

Supporting information:

Experimental activity assays of TLL variants against water-soluble substrate

The hydrolytic activity of the TLL variants was determined against pNP-butyrates and pNP-palmitate emulsified in non-ionic surfactant (Triton X-100) as a function of substrate concentration in solution (Fig. S1). A buffer solution consisting of 50 mM MOPS pH 7.5 + 0.4 % Triton X-100 + 2 mM CaCl₂ was used. Interestingly the wild-type variant displayed the highest specific activity for both substrates tested, in particular at higher concentrations, whereas the Esterase variant displayed extremely low activity. The activity of both the wild-type and Hybrid variants increased at higher substrate concentrations, although this increase was more rapid for the wild-type variant.

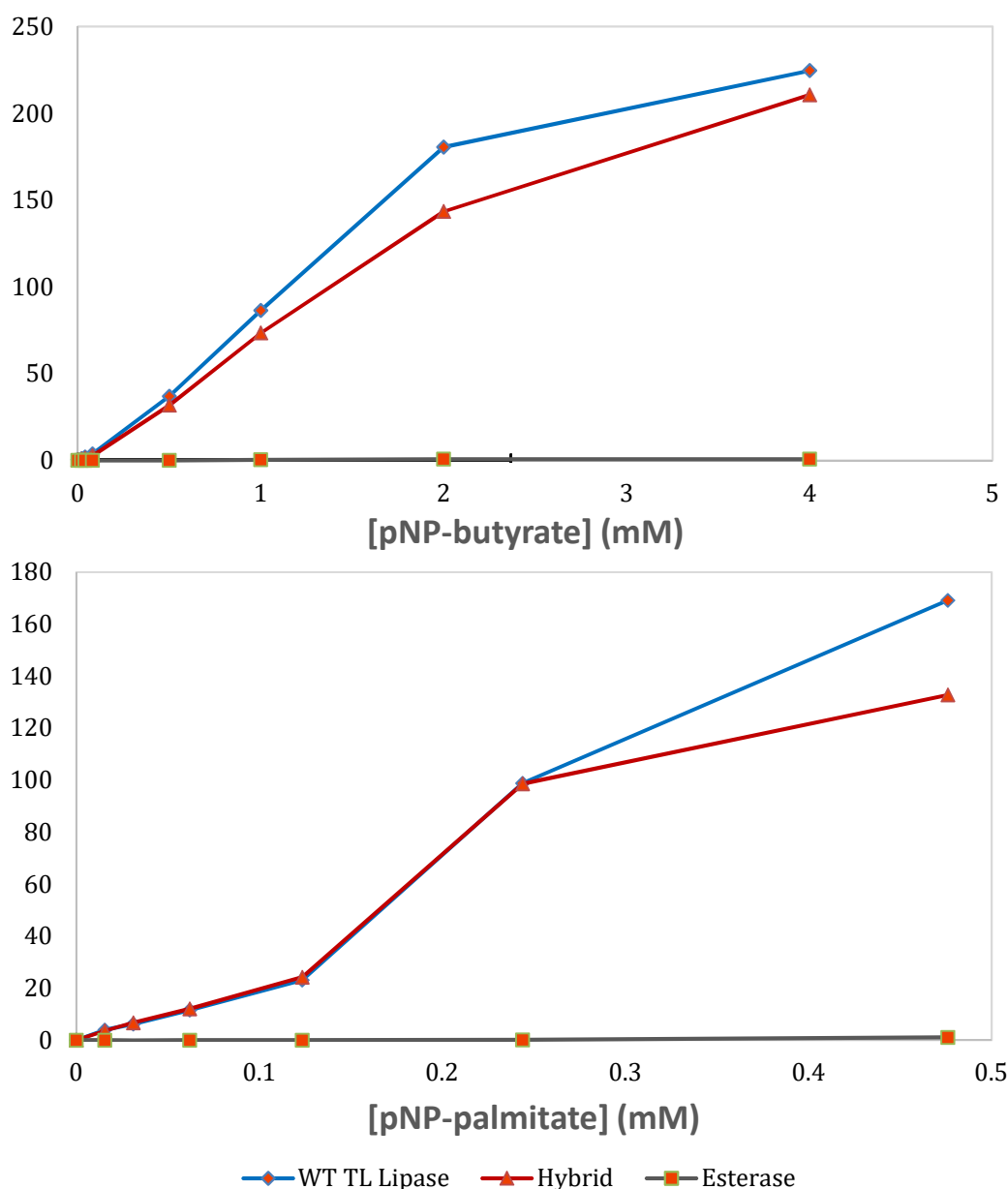


Figure S1: The specific activity of the TLL variants was determined against pNP-palmitate (A) and pNP-butyrates (B) emulsified in non-ionic surfactant as a function of substrate concentration at pH 7.5.

CG simulations of TLL variants with a triglyceride interface

5 repeat simulations were performed of three TLL variants (wild-type open, wild-type closed, Esterase and Hybrid variants) positioned in three different orientations above a triglyceride surface, totalling 15 simulations per variant. The number of binding events within the replicate ensembles is shown in Table S1. One binding event is defined as the association of the lipase molecule with the triglyceride interface during one simulation.

