Published in final edited form as:

J Phys Chem Lett. 2018 November 15; 9(22): 6444–6449. doi:10.1021/acs.jpclett.8b02945.

Impact of Mutations on the Binding Pocket of Soybean Lipoxygenase: Implications for Proton-Coupled Electron Transfer

Pengfei Li, Alexander V. Soudackov, and Sharon Hammes-Schiffer*

Department of Chemistry, Yale University, 225 Prospect Street, New Haven, Connecticut 06520

Abstract

Soybean lipoxygenase catalyzes a proton-coupled electron transfer (PCET) reaction and serves as a prototype for hydrogen tunneling in enzymes due to the unusually high kinetic isotope effect and significant modulation of the rate constant and kinetic isotope effect by mutation. Herein these experimental observations are interpreted in the context of changes to the substrate binding pocket in microsecond molecular dynamics simulations of wild-type and mutant soybean lipoxygenase. The binding pocket exhibits an hourglass shape with residues L546 and L754 bracketing the bottleneck, positioning the linoleic acid substrate for PCET. Mutation of I553 to less bulky residues slightly increases the width of the bottleneck and the volume of the binding pocket. Mutating L546 or L754 to a smaller residue further enlarges this width and volume, and mutating both has an even more dramatic effect. This analysis illustrates how mutation of the substrate binding pocket can be used as a strategy to tune the kinetics.

TOC Graphic

*Corresponding Author sharon.hammes-schiffer@yale.edu.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Details of protocols for binding pocket volume analysis, docking, and molecular dynamics simulations, force field parameters, supplementary figures and tables (PDF)

The authors declare no competing financial interest.

Lipoxygenases play vital roles in various life processes such as plant defense and growth¹ and animal cell differentiation and maturation.² Soybean lipoxygenase-1 (SLO) catalyzes the peroxidation of linoleic acid through a mechanism that involves proton-coupled electron transfer (PCET), which is rate-limiting for temperatures above 32°.³ In this PCET reaction, the proton transfers from C11 of the linoleic acid substrate to the hydroxyl group coordinated to the iron of the cofactor, while the electron transfers from the π -backbone of the linoleic acid to this iron.⁴ The kinetic isotope effect (KIE) for wild-type (WT) SLO, where only the transferring hydrogen was substituted with deuterium, was found to be ~80 at room temperature,⁴ and various single and double mutants were found to significantly modulate the magnitude and temperature dependence of this KIE. In particular, a series of I553 mutants indicated that the magnitude and temperature dependence of the KIE increase as the size of this side chain decreases (Table 1).⁵ Moreover, the L546A/L754A double mutant (DM) was found to exhibit a KIE of ~700 at room temperature with relatively weak temperature dependence.^{6–7} These two hydrophobic residues, L546 and L754, are highly conserved throughout the iron lipoxygenase family⁸ and have been presumed to confine the positioning of the reactive C11 of the bound substrate through direct steric interactions.^{9–10} Residue I553 is further away but is still part of the extended substrate binding pocket. Due to these intriguing experimental KIE data on SLO and its mutants, this enzyme serves as a prototype for hydrogen tunneling in enzymes.

Despite a substantial number of theoretical studies aimed at understanding the physical basis for these unusually high KIEs,^{5–7,9,11–18} a comprehensive atomic-level understanding of these experimental results is still not fully established. Recent quantum mechanical/molecular mechanical (QM/MM) free energy simulations,¹⁸ in conjunction with a vibronically nonadiabatic PCET theory,¹⁹ suggest that the larger KIE for the DM compared to WT SLO arises from differences in the substrate binding to the enzyme.²⁰ Prior ENDOR experiments²¹ highlighted these differences in substrate binding, as well as the need for stochastic conformational sampling to achieve reactive geometries, and analysis of the X-ray

crystal structures indicated a slightly expanded active site cavity for the DM.⁶ To further investigate these issues, herein we present microsecond classical molecular dynamics (MD) simulations of WT and mutant SLO, including I553V, I553A, I553G, L546A, L754A, and the DM, along with an analysis of the binding pocket residues. This analysis includes the calculation of the pocket volume and substrate docking, as well as the evaluation of distances between key residues near the catalytic region. This study provides fundamental insights into the role of binding pocket structure and fluctuations in regulating PCET reactions in enzymatic reactions.

