

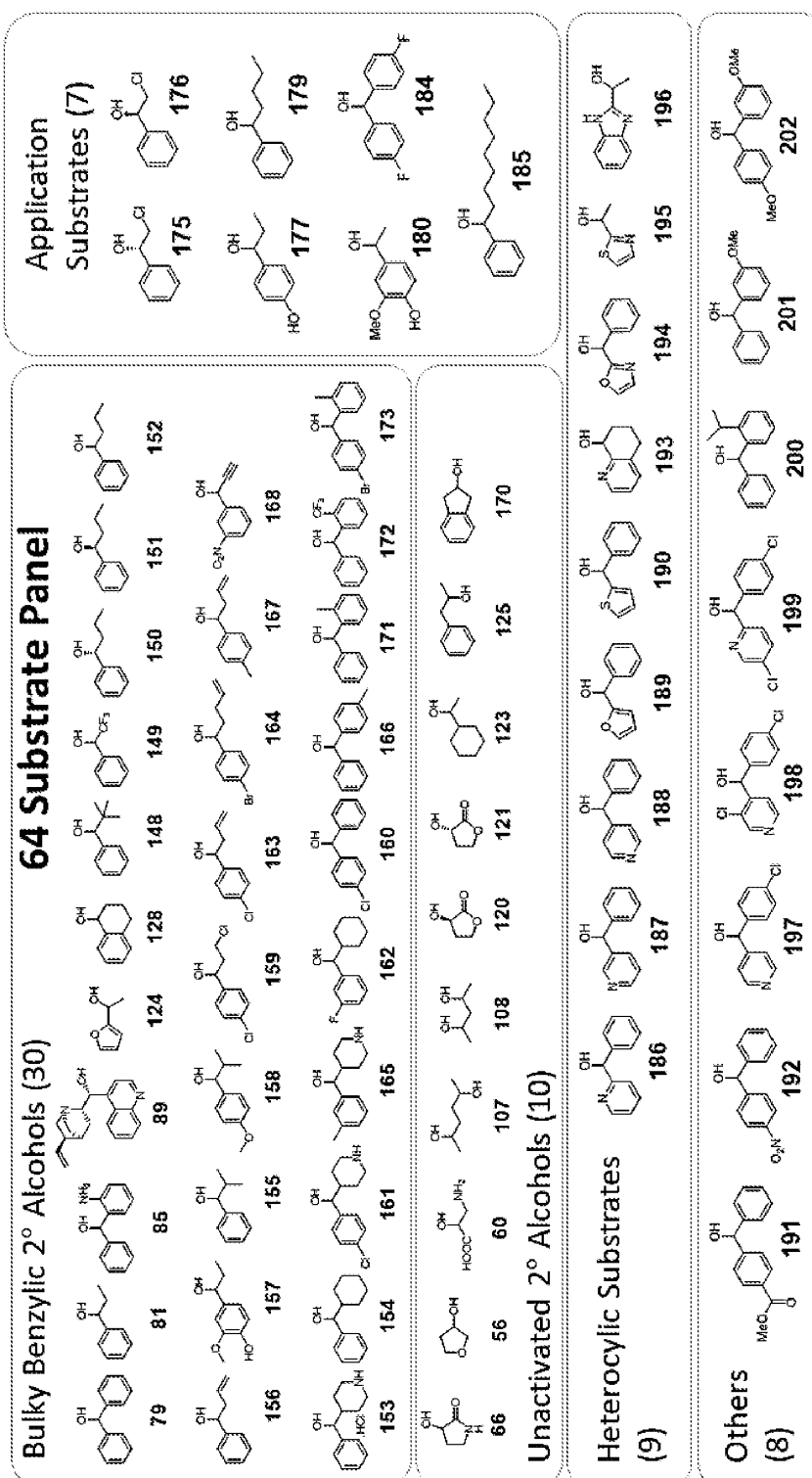
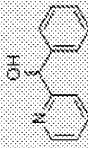
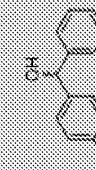


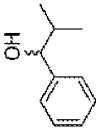
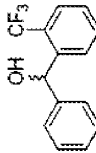
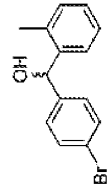
Fig. 1