Simulated system	Binding events
Wild-type: open	10/15
Wild-type: closed	13/15
Esterase	9/15
Hybrid	14/15

Table S1: The number of binding events shown for CG-MD simulations of TLL variants (Esterase, Hybrid, wild-type) with a triglyceride layer. One binding event represents the association of the lipase variant with the interface during one simulation. Once bound, the lipase did not dissociate from the interface in any of the simulations (2 μ s). 15 replicates were simulated per variant.

The rotational and translation motions exhibited by the lipase variants during selected simulations are shown in Fig. S1. These motions were described by calculation of rotation matrices, as described in the main text.

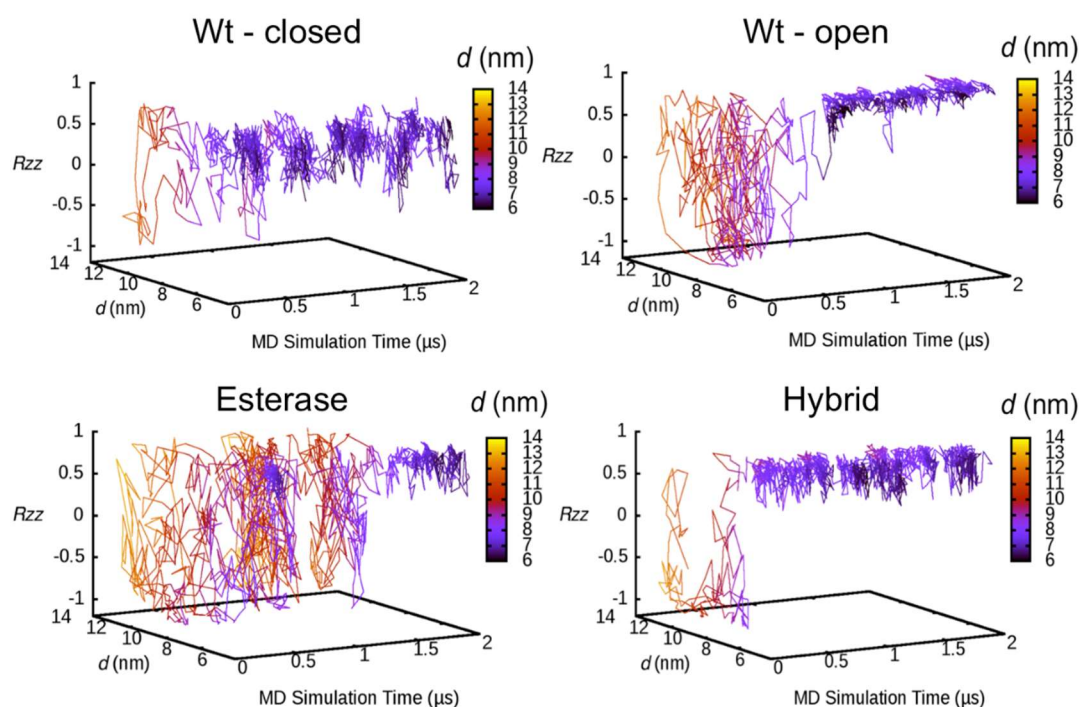


Figure S2: Time evolution of d and R_{zz} for representative CG-MD simulations of lipase variants with a triglyceride surface. R_{zz} defines the transition of the lipase variant relative to the reference orientation; when $R_{zz} = 1$, the lipase variant adopts the orientation of the reference structure.

Atomistic simulations of TLL variants with a tributyrin layer

Three repeat simulations of the wild-type, Esterase, and Hybrid TLL variants were simulated with a tributyrin interface. Both initially closed and open structures were investigated. The final structure of the lipase variants and time evolution of the root mean squared deviation (RMSD) are shown in Fig. S2 and S3. Corresponding to the results reported in the main text, the initially closed wild-type variant showed partial interfacial activation during association with the interface for 2/3 of the repeat simulations, whereas the lid remained in the closed position during Run 3 (Fig. S3A). The initially closed Esterase and Hybrid variants simulations also correspond to the simulations shown in the main text, where Esterase did not exhibit any interfacial activation, and 2/3 repeat simulations of Hybrid resulted in partial activation (Fig. S3B and C).