The initial coordinates for the WT, I553V, I553A, I553G, L546A, L754A, and DM systems were obtained from crystal structures corresponding to PDB entries 3PZW,²² 3BND,⁵ 3BNE,⁵ 3BNC,⁵ 5TQN,²³ 5TR0,²⁴ and 5TQO,²⁵ respectively. AutoDockTools²⁶ and AutoDock Vina²⁷ were employed to investigate substrate docking to WT and DM SLO with the Fe(III)OH state of the cofactor. We also performed classical MD simulations of WT SLO and six mutants with the Fe(II)H₂O state of the cofactor and no substrate bound because the substrate has been found to dissociate during equilibration as a result of weak binding interactions. Moreover, crystal structures of SLO with bound substrate are not available, and studying the binding pocket of the protein without substrate avoids the bias inherent to the substrate docking procedure. For the classical MD, the AMBER ff14SB force field was used to model the protein,²⁸ in conjunction with additional parameterization of the metal site via the MCPB.py program²⁹ and van der Waals parameters of the Fe²⁺ ion obtained from the ion-oxygen distance (IOD) parameter set.³⁰ The TIP3P water model³¹ was used to model the solvent with ~0.15 M NaCl, where the van der Waals parameters of the Na⁺ and Cl⁻ ions were obtained from the hydration free energy (HFE) parameter set for the TIP3P water model.³² The production MD trajectories were conducted using pmemd.cuda³³ to propagate 1 μ s for each of the seven systems. Finally, the POVME2 tool³⁴ was used to evaluate the binding pocket volume based on these trajectories. Additional details of these simulations are provided in the Supporting Information (SI).

Using the POVME2 tool³⁴ to analyze the binding pocket volume of WT SLO, the binding pocket was found to exhibit the shape of an hourglass with L546 and L754 bracketing the bottleneck (Figure 1A). This observation is consistent with the initial analysis of the 1YGE crystal structure of WT SLO.³⁵ In the crystal structures of WT, I553L, I553V, I553A, I553G SLO (PDB entries 3PZW²²/5T5V,³⁶ 3BNB,⁵ 3BND,⁵ 3BNE,⁵ 3BNC,⁵ respectively), the side chains of residues L546 and L754 are perpendicular to each other (i.e., the plane containing the C _{γ} , C _{δ 1}, C _{δ 2} atoms in the L546 residue is perpendicular to the corresponding plane of the L754 residue, as depicted in Figure S1). Our MD simulations illustrate that this angle fluctuates about an average value of slightly lower than 90° for WT and I553V, while its average value is slightly higher for I553A and I553G (Figure S1). These two methyl groups on each residue bracketing the bottleneck provide steric interactions that may assist in positioning the substrate in the binding pocket, as proposed previously.^{5, 10}

To further investigate the roles of L546 and L754 at the bottleneck of the binding pocket, we performed docking calculations for linoleic acid binding to WT and DM SLO. In these calculations, the substrate was flexible, but the protein remained rigid with the coordinates obtained from an equilibrated conformation with the Fe(III)OH state of the cofactor. From

the 1998 docking poses generated for WT SLO and 2000 docking poses for the DM, we identified the poses satisfying criteria designed to select conformations suitable for PCET. These criteria are as follows: C11-O distance ≤ 3.5 Å, C11-Hs-O angle $\geq 120^\circ$, and Hs-O-Ho angle $\geq 100^\circ$, where Hs is the pro-*S* hydrogen that transfers from C11 of the linoleic acid to the oxygen of the hydroxyl group, and Ho is the hydrogen atom of the hydroxyl group.

For WT SLO, we found that only nine structures satisfied all of these geometric criteria, and the five structures with the highest affinities were oriented with the carboxylate group of the linoleic acid hydrogen-bonded to R707, previously denoted as the carboxylate-in conformation (Figure 1B).²¹ The remaining four structures satisfying these criteria were orientated in the carboxylate-out conformation (Figure S2), with the tail occupying the channel that has been proposed to be involved in oxygen transport (Figure 1A). In all five of the structures corresponding to the carboxylate-in conformation, the C9-C10 double bond was pinched by the sidechains of L546 and L754 at the bottleneck of the binding pocket (Figure 1B and Table S1). These binding modes are similar to the binding mode observed in our previous QM/MM free energy simulations that applied restraints at the proton transfer interface, which ensured that the substrate remained bound¹⁸ (Figure S3). A channel analysis using the CAVER Analyst program (Figure S4)³⁷ identified several potential oxygen channels for these conformations that would yield the correct stereochemistry for the product, generating 13*S*-hydroperoxyoctadecadienoic acid. These results support the previous hypothesis that linoleic acid utilizes both subcavities for binding³⁵ and suggest that L546 and L754 may play significant roles in positioning the substrate in the binding pocket as well as orienting it for the PCET reaction and the subsequent reaction with molecular oxygen.

