Supporting Information for: Origin of Regio- and Stereospecific Catalysis by 8-Lipoxygenase

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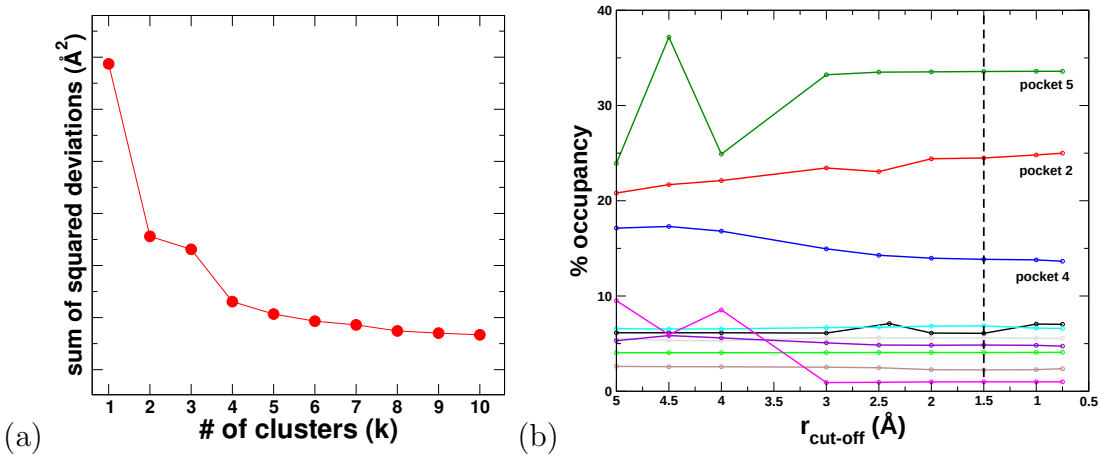


Figure S1: The number of clusters for the $k-$means clustering was decided by monitoring the sum-of-squared deviation (SSD) of the cluster members from the corresponding cluster center for an increasing number of clusters. The SSD attains a plateau at $k = 10$ (a). The other important parameter of $k-$means clustering is the cut-off distance between the cluster center and a new data point, below which the new data point is included to the cluster and a new cluster center is determined. The occupancy of different pockets as a function of cut-off distance is shown in (b). This shows that the population distribution converges for cut-off < 2.5 Å. A cut-off distance of 1.5 Å is chosen for the present analysis.

Table S1: Cluster center and variance along the three Cartesian coordinates and the corresponding pocket population in wt-lox. The pockets near the protein periphery (such as, 2, 4, and 10) show large variance.

Pocket	Cluster Center			Variance			Population
	X	Y	Z	X	Y	Z	in %
1	-0.94	7.16	7.74	0.59	0.48	1.02	6
2	-13.62	10.29	0.32	4.20	4.87	1.84	25
3	-2.60	6.47	13.02	2.13	1.33	1.90	4
4	-17.99	13.59	-0.25	9.35	4.98	4.96	14
5	3.15	12.20	-1.12	3.01	0.91	1.80	33
6	-7.96	7.43	6.07	1.81	1.94	2.62	2
7	-16.86	12.96	-4.94	0.77	0.54	0.80	5
8	-1.29	13.02	4.26	2.37	2.36	2.14	5
9	-4.50	10.31	5.86	2.08	2.59	2.06	6
10	12.26	15.80	0.53	5.16	1.56	1.38	1