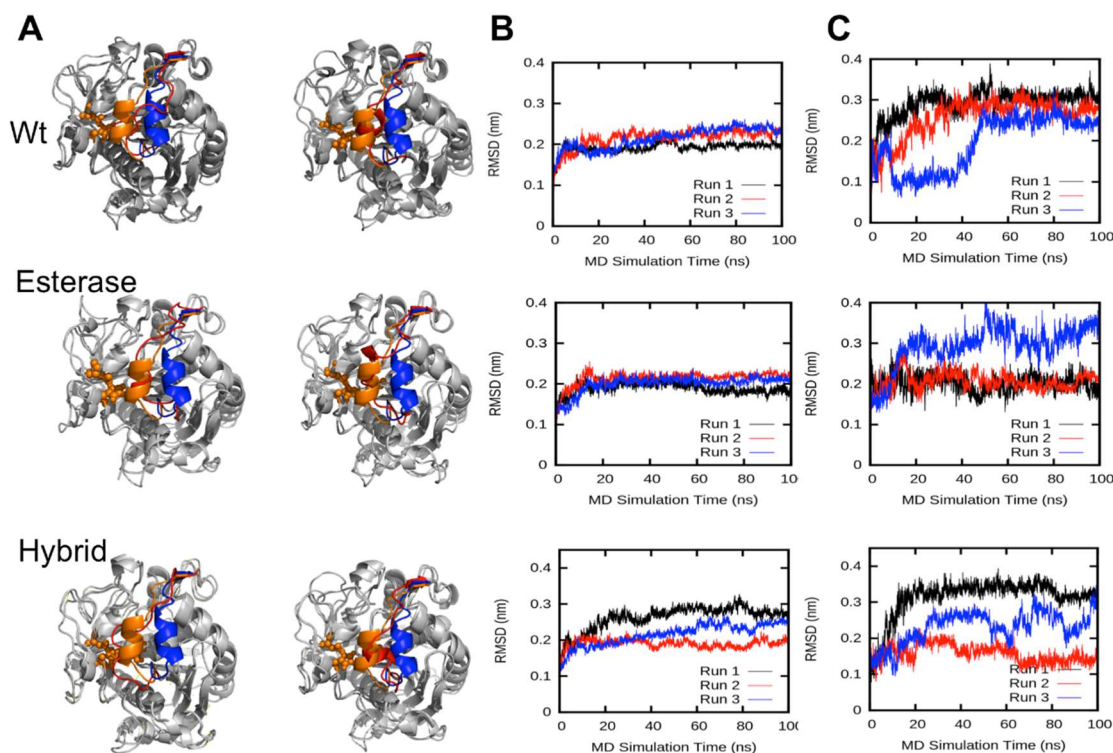


Figure S3: (A) Top down view of the initially closed TLL variant lipase structures after simulation of a tributyrin interface (100 ns; lid coloured red) aligned with the closed (lid coloured orange) and open (lid coloured blue) crystal structures of wild-type TLL. The structures represent Run 1 and 3 of 3 repeat simulations for the wild-type lipase, and Run 2 and 3 for the 1L and 3L variants. (B) Time evolution of the RMSD of the Cα atoms of the lipase structures during simulation. (C) Time evolution of the Cα atoms within the lid region of the TLL variants during simulation.

Simulations of the initially open wild-type variant resulted in almost immediate closure of the lid region. This most likely results from exposure of the hydrophobic lid residues to the surrounding water molecules prior to lipase association with the interface (Fig. S3A). Conversely, the lid region of the 1L variant tended to remain in the open position, and could be related to the reduced flexibility imparted by the particular substitutions made, as is discussed in the main text (Fig. S3B).

Similarly, the lid region of 3L seemed to be most stable in the open position during all of the repeat simulations (Fig. S3C). This correlates well with the experimentally observed increase in interfacial lipase activity for this variant (Skjold-Jørgensen et al., 2014).

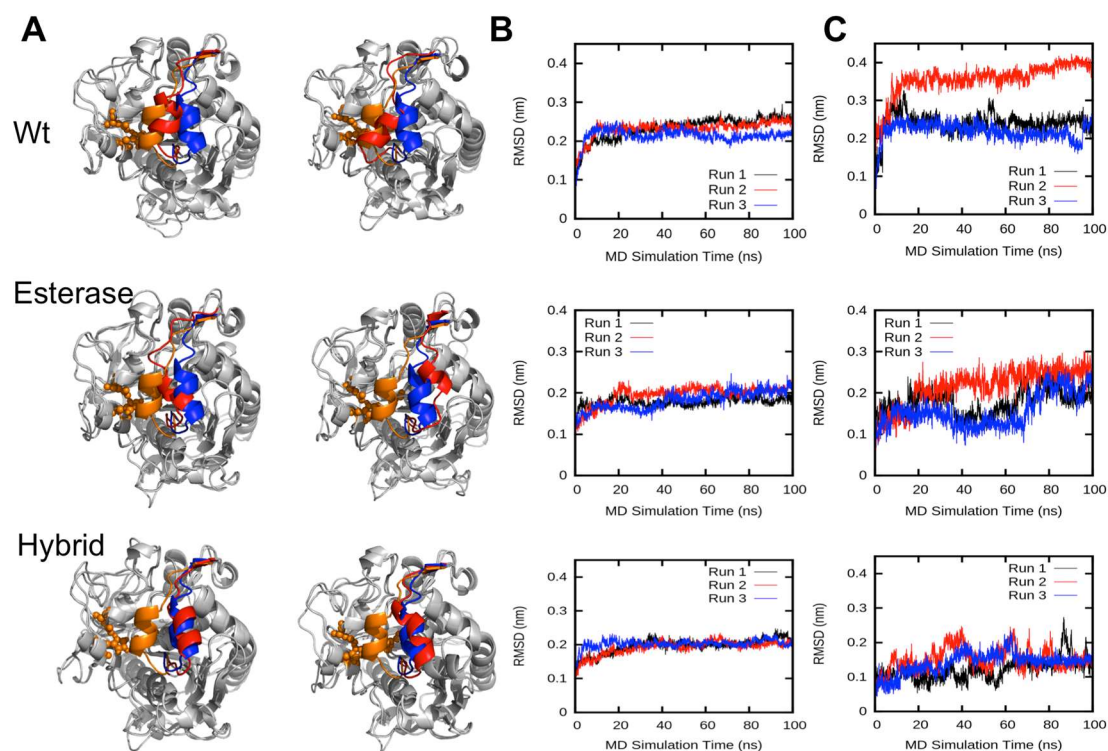


Figure S4: (A) Top down view of the initially open TLL variant lipase structures after simulation of a tributyrin interface (100 ns; lid coloured red) aligned with the closed (lid coloured orange) and open (lid coloured blue) crystal structures of wild-type TLL. The structures represent Run 2 and 3 of 3 repeat simulations. (B) Time evolution of the RMSD of the Ca atoms of the lipase variants during simulation. (C) Time evolution of the RMSD of the Ca atoms within the lid region of the TLL variants during simulation.