For the DM, we found that ten structures satisfied the geometric criteria that selected conformations suitable for PCET. In contrast to WT SLO, these ten structures could not be classified into two distinct groups, such as carboxylate-in and carboxylate-out, because they exhibited a much wider range of conformations. Five different examples of these binding modes are illustrated in Figure S5. This wider range of conformations found for the DM is consistent with the enlarged substrate binding pocket in the DM, providing much more freedom for substrate binding.

Our microsecond MD simulations of WT SLO, as well as the I553 mutants, illustrate that the mutation of I553 to a smaller residue increases the width of the bottleneck bracketed by L546 and L754 and increases the binding pocket volume. The width of the bottleneck is analyzed by calculating the distance between the midpoint of the sidechain C₈₁ and C₈₂ atoms in L546 and the analogous midpoint in L754. Figure 2 depicts the distribution of this distance for the WT, I553V, I553A, and I553G systems, illustrating that the average distance increases and the distribution becomes broader as residue 553 becomes smaller. The MD data cannot differentiate between all of the systems but show clear differences between the larger residues (WT and I553V) and the smaller residues (I553A and I553G). Specifically, the average distances are 4.80, 4.76, 5.09, and 5.03 Å with half peak widths of 0.75, 0.77, 1.02, and 1.10 Å for WT, I553V, I553A and I553G, respectively. Moreover, the root-mean-square fluctuations (RMSFs) of the C_α atoms of residues 541–546 are greater for I553A and I553G than for WT and I553V (Figure S6), indicative of enhanced flexibility on the L546

side of the bottleneck. However, the relative orientation of residues 546 and 754 is maintained along this series of mutants; the angle between the plane containing the C_γ, C_{δ1}, and C_{δ2} atoms in L546 and the corresponding plane in L754 shifts only slightly from 90° as the sidechain of 553 becomes smaller (Figure S1). Consistent with these trends in the bottleneck width, Table 2 indicates that the binding pocket volume is greater for I553A and I553G than for WT and I553V.

These results for the I553 series of mutants are consistent with the experimental observations⁵ that the rate constant changes by only a factor of five or less, the KIE increases from 80 to 180, and the temperature dependence of the KIE increases significantly as residue 553 becomes smaller (Table 1). Because the orientation and distance between L546 and L754 is only slightly perturbed, these residues are still able to position the substrate in an orientation suitable for PCET, leading to only modest changes in the catalytic rate constant. The slightly larger width of the bottleneck provides more freedom for the part of the substrate around the C9-C10 double bond to move. This enhanced mobility could lead to a slightly larger equilibrium distance between the proton donor and acceptor (i.e., C11 of the substrate and the oxygen of the hydroxyl group) and a broader sampling distribution associated with this distance. According to nonadiabatic PCET theory,¹⁹ a larger equilibrium proton donor-acceptor distance typically leads to a higher KIE when the ground vibronic states dominate, as observed previously for SLO,^{11, 19} and a broader sampling distribution associated with this distance typically leads to greater temperature dependence of the KIE.

Mutation of L546 and L754 to smaller residues impacts the bottleneck width and the binding pocket volume in a more direct manner. As in the analysis of the I553 mutants, the bottleneck width is represented by the distance between two points associated with the sidechains of residues 546 and 754. These points correspond to the midpoint of the C_{δ1} and C_{δ2} atoms for leucine and the C_β atom for alanine. This distance and the binding pocket volume increase along the series of WT to L546A and L754A, which are similar, to the DM (Figure 3 and Table 2). The slightly larger distance for L754A compared to L546A (Figure 3) can also be inferred from the crystal structures (Figure S7). Similar to the I553 mutants, we found that the RMSFs of the backbone Ca atoms of residues 541–545 are greater for this set of mutants than for WT SLO (Figure S8), indicating enhanced mobility of this region caused by these mutations. Moreover, analysis of these MD trajectories illustrated that more water molecules were located within 10 Å of the Fe²⁺ ion in the L754A mutant and the DM than in the WT and L546A mutant systems (Figure S9).

