chain triglycerides with a broad range of substrate specificity (e.g., aliphatic, aliphatic, bicyclic and aromatic esters, thioesters, activated amines) while maintaining high regioselectivity,

chemoselectivity, and enantioselectivity. As lipases are remarkably stable in organic solvent, they are largely employed in organic synthesis: esterification, transesterification, aminolysis, and oximolysis reactions.^{2,3} Such properties make lipases attractive enzymes for industrial applications. They are used to make detergents, food, textiles, pharmaceuticals, and paper.^{1,4–6}

Most lipases adopt a common α/β hydrolase fold irrespective of their sequence.^{7,8} Lipases are serine hydrolases, containing a catalytic triad of Ser, His and Asp/Glu residues. On the basis of structural studies of lipases, it was found that most of the active site pocket is covered by a mobile domain (lid or flap) consisting of an α -helical fragment that controls the access of substrate to the active site.^{9,10} The active site is protected by the lid when in its closed conformation, and opening the lid exposes the active site. The presence of the lid is one of the important features in lipase activation, termed interfacial activation. The major event that takes place during interfacial activation is the displacement of the lid covering the active site by a lipid-water interface, which acts as the key feature that triggers the structural rearrangement of the α -helical domain.⁹ Previously, in the study of a thermostable lipase from *Bacillus stearothermophilus* (L1 lipase), a new mode of activation, termed “Temperature Switch” activation, was identified.¹¹ In this activation process, the lid displacement is triggered by a temperature switch; the lid will move and uncover the active site only when the temperature reaches a critical value. Although many studies have been performed to better understand the mode of action at the molecular level, few studies have been carried out using a closed conformation structure because it is difficult to establish the accuracy of the structure and difficult to relate the results to experimental data.

Recently, a thermoalkalophilic lipase from *Geobacillus zalihae*, known as T1 lipase, has become a model lipase in the study of enzyme activation using MD simulations.^{12,13} Like BTL2 lipase from *Geobacillus thermocatenulatus*,¹⁴ T1 lipase belongs to the I.5 lipase family¹⁵ and exhibits optimal activity at 70°C and pH 9.¹² T1 lipase was recently crystallized, and the structure was solved at 1.5-Å resolution. The crystal structure is in a closed conformation structure, and no active or open crystal structure has been reported as of yet.¹⁶ The catalytic triad of T1 lipase consists of Ser113, Asp317 and His358.¹⁶ Of the I.5 lipase family lipases, only the structure of BTL2 lipase has been solved in an open conformation.¹⁷ The unavailability of the active or open conformation structure of T1 lipase is a major obstacle in the study of enzyme activation. Recently, Wang *et al.* (2010) reported on the mechanism of T1 lipase activation in water using MD simulations.¹³ However, they do not take into account the substrate environment in their molecular dynamics simulations. To fully understand

the interfacial activation of T1 lipase at the molecular level, molecular dynamics (MD) simulations were performed with explicit water and water-octane micelles to mimic the lipid-water interface at different temperatures. These simulations help to unlock the mystery of catalysis.

Results and Discussion

To understand how an enzyme works, we must have a good understanding and in-depth knowledge of its protein structure.^{18,19} Protein crystal structure is the key element in the field of structural biology and protein chemistry.²⁰ Although significant effort has been put into deciphering protein structures, only a few thousand protein structures have been solved. Despite the fact that T1 lipase has been successfully crystallized, no open conformation structure had been reported.^{16,21} Without the open conformation or protein-substrate complex structure, it is difficult to fully understand the mode of action of the lipases. Lipase activity is greatly enhanced when acting at the water-lipid interface. This interfacial activation involves a structural rearrangement of the lid domains that exposes the catalytic residues to the substrate.⁹ Recently, Wang *et al.*¹³ reported that T1 lipase might undergo aqueous activation instead of interfacial activation. They also proposed a double-flap mechanism¹³ that regulates the access of the substrate to the active site pocket, which differs from the unidirectional opening mechanism previously thought to control the lid in I.5 family lipases such as the BTL2 and L1 lipases.^{17,22} It is important to validate this experiment because few studies were carried out regarding the T1 lipase activation process. To date, there is still no open conformation for T1 lipase, and the lid activation mechanism has not been experimentally determined yet. However, BTL2 lipase, which is in the same family, is very similar to T1 (96% sequence identity), and its structure has been solved in an open conformation in the presence of detergent molecules.¹⁷ By comparing the sequence alignment and the protein structure (Fig. 1), we can show that the lid domain for T1 lipase comprises 55 amino acids (Asp175–Lys229) which are located on $\alpha 6$ and $\alpha 7$ helices and form a helix-loop-helix-like motif. To determine whether T1 lipase activation occurs by interfacial activation⁹ or aqueous activation,¹³ a temperature-dependent or -independent process, MD simulations of 10 ns were conducted in two different solvent environments (water and water-octane interface) and at five different temperatures (20, 50, 70, 80 and 100°C).