Conformational Dynamics of Lipase Variants in Water

In order to compare interfacial behaviour of the lipase molecules to purely aqueous environment, three repeat simulations of the lipase variants either initially closed or open were performed in water. Structural analysis is shown in Fig. S4 and Fig. S5 for the initially closed lipase variants; Fig. S6 and Fig. S7 shows analysis for simulations of the initially open lipase variants.

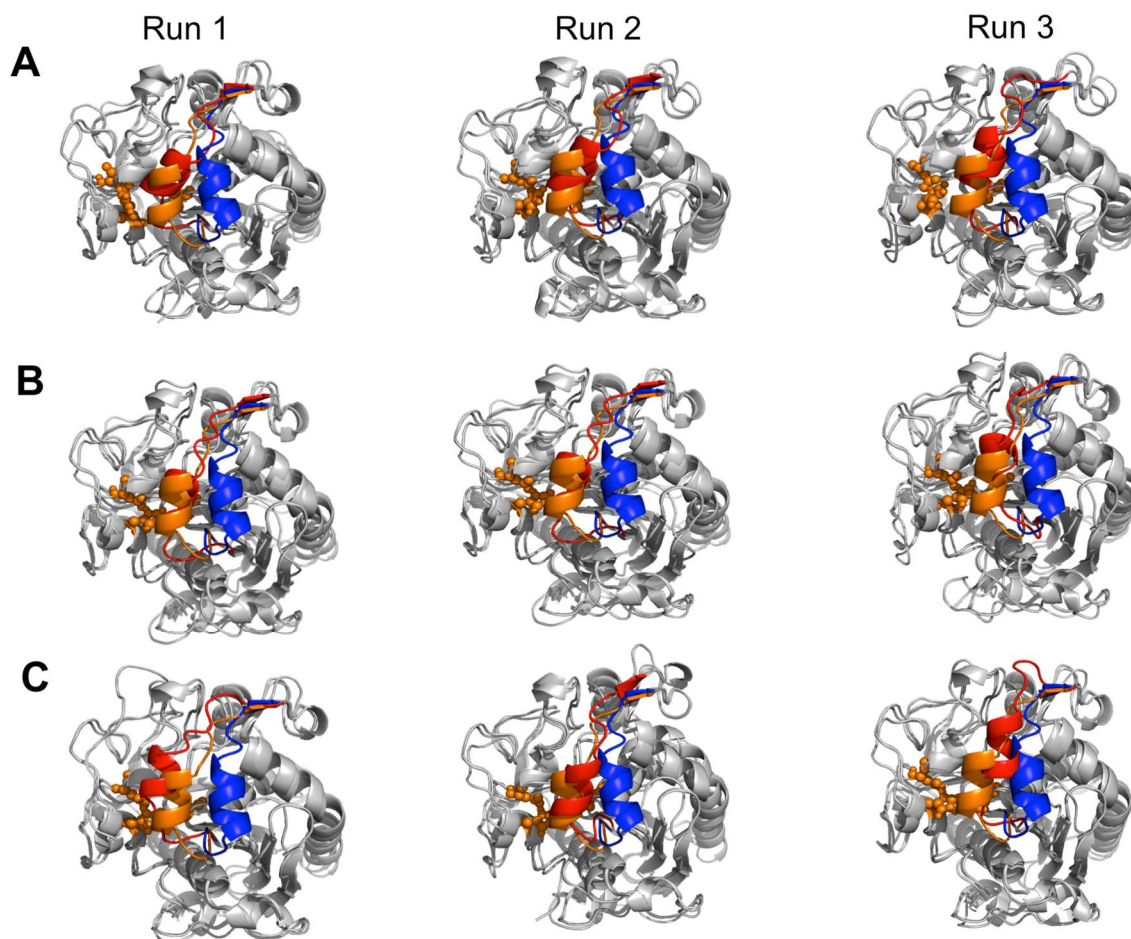


Figure S5: Top down view of initially closed lipase structures extracted from the final frame of AT-MD simulations in the lipase variants in water. The simulated structures of the wild-type (**A**), Esterase (**B**) and Hybrid (**C**) lipase variants (100 ns; lid coloured red) are aligned with the closed (lid coloured orange) and open (lid coloured blue) crystal structures of wild-type TLL.