As a result of the wider bottleneck and greater volume, the equilibrium proton donor-acceptor distance is expected to increase along this series. As mentioned above, according to nonadiabatic PCET theory,¹⁹ this greater equilibrium proton donor-acceptor distance is typically associated with a decrease in the PCET rate constant and an increase in the KIE when the ground vibronic states dominate.^{11, 19} In addition, the equilibrium constant associated with the substrate binding in an active conformation^{18, 21} is also expected to decrease when the binding pocket volume increases. Thus, this analysis of the MD simulations is consistent with the experimentally observed^{6, 9} decrease in the catalytic rate constant and increase in the KIE along this series. Because the KIE is influenced by many factors in addition to the binding pocket volume and bottleneck width, the trends within the

I553 mutant series cannot be meaningfully compared to the trends within the L546A/L754A set of mutants. Moreover, the binding pocket volumes for the I553G, I553A, L546A, and L754A mutants are the same to within the accuracy of the calculations, as evident from the standard deviations reported in Table 2. The temperature dependence of the KIE for the DM appears to arise from a balance among several factors, which could include effects on the structure and conformational motions, and is not easily explained in terms of only substrate binding.

These differences between WT and DM SLO are consistent with our previous QM/MM free energy simulations.¹⁸ These previous simulations indicated that the equilibrium proton donor-acceptor distance (i.e., the C11-O distance) is ~ 0.2 Å greater for the DM compared to WT SLO. Moreover, this previous work also illustrated that the substrate backbone could adopt different configurations for the DM in which the substrate backbone moves into the region previously occupied by the leucine side chain. These different configurations lead to a less optimal orientation of the proton transfer interface, a larger proton donor-acceptor distance, and a lower probability of sampling shorter proton donor-acceptor distances due to the constrained environment. These combined data suggest that the substrate is bound to the DM in a manner that is less conducive to PCET than for WT SLO. These differences in substrate binding lead to less effective sampling of shorter proton donor-acceptor distances for the DM and explain the higher KIE for the DM. All of these observations are consistent with the present work showing the significantly enlarged substrate binding cavity and bottleneck width for the DM compared to WT SLO.

The MD simulations presented herein provide a plausible explanation for the experimentally observed effects of mutations on the rate constant and KIE of SLO in terms of changes to the shape and volume of the substrate binding pocket. The binding pocket of SLO is found to exhibit an hourglass shape with residues L546 and L754 bracketing the bottleneck. These two residues are proposed to play an important role in positioning the substrate by pinching its double bond and maintaining a suitable orientation for PCET and the subsequent reaction with molecular oxygen. The MD simulations illustrated that mutating I553 to smaller residues slightly increases the width of the bottleneck and the volume of the binding pocket, explaining the somewhat higher KIEs and greater temperature dependencies of the KIEs. Mutating L546 or L754 to a smaller residue exerts a more direct effect and enlarges the bottleneck width and binding pocket even more, leading to lower catalytic rate constants and higher KIEs. Moreover, the DM, in which both L754 and L546 are mutated to alanine, exhibits an even larger bottleneck width and binding pocket volume, providing an explanation for the more dramatic increase of the KIE to ~ 700 .^{6, 18} These simulations and analyses illustrate how the kinetics of PCET reactions may be strongly influenced by the characteristics of the substrate binding pocket, which can be finely tuned through specific mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

We acknowledge the financial support from the National Institutes of Health Grant GM056207. This work used the Extreme Science and Engineering Discovery Environment (XSEDE),³⁹ which is supported by National Science Foundation grant number ACI-1548562. Specifically, this work used Comet at the San Diego Supercomputer Center through allocation TG-MCB120097. We are also grateful for helpful discussions with David Stevens.