Simulation of closed T1 lipase in an aqueous environment

MD simulations of T1 lipase for 10 ns at five different temperatures (20, 50, 70, 80, and 100°C) were

Figure 1. Structure superposition of T1 lipase with BTL2 lipase. RMSD value of 0.545 Å was obtained from the superimposition. The helices that formed the lid for BTL2 are colored in green ($\alpha 6$ helix) and yellow ($\alpha 7$ helix), whereas the proposed helices that formed the lid for T1 are colored in blue ($\alpha 6$ helix) and brown ($\alpha 7$ helix). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

conducted to determine if there is any possibility for T1 lipase to undergo aqueous activation (displacement of the lid domain) under the influence of temperature.¹³ The 10-ns trajectories were analyzed (Fig. 2) and compared with the previously reported results by Wang *et al.* (2010).¹³ We found that there was no significant conformational change after running the MD simulations in explicit water at various temperatures except at 100°C. There was no significant difference when comparing the root mean square deviation (RMSD) of the C α backbone for the simulations run at different temperatures with the exception of MD simulations at 100°C [Fig. 2(A)]. Throughout these experiments, the RMSD only ranged from 0.9 to 1.7 Å except for simulations at 100°C. For lid displacement to take place, a larger value of backbone RMSD must be observed. The radius of gyration [Fig. 2(C)] and solvent accessible surface [Fig. 3(A)] showed no significant increase during the simulations, proving that structural rearrangement of the lid region did not occur. Such a rearrangement would cause T1 lipase to lose its notable compactness (2-Å increment). This was not

Figure 2. Quantitative analysis of conformational changes for closed T1 lipase during MD simulations in different environments and temperatures. A: RMSD of T1 lipase C α -backbone atoms as a function of time during simulations of 10 ns in aqueous environment at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green) and 100°C (purple); B: RMSD of T1 lipase C α -backbone atoms as a function of time during simulations of 10 ns in water-octane interface at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green), and 100°C (purple); C: Radius of gyration as a function of time during simulations of 10 ns of closed T1 lipase in aqueous environment at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green), and 100°C (purple); D: Radius of gyration as a function of time during simulations of 10 ns of closed T1 lipase in water-octane interface at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green), and 100°C (purple). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3. Time variation of the solvent accessible surface area of closed T1 lipase in distinct environments and temperatures. A: T1 lipase in aqueous environment at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green), and 100°C (purple) during simulations of 10 ns; B: T1 lipase in water-octane interface at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green), and 100°C (purple) during simulations of 10 ns. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed, and T1 lipase was not well exposed to the solvent. At 100°C, greater RMSD was observed, since at this temperature and beyond, the protein started to denature and because of the excessive amount of the heat in the system, many bonds begin to break apart.

The calculated B-factors per residue [Fig. 4(A)] also showed a similar trend, with no significant result supporting the hypothesis that T1 lipase could

be activated by the aqueous environment. By analyzing all the results for the MD simulations in the aqueous environment, we found that there was no significant result to prove aqueous activation of the lipase. However, a significant temperature effect on the structure of T1 lipase was observed in the system. T1 lipase is a thermostable enzyme that has an optimal temperature of 70°C and shows a significant decrease in activity at 80°C, with a low activity at