Selected Top Variant Examples

Substrate	Name	Mutation vs M3-5	Activity Fold vs M3-5*
 190	GOh2044	F217A, N268W, A24H	14,700
 186	GOh2012	F217A, N268W, A195T	14,400
 79	GOh2012	F217A, N268W, A195T	9,700
 184	GOh2044	F217A, N268W, A24H	8,600
 160	GOh2070	F217A, N268W, D239E	7,400

* rounded to the nearest 100

Fig. 2

Mutant List	HPLC Yields		
Backbone = M3-5			
GOh1052 – F217A + N268W	155	172	173
Backbone = GOh1052			
- GOh2012 – A195T - GOh2027 – Y352W - GOh2037 – F487R - GOh2039 – E218K - GOh3001 – A195T, Y352W	GOh3001 – 10% GOh2012 – 5% GOh2027 – 5% GOh2039 – 5%	GOh3001 – 35% GOh2037 – 25% GOh2039 – 19% GOh2012 – 18% GOh2027 – 18%	GOh3001 – 95% GOh2039 – 90% GOh2027 – 87%
	Benchmark		
	GOh1052 – 4%	GOh1052 – 14%	GOh1052 – 75%

Conditions: Purified protein (50 μL, 400 μg/mL), 5 μL HRP (1 mg/mL), 5% DMSO v/v, 1 mM substrate, 25 °C, 220 rpm, topped up to 200 μL with 100 mM sodium phosphate (pH 7.0) buffer (in 2 mL HPLC vials), 24 hours.

Fig. 3

List of Best Mutants

Variant	Mutation	Comment	Activity Fold Change*	Residual Activity*	Relative Expression	Soluble/ Insoluble
GOh1052	Backbone	F217A, N268W from M3-5	1.00	56%	1.00	0.43
GOh2001	A195S	Generalist activity, Best relaxed enantioselectivity	1.24	39%	0.68	0.45
GOh2008	N259T	Generalist activity	1.12	84%	0.68	0.41
GOh2012	A195T	Generalist activity	1.80	3%	0.95	0.50
GOh2044	A24H	Good activity	2.00	74%	0.69	0.25
GOh2081	A195V	Good activity	2.04	19%	1.15	0.80
GOh2082	E218F	Best 25 °C activity	2.68	78%	1.03	1.24
GOh3001	A195T, Y352W	Good activity, stability, solubility	2.00	3%	0.66	0.62
GOh3002	N341W, Y352T	Best expression	1.21	66%	10.09	0.43
GOh3006	K389I, I28R	Best 40 °C activity, Best thermostability	2.00	92%	0.93	0.34
GOh3013	I28R, A195T, N259T, K389I	Good activity, solubility	2.36	57%	0.97	0.99
GOh3023	I28R, A195T, N341W, K389I	Good activity, Best solubility	2.16	52%	0.45	2.01
GOh3026	I28R, A195T, N259T, T447A	Good activity, solubility	2.28	58%	0.35	0.61
GOh3038	I28R, A195T, N259T, N341W, K389I	Good activity	2.24	62%	0.59	0.42

*Measured with substrate 152 (1-phenyl-1-butanol). Activity measured by high performance liquid chromatography (HPLC) conversions.