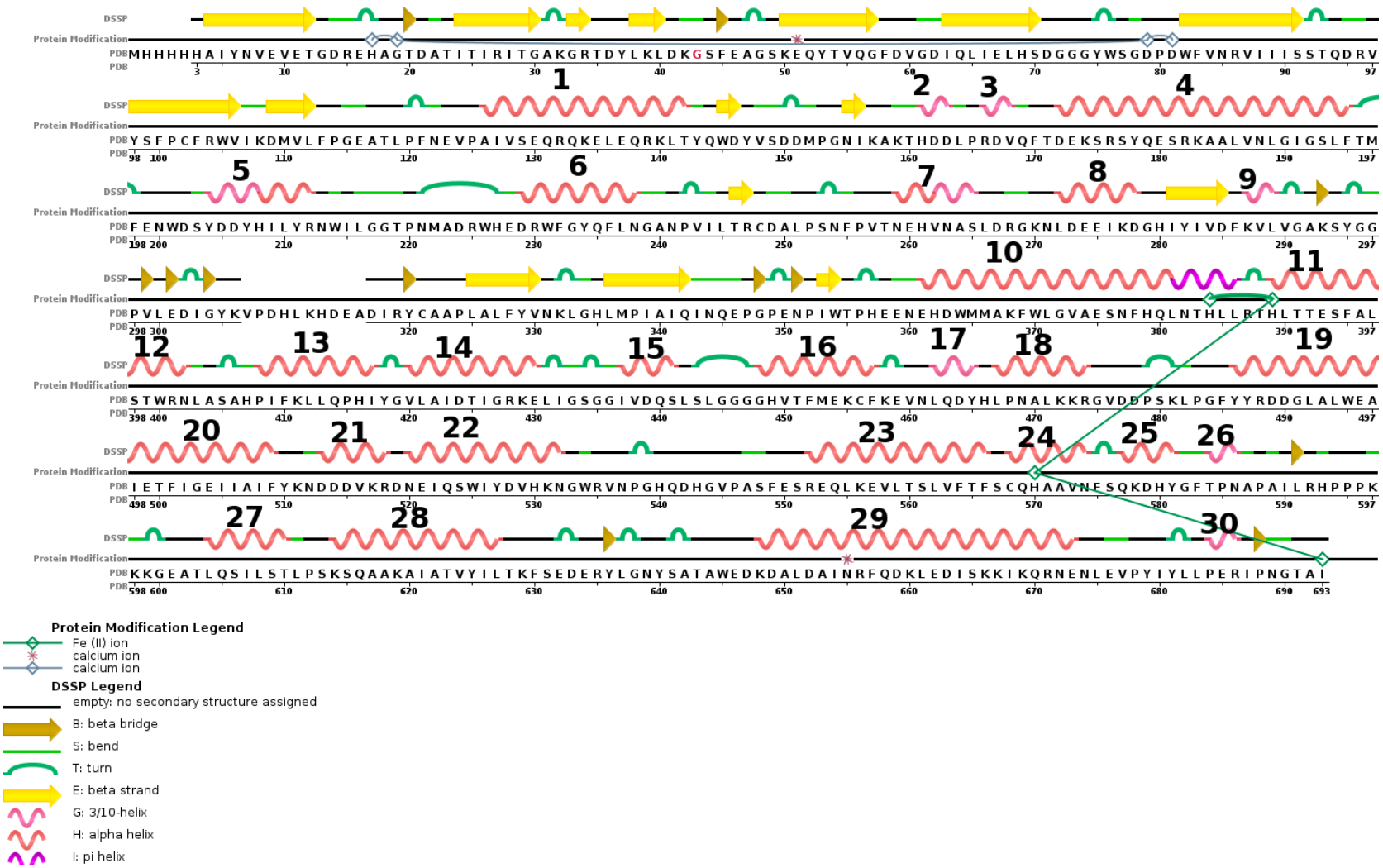
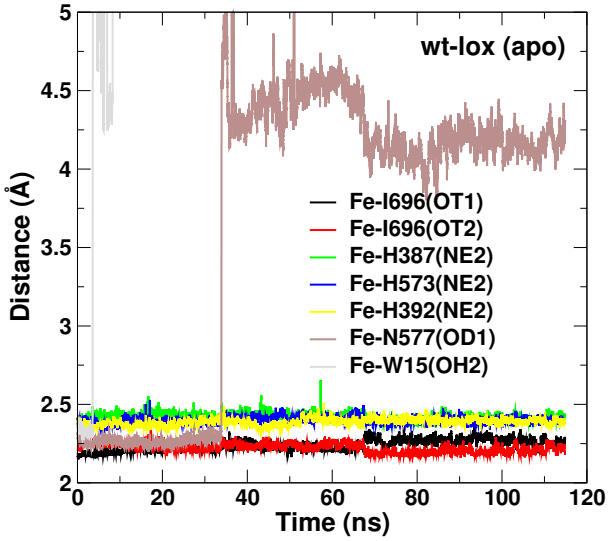
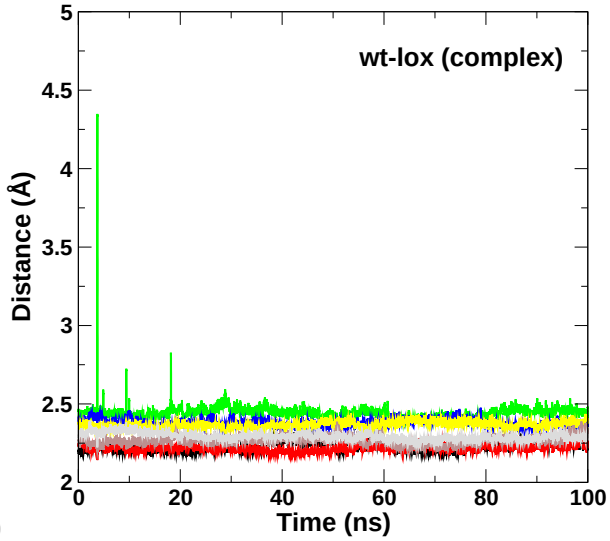


Figure S2: Secondary structure (from DSSP algorithm) of the X-ray crystal structure of 8-lipoxygenase obtained from the protein data bank (PDBID 4QWT). Helix numbers are indicated in the figure.



(a)



(b)

Figure S3: Distance of the active site residues from Fe atom during the MD simulation of wt-lox (a) in the absence of substrate ACD and (b) in its presence.