Figure 4. Flexibility and lid movement of closed T1 lipase in terms of B-factor. A: Calculated B-factors of T1 lipase residues from MD simulations carried out in aqueous environment at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green), and 100°C (purple); B: Calculated B-factors of T1 lipase residues from MD simulations carried out in water-octane interface at 20°C (red), 50°C (yellow), 70°C (blue), 80°C (green), and 100°C (purple). The secondary structure of T1 lipase is shown on the graph for reference. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 5. The lid domain of T1 lipase. The lid of T1 lipase consists of $\alpha 6$ and $\alpha 7$ helices connected by a loop and forming a helix-loop-helix motif. The hydrophobic residues of the lid domain are colored in red. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

temperatures below 50°C.¹² As a thermostable enzyme, T1 lipase is more rigid and less flexible when compared with non-thermostable enzymes.²² From the RMSD data [Fig. 2(A)], we noticed that at 100°C, T1 lipase was less stable because simulations fluctuated several times in the time interval. However, at 20°C, the structure was more stable and deviated less from the initial value, staying well converged throughout the simulation period. At high temperature, the enzyme begins to unfold and become less compact, more flexible, and more unstable.²³ In contrast, at low temperature, T1 lipase is more rigid, more compact and less flexible, which makes it difficult for the enzyme to interact with the substrate (the enzyme is in an inactive state).

Simulation of closed T1 lipase at a water-octane interface

Simulations of closed T1 lipase at different temperatures in the presence of a water-octane interface caused significant lid domain displacements and were greatly affected by temperature. However, the stabilities of the simulated T1 lipases differed according to their respective temperatures. The fact that the C α backbone RMSD for the 100°C simulation [Fig. 2(B)] showed the highest fluctuation indicates that T1 lipase was unstable at temperatures higher than 70°C. This observation supports the previous finding that T1 lipase is highly active at 70°C but shows a drastic decrease in activities at temperatures beyond its optimum.^{12,23} The computed radius of gyration [Fig. 2(D)] and solvent accessible surface [Fig. 3(B)] values from the T1 lipase simulations with a water-octane interface showed a trend of increasing with temperature, with 100°C giving

the highest values of these parameters throughout the entire simulation period. This means that the T1 lipase structure became less compact and that more surface area was exposed to the solvent than in the octane-free system, suggesting that a lid movement occurred in the simulations with octane. To confirm the lid movement and the theory of interfacial activation, the root mean square of fluctuation per residue was computed in terms of the B-factor [Fig. 4(B)].^{9,24,25} Residues 175 to 190, which form the $\alpha 6$ helix, showed a large fluctuation ($\sim 500\text{\AA}^2 - 2500\text{\AA}^2$) from the initially closed T1 lipase after 10 ns of simulation. A noteworthy fluctuation was observed also for residues 220-229 ($\alpha 7$ helix). The B-factor per residue data showed that the $\alpha 6$ and $\alpha 7$ helices were the key domains forming the helix-loop-helix motif (Fig. 5) that regulates access to the active site pocket, as previously proposed. However, the $\alpha 9$ helix, which has recently been reported to be one of the lids, showed a very low fluctuation and could not have acted significantly as a mobile lid during enzyme activation. Although the $\alpha 4$ and $\alpha 5$ helices showed significant fluctuations at 100°C, they were not regarded as lids because their large fluctuations might be due to the instability of the protein structure at high temperatures.²³

T1 lipase activation

To date, most lipases are known to be activated in the presence of a lipid-water interface in a process called interfacial activation.⁹ Despite the existence of this theory, some researchers have proposed other modes of activation, such as aqueous activation¹³ and temperature switch activation.¹¹ To understand how lipases are activated, significant efforts have been put into solving the crystal structures in an open conformation,²⁶⁻²⁸ simulating the activation event through MD simulations^{24,25,28,30} and utilizing spectroscopy techniques such as electron paramagnetic resonance (EPR).³¹

One of the most important features of most lipases is the presence of a mobile domain regulating access to the active site. This mobile domain is generally known as the lid. Structural alignment with BTL2 lipase¹⁷ and L1 lipase¹¹ revealed that the $\alpha 6$ and $\alpha 7$ helices are the lids of T1 lipase (Fig. 5). The bipartite distribution of hydrophobic residues on the $\alpha 6$ helix and the existence of an adjustable loop connecting both helices ($\alpha 6$ and $\alpha 7$) led to the proposed unidirectional lid-opening mechanism for L1 lipase.¹¹ This theory contradicted another proposed mechanism for BTL2 lipase because it suggested a strong contribution from helix $\alpha 7$ and stated that activation was controlled by the presence of two lids.¹⁷ Detailed analysis of the data from our experiment suggested that T1 lipase activation is governed by a unidirectional lid-opening mechanism¹¹ (Fig. 6). The lid domain consisted of $\alpha 6$ and $\alpha 7$ helices