Funding Sources

National Institutes of Health Grant GM056207

References

1. Grechkin A, Recent developments in biochemistry of the plant lipoxygenase pathway. *Prog. Lipid Res.* 1998, 37, 317–352. [PubMed: 10209652]
2. Schewe T; Ku H, Do 15-lipoxygenases have a common biological role? *Trends Biochem. Sci* 1991, 16, 369–373. [PubMed: 1785138]
3. Glickman MH; Klinman JP, Nature of rate-limiting steps in the soybean lipoxygenase- 1 reaction. *Biochem.* 1995, 34, 14077–14092. [PubMed: 7578005]
4. Rickert KW; Klinman JP, Nature of hydrogen transfer in soybean lipoxygenase 1: separation of primary and secondary isotope effects. *Biochem.* 1999, 38, 12218–12228. [PubMed: 10493789]
5. Meyer MP; Tomchick DR; Klinman JP, Enzyme structure and dynamics affect hydrogen tunneling: The impact of a remote side chain (I553) in soybean lipoxygenase-1. *Proc. Natl. Acad. Sci. U.S.A.* 2008, 105, 1146–1151. [PubMed: 18216254]
6. Hu S; Sharma SC; Scouras AD; Soudackov AV; Carr CAM; Hammes-Schiffer S; Alber T; Klinman JP, Extremely elevated room-temperature kinetic isotope effects quantify the critical role of barrier width in enzymatic C-H activation. *J. Am. Chem. Soc.* 2014, 136, 8157–8160. [PubMed: 24884374]
7. Hu S; Soudackov AV; Hammes-Schiffer S; Klinman JP, Enhanced Rigidification within a Double Mutant of Soybean Lipoxygenase Provides Experimental Support for Vibronically Nonadiabatic Proton-Coupled Electron Transfer Models. *ACS Catal.* 2017, 7, 3569–3574. [PubMed: 29250456]
8. Newcomer ME; Brash AR, The structural basis for specificity in lipoxygenase catalysis. *Protein Sci.* 2015, 24, 298–309. [PubMed: 25524168]
9. Knapp MJ; Rickert K; Klinman JP, Temperature-dependent isotope effects in soybean lipoxygenase-1: correlating hydrogen tunneling with protein dynamics. *J. Am. Chem. Soc.* 2002, 124, 3865–3874. [PubMed: 11942823]
10. Klinman JP; Offenbacher AR; Hu S, Origins of Enzyme Catalysis: Experimental Findings for C-H Activation, New Models, and Their Relevance to Prevailing Theoretical Constructs. *J. Am. Chem. Soc.* 2017, 139, 18409–18427. [PubMed: 29244501]
11. Hatcher E; Soudackov AV; Hammes-Schiffer S, Proton-coupled electron transfer in soybean lipoxygenase. *J. Am. Chem. Soc.* 2004, 126, 5763–5775. [PubMed: 15125669]
12. Tejero I; Garcia-Viloca M; Gonzalez-Lafont A; Lluch JM; York DM, Enzyme dynamics and tunneling enhanced by compression in the hydrogen abstraction catalyzed by soybean lipoxygenase-1. *J. Phys. Chem. B* 2006, 110, 24708–24719. [PubMed: 17134234]
13. Hatcher E; Soudackov AV; Hammes-Schiffer S, Proton-coupled electron transfer in soybean lipoxygenase: dynamical behavior and temperature dependence of kinetic isotope effects. *J. Am. Chem. Soc.* 2007, 129, 187–196. [PubMed: 17199298]
14. Mavri J; Liu H; Olsson MH; Warshel A, Simulation of tunneling in enzyme catalysis by combining a biased propagation approach and the quantum classical path method: application to lipoxygenase. *J. Phys. Chem. B* 2008, 112, 5950–5954. [PubMed: 18069813]
15. Edwards SJ; Soudackov AV; Hammes-Schiffer S, Impact of distal mutation on hydrogen transfer interface and substrate conformation in soybean lipoxygenase. *J. Phys. Chem. B* 2010, 114, 6653–6660. [PubMed: 20423074]
16. Phatak P; Venderley J; Debrotta J; Li J; Iyengar SS, Active Site Dynamical Effects in the Hydrogen Transfer Rate-limiting Step in the Catalysis of Linoleic Acid by Soybean Lipoxygenase-1