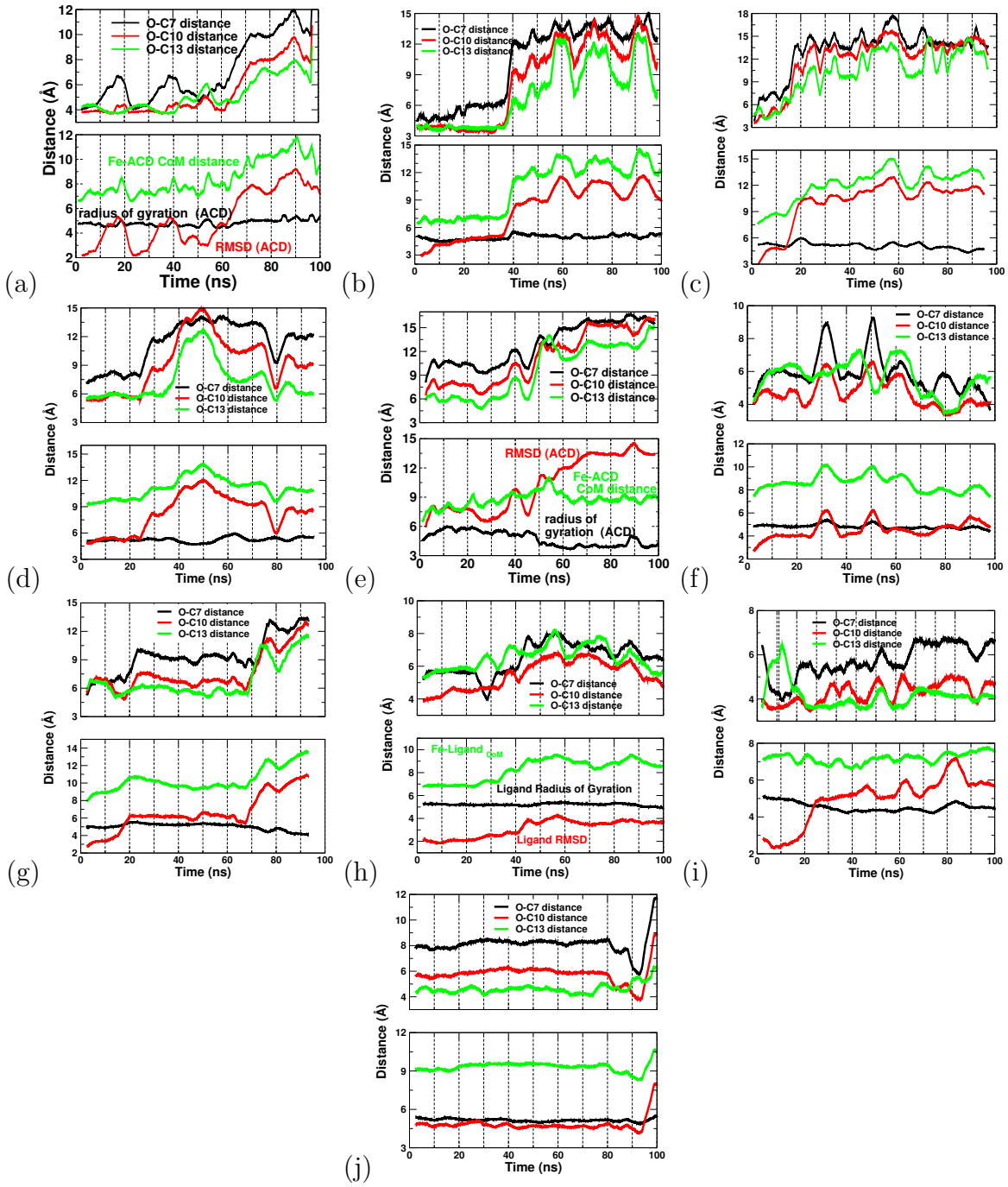


Figure S4: Time evolution of distances of the double allylic carbons (C7, C10, and C13) of ACD from the metal coordinated catalytic active water molecule (upper panel); time evolution of radius of gyration of ACD, RMSD of ACD, and distance between the center of mass of ACD from Fe (lower panel) for a trajectory of wt-lox (a), three trajectories of R185A mutant (b-d), three trajectories for A592M mutant (e-g), and three trajectories of A623H mutant of lox (h-j).