Fig. 4

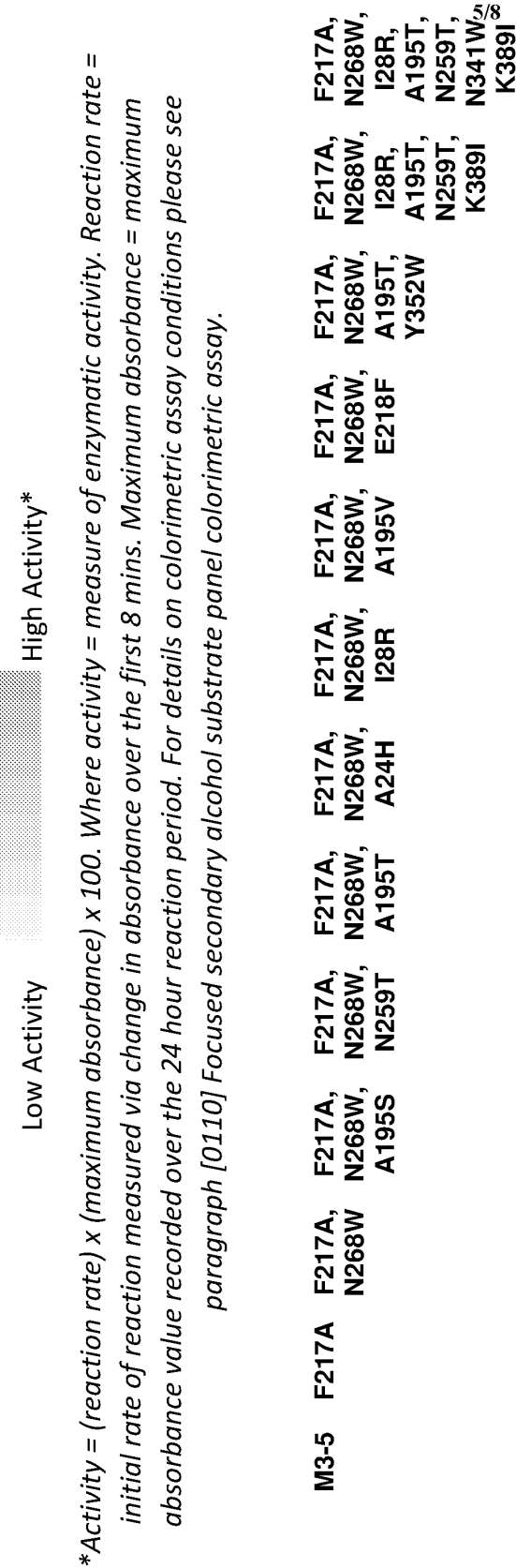


Fig. 5

158	0.5	0.2	0.5	2	3	1.1	0.7	1.3	1.6	4.2	1	1.6	3.9
159	5.5	793.8	2550.6	3613.4	3550	1802.9	4000.3	3872.4	4093.6	6913.9	3910.2	7088.9	7541.2
164	268.2	996.4	2993.4	3360.9	2560.3	3810.9	4089.3	3865.2	4140	5015.2	4459	5081.8	5406.2
167	63.6	37.5	352	499.7	1135.1	343.1	783.9	846.8	657.4	1194.4	834.3	1267.1	1301
168	6306.8	124.1	5769.1	2230.2	142.6	1159.9	36271.7	33071.3	39774.4	1465.7	1856.2	42874.7	1042.1
153	0.4	3.1	1.7	3.3	14	4.4	3.1	4.8	3.8	2.7	4.9	6.7	7.6
154	0.4	356.3	203.8	411.5	719.4	564.2	253.9	333.9	312.6	564.3	473.4	635.9	690
160	1.5	6921.1	5884.1	5996.2	6529.3	6393.3	8582.4	6469.7	10517	14582.4	3621.3	11659.1	11204.9
161	0.5	0	0	0.7	0.5	1.3	0	0	2.4	1.5	5.4	0.9	1.1
165	0.4	0.1	0.2	0.1	0.2	0.1	0	0	0	0	0.1	0.1	0.1
166	0.8	2451.3	2773.5	3860.5	4051.5	4768.5	4569.2	4065.2	4038.3	5766	4555.5	6200.7	6414.2
155	0.7	2	1.8	4.5	16.2	2.8	0.9	1.4	0.4	4.7	0.8	1.5	1.3
162	0.5	0.1	0.3	1.3	1.1	2	0.7	0.5	0.2	0.3	0.4	0.1	0.4
163	48.6	57.2	102.7	1387.8	695.1	2524.1	2611.6	2473.1	2847.8	4059	1522.8	5024.6	5292.5
173	0.7	267.7	127.5	198.7	158.4	214.8	169.7	198.6	256.5	300.6	361.1	351.8	360
171	0.4	872.3	593.3	726.4	813.8	700.1	705.4	811.1	735.7	1337.3	1422.9	1319	1362.9
172	0.4	6.4	15	18.3	17.5	27.1	21.6	29.4	15.5	49.4	13.1	60.5	76.6
128	2.8	287.3	43.8	899.6	569.5	307.7	570.7	679.9	718.6	1038.3	936.8	1209.4	1220.4
66	2.6	3.4	0.7	4.5	10.1	5	9	12.4	4.2	2.6	3.9	21.7	24.7
56	26.3	1.2	1.3	0.8	0.7	0.4	0.6	1.1	1.2	1.5	0.6	1.2	1.5
60	3.1	0.2	0.1	0.1	0.1	0	0	0	0	0.1	0.1	0.3	0.1
107	28	3	3.2	3.9	5.9	2.8	5.2	7.2	7.2	16.5	5.6	11.7	8.5
108	4	0.5	0.5	1	1.5	0.6	1.7	2.1	2	3.7	1.8	4.7	3.5
120	134.8	4.5	2.9	4.5	8.3	5.9	24.7	29.8	8.9	4.8	14.5	45.8	47.7
121	8.9	25.7	1.3	2	5.1	1.6	13	14.5	3.7	3.7	2.9	14.7	14.7
123	1.2	0.1	0.1	1.4	0.7	0.5	0.4	0.6	0.7	3.9	0.6	1.5	1.1
125	4	0.2	0.3	4.5	3.6	1.3	3	3.2	2.7	2.6	5.7	6	6.3
175	65.6	1132.1	1405.4	909.5	1172.9	1779.9	3190.3	2859.9	2738.6	6201.9	2054.4	3628.2	3975.9