- (SLO-1): Primary and Secondary Isotope Contributions. *J. Phys. Chem. B* 2015, 119, 9532–9546. [PubMed: 26079999]
17. Salna B; Benabbas A; Russo D; Champion PM, Tunneling Kinetics and Non-Adiabatic Proton-Coupled Electron Transfer in Proteins: The Effect of Electric Fields and Anharmonic Donor-Acceptor Interactions. *J. Phys. Chem. B* 2017, 121, 6869–6881. [PubMed: 28628313]
18. Li P; Soudackov AV; Hammes-Schiffer S, Fundamental Insights into Proton-Coupled Electron Transfer in Soybean Lipoxygenase from Quantum Mechanical/Molecular Mechanical Free Energy Simulations. *J. Am. Chem. Soc.* 2018, 140, 3068–3076. [PubMed: 29392938]
19. Hammes-Schiffer S; Soudackov AV, Proton-coupled electron transfer in solution, proteins, and electrochemistry. *J. Phys. Chem. B* 2008, 112, 14108–14123. [PubMed: 18842015]
20. Sharma SC; Klinman JP, Kinetic Detection of Orthogonal Protein and Chemical Coordinates in Enzyme Catalysis: Double Mutants of Soybean Lipoxygenase. *Biochem.* 2015, 54, 5447–5456. [PubMed: 26154975]
21. zHoritani M; Offenbacher AR; Carr CAM; Yu T; Hoeke V; Cutsail III GE; Hammes-Schiffer S; Klinman JP; Hoffman BM, ¹³C ENDOR Spectroscopy of Lipoxygenase-Substrate Complexes Reveals the Structural Basis for C-H Activation by Tunneling. *J. Am. Chem. Soc.* 2017, 139, 1984–1997. [PubMed: 28121140]
22. Chruszcz M; Wlodawer A; Minor W, Determination of protein structures—a series of fortunate events. *Biophys. J.* 2008, 95, 1–9. [PubMed: 18441029]
23. Poss EM; Fraser JS; Gee C, The Structure of Lipoxygenase-1 (Soybean) L546A Mutant at 293K. 2017.
24. Poss EM; Fraser JS; Gee C, The Structure of Lipoxygenase-1 (Soybean) L754A Mutant at 293K. 2017.
25. Poss EM; Fraser JS; Gee C, Lipoxygenase-1 (Soybean) L546A/L754A Mutant at 300K. 2017.
26. Morris GM; Huey R; Lindstrom W; Sanner MF; Belew RK; Goodsell DS; Olson AJ, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* 2009, 30, 2785–2791. [PubMed: 19399780]
27. Trott O; Olson AJ, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010, 31, 455–461. [PubMed: 19499576]
28. Maier JA; Martinez C; Kasavajhala K; Wickstrom L; Hauser KE; Simmerling C, ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. *J. Chem. Theory Comput.* 2015, 11, 3696–3713. [PubMed: 26574453]
29. Li P; Merz KM, Jr, MCPB.py: A Python Based Metal Center Parameter Builder. *J. Chem. Inf. Model.* 2016, 56, 599–604. [PubMed: 26913476]
30. Li P; Roberts BP; Chakravorty DK; Merz KM, Jr, Rational Design of Particle Mesh Ewald Compatible Lennard-Jones Parameters for +2 Metal Cations in Explicit Solvent. *J. Chem. Theory Comput.* 2013, 9, 2733–2748. [PubMed: 23914143]
31. Jorgensen WL; Chandrasekhar J; Madura JD; Impey RW; Klein ML, Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 1983, 79, 926–935.
32. Li P; Song LF; Merz KM, Jr, Systematic Parameterization of Monovalent Ions Employing the Nonbonded Model. *J. Chem. Theory Comput.* 2015, 11, 1645–1657. [PubMed: 26574374]
33. Salomon-Ferrer R; Götz AW; Poole D; Le Grand S; Walker RC, Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 2. Explicit Solvent Particle Mesh Ewald. *J. Chem. Theory Comput.* 2013, 9, 3878–3888. [PubMed: 26592383]
34. Durrant JD; Votapka L; Sorensen J; Amaro RE, POVME 2.0: an enhanced tool for determining pocket shape and volume characteristics. *J. Chem. Theory Comput.* 2014, 10, 5047–5056. [PubMed: 25400521]
35. Minor W; Steczko J; Stec B; Otwinowski Z; Bolin JT; Walter R; Axelrod B, Crystal structure of soybean lipoxygenase L-1 at 1.4 Å resolution. *Biochem.* 1996, 35, 10687–10701. [PubMed: 8718858]
36. Offenbacher AR; Hu S; Poss EM; Carr CAM; Scouras AD; Prigozhin DM; Iavarone AT; Palla A; Alber T; Fraser JS; Klinman JP, Hydrogen-Deuterium Exchange of Lipoxygenase Uncovers a Relationship between Distal, Solvent Exposed Protein Motions and the Thermal Activation Barrier