Figure 6. Conformation of closed T1 lipase before and after MD simulations in the presence of water-octane interface at 70°C. A: Surface view of initial structure of closed T1 lipase before MD simulations; B: Surface view of final structure of open T1 lipase after MD simulations. The lid domain is colored in green, whereas, the active site is colored in red. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

connected by a loop, thus forming a helix-loop-helix motif of around 55 amino acids (Asp175 – Lys229). Upon lid displacement, the hydrophobic residues (Phe176, Phe180, Phe181, Leu183, Ala184, Val185, Leu186, Ala188, Ala189, Ala190, Val 191, Ala192, Val195 and Pro196) of the lid were overexposed, disrupting the strong hydrophobic interaction that usually maintains the large hydrophobic cavity burying the catalytic residues. A large structural displacement of the lid domain was observed from 1 ns until the end of the simulation in the water-octane interface. When studying the effects of temperature variation on T1 lipase activation, we found that temperature was a critical factor for lid displacement. The degree of lid displacement was greatly influenced by temperature change, in agreement with the temperature switch mechanism¹¹ previously proposed, which states that the lid can only be moved when the temperature is increased to a certain value and cannot be opened at low temperatures even in the presence of lipid substrates. Nevertheless, an open conformation of thermostable lipase (BTL2) has been successfully crystallized at low temperature (25°C), and the lid remained open in this crystal structure.¹⁷ Our results suggest that the lid can remain slightly open even at a lower temperature (20°C), with a maximum displacement of ~ 1.5 Å (Table I). Typically, as observed in other lipases, upon activation, the lid will move away more than 10 Å. However, the extent of lid opening differed at various

temperatures (20, 50, 70, 80, and 100°C), with 100°C giving the highest displacement (~ 19 Å). From our previous study,¹¹ we know that enzymatic activity increases as the temperature increases until 70°C is reached. Temperature increases beyond that would result in decreased activity because the protein structure becomes unstable and begins to unfold at such a high temperature. To catalyze a hydrolysis reaction, a hydrogen bond forms between Asp317 and His358, thus increasing the pKa of the imidazole side chain of His358. In the next step, Ser113 is deprotonated by His358 (general base) and acts as a nucleophile to attack the carbonyl carbon of the ester bond of the substrate. Based on the experimental data, we found out that the critical distance between the catalytic residues (Asp317-His358-Ser113) was very important in the catalytic machinery. The efficiency of the catalysis process greatly depends on this critical distance. In our study, we found that the distances between these residues are affected by the change in temperature (Fig. 7). At 80°C, the distance between catalytic Ser113 and His358 was a bit far (5.75 Å) compared with 70°C (2.62 Å), which is the optimal temperature of T1 lipase. Examining this distance at various temperatures will help to explain why the enzymatic activity of T1 lipase decreases as the temperature increases above its optimum (70°C).

The details of lid movement during the simulation period are shown in Figures 8 and 9. The

Table I. Lid Displacement of Closed T1 Lipase

Environments Temperatures (°C)	Water interface					Octane-water interface				
	20	50	70	80	100	20	50	70	80	100
Lid displacement (Å)	0.92	1.21	1.87	1.73	1.81	1.59	10.39	15.15	18.87	19.33

The lid displacement was measured by the displacement of Leu183 C α atom between initial and 10 ns structures.

Figure 7. The distance between the catalytic residues (Asp317-His358-Ser113) of T1 lipase at two different temperatures in water-octane interface. A: MD simulation at 70°C; B: MD simulation at 80°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

movements show that the lid gradually opened from its initial closed conformation in the presence of the water-octane interface. Protein-solvent interaction has been proposed as the major driving force enabling large displacement of the lid and major changes in the hydrophobicity of the exposed surface.³² A broad distribution of the hydrophobic resi-