Fig. 5 (cont.)

176	4.2	461.7	451.5	462.7	648	582.3	1468.5	1328.2	1008.2	2028.4	1355	1680.3	1811.6
179	1.7	624.6	1024.1	1073.4	1364	1546.4	1786.4	1724.1	1770.4	2607.9	2673.2	2460.3	2669.2
184	4.3	5196.8	4896.5	5121.9	5486.3	3679.6	36824.6	33113.1	13104.4	37389.4	24662.9	42443.9	42171.6
185	1.6	4.8	14.5	3.2	21.5	4.9	33.7	33	50.2	74.2	56.9	73.8	71.4
186	0.5	5194.2	1611.7	765.6	2709.3	7372.3	5344.8	4562	4651.3	5750.4	3704.2	6055.4	6334.4
187	2.7	5328	3966.2	1610	5294.5	6529.8	7967.2	6943.9	7118.5	14773.3	3666.3	11405.2	11418.3
188	5.6	6390.3	3460	1502	4641	4427.5	7234.5	6234.8	6145.6	13542	3592.1	10572.9	10923.1
189	6.2	7609.5	5197.9	2204.1	6187.6	3579.5	35879.3	31598.7	13079.7	37008.2	2760	39615.5	40537.4
190	2.5	10880.4	4862.3	2125.4	5394.2	2874.3	36573.1	9346.6	12773	34572.6	3285.6	41026	40887.3
191	2.3	4892.3	1997.9	1142.6	2662.7	2075.7	3560.9	3567.3	3066.3	6841.2	5178.7	5490.7	5708.4
192	6.9	9990	4507.4	4446.5	5393.4	3860.9	11662.5	10369.5	10102.2	14326.5	4104.4	12296.6	11545.4
193	121.2	2438.6	1287.4	585	2052	629.1	5473.4	6044.4	5979.5	6157	2120.6	8513.1	7666
194	58.8	8628.4	4889.1	1168.2	6404	5651.1	37926.7	35941.4	12265.6	38840.3	4135.3	41919.5	41775.2
195	562.4	243.7	762.8	114.9	639.4	966.1	1228.8	1240.2	1205.9	2639.1	1740.4	1940.1	2001.9
196	1.6	6.4	68.7	31.8	90	69.1	102.8	85.8	110.1	90.2	117.6	156.2	169.8
197	22.1	4368.8	4339	3389.6	4535	4484.1	10884.4	10398.9	10435.3	33967.2	2193.3	11129.9	10837.7
198	195.1	12524.7	5827.3	3983.6	4587	3742.1	37136.6	35636	11565.5	38413.9	2437.5	42362.3	42404.5
199	10.9	5764.6	4551	3627.8	4396.3	3551.8	34491.3	9491.2	12537.6	37792.8	2546.8	39605.4	40834.8
200	0.1	114.7	8.9	4.9	6	6.3	11.7	17.3	10.2	31.1	35.9	29.8	30.5
201	1.3	6527.6	2450.6	2110.2	2468.3	2617.8	5377.8	5102.6	4278.5	8880.2	4594.8	8114.1	8282.8
202	8	5243.8	3493.1	3560.2	3792.2	3755.9	9088.7	7836	6174.9	12697.9	5104.8	11532	11552.6

