

Kinetic Detection of Orthogonal Protein and Chemical Coordinates in Enzyme Catalysis: Double Mutants of Soybean Lipoxygenase

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Supporting Information

Table S1. Empirical rate constant for Ile553Ala/Leu754Ala SLO-1 with HLA in 0.1 M borate, pH 9.0

Temperature, °C	k_{cat} , s ⁻¹	k_{cat} error	K_{M} , μM	K_{M} error
5	0.16028	0.007316	9.08	1.30
10	0.21550	0.013856	6.99	1.37
15	0.26156	0.014112	6.61	1.11
20	0.31848	0.013792	8.62	1.02
25	0.41816	0.020800	6.70	1.08
30	0.55739	0.026453	12.3	1.63
35	0.60702	0.027420	11.8	1.48
40	0.72668	0.036991	10.7	1.61
45	0.81505	0.033855	10.9	1.38
50	0.97010	0.066138	13.6	2.07

Table S2. Empirical rate constant for Ile553Ala/Leu754Ala SLO-1 with DLA in 0.1 M borate, pH 9.0

Temperature, °C	k_{cat} , s ⁻¹	k_{cat} error	K_{M} , μM	K_{M} error
15	0.002446	0.000163	10.1	1.50
20	0.003077	0.000196	9.01	1.40
25	0.004460	0.000179	9.43	1.37
30	0.006553	0.000313	19.9	1.60
35	0.007664	0.000444	18.9	4.15
40	0.011180	0.000778	20.2	4.50
45	0.012617	0.000778	24.1	2.10

Table S3. Empirical rate constant for WT SLO-1 in 0.1 M CHES, pH 9.0 at 20 °C, from viscogen studies.

Glucose % (wt)	k_{cat} , s ⁻¹	k_{cat} error	K_{M} , μM	K_{M} error
0	209	11	28	4
8	218	10	31	5
14	227	11	33	4
21.5	226	12	32	4
26	260	15	37	5
30	257	17	38	5

Table S4. Empirical rate constant for Ile553Ala/Leu754Ala SLO-1 in 0.1 M CHES, pH 9.0 at 20 °C, from viscogen studies.

Glucose % (wt)	k_{cat} , s ⁻¹	k_{cat} error	K_{M} , μM	K_{M} error
0	0.55618	0.019914	22.6	1.5
8	0.47901	0.068112	22	5.8
14	0.43416	0.032469	18.6	2.9
21.5	0.37811	0.041418	14.6	3.2
30	0.32074	0.019515	10.1	1.7

Table S5. Empirical rate constant for Leu546Ala/Leu754Ala SLO-1 in 0.1 M CHES, pH 9.0 at 20 °C, from viscogen studies.

Glucose % (wt)	k_{cat} , s ⁻¹	k_{cat} error	K_{M} , μM	K_{M} error
0	0.014074	0.001155	9.59	2.06
8	0.014468	0.001901	11.24	3.64
14	0.014412	0.001537	11.15	2.94
21.5	0.016624	0.001532	11.49	2.57
30	0.016572	0.003797	13.32	6.29

Figure S1. Calibration curve for HPOD signal at 234 nm in HPLC assay showing linearity of signal with concentration of HPOD. A 100 μ L of HPOD aliquot of varying concentrations were loaded onto the HPLC analytical column (Phenomenex, Luna 5 micron).