dues on the lid domain were identified as the key element in this structural rearrangement. Previously, in the closed state, the hydrophobic residues were shielded from the water, and the hydrophilic residues were exposed to the solvent. In the water-octane interface, the situation was reversed: the hydrophobic region was overexposed because of its high affinity for non-polar solvents, and the hydrophilic side became partially buried in the exposed hydrophobic pocket. The lid opening was mainly stabilized by the protein-solvent interaction, in which the hydrophobic residues of the lid interacted strongly with the non-polar solvent (lipid environment).³² Additional stabilization forces were contributed by the formation of new hydrogen bonding networks³³ and salt bridges.^{34,35} Five new additional salt bridges were observed, but only two interactions (Asp205-Arg214 and Asp209-Arg214) were directly involved in stabilizing the lid domain (Fig. 10), which was located in the loop connecting the $\alpha 6$ and $\alpha 7$ helices. Thus, at this moment, the enzyme could be said to be in the activated state, ready to attack incoming triglyceride molecules. Figure 11 illustrates the effects of solvent environment and temperature on T1 lipase activation.

Lid-closed simulation

To further investigate the dynamics of the lid movement of T1 lipase, we performed a lid-closed MD simulation using the open conformation structure obtained from the previous 10 ns MD simulation in water-octane interface at 70°C. The protein

Figure 8. Conformational transition of the lid during simulations of 10 ns in different environments at 70°C. A: Conformation of closed T1 lipase in water; B: Conformation of closed T1 lipase in water-octane interface. The lid gradually opens in water-octane interface but remains closed in water. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 9. Superposition of the final T1 lipase structure after the MD simulations with the initial structure. A: Superposed images of T1 lipase after MD simulations in water at 20, 50, 70, 80, and 100°C; B: Superposed structures of T1 lipase after MD simulations in water-octane interface at 20, 50, 70, 80, and 100°C. The $\alpha 6$ and $\alpha 7$ helices of the initial structure are colored in cyan and yellow, whereas in final structure those helices are colored in blue and brown. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

structure was solvated in explicit water, and a 10 ns MD simulation was conducted at 70°C under constant pressure, as described in the methodology section. Based on the trajectory analysis, a significant lid movement event was observed after 1 ns (Fig. 12). Fluctuation of the C α backbone was observed after 1 ns and the position of the backbone converged throughout the simulation time [Fig. 12(A)]. Significant decreases in solvent accessible surface [Fig. 12(B)] and radius of gyration [Fig. 12(C)] values were observed, suggesting that the lid began to completely close after 1 ns and remained in the closed state until the end of the simulation. The calculated B-factors per residue [Fig. 12(D)] showed a notable fluctuation around the residues that form the lid domain (Asp175 – Lys229), supporting the idea that a lid movement occurred. Details on the lid movement can be seen in the simulation snapshots that were taken at 0 ns, 1 ns, 5 ns and 10 ns (Fig. 13).

Even though lipase activation has been extensively studied, the details of the T1 lipase activation mechanism are still poorly understood due to the lack of an open conformation structure. This lack of structural data has led to many assumptions and postulations. As has been proven by our MD simulations of T1 lipase in two different solvent interfaces (water and water-octane interface) at five different temperatures (20, 50, 70, 80, and 100°C), T1 lipase activation is governed by interfacial activation coupled with temperature activation and neither aqueous activation nor temperature switch activation alone could significantly activate the T1 lipase.

The key element that enables this event is the presence of a lid domain formed by helices $\alpha 6$ and $\alpha 7$, which are connected by a loop to form a helix-loop-helix motif. The lid gradually opens to its maximum extend (>10 Å) as the buried hydrophobic residues become overexposed and take part in strong hydrophobic interactions with the nonpolar solvent octane. The insights into the activation mechanism of T1 lipase that we have reported here will help explain how the enzyme works and behaves in nature, thus providing researchers with an understanding of

Figure 10. Lid opening structure stabilization by salt bridges formation. After the lid was displaced, two salt bridges between Asp205-Arg214 and Asp209-Arg214 were formed. The salt bridges within the lid region stabilized the opening structure of the lid. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 11. Schematic illustration of T1 lipase activation. The unidirectional lid (yellow) movements occurred in the presence of octane micelles and were enhanced with increases in temperature. At lower temperature and with the presence of octane micelles, the lid only opened slightly. In the absence of octane micelles, the lid remained closed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 12. Quantitative analysis of conformational changes for lid-closed simulation of T1 lipase in aqueous environment at 70°C. A: RMSD of T1 C α -backbone atoms as a function of time during simulations of 10 ns; B: Time variation of the solvent accessible surface area during simulations of 10 ns; C: Radius of gyration as a function of time during simulations of 10 ns; D: Calculated B-factors of T1 lipase residues during simulations of 10 ns. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 13. Conformational transition of the lid during lid-closed simulations of 10 ns in aqueous environment at 70°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

activation mechanism while waiting for an open conformation structure. This study also provides a fresh start to those working on engineering the protein via rational design or biomimicry.