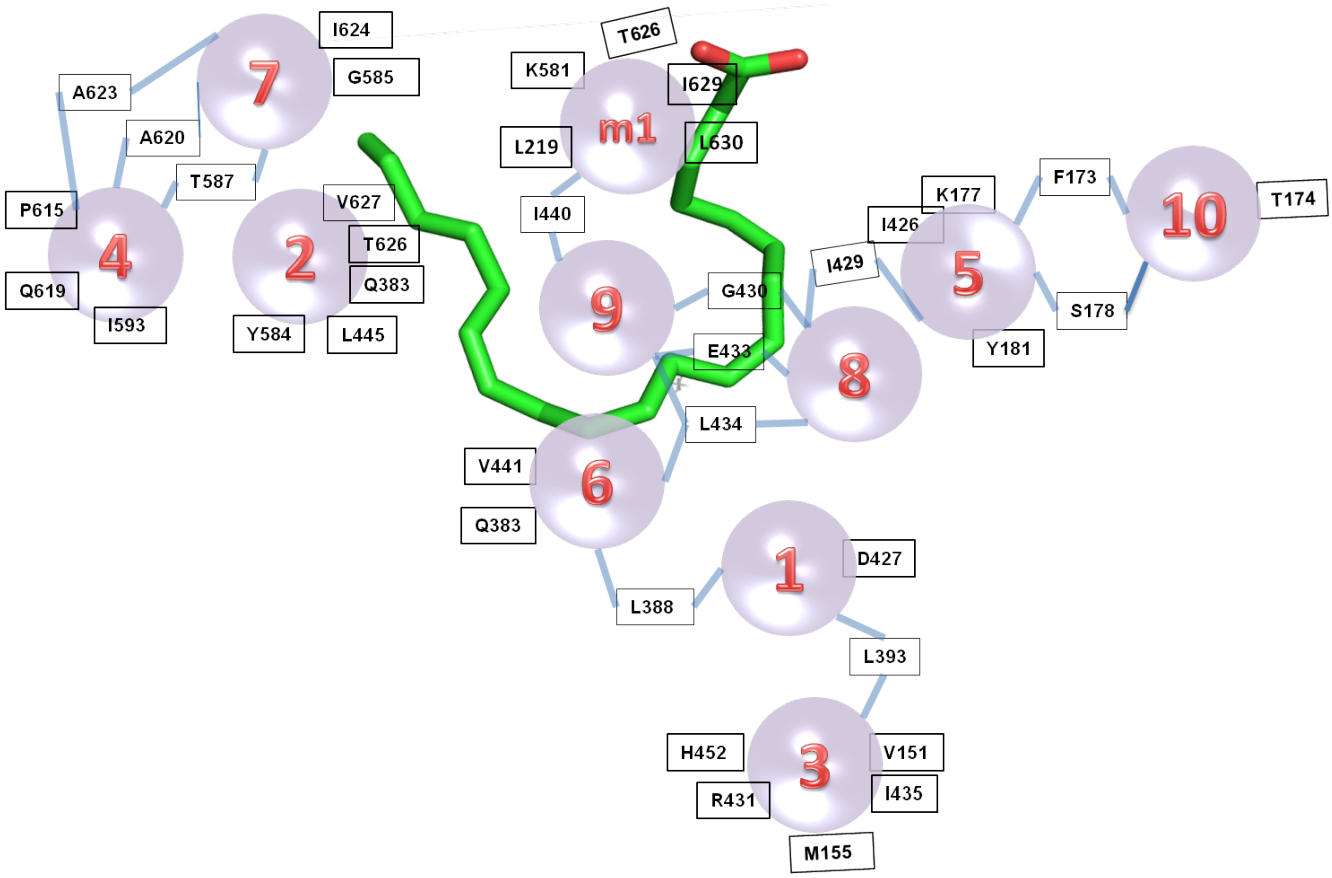


Figure S5: Residues defining the oxygen pockets in 8-lox. The substrate ACD (shown as sticks) is overlaid to indicate the relative positions of the oxygen pockets.