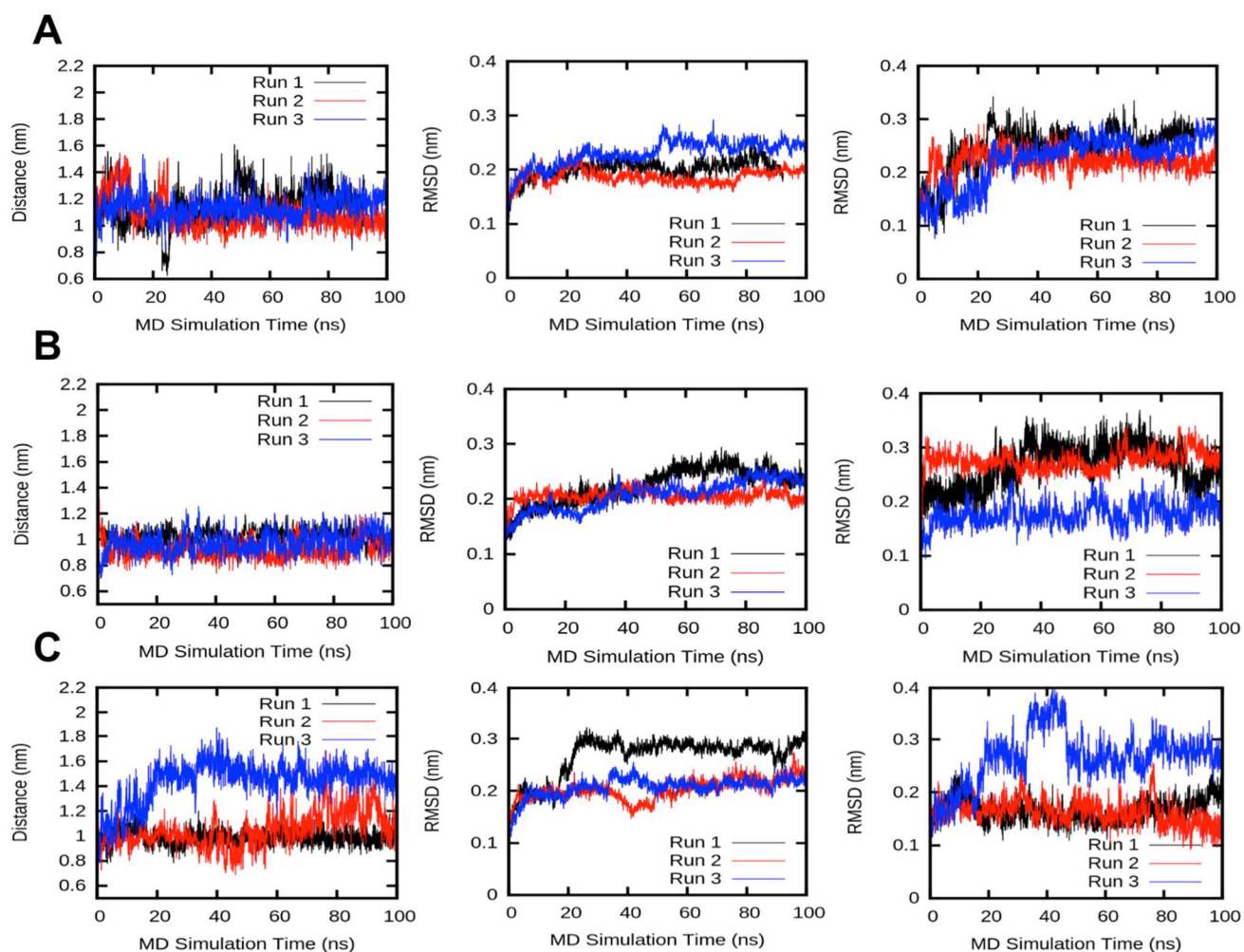


Figure S6: Structural analysis for AT-MD simulations of initially closed wild-type (A), Esterase (B), and Hybrid variants (C) in water. Left: Time evolution of the distance between the C α atoms of residue 87 (within lid region) and residue 255 (neighbouring region) measuring the closed-open transition. Middle: Time evolution of the RMSD of protein C α atoms. Right: Time evolution of the C α atoms within the lid region of the protein.