- for Catalytic Proton-Coupled Electron Tunneling. *ACS Cent. Sci.* 2017, 3, 570–579. [PubMed: 28691068]
37. Kozlikova B; Sebestova E; Sustr V; Brezovsky J; Strnad O; Daniel L; Bednar D; Pavelka A; Manak M; Bezdeka M, CAVER Analyst 1.0: graphic tool for interactive visualization and analysis of tunnels and channels in protein structures. *Bioinformatics* 2014, 30, 2684–2685. [PubMed: 24876375]
38. Collazo L; Klinman JP, Control of the Position of Oxygen Delivery in Soybean Lipoxygenase-1 by Amino Acid Side Chains within a Gas Migration Channel. *J. Biol. Chem.* 2016, 291, 9052–9059. [PubMed: 26867580]
39. Towns J; Cockerill T; Dahan M; Foster I; Gaither K; Grimshaw A; Hazlewood V; Lathrop S; Lifka D; Peterson GD, XSEDE: accelerating scientific discovery. *Comput. Sci. Eng.* 2014, 16, 62–74.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

(A) Depiction of the binding pocket volume, shown by pink spheres, illustrating the hourglass shape of the pocket, as well as L546 and L754 bracketing the bottleneck region for WT SLO with the cofactor in the Fe(II)H₂O state. The channel proposed to be involved in the transport of molecular oxygen is identified with a black arrow.³⁸ The binding pocket was generated by the POVME2 tool³⁴ with each grid point separated by 0.5 Å. (B) The linoleic acid substrate bound to WT SLO with the cofactor in the Fe(III)OH state for one of the five carboxylate-in structures found to have the highest binding affinity with the AutoDock Vina program. To avoid crowding, the water and hydroxyl groups bound to the iron are omitted. An alternative carboxylate-out substrate binding mode is shown in Figure S2 of the SI, and a comparison to the previous substrate binding mode observed in QM/MM free energy simulations¹⁸ is shown in Figure S3 of the SI.

Figure 2.

The probability distribution for the distance between the midpoint of the $C_{\delta 1}$ and $C_{\delta 2}$ atoms in the L546 sidechain and the corresponding midpoint in the L754 sidechain for the WT, I553V, I553A, and I553G SLO systems. These data were obtained from a 1 μ s MD trajectory for each system.

Figure 3.

The probability distribution for the distance between the L546 sidechain and the L754 sidechain for the WT, L546A, L754A, and DM SLO systems. The points used to compute this distance are the midpoint between the $C_{\delta 1}$ and $C_{\delta 2}$ atoms for leucine and the C_{β} atom for alanine. These data were obtained from a 1 μ s MD trajectory for each system.

Table 1.Kinetic Properties of WT SLO and Six Mutants^a

	k_{cat} (s^{-1}) at 30 °C	$E_a(\text{H})^b$ (kcal/mol)	KIE (at 30 °C)	E_a^c (kcal/mol)
WT	297	2.1	81	0.9
I553V	91	2.4	77	2.6
I553A	280	1.9	93	4.0
I553G	58	0.03	178	5.3
L546A	4.8	4.1	93	1.9
L754A	0.31	4.1	112	2.0
DM	0.025	9.9	692	0.3

^aThese data are from Ref 10, which cites the original work.^bEffective activation energy for H obtained from fitting to Arrhenius equation.^cDifference in effective activation energies for H and D.

Table 2.Binding Pocket Volume Calculated for WT and Mutant SLO^a

System	Volume (Å ³)	Standard Deviation (Å ³)
WT	105	17
I553V	101	17
I553A	134	24
I553G	129	27
L546A	138	24
L754A	130	22
DM	171	26

^a20,000 frames evenly distributed along a 1 μs MD trajectory were used for each system. The results are identical to within 0.5 Å³ for two independent sets of 10,000 frames, indicating convergence.