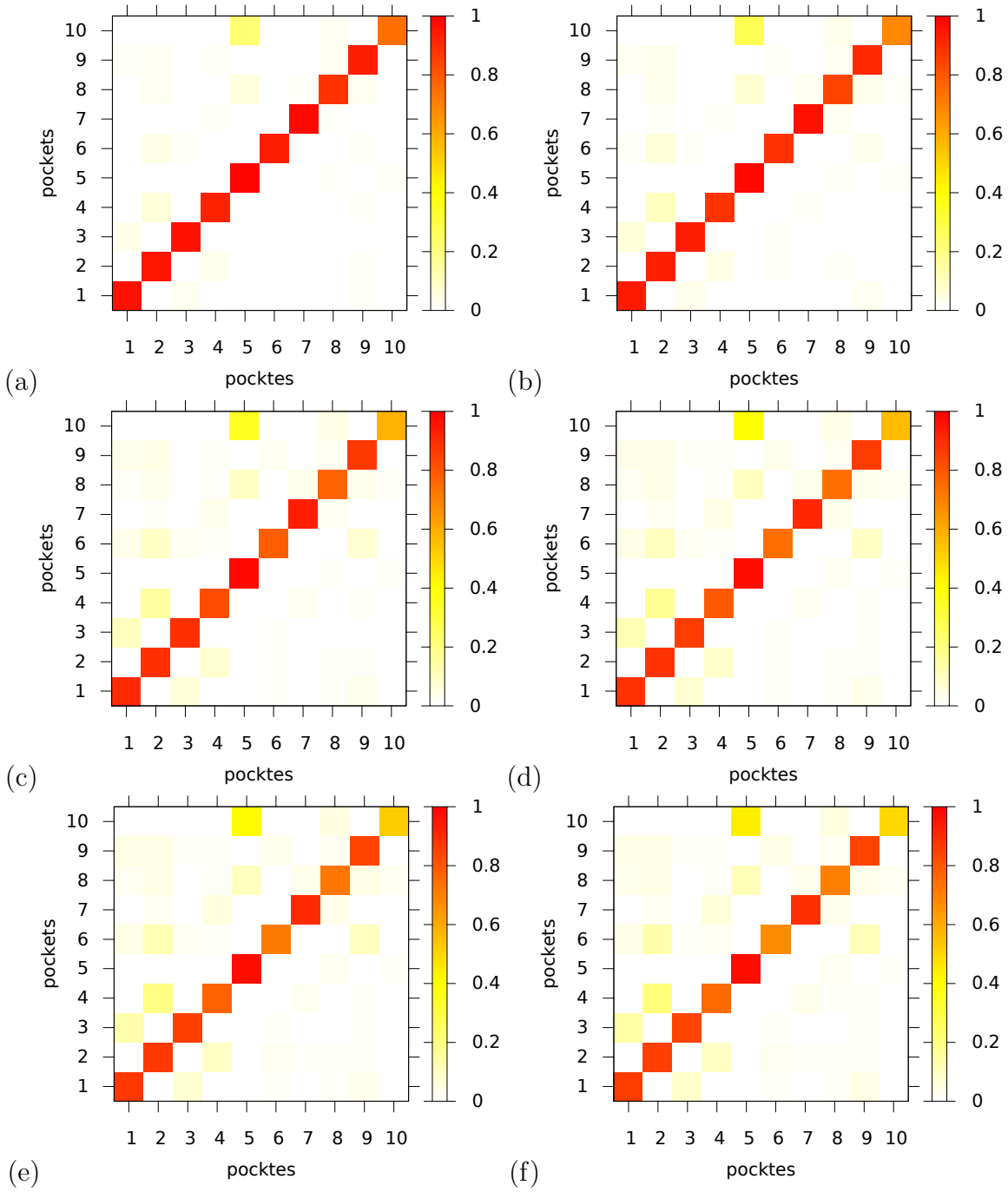


Figure S6: The transition matrices for oxygen migration between the pockets of wt-lox for lag times of (a-f) 10, 20, 50, 100, 200, 500 ps, respectively.

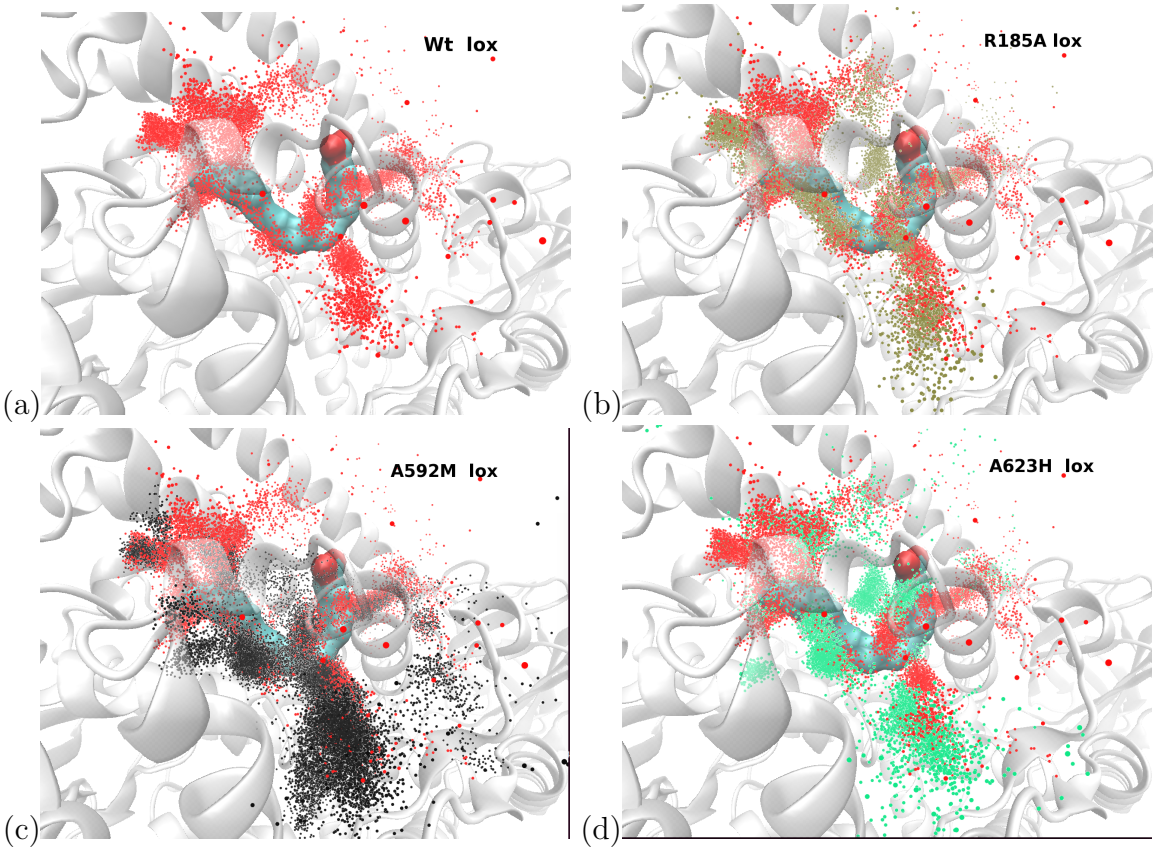


Figure S7: Distribution of oxygen (shown as dots) in wt-lox and mutants. The protein (as cartoon) and substrate (as sphere) are shown in their X-ray crystal structure conformation. The distribution of oxygen in wt-lox is also shown in the figures for mutants for comparison.