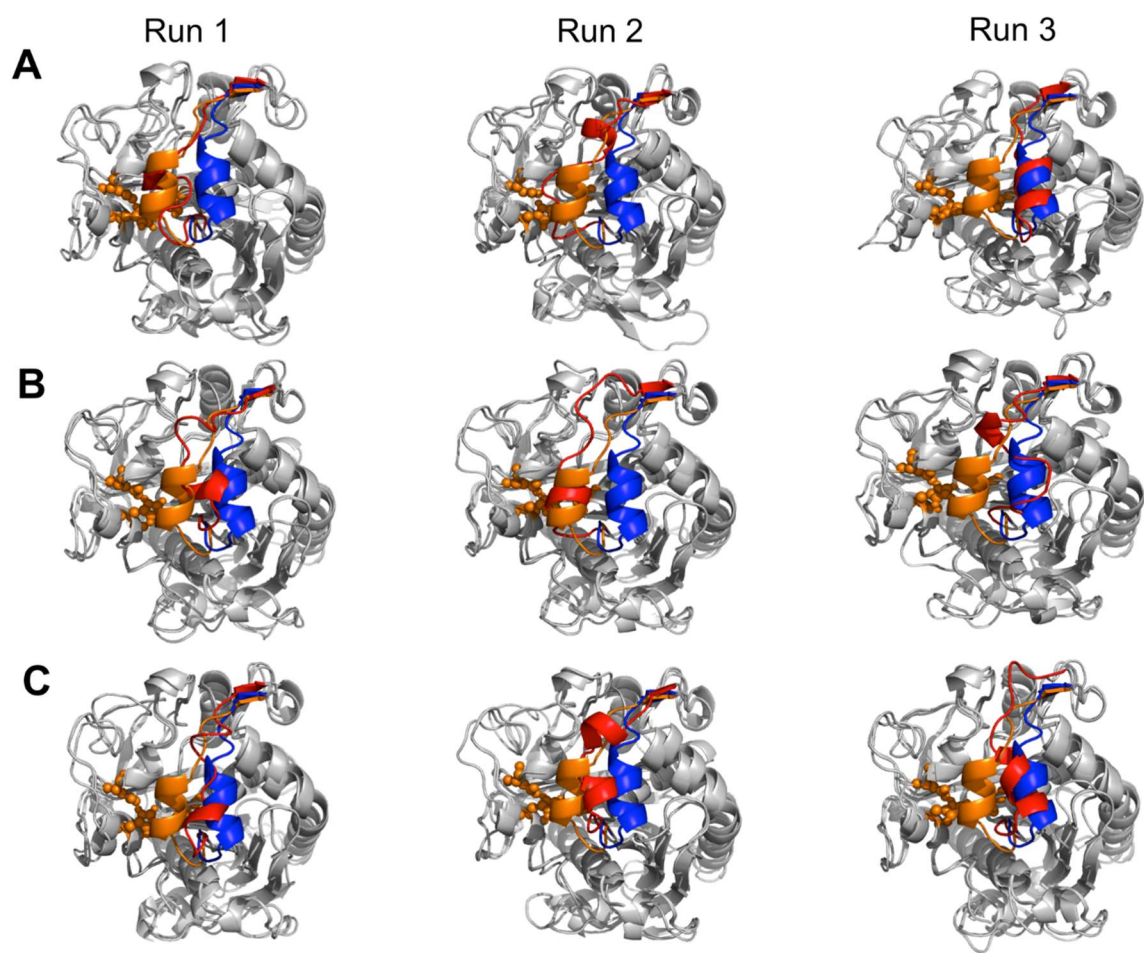


Figure S7: Top down view of initially open lipase structures extracted from the final frame of AT-MD simulations in the lipase variants in water. The simulated structures of the wild-type (**A**), Esterase (**B**) and Hybrid (**C**) lipase variants (100 ns; lid coloured red) are aligned with the closed (lid coloured orange) and open (lid coloured blue) crystal structures of wild-type TLL.