Materials and Methods

Structure analysis

Closed T1 (PDB ID: 2DSN) and open BTL2 (PDB ID: 2W22) structures were obtained from the Protein Data Bank. These two structures were superimposed and analyzed. Similarities between the protein domains and motifs were observed in order to determine the lid domain for T1 lipase.

Molecular dynamics simulation

MD simulations were conducted on a closed conformation of T1 lipase that was obtained from the Protein Data Bank (PDB ID: 2DSN) and chain A was selected for this study. Simulations were carried out using YASARA program^{36,37} in two different explicit solvent environments which were in the water interface and in water-octane interface at different temperatures (20, 50, 70, 80, and 100°C). Metal ions such as zinc and calcium that present in the T1 lipase structure were conserved in the model and were parameterized according to GAFF (General AMBER Force Field) atom types.³⁸ All subsequent MD simulations were performed using the YASARA program^{36,37} and the simulations were done in a truncated orthorhombic cell under periodic boundary conditions. AMBER03 force field³⁹ was applied to all atoms in the system with 7.86 Å cutoff with non-bonded interaction. The pKa values for Asp, Glu, His and Lys residues were predicted and the protonation states were assigned according to pH 9, the optimum pH of T1 lipase.⁴⁰ Then the simulation box is filled with water molecules, and Na⁺ or Cl⁻ counter ions are placed at the locations of the lowest or highest electrostatic potential until the cell is neutral and 0.9% NaCl concentration is reached (~26 Na⁺ and ~22 Cl⁻). A short MD simulation is run for the solvent, and water molecules are subsequently deleted until the water density has reached 0.997 g/L.

The water-octane solvent cell was created by using 240 octane molecules as the starting structure and was solvated with 16,892 water molecules. Appropriate point charges and parameterization of octane molecules were achieved by using YASARA AutoSMILES algorithm. AutoSMILES is an automatic force field parameter assignment for organic molecules that involves in assignment of AM1BCC atom and bond types,⁴¹ assignment of GAFF atom types³⁸ and remaining force field parameters. The octane box was subjected to 5000 steps of steepest descent minimization with the AMBER03 force field³⁹ to remove bumps and correct the covalent geometry. After the minimization step, the system was equilibrated to 298 K for 100 ps and the pressure was set to 1 bar. Temperature and pressure were controlled with Berendsen thermostat⁴² and manometer respectively. For MD simulations under water-octane interface, the octane box was laid over the water box containing the protein and the energy was minimized until convergence to remove the region with strong bumps and conformational stress. The system was then equilibrated to 298 K for 100 ps and the pressure was set to 1 bar. Berendsen thermostat⁴² and manometer were used to control the temperature and pressure. For the subsequent MD simulations experiments under different solvent environments and temperatures, initial minimization steps (steepest descent and simulated annealing methods) were conducted until convergence was reached before a short position-restrained equilibration procedure of the system is carried out. Particle Mesh Ewald algorithm⁴³ was used to treat long range electrostatic interactions with 10 Å of non-bonded cutoff. LINCS algorithm⁴⁴ was used to constrain all bonds involving hydrogen atoms to enable 2 fs time steps. The system was then equilibrated to 298 K for 100 ps and the pressure was set to 1 bar and was controlled using Berendsen thermostat⁴² and manometer. The production runs of the simulations were carried out according to the solvent environments (water interface and water-octane interface) and temperatures (20°C, 50°C, 70°C, 80°C and 100°C) under study for 10 ns with the position restraints and pressure

coupling are turned off. Simulation snapshots were saved for every 25 ps for trajectory analysis and all the MD simulations experiments were repeated at least twice by using different starting initial velocities. The resulting trajectories were analyzed for the root-mean squared deviation (RMSD), radius of gyration, solvent accessible area, residue positional fluctuation (B-factor) and atom–atom distances using YASARA.^{36,37}

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