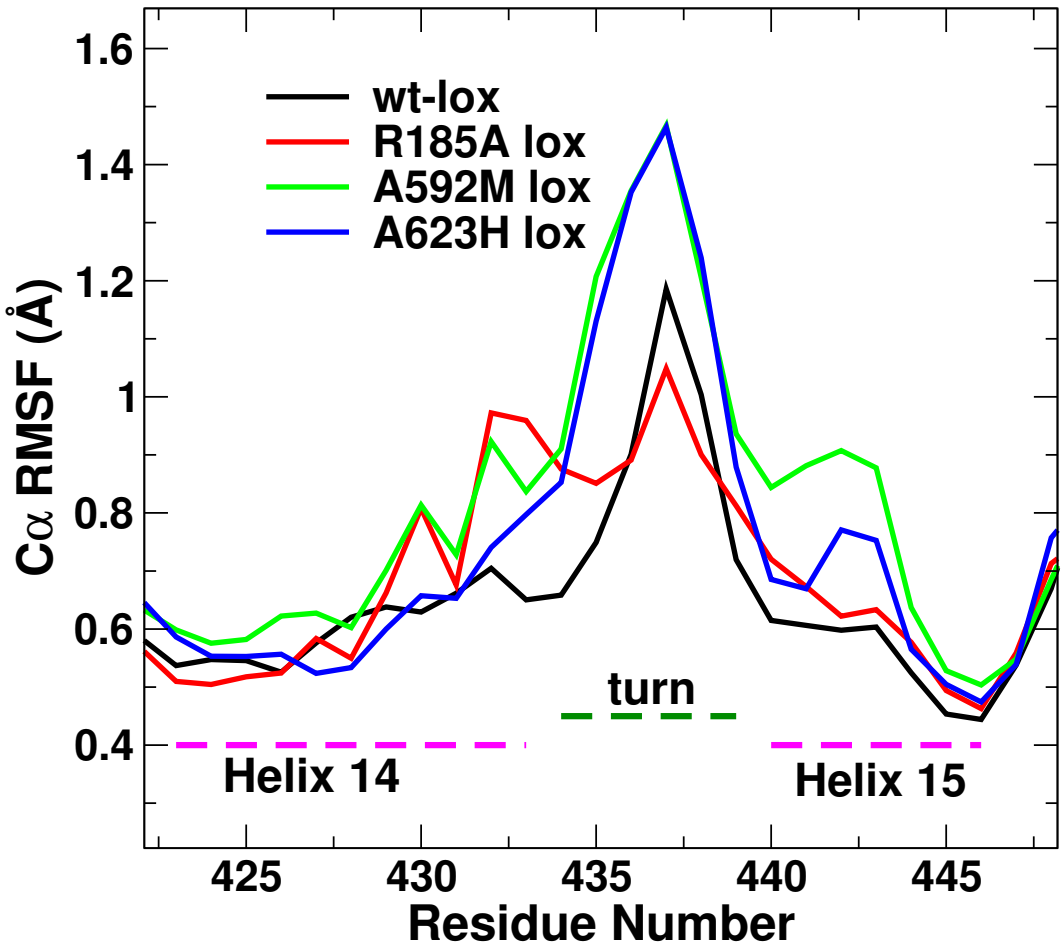


Figure S8: Root mean squared fluctuation of residues of helices 14 and 15, and the turn between them. Greater RMSF of helix 15 in A592M and A623H mutants are due to repulsion between bulkier residues at 592 and 623 positions and the helix 15 as well as the turn between helices 14 and 15.