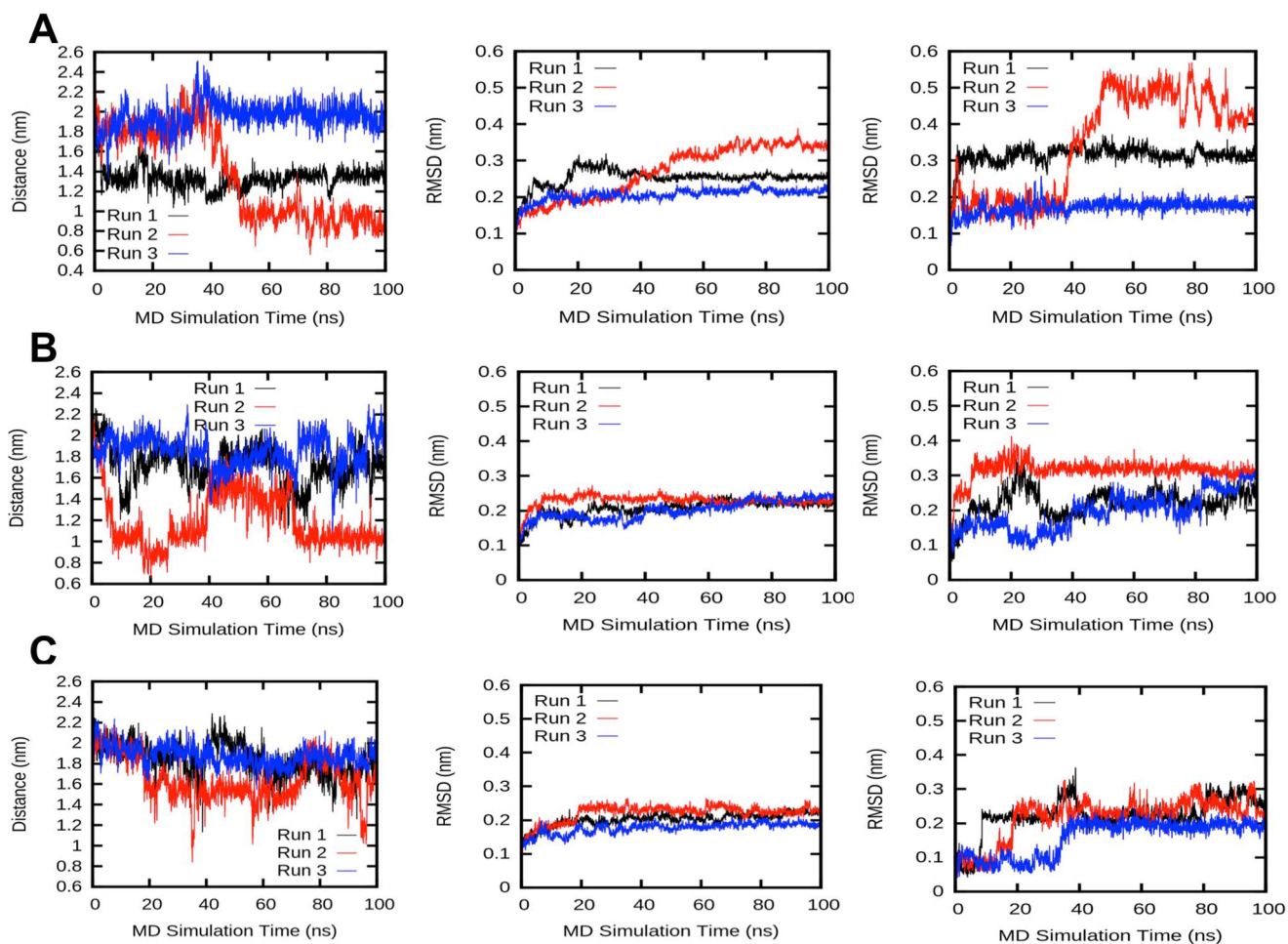


Figure S8: Structural analysis for AT-MD simulations of initially open wild-type (**A**), Esterase (**B**), and Hybrid variants (**C**) in water. Left: Time evolution of the distance between the C α atoms of residue 87 (within lid region) and residue 255 (neighbouring region) measuring the closed-open transition. Middle: Time evolution of the RMSD of protein C α atoms. Right: Time evolution of the C α atoms within the lid region of the protein.

Steered MD simulations of TLL variants in water

In order to investigate the energetics underlying lid displacement in the TLL variants, SMD simulations were performed using a distance CV. Contact analysis between the lid residues and the surrounding protein environment are shown in Fig. S7. Furthermore, the orientation of the amino acid side chains at the end of the SMD simulations are shown in Fig. S8.

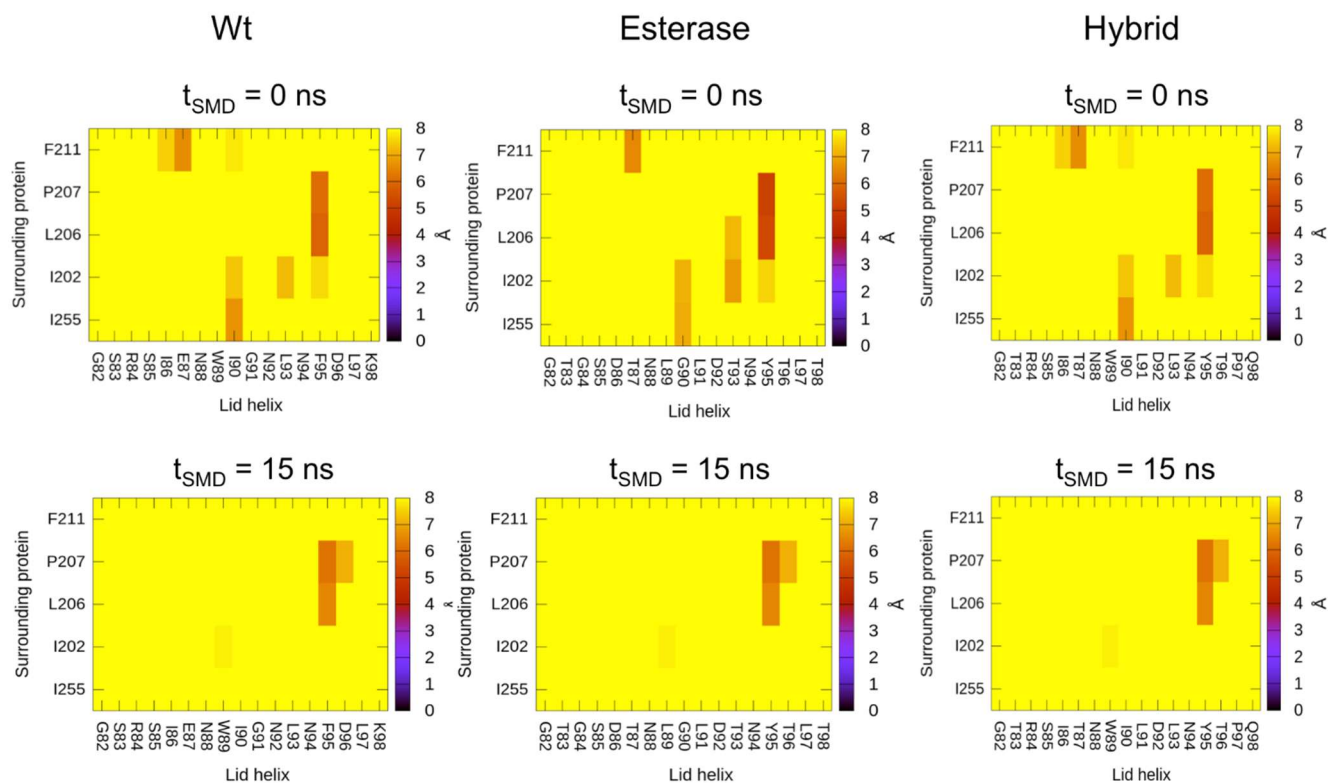


Figure S9: Contacts calculated between the lid region of TLL variants and surrounding protein residues during SMD simulations of the variants in water. The matrices show contacts for the first (*top panel*) and final (*bottom panel*) frames of the SMD simulations. A 4 Å cut-off was used.