Fig. 5 (cont.)

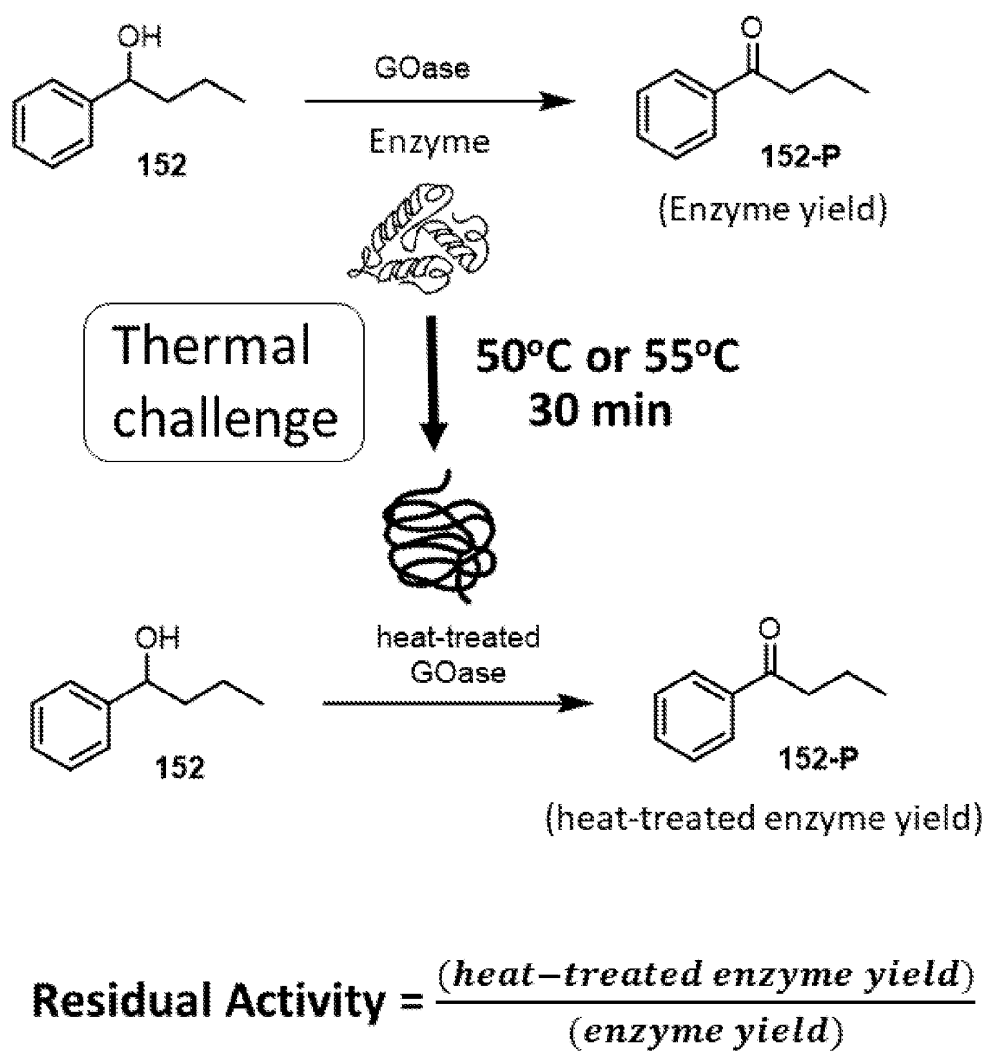


Fig. 6