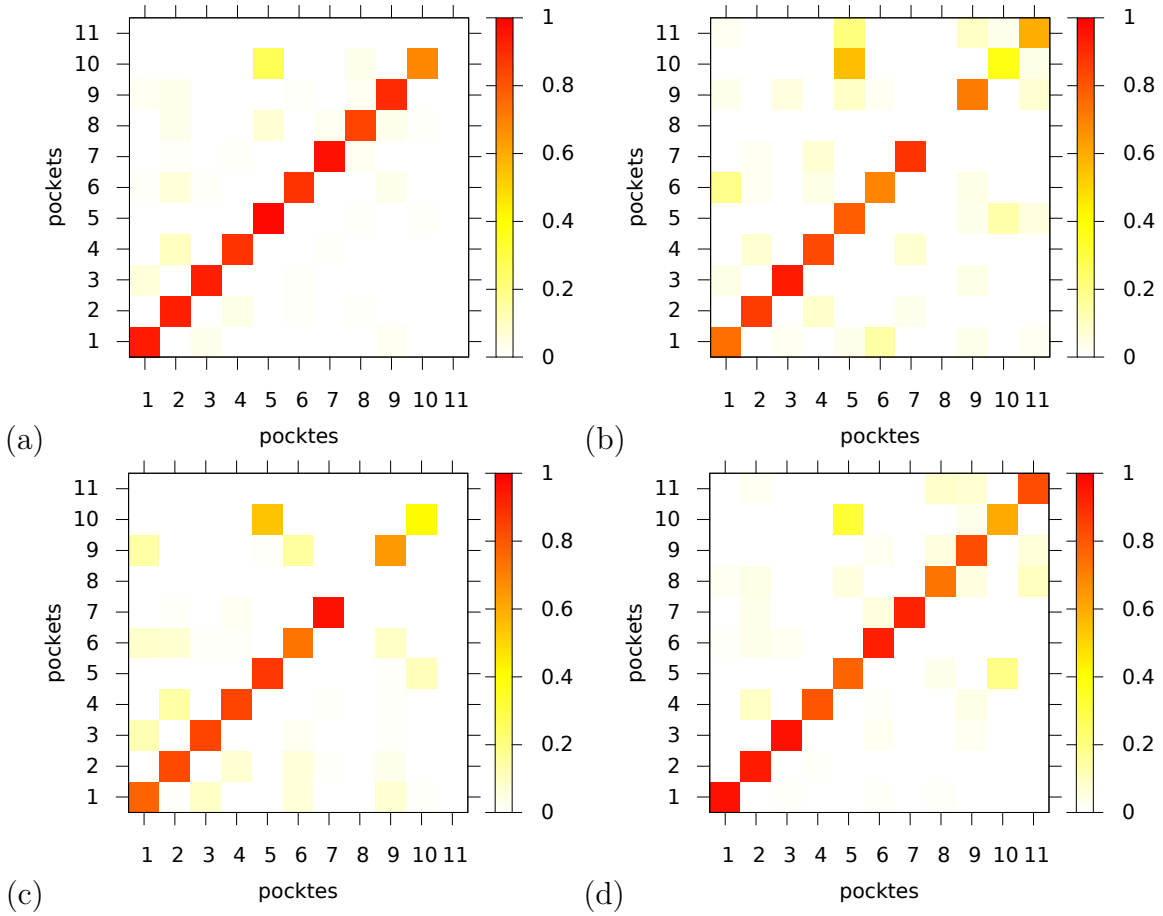


Figure S9: The transition matrices for oxygen migration between the pockets of (a) wt-lox, (b) R185A, (c) A592M, and (d) A623H mutants for lag time of 50 ps. The pocket m1, observed only in R185A and A623H mutants, is denoted as 11 in the figure.

Table S2: Transition matrix of oxygen migration wt-lox and mutants.

Pockets	1	2	3	4	5	6	7	8	9	10	m1
					wt-lox						
$\tau = 10$ ps											
1	0.96	0	0.02	0	0	0	0	0	0.01	0	0
2	0	0.95	0	0.03	0	0	0	0	0.01	0	0
3	0.03	0	0.96	0	0	0	0	0	0	0	0
4	0	0.06	0	0.92	0	0	0	0	0.01	0	0
5	0	0	0	0	0.99	0	0	0.01	0	0.01	0
6	0	0.04	0.01	0	0	0.93	0	0	0.01	0	0
7	0	0	0	0.01	0	0	0.98	0.01	0	0	0
8	0	0.02	0	0	0.05	0	0.01	0.88	0.02	0	0
9	0.01	0.02	0	0.01	0	0	0	0.02	0.93	0	0
10	0	0	0	0	0.22	0	0	0.02	0	0.74	0
m1	0	0	0	0	0	0	0	0	0	0	0
$\tau = 50$ ps											
1	0.94	0	0.03	0	0	0	0	0	0.02	0	0
2	0	0.93	0	0.04	0	0.01	0	0.01	0.01	0	0
3	0.06	0	0.93	0	0	0.01	0	0	0	0	0
4	0	0.10	0	0.88	0	0	0.01	0	0.01	0	0
5	0	0	0	0	0.98	0	0	0.01	0	0.01	0
6	0.01	0.06	0.01	0	0	0.88	0	0	0.03	0	0
7	0	0.01	0	0.01	0	0	0.96	0.02	0	0	0
8	0.01	0.03	0	0	0.07	0	0.02	0.84	0.03	0.01	0
9	0.02	0.03	0	0.01	0	0.01	0	0.02	0.90	0	0
10	0.01	0	0	0	0.27	0	0	0.03	0	0.68	0
m1	0	0	0	0	0	0	0	0	0	0	0
					R185A lox						

Table S2: Transition matrix of oxygen migration wt-lox and mutants.