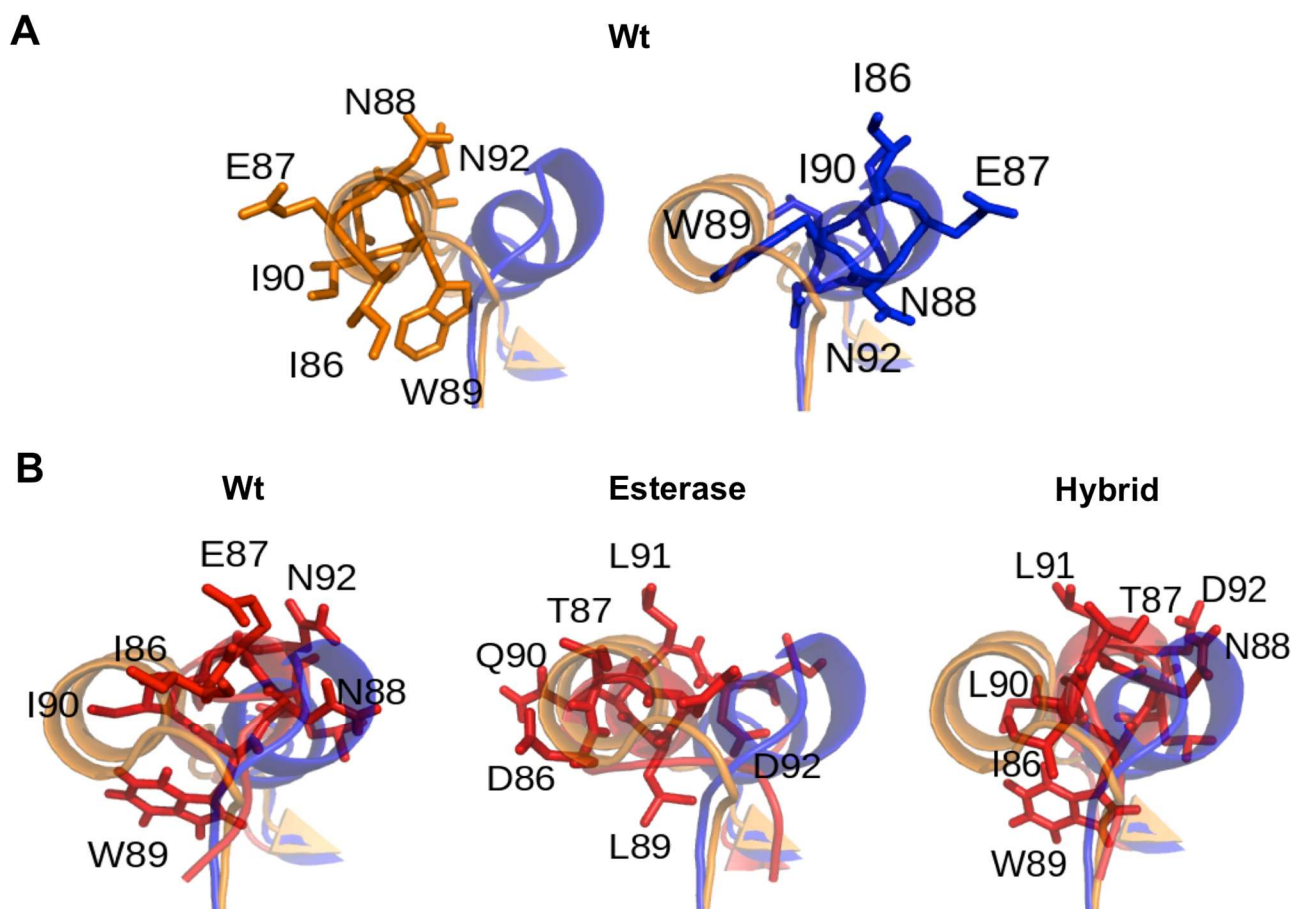


Figure S10: (A) Position of amino acid side chains within the lid region of the closed (orange) and open (blue) crystal structures of wild-type TLL. Only the lid region (cartoon representation) is shown for the aligned structures. The residues are shown as sticks. (B) Position of amino acid side chains within the lid regions of lipase variants after SMD simulations (15 ns). The lid region and residues are coloured in red. These are aligned with the closed (orange) and open (blue) positions of the lid region in the crystal structures of TLL.

As can be seen from Fig. S9B, the Esterase lid region was not significantly displaced during the SMD simulation. Instead, a neighbouring loop region was shifted, resulting in evolution of the distance CV used during the SMD simulation (Fig. 8B). The start and end positions of the shifted loop region and the lid region of the Esterase variant are shown in Fig. S10, confirming that evolution of the CV evolution can be attributed to the movement of this neighbouring loop region rather than the lid helix.