Pockets	1	2	3	4	5	6	7	8	9	10	m1
$\tau = 10$ ps											
1	0.82	0	0.01	0	0.02	0.08	0	0	0.03	0	0
2	0	0.89	0	0.07	0	0	0.02	0	0	0	0
3	0.06	0	0.96	0	0	0	0	0	0.03	0	0
4	0	0.06	0	0.88	0	0	0.05	0	0	0	0
5	0	0	0	0	0.84	0	0	0	0.03	0.10	0.03
6	0.11	0.01	0	0	0	0.82	0	0	0.04	0	0
7	0	0.01	0	0.05	0	0	0.92	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0.02	0	0.04	0	0.07	0.03	0	0	0.77	0	0.07
10	0	0	0	0	0.44	0	0	0	0	0.54	0
m1	0.02	0	0	0	0.13	0	0	0	0.08	0	0.71
$\tau = 50$ ps											
1	0.74	0	0.02	0	0.03	0.14	0	0	0.03	0	0.02
2	0	0.86	0	0.08	0	0	0.03	0	0	0	0
3	0.04	0	0.94	0	0	0	0	0	0.04	0	0
4	0	0.07	0	0.83	0	0	0.07	0	0	0	0
5	0	0	0	0	0.78	0	0	0	0.03	0.13	0.05
6	0.18	0.02	0	0.04	0	0.69	0	0	0.04	0	0
7	0	0.02	0	0.07	0	0	0.88	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0.03	0	0.05	0	0.09	0.02	0	0	0.71	0	0.07
10	0	0	0	0	0.55	0	0	0	0	0.37	0.04
m1	0.02	0	0	0	0.20	0	0.06	0	0.09	0.03	0.59
A592M lox											
$\tau = 10$ ps											

Table S2: Transition matrix of oxygen migration wt-lox and mutants.

Pockets	1	2	3	4	5	6	7	8	9	10	m1
1	0.88	0	0.04	0	0	0.02	0	0	0.05	0	0
2	0	0.87	0	0.07	0	0.04	0	0	0	0	0
3	0.05	0	0.93	0	0	0	0	0	0	0	0
4	0	0.12	0	0.86	0	0	0.01	0	0	0	0
5	0	0	0	0	0.91	0	0	0	0	0.09	0
6	0.02	0.04	0	0	0	0.85	0	0	0.07	0	0
7	0	0.01	0	0.02	0	0	0.97	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0.11	0.04	0	0	0.01	0.11	0	0	0.73	0	0
10	0	0	0	0	0.42	0	0	0	0	0.55	0
m1	0	0	0	0	0	0	0	0	0	0	0
$\tau = 50$ ps											
1	0.77	0.01	0.09	0	0	0.06	0	0	0.07	0.01	0
2	0	0.83	0	0.07	0	0.06	0.01	0	0.03	0	0
3	0.12	0	0.84	0	0	0.02	0	0	0.01	0	0
4	0	0.14	0	0.84	0	0	0.01	0	0.01	0	0
5	0	0	0	0	0.87	0	0	0	0.01	0.11	0
6	0.08	0.07	0.01	0.01	0	0.73	0	0	0.09	0	0
7	0	0.01	0	0.02	0	0	0.96	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0.14	0	0	0	0.01	0.15	0	0	0.64	0	0
10	0	0	0	0	0.54	0	0	0	0	0.41	0
m1	0	0	0	0	0	0	0	0	0	0	0
A623H lox											
$\tau = 10$ ps											
1	0.97	0	0.01	0	0	0.01	0	0.01	0.01	0	0

Table S2: Transition matrix of oxygen migration wt-lox and mutants.

Pockets	1	2	3	4	5	6	7	8	9	10	m1
2	0	0.97	0	0	0	0	0	0	0	0	0
3	0.01	0	0.97	0	0	0	0	0	0	0	0
4	0	0.05	0	0.88	0	0	0	0	0	0	0.02
5	0	0	0	0	0.85	0	0	0.03	0	0.12	0
6	0	0	0.01	0	0	0.96	0	0	0.01	0	0
7	0	0.03	0	0	0	0	0.96	0	0	0	0
8	0.02	0	0	0	0.03	0	0	0.79	0.04	0	0.09
9	0	0	0.01	0	0	0	0	0.03	0.88	0	0.05
10	0	0	0	0	0.21	0	0	0	0.01	0.76	0
m1	0	0	0	0	0	0	0	0.06	0.05	0	0.87
$\tau = 50$ ps											
1	0.96	0	0.01	0	0	0.01	0	0.01	0	0	0
2	0	0.94	0	0.01	0	0	0	0	0	0	0
3	0	0	0.96	0	0	0.02	0	0	0.02	0	0
4	0	0.09	0	0.80	0	0.01	0	0	0.04	0	0
5	0	0	0	0	0.77	0	0	0.03	0	0.19	0
6	0.01	0.03	0.02	0	0	0.93	0	0	0	0	0
7	0	0.03	0	0	0	0.05	0.92	0	0	0	0
8	0.02	0.04	0	0	0.05	0	0	0.73	0.05	0	0.10
9	0	0	0	0	0	0.02	0	0.05	0.82	0	0.06
10	0	0	0	0	0.32	0	0	0	0.03	0.60	0
m1	0	0.02	0	0	0	0	0	0.08	0.07	0	0.82